An investigation of environmental impacts on sediments by marine cage fish farms using long term metadata analysis.

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Declaration

I declare that this thesis has been compiled by myself and is the result of my own investigations. It has not been submitted for any other degree and all sources of information have been duly acknowledged.

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Abstract

Many studies have investigated the impacts of marine cage fish farming on seabed sediments. Most of these studies have focused on organic loading or toxic chemicals used for the treatment of disease, normally for a single or a small number of sites over short time periods. Only very rarely has there been the opportunity to use large data sets consisting of a large number of fish farm sites over a long time scale. In Scotland, localised nutrient impacts have been well documented for marine cage salmon farms, but mixed effects of nutrient and chemicals such as SLICE (the active ingredient of which is emamectin benzoate) have not been investigated in the long term. The aim of this project was to investigate the ecological impacts on sediments from farming activities using very large spatial and temporal data to investigate the long term effects of nutrient and chemical waste.

This was achieved using a metadata set collected from 403 sampling stations at 31 fish farms on the west coast of Scotland over a 9 year period. Data consisted of sediment macrofauna, carbon and nitrogen levels, redox potential, particle size for sediment characterisation and sediment concentrations of SLICE. The data was analysed for trends using statistical and multivariate analysis to look for changes in sediment community and related conditions, and the relationships between these parameters were investigated.

At sampling stations that were less than 50 metres from the sea cages, 72% of the macrofauna communities were correlated with regard to their species composition and abundance. A significant relationship between the concentration of SLICE and sediment characteristics was represented as:

SLICE= 0.000644(median size particle size) + 0.0311*(C %) – 0.00213*(redox potential) + 1.453.*

Annelids were the most sensitive to the presence of emamectin benzoate, with the sipunculid *Phascolion strombi*, the echinoderm *Ophiura affinis*, and the custaceans *Iphinoe*, *Diastylis* and *Iphimedia* also showing sensitivity. During the data period, there was a clear change in species composition associated with improved seabed conditions. This correlated with biomass changes at the relevant sites, where there was a consequent decrease in nutrient input and SLICE usage.

The statistical comparison of the AMBI and ITI indices indicated a 68.9% correlation, but they differed in their ability to indicate levels of organic disturbance. AMBI was shown to correlate more closely with conditions and thus a more reliable index when working with large databases.

Univariate and multivariate analysis indicated that a combination of abundance (N), Shannon Wiener (H') and AMBI, as biological indices for describing the status of the ecological level associated with the carbon percentage and redox potential of sediments gave the most reliable representation of environmental change over a series of sampling stations.

In conclusion, the overall results suggest that, in the long-term, sampling stations which contained significant levels of SLICE had a higher impact status than those affected only by nutrient inputs. The accuracy of multiple regression models were increased by adding biotic and abiotic parameters, though fish biomass at the sites were not

considered be as important factor for the prediction of impacts. However, this model could be sensitive to natural environmental conditions and variations. In light of these results and conclusions, recommendations can be made both for updating the existed environmental regulation of marine fish farms and in the development of meaningful models to relate sediment conditions to accurate estimations of overall environmental impacts.

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1.1 Extensive approach of the environmental impacts caused by aquaculture chemicals. The monitoring processes established by environmental bodies and the interaction of aquaculture biomass with the local ecosystems.

1.1.1 The environmental impacts caused by aquaculture chemicals

As with nearly all forms of aquaculture and agriculture, marine fish farming sites generate considerable amounts of waste including nutrients, waste feed and faeces, and by-products such as chemical residues. The environmental effects of marine cage fish farming are generally most prevalent within close proximity to the cage groups. Consequently, much of the research activity into these impacts has concentrated on the immediate local environment. A number of monitoring strategies to assess the health of the benthos have been proposed by SEPA (Scottish Environment Protection Agency) and existing farms are now regulated and are monitored on a regular basis to evaluate the activity of each site and their risk to the local environment (Wells *et al.*, 2008). From studies that have focused on biodiversity indices of natural habitats and the response of particular species within to certain elevated levels of chemicals found in farm vicinities, it is known that the abundance of some species can be altered by exposure to these chemical treatments (Pfleeger & Zobel, 1995). Copepods are the main impacted phyla (such as the caligids *Lepeophtheirus salmonis* and *Caligus elongatus* in Scotland) due to the action of the parasiticides, eliminating them during fish farm treatments but other phyla are also infected such as haemoflagellates (*e.g. Cryptobia*

salmositica) and microsporidians such as *Loma salmonae*, among others (Guo & Woo, 2009).

It is known that the aquaculture industry produces 110 million tonnes annually (FAO, 2009) but this culture process also exerts effects on the environment. These effects come from various sources; feeding activity is one of the main factors. The impacts caused by aquaculture are shown in organic, nutrient and toxic pollution (Black, 2001). The toxic input from aquaculture is one of the main concerns addressed in this study which will focus on the parasiticides used within farms. The accumulation of toxic substances in the seabed results from the various chemicals that are used by farming companies to ensure the well-being of their fish stocks. These chemicals may include those used for sea lice treatments, growth catalysts (hormones) and enhancers of the immune system (antibiotics). These chemicals are diluted in the water column beneath cages, with some of these chemicals quickly reaching the seabed where they may subsequently accumulate and / or cause enrichment. For those chemical residues that accumulate in the seabed, a potential process of biological degradation can occur and, eventually, pollution resulting (Horst & Walker, 1996). SEPA has the duty to identify possible pollution impacts but their most valuable role is also to regulate the chemicals used by fish farms to avoid potential effects before they occur. Parasite infections, *e.g.* sea lice, on fish farms require immediate handling. Because of the high density of fish within cages, infections can spread rapidly, which if not treated can result in mortalities and financial losses. Sea lice remain as one of the one major health problems in the marine salmonid industry which spans four decades. The main species of sea lice causing problems are: *Lepeophtheirus salmonis* and *Caligus elongatus* in the northern hemisphere, whilst *Caligus teres* and *Caligus rogercresseyi* are a significant

problem in South America. *Lepeophtheirus salmonis* only infects salmonid species and is commonly referred to as the salmon louse. The other species, those belonging to the genus *Caligus* can infect a wide range of marine fish species (SEPA, 1999).

Salmon aquaculture is an important industrial activity in Norway, Scotland, Chile and elsewhere such as Canada *etc*. The continuous infection of sea lice (*L. salmonis* and various *Caligus* species) has led to increased use in parasiticides to control numbers of lice on fish. While pesticides are used to combat sea lice infections, the use of disinfectants also help to prevent the spread of disease agents. These chemicals are commonly incorporated into feed, which are ingested and the drug absorbed and metabolised ultimately reaching the lice that feed upon the fish. These substances contribute to the input of chemical wastes into the environment, which may have a negative impact on human health, the health of the cultured aquatic animals, habitats and indigenous organisms (Rosenthal *et al.*, 1993; Ervik *et al*., 1994; Haya *et al.*, 2001). Those chemicals are used throughout the world and especially in countries with extensive production of fish from farming, such as Scotland, Canada, Greece, *etc*. In Canada and Scotland, the use of chemicals, besides the use of pesticides, include feed additives, chemotherapeutants, and antifouling agents (Zitko, 1994).

The parasiticides are used to decrease or eliminate the populations of the parasitic copepods in the fish farms. The parasiticide impairs the parasites nervous system, by causing paralysis and death. The main categories of compound used are: organo-phosphate based chemicals, hydroxen peroxide, ivermectin, cypermethrin, benzyl-ureas (Treves-Brown, 2000). The sea lice treatments that are commonly used in Scotland are azamethiphos (Salmosan), cypermethrin (Excis), emamectin benzoate

(SLICE), hydrogen peroxide (Salartect, Paramove) and teflubenzuron (Calicide) (SEPA's policies papers). Although, Excis and Calicide were commonly used until fairly recently (Medina, 2004; SEPA, Policy No. 29), SLICE is now the compound that is widely used throughout Scotland (SEPA, Policy No. 30). As these compounds have been developed by the pharmaceutical industry for the treatment of lice, their mode of action has been specified to avoid harm and other potential chemical threats to fish (Treves-Brown, 2000).

The effects of the chemical wastes are mostly causing degradation on local fisheries resources, indigenous species and fish habitats (Kalantzi & Karakassis, 2006). The distribution and fate of many of the aquaculture industry associated chemical wastes are largely unknown. Persistent chemicals may accumulate in sediments, as a consequence of excess feed or faeces sinking to the bottom. According to the industry, recent improvements in feeding technology have significantly decreased the amount of excess feed that falls through the cages (Haya *et al.,* 2001). Less feed per kilogram of fish is required as new feed formulations and real-time video monitoring of feeding behaviour improve feed utilisation (Haya *et al.*, 2001).

The released organic and nutrient enrichment of sediments in fish farms originates primary from fish faeces and uneaten food, which is diluted underneath the fish biomass towards the seabed (Beveridge, 2004). The methods used to assess impacts are those required for an Environmental Impact Assessment (EIA) for each site. These methods were established by SEPA for fish farms, and they include determinations on the percentage of carbon and nitrogen, redox potentials, particle sizing analysis and

macrofauna data along a specified length of the seabed within the immediate vicinities of cages (SEPA, 2000).

1.1.2 The monitoring processes established by environmental bodies

The aquaculture industry is regulated in many countries with the primary objectives of ensuring the protection of the environment and of human health. Within the European Union (EU), environmental impacts are regulated by a variety of European directives and international conventions; these directives and conventions aid the development and implementation of national legislation and regulations within individual countries (Read & Fernandes, 2003). In order to comply with the regulations, it is essential to measure and monitor a variety of ecological factors which include physical, chemical, biological and geological. In Scotland, this monitoring is regulated by a number of governmental and other related organisations, including the Crown Estates, the Scottish Executive Environment and Rural Affairs Department (SEERAD) and SEPA. The existing aquaculture sites must provide data on benthic communities, sediment chemistry and water quality; the degree and rate of sampling is dependent on the biomass (Gillibrand & Turrell, 1997; Cromey *et al.,* 2002; SEPA, 2005). In areas of low dispersion with large biomasses, then more monitoring may be necessary. The methods for assessing sediment quality are not as well developed as those for assessing water quality, however, SEPA have set criteria for sediment quality with values for selected sediment measurements with action levels. Sites typically have redox potential values of less than - 150mV (as a depth average profile) but for sites with values lower than - 125mV (in surface sediments 0 – 3 cm) and total organic carbon levels of 9% or lower,

then these sites are considered to be unpolluted by the environmental quality standards (EQS) (SEPA, 2000).

The term quality criterion, rather than standard, is applied to sediments since the methods for deriving the protective limits are less well established and validated than those for waters. Frequently, the results of chemical analysis for sediment samples will be compared with those for uncontaminated reference sites and indices are set with additional information from EQS as guides (SEPA, 2000).

Univariate analysis is frequently used in ecological research to compare single parameters between sites. For an impact study, it is important to determine the ecological indices at various points around farm sites, these include measurements at the site (0 m), adjacent to it (up to 25 m away) and at distances of up to 100 m away. In SEPA's marine fish farm manual (SEPA, 2000), the methodologies and the evaluation of the indices that are used to determine ecological trends are described in detail. The analysis of quantitative biological data using appropriate numerical and statistical methods is a crucial step in any assessment of data obtained from monitoring. Unprocessed biological data usually consists of matrices containing the abundance of each species (or taxa) at multiple sampling stations, or alternatively they consist of the abundance of species at one sampling station over time. This data requires further analysis to aid interpretation, to simplify presentation and to permit comparison with biological standards. Some of the main biological data analysis techniques are outlined in the marine fish farm manual in detail, notably within the appendices (SEPA, 2000).

1.1.3 The interaction of aquaculture biomass with the local ecosystem

The organic load discharged by cage fish farms consists of uneaten food and faeces which settle on the seabed in the vicinity of the cages. In highly energetic areas, this material may be dispersed and assimilated by the benthic fauna with relatively little detectable accumulation or effects. In lower energy areas, the sea bed may become organically enriched and anoxic causing distortions in the structure of the benthic fauna; microbial films of *Beggiatoa* may develop on the sediment surface (SEPA, 2005). In these situations, the effects may be more intense but cover a smaller surface area (SEPA's fish farming manual, 2000).

Unlike some other effects such as nutrient enrichment, the effects of organic pollution on the sea bed are usually localised., Monitoring, therefore, should focus on the vicinity of the farm and, for this reason, some of the sea bed monitoring can be conducted by the farm operator or his consultants. Small biomass farms in dispersive areas are unlikely to cause problems so a biomass / sensitivity should be prepared to ensure that monitoring effort is targeted where the risk is greatest, *i.e.* at sensitive sites with a large biomass. In this case, the biomass of an individual farm, and its sensitivity is based on the water current speed underneath the farm and the farm operator should determine this and send it to environmental bodies for assessment (the data is usually combined with hydrography data) (Environmental Services, 2007).

Aquaculture wastes include food, excretory products (faecal and urinary) and chemicals, however, aquaculture does not always result in changes in the sediment chemistry or in macrobenthic ecology, the degree of nutrient enrichment depends on a

number of different factors including the species resident in the vicinity of cages, the food being administered, management, currents and depth (Beveridge, 2004). The techniques used to measure and analyse any potential impacts must, therefore also take these factors into account. Read & Fernandes (2003) observed that the best monitoring programme would be one which would give an indication of the environmental status of an area by using sufficient variables and sampling. This monitoring programme would take into account the natural conditions of the area and from that it could assess whether the fish farm has had an impact on the environment. This is important, as some aquatic environments have natural inputs of organic material (*e.g.* leaf litter) or human inputs that are not related to aquaculture (*e.g.* agricultural run-off). If these inputs are not acknowledged, then aquaculture could, unfairly, be blamed for this additional organic loading. If the sites are monitored and sampled against a reference or control site, then as long as the reference site is within the area of the farm under assessment, then hopefully this additional organic loading would be picked up and accounted for (Beveridge, 2004).

1.2 General review of the sea lice problem in Scotland and the parasiticides that are used. The modeling strategies developed for their prediction and fate on the marine sediment.

1.2.1 Sea lice problem in Scotland

Sea lice infection is a common problem in Scottish fish farms but infections are common on wild fish, some of the earliest reports dating back to the 1940's (White,

1940). Depending on the severity of infection, sea lice can exert a range of effects on their hosts ranging from superficial damage to the epithelium on which they feed to larger, deep wounds that can result in mortalities. The industry attempts to address the problem of sea lice through a range of methodical approaches. The main approach is through the use of antiparasitic chemicals, bath or in-feed compounds, which target the lice directly (SP, 2000). While these chemicals are used to relieve infected farmed fish from lice, they can cause effects in the marine ecosystem in terms of water quality and in the number of the species living in habitats in the seabed beneath cages (SEPA, 2000). Modelling the fate and the dispersion of these chemicals is an essential tool, providing information on how to regulate the dose and the treatment period (Perez *et al.*, 2002). Modelling is also used to identify possible changes to the ecosystem, such as increases or decreases in the number of species which reflect eutrophic or oligotrophic conditions beneath cages (Beveridge, 2004).

Investigating the biology and ecology of sea lice is used in dealing with the infection problem properly. The major species infecting wild fish populations are *Lepeophtheirus salmonis, Caligus elongatus, Caligus curtus, Caligus clemensi, Caligus rogercresseyi and Caligus teres* (White, 1940). As for their ecology, the important issues are the time and the environmental conditions in regard to the infection on fish (Johnson *et al.*, 2004). *Lepeophtheirus salmonis* has a wide range of distribution, occurring on salmonids in the North Atlantic and North Pacific. *Lepeophtheirus salmonis* causes infections in populations located on Canada, USA, Japan and Europe (White, 1940). *Lepeophtheirus salmonis* appears to be specific to salmonids; laboratory tests of infections have been reported on Atlantic salmon *Salmo salar,* Pacific salmon, *Oncorhynchus* sp. and on Arctic charr *Salvelinus alpinus. Caligus elongatus,* however,

shows broad host specificity and has been recorded from over 80 marine fish species. This species has a distribution throughout the North Atlantic and infects farmed salmon stocks on the Atlantic coasts of Canada, Ireland, and Scotland. According to Stone *et al.* (1999), Johnson *et al.* (2004), and Krkosek *et al.* (2005) the species that infect farmed Atlantic salmon in Scotland are *L. salmonis* and *C. elongates.* In Canada, another species, *Caligus curtus*, is also known to infect a range of fish species including, occasionally, salmonids (Boxshall & Defaye, 2003). A summary of the main species of sea lice infecting farmed stocks of fish are summarised in Table 1.1.

Table 1.1. A summary of the main species of sea lice infecting farmed stocks of fish (SP, 1998).

Species	**Known range**	**Hosts**
Lepeophtheirus salmonis	North Atlantic and North Pacific affecting farms in Canada, USA, Japan & Europe	Specific to salmonid species *e.g.* Atlantic salmon *Salmo salar*, Pacific salmon, *Onchorhynchus sp*, Arctic charr *Alpinus*
Caligus elongatus	Atlantic coast of Canada, Ireland, Scotland	Not host specific – found on over 80 species of fish including salmonids
Caligus curtus	Atlantic coast of Canada	Not host specific - occasionally found on salmonids
Caligus clemensi	Pacific coast of Canada, USA	Not host specific - occasionally found on salmonids
Caligus rogercresseyi	Pacific coast – Chile	Not host specific - the dominant species found on salmonids in Chile
Caligus teres	Pacific coast – Chile	Occasionally found on salmonids in Chile

The biological issues directly related to the sea lice infections on farmed fish are their life-cycle, reproduction and host locations (Boxshall & Defaye, 2003). Sea lice

have a flattened body design and are equipped with numerous swimming appendages and specialised feeding structures (Images 1 and 2). The life-cycle of *Lepeophtheirus salmonis* consists of three phases as it is presented in Boxshall & Defaye (1993) and can be summarized as follows:

• Free-swimming larval stages – nauplius I, nauplius II and copepodid;
• Immature attached stages – copepodid, chalimus I, II, III and IV;
• Motile, sexually dimorphic stages – pre-adult I, pre-adult II and mature adults.

The life-cycle of *Caligus* is similar although *Caligus* has no pre-adult stages. Interference with chitin synthesis and the disruption of the moulting process plays an important role in some sea lice treatments (*e.g*. Diflubenzuron). The infective copepodid on locating a suitable host, attaches to and grips the host using its maxillipeds, which are used for leverage, while the hooked antennae are driven into the epidermis of the host. Shortly thereafter, the copepodid moults into the first chalimus stage which produces a frontal filament which is used to securely anchor it to the host through the next four, chalimus, stages. The filament is produced through an extension of the cuticle into the epidermis of the fish to produce a firm anchor. The louse moults through four chalimus stages, feeding on the host skin around the filament attachment point (Boxshall & Defaye, 1993). It is the frontal filament which prevents the chalimus stages from being removed during hydrogen peroxide treatments; lice may subsequently recover. Once the lice become pre-adults, they can move freely over the host fish, they are then referred to as motile lice. For *L. salmonis,* the lice tend to remain feeding in one location for some time and, as a result, an oval imprint may often be seen where the lice has been attached. The main mechanism for attachment in motile lice is a sucker-

like seal produced by a thin membrane on the outer margin of the cephalothorax (anterior body section) of the sea lice, aided by the antennae and the sternal furca which dig into the epithelium. The front edge of the louse's carapace may also be wedged under fish scales (Boxshall & Defaye, 1993).

Caligus elongatus tends to show a higher level of activity on the fish than *L. salmonis* and this may, in part, account for the more widespread but less severe lesions seen on fish infected by this species. Both *L. salmonis* and *C. elongatus* can transfer between host fish. As a result other infected farmed fish or wild fish passing through the area may act as a source of infection of *Caligus*. In Scotland, *C. elongatus* is commonly found on wild fish such as saithe, *Pollachius virens,* and herring *Clupea harengus,* and these fish are often found in the vicinity of commercial salmon farms. Male and female lice can be easily distinguished from the first pre-adult stage onwards. As a general rule, the smallest motile stages are pre-adult I males. Pre-adult II males are similar in size to pre-adult I females, and pre-adult II females are similar to adult males. Adult females are the largest stage and, once mature, have a large genital segment where the eggs develop before being extruded as egg strings. Immature adult females, however, have a relatively small genital segment and, for this reason they are sometimes confused with pre-adult females although the cephalothorax is similar in size to that of mature adult females (Boxshall & Defaye, 1993).

Infection of fish depends on the ability of the copepodid to locate a suitable host before its energy reserves are depleted. Copepodids respond positively to light and possibly to areas of low water pressure causing them to aggregate near the surface of the water. This may explain why farmed fish held in pens in the upper layers of the

water are so susceptible to sea lice. Copepodids also respond positively to vibration; this may induce their movement towards actively swimming fish. *Lepeophtheirus salmonis* copepodids respond to non-salmonid hosts when stimulated by fish swimming past, but fail to attach even when contact is made, suggesting that chemical recognition of the host species probably determines whether settlement occurs. Responses to chemical cues produced by host fish in the water, however, have not yet been confirmed (Boxshall & Defaye, 1993).

A number of factors have been suggested which might influence the susceptibility of fish to sea lice. It is also possible that different genetic strains of fish differ in their susceptibility to sea lice. Damage to the host fish is caused by the feeding activity of sea lice. The most damaging stage of *L. salmonis* tends to be the pre-adults, particularly as these concentrate on the head region which has no protective scales. Although adult female *L. salmonis* are the largest stage and can cause more damage, once they become gravid the majority tend to move to a position behind the dorsal and anal fins where damage is less severe and are not so detrimental to fish (Boxshall & Defaye, 1993).

The effects on farmed fish vary from local skin damage (Image 3), a generalized chronic stress response in fish since feeding and attachment cause changes in the mucus consistency, up to damaging the epithelium resulting in loss of blood and fluids, electrolyte changes, and cortisol release (Ross *et al.*, 2000). The seven impacts or levels of damage exerted by lice are described in a study by Ross *et al.* (2000) and could be grouped as follows:

• *Direct damage* – Chalimus may cause localised areas of damage around the attachment point. Feeding activity by adult and pre-adult sea lice results in erosion of the protective mucus and skin layers with the loss of scales. In severe cases, there is haemorrhaging and the underlying muscle may be exposed. Where deep lesions establish, adult lice may feed on host blood.

• *Indirect damage* – Caused by fish scraping or jumping against nets in response to the irritation caused by sea lice.

• *Secondary infections* – The protective layer of mucus may be lost through lice grazing on the skin and this may be associated with secondary bacterial infections. Erosion of the eyes can lead to corneal ulceration and secondary infection causing blindness and cataract formation.

• *Stress* – Reduces immunity and resistance of fish to other infections.

• *Osmoregulatory failure* – Damage to the skin results in changes in the fish's blood chemistry so they are no longer able to maintain salt and water balance with the environment. This also causes stress which may further reduce the resistance of the fish to disease.

• *Loss of appetite and reduced growth* – Fish that are heavily affected by sea lice show a reduced feeding response and, although it has not yet been proven, this may have a direct effect on feed conversion and fish growth rates.

• *Mortality* – May result from any of the above factors. In Scotland alone, the annual cost to the industry through fish mortality is estimated at $7 million. In some cases, mortality may also result from the additional stresses imposed by bath treatments and through accidental overdose, and such losses have been estimated at a further $2.8 million per year.

1.2.2 Parasiticides and impacts on the seabed

The treatments used against sea lice have been examined in various papers regarding their toxic attributes, and both laboratories tests and field sampling projects performed to show if impacts occur in the species after the treatment tests (Davies *et al*., 1997; Roy *et al.*, 2000; Willis & Ling, 2003; Medina *et al.*, 2004). The factors assessed on them are the effects of the treatments directly to the farmed fish (Roy *et al.*, 2000; Velisek *et al.,* 2006b) and to the local ecosystem around the farms, mostly in terms of the local fauna (Pahl & Opitz, 1999; Medina *et al.*, 2004). However, indicative studies made to identify possible overdose effects by using the chemicals in high doses, showed them to be harmless to fish (Davies *et al.*, 1997; Treves-Brown, 2000).

The toxic effects of various parasiticides and their environmental impact, has been studied in two ways: 1) through studies and surveys conducted at farm sites; and, 2) through toxicity tests conducted in the laboratory on copepods and other species. Most of the impacts have presented a potential impact to the non-targeting species, directly associated to the copepods, but also to species not included to the same families, such as lobsters (Pahl & Opitz, 1999).

In a study by Willis & Ling (2003), the exposure of copepods (*Acartia clausi*, *Pseudocalanus elongatus, Temora longicornis* and *Oithona similis*) to cypermethrin caused mortality at concentrations considerably lower (EC50 values ranged from 0.12 μg L^{-1} for P. elongatus nauplii to 232 μg L^{-1} for *O. similis* adults) than the recommended sea lice treatment concentration of 5 mg L^{-1}. Overall, the cyclopoid copepod *Oithona similis* was the most sensitive species with the nauplii being the most

sensitive life stage, with 48 h EC50 values of 0.12 and 0.14 mg L^{-1} for nauplii of *T. longicornis* and *O. similis* respectively. The variation in response between life stages and species may be related to size differences and the mode of action of cypermethrin. In a study by Medina *et al.* (2004), the impacts of cypermethrin on the copepod *Acartia tonsa* were investigated, with the study demonstrating negative impacts on growth. Short-term exposures to cypermethrin (a single dose of cypermethrin was applied to the treatment enclosures 5 days after the experiment was set up) reduced copepod's feeding rates at concentrations well below those affecting egg production rates and survival of eggs and adult stages, and lethal effects on naupliar stages occurred at lower concentrations than any other effect observed on eggs or adults. Life-table sensitivities of the intrinsic rate of increase (r_m) to cypermethrin were similar to those observed in short-term exposures. More specifically, exposure to cypermethrin impaired *r*m responses at concentrations (7.4 ng L^{-1}) that also affected feeding and naupliar responses probably through sublethal effects on feeding.

Toxicity studies on the use of other sea lice treatments have been assessed against other crustacean and copepod species. An investigation by Pahl & Opitz (1999) on the toxicity of azamethiphos on lobster, *Hommarus americanus*, larvae found that the use of the recommended dose for an hour resulted in significant mortalities. Davies *et al.* (1997), investigated the impact of ivermectin on marine organisms and concluded that mussels, such as *Mytilus edulis*, growing in the vicinity of fish farms are unlikely to accumulate detectable concentrations of ivermectin.

The chemotherapeutants most commonly used in Scotland for the treatment of sea lice are azamethiphos (Salmosan), teflubenzuron (Calicide), cypermethrin (EXCIS)

and emamectin benzoate (SLICE). Calicide, an in feed solution, and Excis, a bath based pyrethroid that is toxic to Crustacea but given its high solubility (Medina, 2002), does not pose a serious environmental impact, were until recently, the two most commonly used treatments in Scotland. Salmosan, administered as a bath treatment, is also toxic to Crustacea including lobster, prawn, crab, and shrimp (Beveridge, 2004), but has been shown to have a low level of absorption by the seabed. Salmosan was one of the early compounds granted permission for use (SEPA, 2000).

Emamectin benzoate, the active ingredient of SLICE produced by Schering Plough, after 1999 emerged as the most commonly used sea louse treatment in Scotland. The formulation of SLICE consists of 0.2% emamectin benzoate (*i.e.* 9 parts 4-epimethyamino-4-deoxyavermectin B1a benzoate to < 1 part 4-epimethyamino-4-deoxyavermectin B1b benzoate), 0.01% butylated hydroxyanisole, 2.5% propylene glycol, 47.4% maltodextrin and corn starch (*qs* to 100%). It is recommended that no more than three treatments are given in any 12 calendar month, and no more than five treatments are given in any two year growth cycle. The treatment regime is as follows: SLICE will be fed at a rate of 50 ug per kg of fish per day for seven days. The dose will be administered as SLICE pre-mix coated on to feed. The Environmental Quality Standards (E.Q.S.) for SLICE as given by SEPA is 4 ng/g. SLICE follows the digestion activities of the fish body and is finally excreted in faeces and follows a course towards to the water column and to the seabed. For this reason, SEPA have implemented a series of monitoring strategies to measure the concentration of SLICE in the seabed (SEPA, 1999, 2000; Wells *et al.*, 2008). SEPA has created a protocol, with annual application and farms must measure SLICE in samples that must be taken between 110 and 130 days after the cessation of the SLICE treatment. For assessment, measurements

must be taken at three different stations: one underneath the cages at 0 m, one at a distance of 25 m from the cages and one at a distance of 150 m away from the cages. This strategy was modified in 2007 and the number of measurement stations reduced from three to two, *i.e.* one beneath the cages and one at a distance of 100 m away (SEPA, 2007).

In zooplankton, copepods are the most sensitive to the parasiticides used for the treatment of sea lice, while phytoplankton interacts indirectly with the compounds. Studies made on zooplankton, as presented in the final report ordered by the Department for Environment, Food and Rural Affairs (Defra) and contracted to SAMS, (Scottish Association for Marine Science) (SAMS *et al.*, 2005). A 5-year study found that zooplankton have a low risk of exposure to SLICE administered as a component in salmon feed. The most plausible exposure route is associated with the ingestion of feed and faecal particles. Excretion of SLICE by salmon continues for an extended period post-treatment, exposing the seabed to SLICE-associated particulates over a long period of time (Willis, 2005). The resuspension of freshly deposited material will move SLICE back into the water column for a considerable period post-treatment, making it potentially available for ingestion or absorption by a range of marine organisms. Despite all the sea lice treatments that were administered during the 5-year study, no adverse effects on zooplankton were detected at either the species or community level. Changes observed were naturally occurring, with patchiness in distribution, life history characteristics, and water currents being the most influential factors affecting zooplankton distribution and community composition (SAMS *et al.*, 2005).

Phytoplankton plays an important role in marine ecosystems. Their growth is directly influenced by physical factors such as temperature, salinity, light and nutrients, and they are the first link in the marine food chain. Information on seasonal changes in phytoplankton species and abundance is vital as it allows identification of responses that may be caused by the use of sea lice treatment agents, as opposed to those caused by physical factors such as changing salinity, temperature, light and nutrients, or biological variables such as grazing pressure. Despite differing physical conditions and sea lice treatment histories, the comparison of four sea loch phytoplankton communities (Lochs Sunart, Diabaig, Kishorn and Craignish) revealed a significant similarity of 76% between them (SAMS *et al.,* 2005). In all lochs, the phytoplankton community was typically dominated by a relatively small number of species with many other species present in low numbers at different times of the year. Some of the more common (and bloom forming) species were observed all year round at all sites (small *Chaetoceros* sp., *Skeletonema costatum*, *Gymnodinium* sp., and unidentified cryptophytes). Phytoplankton blooms occurred at a normal frequency and duration for Scottish coastal waters and were caused by species commonly observed at all sites (SAMS *et al.,* 2005).

Long-term zooplankton and phytoplankton sampling campaigns confirmed that sea lice treatments did not alter natural seasonal trends as the processes of species succession and population dynamics were well within the range of what might be expected or predicted for fjordic sea loch systems (SAMS *et al.,* 2005). Similarly, the study of organism settlement on sub-littoral arrays yielded a clear picture of natural seasonal and annual species successions and abundances, beginning with the spring settlement of barnacles each year (SAMS *et al.,* 2005).

1.2.3 Modelling strategies

In order to predict and record environmental impacts, a range of models have been developed. The major models for predicting the fate and the dispersion of parasiticides include GIS software tool (Perez *et al.,* 2002) and DEPOMOD (Cromey *et al.,* 2002). The parameters that are used and interact in a model is an important issue and much consideration is taken in order to distinguish the factors that will not be included in the final model (Ford, 1999). As an example, for a solid waste dispersion model, knowledge on the quantities and composition of wastes and settling velocities is essential (Chen *et al.,* 2003).

In the study of Gillibrand & Turrell (1997), the use of simple models in the regulation of impacts on Scottish sea lochs was investigated. According to the study, simulations of the dispersion of chemicals following treatments for sea lice infections, allowed for regulations on the use of these chemicals to be set which comply with EQS. The models make many simplifying assumptions about the underlying hydrography of sea lochs. They do, however, provide a first estimate of possible effects, and as such, have proven a useful management tool. In another study conducted by Cromey *et al.* (1998), the environmental modelling issue in Scottish sea lochs was addressed in a more mathematical way when compared to that proposed by Gillibrand & Turrell (1997). In Cromey *et al.* (1998), the impacts of particulate organic carbon on marine benthic ecosystems were modelled mathematically. The principal model that was used, BenOss (Biological Effects and Organic Solids Sedimentation), links other various modules as such: particle tracking; a re-suspension module which accounts for both deposition and re-suspension of carbon at the sea bed; a chemical module which

removes organic carbon according to its degradability using the G-model; and a biological module which predicts benthic community structure on the basis of carbon availability. The model predicts the benthic effects of reducing carbon input.

The need for further studies on the dispersion and the fate of the parasiticides used by the Scottish fish farm industry made the development of enhanced software an essential requirement. From the MERAMED project (Carroll *et al.,* 2003), which set out to study the impacts of fish farms in the Mediterranean Sea, a new software tool DEPOMOD was developed. DEPOMOD was used to model the dispersion of waste but then was later modified to model the dispersion of parasiticides in the seabed (Cromey *et al.,* 2002). The function of DEPOMOD is described in detail in Cromey *et al.* (2002). The accumulated solids produced by fish farms and the changes in the benthic fauna community are predicted by DEPOMOD. There are three models in DEPOMOD: 1) a grid generation model; 2) a particle tracking model; and, 3) a re-suspension model. For the first model, the user receives the grid information on depth, cage and the position of the sampling stations within the test area. For the second model, the deposition of particles on the seabed can be predicted with the input of wastage rates of fish food, faeces and the hydrodynamics of the area. The re-suspension model, then calculates the redistribution of particles by near bed current flow fields to predict the net solids accumulated on the seabed within the grid area. The prediction of the impact level on the seabed can be made from the quantitative relationship between solid accumulation and the benthic community descriptors (Cromey *et al.,* 2002).

Another approach to determining the waste distribution beneath farm cages is through the use of a GIS-model (Perez *et al.,* 2002). In this approach, a GIS tool is

combined with a spreadsheet of the data needed to describe the environmental impacts. In brief, the model uses existing distribution algorithms and also incorporates functions to calculate the feed loading for each and every cage within a pontoon, spreads the input load over the whole cage area and simulates post-depositional distribution of the carbon. The model uses approximate estimates of feed and faecal waste (mass balance model) and separate, unique settling velocities for waste feed and faecal particles. Output from the model is in the form of a contour plot of organic carbon showing distribution of the particulate organic carbon material as deposited on the sea-bed.

1.3 Methods used in studying the ecological status of the seabed

1.3.1 General description and background

The methodology of monitoring the seabed is based on measuring the biological parameters in the seabed by unofficially dividing it into two sectors, the sampling and the analysis sectors. During sampling, the biotic and abiotic attributes are collected in order to study the ecosystem by assessing it over a whole spectrum of biological changes. The biotic factor contains the macrofauna (species caught in the seabed by dragging samplers) whilst the abiotic factor contains the physico-chemical parameters of the sampling area within which the macrofauna were collected *e.g.* the redox potential, carbon and nutrient percentage, particle size, *etc* (SEPA Fish Farm Manual, 2000).

After the measurements are typed into spreadsheets, they are compared with EQS directly or are subjected to further analysis with the help of statistical programs. The results are in various forms, according to the software used, but are either results from univariate and / or multivariate statistical tests, from simple and more complex multiple regressions.

1.3.2 Statistical approach using biological indices and their results

The biological indices are a pack of ecological tools used to determine the environmental impacts in various farm areas. These indices are used to give information about the biotic activity and situation of the farmed seabed sites with a particular emphasis on the trophic processing and scaling of the macrofauna. The results from these indices are presented as simple values without units but in a scale of exact numbers that are part of a complete numeric formulation that classify the effects into different levels (Maurer *et al.*, 1999).

The biological indices that will be studied in this project are the benthic biotic indices. Azti marine biotic index (AMBI) and infaunal trophic index (ITI) are two of the benthic indices that are widely used by a range of research bodies. SEPA requires ITI to be the core of the univariate analysis for EIA papers, which are also to be conducted by Scottish fish farms, whilst AMBI is a tool used in research centres to monitor the benthic ecological situation.

In ITI, the formula that is used to calculate the results are more obvious to the user, because of its manual application to the macrofauna datasheet and use of

distributive formulae, whilst AMBI is applied to a user-interface as part of the software designed by AZTI (www.azti.es). Essentially, their application in terms of biological sequence is based on the classification of the trophic properties of the species but also in their current abundance.

ITI has a scale of effects ranging from 0-100, where a high value indicates positive progress concerning the ecological fate of the site, whilst AMBI has a 7 point scale where a high value indicates an increasing negative effect on the condition of the area. Given the different scales used by these indices, there is a need to define a correlation between them if they are to be compared. A comparison of indices is frequently made to identify which are the better to use and whether their combination produces a better estimation of the site than using a single index alone (Borja *et al.,* 2000).

1.3.3 Critical review for verification in the use of the indices

The indices derived from separate univariate analyses and their combination with each other and with other ecological trends of the seabed have been extensively tested in the past in order to obtain an accurate status of the biotopes. The principal regulatory establishment dealing with the marine ecological status is SEPA whose implementation of EQS and EIA are decisive for the environment protection. The statistical approaches on discriminating spatial variation in species diversity, in relation to the diversity indices used for analysing environmental data from marine fish farms was studied by Cheng (2004), who set out to compare the effectiveness of various statistical approaches and

then to present the best strategy for discriminating the spatial variations in species diversity. It was concluded that the most powerful tools for discriminating the spatial variations in species diversity were multivariate approaches. Among the multivariate methods that have been considered, ordination by non-metric multi-dimensional scaling is preferable, and its superimposition with cluster analysis is recommended in order to obtain more information regarding the relationship between sites.

The combination of benthic indices was also considered in the study of Van Dolah *et al.* (1999) where a benthic index of biotic integrity was developed for use in estuaries in south-eastern USA. The final combined index correctly classified 93% of stations, province-wide, in the developmental data set and 75% of stations in the validation data set. Comparison of the index results with those of individual benthic measurement methods and sediment bioassays from stations sampled in 1993 and 1995 showed that the index detected a higher percentage of samples where bioeffects were expected (based on sediment chemistry) than did any of these other measurement methods individually (Van Dolah *et al.*, 1999).

Chainho *et al.* (2007) studied the influence of seasonal variability in benthic invertebrate community structure on the use of biotic indices to assess the ecological status of a Portuguese estuary. The outcome was that the diversity indices were better correlated to eutrophication-related variables than they were to AMBI and abundance-biomass comparison (ABC) methods.

1.4 Project objectives

1.4.1 Primary objectives

This study sets out to study the properties of emamectin benzoate (SLICE) in the marine environment with two main interests: 1) To determine the impacts of SLICE, if any, in marine farmed ecosystems with particular emphasis paid to potential changes in the seabed; 2) To study the fate and dispersion of SLICE in these ecosystems. To investigate these, two hypothetical questions were posed: 1) Is the use of SLICE responsible for the observed environmental impacts to the seabed around certain Scottish farm sites? and, 2) What is the fate of the SLICE that is dispersed in the seabed around Scottish farm sites?

This study set out to answer these two questions using a range of statistical tools, as well as the biological indices and methods that have been created to assess the marine environment and any changes to it.

1.4.2 Secondary objectives

This study also sets out to use a combination of indices to determine which methods permit the best classification of the seabed. Specifically, which combination of benthic indices with other trends provides the most accurate assessment of the seabed underneath farm cages and in the areas adjacent to it? It is anticipated, that this will allow for recommendations on a comprehensive sampling methodology for future use that will have the additional benefit of reducing current sampling times. Additionally,

this will make a contribution by updating the monitoring and modelling procedures and requirements used to identify the current status of the farming biotopes. The same applies for the comparison of the two biotic and trophic indices: ITI and AMBI.

A secondary research was made to complete and enhance the SLICE modelling, by investigating the biomass attributes in SLICE modelling and testing the biotic and abiotic parameters interaction. This project results are crucial to know, to determine whether the biomass is capable and accurate to predict the SLICE behaviour on its own, in terms of modelling accuracy since the use of DEPOMOD which is biomass based, is suggested by SEPA. Also, this study has as purpose to provide accuracy results about the indices and the methods used by the marine scientists, to assess and analyse the ecological attributes of the seabed, and to provide verification regarding the regulations used for completing scientific studies such as EIA and EQS.

Image 1. A gravid female *Lepeophtheirus salmonis* (image taken from the Sea Lice Technical Monograph, 2000).

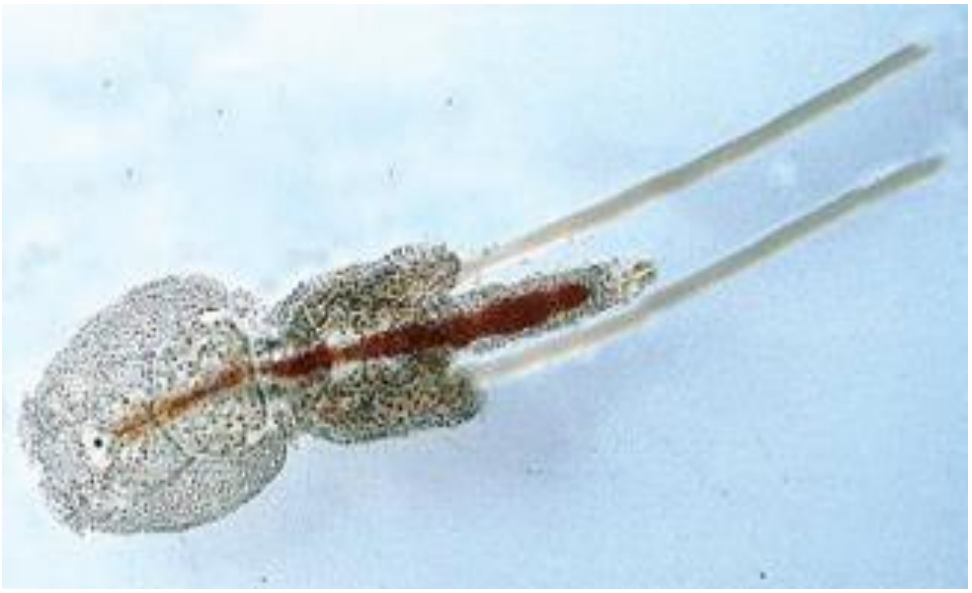


Image 2. Life-cycle of *Lepeophtheirus salmonis* (not to scale) which consists of two naupliar stages (only one shown), an infective copepodite, four chalimus stages which are attached to the host by a frontal filament, two sexually dimorphic pre-adult stages (only one louse for each stage shown) and then the adults (both male and gravid female shown). (Image taken from the Sea Lice Technical Monograph, 2000).

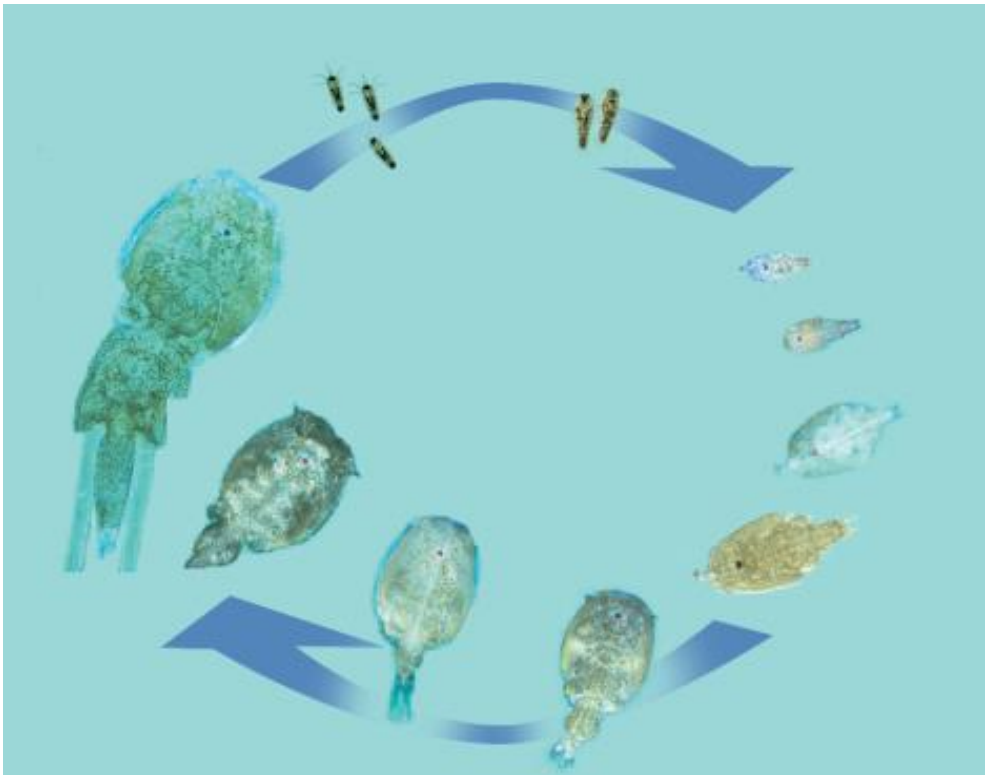


Image 3. Gravid female *Lepeophtheirus salmonis* tend to aggregate in regions behind the dorsal and adipose fins (top image) and around the anus and anal fins (lower image) (images taken from the Sea Lice Technical Monograph, 2000).

Chapter 2
An assessment of the fish biomass influence on environmental impact of marine cage farms through the analysis of long term metadata

2.1. INTRODUCTION

2.1.1 Background

One of the most important activities for the protection of the marine environment is to assess accurately changes of ecological activities and their interaction. These changes are often natural within the dynamic environment, but can also be caused as a result of impacts due to anthropogenic disturbance. Monitoring these changes is often more complicated in aquatic environments than in terrestrial ones, as impacts are less visible and are not immediately apparent (Telfer & Beveridge, 2001a). An anthropogenic operation, which is considered having significant localised impacts of the marine environment, is the marine cage farming of fish (Beveridge, 2004).

As with nearly all forms of aquaculture (and agriculture), marine fish farming sites generate considerable amounts of waste including nutrients, such as uneaten feed and faeces, and chemical residues. These are often released directly to the environment in a diffuse manner and dispersed by tidal and wind action. The environmental effects of marine cage fish farming are generally most prevalent within close proximity to the cage groups. Consequently, much of the research activity into these impacts has concentrated on the immediate, local environment (Beveridge, 2004), including seabed communities – the benthos. Several monitoring strategies to assess the health of the benthos surrounding both proposed and existing fish farm sites have been designed to examine this risk to the local environment (Beveridge, 2004; SEPA, 2005; Wells *et al.*, 2008).

The term quality criterion rather than quality standard is often applied to sediments since the methods for deriving the protective limits are less well established and validated than those for the water column, which usually employ more objective and directly measurable chemical parameters (Telfer & Beveridge, 2001b). Though chemical indicators are used for assessing impacts of aquaculture on sediments, biological or benthic community level changes are used as ultimate measures of impact. However, changes in community structure are difficult to quantify and employ univariate measures such as diversity indices, or multivariate measures addressing community function (Krebbs, 1999).

Indices are used by environmental regulators throughout the world as standards or for defining certain environmental criteria for sediment quality. Sediment samples are subjected to a variety of different univariate indices as each has different strengths and weaknesses in defining sediment impact (SEPA, 2007). These are: number of taxa, abundance, Shannon–Weiner diversity, and Infaunal Trophic Index (ITI). In addition, these are compared with chemical measures relevant to inputs of nutrient or chemical waste into sediments, such as total organic carbon, redox potential, and free and total sulphides (SEPA 2007).

The analysis of quantitative biological data using appropriate numerical and statistical methods is a crucial step in any assessment of data obtained from monitoring, using these indices. Raw biological data usually consists of matrices containing abundances of species (or taxa) at sampling stations at pre-defined positions around the fish farm, or alternately they consist of abundance of species at single sampling stations

over time. These data require further analysis to aid interpretation, to simplify presentation and for comparison with biological standards (SEPA 2007).

Grizzle & Penniman (1991) studied the effects of organic enrichment on estuarine macrofauna using various univariate and multivariate analyses of benthic and sedimentary data. They showed substantial and predictable changes along an estuarine nutrient pollution gradient, which were similar to those reported from coastal waters affected by organic wastes (Pearson & Rosenberg, 1978), where an area nearest the pollution source has the lowest numbers of taxa (S), but the highest abundances (A) and biomass (B). Conversely, with toxic pollutants, such as pesticides, the trend is different where S is again decreased near the pollution source, but this is associated with low A and B near the source as well (Kingston, 1992). Community structure and diversity can be used to relate the levels of pollutants (nutrient or toxic) to environmental impacts. With marine fish cage aquaculture, the level of inputs such as feed and chemicals are depended on the level of fish production (or more directly, standing biomass) (Telfer *et al.,* 2006).

2.1.2 Aims and objectives

The aim of this study is to use metadata to investigate the relationship between changes on benthic communities and fish biomass over a spatial range encompassing the whole of the west coast of Scotland including the islands, over a three year period (2003 – 2006). The changes to the biological and chemical data will be tested by analysing the benthic indices and comparing them to sediment chemistry. Correlation to fish biomass over the three-year period will allow investigation of the effects of aquaculture

production, using the hypothesis that the fish farm waste levels, related to change in fish biomass, have a significant relationship on changes in benthic communities.

2.2 MATERIALS AND METHODS

2.2.1 Project design

Project methods followed three major stages in sequence: 1) data collection, 2) data input and formatting to spreadsheets, and 3) analysis using statistical software. Environmental data were obtained from commercial monitoring by the Institute of Aquaculture (unpublished), and from SEPA, using data from statutory regulatory environmental monitoring studies at marine fish farms, between 2003 and 2006. Data analysed includes physical and chemical parameters associated with bottom sediments (location coordinates, current speed, total organic carbon (TOC), redox potential, particle size analysis, and sediment nitrogen) and macrofauna data (species richness and abundance counts per unit area) from approximate 19 fish farm sites, containing 403 samples (spatial and temporal), around the Scottish coast (Table 1). Data were measured according to the methods required under SEPA's regulations (SEPA, 2007). Original data were provided, either as spreadsheets (MS Excel), databases (MS Access), or as hard-copy paper formats. This was collated in an appropriate format for further analyses into three spreadsheets (MS Excel); one for physico-chemical data, one for macrofaunal data and one for standing biomass.

2.2.2 Data input in spreadsheet

Three spreadsheets were created for interpretation and further long terms analysis of results:

- **Spreadsheet 1:** a two-way matrix containing redox potential (Eh) at 2 cm sediment depth, median particle size (μm), fraction of silt/clay particles within sediments (% <64μm by dry weight sediment), and total organic carbon and

nitrogen levels (% by dry weight sediment) as rows, and sampling stations as columns.

- **Spreadsheet 2:** is a two-way matrix of macrofaunal data (no of individuals/m^2 for each taxa) containing species/taxa as rows and sampling stations (same order as Spreadsheet 1) as columns.
- **Spreadsheet 3:** maximum standing biomass at each of the fish farms investigated (tonnes)

Results were available for sampling stations taken at five distances from fish farms (directly beneath cages edge at 0, 25, 150 and >500 m) annually or biannually, over the three years. The spreadsheet titles and summary lists of sampling stations and locations are given in the Appendix 1 (Tables A3 and A4).

Compilation of the data into the final spreadsheets was complex and time consuming. Data were imported and arranged in the format given previously by the raw data (either xls or converted to xls) from the databases. If multiple measurements for the parameters were given for a particular sampling station, the mean value was used. Physical-chemical data in hard copy format were entered by hand onto summary spreadsheets that were then copied and incorporated into final Spreadsheet 1. Macrofauna data were entered into database format using the computer programme WORMS (Moore, personal communication), compiled into systematic order and total abundance for each sampling station, and imported into xls format for each station for compilation into final Spreadsheet 2.

2.2.3 Data analysis

Univariate and multivariate analysis of the macrofauna and the physico-chemical data was performed to investigate the species diversity and/or evenness for each of the sampling stations in order to compare with physico-chemical parameters and standing biomass, and to compare community level data its relationships to physico-chemical trends and change in biomass.

Univariate measures are methods of reconciling complex systems into a single indicative or representative number or index (Krebs, 1999); such measures include species diversity and species evenness, and trophic indices. The following univariate analyses were performed on the macrofauna data (Spreadsheet 2):

- Infaunal Trophic Index (ITI) (Word, 1978; Codling and Ashley, 1992). ITI is a biotic index with a score ranging between 0 and 100. In nutrient influenced conditions, such as estuaries, a value of 0 to 30 is considered highly disturbed, 30 to 60, moderately disturbed and 60 to 100, indicative of background (undisturbed) conditions.
- AZTI's Marine Biological Index (AMBI) (Borja *et al.,* 2000; Borja & Muxika, 2005). Like ITI, AMBI assigns a score on the basis of interactions and presence of species from different trophic levels. The score is related directly to good or poor quality environmental conditions and ranges between one and seven.

- Simpsons Index (D) measures the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species) (see Krebbs, 1999).
- Brillouins Index (Hb). An index of diversity used when the randomness of a sample cannot be guaranteed. The value of Hb is usually less than 4.5 (see Pielou, 1966; Krebbs, 1999).
- Shannon Wiener (Hs or H') is one of several diversity indices used to measure diversity in categorical data. It is simply the information entropy of the distribution, treating species as symbols and their relative population sizes as the probability (see Pielou, 1966; Krebbs, 1999).
- Pielou Evenness (P). Was derived from the Shannon Index by Pielou in 1966. Pielou's Index is calculated from the information supplied by a sample of a point-to-nearest-plant distances and a second sample of quadrat counts. The first sample is obtained by selecting *n* random points within the sample area and measuring the distance from each of these points to its nearest plant. The values are between 0 – 1. When the value is closer to one, the individuals are distributed more equally between species (see Pielou, 1966).
- Heip Evenness (Eh) is a measure of how similar the abundances of different species are. When there are similar proportions of all subspecies then evenness is one, but when the abundances are very dissimilar (some rare and some common species) then the value increases Its maximum value is 1, when $H =$ InS (see Heip, 1974).

Community level data was also analysed using multivariate analysis, where species level data is summarised in a manner allowing further objective statistical comparison with environmental parameters (Kent & Coker, 1992). This has the advantage of using all of the data in the analyses. Two methods of multivariate analysis were used; classification by cluster analysis (Kent & Coker, 1992) and ordination by canonical correspondence analysis (CCA) (Ter Braak & Prentice, 1988).

Both univariate and multivariate analysis was performed by using MVSP 3.1 edition (KCS Ltd, Angelsey). The multivariate analysis is important because it studies a spectrum of various statistical approaches for each of the factors, species and stations each alone or in relation according to the needs and requirements of the study. For this to accomplish a series of calculations are being made by the programme. The two types of the multivariate analysis used in this project were cluster and canonical correspondence analysis (CCA). In cluster analysis, the output is normally in the form of a dendrogram showing the relationships between the community composition at the different sampling stations/occasions by grouping similar stations in a hierarchical manner. For the macrofauna datasheet, the data were not transformed from the original datasheet, neither during the analysis. The method used for clustering was the unweighted pair group method with arithmetic mean (UPGMA) and the similarity matrix based upon the Euclidean distance or similarity method.

CCA is a multivariate extension of weighted averaging ordination, which is a simple method for arranging species along environmental variables. CCA constructs linear combinations of environmental variables along which distributions of species are maximally separated; the significance of which are indicated through the eigenvalues

generated. Within CCA the axes of the correspondence analysis are constrained to be linear combinations of environmental variables, so the ordination bi-plot visualises both a pattern of community variation (as in standard ordination) but also the main features of the distributions of species along the environmental variables. CCA can therefore be used for detecting species-environment relations, and for investigating specific questions about the response of species to environmental variables (see Ter Braak, 1987). Direct multivariate comparison of species distributions and environmental variables with fish biomass were made where the latter two were used as constraints.

Post hoc statistical comparisons between univariate measures and environmental variable and biomass and multivariate scores and biomass were achieved using non-parametric correlation and regression analysis using Sigmastat (3.1 edition) and Microsoft Office Excel (2007 edition).

2.3 RESULTS

Table 2.1 shows the sites tested for impacts the years 2003 and 2006 along with their labels. The sites are seen along with their coded labels used for the rest of the analysis and are the same in the rest figures and tables of this chapter. For better understanding of the sites, a map of Scotland with the sites pointed out as circle dot placemarks can be seen in the map in Figure 2.1.

Table 2.1. Table shows the labels of the sites tested for impacts the years 2003 and 2006. The sites location can be seen on Figure 2.1.

Fish farm site name	**label**
Basta Voe South	site1
Strome	site2
Portree	site3
Port na Moine	site4
Sgeir Mhor	site5
Inchkenneth	site6
Bow of Hascosay	site7
Vatsetter	site8
Bagh Dail nan Ceann North (BDNC N)	site9
Greinham	site10
Loch Etive East	site11
Leinish	site12
Sian	site13
Bay of Vady	site14
Basta Voe North	site15
Port nan Seannag aka Lunga (east side)	site16
Kirk Noust	site17
Stead of Aithness	site18
Aird	site19

The results include mostly univariate analysis results because those focus on the biological results and show the current situation occurring in seabed. Regressions, paired t tests and cluster analysis were also performed to the data as well as CCA (canonical correspondence analysis). The results taken for biological indicators by univariate analysis are shown in the Table 2.2.

Table 2.3 includes fewer sites than Table 2.2, but includes the carbon concentration in g/sample and redox potential measured in mV per site (average values extracted from the summary of all the stations).

Figure 2.1 Map of Scotland showing the location of the tested sites from Table 1.

Table 2.2. Univariate analysis results for year 2003 (1) and for year 2006 (2). The biomass is measured in tonnes/year. Where N is the number of individuals, S the number of the species, H the Shannon Wiener index, AMBI the azti marine biotic index.

label	N_1	N_2	S_1	S_2	H_1	H_2	AMBI 1	AMBI 2	biomass 1	biomass 2
Basta Voe South	137.50	411.00	8.17	33.33	1.32	2.83	2.67	3.50	936	1060
Strome	633.17	783.50	23.17	23.50	2.08	2.61	4.33	2.67	1344	1328
Portree	199.33	652.17	17.83	25.83	2.33	2.71	4.00	3.50	764	1269
Port na Moine	1448.00	1048.80	7.00	24.80	0.85	1.62	4.60	3.60	658	375
Sgeir Mhor	23.33	835.67	11.67	4.33	3.35	1.11	2.00	2.33	241	411
Inchkenneth	370.86	1947.00	18.00	13.57	2.42	2.11	3.86	3.43	688	675
Bow of Hascosay	33.00	370.63	4.25	13.13	1.18	1.64	2.63	3.38	1070	468
Vatsetter	354.75	217.13	28.50	37.63	3.51	3.37	2.25	2.75	1056	720
Bagh Dail nan Ceann N	59.00	337.25	11.67	22.00	2.65	2.48	2.67	3.25	810	743
Greinham	440.25	691.63	10.00	6.88	1.87	1.83	2.38	3.13	309	711
Loch Etive East	119.67	103.00	16.67	3.67	2.42	1.03	3.33	3.33	105	249
Leinish	60.29	361.57	6.57	15.43	1.78	2.46	2.29	2.71	790	337
Sian	123.00	4443.00	9.00	5.33	1.34	1.27	4.33	3.00	752	746
Bay of Vady	16.29	639.86	4.86	8.71	1.76	1.07	2.57	3.86	434	830
Basta Voe North	91.33	124.67	5.67	3.67	1.17	0.86	4.67	2.00	197	733
Lunga	8.50	108.50	3.00	4.75	1.18	1.21	2.00	2.50	408	574
Kirk Noust	47.57	886.86	7.14	46.57	1.89	2.80	2.29	3.71	645	274
Stead of Aithness	88.75	168.00	11.00	3.75	2.58	0.61	2.00	2.25	990	682
Aird	114.17	4099.00	15.00	32.43	2.61	2.44	2.50	3.29	1326	1031

total	4368.76	18229	219.17	329.31	38.21	36.06	57.37	58.19	13523	13216
average	229.93	959.4	11.5	17.3	2.01	1.9	3.01	3.06	711.7	695.6

Table 2.3. Selected factors from the univariate analysis and biomass along with the carbon and redox potential in the sites tested for impacts in 2003 and 2006. The variables are the same as Table 2.2 with the addition of redox potential (mV) and carbon percentage (in dry weight). The average Biomas1 is 623.7 and Biomas2 543.5.

label	N 1	N 2	S 1	S 2	H1	H2	AMBI1	AMBI2	Biomas1	Biomas2	Redox 1	Redox 2	C1	C2
Port na Moine	1448.00	1048.80	7.00	24.80	0.85	1.62	4.60	3.60	658	375	163	226	6.08	11.93
Inchkenneth	370.86	1947.00	18.00	13.57	2.42	2.11	3.86	3.43	688	675	225	202	3.80	12.15
Bow of Hascosay	33.00	370.63	4.25	13.13	1.18	1.64	2.63	3.38	1070	468	161	227	2.77	1.56
Vatsetter	354.75	217.13	28.50	37.63	3.51	3.37	2.25	2.75	1056	720	223	225	2.31	1.15
Bagh Dail nan Ceann	59.00	337.25	11.67	22.00	2.65	2.48	2.67	3.25	810	743	426	191	3.68	5.50
Loch Etive East	119.67	103.00	16.67	3.67	2.42	1.03	3.33	3.33	105	249	49	207	0.61	11.83
Leinish	60.29	361.57	6.57	15.43	1.78	2.46	2.29	2.71	790	337	117	215	1.99	7.27
Bay of Vady	16.29	639.86	4.86	8.71	1.76	1.07	2.57	3.86	434	830	256	253	5.48	2.18
Basta Voe North	91.33	124.67	5.67	3.67	1.17	0.86	4.67	2.00	197	733	41	122	12.18	8.75
Lunga (east side)	8.50	108.50	3.00	4.75	1.18	1.21	2.00	2.50	408	574	255	206	6.96	1.97
Kirk Noust	47.57	886.86	7.14	46.57	1.89	2.80	2.29	3.71	645	274	191	247	7.70	2.18
total	2609.26	6145.27	113.33	193.93	20.81	20.65	33.16	34.52	6861	5978	2107	2321	53.56	66.47
average	237.21	558.66	10.30	17.63	1.89	1.88	3.01	3.14	623.73	543.45	191.55	211.00	4.87	6.04

The descriptive analysis from SIGMASTAT can be seen in Appendix 1, where Table A1 is the resulting output of the Table 2.2 and shows the results coming from the statistical analysis of the univariate indicators descriptive statistics (from the Table 2.2). In detail, Table 2.2 presents the mean values of each of the diversity indices and the biomass values. The total numbers of individuals (N) found in sites for 2006 were highest in three sites, and in 2003 only one site presents a high N value, which means the N number in 2006 is better. The species numbers accordingly (S) was low in four sites in 2006 while three sites found to have low S values in 2003. This result means the difference was low in S values between these years and the majority of the sites had a high S value, which indicates an equal macrofauna species distribution. The Shannon Wiener Index H' is low at three sites in 2003, while the 2006 results show that three sites had low values of H' and that is not a significant difference. The AMBI score in 2003 was high in three sites while three sites had high AMBI value in 2006. The biomass was high in 2003 in three sites and three sites also had high biomass values in
2006.

Table 2.3 created to compare the accuracy of the indices combination. This comparison realised statistically by using the radical values of carbon percentage and redox potential from Table 2.2. Table 2.3 shows two sites to have a combination of high number of individuals N, while their number species S is low, which means an ecosystem with undisturbed conditions. The combination of low S and H' values means that species diversity is low and the species are unevenly distributed, while the opposite situation of high S and H' appeared for only one site in 2003 and one site in 2006. The sites which had low values of N, S, and H' in 2003 and in 2006 had high value of carbon percentage in 2003 but not in 2006. The previous combinations for

these stations indicate that the species were affected by the carbon percentage negatively and particurarly caused disturbed sediment conditions.

Table 2.4 is a detailed summary of the univariate analysis for the individual sampling stations which are identified in terms of their site number and distance (m) and direction (compass direction) from the fish cages. This table shows the results of the stations that have the higher range for any diversity index (or more for the same year) and its purpose is for creating Figures 2.2 to 2.5.

Table 2.5 presents the mean values of all sites for univariate indices in relation to their distance from the cages. It can be seen that at 0m stations had higher values in N and H' in 2006 than in 2003. At the 25m stations also had lower S and H' and also low AMBI score in 2006 than in 2003. The 50m stations had only lower H' values in 2006 than in 2003. There was no specific difference between the two dates at stations from 150m to the reference stations.

Table A2 (see Appendix 1) shows descriptive analysis for the biological indicators for the stations (distance from the cages). Table A2 and Table A3 in the Appendix 2, show results of the paired tests from sites and sampling stations respectively and are comparisons of the properties between 2003 to 2006.

Table 2.4. Selected univariate measures for individual sampling stations for 2003 and 2006.

STATION	N 2003	H' 2003	AMBI 2003	N 2006	H' 2006	AMBI 2006
Basta Voe South 25 NW	40	0.83	2	693	0.93	6
Basta Voe South 50 NW	41	1.64	2	509	3.11	3
Basta Voe South REF 1	118	1.53	4	212	4.21	2
Basta Voe South REF 2	84	2.66	1	314	4.08	2
Basta Voe South 0m SE	95	0.42	5	161	4	2
Basta Voe South 150m SE	447	0.85	2	577	0.65	6
Strome 1400m S	512	1.03	6	252	4.44	2
Strome 800m SW	811	0.28	6	46	2.71	2
Strome 0m SW	1640	0.41	6	1417	0.96	3
Strome 150m SW	548	1.55	5	77	3.19	2
Strome 25m NE	211	4.45	2	131	3.2	2
Strome 25m SW	77	4.74	1	2778	1.14	5
Portree 1100m NE	3	0.92	6	258	3.33	2
Portree 1300m SW	125	2.96	2	195	3.59	2
Portree 0m SW	137	3.55	2	1235	1.24	6
Portree 150m SW	429	0.92	6	1113	1.89	5
Portree 25m NE	362	1.33	6	816	2.63	3
Portree 25m SW	140	4.3	2	296	3.58	3
Port na Moine 25m NB	2626	0.06	6	107	1.28	4
Port na Moine 0m NB	2498	0.05	6	2920	2.19	4
Port na Moine Ref 1	1582	0.11	5	16	2.7	2
Port na Moine Ref 2	146	2.23	3	7	1.84	2
Port na Moine 25m SB	388	1.81	3	2194	0.08	6
Sgeir Mhor 750m SW	25	3.56	2	50	1.73	2

Sgeir Mhor 0m E	29	3.17	2	2403	0.25	3
Sgeir Mhor 25m E	16	3.33	2	54	1.36	2
Inchkenneth 550m NE	1141	0.94	6	60	2.65	2
Inchkenneth 550m NW	638	1.13	6	67	4.05	2
Inchkenneth 0m S	156	2.03	6	6622	1.22	4
Inchkenneth 25m N	80	4.62	2	100	2.84	3
Inchkenneth 25m S	218	2.48	3	3119	1.13	4
Inchkenneth 50m N	170	3.37	2	1766	1.3	5
Inchkenneth 50m S	193	2.37	2	1895	1.56	4
Bow of Hascosay 0m N	15	1.78	5	738	0.33	6
Bow of Hascosay 150m N	49	1.6	1	215	1.82	2
Bow of Hascosay 25m N	28	0.81	2	257	2.23	2
Bow of Hascosay 50m N	50	0.84	2	197	1.58	2
Bow of Hascosay REF 1	12	1.04	2	248	3.31	2
Bow of Hascosay REF 2	24	0.74	2	266	3.18	2
Bow of Hascosay 25m S	67	1.17	4	621	0.26	6
Bow of Hascosay 50m S	19	1.47	3	423	0.42	5
Vatsetter 0m	718	2.22	4	407	5.44	2
Vatsetter 0m	297	3.74	2	357	5.01	2
Vatsetter 0m NE	160	3.32	2	319	0.12	6
Vatsetter 150m NE	263	5.14	2	200	5.23	2
Vatsetter 25m NE	145	3.84	2	122	3.07	1
Vatsetter 50m NE	125	3.66	2	122	3.81	2
Vatsetter 25m SW	243	3.3	2	115	1.47	5
Vatsetter 50m SW	887	2.89	2	95	2.83	2
Bagh Dail nan Ceann 25m SW	67	3	2	595	1.29	3
Bagh Dail nan Ceann 0m	74	2.95	2	404	0.36	6

Bagh Dail nan Ceann Ref 1	49	2.74	3	40	3.36	2
Bagh Dail nan Ceann Ref 2	54	2.25	3	310	4.89	2
Greinham 150m NW	112	2.31	2	29	3.66	2
Greinham 25m NW	58	2.26	3	422	1.04	4
Greinham 25m SE	316	1.26	2	1788	1.01	4
Greinham 50m NW	133	1.64	2	21	1.78	4
Greinham 50m SE	2786	0.15	4	345	0.99	3
Greinham NW 0m	60	1.75	2	2872	0.99	4
Greinham Ref 1	35	2.76	2	8	2.16	2
Greinham Ref 2	22	2.85	2	48	2.98	2
Loch Etive East 25m E	66	3.63	2	107	0.87	3
Loch Etive East 0m E	174	0.66	6	194	0.05	5
Loch Etive East Ref 1	119	2.96	2	8	2.16	2
Leinish 25m N	61	2.54	4	134	2.86	2
Leinish 25m S	32	0.34	2	73	3.29	2
Leinish 50m N	80	3.14	2	303	1.22	4
Leinish 50m S	57	1.77	2	183	2.91	2
Leinish 0m N	65	1.82	2	201	3.17	2
Leinish Ref 1	66	1.39	2	395	3.08	2
Leinish Ref 2	61	1.47	2	1242	0.71	5
Sian 500mNE	229	0.96	6	5	1.92	1
Sian 500mS	134	1.28	5	37	1.87	2
Sian 0mN	6	1.79	2	13287	0.02	6
Bay of Vady 25m N	4	1.5	6	850	0.14	6
Bay of Vady 50m N	15	2.15	1	1351	0.3	6
Bay of Vady REF 1	23	2.33	1	43	2.19	2
Bay of Vady REF 2	27	1.48	1	48	2.52	2

Bay of Vady 0m S	29	1	5	1324	0.1	6
Bay of Vady 25m S	7	1.38	2	546	0.98	3
Bay of Vady 50m S	9	2.5	2	317	1.27	2
Basta Voe North 0m N	110	1.04	5	137	0.83	2
Basta Voe North 25m N	30	2.02	3	158	0.56	2
Basta Voe North REF 2	134	0.44	6	79	1.19	2
Lunga (east side) 25m N	7	1.38	2	65	0.11	3
Lunga (east side) 0m N	7	0.59	2	336	0.46	3
Lunga (east side) Ref 1	13	1.35	2	20	1.86	2
Lunga (east side) Ref 2	7	1.38	2	13	2.41	2
Kirk Noust 25m N	36	2.45	3	416	2.19	4
Kirk Noust 50m N	57	1.55	2	231	5.01	1
Kirk Noust REF 1	38	1.58	2	243	4.66	2
Kirk Noust REF 2	55	2.21	2	223	5.08	2
Kirk Noust 0m S	61	1.47	2	2265	0.33	6
Kirk Noust 25m S	19	1.72	3	843	1.64	5
Kirk Noust 50m S	67	2.28	2	1987	0.72	6
Stead of Aithness 0m N	132	2.58	2	434	0.02	3
Stead of Aithness 25m N	82	2.25	2	163	1.54	2
Stead of Aithness REF 1	33	2.8	2	26	0.74	2
Stead of Aithness REF 2	108	2.68	2	49	0.14	2
Aird 0m NW	38	1.35	3	7569	1.03	5
Aird 0m SE	123	1.54	4	309	3.16	3
Aird 150m NW	334	2.86	2	381	5.34	2
Aird 25m NW	120	3.65	2	18115	1.47	4
Aird 25m SE	16	3.33	2	84	4.21	2
Aird 50m NW	54	2.95	2	2186	1.7	5

Mean	**243.23**	**2.03**	**2.93**	**962.13**	**2.07**	**3.15**

Table 2.5. Mean values of all sites for univariate factors regarding their distance from the cages.

stations	N 2003	N 2006	Hs 2003	Hs 2006	AMBI 2003	AMBI 2006
0 m	288.74	2154.38	1.67	1.13	3.53	4.14
25 m	191.79	1233.17	2.41	1.67	2.72	3.48
50 m	296.44	745.69	2.15	1.91	2.13	3.50
150 m	311.71	370.29	2.18	3.11	2.86	3.00
Ref (varying metres, >800m)	209.14	179.65	1.79	2.96	3.00	2.06

Figures 2.2 to 2.8 show the relationships between 2003-2006 for each of the indicators and environmental factors for the sites as extracted from Table 2.3. The figures show the mean values for both 2003 and 2006. Figure 2.2 shows that two sites had the highest value of N in 2006 and they are also sites that presented the largest difference in range between the years in N values, in 2003 and 2006 (increasing rate). The lower N values are for ten sites, in 2006 and that result shows that the species evenness this year was better than 2003.

Figure 2.2, is the species richness (S) plotted for the tested sites. There were five sites (Sgeir Mhor, L. Etive E., Basta Voe N., Lunga E., and Aithness) that presented the lowest values of S in 2006 and four sites (Basta Voe S., Vatsetter, Kirk Noust and Aird) that had the highest S values the same year and these sites are different

regarding the year, so that there was not a continuous impact. It is also noted that three sites (Basta Voe S., Kirknoust and Aird) presented a wide range in species richness between 2003 and 2006 years as seen for their S values.

In Figure 2.3, the H' values are shown for the different sites and times. The sites showed little difference in H' between 2003 and 2006, though only two sites (Sgeir Mhor and Vatsetter) had slightly higher levels of H' and two sites (Aithness and Port na Moine) presented low values of H' in between the years. The same equitability, with a low deviation, can be seen in Figure 2.4 where AMBI values are high in three sites (Port na Moine, Sian and Basta Voe S.) for 2003 and none was high enough in 2006, an indication for both years that the stations had slight to moderate disturbance. It is also noted that site 15 presented a large-scale difference in AMBI by being decreasing during the years.

Figure 2.5 is the average annual biomass in sites for 2003 and 2006. The difference in biomass can be seen both with low and high deviation. The highest difference was for six sites (Portree, Hascosay, Greinham, Leinish, Basta Voe N. and Kirknoust) and site Sian presented a lower difference in biomass value. The highest biomass value was noted at three sites (Strome, Portree and Basta Voe S.) for 2006 while lower biomass values were noted at three sites (Loch Etive, Port na Moine and Kirk Noust) for the same year. Furthermore, sites Loch Etive E. and Sgeir Mhor had a low biomass both in 2003 and 2006. In terms of the change in biomass between the years, it means that the species diversity presented a positive reaction expressed by equal combination of the N, S and biotic indices.

Figure 2.6 is the redox potential values alongside the sites. The redox value was highest in site Bagh Dail nan Ceann for 2003 and lowest at sites Loch Etive and Basta Voe South in the same year. For 2006, the redox potential values showed an even distribution ranging approximately at 200 mV apart from site Basta Voe South, which was much lower than 200 mV.

In Figure 2.7, the mean carbon percentage value (C % by dry weight sediment) is plotted in relation to the sites. The highest C% value is seen at three sites (Port na moine, Inchkenneth and Loch Etive) and the lowest values at four sites (Hascossay, Vatsetter, Vady and Kirknoust) for 2006. Sites Loch Etive and Inchkenneth showed the highest range of difference in C% for the years between 2003 and 2006 where both of these sites presented a low level in 2003 and higher in 2006. The lowest range of differences in C% value were for sites 7 and 8 and the lowest of all sites, while site 15 presented the highest values in C% for both years.

Figures 2.8, 2.9 and 2.10 show the range of diversity indices as increasing distance from cages measured in metres as extracted from Table 2.5.

Figure 2.2. Bar chart of the number of individual species in sites in 2003 and 2006.

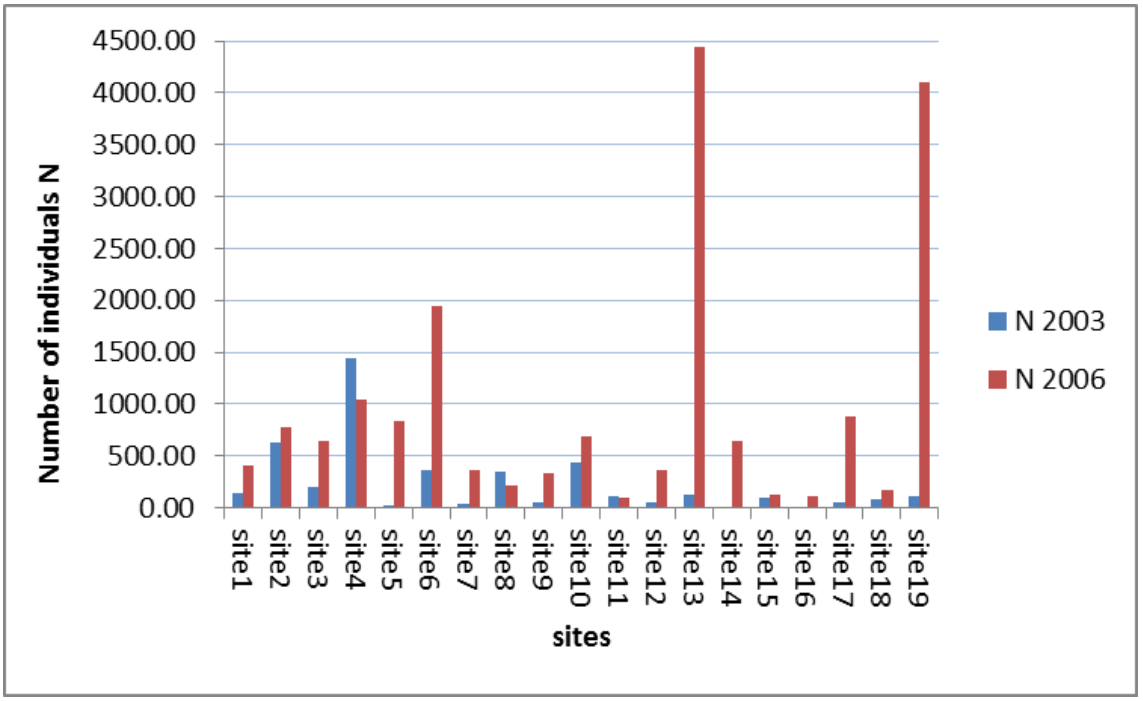


Figure 2.3. Bar graph shows the number of the species S at each site for 2003 and 2006.

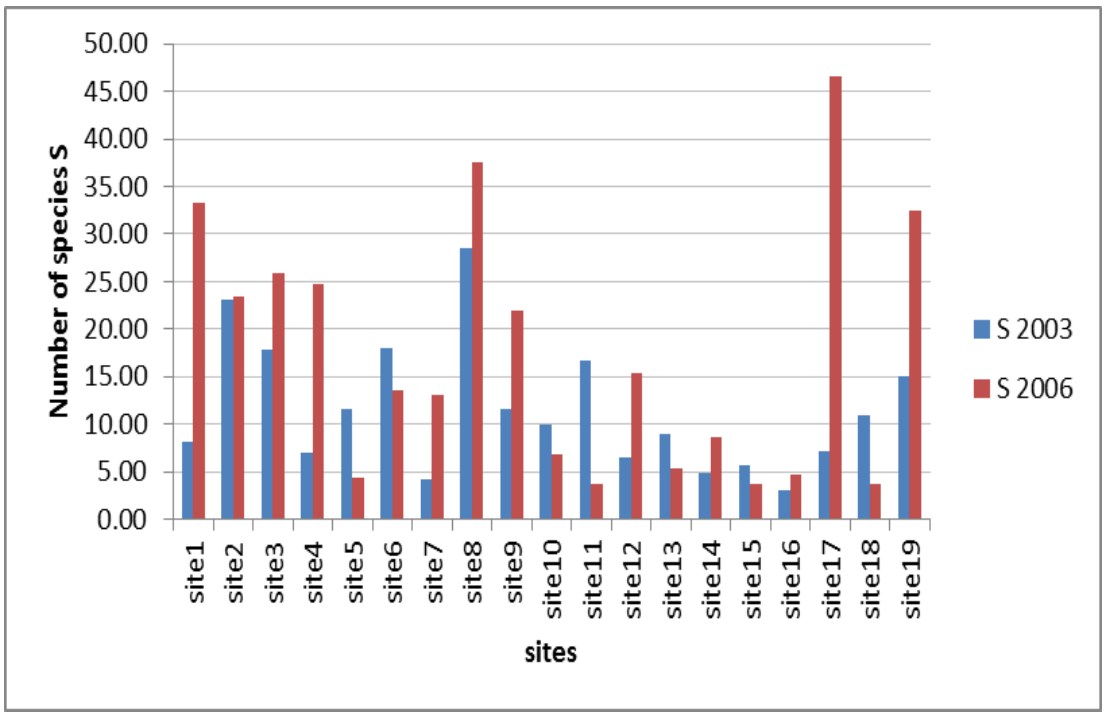


Figure 2.4. Bar chart shows the Shannon Wiener Index average values from the stations for each site between the years 2003 and 2006.

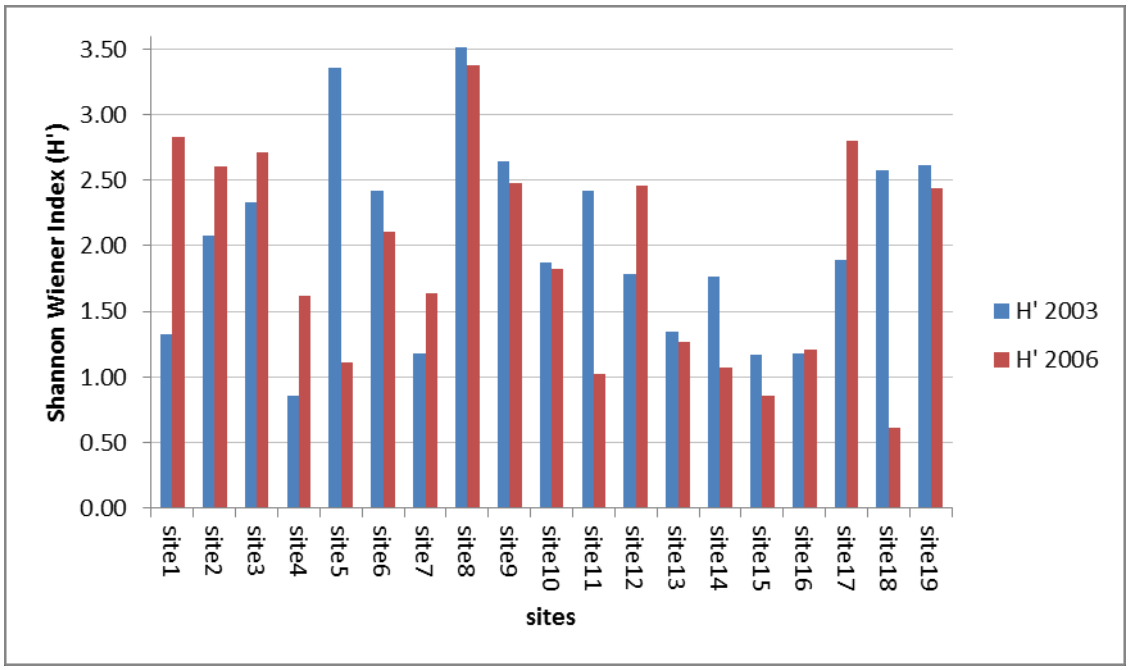


Figure 2.5. Bar chart of the average AMBI scores for each site for 2003 and 2006.

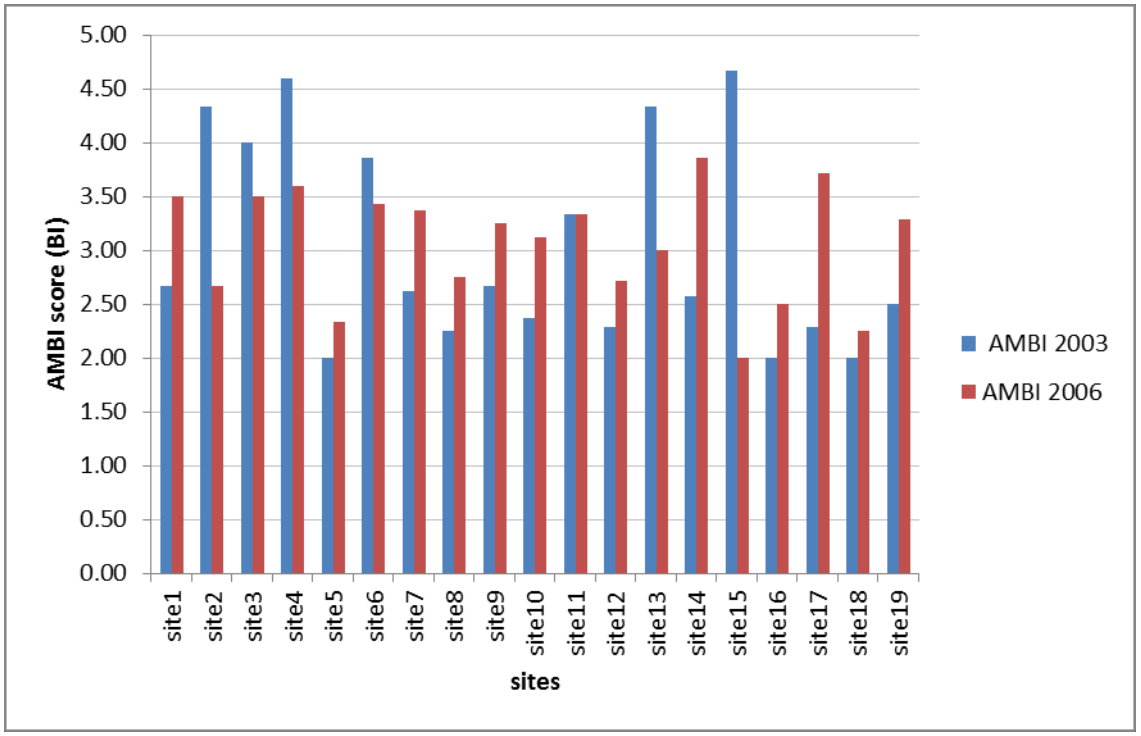


Figure 2.6. Bar chart of the average annual peak biomass at each site in 2003 and 2006.

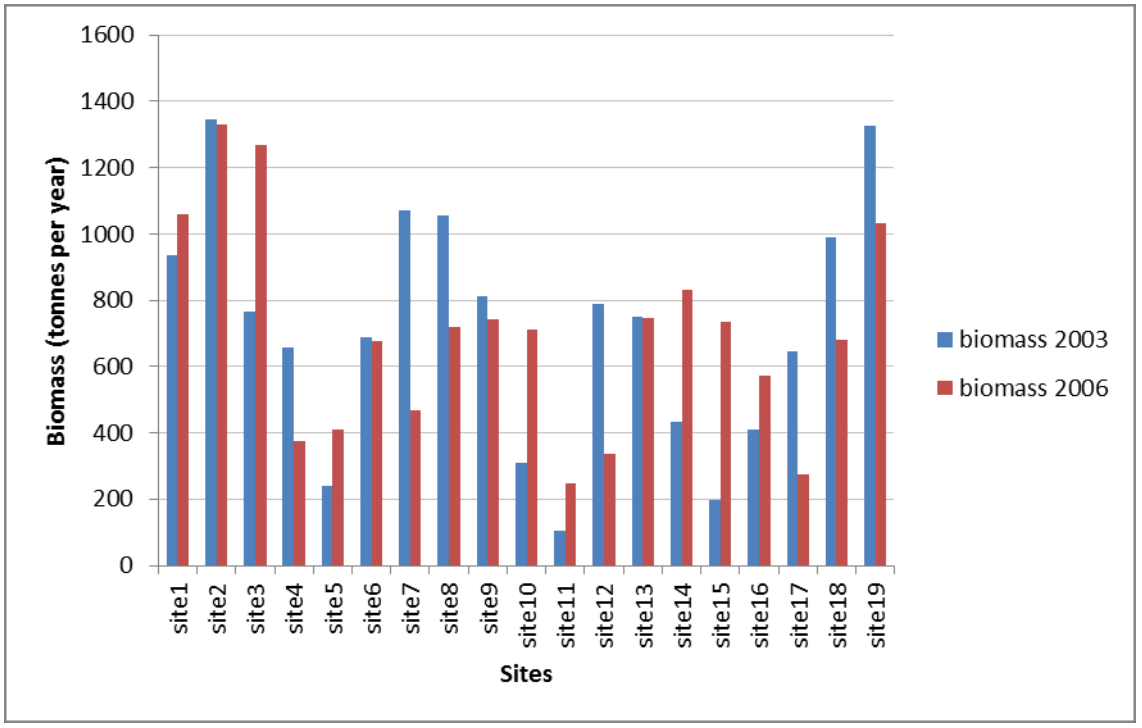


Figure 2.7. Bar chart of average value redox potential for each site for 2003 and 2006.

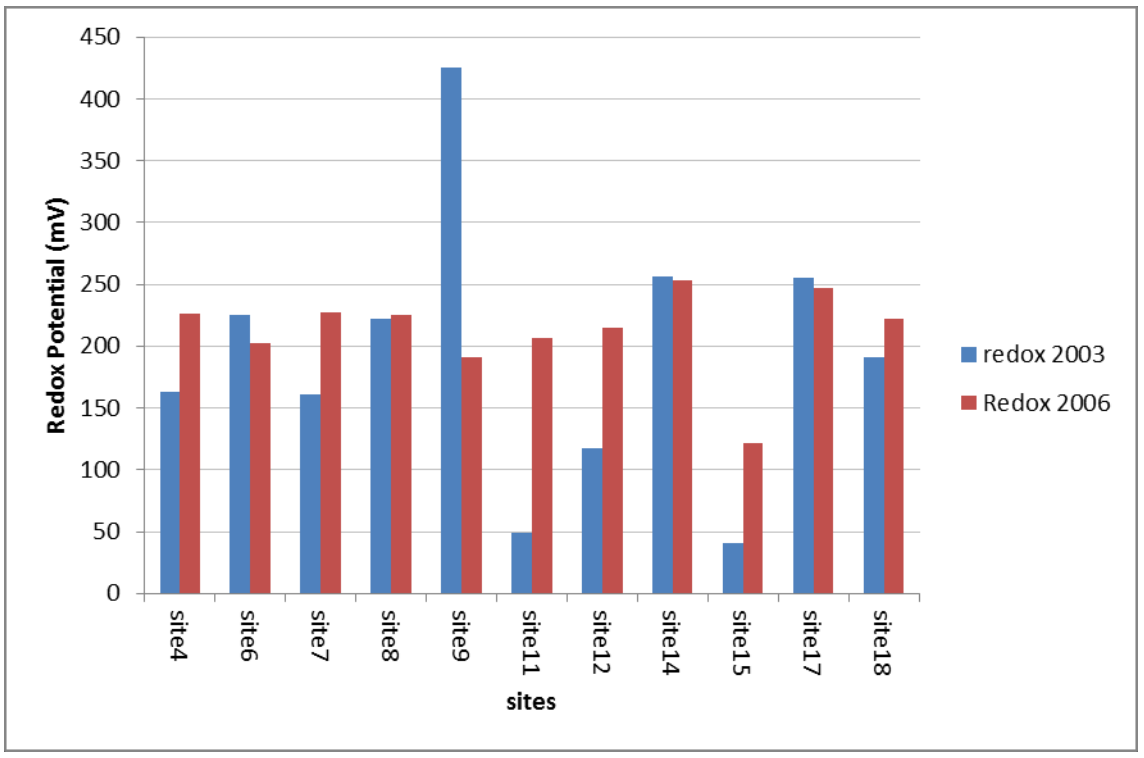


Figure 2.8. Mean value in carbon concentration at each site for 2003 and 2006.

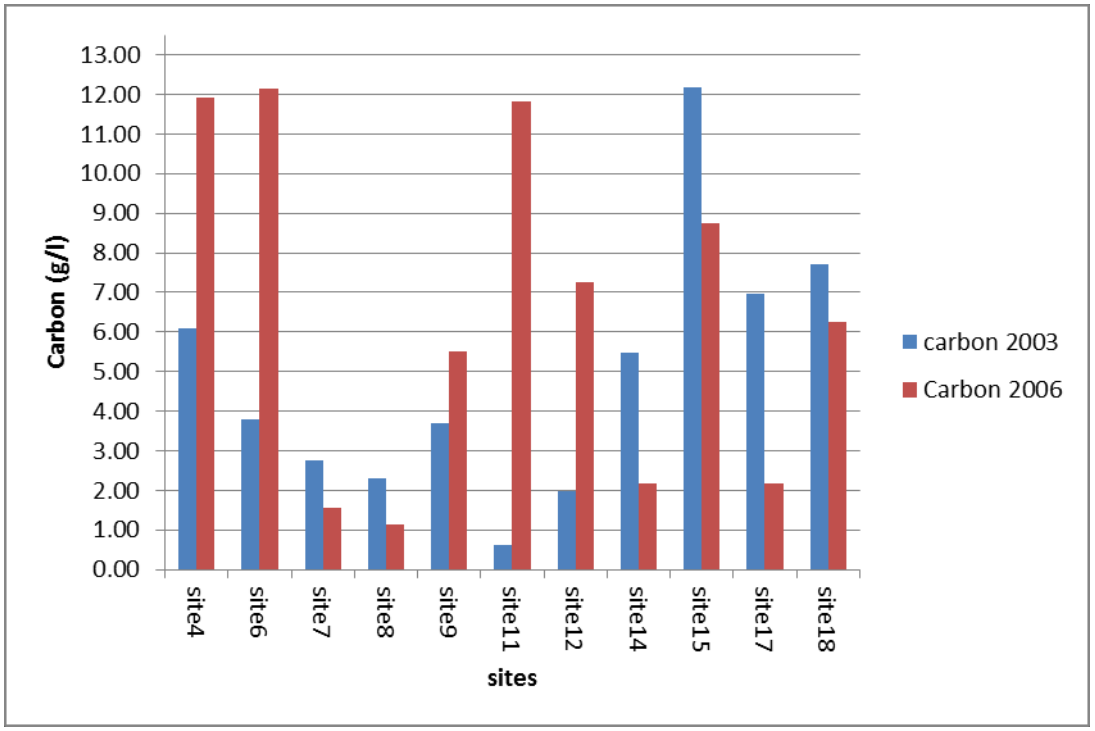


Figure 2.9. Bar chart of the number of individuals (individuals/m^2) recorded at the various sampling distances (m) from cages.

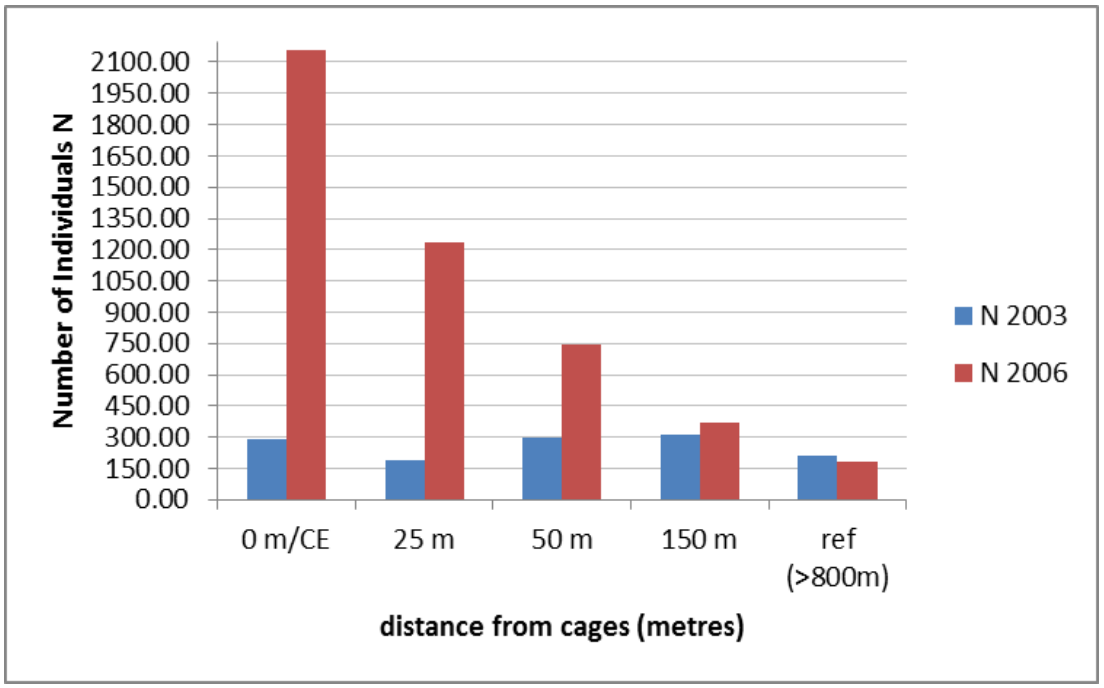


Figure 2.10. Bar chart of mean Shannon Wiener Index (H') in relation to the distance from cages.

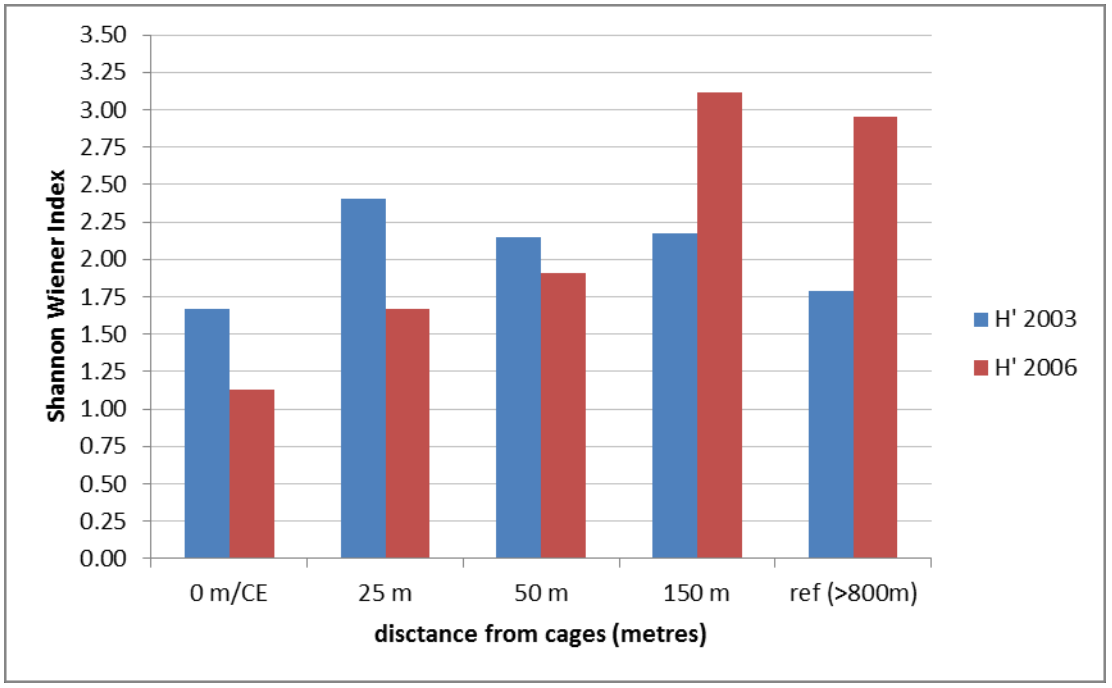
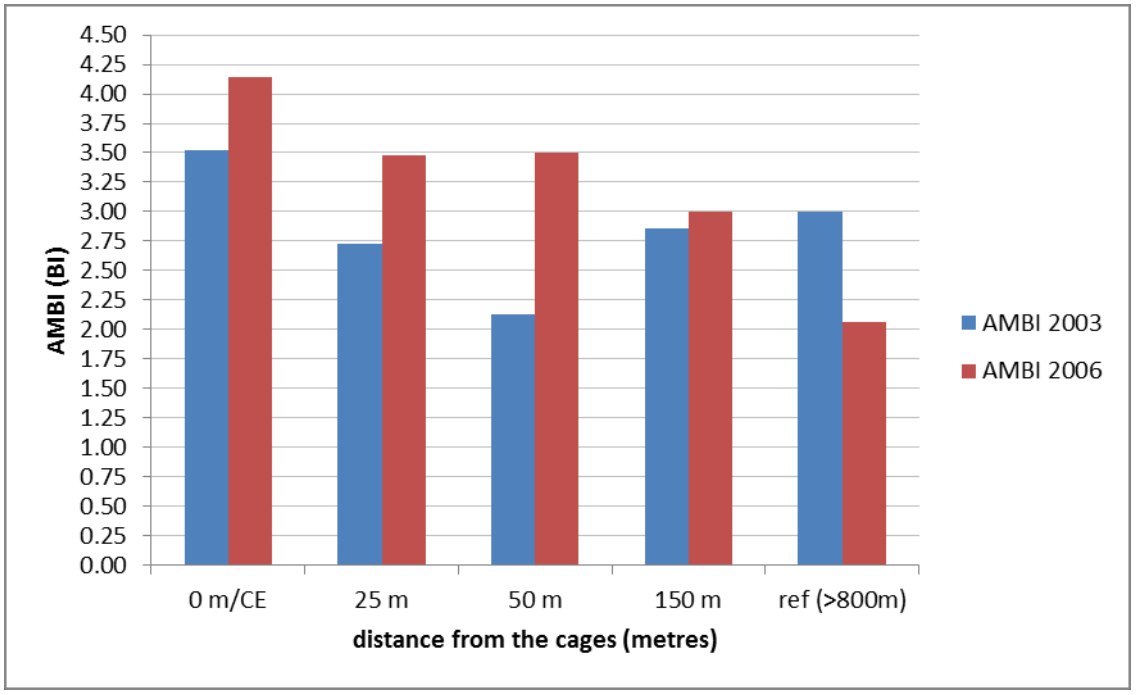


Figure 2.11. Bar chart of the mean AMBI scores in relation to the distance (m) from the cages.



Figures 2.12 to 2.15 show the plot graphs along with their trend lines for the relationship of the biomass with each of the biological indicators. In these figures, the results in terms of the change in biomass between the years and distance from the cages mean that there is variation near to the fish cages in diversity and species richness, but there is little difference further away from the cages. These indices do show a difference where the waste input to sediments is high, but little change where the waste input is low. For Figures 2.12 to 2.15, these relationships are quite weak, though this may be due to using all of the data when we think that the biomass/effect relationship only occurs to between 50 and 150 m. Maybe these correlations would be stronger if the 0, 25 and 50 m data had been used and a separate plot of the reference stations as a control.

Figure 2.12. Graph of the peak biomass in relation with the number of individuals at sites.

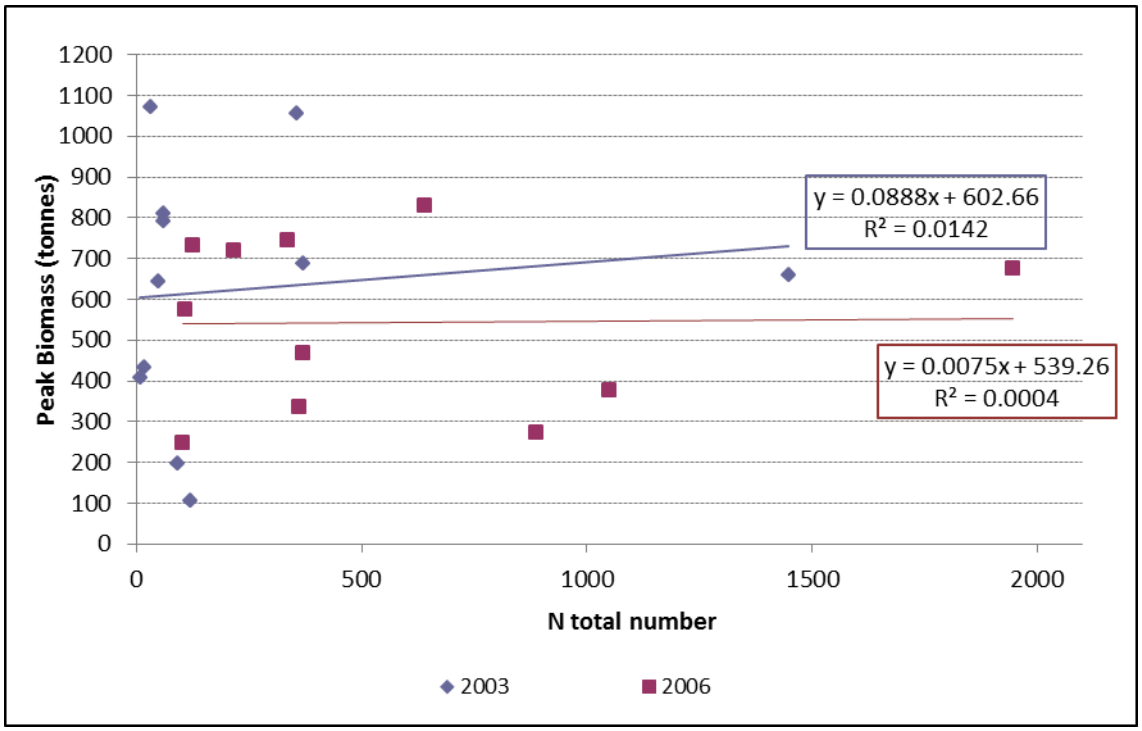


Figure 2.13. Graph of the peak biomass (tonnes) with Shannon Wiener Index.

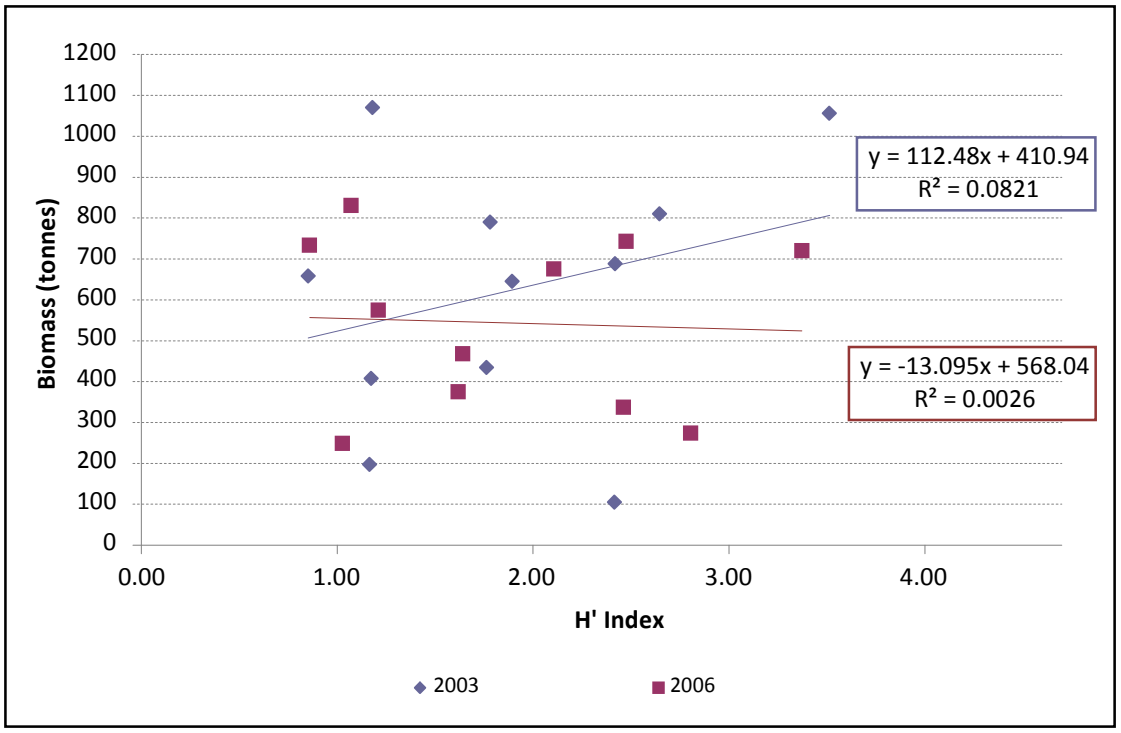


Figure 2.14. Graph of the peak biomass (tonnes) with AMBI score for each site.

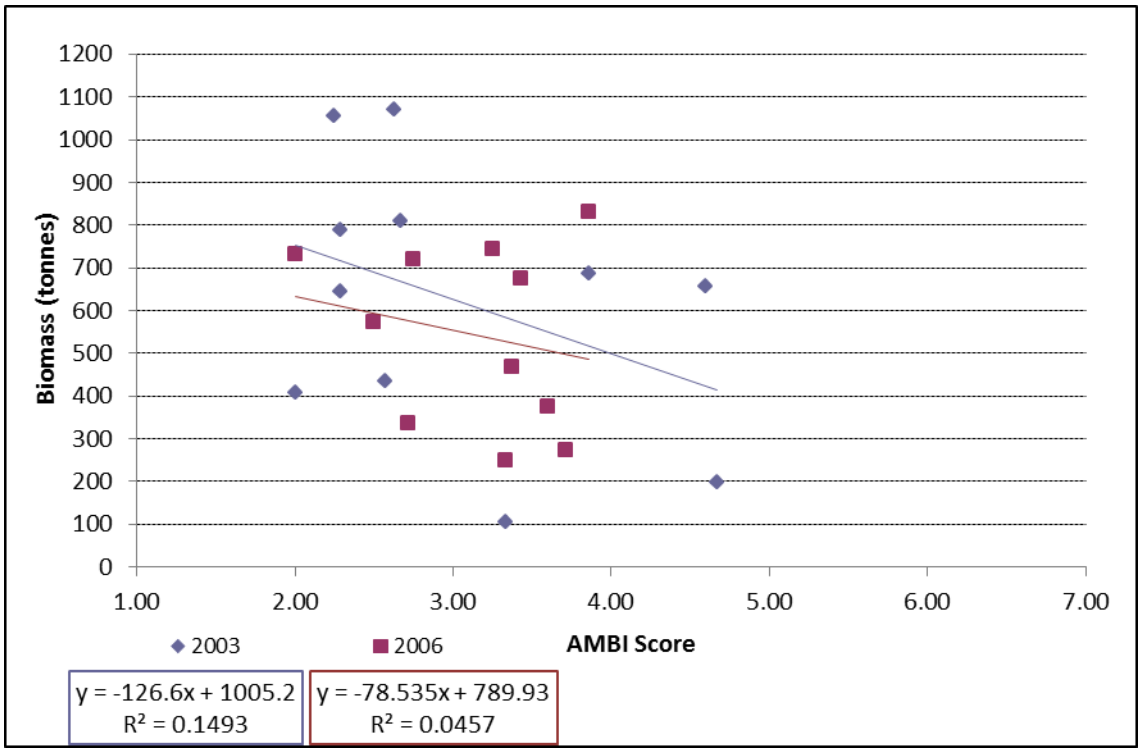
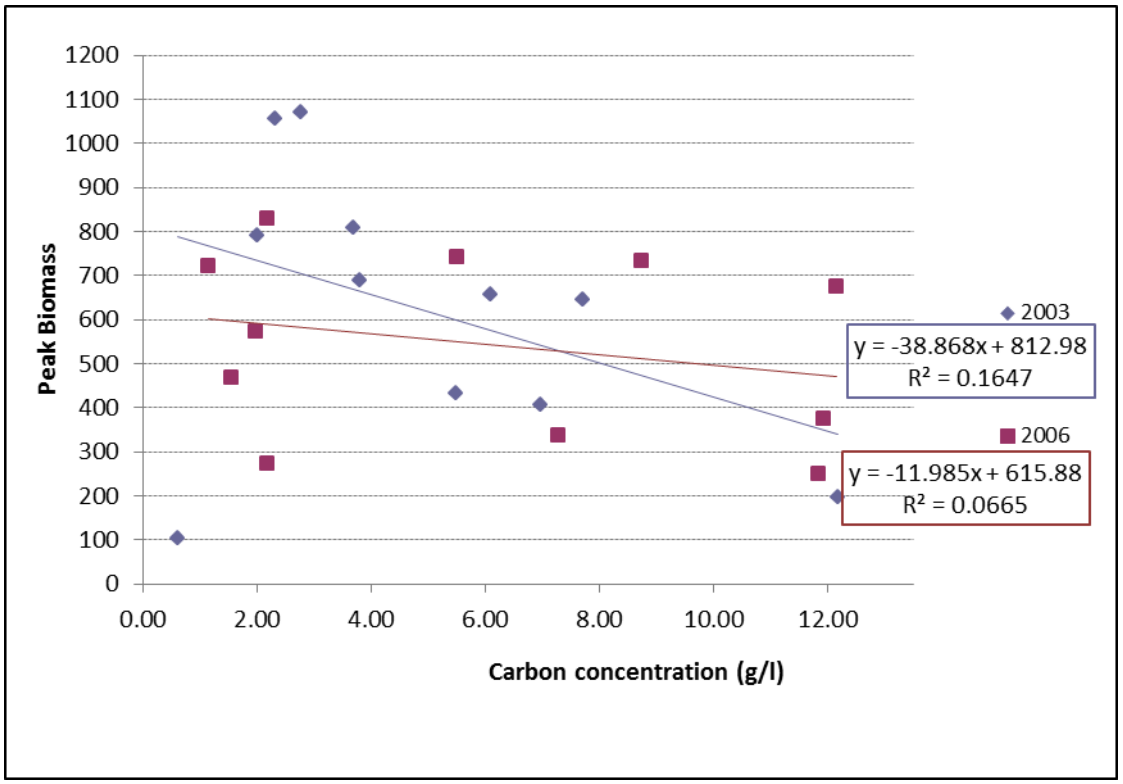


Figure 2.15. Graph of the peak biomass (tonnes) with the carbon concentration at each site.



There is clearly an increase in the number of individuals between 2003 and 2006 at 0 m to 50 m, but not beyond this area as seen in Figure 2.12. This is mirrored by an increase in AMBI up to 50 m (Figure 14) and a decrease in H' (Figure 13) over the same distance between these years. As biomass changes over the same time point (Figure 2.15), there is a chance that this is responsible for these results.

Figures 2.16 to 2.18 present the multivariate analysis figures of the initial spreadsheets (macrofauna and environmental data). Figure 2.16 is a dendrogram showing the similarity between stations based on biological and chemical parameters. Figure 2.16 shows the CCA analysis graph for 2006 and Figure 2.17 the one for the year 2003. These graphs include both the macrofauna data and the biological indicators

together with their alterations connection. Some general results can be seen in Table A1 (see Appendix 1).

In Figure 2.16, the dendrogram shows three main grouping of sites: Group A includes three sites (11, 15 and 17), a larger Group B with 8 sites (7, 12, 8, 9, 14 and 18) and the smallest Group C with 2 sites (4, 6). The first group A includes the sites 11, 15 and 17. Even though a similarity is shown on the dendrogram, they do not present similarities within the other tested parameters, such as the diversity indices. Sites 11, 15 and 17 have a similarity between them, which is the low value in the N number. Groups A and B have a similarity which is a combination of low N number and high C%. The same combination observed for groups B and C which included more sites, and presented a similarity in low values of N and high values in C%. The third group C includes sites 4 and 6. These sites have high C% and N value, but AMBI was also high, which is an indication of degraded ecological status.

Figures 2.17 and 2.18 show the CCA of the biological factors in relation to the sites' full macrofauna data. In Figure 2.17, which represents the state of the parameters in 2006, the AMBI and N number had a close connection. H' appears to indicate a different trend in the data showing a gradient in a different quadrant of the figure. Figure 18 shows the same trends for 2003 though an even closer association between AMBI and N. The vector scaling of these two figures is an indicator of the strength of the trend. In Figure 2.18 the sites are situated between the N and AMBI axes are Portree (25m), Port na Moine (25m) and Vady with two stations (0m and 25m). The sites situated between N and AMBI axes in Figure 2.17 are Basta Voe South (150m),

Hascossay (0m), Sian (0m), Kirk noust with stations (0m and 50m) and Vady with three stations (0m, 25m and 50m). These sites cannot be correlated with the cluster analysis from Figure 2.16 because the sites do not belong in the same groups. Vady site had two stations common in both figures which is an indication that all the stations of this site were improved ecologically during the years from 2003 to 2006. The variety of the stations from sites in Figures 2.16 and 2.17 shows a high correlation of N and AMBI for 2003 and 2006. A good ecological condition can be seen from these figures. The stations at 0m and 50m are situated underneath or adjacent to the main area that organic enrichment usually occurs but the stations at 0m and 50m are present with good N and AMBI relationship for both years.

Figure 2.16. Cluster analysis of the sites regarding their attributes (C, redox, H', AMBI, Biomass, N, S).

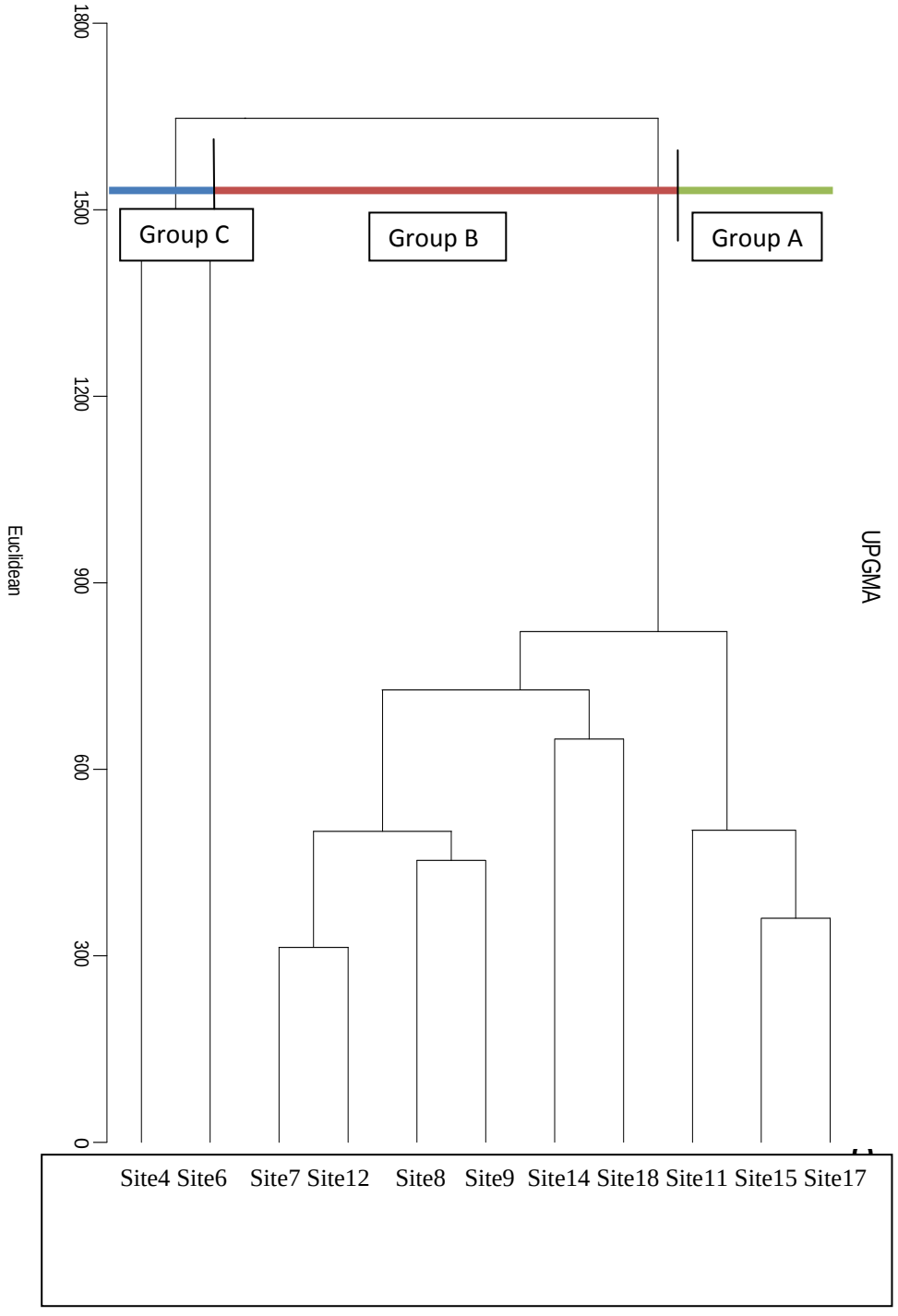


Figure 2.17. CCA of the sites in 2006 (regular data macrofauna and environmental: AMBI, H', N).

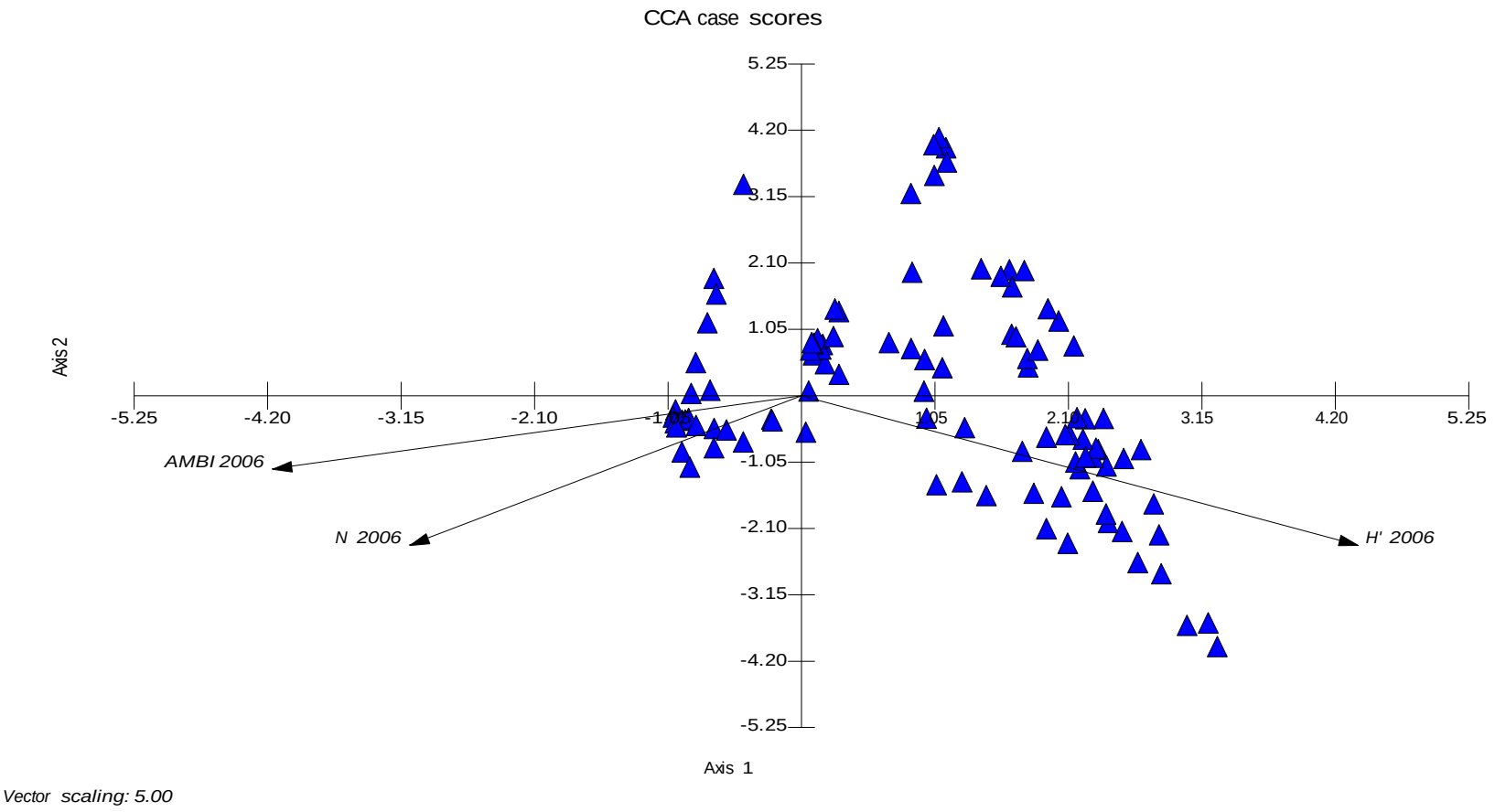
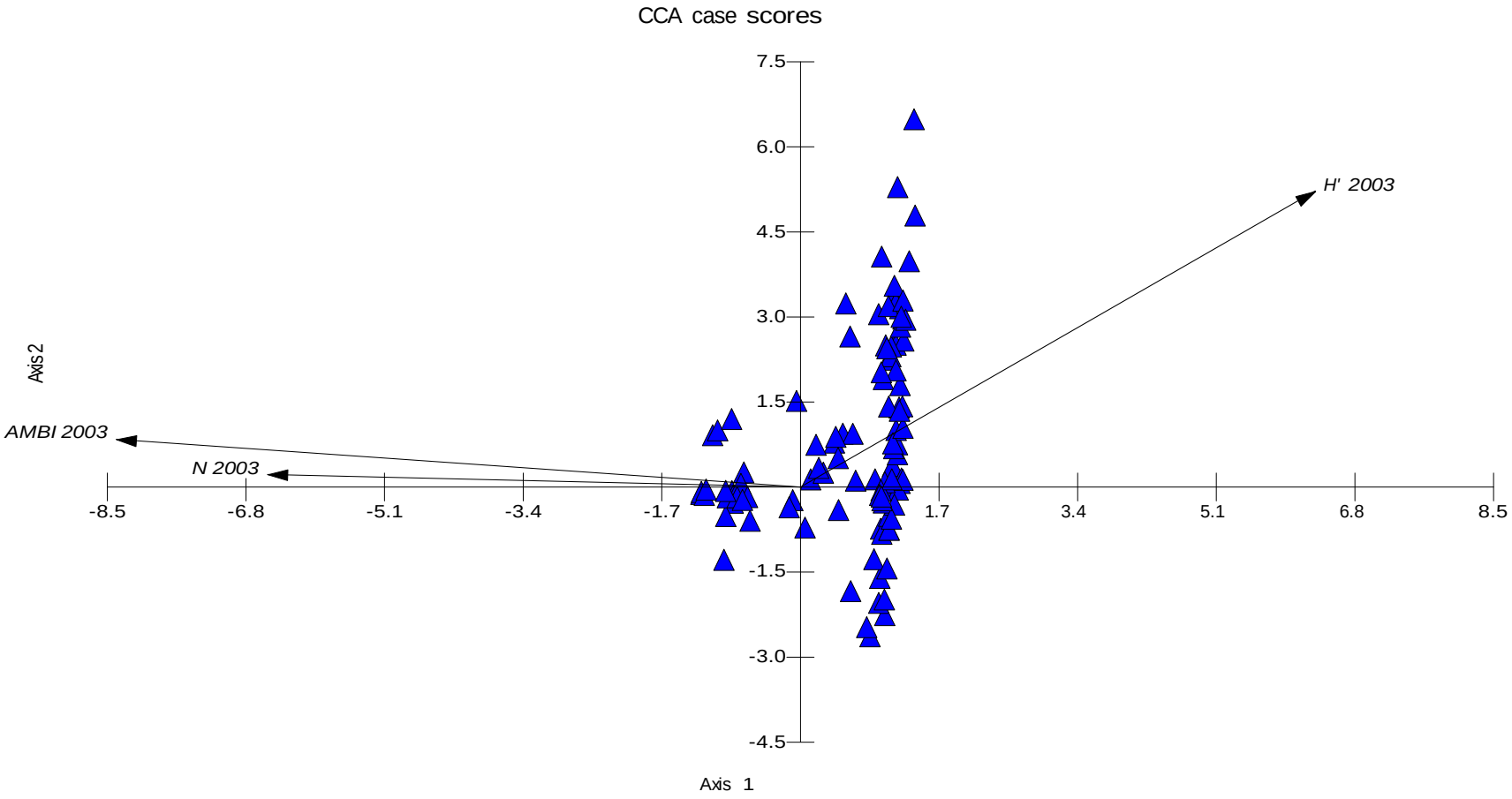


Figure 2.18. CCA of the sites for the year 2003 for (regular macrofauna and environmental data) AMBI, H', N.



2.4 DISCUSSION

2.4.1 Site changes

The average biomass has been reduced from 711.7 tonnes to 695.6 tonnes between 2003 and 2006 as seen the averages row in Tables 2.2 and 2.3. There is a wide variation of 1070 tonnes maximum to 105 tonnes minimum, with a range of 965 tonnes. The total number of individual species (N) was 229.3 in 2003 and 959.4 in 2006, having a range of 1439 and 1844 respectively and the maximum value was in 2006. The species number S in the sites was higher in 2006 with a mean of 11.5 and a 17.3 value in 2003 and the range in 2006 was higher too, while the values in 2003 were nearly the same (3, 3.6) the values in 2006 are not near (25.5, 42.9). The Shannon Wiener Index H' mean values were highly matched for 2003 and in 2006 having values of approximately 2.0 (2.01 and 1.90 respectively) showing a slight increase of the H' in 2003. The maximum values for H' were 3.5 in 2003 and 3.37 in 2006. AMBI values for 2003 were 3.01 and 3.06 in 2006. The maximum values were 4.7 and 3.9 respectively while the minimum value both for 2003 and 2006 was 2.0. Redox potential was higher in 2006 (~211 mV) than in 2003 (~191) while there is a wide range of the values in 2003 (384.7). Carbon concentration in the sediments was 4.87 g in 2003 and 6.04 g in 2006, hence 1.13 g higher in 2006, and the range of the values was 11.57 percentage units for 2003 and 11 percentage units for 2006.

In Table A2 (Appendix 1) the descriptive statistics for the stations are shown. In this, the N mean values were significantly higher in 2006 (962.13) than in 2003 (243.3) with a range of 18110 in the values in 2006. The H' values were approximately equal in 2003 and in 2006 (~ 2), and that equality was reflected for the range of the values that

were 0.85 to 3.51 for 2003 and 1.03 to 3.37 for 2006. AMBI mean values were close to each other in 2003 (2.93) and in 2006 (3.15) and the range of values were equal to five in both cases.

In Figure 2.2, the higher abunadance for species (N) is mostly present for 2006 in 8 out of 11 sites. Sites Sian and Aird showed the highest N number in 2006 and only site Port na Moine had a higher value for the year 2006. Figure 2.3 shows the total species number (S) in sites and for 2006 the (S) was higher in 8 sites, with one site (Loch Etive East) to have less (S) in 2006. Sites Strome and Aithness had the highest value in range for 2006. The Figure 2.4 plot shows an outlook of the H' values from the sites. Here it appears that H' had an equal correlation in 2003 and in 2006. However, sites Sgeir Mhor and Vatsetter had higher H' values in 2003 and sites Basta Voe South, Leinish, and Kirknoust had higher H' in 2006. In Figure 2.5 the AMBI scores do not grow higher than the 5 value both in 2003 and 2006. This good ecological condition can be enhanced for 2006 because the values are lower than 4 in the Figure 2.5, while one site does not have any change. Figure 2.6 shows the biomass in sites. It is seen in the graph that 10 out of 19 sites in 2003 had higher biomasses. The most notable ones are sites Hascossay, Leinish and Aird while sites Portree, Greinham and Basta Voe South had notably higher biomasses in 2006 while site Loch Etive had a low biomass value. Figure 2.7 shows the redox potential in the sites. Sites Loch Etive and Basta Voe South in 2003 presented the lowest values in redox (higher is better) and sites Bagh Dail nan Ceann and Loch Etive had the highest range in values. Site Vatsetter values have not changed during the years. Figure 2.8 shows the carbon concentration throughout the sites. Site Basta Voe South had a higher value in carbon in 2003 and sites Port na Moine, Inchkenneth and Loch Etive had higher value in carbon in 2006. It is also

notable that site Loch Etive had a high variance in its carbon value (noted above as lowest biomass).

The results for univariate analysis in relation to the distance from the cage edge can be seen in the Figures 2.9, 2.10 and 2.11. It is obvious that the N number in 2006 gradually decreases as you move away from the cage edge. For 2006 the highest number occurs underneath the cages and the lowest in the references stations (which they vary in distance from 500m to 1000m). On the other hand, that decrease cannot be seen for the 2003 values. There the values show that 150m stations had the highest N and 25m the lowest. The cage edge at 0m presents a large scale of N during the years, where N is higher in 2006 and increased than 2003. Figure 2.10 shows the H' index in relation with the distance from the cages between the years 2003 and 2006. The columns in 2006 show that H' has a tendency to increase moving away from the cages with a small variation to the ref stations (850m). The H' in 2003 had a mixed variation on its values and does not have a clear tendency but the values were varied in a range of 1.7 to 2.4 that makes H' more stable. Notable comparisons in terms of scaling variation are: the cage edge stations at 0m presented higher values in 2003 than 2006 and the 150m stations presented higher values in 2006 than 2003. Figure 2.11 is the graph for AMBI scale in relation to the distance during time. The columns for 2006 show the decrease of AMBI as moving away from the cages. The columns for 2003 do not present a stable tendency and if the 50m stations were excluded the variation scale lowers to values around 3. The 50m stations have a wide range in change of the AMBI scale from 2 in 2003 to 3.5 in 2006 and the opposite is noted in 150m stations.

It is also seen from the figures that the minimum N number in 2003 occurs in 25m and the minimum in 2006 in the ref stations, while the maximum number in 2003 was at 150m and the maximum was at 2006 in 0m. The minimum H' number in 2003 occurs at 0m and minimum in 2006 at 0m while the maximum H' for 2003 was at 25m and the maximum in 2006 was at 150m. Similarly, the AMBI score was minimal in 2003 at 50m and at the ref stations in 2006 while it was maximal at 0m in 2003 and in 2006.

As mentioned prior in the results, these indices show a difference when the waste input to sediments was high and none when the waste input was low. This could be used to define a zone of effect, for the influence of biomass change on sediment quality. Particurarly, this zone effect only seems to happen somewhere between 50 and 150 m from the cages.

The results of the biomass itself can be seen in Figures 2.12 to 2.15. These equations in terms of representing the trend are strong, as it seems from their r square values (correlation coefficient), where they are near to 1 ($r^2$of 1.0 indicates that the regression line perfectly fits the data). In Figure 2.12 plot, the peak biomass (PB) in relation to the N number is shown through the trend lines for 2003 (11.1) and 2006 (11.2):

PB= 0.089*N+602.67, r^2=0.0142 (11.1) and

PB= 0.007*N+539.26, r^2=0.000398 (11.2), where PB is the peak biomass and N the total number of the species.

In Figure 2.13 the peak biomass is plotted with H' and the trend lines are as follows for 2003 (12.1) and 2006 (12.2):

PB= 112.5*H'+411, r^2= 0.0821 (12.1) and

PB= -13.1*H'+568, r^2= 0.026 (12.2), where H' the Shannon Wiener index

Figure 2.14 shows the plot of the peak biomass with the AMBI score. The trend lines from this plot show the relationship 2003 (13.1) and 2006 (13.2) below:

PB= -126.6*AMBI+1005, r^2= 0.1493 (13.1) and

PB= -78.5*AMBI+790, r^2= 0.0457 (13.2)

The total carbon levels of the sediment samples relation with the peak biomass can be seen in the trend lines (14.1) and (14.2) for 2003 and 2006, as extracted from Figure 2.15:

PB= -38.9*C+813, r^2= 0.1647 (14.1) and

PB= -11.9*C+616, r^2= 0.0655 (14.2), where C is the carbon concentration.

These trends show an increase in the number of individuals between 2003 and 2006 at 0 m to 50 m, but not beyond this area. This is reflected by an increase in AMBI up to 50 m and a decrease in H' (over the same distance) between these years. As biomass changes over the same time point, there is a chance that this caused those results. There are two conclusions from these results. 1) The biomass variation may be related to the change in numbers of individuals/m2 and diversity, and 2) this effect only seems to happen to somewhere between 50 and 150 m from the cages.

The aim of this research was to study the impacts that occurred over a three year period between 2003 and 2006 of the seabed in areas within fish farms. The general results coming from the data do not show any significant impacts on the ecosystems of the tested sites. The average values for each of the indicators show a good environmental condition. That condition was highly seen by the indicators chosen from the variety of the univariate indicators. The idea was to receive a holistic view of the ecological status which occurred these years. The features of the study are focused mainly to the environmental properties of the seabed in 2006 biomass changes. With this as guide, the various biological and chemical trends were plotted to describe the changes and if they lead to impacts.

The peak standing biomass values ranged from 105 tonnes to 1070 tonnes, but these values both apply to the whole dataset of analysis, hence, for both 2003 and 2006 years. For 2003 the results suggest a mixed sediment condition between both sites and sampling stations. This may happen because of the instability the increasing biomass brings to the ecosystem. This increase results to enrichment of carbon and nutrients when the uneaten food and the faeces are decomposing and combined with other various chemicals used by the fish farmers.

The biological and chemical indicators constitute a strong case that biomass changes between 2003 and 2006 did not cause environmental impacts. Specifically, the H’ values in 2003 and 2006 are slightly changed around the average value of ±3.4, which is a good range for H’. Being closer to 4 value of H’ the ecosystem condition is better.

The chemical indicators change proportionally to the biomass changes. When the biomass is reduced less anoxic conditions apply to the seabed and the carbon enrichment is decreased due to fewer decomposition products. The redox potential decreased when total carbon levels increase and the carbon increased levels occur with high fish biomass. This is linked to the level of dissolved oxygen within sediments, which can be used as a proxy for sediment productivity (Beveridge, 2004). The amounts in 2003 have not lead to potential impacts yet because of the good-levelled changes that occurred. There was no significant overall change in sediment carbon levels between 2003 and 2006, showing the overall decrease in biomass during this period had little impact on sediment carbon levels.

2.4.2 Station changes

The individually sampled stations at various distances from the fish cages show a better view of the biological trends since there is a tendency for the indices to present an alteration potentiality as the distance changes. This is suggested by the Figures 2.3, 2.4 and 2.5, where the changes in 2003 are not stable, probably, due to significant increased biomass in 2003. The score for AMBI and the diversity indices in those stations showed that species had natural distribution of evenness and equitability and the ecological parameters, such as carbon percentage, ranged within the quality standards. It seems that the stations at 0m from the cage edge had the highest N values. It was also observed, that diversity is often highest at an intermediate distance from the cages where nutrient input is still high but there is limited environmental degradation regarding the benthic condition (Wells *et al.*, 2008). As shown in the sites section the 2003 H' values are indicative of disturbed sediments, with 0m stations (CE) having the

lowest values and the 25m ones the highest and that difference is remarkable within 25m. The 2006 view of the sites is more stable with H' to be less in zero and higher in 150m, a logic output since the organic enrichment is less as the distance is further and the species present the natural equitability following higher H'. The case of "the lower biomass, the better for the ecosystem" is raised again here. The same issues apply to AMBI values in stations between 2003 and 2006, where the values in 2003 are higher at the 0m and the reference stations. In this, all the stations in 2003 (besides 50 m) have the same scale for AMBI values in between 2.8 and 3.5, a classification describing the condition as "slightly disturbed". Whereas values for 2006 present a score representing stabilised conditions suggesting that higher biomass increases the biotic condition in the areas most impacted by cage wastes, *i.e.* the cage edge.

2.4.3 Trend relationships

In general terms, as the tables of the situation in 2003 compared to 2006 suggests (using paired t-tests), the biomass in the years 2003 to 2006 was reduced in tonnes at a 12.8% rate which constitutes a significant change ($t = 0.732$: $p = 0.481$). Given this result, the H' index was slightly reduced to 0.8%, which is insignificant ($t=0.07$; $p=0.945$), but AMBI, redox and carbon increased by 4% ($t=-0.36$; $p=0.727$), 9.3% ($t=-0.629$; $p=0.543$) and 19.4% ($t=-0.681$; $p=0.511$) respectively. AMBI's score remained within the same classification scale while carbon also changed insignificantly (of ~1.2g). The redox presented a change of 20%, which is slightly significant. By applying SIGMASTAT in these factors, multiple regressions were extracted for all of them:

BM= -26.35*C-85.03*AMBI+19.82*H'+971. (MR1) –year 2003

BM=-13.54*C-72.28*AMBI-30.43*H’+909. (MR2) - year 2006

BM= -18.2*C-87.23*AMBI-18.9*H’+0.62*redox+863 (MR)

The relationship between the redox potential (R) and the carbon (C) can be seen in the regressions below:

C= -0.00409*R+5.652 (C/R in 2003)

C= -0.0522*R +17.06 (C/R in 2006)

And for carbon and redox in a sum of both years: C= 0.0076*R+6.98 (C/R reg.)

The trends tested are some of biological and chemical nature of the seabed samples chosen for their ability to identify various changes that potentially lead to impacts. It was mentioned that the overall condition of the sites and their stations was good and that the results of the biomass reduction were positive results within the individual local status of the farms’ seabed. The trends were plotted in the CCA in relation to their macrofauna data which previewed a one way close relationship with two of the trends (N and AMBI) and a distanced relationship with the third one (H’). The close relationship of the biotic status along with the overall number of the species implies the strong connection of the number of the species regarding their biotic situation. Therefore, when a model is about to be constructed in order to predict the ecological status of an area, both of these trends must be included to make it accurate in terms of the estimation and their description.

In a paper made to test the hypothesis for a model including only the carbon and the redox potential, aiming to predict the ecological conditions of areas within sea farms a regression was made available: y= -0.0076X+6.44 (Lynn, 2006). The regression extracted from this study is: y= -0.0082X+7.4. The redox potential and the carbon are highly correlated to each other, differing only in deviation numbers. The major point is that carbon and redox are not enough to constitute a precise output of the status. Other trends also need to be included. In a sequence to this, it was seen that the AMBI and N number would be a more accurate addition to a redox and carbon regression model and a multiple model would also be a better prediction strategy.

As for the rest of the trends, the results from the correlation analysis suggest that no significant relationships between any pair of the trends occurred in the full datasheet. By the correlation tables it is evident that none of the trends has any significant correlation, but only minor ones appear among the trends during the period 2003 to 2006. The trends which, as a matter of principle, present a correlation are: N and AMBI (also seen in CCA), especially in 2003, the S with H', the H' with biomass, AMBI with carbon and AMBI with redox. The aim to test the hypothesis initially set, if the trends variation can show any changes within a time sequence of three years, can be answered as the results and the regressions (especially the carbon ones) show a slight alteration of the trends during these years, because of the change in biomass. The only change is in the redox potential, which in 2003 was less and in 2006 was higher, an outcome that is understandable, since the biomass reduction led to oxygen increase due to less biochemical reactions in the seabed. The multiple regression, with all the included ecological trends, shows a significant reduction in carbon during the three-year

period, while the diversity factors were naturally and equally plotted except the H' (and redox potential) that should have also increased.

Another question raised is whether these results, which generally show good ecological conditions, would still be the same if the biomass was higher. In this case, mean values would be helpful to be analysed. AMBI has 3.13, H' 1.9, redox 211 mV and carbon 6 g. and all of these means represent the worst case scenario. In case the biomass is increased by 10% then all the trends will be increased or reduced respectively. In this case these values would be: AMBI 3.4, H'1.71, redox 189 and carbon percentage 6.6 and they are all good except H' and redox which are slightly decreased. That shows that if the biomass would be for instance 10% higher (10% higher means biomass of 686 t from 623), the condition would be at the same good level.

2.5 CONCLUSIONS

The sites examined in this project are situated in the west coast of Scotland where the aquaculture industry provides salmon over extensive farming (SEPA, 2007). The increase or stable high level of farming is often strictly associated with the biomass and its changes, resulting in an equal change in the seabed (Beveridge, 2004). The sites general outlook regarding their status, throughout the three-year period, was good enough even though the chemicals used from the farms for the welfare of the fish were not included and not tested. The overall conclusion for these areas is that the environmental situation was good, with no excessively polluted levels and ranging in

terms from slightly to moderately disturbed biotic status of the species and, as far as their richness and equitability is concerned, in a medium level of distribution.

As for the interaction between the biomass and the ecological trends, the conclusions are generally summarised in three combined results: (A) the increasing biomass leads to moderately disturbed conditions within the trends for species numbers, richness and equitability. This was mostly observed to sites where the biomass was higher than 650 t. (B) The decreasing biomass produces a stable condition to the trends, thus to the seabed status. When this occurs, the seabed tends to develop a trend to equally enrich the sediment with carbon and nitrogen, so that the pollution potentiality may gradually decrease. This was observed in sites where the biomass ranged between 249 and 830 t. (C). The level of the changes shows that if the biomass had been slightly higher (≤10%), the local ecosystems would have also been undisturbed, provided that the husbandry and welfare chemicals would not be used within this three-year period.

In relation to the internal ecosystem of the sites, the above conclusions apply. When studying the behaviour of the trends in relation to distance, two major conclusions can be drawn: (A) As observed in past papers, the 25m stations have less numbers in species than the stations underneath the cages, and (B) the trends follow a logical course: The H' increases when the distance of the cages increases, and AMBI decreases when the distance from the cage edge increases. Both of these indicate that underneath or adjacent to the cages the conditions are not the optimal ones as occurred in 2006 in a 50-metre distance from the cages.

In conclusion, only four (out of 19) sites presented higher change levels in all measured factors, but it is not certain by the analysis if any impacts occurred there. This observation led to issues questioning whether the biomass had caused them, but it is more possible that external factors, such as the use of chemicals (like parasiticides), in combination with the biomass, have caused these impacts.

Chapter 3
A comparison of the effectiveness of diversity indices for analysing environmental data from marine fish farms by long term metadata analysis

3.1 INTRODUCTION

There is considerable effort and research into the detailed biology and chemistry of changes within the sediment, with the measurement and assessment of a wide range parameters to determine the degree and extent of risk. These are based on data and detailed case studies on the organic impacts from fish farms and other discharge sources (SEPA, 2005). In order to assess the benthic impacts of marine cage farms, SEPA currently requires operators to monitor a set number of parameters which describe the biological and physico-chemical status of the seabed. The use of underwater video and photographs, where necessary, are sometimes used to provide additional information on the extent of various impacts, the location of previous cage positions and / or the effects on hard substrates. The value of these visual analyses, however, is limited when attempting to determine the extent of impact beneath the sediment surface (Wells, 2008).

An impacts study is analysed by two major approaches *i.e.* the use of univariate and multivariate analyses (Environmental Services, 2007). In a multivariate analysis, the stations are plotted with the species and any subsequent grouping of stations or species that are identified can provide information regarding the similarities of the impacts (Environmental Services, 2007). The univariate analysis, that is the core of analysis in the present project, is a study of the sediment diversity indices as established for impacts assessment by the environmental bodies.

Primary measures are simply the number of taxa and number of individuals at each site, whereas univariate methods refer to the calculation of diversity indices. A variety of indices have been proposed but one of the most widely used is the Shannon-Wiener index (H', using logs to the base 2) which was recommended by both Pearson & Rosenberg (1978) and by Rees *et al.* (1990). Diversity is considered to have two components; species richness and equitability. Equitability can be measured using Pielou's Evenness Index (Pielou, 1966). These single figures measures represent a major simplification of the biological data and consequently, if used without other complementary methods, may lead to misinterpretation. They can be extremely useful, however, if a gradient of effect is apparent or expected. In addition their simplicity suggests that the derivation of biological standards may be practical (SEPA, 2007).

SEPA has conducted numerous studies regarding environmental changes in the marine ecosystem in areas that are occupied by fish farms. The established methods for assessing these changes typically are based on univariate analysis, which are used to identify and categorise the occurring impacts (SEPA, 2005). This is achieved by using a series of indices that permit changes within sediments and its associated species fauna to be calculated. Species can change in abundance on the seabed and thus, their local ecosystem can change respectively (Ponti & Abbiati, 2004). The indices that are used, based on the measurement of various parameters and then compared to data from past studies, allow for the impact to be calculated. These parameters include determinations on species richness, their distribution to each other within the study area, and their trophic attributes (SEPA, 2000).

An important parameter within the univariate analyses is the Infaunal Trophic Index (ITI) (SEPA, 2000). ITI is the index that is calculated by using the species found within a measured area and then, are categorised from the index by applying a particular score of the species' trophic habit (Maurer *et al.,* 1999). Another index analysed by univariate analysis is the Azti's Marine Biotic Index (www.azti.es) (AMBI) (Muniz *et al.,* 2005a; Muxica *et al.,* 2005). This is a biotic index that is calculated using software into which the user enters species data. AMBI then calculates the attributes of the station as a whole by testing the biotic factors of the species. Both indices, ITI and AMBI, present their results in the form of a numeric scale, the score of which is used to characterise the ecological status of the station, and the extent of its degradation (Muniz *et al.,* 2005a; Muxica *et al.,* 2005).

ITI is an ecological community index. Data on species abundance is entered into a formula which provides a value on a 0 to 100 scale that suggests the level of the trophic situation within the study area. This index relies on the assessment of the changes in the feeding (trophic) mode of benthic organisms in areas subject to elevated levels of organic enrichment (Codling & Ashley, 1992). The index was developed from a system originally devised for use in California (Word, 1978). This index differs from the numerical methods in that knowledge of the ecology of the taxa involved is also required. Thus it forms a useful complement to the numerical methods in that the data are considered from an alternative perspective. The index was found to respond satisfactorily to pollution gradients from a variety of sources including sewage and industrial discharges (SEPA, 2007).

The AZTI's Marine Biotic Index (AMBI) (Borja *et al.,* 2000) is widely used along the European (Borja *et al.,* 2000; Ponti & Abbiati, 2004; Borja *et al.,* 2005; Bald *et al.,* 2005) and American (Muniz *et al.,* 2005a) coasts. The concept is similar to that of ITI (abundance of each group), it is based on the distribution of the abundance of each species, into one of five ecological groups (EG) (sensitive to pollution, indifferent, tolerant, and second and first-order opportunistic species): I: very sensitive, II: indifferent, III: tolerant, IV: 2nd order opportunistic, and V: 1st order opportunistic. A new update of the software has added two further categories, and the new categorisation is: 1-2 levels: slightly disturbed; 3-4 levels: moderately disturbed; 5-6 levels: heavily disturbed and level 7: extremely disturbed (Borja *et al.,* 2000; Muniz *et al.,* 2005a). The distribution of these EGs, according to their sensitivity to pollution stress, provides a biotic coefficient that was adapted to provide an estimation of the Quality Status (*EcoQ*) of ecosystems, according to the *Water Framework Directive* (*WFD*) (Borja *et al.,* 2003b, 2005). For the index calculation, the AMBI software was used; this software is freely available on http://www.azti.es.

To date, the ITI and the AMBI indices have not been compared, presumably due to the fact that the use of AMBI is limited. A limited number of other studies (Word, 1978; Borja *et al.,* 2000), however, have compared ITI with other indices. In a study by Word (1978), ITI values were compared with various other indices and sets of measurements to determine the relative effectiveness of each method in describing ecological conditions. In Figure 3 in Word's (1978) study, index values for stations all along the southern California coast are compared with measurements of infaunal diversity, biomass, and number of individuals and with sediment levels of BOD at the same stations. Each set of measurements gives an indication of the variation in

conditions along the coast, but the ITI is the most sensitive measure of changes in the structure of infaunal communities, that contain enhanced levels of organic material (Word, 1978).

The way in which the two indices (ITI and AMBI) are compared is mainly described in detail in the methodology chapter (Chapter 2), but the comparison of these two focuses on assessing their accuracy of use when compared with each other and with two constants that are hypothesised to be accurate. Their comparison to one another is through alignment of their scales and their correlation which is tested statistically. Thereafter, they are compared with carbon percentage (C%) and the Pielou Index, so that a more comprehensive analysis can be made to assess the indices and their potential as ecological descriptors.

This study, therefore, results from the need to test a biotic index for enhancing the univariate analysis both in terms of validity and accuracy. In addition, this study set out to identify a substitute for the use of ITI that is accurate enough to be used so that the users are able to obtain the same or better (*i.e.* more accurate) results by avoiding the calculations of ITI that are long and time consuming. The hypothesis of this study is that the AMBI results will be as valid and as accurate in describing the impacts of the sediments as the use of ITI. The study also aims to establish a comparison protocol for the two indices with each other and with the test constants that are used as part of the evaluation.

3.2. METHODS

3.2.1 General methods

A total of 730 stations were considered for testing in the first round of analysis. Some stations were subsequently removed because the data were incomplete, reducing the number to 310. After data entry into a spreadsheet, the tools used for analysis were Excel (Microsoft Office, 2007), SigmaStat 3.0 and MVSP 3.2. From these, Excel was used for the comparative regressions, SigmaStat for the descriptive analysis, and MVSP for further information on the correlation of the indices.

ITI and AMBI were chosen for testing as they based on the same ecological concepts. In addition, the Pielou Index was included as a constant as this assisted in the comparison and is considered to be precise in symmetry and statistically correct. From the analysis conducted in Excel, a summary of graphs were produced where the two indices are compared with each other and their linear regressions determined. SigmaStat was only used for the multiple regression of ITI and AMBI with Pielou Index and carbon percentage because it provides better statistical application and analytical results. The third set of results was from the use of MSVP to generate tables that provide more details on the correlation of indices, in testing their radical values.

3.2.2 Background to the indices used

The ITI has great potential with regard to standards being set. The index has values that range from 0 to 100 and the results can be interpreted as follows:

Index Value Assessment:

- 60 to 100 Community ‘Normal’
- 30 to 60 Community ‘Changed’
- < 30 Community ‘Degraded’

The purpose of the Infaunal Trophic Index (ITI) is to describe the feeding behaviour of soft bottom benthic communities in terms of a single understandable parameter. These animals fall into four groups; they are either suspension or deposit feeders that feed above, on or below the mud surface. ITI was developed in California, USA and first published in 1979 (Word, 1978). Since then it has been adapted for use in UK waters (Codling & Ashley, 1992) but the principles remain the same. Invertebrates have been divided into four groups based on what type of food is eaten, where it is obtained and how it is obtained. ITI trophic group 1 are suspension feeders *e.g. Mya arenaria*. Group 2 are surface detritus feeders. Group 3 are surface deposit feeders, and group 4 are sub–surface deposit feeders such as *Capitella capitata.*

Tested mostly for soft sediment communities and is known to have limitations when coarse sediment communities are considered. In addition, ITI needs to be interpreted with care when the diversity value is low (*i.e.* the number of species ≤ 5). The formula below (SEPA, 2000) presents the calculation of ITI.

$$ITI = 100 - 33.3\left(\frac{0N_1 + 1N_2 + 2N_3 + 3N_4}{N_1 + N_2 + N_3 + N_4}\right)$$

where N_i is abundance of organisms in trophic group i.

AMBI is a software tool that after the input of the macrofaunal spreadsheet, releases the information of the biotic situation in each of the stations individually. It shows the extent to which each community is disturbed at each of the test stations. The privilege of this programme is that the results can be previewed before their release to a spreadsheet. This preview is in two forms: 1) a preview of individual situations; and, 2) a combined preview of several stations and the extent of their disturbance. This then facilitates the correlation of the effects with a holistic approach of the impacts individually or in areas. This in combination with the cluster analysis available in programmes like MVSP (or other multivariate tools), permits a complete profile to be drawn for a large ecological area to be provided. It also allows for the current situation in a small area to be determined.

AMBI is a tool based on the calculation of the species biotic situation. The output of the results is shown on a 1-7 scale which scores the effect at each station. A score of "1" is regarded to be the optimum situation (*i.e.* no ecological disturbance), whilst a score of "7" suggests that the station is extremely disturbed. AMBI can also provide an Excel spreadsheet with all the results from the tested stations and also with the rest statistical calculations and analysis.

The Pielou Index is an important index exported automatically by running the WORMS software (obtained from Colin Moore after personal communication) along with other indices. Diversity is considered to have two components; species richness and equitability. Equitability can be measured using Pielou's Evenness Index (Pielou, 1966), which is defined as the extent to which the individuals are equally portioned

among all species. The less variation there is in communities between species, the higher the Pielou value is.

The organic enrichment of at a station is calculated by measuring the carbon percentage (C%) extracted by the samples in the seabed. C% is a good reflection of the level of organic pollution. Along with the nitrogen percentage, C% can reveal information regarding the organic and nutrient level of pollution within the test sites. In this project, C% was selected as the indicator of organic pollution used in parallel with the biotic indices used at the test stations. As it was mentioned the indices are based on the carbon values where carbon percentage is included to their calculation formulas. Using this approach, it is possible to obtain a greater number of relevant degrees of organic pollution that the indices have, so that a quality comparison to be made.

3.2.3 Data input and analysis

Macrofauna data, from three main sources, were included in the final spreadsheet. The primary, raw data originated from the Institute of Aquaculture (IoA), from the Scottish Association for Marine Scientists (SAMS) project (SAMS *et al.*, 2005) and from SEPA. The exact locations of the stations and sites are given in Appendix 2, Table A1. Only AMBI and ITI for each station and site are compared; no other analysis of these sites is considered here. The carbon percentage values from the same sites *etc*, originating from the same information sources were also added to the comparison table.

The raw data were entered in to spreadsheet in Excel 2007 following the general output for macrofauna data analysis. This is a table with one column for species data for each station were entered into each row. To simplify the data within the spreadsheet, species were grouped into families and/or were combined into dominating species. For example, *Capitella sp.* and *Capitella capitata* were combined as *Capitella capitata.* Thereafter, the software tool WORMS, (a tool that with the input of the macrofauna datasheet, calculates the ecological indices and the result is extracted in a new spreadsheet), was used to extract the univariate indices, and the AMBI software tool was used to determine the AMBI scores for each station. The univariate indices are biological indices that are calculated automatically by the tool and show the biological occurrence at each station. The output is table listing the different indices in the top row and their values in relation to the stations in the top column. The most important indices are a combination of the required tests according to the nature of the research made each time. For this study, the ITI and AMBI scores are compared, and those for the Pielou Index are also considered for their comparison.

The species list in the final macrofauna table contained 750 species. Having such a large number of species may cause a deviation from the species list, when extracting ITI scores, before running the formula. After the ITI scores were determined, these were then transferred to a new table with the AMBI scores for comparison.

In the new spreadsheet containing both AMBI and ITI scores, the ITI scores were then transformed to AMBI's scale. This is important given the way the scores refer to the level of impact *i.e.* they use opposing scales. ITI has a scale of 0-100, where

zero represents the worst situation and 100 the best, whereas AMBI uses a score of 1 to represent the best condition of a site and 7 as the worst. After that, both the AMBI and ITI scales had a similar increasing negative effect output. To accomplish symmetry, all the new values from ITI were fitted into a comparative "1-7 AMBI scale" by multiplying each value with 7/100. This column of values was then added to the comparison table with the values from the Pielou Index and the C% for subsequent analysis.

The C% was included in the analysis to determine whether one index over another was better able to accurately reflect the level of organic enrichment at a station. The correlation and regression of C% together with the ITI and AMBI were examined using SigmaStat 3.0.

The results were obtained using Excel and Sigma and then MVSP for the cluster analysis of the stations. Excel provided the Tables, the regressions and the column comparison graph, while Sigma was used for the correlation analyses. To validate the analysis, Pielou values for the tested stations were put into a new spreadsheet and then correlated with the ITI and AMBI scores.

3.3 RESULTS

In Table 3.1 the descriptive analysis and the correlation analysis for AMBI and ITI are shown. A Pearson's correlation coefficient of 0.317 ($p = 0.000498$) and a Spearman's coefficient of 0.272 ($p = 0.00311$) were determined. An ANOVA suggests a correlation

of 68.875 with 1 degree of freedom, (P = <0.001). Further information shows that the mean value for AMBI is 2.504 ± 1.4 whilst for ITI it is 4.115 ± 1.2 For AMBI 2.5 value means that the tested stations are categorized as slightly disturbed and for ITI 4.1 means that the stations are moderately disturbed.

Table 3.1. Descriptive summary statistical analysis for AMBI and ITI (output from SigmaStat^{TM}).

Descriptive Statistics	**AMBI**	**ITI**
Missing	0	0
Mean	2.504	4.115
Std.dev	1.412	1.19
Std. error	0.131	0.11
C.I. of mean	0.259	0.218
Range	5	6.084
Max	6	6.999
Min	1	0.916
Median	2	4.355
25%	2	3.306
75%	3	4.666
Skewness	1.291	0.133
Kurtosis	0.707	1.018
K-S Dist.	0.366	0.21
K-S Prob.	<0.001	<0.001
Sum	293	481.43
Sum of squares	965	2145.364
METHOD	Spearman's Correlation	Pearson's Correlation
INDEX	ITI/AMBI	ITI/AMBI
Correlation Coefficient	0.272	0.317
P Value	0.00311	0.000498
Number of Samples	117	117

Summary output

Group	**N**	**Missing**	**Median**	**25%**	**75%**
AMBI	117	0	2	2	3
ITI	117	0	4.355	3.306	4.666
H = 68.875 with 1 degrees of freedom. (P = <0.001)	ONE WAY ANOVA				

Figure 3.1 shows the relationship between the AMBI and ITI scores as a regression analysis. The relationship was determined to be: ITI= 0.62*AMBI+0.76. Calculation of the overall means of the two indices shows that AMBI has an overall smaller value than ITI of a 0.82 units difference (*i.e.* they have an 82% correlation).

Figure 3.1. A scatterplot comparison of ITI and AMBI with their linear regression.

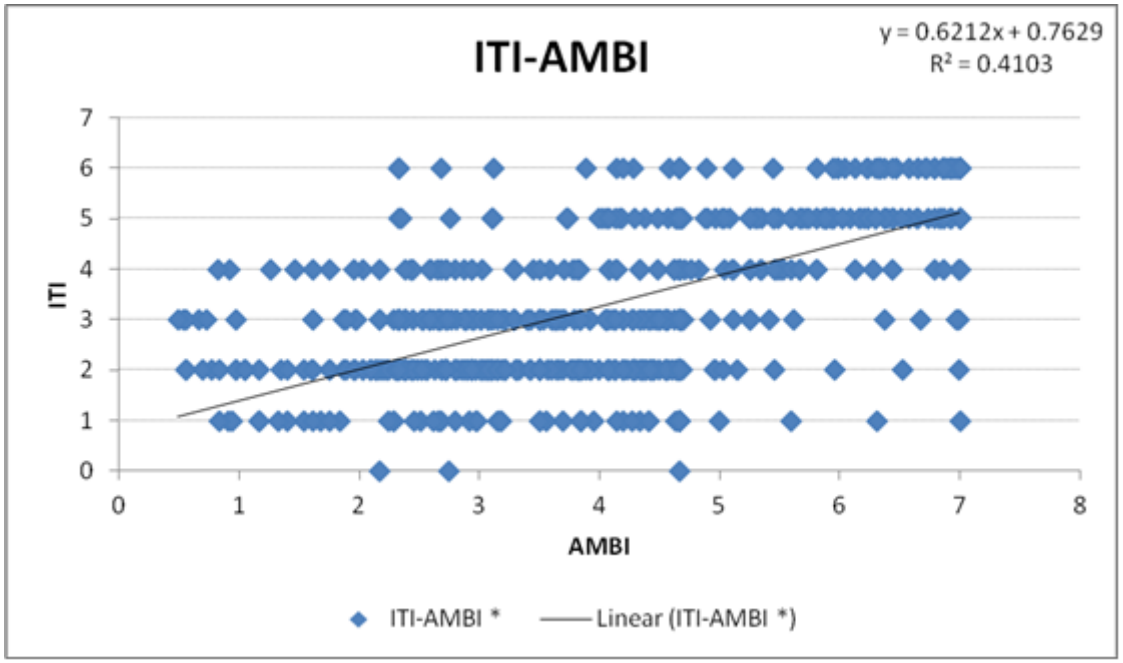
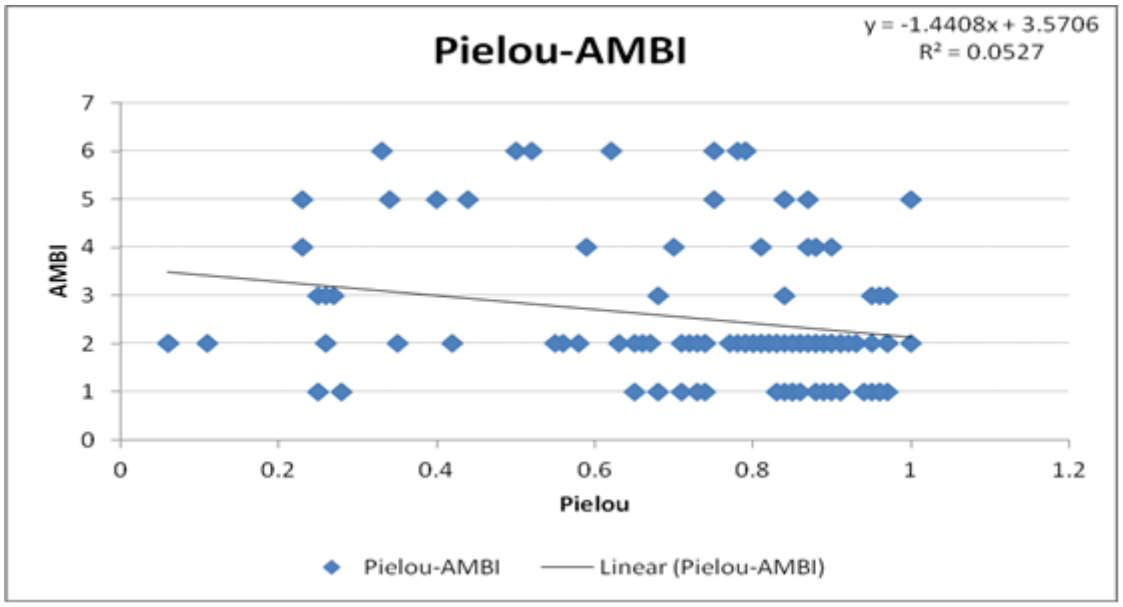
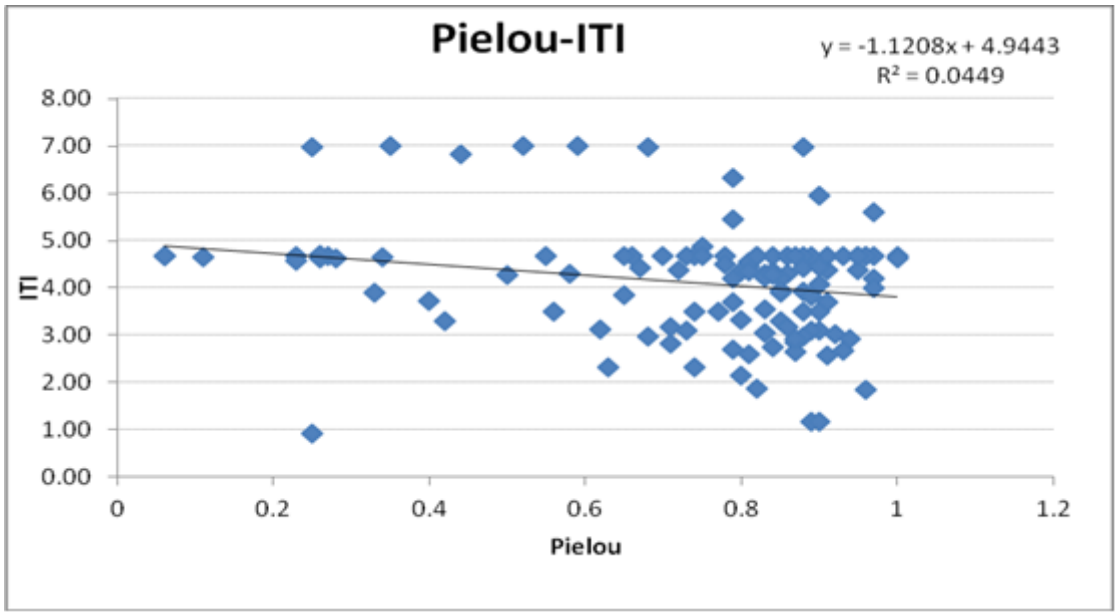


Figure 3.2. A scatterplot comparison of Pielou Evenness with AMBI.



In Figure 3.2, AMBI decreases with Pielou; this is also seen in the ITI plot with Pielou in Figure 3.3. The respective regression analyses for these were AMBI= -1.44*P+3.5 and ITI= -1.12*P+5.

Figure 3.3. A scatterplot comparison of ITI and Pielou Evenness.



Figures 3.4 and 3.5 are scatterplots showing the plot of C% with ITI and AMBI respectively. In Figure 3.4, the trend line of AMBI with C% gives a relationship defined by y = 0.2441x + 4.271 (r2 = 0.0138), which suggests that as C% increases, so does AMBI. In contrast to this, the plot shown in Figure 3.5, of C% with ITI suggests that one decreases, so does the other; this is defined by the relationship y = -0.271x + 5.997 (r2 = 0.0121). The trend line in Figure 3.5 appears the more accurate of the two suggested by the r2 value that is closer to zero, suggesting lower deviation. Figure 3.6 is the connection of AMBI and ITI with the carbon percentage. In Figure 3.6 the carbon percentage increases with AMBI, but decreases with ITI. The regression for AMBI is C%= 0.24*AMBI+4.3, whilst for ITI is C%= -0.27*ITI+6. The AMBI regression reflects the condition better than ITI, because when the carbon percentage is increased in the sediments, the possibility of disturbed condition is also increased.

Figure 3.4. A scatter plot of AMBI with the C% in the sediment for all the tested stations. AMBI values in the figure are scaled from 1 to 7 (AMBI levels) and C% values range from 0 to 12% (% dry weight sediment).

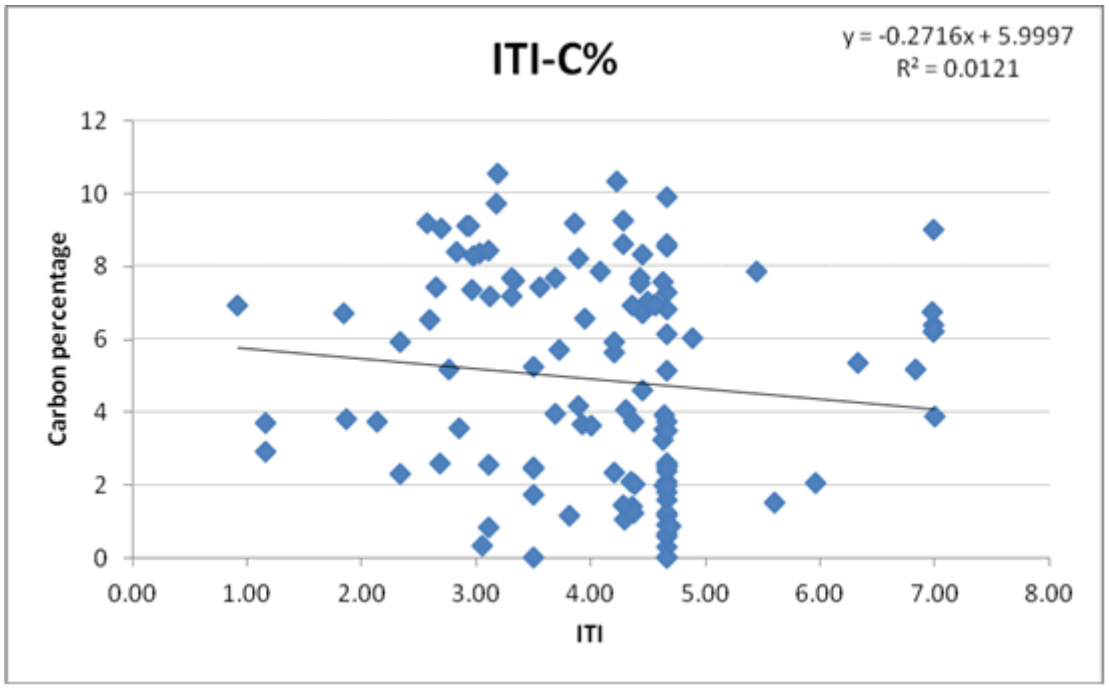


Figure 3.5. A scatterplot comparison of C% (% dry weight sediment) in the sediment with ITI for all the tested stations. ITI values in the figure are scaled from 1 to 7 (AMBI levels) and C% values range from 0 to 12% (% dry weight sediment).

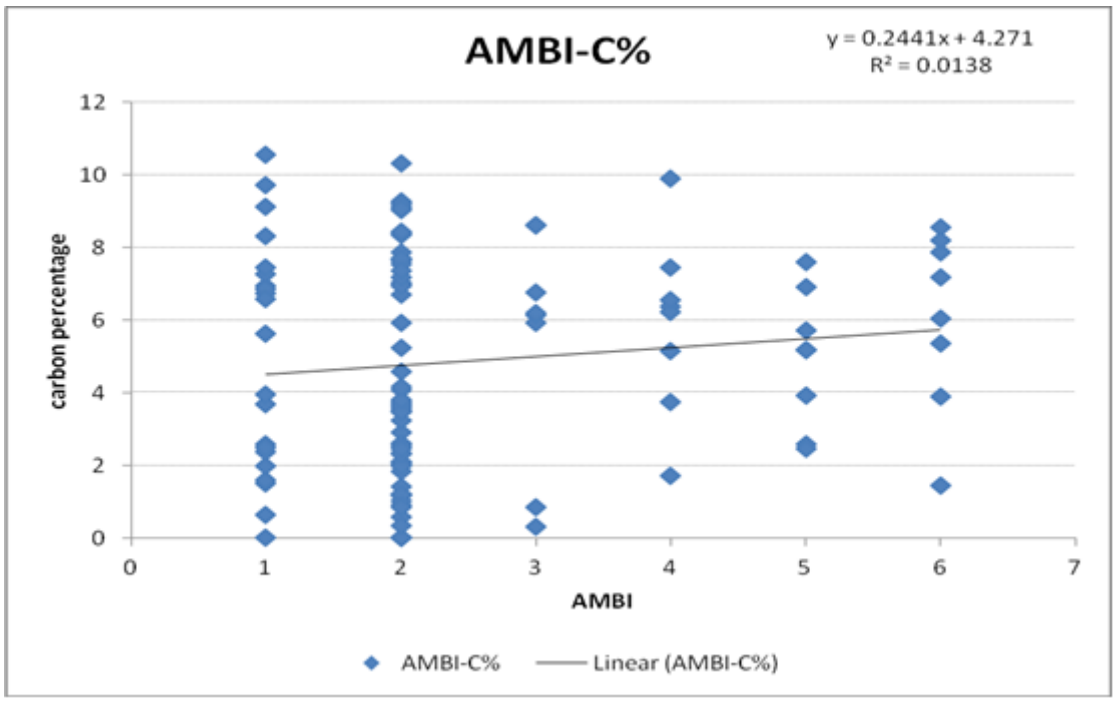


Figure 3.6 is a general overview of the indices shown alongside C% and Pielou. From this figure, Table 3.2 was created recording the highest and lowest values for each station. Table 3.2A shows the lowest and highest values of carbon percentage in parallel comparison with AMBI and ITI. In this Table, AMBI and ITI presents a rather low correlation with C%. AMBI has a higher correlation with C% in comparison to ITI, with AMBI shown to be better with lower carbon percentage values and ITI to be better with the higher crabon percentage values. Table 3.2B has the lowest and highest values of AMBI in relation to C%. It is shown that approximately 7 values out of 10 are correlated with C%. Table 3.2C shows the ITI lowest and highest values in relation to their C%, with approximately 5 values correlating with each other.

Table 3.2A. Lowest and highest ranges; carbon (% dry weight sediment) values with AMBI and ITI.

Stations **Site, year, location**	**carbon %**	**AMBI**	**ITI**
Lowest values within Figure 3.6			
Geasgill 2006 50m N	0	2	4.67
Bow of Hascosay 2006 25m N	0	2	3.5
Vatsetter 2006 50m N	0	1	4.67
ardmaddy 2006 850m N	0	2	4.67
Geasgill 2006 50m N	0.31	3	4.67
Highest values within Figure 3.6			
Cornaig 2003 0m	10.57	1	3.2
Rubh 2003 850m N	9.05	2	2.7
Stead of Aithness 2007 0m	10.33	2	4.23
Druimyeon Bay 2006 850m N	9.27	2	4.3
Cornaig 2003 150m N	9.72	1	3.17

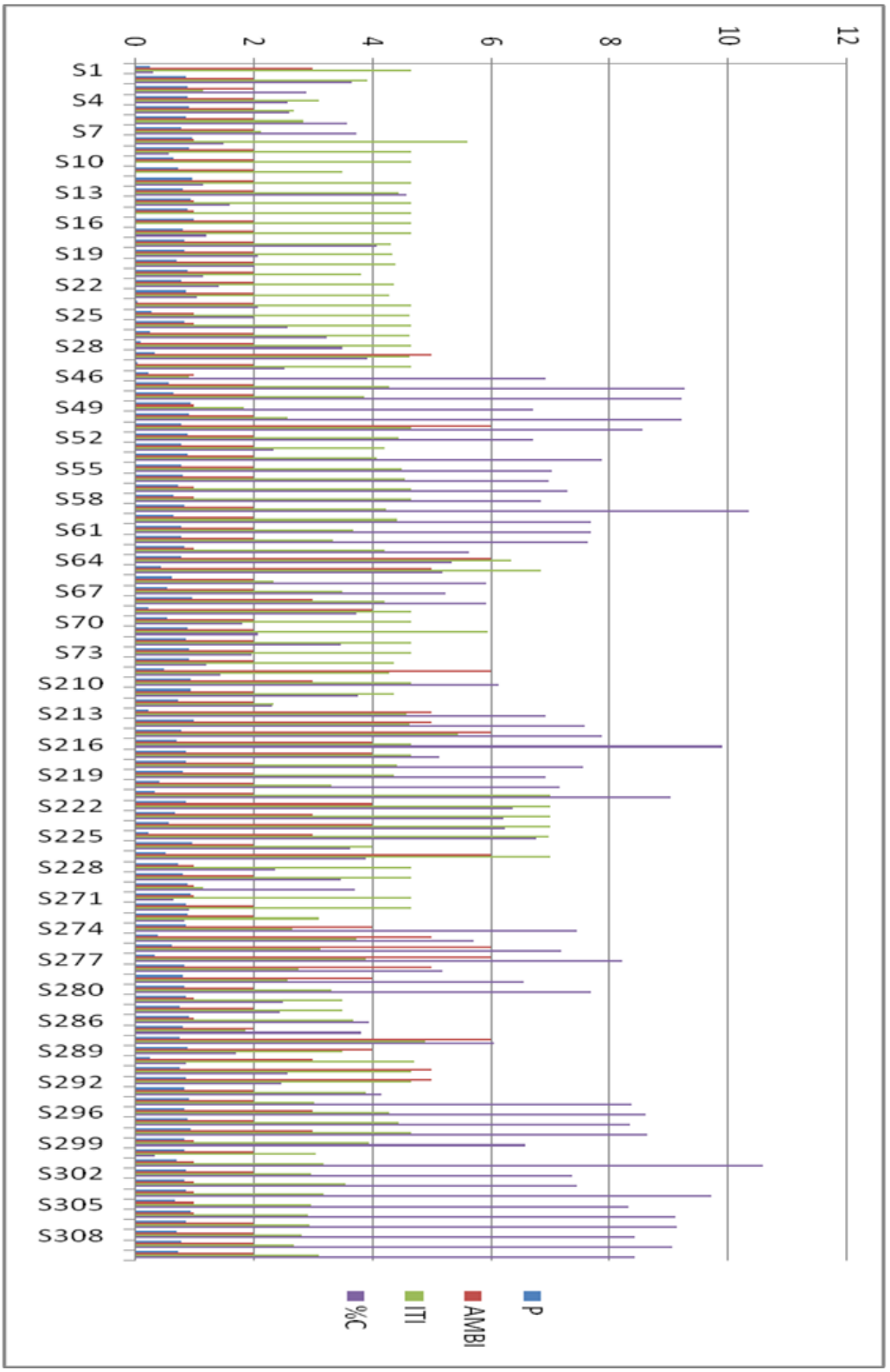
Table 3.2B. Lowest and highest value of AMBI compared with those of carbon (% dry weight sediment).

Stations **Site, year, location**	**AMBI**	**C%**
Lowest values within Figure 3.6		
Port na moine 2006 850m N	1	1.51
Flotta 2006 0m	1	1.6
Kilbn 2005 100m S	1	0.65
Lingay 2003 850m S	1	8.31
Lingay 2003 0m	1	9.11
Highest values within Figure 3.6		
Brunnaness 2007 0m	6	8.55
Sian Bay 2007 25m S	6	5.35
Port na cro 2003 50m S	6	3.89
Kirkaldy 2005 0m	6	8.21
Kenmore 2003 0m N	6	6.04

Table 3.2C. Lowest and highest value of ITI compared with those of carbon (% dry weight sediment).

Stations **Site, year, location**	**ITI**	**C%**
Lowest values within Figure 3.6		
Ardyne 2006 50m N	1.17	2.9
Uiskevagh South 2006 50m N	0.92	6.93
Vidlin North 05 150m N	1.84	6.72
Vady 2003 850m N	1.17	3.7
West fara 2005 50m S	1.87	3.82
Highest values within Figure 3.6		
Sian Bay 2007 25m S	6.33	5.35
Vady 2003 0m	6.83	5.18
Kirkaldy 2003 50m S	6.99	9.03
Kirkaldy 2003 25m N	6.99	6.23
Port na cro 2003 50m S	7	3.89

Figure 3.6. Combination bar chart showing a direct comparison between the results for Pielou Evenness, carbon (% dry weight sediment), ITI and AMBI. The labels mean the tested stations and they can be seen in detail at the Appendix 2, Table A1.



3.4. DISCUSSION

The results are organised in such a way to release useful ecological information about the two indices, AMBI and ITI. The aim was to analyse these to determine: 1) which was the more accurate; 2) to identify any differences or similarities in related sectors; 3) to make observations on the technical issues regarding the use of the software; and, 4) to optimise the general use of the related indices in terms of future improvement in marine ecological studies.

Initially, it can be concluded that ITI requires more time in the preparation of the spreadsheets and obtaining results. AMBI, however, is much easier to be use because of the way the software is constructed and the only work required is the input of the spreadsheet into the software and a quick check of the species list guided by the software during the analysis. Thus, the two main advantages appear to be: (A) The AMBI score is more reliable than (manually) calculating the ITI values because of the possible remaining gaps to the species trophic marks; and, (B) when working with large databases or a quick analysis is required, then AMBI is better.

AMBI can present a better understanding of the ecological context of an area because it has 5 or 7, depending on the version used, increasing effects scale, while ITI has a lower number of major classes. ITI is also defective because the values are within a wide range of impacts and as a result there is no precise summary of the situation. AMBI's seven degrees of pollution leads to better comprehension of the situation in the area because the scale is extended and thus provides a more precise description of the

impacts. For example, when a station presents an AMBI score equal to 6 and an ITI score equal to 15, for ITI it is not possible to identify if the impact is closer to 30 or 0 (where 30 is the limit for moderate impact and zero for high impact). For AMBI, however, a score of 6 indicates a heavily disturbed ecological community, with the possibility of subsequently checking the standard deviation of the value and other information such as the species number and abundance from AMBI's exported Excel file.

Statistical comparison of the indices is more accurate if both indices use similar scales of values. It is for this reason that the ITI was transformed so that it was similar to that of AMBI. As AMBI has 7 levels of scale and ITI 3, ITI was transformed to AMBI's scale (the reasons for this are discussed earlier in this chapter). This may have caused a low deviation from ITI real values. The comparison of the indices with each other can be seen in Table 3.1 where an ANOVA indicates a 68.9% correlation, which is a strong correlation. Considering these, it is difficult to say which one is more accurate in terms of approaching objectively the effects of the marine sites. It must be considered, however, that the ITI values presented a lower level of the objective level because few of the species were not obtained with a trophic mark in the spreadsheet and are not calculated in the final formula. This would have led to higher values of ITI (so the mean would be even higher than 4.1).

The correlation between the two indices is high but there is the issue of which is the better to use when concerning the quality standards set for identifying impacts in marine ecosystems. For this study a series of figures and tables were created to assist in

determining which the better to use is. The creation of the figures was based on previewing the correlation of the indices along with the carbon percentage (abiotic factor) and Pielou Index (biotic factor), to investigate for changes within the stations and their sediment ecological status. The Pielou Index is an indicator of the alteration in species and precisely how evenly the community species is distributed in terms of their domination in a specified area. When the species are equally distributed then the Pielou Index values approach zero and when the Pielou values are high that means the ecological area is degraded. That should give a different outcome of the indices because when AMBI and ITI levels are high, the worst conditions occur. This appeared to the Figures 3.4 and 3.5 containing the compared indices, both AMBI and ITI presented a high degree of response to the environmental effects and reflected the conditions accurately.

Further analysis regarding the pollution of the sites and the identification role of the indices was needed. For this reason, C% was put into the figures along with the indices but also tables containing their radical values were created. Carbon percentage is an indication of the organic enrichment within seabed samples. The indices approximately calculated similar carbon enrichments and this makes their connection obvious. The figures from the C% suggested an uneven change with each other. The logical outcome of the Figures 3.4 and 3.5 should have been a rise in their amount, because the carbon percentage in the seabed increased and also increased the possibility for disturbed conditions within the sediment species. This can be seen in the AMBI graph (Figure 3.4) but not in ITI (Figure 3.5) where ITI gave a declining regression with C%. The carbon values would normally show a level of impact independently of what AMBI or ITI scores would be. From this outcome, the ITI did not present the logic

behaviour for the regression and its trend line was raised negatively instead of positively. AMBI appears to be fairly accurate when investigating biotic effects in relation to the trophic behaviour of the species. This is not correlated with the main comparison because the ITI showed a mean value higher than AMBI. It would be more logical, if the carbon percentage in relation with ITI had been positively correlated instead of negatively and in this occasion ITI was more inaccurate than AMBI.

To further investigate the alternative results from ITI with C%, tables containing the radical values for each of the tested parameters were created (Tables 3.2A, 3.2B and 3.2C). The assumption was that C% decreased the ITI levels in the trend line from Figure 3.5, because of a level of nutrient or toxic enrichment had occurred to the stations or the stations had initially low pollution and a natural disorder was happening. In the Tables 3.2A, 3.2B and 3.2C, the ITI values were not correlated in a high level with their carbon values for the tested stations, but AMBI values had higher correlation to their carbon values and the ecological conditions occurred in the tested stations. Though it is difficult to estimate a good conclusion from these tables, because they include only one factor of pollution (organic) and not other pollution parameters (nutrients); AMBI has approximately 7 cases correlated out of 10 and ITI 5.

In conclusion, the study objectives can be answered. AMBI was proven to be better index when working with large databases as the scores are better able to describe the ecological impacts than are the scores derived from ITI. Both AMBI and ITI are similar in that they can both present the species alteration within an ecological area, but they differ in their ability to correctly identify organic pollution. Of these two, AMBI is

the better. This study did not experience any technical issues regarding the use of the software, but it is worth mentioning that ITI is a manual process that requires numerous calculations in Excel to be made, while AMBI is a free pc tool which provides additional useful information. In an ecological approach, the use of the AMBI index is better because it has 7 levels of disturbance while ITI has 3 large levels that fail to describe accurately the quality of an area.

Chapter 4
A combination of selected indices for assessing the environmental impact of marine fish farms using long term metadata analysis.

4.1 INTRODUCTION

4.1.1 General background

Given the rise in anthropogenic pollution due to culturing and recreational activities many tools have been created to quantify the levels of pollution within a specified area (Beveridge, 2004). Aquaculture is an activity which leads to increased enrichment beneath sea cages and potential ecological changes in the sediments require monitoring; this need for assessment led to the creation of biotic indices (Telfer & Beveridge, 2001a). There are a range of indices including species richness, species abundance, trophic indices *etc*. along with a range of abiotic indices (mostly physico-chemical measurements) which when combined represent a complete tool for studying the environmental attributes of a cultured area (SEPA, 2007).

The biological indices are statistical ecological tools used to determine the environmental impacts in various farm areas and they consist of the univariate analysis for assessing the sediments. Those indices are applied for assessing the diversity of seabed species in order to study the sediment impacts, but are also used to environmental regulation and its policy papers (SEPA, 2000). The biological indices to be studied in this project are the benthic biotic indices. Azti's (www.azti.es) Marine Biotic Index (AMBI) and Infaunal Trophic Index (ITI) are two of the benthic indices used for the biotic and trophic status by the ecological research bodies (Lazaro *et al.*, 2005; SEPA, 2007). The Scottish environmental protection agency (SEPA) requires ITI to be the core of the univariate analysis for the environmental impact assessment (EIA)

papers conducted by the fish farms alongside Scotland (Environmental Services, 2007) and AMBI is one popular tool used by industrial and research centres in order to monitor the benthic ecological situation along with N, H' and the chemical trends: carbon, nitrogen percentages and redox potential (Lazaro *et al.,* 2005).

These indices are used to give information about the biotic activity and situation of the farmed seabed sites and they particularly emphasise the trophic and distributed processing and scaling of the species (Maurer *et al.,* 1999; Borja *et al.,* 2000). The results coming from these indices are presented in simple values without units but in a scale of exact numbers that are part of a complete numeric formulation counting the effects into levels (Maurer *et al.,* 1999; Borja *et al.,* 2000).

4.1.2 Enrichment and pollution

The organic load discharged by cage fish farms consists of uneaten food and faeces which settle to the seabed in the vicinity of the cages (Beveridge, 2004). In highly energetic areas this material may be dispersed and assimilated by the benthic fauna with relatively little detectable accumulation or effects. In lower energy areas the sea bed may become organically enriched and anoxic causing distortions in the structure of the benthic fauna and development of microbial films of *Beggiatoa* on the sediment surface. In these more quiescent situations, the effects may be more intense but cover a smaller surface area (as described at SEPA's farming manual) (SEPA, 2007).

Unlike some other effects such as nutrient enrichment, the effects of organic pollution on the sea bed are usually localised. Therefore, monitoring should focus on

the vicinity of the farm and for this reason; some sea bed monitoring lends itself well to self-monitoring by the operator or his consultants (SEPA 2007). Small biomass farms in dispersive areas are unlikely to cause problems so a biomass/sensitivity should be prepared to ensure that monitoring effort is targeted where the risk is greatest, such as at sensitive sites with a large biomass (SEPA, 2007). In this case, the biomass is that of the individual farm, and the sensitivity is based on current speed at the farm as supplied by the operator and accepted by SEPA (SEPA, 2000).

4.1.3 Monitoring and indices

SEPA, as the Scottish regulatory authority, having identified existing and potential uses, will establish its Environmental Quality Objectives (EQOs) for the water body in question (SEPA, 2007). Environmental Quality Standards (EQSs) are set to protect these given water uses. These standards are often concentration limits for specific chemicals of concern, although various biological standards have also been derived. EQSs may be set on the UK-wide or, in some cases, on a more local basis depending upon the priority of the parameters involved (SEPA, 2007). In situations where a number of uses have been identified for a given water body, and where various standards (EQSs) have been set to protect these uses, *e.g.* the concentration limits for a given substance may vary according to water use, the most stringent of these standards must be applied (SEPA 2005). Methods for deriving quality criteria for sediments are less well developed than those for water and accepted sediment quality standards do not yet exist (Den Besten *et al.,* 2003). The term quality criterion rather than standard is applied to sediments since the methods for deriving the protective limits are less well established and validated than those for waters. Frequently, the results of chemical analysis for sediment samples will be compared with those for uncontaminated

reference sites (SEPA, 2007). The sediment samples are tested in the following parts of indices as extracted by the Table a7 in SEPA's paper for monitoring the sediment quality: number of taxa, abundance, Shannon–Weiner Diversity, infaunal trophic Index (ITI), organic carbon, redox potential, and loss on ignition (SEPA, 2007).

The ITI and AMBI indices were described and assessed in detail in chapter 2. The univariate analysis is made only for the macrofauna data for the extraction of the ecological indices (SEPA, 2005). In this, ITI is the suggested results output. Along with ITI, Simpsons Index D, Brillouins Index Hb, Shannon Wiener Hs, Pielou Evenness P and Heip Evenness Eh are also measured and give important information about the ecological state of the stations. Evenness (or Equitability): this is a measure of how evenly individuals are distributed among the different species. Infaunal Trophic Index (ITI) is a biotic index, which was developed by the Water Research Centre and varies between 0 and 100. It relies on the assessment of the changes in the feeding (trophic) mode of benthic organisms in areas subject to increase organic enrichment. SEPA (2003) adopts the following classification to interpret ITI values with regard to benthic communities around fish farm sites: 60 to 100 values: Community is 'normal'; 30 to 60 value: community is 'changed'; less than 30 values: community is 'degraded'. Number of individuals (N): The abundance or number of individuals in a population. Number of species (S): The number of species in a sample or group of samples. Pielou's evenness index (P): The calculation of evenness or equitability within a community, which is defined by the degree to which the individuals are evenly portioned among all species. Shannon-Wiener's diversity index (H'): This is the measure of the diversity of a community which incorporates both species richness and equitability components. The higher the Shannon-Weiner value, the more diverse the community is. Simpson's

dominance index (D): this is essentially the reverse of evenness. If a sample has a high dominance value it is highly dominated by one species. Standard deviation: a measure of the average amount by which each observation in a series of observations differs with the mean.

4.1.4 Critical review

The indices and their combination with each other and with other ecological trends of the seabed were tested in the past in order to obtain the accurate status of the biotopes (Van dolah *et al.*, 1999; Cheng, 2004; Beyrem *et al.*, 2007; Chainho *et al.*, 2007). The body mainly dealing with these issues –besides the research institutes and schools- in Scotland is SEPA, whose contribution by creating the EQS and EIA is decisive for the environment protection (SEPA, 2005). The main issues dealing with this study have been raised in the prior paragraphs. These notable papers strictly dealing with the combination of indices arise from various origins regarding the seabed study.

The statistical approaches on discriminating spatial variation of species diversity in relation to the indices was studied in Cheng's paper (2004) where the main purpose was to compare the effectiveness of various statistical approaches and then present the best strategy for discriminating the spatial variations of species diversity. It is concluded that the most powerful tools for discriminating the spatial variations of species diversity are in the multivariate category. Among multivariate methods, ordination by non-metric multi-dimensional scaling is preferable, and its superimposition with cluster analysis is recommended in order to obtain more information regarding the relationship between sites. In Beyrem *et al.* (2007) the individual and combined effects of cadmium and diesel on a nematode community in a

laboratory microcosm experiment were studied. Total nematode abundance (I), mean individual weight (bi), Shannon–Weaver index H0, species richness (d), evenness (J0) and number of species (S) decreased significantly in microcosms contaminated with both cadmium and diesel. Results from multivariate analyses of the species abundance data demonstrated that responses of nematode species to the cadmium–diesel treatments were varied.

The combination of benthic indices was also mentioned widely in the paper made by Van Dolah *et al.* (1999) where a benthic index of biotic integrity was developed for use in estuaries of the south eastern USA. The final combined index correctly classified 93% of stations province-wide in the developmental data set and 75% of stations in the validation data set. Comparison of the index results with those of individual benthic measures and sediment bioassays from stations sampled in 1993 and 1995 showed that the index detected a higher percentage of samples where bioeffects were expected (based on sediment chemistry) than did any of these other measures individually.

Chainho *et al.* (2007) studied the influence of seasonal variability in benthic invertebrate community structure on the use of biotic indices to assess the ecological status of a Portuguese estuary. The outcome was the diversity indices were better correlated to eutrophication related variables than AMBI and ABC method. Predictable responses of benthic indices to anthropogenic stress symptoms were stronger during the dry period.

4.1.5 Aims and objectives

The combination of indices in order to obtain the optimum status of the seabed is the aim of this project. The hypothesis is, if there is a combination of benthic indices with other ecological trends in order to present an accurate and useful conclusion of the seabed underneath and adjacent fish farms, so that a recommendation for a better methodology to future research will be available, a shortening in the sampling time and an update of the present monitoring procedures and requirements, so that the current status of the farming biotopes will be identified and then described more accurately.

4.2 METHODS

In previous studies related to using a combination of indices, the methodology used is largely similar (Van Dolah, 1999; Cheng, 2004; Lazaro *et al.,* 2005; Chainho *et al.,* 2007). That includes a series of actions fulfilled in a way to make a comparison possible often by comparing the indices with other factors using statistics extracted from tables and figures. The design of this project is consisted of two main parts. Initially, the data selection and collection followed by its input into common spreadsheets. Secondly, the statistical analysis led to results plotting and finally the analyses of the results.

4.2.1 Data processing

The data originated from Environmental Services at the Institute of Aquaculture (IoA), of University of Stirling. The data were collected from various farm sites across Scotland and for various years (2003-2006) and stations (0, 25, 50, 150 m and reference stations). There is an analytical table in the annex that shows the stations, time and

location. After the data were put together in the macrofaunal spreadsheet, all the species were present to the raw datasheets. Eventually after the editing, the first column list consisted of 655 different species and the top raw had their 119 identical and unique stations. After completing the macrofaunal spreadsheet, a second was created for the physico-chemical measurements (P-C) of these benthic stations. The stations in both spreadsheets were identical and unique; the data for the second datasheet originated from IoA and included measurements on median particle size analysis (MPSA), carbon percentage (C%), nitrogen percentage (N%) and redox potential.

The data were processed for univariate and multivariate analysis and the spreadsheets imported in a number of software tools. Some of the issues regarding the software are that all blank cells were turned to zero for the multivariate analysis so there were no missing data in the final datasheets, to avoid wrong interpretation of the results. For the spreadsheets, Excel 2003 edition was used for their creation and SigmaStat 3 for the statistical analysis.

4.2.2 Data analysis

To analyse the data, software tools were applied. Besides Excel for simple statistical regressions and column diagrams, MVSP 3.13c and SigmaStat 3.1 were also used to perform statistical analyses. A DOS-based programme called WORMS was used to obtain the results of the indices from the macrofaunal sheet. The indices obtained from this programme along with ITI, Simpsons Index D, Brillouins Index Hb, Shannon Wiener Hs, Pielou Evenness P are also measured and give important information about

the ecological state of the stations. The analysis of this data is fully described in Chapter 2.

For the final analysis Pielou, Shannon Wiener Index and ITI were utilised. The index AMBI was also used. AMBI's scores come individually and separately from AMBI Version 3 which is released by AZTI. AMBI is a tool based on the calculation of the species biotic situation. The output of the results is shown in a 1-7 score scale as a mark of the effects that occurs at the stations. One is the optimum situation (no ecological disturbance) and seven is the worst situation (extremely disturbed station). Along with the software output, the tool provides an Excel spreadsheet with all the scale values along with the statistical process and analysis.

After running the software, all the index values were copied into a new spreadsheet to create the final results. To obtain a better correlation the ITI scores were rescaled so that they were on the same range as the other indices. This was achieved by deducting 100 from all the ITI values and then multipling by 0.07 (to approach the AMBI scaling correlation). That spreadsheet was then ready for analysis, regressions were produced which were then combined using Sigmastat. Descriptive analyses, correlation tests (Pearson's and One way ANOVA) and their summary diagrams were obtained.

MVSP was used to obtain cluster analysis and to conduct Canonical Correspondence Analysis (CCA). The most important part of this research is the cluster

analysis which made for the macrofauna species, in order to find similarities in their abundance within the various stations. The CCA was a cluster dendrogram of the macrofauna data combined with the P-C factors and it was performed twice for both species and stations. Cluster analysis was also performed to find the P-C attributes and their combined similarity within the stations.

The results of the univariate and multivariate analysis were combined to give information regarding the ecological state and look at their common properties, in order to suggest an accurate statistical method for modeling the sediment ecology. This would be achieved by making a combination table, including all the diversity indices and the physic-chemical factors. Eventually, performing a new statistical analysis to test the selected parameters for the specific sites, would confirm the accuracy of the new model.

4.3 RESULTS

In Table 4.1 the descriptive analysis of the univariate analysis is shown. The diversity indices on the first column are the total number of species N, the species richness diversity S, the Simpsons Index D, Brillouins Index Hb, Shannon Wiener Hs, Pielou Evenness P, Heip Evenness Eh, infaunal trophic index ITI and Azti marine biotic index AMBI. The second column is the total size of the tested stations, the third column is the mean values of the indices, the forth is the standard deviation value from the original mean value, the fifth column has the standard error of the standard deviation, the sixth column is the range of the values for the indices, the seventh column contains the maximum value of the index and the eighth column its minimum value within the

sample, the ninth column contains the median value of the range and the last column is the summary of the values for the index. The data used is presented on the Appendix 3, Table A1. In this table, the mean N number is 84.96 with a maximum number of 1407 and a minimum of 5 (a range of 1402) individuals. The S number has a mean of 10.32 with a maximum value of 24 and a minimum of 2 (a range of 22) different species. The H' mean is set to 2.4 with a maximum value of 3.75 and a minimum of 0.12 (a range of 3.63) units. The AMBI score had a mean value equal to 2.53 ranging 5 class levels from 1 to 6. ITI had a mean of 4.135 (following its transmission to an equivalent scale as that used for AMBI). In Table 4.2, the descriptive analysis of the chemical parameters is shown. The first column presents the tested parameter (MPSA, carbon percentage, nitrogen percentage and redoc potential). Then is the second column that is the total size of the tested stations, the third column contains the missing data for each of the parameters, the fourth column is the mean values of the parameters, the fifth is the standard deviation value from the original mean value, the sixth column has the standard error of the standard deviation, the seventh column is the range of the values for the parameters, the eighth column contains the maximum value of the parameter and the ninth column its minimum value within the sample, the tenth column contains the median value of the range and the last column is the summary of the values for the parameter. The data used is presented on the Appendix 3, Table A2. Median PSA had a mean value of 385.9 ranging between 3533 and 82. The mean carbon percentage is 4.9 with values ranging from 10.57 to zero. The nitrogen percentage had a mean value of 0.164 with values ranging from 1.17 units to zero. The redox potential mean was 304.9 with a maximum value of 540 and a minimum value of zero.

Table 4.1. Descriptive analysis of the diversity indices for the macrofauna data in sediments table from the 119 sampling stations. N = Total number of individuals, S = total number of species, D = Simpson's Index, Hb = Brillouins Index, Hs = Shannon Wiener Index, P = Pielou Evenness, Eh = Heip Evenness., ITI = infaunal trophic index, AMBI = Azti marine biotic index.

Index	Size	Mean	Std Dev	Std error	Range	Max	Min	Median	Sum
N	119	84.958	198.113	18.161	1402	1407	5	39	10110
S	119	10.319	4.661	0.427	22	24	2	10	1228
D	119	0.72	0.231	0.0212	0.97	1	0.03	0.82	85.71
Hb	119	1.348	0.506	0.0464	2.16	2.24	0.08	1.43	160.41
H'	119	2.386	0.885	0.0811	3.63	3.75	0.12	2.58	283.96
P	119	0.742	0.224	0.0205	0.94	1	0.06	0.82	88.31
Eh	119	0.574	0.253	0.0232	0.98	1	0.02	0.63	68.25
AMBI	119	2.529	1.419	0.13	5	6	1	2	301
ITI	119	4.135	1.203	0.11	6.084	6.999	0.914	4.355	492.1

Table 4.2. Descriptive analysis of the chemical trends (environmental parameters) for the macrofauna data from the sampling stations. N = Total number of individuals, S = total number of species, D = Simpson's Index, Hb = Brillouins Index, Hs = Shannon Wiener Index, P = Pielou Evenness, Eh = Heip Evenness., ITI = infaunal trophic index, AMBI = Azti marine biotic index.

Parameter	Size	Missing	Mean	std Dev	Std error	Range	Max	Min	Median	Sum
Median PSA mV	119	1	385.844	419.924	38.557	3451.647	3533.497	81.85	249.95	45529.6
C%	119	2	4.882	2.936	0.271	10.57	10.57	0	5.14	571.22
N%	119	2	0.164	0.188	0.0174	1.17	1.17	0	0.09	19.21
Redox µm	119	12	304.888	113.107	10.395	540	540	0	287.5	32623

Table 4.3 is the one way ANOVA, and Kruskal-Wallis one way analysis of variance on ranks of the diversity indices for the macrodauna data from the tested stations. The diversity indices on the first column are the total number of species N, the species richness diversity S, the Simpsons Index D, Brillouins Index Hb, Shannon Wiener Hs, Pielou Evenness P, Heip Evenness Eh, infaunal trophic index ITI and Azti marine biotic index AMBI. The next column is the (Ns) total number of the stations tested, then the missing data for the analysis column, next column is the median value of the each of the indices and the last two columns is the 25 and 75 pecentages of correlation the indices have with each other. The data used is presented on the Appendix 3, Table A1. In this table N is strongly correlated with S, D, Hb, Hs or H', and Eh. The S index is correlated with the D index. The Eh and Hs (or H') are correlated with AMBI. The ITI is correlated with all the other indices except the N.

Table 4.3. One way ANOVA, Kruskal-Wallis One Way Analysis of Variance on Ranks of the diversity indices for the macrofauna data from the tested stations. N = Total number of individuals, S = total number of species, D = Simpson's Index, Hb = Brillouins Index, Hs = Shannon Wiener Index, P = Pielou Evenness, Eh = Heip Evenness., ITI = infaunal trophic index, AMBI = Azti marine biotic index.

Indices	**Ns**	**Missing**	**Median**	**25%**	**75%**
N	119	0	39	21	62
S	119	0	10	7	13
D	119	0	0.82	0.647	0.88
Hb	119	0	1.43	1.043	1.738
H'	119	0	2.58	1.858	3.037
P	119	0	0.82	0.68	0.89
Eh	119	0	0.63	0.422	0.76
AMBI	119	0	2	2	3
ITI	119	0	4.355	3.313	4.666

Table 4.4 is the one way analysis of variance and correlation in chemical trends including Kw analysis, Dunn's multiple comparison and Pearson's correlation, for the physico-chemical parameters from the tested stations. The parameters are the median

particle size analysis (MPSA measured in μm), the carbon and nitrogen percentages (% of dry weight sampling) and redox potential measured in mV. Kruskal-Wallis One Way Analysis of Variance on Ranks of the physico-chemical parameters at all stations, H = 376.552 with 3 degrees of freedom. (P = <0.001), Normality Test: Failed (P < 0.050). The parameters are given in the first column, the (N) total number of the stations tested, then the missing data for the analysis column, next column is the median value of the each of the indices and the last two columns is the 25 and 75 pecentages of correlation the indices have with each other. In this table, all the trends are correlated besides redox and median PSA. Table 4.5 is a summary of the redox potential, carbon, and nitrogen percentage compared with each other. There is similarity of the correlation which is applied in these three associated factors. Table 4.6 is the correlation of the factors which tested finally. Carbon percentage with H', and H' with AMBI are the only pairs among the others, that they were not correlated.

Table 4.4. One way analysis of variance and correlation in chemical trends, including Kw analysis, Dunn's multiple comparison and Pearson's correlation, the physico-chemical parameters from the tested stations. MPSA = Median partical size, %C = percentage carbon in sediment by dry weight, %N = percentage nitrogen in sediment by dry weight, Redox = redox potential (mV).

Environmental parameters	**N**	**Missing**	**Median**	**25%**	**75%**
MPSA	119	1	249.95	179.19	493.14
%C	119	2	5.14	2.34	7.45
%N	119	2	0.09	0.04	0.24
REDOX	119	12	287.5	225.25	398.75

All Pairwise Multiple Comparison Procedures (Dunn's Method) of the physico-chemical parameters at all stations:

Comparison of the parameters	**Diff of Ranks**	**Q**	**P<0.05**
REDOX *vs* %N	284.073	16.01	Yes

REDOX *vs* %C	175.685	9.901	Yes
REDOX *vs* PSA	4.538	0.256	No
MPSA *vs* %N	279.536	16.15	Yes
MPSA *vs* %C	171.147	9.889	Yes
%C *vs* %N	108.389	6.25	Yes

Pearson Product Moment Correlation of the environmental parameters at all stations:

	%C	%N	REDOX	
PSA	-0.0797	-0.0947	0.164	Correlation Coefficient
	0.395	0.312	0.0926	P Value
	116	116	106	Number of Samples
%C		0.585	-0.317	Correlation Coefficient
		4.49E-12	0.000917	P Value
		117	106	Number of Samples
%N			-0.322	Correlation Coefficient
			0.000754	P value
			106	Number of Samples

Table 4.5. Comparison of the correlation of the chemical trends (environmental parameters) with each other for the macrofauna data from the tested stations by using Multiple Comparison Procedures (Dunn's Method).

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Comparison of the parameters	**Diff of Ranks**	**Q**	**P<0.05**
REDOX *vs* %N	221.569	16.802	Yes
REDOX *vs* %C	113.18	8.583	Yes
%C *vs* %N	108.389	8.409	Yes

Table 4.6. Comparison of the correlation of the chemical trends with the benthic diversity indices for the macrofauna data from the tested stations by using Multiple Comparison Procedures (Dunn's Method).

Comparison of the parameters	**Diff of Ranks**	**q**	**P<0.05**
REDOX *vs* %N	490	25.44	Yes
REDOX *vs* S	445	23.103	Yes

REDOX *vs* AMBI	252	13.083	Yes
REDOX *vs* H'	216	11.214	Yes
REDOX *vs* %C	157	8.151	Yes
%C *vs* %N	333	17.288	Yes
%C *vs* S	288	14.952	Yes
%C *vs* AMBI	95	4.932	Yes
%C *vs* H'	59	3.063	No
H' *vs* %N	274	14.225	Yes
H' *vs* S	229	11.889	Yes
H' *vs* AMBI	36	1.869	No
AMBI *vs* %N	238	12.356	Yes
AMBI *vs* S	193	10.02	Yes
S/N *vs* %N	45	2.336	No

Table 4.7 provides a comparative table of the maximum and minimum values of the benthic diversity indices and chemical parameters of the stations. The maximum N and S, and H' maximum values columns show the good ecological conditions for the stations in their sediment. The minimum AMBI and minimum carbon values preview the best ecological conditions for the stations. The higher redox in the stations is a suggestion of good conditions concerning the chemical trends. Table 4.6 shows that these 5 trends are highly correlated, since the upgrading or downgrading conditions are in agreement within this table.

Table 4.7. Comparative table of the maximum and minimum values of the benthic diversity indices and chemical parameters of the stations (labels meaning can be shown in Appendix 2, Table A1) that are selected for combination testing. N is the total individual number of the species, Hs the Shannon Wiener index, C% the carbon percentage (% dry weight sample) and redox potential (mV). The data used is presented on the Appendix 3, Table A1.

N		Hs	
max	min	max	min
Vatsetter 06 50m Vady 03 850m Portnacro 03 50m Flotta 06 0m	Meavaig 06 50m Kirknoust 03 25m Tolsta 06 0m Kirknoust 05 50m Kenmore 03 0m	Vidlin North 06 50m PortNacro S 06 50m Vatsetter 03 150m West Fara 05 50m West Fara 05 850m	Groatay 06 850m Meavaig 06 50m Vady 03 850m Portnacro 03 50m Sian 06 25m
C%		Redox	
max	min	max	min
Cornaig 03 0m Aithness 07 0m Torgawn 05 25m Lingay 03 850m Lingay 03 0m	Cornaig 03 850m Sgeir Mhor 06 850m Kilbane 05 100m Kilbane 05 850m Kirknoust 05 850m	Kirknoust 05 850m Kilbane 05 850m Kilbane 05 100m Vady 03 0m West Fara 05 25m	Torgawn 04 850m Torgawn 04 50m Ardinish 03 25m Ardvourlie 06 850m Meavaig 06 50m
AMBI			
max	min		
Brunnaness 07 0m Sian Bay 07 25m Vatsetter 03 0m Torgawn 05 0m Portnacro 03 50m Kenmore 03 0m Kirknoust 05 25m Kirknoust 05 0m	Flotta 06 0m Portnacro 03 150m Kilbane 05 100m West Fara 05 50m Cornaig 03 850m		

Figure 4.1 is a line plot of the results as extracted by the univariate analysis and shows the maximum-minimum and the full range of the univariate range of trends. Some of these results are better seen in Table 4.7. It shows that ITI is the index having a range of the highest values and thus the worst effects in these stations during the statistical analysis. AMBI has a lower scale and for this reason was the one of the two

biotic-trophic indices that were chosen for further analysis. The ITI results are not highly correlated with the other results as they result from the descriptive analysis in Tables 4.1 and 4.2. P and H' are highly correlated and H' was chosen for further research because H' indicates the Pielou information and also the equitability of the species.

Figure 4.1. A line plot for comparison of values for the benthic diversity indices tested from the macrofauna data for all the stations as designed by the data used and presented in Appendix 3, Table A1.

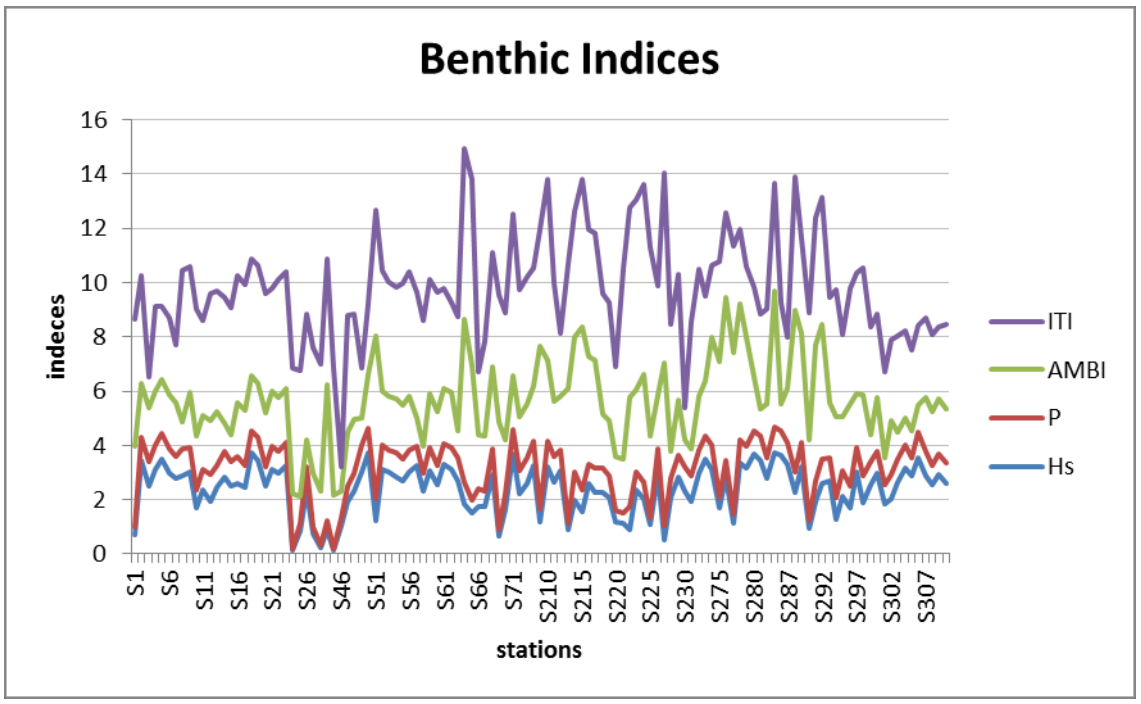


Figure 4.2 presents a line graph of the redox potential and median particle size analysis (MPSA). Redox potential would be a better addition in a combining table than the other biological trends, because the oxygen is an important factor within the seabed since regulates the anoxic conditions much better than the rest chemical trends.

Figure 4.2. Redox potential (mV) and median particles sizing (μm) line graph from the macrofauna data for all the stations as designed from the contents of Appendix 3, Table A2.

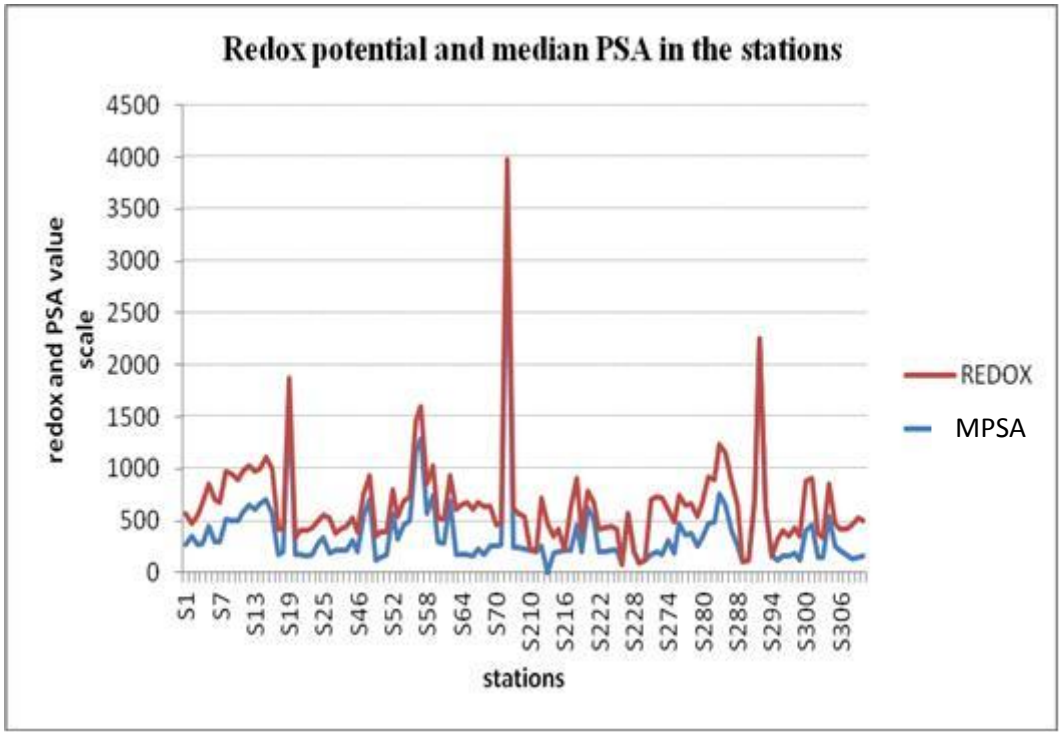
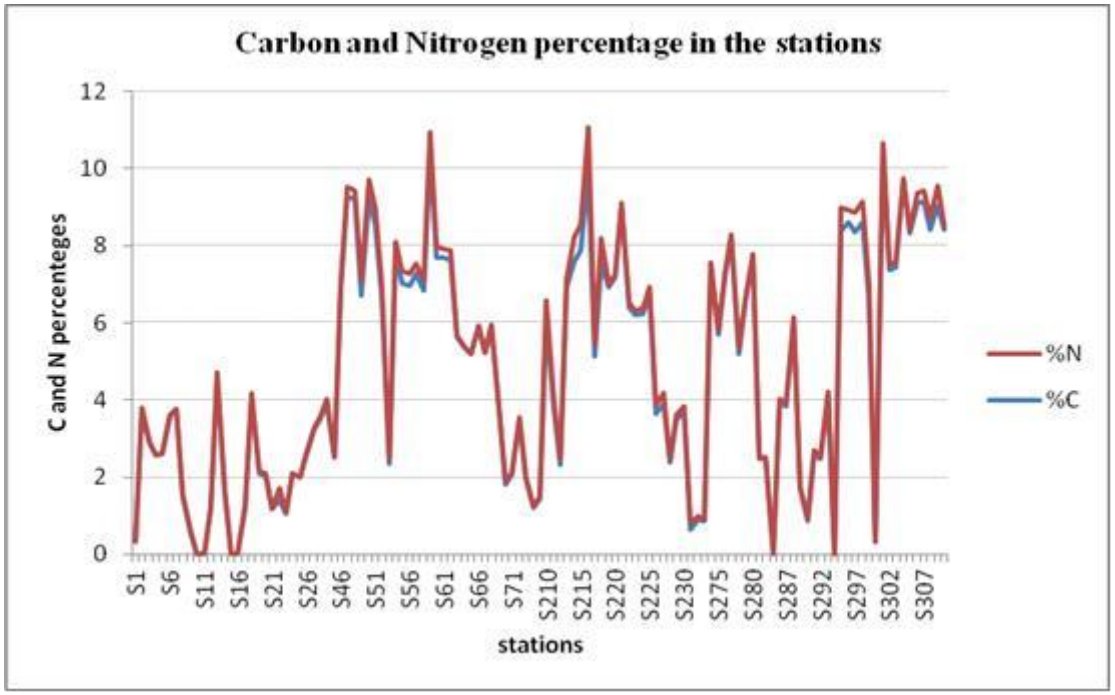


Figure 4.3 shows a line graph of the range of the organic and nutrient enrichments percentage which appears to be extremely correlated. Carbon percentage has a high quality correlation with all the biological trends, as well as with redox potential. For this reason, the carbon percentage was chosen to continue on further combination testing.

Figure 4.3. Carbon and nitrogen percentage comparison graph for all the stations as designed from the data on the Appendix 3, Table A1.



Cluster analysis was used to analyse the environmental parameters (MPSA, C%, N% and redox potential) with the stations to look for natural grouping within the data (Figures 4.4 and 4.5). Given the number of stations that were analysed (119), these labels cannot be sensibly displayed on the X-axis but Figure 4.4 suggests that there are, broadly, three main clusters (as labelled on the figure) which group the stations by different common factors. Cluster 1 (see Figure 4.4) groups sites by common redox potential which separated them from Cluster 2 by common carbon percentage, whilst Cluster 3 has no apparent basis for their grouping other than these sites remaining from the other two clusters. Figure 4.5, which presents a UPGMA cluster analysis of all the stations in relation to their macrofauna data (species number and diversity). This is of less use with no major clusters evident. For Figure 4.5, it would appear that the attributes of each site and station meant no common factors to group sites could be found.

Figure 4.4. Cluster analysis dendrogram for the similarity of all the stations in relation to their physico-chemical parameters. The parameters are MPSA (μm), C%, N% and redox potential (mV).

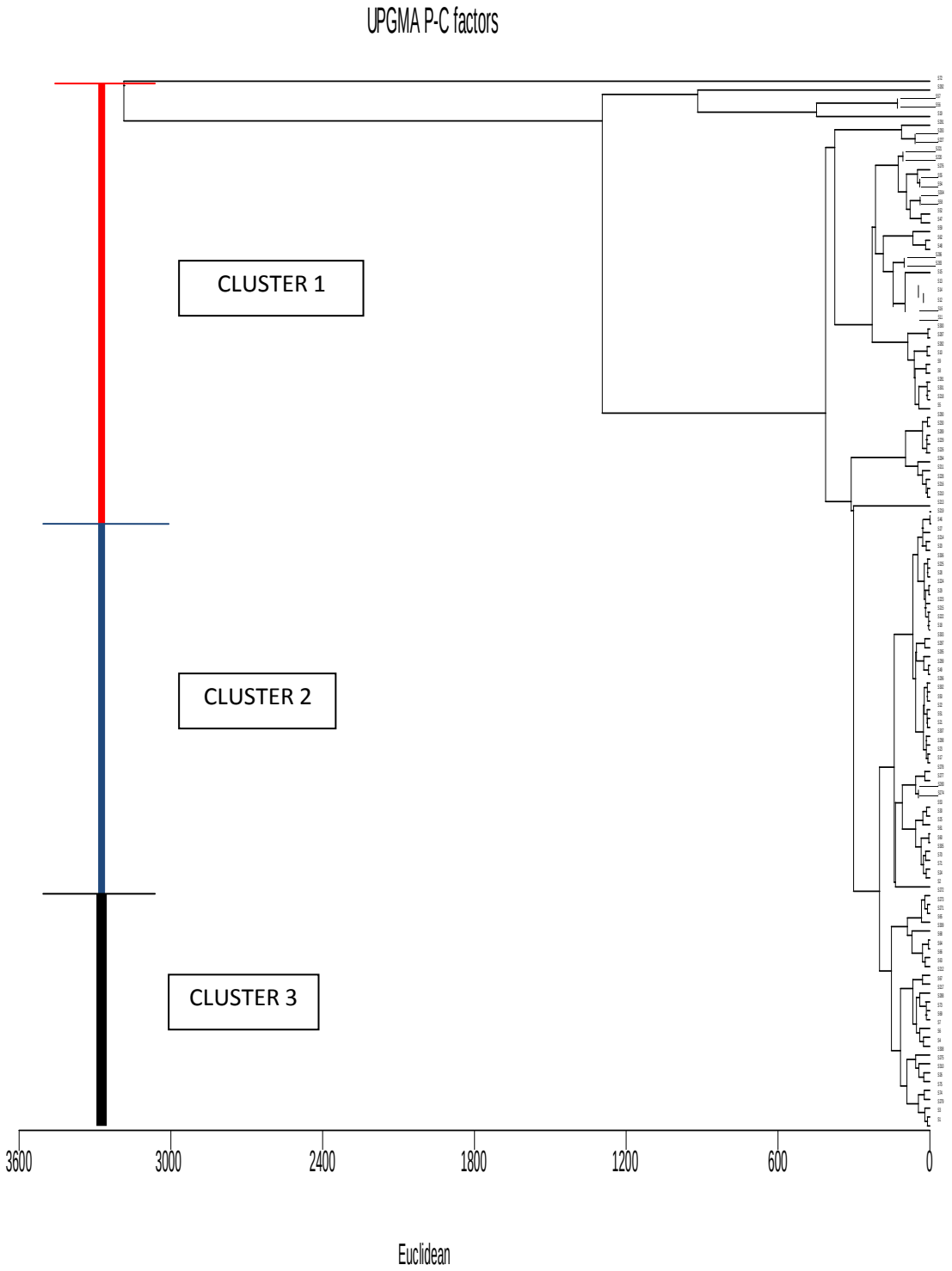


Figure 4.5. Cluster analysis dendrogram showing the grouping of all the stations in relation to their macrofauna species number and diversity. It would appear that the attributes of each site and station meant no common factors and group sites can be identified.

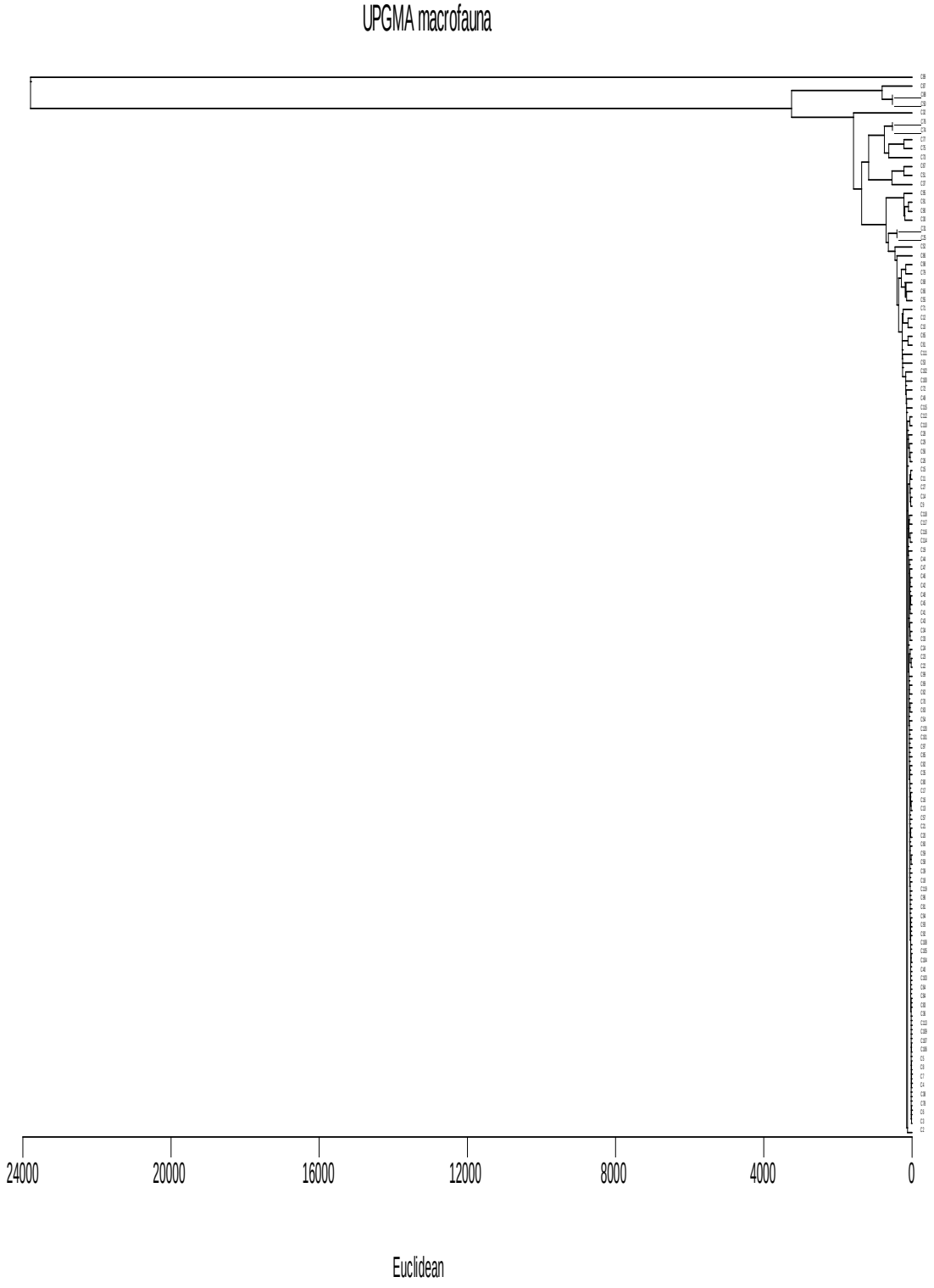


Figure 4.6 is the plot of the column means as extracted from Table 4.1. It is obvious that S, H', AMBI are quite related in their mean values and it is suggested to be present for a parameters combination. It is seen by the graph that the range of the deviations (error) are very low and there is a range value of the units of the trends.

Figure 4.6. Scatter plot graph of the indices means (where N is the individuals number, S the species numbers, D the Simpson's dominance index, Hb the Brillouins Index, Hs the Shannon Wiener, P the Pielou index and Eh the Heip Evenness) with their standard deviation. X data axis is the diversity indices and Y the means value of the mean column from Table 4.1, transported to a logarithmic scale.

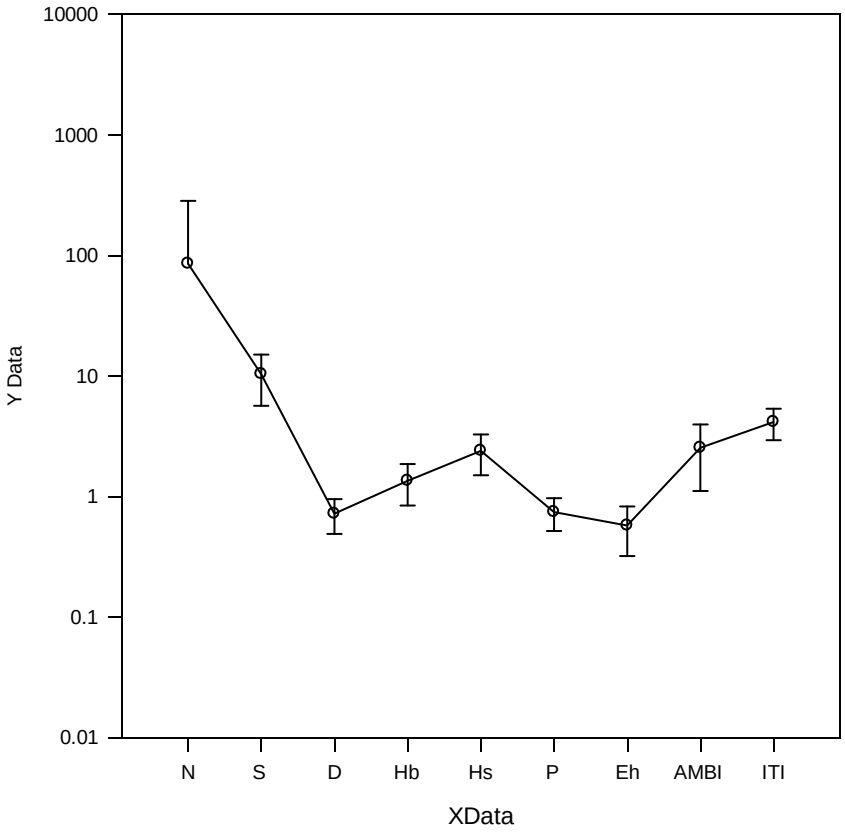
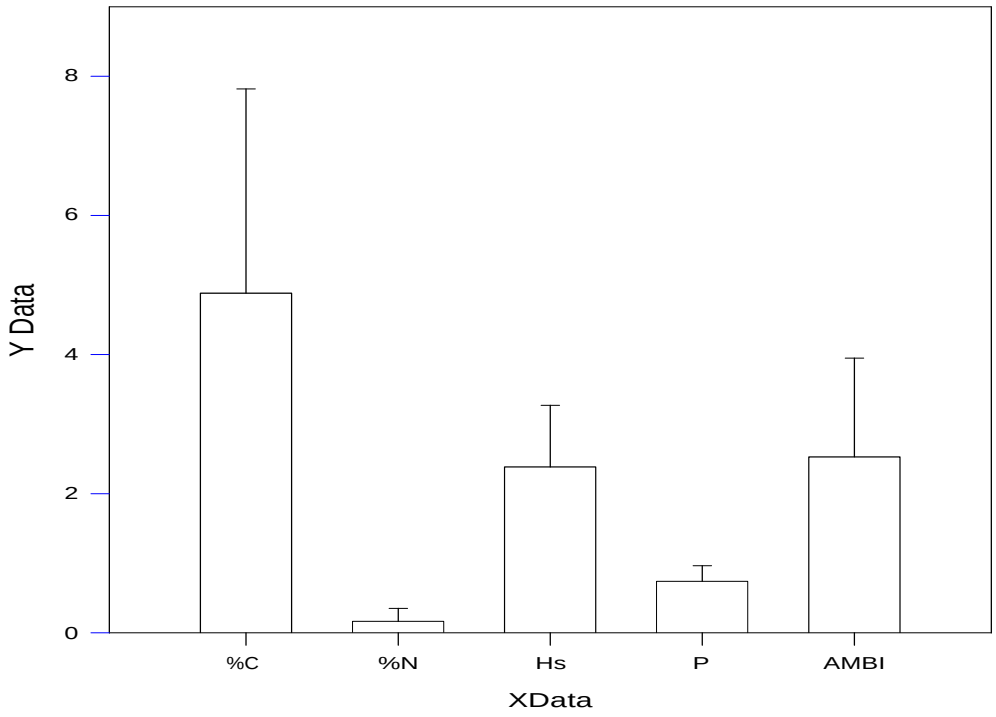


Figure 4.7 is a bar chart showing the means of C%, N%, AMBI, P (Pielou index) and H' (Shannon Wiener), in which their column means are extremely connected. As seen from the previous figure (Figure 4.6), the trends are also related and when excluding N and P, the C%, AMBI and Hs is important to justify a common range of the trends means.

Figure 4.7. Bar graph of the physico-chemical parameters means combined with indicative univariate diversity indices. In X axis where %C is the carbon percentage measured in % of total dry weight, %N is the nitrogen percentage measured in % of total dry weight, Hs is the Shannon Wiener index, and P is the Pielou index). Y axis is the means from table 4.1.



4.4 DISCUSSION

This study focuses on the possibility of creating a model in which indices are combined along with other trends (chemical or physical) to provide the best description of the benthic status in the tested areas. To do this the figures and tables from the analysis help to the comprehension of an accurate way of choosing the factors to describe the environmental attributes and the changes in a higher level. While searching into the statistical results (quantitative analysis approach), a qualitative approach also took place, in an effort to distinguish and further clarify the proper combination and describe the changes application. From a previous combination made regarding the redox values and whether they can predict the environmental impacts using the carbon as a second variable (Falconer, 2007), the results did not show that this is accurate in a high degree. Only two trends are not adequate to give the outmost information of the status in an area and more must be included. Furthermore, the benthic indices alone fail to describe the chemical status of the site and if, for example, a chemical had been applied by the farmer, it cannot be identified as the reason of a possible degradation. On the contrary, in a model when only the chemical parameters are included, any increase in the carbon or the redox numbers does not suggest an immediate or high change in species as benthic indices must also be included.

The indices count the species and give statistical results deriving from a variety of calculations. The major approach is that the benthic indices measure the equitability and the richness of the species which are present in the sample (thus in the sediment). The indices are extracted by taking into account the numbers of the individual species both individually

and combined. Moreover the indices count if the species found in the sample are equally distributed in terms of quality and quantity. The trophic situation (ITI) gives an extra result regarding the status of the benthos under a polluting approach of the species' feeding activity. The AMBI is another much easier to use index and also important for the classification of an area and its biotic status.

From all the indices extracted by the software tools, only the accurate ones would be included in a final combination output, which would be represented in a table showing them and their values. In this approach, descriptive statistics were used along with line graphs and plot figures. In this study, no regression analysis was obtained since the future changes of the trends with each other were not the objective and it was therefore not needed to identify the quality of the indices. The accurate indices will be put in a table that will describe the levels of the changes currently in the present inside the benthic sites.

Initially the research was about studying whether some of the indices are important enough to be part of a later staged research. If a closer look to the definition is to be made, some of them have such a similarity that can be excluded from a better index. The indices remained to be tested are: N, S, P, Hs, ITI and AMBI. Figures made to preview the resemblance of the selected indices and the line graphs representations showed further resemblance. The number of the species variety (S) and their actual number (N) were not added in the following steps individually but their N/S relation was. That was obtained by dividing them and the values ranging from 1 to 0 and the optimal percentage in species

distribution in the stations is 1. The ITI and the AMBI are not equally plotted but the AMBI has higher levels of results and changes than the ITI, which it was more evenly matched to the real situation. At this point, the alteration of ITI from its original scale to the level of 1-7 in order to match the AMBI's must be underlined, and the analysis would be more correlated and this might have caused a disorder for ITI and its extracted results. Eventually ITI was released from the later stages of the concluding comparison of the indices. From the Pielou and Shannon-Wiener, only H' was determined to be used further since it is a more descriptive index than P. Since PSA presents a slight interaction in describing the sediment status, it was excluded from the model. Eventually, the indices were essentially chosen for accuracy of the effects describing were: H', AMBI, and N.

The next step was to check if the indices were correlated. The Tables 4.5 and 4.6 show that the correlation occurred in these indices happened in a high degree. At this point a comparison of the results regarding the current status description had to be done to determine if the selected indices match the effects or not, and if modification was needed. For this, carbon and nitrogen percentages, redox potential, and PSA were put into tables and descriptive statistics along with correlations were extracted by the statistical analysis. The line graphs also obtained from the tables showed that PSA is more correlated with C and N, and because of the slight relation of the PSA in describing the general status of the sediment characterization, it was finally excluded. Carbon and nitrogen percentages were so highly correlated with the line graphs that one of them had to be included. It was carbon that was chosen to carry on due to its attributes related to the organic enrichment and its multiple effects in the seabed, as well as its tendency to cause wider range of impacts.

After the carbon and the redox had been decided to be in the final combination table, the outlook of the stations was examined as a whole outcome. According to the chemical trends, the stations presented a good level of non-degraded environmental conditions, a result also obtained by the benthic indices. This argument was useful to be carried on for the important test for the correlation of the biological indices along with the chemical parameters.

The correlation occurring in all of the chosen factors would be further tested in more detail. As seen in Table 4.7, the stations are in agreement regarding their ecological status. This proves that the combination of these indices (H' and AMBI) can approach and preview the current status more accurately and extensively than the others. It is underlined that the close relationship between the benthic indices and the carbon and oxygen is due to their quantity in the seabed being responsible for the species growth which translates into richness, equitability and biotic levels. A table with these 4 or 5 trends would be a concise table (yet handy and easy) that previews the status of the ecological level in a high degree.

The effort of combining the indices as a way to receive accurate results for explaining the impacts and changes in the seabed requires a long and continuous study. Furthermore, the combination of these indices is not only limited to the biological attributes but also to one of the chemical properties that has been tested for the same purpose throughout the years. Falconer (2007) suggested that it is possible to only use redox potential

to describe the impacts. To do that, regressions were extracted and put into a confirming process to test their accuracy. However, it was not possible to fully describe the impacts at a high level. In comparison to the research made here, is recommended that the redox-carbon relationship could be enhanced and upgraded by the addition of the AMBI and H' indices. The combination of the two would give a quality boost to the extracted regressions since the species interaction in the seabed is important to be included in any model that aims to predict the level of impacts in the seabed.

In other papers made to study the combination of trends, it is recommended that the cluster analysis and the MDS (Cheng, 2004) are more productive in order to approach the diversity of the species with a more accurate and descriptive similarities and differences technique. In a more holistic approach however, as resulting from the present research, when testing the impacts of the stations in an area, the multivariate analysis is a good tool to enhance the relationships of the trends but the analysis of the trends (seen by a statistic frame) is more productive itself. In a paper made to study the combined effects of the nematode species after their infection by heavy metals (Beyrem *et al.,* 2007), the indices, and particularly the S, N and H', showed a proportional change when the metals concentration increases which is another view of the use of the combined indices when analysing and testing effects in species. Another paper has revealed that salinity is not the factor to be correlated with the biological indices to present a better picture of the impacts (Ismael & Dorgham, 2003), referring that carbon and redox would be the most appropriate trends to be correlated with the biotic indices. The idea of gathering the stations together and extract the information using the indices, leads to a more accurate statistical explanation of the benthic

attributes, in contrast to analysing each station individually as suggested in Dolah *et al.*, (1999) finds agreement in this study as well.

4.5 CONCLUSION

The combination of indices in order to obtain the best predictions of the seabed was the aim of this project. After having tested, related and compared the indices and having installed the chemical trends as indication by setting them as constant for comparison analysis, the benthic indices were reduced to AMBI, H' and N. The excluded indices were the S, D, Hb, P, Eh and ITI, as giving same outcome in a large degree and overlap for the specific purpose of combing the indices. In that way to only the most descriptive ones are obtained, in an effort to decrease the analysis process and thus achieving time economy, a very important factor in research generally.

Chapter 5

Investigation of the impacts of emamectin benzoate on marine sediments by long term metadata analysis

5.1 INTRODUCTION

5.1.1 General approach

An aspect of the Scottish salmon aquaculture industry is to defend its product against natural factors, such as the infections of sea lice that occur in the North Atlantic Ocean (SEPA, 2000). These infections cause a massive impact on the salmon culture in Scotland and the major strategy to manage these is through the application of parasiticides to reduce their numbers (Boxshall & Defaye, 1993). The parasiticides that are used are chemical substances that the farmers administer by either an in-feed or bath method. These chemicals, however, are not only efficacious in removing lice but also they have the strong potentiality of interacting with the local biological fauna within the area of the farmed ecosystem (Treves-Brown, 2000). This interaction alters the seabed in various ways causing from small components enrichment to high pollution (Beveridge, 2004). Hence, there is an important need in studying these parasiticides to obtain key information that may assist in the protection and development of the marine environment in these farmed areas.

5.1.2 Background and critical review

It is known that the global aquaculture industry produces 110 million tonnes of fish annually (FAO, 2009) but this culture process exerts effects on the environment (Kalantzi & Karakassis, 2006). These effects can result from a variety of sources, feeding activity being one of the main factors (Kalantzi & Karakassis, 2006). The impacts caused by aquaculture can be seen in organic, nutrient and toxic enrichment (BIOFAQS, 2001). The toxic input is

one of the main concerns of this project because the chemical of interest in this study is an extensively used parasiticide for the treatment of sea lice. Toxic enrichment in the seabed is a result of various chemicals being used to uphold the health and welfare of fish being reared in cages (Costello *et al.,* 2001). A wide range of chemicals, however, are used in the aquaculture industry and these may include growth catalysts, hormones, enhancers of the immune system and antibiotics (Willis *et al.,* 2005). These chemicals are diluted beneath cages in the water column with a proportion of this reaching the seabed where they may accumulate. Should accumulation within the seabed occur, then a potential process of biological degradation can take place which eventually can lead to pollution (Gillibrand *et al.,* 2002). SEPA (Scottish Environmental Protection Agency) has the duty to identify possible pollution impacts but its most valuable role is to regulate the use of key chemicals that are used to avoid any potential deleterious effects before they occur (SEPA, 2007).

The biomass of a farm releases organic and nutrient enrichment both of which primarily originated from faeces and the proportion of uneaten food (Beveridge, 2004; Kalantzi & Karakassis, 2006). The methods used to determine impacts are through the measurements demanded for environmental impact assessment (EIA) that were established and are set by SEPA for fish farms, and these include measurements on the carbon and nitrogen percentage, redox potential, particle sizing analysis along with information on the macrofauna data from the same area of seabed (SEPA, 2007).

The chemicals that are used to control sea lice numbers (and those for the management of any parasite) are called parasiticides, which following their release, either as

a bath or as an in-feed, are subsequently released into the aquatic environment where they may have impacts on other aquatic organisms and their habitat (Treves- Brown, 2000). Scotland, as a major salmon producer, has to manage sea lice infections on their stock to protect their production (Johnson *et al.*, 2004). These chemotherapeutants not only have the potential to negatively impact on the environment through their effects on sensitive non-target organisms, but also they may alter the population structures of the fauna in the immediate environments (Treves- Brown, 2000). The chemotherapeutants that most commonly used in Scotland are: azamethiphos marketed as "Salmosan", teflubenzuron sold as "Calicide, cypermethrin as "Excis" and emamectin benzoate sold as "SLICE" (SEPA, 2005). Calicide is an in-feed solution, initially used in Scotland in 1999 which with Excis, are two of the most commonly used parasiticides by Scottish fish farms (SEPA, 2007). Excis is a bath-based pyrethroid that was also first used in 1999 and is toxic to Crustacea, however, its fate in the environment does not represent a serious problem because of its high solubility (SEPA, 2007). Salmosan is also toxic to Crustacea and is applied as a bath treatment. Salmosan has been shown to have a level of absorption by the seabed, however, this chemical was licenced for use before the other commonly used chemotherapeutants (Pahl & Opitz, 1999).

Emamectin benzoate (E.B.) is the active ingredient of SLICE and is one of the more recently licenced products for use by the aquaculture industry (SEPA, 2005). Following its release, Schering Plough (SP) conducted and released a paper dealing with laboratory and field based toxicity studies regarding the environmental issues and made suggestions regarding the quantities that should be used and the duration time for its application

(Schering Plough Animal Health, 1998). Later, SEPA's regulation papers agreed with the paper released by SP for its use by Scottish salmon farms (SEPA, 2005).

The chemical type of the SLICE is:

• = > 90% of 4-epimethyamino-4 -deoxyavermectin B1a benzoate (MAB1a).

• = < 10% of 4-epimethyamino-4-deoxyavermectin B1b benzoate (MAB1b).

In addition this formulation also contains butylated hydroxyanisole (0.01%), propylene glycol (2.5%), maltodextrin (47.40%) and corn starch (*qs* to 100%). The applicable dose is three treatments in any 12 calendar months, and five treatments in any two year growth cycle. The treatment regime is as follows: emamectin benzoate will be fed at a rate of 50 ug per kg of fish per day for seven days (Schering Plough Animal Health, 1998). The dose will be administered as SLICE pre-mix coated on to feed. The E.Q.S. for SLICE as given by SEPA is 4 ng/g (SEPA, 2007). Following its digestion by fish, SLICE is finally excreted in faeces, into the water column and then to the seabed; it is for this reason, SEPA has conducted numerous studies on measuring SLICE and monitoring it in the seabed. SEPA created a protocol whereby farms must apply to them to use it and additionally, must measure SLICE residues in the seabed sediments in samples taken between 110 and 130 days following the cessation of the treatment (SEPA, 2000). These measurements must be taken at three different stations: one underneath the cages, one at a distance of 25 m and one at a distance of 150 m from the cages. This strategy was modified in 2007 and the number of stations was reduced from three to two: one underneath the cages and one at a distance of 100 m away from the cages (Environmental Services, 2007).

The research made to study the SLICE properties follows SEPA's tactics. In Scotland two major projects, excluding those conducted by SEPA, were made to study SLICE closely. The SAMS project (Scottish Association for Marine Science) was a 5 year project (1999-2004) investigating the effects of SLICE in four Scottish sea lochs which introduced DEPOMOD as a modelling tool for the prediction of SLICE (SAMS *et al.,* 2005). This study concluded without identifying polluting effects in lochs but gave future predictions for SLICE in these lochs using DEPOMOD. Later, SEPA investigated the treatment patterns and residues and found that there was a difference of 7.5 ug/kg in the values predicted by DEPOMOD to the actual levels that were determined (see Figure 3 in Wells *et al.,* 2008). SEPA has also made research on the effects of SLICE producing useful information for its toxicity on a range of species. Furthermore, the environmental risk assessment (E.R.A.) and E.I.A. that were made, demonstrated the toxicity SLICE has to species and ecosystems. Another study conducted by Telfer *et al.* (2006) in Loch Duich did not show disturbed trends in sediment faunal composition, or uptake in sentinel species that could be related to environmental discharge, caused by the levels of emamectin from the nearby fish cages.

5.1.3 Aims and objectives

This project was made to study the properties of SLICE in the marine environment. The project has two main interests. Firstly, to look for the impacts SLICE may have (if any) in the marine farmed ecosystems emphasising any changes in the seabed. Secondly, to study the fate and dispersion of the SLICE in these ecosystems. For the search of these matters two hypothetical questions were posed:

- Is SLICE responsible for environmental impacts to the seabed at Scottish fish farm sites?

- What is the fate of the SLICE dispersed in the seabed of Scottish fish farm sites?

The objective of the project is to provide information that help answer these two questions by using statistical tools and also biological indices and methods created to measure changes in the marine environment and identify its possible causes.

5.2 METHODS

5.2.1 Project design

The project was comprised of three main stages. Data collection, data input to spreadsheets and data analysis. The data were obtained both from the Institute of Aquaculture (Environmental Services) and the Scottish Environmental Protection Agency (SEPA). Additional data was also requested from the Department of the Environment, Food and Rural Affairs (Defra) regarding a project they funded (SAMS *et al.*, 2005) concerning the ecological status of the lochs which have farming activity within them. The data includes environmental and macrofauna data from various farm sites across Scotland and SLICE data from same sites. Following data entry into spreadsheets, the data were analysed by a variety of different approaches to test the original hypothesis.

5.2.2 DATA input

The data SEPA requires for monitoring are the same as those used here. The data were collected from three sources: IoA Environmental Services, SEPA and the SAMS report. Two final spreadsheets were created to permit analysis of the data. The first spreadsheet combines environmental data and the SLICE measurements. The final environmental factors-based spreadsheet contains redox potential, median particle size analysis (MPSA), carbon percentage C% and SLICE. The second spreadsheet has the macrofauna data from the same sites and stations and the spreadsheets are identical regarding the sites and stations, as the first spreadsheet has the environmental factors exclusively. During the process of the creation of the final analysed spreadsheets, others were created to assist in the final concluding output. The methodology and the steps used are being described analytically throughout this chapter.

For a SLICE study project, it is essential to have emamectin benzoate data to correlate the other factors against. The data selection followed the SEPA instructions and recommendations as given in their marine fish farm manual (2000). For that, data related to carbon and nitrate percentage, particle size analysed data, redox potential, emamectin benzoate measurements and macrofauna data were included. After collecting these data, the next step was to evaluate the requirements to accomplish the analysis purpose. All the possible data were input into two spreadsheets. The first was in a table form, in which the columns contain the stations names and the rows contain the measurements of redox potential, carbon and nitrogen percentages and particle size analysis. The following rows

have the measurements according to the stations. The measurement units for the factors are: for redox potential in mV, for carbon and nitrogen percentages and for MPSA μm. The redox potential measurements that were included in this spreadsheet were from the 2 m statios under the water surface, in order to obtain the average tested depth (all the tested depths were in a scale from 0 to 5 m from the sea surface). Particle size analysis measurements that were included in the spreadsheet were those from the 63 μm sieving method, which constitute the average of the measurements of all the sieving process. All the stations in both spreadsheets are identical and unique. The same method was followed for the creation of the second spreadsheet which contained the macrofauna data. The top row contains the stations. The first column contains the species. The tested spredsheet contains the species abundance identical and unique with all the available stations.

These speadsheets were combined into a final one to provide an analysis of SLICE using various software tools. These tools are univariate analysis according to SEPA's directions (ITI) and multivariate analysis for identifying the correlation of SLICE with all the other environmental factors, as well as SLICE and its interactions within the stations and species. A series of other tests and analysis were also available from this spreadsheet such as regressions and AMBI software tool.

The data which were included in the spreadsheets in their final form were from various salmon farm sites across West Scotland, Orkney and the Shetland Islands. Figures 5.1 and 5.2 show the sites that were included in the final spreadsheets. The table also includes the stations so that a first approach of the areas with the measured SLICE will be

possible. In Appendix 4, Table A2 represents a more detailed table giving the labels for each of the sites and stations, permitting the reader to examine the results in a detailed way.

As commented upon in SEPA's guidance papers, after 2007 the method for measuring SLICE was changed. The stations measured for the chemical are 0 and 100 m. That meant that the data collected from stations before 2007 could not be correlated with those now collected. Therefore, the stations close to 100 m, *i.e.* the 50 m and the 150 m analysis points, were correlated to ascertain whether either could be used. To determine this, the top 5 species at the 50 and 150 m stations (and the ones including SLICE only) were tested. The results showed a 60% correlation of the species occur in these stations and a 72.2 % correlation in their abundance. This comparison is statistically acceptable, so the macrofauna and environmental data collected at the 150 m stations were added to the spreadsheet to fill the gap of the missing 100 m stations. The statistical results from this method can be seen in Appendix 4, Table A3.

For SAMS data, the SLICE value measurements were grouped into 3 major categories: no detectable value (*i.e.* zero), trace (0.03-1.9 ng/g) and its actual measurement value, *e.g.* 2.3 ng /g. To make the spreadsheet accurate, the worst case scenario was adopted and eventually used in the final spreadsheets. In that case, 16 stations finally added in the spreadsheet have a SLICE value of 1.9 ng /g, which is the highest value in the trace scale. It should be noted that redox potential data were not included in the SAMS data collected (as a result these data were uncorrelated with the rest of the data came from different sources that did have redox potential values). For the multivariate analysis, 34 sites were tested

(including 128 sampling stations).

The second spreadsheet took longer to create, because this contained more data species. The final spreadsheet was created by removing any duplicate or similar species for analysis accuracy. Since the stations were identical and unique in both of the sheets, labels were created in order to run the MVSP software as required by the program. The species included in the final spreadsheet were classified by using the software WORMS, which provides a classified list of species.

5.2.3 Data analysis

The core of the analysis is the determination of the impacts of SLICE and its properties in the marine environment in the area around salmon farms. The strategy for studying SLICE in this project was to use univariate and multivariate analyses available in computer programmes. The methods used for this part of the study follow those described in SEPA's papers, in addition to a range of other simple statistical tools and programmes not used by SEPA. SEPA's guidance for monitoring is based on two major statistical classes: univariate and multivariate analysis of the macrofauna and the physico-chemical data. Only the macrofauna data was analysed by univariate analyses. In this, ITI is the suggested results output. Along with ITI, Simpsons Index D, Brillouins Index Hb, Shannon Wiener Hs or H', Pielou Evenness P and Heip Evenness Eh, these provide important information regarding the ecological state of the stations (the method is described in detail in Chapters 3 and 4). For this project, the univariate analysis was conducted in the Excel spreadsheet by using the WORMS programme. This table of results relates to the Infaunal Trophic Index (ITI) and the

other indices for each station. ITI is measured because it provides a view of the station community regarding its degrading state, such as pollution and leads to important information about the trophic conditions within the community.

Multivariate analysis was applied to both spreadsheets. Cluster analysis and Canonical Correspondence Analysis (CCA) are two statistical features that SEPA considers essential. With this method, there is statistical information about the interaction of the physico-chemical factors (and SLICE) in relation to the species and their behaviour was studied using a variaty of factors. Multivariate analysis was performed by using MVSP 3.1 edition. The multivariate analysis is important because it studies a spectrum of various statistical approaches for each of the factors, species and stations alone or in combination with one another according to the needs and requirements of the study. From the cluster analysis, a dendrogram is produced which shows the correlation of the data in relation to the environmental factors and SLICE.

In CCA, the data are spread within axes and arrows of each factor are oriented to the direction of the impacts. The macrofauna data of the stations is correlated with the environmental factors. The stations inside (or adjacent) to the SLICE arrow are the ones affected by the chemical. Then, the stations and their species can be separated from the whole figure and analysed further, to provide further results about the behaviour of SLICE. This is seen in combination with the species, to study the factors attributes and predict the fate of the SLICE in the marine environment. As part of the univariate analysis and for enhancing the results in quality, AMBI was used. Its property to calculate the biotic index

and express it in a detailed output is very helpful to identify the changes in the stations in terms of the species disturbance and their biotic and ecological profile (more details provided in previous Chapters 3 and 4).

The remaining figures were made in Sigmastat (3.1 edition) and Microsoft Office Excel (2007 edition) and provided a series of regressions and data plots testing various parameters. Those parameters tested the SLICE fate on the ecosystem, such as time and distance from the sea cages and their correlation with the physico-chemical parameters, such as carbon percentage, redox potential and median size particles. Additional regressions were made to calculate the interactions of the *Capitella* sp. group along with nutrient, organic and redox co-efficients.

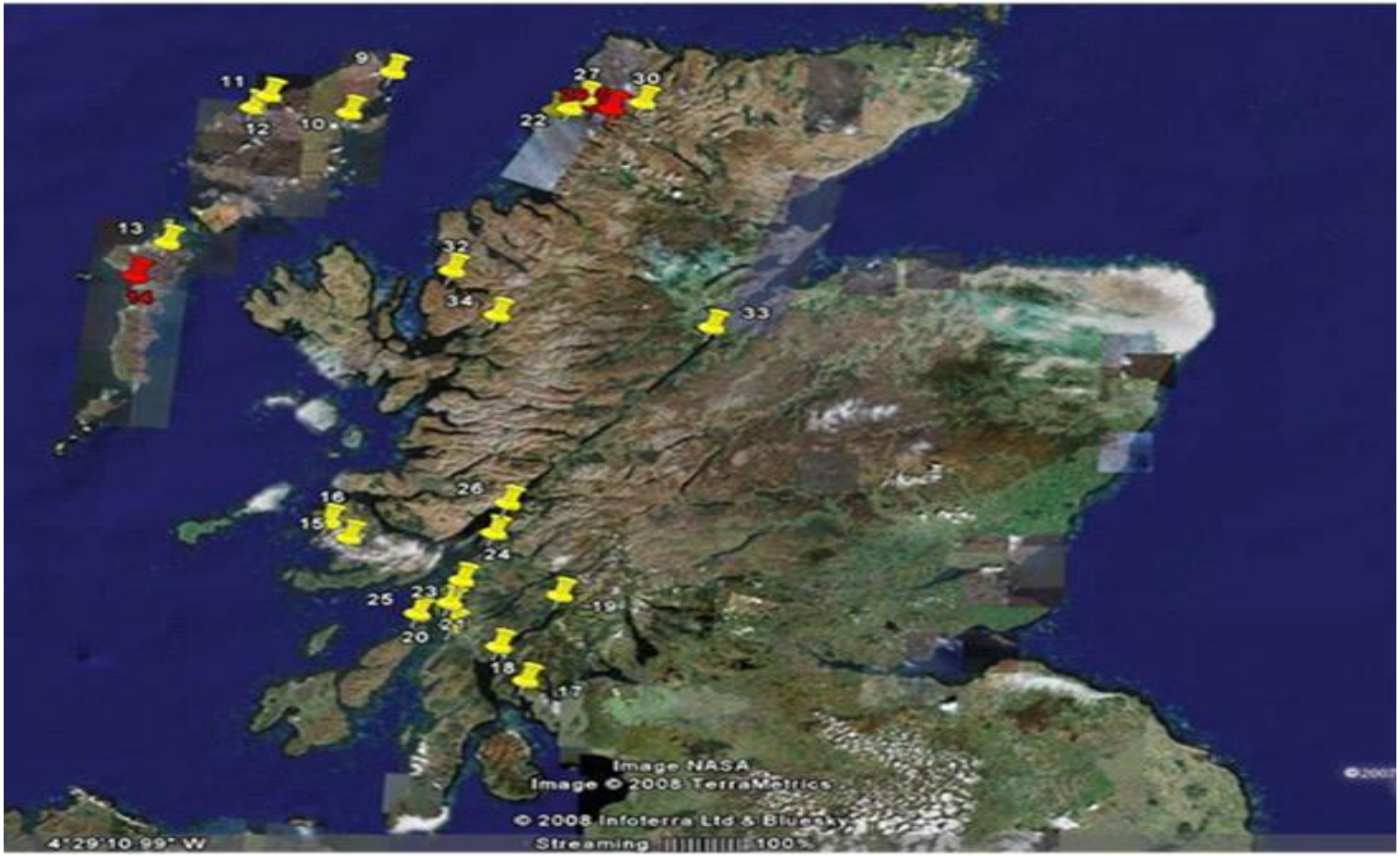
5.3 Results

The results give information regarding three important aspects related to SLICE. The effects from the treatment to the marine environment, the fate of the chemical in terms of the dispersion of the residues to the seabed and predict its quantity within these areas. Further investigation of the *Capitella* sp. group, with nutrients and organic enrichment are also presented in order to investigate any impacts on sediment species.

The sites from Tables 5.1 and 5.2 (being across Scotland) are plotted on a map by using the Google Earth free net-based software and can be seen in the Figures 5.1 and 5.2. Figure 5.1 shows a map of Scotland with the sites where SLICE is present marked by yellow

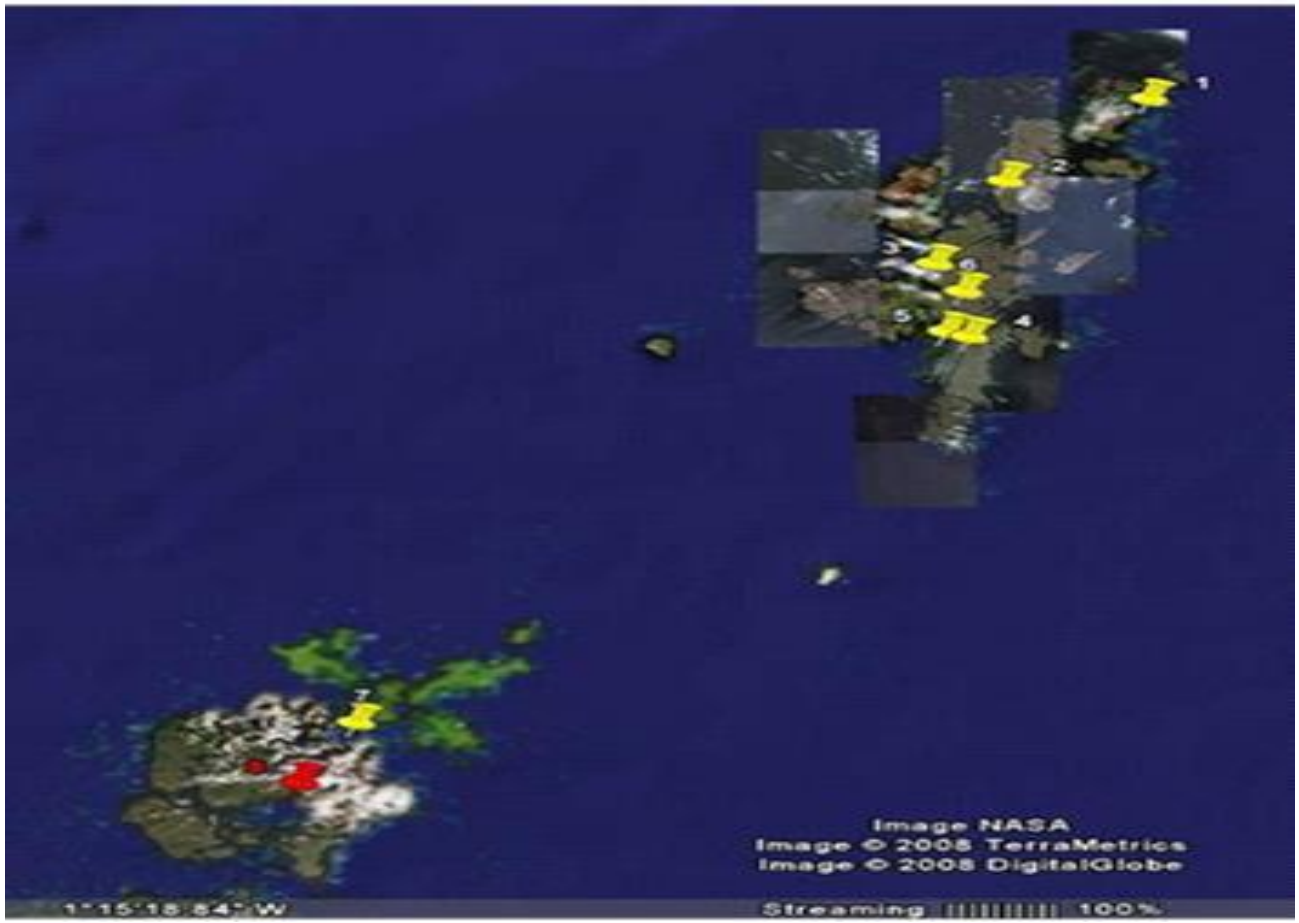
and red pins. The yellow pins indicate where the quantity of SLICE is present up to 3 ng/g, while the red pins show sites with values of more than 3 ng/g. Figure 5.2, is a map of the Orkney Island and the Shetland Isles. Again, the sites with SLICE are marked by using yellow and red pins following the same system as given above. The Physico-chemical parameters sheet (Appendix 4, Table A4) has the SLICE quantity in the stations across the sites along with carbon percentage, redox potential, and median particle size analysis. The units for each of the factors are: ng/g for SLICE, percentage for carbon, redox potential is measured in mV and median size of the MPSA in m particles. The macrofauna data sheet shows these stations with their species quantity (this sheet has been added as Appendix 4, for convenience because of its length) and in the results section Table A5 (see Appendix 4) has been added, with the univariate results shown. In Table A5, the values of all indices have been transformed to percentages so that there is a common basis of the creation of the figures.

Figure 5.1. Map of Scotland with the sites where sediment residues for SLICE were found. Yellow sites have < 3 ng/g dry weight sediment whilst red sites have >3ng/g dry weight sediment.



Key to sample sites: 9 Tolsta; 10 Sgeir Mhor; 11 Port Na Cro; 12 Greinham; 13 Loch Fada; 14 Droigniche; 15 Inch Kenneth; 16 Geasgill; 17 West Kyles; 18 Ardcastle; 19 Furnace; 20 Port Na moine; 21 Port na Gile; 22 Nedd; 23 Ardmaddy; 24 Connel; 25 Aird; 26 Shian Bay; 27 Reintraid; 28 Torgawn; 29 Drumbeg; 30 Kempi Bay; 31 Oldany; 32 Kenmore; 33 Creag na Hiolaire; 34 Kishorn.

Figure 5.2. Map of Orkney and the Shetland Isles showing sites where sediment residues for SLICE were found. Yellow sites have < 3 ng/g dry weight sediment whilst red sites have >3ng/g dry weight sediment.



Key to sample sites: 1 Vatsetter; 2 Setterness; 3 Selie Ness; 4 Merry Holm; 5 Stead of Aith; 6 Djuba Wick; 7 Hascossay; 8 Chalmers Hop

Figures 5.3-5.7 extracted from Table A4 (see Appendix 4), and Figure 5.8 from Table A5 (see Appendix 4) shows the interaction of the chemical with the biological indices received from the univariate analysis made by the macrofauna spreadsheet. The prediction of the fate of SLICE in relation with the environmental factors can be seen in the following regressions extracted from Figures 5.3-5.7. The SLICE behaviour alongside the distance from the cages can be seen in Figure 5.3. At 0m the amount of SLICE is zero and at the next year is increased to 0.63 ng/g. The SLICE amount at 25m before is more than the second year amount, which ranged from 0.73 to 0.52. The SLICE at the 150 m stations from the first year was increased from 0.62 to 0.64 ng/g.

Figure 5.4, shows SLICE in relation with the median particle size analysis. The size used in the original spreadsheet is the same as in Figure 5.4, the median size measured in μm particles. Here SLICE increases as the median size of the particles also increases. The resultant regression analysis provides this in the formula: SLICE= 0.0008*(median size) + 1.004.

Figure 5.5 presents SLICE in relation to redox potential. This relationship was also addressed in the MVSP Figures 5.9 and 5.11 and showed an opposite relation, as it is also suggested in Figure 5.5. SLICE is reduced as the redox potential is increased and the reduction is given by the regression: SLICE= -0.0022*Redox + 1.7522.

Figure 5.6 is the relationship between SLICE and the carbon percentage. The carbon levels rise as SLICE increases. The regression for this relationship is: SLICE= 0.0546*C% + 0.8658.

In Figure 5.7, SLICE is plotted along with a number of other factors into a multiple regression analysis graph and the regression formula extracted to be: SLICE = 1.453 + (0.000644 * Median) + (0.0311 * %C) - (0.00213 * redox), a model which summarises all the above into one formula that can be used in multiple ways.

Figure 5.3. Mean sediment concentrations of SLICE (ng/g) for the sites surveyed with distance (in metres) from the cage edge.

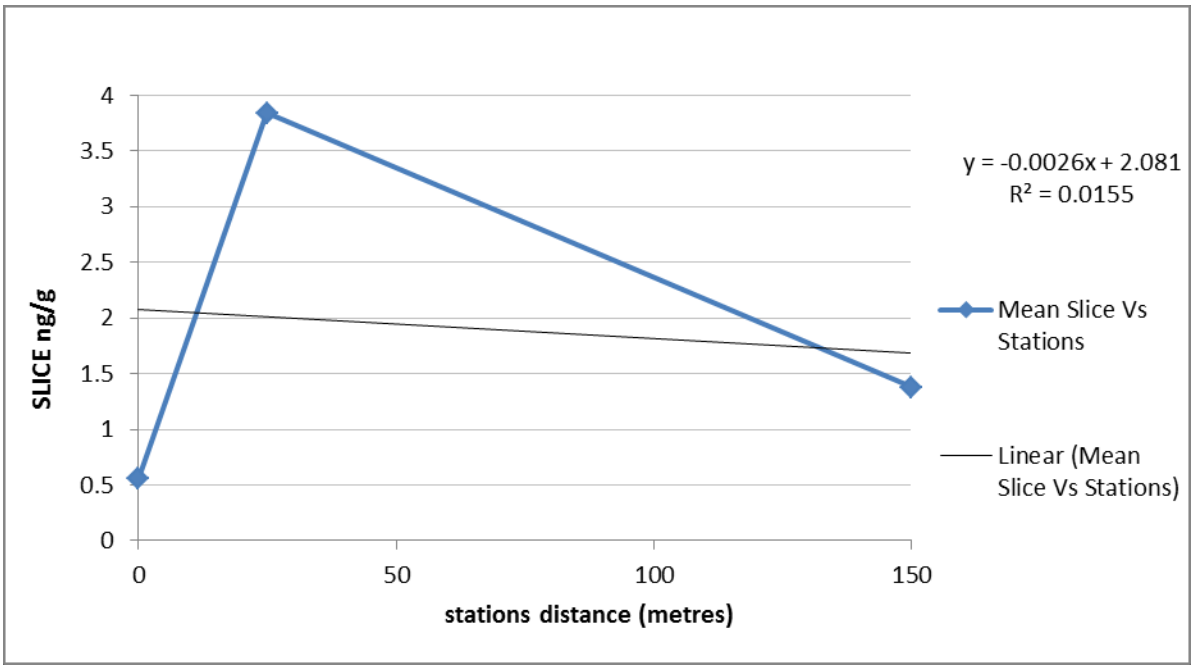


Figure 5.4. Linear regression plot of sediment concentrations of SLICE (ng/g) with median particle analysis MPSA (µm.)

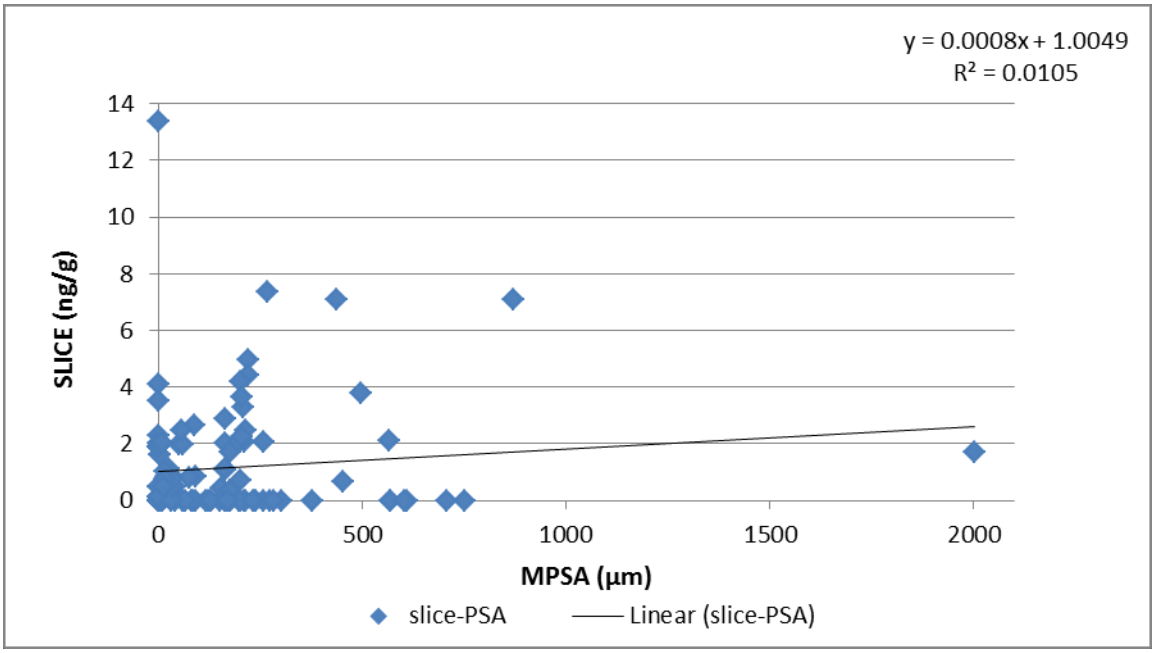


Figure 5.5. Linear regression plot of sediment concentrations of SLICE (ng/g) with redox potential (mV).

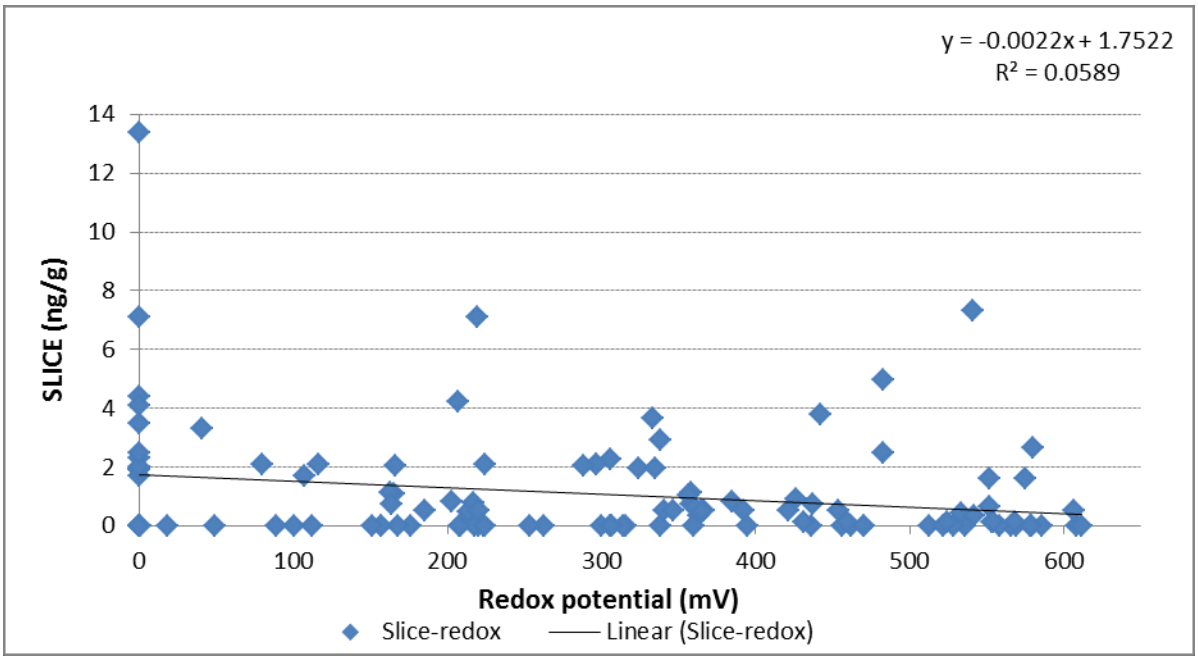


Figure 5.6. Linear regression plot of sediment concentrations of SLICE (ng/g) with the percentage of organic carbon in sediments.

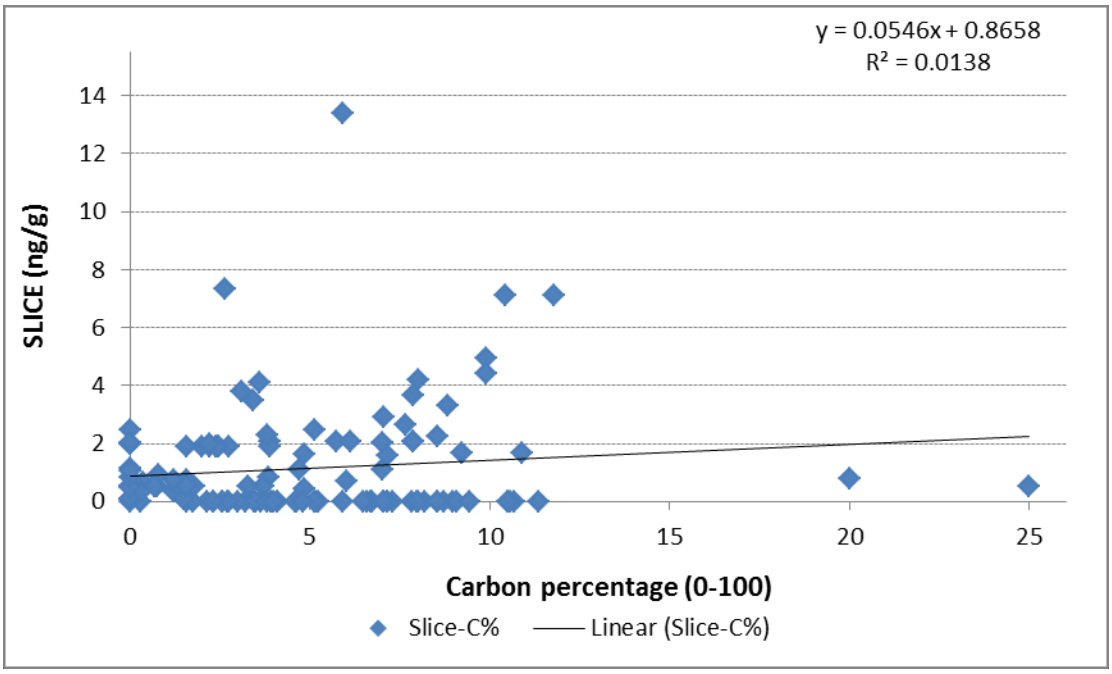


Figure 5.7. Multiple regression and scatter plot of sediment concentrations of SLICE with the environmental parameters: C%, redox potential (mV) and MPSA (µm).

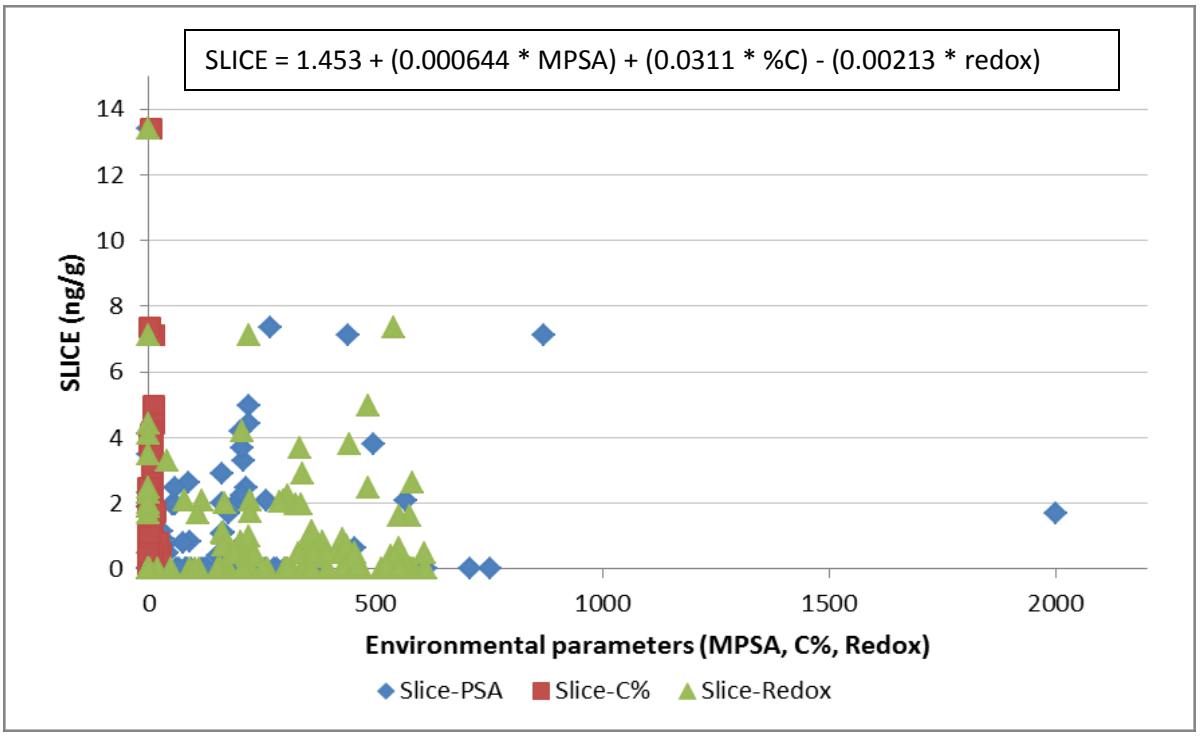


Figure 5.8 is the scatter plot and the regressions from SLICE with all the biological indices. From this, the SLICE relation with each of the indices is shown.

SLICE-ITI: SLICE= 0.25*ITI+1.52

SLICE-AMBI: SLICE= 2.1*AMBI+0.67

SLICE-Pielou: SLICE= 0.15*P+1.62

SLICE-D: SLICE=0.09*D+1.665

These regressions show in detail the SLICE relationship with each of the biological factors. SLICE increases the biological indices in terms of the number of species (individually and in abundance) while the trophic and biotic behaviour of the ecosystems decreases in quality (as AMBI and ITI are increased).

The effects in the environment can also be enhanced by the MVSP Figures 5.9-5.11. In these, the cluster analysis and the CCA analysis are plotted as graphs. Figure 5.10 shows the dendrogram of the cluster analysis as extracted from the macrofauna datasheet. The stations are split into 2 major groups as shown in the Figure 5.10. Table 5.3 identifies the stations because these cannot be seen in Figure 5.10. Figure 5.9, is a CCA of the stations with SLICE and the environmental parameters, with SLICE and redox emerging on opposing axes. This shows that when emamectin benzoate increases, the redox potential is reduced. Table 5.1 shows the stations grouped in the SLICE and redox axes. The species in these axes are shown in Table 5.2. When the effects were analysed, these trends (SLICE and redox) were assumed as common and suggested which species in these stations were infected more (SLICE axis) by SLICE and redox

(redox axis). The same result regarding the SLICE-redox relationship can also been seen in Figure 5.11, where a CCA looked for the impact of SLICE on the macrofauna species and shows that 35 species are affected by SLICE in terms of their evenness, as indicated on Table A5. From Figure 5.11, Table 5.4 was produced to provide the names of the species affected by SLICE, since they are not visible in the Figure 5.11.

Figure 5.8. Scatter plot and regression analysis of the SLICE concentrations compared with the diversity indices using the data from Table A5 (see Appendix 4). The indices are Infaunal Trophic Index (ITI), Azti's Marine Biotic Index, Pielou index and Simpson's Index. The SLICE was measured in ng/g per sample and the indices scale extends from zero to one.

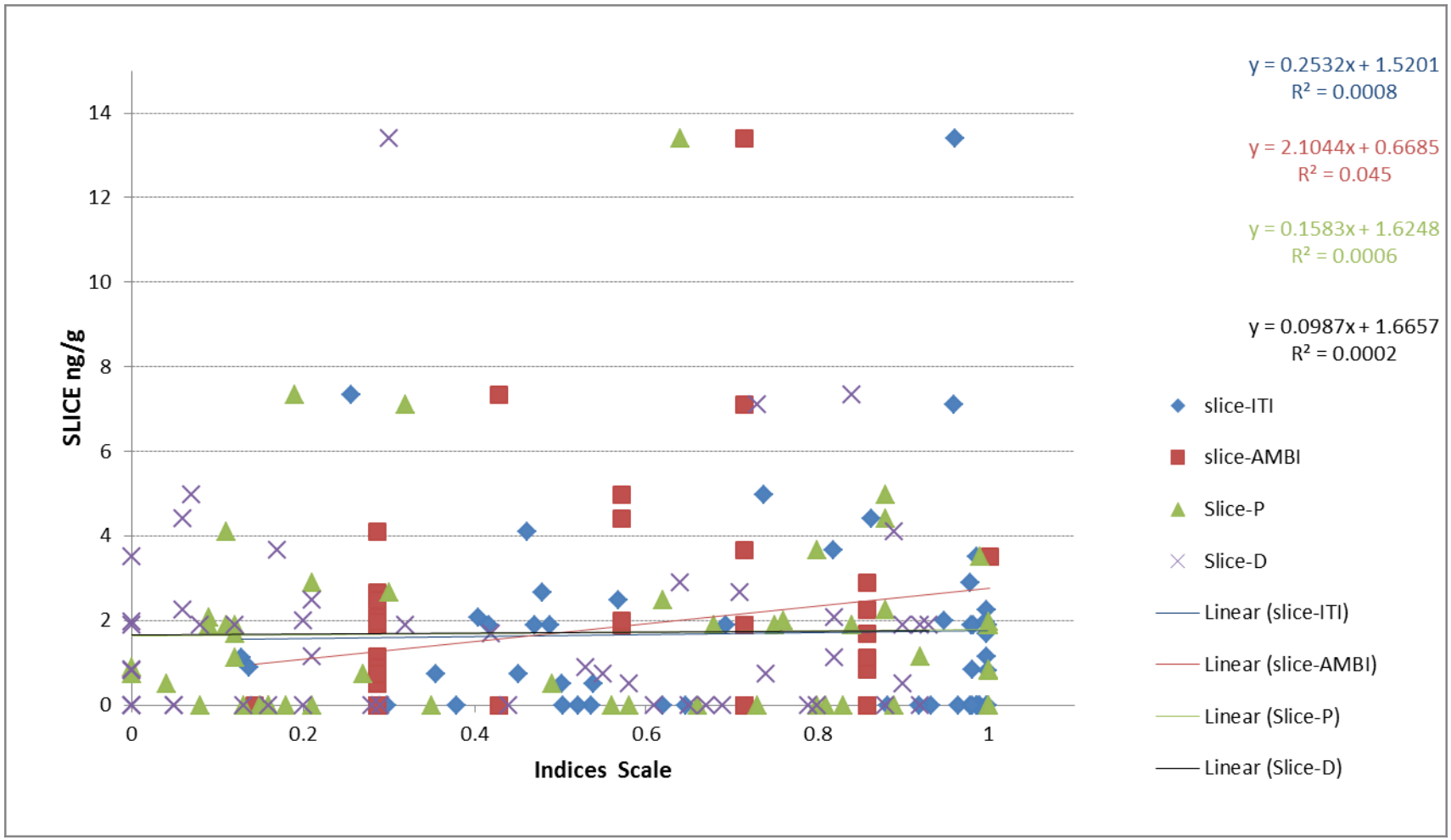
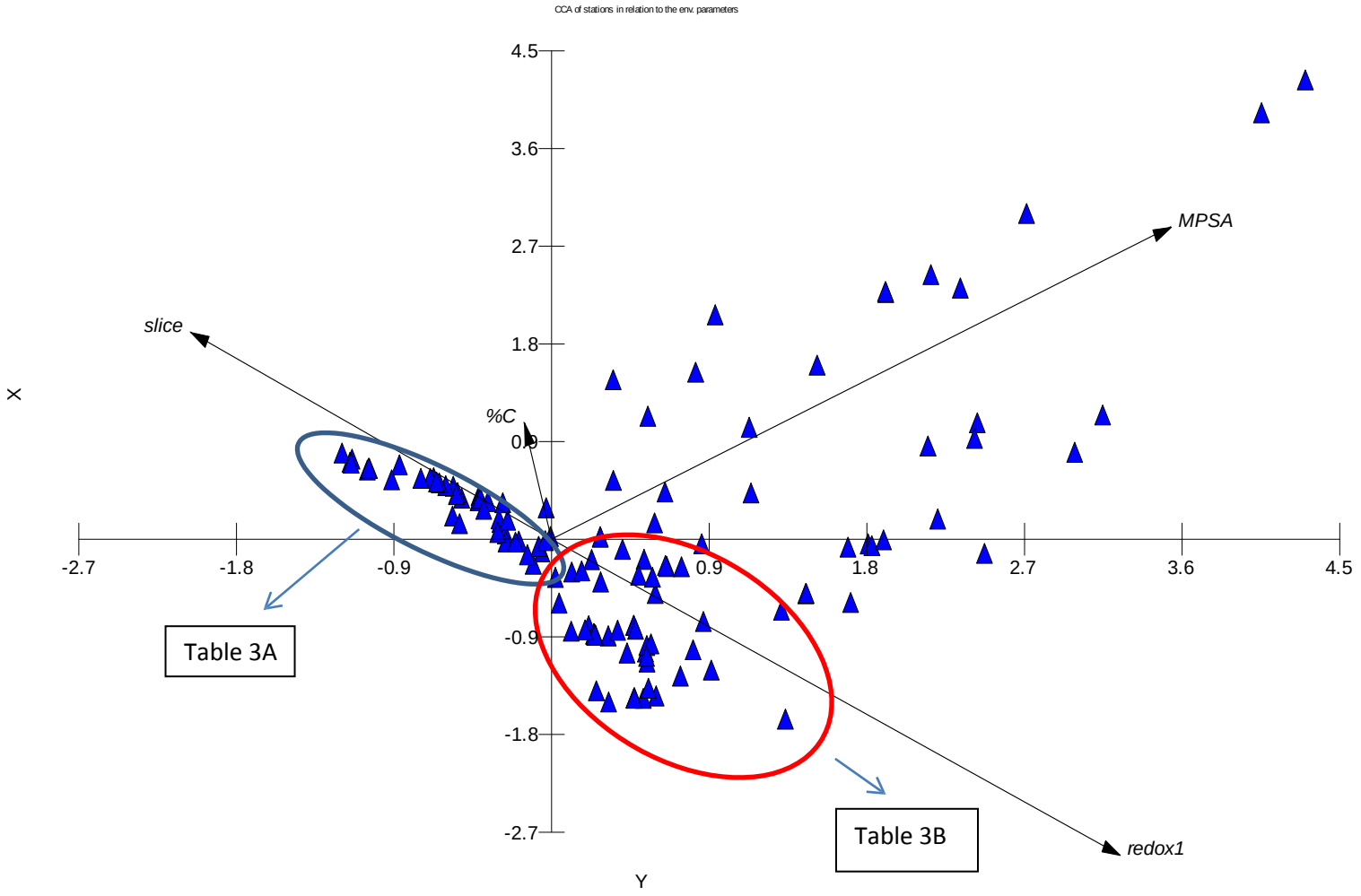


Figure 5.9. CCA graph of the stations in the macrofauna sheet correlated with the environmental parameters SLICE (ng/g), MPSA (μm), and redox potential (mV). The ellipses highlight sites that are affected by SLICE and the details of these are shown in Tables 3A and 3B.



Vector scaling: 9.06

Figure 5.10. Cluster diagram shows the similarities in station as analysed from the macrofauna datasheet. Groupings of stations A and B are highlighted for further analysis.

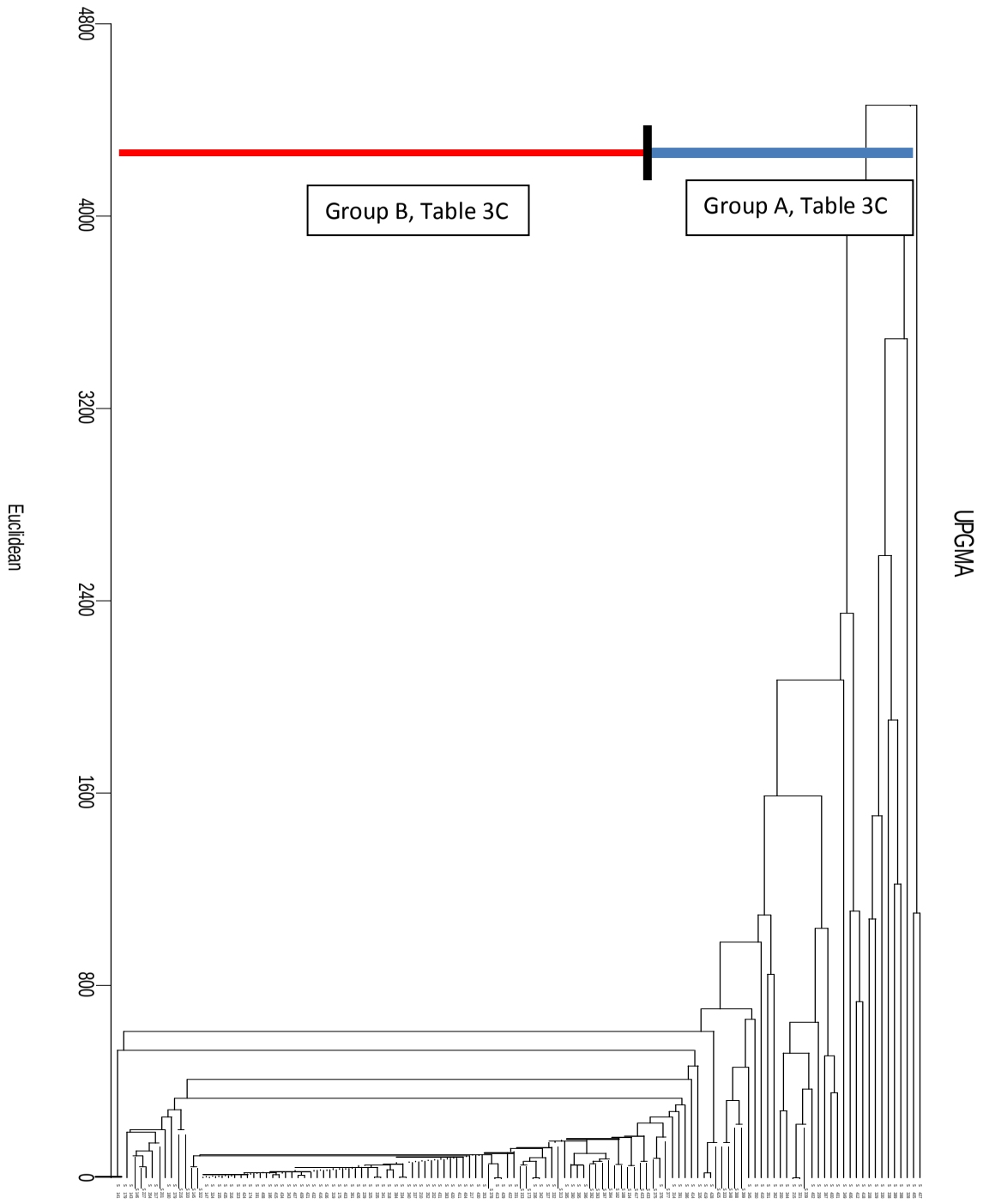


Figure 5.11. CCA graph of the species in macrofauna sheet correlated with the environmental parameters SLICE (ng/g), MPSA (μm), Redox potential (mV). The ellipse highlights sites that are affected by SLICE and are shown in Table 4.

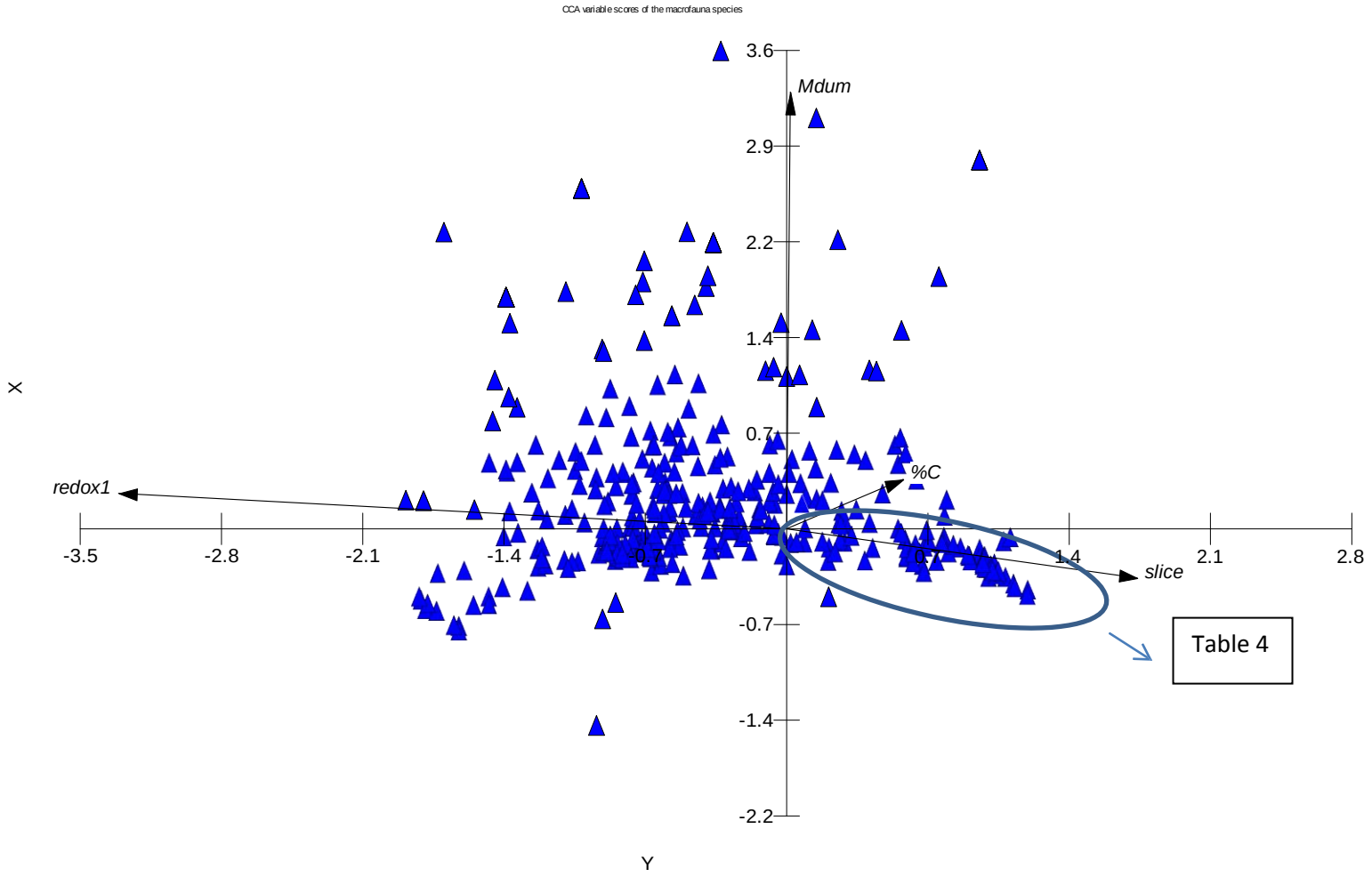


Table 5.1. Table shows the CCA results using MVSP from the macrofauna and environmental parameter datasheets extracted from Figure 5.9, showing the division of grouping defined by the analysis of the SLICE axis. There are a total of 28 stations with the majority of 0m and 25m stations affected by SLICE.

Labels shown in the Figure 5.9 of the SLICE axis	**Stations** **Site/year/cage distance/direction**
S209	Torgawn 04 25m SE
S317	Nedd 05 25m N
S320	Torgawn 05 25m S
S328	Port na moine 03 0m N
S329	Port na moine 03 25m N
S330	Port na moine 03 150m N
S333	HascosaY 03 0m S
S338	VatsetteR 03 25m N
S339	VatsetteR 03 0m S
S344	BaghDialnanCaenn 05 25m S
S345	BaghDialnanCaenn 05 150m S
S348	DjubaWick 05 0m N
S354	Selie Ness 05 0m N
S384	WestKyles 05 150m S
S387	Kishorn 01 0m N
S389	Kishorn 01 150m N
S391	Kishorn 01 25m N
S393	Kishorn 01 0m S
S394	Kishorn 01 25m S
S395	Kishorn 01 150m S
S396	Kishorn 01 0m E
S397	Kishorn 01 25m E
S398	Kishorn 02 0m N
S400	Kishorn 02 150m N
S401	Kishorn 04 0m N
S402	Kishorn 04 25m N
S403	Kishorn 04 150m N
S422	Tolsta 06 25m N

Table 5.2. Table shows the CCA results using MVSP from the macrofauna and environmental parameter datasheets extracted from Figure 5.9, showing the division of grouping defined by the analysis of the redox axis. A total of 22 stations are shown.

Labels shown in the Figure 5.9 of the Redox axis	**Stations** **Site / year / cage distance / direction**
S154	Nedd03 25m S
S174	Torgawn 03 25m E
S175	Torgawn 03 50m E
S202	Reintraid 04 50m SE
S208	Torgawn 04 0m NW
S216	Torgawn 05 25m SE
S319	Torgawn 05 150m NW
S325	Oldany 05 150m NW
S326	Oldany 05 25m NW
S331	HascosaY 03 150m NW
S332	HascosaY 03 25m NW
S378	InchKenneth 05 150m NW
S383	Chalmershope 05 0m NW
S407	Inchkenneth 06 0m S
S420	Tolsta 06 0m NW
S421	Tolsta 06 150m S
S424	Ardcastle 07 0m SW
S429	Setterness West 06 0m N
S430	Poll na Gile 06 150m S
S431	Aird 07 0m NW
S432	Aird 07 150m NW
S435	Kempi Bay 07 150m N

Table 5.3. Cluster analysis of the stations and their similarity to one onother as determined from the macrofauna datasheets, showing the grouping of the two groups (A and B) as determined by the cluster analysis presentein Figure 5.10. The first group contains 35 and the second 93 stations.

Labels in Group A **Extracted from Figure 5.10**	**Stations** **Site / year / cage distance / direction**
S427	Furnace 07 0m SW
S406	Sgeir mhor 06 0m E
S328	Port na moine 03 0m E
S398	Kishorn 02 0m N
S389	Kishorn 01 150m N
S338	VatsetteR 03 25m E
S330	Port na moine 03 150m
S329	Port na moine 03 25m
S208	Torgawn 04 0m NW
S418	Greinham 06 25m NW
S412	Bow of Hascosay 06 25m N
S406	Sgeir mhor 06 0m E
S349	Stead of Aith 05 150m
S401	Kishorn 04 0m N
S400	Kishorn 02 150m N
S387	Kishorn 01 0m N
S209	Torgawn 04 25m SE
S402	Kishorn 04 25m N
S339	VatsetteR 03 CE
S321	Torgawn 05 0m
S215	Torgawn 05 0m NW
S344	BaghDialnanCaenn 05 25m
S200	Reintraid 04 0m NW
S362	ConNel 05 0m
S351	Stead of Aith 05 0m
S410	Bow of Hascosay 06 0m N
S350	Stead of Aith 05 25m
S345	BaghDialnanCaenn 05 150m
S399	Kishorn 02 25m N
S388	Kishorn 01 25m N
S348	DjubaWick 05 CE
S333	HascosaY 03 CE
S425	Creag na h-iolaire 07 0m SW
S428	Kenmore Point 07 0m SW
S419	Greinham 06 CE NW

Labels in Group B **Extracted from Figure 5.10**	**Stations** **Site / year / cage distance / direction**
S326	Oldany 05 25m S

S414	Poll na gile 06 150m S
S386	WestKyles 05 0m N
S391	Kishorn 01 25m N
S314	Reintraid 05 25m S
S377	Ardmaddy 05 150m N
S376	Ardmaddy 05 25m S
S375	Ardmaddy 05 0m S
S433	Aird 07 25m NW
S423	Ardcastle 07 0m SW
S417	Greinham 06 150m NW
S154	Nedd 03 25m S
S188	Drumberg 04 25m SW
S182	Droigniche 04 50m S
S394	Kishorn 01 25m S
S397	Kishorn 01 25m E
S393	Kishorn 01 0m S
S392	Kishorn 01 150m N
S396	Kishorn 01 0m E
S395	Kishorn 01 150m S
S390	Kishorn 01 0m N
S385	WestKyles 05 25m
S313	Reintraid 05 150m
S332	HascosaY 03 25m
S327	Oldany 05 0m N
S342	SteadAithness 03 0m N
S336	SelieNess 03 0m S
S173	Torgawn 03 0m W
S153	Nedd 03 0m N
S331	HascosaY 03 150m N
S422	Tolsta 06 25m S
S429	Setterness West 06 0m N
S413	Poll na gile 06 0m S
S378	InchKenneth 05 150m S
S353	Selie Ness 05 25m S
S430	Poll na Gile 06 150m S
S217	Torgawn 05 50m SE
S404	Ardmaddy 2006 150m N
S411	Bow of Hascosay 2006 150m N
S420	Tolsta 06 0m NW
S383	Chalmershope 05 0m S
S431	Aird 07 0m NW
S202	Reintraid 04 50m SE
S352	Selie Ness 05 150m S
S210	Torgawn 04 50m SE
S337	VatsetterR 03 150m N
S340	SteadAithness 03 150m N
S334	SelieNess 03 150m N

S384	WestKyles 05 150m S
S318	Nedd 05 0m N
S341	SteadAithness 03 25m N
S335	SeleiNess 03 25m N
S325	Oldany 05 150m S
S229	Portnacro 03 Ref (850m) S
S426	merry Holm 07 0m NW
S361	CoNnel 05 150m S
S403	Kishorn 04 150m N
S175	Torgawn 03 50m E
S319	Torgawn 05 150m S
S436	Sian Bay 07 0m N S
S416	BDNC N 06 CE NW
S432	Aird 07 150m NW
S421	Tolsta 06 150m S
S409	Geasgill 06 150m W
S407	Inchkenneth 06 0m S
S343	BaghDialnanCaenn 05 0m S
S434	Kempi Bay 07 0m N
S415	BDNC 06 0m NW
S186	Drumberg 04 25m NE
S408	Geasgill 06 0m W
S191	Nedd 04 50m N
S174	Torgawn 03 25m E
S424	Ardcastle 07 0m SW
S323	Drumbeg 05 25m S
S316	Nedd 05 150m N
S435	Kempi Bay 07 150m N
S155	Nedd 03 50m S
S324	Drumbeg 05 0m S
S147	Drumbeg 03 50m SW
S322	Drumbeg 05 150m S
S145	Drumbeg 03 0m SW
S320	Torgawn 05 25m SW
S216	Torgawn 05 25m SE
S379	InchKenneth 05 25m SW
S180	Droigniche 04 25m S
S201	Reintraid 04 25m SE
S317	Nedd 05 25m N
S354	Selie Ness 05 0m S
S227	Portnacro 03 50m S
S146	Drumbeg 03 25m SW
S143	Droigniche 03 REF (850m) S
S179	Droigniche 04 0m N
S141	Droigniche 03 0m N

Table 5.4. CCA results using MVSP from the macrofauna and environmental datasheets based on the species data extracted from Figure 5.11 and shows the species that are mostly affected by SLICE.

SLICE axis	*Neoamphitrite figulus, Phyllodoce maculates, Ascidiscia aspersa, Montacuta substriata, Phascolion strombi, Diastyllis lucifera, Ophiura sp., Caulleria alata, Gattyana cirrosa, Amphiura sp., Cucumariidae sp., Caulleria zetlandica, Iphinoe tenella, Lumbrinereis latreilli, Thyasira sp., Ophiura aphinis, Cerianthus lloydii, Diastyllis laevis, Diplocirrus glaucus, Mysella bidentata, Iphinoe sp., Eumida sp., Nematoda sp., Malmgrenia glabra, Ophiuroidae sp., Amphicteis gunneri, Ophiodromus flexuosus, Phyllodoce groenladica, Capitella sp., Glycinde nordmanii, Aonides oxycephala Tmetomyx cicada, Glycera alba, Terebellides stroemi, Leptosynapta inhaerens, Iphimedia minuta*
Redox axis	*Leptosynapta inhaerens, Malacoceros tetracerus, Arenicola marina, Euclymene lumbricoides, Anaitides maculatus, Ampharete acutifrons, Glycera rouxi, Magelona mirabilis, Nephtis sp., Demonax sp., Ampelisca tenuicornis, Angulus tenuis, Corophium bonnelii, Ophiura albida, Nematonereis unicornis, Platynereis dumerili, Turitella communis, Mysta picta, Scobicularia plana, Kefersteinia cirratus, Asychis sp., Ampelisca diadema, Anaitides mucosa, Pholoe inornata*

The abundance *of Capitella* sp. and its interactions are summarised in Table 5.5 where an analytical approach of the abundance of the species in each of the SLICE stations can be seen. In Table 5.5, there is a preview of the *Capitella* sp. abundance with their environmental parameters, as found in the ambient seabed, in an effort to identify any information regarding the effects of the nutrient levels at these sites. Figures 5.12-5.14 present this information. In Figure 5.12, nutrients increases as the abundance of the *Capitella* group increases in a figure where the *Capitella* numbers are plotted in relation

to the nitrogen percentage. The nitrogen percentage regression is given by the formula: N%= 0.006*abundance + 0.27.

Figure 5.13 is the regression of *Capitella* abundance with carbon percentage where as Figure 5.14 is the *Capitella* abundance with redox potential. In Figure 5.13, the regression shows an increase in the number of *Capitella* species as the carbon percentage also increases and the regression relationship between C% and *Capitella* abundance is C%= 64.4*abundance + 184.5. In Figure 5.14, the redox potential is low but the *Capitella* species number increases, following the trend line of the plot which is: Redox= -1.17*abundance+833.5.

Figure 5.12. Regression analysis of *Capitella* group (abundance) against nitrogen concentration (N% dry weight sediment).

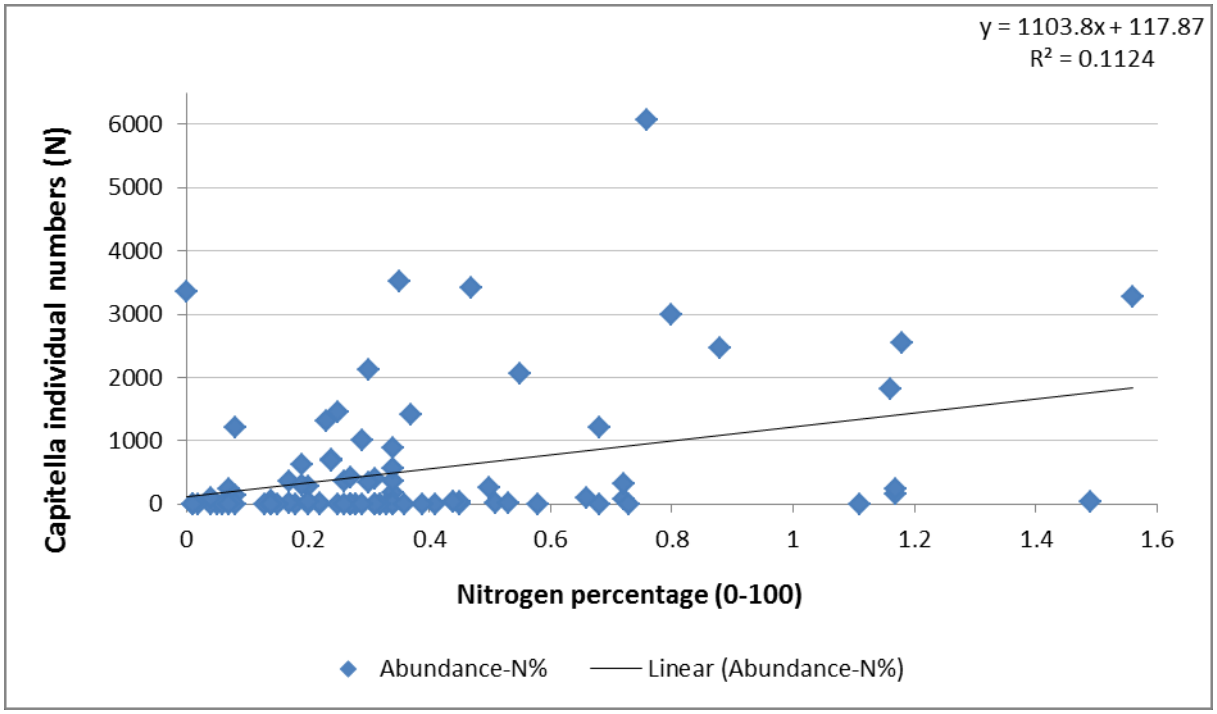


Figure 5.13. *Capitella* numbers (N) in relation to the carbon percentage (C% dry weight sediment) with their regression analysis.

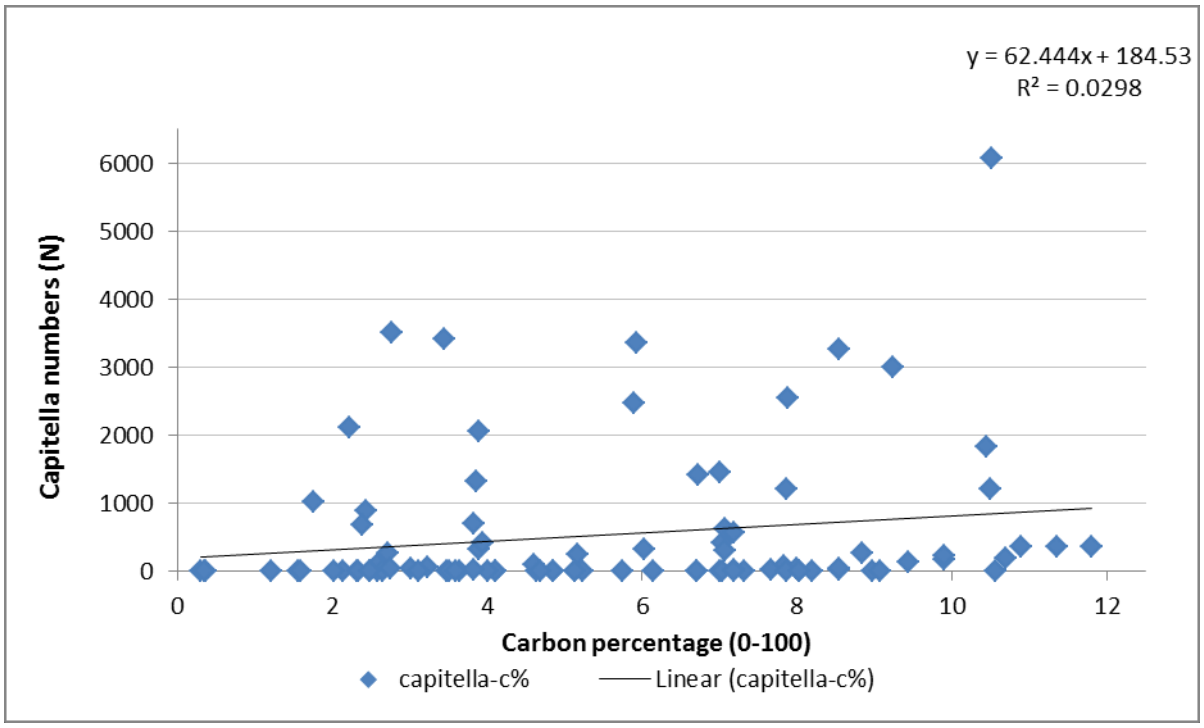
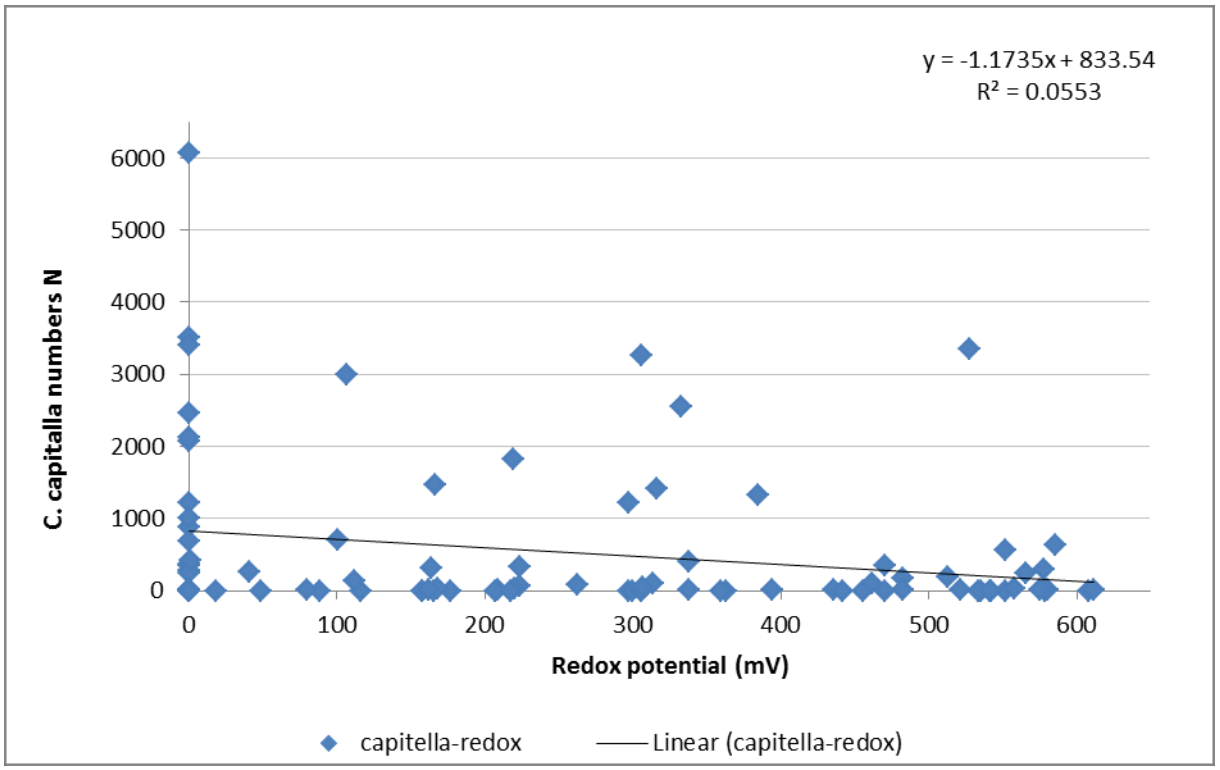


Figure 5.14. *Capitella* numbers (N) in relation to the redox potential (mV) with their regression analysis.



5.4 Discussion

5.4.1 General discussion

The collection of the data was based on the needs of the project. Initially, that was addressed by data input with physico-chemical, environmental parameters and macrofauna data. In the later stages, the data with SLICE was inserted in the spreadsheet in order to search its properties in the environment, both in monitoring its possible environmental effects and impacts but also in modeling its quantity along with the interactions it presents with the related physico-chemical factors. Additionally, data records were kept for analysis for the SLICE and its properties concerning time and distance. This was the designed plan in searching the fate and dispersion of SLICE as well as its impacts in the local studied ecosystems.

The two major parts of this project are discussed here. Figures 5.3 to 5.11 and Tables 5.1 to 5.4 show the fate and dispersion of SLICE, and present the impacts of the stations on the farm sites where SLICE data was available and applied. An overview of these sites is shown in Figures 5.1 and 5.2. The exact data from the sites appearing in the maps can be seen in Appendix 4 where the full data labels are shown in Table 5.A2. Before the main analysis of the results, it is crucial to underline that the data have been obtained from various sources and the measuring techniques often vary. During this project the results are slightly edited in order to have a complete similarity within the spreadsheets. The first edit is the application of the "worst case scenario" in the data coming from the SAMS. There, the SLICE values are split into not detectable (N/D=0), trace (<1.9 ug/kg), and actual values. However, values higher than 0.5 ug/kg (or ng/g)

are actual values (and not traces) and since the aim of the project is to search the exact and precise properties of the SLICE, the traces inputs were replaced with the higher limit of the scale given by the authors (1.9 ug/kg). Then the data from the SEPA received after 2006 present asymmetry and should be correlated to meet similarity with the rest of the spreadsheets. The SLICE data that SEPA expects from the industry to provide come from station 0 m (cage edge) and the stations located 100 m away from the cage. At the same time, the physico-chemical stations do not include the 100 m stations. That practically makes the 100 m SLICE data uncorrelated with macrofauna and physico-chemical data, therefore, analysis of results within impacts and effects is not possible. To avoid this discord, the macrofauna data of the 50 and 150 m stations were correlated. The correlation applied in the species similarity of these stations in terms of their numbers and abundance. That led to an efficient correlation for the 150 m stations parameters to be used in the spreadsheets instead.

5.4.2 Fate and dispersion

The main part of the analysis of the SLICE fate comes from the multivariate results. Initially, the stations were analysed using cluster analysis to determine whether there are similarities among the stations and in what degree. Figure 5.10 shows the stations grouped in two major groups. The upper group contains stations that are fewer in number than those in the other group but show a smaller similarity. The lower group contains stations closely related to each other. This graph shows that the analysed stations are strongly similar under the interactions of the same physico-chemical and macrofaunal parameters and SLICE. The two groups given in Tables 5.1 and 5.3B show 16 stations in group A belonging to the SLICE arrow group of stations and 12 stations in group B. Accordingly, group A contains one station from the redox arrow and group

B contains 23. Cluster analysis grouped the stations in 2 main groups. This shows that the similarity of the stations with each other is strongly correlated and the possible effects are approximately the same in these stations. At this point of the study, behaviour of the stations is important to be mentioned because both the fate and the dispersion of SLICE are shown.

In Figure 5.9 the Canonical Correspondence Analysis is shownin a graph summarising the fate of the SLICE given by its interaction with the carbon percentage, redox potential and median size of the particles. The stations from macrofauna data are also included in the plot and are correlated in the arrows. As shown in the graph, the redox potential is inversely proportional to the SLICE arrow. As SLICE is increased the redox potential is decreased. The rest of the factors are increased (C%, particles median size) while SLICE is increased, but the relationship is not as obvious as shown in the regressions. In Figure 5.10, the species were plotted against the physico-chemical factors in order to find which species interacted with SLICE. These species are shown in Table 5.4 and indicate that SLICE interacts with them regarding their general abundance in terms of species variability but also their individual occurrence number.

The fate of SLICE alongside the stations which directly interact with SLICE is shown in Table 5.1. This relation is very important because the fate of SLICE there can alter the biological community individually, but also release general information regarding the dispersion when the effects are grouped together after the analysis. For these stations a detailed study will be presented in the discussion in the environmental effects section.

Closer looks to the figures that have the regressions give more information about the SLICE fate interacting with the environmental factors. In Figure 5.4, the regression SLICE= 0.0008*(MPSA) + 1.0049 shows that SLICE increases the size of the particles when it is applied. The same increase in the carbon percentage can be seen in the regression in Figure 5.6, SLICE= 0.0546*(C%) + 0.8658 which shows that SLICE application increases carbon percentage in the seabed. When carbon percentage is increased it indicates a situation of organic enrichment to the site. However, it is difficult to identify whether there is also a level (low or high) of toxic pollution caused by SLICE compounds inside the organic pollution. The redox potential is shown in the regression in Figure 5.5, to be inversely proportional to the SLICE application. When SLICE is increased (or being present) the redox potential is decreased. The regression SLICE= -0.0022*(redox potential) +1.7522 is an indication that SLICE dispersion can cause anoxic conditions due to the lack of oxygen inside the sediments.

Figure 5.5 shows the connection between SLICE and the redox potential. The regression shows that the presence of SLICE reduces the amount of the available oxygen necessary for the ecological processes inside the seabed but also in its upper layers. In both cases, biological processes occur as part of the natural cycles and a strong potentiality of pollution is raised. At this point there is an indication that the SLICE may cause a loss in redox potential that will eventually lead to anoxic conditions in the seabed areas adjacent to sea cages. This is not abnormal, since the SLICE could cause those results, especially in a long term and high dose application, as most of the chemicals behave in a similar manner.

All of the above are summarised in the multiple regression analysis deriving from Sigmastat:

SLICE= 0.000644*(MPSA) + 0.0311*(C %) – 0.00213*(redox potential) + 1.453

In this multiple regression analysis the output of the single linear regressions is the same in the fate of SLICE regarding the changes and the reaction of the studied trends. The carbon percentage and the particles' median size are increased in the multiple regression formula but the redox potential presents the respective decrease.

Multiple regressions are important because they summarise and assemble the interacting factors in a time period. In maths, the multiple regressions are crucial because they produce models for a specific variable. Another advantage of the multiple regression is its ability to transfer the variables to constants and vice versa. The same properties are applied in this case as well. SLICE is the variable given by three different constants. There is a model of the SLICE fate in the ecosystem in terms of its quantity and is always available for any time and area. This regression does not include the biomass or the hydrography of the area. The fact that the measurements came from areas with various biomass quantities (the regression is balanced to a biomass average) is an advantage of the regression because the biomass parameter is not a factor that alters the SLICE directly or potentially. The alteration is applied mostly to the organic and nutrient enrichment. The initial variability in the time and area (space) is also a key factor in making hydrography a not important parameter in the regression. Individually in the lochs that situate a fish farm, the flushing rate can be added in a small deviation

percentage in SLICE amount by the farmer. If, for example, a loch has a slow flushing rate, such as the Kishorn and Graignish lochs (Edwards & Sharples, 1986), then the SLICE should be slightly more than the quantity the formula suggests. The opposite applies to a farm that is situated in a loch with a quick flushing rate, such as Carloway and Etive (Edwards & Sharples, 1986). SLICE should be slightly less than the predicted. At this level, the observation that the SLICE quantity is not altered by the mentioned parameters is underlined again, since the variability and the large amount of measurements included in the spreadsheets reduce that possibility.

The direct applications of this general formula are: 1) the assistance for the use of SLICE in future regulations; 2) a guide to identify possible environmental impacts during the combination of chemicals combined with SLICE (hormones and antibiotics); and, 3) the calculation of the new SLICE quantity when a farm plans to make an increase or decrease of its existing biomass. It is assumed for this case that a fish farm is planning to increase the biomass in one or more of its cages and needs to know how the SLICE will be altered. There will be an increase of the organic enrichment, as result of the new biomass, due to faeces and uneaten food decomposition in the water column and on the seabed. Then the percentage of carbon will also be increased and, according to the formula, SLICE will be increased in quantity according to the new numbers. In this case, the farm has a predicted SLICE quantity and can see if the new quantity addresses the EQS established for SLICE application and decide accordingly.

SLICE is dispersed in the seabed alongside the stations the SEPA requires for monitoring. The dispersion covers the stations situated 0, 25 and 150 m away from the

cages as shown in Table 5.1 and Figures 5.9 and 5.10. The amount of SLICE dispersed varies from site to site in these stations. However, there is a reduction throughout for the sites during the years that data was available since data among the years were mismatched. In this sector, it is difficult to correlate previous data from the SEPA, since the monitoring method was changed in 2007 (2 stations instead of 3 and in different distance from the cages without relation to the physico-chemical and macrofauna parameters). The dispersion of SLICE is mostly a relative matter related to the time it is measured; moreover the data cannot describe how the fish were treated during the high values of SLICE or the previous status of the sites. Another subjective parameter is the variability of the sites where SLICE is applied, making its dispersion unbalanced with ataxia. This variability consists of factors such as the hydrography and flushing rate of the lochs, and even the geomorphology and the previous pollutant profile (if any) of these sites.

The tested stations presented SLICE amounts varying from zero to traces and to actual values (till high numbers exceeding the EQS). This dispersion shows that SLICE is not properly regulated if the wanted level of its amount should be eventually 0. The SLICE in 0, 25 and 150 m stations showed an overall reduction in quantity compared to the 25 to 150 m stations which is a logical conclusion, taking into account the distance from the initial application point (cages). Moreover, Table A4 (see Appendix 4) and Figure 5.3 show that the 25 m stations are the points where most of SLICE is present and not the 0m stations . That is not very obvious, considering that the amount is concentrated only around the bottom of the application point and is not dispersed farther. A further analysis of Table A4 (see Appendix) and Figure 5.3, shows presence of SLICE amounts 150 m away from the cages. This is quite unexpected since this is a

long distance for a chemical to be present. The SEPA would not have otherwise deducted the measurement distance from 150 to 100 m. There is an increase of the SLICE at the 150 stations, an indication that SLICE may have a tendency to increase, remain or slightly decrease its amounts, leading to accumulation of its amount and possibly causing biological impacts. It is fair to mention that accumulation does not necessarily lead to negative impacts. It may be present on the seabed but not having any effects and also, considering the fact that if analysis were still to be carried on for these stations during the following years, SLICE could be nearly zero.

Figure 5.3 is important in SLICE fate because the initial quantity in an application is higher in the 25 m stations and is reduced to 150 m ones, but in time, SLICE is higher in the 150 m stations having a peak decrease at 25 m. There is a general fate setting that SLICE is reduced with time, without basic components and independently within different distance scaling. It is also noted that its dispersion reaches 150 m away from the sea cages having a peak at 25 m away from them, without being at significally high levels at the seabed underneath the cages as was initially expected.

5.4.3 Environmental effects

The stations whose columns approach or balance value 1 in the graphs are the stations presenting biological disorder. As the value of the columns rises, the indices preview the level of the disorder, since the graph is designed to increase the disorder from zero (no effect) to 1 (extremely affected). The stations are split into Tables 5.10 and 5.11 below which show the physico-chemical interaction with each other.

In Tables 5.1 and 5.2, it is shown that the stations Port na Moine (2003) 0 and 25 m, Kishorn (2002) 0 m and Torgawn (2004) 0 m (S208, S328, S329 and S398), present SLICE in a noticeable trace level while the carbon percentage is higher than that of the stations with the less disorder in the Table. The stations Port na Moine (2003) 0 and 25 m, also have an AMBI high mark of 6 (heavily disturbed) but for the Kishorn (2002) 0 m station (marked 7) the biotic index is extremely disturbed. It is also noticed that the *Capitella* species in that station are present in high value, while the station is present in the SLICE arrow in the CCA analysis, in Figure 5.9. This station presents the highest AMBI value of the spreadsheet in total. There, the species are only 2 in number and are found in high concentration levels. The SLICE in that station was measured to be 3.4 ng/g and the EQS for the SLICE quantity was 4. The minimal level of the AMBI score in mainstream site at 150m station had SLICE quantity zero and 7 different species were measured.

As an indication of the present situation of the tested stations, for the sites across Scotland, the average amount of those affected by the AMBI was calculated, as shown in Table A5 (see Appendix 4). There were 74 stations with a score of 1-3 (lightly affected) and a percentage of 58.2%. In the moderately affected stations (score 4), 7 are in a 5% percentage while 46 stations show a high impact level (score 5-7) and a percentage of 36.2%. That generally shows a good situation in terms of the effects in a large scale percentage. However, the percentage of the highly affected stations in the sites shows pollution of either an organic, nutrient or toxic background, or a combination of them.

Table 5.5. Table that shows which stations had the highest SLICE concentrations in relation to AMBI and species abundance (S).

Stations Site-year-distance	**SLICE ng/g**	**AMBI (range 1-7)**	**Species abundance (S)**
Torgawn05 25m	4.96	4	6
Droigniche04 25m	7.11	6	0
Torgawn04 25m	7.11	5	11
Chalmers hope05 0m	7.34	3	13
kishorn04 0m	13.4	5	5

This observation can be enhanced when taking into account the interaction of SLICE with the AMBI score. In Table 5.5, the stations which present the highest SLICE values have been placed together with species abundant numbers and AMBI as shown in Table 5.5, and indicate that only one of the stations is characterised as heavily disturbed (Droigniche, 2004, 25 m), while the rest scale around moderately disturbed (Kishorn, 2004, 0 m and Torgawn, 2004, 25 m), where the station with the highest SLICE is included. Additionally, the other two stations (Chalmers hope, 2005, 0 m and Torgawn, 2005, 25 m) do not present a high AMBI score, even when the SLICE residues are close to 5 and 7.5 ng/g. There was a low number of species where the habitat in the local farm sites was tested and SLICE had been applied. It is also noted that none of the stations, where SLICE was applied in a quantity of more than 4 ng/g, gains any AMBI score below 3. On the contrary, the species variation in stations Torgawn (2005) 25 m and Kishorn (2004) 0 m is 6 and 5 respectively, while in station Droigniche (2004) 25 m no species are found at all. It is therefore difficult to establish a clear toxic pollution effect deriving from the SLICE treatment, but the disorder, in terms of the biotic level and the species variety, is obvious and appears in the low

numbers of species in these stations. This argument shows a common situation in the seabed biotopes situated below farm cages which comes from the enrichment of the farm process, and is matched by considering the distance of these stations from the cage edge. For example, the stations mentioned above, Droigniche (2004) 25 m, Torgawn (2004) 25 m and Torgawn (2005) 25 m are 25 m located away from the cage and not exactly underneath the sea cages where the feeding and chemical waste is transformed into pollute enrichment.

The above observations can be enhanced by the regressions coming from the plots of the SLICE with the indices. The most important of these is the SLICE- AMBI relation because it is easier to be correlated with the output from the AMBI software, where the stations detailed analysis is applied. The equation of SLICE with the AMBI [SLICE= 2.1044* (AMBI score) + 0.6685] clearly shows that the AMBI score is increased when SLICE is increased. The increase in AMBI scores leads to conditions with heavily and extremely disturbed results, according to the AMBI scale of effects. Therefore, as seen in the table for the higher numbers of SLICE in stations, there is a score starting from 3 in AMBI. This means that SLICE assisted in the raise of the biotic disturbance of these stations, hence there is an environmental impact regarding to the balance of the species in those biotopes, appearing with the form of high abundance for less species.

Additionally, the other indices are also increased with the increase of SLICE, as the regressions in Figure 5.2 show: SLICE= 0.2532* (ITI) + 1.5201 (1), SLICE= 0.1583*P + 1.6248 (2) and SLICE= 0.0987*D + 1.6657 (3) (where D is the Simpson's

Index). When the Simpson's index is high, the site is dominated by less species. Regression (3) shows that SLICE causes a slight increase in the Simpson's index, which means that there is a low evenness in the sites where SLICE is applied. As mentioned previously, the stations presented a low degree of species evenness while, at the same time, species such as the *Capitella* increased their numbers. This is additionally confirmed by formula (3), which presents the increase of the D. This increase shows the decrease of the evenness for the stations and their sites in general. There was initially the indication of a lack of species variety and biotic disturbance in the stations with high SLICE amount, and secondly after the relation formula of SLICE and D for the stations in their summary, it is obvious that where SLICE was present (regardless of the amount), the sites presented an increase in D, meaning that the richness of the species was low. That constitutes a slightly negative case regarding the SLICE application in the farmed sites, not only seen in the prospective of forcing the local ecosystems to a species richness decrease and a biotic disorder, but also in general terms, to show there exists a potentiality for negative impacts in the biological status of areas adjacent to fish farms.

As the Pielou index is increased, SLICE is higher, as given by regression (2). Under normal circumstances, the Pielou index is nearly parallel to the axis showing the balance of the species with each other. In a normal ecosystem the species are equally distributed in terms of their individual numbers and variety evenness. When the Pielou index is high, the species are not balanced but they occur irregularly depending on the impact. This is shown in formula (2). The regression shows not a stable parallel line but a slightly increased one. That indicated the potentiality of the species to grow unstably in quantity, yet not a tremendous impact within these site areas. The impacts come from various causes but this spreadsheet includes SLICE quantities that apply in noticeable amount and are present in high percentage in some of the stations. Finalising the

analysis of the SLICE effects in the tested farmed sites, the ITI index is shown in regression (1) and is seen to be increased when SLICE increases. The ITI is an infaunal trophic index which shows the situation of an area regarding the trophic activities of its species. When the ITI is low, the situation is worse and when it is high, the situation is improved. However, in this regression the ITI scores were altered to be compatible with the foundation idea of the statistical analysis follow that zero is the optimal level and 1 is the most degraded. Therefore, the higher the levels are, the worse the trophic index is, as shown in regression (1).

At this level of the discussion, the results of the *Capitella* group are assessed. By focusing into a species indicator, the SLICE properties are revealed within the ecosystem. Figure 5.5 shows the abundance of the *Capitella* group alongside the stations of the sites with SLICE. In this graph, the SLICE value is not included. The carbon percentage and redox potential are included however, along with the number of the *Capitella* in each of the stations. The stations that have the highest numbers of *Capitella* are shown in Table 5.6 below:

Table 5.6. Highest number of *Capitella* sp. (at stations) compared to the distance from the cages and species abundance.

	S328	**S329**	**S330**	**S389**	**S398**	**S338**	**S401**	**S208**
***Capitella* number (N)**	6064	3271	2553	3512	3410	3356	2468	2998
Distance from cages (m)	CE	25	150	150	CE	25	CE	CE
AMBI	6	6	5	2	7	6	5	6
Number of present species (S)	4	4	10	4	2	5	5	2

Abbreviations: CE refers to cage edge. S328 is Port na Moine 2003 0m, S329 is Port na Moine 2003 25m, S330 is Port na Moine 2003 150m, S389 is Kishorn 2001 150m, S398 is Kishorn 2002 0m, S338 is Vatsetter 2003 25m, S401 is Kishorn 2004 0m and S208 is Torgawn 2004 0m.

In these stations, the AMBI score is not less than 2, while the general image of the stations scales from moderately disturbed to extremely disturbed. The number of the species occurring in the stations is low. In only one of them 10 different species occur and in the rest the number does not exceed a maximum of 5. The *Capitella* spp. were present in high numbers in the full variation of the measured stations. As seen in Table 5.6, cage edge, 25 m and 150 m include *Capitella* species in high numbers. This is an indication that the SLICE residues are causing those high numbers, since it is not very common for high values of *Capitella* to be observed in areas situated 150 m away from fish cages. This table contains the 2 stations mentioned when analysing the SLICE immediate effects to stations' ecosystems. Stations Kishorn 0m (2004) and Kishorn 0m (2002) are present in this table as well. Kishorn station 2004 presented the highest value in SLICE and Kishorn station 2002, the highest AMBI score (=7). Both stations are situated at the cage edge of the farms.

Figures 5.13 and 5.14 show the relationship between the numbers of *Capitella* with the carbon percentage and the redox potential respectively. In figure 13, the numbers increase when the carbon percentage increases, which is logical deduction, knowing that organic enrichment increases the *Capitella* numbers. In Figure 5.14, the redox potential decreases when the *Capitella* numbers increase. This enhances the results of the previous paragraph which showed that the redox is decreased with an immediate increase of SLICE. In general terms, the carbon percentage and redox potential are indicators of the effects of an ecosystem regarding its response to chemicals. The organic enrichment with the parallel reduction of the existing oxygen, which leads to high numbers of *Capitella* in these areas, is a combination of a potentially negative impact on the ecosystems of these stations. This becomes obvious

by the high levels of the AMBI score and the diminished variety species in these sites.

Figure 5.12 shows the regression analysis of the nutrients placed together with the number of *Capitella*. Even if the R square of the trend line is high, it is obvious that the nutrients increase the numbers of *Capitella*; a logical observation, since the high level of nutrients is what causes the growth and domination of the *Capitella* in the seabed. It is not accurate to claim that nutrients are increased by the SLICE amount in these stations since no graphs in this project show that. In general terms however, the nutrient percentage follows the carbon percentage in behaviour. This assumption in combination with the biotic disorder and the low number of evenness of the stations tested for having *Capitella* numbers in abundance, constitute a strong impact to the normal abundance in number and variety of the species in the stations with SLICE.

As the impacts on the ecological parameters have been analysed by correlating SLICE, the analysis of these factors in canonical correspondence showed that the species mostly affected by SLICE are the following (also seen in Table 5.4, ascending list): *Neoamphitrite figulus, Phyllodoce maculates, Ascidiscia aspersa, Montacuta substriata, Phascolion strombi, Diastyllis lucifera, Ophiura sp., Caullerya alata, Gattyana cirrosa, Amphiura sp., Cucumariidae sp., Caullerya zetlandica, Iphinoe tenella, Lumbrineris latreilli, Thyasira sp., Ophiura aphinis, Cerianthus lloydii, Diastylis laevis, Diplocirrus glaucus, Mysella bidentata, Iphinoe sp., Eumida sp., Nematoda sp., Malmgrenia glabra, Ophiuroidae sp., Amphicteis gunneri, Ophiodromus flexuosus, Phyllodoce groenladica, Capitella sp., Glycinde nordmani, Aonides oxycephala, Tmetomyx cicada, Glycera alba, Terebellides stroemi, Iphimedia minuta.* For some of these sediment species, the research in the laboratories as well as in the

field has already shown an impact from SLICE. Consequently, in Table 5.4 the species suffering the greater impact are the ones from the polychaeta class of the Annelida phylum and the Crustacea subphylum of the Arthropoda phylum (such as *Iphinoe sp.*, *Diastylis Sp.* and *Iphimedia sp.*). Some of the copepod species from the past studies are: *Acartia clausi, Pseudocalanus elongatus, Temora longicornis* and *Oithona similis* (Willis & Ling, 2003).

Chapter 6
General discussion

6.1 GENERAL DISCUSSION

In this chapter, the outcomes of the project, as presented within the individual chapters, will be reviewed and overall conclusions regarding potential longer term effects of emamectin benzoate, the use of the biological indices in investigating long terms environmental effects of cage aquaculture will be assessed. Revisiting the initial aims of this project, it is about a complete study of the longer term effects of the chemical emamectin benzoate be analysing a large temporal meta-data set. However, in consequent the impacts of fish biomass and investigation of methods of numerical assessment have been investigated. This accomplishment is mostly based on the idea of the investigation on long term levels of SLICE in sediments and investigating the methods for numerical analysis and importance of biomass. Eventually the outcome showed that this data can be used for validation and improvement of long term temporal modelling. The side research regarding the indices and their use in describing the ecological status of the seabed was tested, using various statistical methods. The biomass role in models for predicting the fate and dispersion of SLICE has been studied as well, using statistical methods and with data ranging within three years.

For a better understanding of the project's processes and its timeline, some interpretation is needed. Modelling of the fate and dispersion of SLICE raised an important question which required testing. Firstly, how robust are univariate indices in interpreting environmental impacts of cage fish farming including the effects of chemical wastes, such as SLICE, and are they being used most effectively? In testing this, another included an assessment of the accuracy of the two widely used trophic indices and their use in a impact evaluation; AMBI and ITI. These indices are based on

classification of trophic status of a community. These indices were used in the assessment of influence of changing fish biomass on benthic communities over time, and on assessing temporal effects of SLICE. Eventually, these conclusions produced the findings that were analysed in individual chapters and will be discussed in this chapter.

6.2 Fate and prediction modelling for SLICE

The data from SEPA after the year 2006, present asymmetry with the prior data and should be correlated to meet similarity. The SLICE data provided by the Scottish aquaculture industry to SEPA are from sampling stations at the cage edge and 100 metres away from the cage (SEPA, 2007). The physicochemical monitoring requirements do not include the 100 metres stations making correlations between SLICE concentrations in sediment and macrofauna/physicochemical data at 100 m difficult. To avoid this discord the macrofauna data of the 50 and 150 metres stations were correlated, with 100 m SLICE data in terms of their species numbers and abundances. These results led to high correlations for the 150 metres stations parameters which were then used for further analysis of the data. Clearly this is an issue which regulatory agencies, such as SEPA, should take in account when structuring monitoring and environmental assessment methodology, e.g. macrofauna and physicochemical datasets must be acquired from samples taken at the same time and location for all parameters.

An initial conclusion is that for spaces less of 50 metres (100-150 m in this case) the stations within the seabed are correlated with their macrofauna species abundance in a high percentage (~ 72%). This correlation leads to the conclusion that same attributes with regard to species and seabed toxicology apply within this space spectrum (Walker, 2003), and the biological activities and toxic outcomes are similar between 100 and 150 metres from the cages. Therefore any even low levels of emamectin benzoate or organic pollutants found at this distance have the potential to accumulate and cause adverse and potentially toxic impacts over time.

Stations undergoing data analysis were shown to have similar interactions between physicochemical and macrofaunal parameters, and SLICE. This was confirmed by a significant by the multiple regression analysis:

SLICE= 0.000644(median size particle size) + 0.0311*(C %) – 0.00213*(redox potential) + 1.453.*

In this regression SLICE was reduced with time, without taking in account the distance from the cages. Hydrographical models indicate that dispersion of SLICE reaches up to 150 metres away from the sea cages, peaking at 25 metres from them rather than beneath the cages as would be expected from measurements of organic loading from feeding activity (Beveridge, 2004).

6.3 SLICE effects on sediment ecology

While it is difficult to establish a clear toxic pollution effect coming from chemicals such as SLICE, community level data can be used as indicators of affect based on composition and species number at the different sampling stations. Comparison of these factors over distance and time can give an indication of spatial and temporal impacts (Black, 2001).

The fact that research came up with a 36.2% of the stations as "strongly affected" and 5% as "medium affected" shows that SLICE itself or a combination of other toxic factors has negative impacts on local areas widely seen against to what the previous literature has shown. The separation of the effects of SLICE from the other factors cannot be seen in the present analysis however the combination of SLICE in relation with the other factors causes a medium to strong affection degree of macrofauna disturbance. Furthermore, the non-identification of the impacts shown in previous papers may have been due to the small data sets used in the analysis and for local areas. This study improves on this by use of a large meta-data set from the west-coast of Scotland (654 species at 403 stations; 5 environmental variables at 403 stations).

ITI is an infaunal trophic index which shows the situation of an area regarding its species trophic activities (Maurer, 1999). When ITI is low the ecological situation in the sediment is worse and when it is up, the situation is better (Word, 1978). In this study the calculated ITI scores were re-scaled within the regression analysis where a zero score was high environmental quality and 1 is the lowest environmental quality.

From the previous conclusions a recommendation for less qualitative and quantitative use of the chemicals in the sea farms is expressed. The use of SLICE along with other chemicals on the same time is mostly a negative use from a farm towards the sediment ecology. This policy not only it degrades the local ecosystem which also has a negative impact to the biomass overall welfare but also the whole area acquires a toxic behaviour due to unbalanced oxygen release that affects directly the fish respiration.

As seen in Chapter 5, the most affected species are annelids as indicated by the AMBI scores at individual sampling stations, though, as seen in Chapter 5 emamectin benzoate also interacts negatively with other phyla, such as Sipuncula (*Phascolion Strombi*), Arthropoda (*Diastyllis lucifera*) and Echinodermata (*Ophiura affinis*), with the potential for toxic impact on these if the quality of SLICE found in sediments exceeds regulatory standards. Regarding the Arthropoda, which are common in benthic faunal communities, would be particularly sensitive as emamectin is designed to be toxic to these organisms (*e.g.* copepodic sea lice) (Telfer *et al.*, 2006) as expressed by past papers (mentioned in Chapter 5) such as Willis & Ling (2003) and these findings are backed up from the current study and particularly for the Crustacea genera *Iphinoe, Diastylis* and *Iphimedia* (shown in Chapter 5 and particularly in Figure 5.11 and Table 5.4).

6.4 General conclusions about the modelling of SLICE

The fate of SLICE in the seabed is the dispersion it has on the sediments. That came out of the regressions analyses made for the SLICE fate and dispersion. The investigation

of the Emamectin benzoate showed that the level of identifiable toxicity is proportional to its quantity in seabed sediments and its fate within the ecosystem should be incorporated into models which link the fate and toxic effect of SLICE, the pragmatic trends must be added and the assumptions must also be in the level of causing the least effect on the model. This has been assumed for such models but not actually shown before over a long term period. The main difference shown in this study is that impact is shown at sampling stations after the application of SLICE compared to previous effects and controls. This is due to three main reasons: 1) the stations tested were analysed using a greater variety of more sensitive environmental indices and for more environmental variables than in previous studies, and there was no mismatch in the sampling areas between the biological and physical measurements in relation to their chemical residues. For example redox potential was included here but has not been used in other papers. 2) The data set used in the analyses was very large - 403 stations, sampled over an eight year period, with 654 species compared with 5 different environmental variables (MPSA, C%, N%, redox pot and SLICE concentration) - making it possible to define more subtle spatial and temporal trends in the data than previous studies. 3) The initial results were analysed more rigorously using more accurate statistical models developed as part of this study.

While testing that part of the project the question of which ecological parameters should be included both biotic and abiotic, and if these would be capable of defining the relationship between impacts and fish biomass was also considered. The extensive data set considered biomass in detail and investigated the contribution this made to the overall impacts indicated through the benthic community compositions at the sites with space and time. Results showed there was considerable relationship

between biomass and effect, but when the carbon percentage (and the relationship C-N percentage) is used in the model the biomass trend does not add more accuracy.

It was mentioned in the discussion part of the Chapter 5 that is difficult to identify clear SLICE impacts on the sediment due to technical issues, such as the limited background data on chemical residues and the level of organic and nutrient impacts interacting with the toxicity levels of SLICE prior to the process of the present study. The approach to identify the level of SLICE, as well as its impacts, on the Scottish sea lochs was primary based on the ecological data collected in the past, in an effort to model them altogether and thus grouping SLICE any impacts.

There is no model to describe a complete ecological system. Though as more data becomes available models will improve and new models created. In the statistical models developed and used in this study some parameters not included e.g. hydrography and the flushing rates of the lochs were not used to calculate dispersal and dilution. This addition in future will enable the multiple regression models developed to be specialised for each of the lochs and more accurate outcomes for specific sites to be derived.

A unique feature of this study was that it used a holistic approach for multiple sites throughout Scotland over time regarding the SLICE application. Another is the testing of the AMBI index as a tool to investigate impacts, a method lacking in previous studies, to enable a new outlook of the SLICE effects in combination with every other enrichment that may have been already present in the lochs. A key finding was that the sampling strategy used for regulations and EIA are not detailed enough or sensitive

enough to pick up effects of SLICE, and may be a multiple regression approach should be used like the one developed here. The proposed stations for measuring the SLICE residues, as extracted by the present study, should be the cage edge (zero metres), 25 metres and 150 metres along with the reference sites compulsory (and for optimum results another station at 75 metres) by using the other methodology exactly as is set by SEPA.

There is also confirmation regarding the EQS value, set by SEPA, at 7 ng/g DW for SLICE residues in sediments, that is suitable because the univariate analysis with AMBI and the MVA results showed in a high degree a good to moderately good condition for the sites in this value (7 ng/g DW), taking in account that any impacts shown to macrofauna in sediments may not due to SLICE completely. Only 4 sites were found to have values above the SLICE EQS but they did not present indications of strong ecological degradation because the analysis showed good indicators for these stations.

6.5 The potentiality of using benthic indices combination to assess the sediment condition

This project developed and tested combinations of environmental indices to explain the impacts and changes in the seabed and compare the sensitivity and accuracy of using single indices. Furthermore, combination of these indices was not limited only to biological attributes but also to chemical properties over time. Several bio-indices which have been used extensively for assessing environmental impact in marine

environments were tested and included in studies as recommended (Chaino, 2007; Ismael, 2003)

Combining indices to provide a modelling (assessing) tool had two novelties. Originally, for the model development, AMBI was used. This was a new strategy to use alternate to ITI, which is the core to the SEPAs regulations for the univariate analysis. The second was the use of multivariate analysis in assessing the univariate analysis and its properties within the indices and the level of their similarity and correlation. After this process an important outcome was the extraction of results without using the whole spectrum of the indices by deducting the less descriptive indices. Those novelties led to the study being more effective and improved modelling accuracy and validation using a number of parameters to constitute a model package not only with indices but with chemical factors as well. The outcome of this study was a recommendation for using both chemical factors with biological indices in the sediment modelling to give greater precision and sensitivity when assessing the environmental conditions of the seabed.

6.6 Trophic Indices comparison: ITI versus AMBI

There is a considerable lack of studies which compare the performance of univariate indices for assessing environmental conditions with marine cage aquaculture. With the advances on the farmed chemical development area, the past indices and methods must be revised and tested to prove if they are still appropriate for such studies. The AMBI and ITI indices were compared statistically so that the strongest could then be used in future core analysis and model input for analysis of impacts of SLICE. Both indices

provide results related to seabed condition and their results description is merely the same; three classes of effects for ITI, 7 for AMBI. In terms of defining their core calculation, it is trophic habits against biotic conditions. Those are highly correlated because the biotic condition is related mostly to the productivity of the species and thus with the level of their trophic consumption.

This study outcome is that AMBI should be included in the univariate analysis and this is a recommendation to be made to the environmental bodies when assessing the impacts.

6.7 Biomass impacts to sea farms over time

The results on the effects on the relationship on impact and biomass are based on a three year period (2006 to 2008) during which the overall biomass of fish the sites decreased. The results show an improvement of seabed condition as illustrated by the indices used, and through the multivariate analysis of the community level data. This confirms that biomass level (thus production parameters) has a significant effect of the status of the seabed at fish farm sites and often this can be more important in defining the changing nature of seabed sediments that chemical inputs defined around treatment times (Telfer *et al.,* 2006).

This study has the unique factor of using long datasets for the ecological parameters as well as the wide macrofauna species distribution. The dataset provided a series of figures that they were used statistically to provide results concerning the

sediment impacts as seen by the organic and nutrient enrichment spectrum. Those impacts were finally effects, that addressed in a high degree to the main outcome that no strong and acute impact happened by the biomass at the seabed.

A disadvantage of this study is that the models do not again include any geophysical data of the lochs tested such as hydrography and flushing rates. Therefore the models developed describe the biomass ecologically effects over time, rather than spatially. The geomorphology and the geophysical attributes of the sites do not change dynamically in such a little time scale, so even if they are not included as parameters inside the models, their presence would not have changed the results.

This study showed that regulating standing biomass, used by SEPA as standard (SEPA, 2007), is an appropriate approach to regulation of the marine environment with regards to marine fish cages. However, further research needs to be undertaken to confirm the results of this study for larger cage systems in excess of 600 tonnes maximum biomass.

6.8 Conclusions

The fate of Emamectin benzoate in the seabed is its dispersion levels and its impacts on the local farm ecosystems cannot be identified fully due to the interaction with the other ecological factors and the organic enrichment from the feeding process. For assessing these impacts, separate and common eco-parameters are needed such as carbon

percentage, redox, and AMBI. The modelling of fate strongly related with the quantity of Emamectin benzoate is the following:

SLICE= 0.000644(median size particle size) + 0.0311*(C %) – 0.00213*(redox potential) + 1.453*

And the model for identifying the level of impacts Emamectin benzoate has on the seabed of farms that is applied the following:

*SLICE = 0.654 + (0.316 * AMBI Score) - (1.470 * P) + (0.583 * H')*

While combinations of the two univariate measure provided accurate indications of environmental impacts related to SLICE of the two, AMBI was easier and more accurate for use. This study developed a complete modelling outcome for use. The biomass effects in predictive models did not show any use in modelling (regression analysis) and thus the biomass can be excluded from a prediction model.

According to the multiple regression models, regarding the dispersion in terms of quantity, when emamectin benzoate is raised then the particle size is larger and the carbon percentage increases, while the oxygen levels decreases. The fate of emamectin benzoate in seabed impacts, as seen in the multiple regression, causes increasing in AMBI and Shannon Wiener values when Emamectin benzoate increases, and species most sensitive appear to be the annelids, copepods and Sipunculidae.

The most effective approach to assessing the impacts of SLICE is a multiple regression method as studied and previewed in this project, including data for physical parameters such as redox potential and environmental indices such as AMBI. It is recommended that this methodology be employed in routine assessments for regulation and environmental management of marine cage aquaculture. Included in this should be consistency of sampling, for example results from this study suggest that, since the 100m stations are correlated in 72% with the 150m stations, only the stations 150m need to be tested for physico-chemical and biological parameters and SLICE concentrations.

The main conclusions of this study can be summarised as:

- Species abundance and redox potential and their use in multiple regression models are an accurate method of assessing impact of SLICE and its distribution
- Both biotic and abiotic indices must be included in biological assessment methodology for greater accuracy
- AMBI is the best trophic-based index and should be included to the modelling,
- Biomass is a useful tool for analysing issues on the ambient farm ecosystem and the impacts of emamectin benzoate, but is not needed when carbon percentage is included in the model.

Chapter 7
References

7. REFERENCES

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APPENDICES

APPENDIX 1

Tables of raw data and statistical analysis taken from Chapter 2, "An assessment of the influence of fish biomass on environmental impact of marine cage farms by analysis long term metadata".

Table A1. Descriptive statistics for sites including selected univariate factors with biomass and carbon, redox as coming by applying Sigmastat for the Table A3 and A4. Where before means the 2003 and after 2006 years. The diversity indices on the first column are the total number of individuals N, the species richness diversity S, the Simpsons Index D, Brillouins Index Hb, Shannon Wiener Hs, Pielou Evenness P, Heip Evenness Eh, infaunal trophic index ITI and Azti marine biotic index AMBI. Then is the second column that is the total size of the tested stations, the third column contains the missing data for each of the indices, the fourth column is the mean values of the indices, the fifth is the standard deviation value from the original mean value, the sixth column has the standard error of the standard deviation, the seventh column is the range of the values for the indices, the eighth column contains the maximum value of the index and the ninth column its minimum value within the sample, the tenth column contains the median value of the range and the last column is the summary of the values for the index.

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
N before	11	0	237.205	421.263	127.016	283.009
N after	11	0	558.659	558.616	168.429	375.284
S before	11	0	10.302	7.802	2.352	5.241
S after	11	0	17.629	14.089	4.248	9.465
Hs before	11	0	1.892	0.801	0.241	0.538
Hs after	11	0	1.877	0.826	0.249	0.555
before AMBI	11	0	3.013	0.959	0.289	0.644
after AMBI	11	0	3.138	0.573	0.173	0.385
biomass before	11	0	623.727	314.298	94.764	211.148
biomass after	11	0	543.455	210.538	63.480	141.441
redox before	11	0	191.411	107.428	32.391	72.171
Redox after	11	0	210.989	34.926	10.531	23.464
carbon before	11	0	4.869	3.281	0.989	2.204
Carbon after	11	0	6.043	4.529	1.366	3.043

Column	Range	Max	Min	Median	25%	75%
N before	1439.500	1448.000	8.500	60.286	36.643	295.979
N after	1844.000	1947.000	103.000	361.571	147.781	825.107
S before	25.500	28.500	3.000	7.000	5.060	15.417
S after	42.905	46.571	3.667	13.571	5.741	24.100
Hs before	2.662	3.514	0.852	1.781	1.177	2.419
Hs after	2.513	3.373	0.860	1.641	1.106	2.472
before AMBI	2.667	4.667	2.000	2.625	2.286	3.726
after AMBI	1.857	3.857	2.000	3.333	2.723	3.557
biomass before	965.000	1070.000	105.000	658.000	414.500	805.000
biomass after	581.000	830.000	249.000	574.000	346.500	729.750
redox before	384.750	425.750	41.000	190.778	127.861	247.500
Redox after	131.000	253.000	122.000	215.000	202.906	226.750
carbon before	11.575	12.180	0.605	3.800	2.426	6.739
Carbon after	10.997	12.150	1.152	5.500	2.025	11.062

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Sum	Sum of Squares
N before	2.820	8.411	0.337	<0.001	2609.250	2393553.354
N after	1.751	3.219	0.268	0.026	6145.252	6553623.840
S before	1.480	1.809	0.294	0.009	113.321	1776.108
S after	1.059	0.371	0.198	0.254	193.919	5403.459
Hs before	0.655	0.0117	0.176	0.409	20.811	45.780
Hs after	0.444	-0.935	0.158	0.554	20.649	45.586
before AMBI	0.930	-0.621	0.277	0.018	33.142	109.052
after AMBI	-0.755	-0.203	0.214	0.171	34.523	111.633
biomass before	-0.209	-0.680	0.163	0.511	6861.000	5267223.000
biomass after	-0.170	-1.710	0.188	0.320	5978.000	3692034.000
redox before	0.662	1.346	0.183	0.359	2105.517	518425.826
Redox after	-1.671	4.191	0.215	0.163	2320.875	501876.516
carbon before	1.018	1.223	0.173	0.433	53.559	368.441
Carbon after	0.339	-1.782	0.258	0.039	66.475	606.829

Table A2. Descriptive statistics in selected univariate factors in stations as coming by applying Sigmastat for the Table A3 and A4. Where before means the 2003 and after 2006 years.

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
before N	105	0	243.229	499.078	48.705	96.584
before Hs	105	0	2.025	1.132	0.111	0.219
before AMBI	105	0	2.933	1.558	0.152	0.302
N after	105	0	962.133	2392.543	233.488	463.016
Hs after	105	0	2.073	1.460	0.143	0.283
after AMBI	105	0	3.152	1.518	0.148	0.294

Column	Range	Max	Min	Median	25%	75%
before N	2783.000	2786.000	3.000	74.000	32.750	171.000
before Hs	5.090	5.140	0.0500	1.790	1.238	2.853
before AMBI	5.000	6.000	1.000	2.000	2.000	4.000
N after	18110.000	18115.000	5.000	257.000	92.250	704.250
Hs after	5.420	5.440	0.0200	1.820	0.975	3.162
after AMBI	5.000	6.000	1.000	2.000	2.000	4.000

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Sum	Sum of Squares
before N	3.740	14.650	0.317	<0.001	25539.000	32116063.000
before Hs	0.448	-0.263	0.0957	0.019	212.650	564.044
before AMBI	1.061	-0.339	0.354	<0.001	308.000	1156.000
N after	5.291	31.941	0.345	<0.001	101024.000	692521754.000
Hs after	0.527	-0.620	0.102	0.009	217.710	673.157
after AMBI	0.797	-0.786	0.300	<0.001	331.000	1283.000

Table A3. The full univariate analysis for the year 2003. The diversity indices, as shown on the sequence of the columns, are the number of individual species N, the species richness diversity S, the Simpsons Index D, Brillouins Index Hb, Shannon Wiener Hs, Pielou Evenness P, Heip Evenness Eh and Azti marine biotic index. The sites can be seen with their labels it tables A5 and A6.

STATION	N	S	D	Hb	Hs	P	Eh	before AMBI
27 NW25	40	6	0.24	0.45	0.83	0.32	0.16	2
27 NW50	41	7	0.52	0.96	1.64	0.59	0.35	2
27 REF 1	118	9	0.46	0.96	1.53	0.48	0.24	4
27 REF 2	84	10	0.8	1.68	2.66	0.8	0.59	1
27 SE0	95	3	0.14	0.26	0.42	0.27	0.17	5
27 SE150	447	14	0.22	0.55	0.85	0.22	0.06	2
30 1400m S	512	9	0.34	0.69	1.03	0.33	0.13	6
30 800m SW	811	5	0.07	0.18	0.28	0.12	0.05	6
30 0m SW	1640	11	0.1	0.27	0.41	0.12	0.03	6
30 150m SW	548	28	0.4	1.01	1.55	0.32	0.07	5
30 25m NE	211	45	0.93	2.8	4.45	0.81	0.47	2
30 25m SW	77	41	0.94	2.71	4.74	0.89	0.64	1
31 1100m NE	3	2	0.67	0.37	0.92	0.92	0.89	6
31 1300mSW	125	21	0.78	1.84	2.96	0.67	0.34	2
31 0mSW	137	29	0.84	2.19	3.55	0.73	0.38	2
31 150mSW	429	9	0.33	0.61	0.92	0.29	0.11	6
31 25mNE	362	14	0.39	0.87	1.33	0.35	0.12	6
31 25mSW	140	32	0.94	2.67	4.3	0.86	0.6	2
32 NB 25m	2626	2	0.01	0.04	0.06	0.06	0.04	6
32 NB CE	2498	3	0.01	0.03	0.05	0.03	0.02	6
32 Ref 1	1582	5	0.02	0.07	0.11	0.05	0.02	5
32 Ref 2	146	13	0.64	1.42	2.23	0.6	0.31	3
32 SB 25m	388	12	0.56	1.2	1.81	0.5	0.23	3
33 750mSW	25	13	0.95	1.92	3.56	0.96	0.9	2
33 0mE	29	11	0.89	1.77	3.17	0.92	0.8	2

33 25mE	16	11	0.95	1.68	3.33	0.96	0.9	2
34 550mNE	1141	5	0.33	0.64	0.94	0.4	0.23	6
34 550mNW	638	7	0.39	0.76	1.13	0.4	0.2	6
34 0mS	156	10	0.66	1.32	2.03	0.61	0.34	6
34 25mN	80	34	0.96	2.71	4.62	0.91	0.71	2
34 25mS	218	20	0.73	1.6	2.48	0.57	0.24	3
34 50mN	170	29	0.82	2.11	3.37	0.69	0.33	2
34 50mS	193	21	0.63	1.5	2.37	0.54	0.21	2
45 N0	15	4	0.73	0.98	1.78	0.89	0.81	5
45 N150	49	5	0.63	0.99	1.6	0.69	0.51	1
45 N25	28	3	0.31	0.47	0.81	0.51	0.37	2
45 N50	50	5	0.26	0.49	0.84	0.36	0.2	2
45 REF 1	12	3	0.44	0.54	1.04	0.66	0.53	2
45 REF 2	24	4	0.24	0.39	0.74	0.37	0.22	2
45 S25	67	5	0.47	0.74	1.17	0.51	0.31	4
45 S50	19	5	0.53	0.79	1.47	0.63	0.44	3
46 C1	718	17	0.7	1.5	2.22	0.54	0.23	4
46 C2	297	31	0.9	2.44	3.74	0.76	0.41	2
46 NE0	160	24	0.85	2.1	3.32	0.72	0.39	2
46 NE150	263	57	0.96	3.25	5.14	0.88	0.61	2
46 NE25	145	26	0.9	2.41	3.84	0.82	0.53	2
46 NE50	125	21	0.9	2.3	3.66	0.83	0.58	2
46 SW25	243	22	0.87	2.15	3.3	0.74	0.42	2
46 SW50	887	30	0.8	1.95	2.89	0.59	0.22	2
50 25m SW	67	14	0.83	1.82	3	0.79	0.54	2
50 CE	74	15	0.81	1.79	2.95	0.75	0.48	2
50 Ref 1	49	11	0.82	1.64	2.74	0.79	0.57	3
50 Ref 2	54	9	0.72	1.36	2.25	0.71	0.47	3
56 150m NW	112	12	0.7	1.46	2.31	0.65	0.36	2
56 25m NW	58	10	0.7	1.37	2.26	0.68	0.42	3
56 25m SE	316	10	0.38	0.83	1.26	0.38	0.15	2

56 50m NW	133	12	0.57	1.04	1.64	0.46	0.19	2
56 50m SE	2786	7	0.03	0.1	0.15	0.05	0.02	4
56 CE NW	60	8	0.58	1.06	1.75	0.58	0.34	2
56 Ref 1	35	12	0.78	1.55	2.76	0.77	0.53	2
56 Ref 2	22	9	0.87	1.55	2.85	0.9	0.77	2
58 25m E	66	20	0.89	2.15	3.63	0.84	0.6	2
58 CE E	174	9	0.16	0.4	0.66	0.21	0.07	6
58 Ref 1	119	21	0.78	1.83	2.96	0.67	0.34	2
59 25m N	61	8	0.81	1.58	2.54	0.85	0.69	4
59 25m S	32	2	0.12	0.19	0.34	0.34	0.26	2
59 50m N	80	13	0.87	1.95	3.14	0.85	0.65	2
59 50m S	57	6	0.64	1.1	1.77	0.68	0.48	2
59 CE N	65	6	0.69	1.15	1.82	0.7	0.51	2
59 Ref 1	66	6	0.49	0.86	1.39	0.54	0.32	2
59 Ref 2	61	5	0.53	0.92	1.47	0.63	0.44	2
61 500mNE	229	13	0.24	0.6	0.96	0.26	0.08	6
61 500mS	134	10	0.36	0.8	1.28	0.39	0.16	5
61 0mN	6	4	0.8	0.8	1.79	0.9	0.82	2
67 N25	4	3	0.83	0.62	1.5	0.95	0.91	6
67 N50	15	6	0.76	1.13	2.15	0.83	0.69	1
67 REF 1	23	6	0.81	1.33	2.33	0.9	0.81	1
67 REF 2	27	7	0.46	0.8	1.48	0.53	0.3	1
67 S0	29	3	0.43	0.6	1	0.63	0.5	5
67 S25	7	3	0.67	0.66	1.38	0.87	0.8	2
67 S50	9	6	0.92	1.19	2.5	0.97	0.93	2
81 N0	110	6	0.35	0.65	1.04	0.4	0.21	5
81 N25	30	6	0.69	1.17	2.02	0.78	0.61	3
81 REF 2	134	5	0.12	0.27	0.44	0.19	0.09	6
92 25m N	7	3	0.67	0.66	1.38	0.87	0.8	2
92 CE N	7	2	0.29	0.28	0.59	0.59	0.51	2
92 Ref 1	13	4	0.53	0.7	1.35	0.68	0.52	2

92 Ref 2	7	3	0.67	0.66	1.38	0.87	0.8	2
97 N25	36	8	0.79	1.44	2.45	0.82	0.64	3
97 N50	57	9	0.45	0.91	1.55	0.49	0.24	2
97 REF 1	38	5	0.58	0.95	1.58	0.68	0.5	2
97 REF 2	55	8	0.69	1.34	2.21	0.74	0.52	2
97 S0	61	7	0.53	0.9	1.47	0.52	0.29	2
97 S25	19	5	0.67	0.95	1.72	0.74	0.57	3
97 S50	67	8	0.76	1.43	2.28	0.76	0.55	2
98 N0	132	12	0.78	1.66	2.58	0.72	0.45	2
98 N25	82	11	0.68	1.39	2.25	0.65	0.38	2
98 REF 1	33	9	0.86	1.62	2.8	0.88	0.75	2
98 REF 2	108	12	0.8	1.7	2.68	0.75	0.49	2
99 0m NW	38	5	0.5	0.8	1.35	0.58	0.39	3
99 0m SE	123	9	0.5	0.98	1.54	0.48	0.24	4
99 150m NW	334	25	0.76	1.87	2.86	0.62	0.26	2
99 25m NW	120	24	0.87	2.26	3.65	0.8	0.5	2
99 25m SE	16	11	0.95	1.68	3.33	0.96	0.9	2
99 50m NW	54	16	0.81	1.72	2.95	0.74	0.45	2

Table A4. The full univariate analysis of all the stations for the year 2006.

STATION	N	S	D	Hb	Hs	P	Eh	after AMBI
27 NW25	693	16	0.26	0.61	0.93	0.23	0.06	6
27 NW50	509	40	0.8	2.04	3.11	0.58	0.2	3
27 REF 1	212	43	0.9	2.65	4.21	0.78	0.42	2
27 REF 2	314	52	0.87	2.61	4.08	0.72	0.31	2
27 SE0	161	37	0.89	2.48	4	0.77	0.42	2
27 SE150	577	12	0.19	0.43	0.65	0.18	0.05	6
30 1400m S	252	50	0.91	2.81	4.44	0.79	0.42	2

30 800m SW	46	13	0.78	1.57	2.71	0.73	0.46	2
30 0m SW	1417	4	0.46	0.66	0.96	0.48	0.32	3
30 150m SW	77	19	0.83	1.92	3.19	0.75	0.45	2
30 25m NE	131	25	0.75	1.97	3.2	0.69	0.34	2
30 25m SW	2778	30	0.43	0.77	1.14	0.23	0.04	5
31 1100m NE	258	30	0.81	2.14	3.33	0.68	0.31	2
31 1300mSW	195	24	0.87	2.3	3.59	0.78	0.48	2
31 0mSW	1235	21	0.34	0.83	1.24	0.28	0.07	6
31 150mSW	1113	20	0.62	1.28	1.89	0.44	0.14	5
31 25mNE	816	32	0.7	1.76	2.63	0.53	0.17	3
31 25mSW	296	28	0.85	2.33	3.58	0.74	0.4	3
32 NB 25m	107	3	0.56	0.84	1.28	0.81	0.71	4
32 NB CE	2920	106	0.46	1.46	2.19	0.33	0.03	4
32 Ref 1	16	7	0.89	1.43	2.7	0.96	0.92	2
32 Ref 2	7	4	0.81	0.86	1.84	0.92	0.86	2
32 SB 25m	2194	4	0.02	0.06	0.08	0.04	0.02	6
33 750mSW	50	5	0.64	1.07	1.73	0.75	0.58	2
33 0mE	2403	4	0.08	0.17	0.25	0.13	0.06	3
33 25mE	54	4	0.57	0.85	1.36	0.68	0.52	2
34 550mNE	60	15	0.71	1.55	2.65	0.68	0.38	2
34 550mNW	67	24	0.93	2.38	4.05	0.88	0.68	2
34 0mS	6622	8	0.54	0.84	1.22	0.41	0.19	4
34 25mN	100	12	0.83	1.8	2.84	0.79	0.56	3
34 25mS	3119	11	0.51	0.78	1.13	0.33	0.12	4
34 50mN	1766	11	0.5	0.89	1.3	0.38	0.15	5
34 50mS	1895	14	0.58	1.07	1.56	0.41	0.15	4
45 N0	738	6	0.09	0.22	0.33	0.13	0.05	6
45 N150	215	15	0.5	1.17	1.82	0.47	0.18	2
45 N25	257	12	0.72	1.48	2.23	0.62	0.34	2
45 N50	197	14	0.42	1	1.58	0.41	0.15	2
45 REF 1	248	32	0.79	2.12	3.31	0.66	0.29	2

45 REF 2	266	17	0.86	2.1	3.18	0.78	0.5	2
45 S25	621	4	0.08	0.18	0.26	0.13	0.07	6
45 S50	423	5	0.13	0.28	0.42	0.18	0.08	5
46 C1	407	97	0.96	3.46	5.44	0.82	0.44	2
46 C2	357	68	0.95	3.2	5.01	0.82	0.47	2
46 NE0	319	4	0.02	0.07	0.12	0.06	0.03	6
46 NE150	200	54	0.97	3.26	5.23	0.91	0.69	2
46 NE25	122	24	0.76	1.89	3.07	0.67	0.32	1
46 NE50	122	27	0.9	2.36	3.81	0.8	0.5	2
46 SW25	115	13	0.39	0.89	1.47	0.4	0.15	5
46 SW50	95	14	0.81	1.77	2.83	0.74	0.47	2
50 25m SW	595	6	0.54	0.88	1.29	0.5	0.29	3
50 CE	404	4	0.12	0.24	0.36	0.18	0.1	6
50 Ref 1	40	18	0.86	1.87	3.36	0.81	0.54	2
50 Ref 2	310	60	0.94	3.12	4.89	0.83	0.49	2
56 150m NW	29	15	0.94	1.99	3.66	0.94	0.83	2
56 25m NW	422	5	0.49	0.71	1.04	0.45	0.27	4
56 25m SE	1788	3	0.5	0.7	1.01	0.64	0.51	4
56 50m NW	21	6	0.61	0.97	1.78	0.69	0.49	4
56 50m SE	345	6	0.39	0.66	0.99	0.38	0.2	3
56 CE NW	2872	2	0.5	0.69	0.99	0.99	0.99	4
56 Ref 1	8	5	0.86	1.01	2.16	0.93	0.86	2
56 Ref 2	48	13	0.84	1.75	2.98	0.81	0.57	2
58 25m E	107	4	0.32	0.56	0.87	0.43	0.27	3
58 CE E	194	2	0.01	0.03	0.05	0.05	0.03	5
58 Ref 1	8	5	0.86	1.01	2.16	0.93	0.86	2
59 25m N	134	20	0.71	1.78	2.86	0.66	0.33	2
59 25m S	73	16	0.86	2	3.29	0.82	0.58	2
59 50m N	303	9	0.35	0.8	1.22	0.38	0.17	4
59 50m S	183	19	0.79	1.87	2.91	0.69	0.36	2
59 CE N	201	22	0.84	2.04	3.17	0.71	0.38	2

59 Ref 1	395	20	0.83	2.05	3.08	0.71	0.39	2
59 Ref 2	1242	2	0.31	0.49	0.71	0.71	0.64	5
61 500mNE	5	4	0.9	0.82	1.92	0.96	0.93	1
61 500mS	37	8	0.6	1.07	1.87	0.62	0.38	2
61 0mN	13287	4	0	0.02	0.02	0.01	0.01	6
67 N25	850	4	0.03	0.09	0.14	0.07	0.03	6
67 N50	1351	10	0.08	0.2	0.3	0.09	0.03	6
67 REF 1	43	9	0.7	1.29	2.19	0.69	0.45	2
67 REF 2	48	10	0.76	1.5	2.52	0.76	0.53	2
67 S0	1324	5	0.02	0.07	0.1	0.04	0.02	6
67 S25	546	12	0.28	0.65	0.98	0.27	0.09	3
67 S50	317	11	0.36	0.83	1.27	0.37	0.14	2
81 N0	137	4	0.28	0.54	0.83	0.41	0.26	2
81 N25	158	3	0.19	0.37	0.56	0.36	0.24	2
81 REF 2	79	4	0.44	0.76	1.19	0.6	0.43	2
92 25m N	65	2	0.03	0.06	0.11	0.11	0.08	3
92 CE N	336	7	0.11	0.29	0.46	0.16	0.06	3
92 Ref 1	20	4	0.74	1.07	1.86	0.93	0.88	2
92 Ref 2	13	6	0.86	1.25	2.41	0.93	0.86	2
97 N25	416	39	0.52	1.4	2.19	0.41	0.09	4
97 N50	231	72	0.93	3.09	5.01	0.81	0.44	1
97 REF 1	243	52	0.94	2.94	4.66	0.82	0.48	2
97 REF 2	223	58	0.96	3.17	5.08	0.87	0.57	2
97 S0	2265	17	0.08	0.22	0.33	0.08	0.02	6
97 S25	843	46	0.37	1.07	1.64	0.3	0.05	5
97 S50	1987	42	0.15	0.48	0.72	0.13	0.02	6
98 N0	434	2	0	0.01	0.02	0.02	0.02	3
98 N25	163	8	0.51	1	1.54	0.51	0.27	2
98 REF 1	26	3	0.28	0.42	0.74	0.47	0.34	2
98 REF 2	49	2	0.04	0.08	0.14	0.14	0.1	2
99 0m NW	7569	4	0.42	0.71	1.03	0.51	0.35	5

99 0m SE	309	27	0.84	2.07	3.16	0.66	0.3	3
99 150m NW	381	77	0.96	3.42	5.34	0.85	0.52	2
99 25m NW	18115	34	0.56	1.02	1.47	0.29	0.05	4
99 25m SE	84	34	0.9	2.46	4.21	0.83	0.53	2
99 50m NW	2186	49	0.44	1.14	1.7	0.3	0.05	5

Table A5. The selected sites with their labels and their 2003univariate analysis, as coming from testing the macrofauna data.

label	Fish farm site name	N	S	Hs	before AMBI
27	Basta Voe South	137.50	8.17	1.32	2.67
30	Strome	633.17	23.17	2.08	4.33
31	Portree	199.33	17.83	2.33	4.00
32	Port na Moine	1448.00	7.00	0.85	4.60
33	Sgeir Mhor	23.33	11.67	3.35	2.00
34	Inchkenneth	370.86	18.00	2.42	3.86
45	Bow of Hascosay	33.00	4.25	1.18	2.63
46	Vatsetter	354.75	28.50	3.51	2.25
50	Bagh Dail nan Ceann North (BDNC N)	59.00	11.67	2.65	2.67
56	Greinham	440.25	10.00	1.87	2.38
58	Loch Etive East	119.67	16.67	2.42	3.33
59	Leinish	60.29	6.57	1.78	2.29
61	Sian	123.00	9.00	1.34	4.33
67	Bay of Vady	16.29	4.86	1.76	2.57
81	Basta Voe North	91.33	5.67	1.17	4.67
92	Port nan Seannag aka Lunga (east side)	8.50	3.00	1.18	2.00
97	Kirk Noust	47.57	7.14	1.89	2.29
98	Stead of Aithness	88.75	11.00	2.58	2.00
99	Aird	114.17	15.00	2.61	2.50

Table A6. The selected sites with their labels and their 2006univariate analysis, as coming from testing the macrofauna data.

Label	Fish farm site name	N	S	Hs	after AMBI
27	Basta Voe South	411.00	33.33	2.83	3.50
30	Strome	783.50	23.50	2.61	2.67

31	Portree	652.17	25.83	2.71	3.50
32	Port na Moine	1048.80	24.80	1.62	3.60
33	Sgeir Mhor	835.67	4.33	1.11	2.33
34	Inchkenneth	1947.00	13.57	2.11	3.43
45	Bow of Hascosay	370.63	13.13	1.64	3.38
46	Vatsetter	217.13	37.63	3.37	2.75
50	Bagh Dail nan Ceann North (BDNC N)	337.25	22.00	2.48	3.25
56	Greinham	691.63	6.88	1.83	3.13
58	Loch Etive East	103.00	3.67	1.03	3.33
59	Leinish	361.57	15.43	2.46	2.71
61	Sian	4443.00	5.33	1.27	3.00
67	Bay of Vady	639.86	8.71	1.07	3.86
81	Basta Voe North	124.67	3.67	0.86	2.00
92	Port nan Seannag aka Lunga (east side)	108.50	4.75	1.21	2.50
97	Kirk Noust	886.86	46.57	2.80	3.71
98	Stead of Aithness	168.00	3.75	0.61	2.25
99	Aird	4099.00	32.43	2.44	3.29

APPENDIX 2

Tables of raw data and statistical analysis taken from Chapter 3, "A comparison of the effectiveness of diversity indices for analysing environmental data from marine fish farms by long term metadata analysis".

Table A1. The labels and the meaning of their sites and stations. The stations column is the distance from the cages, T1 and T2 the trials for each of the station and CE the cage edge (0m). The sites column has the site name and the year tested. For the labels S288-S293, the indicators on the stations column 1 is for 0m, 2 is for 25m, 3 for 150m and 4 for the reference stations (850m).

label	stations	Sites
S1	Ref1 (850m)	ardmaddy2006
S2	Ref2 (850m)	ardvourlie2006
S3	T1.50m	ardyne2006
S4	T1.25m	Basta Voe South2006
S5	CE	Djubawick2006
S6	T2.25m	Strome2006
S7	T2.50m	Portree2006
S8	Ref 1 (850m)	Port na moine2006
S9	Ref 2 (850m)	Sgeir Mhor2006
S10	T1 150m	Inchkenneth2006
S11	T1 50m	Geasgill2006
S12	T1 25m	Strone2006
S13	T1 CE	Shuna castle bay2006
S14	T2 CE	Flotta2006
S15	T2 25m	Bow of Hascosay2006
S16	T2 50m	Vatsetter2006
S17	Ref1 (850m)	Poll na gile2006
S18	T1 50m	BDNC S2006
S19	T1 25m	BDNC N2006
S20	CE	Boisdale2006
S21	T2 25m	Eilean Haey2006
S22	T2 50m	Ornish2006
S23	Ref2 (850m)	Greinham2006

S24	Ref 1 (850m)	Groatay2006
S25	Ref 2 (850m)	Loch Etive East2006
S26	T1 150m	Leinish2006
S27	T1 50m	Meavaig2006
S28	T1 25m	Sian2006
S29	T1 CE	Tolsta2006
S30	T2 CE	Vacasay2006
S31	T2 25m	Bay of Vady2007
S32	T2 50m	Ardcastle2007
S33	Ref 1 (850m)	Binna Ness2006
S34	Ref 2 (850m)	Creag na h-iolaire2007
S35	T1 50m	Merry Holm2007
S36	T1 25m	Furnace2007
S37	T1 CE	Kenmore Point2007
S38	T2 25m	Quarry Point2007
S39	T2 50m	Setterness West2006
S40	Ref 1 (850m)	Loch Etive East2006
S41	Ref 2 (850m)	Poll na Gile2006
S42	T1 50m	Linnhe2007
S43	T1 25m	West Loch Tarbert 2007
S44	CE	Basta Voe North2007
S45	T2 25m	TREANAY2006
S46	T2 50m	Uiskevagh South2006
S47	Ref 1 (850m)	Druimyeon Bay2006
S48	Ref 2 (850m)	Winnaness2007
S49	T1 150m	Vidlin North 05
S50	T1 50m	Vidlin North 06
S51	T2 CE	Brunnaness2007
S52	T2 25m	Linnhe2007
S53	T2 50m	Kirkabister2006

S54	REF 1 (850m)	Port nan Seannag aka Lunga 2007
S55	Ref 2 (850m)	Earnsaig NEVIS A2006
S56	T1 150m	Stoull NEVIS B2006
S57	T1 50m	Ardintigh Bay NEVIS C2006
S58	T1 25m	Kirk Noust2007
S59	T1 CE	Stead of Aithness2007
S60	T2 CE	Aird2007
S61	T2 25m	Camas an Eilean2007
S62	T2 50m	Kenmore2007
S63	T1 50m	Kempi Bay2007
S64	T1 25m	Sian Bay2007
S65	Cage Edge	vady03
S66	T2 25m	vady03
S67	T2 50m	vady03
S68	Ref 2 (850m)	vady03
S69	Ref 1 (850m)	vatset03
S70	REF2 (850m)	vatset03
S71	T1 150m	vatset03
S72	T1 50m	vatset03
S73	T1 25m	vatset03
S74	T1 CE	vatset03
S75	T2 CE	vatset03
S76	T2 25m	vatset03
S77	T2 50m	vatset03
S78	Ref 1 (850m)	westfara03
S79	Ref 2 (850m)	westfara03
S80	T1 50m	westfara03
S81	T1 25m	westfara03
S82	CE	westfara03

S83	T2 25m	westfara03
S84	REF1 (850m)	stead Airthness 05
S85	REF 2 (850m)	stead Airthness 05
S86	T1 150m	stead Airthness 05
S87	T1 50m	stead Airthness 05
S88	T1 25m	stead Airthness 05
S89	T1 CE	stead Airthness 05
S90	T2 25m	stead Airthness 05
S91	T2 50m	stead Airthness 05
S92	contN (850m)	torgawn01
S93	150m N	torgawn01
S94	50m N	torgawn01
S95	25m N	torgawn01
S96	0m N	torgawn01
S97	0m S	torgawn01
S98	25m S	torgawn01
S99	50m S	torgawn01
S100	Cont Sth (850m)	torgawn01
S101	150MW	droig02
S102	50m N	droig02
S103	25m N	droig02
S104	0m N	droig02
S105	50m S	droig02
S106	Cont S (850m)	droig02
S107	150mN	drumbeg02
S108	50MW	drumbeg02
S109	25MW	drumbeg02
S110	0MW	drumbeg02
S111	0ME	drumbeg02
S112	25ME	drumbeg02

S113	50ME	drumbeg02
S114	CONTW (850m)	nedd02
S115	50mN	nedd02
S116	25mN	nedd02
S117	0mN	nedd02
S118	0mS	nedd02
S119	25mS	nedd02
S120	50mS	nedd02
S121	ContS (850m)	nedd02
S122	ContN (850m)	oldany02
S123	150MW	oldany02
S124	50MW	oldany02
S125	25MW	oldany02
S126	0MW	oldany02
S127	0ME	oldany02
S128	25ME	oldany02
S129	50ME	oldany02
S130	Cont N (850m)	torg02
S131	150mN	torg02
S132	50mN	torg02
S133	25mN	torg02
S134	0mN	torg02
S135	0mS	torg02
S136	25mS	torg02
S137	50mS	torg02
S138	ContS (850m)	torg02
S139	50m N	droig03
S140	25m N	droig03
S141	0m N	droig03
S142	50m NE	droig03

S143	Cont S (850m)	droig03
S144	25m NE	drum03
S145	0m SW	drum03
S146	25m SW	drum03
S147	50m SW	drum03
S148	Cont SW (850m)	drum03
S149	Cont N (850m)	drum03
S150	Cont N (850m)	nedd03
S151	50m N	nedd03
S152	25m N	nedd03
S153	0m N	nedd03
S154	25m S	nedd03
S155	50m S	nedd03
S156	Cont S (850m)	nedd03
S157	Cont W (850m)	oldany03
S158	50m N	oldany03
S159	25m N	oldany03
S160	0m S	oldany03
S161	25m S	oldany03
S162	50m S	oldany03
S163	Cont W (850m)	reintreid03
S164	50m W	reintreid03
S165	25m W	reintreid03
S166	0m W	reintreid03
S167	25m E	reintreid03
S168	50m E	reintreid03
S169	Cont E (850m)	reintreid03
S170	150m N	torgawn03
S171	50m W	torgawn03
S172	25m W	torgawn03

S173	0m W	torgawn03
S174	25m E	torgawn03
S175	50m E	torgawn03
S176	Cont E (850m)	torgawn03
S177	50m N	droig04
S178	25m N	droig04
S179	0m N	droig04
S180	25m S	droig04
S181	Cont S (850m)	droig04
S182	50m S	droig04
S183	Cont NE (850m)	droig04
S184	Cont N (850m)	drumbeg04
S185	50m NE	drumbeg04
S186	25m NE	drumbeg04
S187	0m NE	drumbeg04
S188	25m SW	drumbeg04
S189	50m SW	drumbeg04
S190	Cont NW (850m)	nedd04
S191	50m N	nedd04
S192	25m N	nedd04
S193	0m N	nedd04
S194	25m S	nedd04
S195	50m S	nedd04
S196	Cont S	nedd04
S197	Cont NW (850m)	reintreid04
S198	50m NW	reintreid04
S199	25m NW	reintreid04
S200	0m NW	reintreid04
S201	25m SE	reintreid04
S202	50m SE	reintreid04

S203	Cont SE (850m)	reintreid04
S204	Cont NW (850m)	torgawn04
S205	150m NW	torgawn04
S206	50m NW	torgawn04
S207	25m NW	torgawn04
S208	0m NW	torgawn04
S209	25m SE	torgawn04
S210	50m SE	torgawn04
S211	Cont SE (850m)	torgawn04
S212	R1	torgawn05
S213	50m NW	torgawn05
S214	25m NW	torgawn05
S215	0m NW	torgawn05
S216	25m SE	torgawn05
S217	50m SE	torgawn05
S218	Cont SE (850m)	torgawn05
S219	Ref N (850m)	kirk03
S220	Ref 2 (850m)	kirk03
S221	T1 50m	kirk03
S222	T1 25m	kirk03
S223	CE	kirk03
S224	T2 25m	kirk03
S225	T2 50m	kirk03
S226	50m N	portnacro03
S227	50m S	portnacro03
S228	150m S	portnacro03
S229	Ref S (850m)	portnacro03
S230	Ref 1 (850m)	vady03
S231	Ref 2 (850m)	vady03
S232	T1 50m	vady03

S233	T1 25m	vady03
S234	Cage Edge	vady03
S235	T2 25m	vady03
S236	T2 50m	vady03
S237	CONTW (850m)	carlaim02
S238	50MW	carlaim02
S239	25MW	carlaim02
S240	0MW	carlaim02
S241	25ME	carlaim02
S242	50ME	carlaim02
S243	CONTE (850m)	carlaim02
S244	CONT1 (850m)	leinish03
S245	CONT2 (850m)	leinish03
S246	150MN	leinish03
S247	50MN	leinish03
S248	25MN	leinish03
S249	0MN	leinish03
S250	0MS	leinish03
S251	25MS	leinish03
S252	50MS	leinish03
S253	CONT1 (850m)	pooltiel03
S254	SE150M	pooltiel03
S255	SW50M	pooltiel03
S256	SE25M	pooltiel03
S257	SE0M	pooltiel03
S258	NE0M	pooltiel03
S259	NE25M	pooltiel03
S260	NE50M	pooltiel03
S261	0m	lake portnamoine 03
S262	25m	lake portnamoine 03

S263	150m	lake portnamoine 03
S264	ContN (850m)	lake portnamoine 03
S265	ContS (850m)	lake portnamoine 03
S266	Ref N (850m)	kilbn05
S267	100m N	kilbn05
S268	50m N	kilbn05
S269	Centre	kilbn05
S270	50m S	kilbn05
S271	100m S	kilbn05
S272	Cont N (850m)	kilbn05
S273	Ref 1 (850m)	kirk05
S274	Ref 2 (850m)	kirk05
S275	T1 50m	kirk05
S276	T1 25m	kirk05
S277	T1 CE	kirk05
S278	T2 25m	kirk05
S279	T2 50m	kirk05
S280	REF 1 (850m)	west fara05
S281	REF 2 (850m)	west fara05
S282	T1 150m	west fara05
S283	T1 50m	west fara05
S284	T1 25m	west fara05
S285	T1 CE	west fara05
S286	T2 25m	west fara05
S287	T2 50m	west fara05
S288	CN1	kenmore03
S289	CN3	kenmore03
S290	K1	kenmore03
S291	K2	kenmore03
S292	K3	kenmore03

S293	K4	kenmore03
S294	Ref 1 (850m)	ardinish03
S295	Ref 2 (850m)	ardinish03
S296	0m	ardinish03
S297	25m	ardinish03
S298	150m	ardinish03
S299	Ref 1 (850m)	cornaig03
S300	Ref 2 (850m)	cornaig03
S301	0m	cornaig03
S302	25m	cornaig03
S303	150m	cornaig03
S304	Ref A (850m)	lingay03
S305	Ref B (850m)	lingay03
S306	0m	lingay03
S307	25m	lingay03
S308	150m	lingay03
S309	Ref B (850m)	rubh03
S310	0m	rubh03

Table A2. Univariate analysis performed for the macrofauna data of all the tested stations. The first column contains the stations in label form, the second has the Pielou index, the second the Azti marine biotic index (AMBI), the fourth the infaunal trophic index (ITI) and the fifth the carbon percentage measured from % of the dry weight of the sample.

STATION	**P**	**AMBI**	**ITI**	**%C**
S1	0.27	3	4.67	0.31
S2	0.88	2	3.93	3.67
S3	0.89	2	1.17	2.9
S4	0.9	2	3.11	2.57
S5	0.93	2	2.68	2.6
S6	0.87	2	2.85	3.58
S7	0.8	2	2.14	3.73
S8	0.97	1	5.60	1.51
S9	0.91	2	4.67	0.59
S10	0.66	2	4.67	0

S11	0.74	2	3.50	0
S12	0.97	2	4.67	1.17
S13	0.81	2	4.45	4.59
S14	0.95	1	4.67	1.6
S15	0.89	1	4.67	0
S16	1	2	4.67	0
S17	0.82	2	4.67	1.22
S18	0.83	2	4.31	4.07
S19	0.84	2	4.34	2.09
S20	0.72	2	4.39	2.02
S21	0.89	2	3.82	1.17
S22	0.8	2	4.36	1.41
S23	0.86	2	4.29	1.04
S24	0.06	2	4.66	2.08
S25	0.28	1	4.64	1.99
S26	0.84	1	4.67	2.58
S27	0.26	2	4.63	3.23
S28	0.11	2	4.65	3.51
S29	0.34	5	4.64	3.93
S30	0.06	2	4.67	2.52
S46	0.25	1	0.92	6.93
S47	0.58	2	4.29	9.27
S48	0.65	2	3.86	9.2
S49	0.96	1	1.84	6.72
S50	0.91	2	2.57	9.2
S51	0.78	6	4.67	8.55
S52	0.9	2	4.45	6.71
S53	0.79	2	4.20	2.35
S54	0.9	2	4.08	7.86
S55	0.78	2	4.50	7.03
S56	0.81	2	4.56	6.97
S57	0.74	1	4.67	7.28
S58	0.65	1	4.67	6.84
S59	0.83	2	4.23	10.33
S60	0.67	2	4.42	7.69
S61	0.79	2	3.69	7.68
S62	0.8	2	3.33	7.63
S63	0.85	1	4.20	5.64
S64	0.79	6	6.33	5.35
S65	0.44	5	6.83	5.18
S66	0.63	2	2.33	5.93
S67	0.56	2	3.50	5.23
S68	0.97	3	4.20	5.92

S69	0.23	4	4.67	3.74
S70	0.55	2	4.67	1.82
S71	0.9	2	5.96	2.07
S72	0.86	2	4.67	3.48
S73	0.93	2	4.67	1.97
S74	0.91	2	4.37	1.22
S75	0.5	6	4.28	1.44
S210	0.96	3	4.67	6.14
S211	0.95	2	4.37	3.76
S212	0.74	2	2.33	2.31
S213	0.23	5	4.57	6.92
S214	1	5	4.63	7.59
S215	0.79	6	5.44	7.87
S216	0.7	4	4.67	9.9
S217	0.88	4	4.67	5.14
S218	0.88	2	4.43	7.54
S219	0.81	2	4.36	6.93
S220	0.42	2	3.31	7.17
S221	0.35	2	6.99	9.03
S222	0.88	4	6.99	6.38
S223	0.68	3	6.99	6.2
S224	0.59	4	6.99	6.23
S225	0.25	3	6.97	6.77
S226	0.97	2	4.00	3.64
S227	0.52	6	7.00	3.89
S228	0.73	1	4.67	2.38
S229	0.82	2	4.67	3.48
S230	0.9	1	1.17	3.7
S271	0.96	1	4.67	0.65
S272	0.86	2	4.67	0.91
S273	0.89	2	3.11	0.85
S274	0.87	4	2.65	7.45
S275	0.4	5	3.72	5.71
S276	0.62	6	3.12	7.19
S277	0.33	6	3.89	8.21
S278	0.84	5	2.76	5.19
S279	0.81	4	2.59	6.54
S280	0.85	2	3.31	7.69
S281	0.88	1	3.50	2.49
S282	0.77	2	3.50	2.46
S286	0.91	1	3.69	3.95
S287	0.82	2	1.87	3.82
S288	0.75	6	4.89	6.04

S289	0.9	4	3.50	1.72
S290	0.26	3	4.70	0.86
S291	0.75	5	4.67	2.58
S292	0.87	5	4.67	2.47
S293	0.85	2	3.89	4.16
S295	0.92	2	3.03	8.38
S296	0.84	3	4.28	8.6
S297	0.9	2	4.45	8.34
S298	0.95	3	4.67	8.62
S299	0.85	1	3.95	6.58
S300	0.83	2	3.05	0.33
S301	0.71	1	3.18	10.57
S302	0.88	2	2.97	7.36
S303	0.83	1	3.56	7.45
S304	0.86	1	3.17	9.72
S305	0.68	1	2.97	8.31
S306	0.94	1	2.92	9.11
S307	0.87	2	2.94	9.13
S308	0.71	2	2.82	8.42
S309	0.79	2	2.69	9.05
S310	0.73	2	3.11	8.43

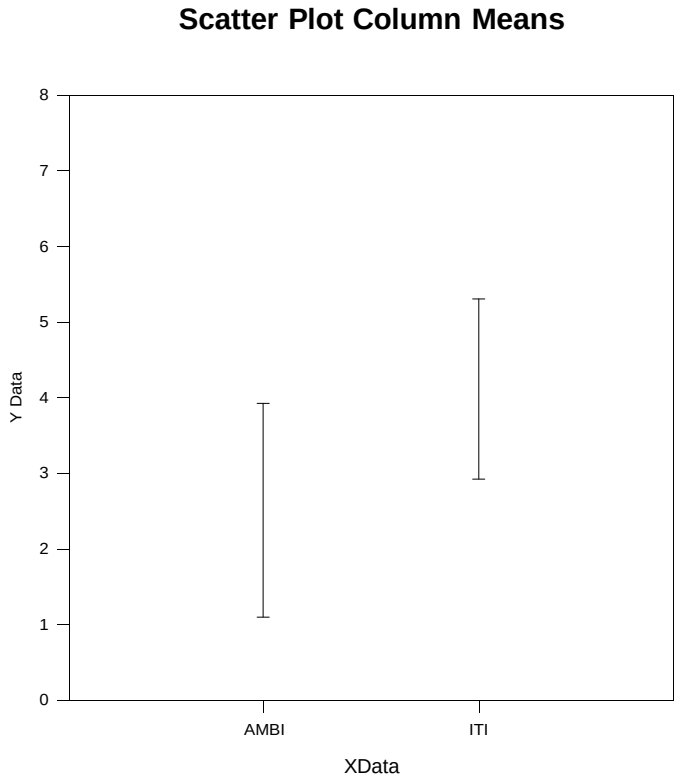
Table A3. Descriptive Statistics for the comparison of ITI and AMBI. The outlook is from the Sigmastat result page

Column	**Size**	**Missing**	**Mean**	**Std Dev**	**Std. Error**	**C.I. of Mean**
AMBI	117	0	2.504	1.412	0.131	0.259
ITI	117	0	4.115	1.190	0.110	0.218

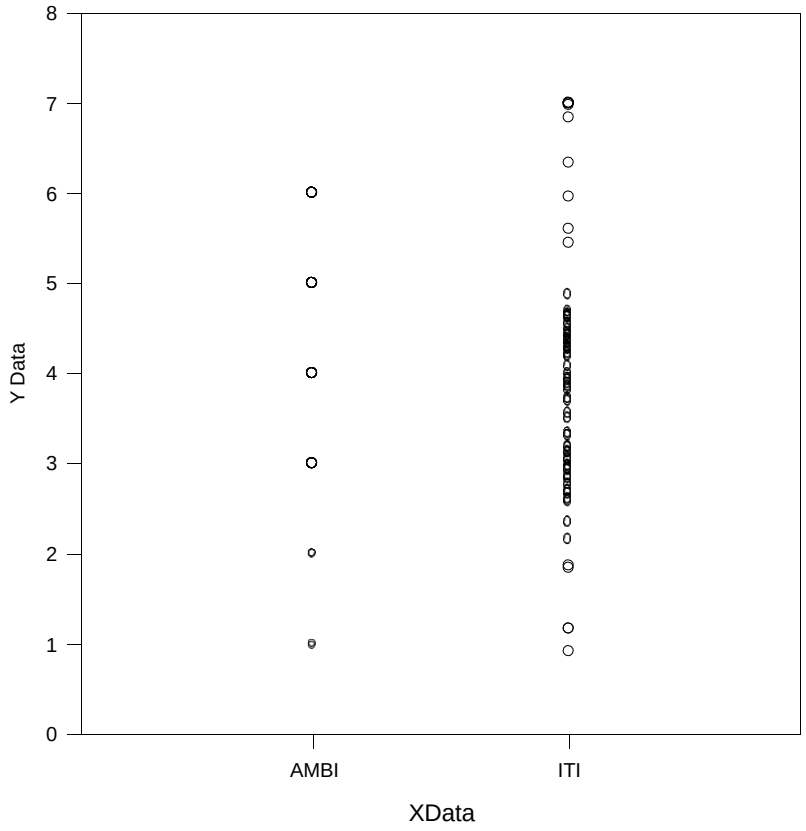
Column	**Range**	**Max**	**Min**	**Median**	**25%**	**75%**
AMBI	5.000	6.000	1.000	2.000	2.000	3.000

ITI	6.084	6.999	0.916	4.355	3.306	4.666

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Sum	Sum of Squares
AMBI	1.291	0.707	0.366	<0.001	293.000	965.000
ITI	0.133	1.018	0.210	<0.001	481.430	2145.364



Point Plot



Normality Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Sunday, September 28, 2008, 22:10:58

Data source: Data 1 in Notebook 1

Group	N	Missing	Median	25%	75%
AMBI	117	0	2.000	2.000	3.000
ITI	117	0	4.355	3.306	4.666

T = 9500.000 n(small)= 117 n(big)= 117 (P = <0.001)

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

APPENDIX 3

Tables of raw data and statistical analysis taken from Chapter 4, "The application of combinations of diversity measures for assessment of environmental impact of marine fish farms by long term metadata analysis".

Table A1 Univariate measures calculated for all sampling stations (spatial and temporal). The diversity indices, as shown on the sequence of the columns, are the individual species N, the species richness diversity S, the Simpsons Index D, Brillouins Index Hb, Shannon Wiener Hs, Pielou Evenness P, Heip Evenness Eh Azti marine biotic index AMBI and the infaunal trophic index ITI. The labels meaning can be seen in Appendix 2, Table A1.

STATION	N	S	D	Hb	Hs	P	Eh	AMBI	ITI
S1	113	6	0.2	0.43	0.7	0.27	0.12	3	4.6662
S2	49	15	0.9	2.01	3.43	0.88	0.7	2	3.9291
S3	47	7	0.82	1.52	2.49	0.89	0.77	2	1.1669
S4	55	11	0.88	1.88	3.11	0.9	0.76	2	3.1108
S5	39	14	0.92	2.02	3.52	0.93	0.81	2	2.6831
S6	33	11	0.87	1.71	2.99	0.87	0.7	2	2.8518
S7	52	11	0.83	1.67	2.78	0.8	0.59	2	2.1385
S8	14	8	0.92	1.47	2.9	0.97	0.92	1	5.5993
S9	23	10	0.89	1.65	3.03	0.91	0.8	2	4.6662
S10	23	6	0.57	0.94	1.71	0.66	0.46	2	4.6662
S11	36	9	0.74	1.36	2.36	0.74	0.52	2	3.5
S12	11	4	0.8	1.01	1.94	0.97	0.94	2	4.6662
S13	23	8	0.78	1.34	2.44	0.81	0.63	2	4.4541
S14	11	8	0.93	1.37	2.85	0.95	0.88	1	4.6662
S15	15	7	0.85	1.3	2.5	0.89	0.78	1	4.6662
S16	6	6	1	1.1	2.58	1	1	2	4.6662
S17	18	8	0.79	1.29	2.46	0.82	0.64	2	4.6662
S18	48	22	0.88	2.09	3.72	0.83	0.58	2	4.3071
S19	48	17	0.88	1.99	3.45	0.84	0.62	2	4.3442
S20	40	11	0.71	1.43	2.5	0.72	0.46	2	4.3862
S21	36	11	0.88	1.79	3.1	0.89	0.75	2	3.8178
S22	34	13	0.84	1.66	2.97	0.8	0.57	2	4.3554
S23	47	14	0.88	1.91	3.26	0.86	0.66	2	4.2931
S24	839	6	0.03	0.09	0.14	0.06	0.02	2	4.6634
S25	84	8	0.22	0.49	0.84	0.28	0.11	1	4.6361
S26	19	7	0.8	1.28	2.35	0.84	0.68	1	4.6662
S27	77	7	0.2	0.42	0.73	0.26	0.11	2	4.6333
S28	112	4	0.05	0.13	0.22	0.11	0.06	2	4.6452
S29	108	6	0.29	0.55	0.88	0.34	0.17	5	4.641
S30	433	4	0.03	0.08	0.12	0.06	0.03	2	4.6662
S46	1404	18	0.37	0.71	1.06	0.25	0.06	1	0.915537
S47	89	10	0.57	1.19	1.92	0.58	0.31	2	4.288151
S48	56	12	0.67	1.37	2.31	0.65	0.36	2	3.859908

S49	14	9	0.93	1.52	3.04	0.96	0.9	1	1.840192
S50	32	17	0.93	2.03	3.72	0.91	0.76	2	2.568767
S51	13	3	0.56	0.67	1.24	0.78	0.68	6	4.6662
S52	20	11	0.89	1.63	3.11	0.9	0.76	2	4.4541
S53	33	14	0.81	1.66	3.02	0.79	0.55	2	4.1993
S54	23	9	0.87	1.56	2.84	0.9	0.77	2	4.0831
S55	84	11	0.81	1.7	2.71	0.78	0.56	2	4.4996
S56	53	13	0.85	1.79	3.01	0.81	0.59	2	4.5605
S57	120	21	0.83	2.03	3.25	0.74	0.43	1	4.6662
S58	84	12	0.7	1.43	2.31	0.65	0.36	1	4.6662
S59	49	13	0.85	1.82	3.08	0.83	0.62	2	4.2287
S60	46	14	0.73	1.47	2.57	0.67	0.38	2	4.4247
S61	47	18	0.84	1.88	3.29	0.79	0.52	2	3.6939
S62	54	15	0.85	1.85	3.14	0.8	0.56	2	3.3327
S63	24	9	0.84	1.49	2.7	0.85	0.69	1	4.1993
S64	22	5	0.69	1.05	1.84	0.79	0.64	6	6.3329
S65	125	11	0.44	0.95	1.52	0.44	0.19	5	6.8341
S66	56	7	0.62	1.08	1.76	0.63	0.4	2	2.3331
S67	70	9	0.62	1.08	1.77	0.56	0.3	2	3.5
S68	10	8	0.96	1.37	2.92	0.97	0.94	3	4.1993
S69	244	7	0.2	0.42	0.66	0.23	0.1	4	4.6662
S70	97	8	0.59	1.05	1.66	0.55	0.31	2	4.6662
S71	75	17	0.92	2.24	3.67	0.9	0.73	2	5.957
S72	41	6	0.77	1.35	2.22	0.86	0.73	2	4.6662
S73	23	7	0.86	1.47	2.62	0.93	0.86	2	4.6662
S74	27	12	0.91	1.8	3.27	0.91	0.79	2	4.3743
S75	30	5	0.4	0.66	1.17	0.5	0.31	6	4.2777
S210	16	10	0.94	1.63	3.2	0.96	0.91	3	6.6703
S211	11	7	0.91	1.3	2.66	0.95	0.89	2	4.3743
S212	61	18	0.81	1.81	3.08	0.74	0.44	2	2.3331
S213	331	14	0.22	0.57	0.89	0.23	0.07	5	4.5668
S214	8	4	0.86	0.98	2	1	1	5	4.6312
S215	10	4	0.64	0.78	1.57	0.79	0.66	6	5.4439
S216	39	13	0.71	1.45	2.59	0.7	0.42	4	4.6662
S217	12	6	0.82	1.15	2.28	0.88	0.77	4	4.6662
S218	12	6	0.82	1.15	2.28	0.88	0.77	2	4.4331
S219	17	6	0.74	1.12	2.09	0.81	0.65	2	4.3554
S220	62	7	0.35	0.7	1.18	0.42	0.21	2	3.3068
S221	152	10	0.33	0.72	1.15	0.35	0.14	2	6.9944
S222	74	2	0.42	0.58	0.88	0.88	0.84	4	6.9916
S223	189	11	0.73	1.54	2.36	0.68	0.41	3	6.9909
S224	54	11	0.58	1.19	2.04	0.59	0.31	4	6.993

S225	1407	20	0.38	0.73	1.08	0.25	0.06	3	6.9734
S226	11	8	0.95	1.4	2.91	0.97	0.93	2	3.9998
S227	17	2	0.22	0.29	0.52	0.52	0.44	6	6.9993
S228	21	7	0.67	1.11	2.06	0.73	0.53	1	4.6662
S229	21	11	0.82	1.49	2.84	0.82	0.62	2	4.6662
S230	10	6	0.84	1.12	2.32	0.9	0.8	1	1.1669
S271	5	4	0.9	0.82	1.92	0.96	0.93	1	4.6662
S272	24	11	0.86	1.6	2.97	0.86	0.68	2	4.6662
S273	38	15	0.91	1.98	3.48	0.89	0.72	2	3.1108
S274	29	12	0.88	1.74	3.13	0.87	0.71	4	2.6516
S275	309	18	0.43	1.09	1.68	0.4	0.13	5	3.724
S276	150	24	0.71	1.76	2.83	0.62	0.27	6	3.1227
S277	112	10	0.29	0.67	1.11	0.33	0.13	6	3.8885
S278	61	16	0.88	2.01	3.37	0.84	0.62	5	2.7573
S279	82	15	0.87	1.96	3.18	0.81	0.58	4	2.5921
S280	62	20	0.9	2.18	3.69	0.85	0.63	2	3.3054
S281	46	15	0.9	2.01	3.45	0.88	0.71	1	3.5
S282	51	12	0.81	1.65	2.77	0.77	0.53	2	3.5
S283	43	17	0.93	2.14	3.75	0.92	0.78	5	3.9998
S286	44	16	0.92	2.1	3.64	0.91	0.76	1	3.6939
S287	104	16	0.86	2.06	3.29	0.82	0.59	2	1.8662
S288	54	8	0.74	1.38	2.25	0.75	0.54	6	4.8881
S289	20	12	0.9	1.66	3.21	0.9	0.75	4	3.5
S290	161	12	0.23	0.58	0.95	0.26	0.08	3	4.6977
S291	14	6	0.68	0.99	1.95	0.75	0.57	5	4.6662
S292	15	8	0.84	1.33	2.61	0.87	0.73	5	4.6662
S293	27	9	0.83	1.52	2.71	0.85	0.69	2	3.8885
S294	14	3	0.58	0.7	1.26	0.8	0.7	3	4.6662
S295	18	5	0.8	1.19	2.13	0.92	0.85	2	3.0331
S296	11	4	0.71	0.87	1.68	0.84	0.73	3	4.2777
S297	25	10	0.88	1.65	3	0.9	0.78	2	4.4541
S298	8	4	0.82	0.93	1.91	0.95	0.92	3	4.6662
S299	26	8	0.82	1.45	2.56	0.85	0.7	1	3.948
S300	50	12	0.85	1.76	2.96	0.83	0.62	2	3.0541
S301	26	6	0.64	1.04	1.83	0.71	0.51	1	3.1815
S302	37	5	0.74	1.24	2.04	0.88	0.78	2	2.9652
S303	22	9	0.83	1.44	2.65	0.83	0.66	1	3.5553
S304	34	13	0.87	1.79	3.17	0.86	0.67	1	3.1731
S305	90	19	0.75	1.74	2.87	0.68	0.35	1	2.9694
S306	27	14	0.94	1.93	3.56	0.94	0.83	1	2.9162
S307	24	10	0.86	1.58	2.89	0.87	0.71	2	2.9351
S308	52	12	0.74	1.49	2.53	0.71	0.43	2	2.8245

S309	52	13	0.84	1.73	2.91	0.79	0.54	2	2.6908
S310	50	12	0.75	1.54	2.62	0.73	0.47	2	3.1108

Table A2 Environmental parameters used for validation of combinations of indice. The first label is the stations (The labels meaning can be seen in Appendix 2, Table A1). The second is the Median particle size analysis measure in μm (MPSA), the third id the carbon percentage and the fourth the nitrogen percentage (both measured in percentage of dry weight from the sample) and the fifth column is the redox potential measured in mV.

STATION	**MPSA**	**%C**	**%N**	**REDOX**
S1	271.64	0.31	0.03	294.5
S2	348.66	3.67	0.12	124
S3	267.9	2.9	0.03	287.5
S4	277.35	2.57	0.01	395
S5	444.42	2.6	0.03	413.5
S6	291.14	3.58	0.04	417
S7	301.41	3.73	0.05	372.5
S8	517.66	1.51	0.06	459.5
S9	507.01	0.59	0.05	448.5
S10	500.02	0	0	392
S11	582.43	0	0	390.5
S12	655.29	1.17	0.04	369.5
S13	615.65	4.59	0.12	356.5
S14	664.44	1.6	0.06	345.5
S15	707.23	0	0	400
S16	574.41	0	0	433
S17	174.28	1.22	0.05	250.5
S18	203.01	4.07	0.11	212
S19	1667.18	2.09	0.06	212
S20	174.28	2.02	0.08	162.5
S21	176.72	1.17	0.02	227
S22	167.18	1.41	0.31	232
S23	177.95	1.04	0.05	256
S24	269.76	2.08	0.02	218
S25	341.48	1.99	0.01	211.5
S26	189.41	2.58	0.04	341.5
S27	214.59	3.23	0.05	160
S28	217.58	3.51	0.06	201
S29	217.58	3.93	0.08	226.5

S30	314.21	2.52	0.02	213
S46	204.42	6.93	0.11	182.5
S47	535.93	9.27	0.25	229
S48	707.23	9.2	0.24	228
S49	115.77	6.72	0.37	236
S50	153.83	9.2	0.51	244.5
S51	170.7	8.55	0.44	226.5
S52	570.44	6.71	0.32	226
S53	318.6	2.35	0.12	225
S54	460.1	7.86	0.23	229
S55	500.02	7.03	0.31	228
S56	1165.15	6.97	0.31	306.5
S57	1292.86	7.28	0.25	303
S58	566.49	6.84	0.28	287.5
S59	747.57	10.33	0.6	282.5
S60	291.14	7.69	0.27	231
S61	287.13	7.68	0.22	229.5
S62	697.49	7.63	0.24	240
S63	174.28	5.64	0.04	431
S64	179.19	5.35	0.04	468
S65	174.18	5.18	0.01	504
S66	167.18	5.93	0	448
S67	233.2	5.23	0.01	441.5
S68	174.28	5.92	0.02	465.5
S69	249.95	3.74	0.07	386
S70	249.9	1.82	0.09	212
S71	275.43	2.07	0.03	205.5
S72	3533.497	3.48	0.06	447.5
S73	241.43	1.97	0.02	380.5
S74	241.43	1.22	0.01	333
S75	234.83	1.44	0.05	312
S210	211.63	6.14	0.45	0
S211	198.83	3.76	0.23	25
S212	258.77	2.31	0.15	455.5
S213	*	6.92	0.23	471
S214	186.8	7.59	0.63	167.5
S215	198.83	7.87	0.68	217
S216	219.1	9.9	1.17	*
S217	214.59	5.14	0.31	402.5
S218	463.3	7.54	0.67	449.5
S219	204.42	6.93	0.11	182.5
S220	624.25	7.17	0.07	166

S221	517.66	9.03	0.07	157
S222	203.01	6.38	0.14	211
S223	203.01	6.2	0.09	226.5
S224	217.58	6.23	0.15	225
S225	211.63	6.77	0.15	202
S226	81.85	3.64	0.24	*
S227	566.4944	3.89	0.3	*
S228	201.6	2.38	0.06	*
S229	92.73	3.48	0.14	*
S230	124.94	3.7	0.13	*
S271	176.72	0.65	0.16	530.5
S272	196.09	0.91	0.09	531
S273	179.19	0.85	0.03	540
S274	307.75	7.45	0.1	302
S275	190.72	5.71	0.08	297.5
S276	469.77	7.19	0.08	272
S277	358.46	8.21	0.09	288.5
S278	378.91	5.19	0.13	290
S279	251.69	6.54	0.05	284.5
S280	343.85	7.69	0.08	330
S281	469.77	2.49	0.04	454.5
S282	493.14	2.46	0.04	401
S283	752.77	*	*	475.5
S286	655.29	3.95	0.08	501
S287	411.79	3.82	0.1	471.5
S288	260.27	6.04	0.09	388.5
S289	100.78	1.72	0.02	*
S290	116.57	0.86	0.07	*
S291	707.23	2.58	0.13	*
S292	2249.95	2.47	0.08	*
S293	624.25	4.16	0.06	*
S294	162.61	*	*	*
S295	114.97	8.38	0.6	211
S296	161.49	8.6	0.31	238
S297	161.49	8.34	0.52	185
S298	188.1	8.62	0.53	246
S299	116.57	6.58	0.4	231.5
S300	406.11	0.33	0.01	474.5
S301	456.92	10.57	0.09	452.5
S302	148.59	7.36	0.14	236.5
S303	143.53	7.45	0.13	193
S304	543.41	9.72	0.04	316.5

S305	249.95	8.31	0.11	226
S306	217.58	9.11	0.26	207.5
S307	176.72	9.13	0.29	241.5
S308	129.35	8.42	0.31	332
S309	143.53	9.05	0.5	390
S310	167.18	8.43	0.08	334

APPENDIX 4

Tables of raw data and statistical analysis taken from Chapter 5, "Investigation of the impacts of emamectin benzoate on marine sediments by long term metadata analysis".

Table A1. Univariate measures for the sampling stations (spatial and temporal) used in analysis of long terms effects of emamectin on benthic communities. The first column has the station labels (their meaning can be seen in Appendix 4, Table A2). The diversity indices as a sequence of the columns are the number of individual species N, the species richness diversity S, the Simpsons Index D, Brillouins Index Hb, Shannon Wiener Hs, Pielou Evenness P, Heip Evenness Eh, and the infaunal trophic index ITI.

station	N	S	D	Hb	Hs	P	Eh	ITI
S141	67	6	0.35	0.68	1.12	0.43	0.24	1.91
S143	32	10	0.84	1.6	2.8	0.84	0.66	6.97
S145	11	2	0.18	0.22	0.44	0.44	0.36	29.66
S146	0	0	0	0	0	0	0	0.10
S153	17	2	0.12	0.17	0.32	0.32	0.25	0.34
S154	33	4	0.28	0.48	0.84	0.42	0.27	2.16
S155	7	5	0.9	1.02	2.24	0.96	0.93	46.21
S173	36	3	0.44	0.64	1.05	0.66	0.54	1.77
S174	13	7	0.79	1.18	2.35	0.84	0.69	49.64
S175	8	3	0.61	0.64	1.3	0.82	0.73	62.09
S179	67	6	0.35	0.68	1.12	0.43	0.24	1.64
S180	0	0	0	0	0	0	0	0.26
S182	28	6	0.75	1.23	2.12	0.82	0.67	66.93
S186	5	2	0.4	0.32	0.72	0.72	0.65	57.19
S188	10	4	0.78	0.92	1.85	0.92	0.87	74.76
S191	9	4	0.58	0.69	1.45	0.72	0.58	61.30
S200	8	4	0.86	0.98	2	1	1	0.62
S201	10	4	0.64	0.78	1.57	0.79	0.66	2.15
S202	39	13	0.71	1.45	2.59	0.7	0.42	52.04
S208	74	2	0.42	0.58	0.88	0.88	0.84	0.22
S209	189	11	0.73	1.54	2.36	0.68	0.41	4.10
S210	54	11	0.58	1.19	2.04	0.59	0.31	26.70
S215	34	4	0.44	0.71	1.2	0.6	0.43	0.33
S216	196	6	0.07	0.18	0.3	0.12	0.05	26.29
S217	10	7	0.87	1.19	2.52	0.9	0.79	25.81
S227	17	2	0.22	0.29	0.52	0.52	0.44	1.58
S229	21	11	0.82	1.49	2.84	0.82	0.62	77.23
S313	53	7	0.5	0.93	1.56	0.55	0.32	46.40
S314	81	2	0.16	0.27	0.42	0.42	0.34	5.96
S316	0	0	0	0	0	0	0	18.03
S317	92	6	0.13	0.29	0.49	0.19	0.08	0.95
S318	51	2	0.04	0.08	0.14	0.14	0.1	2.32

S319	11	5	0.82	1.08	2.12	0.91	0.84	59.56
S320	202	4	0.06	0.14	0.24	0.12	0.06	13.70
S321	34	4	0.44	0.71	1.2	0.6	0.43	0.33
S322	3	2	0.67	0.37	0.92	0.92	0.89	2.32
S323	0	0	0	0	0	0	0	0.10
S324	0	0	0	0	0	0	0	0.10
S325	48	13	0.88	1.86	3.15	0.85	0.66	46.35
S326	440	12	0.2	0.47	0.73	0.2	0.06	6.68
S327	30	2	0.07	0.11	0.21	0.21	0.16	5.47
S328	324	4	0.16	0.36	0.54	0.27	0.15	0.23
S329	1047	4	0.06	0.17	0.25	0.12	0.06	0.22
S330	996	10	0.17	0.44	0.65	0.2	0.06	18.14
S331	19	7	0.8	1.28	2.35	0.84	0.68	70.22
S332	112	4	0.05	0.13	0.22	0.11	0.06	38.01
S333	108	6	0.29	0.55	0.88	0.34	0.17	8.07
S334	14	9	0.93	1.52	3.04	0.96	0.9	76.26
S335	45	11	0.74	1.52	2.6	0.75	0.51	51.66
S336	27	7	0.59	1.01	1.81	0.65	0.42	19.20
S337	10	6	0.84	1.12	2.32	0.9	0.8	68.64
S338	22	5	0.69	1.05	1.84	0.79	0.64	1.13
S339	125	11	0.44	0.95	1.52	0.44	0.19	3.50
S340	14	9	0.93	1.52	3.04	0.96	0.9	76.17
S341	45	11	0.74	1.52	2.6	0.75	0.51	51.66
S342	27	7	0.59	1.01	1.81	0.65	0.42	19.20
S343	8	5	0.86	1.01	2.16	0.93	0.86	54.40
S344	0	0	0	0	0	0	0	1.90
S345	3	2	0.67	0.37	0.92	0.92	0.89	1.40
S348	81	2	0.05	0.1	0.17	0.17	0.12	1.40
S349	92	19	0.78	1.75	2.88	0.68	0.35	64.86
S350	28	12	0.89	1.75	3.18	0.89	0.73	35.65
S351	0	0	0	0	0	0	0	51.09
S352	75	21	0.84	2.08	3.5	0.8	0.51	51.10
S353	97	12	0.49	1.11	1.82	0.51	0.23	44.16
S354	0	0	0	0	0	0	0	1.84
S361	26	13	0.94	1.89	3.49	0.94	0.85	71.80
S362	184	7	0.28	0.57	0.88	0.31	0.14	28.34
S375	0	0	0	0	0	0	0	66.70
S376	4	2	0.5	0.35	0.81	0.81	0.75	66.03
S377	0	0	0	0	0	0	0	64.83
S378	19	7	0.82	1.34	2.46	0.88	0.75	87.17
S379	62	6	0.63	1.03	1.66	0.64	0.43	2.76
S383	44	13	0.84	1.75	3.01	0.81	0.59	74.43

S384	0	0	0	0	0	0	0	0.10
S385	133	2	0.02	0.04	0.06	0.06	0.05	3.22
S386	240	3	0.02	0.05	0.08	0.05	0.03	39.88
S387	656	3	0.12	0.26	0.39	0.25	0.16	2.00
S388	216	2	0.07	0.15	0.23	0.23	0.17	2.35
S389	1328	4	0.08	0.21	0.31	0.16	0.08	1.80
S390	21	11	0.91	1.7	3.2	0.92	0.82	39.26
S391	182	16	0.32	0.79	1.29	0.32	0.1	30.73
S392	77	13	0.83	1.78	2.88	0.78	0.53	43.07
S393	76	20	0.92	2.32	3.84	0.89	0.7	51.18
S394	68	19	0.92	2.22	3.7	0.87	0.67	47.84
S395	65	17	0.9	2.15	3.59	0.88	0.69	58.28
S396	34	11	0.89	1.77	3.08	0.89	0.74	53.80
S397	67	18	0.93	2.29	3.82	0.91	0.77	53.02
S398	2461	2	0	0	0.01	0.01	0	1.41
S400	0	0	0	0	0	0	0	0.13
S401	431	5	0.3	0.57	0.85	0.36	0.2	3.89
S402	373	7	0.2	0.44	0.67	0.24	0.1	5.16
S403	297	9	0.65	1.38	2.08	0.65	0.4	11.73
S404	12	6	0.82	1.15	2.28	0.88	0.77	51.26
S405	44	10	0.72	1.39	2.37	0.71	0.46	48.84
S406	0	0	0	0	0	0	0	66.70
S407	0	0	0	0	0	0	0	0.42
S408	17	2	0.44	0.51	0.87	0.87	0.83	59.30
S409	16	3	0.63	0.81	1.42	0.9	0.84	77.56
S410	27	2	0.07	0.12	0.23	0.23	0.17	47.28
S411	28	6	0.53	0.91	1.61	0.62	0.41	68.85
S412	18	3	0.22	0.32	0.61	0.39	0.27	59.63
S413	0	0	0	0	0	0	0	0.11
S414	18	2	0.53	0.6	1	1	1	86.32
S415	0	0	0	0	0	0	0	66.79
S416	0	0	0	0	0	0	0	66.70
S417	5	5	1	0.96	2.32	1	1	57.45
S418	14	2	0.14	0.19	0.37	0.37	0.29	3.57
S419	0	0	0	0	0	0	0	0.62
S420	4	0.089	0.21	0.31	0.16	0.08	0	0.28
S421	5	4	0.9	0.82	1.92	0.96	0.93	49.80
S422	27	3	0.21	0.34	0.61	0.38	0.26	43.24
S423	0	0	0	0	0	0	0	84.86
S424	0	0	0	0	0	0	0	35.36
S425	4	3	0.83	0.62	1.5	0.95	0.91	48.20
S426	2	2	1	0.35	1	1	1	0.24

S427	11	3	0.73	0.85	1.57	0.99	0.99	16.11
S428	0	0	0	0	0	0	0	66.70
S429	0	0	0	0	0	0	0	0.10
S430	18	2	0.53	0.6	1	1	1	86.32
S431	98	8	0.58	0.96	1.52	0.51	0.27	46.10
S432	76	11	0.74	1.56	2.53	0.73	0.48	64.48
S433	46	7	0.8	1.47	2.41	0.86	0.72	37.29
S434	42	6	0.65	1.12	1.85	0.72	0.52	42.55
S435	12	2	0.55	0.57	1	1	1	54.88
S436	8	2	0.54	0.5	0.95	0.95	0.94	61.94
S437	4	2	0.67	0.45	1	1	1	84.41

Table A2. The stations and sites as labelled for both the univariate and multivariate analysis in Chapter 5.The first column has the labels used in the text of Chapter 5, the second column has the stations distance from the cage edge and its direction and the third column the site name.

stations to final sheet	detailed stations in IoA sheet	site name
S141	0m N	droig03
S143	Cont (850m) S	droig03
S145	0m SW	drum03
S146	25m SW	drum03
S153	0m N	nedd03
S154	25m S	nedd03
S155	50m S	nedd03
S173	0m W	torg03
S174	25m E	torg03
S175	50m E	torg03
S179	0m N	droig04
S180	25m S	droig04
S182	50m S	droig04
S186	25m NE	drum04
S188	25m SW	drum04
S191	50m N	nedd04
S200	0m NW	rein04

S201	25m SE	rein04
S202	50m SE	rein04
S208	0m NW	torg04
S209	25m SE	torg04
S210	50m SE	torg04
S215	0m NW	torg05
S216	25m SE	torg05
S217	50m SE	torg05
S227	50m S	Port nacro 03
S229	Ref (850m) S	portnc03
S313	150m S	Reintraid 05
S314	25m S	Nedd05
S316	150m N	Nedd05
S317	25m N	Nedd05
S318	0m N	Nedd05
S319	150m S	Torgawn05
S320	25m S	Torgawn05
S321	0m	Torgawn05
S322	150m S	Drumbeg05
S323	25m S	Drumbeg05
S324	0m S	Drumbeg05
S325	150m S	Oldany05
S326	25m S	Oldany05
S327	0m	Oldany05
S328	0m	Port na Moine 03
S329	25m N	Port na Moine 03
S330	150m N	Port na Moine 03
S331	58.HY T1 150m N	mainstream03
S332	58.HY T1 25m N	mainstream03
S333	58.HY T1 CE	mainstream03
S334	150m N	SelieNess 03

S335	25m N	SelieNess 03
S336	0m S	SelieNess 03
S337	150m N	Vatsetter 03
S338	25m N	Vatsetter 03
S339	0m	Vatsetter 03
S340	58.ST 150m N	mainstream03
S341	58.ST 25m N	mainstream03
S342	58.ST 0m N	mainstream03
S343	59.BDnC 0m	lakeland05
S344	59.BDnC 25m	lakeland05
S345	59.BDnC 150m	lakeland05
S348	60.D/Wick C/E	mainstream05
S349	60.Stead of Aith 19/04/05 150m	mainstream05
S350	60.Stead of Aith 19/04/05 25m	mainstream05
S351	60.Stead of Aith 18/04/05 CE	mainstream05
S352	60.Selie Ness T 150m	mainstream05
S353	60.Selie Ness T 25m	mainstream05
S354	60.Selie Ness T CE	mainstream05
S361	61.CN July 05 150m	panfish05
S362	61.CN July 05 Cage	panfish05
S375	61.Ard 1	panfish05
S376	61.Ard 2	panfish05
S377	61.Ard 3	panfish05
S378	61.IK 150	panfish05
S404	24, 150m N	ardmaddy2006
S405	29, 0m	Djubawick2006
S406	33, ME	Sgeir Mhor2006
S407	34, 0MS	Inchkenneth2006
S408	35, 0MW	Geasgill2006
S409	35,150MW	Geasgill2007
S410	45, N0	Bow of Hascosay2006

S411	45, N150	Bow of Hascosay2006
S412	45, N25	Bow of Hascosay2006
S413	47, 0MS	Poll na gile2006
S414	47, 150MS	Poll na gile2006
S415	49, CE	BDNC S2006
S416	50, CE	BDNC N2006
S417	56, 150M NW	Greinham2006
S418	56, 25M NW	Greinham2006
S419	56, CE NW	Greinham2006
S420	62, CE	Tolsta2006
S421	62, 150MT1	Tolsta2006
S422	62, 25MT1	Tolsta2006
S423	68, 0MSW	Ardcastle2007
S424	68, 73MSW	Ardcastle2007
S425	70, OMSW	Creag na h-iolaire2007
S426	71,0NW0	Merry Holm2007
S427	72,OSW	Furnace2007
S428	73, 0MSW	Kenmore Point2007
S429	76, N0	Setterness West2006
S430	78, 150MS	Poll na Gile2006
S431	99, 0M NW	Aird2007
S432	99, 150MNW	Aird2007
S433	99, 25MNW	Aird2007
S434	102, OMN	Kempi Bay2007
S435	102, 150M N	Kempi Bay2007
S436	103, 0MN	Sian Bay2007
S437	103, 150MN	Sian Bay2007

Table A3. The statistic analysis for the correlation of the species within the 50m and 150 m stations. The outlook of the table is from sigmastat result page.

50 metres stations

STATION k1

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Glycinde nordmani	10	47.62	47.62
2	Pholoe inornata	3	14.29	61.9
3	Sthenelais bo	2	9.52	71.43
4	Golfingia sp.	1	4.76	76.19
5	Gattyana cirros	1	4.76	80.95
6	Harmothoe impa	1	4.76	85.71
7	Eteone long	1	4.76	90.48
8	Nereimyra punctat	1	4.76	95.24
9	Nereis pelagic	1	4.76	100

STATION k2

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Glycera tridactyl	4	36.36	36.36
2	Eumida bahusiensi	2	18.18	54.55
3	Ophiodromus flexuosus	2	18.18	72.73
4	Tubulanus sp.	1	9.09	81.82
5	Cerebratulidae sp.	1	9.09	90.91
6	Goniada maculat	1	9.09	100

STATION k4

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	NEMATODA spp	16	33.33	33.33
2	Nemertea sp.	14	29.17	62.5
3	Pholoe inornata	4	8.33	70.83
4	Glycera alba	3	6.25	77.08

5	Nephtys homberg	3	6.25	83.33
6	Edwardsia claparedii	1	2.08	85.42
7	Cerianthus sp.	1	2.08	87.5
8	Priapulus caudatu	1	2.08	89.58
9	Aphrodita aculeata	1	2.08	91.67
10	Eteone long	1	2.08	93.75

STATION k5

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	NEMATODA spp	365	95.3	95.3
2	Anaitides mucosa	10	2.61	97.91
3	Eteone long	5	1.31	99.22
4	Eumida bahusiensi	2	0.52	99.74
5	Nemertea sp.	1	0.26	100

STATION k6

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	16	69.57	69.57
2	Nephtys homberg	3	13.04	82.61
3	Virgularia mirabilis	1	4.35	86.96
4	Priapulus caudatu	1	4.35	91.3
5	Pholoe synophthalmica	1	4.35	95.65
6	Nephtys kersivalensi	1	4.35	100

STATION k7

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Trypanosyllis coeliaca	19	28.79	28.79
2	Glycera lapidum	12	18.18	46.97

3	Sphaerosyllis taylori	11	16.67	63.64
4	Astrorhiza limicola	6	9.09	72.73
5	Anaitides mucosa	5	7.58	80.3
6	Cerebratulidae sp.	3	4.55	84.85
7	Malmgrenia ljungmani	2	3.03	87.88
8	Pholoe synophthalmica	2	3.03	90.91
9	Eusyllis blomstrandi	2	3.03	93.94
10	Oerstedia dorsalis	1	1.52	95.45

STATION k8

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	8	88.89	88.89
2	Nephtys sp.	1	11.11	100

STATION k9

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	NEMATODA spp	18	34.62	34.62
2	Nephtys incisa	7	13.46	48.08
3	Pholoe inornata	6	11.54	59.62
4	Tubulanus albocapitatus	5	9.62	69.23
5	Golfingia sp.	3	5.77	75
6	Edwardsia claparedii	2	3.85	78.85
7	Cerianthus lloydi	2	3.85	82.69
8	Harmothoe sp.	2	3.85	86.54
9	Eteone sp.	2	3.85	90.38
10	Glycera alba	2	3.85	94.23

STATION k10

RANK	SPECIES	NUMBER	PERCENT	CUM

				PERC
1	NEMATODA spp	564	98.6	98.6
2	Anaitides mucosa	7	1.22	99.83
3	Pholoe inornata	1	0.17	100

STATION k11

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	NEMATODA spp	22	88	88
2	Harmothoe sp.	1	4	92
3	Anaitides mucosa	1	4	96
4	Glycera alba	1	4	100

STATION k12

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Glycinde nordmani	3	37.5	37.5
2	Nemertea sp.	2	25	62.5
3	Pholoe inornata	2	25	87.5
4	Kefersteinia cirrata	1	12.5	100

STATION k13

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Glycinde nordmani	5	35.71	35.71
2	Glycera lapidum	4	28.57	64.29
3	Nemertea sp.	3	21.43	85.71
4	Cerianthus lloydi	2	14.29	100

STATION k14

RANK	SPECIES	NUMBER	PERCENT	CUM PERC

1	Tubulanus polymorphus	20	33.33	33.33
2	Edwardsia claparedii	10	16.67	50
3	Phascolion strombus	10	16.67	66.67
4	Pholoe synophthalmica	6	10	76.67
5	NEMATODA spp	5	8.33	85
6	POLYNOIDAE sp.	2	3.33	88.33
7	Anaitides mucosa	2	3.33	91.67
8	Ophiodromus flexuosus	2	3.33	95
9	Pholoe inornata	1	1.67	96.67
10	Glycera lapidum	1	1.67	98.33

STATION k15

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	NEMATODA spp	66	44.59	44.59
2	Pholoe synophthalmica	40	27.03	71.62
3	POLYNOIDAE sp.	12	8.11	79.73
4	Chrysopetalum debile	9	6.08	85.81
5	Eumida sanguinea	4	2.7	88.51
6	SIPUNCULA sp.	2	1.35	89.86
7	Phascolion strombus	2	1.35	91.22
8	Alentia gelatinosa	2	1.35	92.57
9	Eumida sp.	2	1.35	93.92
10	Glycera lapidum	2	1.35	95.27

STATION k16

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	9	52.94	52.94
2	Exogone naidina	4	23.53	76.47
3	Exogone hebes	3	17.65	94.12

4	Nephtys sp.	1	5.88	100

STATION k17

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	22	81.48	81.48
2	Nephtys sp.	5	18.52	100

STATION k18

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys incisa	4	40	40
2	Cerebratulidae sp.	3	30	70
3	Pholoe inornata	1	10	80
4	Pholoe synophthalmica	1	10	90
5	Ophiodromus flexuosus	1	10	100

STATION k19

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	4	57.14	57.14
2	Cerebratulidae sp.	1	14.29	71.43
3	Pholoe synophthalmica	1	14.29	85.71
4	Ophiodromus flexuosus	1	14.29	100

STATION k20

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys incisa	13	86.67	86.67
2	Carinomidae	1	6.67	93.33
3	Ophiodromus flexuosus	1	6.67	100

STATION k21

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys incisa	5	41.67	41.67
2	Pholoe inornata	4	33.33	75
3	Edwardsia claparedii	2	16.67	91.67
4	Tubulanus albocapitatus	1	8.33	100

STATION k22

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys incisa	7	50	50
2	Golfingia sp.	2	14.29	64.29
3	Ophiodromus flexuosus	2	14.29	78.57
4	Actiniidae sp.	1	7.14	85.71
5	Tubulanus albocapitatus	1	7.14	92.86
6	Harmothoe marphysae	1	7.14	100

STATION k23

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	1	33.33	33.33
2	Glycera rouxii	1	33.33	66.67
3	Ophiodromus flexuosus	1	33.33	100

STATION k24

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	76	96.2	96.2
2	Glycera rouxii	2	2.53	98.73
3	Ophiodromus flexuosus	1	1.27	100

STATION k25

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Pholoe inornata	6	28.57	28.57
2	Harmothoe marphysae	3	14.29	42.86
3	Golfingia sp.	2	9.52	52.38
4	Glycinde nordmanni	2	9.52	61.9
5	Edwardsia claparedii	1	4.76	66.67
6	Cerianthus lloydi	1	4.76	71.43
7	Tubulanus albocapitatus	1	4.76	76.19
8	Phascolion strombus	1	4.76	80.95
9	Aphrodita aculeata	1	4.76	85.71
10	Anaitides mucosa	1	4.76	90.48

STATION k26

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Golfingia sp.	9	32.14	32.14
2	Pholoe inornata	5	17.86	50
3	Nephtys incisa	5	17.86	67.86
4	Harmothoe marphysae	3	10.71	78.57
5	Glycera rouxii	3	10.71	89.29
6	Tubulanus albocapitatus	2	7.14	96.43
7	Edwardsia claparedii	1	3.57	100

STATION k27

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Ophiodromus flexuosus	9	45	45
2	Edwardsia claparedii	3	15	60
3	Tubulanus albocapitatus	2	10	70

4	Aphrodita aculeata	2	10	80
5	Pholoe inornata	2	10	90
6	Phascolion strombus	1	5	95
7	Anaitides mucosa	1	5	100

STATION k28

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Edwardsia claparedii	21	45.65	45.65
2	Tubulanus albocapitatus	10	21.74	67.39
3	Cerianthus lloydi	8	17.39	84.78
4	Ophiodromus flexuosus	3	6.52	91.3
5	Pholoe inornata	2	4.35	95.65
6	Aphrodita aculeata	1	2.17	97.83
7	Kefersteinia cirrata	1	2.17	100

STATION k29

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys cirrosa	16	53.33	53.33
2	Golfingia vulgari	4	13.33	66.67
3	Pholoe inornata	3	10	76.67
4	Edwardsia claparedii	2	6.67	83.33
5	Harmothoe antilope	2	6.67	90
6	Oerstedia dorsalis	1	3.33	93.33
7	Shtenelais sp.	1	3.33	96.67
8	Glycinde nordmani	1	3.33	100

STATION k30

RANK	SPECIES	NUMBER	PERCENT	CUM PERC

1	Oerstedia dorsalis	11	33.33	33.33
2	Pholoe inornata	9	27.27	60.61
3	Golfingia vulgari	5	15.15	75.76
4	Harmothoe antilope	4	12.12	87.88
5	Shtenelais sp.	2	6.06	93.94
6	Edwardsia claparedii	1	3.03	96.97
7	Nephtys cirrosa	1	3.03	100

STATION k31

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Pholoe inornata	4	28.57	28.57
2	Glycera alba	3	21.43	50
3	Nephtys cirrosa	2	14.29	64.29
4	Edwardsia claparedii	1	7.14	71.43
5	Cerianthus lloydi	1	7.14	78.57
6	Oerstedia dorsalis	1	7.14	85.71
7	Virgularia sp.	1	7.14	92.86
8	Harmothoe antilope	1	7.14	100

STATION k32

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Pholoe inornata	6	20	20
2	Edwardsia claparedii	5	16.67	36.67
3	Virgularia sp.	5	16.67	53.33
4	Glycera alba	5	16.67	70
5	Oerstedia dorsalis	3	10	80
6	Golfingia vulgari	1	3.33	83.33
7	Eumida sanguinea	1	3.33	86.67
8	Glycinde nordmani	1	3.33	90

9	Phyllodocidae sp.	1	3.33	93.33
10	Goniada sp.	1	3.33	96.67

STATION k33

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Edwardsia claparedii	4	57.14	57.14
2	Nephtys incisa	3	42.86	100

STATION k34

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Edwardsia claparedii	2	40	40
2	Tubulanus albocapitatus	1	20	60
3	Virgularia mirabilis	1	20	80
4	Nephtys incisa	1	20	100

STATION k35

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	NEMATODA spp	39	60	60
2	Anaitides mucosa	24	36.92	96.92
3	Cerebratulidae sp.	2	3.08	100

STATION k36

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	ACTINARIA sp.	4	23.53	23.53
2	Cerebratulidae sp.	4	23.53	47.06
3	Pholoe synophthalmica	2	11.76	58.82
4	Exogone hebes	2	11.76	70.59
5	Exogone naidina	2	11.76	82.35

6	Nephtys hombergii	2	11.76	94.12
7	Anaitides mucosa	1	5.88	100

STATION k37

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Harmothoe sp.	3	60	60
2	Anaitides mucosa	1	20	80
3	Glycera alba	1	20	100

STATION k38

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Tubulanus polymorphus	8	38.1	38.1
2	Glycera alba	7	33.33	71.43
3	Nephtys hombergii	2	9.52	80.95
4	Astrorhiza limicola	1	4.76	85.71
5	Eumida ockelmanni	1	4.76	90.48
6	Glycinde nordmanni	1	4.76	95.24
7	Ophiodromus flexuosus	1	4.76	100

STATION k40

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Glycera alba	3	60	60
2	Eteone long	1	20	80
3	Nephtys cirrosa	1	20	100

STATION k41

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	17	85	85

2	Glycera alba	2	10	95
3	Nephtys hombergii	1	5	100

STATION k42

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	4	57.14	57.14
2	Cerebratulidae sp.	1	14.29	71.43
3	Pholoe synophthalmica	1	14.29	85.71
4	Ophiodromus flexuosus	1	14.29	100

STATION k43

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys incisa	2	50	50
2	Cerebratulidae sp.	1	25	75
3	Nephtys hombergii	1	25	100

STATION k44

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Pholoe inornata	4	19.05	19.05
2	Nephtys cirrosa	3	14.29	33.33
3	Virgularia mirabilis	1	4.76	38.1
4	Lepidonotus sp.	1	4.76	42.86
5	Priapulus caudatu	1	4.76	47.62
6	Phascolion strombus	1	4.76	52.38
7	Harmothoe antilope	1	4.76	57.14
8	ANNELIDA sp.	1	4.76	61.9
9	Glycera alba	1	4.76	66.67
10	Musculus sp.	1	4.76	71.43

STATION k45

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	NEMATODA spp	4	50	50
2	Cerebratulidae sp.	2	25	75
3	Glycera lapidum	1	12.5	87.5
4	Exogone hebes	1	12.5	100

STATION k46

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	21	87.5	87.5
2	Exogone naidina	2	8.33	95.83
3	Cerebratulidae sp.	1	4.17	100

STATION k47

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	8	42.11	42.11
2	Harmothoe sp.	3	15.79	57.89
3	Nephtys cirrosa	3	15.79	73.68
4	Pholoe inornata	2	10.53	84.21
5	Actiniidae sp.	1	5.26	89.47
6	Eteone sp.	1	5.26	94.74
7	Ophiodromus flexuosus	1	5.26	100

STATION k48

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys hombergii	21	87.5	87.5
2	Anaitides mucosa	2	8.33	95.83

3	Pholoe inornata	1	4.17	100

STATION k49

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys hombergii	9	81.82	81.82
2	Eteone sp.	1	9.09	90.91
3	Anaitides mucosa	1	9.09	100

STATION k50

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Sphaerosyllis taylori	21	25.3	25.3
2	Tubulanus polymorphus	19	22.89	48.19
3	Exogone naidina	17	20.48	68.67
4	Eumida ockelmanni	8	9.64	78.31
5	Pholoe inornata	5	6.02	84.34
6	Edwardsia claparedii	4	4.82	89.16
7	Cerebratulidae sp.	4	4.82	93.98
8	Glycera alba	3	3.61	97.59
9	Aphrodita aculeata	1	1.2	98.8
10	Anaitides mucosa	1	1.2	100

STATION k51

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Tubulanus polymorphus	8	44.44	44.44
2	Nephtys hombergii	3	16.67	61.11
3	Glycera alba	2	11.11	72.22
4	Exogone naidina	2	11.11	83.33
5	Hesionidae sp.	1	5.56	88.89

6	Ophiodromus flexuosus	1	5.56	94.44
7	Nephtys sp.	1	5.56	100

STATION k52

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Exogone verugera	36	48.65	48.65
2	Glycera lapidum	21	28.38	77.03
3	Astrorhiza limicola	6	8.11	85.14
4	Sphaerosyllis taylori	6	8.11	93.24
5	Exogone naidina	4	5.41	98.65
6	Tubulanus polymorphus	1	1.35	100

STATION k53

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	29	78.38	78.38
2	Exogone hebes	3	8.11	86.49
3	Exogone verugera	2	5.41	91.89
4	Pholoe inornata	1	2.7	94.59
5	Exogone naidina	1	2.7	97.3
6	Sphaerosyllis taylori	1	2.7	100

STATION k54

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Pholoe synophthalmica	4	30.77	30.77
2	Glycera alba	3	23.08	53.85
3	Phascolion strombus	2	15.38	69.23
4	Sphaerosyllis tetralix	2	15.38	84.62
5	Ophiodromus flexuosus	1	7.69	92.31

6	Sphaerosyllis taylori	1	7.69	100

STATION k55

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Anaitides mucosa	2	33.33	33.33
2	Edwardsia claparedii	1	16.67	50
3	Phascolion strombus	1	16.67	66.67
4	Sphaerosyllis tetralix	1	16.67	83.33
5	Sphaerosyllis taylori	1	16.67	100

STATION k58

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Glycera alba	3	30	30
2	Pholoe synophthalmica	2	20	50
3	Edwardsia claparedii	1	10	60
4	Phascolion strombus	1	10	70
5	Exogone hebes	1	10	80
6	Exogone naidina	1	10	90
7	Exogone verugera	1	10	100

STATION k59

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Glycera alba	1	25	25
2	Glycinde nordmanni	1	25	50
3	Eusyllis blomstrandi	1	25	75
4	Sphaerosyllis taylori	1	25	100

STATION k60

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	NEMATODA spp	18	34.62	34.62
2	Nephtys incisa	7	13.46	48.08
3	Pholoe inornata	6	11.54	59.62
4	Tubulanus albocapitatus	5	9.62	69.23
5	Golfingia sp.	3	5.77	75
6	Edwardsia claparedii	2	3.85	78.85
7	Cerianthus lloydi	2	3.85	82.69
8	Harmothoe sp.	2	3.85	86.54
9	Eteone sp.	2	3.85	90.38
10	Glycera alba	2	3.85	94.23

150 m stations

STATION m1

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Glycinde nordmani	5	27.78	27.78
2	Golfingia sp.	4	22.22	50
3	Sthenelais bo	2	11.11	61.11
4	Nephtys caec	2	11.11	72.22
5	Edwardsia claparedii	1	5.56	77.78
6	Nemertea sp.	1	5.56	83.33
7	Eteone long	1	5.56	88.89
8	Glycera lapidum	1	5.56	94.44
9	Nereis pelagic	1	5.56	100

STATION m3

RANK	SPECIES	NUMBER	PERCENT	CUM PERC

1	NEMATODA spp	56	72.73	72.73
2	Nemertea sp.	7	9.09	81.82
3	Eteone long	5	6.49	88.31
4	Priapulus caudatu	3	3.9	92.21
5	Anaitides groenlandic	3	3.9	96.1
6	Cerebratulidae sp.	1	1.3	97.4
7	Pholoe inornata	1	1.3	98.7
8	Nereis longissim	1	1.3	100

STATION m4

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys homberg	2	40	40
2	Nemertea sp.	1	20	60
3	Pholoe synophthalmica	1	20	80
4	Platynereis dumerili	1	20	100

STATION m5

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys incisa	5	33.33	33.33
2	Pholoe inornata	4	26.67	60
3	Glycera alba	2	13.33	73.33
4	Edwardsia claparedii	1	6.67	80
5	Golfingia sp.	1	6.67	86.67
6	Harmothoe sp.	1	6.67	93.33
7	Eteone sp.	1	6.67	100

STATION m6

RANK	SPECIES	NUMBER	PERCENT	CUM PERC

1	Nemertea sp.	4	50	50
2	Glycinde nordmani	3	37.5	87.5
3	Glycera lapidum	1	12.5	100

STATION m7

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Pholoe inornata	9	50	50
2	Nephtys incisa	9	50	100

STATION m8

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Tubulanus albocapitatus	5	19.23	19.23
2	Nephtys incisa	4	15.38	34.62
3	Actiniidae sp.	3	11.54	46.15
4	NEMATODA spp	3	11.54	57.69
5	Golfingia sp.	3	11.54	69.23
6	Harmothoe marphysae	3	11.54	80.77
7	Aphrodita aculeata	2	7.69	88.46
8	Tubulanidae spp	1	3.85	92.31
9	Pholoe inornata	1	3.85	96.15
10	Glycera rouxii	1	3.85	100

STATION m9

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Edwardsia claparedii	1	16.67	16.67
2	Tubulanus albocapitatus	1	16.67	33.33
3	Pholoe inornata	1	16.67	50
4	Glycera rouxii	1	16.67	66.67

5	Glycinde nordmanni	1	16.67	83.33
6	Ophiodromus flexuosus	1	16.67	100

STATION m10

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Golfingia sp.	8	33.33	33.33
2	Edwardsia claparedii	5	20.83	54.17
3	Pholoe inornata	4	16.67	70.83
4	Glycera rouxii	3	12.5	83.33
5	Harmothoe marphysae	2	8.33	91.67
6	Tubulanus albocapitatus	1	4.17	95.83
7	Nephtys incisa	1	4.17	100

STATION m11

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Nephtys incisa	2	40	40
2	Edwardsia claparedii	1	20	60
3	Tubulanus albocapitatus	1	20	80
4	Ophiodromus flexuosus	1	20	100

STATION m12

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Pholoe inornata	9	50	50
2	Nephtys incisa	9	50	100

STATION m13

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Golfingia vulgari	5	25	25

2	Oerstedia dorsalis	4	20	45
3	Nephtys cirrosa	3	15	60
4	Edwardsia claparedii	1	5	65
5	Cerianthus lloydi	1	5	70
6	Priapulus caudatu	1	5	75
7	Pholoe inornata	1	5	80
8	Eteone long	1	5	85
9	Glycera alba	1	5	90
10	Glycinde nordmani	1	5	95

STATION m15

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Oerstedia dorsalis	6	30	30
2	Golfingia vulgari	4	20	50
3	Goniada maculat	2	10	60
4	Edwardsia claparedii	1	5	65
5	Cerianthus lloydi	1	5	70
6	Phascolion strombus	1	5	75
7	Aphrodita aculeata	1	5	80
8	Sthenelais sp.	1	5	85
9	Eumida sanguinea	1	5	90
10	Sphaerodorum gracili	1	5	95

STATION m16

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Phascolion strombus	4	33.33	33.33
2	Glycera alba	2	16.67	50
3	Aphrodita aculeata	1	8.33	58.33
4	Pholoe synophthalmica	1	8.33	66.67

5	Eumida sanguinea	1	8.33	75
6	Exogone hebes	1	8.33	83.33
7	Sphaerosyllis tetralix	1	8.33	91.67
8	Sphaerosyllis taylori	1	8.33	100

STATION m17

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Phascolion strombus	1	33.33	33.33
2	Anaitides mucosa	1	33.33	66.67
3	Exogone hebes	1	33.33	100

STATION m18

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Pholoe synophthalmica	3	30	30
2	Glycera alba	2	20	50
3	Exogone naidina	2	20	70
4	Exogone verugera	2	20	90
5	Phascolion strombus	1	10	100

STATION m19

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	NEMATODA spp	44	48.35	48.35
2	Actiniidae sp.	11	12.09	60.44
3	Edwardsia sp.	8	8.79	69.23
4	Kefersteinia cirrata	6	6.59	75.82
5	Sipunculan sp.	5	5.49	81.32
6	Glycera alba	5	5.49	86.81
7	Nemertea sp.	4	4.4	91.21

8	Pholoe inornata	2	2.2	93.41
9	Glycinde nordmanni	2	2.2	95.6
10	Harmothoe sp.	1	1.1	96.7

STATION m20

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Glycera sp.	6	37.5	37.5
2	Glycera alba	6	37.5	75
3	Harmothoe antilope	2	12.5	87.5
4	Virgularia sp.	1	6.25	93.75
5	Hesiospina sp.	1	6.25	100

STATION m21

RANK	SPECIES	NUMBER	PERCENT	CUM PERC
1	Hesiospina sp.	35	87.5	87.5
2	Glycera sp.	2	5	92.5
3	Glycera alba	2	5	97.5
4	Virgularia sp.	1	2.5	100

Excel raw statistic analysis

	150m	50m	
total	52	72	0.722222
top 5	nematoda	nematoda	0.60
	hesiospinae	anaitides	
	pholoe	exogone	
	nephtys	neptyhs	
	golfingia	pholoe	

Table A4. Table of the median particle size analysis, carbon percentage, redox potential (environmental parameters) and SLICE concentrations from the sampling stations (temporal and spatial). For the Kishorn sites at the first column, a, b, c and d indicators mean the four separate sample trials.

Stations **Site / year /cage distance / direction**	**MPSA** **µm**	**%C**	**Redox** **mV**	**SLICE** **ng/g**
Droigniche 03 0m N	752.77	11.36	470	0
Droigniche 03 850m S	378.91	10.7	512.5	0
Drumbeg 03 0m SW	239.76	8.97	49	0
Drumbeg 03 25m SW	208.72	8.84	41	3.3
Drumbeg 03 50m SW	176.72	8.72	569.5	0
Nedd 03 0m N	210.17	3.22	223.5	0
Nedd 03 25m S	163.74	3.64	394.5	0
Nedd 03 50m S	171.88	4.11	608.5	0
Torgawn 03 0m W	152.77	7.83	262.5	0
Torgawn 03 25m E	234.85	8.19	176.5	0
Torgawn 03 50m E	198.83	4.64	579	0
Droigniche 04 0m N	2001.11	10.89	0	1.68
Droigniche 04 25m S	870.77	11.8	0	7.11
Droigniche 04 50m S	258.77	5.74	116	2.07
Drumberg 04 25m NE	214.59	7.32	89	0
Drumberg 04 25m SW	203.01	8.03	157.5	0
Nedd 04 50m N	707.23	10.56	18	0
Reintraid 04 0m NW	162.61	7.01	166.5	2.02
Reintraid 04 25m SE	162.61	7.04	338	2.9
Reintraid 04 50m SE	88.34	7.66	580	2.65
Torgawn 04 0m NW	176.72	9.24	107	1.68
Torgawn 04 25m SE	438.3	10.44	219	7.11
Torgawn 04 50m SE	211.63	6.14	80	2.07
Torgawn 05 0m NW	198.83	7.87	297	2.07
Torgawn 05a 25m SE	219.1	9.9	482.5	4.96

Torgawn 05a 50m SE	214.59	5.14	482.5	2.48
Portnacro 03 50m S	566.49	3.89	224	2.09
Portnacro 03 850m S	92.73	3.48	209	0
Reintraid 05 150m	162.61	7.01	165.4	1.08
Reintraid 05 25m	162.61	7.04	338	0
Nedd 05 150m	0.91	4.11	456	0
Nedd 05 25m	0.71	2.72	0	0
Nedd 05 0m	301.41	7.99	168	0
Torgawn 05 150m	198.83	7.87	297	2.07
Torgawn 05 25m	219.1	9.9	0	4.41
Torgawn 05 0m	1.81	10.5	0	0
Drumbeg 05 150m	176.72	9.08	157.5	0
Drumbeg 05 25m	203.01	8.03	207	4.2
Drumbeg 05 0m	258.77	2.31	535.5	0
Oldany 05 150m	0.13	3.01	579	0
Oldany 05 25m	0.22	5.16	566	0
Oldany 05 0m	81.85	9.43	112	0
Port na moine 03 0m	0.02	10.52	0	0
Port na moine 03 25m	204.42	8.55	305.5	2.26
Port na moine 03 150m	204.42	7.88	333	3.67
HascosaY 03 150m	9.13	2.58	300	0
HascosaY 03 25m	9.11	3.51	436	0
HascosaY 03 0m	203.01	7.07	585.5	0
SelieNess 03 150m	570.44	6.71	306	0
SeleiNess 03 25m	170.7	8.55	306.5	0
SelieNess 03 0m	10.58	2.58	462	0
VatsetterR 03 150m	233.2	5.23	521.5	0
VatsetteR 03 25m	167.18	5.93	528	0
VatsetteR 0m	115.77	6.72	316	0

SteadAithness 03 150m	153.83	4.85	533.5	0.42
SteadAithness 03 25m	128.46	7.18	220.5	0
SteadAithness 03 0m	123.22	4.61	314	0
BaghDialnanCaenn 05 0m	611.39	5.13	611.5	0
BaghDialnanCaenn 05 25m	91.45	3.86	384.5	0.83
BaghDialnanCaenn 05 150m	85.33	3.94	1	0
DjubaWick 05 0m	82.42	3.83	101	0
Stead of Aith 05 150m	3.75	4.85	575	1.62
Stead of Aith 05 25m	2.34	7.18	552	1.6
Stead of Aith 05 0m	128.46	7.18	218	0
Selie Ness 05 150m	570.44	6.71	306	0
Selie Ness 05 25m	170.7	8.55	306.5	0
Selie Ness 05 0m	3.29	7.07	578	0
CoNnel 2005 150m	453.76	0.37	552	0.64
ConNel 2005 Cage	602.97	0.29	470	0
Ardmaddy 2005 0m	175.5	0.34	542	0.35
Ardmaddy 2005 25m	175.5	1.21	363	0.35
Ardmaddy 2005 150m	169.52	2.14	360	0
InchKenneth 2005 150m	163.74	4.69	162.6	1.11
InchKenneth 2005 25m	203.01	6.03	164	0.72
Chalmershope 2005 0m	267.9	2.65	541	7.34
WestKyles 2005 150m	275.43	4.01	0	0
WestKyles 2005 25m	283.18	2.74	558	0
WestKyles 2005 0m	496.57	3.11	442	3.8
Kishorn 2001a 0m	0.03	3.89	0	1.9
Kishorn 2001a 25m	0.04	2.44	0	1.9
Kishorn 2001a 150m	0.13	2.76	0	1.9
Kishorn 2001b 0m	0.03	3.83	0	2.3
Kishorn 2001b 25m	0.52	2.02	0	1.9

Kishorn 2001b 150m	0.18	1.56	0	0
Kishorn 2001c 0m	0.04	2.48	0	1.9
Kishorn 2001c 25m	0.06	2.34	0	0
Kishorn 2001c 150m	0.07	2.48	0	1.9
Kishorn 2001d 0m	0.06	3.6	0	4.1
Kishorn 2001d 25m	0.15	1.59	0	1.9
Kishorn 2002 0m	0.04	3.44	0	3.5
Kishorn 2002 25m	0.04	2.2	0	1.9
Kishorn 2002 150m	0.04	2.38	0	1.9
Kishorn 2004 0m	0.03	5.9	0	13.4
Kishorn 2004 25m	0.06	2.21	0	2
Kishorn 2004 150m	0.11	1.75	0	0
Ardmaddy 2006 150m N	61	0	151.5	0
Djubawick 2006 0m S	33	3.72	453.5	0.5
Sgeir mhor 2006 0m E	2	0	568	0.1
Inchkenneth 2006 0m S	52	0	324	1.967
Geasgill 2006 0m W	38	1.34	421.5	0.5
Geasgill 2006 150m W	15	0	220	0.533
Bow of Hascosay 2006 0m N	16	0	340.5	0.5
Bow of Hascosay 2006 150m N	34	25	346.5	0.5
Bow of Hascosay 2006 25m N	0	0	392.5	0.5
Poll na gile 2006 0m S	2	1.8	606.5	0.5
Poll na gile 2006 150m S	59	0	334.5	1.9 67
BDNC 2005 0m NW	2	0	553	0.1
BDNC 2006 0m NW	2	0	524	0.1
Greinham 2006 150m NW	1	0	431.5	0.1
Greinham 2006 25m NW	36	0	459.5	0.1
Greinham 2006 0m NW	1	1.5	554	0.1
Tolsta 2006 0m NW	26	0	358	1.133

Tolsta 2006 150m	41	3.3	366.5	0.5
Tolsta 2006 25m	58	0	0	2.48
Ardcastle 2007 0m SW	39	0	214	0.49
Ardcastle 2007 0m SW	41	6.6	253.5	0
Creag na hiolaire 2007 0m NW	75	20	217.25	0.78
merry Holm 2007 0m NW	11	0	288	2.037
Furnace 2007 0m SW	66	6.5	224	0
Kenmore Point 2007 0m SW	32	4.8	207.5	0
Setterness West 2006 0m N	19	0	203	0.823
Poll na Gile 2006 150m S	31	0.8	426.5	0.9
Aird 2007 0m NW	35	0.76	364.5	0.5
Aird 2007 150m NW	35	1.56	358	0.733
Aird 2007 25m NW	16	0	356.5	1.033
Kempi Bay 2007 0m N	19	1.57	220.5	0.5
Kempi Bay 2007 150m N	17	1.23	437	0.73
Sian Bay 2007 0m N	12	0.7	185.5	0.5

Table A5. Table of univariate measures calculated for the sampling stations used for comparison of Emamectin benzoate sediment concentrations. The table contains the stations along with Wiener index (Hs), Pielou index (P), Heip Evenness (Eh), Infaunal trophic index (ITI) and Azti's marine biotic index (AMBI). For the Kishorn sites at the first column, a, b, c and d indicators mean the four separate sample trials.

Station **Site/year/cage distance/direction**	**Hs**	**P**	**Eh**	**ITI**	**AMBI**
Droigniche 03 0m N	1.12	0.43	0.24	1.91	6
Droigniche 03 850m S	2.8	0.84	0.66	6.97	5
Drumbeg 03 0m SW	0.44	0.44	0.36	29.66	4
Drumbeg 03 25m SW	0	0	0	0.10	6
Drumbeg 03 50m SW	0.32	0.32	0.25	0.34	6
Nedd 03 0m N	0.84	0.42	0.27	2.16	5
Nedd 03 25m S	2.24	0.96	0.93	46.21	2
Nedd 03 50m S	1.05	0.66	0.54	1.77	5
Torgawn 03 0m W	2.35	0.84	0.69	49.64	2
Torgawn 03 25m E	1.3	0.82	0.73	62.09	2
Torgawn 03 50m E	1.12	0.43	0.24	1.64	6
Droigniche 04 0m N	0	0	0	0.26	6
Droigniche 04 25m S	2.12	0.82	0.67	66.93	2
Droigniche 04 50m S	0.72	0.72	0.65	57.19	2
Drumberg 04 25m NE	1.85	0.92	0.87	74.76	2
Drumberg 04 25m SW	1.45	0.72	0.58	61.30	2
Nedd 04 50m N	2	1	1	0.62	6
Reintraid 04 0m NW	1.57	0.79	0.66	2.15	6
Reintraid 04 25m SE	2.59	0.7	0.42	52.04	2
Reintraid 04 50m SE	0.88	0.88	0.84	0.22	6
Torgawn 04 0m NW	2.36	0.68	0.41	4.10	5
Torgawn 04 25m SE	2.04	0.59	0.31	26.70	2
Torgawn 04 50m SE	1.2	0.6	0.43	0.33	6
Torgawn 05 0m NW	0.3	0.12	0.05	26.29	4

Torgawn 05a 25m SE	2.52	0.9	0.79	25.81	3
Torgawn 05a 50m SE	0.52	0.52	0.44	1.58	6
Portnacro 03 50m S	2.84	0.82	0.62	77.23	2
Portnacro 03 Ref S	1.56	0.55	0.32	46.40	3
Reintraid 05 150m	0.42	0.42	0.34	5.96	5
Reintraid 05 25m	0	0	0	18.03	3
Nedd 05 150m	0.49	0.19	0.08	0.95	5
Nedd 05 25m	0.14	0.14	0.1	2.32	3
Nedd 05 0m	2.12	0.91	0.84	59.56	2
Torgawn 05 150m	0.24	0.12	0.06	13.70	4
Torgawn 05 25m	1.2	0.6	0.43	0.33	6
Torgawn 05 0m	0.92	0.92	0.89	2.32	6
Drumbeg 05 150m	0	0	0	0.10	3
Drumbeg 05 25m	0	0	0	0.10	6
Drumbeg 05 0m	3.15	0.85	0.66	46.35	2
Oldany 05 150m	0.73	0.2	0.06	6.68	2
Oldany 05 25m	0.21	0.21	0.16	5.47	5
Oldany 05 0m	0.54	0.27	0.15	0.23	6
Port na moine 03 0m	0.25	0.12	0.06	0.22	6
Port na moine 03 25m	0.65	0.2	0.06	18.14	5
Port na moine 03 150m	2.35	0.84	0.68	70.22	1
HascosaY 03 150m	0.22	0.11	0.06	38.01	2
HascosaY 03 25m	0.88	0.34	0.17	8.07	5
HascosaY 03 CE	3.04	0.96	0.9	76.26	2
SelieNess 03 150m	2.6	0.75	0.51	51.66	2
SeleiNess 03 25m	1.81	0.65	0.42	19.20	5
SelieNess 03 CE	2.32	0.9	0.8	68.64	2
VatsetterR 03 150m	1.84	0.79	0.64	1.13	6
VatsetteR 03 25m	1.52	0.44	0.19	3.50	5

VatsetteR 03 CE	3.04	0.96	0.9	76.17	2
SteadAithness 03 150m	2.6	0.75	0.51	51.66	2
SteadAithness 03 25m	1.81	0.65	0.42	19.20	5
SteadAithness 03 CE	2.16	0.93	0.86	54.40	3
BaghDialnanCaenn 05 0m	0	0	0	1.90	6
BaghDialnanCaenn 05 25m	0.92	0.92	0.89	1.40	6
BaghDialnanCaenn 05 150m	0.17	0.17	0.12	1.40	3
DjubaWick 05 CE	2.88	0.68	0.35	64.86	4
Stead of Aith 05 150m	3.18	0.89	0.73	35.65	2
Stead of Aith 05 25m	0	0	0	51.09	2
Stead of Aith 05 CE	3.5	0.8	0.51	51.10	2
Selie Ness 05 150m	1.82	0.51	0.23	44.16	6
Selie Ness 05 25m	0	0	0	1.84	2
Selie Ness 05 0m	3.49	0.94	0.85	71.80	2
CoNnel 05 150m	0.88	0.31	0.14	28.34	2
ConNel 05 0m	0	0	0	66.70	2
Ardmaddy 05 0m	0.81	0.81	0.75	66.03	2
Ardmaddy 05 25m	0	0	0	64.83	2
Ardmaddy 05 150m	2.46	0.88	0.75	87.17	6
InchKenneth 05 150m	1.66	0.64	0.43	2.76	2
InchKenneth 05 25m	3.01	0.81	0.59	74.43	3
Chalmershope 05 0m	0	0	0	0.10	3
WestKyles 05 150m	0.06	0.06	0.05	3.22	2
WestKyles 05 25m	0.08	0.05	0.03	39.88	5
WestKyles 05 CE	0.39	0.25	0.16	2.00	5
Kishorn 01a 0m	0.23	0.23	0.17	2.35	5
Kishorn 01a 25m	0.31	0.16	0.08	1.80	2
Kishorn 01a 150m	3.2	0.92	0.82	39.26	2
Kishorn 01b 0m	1.29	0.32	0.1	30.73	2

Kishorn 01b 25m	2.88	0.78	0.53	43.07	2
Kishorn 01b 150m	3.84	0.89	0.7	51.18	2
Kishorn 01c 0m	3.7	0.87	0.67	47.84	2
Kishorn 01c 25m	3.59	0.88	0.69	58.28	2
Kishorn 01c 150m	3.08	0.89	0.74	53.80	2
Kishorn 01d 0m	3.82	0.91	0.77	53.02	4
Kishorn 01d 25m	0.01	0.01	0	1.41	7
Kishorn 02 0m	0	0	0	0.13	5
Kishorn 02 25m	0.85	0.36	0.2	3.89	5
Kishorn 02 150m	0.67	0.24	0.1	5.16	4
Kishorn 04 0m	2.08	0.65	0.4	11.73	2
Kishorn 04 25m	2.28	0.88	0.77	51.26	2
Kishorn 04 150m	2.37	0.71	0.46	48.84	2
Ardmaddy 2006 150m N	0	0	0	66.70	6
Djubawick 2006 0m S	0	0	0	0.42	2
Sgeir mhor 2006 0m E	0.87	0.87	0.83	59.30	2
Inchkenneth 2006 0m S	1.42	0.9	0.84	77.56	2
Geasgill 2006 0m W	0.23	0.23	0.17	47.28	2
Geasgill 2006 150m W	1.61	0.62	0.41	68.85	2
Bow of Hascosay 2006 0 m N	0.61	0.39	0.27	59.63	6
Bow of Hascosay 2006 150m N	0	0	0	0.11	2
Bow of Hascosay 2006 25m N	1	1	1	86.32	2
Poll na gile 2006 0m S	0	0	0	66.79	2
Poll na gile 2006 150m S	0	0	0	66.70	2
BDNC S 2006 CE NW	2.32	1	1	57.45	6
BDNC N 2006 CE NW	0.37	0.37	0.29	3.57	6
Greinham 2006 150m NW	0	0	0	0.62	6
Greinham 2006 25m NW	0.16	0.08	0	0.28	2
Greinham 2006 CE NW	1.92	0.96	0.93	49.80	2

Tolsta 2006 CE NW	0.61	0.38	0.26	43.24	2
Tolsta 2006 150m	0	0	0	84.86	2
Tolsta 2006 25m	0	0	0	35.36	2
Ardcastle 2007 0m SW	1.5	0.95	0.91	48.20	6
Ardcastle 2007 0m SW	1	1	1	0.24	4
Creag na h-iolaire 2007 0m SW	1.57	0.99	0.99	16.11	2
merry Holm 2007 0m NW	0	0	0	66.70	6
Furnace 2007 0m SW	0	0	0	0.10	2
Kenmore Point 2007 0m SW	1	1	1	86.32	2
Setterness West 2006 0m N	1.52	0.51	0.27	46.10	2
Poll na Gile 2006 150m S	2.53	0.73	0.48	64.48	2
Aird 2007 0m NW	2.41	0.86	0.72	37.29	3
Aird 2007 150m NW	1.85	0.72	0.52	42.55	2
Aird 2007 25m NW	1	1	1	54.88	2
Kempi Bay 2007 0m N	0.95	0.95	0.94	61.94	2
Kempi Bay 2007 150m N	1	1	1	84.41	6

APPENDIX 5

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A combination of selected indexes for assessing the environmental impact of marine fish farms using long term metadata analysis

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Abstract

Several biological indexes can be used to assess environmental impacts of aquaculture in the aquatic ecosystem. Some biological indices are used within environmental legislative and policy frameworks which aim to monitor the impact of marine aquaculture and regulate the operation of fish farms. In Scotland, the impact of fish farms is assessed according to benthic ecosystem status compared with modeled organic loading. The purpose of this paper is to evaluate the benefits of using an optimal combination of a minimal number of selected benthic and aquatic parameters which can provide accurate and reliable information about the benthic status around the fish farm sites in Scotland. The data analyzed in this paper were obtained from the Institute of Aquaculture (IoA), of University of Stirling, and were collected from various fish farm sites across Scotland over several years. Macrofaunal and physico-chemical parameters included in the analysis were: Median Particle Size Analysis (MPSA); total sediment Carbon (C% by dw); total sediment Nitrogen (N% by dw) and Redox Potential (Eh). In this analysis a number of diversity and trophic level based indices were also used - including the Shannon Index (H'), the Infaunal Trophic Index (ITI) and the Azti's Marine Biotic Index (AMBI) - to asses the biotic status of the sites. Univariate and multivariate analysis of the data indicated that a combination of Abundance (N), H' and AMBI as biological indexes for describing the status of the ecological level along with the carbon percentage and redox potential appeared to be the give the best representation of change. This combination is even more accurate over a series of sampling stations and time points, rather than for a single site only, offering a convenient method for assessing the risk of aquaculture pollution of biotopes bellow or adjacent to floating marine fish farm cages.

Keywords: Environmental impact, Marine fish farms, Long term metadata analysis

Introduction

Many tools have been created to identify the level of pollution impacts on the marine environment due to the increase in anthropogenic activities, such as aquaculture. Aquaculture is an activity which increases nutrient enrichment in sediments beneath sea cages (Karakassis et al. 2000). Environmental changes due to this enrichment can be monitored using a range of direct physico-chemical measurements (SEPA 2005) combined with calculation of biotic indices based on invertebrate community structure (Telfer and Beveridge 2001a).

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A number of such indices are used including simple species richness, species/abundance diversity measures and trophic indices. These measurements are also used widely for defining environmental quality standards (EQSs) by environmental regulators and legislators. For example, the Scottish Environment Protection Agency (SEPA) has a requirement for the Infaunal Trophic Index (ITI) (Codling and Ashley 1992) to be included in annual or bi-annual monitoring assessments. AMBI is a popular numerical tool used in many industrial and research centres in order to monitor benthic ecological quality.

This is often used along with abundance (N), Shannon Index (H') and the chemical measurements, such as carbon, and nitrogen and redox potential (Lazaro et al. 2005). These indices are used to give information about the biotic community present at seabed sites and they particularly emphasise the trophic and distributions of species and their relative abundance, which can be used as an indication of environmental quality (Borja et al. 2000; Maurer et al. 1999).

This study aims to evaluate a combination of indexes and identify subsets of parameters that best describe environmental conditions and biological traits in marine salmon farming. The results are discussed in the context of improving the methodology for assessing the environmental conditions in marine aquaculture sites.

Materials and methods

The data used in the present paper were obtained from the Institute of Aquaculture (IoA), University of Stirling and were collected from 309 sampling stations around Scottish marine cage fish farms in accordance to the SEPA policy of statutory regulatory environmental monitoring studies at marine fish farms. Medium Particle Size Analysis (MPSA), carbon percentage (C%), nitrogen percentage (N%) and redoxpotential (Eh) at each sampling stations were measured using standard methods (SEPA 2005). Macrofauna were sampled using a standard size grab sampler (Van Veen 0.025 m^2) as five replicates for each stations and the species richness and abundance counts per unit area calculated after sorting by eye. Using the macrofauna data, the values of the following biological indicators was calculated:

- Number of individuals (N) in five replicates per station
- Number of species (S) in five replicates per station
- Infaunal Trophic Index (ITI)
- AZTI's MBI
- Simpsons Index (D)
- Brillouins Index (Hb)
- Shannon Index (H')
- Pielou Evenness (P)
- Heip Evenness (Eh)

ITI is a biotic index with a score between 0 and 100. In nutrient influenced conditions, such as estuaries, a value of 0 to 30 is considered highly disturbed, 30 to 60, moderately disturbed and 60 to 100, indicative of background (undisturbed) conditions (Word 1987; Codling and Ashley 1992). In the present work, in order to obtain comparable range of ITI values with the other biological indices, the ITI scores were altered by deducting 100 from all the ITI values and then multiplied by 0.07 (to approach the AMBI scaling correlation).

The AZTI Marine Biological Index (AMBI) (Borja et al. 2000; Borja and Muxika 2005) assigns a score on the basis of interactions and presence of species from different trophic levels. The score is directly related to good or poor quality environmental conditions (Borja et al. 2000; Borja and Muxika 2005). The Simpsons Index (D) is based on sample measurements that account for both richness and proportion (percent) of each species from a sample within an area. The index assumes that the proportion of individuals in an area indicate their contribution to overall diversity. If a sample has a high dominance value it is highly dominated by one species (Krebs 1992).

The Brillouin index (Hb) measures the diversity of a over a whole species population allowing for all of the data to be used rather than a statistical measure of probability of occurrence within a population (Pielou 1966; Krebs 1992). The Shannon Index (H') is based on the proportional abundance of the species present in an ecosystem. This diversity index measures the order (or disorder) observed within a particular system according to the number of individuals observed for each subspecies in a sample plot (Pielou 1966; Krebs 1992). The Pielou Evenness index (P) is based on the ratio of the Shannon Index of diversity/ species richness. Pielou Evenness index provides an estimation of the the evenness of distribution in different areas. Heip's Evenness (E^h) is a measure of

how similar the abundances of different species are. When there are similar proportions of all subspecies then evenness is one, but when the abundances are very dissimilar (some rare and some common species) then the value increases (Heip 1974).

The biological indices and the water chemistry data were used for a Hierarchical cluster analysis, and the similarity between two sites was estimated according to the Euclidean distance. The Euclidean distance provides a good index of the similarity between two samples, sites with the highest similarity are characterized by the shortest distance between them (Howard 1991).

Results and discussion

Biological indices and the chemical data are presented in Tables 1 and 2, respectively. Both categories of indices (chemical and biological) exhibited wide variability between sampling stations. This variability is commonly observed in aquaculture sites and is partially a result of a variability in a range of parameters including the distance from the source of pollution (i.e. the fish cage) and a seasonal range of currents and water exchange (Borja et al. 2009).

Table 1. Average values (+/- SD) and range of the biological indexes

Index	Average (SD)	Range
ITI	4.13 (1.20)	0.91-6.99
AMBI	2.53 (1.41)	1-6
Simpsons Index (D)	0.72 (0.23)	0.03-1.00
Brillouins Index (Hb)	1.34 (0.50)	0.08-2.24
Shannon Index (H')	10.32	2.00-24.00
Pielou Evenness (P)	0.74 (0.22)	0.06-1.00
Heip Evenness (Eh)	0.57 (0.25)	0.02-1.00

ITI was the index which exhibited the highest range of values, conversely AMBI exhibited a lower range and was therefore selected to be used for further data analysis. The correlation between the different parameters is presented in Tables 3 and 4. Carbon and Nitrogen % correlated with Redox Potential, whereas Median Partical Size Diameter did not correlated with any of the other parameters.

There was a good correlation between the biological indices, the exception being between N with Hb and H' and between the S and P. A further analysis revealed that Hs and Hb correlated with AMBI and ITI, whereas P and Hs were highly correlated. For this reason Hs was chosen for further analysis as it can account for both Pielou evenness and equitability of the species.

Table 2. Average values (+/- SD) and range of the chemical parameters

Parameter	Average (SD)	Range
Median Particle Size	385.84 (419.92)	82 -3533
Carbon %	4.88 (2.93)	0 -10.57
Nitrogen %	0.16 (0.18)	0 - 1.17
Redox Potential	304.80 (113.10)	0 - 540

Interestingly, the results indicate that among the other biological indices, ITI, AMBI, and H' were good indicators of benthic status, but the Shannon and AMBI indices were highlighted on the basis of how accurately they described the status of the disturbance.

The stations with clearly non-degraded environmental conditions could be easily discriminated according to the chemical and biological index analysis, nevertheless a good correlation of the biological indices with the chemical parameters was exhibited between the benthic indices and carbon and oxygen. This is due to the fact that presence of both carbon and oxygen in the benthic environment are required for high species richness, equitability and diversity. Further analysis of the data was required to evaluate the relative significance of each parameter in

providing accurate information on the environmental status of aquaculture sites. These analytic methods may include multivariate analysis using ordination by non-metric multi-dimensional scaling (MDS) as Cheng et al. (2004) suggested.

Table 3. Pearson product moment correlation between chemical parameters

	C		N		Median particle size	
	Correlation	P-value	Correlation	P-value	Correlation	P-value
Redox	-0.317	<0.01	-0.322	<0.01	0.164	0.090
C			0.585	<0.01	-0.079	0.395
N					-0.094	0.312

Table 4. Pearson product moment correlation between the biological indexes of benthic status. An asterisk indicates a highly significant correlation ($P < 0.01$)

	S		D		Hb		Hs		P	
	Correlation	P-value	Correlation	P-value	Correlation	P-value	Correlation	P-value	Correlation	P-value
N	0.475	*	-0.459	*	0.026	0.077	-0.15	0.103	-0.698	*
S			0.354	*	0.751	*	0.675	*	-0.513	0.579
D					0.76	*	0.874	*	0.87	*
Hb							0.967	*	0.462	*
H									0.601	*

The use of a combination of benthic indices has the potential to reduce the error (Van Dolah et al. 1999), contrary to using a single index, and thus it can more accurately reflect the range of benthic ecological conditions.

In conclusion, the results indicate that a combination of two chemical parameters: the Redox Potential and C% with AMBI or H' would accurately predict the level of disturbance of benthic ecosystems around the aquaculture sites.

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