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APPROACHES AND METHODS USED TO ASSESS ECOLOGICAL QUALITY UNDER EUROPEAN ENVIRONMENTAL LEGISLATION

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A thesis submitted to the Department of Zoology, School of Natural Sciences, National University of Ireland, Galway in fulfilment for the degree of Doctor of Philosophy, September 2012.

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ABSTRACT

Benthic habitats and the macrobenthic communities they support form an integral component of ecological quality assessments in marine and estuarine waters. Good conceptual understanding of the changes in benthic community structure in relation to environmental disturbances has underpinned the development of a wide range of techniques to detect, describe and interpret change in habitat quality. Many of these techniques have been proposed as monitoring tools under European Environmental Directives (e.g. Habitats Directive, HD; Water Framework Directive, WFD; Marine Strategy Framework Directive, MSFD). The primary aim of this thesis was to build on current scientific understanding of the performance and sensitivity of a range of techniques currently used to meet the provisions of environmental legislation. This body of work is comprised of four separate, yet related, studies.

The HD and WFD both share the common overall goal of conserving biodiversity and good habitat quality, and focus on aspects of coastal and transitional water systems. This thesis proposes a pragmatic approach for the integration of habitat classification (sensu the HD) and Ecological Status (ES) assessments (sensu the WFD) in sedimentary habitats. Integration of the HD and WFD has the potential to strengthen both Directives by mutual enlightenment between assessment outputs. The proposed approach also has potential to provide a framework for applying Before-After Control-Impact (BACI) designs for assessing change at a regional level for the WFD and HD. Natural spatial and temporal variability in monitoring data has been shown to have a confounding effect on the reliability of ES assessments using WFD Ecological Quality Ratios (EQRs). BACI designs have the potential to improve current environmental assessments by objectively accounting for the effects of natural variability thus allowing the effects of anthropogenic impacts to be discriminated more accurately and reliably. Similarly, increasing the reliability and robustness of habitat classification and maps revealing their spatial distribution have potential to improve the effectiveness of environmental management, particularly the HD which in part focuses on the management of conservation sites. This thesis determined the performance of different sediment particle size analysis techniques in terms of the ability of their resulting sediment distributions to explain variance and patterns in faunal datasets. Using sediment distribution data that best explains the underlying pattern within faunal datasets will allow communities and biotopes to be resolved and mapped with greater confidence. These elements have the potential to improve the effectiveness of environmental management. This thesis also highlights the implications of changing sampling strategies on the allocation of ES (sensu the WFD). Adopting single sample survey designs cause ES to be classified as lower when compared to a multiple sample survey design. Consequently, the effects of sampling design must be considered when assessing ES, particularly if assessments form the basis of scientific advice used to inform management decisions. Furthermore, the reliability of EQRs must be determined before the indices are used to assess ES in geographical locations (and conditions) for which the techniques were not originally designed. This thesis proposes a novel approach of using abundances of taxa higher than species with EQRs as an effective way of facilitating the transfer of WFD-type management to a new geographic region at Hong Kong, China. A number of member states, including Ireland, have expressed interest in adopting OSPAR’s Ecological Quality Objective (EcoQO) framework to help meet their obligations under the MSFD, however, there are concerns as to the performance of EcoQOs in a real world situation. This thesis used time-series data to validate the application of OSPAR’s EcoQO for tributyltin contamination in Irish waters. It is suggested that the quantified temporal responses of the EcoQO provided by the time-series analysis could be potentially used to objectively track change in environmental quality over time.
DECLARATION

I certify that this thesis which I now submit for examination for the award of PhD, is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

This thesis was prepared according to the regulations for postgraduate study by research of the National University of Ireland, Galway and has not been submitted in whole or in part for an award in any other Institute or University.

The work reported on in this thesis conforms to the principles and requirements of the University’s guidelines for research.

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Signature ____________________________ Date ______________
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6 DISCUSSION

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1 INTRODUCTION

Due to increasing adverse impacts of human activities on marine and estuarine ecosystems the need for environmental monitoring has become increasingly important (Reiss and Kröncke, 2005; Quintino et al., 2006). In general, marine monitoring programs are designed to detect significant changes to ecological quality associated with an impact. Where adverse and undesirable change exists remedial management action may be initiated to address the causes of change and so alleviate impacts on the environment (Elliot, 1994; Quintino et al., 2006; Whomersley et al., 2008; Borja et al., 2010). Benthic faunal communities have been shown to respond quickly to both anthropogenic and natural stress, and in particularly those stressors that influence the structure, chemistry and quality of sediments (Pearson and Rosenberg, 1978; Dauer, 1993; Borja et al., 2000; Quintino et al., 2006). As a result, monitoring macrobenthic species, their communities and habitats have become integral components of assessing change in ecological quality in marine and estuarine waters (Bilyard, 1987; Warwick et al., 1990; McLusky and Elliot, 2004; Quintino et al., 2006; Kennedy et al., 2011; Forde et al., 2012).

The macrobenthos play an important role in modifying and maintaining sediment habitat quality through the structuring of sediment properties and the cycling of nutrients and material between the sediment and the water column (Dauer, 1993; Clarke, 1993; Aller, 1980; Aller and Aller, 1998; Borja et al., 2000, 2008; Rosenberg et al., 2002; Anderson et al., 2008). Dauer (1993) attributed the ability of the benthos to reflect sediment quality to aspects of their biology. Many macrobenthic species reside at the sediment/water interface where many pollutants accumulate. Benthic macrofauna are relatively sedentary and are forced to tolerate local adverse conditions. Many species have relatively long life spans (ranging from a few months to a few years) and exhibit temporal responses to long term disturbances. Macrobenthic communities consist of different species that exhibit different tolerances to stress. Consequently, community level responses are often more sensitive to environmental disturbances than
individual species or biological indices (e.g. measures of diversity) (Underwood and Peterson, 1988; Elliot, 1994; Clarke, 1993; Anderson et al., 2008). The macrobenthos as a tool for monitoring environmental change has the added benefit that there is a good conceptual understanding of the changes in benthic community structure in relation to environmental disturbances (Quintino et al., 2006) in particular organic enrichment and oxygen deficiency (e.g. Pearson and Rosenberg, 1976, 1978), and physical disturbance (Rhoads and Germano, 1986; Rhoads et al., 1978).

Early descriptions of macrobenthic communities were based on the relationships between benthic fauna and sediments under relatively stable environmental conditions (e.g. Peterson, 1913; Thorson, 1957). Under stable conditions communities are characterised by species that are temporally and spatially persistent, dominate in biomass and are relatively long-lived (Peterson, 1913; Thorson, 1957). In these instances, benthic communities, whose component species have evolved to endure local variations in environmental conditions, undergo relatively minor changes over time (Rosenberg et al., 2002). Rhoads and Boyer (1982) later described the species characterising these communities as “equilibrium species” living in sediments where disturbance is uncommon.

A more ecologically realistic concept was proposed by Johnson (1971, 1972) who suggested that benthic communities are a temporal and spatial mosaic, “parts of which are at different levels of succession...in this view the community is a collection of relics of former disasters”. The term succession used by Johnson (1971, 1972) was adopted from the concept of community succession in plant ecology, as popularised by Clements (1916), who suggested that the development of plant communities followed a predictable series of succession stages up to a stable climax stage, and that all stages were environmentally controlled. Significant ecological disturbance causes change to the structure of benthic communities in terms of species composition, abundance and biomass (Johnson 1971, 1972; Rosenberg et al., 2004). Huston (1994) described communities to be a mosaic of parts that
Johnson’s (1971) concept of succession in benthic communities following disturbance was expanded in the generalised models of Pearson and Rosenberg (1976, 1978) who described succession in benthic communities in response to organic enrichment and oxygen deficiency. Pearson and Rosenberg (1976, 1978) showed that the structure of benthic communities changed in a predictable fashion in relation to distance from sources of disturbance. In general, benthic communities exhibit declines in species diversity, abundance and biomass with increasing levels of disturbance. Pearson and Rosenberg (1978) suggested that as a particular disturbance increases it successively affects individuals and then species. In this view, it was argued that individuals adapt to cope with a disturbance up to a point where it is outcompeted and replaced by another individual that is better adapted. As the intensity of disturbance increases beyond the species capacity to cope, the species will be replaced by another species or group of species. Consequently, changes in the composition of faunal communities across a disturbance gradient can be viewed as a continuum punctuated by steps where changes in species composition occur (Pearson and Rosenberg, 1978; Rosenberg et al., 2004). This thesis was further developed by Rhoads and Germano (1986) who highlighted the use of succession models for understanding changes in faunal distributions along physical disturbance gradients. Heip (1995) suggested that the generalised models of succession were generally applicable to disturbed sublittoral habitats. While the generalised models showed change in faunal composition along a gradient of disturbance as a continuum, Pearson and Rosenberg (1978) and, Rhoads and Germano (1986) identified four, largely equivalent, succession Stages along a gradient extending from severely disturbed to undisturbed (or normal) conditions. The succession Stages described by Pearson and Rosenberg (1978) and, Rhoads and Germano (1986) can be classified as Stage 0 (or grossly polluted/disturbed), Stage I (polluted/disturbed), Stage II (transitionary) and Stage III (unpolluted/undisturbed) (Rosenberg et al., 2002).
The initial stage following a major disturbance, Stage 0, sees the disappearance of benthic species and the sediment becoming azoic. During this stage space is made available for colonisation, however, environmental conditions may be extremely inhospitable (Pearson and Rosenberg, 1978; Rhoads and Germano, 1986). Stage I sees the establishment of pioneering communities with free space becoming increasingly occupied by small bodied, fast growing, stress tolerant species. These opportunistic species can be described as r-selected species in that they have short life cycles and high turnover rates. In subsequent succession Stages, benthic communities become increasingly dominated by stress sensitive, long lived, slow growing species which produce fewer offspring (i.e. K-selected species). Typical opportunistic species are small tube-dwelling polychaetes, such as species of the genera *Capitella* and *Scolepis*, which may occur at high densities (Pearson and Rosenberg, 1978). Rhoads and Boyer (1982) reported small tube dwelling polychaetes occurring at densities in excess of $10^5$ m$^{-2}$ in Stage I pioneering communities. Feeding, burrowing and tube construction activity acts to rework sediment and influence particle (and contaminant) transport, while tube irrigation and ventilation results in increased water and solute transport, and increased oxygenation of sediments (Aller, 1980; Aller and Aller, 1998; Yingst and Rhoads, 1980).

At Stage II the increased oxygenation of sediments by opportunistic species enhances habitat conditions allowing the establishment of free-living taxa and larger tube dwellers. These taxa typically include burrowing deposit feeding bivalves of the genera *Corbula* and *Thyasira*, scavenging polychaetes *Anaitides* and *Goniada*, the burrowing deposit feeding polychaete *Pholoe* and tube dwelling polychaetes such as *Pectinaria*. These larger taxa cause more intensive mixing of sediment particles and water over vertical distances of up to 10 – 20 cm (Rhoads and Boyer, 1982). The resulting physical and chemical changes further enhance sediment conditions. The absence of further disturbance allows the development of Stage III communities which exhibit increasingly greater levels of diversity and abundance of stress sensitive species (Pearson and Rosenberg, 1978; Rhoads and Germano, 1986). Rhoads and Boyer (1982) described late succession Stage
communities to be dominated by large bodied, free living, infaunal deposit feeders.

Classifying faunal communities into succession Stages can be problematic. Ecological data tend to contain elements from more that one succession Stage. For instance, where a physical disturbance is limited to surficial sediments deeper burrowing species which characterise a Stage III community may coexist with Stage I opportunists colonising surficial sediments (Rhoads and Germano, 1986). Similarly, in the presence of labile organic material it is common for Stage I opportunistic species to persist into later succession Stage communities (Rhoads and Germano, 1986). Consequently, the succession Stages outlined above should be viewed as idealised states against which faunal community structure can be compared.

1.1 BENTHIC INVERTEBRATES AS INDICATORS OF ECOLOGICAL QUALITY

The ecological paradigms proposed by Pearson and Rosenberg (1976, 1978) and, Rhoads and Germano (1986) have resulted in a good conceptual understanding of the spatial and temporal responses of benthic communities in relation to disturbance. This understanding has underpinned the development of a wide range of numerical techniques to detect, describe and interpret change in benthic communities and habitat quality (Elliot, 1994; Heip et al., 1998; Borja et al., 2000, 2009; Clarke and Warwick, 2001; Rosenberg et al., 2004; Quintino et al., 2006; Whomersley et al., 2008). Macrobenthic monitoring surveys are designed to detect significant changes in habitat quality associated with ecological disturbances or remedial management measures. Benthic surveys often produce complex data matrices describing species presence and abundance (and possibly biomass), and local environmental conditions (e.g. sediment characteristics, ecological disturbances) at a series of sites (Elliot, 1994; Clarke and Warwick, 2001; Clarke and Gorley, 2006; Whomersley et al., 2008). Aubrey and Elliot (2006) emphasised the need for methods to simplify, summarise, quantify and communicate complex ecological information.
Biological survey data can be used to produce a range of univariate and multimetric biological indices (Heip et al., 1994; Clark and Warwick, 2001; Quintino et al., 2006). Univariate biological indices are typically based on the relative abundance of species in samples, and are designed to condense species data in a sample into a single coefficient that provides quantitative estimates of biological variability (Heip et al., 1998; Clarke and Warwick, 2001). Univariate indices can be categorised as primary or derived indices (Whomersley et al., 2008). Primary biological indices include number of individuals (N), numbers of species (S) and measures of biomass while derived biological indices include Simpson’s evenness \((1-\lambda')\), Margalef’s species richness index \((d)\), Shannon–Wiener species diversity \((H')\) and taxonomic distinctness (Heip et al., 1998; Clarke and Warwick, 2001). Univariate indices are particularly useful where inter- and/or intra-site sample data can be analysed for significant spatial (or temporal) change in species diversity, abundance and biomass in relation to a disturbance using parametric (e.g. Analysis of Variance; ANOVA) and non-parametric (e.g. Kruskal-Wallis) univariate statistical analyses (Elliot, 1994; Warwick and Clarke, 2001; Whomersley et al., 2008). Simple (or multiple) regression techniques can also be used to model the relationship between biological indices and measured environmental parameters (and/or time). In these instances the indices are used as the dependent variable and the environmental descriptors (and/or time) as independent variable(s) (Clarke and Warwick, 2001; Burnham and Anderson, 2002, Zuur et al., 2007). Univariate diversity indices were commonly used in the past (Heip, 1994; Clarke and Warwick, 2001) but recently more complex multimetric indices have been proposed as more sensible tools for the assessment of benthic ecological quality (Krönke and Reiss, 2010).

The last decade has seen the development of an abundance of biological multimetric indices based on the sensitivities or tolerances of benthic species to disturbance (for review see Borja et al., 2009). These multimetric benthic indices have been largely developed to address environmental legislation which require the derivation and use of multimetrics to assess environmental quality e.g. the European Water Framework Directive (WFD, 2000/60/EC),
the US Clean Water Act (USEPA, 2002) (Quintino et al., 2006). In general, these indices examine relative changes in the proportions of disturbance sensitive and tolerant taxa to determine a measure of quality. Multimetric indices can be investigated for spatial and temporal patterns using the parametric and non-parametric univariate statistical analyses mentioned above. Under the WFD multimetric indices are used to measure progress toward achieving the Directives main goal of Good Ecological Status (GES) for all water bodies by 2015. WFD multimetrics are a recurring topic in this thesis (i.e. Chapters 2, 3 and 4) and are discussed later in the context of the Directive.

There is increasing use of multivariate techniques in benthic ecological studies. Macrobenthic communities are comprised of different species that exhibit different tolerances to stress. Consequently, community level responses are often more sensitive to environmental disturbances than individual species or diversity indices (Underwood and Peterson, 1988; Clarke, 1993; Anderson et al., 2008). Multivariate methods are considered more powerful than univariate techniques as they retain all information regarding the samples (e.g. presence or absence of species, species abundance and biomass) and permit testing the effects of disturbances on the whole community assemblage at once (Clarke and Warwick, 2001; Anderson et al., 2008). In addition, multivariate methods are frequently used to investigate the extent to which information on specific local environmental variables is related to the patterns within faunal community distribution data. Chapters 3 and 4 of this thesis use multivariate techniques to highlight the ability of environmental parameters (e.g. sediment granulometry, interstitial salinity) to explain variance in benthic community datasets and thus support habitat/biotope classification and mapping for marine monitoring. Habitat and biotope classification is a requirement for the European Habitats Directive (HD; 92/43/EEC). The main aim of the HD is the protection of defined habitats, biotopes and species. Habitat and biotope classification is discussed below in the context of the HD.
There is a suite of publications available that provide excellent overviews of the theoretical basis and practical application of multivariate techniques to ecological monitoring data (e.g. Clarke and Warwick, 2001; Clarke and Gorley, 2006; Anderson et al., 2008). Here, we present some multivariate techniques commonly used in marine monitoring (and within this thesis), with particular reference to the statistical program PRIMER v6 (Clarke and Gorley, 2006) and the add-on package PERMANOVA+ (Anderson et al., 2008).

A common starting point for many multivariate techniques is the computation of a triangular similarity matrix of similarly coefficients (e.g. Bray Curtis similarity) between every pair of samples. The similarity matrix can be based on biological data (e.g. species distribution data) or on environmental variables (e.g. sediment parameters). Various ordination and clustering techniques can then be applied to the similarity matrix to determine the relationship between the samples. Multidimensional scaling (MDS) is a technique that ordnates samples as points in 2D or 3D space based on similarity in species distribution data (or environmental variables). Hierarchical Agglomerative Clustering (HAC) is used to cluster samples based on between-sample similarities into groups in dendrograms. Similarity Profiling (SIMPROF) is used to test if differences between HAC derived similarity based clusters are significant. Similarity Percentages (SIMPER) analysis can be used to determine the characterising species of each cluster of stations identified either arbitrarily (by eye) from HAC dendrograms or statistically using SIMPROF testing (Clarke and Warwick, 2001; Clarke and Gorley, 2006; Anderson et al., 2008).

Distance-based Linear Modelling (DistLM) can be used to assess the power of environmental variables to model and explain variance in multivariate faunal distributions. DistLM is a data analysis technique that performs linear regression using inter-point similarities in the similarity matrix as the response variable and (in this case) with environmental variables as predictors. DistLM also allows for the selection of optimal subsets of predictor variables that provides the best explanatory power (i.e. the subset of variables that account for the maximum amount of variance in faunal
Distance-based Redundancy Analysis (dbRDA) is an ordination technique for visualising the outputs of DistLM. This ordination technique plots fitted values of between-sample Bray-Curtis similarity from the prediction of the linear model given by DistLM. In these ordinations a vector overlay of the predictor variables can be used to illustrate the relative influence of each predictor variable (Clarke and Warwick, 2001; Clarke and Gorley, 2006; Anderson et al., 2008).

Marine monitoring studies have shown that certain benthic species have specific responses to certain disturbances including oil, chemical and heavy metal contamination. In such instances, species are very useful biological indicators (or bio-indicators) of specific ecological disturbances (Dauvin et al., 2003). The European Marine Strategy Framework Directive (MSFD; 2008/56/EC) requires Member States (MS) to develop sets of ecological indicators and related targets by 2012. These indicators and their associated targets are used to assess progress towards reaching the Directives fundamental aim of Good Environmental Status (GEnS) for all water bodies by 2020 (Heslenfeld and Enserink, 2008; van Hoey et al., 2010; Fleming-Lehtinen, 2011; Long, 2011). A number of benthic species have been suggested as potential indicators for monitoring contaminants under the MSFD (Heslenfeld and Enserik, 2008). Chapter 5 of this thesis presents an Irish case study of a potential MSFD bio-indicator species. The MSFD is discussed below with particular reference to bio-indicators.

Quintino et al (2006) emphasised the continued need to question the ecological basis of the paradigms upon which biological indices, indicators, and numerical techniques are based. In addition, there is need to determine the performance of the indices and numerical techniques when used to assess the impacts of different types of disturbance, and in new geographical regions, for which the techniques were not originally developed. This is especially important where these techniques form the basis of scientific advice used to inform management decisions.
This thesis aims to contribute to the current scientific understanding of the performance and sensitivity of a range of techniques and methods used to meet the provisions of environmental legislation. Some of the primary objectives of the thesis Chapters are highlighted in turn below in the context of European Directives to which they most readily apply. Within each chapter of this thesis possible directions of future research are highlighted. The final chapter of this thesis provides a critical assessment of techniques and methods and further illustrates the scientific contributions made within this body of work. The final chapter also makes further suggestions for the direction of future research.

1.2 WATER FRAMEWORK DIRECTIVE (WFD; 2000/60/EC)

The WFD, which entered into force in December 2000, has a broad scope for action and covers the protection of all surface water bodies including lakes, rivers, their basins and catchments, through to transitional (i.e. estuaries and lagoons) and coastal waters. The WFD covers a narrow band of coastal waters extending 1 nautical mile (nm) from the high water mark. Under the WFD transitional water bodies are defined as surface waters that are in the vicinity of a river mouth that are partially saline due to the influence of coastal waters. The principal objective of the WFD is to achieve at least GES for all water bodies by 2015. Assessment of Ecological Status (ES) is based on assessment of the status of structural components of the ecosystem. These components include physical, chemical, hydromorphological and biological quality elements, with the latter being of particular importance (Rosenberg et al., 2004; Borja, 2005).

In Ireland two of the most commonly used multimetric benthic indices for assessing ES are the Multivariate-AZTI Marine Biotic Index (M-AMBI) (Borja et al., 2007; Muxika et al., 2007a,b) and the UK and Ireland Infaunal Quality Index (IQI) (Prior et al., 2004; Borja et al., 2007, 2009; Mackie, 2009; Forde et al., 2012). These benthic indices are largely based on the Pearson Rosenberg (1978) model for organic enrichment and oxygen deficiency (i.e. where disturbance increases sensitive species disappear and are replaced
by more stress tolerant, opportunistic species). M-AMBI and IQI include measures of diversity and abundance of invertebrate taxa, and measures of the proportions of five Ecological Groups (EG) to which benthic species are allocated depending on their tolerance to disturbance (Borja et al., 2009). Before assessments can be made High and Bad status reference conditions must be described. Reference conditions are often set using the highest and lowest values in the dataset for each of the metrics used in the calculation of the index (e.g. for M-AMBI; Borja et al., 2009; Kennedy et al., 2011) (e.g. for IQI; Forde et al., 2012). Monitoring data for each element are compared to their reference conditions to derive an Ecological Quality Ratio (EQR). EQRs range between 0 and 1, with High status represented by values close to 1 and Bad status close to 0. The EQR scale is sub-divided into five ES classes by assigning a numerical value to each of the class boundaries. More detailed descriptions of the calculation of M-AMBI and IQI are given in Chapters 2, 3 and 4.

The assignment of species to EG in M-AMBI and IQI is based on the extensive literature describing North Atlantic species and, where the existing literature is lacking, consensus expert judgement (Borja et al, 2000; Borja et al., 2003; Teixeira et al., 2010). Provided that the sensitivities of taxa are known M-AMBI is useful for assessing ES. However, the sensitivity of many tropical/sub-tropical taxa has not yet been determined. This gap in knowledge presents a significant barrier to the direct transfer of WFD EQRs and the ideas underpinning them to the management of coastal waters beyond Europe. It has been proposed that faunal tolerances should be analysed at the species level as different species within the same genera may not exhibit the same sensitivity or tolerance to disturbance (Rosenberg et al., 2004). However, numerous studies have indicated many benthic congeners are conservative in terms of their sensitivities or tolerances to disturbances (Dauvin et al., 2003). Furthermore, monitoring studies have shown that species level data carries a considerable degree of redundancy and that community level response to environmental disturbance can be detected at higher taxonomic levels (e.g. Warwick, 1988a,b; Oslgard et al., 1997, 1998). Based on this evidence the overall aim of Chapter 2 was to
investigate the potential of using higher taxonomic (i.e. family) level data with M-AMB for assessing ES in a new bio-geographical region at sub-tropical Hong Kong and surrounding waters. The first objective of Chapter 2 was to present a methodology for assigning family taxa to EG based on the sensitivities of species within the parent family. The second objective was to determine the effects of using higher taxonomic level data with M-AMBI in European waters where the EQR was originally developed. Another objective was to investigate the performance of family level M-AMBI when used to detect change in ES at Hong Kong. Chapter 2 highlights the use of higher taxonomic data with M-AMBI as a means of facilitating the transfer of WFD EQRs for ES assessment to new geographical regions, the proposed methodology. The approach also has potential in European monitoring. In this context, Chapter 2 makes a major contribution by showing that monitoring ES for the WFD may be conducted using family level data and M-AMBI. By relying on family level identification, instead of relatively more expensive and time consuming species level identification (Trigal-Domínguez et al., 2010), the proposed methodology, in part, meets the need for more rapid and cost-effective approaches to marine monitoring (Dimitriou et al., 2012).

Another facet of benthic monitoring that has received attention in the literature is the adequacy of data used for generating indices for making management decisions. Generation of data in benthic monitoring programs is labour intensive and often there is a compromise between “scientific and statistical rigour” and, “practical and financial constraints” (Elliot, 1994). Due to the increased need for monitoring as imposed by legislation, environmental managers are now often forced to allow the latter take priority. For instance, as a means of reducing cost benthic monitoring programs often adopt single grab sample surveys instead of multiple grab designs (Kennedy, 2008; Seiderer and Newell, 1999; Forde et al., 2012). Typically in these single sample surveys a sediment subsample is first removed from the grab for sediment analyses (e.g. sediment particle distribution, porosity, organic content) while the remainder of the grab is used for faunal analysis. Studies have shown that diversity indices are influenced by changes in sampling
design (Kennedy et al., 2011) and sample size (Clarke and Warwick, 2001). Despite this the effect of changing sampling strategies on the determination of ES using EQRs, which incorporate measures of diversity, has not been determined. An objective of Chapter 3 was to address this gap in knowledge by determining the effects of sub-sampling on IQI component indices, EQR values and classification of ES. The findings outlined in Chapter 3 allow evidence-based recommendations to be made regarding sampling strategies used for ES assessment (sensu the WFD) (Forde et al., 2012).

1.3 HABITATS DIRECTIVE (HD; 92/43/EEC)

The main aim of the HD, which came into affect in May 1992, is to promote the maintenance of biodiversity. The Directive concentrates on the protection of defined habitats and species. The HD makes provisions for MS to develop measures to maintain (and where practical restore) habitats and species at Favourable Conservation Status (FCS) through the designation and management of conservation sites (Dauvin et al., 2008a,b; Fleming-Lehtinen, 2011; Fraschetti et al., 2011). Reliable and robust classification of benthic habitats and biotopes, and maps revealing their spatial distribution are important preliminary steps for the effective management of marine habitats and species under the HD (Fraschetti et al., 2008, 2011).

A current trend among benthic monitoring studies involves the use of local environmental variables, such as sediment particle distribution data, to help explain patterns in benthic macrofaunal community distribution data and so assist habitat and biotope classification (Clarke and Warwick, 2001; Clarke and Gorley, 2006; Forde et al., 2012). Monitoring programs often use different granulometric methods to determine sediment particle distributions. Some granulometric methods measure different sediment characteristics and so invariably produce different sediment distributions (Eleftheriou and McIntyre, 2005; Rodríguez and Uriarte, 2009; Forde et al., 2012). The performance of different granulometric methods in terms of the ability of the sediment distributions they produce to explain variance in faunal datasets has not been determined. Adopting the granulometric method that best
explains patterns in faunal distributions has the potential to produce more reliable and robust habitat and biotope classifications. An objective of Chapter 3 was to assess the ability of sediment distributions derived using different granulometric methods to explain variance in multivariate faunal datasets.

In attempts to meet the provisions of the HD a number of biotope classification systems have been developed (Dauvin et al., 2008a,b; de Paz et al., 2008). Marine monitoring programs are increasingly using the EU Nature Information System (EUNIS) habitat classification system (Galparsoro et al., 2008, 2010). Under EUNIS (2002) biotopes are typically classified using a bottom-up taxonomic approach where characterising species within biotic communities are identified. These biological communities are then compared to core records of (or standard) communities and their most commonly associated habitats to identify biotopes (Connor et al., 2004). There are a number of issues with using a bottom-up approach for the classification of biotopes for the HD. The most significant of these issues are that biotopes are defined based on a temporal snapshot of variable benthic communities and so do not accurately reflect biological variability in the community or the spatial distribution of the habitat. Chapter 4 presents an alternative approach to defining habitats and biotopes for the HD which consists of using environmental parameters (e.g. sediment parameters, interstitial salinity) as top-down habitat classifiers. Faunal assemblages within the top-down habitats are then characterised to produce top-down classified biotopes. These biotopes more accurately reflect the spatial extent of the habitats (or resources) while accounting for the variability of the overall biological communities within the identified habitats. The performance of the proposed top-down classification is compared to EUNIS bottom-up system in two Irish estuaries and marine embayments. Chapter 4 also demonstrates how faunal data within the top-down habitats (classified for the purposes of the HD) can be used in ES assessment for the WFD.
1.4 MARINE STRATEGY FRAMEWORK DIRECTIVE (MSFD; 2008/56/EC)

The MSFD was adopted in December 2008 and is based on the concept of GEnS which involves protecting the marine environment while promoting sustainable use of marine resources (van Hoey et al., 2010; Long, 2011). The MSFD states that by 2012 MS must assess the current condition of their marine waters, define what GEnS would be for their waters and develop targets, indicators and programmes that will allow GEnS to be achieved by 2020. Under the MSFD assessment of EnS (Environmental Status) is based on eleven high level descriptors that reflect ecosystem functioning. These descriptors include biological diversity, non-indigenous species, commercial species, marine food webs, eutrophication, sea-floor integrity, hydrographical conditions, contaminants in fish and seafood, marine litter, environmental contaminants, and underwater noise. In comparison to the WFD and HD, the MSFD is more regional in its approach, including provisions that neighbouring countries need to define common indicators for ecosystem descriptors (Rice et al., 2010; van Hoey et al., 2010). By taking into consideration human impacts on all parts of the ecosystem the MSFD is regarded as the European Union's first attempt at ecosystem based management (van Hoey et al., 2010; Long, 2011).

The Directive states that MS should endeavour to integrate established environmental monitoring programs. One such monitoring program is OSPAR's Ecological Quality Objective (EcoQO) system. OSPAR is an agreement signed by fifteen governments, including Ireland, along the western coast of Europe whose aim is to protect the marine environment of the North-East Atlantic. In 1992 OSPAR identified nine ecological issues which were related to specific components of the ecosystem. For each ecological issue one or more EcoQOs were developed. The main aim of these EcoQO was to provide defined and measurable objectives to help guide progress towards OSPAR's overall goal of a healthy and sustainable ecosystem (Heslenfeld and Enserink, 2008; OSPAR, 2009). OSPAR's EcoQO system is widely regarded as a successful and robust model of how
indicators can be used for assessing, monitoring and understanding environmental status. Testing of OSPAR’s EcoQOs has been largely confined to North Sea countries and there are concerns as to the performance of EcoQOs in other geographical areas. Despite this increasingly more countries have expressed interest in adopting the EcoQO framework to help meet their obligations under the MSFD (Heslenfeld and Enserink, 2008).

OSPAR has developed an EcoQO for monitoring tributyltin tin (TBT) contamination in the marine environment based on the biological response of dog whelk *Nucella lapillus* (OSPAR, 2009). The rationale for this EcoQO is that female dog whelks are particularly sensitive to TBT which has been widely used as the active ingredient in anti-fouling treatments. TBT has been linked to the development of imposex in dog whelk. Impos sex is the irreversible imposition of non-functioning male sexual characteristics which acts to reduce reproductive capabilities and can eventually lead to reproductive failure and serious population declines (Bryan et al., 1986; Minchin et al., 1995, 1996; Oehlmann et al., 1991; 1998; OSPAR, 2009; Guðmundóttir et al., 2011). Impos sex in *N. lapillus* has been used to monitor TBT contamination in Irish waters since the 1980s (e.g. Duggan et al, 1988; Minchin et al., 1995, 1997; Minchin, 2003). The objective of Chapter 5 of this thesis was to build on the Irish literature base using OSPAR’s EcoQO to describe temporal and spatial trends in TBT contamination in Irish waters since the introduction of a partial ban on TBT based antifoulants in 1987 (Anon, 1987). Chapter 5 also determines the validity of OSPAR’s EcoQO threshold values for imposex in *N. lapillus* in Irish waters.
2 M-AMBI DERIVED FROM TAXONOMIC LEVELS HIGHER THAN SPECIES ALLOWS ECOLOGICAL STATUS ASSESSMENTS OF BENTHIC HABITATS IN NEW GEOGRAPHICAL AREAS

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Ecological Indicators (In Press)
ABSTRACT

Under the Water Framework Directive (WFD) benthic Ecological Quality Ratios (EQRs) are important tools for assessing Ecological Status (ES) of coastal and transitional waters. Calculation of the Multivariate-AZTI Marine Biotic Index (M-AMBI) EQR is based on the proportions of sensitive and stress tolerant benthic invertebrate species, number of species and Shannon-Wiener diversity. The sensitivity of many tropical/sub-tropical taxa has not yet been determined, presenting a barrier to the direct transfer of WFD EQRs, and the ideas underpinning them, to the management of coastal waters beyond Europe. To overcome this we examine using higher taxonomic level data with M-AMBI.

Before applying such approaches to assessing ES in new geographical regions it is essential to determine the effects of using higher taxonomic level data on M-AMBI in areas where the tool was developed. To this end, we use macrofaunal data from three well studied sites in northwestern Europe to examine the effects of using taxonomic level data higher than species on M-AMBI. Using the European datasets M-AMBI ES classification was shown to be robust to changes in taxonomic level data. We test the suitability of family-level M-AMBI for assessing ES in subtropical Hong Kong waters. Family level M-AMBI was useful in detecting stress in Hong Kong, where it successfully detected temporal and spatial shifts in ES in response to seasonal hypoxia and salinity variability, and anthropogenic organic enrichment.
2.1 INTRODUCTION

Benthic habitats support diverse macrofaunal communities that are comprised of species that exhibit different responses to anthropogenic and natural stresses. Analysing change in community structure at the species level is an effective way of assessing spatial and temporal change in Ecological Status (ES) due to disturbance (Pearson and Rosenberg, 1978; Underwood and Peterson, 1988; Borja et al., 2000; Anderson et al., 2008; Kennedy et al., 2011; Forde et al., 2012).

The European Water Framework Directive (WFD, 2000/60/EC) provides a framework for the protection and improvement of the ES of all surface and ground waters including transitional and coastal waters. Under the WFD EU Member States (MS) are obliged to classify, assess and monitor the ES of their water bodies with the overall objective of achieving Good ES by 2015. ES is assigned to a water body through the assessment of hydromorphological, physico-chemical and biological quality elements. ES is determined by comparing ecological monitoring data to reference undisturbed conditions to calculate an Ecological Quality Ratio (EQR). EQR values range between 0 and 1, with High status represented by values close to 1 and Bad status being close to 0 (Borja et al., 2000; Borja, 2005).

To meet the provisions of the WFD a number of MS have proposed indices for assessing environmental quality based on evaluations of benthic communities and their sedimentary habitats (for review see Borja et al., 2009). Among the many proposed tools the AZTI Marine Biotic Index (AMBI, Borja et al., 2000) and its EQR tool Multivariate-AMBI (M-AMBI, Borja et al., 2007; Muxika et al., 2007a,b) are the most commonly reported (Aguado-Gimenez et al., 2007; Borja et al., 2009; Carvalho et al., 2006; Dauvin et al., 2007; Simboura and Reizopoulou, 2007, 2008; Reiss and Kröncke, 2005; Zettler et al., 2007). AMBI assigns macrofaunal species to one of five Ecological Groups (EGs) depending on their sensitivity to an increasing stress gradient (i.e. organic matter enrichment). The assignment of species to EGs is based on the extensive literature describing North Atlantic species
and, where the existing literature is lacking, consensus expert judgement (Borja et al, 2000, 2003; Teixeira et al., 2010). AMBI ES scores are based on the relative proportion of individuals across the five EGs. Provided that the sensitivities of taxa are known AMBI-based tools are useful as they allow ES to be assessed without the need for spatial and temporal references at the site in question (Kennedy et al., 2011). Many previous studies have highlighted the ability of the AMBI-based methodologies to successfully discriminate macrobenthic community responses, and hence ES, across a wide range of natural and anthropogenic environmental disturbances, in both coastal and transitional waters, and throughout different regions (Borja et al., 2003, 2011; Carvalho et al., 2006; Dauvin et al., 2007; Fleischer et al., 2007; Muniz et al., 2005; Muxika et al., 2005; Solís-Weiss et al., 2004).

Traditionally ecological assessment programs, including those using the AMBI-based tools, have relied on expensive and time consuming identification of macrobenthic organisms to species level (Trigal-Domínguez et al., 2010). However, in response to increasing anthropogenic disturbances to marine systems there has been a pressing need for more rapid and cost-effective approaches to monitoring (Dimitriou et al., 2012). To this end, there has been renewed interest in Taxonomic Sufficiency (TS) (Ellis, 1985) which is based on the concept of using coarser taxonomic levels (e.g. genus, family) to derive information that is sufficient to meet the objectives of monitoring studies. TS is based on the thesis that as environmental disturbance increases its effects cause detectable changes in community assemblage structure at increasingly higher levels within the taxonomic hierarchy i.e. when a given stress increases, it successively affects individuals, species, genera and families (Dauvin et al., 2003). This concept is now known as the “hierarchical response-to-stress” (Ferraro and Cole, 1990; Warwick, 1988a). It has been demonstrated that there is a considerable degree of redundancy in species level data (Clarke and Warwick, 1998; Oslgard et al., 1998). Analysis at higher taxonomic levels might act to reduce the confounding effects of species level responses to natural variation and allow the effects of anthropogenic disturbance to be more clearly detected (Dauvin et al., 2003). However, as is the case when
aggregating species data to AMBI based EGs, using higher taxonomic resolution data imparts the risk that information, which can only be obtained from species level community analyses, may be lost (Dauvin et al., 2003; Maurer, 2000).

AMBI tools have been successfully applied to geographical areas outside of Europe including China (Cai et al., 2003, 2012; Wu et al., 2012), South America (Muniz et al., 2005), North America (Borja et al., 2008; Borja and Tunberg, 2011) and the Indian Ocean (Bigot et al., 2008). Bigot et al. (2008) modified the AMBI based methodologies for ES assessment of tropical soft bottom habitats in the Indian Ocean. Modifications included assigning species that were novel to the AMBI list to EGs, reassigning species to different AMBI EGs and aggregating species to higher taxonomic levels to facilitate EG assignments. Despite these modifications AMBI’s need for species level data resulted in almost 12% of the data been omitted from the assessment (Bigot et al., 2008).

While we wait for tropical species to be assigned to EGs an alternative approach to adapting M-AMBI for ES assessment in new geographical regions would be to use higher taxonomic EGs. This approach has potential as a temporary, yet effective, way of facilitating the transfer of WFD-type management to new areas. However, before applying such approaches to new regions it is essential to determine the effects of using abundances of taxa higher than species on M-AMBI in areas where the tool was developed.

The objectives of this study was to (1) determine the effect of using higher taxonomic data on M-AMBI in areas where the EQR was developed and (2) examine the suitability of using family level M-AMBI to assess ES in subtropical Hong Kong, China. Here, we use a 103 station spatial dataset of benthic macrofauna from three well described NW European transitional and coastal water bodies to demonstrate the ability of M-AMBI to successfully determine ES using genus and family level taxonomic data. The European studies were selected to cover a comprehensive range of habitats exposed to natural and anthropogenic pressures including salinity variability, urban
settlement, port activity, oil exploration, etc. We then used family level taxonomic data with M-AMBI to detect temporal and spatial changes in ES at 119 stations in a subtropical coastal and transitional system at Hong Kong Harbour and its surrounding waters, China. At the China study site natural pressures included seasonal hypoxia and salinity variability while anthropogenic pressures included domestic and industrial effluents as well as dredging and trawling activities. The approach presented here provides an objective means for classifying family EGs based on established species EG classifications. In addition, calculation of M-AMBI using higher taxonomic level data has the inherent convenience and relatively lower cost of identification of fauna at higher taxonomic levels.

2.2 MATERIALS AND METHODS

2.2.1 Case study descriptions, pressures and sampling

2.2.1.1 European case studies

The Irish study site comprised of the Bandon and Belgooly estuaries and their respective harbours at Kinsale and Oysterhaven. The estuaries and harbours are exposed to natural estuarine pressures and anthropogenic disturbances typical of human settlement and port activities. Kinsale town is located in the inner part of Kinsale Harbour and has a resident population of approximately 2500, though this increases substantially in the summer months. The inner harbour also contains two yachting marinas and a commercial fishing pier that is base for approximately ten trawlers and facilitates transfers of approximately 150,000 t of cargo annually. Shipping channels in the inner harbour are dredged approximately every two years. Kinsale has a secondary sewage treatment plant which discharges effluent into the lower Bandon at Commoge. A pharmaceutical company discharges treated effluent into the outer harbour at Sandy Cove Island via a subtidal outfall pipe. By comparison to Kinsale, Oysterhaven is relatively sparsely populated and is exposed to significantly lower levels of anthropogenic pressures. The most significant pressures at Oysterhaven are domestic sewage outfalls distributed along the river and harbour. Previous studies
have shown that while benthic habitat quality at Kinsale and Oysterhaven is generally Good or High, habitat quality in the inner harbour area at Kinsale town ranges between Poor and Moderate (Kennedy et al., 2011; Forde et al., 2012). Forde et al. (2012) provides detailed descriptions of benthic habitats, communities and habitat ES. In May 2011, 41 stations at Kinsale and 11 stations at Oysterhaven were sampled. All sampling stations were shallow (< 15m) and covered a wide range of transitional and coastal soft sediment habitats. At each station a 0.1 m² Day grab was retrieved for macrofaunal analysis.

The Ekofisk oilfield is located in the North Sea 320km southwest of Stavanger, Norway. Oil exploration began in the area in the late 1960s. The first environmental impact survey was carried out in 1973 (Gray et al., 1990). The 1973 environmental survey comprised of 38 sampling stations distributed across five radial transects extending approximately 8km from the main platform complex. An additional reference station was sampled 30 km to the east of the platform complex. Sampling stations were characterised by fine or very fine sands. At each sampling station three replicate 0.1 m² Day grab samples were taken for macrofaunal analysis. Gray et al. (1990) showed significant changes in benthic community structure in response to increasing hydrocarbon stress. These changes included reductions in species abundances and diversity, and increased dominance of opportunistic species. Multivariate analyses showed that stations formed clear groupings based on community structure in relation to oil activities. Furthermore these groupings were detectable after aggregating species data to higher levels within the taxonomic hierarchy.

The Clyde sewage-sludge dump-ground is located east of the Isle of Arran in the Firth of Clyde, Scotland. Dumping of sewage-sludge began at the site in 1974. In 1983, macrofaunal grab samples were retrieved at 12 stations along a west-east transect across the dump-ground. Pearson and Blackstock (1984) described significant loss in community diversity and increased dominance by opportunistic species in response to increasing levels of organic and heavy metal pollution (Warwick et al., 1987).
2.2.1.2 Hong Kong, China case study

Hong Kong is located on the south coast of China and is comprised of Hong Kong Island, Kowloon Peninsula, the New Territories, and approximately 230 islands (Figure 2.1; Morton and Blackmore, 2001; Shin and Ellingsen, 2004). Hong Kong has a subtropical climate with a hot and wet season from May to September and a cool and dry season from November to March (Hodgkiss, 1984). Due to rapid industrialisation and high population density the waters surrounding Hong Kong are subject to considerable anthropogenic pressures including agricultural and industrial effluent pollution, domestic sewage discharge and physical disturbance by trawling and dredging (Morton, 1996; Shin et al., 2004; Shin and Ellingsen, 2004). A baseline survey of the transitional and inshore soft-bottom macrobenthos sampled 119 stations in summer (June–July) and winter (November–December) in 2001. At each station five replicate 0.1 m$^2$ van-Veen grabs were retrieved for macrofaunal analysis. Shin et al. (2004) described seasonal changes in macrobenthic community structure. These seasonal changes were related to increased summer river discharge affecting local salinity in Deep Bay (Figure 2.1). Similarly, summer river discharge produced hypoxic conditions at Tolo Harbour. Seasonal changes were also observed in benthic communities subjected to eutrophication stress near Victoria Harbour.

2.2.1.3 Macrofaunal sample and data processing

In the studies described above all macrofaunal grab samples were sieved through a 1mm sieve. The retained material was fixed in 5-10% buffered formalin and stored in 70% IMS. In the laboratory the macrofauna was stained, sorted, enumerated, and identified to species, or lowest possible taxonomic level, using standard keys.

Here, prior to analysis all non-infaunal species were removed from the datasets. Faunal datasets from the European case studies (i.e. Ekofisk, Kinsale and Oysterhaven, and Clyde) were combined to produce a single large-spatial scale dataset comprised of 103 stations. The summer and winter Hong Kong surveys were not combined. All datasets were checked for
nomenclatural inconsistencies and synonyms using the TREx (Taxonomic Routines for Excel®) tool developed by Thompson Unicomarine Ltd, and the online Taxon Match tool in the World Register of Marine Species (WoRMS; www.marinenspecies.org/aphia.php?p=match). These tools were also used to resolve higher taxonomic level classifications. Using the Aggregate routine in PRIMER v6 (Clarke and Gorley, 2006) each species dataset was aggregated to higher taxonomic levels to produce genus and family level datasets (i.e. where genus and family are lowest possible taxonomic level in the aggregated datasets respectively).

Figure 2.1: Map showing benthic sampling station at Hong Kong and surrounding waters, China. Stations were sampled in both summer and winter 2001.
2.2.1.4 Macrofaunal dataset characteristics

The European dataset was comprised of 36,456, individuals while the winter and summer Hong Kong datasets were comprised of 31,192 and 25,646 individuals respectively. A summary of the relative proportions of major faunal groups characterising the datasets are shown in Table 2.1. The European and the winter Hong Kong datasets were dominated by Polychaetes followed by Oligochaetes, Molluscs, Echinoderms and Crustaceans. The summer Hong Kong dataset was also dominated by Polychaetes, however, Molluscs were the second most abundant group, followed by Oligochaetes, Echinoderms and finally Crustaceans. In all three datasets minor faunal groups included Nematoda, Nemertina, Cnidaria, Phoronida and Sipuncula. These minor faunal groups accounted for between 5% and 1% of the total faunal abundances in the datasets.

Table 2.1: Summary of major fauna groups characterising European and Hong Kong datasets. Values are percentage contributions based on species abundances.

<table>
<thead>
<tr>
<th>Group</th>
<th>European</th>
<th>Hong Kong</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Winter</td>
<td>Summer</td>
</tr>
<tr>
<td>Polychaetes</td>
<td>49.28</td>
<td>71.25</td>
</tr>
<tr>
<td>Oligochaetes</td>
<td>25.18</td>
<td>11.63</td>
</tr>
<tr>
<td>Molluscs</td>
<td>9.66</td>
<td>2.09</td>
</tr>
<tr>
<td>Echinoderms</td>
<td>6.08</td>
<td>4.27</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>4.78</td>
<td>4.40</td>
</tr>
<tr>
<td>Cumulative %</td>
<td>94.98</td>
<td>93.64</td>
</tr>
</tbody>
</table>

The proportion of fauna in each taxonomic level for the species level datasets and the aggregated genus and family level datasets (see section 2.2.1.3) are presented in Table 2.2. In the European species level dataset the total number of taxa was 348. Aggregating the species data to genus and family taxonomic levels produced 266 and 135 taxa respectively. Similarly aggregating the winter and summer Hong Kong species datasets to family level reduced the number of taxa from 314 to 103 and from 328 to 93 respectively.
Table 2.2: Summary showing number of taxa within each taxonomic level in species, genus and family aggregated datasets for the European case study (2.2a) and Hong Kong surveys (2.2b). Numbers in parenthesis is the number of taxa successfully assigned to an Ecological Group using the AMBI library (v2 Feb 2010).

### 2.2a: European dataset

<table>
<thead>
<tr>
<th>Level</th>
<th>Species</th>
<th>Genus</th>
<th>Family</th>
<th>Order</th>
<th>Class</th>
<th>Phylum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species level</td>
<td>284 (279)</td>
<td>45 (40)</td>
<td>10 (6)</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>6 (6)</td>
<td>348</td>
</tr>
<tr>
<td>Genus level</td>
<td>247 (216)</td>
<td>10 (6)</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>6 (6)</td>
<td>266</td>
<td></td>
</tr>
<tr>
<td>Family level</td>
<td>126 (43)</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>6 (6)</td>
<td>135</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.2b: Hong Kong datasets

<table>
<thead>
<tr>
<th>Season</th>
<th>Level</th>
<th>Species</th>
<th>Genus</th>
<th>Family</th>
<th>Order</th>
<th>Class</th>
<th>Phylum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>Species level</td>
<td>197</td>
<td>101</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>314</td>
</tr>
<tr>
<td></td>
<td>Family level</td>
<td>98 (22)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5 (5)</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>Species level</td>
<td>203</td>
<td>107</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>328</td>
</tr>
<tr>
<td></td>
<td>Family level</td>
<td>88 (28)</td>
<td>-</td>
<td>5 (5)</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.2.2 Intercalibration of M-AMBI across taxonomic levels using European data

AMBI software and guidelines from the authors concerning its calculation (Borja and Muxika, 2005; Muxika et al., 2007b) are available free from AZTI (http://ambi.azti.es). AMBI values are calculated based on the proportion of species within a sample across five EGs according to their sensitivity to disturbance (Borja et al., 2000). Calculation of M-AMBI EQR values involves ordination of samples based on the values of AMBI, number of species and Shannon-Wiener diversity. This ordination analysis is followed by Factor Analysis to determine the position of the sample along a scale linking the High and Bad reference stations (i.e. station EQR values are expressed as values between 1 and 0). In the current study reference conditions were set using the highest and lowest values in the datasets for each of the metrics used the calculation of M-AMBI (Borja et al., 2009). The EQR scale is divided into five ES classes (i.e. High, Good, Moderate, Poor, and Bad) by assigning a numerical value to each of the class boundaries allowing ES to be assigned to samples (Muxika et al., 2007b).
For the European case study, M-AMBI ES was determined using species, genus and family level datasets. The AMBI EG library used was v2 February 2010. Using the European species level data 338 of the 348 taxa (97.1%) were successfully ascribed an EG based on classifications provided within the AMBI EG library (Table 2.2a). Taxa that were not ascribed an EG based on the AMBI EG library were designated as “not assigned” in accordance to the recommendations of the authors (Borja and Muxika, 2005; Muxika et al., 2007b).

Using European genus and family level datasets 235 of 266 taxa and 42 of 135 taxa were ascribed an EG based on the AMBI library v2 Feb 2010 (Table 2.2a). Taxa in the genus and family level datasets that were novel to the AMBI taxa were assigned to EGs based on median EG values of all AMBI entries within the parent taxa (Table 2.3a and 2.3b respectively). For instance, the genus *Prionospio* was assigned to EG IV (i.e. median EG value based on 4 species in EG II, 1 species in EG III and 20 species in EG IV).

**Table 2.3:** List of genera and families novel to AMBI list (v2 Feb 2010) together with proposed Ecological Grouping (EG). Proposed genus (2.3a) and family (2.3b). EG values are the median AMBI EG of all congeners within the parent genus and family respectively. Faunal groups in parenthesis.

**2.3a: Genera**

<table>
<thead>
<tr>
<th>Taxon Name</th>
<th>EG</th>
<th>Taxon Name</th>
<th>EG</th>
<th>Taxon Name</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abyssoninoe (Pol)</td>
<td>I</td>
<td>Myrianida (Pol)</td>
<td>II</td>
<td>Nuculana (Biv)</td>
<td>I</td>
</tr>
<tr>
<td>Alitta (Pol)</td>
<td>I</td>
<td>Pirakia (Pol)</td>
<td>II</td>
<td>Nuculoma (Biv)</td>
<td>II</td>
</tr>
<tr>
<td>Amphitritides (Pol)</td>
<td>I</td>
<td>Pistella (Pol)</td>
<td>I</td>
<td>Tellimyia (Biv)</td>
<td>II</td>
</tr>
<tr>
<td>Chaetoparia (Pol)</td>
<td>II</td>
<td>Prionospio (Pol)</td>
<td>IV</td>
<td>Medicorophium (Cru)</td>
<td>III</td>
</tr>
<tr>
<td>Ephesiella (Pol)</td>
<td>I</td>
<td>Protodorvillea (Pol)</td>
<td>II</td>
<td>Palaemon (Cru)</td>
<td>I</td>
</tr>
<tr>
<td>Hediste (Pol)</td>
<td>III</td>
<td>Pseudomystide (Pol)</td>
<td>II</td>
<td>Acrocnida (Ech)</td>
<td>I</td>
</tr>
<tr>
<td>Lipobranchius (Pol)</td>
<td>III</td>
<td>Angulus (Biv)</td>
<td>I</td>
<td>Ophiocten (Ech)</td>
<td>II</td>
</tr>
<tr>
<td>Litocorsa (Pol)</td>
<td>III</td>
<td>Moerella (Biv)</td>
<td>I</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**2.3b: Family**

<table>
<thead>
<tr>
<th>Taxon Name</th>
<th>EG</th>
<th>Taxon Name</th>
<th>EG</th>
<th>Taxon Name</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nymphonidae (Ath)</td>
<td>I</td>
<td>Euryplacidae (Cru)</td>
<td>II</td>
<td>Aphroditidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Phoxichilidiidae (Ath)</td>
<td>II</td>
<td>Idoteidae (Cru)</td>
<td>II</td>
<td>Apistobranchiidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Arcidae (Biv)</td>
<td>I</td>
<td>Iphimediidae (Cru)</td>
<td>I</td>
<td>Arenicolidae (Pol)</td>
<td>I</td>
</tr>
</tbody>
</table>
### 2.3b cont: Family

<table>
<thead>
<tr>
<th>Taxon Name</th>
<th>EG</th>
<th>Taxon Name</th>
<th>EG</th>
<th>Taxon Name</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcticidae (Biv)</td>
<td>III</td>
<td>Ischyroceridae (Cru)</td>
<td>II</td>
<td>Chaetopteridae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Corbulidae (Biv)</td>
<td>IV</td>
<td>Leucothoidae (Cru)</td>
<td>I</td>
<td>Chrysopetalidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Donacidae (Biv)</td>
<td>I</td>
<td>Liljeborgiidae (Cru)</td>
<td>I</td>
<td>Cossuridae (Pol)</td>
<td>IV</td>
</tr>
<tr>
<td>Galeommatidae (Biv)</td>
<td>I</td>
<td>Maeridae (Cru)</td>
<td>I</td>
<td>Dorvilleidae (Pol)</td>
<td>IV</td>
</tr>
<tr>
<td>Kellicidae (Biv)</td>
<td>I</td>
<td>Megaluropidae (Cru)</td>
<td>I</td>
<td>Eulepethidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Lucinidae (Biv)</td>
<td>I</td>
<td>Melitidae (Cru)</td>
<td>I</td>
<td>Flabelligeridae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Montacutidae (Biv)</td>
<td>II</td>
<td>Nebaliidae (Cru)</td>
<td>IV</td>
<td>Glyceridae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Myidae (Biv)</td>
<td>II</td>
<td>Oedicerotidae (Cru)</td>
<td>I</td>
<td>Goniidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Mytilidae (Biv)</td>
<td>I</td>
<td>Ogyrididae (Cru)</td>
<td>I</td>
<td>Heterospionidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Noetiidae (Biv)</td>
<td>I</td>
<td>Oregoniidae (Cru)</td>
<td>I</td>
<td>Lumbrineridae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Nuculanidae (Biv)</td>
<td>I</td>
<td>Palaemonidae (Cru)</td>
<td>I</td>
<td>Magelonidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Nuculidae (Biv)</td>
<td>II</td>
<td>Pasiphaeidae (Cru)</td>
<td>III</td>
<td>Maldanidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Semelidae (Biv)</td>
<td>III</td>
<td>Photidae (Cru)</td>
<td>I</td>
<td>Nephtyidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Thraciidae (Biv)</td>
<td>I</td>
<td>Phoxocephalidae (Cru)</td>
<td>I</td>
<td>Nereididae (Pol)</td>
<td>III</td>
</tr>
<tr>
<td>Thyasiridae (Biv)</td>
<td>II</td>
<td>Polybiidae (Cru)</td>
<td>I</td>
<td>Oenonidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Ungulinidae (Biv)</td>
<td>II</td>
<td>Pontoporeiidae (Cru)</td>
<td>I</td>
<td>Ophelidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Veneridae (Biv)</td>
<td>I</td>
<td>Porcellanidae (Cru)</td>
<td>I</td>
<td>Orbinidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Yoldiidae (Biv)</td>
<td>I</td>
<td>Processidae (Cru)</td>
<td>I</td>
<td>Oweniidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Cerianthidae (Cni)</td>
<td>I</td>
<td>Uristidae (Cru)</td>
<td>II</td>
<td>Paraonidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Veretillidae (Cni)</td>
<td>I</td>
<td>Urothoidae (Cru)</td>
<td>I</td>
<td>Pectinariidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Virgulariidae (Cni)</td>
<td>I</td>
<td>Asteriidae (Ech)</td>
<td>I</td>
<td>Pholoidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Alpheidae (Cru)</td>
<td>II</td>
<td>Caudinidae (Ech)</td>
<td>I</td>
<td>Phyllodocidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Ammomegacanthus (Cru)</td>
<td>I</td>
<td>Phyllophoridae (Ech)</td>
<td>I</td>
<td>Pilargidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Amphiocochliidae (Cru)</td>
<td>II</td>
<td>Synaptidae (Ech)</td>
<td>I</td>
<td>Poecilocheiridae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Ampithoides (Cru)</td>
<td>II</td>
<td>Cylchinidae (Gas)</td>
<td>II</td>
<td>Polynoida (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Arcturidae (Cru)</td>
<td>I</td>
<td>Dotidae (Gas)</td>
<td>I</td>
<td>Scalibregmatidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Argissidae (Cru)</td>
<td>II</td>
<td>Eulimididae (Gas)</td>
<td>I</td>
<td>Sphaerodoridae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Atylidae (Cru)</td>
<td>I</td>
<td>Flabellinidae (Gas)</td>
<td>II</td>
<td>Spionidae (Pol)</td>
<td>III</td>
</tr>
<tr>
<td>Axilidae (Cru)</td>
<td>II</td>
<td>Nassariidae (Gas)</td>
<td>II</td>
<td>Syllidae (Pol)</td>
<td>II</td>
</tr>
<tr>
<td>Callianassidae (Cru)</td>
<td>III</td>
<td>Onchidorididae (Gas)</td>
<td>I</td>
<td>Terebellidae (Pol)</td>
<td>I</td>
</tr>
<tr>
<td>Calliopiidae (Cru)</td>
<td>I</td>
<td>Philinidae (Gas)</td>
<td>II</td>
<td>Ischnochitonidae (Ppc)</td>
<td>I</td>
</tr>
<tr>
<td>Cheirocratidae (Cru)</td>
<td>I</td>
<td>Acoetidae (Gas)</td>
<td>II</td>
<td>Lepidochitonidae (Ppc)</td>
<td>II</td>
</tr>
<tr>
<td>Corophiidae (Cru)</td>
<td>III</td>
<td>Ampharetidae (Gas)</td>
<td>II</td>
<td>Dentaliidae (Sca)</td>
<td>II</td>
</tr>
<tr>
<td>Dexaminidae (Cru)</td>
<td>III</td>
<td>Aphanomidae (Gas)</td>
<td>II</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Groups:** Ath, Arthropoda; Biv, Bivalves; Cni Cnidarians; Cru, Crustaceans; Ech, Echinoderms; Gas, Gastropods; Pol, Polychaetes; Ppc, Polyplacophora; Sca, Scaphopoda.
The agreement between ES classifications at sampling stations across taxonomic levels (i.e. species, genus and family level data) using standard inter-calibrated ES boundaries (High/Good 0.77; Good/Moderate 0.53; Moderate/Poor 0.39; Poor/Bad 0.2; (EC, 2008; Borja et al., 2009) was determined using a Kappa analysis (Cohen, 1960; Landis and Koch, 1977) applying the method presented in Borja et al. (2007) and Simboura and Reizopoulou. (2008). This methodology applies a weighting to misclassifications to down-weight the importance of misclassification between adjacent classes, while misclassifications between non-adjacent classes are assigned considerable importance (Kennedy et al., 2011; Forde et al., 2012). To maximise the agreement in ES classifications (i.e. to inter-calibrate ES outputs) across the different taxonomic level datasets the standard ES boundaries were modified using the approach outlined in Borja et al. (2007).

A Second-stage Multi-Dimensional Scaling (MDS) analysis (Somerfield and Clarke, 1995) was used to compare the multivariate patterns in faunal abundance data derived from each taxonomic level to each other and to the pattern derived from data aggregated to AMBI EGs level. The abundance data were square root transformed to down-weight the influence of the most dominant taxa. The transformed data were used to construct a Bray-Curtis similarity matrix for each taxonomic level and for the data that had been aggregated to EG. Spearman rank correlations were calculated between each pair of similarity matrices. These pairwise correlations were used as the distance metric in a second stage resemblance matrix, which was used as input for a Multi Dimensional Scaling (MDS) ordination (Kruskal and Wish, 1978; Clarke and Green, 1988). The relative distances between points on the second-stage MDS plot indicate how similar the multivariate patterns at each taxonomic level are to each other and to the pattern in the EG matrix.

2.2.3 Calculation of Family level M-AMBI at Hong Kong, China

Few species or genera in the Hong Kong datasets were listed in the AMBI database. Consequently, M-AMBI was calculated for the Hong Kong datasets
using family level data only. Using the winter and summer Hong Kong family level datasets 27 of 103 taxa and 33 of 93 taxa were successfully ascribed an EG using the existing AMBI library (Table 2.2b). For each dataset AMBI-novel taxa were assigned median EG values using the method outlined in section 2.2. Proposed EG values are presented in Table 2.3b. The ES classification boundaries used were the family level boundaries derived from the inter-calibration exercise using the European datasets (see section 2.2.2).

For the winter and summer surveys ArcVIEW 10 was used to interpolate EQR point data to create continuous surface maps with coastline polygons used as boundaries. The inter-calibrated family level ES classification boundaries were applied to interpolated EQR surfaces to create maps depicting ES.

2.3 RESULTS

2.3.1 Calculation and intercalibration of M-AMBI across taxonomic levels using European data

The proportions of stations assigned to the AMBI ES classes for the species level dataset and the aggregated genus and family level datasets are presented in Table 2.4a. Weighted Kappa analysis of the species and genus level M-AMBI EQR outputs showed “Almost perfect” agreement between the ES classifications (Kappa value = 0.95%; % Problem = 2.9). This level of agreement could not be improved upon by modifying the genus EQR ES boundaries.

Similarly using standard ES classification boundaries for species and family EQR outputs resulted in “Almost Perfect” agreement between ES classifications (Kappa value = 0.92; % Problem = 3.9). However, adjusting (or intercalibrating) the ES boundaries for family level EQR outputs had a small positive effect on the agreement between species and family ES classifications (Kappa value = 0.947; % Problem classifications = 1.0). Intercalibrated family level ES class boundaries were High/Good 0.75,
Good/Moderate 0.49, Moderate/Poor 0.34 and Poor/Bad 0.19. The proportions of stations assigned to the AMBI ES classes for the family level dataset using the intercalibrated ES boundaries are presented in Table 2.4a.

Table 2.4: Summary of assignments of stations to Ecological Status (ES) classes using M-AMBI (AMBI library v2 Feb 2010).

2.4a: Counts of European stations assigned to ES classes using datasets aggregated to species, genus and family level.

<table>
<thead>
<tr>
<th>Aggregation Level</th>
<th>Species*</th>
<th>Genus*</th>
<th>Family**</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>36</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>Good</td>
<td>45</td>
<td>48</td>
<td>45</td>
</tr>
<tr>
<td>Moderate</td>
<td>10</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Poor</td>
<td>11</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Bad</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>103</td>
<td>103</td>
</tr>
</tbody>
</table>

2.4b: Counts of Hong Kong sampling station assigned to ES classes using datasets aggregated to species, genus and family level.

<table>
<thead>
<tr>
<th>Survey</th>
<th>Winter**</th>
<th>Summer**</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Good</td>
<td>81</td>
<td>73</td>
</tr>
<tr>
<td>Moderate</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Poor</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Bad</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>119</td>
</tr>
</tbody>
</table>

* Standard ES class boundaries used; High/Good 0.77, Good/Moderate 0.53, Moderate/Poor 0.39 and Poor/Bad 0.2 (*sensu* EC 2008; Borja et al., 2009).

** Intercalibrated ES class boundaries used; High/Good 0.75, Good/Moderate 0.49, Moderate/Poor 0.34 and Poor/Bad 0.19.

For each of the Bray-Curtis similarity matrices used in the Second-stage MDS analysis the number of taxa within each taxonomic level is presented in Table 2.5. Spearman pairwise rank correlations between Bray-Curtis similarity matrices for each taxonomic level and EG level were all $>0.68$ (Table 2.6). Second-stage MDS ordination (Figure 2.2) of these pairwise correlations shows that the correlation in multivariate patterns between each
taxonomic level to the pattern in the EG matrix increases as taxonomic level increases. For example, EG matrix is more highly correlated with that of family than with species or genus. Similarly EG is more highly correlated with Class and Phylum than to species, genus and Family (Figure 2.2).

Table 2.5: Summary of the number of taxa within each taxonomic level in the Bray-Cutis similarity matrices used in the Second-stage MDS analysis.

<table>
<thead>
<tr>
<th>Aggregation Level</th>
<th>Species</th>
<th>Genus</th>
<th>Family</th>
<th>Order</th>
<th>Class</th>
<th>Phylum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>284</td>
<td>45</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>348</td>
</tr>
<tr>
<td>Genus</td>
<td>247</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td></td>
<td>266</td>
</tr>
<tr>
<td>Family</td>
<td>126</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td></td>
<td></td>
<td>135</td>
</tr>
<tr>
<td>Order</td>
<td>40</td>
<td>2</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
<td>48</td>
</tr>
<tr>
<td>Class</td>
<td></td>
<td></td>
<td>14</td>
<td>6</td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Phylum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2.6: Pairwise Spearman rank correlations between similarity matrices derived from European case study abundance data aggregated to a range of taxonomic levels and AMBI Ecological Grouping (EG).

<table>
<thead>
<tr>
<th>Aggregation Level</th>
<th>Species</th>
<th>Genus</th>
<th>Family</th>
<th>Class</th>
<th>Phylum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus</td>
<td>0.871</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>0.806</td>
<td>0.945</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class</td>
<td>0.720</td>
<td>0.796</td>
<td>0.817</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phylum</td>
<td>0.703</td>
<td>0.781</td>
<td>0.807</td>
<td>0.977</td>
<td></td>
</tr>
<tr>
<td>EG</td>
<td>0.683</td>
<td>0.755</td>
<td>0.788</td>
<td>0.829</td>
<td>0.830</td>
</tr>
</tbody>
</table>
Figure 2.2: Second stage MDS ordination of pairwise Pearson rank correlations between similarity matrices derived from European case study abundance data aggregated to a range of taxonomic levels and AMBI Ecological Grouping (EG).

2.3.2 Calculation of Family level M-AMBI at Hong Kong, China

For both surveys ES was calculated using the family level dataset and the intercalibrated ES boundaries (described in Section 2.4.1). ES was generally Good to Moderate in both summer and winter (Figures 2.3a and 2.3b respectively; Table 2.4b). In summer 78% of stations met or exceeded Good ES while the remainder were classified as Moderate or worse. In winter there was a general improvement in ES with almost 88% of stations attaining Good ES or better. This winter improvement in ES was most apparent at Deep Bay to the north-west, in Victoria Harbour near Hong Kong Island and at Tolo Harbour to the north-east (Figures 2.3a and 2.3b).
Figure 2.3: Map showing family level M-AMBI Ecological Status (ES) in summer (2.3a) and winter (2.3b) in 2001 at Hong Kong and surrounding waters, China.
2.4 DISCUSSION

2.4.1 Taxonomic sufficiency and M-AMBI in marine monitoring programs

Taxonomic sufficiency (TS) in ecological monitoring programs significantly reduces costs, time and the expertise needed for species level identification while avoiding the loss of relevant ecological information (Ferraro and Cole, 1990, 1995; Dauvin et al., 2003; Belvilacqua et al., 2009; Trigal-Domínguez et al., 2010). TS also has the added advantage of reducing the risk of errors in taxonomic identification at higher taxonomic levels (Olsgard and Somerfield, 2000; Dauvin et al., 2003). According to the “hierarchical-response-to-stress” principle the taxonomic level required to discriminate changes in benthic communities could depend on the strength of the anthropogenic disturbance (Dauvin et al., 2003). Variation in natural environmental conditions are thought to influence community assemblage structure by forcing species replacement while relatively more severe anthropogenic disturbance forces changes in the proportion of higher level taxa (Warwick, 1988a,b; Ferraro and Cole, 1990, 1995; Dauvin et al., 2003). Studies have shown that in soft-bottom benthic communities higher taxonomic levels, particularly the family level, are sufficient to reflect species level responses across strong anthropogenic disturbance gradients (e.g. heavy industrial and domestic discharge, oil and heavy metal pollution) (Warwick, 1988a,b; Ferraro and Cole, 1990, 1995; Somerfield and Clarke, 1995; Vanderklift et al., 1996; Olsgard and Somerfield, 2000; Olsgard et al., 1997, 1998; Dauvin et al., 2003; Belvilacqua et al., 2009; Soares-Gomes et al., 2012). Fewer studies have tested the effects of TS across weak or intermediate anthropogenic disturbance gradients (Bevilacqua et al., 2009; Bacci et al., 2009). This gap in knowledge has led to concerns among authors as to whether higher taxonomic levels can accurately reflect the changes in community structure associated with less severe disturbance gradients (Dauvin et al., 2003; Bevilacqua et al., 2009). However, some studies have shown that family level identification is a good indicator of
species level community variation across weak natural environmental gradients (Dethier and Schoch, 2006; Bevilacqua et al., 2009).

Another concern regarding using TS in monitoring programs is the loss of ecologically relevant information by the exclusion of species that may be useful indicators of particular types of disturbance (Mauer, 2000; Dauvin et al., 2003). While this is a real concern in freshwater systems where congeners may exhibit wide responses to pollution and disturbance (Resh and Unzicker, 1975), many marine invertebrate congeners appear to exhibit similar tolerances to pollution (Dauvin et al., 2003). However, some marine monitoring studies have shown that species within certain genera and families have specific responses to pollution disturbances such as oil, organic matter and metal contamination. For example, the amphipod *Ampelisca sarsi* has been shown to survive at low densities after oil spills when all other species of the genus disappear completely (Dauvin, 1998). Furthermore, studies have shown that species may exhibit different response to particular disturbances depending on habitat/geography (Grémare et al., 2009; Teixeira et al., 2010, 2012). In such instances, species are very useful bio-indicators (Dauvin et al., 2003). Consequently, there is a risk that the response of the benthos to disturbance may be overlooked when identifications are carried out at higher taxonomic levels (Trigal-Domínguez et al., 2010).

Most biotic EQRs used for monitoring ES in the marine environment are based on changes in the relative proportions of stress sensitive and tolerant taxa (Dauvin et al, 2008; Borja et al., 2011). Consequently, a crucial step in the calculation of EQRs such as M-AMBI is the allocation of taxa to appropriate EGs. The conservative nature of some marine invertebrate congeners in terms of their response to disturbance (Dauvin et al., 2003) is reflected in how the AMBI lists assigns EGs to taxa even at the level of family, Class and Phylum. This is based on expert judgement and consensus that all members of a particular family, Class or Phylum are similar. For example, in the AMBI list (v2 February 2010) EGs have been assigned to polychaete families such as Hesionidae (II) and Sabellidae (I), echinoderm Classes such as Holothuroidea (II) and Ophiuroidea (II) and Phyla including
Cnidaria (I) and Nemertina (III). In the current study higher taxa were ascribed an EG based on the median values for AMBI entries within the genus or family in question. Because EG are an ordinal classification of sensitivity to disturbance, median values were thought to be the most accurate measure of the central tendency of the EG values within the taxa.

In a recent study, Dimitriou et al. (2012) investigated the effect of using higher taxonomic level data on the Benthic Quality Index (BQI) (Rosenberg et al., 2004; Leonardsson et al., 2009). Dimitriou et al. (2012) showed BQI ES calculated using family level data was significantly and highly correlated to BQI, AMBI (Borja et al., 2000), Shannon diversity H’ (Shannon and Weaver, 1949) and BENTIX (Simboura and Zenetos, 2002) ES calculated using species level data. The significant correlation between ES values indicated the BQI tool was robust to changes in taxonomic level data (Dimitriou et al., 2012). Similarly, in the European case studies used here, classification of ES using M-AMBI was robust to aggregation to genus level and required only minor adjustment of the ES class boundaries to produce the optimal match with species level M-AMBI ES when family level data were used. This adjustment may have been necessary because of lower values of Shannon diversity and number of taxa at the family level, in addition to changes in the value of AMBI. Here, the robustness of M-AMBI ES classification was taken as confirmation that family level data from Hong Kong could be used to determine M-AMBI ES with some degree of confidence.

Other studies have investigated the effect of calculating AMBI with modified data. Kennedy et al. (2011) demonstrated that in Kinsale Harbour, M-AMBI was robust to changes in the sieve size used to retain macrofauna, and to the omission of groups other than Polychaetes, Molluscs and Echinoderms from the analysis. Warwick et al. (2010) investigated the effect of calculating AMBI using transformed abundance data, biomass data and production data when compared to a disturbance axis generated from meta-analysis of several previously described impacts. They found that all versions of AMBI calculated provided a better fit to the disturbance axis than standard diversity indices, and that AMBI derived from moderately (square root) transformed
abundance data provided the best fit. These studies verify that “the disturbance response in macrobenthic community structure is robust to some degrees and some types of simplification” (Kennedy et al., 2011). In a recent study Muxika et al. (2012) showed AMBI could be successfully applied using functional measures of the macrobenthos, such as biomass and production.

The premise behind aggregation to higher taxonomic levels is that, in a broad sense, significant changes in the distribution of genera and families is an indicator of significant disturbance, because many members of macrofaunal families share similar sensitivities (Dauvin et al., 2003). Assigning EG takes a different approach, aggregating the species into groups with similar sensitivities without reference to taxonomic relatedness. In this study, using the European dataset we investigated how aggregation to higher taxonomic levels compared to aggregation to EG by comparing the multivariate distributions. EG distribution was more highly correlated with the distribution of families than with the distribution of species. This may indicate that aggregation to family level is similar to aggregation to EG, because they both integrate the sensitivities of the original species. Another possible explanation is that aggregation to family level simply reduces the number of variables used to produce the Bray Curtis similarity index, smoothing out the relationship between the samples (i.e. as the taxonomic resolution of the datasets is reduced, the datasets begin to resemble one another more in terms of their multivariate structure). EG is perhaps the most severe aggregation applied to species data in this study, reducing the number of taxa to 5. The number of taxa in the other matrices was 348 for species, 266 for genus, 135 for family, 48 for Order, 20 for Class and 10 for Phyla. EG was most correlated with class and phyla, the levels with the fewest taxa. While it is intuitively acceptable (and verified by the AMBI library in some cases) that the species within many families are largely the same in terms of sensitivity, this is unlikely to be the case for the species within a Class or Phylum. Further research to establish the linkage between EG and taxonomic aggregation using larger datasets with different disturbance gradients would be useful to clarify this issue.
Until more species are assigned to EGs, using higher taxonomic level data with M-AMBI may be useful in extending the use EQRs and WFD type approaches to management in new geographical regions. This is particularly important in areas where the current lack of knowledge of the composition and ecology of benthic macrofauna communities for the moment prohibits the development of local environmental management tools. However, the use of TS in monitoring programs is likely to be most advantageous when supported by periodic species level studies to reduce the risk of missing subtle species level responses to disturbance (Ferraro and Cole, 1990; Olsgard et al., 1998).

2.4.2 Suitability of Family level M-AMBI in Hong Kong waters

AMBI based methodologies for ES assessments are based on the sensitivity of species to organic matter enrichment; however, the sensitivity of many Asian taxa has not been designated at this point. To overcome this and facilitate M-AMBI ES analyses, the Hong Kong species lists were aggregated to the higher taxonomic level of family. In this study, the suitability of family level M-AMBI for ES assessment in sub-tropical inshore and coastal areas was tested based on its ability to account for previously described patterns in benthic communities. In a baseline study at Hong Kong and its surrounding waters, Shin et al. (2004) described distinct seasonal changes in community structure. During summer months, Deep Bay (northwest of Hong Kong) was characterised by a low diversity benthic community dominated by stress tolerant species. The dominance of stress tolerant species was attributed to local salinity stresses caused by increased wet season river discharge in the Pearl estuary (Morton and Wu, 1975; Shin et al., 2004; Shin and Ellingsen, 2004). Here, family level M-AMBI classified ES at Deep Bay between Bad and Moderate, reflecting the status of the stressed summer community. In winter, with the exception of one sampling station at the mouth of Deep Bay, ES in the area improved. This winter improvement in ES reflects a local temporal shift in community structure described by Shin et al. (2004). The shift in community structure was attributed to increases in salinity owing to reduced winter river flow. This reduction in salinity stress produced more
favourable environmental conditions which allowed temporal increases in community diversity (Shin et al., 2004). The ability of family level M-AMBI to detect changes in ES associated with salinity stress reflects the typical response of species level M-AMBI to salinity in estuaries (Zettler et al., 2007). Previous studies have shown that Victoria Harbour to the north of Hong Kong Island is influenced by domestic sewage discharge (Wu, 1982; Thompson and Shin, 1983) while Tolo Harbour and Tolo Channel to the northeast are subject to seasonal hypoxia events (Shin et al., 2004; Fleddum et al., 2011). In the current study M-AMBI classified the organically enriched areas around Victoria Harbour as ranging between Bad and Moderate in both summer and winter. M-AMBI did highlight a slight seasonal improvement in ES at Victoria Harbour during the winter season. This relative temporal improvement may be due to increased environmental stability in winter due to seasonal reductions in freshwater discharge. Previous studies at Tolo Harbour and Tolo Channel have related increased dominance by stress tolerant species and reduced community diversity to low dissolved oxygen levels experienced during summer hypoxia events. In winter dissolved oxygen levels in the area increase allowing the reestablishment of sensitive species (Shin et al., 2004; Fleddum et al., 2011; Shin and Ellingsen, 2004). In the current study, changes in M-AMBI ES reflected this seasonal shift in benthic community structure, changing from Bad and Moderate in the summer season to Moderate and Good in winter. Cheung et al. (2008) demonstrated the use of AMBI on polychaete fauna in reflecting changes in sediment quality in Victoria Harbour. The present study further confirmed the applicability of such an index in subtropical waters.

The current study represents the first attempt at investigating the effect of explicitly using abundances of taxa higher than species to derive M-AMBI EQR in sub-tropical waters. In general, classification of ES by M-AMBI was robust to data changes in the taxonomic level to which data were aggregated. This ability of M-AMBI to consistently classify ES at increasingly higher taxonomic level supports a TS approach to monitoring using M-AMBI. This approach may facilitate the transfer of WFD management techniques to
new geographical areas, as demonstrated here in the case of subtropical Hong Kong.

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2.6 CONTRIBUTIONS

JF and RK participated in survey work at Kinsale. JF assisted Patrick Colman Collins and Adrian Patterson on laboratory analysis of Kinsale faunal samples. PKSS provided the Hong Kong dataset while PJS provided the Clyde and Ekofisk datasets. JF and BK collaborated on statistical and spatial analyses. JF was the primary author of this manuscript.
3 COMPARISON OF GRANULOMETRIC METHODS AND SAMPLING STRATEGIES USED IN MARINE HABITAT CLASSIFICATION AND ECOLOGICAL STATUS ASSESSMENT

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ABSTRACT

Sediment particle size analysis (PSA) is routinely used to support benthic macrofaunal community distribution data in habitat mapping and Ecological Status (ES) assessment. Two commonly used granulometric Techniques for characterising sediments are wet/dry-sieving (WDS) or a combination of laser particle sizing (LPS) and WDS. Hydrogen peroxide ($H_2O_2$) pre-treatment is frequently used to remove/digest organic material adhering to the sediment grains prior to PSA. Frequently programs monitoring ES employ a single grab strategy whereby the grab is used for combined sediment particle size and macrofaunal analyses while other programs use independent grabs for particle size and macrofaunal analyses.

Currently, the effects of using different PSA Methods and sampling strategies on the ability of resulting sediment distribution data to explain variability in multivariate macrofaunal distribution data have yet to be identified. Similarly the effect of changing PSA Methods and sampling strategies on ES classification (as calculated using the UK and Ireland Infaunal Quality Index; IQI) and EU Nature Information System sediment classifications has not been examined. To address these gaps in knowledge, we use benthic macrofaunal and PSA grabs from two embayments in the south of Ireland. Four frequently used PSA Methods (i.e. WDS or LPS/WDS Technique with/without $H_2O_2$ pre-treatment) and two common sampling strategies (i.e. single and multiple grab survey designs) are applied.

A combination of LPS and WDS without $H_2O_2$ pre-treatment was identified as the PSA Method that explained the most variance in macrofaunal distributions. IQI ES and EUNIS sediment classification were robust to changes in PSA Method. Fauna and sediment samples returned from the same grab sample significantly decreased macrofaunal variance explained by sediment distribution data and caused ES to be classified as lower. Employing the PSA Method and sampling strategy that maximises the amount of variance explained in macrofaunal distribution has the potential to increase confidence with which benthic habitats can be mapped and help improve benthic ES monitoring.
3.1 INTRODUCTION

Benthic faunal communities play an important role in the cycling of nutrients and material between the sediment and the water column, and have been shown to respond quickly to both anthropogenic and natural stress (Pearson and Rosenberg, 1978; Dauer, 1993; Borja et al., 2000). As a result, investigating benthic faunal communities and their sedimentary habitats is an effective way of assessing changes in ecological quality in coastal and transitional waters (Pearson and Rosenberg, 1978; Rosenberg et al., 2004; Kennedy et al., 2011). The suitability of the macrobenthos as bio-indicators in soft-bottom habitats can be attributed to aspects of their biology. Many macrobenthic species are relatively sedentary and cannot avoid adverse water/sediment conditions. Many species have relatively long life spans (> 2 years) and exhibit temporal responses to long term disturbances (Dauer, 1993). Macrobenthic communities consist of different species that exhibit different tolerances to stress. Consequently community responses are more sensitive to environmental disturbances than single indicator species or diversity indices (Underwood and Peterson, 1988; Clarke, 1993; Anderson et al., 2008).

Animal–sediment relationship studies have long reported strong associations between the structure of benthic faunal communities and the sediments they inhabit (Petersen, 1913; Sanders, 1958; Glémarec, 1973; Buchanan et al., 1978; Flint, 1981; Kunitzer et al., 1992; Mackie et al., 1996; van Dalfsen, et al., 2000). Sediment particle size analysis (PSA) is an environmental variable commonly used by benthic ecologists to explain fauna distributions, and aid classification and mapping of benthic communities (Seiderer and Newell, 1999; Eleftheriou and McIntyre, 2005; Jayaraj et al., 2008).

Two commonly used granulometric techniques for characterising sediments are wet/dry-sieving (WDS) or a combination of laser particle sizing (LPS) and WDS. The two techniques measure different sediment characteristics and so produce different sediment PSA distributions (Eleftheriou and McIntyre, 2005; Rodríguez and Uriarte, 2009). In WDS, sediments are sieved through a
series of sieves of decreasing aperture. The weight retained in each sieve represents discrete fractions. The relative proportions of these fractions are used to build distributions of particles and define the overall sediment class. Because grains are irregular in shape, dry sieving gives a more fine result as the grains pass through the sieves on their smallest axis (Eleftheriou and McIntyre, 2005). LPS is an optical technique which uses the average distribution of measured grain size axes and reports grain size as equivalent spherical volumes. LPS is used to measure the distribution of grain sizes up to a typical maximum size of 1 mm. Sediment fractions coarser than 1 mm are measured using WDS. The LPS (< 1 mm) and WDS (> 1 mm) datasets are later merged to produce the complete PSA distribution (Eleftheriou and McIntyre, 2005; NMBAQC, 2009). Studies comparing the grain size distributions measured by WDS and LPS have showed that WDS produces more fine and more sorted PSA distributions when compared to LPS (Murray and Holtum, 1996; Konert and Vandenberghe, 1997; Rodríguez and Uriarte, 2009).

Typically sediments are completely disaggregated using chemical dispersants (e.g. sodium hexametaphosphate) and/or sonication prior to PSA (Eleftheriou and McIntyre, 2005; NMBAQC, 2009). Disaggregation of natural aggregates into primary sediment particles may destroy important facets of the sediment matrix, such as faecal pellets, with which the resident fauna interact (Fuller and Butman, 1988; Snelgrove and Butman, 1994). Sediment organic carbon is regarded as a major food source for benthic macrofauna (Gunnarsson et al., 2009). Hydrogen peroxide (H$_2$O$_2$) is an oxidising pre-treatment that is frequently used to remove/digest organic material adhering to the sediment grains prior to PSA. H$_2$O$_2$ pre-treatment significantly reduces the mean and median grain size (Allen and Thornley, 2004; Vaasma, 2008). H$_2$O$_2$ digestion of organic material, like disaggregation of biologically important aggregates, may also destroy possible sediment/faunal linkages and so reduce the explanatory power of the sediment PSA dataset (Snelgrove and Butman, 1994). The majority of studies comparing sediment PSA techniques and pre-treatments fail to deal with comprehensive ranges of sediment types and are confined to specific sediment classes e.g. fine and
medium grained sands (Rodríguez and Uriarte, 2009), fine sediment (Allen and Thornley, 2004; Vaasma, 2008). There are no published studies investigating the effect of sediment PSA Method (i.e. WDS or LPS/WDS technique with/without H₂O₂ pre-treatment) on the power of sediment PSA distribution data to explain variance in multivariate macrofaunal data.

The Water Framework Directive (WFD, 2000/60/EC) is a pan-European management directive which provides a framework for the protection and enhancement of the Ecological Status (ES) of water bodies including estuarine/transitional and coastal waters. Under the WFD, member-states are legally required to (1) classify and (2) assess and monitor the water bodies with the overall objective of achieving “Good Ecological Status” for all waters by 2015.

The European Nature Information System (EUNIS) (Connor et al., 2004; Davies et al., 2004) is a classification system that uses both abiotic and biotic factors to identify ecological biotopes. EUNIS classification of biotopes can be either bottom-up or top-down. Bottom-up classification follows a taxonomic approach with biotopes or habitats based on classification of macrofaunal distributions. Top-down classifications are based on physical characteristics such as sediment type (Davies et al., 2004; Connor et al., 2004). EUNIS top-down classification uses a simplification of the sediment classification (Long, 2006) proposed by Folk and Ward (1957). Under Long (2006) PSA distributions are assigned to one of four broad hierarchal sediment classes i.e. muds and sandy muds (Mu), sand and muddy sand (Sa), mixed sediment (Mx), coarse sediment (Cs). These EUNIS sediment classes are used as a major classifier when identifying biotopes (Davies et al., 2004; Connor et al., 2004). The effect of PSA Method on EUNIS sediment classification has not been published in the literature to date.

Under the WFD each member state is obliged to assess the ES of water bodies. Assessment of water status is based on physicochemical and biological elements, with the latter element being of particular importance (Borja, 2005). Ecological monitoring data are compared to undisturbed
reference conditions to derive an Ecological Quality Ratio (EQR). EQRs range between 0 and 1, with high status represented by values close to 1 and Bad status close to 0. The EQR scale is sub-divided into five ES classes (High, Good, Moderate, Poor and Bad). Several multi-metric indices have been proposed by member states for the assessment of ES based on the macrobenthos (Borja et al., 2009). The UK and Ireland Infaunal Quality Index (IQI) is a multi-metric EQR tool that assesses ES based on macrobenthic distributions and PSA in transitional and coastal waters (Prior et al., 2004; Mackie, 2009; Borja et al., 2007, 2011). The macrobenthic elements that are included in IQI ES assessment include measures of diversity and abundance of invertebrate taxa and a measure of the proportion of disturbance-sensitive taxa. Physico-chemical elements include sediment and habitat salinity (i.e. transitional or coastal). The effect of PSA Method on designation of ES using IQI has yet to be determined.

Some programs monitoring ES have employed a sampling strategy where a single grab sample is taken at each station (Seiderer and Newell, 1999; Kennedy, 2008). The grab is sub-sampled for PSA, and the remainder of the grab sample is analysed for macrofaunal. Motivation for single grab survey designs include cost reduction and the rationale that PSA distributions will have more Affinity with the macrofaunal distribution allowing for their communities to be classified with increased levels of confidence. There are possible disadvantages with this approach. Sub-sampling the macrofaunal grab may affect the recording of rare or less numerous species (Clarke and Warwick, 2001). Removing a sub-sample that would have a consistent effect on macrofaunal distribution is intuitively quite difficult. A standard volume of subsample would remove a different percentage of material depending on grab penetration (sample volume). Different types of sediments require different sample volumes for representative samples (British Standards Institution, 1996; Boyd, 2002; Passhier, 2007). The subsample necessary to characterise coarse or mixed sediments may be far larger than that necessary to characterise fine sediments. An independent grab sample for PSA may be a better sampling strategy if the Affinity of the PSA from the independent grab sample to the macrofauna is better than that of the PSA.
sub-sampled from the macrofaunal grab sample. There are no published accounts of experiments to determine the effect of sample Affinity on the linkage between PSA and macrofaunal distributions.

The aim of the current study was to address current gaps in scientific knowledge by determining 1) the effects of using different PSA Methods and sampling strategies on the ability of PSA distribution data to explain variability in multivariate macrofaunal distribution data, and 2) the effect of changing PSA Method and sampling strategies on ES and EUNIS sediment classifications. Here, we use a 59 station spatial dataset of macrofaunal and sediment Day grabs (taken using two sampling strategies) from embayments on the south coast of Ireland. Four PSA Methods are applied and significant difference in the power of the four PSA distributions to explain variance in the multivariate structure of the macrofaunal data is determined. For each station, PSA distributions derived from each of the four different datasets were classified according to the simplified EUNIS classification of Long (2006). The effect of PSA Method on the classification of sediment type is investigated. ES *sensu* the WFD (2000/60/EC) is determined using IQI. The effect of PSA Method on IQI ES classification is determined. We investigate the effect of changing sampling strategy (i.e. sub-sampling) on the power of PSA distributions to explain variance in faunal distributions. The effect of sub-sampling on IQI component indices, EQR and ES is investigated.

### 3.2 MATERIALS AND METHODS

#### 3.2.1 Study area

The study site extends over two embayments on the south coast of Ireland, namely the Bandon and Belgooly estuaries and their harbours at Kinsale and Oysterhaven respectively. In May 2009 a total of 59 shallow stations (<15 m) were sampled. The stations covered a comprehensive range of sediment types, including coarse and mixed sediments, sands and muddy sands, and muds and sandy muds (Figure 3.1). Salinities ranged from fully marine conditions in the outer harbours (> 30 practical salinity units; psu) to polyhaline (30 ≤ psu ≥ 18) in the high estuaries. Basic habitat information for
the estuaries and harbours are shown in Table 3.1. Identified soft bottom communities corresponded well to EUNIS level 4 biotopes (Connor et al., 2004; Table 3.1, Figure 3.2). Habitats 1 and 2 were characterised by species-poor communities typical of estuarine muds and sandy muds. Habitat 1 communities were dominated by polychaetes of the genus Nephtys, the polychaetes Scoloplos armiger and Melinna palmata and the oligochaetes Tubificoides benedii. Communities in Habitats 2 were relatively more species-impoverished than those found in Habitat 1, and were dominated by oligochaetes and the tube dwelling Spionid polychaetes Streblospio shrubsolii and Pygospio elegans. Habitat 3 was characterised by mixed sediments dominated by the Caprellid crustacean Pariambus typicus and polychaetes Eumida bahusiensis and Mediomastus fragilis. Habitat 4 was limited to dredged sites in the Bandon estuary. The mixed sediment communities at these physically impacted areas were dominated by the polychaetes M. palmata and M. fragilis. Habitat 5 was characterised by sandy mud communities dominated by the bivalves Kurtiella bidentata and Abra nitida and the polychaete Chaetozozone gibber. Habitat 6 was characterised highly reduced muddy sediments. The highly impacted nature of the area was reflected by the species-poor community which was exclusively comprised of T. benedii (Oligochaeta) and the polychaete Capitella. The community found in Habitat 7, at the mouth of Kinsale Harbour, was a muddy sand marine community dominated by the ophiuroid Amphiura filliformis and the bivalve K. bidentata. Communities in the sandy sediments of Habitat 8 were dominated by polychaetes of the genus Magelona and bivalves Fabulina fabula and A. nitida. Sediments in Habitats 9 were muddy sands which supported diverse faunal assemblages dominated by bivalves and polychaetes. Resident bivalves included Dosinia lupinus, Thracia phaseolina and F. fabula while polychaetes were represented by members of the genus Nephtys, the Spionid Spiophanes bombyx and the Sabellid Owenia filliformis. The deepest sampling stations were in Habitat 10. This area supported a diverse marine muddy sand community dominated by Lumbrinerid and Mageloniid polychaetes and Phoronids. Habitat 11, the harbour mouth of Oysterhaven, was a high energy habitat characterised by a coarse sandy community dominated by the polychaetes Pisme remota, Glycera convoluta
and *Pista cristata*. The southern regions outside the sampling areas were rocky.

**Figure 3.1:** Map showing EUNIS sediment classifications at sampling stations at Kinsale and Oysterhaven, Co Cork (2009). EUNIS sediment classification assigned using LPS-NP-SA samples; Mu = Mud and sandy mud, Sa = Sand and muddy sand, Mx = Mixed sediment, Cs = Coarse sediment.
**Table 3.1**: Basic habitat information for sampling areas at Kinsale and Oysterhaven, Co Cork (2009).

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Mean Depth</th>
<th>Sediment Type</th>
<th>Mean Salinity</th>
<th>EUNIS Level 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5m</td>
<td>Mu</td>
<td>23</td>
<td>Infralittoral muddy sand</td>
</tr>
<tr>
<td>2</td>
<td>5m</td>
<td>Mu</td>
<td>26</td>
<td>Infralittoral muddy sand</td>
</tr>
<tr>
<td>3</td>
<td>10m</td>
<td>Mx</td>
<td>29</td>
<td>Infralittoral mixed sediment</td>
</tr>
<tr>
<td>4</td>
<td>10m</td>
<td>Mu</td>
<td>29</td>
<td>Infralittoral muddy sand</td>
</tr>
<tr>
<td>5</td>
<td>10m</td>
<td>Mu</td>
<td>29</td>
<td>Infralittoral muddy sand</td>
</tr>
<tr>
<td>6</td>
<td>1.5m</td>
<td>Mu</td>
<td>29</td>
<td>Infralittoral mud</td>
</tr>
<tr>
<td>7</td>
<td>14m</td>
<td>Mu</td>
<td>32</td>
<td>Infralittoral muddy sand</td>
</tr>
<tr>
<td>8</td>
<td>12m</td>
<td>Mu</td>
<td>32</td>
<td>Infralittoral muddy sand</td>
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<tr>
<td>9</td>
<td>10m</td>
<td>Mu</td>
<td>32</td>
<td>Infralittoral muddy sand</td>
</tr>
<tr>
<td>10</td>
<td>18m</td>
<td>Mu</td>
<td>34</td>
<td>Circalittoral muddy sand</td>
</tr>
<tr>
<td>11</td>
<td>12m</td>
<td>Sa</td>
<td>34</td>
<td>Circalittoral sand</td>
</tr>
</tbody>
</table>

Sediment type is the simplified sediment classification (Long, 2006) proposed by Folk and Ward (1957) i.e. Mud and sandy mud (Mu), Sand and muddy sand (Sa), Mixed sediment (Mx), Coarse sediment (Cs). EUNIS Level 4 classification *sensu* Connor et al. (2004).
3.2.2 Sampling design

At each sampling station four replicate 0.1 m$^2$ Day grab samples were taken. Two grabs were retained exclusively for macrofaunal analysis (designated F$_1$ and F$_2$). The third grab retrieved was used for sediment PSA samples; four sediment samples were taken (samples designated S$_A$). The fourth grab retrieved at each station was used for combined sediment PSA and macrofaunal analyses i.e. a single grab sampling strategy. A sub-sample of this grab was taken for sediment PSA (S$_B$) while the remainder of the grab sample was used for macrofaunal analysis (F$_3$). All sediment samples were depth integrated samples and were visually inspected to ensure they were
representative of the whole grab (NMBAQC, 2009). Sediment samples were stored in sealed plastic bags and frozen at –20 °C pending analysis.

3.2.3 Macrofaunal sample processing

The macrofaunal grab samples, F_1, F_2 and F_3, were sieved through a 1mm sieve. Fauna retained on the sieves was fixed in 10% sea water buffered formalin and stored in 70% IMS. In the laboratory the macrofauna was stained using Eosin–Briebich scarlet dye, sorted, enumerated and identified to species level using standard keys/NMBAQCS guidelines (NMBAQC, 2009).

3.2.4 Sediment sample processing

3.2.4.1 WDS of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) pre-treated and non pre-treated sediments

S\textsubscript{A} sediment samples (i.e. those taken from independent grabs) were dried to constant weight at 100 °C. From each dried S\textsubscript{A} sediment, two 25 g subsamples were weighed out. One sub-sample was pre-treated with H\textsubscript{2}O\textsubscript{2} to remove organic material (samples labelled S\textsubscript{A}P) (Allen and Thornley, 2004). The second sub-sample was not pre-treated (S\textsubscript{A}NP). Granulometry was determined for both S\textsubscript{A}P and S\textsubscript{A}NP samples using the traditional WDS technique (Eleftheriou and McIntyre, 2005). The <63 µm fraction was determined by weight loss following wet sieving. Coarser fractions were determined by mechanical dry sieving through a series of full Phi Wentworth sieves ranging between 4 and –2 Phi units. The two granulometric datasets were labelled WDS-S\textsubscript{A}P and WDS-S\textsubscript{A}NP respectively.

3.2.4.2 LPS of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) pre-treated and non pre-treated sediments

Two S\textsubscript{A} sediment samples from each station were wet split at 1 mm and the < 1 mm fraction retained. One <1 mm sediment fraction was pre-treated with H\textsubscript{2}O\textsubscript{2} (labelled S\textsubscript{A}P) (Allen and Thornley, 2004). The second <1 mm sediment
sample was not pre-treated (S\textsubscript{A}NP). LPS was performed on three aliquots of each S\textsubscript{A}P and S\textsubscript{A}NP sediment (NMBAQC, 2009). In all cases the <1 mm fraction aliquot was added to the Hydro-G dispersion unit of a Malvern Mastersizer 2000 until obscuration reached between 15% and 18%. For each aliquot the measurement cycle was 5 X 30,000 scans. The <1 mm LPS data for the S\textsubscript{A}P and S\textsubscript{A}NP samples and their corresponding >1 mm WDS-S\textsubscript{A} data were merged together (i.e. the LPS data was normalised to the <1 mm WDS sieve percentage and then combined with the >1 mm sieve fractions to produce complete PSA distribution) (NMBAQC, 2009).

Granulometry was determined for each S\textsubscript{B} sediment sample using the non pre-treated LPS Method described above; the resulting dataset was labelled LPS-S\textsubscript{B}NP. All PSA data were processed using GRADISTAT (Blott and Pye, 2001) software to derive distribution modality and sediment particle graphic mean (Mz; Folk and Ward, 1957). Mz is a parameter used to describe the mean particle size of a distribution and is analogous to the graphic mean employed with the normal distribution in conventional statistics. Mz values could be used with confidence as approximately 90% of sediment samples exhibited unimodal distributions.

### 3.2.5 Statistical analysis

Analyses were carried out using PRIMER v6 (Clarke and Gorley, 2006) with the add-on package PERMANOVA+ (Anderson et al., 2008) and MINTAB v16.

#### 3.2.5.1 Effect of PSA Method on PSA explanatory power

The F\textsubscript{1} and F\textsubscript{2} faunal datasets (i.e. independent macrofaunal grabs) were averaged, square root transformed and used to generate a zero-adjusted Bray–Curtis similarity matrix (Clarke and Gorley, 2006). Each of the four S\textsubscript{A} PSA datasets were normalised. Distance-based Linear Modelling (DistLM) was used to determine the amount of variance in the multivariate faunal dataset explained by each of the four S\textsubscript{A} PSA datasets. The fit coefficient used was adjusted R\textsuperscript{2} with forwards and backwards selection to find the
optimal regression model. To test for significant differences in the power of the four $S_A$ PSA datasets to explain macrofaunal distribution, the DistLM analysis was bootstrapped using 200 iterations with 2 random samples eliminated. The distributions of adjusted $R^2$ values for each $S_A$ PSA Method were tested for significant differences using a One-way ANOVA with multiple pairwise comparisons made using Tukey’s method.

To explain the pattern in the outputs of the One-way ANOVA of the adjusted $R^2$ distributions, Repeated Measures ANOVA tests were carried out to determine the effects of (1) Technique on the Mz of LPS-$S_A$ and WDS-$S_A$ sample and (2) the effect of $H_2O_2$ Pre-treatment on the Mz of WDS samples.

3.2.5.2 Effect of PSA Method on EUNIS sediment class classification

EUNIS sediment classes sensu Long (2006) were assigned to the sampling stations using each of the four sediment $S_A$ PSA. The effect of PSA Method on the allocation of EUNIS sediment classes was determined using a Chi-square test.

3.2.5.3 Effect of PSA Method on IQI ES classification

IQI version 2 was calculated using the propriety tool in Microsoft Excel developed by the UK Environment Agency (Mackie, 2009). This includes truncation of the species lists, and spelling and synonym standardization. IQI EQR, a continuous variable between 0 and 1, is calculated as follows;

$$IQI = \frac{(0.38 \times \left( \frac{1 - AMBI/7}{1 - AMBI/7}_{\text{max}} \right) + 0.08 \times \left( \frac{1 - \lambda'}{1 - \lambda'}_{\text{max}} \right) + 0.54 \times \left( \frac{S^{0.1}}{S^{0.1}_{\text{max}}} \right) - 0.4}{0.6}$$

where AMBI is the AZTI Marine Biotic Index (Borja et al., 2000), $1 - \lambda'$ is Simpson’s Evenness Index, $S^{0.1}$ is $\log_{10}$ number of species, max parameters are the maximum reference values for the habitat. AMBI is a continuous variable based upon the proportions of five Ecological Groups to which
benthic species are allocated (Borja et al., 2000). AMBI is calculated within version 2 of the IQI tool using the species list current in 2009. Sediment classification and habitat salinity are also used in version 2 of the IQI tool (Mackie, 2009). IQI reference conditions are adjusted for local grain size and salinity using an algorithm within the tool. Salinity was entered as a categorical variable with two levels, either coastal or transitional. This was the same for all Methods. Changing the PSA Method changed the reference conditions for $S^{0.1}$, $1-\lambda'$ and AMBI. IQI EQRs were calculated at each station based on $F_1/F_2$ faunal dataset and each of the four SA PSA datasets in turn. The EQRs were assigned to the five ES classes. The agreement in sampling station ES classifications between different PSA Methods was determined using a Kappa analysis (Cohen, 1960; Landis and Koch, 1977) applying the method presented in Borja et al. (2007) and Simboura and Reizopoulou (2008). This methodology applies a weighting to misclassifications to down-weight the importance of misclassification between adjacent classes, while misclassifications between non-adjacent classes are assigned considerable importance (Kennedy et al., 2011).

3.2.5.4 Effect of sample Affinity on PSA explanatory power

The effect of Affinity on the power of the PSA distributions to explain macrofaunal distribution was tested. The adjusted $R^2$ fit coefficients calculated for sediment and fauna taken from the same grab (i.e. $F_3$ and LPS-S$_b$NP) were compared to sediments and fauna taken from separate grabs (i.e. $F_1$ and LPS-S$_A$NP). Bootstrap DistLM analyses were used to determine the amount of variance in multivariate faunal datasets explained by their corresponding PSA distributions (for method see Section 3.2.5.1). The distributions of adjusted $R^2$ values were tested for significant difference using a One-way ANOVA.
3.2.5.5 Effect of sample Affinity on IQI components, EQR and ES classification

IQI ES was assigned to sampling stations using fauna and sediment taken from separate grabs and, fauna and sediment taken from the same grab. The effect of Affinity on ES classifications was determined using Kappa analysis (described in Section 3.2.5.3). To explain the difference in ES classification caused by Affinity, Repeated Measures ANOVA tests were performed using EQRs and the components indices of IQI (i.e. AMBI, 1–λ', S) as response variables, Station as a random factor and Affinity as a fixed factor.
3.3 RESULTS

3.3.1 Effect of PSA Method on the explanatory power of sediment distributions

One-way ANOVA of the bootstrapped adjusted $R^2$ values indicated highly significant differences existed between the fits of the 4 PSA Methods to the multivariate $F_1/F_2$ faunal dataset (Table 3.2a). Tukey's pairwise comparisons of the mean adjusted $R^2$ values showed that LPS-S$_A$NP and LPS-S$_A$P were the only PSA Methods which did not differ significantly in terms of the amount of macrofaunal variation explained (Table 3.2b). The explanatory power of the WDS-S$_A$NP and WDS-S$_A$P datasets were significantly different (Table 3.2b) indicating that $\text{H}_2\text{O}_2$ pre-treatment had a negative effect on the fit of WDS samples to the $F_1/F_2$ fauna. Repeated Measures ANOVA indicated a highly significant effect of Technique (i.e. LPS or WDS) on sediment Mz (Table 2.3a). The WDS Technique produced a significantly finer PSA distribution than the LPS Technique.

Repeated Measures ANOVA of the Mz values of the WDS-S$_A$ samples indicated a highly significant effect of $\text{H}_2\text{O}_2$ Pre-treatment (Table 3.3b). $\text{H}_2\text{O}_2$ pre-treatment produced a significantly finer PSA distribution when compared to non pre-treated samples.
Table 3.2: One-way ANOVA of DistLM adjusted $R^2$ distributions for 4 PSA Methods with $F_1/F_2$ fauna dataset.

### 3.2a: One-way ANOVA Table of adjusted $R^2$ distributions using Method as a fixed factor.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>3</td>
<td>0.3886</td>
<td>0.3886</td>
<td>0.1300</td>
<td>3234.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>796</td>
<td>0.0319</td>
<td>0.0319</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>799</td>
<td>0.4205</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$S = 0.0063$, $R^2 = 92.42\%$, adjusted. $R^2 = 96.39\%$

### 3.2b: Multiple pairwise comparisons between mean adjusted $R^2$ values of 4 PSA Methods using Tukey’s method. Means that do not share a letter are significantly different.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>WDS-S$_A$-NP</td>
<td>0.301</td>
<td>A</td>
</tr>
<tr>
<td>WDS-S$_A$-P</td>
<td>0.270</td>
<td>B</td>
</tr>
<tr>
<td>LPS-S$_A$-NP</td>
<td>0.324</td>
<td>C</td>
</tr>
<tr>
<td>LPS-S$_A$-P</td>
<td>0.323</td>
<td>C</td>
</tr>
</tbody>
</table>
Table 3.3: Repeated Measure ANOVA analyses of SA sediment sample graphic mean (Mz).

### 3.3a: All SA samples using Technique as a fixed factor, station as a random factor.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technique</td>
<td>1</td>
<td>6.3862</td>
<td>6.3876</td>
<td>78.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Station</td>
<td>58</td>
<td>524.9879</td>
<td>9.0518</td>
<td>111.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>176</td>
<td>14.3559</td>
<td>0.0816</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>235</td>
<td>545.7425</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S = 0.2856, $R^2 = 97.37\%$, adjusted. $R^2 = 96.49\%$

### 3.3b: WDS SA samples using $H_2O_2$ pre-treatment as a fixed factor, station as a random factor.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>1</td>
<td>0.8541</td>
<td>0.8541</td>
<td>16.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Station</td>
<td>58</td>
<td>242.1392</td>
<td>4.1748</td>
<td>80.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>58</td>
<td>2.9929</td>
<td>0.0516</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>245.9863</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S = 0.2272, $R^2 = 98.78\%$, adjusted. $R^2 = 97.55\%$

### 3.3.2 Effect of PSA Method on EUNIS sediment class classification

EUNIS sediment classifications based on the LPS-SA NP PSA dataset are shown in Figure 3.1. Of the 59 sampling stations 31 were classified as muds and muddy sands (Mu), 18 as sands and sandy muds (Sa), 9 as mixed sediments (Mx) and 1 as coarse sediment (Cs). A Chi-square test indicated there was no significant association between PSA Method and EUNIS sediment class allocation (Chi-Sq = 2.282, df = 9, P-value = 0.986).

### 3.3.3 Effect of PSA Method on IQI ES classification

Figure 3.3 shows ES at sampling stations based on the $F_1/F_2$ fauna and LPS-SA NP PSA dataset. Of the 59 stations 47 were classified as High, 6 as Good, 3 as Moderate and 3 as Poor. Weighted Kappa analysis showed “Almost
"perfect" agreement in ES classifications across the four PSA Methods (Table 3.4).

**Figure 3.3:** Map showing Infaunal Quality Index (IQI) Ecological Status (ES) (ver. 2) at sampling stations at Kinsale and Oysterhaven, Co Cork. IQI ES was assigned using the average of two 0.1m$^2$ Day grabs. Sediment particle size data was from an independent grab sample processed by combined laser particle sizing and sieving without peroxide pretreatment. Classification boundaries for Ecological Quality Ratios; Good-High = 0.75, Moderate-Good = 0.64, Poor-Moderate = 0.44, Bad-Poor = 0.24.

**Table 3.4:** Kappa values and percentage mismatch (in parentheses) of IQI ES classifications across four PSA Methods.

<table>
<thead>
<tr>
<th></th>
<th>LPS-S$_A$NP</th>
<th>LPS-S$_A$P</th>
<th>WDS-S$_A$NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>WDS-S$_A$P</td>
<td>0.9947 (0.0)</td>
<td>0.9429 (0.0)</td>
<td>0.9693 (1.7)</td>
</tr>
<tr>
<td>WDS-S$_A$NP</td>
<td>0.9676 (1.7)</td>
<td>0.9238 (1.7)</td>
<td></td>
</tr>
<tr>
<td>WDS-S$_A$P</td>
<td>0.9445 (0.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.3.4 Effect of sample Affinity on explanatory power of sediment distributions

One-way ANOVA of the adjusted $R^2$ values indicated Affinity had a significant effect on explanatory power (Table 3.5). The explanatory power of the sediment sample taken from the same grab as the fauna was significantly lower than that of sediment taken from a separate grab to the fauna.

Table 3.5: One-way ANOVA of DistLM adjusted $R^2$ distributions for $F_1$ fauna vs. LPS-$S_A$NP sediment distributions and $F_3$ fauna vs. LPS-$S_B$NP sediment distributions using Affinity as a fixed factor.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity</td>
<td>1</td>
<td>0.2695</td>
<td>0.2695</td>
<td>0.2695</td>
<td>5920.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>398</td>
<td>0.1811</td>
<td>0.1811</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>399</td>
<td>0.2876</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$S = 0.0067, R^2 = 93.70\%,$ adjusted. $R^2 = 93.69\%$

3.3.5 Effect of sample Affinity on IQI components, EQR, and ES classification

In general, ES classification calculated using sediment and fauna taken from a single grab was lower when compared to that of sediment and fauna taken from separate grabs. Weighted Kappa analysis showed “Low” agreement in ES classifications between different Affinity treatments (Kappa value = 0.248; Percentage mismatch = 12.5). The weighted Kappa analysis identified 7 “problematic” misclassifications which crossed the Good/Moderate boundary. Employing the single grab survey design resulted in less High and Good stations and an increased occurrence of Moderate, Poor and Bad stations. Repeated Measures ANOVA showed a non significant effect of Affinity on EQR, AMBI and $1–\lambda'$ (Table 3.6a, b, c respectively). There was a significant effect of Affinity on $S$ (Table 3.6d). Treatments in which the faunal sample was sub-sampled for PSA had lower numbers of species.
Table 3.6: Repeated Measures ANOVA analyses of IQI components and EQRs for F₁ and F₃ fauna, using Affinity as a fixed factor, Station as a random factor.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6a: Effect of sample Affinity on IQI EQR.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affinity</td>
<td>1</td>
<td>0.04533</td>
<td>0.04533</td>
<td>3.73</td>
<td>0.059</td>
</tr>
<tr>
<td>Station</td>
<td>55</td>
<td>2.02571</td>
<td>0.03683</td>
<td>3.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>55</td>
<td>0.66791</td>
<td>0.01214</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>2.73895</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S = 0.1102, R² = 75.61%, adjusted. R² = 50.79%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.6b: Effect of Affinity on AMBI scores.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
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<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity</td>
<td>1</td>
<td>0.997</td>
<td>0.997</td>
<td>1.33</td>
<td>0.253</td>
</tr>
<tr>
<td>Station</td>
<td>55</td>
<td>166.4714</td>
<td>3.0268</td>
<td>4.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>55</td>
<td>41.1856</td>
<td>0.7488</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>208.6566</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S = 0.8653, R² = 80.26%, adjusted. R² = 60.16%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.6c: Effect of Affinity on Simpsons Evenness Index (1-λ').

<table>
<thead>
<tr>
<th>Source</th>
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<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity</td>
<td>1</td>
<td>0.00182</td>
<td>0.00182</td>
<td>0.12</td>
<td>0.730</td>
</tr>
<tr>
<td>Station</td>
<td>53</td>
<td>2.73157</td>
<td>0.05154</td>
<td>3.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>53</td>
<td>0.79903</td>
<td>0.01508</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
<td>3.53241</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S = 0.1228, R² = 77.38%, adjusted. R² = 54.33%</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

3.6d: Effect of Affinity on Species Richness (S).

<table>
<thead>
<tr>
<th>Source</th>
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<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity</td>
<td>1</td>
<td>725.2</td>
<td>725.2</td>
<td>4.49</td>
<td>0.039</td>
</tr>
<tr>
<td>Station</td>
<td>55</td>
<td>40468.8</td>
<td>735.8</td>
<td>256</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>55</td>
<td>8882.3</td>
<td>161.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>500076.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S = 12.7081, R² = 82.26%, adjusted. R² = 64.20%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.4 DISCUSSION

3.4.1 Relationship of benthic macrofauna to sediment distributions and other environmental variables

Many correlative studies on benthic faunal distributions and sediment distributions have suggested that faunal communities could be characterised based on sediment characteristics alone (Petersen, 1913; Sanders, 1958; Glémarec, 1973; Buchanan et al., 1978; Flint, 1981; Kunitzer et al., 1992; Mackie et al., 1996; van Dalfsen et al., 2000). These correlative studies have led to an incorrect generalisation that distinct associations exist between fauna and specific sediment types. A critical review of the many animal–sediment relationship studies has indicated that very few experiments have demonstrated convincing causative linkage between benthic faunal distributions and sediment PSA alone (Snelgrove and Butman, 1994). The spatial distribution and structure of marine benthic communities are controlled by a myriad of complex interacting abiotic and biotic factors (Posey, 1990; Whomersley et al., 2010; Snelgrove and Butman, 1994; Seiderer and Newell, 1999; Snelgrove et al., 2001).

Proposed factors determining faunal distribution patterns include inter- and intra- specific competition (Moritz et al., 2009), heavy metal pollution (Dauvin, 2008; Sánchez-Moyano et al., 2010), dissolved oxygen (Levin and Gage, 1998; Rosenberg et al., 2001, 2002), organic enrichment (Pearson and Rosenberg, 1978; Weston, 1990; Levin and Gage, 1998) and physical disturbance due to either natural (Probert, 1984; Posey et al., 1996) or anthropogenic events (Newell et al., 1998; Seiderer and Newell, 1999; van Dalfsen et al., 2000). Significant statistical relationships exist between sediment distributions and many of the factors that determine faunal distribution. For instance, sediment characteristics reflect local fluvial and tidal hydrodynamics (dos Santos Brasil and Goncalves da Silva, 2000; Sarkar et al., 2000) which intuitively control the distribution of faunal communities by influencing colonisation of planktonic larval (Cowen and Sponaugle, 2009) and food supply (Tweedle et al., 2005). Organic carbon is
correlated with sediment distribution (Magni et al., 2008) and in turn is a major factor influencing the partitioning and bioavailability of sediment contaminants (Gunnarsson et al., 2009). The covariate relationship that exist between sediment distributions and factors determining faunal distributions makes sediment a suitable and easily measured proxy for the gross environmental conditions influencing faunal distributions (Jansson, 1967; Fresi et al., 1983; Cosentino and Giacobbe, 2006). Anderson (2008) demonstrated the effectiveness of predictive models based on sediment type in characterising multivariate soft-sediment faunal communities.

3.4.2 Effect of PSA Method on explanatory power of sediment distributions

This study is the first published account investigating the effect of PSA Methods on the linkage between sediment and macrofaunal distributions. Sediment distributions derived using LPS technique explained significantly greater levels of variance in multivariate faunal datasets than WDS. The increase in variance explained is due to the significantly different sediment distributions produced by LPS and WDS. Here, the results comparing the effect of PSA Technique on sediment distributions reflect those of Rodríguez and Uriarte (2009); WDS produces a more fine distribution than LPS. The relatively more fine distributions are a product of two factors; sieving selects for the smallest grain size axes and all sediment losses suffered during the weighing of sieved fractions are assigned to the < 63 µm fraction (Eleftheriou and McIntyre, 2005). Using the LPS sediment distributions allows significantly greater levels of natural variability in the multivariate fauna data to be explained. By better explaining the underlying pattern within faunal datasets will allow the communities and biotopes to be resolved with greater confidence and provide more reliable and robust classifications and distributional maps. These elements have the potential to improve the effectiveness of environmental management.

The negative effect of finer distributions on explanatory power is mirrored by the effect of H$_2$O$_2$ pre-treatment. H$_2$O$_2$ pre-treatment digests organic material
adhering to sediment particles, reducing the mean and median grain sizes, and gives rise to finer sediment distributions (Allen and Thornley, 2004; Vaasma, 2008). In this study H\textsubscript{2}O\textsubscript{2} pre-treated WDS samples had significantly finer distributions and exhibited significantly reduced explanatory power when compared to non pre-treated samples. Sediment organic material is an important food source for benthic macrofauna (Gunnarsson et al., 2009). In addition to causing finer PSA distributions the removal of sediment organic material may destroy sediment/faunal linkages, negatively affecting the multivariate fit between faunal and sediment datasets (Snelgrove and Butman, 1994). Previous studies examining only particles < 250 µm have indicated H\textsubscript{2}O\textsubscript{2} pre-treatment is both time and resource consuming, and provides no apparent advantage to investigations on sediment distributions (Allen and Thornley, 2004; Vaasma, 2008). In this study across a wide range of sediment types we have found similar results. When the primary purpose of sediment PSA is to aid classifying macrofaunal communities H\textsubscript{2}O\textsubscript{2} pre-treatment is a deleterious process having negative effects on the explanatory power of sediment distributions. The reduction in explanatory power caused by H\textsubscript{2}O\textsubscript{2} pre-treatment in turn negatively effects classification of macrofaunal communities and may have a potentially negative consequence for the management of benthic systems.

PSA Methods are influenced by factors such as grain shape and density (Blott and Pye, 2001). The interaction between Technique and Pre-treatment in terms of Mz is probably associated with the effect of particle shape on the output of the Techniques. WDS produces finer distributions than LPS because WDS selects the shortest particle axis, whereas LPS characterises the sediment as equivalent spherical diameters derived from the overall distribution of measured particle diameters (Eleftheriou and McIntyre, 2005; Rodríguez and Uriarte, 2009). Particle shape is not only important in relative distributions produced by different PSA Techniques it is also an important property of marine sediments. The initial shape of weathered particles is determined by mineralogy. The angular initial shapes of weathered particles are gradually abraded to being ellipsoidal, cylindrical or spherical by water movement. More rounded particles tend to pack together more effectively
and are more resistant to resuspension. In supporting habitat mapping and ecological assessment, sediment distribution data are acting as a proxy for the current regime in a given area (Snelgrove and Butman, 1994). There is a long history of inferring current parameters from sediment distributions in paleoecology (Ledbetter, 1979, 1986; Johnson et al., 1988; Haskell et al., 1991; Haskell and Johnson, 1993). When reviewing the utility of different grain size measuring techniques McCave et al., (1995) concluded that the more closely a given technique reflected the settling velocity of the particles, the more useful it was for inferring current speed. This is because most of the sorting of sediments by currents into particles that are transported or not occurs on resuspension of sediments. The settling velocity of particles is dependent on shape and composition (Schultz and Zabel, 1999). The relationship between particle composition and shape is further complicated by differing abrasion rates between particle types. The typical shape of terrigenous organic particles entering young marine sediments is irregularly cylindrical with the longest axis approximately twice the length of the shortest (Littke et al., 1991). The organic particles are abraded and decomposed to more rounded forms more quickly than mineral particles, and are more likely to be incorporated in aggregates. Faecal pellets tend to be cylindrical or ellipsoidal, but the exact shape and sinking velocity may depend not only on the species but also on the age or sex of the individual that produced them (Smayda, 1969; Moore, 1931; Wilson et al., 2008). Particle flocs form when mineral and organic particles, and interstitial water, bind to form larger aggregates with lower sinking velocities (Azetsu-Scott and Johnson, 1991). Much of the material in coastal and estuarine sediments may be in the form of flocs (Manning et al., 2007).

In this study we have shown that there is a relationship between PSA Methods and how much variance in macrofaunal community structure their sediment distributions explain. While this shows a very similar pattern to how fine the sediments were determined to be by Mz, this may not be the causal relationship. It may be that the LPS technique by assigning more importance to the average particle diameter more accurately represents the settling velocity distribution of the particles in situ than WDS does. Pre-treatment with
peroxide may further divorce the WDS distribution from the original settling velocity distribution of the sediment by breaking up aggregates and dissolving faecal pellets. The mineral particles remaining after peroxide digestion no longer carry the signal of the processes that led to their deposition in the environment.

3.4.3 Effect of PSA Method on EUNIS sediment classification and IQI ES classification

The WFD requires all participating member countries to classify habitats and assess their ES using comparable methodologies. The EUNIS is a commonly used classification system. In EUNIS top-down classification, habitats are classified, based on sediment distribution data, to one of four broad sediment classes sensu Long (2006) (Connor et al., 2004; Davies et al., 2004; EUNIS, 2004). Under the WFD IQI ES is assessed using macrofaunal and sediment data (Mackie, 2009; Borja et al., 2011). The sediment distributions derived using different Methods (i.e. WDS or LPS technique with/without H₂O₂ pretreatment) did not significantly affect EUNIS sediment classification sensu Long (2006). The consistency observed in the allocation of EUNIS sediment classes is due to the broad nature of the sediment classes. Aggregation of the distribution data to %mud, %sand and %gravel in determining the EUNIS sediment class makes the classification robust to the effect of PSA Method even when there are significant differences between the Methods on a whole phi scale. Similarly, Method did not have a significant effect on ES as determined using the IQI. The main objective of the WFD is long-term monitoring across large spatial areas (Kröncke and Reiss, 2010; Kennedy et al., 2011). The robustness of IQI and EUNIS methodologies suggest that selection of PSA Method is not of major concern in broadscale monitoring programmes, such as national monitoring programmes for the WFD. However, the effectiveness of these monitoring programmes could be potentially improved by employing the PSA Method (i.e. LPS without H₂O₂ pre-treatment) that best accounts for natural variability in macrofaunal distribution.
3.4.4 Effect of sample Affinity on explanatory power of sediment distributions, IQI components, EQR and ES classification

Programs monitoring ES employ different sampling strategies. Some surveys involve replicate grab sampling with separate grabs used for macrofauna and sediment analyses (Dauvin et al., 2007b; Zettler et al., 2007; Reiss and Kröncke, 2005). However, often surveys use single grabs for combined macrofauna and sediment analyses (Seiderer and Newell, 1999; Kennedy, 2008). In this study, the results have shown that using single grabs for combined sediment and faunal analyses has a significant negative effect on the power of sediment distributions to explain variance in the multivariate faunal distribution data. The difference in the amounts of variance explained by sediment distribution in the two Affinity treatments (i.e. sampling strategy) is due to differences in macrofaunal datasets caused by sub-sampling for sediment. Measures of species diversity in macrofaunal benthic communities are highly dependent on sample size (Clarke and Warwick, 2001) and are potentially sensitive to changes in sampling design (Kennedy et al., 2011). In this study the reduction in macrofaunal sample size due to sediment sub-sampling had a significant negative effect on Species richness (S). The loss of rare or less numerous species acted to reduce the linkage between the multivariate fauna and sediment distribution. Non-uniform volume sub-sampling has the potential to further exacerbate the problems associated with sample size effects and the loss of fauna (Clarke and Warwick, 2001).

For instance, representative sub-sampling of different sediment types requires different sample volumes with coarse and mixed sediment requiring a larger volume of material than muds and muddy sands (British Standards Institution, 1996; Boyd, 2002; Passhier, 2007). Independent grab sampling results in a significantly better fit of sediment to fauna, allowing faunal community classification to be performed with greater confidence. This may maximise the potential effectiveness of marine monitoring programmes.

Sediment sub-sampling acted to reduce mean EQR from 0.80 to 0.76; while this effect was found to be non-significant it acted to move samples across the ES boundaries and significantly effected sample ES classification.
ANOVA of the component indices of IQI showed that Affinity had no significant effect on AMBI and $1-\lambda'$ but has a significant negative effect on $S$ (i.e. a sample size effect; Clarke and Warwick, 2001). AMBI is based upon the relative proportions of abundance among five Ecological Groupings of species (Borja et al., 2000). Aggregation of the species data to five groups affords AMBI robustness to losses of rare species due to small changes in sample size (Muxika et al., 2007b). Similarly, the non significant effect of Affinity on $1-\lambda'$ is due to the fact that Evenness is a diversity measure based on the relative number of individuals across species. Rare species by definition represent a small proportion of the number of individuals in a sample. Provided that $S$ and $N$ are both reasonably large the loss of rare species has little effect on the proportional distribution of individuals among the common species. Sub-sampling for sediment significantly reduces $S$ so that it is unlikely to reach reference conditions. Consequently, sediment sub-sampling acts directly to significantly downgrade ES. This has potential to negatively affect the effectiveness of ES monitoring programmes. To avoid such errors and to maximise efficacy, monitoring programmes should employ grab survey designs in which fauna and sediment samples are independent.

Broadscale habitat classification of sedimentary environments is often carried out by remote sensing techniques such as acoustic survey, aerial photography, underwater video by diver or remotely operated vehicle (Parry et al., 2002; Freitas et al., 2003; Kenny et al., 2003; Solan et al., 2003). These surveys are typically validated using a sparse grid of ground-truth bottom samples that are used to characterise the composition of the bottom types delineated (Freitas et al., 2003; Solan et al., 2003). There are no published accounts on the effect of PSA Method used in ground-truthing on the fit of habitat maps derived from remotely sensed data to habitat variability and macrofaunal distribution derived from ground truth samples. Broadscale interpolation of remotely sensed data holds potential to magnify any issues (positive or negative) with the quality of the fit of the sediment distribution data to that of macrofaunal distributions. Further research is needed to determine if the choice of PSA Method is important in ground-truthing acoustic habitat classifications.
The use of sediment characteristics as a top-down classifier of habitats is a common feature in many habitat classification systems such as EUNIS (Connor et al., 2004; Davies et al., 2004). Sediment distributions covary with, and is correlated to, factors such as hydrographic conditions, food supply, physical disturbance regime and larval supply that control macrofaunal distribution (Snelgrove and Butman, 1994). Sediment is relatively easy to sample and measure, and is generally considered to be relatively temporally stable. This makes sediment distributions perhaps the most pragmatic proxy available to environmental managers to account for the distribution of macrofauna and to effectively manage discrete spatial resources. Employing the PSA Method that best accounts for variability in macrofaunal distribution holds potential for improving the effectiveness of environmental management. In this study, we have demonstrated that over a range of sediment types the combined use of laser particle size analysis and wet/dry sieving without peroxide pre-treatment yielded the optimal explanatory power for macrofaunal distribution. Further research focussed on identifying the best PSA Method for providing distribution data to explain the spatial distribution of seabed types delineated by acoustic or optical remote sensing is needed. This may enable the determination of the match of acoustic seafloor classification to macrofaunal distributions that many authors have concluded is necessary (Eastwood et al., 2006; Shumchenia and King, 2010).

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3.6 CONTRIBUTIONS

All listed co-authors participated in survey work at Kinsale. JF assisted PCC and AP on subsequent faunal analyses. JF performed all sediment analyses. JF and BK collaborated on statistical and spatial analyses. JF was the primary author of this manuscript.
DEFINING HABITATS AND REPRESENTATIVE BIOLOGICAL COMMUNITIES IN IRISH COASTAL SITES: A PRACTICAL MANAGEMENT APPROACH INCORPORATING NATURAL VARIABILITY AND HABITAT ECOLOGICAL STATUS

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ABSTRACT

Two important elements of effective marine spatial planning and management efforts are the creation of maps of the distribution of living and non-living resources on the seafloor and, of habitat quality that reflect the degree of anthropogenic disturbance.

Here, we present an approach to biotope classification in transitional and coastal waters that incorporates Ecological Status (ES) assessment (sensu the Water Framework Directive; WFD) and meets the purposes of biotope classification for the Habitats Directive (HD). To this end, we propose a top-down habitat classification system using sediment type and interstitial salinity as classifiers. Species characterising each top-down habitat are determined and linked to the associated habitat characteristics to create biotopes (sensu the HD). Community data within each top-down habitat are then used with the UK and Ireland Infaunal Quality Index (IQI) Ecological Quality Ratio (EQR) tool to determine ES, and its variability, within each classified habitat (sensu the WFD).

This approach produced habitat and biotope distributions, and ES assessments based on realistic measures of faunal community variability within spatial units determined from physical classifiers. This approach will allow management to be conducted more usefully on a habitat level rather than at the level of highly variable macrofaunal communities which are typical of shallow coastal and transitional water systems.
4.1 INTRODUCTION

In recent years the European Community has introduced environmental Directives, such as the Habitats Directive (HD, 92/43/EEC) and the Water Framework Directive (WFD, 2000/60/EC), which provide frameworks for the protection, maintenance and/or restoration of marine ecosystems. The Directives are based on the same simple ecological concept and consist of comparing the current state of ecosystem components to the state which would be expected if the ecosystem was subjected to no, or very minor, disturbances from human activities (Fleming-Lehtinen, 2011).

Benthic habitats and the biological communities they support have been used as indicators of anthropogenic stress in the marine environment for several decades (Pearson and Rosenberg, 1978; Bilyard, 1987; Rosenberg et al., 2004; Quintino et al., 2006; Kennedy et al., 2011; Forde et al., 2012) and play important roles in both Directives (Dauvin et al., 2008b). The HD makes provisions for Member States (MS) to develop measures to maintain (and where practical restore) a list of priority habitats and species at Favourable Conservation Status (FCS) through the designation and management of conservation sites (Dauvin et al., 2008b; Fleming-Lehtinen, 2011; Fraschetti et al., 2011). Classification of habitats and biotopes are central in the implementation of the HD (Galparsoro et al., 2010). The WFD is aimed at achieving Good Ecological Status (GES) for all surface waters, including transitional waters, by 2015 (Borja et al., 2005). The implementation of the WFD requires surface water bodies to be divided into water bodies that must be then monitored for (Ecological Status) ES. Assessment of ES is based on assessment of the status of physicochemical, hydromorphological and biological quality elements, with the latter element being of particular importance (Borja, 2005). The Directives are both concerned with conserving biodiversity and good habitat quality but have different foci. Despite the common goal of the Directives, and their focus on aspects of coastal and transitional systems, no effective means of integrating the Directives has been proposed in the literature. Integration of sampling programs for the Directives would save resources and allow assessments under both
Directives to be conducted on common datasets. This approach would serve to strengthen both Directives by facilitating mutual enlightenment between the outputs of the Directives. In this study, habitat classification (sensu the HD) and ES assessment (sensu the WFD) are integrated in benthic sedimentary habitats in coastal and transitional waters.

4.1.1 Habitat and Biotope Classification (sensu the HD; 92/43/EEC)

A classification system that allows reporting of habitats and biotopes in a comparable manner for use in monitoring and mapping is a vital tool for effective environmental management (Dauvin et al., 2008b; Galparsoro et al., 2010). The importance of a common and unequivocal classification system is reflected by the fact that increasingly more HD and WFD marine monitoring programs are adopting the EU Nature Information System (EUNIS, 2002) habitat classification scheme (Galparsoro et al., 2008). EUNIS habitat classification is a classification scheme that has been designed to provide a framework to describe marine, freshwater and terrestrial habitats using a common set of defined hierarchal habitat units (Galparsoro et al., 2010). Originally EUNIS was organised into 3 hierarchal levels for terrestrial (including freshwater habitats) and 4 levels for marine habitats, however, marine habitats are now defined up to level 6 (Galparsoro et al., 2012). EUNIS marine habitats were initially based on the Marine Classification for Britain and Ireland (Connor et al., 2004). In attempts to increase the applicability of the system across Europe, the list of EUNIS marine habitats has been extended to include specific habitats from the Baltic and Mediterranean (Davies et al., 2004; Galparsoro et al, 2010).

Under EUNIS, marine habitats are defined using both abiotic and biotic features. The first level differentiates between marine habitats (denoted by the code letter A) from terrestrial habitats (B). Level 2 uses biological zones and the presence of rock or sediment as classifiers. For instance, at Level 2 marine sedimentary habitats are classified as littoral sediments (A2) and sublittoral sediments (A5). At Level 3 sediment types are introduced as classifiers. In the case of sublittoral sediments, Level 3 habitats can be
classified as coarse sediment (A5.1), sand (A5.2), mud (A5.3) and mixed sediment (A5.4). At Level 4 sedimentary habitats are further differentiated between using physical and zone characteristics (e.g. Infralittoral muddy sand; A5.24). At Level 5 classifications are based on both physical attributes and biological characteristics (e.g. faunal composition) of the habitats. For instance, a Level 5 habitat commonly found in Irish waters is *Fabulina fabula* and *Magelona mirabilis* in infralittoral compacted fine muddy sand (A5.242). Level 6, the highest discriminant level, classifies habitats based on variation in community structure of Level 5 communities (EUNIS, 2002; Galparso et al., 2008).

Programs monitoring change in soft bottom communities frequently use a bottom-up taxonomic approach to identify biotopes. In this approach species characterising biotic communities are initially identified and are then compared to EUNIS core records (or standard communities) and their most commonly associated habitats to identify biotopes (EUNIS, 2002; Connor et al., 2004). There are a number of issues with using a bottom-up taxonomic approach for the classification of biotopes for the HD. Huston (1994) described benthic communities to be a mosaic of parts that reflect the response of resident fauna to recent disturbance history (Huston, 1994). Where disturbance events are common benthic communities exhibit a great deal of spatial and temporal variability, as is the case in shallow coastal and transitional waters (Elliot and Quintino, 2007). In this view, bottom-up classified biotopes only represent a temporal snapshot of benthic community structure at a particular sampling point and fail to adequately account for biological variation within the habitat.

An alternative approach to bottom-up classification would be to initially define the physical habitat using biologically relevant environmental parameters as top-down classifiers. The fauna within top-down classified habitats may then be characterised to define biotopes. This approach would produce biotopes that would more accurately reflect the spatial extent of the habitat (or resource) while accounting for the variability of the overall biological community associated with the identified habitats.
Studies have shown that faunal distribution patterns are influenced by a myriad of interacting biotic and abiotic factors including inter- and intra-specific competition (Moritz et al., 2009), heavy metal pollution (Dauvin, 2008; Sánchez-Moyano et al., 2010), dissolved oxygen (Levin and Gage, 1998; Rosenberg et al., 2001, 2002), organic enrichment (Pearson and Rosenberg, 1978; Weston, 1990; Levin and Gage, 1998) and physical disturbance (Rhoads and Germano, 1986; Newell et al., 1998; Seiderer and Newell, 1999; van Dalfsen et al., 2000). Significant statistical relationships exist between sediment particle size distributions and many of these factors, making sediment distributions a suitable and easily measured proxy for the gross environmental conditions influencing faunal distributions (Jansson, 1967; Fresi et al., 1983; Cosentino and Giacobbe, 2006; Forde et al., 2012). Many studies have shown multivariate infaunal community structure to be highly correlated with sediment distribution data (e.g. van Dalfsen et al., 2000; Anderson, 2008; Forde et al., 2012). Salinity is considered to be one of the main environmental variables controlling the distribution of estuarine fauna (Bulger et al., 1993; Chapman and Brinkhurst, 1981; Elliot and McLusky, 2002; Rundle et al., 1998). The correlation between the distribution of estuarine communities and salinity gradients has provided the foundation for schemes aimed at partitioning estuaries into manageable parts (Bulger et al., 1993; Elliot and McLusky, 2002; Battaglia, 1959). For example, the Venice Estuarine Classification System (Battaglia, 1959) divides estuaries into five salinity classes based on subjective observations of the distribution of flora and fauna across known estuarine salinity gradients (Bulger et al., 1993). Most estuarine benthic fauna are infaunal rather than epifaunal and so interstitial salinity, and not the salinity of the overlying water column, is recognised as been of more immediate importance (Sanders et al., 1965). Studies have shown multivariate infaunal community composition and species abundance to be highly correlated with interstitial salinity (Dale and Miller, 2008). In this study, interstitial salinity classified according to the Venice System (Battaglia, 1959) and sediment characteristics are demonstrated as biologically relevant top-down habitat classifiers.
In addition to representing the spatial distribution of habitats and accounting for biological variability in the associated communities, this approach to classification is likely to produce small numbers of relatively large contiguous spatial resource units. From a management point of view these large resource units would be more amenable to management than large numbers of units produced by bottom-up classification (i.e. high resolution biotopes based on highly variable benthic communities) (Ferreira et al., 2006; Teixeira et al., 2008; Puente et al., 2008; de Paz et al., 2008).

4.1.2 Assessment of Ecological Status (sensu the WFD; 2000/60/EC)

Under the WFD, ES is assigned to water bodies through the assessment of ecosystem elements (Borja, 2005). Ecological monitoring data are compared to reference (undisturbed) conditions to derive an Ecological Quality Ratio (EQR). EQRs are expressed as a decimal value between zero and one, with High status represented by values close to one and Bad status by values close to zero. EQR scales are divided into five ES classes (High, Good, Moderate, Poor, and Bad) by assigning a numerical value to each of the class boundaries (Borja et al., 2000). The UK and Ireland Infaunal Quality Index (IQI) is a multi-metric EQR tool that is used to assess ES in coastal and transitional waters (Prior et al., 2004; Mackie, 2009; Borja et al., 2007, 2011). Macrobenthic elements included in IQI ES assessment are measures of diversity and abundance of invertebrate taxa, and a measure of the proportion of disturbance-sensitive taxa. Physico-chemical elements used the IQI include sediment particle size distributions and habitat salinity classification.

The WFD states that assessment of ES must be at levels that reflect the spatial extent of habitats while taking into account the variability of the biological communities used to assess ES (Galván et al., 2010). Using biological data at sampling locations within top-down habitats (i.e. classified using interstitial salinity and sediment parameters) would produce a likely range of measures of ES for each habitat and allow ES to be monitored at a scale that reflects the spatial distribution of the habitat. Incorporating ES in
this way would allow communities within habitats to be compared based on
the sensitivity of the fauna to disturbance rather than on species abundance
data. Furthermore, once likely ranges of ES are determined for a habitat
type, deviating from this range could be used as an indication of change
either associated with an ecological impact or due to previously initiated
remedial management action.

Here, we use a 38 station spatial dataset of benthic macrofauna samples and
environmental variables from two estuarine and shallow coastal study sites
on the south coast of Ireland. The main objective of this present study was to
demonstrate an approach for integrating habitat classification (sensu the HD)
and ES assessment (sensu the WFD) in coastal and transitional waters. Here,
subtidal interstitial salinity and sediment distribution data are proposed
as biologically relevant classifiers as demonstrated by their ability to explain
patterns and variance in multivariate faunal distributions. Interstitial salinity is
used, in conjunction with sediment characteristics, as classifiers in top-down
habitat classification. Macrofaunal data within each top-down classified
habitat are then assessed to derive biotopes (sensu the HD). These top-
down classified biotopes are compared to traditional EUNIS bottom-up
biotopes. Macrofaunal, salinity and sediment data collected at stations within
each top-down habitat are used to calculate a range of measures of IQI ES
(sensu the WFD) associated with each habitat.

4.2 MATERIALS AND METHODS

4.2.1 Study sites

The two study sites comprised of 38 sampling stations extending over three
embayments on the south coast of Ireland. The first study site, located in Co.
Cork, included the Bandon and Belgooly estuaries and their harbours at
Kinsale and Oysterhaven respectively (Figure 4.1) while the second study
site, located in Co. Waterford, consisted of the Barrow and Suir River
estuaries and Waterford harbour (Figure 4.2). Sampling stations were
shallow (< 15m) and covered a comprehensive range of estuarine habitats.
Figure 4.1: Map showing EUNIS sediment and interstitial salinity classifications at sampling stations at Kinsale and Oysterhaven, Co. Cork in May 2010. EUNIS sediment classification sensu Long (2006); Mu = Mud and sandy mud (17 stations), Sa = Sand and muddy sand (1 station). Interstitial salinity classification sensu the Venice Estuarine Classification System (Battaglia, 1959); Eu = euhaline (psu > 30; 10 stations), Poly = polyhaline (30 < psu > 18; 7 stations), Meso = mesohaline (18 < psu > 5; 1 station).
Figure 4.2: Map showing EUNIS sediment and interstitial salinity classifications at sampling stations at Waterford in June 2008. EUNIS classification *sensu* Long (2006); Mu = Mud and sandy mud (14 stations), Sa = Sand and muddy sand (5 stations), Cs = Coarse sediment (1 station). Interstitial salinity is classification *sensu* the Venice Estuarine Classification System (Battaglia, 1959); Eu = euhaline (psu > 30; 3 stations), Poly = polyhaline (30 < psu > 18; 7 stations), Meso = mesohaline (18 < psu > 5; 7 station), Oligo = oligohaline (5 < psu > 0.5; 3 stations).
4.2.1.1 Sampling design

In May 2009, 13 stations at Kinsale and 5 stations at Oysterhaven (Figure 4.1) were sampled while in June 2008, 21 stations were sampled at the Waterford site (Figure 4.2). At each station a 0.1 m² Day grab and two replicate Haps cores were retrieved. At each faunal sampling station a single sediment sample was retrieved for sediment analyses.

4.2.1.2 Grab sample processing and faunal analysis

Grab samples were retained for macrofaunal identification. Macrofaunal samples were sieved through a 1mm sieve and the retained material was fixed in 10% buffered formalin and stored in 70% IMS. In the laboratory the macrofauna was stained using Eosin, sorted, enumerated, and identified to species level using standard keys and N MBAQC (2009) guidelines.

4.2.1.3 Core sample processing and salinity analysis

Each replicate Haps core was sectioned vertically at 1–5 cm and 5–10 cm. The 5–10 cm layer was stored in a plastic bag and frozen at −20°C pending extraction of interstitial pore water for interstitial salinity analysis.

Sediment core layer samples were defrosted at room temperature. Interstitial pore water was extracted from representative subsamples of each core layer using a KC pore-water press fitted with GF/C filters. In short, sediment subsamples were placed in pressing cylinders and air pressure was applied over a latex diaphragm. The resulting compression of the sediment matrix caused interstitial water to be filtered through the GF/C filters and expelled through outlets at the base of the pressing cylinders. The filtered interstitial water was collected in glass vials, sealed using Parafilm pending salinity analysis.

Prior to analysis interstitial water samples allowed equilibrate to room temperature before salinity measurements were recorded in triplicate using a handheld Atago ATC- S/Mill-E refractometer (+/−1 psu accuracy). Replicate
core data were used to calculate mean sediment interstitial psu salinities. Sampling stations were assigned salinity classes *sensu* the Venice System (Battaglia, 1959) which are defined as follows, oligohaline (5 < psu ≥ 0.5, 3 stations), mesohaline (18 < psu > 5, 8 stations), polyhaline (30 ≤ psu > 18, 14 stations) and euhaline (psu > 30, 13 stations; Figure 4.1 and 4.2).

4.2.1.4 Sediment analyses

Sediment particle size analysis (PSA) was performed using a combination of wet/dry sieving and laser particle sizing using the methodology outlined in Forde et al. (2012). Sediment distribution data were processed using GRADISTAT (Blott and Pye, 2001) software to derive sediment parameters i.e. Mud:Sand ratio, % Gravel. Using these parameters EUNIS sediment classes *sensu* Long (2006) were assigned to each sampling station. This classification differs from the normal Folk and Ward triangle (Folk and Ward 1954) in that it defines the boundary between muds and sandy mud (Mu) and sands and muddy sands (Sa) as a 4:1 ratio of sand to mud. EUNIS coarse (Cs) sediment corresponds to the normal Folk and Ward (1957) category of coarse sand. Of the 38 sampling stations 31 were classified as muds and muddy sand (Mu), 6 as sands and sandy muds (Sa) and 1 as coarse sediment (Cs) (Figure 4.1 and 4.2).

For each sampling station, sediment organic content was determined by weight loss on ignition (LOI) of 5 g of sediment at 550 °C after 6 hours (Eleftheriou and McIntyre, 2005).

4.2.2 Statistical analysis

Analyses were carried out using PRIMER v6 (Clarke and Gorley, 2006) with the add-on package PERMANOVA+ (Anderson et al., 2008), MINTAB v16 and R.
4.2.2.1 Interstitial salinity and sediment parameters as predictor variables

For each study site, three multivariate predictor datasets were constructed based on environmental data at the sampling stations. The first predictor dataset (PD₁) included percentage organic content (LOI) and sediment parameters used to classify EUNIS sediment classes (i.e. Mud:Sand ratio, % Gravel). The sediment parameters and LOI values used in PD₁ were continuous variables. The second dataset (PD₂) included the variables in PD₁ and interstitial salinity psu as a continuous variable. The third predictor dataset (PD₃) included PD₁ variables and interstitial salinity as categorical salinity classes (as determined using the Venice System; Battaglia, 1959).

The amount of variance in the multivariate faunal datasets from each study site explained by their associated environmental multivariate predictor datasets (i.e. PD₁, PD₂ and PD₃) was determined using the bootstrap distance-based Linear Modelling (DistLM) analysis presented in Forde et al (2012). In short, for each study site the faunal dataset was square root transformed and used to generate a zero-adjusted Bray-Curtis similarity matrix. The environmental predictor datasets were normalised. DistLM was used to model the relationship between the multivariate fauna dataset and the environmental predictor datasets. The fit coefficient used was adjusted $R^2$ with forwards and backwards selection to find the optimal regression model. To test for significant differences in the power of the predictor datasets to explain variance in the macrofaunal distribution datasets, the DistLM analysis was bootstrapped using 100 iterations with 2 random samples eliminated. The distributions of adjusted $R^2$ values, derived from the bootstrapped analysis, for each predictor datasets were tested for significant differences using a One-way ANOVA with multiple pairwise comparisons made using Tukey’s method.

4.2.2.2 Top-down classification

To visualise the clustering of faunal stations at Kinsale and Oysterhaven, and at Waterford based on interstitial salinity and sediment predictor variables,
distance-based redundancy analysis (dbRDA) was used to construct ordination plots of the multivariate macrofaunal variation explained and modelled in DistLM using the PD3 variables (see section 4.2.2.1).

For each study site, PD3 predictor variables were converted to top-down categorical habitat classifiers and added as labels in the dbRDAs. Top-down categorical classifiers were produced as follows; sediment parameters converted to EUNIS sediment classes (sensu Long 2006); LOI continuous variable converted to two categorical classes, <5 LOI % and 5 ≥ LOI % <10; interstitial salinity continuous variable converted to categorical salinity classes (sensu the Venice System; Battaglia 1959).

Using top-down habitat types as a factor in Similarity Percentage (SIMPER) analysis of the macrofaunal data, species characterising the community at each habitat type was determined. Where a habitat type was defined by a single station the species abundance data for the station was examined to identify the charactering species. The characterising species and top-down habitat classifiers were coupled to classify biotopes. Biotopes were critically assessed to determine whether the different biotopes were spatially contiguous and/or represented slightly different versions of the same biotope. For each study site, the spatial distributions of biotopes were mapped using ARCgis 10.

To assess the agreement between the underlying multivariate structures of the faunal datasets and the top-down biotopes Canonical Analysis of Principal Coordinates (CAP) using a leave-one-out cross validation was performed. The success rate in allocation of faunal stations to the identified top-down biotopes was assessed.

4.2.2.3 Bottom-up classification

For each study site, the fauna dataset was square root transformed and used to create a Zero Adjusted Bray-Curtis similarity matrix. The similarity matrix was used to classify the stations into groups of similar community structure in a dendrogram by higher agglomerative clustering (HAC) using group average
linkage. Similarity Profile (SIMPROF) test was used to determine significant differences between clusters based on multivariate structure. The station group structure produced by the SIMPROF test was used as a factor in a SIMPER analysis to determine the characterising species of each station group. The characterising species were compared to the EUNIS comparative tables (Connor et al., 2004) to determine level 5 biotope classifications. Where level 5 classification was not possible SIMPER tables were matched to higher levels (i.e. EUNIS levels 2, 3, 4) using physical habitat parameters (based on PD3 predictor variables). Where a biotope was defined by a single station the species abundance data for the station were examined to identify the characterising species. The degree of agreement between sediment classification in the bottom-up classified biotopes (i.e. biotopes classified sensu Connor et al., 2004) and the actual observed station sediment type was assessed.

4.2.2.4 IQI ES assessment at top-down classified biotopes

For each study site, IQI (ver. 2) was used to determine benthic ES (sensu the WFD) at each sampling station using the propriety tool in Microsoft Excel developed by the NIEA (Mackie, 2009; Borja et al., 2011; Kennedy et al 2011; Forde et al., 2012). IQI (ver. 2) includes truncation of the species lists, and spelling and synonym standardisation. IQI EQR, a continuous variable between 0 and 1, is calculated as follows;

$$IQI = \frac{0.38 \times \left( \frac{1 - AMBI/7}{(1 - AMBI/7)_{max}} \right) + 0.08 \times \left( \frac{1 - \lambda'}{(1 - \lambda')_{max}} \right) + 0.54 \times \left( \frac{S^{0.1}}{S^{0.1}_{max}} \right) - 0.4}{0.6}$$

where AMBI is the AZTI Marine Biotic Index (Borja et al., 2000), 1- \( \lambda' \) is Simpson’s Evenness Index, \( S^{0.1} \) is \( \log_{10} \) (number of species), max parameters are the maximum reference values for the habitat. AMBI is a continuous variable based upon the proportions of five ecological groups (EG) to which benthic species are allocated (Borja et al., 2000). AMBI is
calculated within IQI (ver. 2) tool using the species list current in 2010. Sediment classification and habitat salinity are also used in version 2 of the IQI tool (Mackie, 2009). IQI reference conditions are adjusted for local sediment type and salinity using an algorithm within the tool. Sediment data were entered as particle size distribution variables. Salinity was entered in the IQI tool as a categorical variable (i.e. coastal \(34 \pm 1.8\) psu, transitional \(23.9 \pm 7.5\) psu). In IQI (ver. 2) reliable reference conditions cannot be set for samples characterised by sediments coarser than fine sands and/or by average salinity values <20 psu (Mackie, 2009). Consequently, sampling stations in this study that fulfilled the outlined criteria were omitted from the IQI ES assessment. For each study site, ES classifications at sampling stations within each top-down habitat type were used to calculate mean ES and its variability (i.e. SE).
4.3 RESULTS

4.3.1 Interstitial salinity and sediment parameters as predictor variables

For both the Kinsale and Oysterhaven, and the Waterford study sites One-way ANOVAs of the bootstrapped adjusted R² values indicated that significant differences existed between the fits of the predictor datasets (i.e. PD₁, PD₂ and PD₃) to their respective multivariate faunal datasets (Table 4.1a and 4.2a respectively).

For the Kinsale and Oysterhaven study site, Tukey's pairwise comparisons of mean adjusted R² values showed the explanatory power of all three predictor datasets differed significantly in terms of the amount of macrofaunal variation explained (Table 4.1b). Overall, PD₁ which only included sediment parameters (i.e. Mud:Sand ratio, % Gravel) and LOI values (PD₁) explained least variance at 16.2%, while including salinity classes (PD₂) or salinity values (PD₃) increased the levels of explained multivariate variance to 19.3% and 21.4% respectively (i.e. relative increases of 19.1% and 32.1% respectively; Table 4.1b).

Table 4.1: One-way ANOVA of DistLM adjusted R² distributions for 3 predictor datasets (PD₁, PD₂, PD₃) with the Kinsale and Oysterhaven, Co. Cork faunal dataset (2010).

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predictor Dataset</td>
<td>2</td>
<td>0.137</td>
<td>0.137</td>
<td>0.069</td>
<td>95.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>297</td>
<td>0.213</td>
<td>0.213</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>299</td>
<td>0.350</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S = 0.027, R² = 39.19%, adjusted R² = 38.78%

4.1b: Tukey's multiple pairwise comparisons between mean adjusted R² values of Predictor Datasets. Means that do not share a letter are significantly different.

<table>
<thead>
<tr>
<th>Predictor Dataset</th>
<th>Mean</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD₁ (PSA+LOI)</td>
<td>0.214</td>
<td>A</td>
</tr>
<tr>
<td>PD₂ (PSA+LOI+Sal psu)</td>
<td>0.193</td>
<td>B</td>
</tr>
<tr>
<td>PD₃ (PSA+LOI+Sal class)</td>
<td>0.162</td>
<td>C</td>
</tr>
</tbody>
</table>
In the Waterford dataset Tukey’s pairwise comparisons indicated that the predictor datasets which incorporated salinity values (PD$_2$) or salinity classes (PD$_3$) explained 17.5% and 18.0% of the variance respectively. PD$_2$ and PD$_3$ did not differ significantly in terms of explanatory power (Table 4.2b). The predictor dataset which included sediment parameters and LOI values (PD$_1$) explained the least amount of macrofaunal variance (12.2%; Table 4.2b). Including salinity class (PD$_2$) or salinity values (PD$_3$) as predictor variables increased the relative levels of explained variance by 47.5% and 43.4% respectively.

Table 4.2: One-way ANOVA of DistLM adjusted $R^2$ distributions for 3 predictor datasets (PD$_1$, PD$_2$, PD$_3$) with the Waterford faunal dataset (2008).

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predictor Dataset</td>
<td>2</td>
<td>0.200</td>
<td>0.200</td>
<td>0.100</td>
<td>111.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>297</td>
<td>0.266</td>
<td>0.266</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>299</td>
<td>0.466</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S = 0.030, $R^2 = 42.97\%$, adjusted $R^2 = 42.59\%$

4.2b: Tukey’s multiple pairwise comparisons between mean adjusted $R^2$ values of Predictor Datasets. Means that do not share a letter are significantly different.

<table>
<thead>
<tr>
<th>Predictor Dataset</th>
<th>Mean</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD$_1$ (PSA+LOI)</td>
<td>0.180</td>
<td>A</td>
</tr>
<tr>
<td>PD$_2$ (PSA+LOI+Sal psu)</td>
<td>0.175</td>
<td>A</td>
</tr>
<tr>
<td>PD$_3$ (PSA+LOI+Sal class)</td>
<td>0.122</td>
<td>B</td>
</tr>
</tbody>
</table>
4.3.2 Top-down classification at Kinsale and Oysterhaven

A total of five biotopes, labelled TC1–TC5, were identified at Kinsale and Oysterhaven using top-down classification (Figure 4.3a). The dbRDA plot (Figure 4.4b) showed the sampling stations formed well defined clusters using top-down habitat classifiers. Leave-one-out cross validation analysis indicated that, based on the underlying multivariate faunal structure, faunal stations were correctly allocated to the top-down biotopes 77.8% of the time; indicating that the top-down biotopes were biologically meaningful.

Table 4.3 lists the characterising species for each top-down biotope while the spatial distribution of the biotopes is shown in Figure 4.3a. Biotope TC1 was limited to a single euhaline muddy sand station at Oysterhaven. The community was dominated by the polychaete *Magelona filiformis* and the bivalve *Fabulina fabula*. Biotope TC2 was classified as euhaline sandy muds. This biotope was found at six stations at Kinsale and at one station at Oysterhaven. The diverse marine communities at these stations were comprised of the polychaetes *Nephtys hombergii* and *Lumbrineris gracilis*, the bivalves *Chamelea gallina* and *Mysella bidentata*, the ophiuroid *Amphiura filiformis* and the holothurian *Labidoplax digitata*. Biotope TC3 was found at muddy stations in the inner harbour area at Scilly, near the yachting marina at Kinsale town and in the lower reaches of the Bandon estuary. The community at TC3 was dominated by Tubificid oligochaetes, the polychaetes *N. hombergii* and *Hediste diversicolor*. The sediments at biotope TC4 were classified as polyhaline reduced muds. TC4 was found in the middle reaches of the Bandon and Belgooly estuaries. The community at these stations was dominated by the polychaetes *N. hombergii* and *Scoloplos armiger*, the bivalve *Abra nitida* and the amphipod *Corophium multisetaosum*. Biotope TC5 was limited to a single mesohaline station in the upper reaches of the Bandon River. This biotope was characterised by muddy sediments (Mu) in reduced salinities. The community at TC5 was dominated exclusively by the amphipod *Gammarus salinus*. 
**Figure 4.3a:** Map showing top-down classified biotopes (TC1-TC5) at sampling stations at Kinsale and Oysterhaven, Co. Cork in May 2010. **4.3b:** Distance-based redundancy analysis ordination plot of the multivariate macrofaunal variation explained and modelled by PD2 variables in Distance-based Linear Modelling. PD2 variables include EUNIS sediment PSA parameters (i.e. Mud:Sand ratio, % Gravel), loss on ignition (LOI%) and interstitial salinity psu values as continuous variables. The values in parentheses indicate the percentages of the fitted and total variations explained by each axis.
Table 4.3: Output of Similarity Percentage analysis used to determine species characterising top-down classified biotopes (TC1-TC5) at Kinsale and Oysterhaven, Co. Cork in May 2010. Where a biotope was defined by a single station the species abundance data for the station was examined to identify the charactering species. Mean biotope Infaunal Quality Index (IQI) Ecological Ratios (EQR) (+SE) and Ecological Status (ES) based on station data are included. Biotope TC5 did not meet the criteria for the calculation of IQI (Mackie, 2009).

**Biotope TC1. Euhaline muddy sands. IQI EQR = 1.04, IQI ES = High.**
1 station.

<table>
<thead>
<tr>
<th>Species</th>
<th>Abund</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magelona filiformis</td>
<td>6.48</td>
<td>8.24</td>
<td>8.24</td>
</tr>
<tr>
<td>Fabulina fabula</td>
<td>6.08</td>
<td>7.74</td>
<td>15.98</td>
</tr>
<tr>
<td>Chaetozone gibber</td>
<td>5.83</td>
<td>7.42</td>
<td>23.39</td>
</tr>
<tr>
<td>Magelona johnstoni</td>
<td>5.10</td>
<td>6.49</td>
<td>29.88</td>
</tr>
<tr>
<td>Mediomastus fragilis</td>
<td>4.12</td>
<td>5.24</td>
<td>35.12</td>
</tr>
<tr>
<td>Nemertea</td>
<td>3.74</td>
<td>4.76</td>
<td>39.88</td>
</tr>
<tr>
<td>Ampelisca brevicornis</td>
<td>3.16</td>
<td>4.02</td>
<td>43.90</td>
</tr>
<tr>
<td>Abra alba</td>
<td>3.00</td>
<td>3.82</td>
<td>47.72</td>
</tr>
<tr>
<td>Owenia fusiformis</td>
<td>3.00</td>
<td>3.82</td>
<td>51.54</td>
</tr>
<tr>
<td>Chamelea gallina</td>
<td>2.45</td>
<td>3.12</td>
<td>54.65</td>
</tr>
</tbody>
</table>

**Biotope TC2. Euhaline sandy muds. IQI EQR = 0.92 (+ 0.07), IQI ES = High.**
7 stations. Average similarity: 24.95

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbrineris gracilis</td>
<td>1.91</td>
<td>2.59</td>
<td>1.32</td>
<td>10.36</td>
<td>10.36</td>
</tr>
<tr>
<td>Chamelea gallina</td>
<td>1.64</td>
<td>2.32</td>
<td>0.79</td>
<td>9.3</td>
<td>19.66</td>
</tr>
<tr>
<td>Nemertea</td>
<td>1.51</td>
<td>2.06</td>
<td>1.42</td>
<td>8.25</td>
<td>27.91</td>
</tr>
<tr>
<td>Harpinia antennaria</td>
<td>1.20</td>
<td>1.34</td>
<td>0.79</td>
<td>5.35</td>
<td>33.26</td>
</tr>
<tr>
<td>Amphiura filiformis</td>
<td>2.81</td>
<td>1.32</td>
<td>0.42</td>
<td>5.29</td>
<td>38.55</td>
</tr>
<tr>
<td>Labidoplax digitata</td>
<td>1.40</td>
<td>1.27</td>
<td>0.58</td>
<td>5.07</td>
<td>43.62</td>
</tr>
<tr>
<td>Spiophanes bombyx</td>
<td>0.77</td>
<td>1.22</td>
<td>0.88</td>
<td>4.90</td>
<td>48.52</td>
</tr>
<tr>
<td>Nephtys hombergii</td>
<td>1.30</td>
<td>1.03</td>
<td>0.56</td>
<td>4.15</td>
<td>52.66</td>
</tr>
<tr>
<td>Mysella bidentata</td>
<td>1.61</td>
<td>1.02</td>
<td>0.58</td>
<td>4.07</td>
<td>56.73</td>
</tr>
</tbody>
</table>

**Biotope TC3. Poly/Euhaline muds. IQI EQR = 0.53 (+ 0.05), IQI ES = Moderate.**
2 Stations. Average similarity: 27.25

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubificoides benedii</td>
<td>5.44</td>
<td>11.19</td>
<td>0.83</td>
<td>41.08</td>
<td>41.08</td>
</tr>
<tr>
<td>Nephtys hombergii</td>
<td>1.78</td>
<td>4.54</td>
<td>0.90</td>
<td>16.66</td>
<td>57.74</td>
</tr>
<tr>
<td>Scrobicularia plana</td>
<td>1.87</td>
<td>4.04</td>
<td>0.71</td>
<td>14.84</td>
<td>72.58</td>
</tr>
<tr>
<td>Hediste diversicolor</td>
<td>1.47</td>
<td>3.71</td>
<td>0.85</td>
<td>13.60</td>
<td>86.19</td>
</tr>
<tr>
<td>Tubificoides pseudogaster</td>
<td>1.14</td>
<td>1.53</td>
<td>0.41</td>
<td>5.61</td>
<td>91.79</td>
</tr>
</tbody>
</table>
Table 4.3 cont:

Biotope TC4. Polyhaline muds. IQI EQR = 0.85 (+ 0.05), IQI ES = High.
7 Stations. Average similarity: 18.67

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nephtys hombergii</em></td>
<td>1.99</td>
<td>9.08</td>
<td>0.99</td>
<td>48.64</td>
<td>48.64</td>
</tr>
<tr>
<td><em>Scoloplos armiger</em></td>
<td>1.97</td>
<td>5.11</td>
<td>0.60</td>
<td>27.39</td>
<td>76.03</td>
</tr>
<tr>
<td><em>Abra nitida</em></td>
<td>1.09</td>
<td>1.43</td>
<td>0.32</td>
<td>7.65</td>
<td>83.68</td>
</tr>
</tbody>
</table>

Biotope TC5. Mesohaline muds.

1 Station.

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gammarus salinus</em></td>
<td>4.69</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
4.3.3 Top-down classification at Waterford

The dbRDA plot (Figure 4.4b) showed the sampling stations at Waterford formed well defined clusters using environmental predictor variables. At the Waterford study site top-down classification revealed five biotopes (TW1-TW5) (Figure 4.4a). Leave-one-out cross-validation analysis showed faunal stations were allocated correctly to biotopes 75% of the time, indicating that top-down classified biotopes were biologically relevant.

The species characterising each top-down biotope are presented in Table 4.4. Biotope TW1 was located at two stations in Waterford Harbour. The marine muddy sand community at TW1 was dominated by the polychaetes *M. filiformis* and *N. hombergii*, the amphipod *Ampelisca brevicornis* and the bivalve *F. fabula*. Biotope TW2 was limited to a single polyhaline sandy mud station located in the lower Barrow. The community at TW2 was dominated by the polychaetes *N. hombergii*, *Pygospio elegans* and *Lanice concheliga*, Tubificid oligochaetes and the bivalves *Macoma balthica* and *Mytilus edulis*. Biotope TW3 was identified in the lower reaches of both the Suir and Barrow. The polyhaline mud community at TW3 was characterised by Tubificid oligochaetes, the bivalve *Cerastoderma edule*, and polychaetes of the genera *Pygospio* and *Capitella*. Biotope TW4 was limited to mesohaline mud and muddy sand stations in the middle reaches of the Suir and Barrow. The community at TW4 was characterised by the bivalve *Scrobicularia plana* and Tubificid oligochaetes. Biotope TW5 was limited to oligohaline sediments in the upper reaches of the Barrow. The community at this biotope was comprised of oligochaetes and nematodes.
Figure 4.4a: Map showing top-down classified biotopes (TW1-TW5) at sampling stations at Waterford in June 2008. 4.4b: Distance-based redundancy analysis ordination plot of the multivariate macrofaunal variation explained and modelled by PD$_2$ variables in Distance-based Linear Modelling. PD$_2$ variables include EUNIS sediment PSA parameters (i.e. Mud:Sand ratio, % Gravel), loss on ignition (LOI%) and interstitial salinity psu values as continuous variables. The values in parentheses indicate the percentages of the fitted and total variations explained by each axis.
Table 4.4: Output of Similarity Percentage analysis used to determine species characterising top-down classified biotopes (TW1-TW5) at Waterford in June 2008. Where a biotope was defined by a single station the species abundance data for the station was examined to identify the charactering species. Mean biotope Infaunal Quality Index (IQI) Ecological Ratios (EQR) (+ SE) and Ecological Status (ES) based on station data are included. Biotope TW5 did not meet the criteria for the calculation of IQI.

**Biotope TW1. Euhaline muddy sands. IQI EQR = 0.73 (± 0.01), IQI ES = Good.**

2 stations. Average similarity: 56.36

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fabulina fabula</em></td>
<td>5.45</td>
<td>14.63</td>
<td>25.96</td>
<td>25.96</td>
</tr>
<tr>
<td><em>Nephtys hombergii</em></td>
<td>2.9</td>
<td>7.9</td>
<td>14.02</td>
<td>39.98</td>
</tr>
<tr>
<td><em>Ampelisca brevicornis</em></td>
<td>2.81</td>
<td>7.31</td>
<td>12.98</td>
<td>52.96</td>
</tr>
<tr>
<td><em>Magelona filiformis</em></td>
<td>1.73</td>
<td>5.17</td>
<td>9.18</td>
<td>62.13</td>
</tr>
<tr>
<td><em>Owenia fusiformis</em></td>
<td>2.19</td>
<td>5.17</td>
<td>9.18</td>
<td>71.31</td>
</tr>
<tr>
<td><em>Magelona johnstoni</em></td>
<td>1.71</td>
<td>4.22</td>
<td>7.49</td>
<td>78.81</td>
</tr>
</tbody>
</table>

**Biotope TW2. Euhaline sandy muds. IQI EQR = 0.67, IQI ES = Good.**

1 Station

<table>
<thead>
<tr>
<th>Species</th>
<th>Abund</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nephtys hombergii</em></td>
<td>4.00</td>
<td>16.06</td>
<td>16.06</td>
</tr>
<tr>
<td><em>Autolytus</em></td>
<td>2.24</td>
<td>8.98</td>
<td>25.03</td>
</tr>
<tr>
<td><em>Pygospio elegans</em></td>
<td>2.24</td>
<td>8.98</td>
<td>34.01</td>
</tr>
<tr>
<td><em>Nemertea</em></td>
<td>2.00</td>
<td>8.03</td>
<td>42.04</td>
</tr>
<tr>
<td><em>Crangon crangon</em></td>
<td>1.73</td>
<td>6.95</td>
<td>48.99</td>
</tr>
<tr>
<td><em>Tubificoides pseudogaster</em></td>
<td>1.73</td>
<td>6.95</td>
<td>55.94</td>
</tr>
<tr>
<td><em>Tubificoides</em></td>
<td>1.73</td>
<td>6.95</td>
<td>62.90</td>
</tr>
<tr>
<td><em>Lanice conchilega</em></td>
<td>1.41</td>
<td>5.68</td>
<td>68.57</td>
</tr>
<tr>
<td><em>Macoma balthica</em></td>
<td>1.41</td>
<td>5.68</td>
<td>74.25</td>
</tr>
</tbody>
</table>

**Biotope TW3. Polyhaline muds. IQI EQR = 0.70 (+ 0.06), IQI ES = Good.**

7 stations Average similarity: 25.37

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tubificoides pseudogaster</em></td>
<td>3.13</td>
<td>5.74</td>
<td>1.16</td>
<td>24.54</td>
<td>24.54</td>
</tr>
<tr>
<td><em>Cerastoderma edule</em></td>
<td>0.57</td>
<td>3.42</td>
<td>0.56</td>
<td>14.63</td>
<td>39.18</td>
</tr>
<tr>
<td><em>Capitella</em></td>
<td>1.07</td>
<td>2.77</td>
<td>0.87</td>
<td>11.86</td>
<td>51.03</td>
</tr>
<tr>
<td><em>Corophium volutator</em></td>
<td>1.41</td>
<td>2.36</td>
<td>0.54</td>
<td>10.12</td>
<td>61.15</td>
</tr>
<tr>
<td><em>Pygospio elegans</em></td>
<td>1.17</td>
<td>2.07</td>
<td>0.55</td>
<td>8.84</td>
<td>70.00</td>
</tr>
<tr>
<td><em>Tubificoides benedii</em></td>
<td>0.78</td>
<td>1.67</td>
<td>0.55</td>
<td>7.13</td>
<td>77.13</td>
</tr>
</tbody>
</table>
### Table 4.4 cont:

**Biotope TW4. Mesohaline mud and muddy sand. IQI EQR = 0.68 (+ 0.13), IQI ES = Good**

7 stations. Average similarity: 5.63

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scrobicularia plana</em></td>
<td>0.75</td>
<td>2.88</td>
<td>0.35</td>
<td>51.11</td>
<td>51.11</td>
</tr>
<tr>
<td><em>Tubificoides pseudogaster</em></td>
<td>0.61</td>
<td>2.35</td>
<td>0.33</td>
<td>41.79</td>
<td>92.89</td>
</tr>
</tbody>
</table>

**Biotope TW5. Oligohaline muddy sand and coarse sediments.**

2 stations. Average similarity: 28.78

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enchytraeidae</td>
<td>3.86</td>
<td>4.24</td>
<td>0.58</td>
<td>25.94</td>
<td>25.94</td>
</tr>
<tr>
<td>Nematoda</td>
<td>0.91</td>
<td>3.02</td>
<td>0.58</td>
<td>18.52</td>
<td>44.45</td>
</tr>
<tr>
<td><em>Psammoryctides barbatus</em></td>
<td>0.80</td>
<td>3.02</td>
<td>0.58</td>
<td>18.52</td>
<td>62.97</td>
</tr>
<tr>
<td><em>Tubificoides amplivasatus</em></td>
<td>1.22</td>
<td>3.02</td>
<td>0.58</td>
<td>18.52</td>
<td>81.48</td>
</tr>
<tr>
<td><em>Tubificoides pseudogaster</em></td>
<td>3.37</td>
<td>3.02</td>
<td>0.58</td>
<td>18.52</td>
<td>100.00</td>
</tr>
</tbody>
</table>
4.3.4 Bottom-up classification at Kinsale and Oysterhaven

Bottom-up clustering of faunal sampling stations at the Kinsale and Oysterhaven using HAC and SIMPROF significance tests are shown in Figure 4.5b. Bottom-up classification of the clustered stations using the EUNIS Biotope scheme revealed six biotopes (BC1-BC6) (Figure 4.5a, Table 4.5). Biotope BC1 was identified at a single muddy euhaline station at Oysterhaven. The biotope corresponded to the level 5 EUNIS biotope *F. fabula* and *Magelona* polychaetes in infralittoral compacted fine muddy sand (SS.SSA.IMuSa.FfabMag). Four stations in Kinsale and three stations in Oysterhaven formed biotope BC2, which corresponded reasonably well to the EUNIS level 5 biotope *N. hombergii* and *Tubificoides* in variable salinity infralittoral soft mud (SS.SMU.SMvVS.NhomTubi). Stations in the middle and lower reaches of the Bandon and upper reaches of the Belgooly formed BC3. This biotope was classified as infralittoral muddy sands (level 4 EUNIS SS.SSA.IMuSa) dominated by Tubificid Oligochaetes, the Amphipod *C. multisetosum* and the polychaetes *H. diversicolor* and *Streblospio shrubsolii*. Five muddy sand stations in Kinsale harbour formed biotope BC4. The biotope contained characterising species from two typical circalittoral level 5 biotopes namely, *A. filiformis*, *M. bidentata* and *A. nitida* in circalittoral sandy mud (SS.SMu.CSaMu.AfilMysAnit), and *Mediomastus fragilis* and *Lumbrineris* and venerid bivalves in circalittoral coarse sand or gravel (SS.SCS.CCS.MedLumVen). BC5 was represented by a single muddy marine station in Kinsale harbour. This biotope corresponded well to the level 4 biotope sublittoral muddy sands dominated by the bivalve *C. gallina*, the holothurian *Leptosynapta* and the brittlestar *Amphipholis squamata* (SS.SSa.SMuSa). BC6 was limited to a single station in the upper reaches of the Bandon. The biotope was classified as *Gammarus* species in variable salinity in infralittoral mud (level 5 SS.SSa.SSaVS.NintGam).
Figure 4.5a: Map showing bottom-up classified biotopes (BC1-BC5) at sampling stations at Kinsale and Oysterhaven, Co. Cork in May 2010. 4.5b: Dendrogram of Hierarchical Agglomerative Clustering of square root transformed zero adjusted Bray Curtis similarities between stations. The labelling factor is bottom-up biotope classification assigned using a Similarity Profiling significance test and Similarity Percentage analysis. Groups of samples joined by grey lines are not significantly different, while groups joined by bold black lines are significantly different.
Table 4.5: Output of Similarity Percentage analysis used to determine species characterising bottom-up classified biotopes (BC1-BC6) at Kinsale and Oysterhaven, Co. Cork in May 2010. Bottom-up biotopes based on Hierarchical Agglomerative Clustering and Similarity Profile significance test of outputs of square root transformed zero adjusted Bray Curtis similarities between stations.

### Biotope BC1. Level 5. SS.SSA.IMuSa.FfabMag.

1 Station

<table>
<thead>
<tr>
<th>Species</th>
<th>Abund</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magelona filiformis</td>
<td>6.48</td>
<td>8.24</td>
<td>8.24</td>
</tr>
<tr>
<td>Fabulina fabula</td>
<td>6.08</td>
<td>7.74</td>
<td>15.98</td>
</tr>
<tr>
<td>Chaetozone gibber</td>
<td>5.83</td>
<td>7.42</td>
<td>23.39</td>
</tr>
<tr>
<td>Magelona johnstoni</td>
<td>5.10</td>
<td>6.49</td>
<td>29.88</td>
</tr>
<tr>
<td>Mediomastus fragilis</td>
<td>4.12</td>
<td>5.24</td>
<td>35.12</td>
</tr>
</tbody>
</table>

### Biotope BC2. Level 5. SS.SMU.SMuVS.NhomTubi

7 stations. Average similarity: 30.78

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephtys hombergii</td>
<td>2.94</td>
<td>13.53</td>
<td>3.2</td>
<td>43.98</td>
<td>43.98</td>
</tr>
<tr>
<td>Tubificoides benedii</td>
<td>3.25</td>
<td>4.03</td>
<td>0.48</td>
<td>13.09</td>
<td>57.06</td>
</tr>
<tr>
<td>Abra nitida</td>
<td>1.64</td>
<td>3.88</td>
<td>0.6</td>
<td>12.59</td>
<td>69.65</td>
</tr>
</tbody>
</table>

### Biotope BC3. Level 4SS.SMU.SMuVS

3 Stations. Average similarity 16.97

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubificoides pseudogaster</td>
<td>1.53</td>
<td>5.18</td>
<td>0.58</td>
<td>30.54</td>
<td>30.54</td>
</tr>
<tr>
<td>Corophium multisetalosum</td>
<td>0.80</td>
<td>4.46</td>
<td>0.58</td>
<td>26.26</td>
<td>56.80</td>
</tr>
<tr>
<td>Hediste diversicolor</td>
<td>0.67</td>
<td>3.67</td>
<td>0.58</td>
<td>21.60</td>
<td>78.40</td>
</tr>
<tr>
<td>Streblospio shrubsolii</td>
<td>0.91</td>
<td>3.67</td>
<td>0.58</td>
<td>21.60</td>
<td>100.00</td>
</tr>
</tbody>
</table>

### Biotope BC4. Level 5. SS.SMu.CSaMu.AfilMysAnit & SS.SCS.CCS.MedLumVen

5 stations Average similarity: 36.71

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbrineris gracilis</td>
<td>2.47</td>
<td>3.79</td>
<td>2.62</td>
<td>10.32</td>
<td>10.32</td>
</tr>
<tr>
<td>Nemertea</td>
<td>1.91</td>
<td>2.94</td>
<td>3.76</td>
<td>8.02</td>
<td>18.34</td>
</tr>
<tr>
<td>Harpinia antennaria</td>
<td>1.68</td>
<td>2.79</td>
<td>2.14</td>
<td>7.61</td>
<td>25.95</td>
</tr>
<tr>
<td>Amphiphiara filiformis</td>
<td>3.94</td>
<td>2.75</td>
<td>0.67</td>
<td>7.49</td>
<td>33.44</td>
</tr>
<tr>
<td>Labidoplax digitata</td>
<td>1.96</td>
<td>2.64</td>
<td>1.05</td>
<td>7.20</td>
<td>40.64</td>
</tr>
<tr>
<td>Chamelea galina</td>
<td>1.81</td>
<td>2.24</td>
<td>0.95</td>
<td>6.11</td>
<td>46.75</td>
</tr>
<tr>
<td>Kurtiella bidentata</td>
<td>2.25</td>
<td>2.12</td>
<td>1.04</td>
<td>5.77</td>
<td>52.53</td>
</tr>
</tbody>
</table>
Table 4.5 cont:

1 station.

<table>
<thead>
<tr>
<th>Species</th>
<th>Abund</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chamelea gallina</em></td>
<td>2.45</td>
<td>15.61</td>
<td>15.61</td>
</tr>
<tr>
<td><em>Leptosynapta inhaerens</em></td>
<td>2.00</td>
<td>12.75</td>
<td>28.35</td>
</tr>
<tr>
<td><em>Amphipholis squamata</em></td>
<td>1.41</td>
<td>9.01</td>
<td>37.37</td>
</tr>
<tr>
<td><em>Diplocirrus glaucus</em></td>
<td>1.41</td>
<td>9.01</td>
<td>46.38</td>
</tr>
</tbody>
</table>

### Biotope BC6. Level 5. SS.SSa.SSaVS.NintGam
1 Station.

<table>
<thead>
<tr>
<th>Species</th>
<th>Abund</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gammarus salinus</em></td>
<td>4.69</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
4.3.5 Bottom-up classification at Waterford

EUNIS bottom-up classification at Waterford revealed five biotopes (BW1-BW5) typical of estuarine fine sediments (Figure 4.6a,b; Table 4.6). Biotope BW1 was identified at two sandy euhaline stations in the harbour. The biotope corresponded well to the EUNIS level 5 biotope *F. fabula* bivalves and *M. filiformis* polychaetes in infralittoral compacted fine muddy sand (SS.SSA.IMuSa.FlabMag). BW2 was identified at six muddy stations dispersed across the middle and lower reaches of the Barrow and Suir, and one marine sandy station in the inner area of Waterford harbour. The biotope corresponded to the EUNIS level 4 biotope infralittoral muddy sands (SS.SSa.SMuSa). The biotope supported a diverse community comprised of oligochaetes, polychaetes of the genera *Capitella*, *Nephtys*, *Pygospio*, and *Polydora*, amphipods and the bivalve *S. plana*. BW3 was comprised of six muddy and two sandy stations dispersed along the middle and upper reaches of the Barrow and Suir, and were classified as infralittoral muddy sand (level 4 SS.SSa.SMuSa). The faunal community at biotope BW4 was dominated by Tubificid oligochaetes and the bivalves *S. plana*, *M. balthica* and *C. edule*. In the upper reaches of the Barrow two muddy stations formed biotope BW4. The biotope conformed to the level 5 biotope oligochaetes in variable or reduced salinity infralittoral muddy sediment (SS.SMu.SMuVS.OlVS). One station in the upper Barrow formed biotope BW5 and was classified as the level 4 biotope sublittoral coarse sediment in variable salinity (SS.SCS.SCSVS) and was characterised by robust brackish water tolerant oligochaetes and Platyhelminthes.
Figure 4.6a: Map showing bottom-up classified biotopes (BW1-BW6) at sampling stations at Waterford in June 2008. 4.6b: Dendrogram of Hierarchical Agglomerative Clustering output of square root transformed zero adjusted Bray Curtis similarities between stations. The labelling factor is bottom-up biotope classification assigned using a Similarity Profile significance test and Similarity Percentage analysis. Groups of samples joined by grey lines are not significantly different, while groups joined by bold black lines are significantly different.
Table 4.6: Output of Similarity Percentage analysis used to determine species characterising bottom-up classified biotopes (BW1-BW6) at Waterford in June 2008. Bottom-up biotopes based on Hierarchical Agglomerative Clustering and Similarity Profile significance tests of outputs of square root transformed zero adjusted Bray Curtis similarities between stations

### Biotope BW1. Level 5. SS.SSA.IMuSa.FfabMag
2 stations. Average similarity: 56.36

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabulina fabula</td>
<td>5.45</td>
<td>14.63</td>
<td>25.96</td>
<td>25.96</td>
</tr>
<tr>
<td>Nephtys hombergii</td>
<td>2.90</td>
<td>7.90</td>
<td>14.02</td>
<td>39.98</td>
</tr>
<tr>
<td>Ampelisca brevicornis</td>
<td>2.81</td>
<td>7.31</td>
<td>12.98</td>
<td>52.96</td>
</tr>
<tr>
<td>Magelona filiformis</td>
<td>1.73</td>
<td>5.17</td>
<td>9.18</td>
<td>62.13</td>
</tr>
<tr>
<td>Owenia fusiformis</td>
<td>2.19</td>
<td>5.17</td>
<td>9.18</td>
<td>71.31</td>
</tr>
<tr>
<td>Magelona johnstoni</td>
<td>1.71</td>
<td>4.22</td>
<td>7.49</td>
<td>78.81</td>
</tr>
</tbody>
</table>

### Biotope BW2. Level 4. SS.SSa.IMuSa
7 Stations. Average similarity: 26.88

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubificoides pseudogaster</td>
<td>3.20</td>
<td>6.53</td>
<td>3.64</td>
<td>24.30</td>
<td>24.30</td>
</tr>
<tr>
<td>Pygospio elegans</td>
<td>1.63</td>
<td>4.87</td>
<td>1.23</td>
<td>18.13</td>
<td>42.44</td>
</tr>
<tr>
<td>Corophium volutator</td>
<td>1.55</td>
<td>3.19</td>
<td>0.74</td>
<td>11.88</td>
<td>54.31</td>
</tr>
<tr>
<td>Capitella</td>
<td>1.07</td>
<td>2.77</td>
<td>0.87</td>
<td>10.31</td>
<td>64.62</td>
</tr>
<tr>
<td>Nephtys hombergii</td>
<td>1.24</td>
<td>2.02</td>
<td>0.39</td>
<td>7.50</td>
<td>72.13</td>
</tr>
<tr>
<td>Scrobicularia plana</td>
<td>1.16</td>
<td>1.88</td>
<td>0.60</td>
<td>7.00</td>
<td>79.12</td>
</tr>
<tr>
<td>Polydora cornuta</td>
<td>1.23</td>
<td>1.22</td>
<td>0.35</td>
<td>4.54</td>
<td>83.66</td>
</tr>
<tr>
<td>Gammarus salinus</td>
<td>0.92</td>
<td>0.83</td>
<td>0.38</td>
<td>3.07</td>
<td>86.73</td>
</tr>
</tbody>
</table>

### Biotope BW3. Level
8 Stations. Average similarity 5.99

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubificoides pseudogaster</td>
<td>0.68</td>
<td>2.49</td>
<td>0.33</td>
<td>41.58</td>
<td>41.58</td>
</tr>
<tr>
<td>Scrobicularia plana</td>
<td>0.40</td>
<td>1.20</td>
<td>0.19</td>
<td>19.97</td>
<td>61.54</td>
</tr>
<tr>
<td>Tubificoides benedii</td>
<td>0.30</td>
<td>0.85</td>
<td>0.19</td>
<td>14.16</td>
<td>75.70</td>
</tr>
<tr>
<td>Macoma balthica</td>
<td>0.39</td>
<td>0.82</td>
<td>0.19</td>
<td>13.74</td>
<td>89.45</td>
</tr>
<tr>
<td>Cerastoderma edule</td>
<td>0.25</td>
<td>0.63</td>
<td>0.19</td>
<td>10.55</td>
<td>100.00</td>
</tr>
</tbody>
</table>

### Biotope BW4. Level 5. SS.SMu.SMuVS.OIVS
2 Stations. Average similarity: 36.28

<table>
<thead>
<tr>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nematoda</td>
<td>1.37</td>
<td>9.07</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Psammonryctides barbatus</td>
<td>1.21</td>
<td>9.07</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Tubificoides amplivasatus</td>
<td>1.82</td>
<td>9.07</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>Tubificoides pseudogaster</td>
<td>5.06</td>
<td>9.07</td>
<td>25</td>
<td>100</td>
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</tbody>
</table>
Table 4.6 cont:

<table>
<thead>
<tr>
<th>Biotope BW5. Level 4. SS.SCS.SCSVS 1 Station.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>Enchytraeidae</td>
</tr>
<tr>
<td>Tricladida</td>
</tr>
</tbody>
</table>

4.3.6 IQI ES assessment at top-down classified biotopes

Mean ES (and SE range) for the “top-down” classified biotopes at the Kinsale and Oysterhaven, and at the Waterford study sites are presented in Table 4.3 and 4.4 respectively. Biotope TC5 at Kinsale and Oysterhaven and biotope TW5 at Waterford did not meet the criteria for the calculation IQI ES. Of the four biotopes at Kinsale and Oysterhaven three were designated High while one was designated Moderate. Moderate status was identified at biotope TC3 in the harbour area at Scilly, near the yachting marina at Kinsale town and in the lower reaches of the Bandon estuary. The four biotopes at Waterford were designated Good.
4.4 DISCUSSION

4.4.1 Interstitial salinity as an explanatory variable

This study is the first published account investigating the effect of using interstitial salinity as a parameter to explain variability in multivariate macrofaunal distributions for the purpose of biotope classification (sensu the HD) and ES assessment (sensu the WFD). Interstitial salinity at the Kinsale and Oysterhaven, and the Waterford study sites followed typical estuarine gradients progressing from euhaline conditions in the harbours to polyhaline sites in the lower and middle estuaries, and on into mesohaline and oligohaline habitats in the upper estuaries (Basset et al., 2006; Elliot and McLusky, 2002). Previous studies have shown the multivariate structure of infaunal communities to be highly correlated with interstitial salinity (Chapman and Brinkhurst, 1981; Dale and Miller, 2008). A similar correlative relationship between community structure and interstitial salinity was observed in the present study. Here, using interstitial salinity as a continuous predictor variable or as a categorical predictor variable classified using the Venice System (Battaglia, 1959), in conjunction with routinely used sediment parameters (i.e. sediment and organic content) explained significantly greater levels of variance in the multivariate structure of macrofaunal assemblages than using sediment parameters alone. Increasing levels of explained variance allows benthic communities to be classified and mapped with greater confidence and has potential to improve the effectiveness of environmental management (Forde et al., 2012). The parity between interstitial salinity as a continuous and as a categorical variable in terms of explanatory power provides empirical support for the zonation categories proposed by the Venice System. The latter was formulated based on subjective observations of spatial utilisation of estuaries by organisms (Battaglia, 1959). The performance of the categorical explanatory variables also indicated that the Venice zonation classes are suitable as top-down classifiers for partitioning transitional waters into ecologically relevant habitat types (sensu the HD).
4.4.2 Bottom-up vs. top-down classification and IQI ES assessment

The present study illustrates how biotopes described for the HD can be used to delineate ES (sensu the WFD) on the soft seafloor. Using this approach successfully meets the provisions of the HD and WFD. We show that top-down classification of biotopes using environmental parameters has advantages over using the structure of highly variable faunal communities as the basis for biotope classification. At both study sites bottom-up EUNIS classification produced complex spatial biotope mosaics based on fauna community composition within areas of relatively uniform habitat. This spatial fragmentation of habitats was most apparent with biotopes BW2 and BW3 along the middle and lower reaches of the Barrow and Suir at Waterford and with biotopes BC2 and BC3 in the lower Bandon and inner Kinsale harbour. Here, bottom-up fragmentation precludes representing the spatial distribution of the habitats in their entirety. This fragmentation of habitats into multiple units presents a significant difficulty for environmental management over large spatial scales (Ferreira et al., 2006; Teixeira et al., 2008; Puente et al., 2008; de Paz et al., 2008). Here, EUNIS biotope classification based on faunal community composition generated biotopes whose listed physical attributes did not match the actual environmental habitat conditions. Furthermore, in a number of instances the fauna characterising biotopes did not match any of the core EUNIS records (EUNIS 2002; Connor et al., 2004). This resulted in biotopes being classified to EUNIS level 4 only (i.e. as habitat types). Defining biotopes based on high resolution faunal community structure can produce biotope classifications that are only understood and effectively discriminated between by experts. The use of these technical and applied classifications can represent a significant obstacle to managers, developers and legislators involved in environmental management (Green et al, 2008; Dauvin et al., 2008b).

Here, top-down classification produced biotopes which reflected the spatial extent of the habitats while taking into account the natural variability in the faunal communities. Many of the defined biotopes were common to both study areas, suggesting this approach is suitable for large scale classification.
of habitats across geographical regions. Leave-one-out cross validation analysis showed a high level of consistency in the allocation of stations based on their underlying multivariate faunal structure (i.e. a bottom-up approach) to the top-down classified biotopes. This indicates that using sediment and interstitial salinity as classifiers results in biologically meaningful top-down classified biotopes. The methodology proposed here obviates the need to define fixed core biotope records as is the case under EUNIS. Rather the fauna within the top-down biotope is used to identify the characterising species at the time of sampling. Consequently, there are no issues with transferring this technique to new geographical areas. The generally applicable physical classifiers were suitable for partitioning estuaries into large physical management units that may be easily interpreted and mapped. A further advantage of top-down classification is that it is often easier to obtain physical ecological than to gather biological data (Digby et al., 1998; Galván et al., 2010). In addition classification of habitats using physical data may be more objective than using biological community data (Elliott and McLusky, 2002; Huang et al., 2011). A habitat classification scheme that is user-friendly and is transferable is imperative when the objective is large scale classification of habitats (Green et al., 2008; Krönke and Reiss, 2010; Kennedy et al., 2011).

Habitat classification of sedimentary environments is increasingly performed using remote sensing techniques such as acoustic survey and underwater video, with a rapidly developing array of classification approaches and algorithms available (Brown et al., 2011). These surveys are typically validated using a small number of ground-truth bottom samples. Top down classification schemes based on acoustic survey and physical properties have been found to produce larger, more homogeneous classifications than those incorporating macrofaunal distributions as analysed by multivariate statistics (Eastwood et al., 2006; Shumchenia and King, 2010). In Naragansett Bay, Shumchenia and King (2010) compared top-down and bottom-up classifications. Within the top-down framework, where they tested for differences in macrofauna assemblages among side scan sonar facies, they defined two broad-scale and general habitats. Using the bottom-up
approach, where patterns in abiotic and biotic variables were examined with multivariate statistics, they generated seven biotopes based on the macrofauna abundance, percent sand, water depth, and backscatter standard deviation that corresponded well to, but provided more fine-scale detail than the top-down habitats. They make the conclusion that incorporating the bottom up approach provides a better reflection of animal sediment relationships. This is a valid conclusion, assuming that the macrofaunal distribution remains consistent within the sediments. The advantage of the top-down approach is that it makes fewer assumptions about the consistency of the macrofaunal community structure, and allows that the variability within the top-down may reflect the extent of community variability within the physical habitat.

In the top-down classification system presented here, mean IQI ES (and its range) was calculated for each biotope based on the biological communities present within each habitat type. Basing ES assessment on realistic measures of faunal community variability within spatial units will allow ES to be monitored usefully on a habitat level rather than a highly variable community level (i.e. temporal and spatial snapshots). One of the crucial first steps in effective marine spatial planning and ecosystem-based management efforts is the creation of benthic habitat maps that allow scientists and managers to understand the distribution of living and non-living resources on the seafloor. Another is the creation of maps of ecological status, resilience and habitat quality that reflect the degree of anthropogenic disturbance, threats and pressures (Salomidi et al., 2012). Here, proposed approach to biotope classification and ES assessment would allow the integration of HD and WFD sampling programmes, and facilitate a process of mutual enlightenment between the outputs of both Directives.

4.5 ACKNOWLEDGEMENTS

This work was funded by Eli Lilly SA, Dunderrow, Co. Cork, as part of a long term monitoring program in Kinsale Harbour Ireland. We thank the staff of Eli Lilly for their continued support and interest in this work. The National Parks
and Wildlife Service are thanked for providing support for this research. Thanks to Butch Roberts of M.V Sundance Kid and Martin O’Conghaile of M.V. Sharpshooter for their assistance at sea. Chris Frid, Colin Brown and two anonymous reviewers are thanked for their constructive comments.

4.6 CONTRIBUTIONS

All listed co-authors participated in survey work at Kinsale and Waterford and collaborated on subsequent faunal analyses. JF performed sediment and salinity analyses. JF and BK collaborated on statistical and spatial analyses. JF was the primary author of this manuscript.
5 LONG-TERM TEMPORAL TRENDS (1987–2011) OF ORGANOTIN CONTAMINATION INDUCED IMPOSEX IN THE DOGWHELK NUCELLA LAPILLUS: A CASE STUDY IN KINSALE HARBOUR, SOUTH COAST OF IRELAND

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Marine Pollution Bulletin (In Press)
ABSTRACT

The dog whelk *Nucella lapillus* is particularly sensitive to tributyltin (TBT) which has been widely used as the active ingredient in anti-fouling treatments. TBT has been shown to cause the development of imposex in females. Imposex is the irreversible imposition of non-functioning male sexual characteristics (i.e. penis and vas deferens) which acts to reduce reproductive capabilities and can eventually lead to reproductive failure and possible localised extinctions. Levels of imposex in gastropods have been widely used to monitor TBT contamination in the marine environment. Here, we present the findings of a 24 year study of imposex in *N. lapillus* at Kinsale Harbour, Ireland.

Two indices have been used to quantify population levels of imposex in the dogwhelk. The Vas Deferens Sequence Index (VDSI) is based on the developmental sequence of vas deferens in imposexed females while the Relative Penis Size Index (RPSI) is based on relative measures of male and female imposexed penis size. Here, levels of imposex, as described by both indices, were highest in the two years following the implementation of the Irish ban on TBT in 1987. Between 1987 and 2003 imposex levels fluctuated at high levels. After 2003 both RPSI and VDSI decreased significantly. Regression analysis showed the rate of decline of imposex in the Inner Harbour at Kinsale was different to that of Middle and Outer Areas. This may be due to relatively higher levels of shipping activity and the dredging of navigational channels in the Inner Harbour acting as potential sources of TBT. VDSI levels throughout Kinsale currently meet OSPAR’s EcoQO for TBT contamination (i.e. VDSI in *Nucella* <2). VDSI is still highest in the Inner and Middle Harbour areas. The higher levels of imposex in these areas may be a consequence of regeneration of TBT from buried contaminants during channel dredging.
5.1 INTRODUCTION

Tributyltin (TBT), an organotin compound, has been widely used as an active biocide ingredient in antifoulant preparations for hulls of vessels since the 1960s (Garaventa et al., 2006), and on fish-cage netting since the 1980s (Birchenough et al., 2002a). Erosion and leaching of TBT from antifouling paint on ships and structures is recognised as the major source of TBT in the marine environment (OSPAR, 2011). Effects of TBT antifoulants and their leachates on non-target species were first noted in France in the early 1980’s when the oyster aquaculture sector experienced massive declines in productivity due to reduced spat fall and abnormal adult shell development (Alzieu et al., 1982; Alzieu, 1991; Bryan et al., 1986; Gipperth et al., 2009). The most extensively studied effect of TBT is the irreversible imposition of male sexual characteristics (i.e. penis and vas deferens) onto female marine gastropods, a phenomenon termed imposex. Imposex acts to reduce reproductive capabilities and where levels of TBT contamination are sufficiently high can cause reproductive failure resulting in localised extinctions (Bryan et al., 1986; Minchin et al., 1995, 1996; Oehlmann et al., 1991; 1998). TBT induced imposex has been recorded in over 195 species of molluscs extending from the polar regions to the tropics (Sternberg et al., 2010; Sujatha et al., 1996; Brick and Bolte, 1994; Svavarsson and Skarphédinsdóttir, 1995; Svavarsson, 2000; Gibson and Wilson, 2003; Sternberg, 2010; Guðmundsdóttir et al., 2011).

In response to growing concerns regarding TBT contamination in coastal waters, many European countries, including Ireland in 1987 (Anon, 1987), implemented national restrictions and bans prohibiting the use of TBT based antifoulants on vessels <25m and marine structures (Champ, 2000; Gipperth, 2009). In 1998 the Oslo and Paris Commission (OSPAR), a convention of countries in the North-East Atlantic region whose aim is to protect the marine environment, identified organotin compounds, and TBT in particular, on the OSPAR List of Chemicals for Priority Action. In 2000 OSPAR made recommendations with regard phasing out the use of organotin antifoulants with the target eventual cessation by 2020 (OSPAR, 2008). In 2001 the
International Maritime Organisation (IMO) adopted the Convention on the Control of Harmful Anti-fouling Systems on Ships (AFS Convention). The AFS Convention, which came into force in 2008, calls for an international ban on TBT antifouling paint (Gipperth, 2009). In 2003, in support of the AFS Convention, the European Union implemented legislation prohibiting the marketing and use of TBT antifoulants on EU-flagged vessels and as of 2008 made it an offence for any ship visiting an EU port to have TBT antifouling paint on its hull (EC, 2002/62/EC; Gipperth, 2009; Oliveira et al., 2009; OSPAR, 2011).

TBT induced imposex in the dogwhelk *Nucella lapillus* has been widely used as a biological indicator of TBT contamination and thus a measure of the effectiveness of TBT controls (Minchin et al., 1995, 1996; Guðmunddóttir et al., 2011). Two indices have been used to quantify population levels of imposex in the dogwhelk. The Vas Deferens Sequence Index (VDSI) is based on the developmental sequence of vas deferens in imposexed females while the Relative Penis Size Index (RPSI) is based on relative measures of male and female imposexed penis size (Gibbs et al., 1987; Oehlmann et al., 1991). VDSI is considered to be a better biomarker as it sensitive to early stages of imposex which lack a penis (Oehlmann et al., 1991; Galante-Oliveira et al., 2010). In addition, male penis size exhibits spatial and seasonal variation while female penis size remains relatively constant over time. Variation in male penis size has the potential to introduce error when using RPSI to evaluate TBT contamination levels. Despite the confounding effects of variation in male penis size, RPSI provides valuable information which can complement VDSI in monitoring programmes. RPSI provides a more sensitive indication of relative concentrations of TBT in areas close to release points, particularly where VDSI levels are high (Oehlmann et al., 1991; Galante-Oliveira et al., 2010). Other measures used to investigate temporal changes in the status of *N. lapillus* populations are based on measures of the abundance of dogwhelk on the shore, the adult sex ratio and the relative proportion of juveniles to mature individuals (e.g. Evans et al., 1994; 1996; 2000a; Minchin, 1997; Birchenough et al., 2002a,b).
OSPAR has set an assessment criterion, or an Ecological Quality Objective (EcoQO), based on the biological response of \textit{N. lapillus} to TBT to allow monitoring of TBT contamination in the marine environment. \textit{N. lapillus} is a suitable bio-indicator as they are widely distributed, have limited mobility and are sensitive to TBT at concentrations that can not be routinely measured by most laboratories (Oliveira et al., 2009; OSPAR, 2008). TBT concentrations $< 1 \text{ng Sn l}^{-1}$ are sufficient to induce imposex in \textit{Nucella} while at concentrations $< 3 \text{ng Sn l}^{-1}$ females become sterile (Gibbs and Bryan, 1986; OSPAR, 2008). OSPAR’s EcoQO states that VDSI levels should be consistent with exposure to TBT concentrations that do not pose a risk of causing significant biological effects; for \textit{N. lapillus} this would be a VDSI $< 2$ (OSPAR, 2011).

Here, we use VDSI and RPSI imposex data in the dogwhelk \textit{N. lapillus} to assess temporal and spatial trends in TBT contamination at Kinsale Harbour, Ireland since the introduction of the partial ban on TBT antifoulants in 1987. Time series VDSI data are used to assess if OSPAR’s EcoQO for TBT has been achieved at Kinsale. VDSI data are also used to assess the validity of OSPAR’s EcoQO VDSI threshold of $< 2$ in Irish waters.

5.2 MATERIALS AND METHODS

5.2.1 Study site description and sampling design

The Bandon estuary and Kinsale Harbour are located on the south coast of Ireland (Figure 5.1). The Harbour and estuary are exposed to natural estuarine pressures and typical anthropogenic disturbances associated with human settlement and port activities. Along its course the river Bandon passes a number of villages and towns with a total population of $<10,000$. The Bandon has a mean daily flow rate of 15m$^3$ s$^{-1}$ and discharges into the inner Harbour where it slightly depresses off-bottom salinities. The harbour is classified as partially mixed. Water residence time is estimated at about half a day or one tidal cycle resulting in extensive exchange of water between the harbour and coastal waters (Muylaert and Raine, 1999). Long term benthic monitoring at Kinsale has shown that benthic habitat Ecological Status (\textit{sensu} the European Water Framework Directive) at Kinsale is generally
Good or High with the exception of the inner Harbour area at Kinsale town which ranges between Poor and Moderate. Low habitat quality in the inner Harbour was related to physical dredging disturbance of navigational channels and effluent pollution (Kennedy et al., 2011; Forde et al., 2012).

**Figure 5.1:** Map showing sampling stations (●) in the Inner, Middle and Outer Harbour Areas at Kinsale Harbour, Cork (1987–2011).

For the purpose of assessing temporal and spatial trends in imposex in *N. lapillus* Kinsale Harbour was divided into three broad Areas (i.e. Inner, Middle and Outer Harbour Areas; see Figure. 5.1). The division of the Harbour study Area was based on the relative distribution of possible sources of TBT contamination both past and present. The Inner Harbour Area contains Kinsale town, two yachting marinas and a commercial fishing pier. Kinsale town has a resident population of approximately 2500 which increases substantially during summer months. The fishing pier is a base for
approximately ten trawlers and facilitates approximately 150,000 ton of cargo annually. Cargo is dominated by bulk animal feeds but fertilizers, timber and building materials are also common. Shipping channels in the Inner and Middle Harbour are dredged approximately every two years. Prior to 2006 there were only primary sewage treatment facilities in Kinsale and effluents were discharged at several outfalls distributed in the Inner Harbour. Kinsale now has a secondary sewage treatment plant which discharges effluent into the lower Bandon at Commoge. In the Middle Harbour Area there is a large operational boatyard at Middle Cove. In the Outer Harbour Area a local pharmaceutical company discharges treated effluent into the Outer Harbour at Sandy Cove Island via a subtidal outfall pipe.

With the exception of the Inner Harbour in 1989 and 2003, each Harbour Area was sampled in 1987, 1989, 1992, 1994, 1995, 1996, 2002, 2003 and 2011. Between 1987 and 2011 a total of 57 samples were retrieved within the sampling Areas. In all cases at least 30 adult individuals were collected for imposex assessment. Male:female sex ratios were either equal or slightly male biased. Sampling stations visited within each Area varied over the course of the time-series.

5.2.2 Imposex assessment

In the laboratory, the shell lengths of *N. lapillus* were measured to the nearest 0.1mm using digital vernier callipers. The dogwhelks were de-shelled using a vice, and the tissues removed. Individuals were sexed and examined for indications of imposex under a microscope. Females were distinguished based on the presence of the sperm-ingesting gland behind the capsule gland. The VDSI and the RPSI were determined using the methodologies described by Gibbs et al. (1987).

The VDSI is based on the developmental sequence of the male vas deferens in imposed females. Stage 0 females are normal and do not exhibit male characteristics. In Stage 1 individuals the proximal section of a vas deferens is formed at the opening of the vagina. At Stage 2 a small penis is apparent
and the proximal section of vas deferens is further extended. In Stage 3 individuals the distal section of the vas deferens is formed and the penis is more developed. At Stage 4 the proximal and distal section of the vas deferens are fused while at Stage 5 the vas deferens becomes overdeveloped and seals the genital papilla opening. At Stage 6 aborted egg capsules are visible in the capsule gland. Penis lengths were measured and the RPSI was calculated according to the following equation; \[ \frac{([\text{mean length of female penis}]^3)}{([\text{mean length of male penis}]^3)} \times 100 \] (Gibbs et al., 1987). Laboratory analysis of 1987 individuals predated the publication of VDSI by Gibbs et al. (1987). Consequently, for the 1987 survey only RPSI was determined.

5.2.3 Statistical analyses

Analyses were carried out using MINITAB v16 and R.

5.2.3.1 Temporal and spatial trends in RPSI

As the RPSI data were not normally distributed non-parametric statistical methods were employed. The factors Area and Year were combined into a factor termed RPSI-Sample. The factor RPSI-Sample had 23 unique combinations of Area and Year. Significant differences in median RPSI values across RPSI-Sample levels were investigated using a Kruskal–Wallis test followed by multiple comparisons of the medians using Dunn’s test. To allow multiple comparisons RPSI-Sample levels which did not have replicate data were omitted from the analysis (i.e. levels reduced from 23 to 16 levels).

5.2.3.2 Temporal and spatial trends in VDSI

The VDSI data were checked for temporal autocorrelation using lags from 2 to 16 years. As the partial autocorrelation function was not significantly different from zero (P > 0.05) for any lag, time series analysis techniques were not employed. Temporal trends in VDSI were modeled using quadratic regression with Time (i.e. years post 1987) and \( \text{Time}^2 \) as predictor variables and Area as a nominal covariate. Statistical assumptions of regression
analysis include normally distributed residuals and homogeneity of variances (i.e. the variance within each of the sampling area is equal). Anderson-Darling tests were used to test for normality in the residuals, while homogeneity of residual variances was confirmed using Levene’s test (Zuur et al., 2007).

For the VDSI data Area and Year were combined into a factor termed VDSI-Sample. The VDSI-Sample factor had 21 unique combinations of Area and Year. One-way ANOVA was performed using VDSI as the response and VDSI-Sample as the factor. Multiple pairwise comparisons were made using Tukey’s family error rate to determine where and when mean VDSI became significantly different from those observed in the years closest to the ban.
5.3 RESULTS

5.3.1 Temporal and spatial trends in imposex

Mean RPSI and VDSI imposex values for *N. lapillus* populations sampled in the Inner, Middle and Outer Harbour Areas at Kinsale between 1987 and 2011 are presented in Table 5.1. In each Harbour Area RPSI values were highest in the immediate years following the introduction of the Ireland-wide partial ban on TBT in 1987 (Figure 5.2). In the Inner, Middle and Outer Harbour Areas maximum RPSI values were 42.6, 11.3 and 15.7 respectively. By 2011 RPSI had decreased to < 1 throughout Kinsale Harbour. Similar temporal trends were observed in VDSI values. In the Inner, Middle and Outer Harbour Areas VDSI decreased from 4.11 to 1.9, 4.0 to 1.7 and 3.6 to 0.2 respectively (Figure 5.3).

Table 5.1: Mean Vas Deferens Sequence Index (VDSI) and Relative Penis Size Index (RPSI) values for *Nucella lapillus* in the Inner, Middle and Outer Harbour Areas at Kinsale Harbour, Cork since the introduction of the Irish partial ban on tributyltin based antifoulants in 1987.

<table>
<thead>
<tr>
<th>Year</th>
<th>RPSI Inner</th>
<th>RPSI Middle</th>
<th>RPSI Outer</th>
<th>VDSI Inner</th>
<th>VDSI Middle</th>
<th>VDSI Outer</th>
</tr>
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<tbody>
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<td>7.38</td>
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<td>ND</td>
</tr>
<tr>
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<td>11.30</td>
<td>15.70</td>
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<td>4.00</td>
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</tr>
<tr>
<td>1992</td>
<td>13.49</td>
<td>3.41</td>
<td>4.29</td>
<td>4.11</td>
<td>3.58</td>
<td>3.32</td>
</tr>
<tr>
<td>1994</td>
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<td>3.70</td>
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<td>2.98</td>
</tr>
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<td>1995</td>
<td>8.50</td>
<td>5.95</td>
<td>8.07</td>
<td>3.71</td>
<td>3.85</td>
<td>3.20</td>
</tr>
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<td>3.53</td>
<td>3.85</td>
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</tr>
<tr>
<td>2003</td>
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<td>1.78</td>
<td>5.88</td>
<td>4.10</td>
<td>2.79</td>
<td>3.04</td>
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<td>2011</td>
<td>0.07</td>
<td>0.05</td>
<td>0.00</td>
<td>1.93</td>
<td>1.71</td>
<td>0.24</td>
</tr>
</tbody>
</table>

ND No Data.

1987 sampling predated the publication of the VDSI by Gibbs et al. (1987).
Figure 5.2: Temporal trends in *Nucella lapillus* Relative Penis Size Index (RPSI) values in the Inner, Middle and Outer Harbour Areas at Kinsale Harbour, Cork since the introduction of the Irish partial ban on tributyltin based antifoulants in 1987.

Figure 5.3: Temporal trends in *Nucella lapillus* Vas Deferens Sequence Index (VDSI) imposex values in the Inner, Middle and Outer Harbour Areas at Kinsale Harbour, Cork since the introduction of the Irish partial ban on tributyltin (TBT) based antifoulants in 1987. Reference line for OSPAR's (2011) EcoQO for TBT induced imposex in *Nucella* (VDSI = 2).
The Kruskal–Wallis test indicated significant differences in median RPSI values across RPSI-Sample levels \( (H = 36.01, \ df = 15, \ P = 0.002) \). Dunn’s pairwise comparisons test showed that RPSI values formed two significantly different groups (Table. 5.2). The first group, Group A, was comprised of samples taken between 1987 and 2003 while the second group, Group B, was exclusively comprised of samples taken in 2011. Between 1987 and 2003 RPSI values in the three Harbour Areas remained relatively high and fluctuated over time before deceasing significantly to < 1 by 2011 (Table 5.2; Figure 5.2a)

**Table 5.2**: Dunn's multiple pairwise comparisons between median RPSI values across Year x Area levels. Median RPSI values in italics. Medians that do not share a letter are significantly different. All pairwise comparisons to 2011 samples were significant at \( P < 0.001 \).

<table>
<thead>
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<tr>
<td>Outer</td>
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<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>B*</td>
</tr>
<tr>
<td></td>
<td>8.21</td>
<td>15.7</td>
<td>2.44</td>
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<td>3.50</td>
<td>4.50</td>
<td>0.00</td>
</tr>
<tr>
<td>Middle</td>
<td>ND</td>
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<td>A</td>
<td>A</td>
<td>B*</td>
</tr>
<tr>
<td></td>
<td>3.46</td>
<td>ND</td>
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<td>6.45</td>
<td>1.78</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>ND</td>
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<td>ND</td>
<td>A</td>
<td>A</td>
<td>B*</td>
</tr>
<tr>
<td></td>
<td>13.5</td>
<td>ND</td>
<td>ND</td>
<td>10.65</td>
<td>ND</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND No Data.
* Sample significantly different from Group A.

VDSI data are often not normally distributed and thus statistical analyses are typically confined to non-parametric techniques (for example see Galante-Oliveira et al., 2010; Guðmundóttir et al., 2011). However, in the current study VDSI data satisfied the assumptions of parametric regression analysis (Zuur et al., 2007). Anderson Darling test (test coefficient = 0.537, \( P = 0.161 \)) showed the standardised residual to be normally distributed while Levene’s test indicated homogeneity of variance (test coefficient = 0.701, \( P = 0.501 \)). The presence of overlying influential points in the explanatory variables was investigated using a plot of Cook's distance against observation number. For
the VDSI model (presented in Table 5.3) no appreciable pattern was observed in the standardised residuals vs. fits plots (Zuur et al., 2007).

Regression analysis showed significant temporal decreases in VDSI in the three Harbour Areas ($R^2 = 78.92\%$; $P = < 0.001$; Table 5.3; Figure 5.3). There was no significant difference in VDSI trends in the Outer and Middle Areas ($P = 0.474$). However, the temporal trend in VDSI in the Inner Area was significantly different to those observed in the other sampling Areas ($P = 0.001$).

Table 5.3: Parameter estimates for quadratic regression model for *Nucella lapillus* VDSI imposex values at Inner, Middle and Outer Harbour Areas at Kinsale, Co Cork since the Irish TBT ban in 1987.

<table>
<thead>
<tr>
<th>Source</th>
<th>Coef</th>
<th>SE Coef</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>3.410</td>
<td>0.255</td>
<td>13.281</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time</td>
<td>0.093</td>
<td>0.044</td>
<td>2.114</td>
<td>0.040</td>
</tr>
<tr>
<td>Time Sq</td>
<td>-0.008</td>
<td>0.002</td>
<td>-4.869</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Area Inner</td>
<td>0.414</td>
<td>0.116</td>
<td>3.571</td>
<td>0.001</td>
</tr>
<tr>
<td>Area Middle</td>
<td>0.081</td>
<td>0.112</td>
<td>0.721</td>
<td>0.474</td>
</tr>
</tbody>
</table>

$s = 0.537$, $R^2 = 78.92\%$, adjusted $R^2 = 77.12\%$, PRESS = 17.108

In all Areas VDSI values persisted at high levels, and even showed short term increases until 2003, after which time VDSI imposex levels decreased (Figure 5.3). One-way ANOVA indicated significant differences in mean VDSI values across VDSI-Sample levels ($R^2 = 79.55\%$; $P < 0.001$; Table 5.4). Tukey’s pairwise comparisons indicated that the levels formed 2 major groups in terms of mean VDSI values. Mean VDSI values for the first group (i.e. levels included in Group A) ranged between 4.11 and 2.79; (Table 5.4b). The second group was exclusively comprised of 2011 samples and had mean VDSI ranging between 1.93 and 0.24 (Table 5.4b). These groupings indicate that imposex at Kinsale did not decrease significantly until 24 Years after TBT controls were implemented. In 2011 mean VDSI levels in all Harbour Areas fell below OSPAR’s EcoQO of < 2 (OSPAR, 2011).
Table 5.4: Differences in mean Vas Deferens Sequence Index (VDSI) across all Year x Area levels.

5.4a: Results of One-way ANOVA of using Sample as a fixed factor.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>19</td>
<td>56.019</td>
<td>2.948</td>
<td>11.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>32</td>
<td>8.246</td>
<td>0.258</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S = 0.508, $R^2 = 87.17\%$, adjusted $R^2 = 79.55\%$

5.4b: Tukey's multiple pairwise comparisons between mean VDSI across Year x Area level (Sample factor). Mean VDSI values in *italics*. Means that do not share a letter are significantly different. All pairwise comparisons to 2011 samples were significant at $P < 0.001$.

<table>
<thead>
<tr>
<th>Years Post Ban</th>
<th>Area</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>15</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer</td>
<td></td>
<td>AB</td>
<td>AB</td>
<td>ABC</td>
<td>AB</td>
<td>ABC</td>
<td>AB</td>
<td>$D^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.55</td>
<td>3.32</td>
<td>3.00</td>
<td>3.20</td>
<td>2.90</td>
<td>3.04</td>
<td>0.24</td>
</tr>
<tr>
<td>Middle</td>
<td></td>
<td>AB</td>
<td>AB</td>
<td>ABC</td>
<td>AB</td>
<td>AB</td>
<td>ABC</td>
<td>$C^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.00</td>
<td>3.58</td>
<td>3.24</td>
<td>3.85</td>
<td>3.80</td>
<td>2.79</td>
<td>1.70</td>
</tr>
<tr>
<td>Inner</td>
<td>ND</td>
<td>A</td>
<td>ABC</td>
<td>AB</td>
<td>AB</td>
<td>A</td>
<td>BCD*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.11</td>
<td>3.70</td>
<td>3.60</td>
<td>3.85</td>
<td>4.10</td>
<td>1.93</td>
<td></td>
</tr>
</tbody>
</table>

ND. No Data.

* Sample significantly different from Group A.
Organotins, and in particular TBT, are regarded as the most hazardous pollutants to have been intentionally introduced into the marine environment (Goldberg, 1986; Svavarsson, 2000; Gipperth, 2009). Today organotins are ubiquitous in the marine environment and their effects have been recorded globally (Sousa et al., 2009). In the last two decades various regional and international restrictions and bans prohibiting the use of TBT based antifoulants have been implemented. Overall declining levels of imposex and TBT contamination in many areas provides evidence for the general effectiveness of TBT legislation (Svavarsson, 2000; Birchenough et al., 2002a,b; Oliveira et al., 2009; Guðmundóttir et al., 2011). For example, along the northeast and southwest coast of England and in the north of Scotland *N. lapillus* has recovered in many areas where previously populations were severely impacted or absent (Evans et al., 1994, 1996, 1999; Birchenough et al., 2002a,b). However, high levels of TBT contamination and imposex have been shown to persist in the vicinity of large harbours (Minchin et al., 1995, 1996; Minchin and Minchin 1997; Galante-Oliveira et al., 2006; Germán-Rodríguez et al., 2009; Sousa et al., 2009; Gibbs, 2009; Guðmundóttir et al., 2011).

In Ireland imposex levels in *N. lapillus* have been monitored intermittently since the introduction of the 1987 national bye-law restricting the use of TBT. In 1987 an island-wide baseline survey conducted by Duggan et al. (1988) indicated high levels of imposex in *N. lapillus* throughout most of its range. Imposex levels were highest in the vicinity of fish aquaculture sites, marinas and ports. A comparative study conducted in 1993 highlighted significant declines in imposex in areas near aquaculture and small boat activity (Minchin et al., 1995). However, in the vicinity of shipping and fishing ports imposex levels remained high and in some cases had increased in the years following the ban (Minchin et al., 1995). Minchin et al. (1996) reported high levels of imposex in *N. lapillus* and localised extinctions in the vicinity of Cork Harbour in 1994. Minchin (2003) carried out a survey of TBT effects on molluscs at 6 bays/estuaries in Ireland in 2000, with particular reference to
the potential effects of TBT contamination on shellfish production and marketability. Moderate levels of TBT contamination were reported in enclosed bays while at sites where there was a greater degree of circulation of oceanic waters contamination levels were lower. It was suggested that levels of TBT contamination at the survey sites were unlikely to impact on the local shellfisheries or aquaculture industries (Minchin, 2003).

To the best of our knowledge this contribution represents the first published account outlining detailed temporal trends in RPSI and VDSI at a single Irish location since the introduction of TBT legislation. In the immediate years following the Irish TBT ban mean RPSI at Kinsale ranged between 42.6 and 7.4 while VDSI ranged between 3.6 and 4.0. These values are comparable to those reported for marinas and ports by Duggan et al (1988) in the 1987 Irish baseline survey (Minchin et al., 1995). Here, results show that imposex has decreased significantly in the 24 years since the introduction of the Irish ban on TBT. Despite this overall significant decrease imposex did not decline linearly over time. In this study, there was a general (but non-significant) increase in mean VDSI over the first 10 years following the ban, followed by a decrease in VDSI for all Areas. Numerous studies have shown similar short-term temporal increases in imposex in areas near harbours and important shipping routes (Ten Hallers-Tjabbes et al., 1994; Svavarsson and Skarphédinsdóttir, 1995; Minchin et al., 1995; Santos et al., 2002; Svavarsson et al., 2001). Santos et al. (2002) described increases in the degree of imposex in N. lapillus populations along the Portuguese coast in the five year period following the introduction of national TBT restrictions in 1993. A review of studies and assessments conducted by OSPAR indicated that imposex levels in populations of Nucella generally exhibit no significant change in the immediate years following implementation of TBT restrictions (OSPAR, 2009).

Persistence of imposex in Nucella populations has been attributed to the longevity of TBT in the environment. The distribution and persistence of TBT in the environment is complex due to different degradation rates of TBT in seawater and in sediments (Evans, 1999b). For instance, TBT degrades in
seawater in a matter of days. However, TBT and its derivatives have high affinities to particulate matter and can accumulate in sediments where degradation rates are slower, particularly in anoxic conditions (Langston and Pope, 1995). It has been suggested that TBT that the half life of TBT in sediments can be in the order of years or even decades (Langston et al., 1994; de Mora et al., 1995; Bately, 1996; Evans, 2000). The regeneration of contaminants from harbour sediments following navigational channel dredging and subsequent disposal of spoil are recognised as important local sinks for TBT contamination (de Mora et al., 1995; Galante-Oliveira et al., 2006; Santos et al., 2002). Santos et al. (2004) demonstrated that the frequency and degree of imposex in gastropod populations was related to distance from a TBT contaminated harbour dredge spoil dump off the Oporto coast, Portugal. At Kinsale the regular dredging of navigational channels and potential subsequent regeneration of buried TBT may account for the different rates of decline of imposex in the Inner Harbour Area when compared to the Middle and Outer Areas. Equally, this source of TBT contamination may explain why significant recovery was generally not observed in *N. lapillus* populations until 24 years after the implementation of TBT controls. Persistence of TBT in harbour areas has been attributed to shipyard activities (e.g. removal of TBT coatings from ship hulls) and the continued release of TBT from old painted hulls (Germán-Rodríguez et al., 2009; Gibbs, 2009). In Ireland high levels of TBT contamination have been reported at ports with dry-docks or boat lifting facilities (Minchin et al., 1996; Minchin and Minchin, 1997).

Many of the studies describing the abatement of imposex in the north east Atlantic are of less than 10 years in duration (e.g. Galante-Oliveira et al., 2010; Guðmundsdóttir et al., 2011). However, it has been suggested TBT in sediments may continue to impact local populations for up to 30 years (Langston et al., 1994). This study, which covered 24 years of sampling, collected sufficient data to perform parametric analyses on imposex data from one embayment. This design is different to many other studies that have examined larger areas at a lower spatial and/or temporal resolution and been confined to non-parametric analyses (e.g. Sousa et al., 2009; Guðmundsdóttir
et al., 2011). The time scale of this study allows VDSI to be used to
determine the long term effectiveness of TBT controls at a single location. In
this study, mean VDSI did not become significantly different from the most
impacted (earliest) samples until 2011. This coincided with mean VDSI
decreasing below 2, the threshold level for the EcoQO (OSPAR, 2011). This
confirms the validity of the VDSI threshold as an indicator that TBT
contamination is significantly different from the polluted state using time
series data from an anthropogenically polluted water body.

5.5 ACKNOWLEDGEMENTS

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Sampier.

5.6 CONTRIBUTIONS

JF collected and analysed 2011 samples. JF and BK collaborated on
statistical analyses. JF was the primary author of this manuscript.
Contributors of pre-2011 data are acknowledged above.
6 DISCUSSION

**Biological indicators are a scientific response to the need for reliable and accurate information to assess the condition of the environment** (van Hoey et al., 2010).

The aim of ecological monitoring is to determine whether or not an anthropogenic disturbance has caused a change to the environment and to estimate the degree of its effects (Green, 1979; McIntyre et al., 1984). Studies investigating change in the structure of macrobenthic faunal communities are an integral part of impact assessments in marine and estuarine waters (Bilyard, 1987; Warwick et al., 1990; Elliot, 1994; McLusky and Elliot, 2004; Quintino et al., 2006; Forde et al., 2012). Biological indices and indicators have been developed to provide synoptic information of the state of the environment by summarising data describing the structure and/or functioning of particular aspects of the ecosystem (e.g. diversity, community structure, environmental conditions) (Salas et al., 2006; Quintino et al., 2006; Heslenfeld and Enserink, 2008; Patrício et al., 2009). One of the major criticisms of indices and indicators is that they give an oversimplified representation of the ecosystem being investigated and that valuable underlying data describing biological responses to disturbance is ignored or lost (Warwick and Clarke, 1991; Elliot 1994; Quintino et al., 2006; Patrício et al., 2009). There is also concern at the uncritical use of indices and indicators for assessing the impact of disturbances, and in areas, for which the tools were not originally designed (Quintino et al., 2006). In such instances, there is a danger that management decisions based on these assessments will be flawed (Quintino et al., 2006; Quintino and Elliot, 2008). Consequently, indices and indicators should be chosen based on the type of disturbance to be assessed and with a knowledge of the ecological basis and limitations of the tool in question and, in particular, the adequacy of the data used to derive the tool (Quintino et al., 2006; Patrício et al., 2009; Krönke and Reiss, 2010; Kennedy et al., 2011). The user must also be aware of the applicability and robustness (or sensitivity) of the tool to assess disturbance(s) under different
sets of environmental conditions (Elliot, 1994; Patrício et al., 2009; Krönke and Reiss, 2010).

The implementation of the European Water Framework Directive (WFD) and European Marine Strategy Directive (MSFD) require the derivation and use of ecological indicators (van Hoey et al., 2010). Benthic biological indices developed for assessing Ecological Status (ES) (sensu the WFD) are largely based on the disturbance model of Pearson and Rosenberg (1978), focussing on the proportion of disturbance-sensitive taxa and indices of diversity e.g. the UK and Ireland Infaunal Quality Index (IQI) (Prior et al., 2004; Mackie, 2009; Borja et al., 2007, 2009) and the Multivariate-AZTI Marine Biotic Index (M-AMBI) (Borja et al., 2007; Muxika et al., 2007a,b). These Ecological Quality Ratios (EQRs) are designed and used to differentiate anthropogenically impacted sites from undisturbed reference sites.

There is a trend among ecological assessments of using indices to detect change in environmental quality irrespective of the location of the impacted site or the nature of disturbance (Quintino et al., 2006). As a result EQRs continue to be intercalibrated across a variety of anthropogenic disturbances both within and between ecoregions and habitats (Borja et al., 2007; van Hoey et al., 2007; Borja et al., 2009; Simboura and Argyrou, 2010). In general, these studies have shown a good level of agreement between different EQRs in coastal waters, however, issues remain with the application of EQRs to estuarine and transitional waters (Simboura and Reizopoulou, 2008; Dauvin, 2007; Elliott and Quintino, 2007), and in tropical and subtropical areas were there are strong seasonal effects between wet and dry periods (Borja and Tunberg, 2011).

These issues are largely due to the fact that EQRs are based on the abundances of stress tolerant species which may also be tolerant to naturally occurring stressors (e.g. salinity regime, organic enrichment) (Dauvin and Ruellet, 2008). Estuaries and transitional waters are by their very nature stressed environments due to fluctuations in salinity and the input of
organics. In addition, these systems are frequently exposed to high levels of anthropogenic disturbances (Dauvin, 2007; Elliot and Dauvin, 2007). Benthic communities in estuarine and transitional waters typically exhibit low levels of diversity and are dominated by species which have evolved to tolerate the environmentally stressful conditions (McLusky and Elliott, 2004, 2007). As a result community structure in natural estuaries is very similar to that of anthropogenically stressed estuaries. This similarity makes it very difficult to identify the effects of anthropogenic disturbance (de Paz et al., 2008). This feature of benthic communities is now commonly referred to as the “estuarine quality paradox” (Quintino and Elliot, 2008). In addition, ES monitoring programs frequently apply a single set of reference conditions are used to compare naturally impoverished biological communities characterising variable and low salinity habitats to communities in higher salinity areas (Kennedy et al., 2011; Forde et al., 2012). In such instances, habitats in high estuaries will always be classified as having a worse ES than those further downstream and in full marine conditions (de Paz et al., 2008). Uncritical application of reference conditions for assessing ES under different environmental conditions may result in misclassification of ES and have major implications for management where misclassifications cross the WFD critical Good/Moderate boundary. Where an index misclassifies an area as moderate (or worse), under the WFD remedial management actions must be put in place, usually at considerable financial cost, to bring the area back to a good (or better) status (Quintino et al., 2006). Given the serious implications for management many authors have highlighted the importance of establishing habitat-specific reference conditions in order to avoid misclassification of ES (Muxika et al., 2007a; Borja et al., 2007; Teixeira et al., 2008; de Paz et al., 2006).

As populations and communities change over time in ways we only dimly understand, Krebs (1989) noted that “every manipulative ecological field experiment must have a contemporaneous control” to reliably test if an impact occurs. In this view, without a true understanding of natural variability it is impossible to reliably discriminate anthropogenic induced change using WFD EQRs. In the majority of cases, the derivation of EQRs, and studies
aimed at intercalibrating EQRs, have relied on data from spatial disturbance gradients within impacted areas (Borja et al., 2007, 2008; van Hoey et al., 2007). Furthermore, ES assessments for the WFD are largely confined to sites where disturbance is expected and so very little data are available which describe natural variability in benthic communities at undisturbed (reference) sites. Consequently, few studies have investigated the effects of natural variability on EQRs. Using a 27-year time-series dataset from a coastal area in the southern North Sea, Krönke and Reiss (2010) showed the responses of diversity indices and EQRs were highly variable over time. The study showed natural variability in communities caused ES at sampling stations to change by as much as three (out of five) ES classification units. In a similar study, Kennedy et al. (2011) used a 23-year time series dataset from the south coast of Ireland to assess the performance EQRs over space and time. Using spatial EQR data Kennedy et al. (2011) showed that a significant spatial relationship between IQI and M-AMBI. However, no significant relationship existed between the EQRs in time series. These studies highlight the confounding effects of natural variability which can result in disparate responses in EQRs. Given that the purpose of WFD EQRs is long-term monitoring of ES there is a need to understand the temporal responses of indices due to natural variation in both disturbed and undisturbed (i.e. near pristine or, ideally, pristine) areas if anthropogenic impacts are to be discriminated reliably (Krönke and Reiss, 2011; Kennedy et al., 2011). However, given the lack of areas in the marine environment that can be referred to as pristine our inability to determine variability at reference sites will be a significant factor limiting the effectiveness of current of WFD EQRs. Given this, scientists must accept our current situation and adapt approaches to assessing impacts accordingly.

Monitoring ES for the WFD should move away from using spatial gradients of disturbance for setting reference conditions against which ES is measured and adopt a more habitat specific hypothesis driven, evidence-based approach to assessing change. The long-established Before-After Control-Impact (BACI) design provides a possible model. The BACI approach has typically been presented as a means for testing if an impact occurs by
comparing baseline data collected both before and after an impact (Green, 1979; McIntyre et al., 1984). Given the lack of pristine control sites, using this model in a strict sense in the marine environment would be difficult. However, a BACI approach can be used in areas where impacts have already occurred (Underwood, 1994; Smith, 2002). For instance, however, BACI designs have been successfully used to determine recovery following the cessation of an impact and thus assess the effectiveness of remedial management measures (e.g. Guerra-García et al., 2003; Skilleter et al., 2006; Vitaliano et al., 2007).

Vitaliano et al (2007) used a BACI design to monitor recovery of benthic communities in inner New York Bight following termination of sewage sludge disposal. In the study, benthic community structure at a severely impacted site was compared to that of communities characterising intermediate and minimally impacted sites. A similar approach could be applied to monitor change in ES under the WFD. In this approach ES at the impacted site (or habitat) being investigated may be compared to the ES of similar habitats experiencing lower levels of disturbance (or ideally no disturbance at all) and so allow relative change in ES to be assessed in a statistically robust manner.

The integrated approach to habitat classification (sensu the HD) and ES assessment (sensu the WFD) presented in Chapter 4 may provide a suitable framework for applying a BACI type approach to assessing change at the regional level. In Chapter 4, the proposed top-down approach to habitat classification identified common habitats (and biotopes) both within, and between, estuarine and marine systems, while ES assessments, based on benthic communities within these habitats, allowed expected ranges of ES to be identified. In the case where common habitats are subjected to varying levels of disturbance (or management measures), change in ES and/or community structure could be quantitatively assessed using asymmetrical BACI designs (i.e. more “control” sites than impact sites) as described by Underwood (1994). In a monitoring context, this approach would allow the confounding effect of natural variability on EQRs and/or community structure in specific habitats to be effectively separated from potential impact effects and allow anthropogenic driven change to be quantified. Furthermore,
applying this approach to datasets previously collected for the HD and WFD may allow temporal and regional spatial variability in community structure and EQRs to be determined. This approach would allow anthropogenic impacts to be identified more reliably and accurately.

The importance of understanding temporal variability in monitoring data is also highlighted in this thesis. In Chapter 5 it was shown that imposex levels in *N. lapillus* did not become significantly different from that of the polluted state until 24 years after the implementation of management measures. This coincided with mean VDSI decreasing below 2, the threshold level for OSPAR’s EcoQO (OSPAR, 2011), and so confirms the validity of the EcoQO VDSI threshold as an indicator of TBT contamination. Consequently, the time-series data used in the study clearly indicates that the EcoQO can be directly transferred with confidence to Irish waters. However, the case study also highlights the need for long-term monitoring data if bio-indicators such as OSPAR EcoQO for *N. lapillus* are to be used for establishing management objectives for legislation. Many MS, including Ireland, have expressed interest in adopting OSPAR’s EcoQOs as MSFD indicators to charter progress toward achieving GEnS (Heslenfeld and Enserink, 2008). However, it is imperative that managers fully understand the time-scales involved for specified objectives to be achieved and be cautious in adopting bio-indicators, such as EcoQOs, as management objectives. This is particularly important if MS run the risk of potential legal action for non-compliance with legislation based on the response of bio-indicators. Where long-term monitoring data exists it may also be useful for setting practical and achievable interim targets to monitor recovery over time. The MSFD makes provisions for the implementation of interim targets to track progress towards GEnS (Long, 2011; OSPAR, 2012). In this view, interim targets based on quantified temporal responses could be used to objectively track change in environmental quality and assess whether additional remedial action is required to allow overall final objectives to be reached within set time-frames. Based on the findings of Chapter 5 it is likely that the long-term imposex monitoring data from Kinsale could be successively used to set interim targets for similar estuaries and harbours where imposex persists above
OSPAR’s EcoQO threshold. However, further research is needed to determine the applicability of such interim targets in different areas. For instance, interim targets based on the temporal trends in imposex at Kinsale may not be suitable for *N. lapillus* populations in areas which have been historically exposed to relatively higher levels of TBT contamination and so may exhibit considerably different temporal responses. Similarly, regional variability in the response of *N. lapillus* may also be a significant factor that should be considered before transferring targets to new geographic regions.

The wealth of existing literature describing the intercalibration of WFD EQRs reflects similar concerns regarding transposing indices to new geographical areas (Borja et al., 2007; van Hoey et al., 2007; Borja et al., 2009; Simboura and Argyrou, 2010). However, developing environmental monitoring techniques that are portable between regions have the benefit of allowing established approaches to monitoring and management to be applied to new geographical areas (e.g. Borja et al., 2008; Borja and Tunberg, 2011; Cai et al., 2012, Wu et al., 2012). In general, intercalibration exercises involve shifting EQR class boundaries to increase the agreement between indices in terms of ES classifications with particular importance attached to agreement between indices measures around the Good/Moderate boundary (i.e. the critical WFD boundary). In the absence of habitat specific reference conditions, as discussed above, this method has been used a means of accounting for variability in the responses of different EQRs (Borja et al., 2007; van Hoey et al., 2007; Borja et al., 2009; Simboura and Argyrou, 2010). Chapter 2 of this thesis presents a novel approach to ES assessment using M-AMBI that facilitates the transfer of WFD type management to a new geographic region at sub-tropical Hong Kong, China. The approach involved using the sensitivities of species within the AMBI list to assign family level taxa to EG. Using an intercalibration approach M-AMBI was shown to be robust to changes in the taxonomic resolution of European benthic data. Given that few genera in the Hong Kong datasets were listed in the AMBI EG database Chapter 2 goes on to use intercalibrated EQR boundaries with family level data as a means of assessing temporal and spatial changes in ES at Hong Kong and surrounding waters. The suitability of family level M-
AMBI for ES assessment was tested on the basis of the approach’s ability to account for previously described seasonal patterns in benthic community structure (e.g. Morton and Wu 1975; Shin and Ellingsen, 2004; Shin et al., 2004; Cheung et al 2008; Fleddum et al., 2011).

In Chapter 2 the effect of using taxonomic level data other than family with M-AMBI at Hong Kong was not determined. As very few genera in the Hong Kong datasets were represented in the AMBI EG database it was impossible to investigate the effects of using genus level data with M-AMBI. The ability of family level M-AMBI to successfully detect changes in ES at Hong Kong coupled with the apparent robustness of M-AMBI ES classification using European genus level data may lead to an incorrect generalisation that higher level data (including genera) is suitable for assessing ES in new regions. Instead, scientists should use hypothesis driven investigations to objectively determine the effects of using genus level data on M-AMBI before applying such an approach to new regions. While few AMBI genera were identified in the Hong Kong dataset, this may not be the case in other geographical regions which are not so far removed in terms of faunal composition from the AMBI EG database. Consequently, it is suggested here that further research be conducted to determine the performance and sensitivity of M-AMBI when taxonomic level data other than family is used. There is a wealth of existing data that may be suitable for this research including the datasets used in the European and international intercalibration studies mentioned above.

This discussion has highlighted current and potential approaches for adapting and modifying existing monitoring techniques to increase their applicability to different areas and impacts. This avenue of research is largely driven by the needs of environmental legislation for management tools. Ideally these management tools should allow comparisons to be made across regions but also provide non-technical representations of complex environmental changes that can be understood by scientists, stakeholders and managers alike. However, there is a significant risk that summarising environmental monitoring data for the benefit of legislation and managers will
result in community level data being largely ignored. Elliot (1994) cautioned that our understanding of the biology of marine benthic communities in respect to environmental impacts is in danger of being hampered by the nature of the data analysis employed. In this view, there is a case that future environmental legislation should move toward a more regionalised approach to management. A regionalised approach would be more amenable to accounting for the confounding effects of natural variation on management tools and so potentially allow changes in environmental quality to be better determined.
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