The use of benthic communities in environmental health assessment

Culhane, F.E.

PhD 2012

The use of benthic communities in environmental health assessment

Fiona Elizabeth Culhane

PhD

2012

Edinburgh Napier University

Edinburgh

Abstract

Quality classification of water bodies commonly hinges upon the results of biotic indices. Biotic indices should reliably detect environmental change caused by anthropogenic stress; distinguish between different levels of disturbance; and be applicable in different areas. This study assesses current methods used in the assessment of benthic ecosystem health in transitional and coastal waters. Specifically, this study considers the performance of macrozoobenthos based biotic and diversity indices. Data utilised in the assessment covered a range of sites and environmental gradients including long term monitoring sites in Scotland; sites impacted by fish farms, organic waste discharge, and chemical effluent; estuarine sites; and sites from Galway Bay, Ireland, one of which was impacted by river discharge.

Currently used indices of environmental status are based mainly on structural ecosystem properties and may not encompass all aspects of ecosystem health, such as functioning. Structural and functional based assessment methods were evaluated by comparing the performance of a range of standard benthic abundance indices and approaches focussing on intrinsic biological characteristics.

Indices did not perform consistently in response to different types of impact – organic, chemical and physical, indicating some indices are unsuitable for the detection of multiple stressors. Index quality classifications agreed best in the most impacted sites but performed unpredictably in moderate conditions. Variability of indices increased as disturbance increased, decreasing the statistical certainty and confidence in the index values. Structural indices were found to be more variable than functional indices but the sensitivity of functional indices to anthropogenic disturbance needs further testing to determine whether they are able to detect low level disturbance. Functional indices may not be advantageous in regular monitoring over traditional methods but may provide a more informative assessment of ecosystem health. Use of biological traits may also give an indication of the type or cause of disturbance.

Classification of moderate-good conditions using benthic indices is particularly ambiguous and distinguishing natural from anthropogenic disturbance remains one of the biggest challenges. The results indicate that complementarity of approaches is important in the assessment of quality of coastal and transitional benthic aquatic systems.

Acknowledgements

I would like to express my sincere gratitude to my supervisors Professor Teresa Fernandes, Dr Rob Briers and Dr Paul Tett for giving me the opportunity to carry out a PhD and for giving me the freedom, encouragement, guidance and support to pursue my ideas. I am also grateful for the numerous opportunities I was given to be involved in activities which benefited my academic and personal development such as attending meetings and conferences, and teaching. I would like to thank Professor Mike Elliott who also provided helpful guidance and advice, positive encouragement, and the opportunity to work at IECS for a time.

I am very grateful to the Scottish Environment Protection Agency (SEPA), especially Myles O'Reilly and Lee Heaney, for meeting with me; helping me to obtain numerous and varied datasets; and for allowing me to partake in a benthic sampling trip. I would also like to thank Martin Solan for providing me with a comprehensive dataset.

Thanks to my colleagues and friends at Napier especially Callum and Ana. Ana helped me with Matlab and both always provided personal support and helpful discussions.

I could not have reached this point without the support of some great people. I extend particular thanks to my parents, Maura and Maurice, who have always supported me, encouraged me and put up with me. In addition my family and my friends in Edinburgh and Ireland who have been kind and believed in me, especially Jen, Emily, Laura, Fiona, Susana, Susan and Aideen. They have helped me to always remember the lighter side of life and given me endless moral support over the last few years.

This project was supported by funding from the EU Framework Programme 6 project SPICOSA.

Table of Contents

Abstract i
Acknowledgements iii
Contents iv
List of tables viii
List of figures xi
Acronyms xvi
1 Introduction 1
1.1 Assessment of ecosystem health 1
1.1.1 Ecosystem health in policy 2
1.1.2 What to measure 6
1.1.3 Response of structure and function to stress 11
1.2 Application of indices and assessment approaches 13
1.3 Rationale 17
1.4 Aims 17
2 The performance of benthic indices in long term monitoring sites 19
2.1 Introduction 19
2.1.1 Indices 20
2.1.1 Study sites 28
2.2 Spatial and temporal trends 30
2.2.1 Introduction 30
2.2.2 Methods 34
2.2.3 Results 34
2.2.4 Discussion 42
2.2.5 Conclusion 49
2.3 Index correlations 51
2.3.1 Introduction 51
2.3.2 Methods 54
2.3.3 Results 56
2.3.4 Discussion 59
2.3.5 Conclusion 62
2.4 Sampling effects 63
2.4.1 Introduction 63
2.4.2 Methods 66
2.4.3 Results 66

2.4.4Discussion ... 73
2.4.5 Conclusion ... 75
2.5 General conclusions ... 76
3 Index response to pressure ... 77
3.1 Introduction ... 77
3.2 Methods ... 80
3.2.1 Study sites ... 80
3.2.1.1Barcaldine ... 80
3.2.1.2 Ironrotter Point ... 83
3.2.1.3 Irvine Bay ... 85
3.2.1.4 Fish Farms ... 88
3.2.1.5 Upper Clyde Estuary ... 89
3.2.2 Statistical analysis ... 91
3.3 Results ... 92
3.3.1 Study site descriptions ... 92
3.3.1.1 Barcaldine ... 92
3.3.1.2 Ironrotter ... 98
3.3.1.3Irvine Bay ... 104
3.3.1.4 Fish Farms ... 117
3.3.1.5 Clyde Upper Estuary ... 123
3.3.2 Index correlations with disturbance data ... 130
3.4 Discussion ... 135
3.4.1 Study sites ... 135
3.4.1.1 Barcaldine ... 135
3.4.1.2 Ironrotter ... 137
3.4.1.3Irvine Bay ... 140
3.4.1.4 Fish Farms ... 142
3.4.1.5 Clyde Upper Estuary ... 144
3.4.2 Correlations ... 145
3.5 Conclusions ... 146
4 Comparison of structural and functional approaches ... 150
4.1 Introduction ... 150
4.2 Methods ... 155
4.2.1 Study sites ... 155
4.2.2 Statistical analysis ... 158

4.2.2.1 Structural methods 158
4.2.2.2 Biological traits analysis 158
4.2.2.3 Analysis of trait data 166
4.2.2.3.1 Indices 166
4.2.2.3.2 Rao's Entropy 167
4.2.2.3.3 Comparison of indices.............................. 168
4.2.2.3.4 RLQ analysis.. 168
4.3 Results 169
4.3.1 Spatial and temporal variation 169
4.3.2 Functional diversity.. 179
4.3.3 Rao's Entropy 186
4.3.4 Relationship between structural and functional indices.......... 195
4.3.5 Co-inertia analyses 198
4.3.5.1 Species correspondence analysis............................. 198
4.3.5.2 Traits: fuzzy correspondence analysis .... .200
4.3.5.3 Environmental variables: PCA................................ 202
4.3.5.4 Co-inertia analysis.. 204
4.3.5.4.1 Species versus traits 204
4.3.5.4.2 Species versus environmental variables.... 207
4.3.5.5 RLQ analysis.. 210
4.4 Discussion 215
4.4.1 Structural methods 215
4.4.2 Biological traits 217
4.4.3 Rao's Entropy 218
4.4.4 Comparison of structural and functional indices 220
4.4.5 RLQ analysis... 221
4.5 Conclusion 225
5 Indices, variability and uncertainty in quality classification 229
5.1 Introduction... 229
5.2 Methods... 232
5.3 Results 233
5.3.1 NMMP 233
5.3.2 Pressure data.. 240
5.3.2.1 Fish Farms... 240
5.3.2.2 Ironrotter 242

5.3.2.3 Irvine Bay 246
5.3.2.4 Clyde Upper Estuary 248
5.3.3 Galway Bay 250
5.3.3.1 Abundance .... .250
5.3.3.2 Biomass 257
5.3.4 Impact on sampling regime 260
5.4 Discussion 263
5.4.1 NMMP sites 263
5.4.2 Pressure data 264
5.4.3 Galway Bay 266
5.4.4 Impact on sampling regime 267
5.5 Conclusion 267
6 Discussion 269
6.1 Introduction 269
6.2 Summary of index performance 270
6.3 Application of indices .... .277
6.4 Recommendations 287
7 References 289
8 Appendix 303
Appendix 8.1 BQI 303
Appendix 8.2 Ironrotter sample point locations 319
Appendix 8.3 Sampling dates Clyde Upper Estuary 320
Appendix 8.4 Reference list for biological traits database 321
Appendix 8.5 Creating trait datasets 329
Appendix 8.6 Index results all sites ....CD
Appendix 8.7 Trait database ....CD

List of Tables

Table 2.1 Assigned quality classifications according to index values for some commonly used indices 27

Table 2.2 Details of datasets from long term monitoring stations obtained from SEPA28

Table 2.3 Habitat characteristics of each site 35

Table 2.4 Quality classifications based on average index values across years as determined by five benthic indices at different sites 38

Table 2.5 Pearson product moment correlations between index values and year at different sites 39

Table 2.6 Pearson product moment correlations between all indices and environmental variables 42

Table 2.7 Theoretical groups of associated indices based on underlying focus (adapted from Ruellet & Dauvin, 2007) 55

Table 2.8 Details of data used for index correlation 56

Table 2.9 Pearson product moment correlations between all indices 57

Table 2.10 Date of sampling expedition for each site 66

Table 2.11 Differences between indices calculated with data captured using 500µm and 1000µm sieves from two sites (KH, KC) 70

Table 2.12 Differences between indices calculated with abundance and biomass data from two sites (KH, KC) 72

Table 2.13 A selection of indices showing the effects of sampling method and data type on quality classification 73

Table 3.1 Sampling and impact type details of SEPA datasets used in analysis 80

Table 3.2 Details of sample points and transects at Barcaldine 82

Table 3.3 Details of sample points at Ironrotter Point 84

Table 3.4 Sample details for Irvine Bay and Ayr Bay 87

Table 3.5 Details of fish farm sampling points 88

Table 3.6 Details of upper Clyde estuary sampling points 91

Table3.7 Quality classification based on average index value at each sample point in each year for Barcaldine according to five indices 95

Table 3.8 Correlation between indices and environmental variables at Barcaldine 97

Table 3.9 Quality classification based on average index value at each sample point in each year for Ironrotter Point according to five indices 101

Table 3.10 Correlation between indices and environmental

variables at Ironrotter Point 103

Table 3.11 Quality classification based on average index value at each sample point in each year for Irvine Bay and Ayr Bay according to five indices 112

Table 3.12 Correlation between indices and environmental variables at Irvine and Ayr Bay 114

Table 3.13 Correlation between indices and environmental variables along the Nobel transect 115

Table 3.14 Correlation between indices and environmental variables along the Garnock Valley Sewer transect 116

Table 3.15 Quality classification based on average index value at each sample point for different fish farms according to five indices 121

Table 3.16 Correlation between indices and environmental variables at fish farms 123

Table 3.17 Quality classification based on average index value at each sample point for upper Clyde estuary according to five indices 127

Table 3.18 Correlation between indices and environmental variables at upper Clyde estuary 129

Table 3.19 Pearson product moment correlations between all indices calculated for Barcaldine 131

Table 3.20 Pearson product moment correlations between all indices calculated for Ironrotter 131

Table 3.21 Pearson product moment correlations between all indices calculated for Irvine and Ayr Bay 132

Table 3.22 Pearson product moment correlations between all indices calculated for Nobel transect at Irvine Bay 132

Table 3.23 Pearson product moment correlations between all indices calculated for GVS transect at Irvine Bay 133

Table 3.24 Pearson product moment correlations between all indices calculated for fish farms 133

Table 3.25 Pearson product moment correlations between all indices calculated for upper Clyde estuary 134

Table 4.1 Environmental variables measured at Leverets and Margaretta stations in Galway Bay during the PhD study (Solan, 2000) 157

Table 4.2 Functions of Margaretta and Leverets ecosystems with effect traits (adapted from Bremner et al 2006a and Valiela, 1995) 160

Table 4.3 Factors at Leverets potentially affecting function with response traits 161

Table 4.4 Biological effect and response traits representing functioning at Margaretta and Leverets in Galway Bay 164

Table 4.5 Quality classification, based on average index value from each sample month for Galway Bay according to the five indices 172

Table 4.6 Relationship between sites and between months at each site according to different indices as found using raw abundance data 173

Table 4.7 Pearson product moment correlations between all indices calculated for Galway Bay based on raw species abundance data 174

Table 4.8 Pearson product moment correlations between all indices calculated for Margaretta in Galway Bay based on raw species abundance data............................... 175

Table 4.9 Pearson product moment correlations between all indices calculated for Leverets in Galway Bay based on raw species abundance data................................... 176

Table 4.10 Correlation between indices and water column properties at Galway Bay...... 177

Table 4.11 Correlation between indices and sediment properties at Galway Bay 178

Table 4.12 Pearson product moment correlations between Rao's Entropy traits and functional indices calculated for two sites in Galway Bay based on raw species abundance data 190

Table 4.13 Pearson product moment correlations between Rao's Entropy traits and functional indices calculated for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data 191

Table 4.14 Correlation between functional indices and water column properties at Galway Bay... 193

Table 4.15 Correlation between functional indices and sediment properties at Galway Bay 194

Table 4.16 Pearson product moment correlations between functional indices calculated for two sites in Galway Bay based on raw species abundance data............................. 196

Table 4.17 Pearson product moment correlations between functional indices calculated for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data 197

Table 4.18 Pearson product moment correlations between environmental variables and RLQ axes for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data 213

Table 4.19 Pearson product moment correlations between traits and RLQ axes for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data 214

Table 4.20 Pearson product moment correlations between species (top ten strongest correlations) and RLQ axes for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data 215

Table 5.1 Number of samples required to achieve a given level of error from the mean (L) for different sites when using IQI 260

List of Figures

Figure 1.1 Structural indicator of ecosystem health in response to pressure (Tett et al., 2007) 11

Figure 1.2 Changes in fauna and sediment structure along a gradient of organic enrichment (Pearson and Rosenberg, 1976, 1978)........ 13

Figure 2.1 Location of sample sites around Scotland 29

Figure 2.2 Multidimensional scaling of species abundances between sites and years. 37

Figure 2.3 Mean median phi values with standard deviation over time at each site...... 40

Figure 2.4 Mean silt/clay fraction with standard deviation over time at each site......... 40

Figure 2.5 Mean organic carbon with standard deviation over time at each site........... 41

Figure 2.6 Principal component analysis of all index results........ 58

Figure 2.7 Comparison of the proportional abundance of taxonomic groups of the macrobenthic community composition in KH as captured using 500µm and 1000µm sieves........ 67

Figure 2.8 Comparison of the proportional biomass of taxonomic groups of the macrobenthic community composition in KH as captured using 500µm and 1000µm sieves........ 68

Figure 2.9 Comparison of the proportional abundance of taxonomic groups of the macrobenthic community composition in KC as captured using 500µm and 1000µm sieves........ 68

Figure 2.10 Comparison of the proportional biomass of taxonomic groups of the macrobenthic community composition in KC as captured using 500µm and 1000µm sieves........ 69

Figure 3.1 Location of sample points and transects from Barcaldine alginate processing factory outlet in Loch Creran........ 81

Figure 3.2 Location of transects from the sea outfall at Ironrotter Point........ 83

Figure 3.3 Location of sample transects and discharges in Irvine Bay........ 85

Figure 3.4 Location of sampling transect and discharges in Ayr Bay........ 86

Figure 3.5 Location of sample points along the upper Clyde estuary........ 90

Figure 3.6 MDS plot of samples according to (a) year, (b) transect and (c) distance from outfall at Barcaldine........ 93

Figure 3.7 MDS plot of samples at Barcaldine according to the level of agreement in quality classification between five indices........ 96

Figure 3.8 MDS plot of samples at Ironrotter Point according to (a) year, (b) transect and (c) distance from outfall........ 99

Figure 3.9 MDS plot of samples at Ironrotter Point according to organic matter content in sediment........ 100

Figure 3.10 MDS plot of samples according to the level of agreement between indices at Ironrotter Point 102

Figure 3.11 Organic matter content derived by loss on ignition at Ironrotter Point stations for three years 104

Figure 3.12 MDS plot of samples according to (a) year, (b) transect and (c) type of impact at Irvine and Ayr Bay 105

Figure 3.13 MDS plot of samples sample according to (a) distance, (b) depth and (c) location at Irvine and Ayr Bay 106

Figure 3.14 MDS plot of samples from ICI Nobel Explosives transect in Irvine Bay according to (a) year, (b) location, (c) distance from outfall and (d) depth 108

Figure 3.15 MDS plot of samples from Garnock Valley Sewer transect in Irvine Bay according to (a) year, (b) location, (c) distance from outfall and (d) depth 110

Figure 3.16 MDS plot of samples at Irvine and Ayr Bay according to level of agreement between the index classifications 113

Figure 3.17 MDS plot of samples at fish farm sites according to (a) site of farm, (b) location of sample and (c) use of antifoulant on fish cages 118

Figure 3.18 MDS plot of samples at fish farm sites according to (a) depth, (b) maximum consented tonnes and (c) tonnes of fish at time of survey 119

Figure 3.19 MDS plot of samples at fish farms according to level of agreement between the index classifications: (a) all indices; (b) excluding BQI 122

Figure 3.20 MDS plot of samples at upper Clyde estuary according to distance 124

Figure 3.21 MDS plot of samples at upper Clyde estuary from 2m seawards according to (a) distance downstream, (b) year and (c) month 125

Figure 3.22 MDS plot of samples at upper Clyde estuary from 2m seawards according to (a) top salinity and (b) bottom salinity 126

Figure 3.23 MDS plot of samples at upper Clyde estuary according to level of agreement between the index classifications 128

Figure 4.1 MDS plot of differences between sites (a) and months (b) at Galway Bay based on raw species abundance data 170

Figure 4.2 MDS plot of differences between sites (a) and months (b) at Galway Bay based on biomass data (transformed $\log_{10}$ x+1) 171

Figure 4.3 Functional diversity at two sites in Galway Bay 180

Figure 4.4 Functional diversity in each month at two sites in Galway Bay 181

Figure 4.5 MDS plot of samples based on number of trait modalities for each (a) site and (b) month 182

Figure 4.6 MDS plot of samples based expression of trait modalities by species for each (a) site and (b) month 183

Figure 4.7 MDS plot of samples based expression of trait modalities by species abundance for each (a) site and (b) month 184

Figure 4.8 MDS plot of samples based expression of trait modalities by species biomass for each (a) site and (b) month 185

Figure 4.9 Rao's Entropy calculated using abundance data of different biological traits for two sites in Galway Bay 187

Figure 4.10 Average Rao's Entropy calculated using biomass data (transformed $\log_{10}$ x+1) of different biological traits for two sites in Galway Bay 187

Figure 4.11 Average Rao's Entropy calculated using abundance data for two sites in Galway Bay 188

Figure 4.12 Average Rao's Entropy calculated using biomass data (transformed $\log_{10}$ x+1) for two sites in Galway Bay 188

Figure 4.13 Percentage difference in Rao's Entropy between the month January and March for individual traits calculated using biomass data (transformed $\log_{10}$ x+1) for Leverets 189

Figure 4.14 Biplot of species and sample correspondence analysis (species data used was biomass and transformed ($\log_{10}$ x+1)) 198

Figure 4.15 Plots of correspondence analysis of (a) samples and (b) species 199

Figure 4.16 Biplot of species and traits fuzzy correspondence analysis (species data used was biomass and transformed) 200

Figure 4.17 Plots of fuzzy correspondence analysis of (a) species and (b) traits 201

Figure 4.18 Biplot of environmental variables and samples principal components analysis 202

Figure 4.19 Plots of principal components analysis of (a) samples and (b) environmental variables 203

Figure 4.20 Co-inertia of species and traits 205

Figure 4.21 Plots of co-inertia analysis of (a) samples and (b) traits 206

Figure 4.22 Co-inertia of species and environmental variables 208

Figure 4.23 Plots of co-inertia analysis of (a) species and (b) environmental variables 209

Figure 4.24 RLQ analysis of species (L), functional traits (Q) and environmental variables (R) 211

Figure 4.25 Plots of RLQ analysis of (a) sites, (b) species, (c) environmental variables (R), and (d) functional traits (Q) 212

Figure 5.1 Mean coefficient of variation between replicates across years and sites for each index 234

Figure 5.2 Coefficient of variation between replicates across years at CMT5 (a) and CMT7 (b) for each index 235

Figure 5.3 Coefficient of variation between replicates across years at IBH (a) and LIS (b) for each index 236

Figure 5.4 Coefficient of variation between replicates across years at KH (a) and KC (b) for each index..237

Figure 5.5 Coefficient of variation between replicates across years at RA for each index……………………………………………………………………….238

Figure 5.6 Coefficient of variation between all replicates in all years across NMMP sites for each index……………………………………………………………….…239

Figure 5.7 Coefficient of variation between replicates across 15 fish farm sites at each location…………………………………………………………………………...241

Figure 5.8 Coefficient of variation between replicates across locations for each year of sampling at Ironrotter Point…………………………………………………...…243

Figure 5.9 Coefficient of variation between replicates across the 100m stations for each year of sampling at Ironrotter Point……………………………………………...…244

Figure 5.10 Coefficient of variation between replicates across years (without 1989) for each distance from the outfall at Ironrotter Point……………………………….……..245

Figure 5.11 Coefficient of variation between replicates across years and transects for each type of impact at Irvine and Ayr Bays…………………………………….……..247

Figure 5.12 Coefficient of variation between replicates across years for each distance along the Clyde Upper Estuary for each index……………………………………..….249

Figure 5.13 Coefficient of variation between replicates across months for two sites at Galway Bay for structural indices calculated using abundance……………………….250

Figure 5.14 Coefficient of variation between replicates across months for two sites at Galway Bay for functional indices calculated using abundance…………………….....251

Figure 5.15 Coefficient of variation between replicates across sites at Galway Bay for two types of index calculated using abundance…………………………………….…252

Figure 5.16 Coefficient of variation between replicates across months for two sites at Galway Bay for selected structural and functional indices calculated using abundance………………………………………………………………………..252

Figure 5.17 Coefficient of variation between all replicates in all months across sites at Galway Bay for each structural index……………………………………………....253

Figure 5.18 Coefficient of variation between all replicates in all months across sites at Galway Bay for each functional index……………………………………………..254

Figure 5.19 Coefficient of variation between all replicates in all months and across sites at Galway Bay for each type of index calculated using abundance data……………..255

Figure 5.20 Coefficient of variation between all replicates in all months across structural indices for each site at Galway Bay……………………………………..256

Figure 5.21 Coefficient of variation between all replicates in all months across functional indices for each site at Galway Bay……………………………………….256

Figure 5.22 Coefficient of variation between replicates across months for two sites at Galway Bay for each index calculated using biomass………………………………..257

Figure 5.23 Coefficient of variation between replicates across months for two sites at Galway Bay for each index calculated using biomass………………………………..258

Figure 5.24 Coefficient of variation between replicates across months for two sites at Galway Bay for selected structural and functional indices calculated using biomass...259

Figure 5.25 Coefficient of variation between replicates across sites at Galway Bay for two types of index calculated using biomass……………………………………….259

Figure 5.26 Mean IQI values for each year at NMMP site CMT7 with 95 % confidence interval…………………………………………………………………………...261

Figure 5.27 Coefficient of variation according to IQI for NMMP sites with standard deviation... 262

Acronyms of Abbreviated Indices

1-Lambda': Simpson's Index

A/B: Abundance/Biomass

A/S: Abundance/Species Richness

AMBI: Azti Marine Biotic Index

BOPA: Benthic Opportunistic Polychaete to Amphipod ratio

BQI: Benthic Quality Index

d: Margalef's Diversity Index

Delta, Δ: Taxonomic Diversity

Delta*, Δ*: Taxonomic Distinctness

Delta+, Δ^+: Average Taxonomic Distinctness

EQR: Ecological Quality Ratio

ES (50): Rarefaction, the number of species expected in 50 individuals

H' (loge): The Shannon-Wiener index with natural log base

Hln: The Shannon-Wiener index with natural log base

Hln count a: Shannon-Wiener index, calculated with traits*species richness dataset

Hln sum a: The Shannon-Wiener index calculated with traits*species richness dataset

Hln traits: The Shannon-Wiener index calculated with number of traits

IQI: Infaunal Quality Index

ITI: Infaunal Trophic Index

J': Pielou's Index of Evenness

Lambda+, Λ^+: Variation in Taxonomic Distinctness

MAMBI: Multivariate AMBI

N: Abundance

N1: Hill's diversity

N1 count a: Hill's diversity calculated with traits*species richness dataset

N1 sum a: Hill's diversity calculated with traits*abundance dataset

N1 traits: Hill's diversity calculated with number of traits

Rao's Entropy Trait Abbreviations

- **A:** Degree of attachment
- **B:** Bioturbator/reworking mode

Bd: Burrow depth

Bo: Body type/design

E: Exposure potential

F: Fragility

Fl: Flexibility

Fm: Feeding/resource capture method

Ft: Food type

H: Living habit

L: Lifespan

Ma: Age at maturity

Mv: Movement method

P: Propagule dispersal

R: Reproductive method

Si: Maximum size

Sl: Salinity

So: Sociability

T: Tolerance

S: Species richness

sDelta+, S. Δ^+: Total Taxonomic Distinctness

Chapter 1

Introduction

1.1 Assessment of ecosystem health

Ecosystem health encompasses the structure and functioning of the ecosystem, the ability of the ecosystem to provide ecosystem services and sustain economic activity while maintaining ecological integrity (Rapport et al., 1998). The components of health can be divided into system organisation, resilience, vigour and an absence of distress. These components span the *function* (or vigour) of the ecosystem in terms of activity, metabolism and primary productivity; and the *structure* (or organisation) of the ecosystem in terms of diversity and ecosystem component interactions. In addition, the flexibility and recovery of the ecosystem in the face of stress is the measure of resilience and how well structure and function is maintained in response to stress is the resistance. Structure is the organisation and properties of the ecosystem, for example biodiversity, food webs and biophysical structure (Tett et al., 2007). The functioning of the ecosystem encompasses the processes which occur, for example sediment re-working provided by burrowing macrofauna or cycling of material by microorganisms (Tett et al., 2007).

1.1.1 Ecosystem health in policy

Ecosystem health therefore incorporates ecological, social and economic issues through the relationships between anthropogenically induced ecosystem stresses, resultant modifications in structure and function, increased or decreased capacity of the ecosystem to provide services and the consequential societal response (Rapport et al., 1998). The assessment of ecosystem health can take many forms and encompass an assessment of a wide range of areas and issues, nevertheless, monitoring, carried out to ensure that formulated standards are being maintained, is usually focussed on environmental parameters and species, with economics and social consideration normally used to generate and specify the background of any strategies adopted. Despite the vast body of work done in biomonitoring there continues to be a constant stream of new research and new techniques put forward (e.g. Rosenberg et al., 2004; Bremner et al., 2006b; Dauvin and Ruellet, 2007; Wan Hussin et al., 2012). Drivers for these continuous developments are environmental policies which require comparative work to be carried out across geographical ranges, such as the EC Water Framework Directive (WFD) (EC, 2000) in Europe (Statzner et al., 2001), as well as an emphasis now being placed on functional aspects of ecosystems rather than structure alone, reinforced in the marine environment by the EC Marine Strategy Framework Directive (MSFD) (EC, 2008).

The WFD requires ‘good ecological status’(at least) is established and maintained in water bodies where ‘ecological status’ is expressed as structure and functioning of the ecosystem (EC, 2000). The MSFD requires ‘good environmental status’ for which ecosystems should be allowed to *‘function fully and to maintain their resilience to human-induced environmental change’* (EC, 2008). In addition there is also the requirement of ‘good chemical status’ to be attained under the WFD and ‘favourable conservation status’ under the Habitats Directive (EC, 1992), for concerned habitats. In aquatic habitats, where these pieces of legislation would apply, it is proposed that boundaries or thresholds for Good Environmental Status should coincide with the thresholds for “favourable conservation status” of the Habitats Directive and “good ecological status” and “good chemical status” of the WFD. Current legislatory instruments, such as these, as well as others such as – the UK (UK Marine and Coastal Access Act 2009) and Scottish (Marine (Scotland) Act 2010) Marine Bills, UK Safeguarding Our Seas Strategy (DEFRA, 2002) and UK Marine Monitoring and Assessment Strategy (UKMMAS, 2007), emphasise the 'ecosystem approach' to management (MRAG and UNEP-WCMC, 2007). This approach aims to integrate management of human activities, achieve sustainability and protect ecosystem function and structure and so, the overall health of the system. The recent focus on assessment of ecosystem health (Diaz et al., 2004, Cognetti and Maltagliati, 2008, Raffaelli and Frid, 2010) is therefore primarily policy driven with recently funded EU projects such as SPICOSA (Science and Policy Integration in Coastal System Assessment; www.spicosa.eu) or ELME (European Lifestyles and Marine Ecosystems; www.elme-eu.org) aiming to apply scientific knowledge in order to achieve integrated management. These policies come in response to the increasing realisation of mounting pressures on coastal areas and other ecosystems, the unsustainable nature of many activities and the inability to deal with such pressures (GESAMP, 2001).

Guidance for a common management framework for the implementation of the WFD recommends the use of the DPSIR framework (IMPRESS, 2002). DPSIR stands for Drivers, Pressure, State, Impact and Response. This approach is complementary to the concepts of ecosystem health as the *Drivers* include human activities (as well as natural changes) which exert stress or pressure on the ecosystem. The ecosystem status is described by *State*, although the procedure is not specified. The *Impact* describes a change in state due to pressure and the *Response* is the response of society to the changes. This approach can be used to select indicators and objectives of marine management in a structured way (Rogers and Greenaway, 2005). "*An environmental indicator is a qualitative or quantitative parameter characterising the current condition of an element of the environment or its change over time*" (Aubry and Elliott, 2006). Indicators exist to simplify characterisation of overall ecosystem state by using a small number of selected components of the system; they allow the quantification of the quality of the ecosystem as compared to reference conditions or established thresholds; and are used to communicate information to policy makers and stakeholders (Aubry and Elliott, 2006). To distinguish terminology, indicators is taken here as a broad term which encompasses the components of the ecosystem which can be measured or assessed and which are used to give a sign of the overall state of the ecosystem including, biological components e.g. particular species, overall species richness, abundance of species and percentage cover of species; and physico-chemical components e.g. dissolved oxygen, sediment grain size, temperature, pH and nutrient levels. Indicators could also include Ecological Quality Objectives (EcoQOs) – the desired level of an ecological quality which may be set in relation to a reference level (OSPAR, 2009). EcoQOs were developed as a set of goals to achieve for the state of the health of the North Sea, applying the ecosystem approach. Other indicators include Environmental Quality Standards which are the values for water quality, quantity and habitat structure, which will ensure the right environmental conditions are created to achieve the objectives (EA, 2011). Specifically, water quality standards specify the quantity of a pollutant that can safely be present in the water environment without causing harm to the ecology. Indicators may also include *biotic indices* which are the main focus of this study. Biotic indices incorporate different ecosystem component measurements into an index, integrating the response of components to changes in the environment (Karr, 1999). The value of the index indicates a quality according to given standards or thresholds or relative to other sites where the index has been applied. The components which are incorporated often include measurements of species richness and abundance. Indices are used to simplify a large amount of complicated data into one comprehensible number. Indices can assimilate several aspects of ecosystems such as changes in species diversity e.g. the Shannon-Wiener Index, H’ (Magurran, 2004); changes in trophic composition reflecting the impact of anthropogenic disturbance e.g. the Infaunal Trophic Index (ITI) (Word, 1979); or other attributes and combinations of attributes. While many are routinely used in monitoring, indices have been under much scrutiny recently due to their emphasis and use in management and policy making (Pinto et al., 2009).

Recent emphasis on the ecosystem approach corresponds with the view that it may be more useful for managers to have a less detailed, wider view of the system rather than a reduced but detailed approach focussing on particular components of the system (Elliott, 2002). Achievement of this approach may only be through the management of human activities which have an impact on components of ecosystems (Rogers and Greenaway, 2005). Therefore it is important to continue to develop and improve measurements of ecosystem components and ensure that these measurements are representative of the state of ecosystem health. Early warning signals are easier to detect at the species level, while stress detected at the ecosystem level may already indicate a drastic shift and a collapse in equilibrium of the system (Odum, 1985). This suggests that while it is important to take an ecosystem approach, it is still important to measure components of the ecosystem as these components will indicate early changes and the need for further investigation. Structure and function of ecosystems are intrinsic to the 'ecosystem approach' and indicators should reflect this (MRAG and UNEP-WCMC, 2007).

A number of challenges exist in the implementation of the ecosystem approach (Box 1.1). Crucial to many of these challenges is the identification of appropriate and reliable indicators which can assess structure and function of ecosystems, and which can be related and linked to human activities. Indicators are needed to ensure development of marine environments is sustainable by measuring the extent of impacts of human activities on components of the ecosystem, thus allowing appropriate management of human activities and maintaining ecosystem health (Rogers and Greenaway, 2005). Realistically, the approach to management is likely to be a compromise between the legal requirements, social considerations and best scientific practice (Birk et al., 2012).

Box 1.1 Challenges of applying the ecosystem approach to marine monitoring

- Application to different spatial scales
- Defining ecosystems and management areas
- Providing information on ecosystem health, resilience or good environmental status
- Identifying early warning signs for future trends
- Linking marine monitoring to management objectives
- Understanding societal impacts on the environment
- Producing integrated ecosystem assessments
- Achieving a practical monitoring and assessment system which also answers reporting obligations

From (MRAG and UNEP-WCMC, 2007)

The benthic system is widely used in marine monitoring and assessment of ecological quality (Quintino et al., 2006, Rosenberg et al., 2004, Reiss and Kröncke, 2005). The benthos consists of the flora and fauna which live on or in the seabed. Many components of the benthos have been used as indicators, including fish (e.g. Estuarine Biotic Integrity Index in the USA; Diaz et al., 2004) and macroinvertebrates (e.g. AZTI Marine Biotic Index, AMBI; Borja et al., 2000). Less commonly, macroalgae (e.g. Ecofunctional Quality Index in Italy; Diaz et al., 2004); seagrass (Corbett et al., 2005); microphytobenthos and meiobenthos (Vassallo et al., 2006); and bacteria (Milbrandt, 2005) have been used, or suggested, as potentially useful groups to use in the development of indicators. In addition, the physical and chemical characteristics of the benthic habitat are used as indicators, such as total organic carbon content (Hyland et al., 2005). Quality status assessment is generally relative to reference conditions (or thresholds/standards) which are spatial or historical, and is generally quantified through univariate measurements such as species abundance or richness (Quintino et al., 2006); or multivariate statistical approaches which distinguish patterns in species composition, sometimes in relation to physico-chemical variables e.g. multi-dimensional scaling (MDS) (Clarke and Gorley, 2001). Biotic indices, as described above, are increasingly being used in quality status assessments and management. These include the Ecological Quality Ratio (EQR) (Borja et al., 2007), AMBI (Borja et al., 2000) and the Benthic Quality Index, BQI (Rosenberg et al., 2004). These indices summarise multivariate data into an easily understood score of quality (Diaz et al., 2004). Several of these indices are explained in detail in Chapter 2.

1.1.2 What to measure?

The WFD describes the ecological status as '*the structure and functioning of aquatic ecosystems*' (Article 2, no. 21 EC, 2000). However, the annexes of procedures describe the measurement only of structural properties – diversity, abundance and disturbance sensitive invertebrate taxa (EC, 2000). This implies it is taken that these structural components are also representative of ecosystem function (Solimini et al., 2009, Birk et al., 2012). This has led to substantial development of structural based indices but a comparative lack of development of approaches to indicate function (Birk et al., 2012). However, elements of structure and function may respond independently of each other and linking the two is still a key challenge (Sandin and Solimini, 2009). The relationship between biodiversity, often measured as species richness, and ecosystem functioning has been widely debated (e.g. McNaughton, 1977; Tilman, 1999; Chapin et al., 2000; McCann, 2000; Duffy, 2009, Loreau, 2010). The diversity-stability hypothesis predicts high species diversity (or richness) has a stabilising effect and leads to greater ecosystem resilience (McNaughton, 1977, Tilman, 1999, Chapin et al., 2000) while May's work, in contrast, found increasing numbers of species to have a destabilising effect on populations (May, 1973). Recent reviews reveal that it is largely accepted that biodiversity (species richness) does have a positive effect on ecosystem functioning including stability and production, although biodiversity may not be the driver for this relationship and the mechanisms involved are less clear (McCann, 2000, Loreau, 2010). Others conclude that species richness is important to ecosystem functioning and indeed that the importance of species richness and biodiversity has so far been underestimated (Duffy, 2009). Evidence for the relationship comes from a number of sources but the mechanisms have been less well investigated (Ives and Carpenter, 2007), leading to a lack of ubiquitous consensus.

In terrestrial environments, Tilman et al. (1996) found higher species richness led to lower annual variability, higher resilience and resistance and higher community and ecosystem process stability but not population process stability and positive relationships have been found between productivity and biodiversity (Naeem et al., 1994). In the marine environment, increased biodiversity was found to have a positive relationship with productivity, stability, resistance and resilience (Worm et al., 2006). This may suggest that species richness may be a good proxy for the state of the system – both the structural and functional properties. As the direct measurement of ecosystem functioning is fraught with difficulties, the measurement of structure has often been used as a surrogate (Díaz and Cabido, 2001, Sandin and Solimini, 2009). However this is far from consensus and the relationship between species richness and functioning can often be complex.

Functional diversity relates to functional traits and includes functional richness and composition; functional richness can be measured as the number of functional traits or types (Diaz and Cabido, 2001). Traits of species – characteristics of species life history, morphology and behaviour which influence ecosystem functioning and which are relevant to species responses to the environment (Bremner et al., 2006b, Díaz and Cabido, 2001), are recognised to have a regulatory role in ecosystem functions and processes such as energy cycling (Chapin et al., 2000). The traits present are determined by species identity, species richness, species evenness, species composition (abundance) and interactions, and how these vary over time and space.

Functional diversity is thought to influence ecosystem functioning in terms of resource dynamics and stability (Diaz and Cabido, 2001) including 'selection effect' which suggests that higher species richness leads to a greater probability of species with functionally dominant traits; and 'niche complementarity effect' which suggests higher species richness leads to more efficient resource use due to the greater representation of various functional traits. The diversity-stability hypothesis suggests that high levels of diversity ensure there is a bank of similar functional traits amongst species thus increasing the chance of survival of the traits even if the species composition changes due to pressures (McNaughton, 1977, Tilman, 1999, Chapin et al., 2000). In line with this theory, Walker and colleagues hypothesise that the dominant species are those which have a controlling function at any one time under certain environmental conditions and the rare species may be functionally similar but thrive under different conditions, thereby contributing to the resilience by acting as a buffer if conditions change (Walker et al., 1999). Similarly, the 'insurance hypothesis' indicates that diversity may contribute to ecosystem stability by increasing the probability that some species will respond differently to stress or perturbations meaning some species will be able to replace functionally important species (McCann, 2000). Different tolerances and competitive release can lead to differential responses from species to environmental change leading to overall stability (Hooper et al., 2005).

However, the relationship between species richness and functioning is not always simple and several examples have shown there is not always a direct relationship between biodiversity and ecosystem functioning (Covich et al., 2004). Dominant species may have a strong role in regulating ecosystem functioning but keystone species, which are rare, may also play a large role (Hooper et al., 2005). Invasions have demonstrated the potentially strong impact of a single species on ecosystem function (Hooper et al., 2005). If one or a few species have a strong effect on ecosystem processes then it is not likely that there is a simple relationship between species richness and function (Chapin et al., 2000). Furthermore, it is also the case that the abiotic environment, environmental perturbations and functional traits of dominant species can have greater effects on ecosystems than species richness (Hooper et al., 2005). While increased species richness may lead to increased functional diversity, the range of functional traits may be limited by environmental drivers so that increased species richness would not result in an increase of functional diversity (Hooper et al., 2005). Additionally, a change in species richness may be small or not apparent but this could mask changes in species composition (Stachowicz et al., 2007). In marine systems it was found that species losses occurred at high trophic levels while invasions occurred at low trophic levels resulting in situations with little change in species richness but a trophic skew in the system (Byrnes et al., 2007, Stachowicz et al., 2007). Thus, while species and functional richness are important, species and functional composition are at least as important (Hooper et al., 2005). Loss or gain of species can have variable effects on ecosystem functioning depending on the identity of the species and the functional role they play (Díaz and Cabido, 2001). Some species may be more important to the stability of ecosystems than overall species richness (Ives and Carpenter, 2007) and species composition may be a better predictor of ecosystem processes (Stachowicz et al., 2007).The order of species loss is not generally random and non-random losses can have bigger effects on ecosystem functioning than random losses (Solan et al., 2004, Stachowicz et al., 2007, Duffy, 2009).

There is not usually a simple linear relationship between species richness and niche space occupation in nature as it is more common for a reduction in species to affect some functional types and not others (Diaz and Cabido, 2001). This may not be detected by measuring species richness alone as the number of species is likely to exceed the number of functional types or guilds (Diaz and Cabido, 2001). Accordingly, species richness may not be a good predictor of functional diversity (Díaz and Cabido, 2001, Hillebrand and Matthiessen, 2009).

Functional diversity may have a more direct relationship with ecosystem function and stability than species richness or diversity (Reiss et al., 2009, McCann, 2000). Functional traits are fundamental in controlling ecosystem properties (Hooper et al., 2005). Biles and colleagues found no effect of species richness on ecosystem functioning while functional richness increased functioning in ecosystems (Biles et al., 2003). Odum predicted that function is more resistant than species diversity or richness (Odum, 1985), and it has been found that function could be maintained, even with species loss, until all species within functional guilds were lost (Tilman et al., 1996). Species richness within functional guilds is important as it can contribute a variety of survival strategies and genotypes thereby increasing resistance and resilience (Diaz and Cabido, 2001, Tett et al., 2007).

Many of the theories of the impact of biodiversity on ecosystem functioning rely on the assumption that some species are redundant in the assemblage (Duffy, 2009). This is supported by evidence of saturation points, the saturation effect – a levelling off of ecosystem properties at a certain level of species diversity even as species diversity continues to increase – suggesting redundancy in some species (Reiss et al., 2009, Duffy, 2009). However, it has been suggested that these saturation points are merely an artefact of experimental methods and that actually, diversity has a much bigger role than previously thought (Duffy, 2009). Studies on the effect of biodiversity on ecosystem functions are mainly restricted to species richness and are small-scale, short-term and single process studies but these may not represent realistic processes (Carpenter et al., 2009). It is argued that these studies may underestimate the effects of biodiversity on ecosystem functioning as the species richness required for functioning increases as the number of functions considered increases (Duffy, 2009, Hillebrand and Matthiessen, 2009, Reiss et al., 2009). Greater effects of diversity have been found in longer rather than shorter term studies and in more heterogeneous environments (Hillebrand and Matthiessen, 2009). Thus, biodiversity (species richness), informed by research, could be used as a broad indicator of ecosystem state (Duffy, 2009).

1.1.3 Response of structure and function to stress

Odum (1985) predicted several trends in stressed ecosystems including increased primary production, decrease in size and lifespan of organisms, a decrease of higher trophic levels, a decrease in diversity and increase in dominance (but the reverse if initial diversity is low), and that function is more resistant than structural properties such as species composition. Ecosystems can respond in a slow and linear way to stress but also respond quickly with thresholds and/or non-linear responses (Rapport and Whitford, 1999). The relationship between species and environmental gradients can be asymmetric, non-linear and show heterogeneous scatter indicating complex interactions of several limiting variables (Anderson, 2008). Species richness may show a range of responses to stress or resource availability including the humpbacked curve which shows an increase in diversity with increasing stress or resource availability before decreasing again as stress continues to increase (Connell, 1978, Pearson and Rosenberg, 1978, Odum, 1985, Dodson et al., 2000, Mittelbach et al., 2001, Hooper et al., 2005).

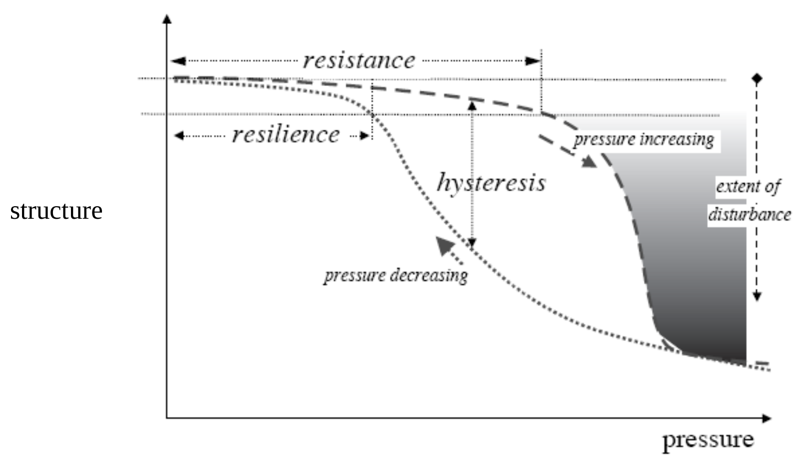


Figure 1.1 Structural indicator of ecosystem health in response to pressure (Tett et al., 2007)

The complex relationship between ecosystem structure and health is hypothesised by Tett et al. (2007) with the undesirable disturbance theory (Fig. 1.1). This shows structural properties can change rapidly beyond a certain threshold where resistance is exceeded. Once structurally damaged to a certain level, resilience is reduced and recovery is impaired (it may be impossible or the path of recovery may be different from the degradation path). Rapport & Whitford (1999) state that recovery may be impossible, that devastated systems do not 'bounce back' and the focus should be on regulating human activities to prevent degradation. Thus, it is important to be able to detect trends towards the threshold so that recovery can remain possible. Anticipating thresholds was identified as a crucial gap in ecosystem assessment as part of the Millennium Ecosystem Assessment (Carpenter et al., 2009). Furthermore, the assessment found that non-linear changes involving accelerating, sudden and irreversible changes are increasing (Ives and Carpenter, 2007).

It may be possible to detect thresholds or small changes by measuring functioning of the ecosystem but difficult when trying to distinguish and interpret small structural changes (Tett et al., 2007). This is particularly due to the high natural variability in structural properties such as species richness while functional properties are expected to be less variable in functionally similar environments. On the other hand, since function may be more resistant than structural properties (Odum, 1985) this suggests function may be a good indicator of the general extent of disturbance in a system but not suitable as an early warning indicator (Paul, 1997).

It is clear the relationship between biodiversity and function is complex and it may be that studies so far have been constrained in estimating the importance of biodiversity. Further research is needed to elucidate the mechanisms behind the observed relationships (McCann, 2000, Ives and Carpenter, 2007) It seems it may be important to consider many aspects of species and functional diversity in assessing the state of ecosystem health. Improving knowledge of non-linear and abrupt changes are important (Carpenter et al., 2009). Ecosystems under stress can show a range of responses and these do not always concur with the biodiversity ecosystem function theory of increased diversity positively impacting ecosystem health properties such as functioning. Justification for this is that described positive biodiversity-ecosystem functioning relationships have been found in environments with similar conditions; under changing environmental conditions or gradients, such as due to anthropogenic stress, other patterns may occur (Hooper et al., 2005). However, the relationship between biodiversity and functioning under stress has not been well examined.

1.2 Application of indices and assessment approaches

Benthic macroinvertebrates of soft substrata are frequently used as the basis in the development of indices. Diaz and colleague's (2004) study on multi-metric indices indicated that 50% were based on macroinvertebrate communities. Macroinvertebrates play an important part in nutrient cycling and are relatively sessile and long lived, they cannot avoid unfavourable conditions, and they integrate changes of conditions over time thereby making good and sensitive indicators (Reiss and Kröncke, 2005). Although several benthic macroinvertebrate biotic indices currently exist, e.g. ITI (Word, 1979), AMBI (Borja et al., 2000), BQI (Rosenberg et al., 2004) and EQR (Borja et al., 2007), these are mostly (although not solely) based on the Pearson and Rosenberg (1978) model (Fig. 1.2) (see Chapter 2 for a detailed account of indices). This model describes a succession of macrofauna from a grossly polluted organic enrichment source to normal conditions in soft sediment habitats. The macrofauna show a predictable response with distance spatially or temporally from the source (Pearson and Rosenberg, 1978).

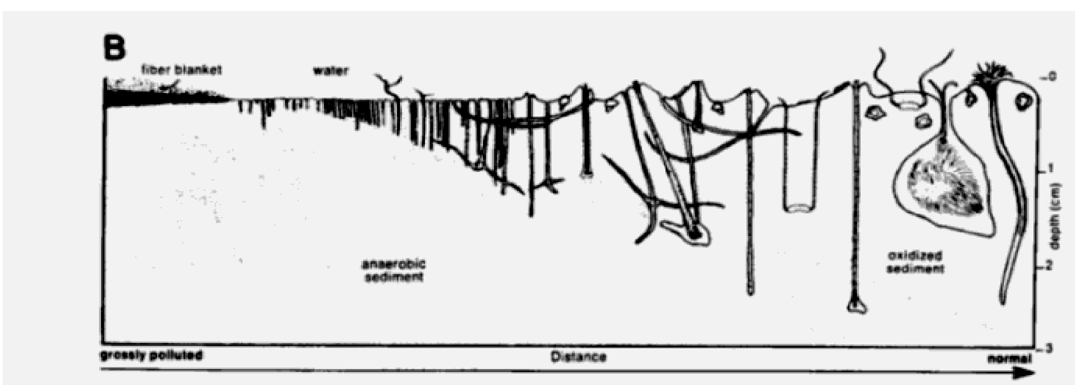


Figure 1.2 Changes in fauna and sediment structure along a gradient of organic enrichment (Pearson and Rosenberg, 1976, 1978)

This response to organic enrichment allows detection of many human caused impacts including sewage, pulp and paper mill waste, oil pollution and organic dredged sediments (Elliott and Quintino, 2007). However, the effectiveness of these indices outside of impacts related to organic enrichment and habitats other than soft sediments is tentative. Recent studies have attempted to test, validate and highlight differences of biological indices (Labrune et al., 2006, Dauvin et al., 2007, Quintino et al., 2006, Fleischer et al., 2007). AMBI has been successful in assessing quality due to a range of causes including anoxic episodes, fish farming and dredging but behaves poorly in assessing effects of sand extraction or in organic poor or naturally stressed environments e.g. low salinity areas of estuaries and subtidal sandbanks (Muxika et al., 2005). Sediment type and subtidal or intertidal location of sampling sites were found to have an effect on the ecological quality status as attributed by the indices AMBI, BENTIX, BQI and H' (Blanchet et al., 2008). As well as indices sensitivity to salinity – such as species number, H' and BQI (Zettler et al., 2007) - studies have found some indices to be sensitive to seasonal variation – such as H' and Hurlbert index (ESn) (Reiss and Kröncke, 2005). These results indicate that coarse sediments as well as some specific pressures, such as human induced physical disturbance as well as natural stress, are overlooked and further validation and testing of impacts due to physical disturbance and chemical pollution is still required (Quintino et al., 2006).

Commonly used indices are mainly structural parameters based on abundance, biomass and species richness, or derivatives thereof (Elliott and Quintino, 2007). There is a need for working measures of ecosystem function (Diaz et al., 2004, Tillin et al., 2008) and improving the ability to use function in the assessment of ecosystem status. Indices including ITI based on feeding guilds, AMBI (Borja et al., 2000), and the BQI (Rosenberg et al., 2004) based on knowledge of species' responses to organic enrichment, are considered functional by some authors (e.g. Elliott and Quintino, 2007) but structural by others (e.g. Tett et al., 2007). These indices do measure function to a certain extent by using typical species responses to certain environmental conditions but have structural components at their core. It is debatable and untested whether these components are an adequate surrogate for functioning. A recent approach, biological traits analysis (BTA), is based on species biological characteristics rather than community structure parameters and as such it makes an explicit link with ecological functioning (Bremner et al., 2006c).

Although some structural indices may act as a proxy for ecosystem functioning, these links are often not clear or well understood. There has been a shift in favour of measuring functional indices or a combination of structural and functional indices in the assessment of ecosystem health (Elliott and Quintino, 2007, Tillin et al., 2008). This is in line with the MSFD approach which requires structural as well as functional approaches are used in the assessment of ecological quality. Bremner et al. (2006b) suggest that functional measurements such as BTA may be able to detect and distinguish types of impact and this method has recently been the focus of a number of studies in an attempt to link environmental quality with biological communities in marine systems (Rachello-Dolmen and Cleary, 2007, Cooper et al., 2008, Marchini et al., 2008, Pranovi et al., 2008, Wan Hussin et al., 2012, Paganelli et al., 2012). Evidence suggests at the very least, that measuring function in addition to structure may allow a more thorough assessment. It was found in several studies of running freshwaters that neither structure nor function alone could detect change due to all sources of anthropogenic disturbance studied but overall, function responded better and may be particularly useful in detecting a response in organisms not directly measured e.g. bacteria (Sandin and Solimini, 2009).

A functional approach to environmental health assessment may be particularly pertinent to the health assessment of transitional waters (Gray and Elliott, 2009). The ‘estuarine quality paradox’ describes the difficulty in distinguishing natural and anthropogenic caused stress in transitional waters as benthic community composition in transitional waters has, arguably, similar characteristics to those found impacted by human activities (Elliott and Quintino, 2007). This is due to the natural variability of salinity, temperature and turbidity, as well as generally high natural levels of organic matter prevalent in these environments. This means that indices measuring structural properties may imply low quality due to the naturally low species richness and high individual abundance in estuaries (Elliott and Quintino, 2007). While disturbance of the ecosystem may be natural, policy defines only anthropogenically originated disturbance as undesirable (Tett et al., 2007). Species number, Shannon-Wiener diversity (H’) and BQI were found to indicate low ecological quality status at low salinities (Zettler et al., 2007) and H’ and BQI obtained for transitional waters were found to indicate degraded conditions when compared to other indices used in the same study (Blanchet et al., 2008). However, these community characteristics are expected in estuaries/transitional waters and are inherent in successful estuarine functioning (Elliott and Quintino, 2007). Correspondingly, although structural indices exist, reference conditions have not been established throughout and it has been proposed that index threshold values are not appropriate for classification, particularly in transitional waters (Ruellet and Dauvin, 2007). This has implications for policy implementation and the objectivity of site assessment (Tett et al., 2003). Quality levels need to be assessed using defined conditions but these conditions are likely to differ in marine and transitional waters due to natural environmental variation. One approach in resolving this issue is the re-adjustment of index threshold values and establishment of appropriate reference conditions (Ruellet and Dauvin, 2007). Methods and theories such as the estuarine quality paradox are largely untested in variable environments such as transitional waters and require development for validation (Birk et al., 2012). Furthermore, assigning threshold values often relies heavily on expert judgement (Birk et al., 2012). However, emphasising functional approaches over structural approaches, as suggested by Elliott and Quintino (2007), may be a more biologically relevant way of overcoming difficulties in assessing ecosystem health in variable environments such as estuaries. Thus, development of functional indicators in the marine environment is crucial.

There are important environmental, legislative and financial implications for policy implementation when the indices used in routine monitoring over-estimate the quality of poor areas or under-estimate quality of good areas (Quintino et al., 2006). The definition of ‘good’ health in the context of the WFD and the MSFD can be open to interpretation and are largely based on human value judgements (Mee et al., 2008). Discrepancies and inconsistencies between indices lead to a lack of confidence in quality assessments (Quintino et al., 2006). Several studies have found various indices over- or under-estimate quality relative to each other e.g. EQR and BQI (Quintino et al., 2006); AMBI, H’ and BQI (Zettler et al., 2007); The benthic opportunistic polychaete to amphipod ratio (BOPA), AMBI, BENTIX, BQI and H’ (Blanchet et al., 2008). These discrepancies are due to differences in the way indices deal with dominant species; assess species tolerance; and ecological quality threshold values (Labrune et al., 2006). A calibration of thresholds for different indices is required and a multi-metric approach, combining different indices, to avoid misclassification may be necessary (Dauvin et al., 2007, Borja et al., 2007). The ability to quantify uncertainty in outcomes of ecosystem health assessment is lacking and requires further development (Carpenter et al., 2009).

1.3 Rationale

Indices should be consistent in their capacity to detect disturbance; in their power to discriminate between anthropogenic and natural disturbance; in their ability to distinguish different levels of disturbance; and in their applicability in different areas and circumstances. While some indices have been shown to be successful within the realm of certain, consistent limitations (e.g. AMBI is limited in organic poor, naturally stressed, taxa poor, low abundance environments; (Muxika et al., 2005, Zettler et al., 2007, Muniz et al., 2005), critical evaluation of indices still needs further development and several issues are outstanding and require resolution. Lack of functional indicator development, operation over salinity ranges and seasons, detection of impacts from physical and chemical stress, multiple pressures and detection of impacts in coarse or mobile sediment habitats are all notable gaps in marine assessment of ecosystem health. Analysis of wide ranging data as well as critical examination and novel use of current techniques may fill gaps and improve current monitoring.

1.4 Aims

The aims of this project were to assess the current methods and approaches used in the assessment of ecosystem health and examine the efficiency of biotic indices in detecting disturbance from a range of sources in transitional and coastal waters.

These aims were investigated in the following chapters through:

- Chapter 2: The examination of spatial and temporal trends of indices; index correlations; and the effect of sampling method on index results; using reference data, in order to assess how indices perform in relation to each other under naturally variable conditions with no strong environmental gradients impacting upon them.

- Chapter 3: Investigation of the pressure response of indices using data from a range of impacted and reference sites to assess how well indices detect different types and intensities of disturbance and how indices perform in relation to each other.
- Chapter 4: Investigation of the performance of structural methods compared to functional methods to assess whether quality assessment is consistent with both approaches and assess the usefulness of different functional approaches as no standard method is currently available for use in the marine environment.
- Chapter 5: Assessment of the variability and uncertainty of index classifications to quantify the level of uncertainty associated with indices in relation to each other and assess how this may impact on sampling regimes.

Chapter 2
The performance of benthic indices in long term monitoring sites

2.1 Introduction

This chapter investigates the performance of indices at various sites with natural background variability to assess the natural variation of indices used in communities which are not impacted by disturbances. A suite of indices which are commonly used in publications; by monitoring agencies; as part of the Water Framework Directive; and some which are readily available on the software programme Primer, were initially chosen (Section 2.1.1). Data used came from sites around Scotland which are part of the National Marine Monitoring Programme (NMMP) and were collected by and obtained from the Scottish Environment Protection Agency (SEPA) (Section 2.1.2). The study is composed of the response of indices to spatial and temporal trends (Section 2.2); the strength of relationships between different indices (Section 2.3); and the impact of some aspects of sampling protocol on index results (Section 2.4). This chapter aimed to investigate the performance and variation in responses of the indices in undisturbed conditions before testing index responses to disturbance in subsequent chapters.

2.1.1 Indices

The following indices were selected to be used as the methods of ecological quality assessment for the datasets.

Species richness (S) i.e. total number of species present; Abundance (N) i.e. total number of individuals; and Ratio of abundance to species richness (A/S) are commonly used univariate indicators in measuring diversity (Quintino et al., 2006).

Margalef's index (d) is a commonly used measure of species richness but is very sensitive to sampling effort (Magurran, 2004).

$$d = \frac{(S-1)}{\ln N}$$

The Brillouin index (HB) is used in situations where it cannot be ensured that the sample is random or when all individuals are counted; and is used to measure a collection rather than a sample (Magurran, 2004).

$$HB = \frac{lnN! - \sum ln\, n_i!}{N}$$

Fisher (α) shows the shape of the species distribution and the fit compared to a log series (Clarke and Gorley, 2001).

$$S = \alpha \ln\left(1 + \frac{N}{\alpha}\right)$$

Rarefaction (ES_n) gives the expected number of species for a given number of individuals (n) for example, the number of species expected in 50 individuals, ES50 (Clarke and Gorley, 2001). This index can be biased when applied to small sample sizes.

$$ES50 = \sum_{i=1}^{S} \frac{(N-N_i)!\,(N-50)!}{(N-N_i-50)!\,N!}$$

The Shannon-Wiener index (H') is one of the mostly commonly used and persistent indices and is incorporated into new indices such as m-AMBI(see below) (Muxika et al., 2007). However, this index is an example of a less than ideal index due to its sensitivity to sample size – being used out of tradition or 'inertia' (Magurran, 2004). Natural log or $\log_2$ is used to calculate the index in different examples but there is no particular reason why either should be used above the other as long as the choice is consistent (Magurran, 2004).

$$H' = -\sum_i pi(\log pi)$$

...where pi is the proportion of individuals
of species i in the total abundance
natural log or $\log_2$ *is used*

Pielou's evenness index (J') is a measure of equitability

$$J' = \frac{H'}{Log(S)}$$

Simpson's index (D or λ) decreases as diversity increases and due to this is often expressed as 1-D (λ). The Simpson's index measures the variance of the species abundance distribution (Magurran, 2004). It has been incorporated into the EQR (see below) (Borja et al., 2007) and is the basis of measures of taxonomic distinctness (Clarke and Gorley, 2001) and is favoured by many as a robust index (Magurran, 2004). λ' is a revised form of Simpson's index which is used when N is small (Clarke and Gorley, 2001).

$$1 - \lambda' = 1 - \sum \left(\frac{ni(ni-1)}{N(N-1)}\right)$$

...where ni is the number of individuals in the ith species
and N is the total number of individuals.

Hill's diversity N1 is a revision of the Shannon-Wiener index which predicts the number of species there would be in a sample if all species were similarly abundant (Magurran, 2004).

$$N1 = exp(H')$$

The Basque research institute AZTI Marine Biotic Index (AMBI) is a continuous biotic coefficient which is derived from the proportions of five ecological groups of organisms based on their sensitivity or tolerance to disturbance (Borja et al., 2000). Groups I and II dominated communities indicate normal benthic community health, Group III indicates unbalanced, going towards pollution, Group IV and V polluted and Group V heavily polluted. A lower score indicates higher quality, hence a community composed only of GI species would yield a result of 0.

$$\begin{aligned} Biotic\ coefficient \\ &= \{(0\ \times \%\ GI) + (1.5\ \times \%GII) + (3\ \times \%GIII) + (4.5\ \times \%GIV) \\ &+ (6\ \times \%GV)\}/100 \end{aligned}$$

Multivariate-AMBI (m-AMBI) is an extension of AMBI which includes richness and Shannon diversity in order to make AMBI more relevant for WFD implementation which requires the assessment of these structural components (Muxika et al., 2007). The ecological quality ratio (EQR) was developed in order to comply with WFD guidelines on quality assessment and intercalibration between different Member States (Borja et al., 2007). It was derived from comparing monitoring data with reference condition data and incorporates Simpson's index and AMBI to give a value between 0 and 1, with 1 being good and 0 bad quality.

$$EQR = \frac{\left(\left(2 \times \left(1 - \left(\frac{AMBI}{7}\right)\right)\right) + (1 - \lambda')\right)}{3} \times \frac{\left(\left(1 - \left(\frac{1}{A}\right)\right) + \left(1 - \left(\frac{1}{S}\right)\right)\right)}{2}$$

The benthic opportunistic polychaete amphipod index (BOPA) examines the ratio of opportunistic species of polychaetes to amphipods using relative frequencies (Dauvin and Ruellet, 2007). A low value of the index indicates good quality as there is a low number of opportunistic species. The proposed advantages of this index included the drastically reduced taxonomic task when compared with AMBI for example, as all amphipods apart from one genus were classified as sensitive species (www.azti.es) and therefore would not need to be identified to species level (Dauvin and Ruellet, 2007). However, more recent species lists show varying degrees of sensitivity within the amphipod group and greater detailed taxonomy is probably required than first envisioned.

$$BOPA = \log_{10}\left[\left(\frac{f_P}{(f_A+1)}\right)+1\right]$$

...where f_P is the frequency of opportunistic polychaetes and f_A is the frequency of amphipods

The infaunal trophic index (ITI) describes the community according to feeding behaviour types I-IV (Word, 1979). Group I is dominated by suspension feeders; Group II by suspension and surface-detritus feeders; Group III by surface deposit feeders; and Group IV by subsurface detritus feeders. The index ranges from 0 to 100 and the value indicates the dominant feeding group. The index can only be applied to soft bottom silty sand or clay areas. This index goes some way to measuring function of the system and has been used by environment agencies such as SEPA for quality assessment. However, the ITI is limited both to the types of pressure and habitat it responds to and as a measure of health (Pinto et al., 2009). Parallels in freshwater systems with functional feeding groups are now considered to be inadequate in detecting stress due to human disturbance in these habitats (Statzner et al., 2005). ITI has been criticised as an index of ecosystem health since the greatest index values would be obtained if all species present in the community were suspension feeders, while a more balanced community of feeding types would result in a lower quality classification (Gamito and Furtado, 2009).

$$ITI = 100 - \left[33 - 1/3\left(\frac{0n_1 + 1n_2 + 2n_3 + 3n_4}{n_1 + n_2 + n_3 + n_4}\right)\right]$$

...where n_i is the number of individuals in Group i

The infaunal quality index (IQI) is a modified version of the EQR revised by the Environment Agency (EA) for implementation in England, Wales and Scotland for monitoring quality of coastal and transitional waters (WFD-UKTAG, 2008).

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

Taxonomic diversity and distinctness measures have been developed by Clarke & Warwick and can be calculated using the statistical package Primer. The theory behind these indices is that an assemblage with greater taxonomic variety will be more diverse than another assemblage with the same species richness and abundance but less varied taxonomy (Magurran, 2004). Clarke & Warwick's taxonomic distinctness is derived from Simpson's index and is considered promising due to the lack of sensitivity to sampling effort which blights many other indices (Magurran, 2004). There are five variations which can be used differing in the measurements made between taxa. Taxonomic Diversity (Δ) is the taxonomic distance between two individuals in the sample and incorporates both species abundance and relatedness. Taxonomic Distinctness (Δ*) is similar to Δ but measures the distance between two individuals as long as they are not the same species. Average Taxonomic Distinctness (AvTD) Δ^+ is used for presence absence data and measures the distance between pairs of individuals in a sample. Total Taxonomic Distinctness (TTD) $S.\Delta^+$ sums the average distances between pairs of species. Variation in Taxonomic Distinctness (VarTD) Λ^+ is the variance of taxonomic distances between each pair of species about the mean and is a measure of the evenness of taxonomic variation.

Taxonomic Diversity (Delta, Δ)

$$\Delta = \frac{\left[\sum\sum_{i<j} \omega_{ij} x_i x_j\right]}{[N(N-1)/2]}$$

Taxonomic Distinctness (Delta*, Δ*)

$$\Delta^* = \frac{\left[\sum\sum_{i<j} \omega_{ij} x_i x_j\right]}{\left[\sum\sum_{i<j} x_i x_j\right]}$$

Average Taxonomic Distinctness (AvTD) (Delta+, Δ^+)

$$\Delta^+ = \frac{\left[\sum\sum_{i<j} \omega_{ij}\right]}{[S(S-1)/2]}$$

Total Taxonomic Distinctness (TTD) (sDelta+, S. Δ^+)

$$S.\Delta^+ = \sum_i \left[\frac{\left(\sum_{j\neq i} \omega_{ij}\right)}{(S-1)}\right]$$

Variation in Taxonomic Distinctness (VarTD) (Lambda+, Λ^+)

$$\Lambda^+ = \frac{\left[\sum\sum_{i<j}\left(\omega_{ij} - \Delta^+\right)^2\right]}{[S(S-1)/2]}$$

…where S is the number of species, ω_{ij} is the taxonomic distances through the classification tree between every pair of species (the first from species i and the second from species j), and the double summation ranges over all pairs i and j of these species (i < j)

The Benthic Quality Index (BQI) is a biotic index developed for the WFD which incorporates rarefaction (ES50), abundance and richness (Rosenberg et al., 2004). This index is proposed as being more objective than indices such as AMBI as the species are assigned an objective tolerance value according to the $ES50_{0.05}$ value. This index has so far not been widely adopted or tested probably due to the difficulty in calculating the $ES50_{0.05}$ (but see (Labrune et al., 2006, Fleischer et al., 2007)). In some cases BQI was calculated without using site specific $ES50_{0.05}$ values (e.g. Quintino et al. 2006). $ES50_{0.05}$ values were calculated for this study (for details see Appendix 8.1)

$$BQI = \left(\sum_{i=0}^{n} \left(\frac{A_i}{totA} \times ES50_{0.05i} \right) \right) \times log_{10}(S+1)$$

...where Ai is the abundance of species i

$ES50_{0.05}$ is the ES50 at 5% of the population of species i

Several indices have associated quality classifications which correspond to Water Framework Directive classifications in some cases (Table 2.1).

Table 2.1 Assigned quality classifications according to index values for some commonly used indices

Index	Boundaries	Quality
AMBI (Borja, 2004)	AMBI≤1.2 1.2<AMBI≤3.3 3.3<AMBI≤4.3 4.3<AMBI≤5.5 AMBI>5.5	High Good Moderate Poor Bad
H’ $\log_2$ (Labrune et al., 2006)	H’>4 3<H’≤4 2<H’≤3 1<H’≤2 H’≤1	High Good Moderate Poor Bad
M-AMBI (Muxika et al., 2007)	≥0.82 0.62≤mAMBI<0.82 0.41≤mAMBI<0.61 0.20≤mAMBI<0.40 <0.20	High Good Moderate Poor Bad
BOPA (Dauvin and Ruellet, 2007)	0.00000 ≤BOPA≤0.04576 0.04576<BOPA≤0.13966 0.13966<BOPA≤0.19382 0.19382<BOPA≤0.26761 0.26761<BOPA≤0.30103	High Good Moderate Poor Bad
ITI (Word, 1979)	≥80-100 ≥60-80 ≥30-60 0-30	Reference conditions Normal conditions Changed conditions Degraded conditions
IQI (WFD-UKTAG, 2008)	≥0.75 0.64≤IQI<0.75 0.44≤IQI<0.64 0.24≤IQI<0.44 IQI<0.24	High Good Moderate Poor Bad
BQI (Appendix 8.1)	>17.28 >12.96≤17.28 >8.64≤12.96 >4.32≤8.64 ≤4.32	High Good Moderate Poor Bad

2.1.2 Study Sites

Datasets from a number of study sites from coastal and transitional waters were obtained from the Scottish Environment Protection Agency (SEPA) (Table 2.2, Fig. 2.1).

Table 2.2 Details of datasets from long term monitoring stations obtained from SEPA

Site	**Site Code**	**Water Body Type**	**Latitude/ Longitude**	**Depth (m)**	**Grab Size (m^2) Mesh Size (µm)**	**Abundance /Biomass**	**Year data available** (no. of replicates)
Clyde Middle Transect Station 5	CMT 5	Coastal	5549.30N 0458.70W	57	0.1 1000	y/y	1993 (9) 1996 (5) 1999-2005 (5)
Clyde Middle Transect Station 7	CMT 7	Coastal	5556.85N 0453.65W	81	0.1 1000	y/y	1993 (9) 1996 (5) 1999-2005 (5)
Lismore Deep	LIS	Coastal	5634.80N 0528.30W	109	0.1 1000	y/y	1999-2005 (5)
Irvine Bay Station H	IBH	Coastal	5535.92N 0447.40W	38	0.1 1000	y/y	1999-2005 (5)
Kingston Hudds	KH	Coastal	5607.41N 0255.80W	30	0.1 1000 and 500	y/y	1999-2005 (5)
Kincardine	KC	Trans-itional	5601.50N 0332.60W	7	0.1 1000 and 500	y/y	2000-2005 (5)
RA	RA	Trans-itional	5602.10N 0338.30W	5	0.1 1000	y/n	1979 (3) 1990-1992 (5) 1993 (9)and (3) 1994 (5) and (3) 1995 (5) 1996 (3) 1997-1999 (5)

The stations have been monitored on a long term basis by SEPA as part of the National Marine Monitoring Programme (NMMP), now known as the Clean Safe Seas Environmental Monitoring Programme (CSEMP) (Table 2.2, Fig. 2.1). In addition, these stations form part of the surveillance monitoring network for the WFD. These study sites are considered to be reference sites as there are no point sources of pollution affecting them, although there may be other far field pressures or direct fishing pressure. These stations were sampled as 5 replicates; from 2006 onwards the sampling regime changed so most recent datasets come from 2005.

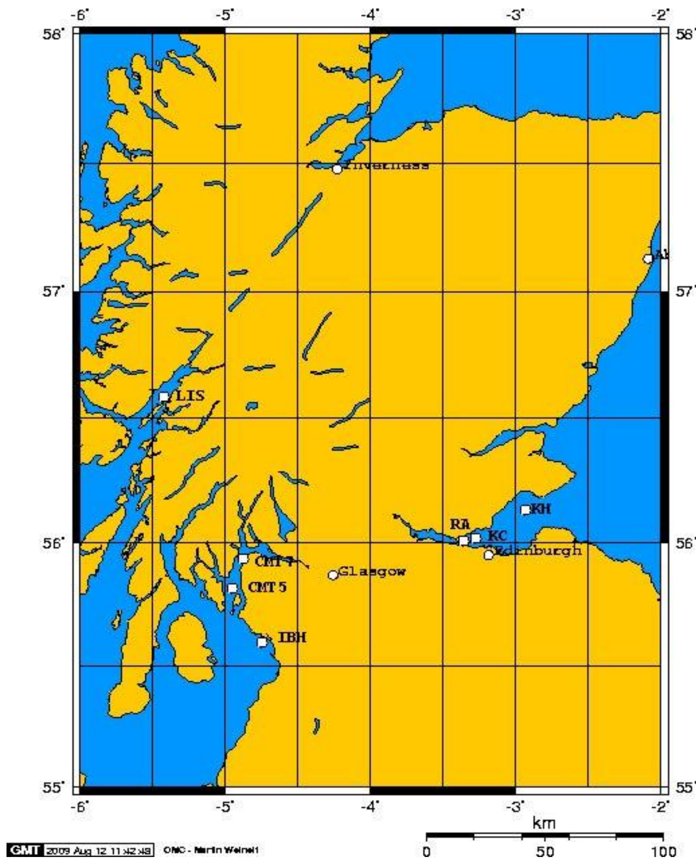


Figure 2.1 Location of sample sites around Scotland

2.2 Spatial and Temporal trends

2.2.1 Introduction

Studies have shown a wide range of factors can influence the variability of benthic communities. These include a range of biological, physical and chemical conditions of the environment including recruitment, food availability, predation, sediment type, salinity, temperature and season (Reiss and Kröncke, 2005). Sediment type can be one of the most important influencing factors. Sediment structure can influence the benthic community by being of direct importance to species, such as those which ingest mud, of indirect importance to others, or as a reflection of other physical, hydrodynamic properties of the system (Buchanan, 1984). It may not always be possible to determine which of these is the principle factor observed in effects of sediment structure. Invertebrate communities were found to be related to depth which was in turn related to a gradient in sediment type (Bigot et al., 2006). In another study sediment type was found to be the main factor influencing the benthic community and this was related to hydrodynamics (Bachelet et al., 1996). In the same study it was also found that salinity and depth had an influence, but to a lesser degree. Sediment type and distance from the open sea were found to have a strong influence on faunal communities but depth had only a slight influence (Blanchet et al., 2005).

Detecting trends over long timescales against noisy background variability can also be difficult. Very long term studies of benthic communities are rare. Frid et al. (2009) carried out analysis on a 33 year dataset. They found a seasonal effect over the whole time period with September having higher diversity than March. Winter temperature and phytoplankton were found to influence communities. Kröncke & Reiss (2010) analysed a 28 year dataset and similarly found cold winters to be a significant factor in change in benthic communities. Other studies have found cyclical changes in benthic communities over periods of 6 – 11 years (Gray and Christie, 1983) which may be related to major atmospheric processes such as the North Atlantic Oscillation (Gray and Elliott, 2009). Sampling protocol can influence the detection of real disturbance gradients from natural patchiness (Armonies, 2000). Communities in a small sample area respond to variations on a local and short term scale, thus sampling a small area may be unrepresentative of the larger area and longer time scales. Current methods allow measurement at a small scale whereas there is a need for methods which can measure community change over greater temporal and spatial scales (Leonard et al., 2006).

Due to the influence of environmental conditions, it is expected that benthic communities in different areas will vary. However, indices should ideally only detect differences which represent an increase or decrease in quality and not natural variation amongst communities. The level of noise in data which occurs naturally and impacts the classification of indices is an important consideration when trying to account for community changes due to anthropogenic disturbances (Kröncke and Reiss, 2010). It is important to know whether indices are influenced by heterogeneity in environmental conditions and by how much, in order to be able to distinguish between natural background variability and anthropogenic disturbance. Studies have found that quality classifications can depend on the index used (Labrune et al., 2006, Quintino et al., 2006, Dauvin et al., 2007, Chainho et al., 2007, Blanchet et al., 2008, Afli et al., 2008). Indices also respond to patchiness, with variability of indices within sites as great as between sites (Quintino et al, 2006). Some authors find indices to perform poorly when conditions are ‘moderate’ as opposed to more clearly ‘good’ or ‘poor’ (Quintino et al., 2006, Puente and Diaz, 2008). This may be due to the difficulty in distinguishing a moderate disturbance trend from natural heterogeneity. Several studies have found indices to be sensitive to natural environmental gradients such as salinity (Dauvin et al., 2007, Teixeira et al., 2008b, Fleischer and Zettler, 2009); sediment type (Dauvin et al., 2007, Blanchet et al., 2008, Teixeira et al., 2008b, Teixeira et al., 2008a); season (Reiss and Kröncke, 2005); and location (Blanchet et al., 2008, Teixeira et al., 2008a). In addition, indices have been found to be sensitive to annual variation (Salas et al., 2004, Kröncke and Reiss, 2010).

Some authors have found univariate indices such as Shannon index, species richness and abundance, Hurlbert’s Index (ES (100)), Pielou’s index, Simpson’s index and Margalef’s index to be more sensitive to temporal and environmental variability than indices such as AMBI, W statistic, BQI, BOPA and taxonomic distinctness (Salas et al., 2004, Reiss and Kröncke, 2005, Chainho et al., 2007, Kröncke and Reiss, 2010). Kröncke and Reiss (2010) assessed performance of several indices over a 28 year timescale which included periods of natural disturbance such as particularly cold winters. They found all indices fluctuated due to these natural disturbance events but variability was higher for univariate indices such as H', Hurlbert's Index and species richness, slightly lower for indices such as BOPA and AMBI and lowest for multimetric indices such as IQI and M-AMBI. They also found that only species richness, abundance and the Norwegian multimetric index were able to detect a general increasing trend over the time period. In contrast, in their 33 year study, Frid et al. (2009) found univariate measures of species richness and abundance did not indicate a long term trend which was evident from multivariate analysis. Small scale variations can impact local communities obscuring larger scale and general spatial or temporal trends (Armonies, 2000).

Indices have been found to attribute lower quality to muddy sediment samples and coarser samples with medium grained samples being classified as having higher quality (Blanchet et al., 2008; Texeira et al., 2008a). Lower quality has also been found due to lower salinity (Texeira et al., 2008a). These environmental factors vary naturally but can also be tightly linked to anthropogenic disturbance. The proportion of clay in sediments can be an indicator of the contaminant load of the sediment due to the greater ability of the finer grains to adsorb contaminants (Horowitz, 1991 in (Szava-Kovats, 2008)). Estuaries have naturally high variability in physico-chemical attributes and macroinvertebrate communities have high abundance and low species richness which can be indicators of disturbance in comparable coastal areas (Elliott and Quintino, 2007). However, estuaries are often a sink for many anthropogenic inputs from land run-off to industrial and sewage effluents. Fishing pressure is widespread and chronic on most areas of the seabed and little useful data is known about the fishing effort or intensity to relate to benthic disturbance (Kaiser et al., 2000). Fishing pressure has been shown to impact benthic communities (Kaiser et al., 2000) but few studies have been carried out and effects could be both direct and indirect. Therefore the extent to which fishing pressure shapes benthic communities is unknown. These examples demonstrate the difficulty in separating natural from anthropogenic disturbance. The natural variability of benthic communities should be considered when assessing quality (Quintino et al., 2006). Methods which compare data to a reference set can incorporate natural variability and have been found to be better than stand alone indices which are overly sensitive to noisy data (Leonard et al., 2006, Lamb et al., 2009). However, reference data are difficult to come by and most sites probably now integrate some level of anthropogenic disturbance.

Macrobenthic communities are well known to respond to changes in environmental conditions. This is one of the reasons they make good bio-indicators of disturbance. However, it is also well known that the marine environment is highly heterogeneous and one limitation of using macroinvertebrates as bio-indicators is trying to distinguish natural variability from change due to anthropogenic disturbance. This analysis uses data which have no known disturbances (although may be impacted by fishing pressure and diffuse pollution). Thus, the variability in index classifications results largely from natural variability and inherent differences in the indices themselves.

Aims

The aims are to assess spatial and temporal patterns at different coastal and transitional sites; to establish the extent of natural variability of the sites; and to assess index performance at different sites.

Null Hypotheses

1. Index quality classifications do not differ in different sites
2. Indices do not detect temporal trends
3. Index quality classifications are not related to natural environmental attributes

2.2.2 Methods

Statistical Analysis

A range of analyses have been carried out with the NMMP data (Section 2.1.2) to investigate spatial and temporal trends. Multidimensional scaling (MDS) and ANOSIM analysis was carried out using Primer 6 statistical software to assess spatial variation of benthic communities between sites and temporal variation within sites. A suite of indices were calculated for each of the sites (Section 2.1.1). Performance of the indices was assessed using Kruskal Wallis to assess differences in index value between sites (carried out using SPSS 18). Pearson product moment correlation was used to assess direction of change in quality over time according to indices and between indices and environmental variables to assess the influence of environmental factors on index results. Only percentage *r* values are given and not p-values to avoid Type I errors due to multiple comparisons. Correlation analyses were carried out using Minitab 15.

2.2.3 Results

A range of depths were represented by the different sites (Table 2.3). All of the sites had silty type sediments although these ranged between coarse and fine. Content of organic carbon ranged from 1.37 to 4.04%.

Table 2.3 Habitat characteristics of each site. Median Phi, Silt/Clay fraction and organic carbon are averaged across all the available data for the site with standard deviation (n values vary due to missing data; for RA only mean values available; see Appendix 8.6 for data).

Site	Median Phi	Silt and Clay Fraction %	% Organic Carbon	Sediment Type	Depth (m)
CMT5	6.37±0.61	89.15±10.98	3.14±1.37	Fine Silt	57
CMT7	5.41±0.99	65.07±16.68	2.56±1.42	Medium Silt	81
LIS	6.81±0.16	97.66±1.35	2.88±1.31	Fine Silt	109
IBH	4.11±0.25	52.19±5.30	1.37±0.72	Coarse Silt	38
KH	4.84±1.23	68.18±16.76	1.95±0.97	Coarse Silt	30
KC	4.68±1.61	61.10±25.74	3.41±1.18	Coarse Silt	7
RA	4.55±0.78	64.19±15.78	4.04±1.77	Coarse Silt	5

Each site had its own distinct species assemblages indicating there was greater variability between sites than within sites (Fig. 2.2, One-way ANOSIM (sites) $R=0.918$, $p<0.001$). CMT5 and CMT7 had greater similarity than any other pair of sites (One-way ANOSIM pairwise comparison $R=0.644$, $p<0.001$). LIS, IBH and KH were all more similar to CMT5 than to any other site (One-way ANOSIM pairwise comparison with CMT5: $R=0.691$, $p<0.001$; $R=0.717$, $p<0.001$; $R=0.942$, $p<0.001$, respectively). KC and RA were more similar to each other than to any of the other sites (One-way ANOSIM pairwise comparison $R=0.836$, $p<0.001$). The greatest differences were found between RA and CMT7, LIS and IBH (One-way ANOSIM pairwise comparison with RA: $R=1$, $p<0.001$ in all cases) and between KC and LIS and IBH (One-way ANOSIM pairwise comparison with KC: $R=1$, $p<0.001$ in all cases).

CMT5 showed significant differences between years (One way ANOSIM (years) $R=0.626$, $p<0.001$) with 1996 having the least similarity to any other year (One way

ANOSIM pairwise comparison with 1996 and all other years R=1, $p<0.01$). 2001 was not significantly different to 2000 or 2005 (One way ANOSIM pairwise comparison, $p>0.05$). In CMT7 several years had quite distinct benthic communities (One way ANOSIM (years) R=0.622, $p<0.001$). Only 1993 and 2004 were not found to be significantly different from each other. At IBH the benthic communities in all years were found to have significant differences from all other years (One way ANOSIM (years) R=0.667, $p<0.001$). LIS showed the least differences between years of all sites (One way ANOSIM (years) R=0.406, $p<0.001$). 2003 and 2005 were the only years to show significant differences to all other years. At KH all years showed significant differences from all other years with each year having fairly distinct benthic communities (One way ANOSIM (years) R=0.678, $p<0.001$). Differences between years were also low at KC compared to other sites (One way ANOSIM (years) R=0.447, $p<0.001$), however, 2002 and 2005 were the only years to show no significant differences; all other years were significantly different from each other. At RA differences were found between most years (One way ANOSIM (years) R=0.719, $p<0.001$). No difference was found between 1994 and 1995. 1979 had a markedly different benthic community from other years at this site and the greatest differences were found between 1979 with all other years and between 1991 with 1996 and 1999.

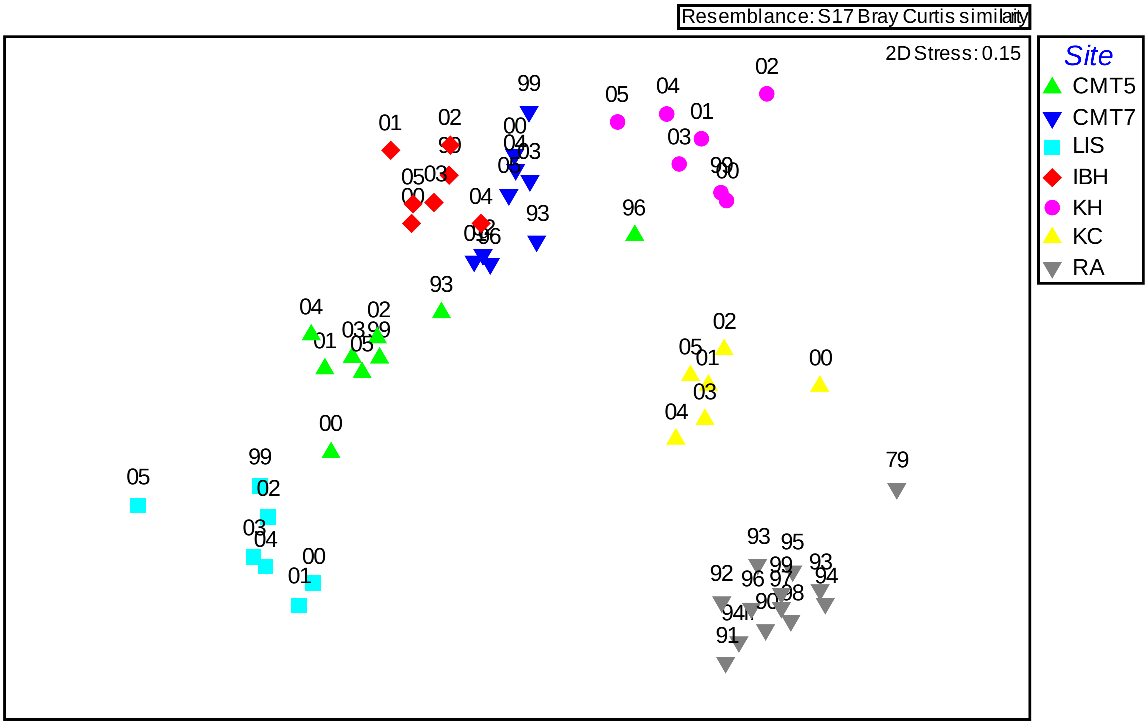


Figure 2.2 Multidimensional scaling of species abundances between sites and years. Numbers refer to year of sampling. Data averaged over replicates (for numbers of replicates see Table 2.2)

Quality classifications at different sites depended on the index used (Table 2.4). AMBI indicated good quality at all sites. However, other indices revealed a greater range of quality classifications. Significant differences were found between sites according to all indices tested (Kruskal-Wallis, $p<0.001$) apart from variation in taxonomic distinctness (Lambda+) (Kruskal-Wallis, $p>0.05$). Lismore appeared to have the worst quality overall while KH and IBH appeared to have the best quality.

Table 2.4 Quality classifications based on average index values across years as determined by five benthic indices at different sites

Site	IQI	BQI	BOPA	AMBI	ITI
CMT5	Good	Moderate	High	Good	Normal
CMT7	Good	Good	Good	Good	Normal
LIS	Moderate	Bad	Moderate	Good	Changed
IBH	High	Good	High	Good	Normal
KH	High	Good	High	Good	Normal
KC	Good	Moderate	High	Good	Changed
RA	Good	Poor	High	Good	Changed

Temporal trends according to different indices at the sites were investigated using Pearson product moment correlation (Table 2.5). Indices showed variable capacities to detect monotonic trends at the sites. Overall, CMT5, LIS, IBH and RA decreased in quality over time. KH and KC increased in quality and CMT7 showed no change. However, within one site some indices indicated an increase in quality while others indicated a decrease. For example, in CMT5 and LIS, evenness (J') and taxonomic distinctness (Delta*) increased indicating an increase in quality while other indices detected a decrease in quality. In CMT7, LIS and KC AMBI (which decreases with increasing quality) detected a trend opposite to that of other indices at the same sites. At IBH ITI, BOPA, average taxonomic distinctness (Delta+) and AMBI indicated increasing quality while other trends in indices indicated a decrease in quality. BOPA (which decreases with increasing quality) also indicated an opposite trend to other indices at the sites KC and RA.

Table 2.5 Pearson product moment correlations between index values and year at different sites with percentage correlation, *r*. Darker colours indicate a stronger relationship.

	CMT5	CMT7	LIS	IBH	KH	KC	RA
S	-47.3	7.1	-53.4	-29.0	66.2	44.0	-48.2
N	-55.7	6.4	-44.9	-25.4	13.9	14.0	-61.3
d	-41.4	7.5	-36.4	-26.8	73.4	51.6	-38.2
J'	33.3	16.0	40.8	-32.9	35.2	58.1	14.9
Brillouin	-48.7	17.1	-52.0	-42.6	54.0	62.7	-27.3
Fisher	-17.5	6.1	6.8	-22.4	73.1	55.7	-28.4
ES(50)	-43.1	9.3	-59.3	-27.3	55.8	64.2	-26.7
H'(loge)	-36.6	16.5	-32.0	-40.4	52.1	65.6	-21.9
Simpson	-7.1	27.0	10.9	-40.2	35.8	61.3	-3.1
N1	-34.8	9.6	-28.2	-39.7	60.1	66.6	-30.4
IQI	-32.0	0.7	23.2	-6.5	57.2	12.4	-9.6
EQR	-6.2	3.7	2.1	42.3	37.2	13.4	-0.4
ITI	10.0	40.2	-20.0	34.8	21.5	-39.3	-19.4
BOPA	-3.3	3.3	-24.7	-61.5	0.8	25.3	-79.3
A/S	-51.7	-1.7	-45.6	-8.2	-17.1	-44.5	-38.6
Delta	6.0	8.3	32.6	-25.2	34.1	45.2	-4.1
Delta *	27.2	-20.3	40.2	-0.1	-27.4	-61.5	-0.4
Delta +	15.3	-12.9	11.7	44.7	64.8	-40.4	11.6
sDelta +	-46.2	5.7	-48.7	-25.5	68.3	43.5	-42.9
Lambda +	-6.3	23.9	-44.7	-24.4	-67.6	39.7	-8.8
AMBI	7.0	34.3	-44.7	-65.0	3.2	66.7	-13.3
BQI	-55.4	20.6	-42.8	-17.6	71.2	53.0	-64.1
MAMBI	-40.9	1.8	5.4	-19.3	67.6	42.9	-35.2
Total biomass	-23.9	-26.1	5.6	35.0	-9.2	4.1	

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Note: A/S, BOPA and AMBI index values have inverse relationships with quality

Environmental variables median phi, the silt/clay fraction and organic carbon were found to vary over time at the sites (Figs 2.3-2.5).

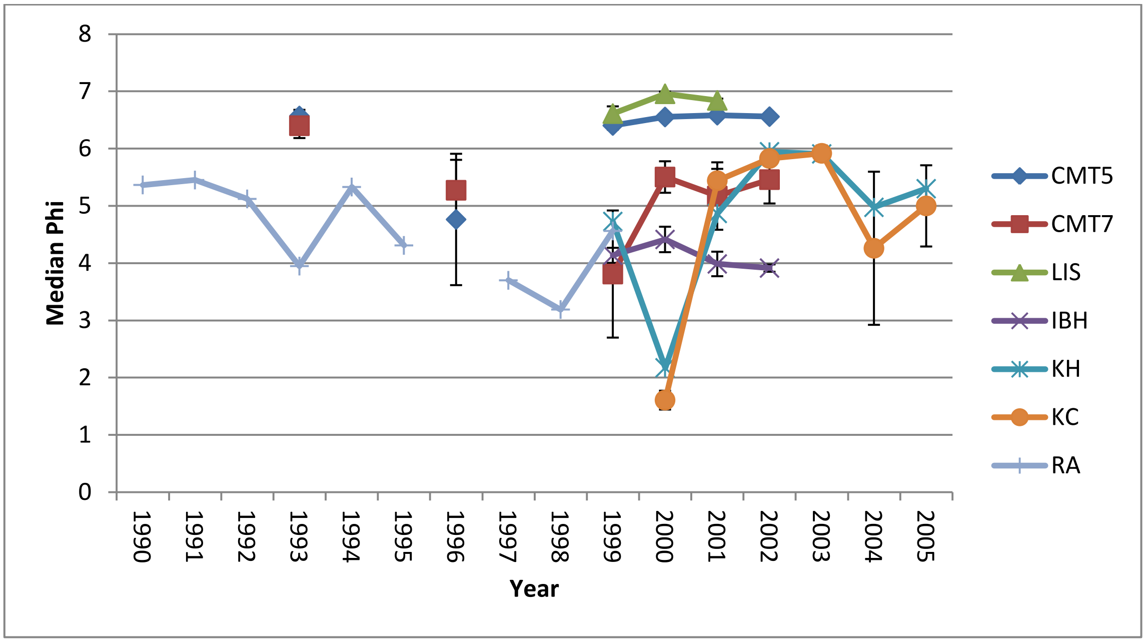


Figure 2.3 Mean median phi values with standard deviation over time at each site (n values vary due to missing data; for RA only mean values available; see Appendix 8.6 for data)

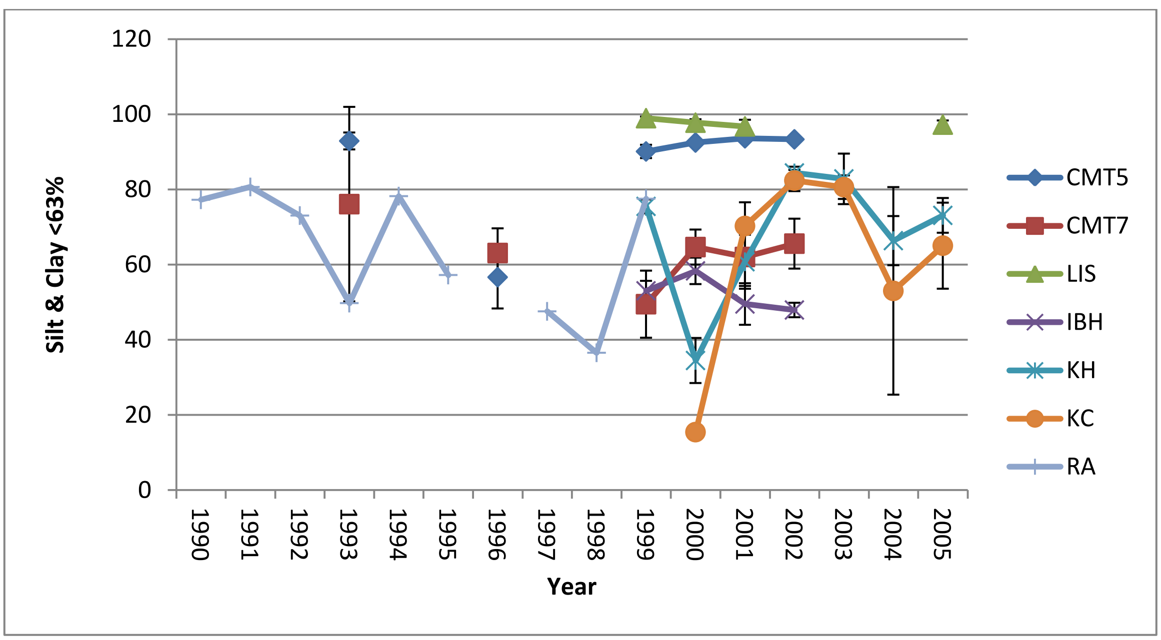


Figure 2.4 Mean silt/clay fraction with standard deviation over time at each site (n values vary due to missing data; for RA only mean values available; see Appendix 8.6 for data)

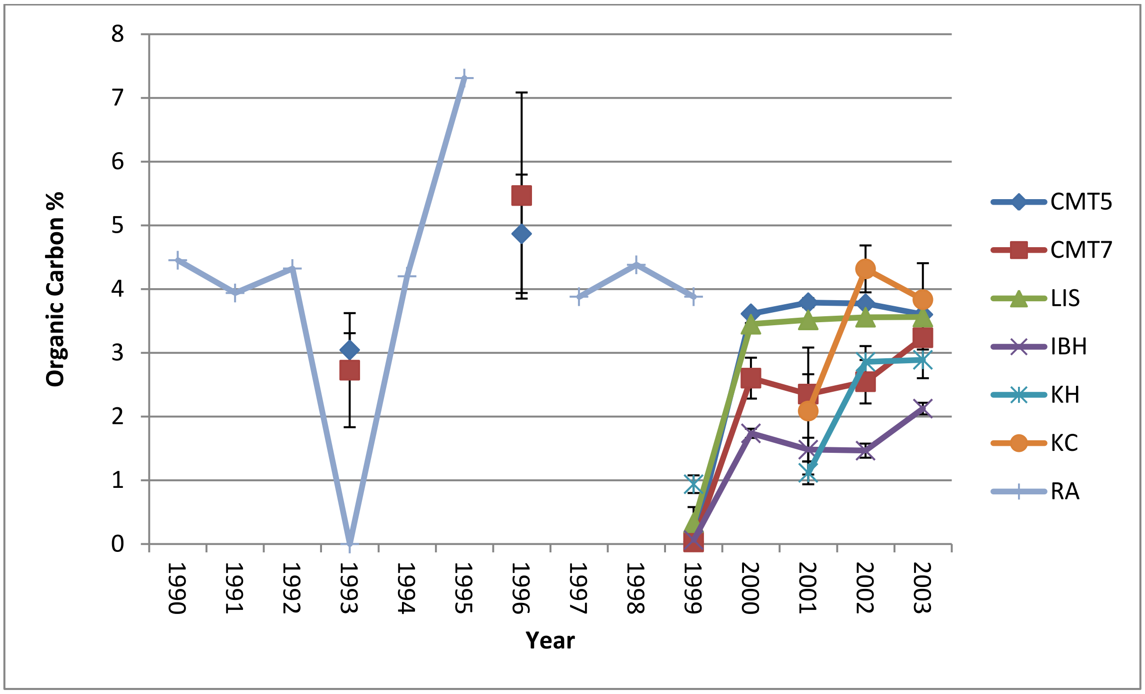


Figure 2.5 Mean organic carbon with standard deviation over time at each site (n values vary due to missing data; for RA only mean values available; see Appendix 8.6 for data)

The correlations between indices and environmental variables revealed mainly weak relationships (Table 2.6). The strongest correlation with an index and an environmental variable was found between BOPA and depth which indicated a lower quality classification by BOPA at deeper sites.

Table 2.6 Pearson product moment correlations between all indices and environmental variables with percentage correlation, *r*. Darker colours indicate a stronger relationship.

	Median Phi	Silt/Clay Fraction	Organic Carbon (%)	Depth (m)
S	-5.2	-13.7	-35.9	-7.3
N	-6.9	-10.3	-28.3	-7.9
d	-2.4	-12.1	-36.1	-5.3
J'	10.5	10.2	9.7	13.0
Brillouin	-4.1	-19.6	-31.6	-8.1
Fisher	3.5	-5.9	-32.5	2.6
ES50	0.3	-14.6	-31.4	-8.0
H' (loge)	0.4	-14.8	-29.6	-4.0
Simpson	6.7	-3.5	-12.1	7.7
N1	-0.5	-11.9	-27.9	-1.9
IQI	-23.8	-31.5	-32.2	-31.3
EQR	-23.2	-32.1	-21.6	-36.6
ITI	1.0	-5.3	-39.8	32.9
BOPA	34.5	35.1	6.2	63.5
A/S	-19.7	-22.0	-18.6	-15.8
Delta	4.6	-7.7	-23.8	15.8
Delta*	-5.1	-12.7	-26.2	14.6
Delta+	-1.7	-13.8	-22.8	22.9
sDelta+	-5.2	-14.1	-36.6	-5.5
Lambda+	15.7	12.7	-0.8	-3.2
AMBI	33.0	31.3	12.0	48.2
BQI	-23.2	-31.2	-27.7	-20.3
MAMBI	-19.3	-28.8	-18.1	-42.4
Total biomass	7.9	7.1	-1.5	5.6
Median Phi		91.9	13.7	49.1
Silt/Clay Fraction			13.9	40.6
Organic Carbon (%)				-19.0

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

2.2.4 Discussion

The sites used in this analysis are part of the National Marine Monitoring Programme (NMMP) which is a programme which aims to study long term trends in British waters. All of the sites have silty sediment – ranging from coarse to fine, but as none of the sites are sandy the benthic assemblages are probably relatively similar compared to other studies or sites which may include coarser grained sediment types. There are a range of depths included however, with the shallowest being 5m and the deepest 109m. Sites are located in areas which represent background levels and are not impacted by point sources of pollution or direct pressures. However, impacts due to diffuse pollution or fishing pressure cannot be controlled for and therefore may impact on the sites. In addition there may be global scale impacts such as climate change impacting these sites.

Therefore, these factors must be considered when interpreting variation in this data as natural background variation.

As has been found in several other studies (e.g. Labrune et al, 2005; Quintino et al, 2006; Dauvin et al, 2007; Chaino et al, 2007; Blanchet et al, 2008; Afli et al, 2008) quality classification depended on the index used, although many indices did agree with each other in some sites (Table 2.4). Interestingly, AMBI classified all sites as good while other indices found a greater range of qualities to attribute to these sites. It was expected that all the sites would have at least good quality as there are no known impacts. However, there appeared to be a gradient of quality amongst the sites with KH, CMT7 and IBH having the best quality, CMT5, KC and RA having worse quality and LIS having the least good quality.

The sensitivity of indices in detecting monotonic temporal trends in quality was tested with correlation and inconsistencies between the indices were found. Some indices detected trends while others did not. Furthermore, some indices detected opposing trends at the same sites. Thus it was not clear if trends detected were actual environmental trends or natural changes in the benthic community. Frid et al (2009) found species richness and abundance were not able to detect temporal trends but found multivariate analysis did. Therefore the correlations and multivariate analysis needed to be considered together to try to determine if trends were real or not. A further problem is whether these trends represented a change in quality or a natural change in community composition and structure.

In CMT5, the multivariate analysis did not indicate a clear trend in any one direction over time. Only data from 1996 were markedly different from any other year. This coincided with a difference in sediment properties at this site with an increase in organic carbon, a decrease in median grain size and an increase in the silt/clay fraction compared to other years (Figs 2.3-2.5). ANOSIM analysis suggested 2001 was not significantly different from 2000 or 2005 while all other years were found to have significantly different community composition. However, many indices detected a trend in quality over time. This could be explained by a decrease in species richness and a decrease in species abundance over time and this being detected by indices sensitive to species richness and abundance. This would also explain the increase in evenness. However, taxonomic distinctness (Delta*) increased over time, indicating that while the community was less species rich, it may not have been less diverse taxonomically. Furthermore, indices based on ecological groups like AMBI, BOPA and ITI found no trend and these results would support the multivariate analysis. Nevertheless, taking into account sediment properties, from 2000 to 2004, this site had a consistent, higher level of organic carbon than at many of the other sites. As the indices also suggested the quality to be lower at CMT5 than some other sites (Table 2.4), the site may be under stress and this could lead to a very gradual decline in quality, as detected by some indices such as species richness, which would be difficult to distinguish from natural background variability.

In CMT7, no trend over time was obvious from the multivariate analysis. While several years' data showed distinct communities, there was no obvious trend in any one direction. Correlation of the indices with time supported this as no strong trends in quality were found. ITI and AMBI detected the strongest trends. ITI detected an increase in quality at this site, while AMBI detected a decrease in quality. Since there appeared to be no apparent trend at this site, it implied that these indices were sensitive to some species level changes in the community. For AMBI this change was due partly to a particularly good sample from 1993 and a worse than usual sample from 2004, and partly due to a slight increase in the proportion of Group III species coinciding with a decrease in Group II species from 1999 onwards. ITI detected an increase in quality due to an increase in the proportion of suspension feeders and a decrease in surface detritus feeders over time from 2000 onwards. The communities at CMT7 and CMT5 were found to be most similar to each other. This could largely be due to geographical proximity of these sites as they are both located along a transect in the Clyde. The organic carbon content at CMT7 was elevated in 1996, although the benthic community was not found to be particularly different in this year compared to other years. In subsequent years the organic carbon content was variable, but lower than levels at CMT5. The general lack of detection of trends in quality by indices is encouraging and suggests the indices were not responding to background variability.

In LIS the multivariate analysis potentially revealed an overall unidirectional trend going from 1999 to 2005. There was a decrease in species richness and abundance from 2002 onwards and this was detected by some indices which are sensitive to species richness and abundance as a decrease in quality. Similarly to CMT5, evenness and taxonomic distinctness increased at this time. In addition, quality according to AMBI increased. AMBI increased because the proportion of Group III species gradually became replaced by the proportion of Group II species. Over time the proportion of Group I species also increased although the proportions of Group IV and V species remained at a fairly high proportion throughout. Although species richness was low (8 was the maximum found in any one sample during the last three years of sampling), very sensitive species persisted in the community during this time. These species included *Glycera rouxi*, *Chaetoderma nitulum*, *Calocaris macandreae* and *Amphiura chaijei*. *G. rouxi* and *C. macandreae* are in AMBI group II and have been found to be highly intolerant of heavy metals (MarLIN, 2011). Other species of *Chaetoderma* sp. have been shown to be sensitive, for example *C. edule* is intolerant of industrial pollution, low oxygen, increased temperatures, hydrocarbon contamination, heavy metals, synthetic chemicals and physical disturbance (MarLIN, 2011). *A. chaijei* is also very sensitive, in AMBI group II, and is highly intolerant of anoxia, heavy metals, physical disturbance, hydrocarbons, decreased salinity and nutrients (MarLIN, 2011). All AMBI Group I species which were present in 2004 and some earlier years were absent in 2005. Despite being a sparsely populated site, the presence of such highly intolerant species suggested the site was not impacted. However, even though the site had relatively low species richness over this whole sampling period, this did further decrease over time and indices which suggested an increase in quality were perhaps misleading in this case. AMBI has been previously found to be unsuitable as an index in sites with low abundance or species number (Muniz et al., 2005).

In IBH multivariate analysis did not suggest a trend in any one direction over time. Some diversity indices detected a decrease in quality while the indices based on other aspects such as ITI, BOPA, average taxonomic distinctness (Delta +) and AMBI detected an increase in quality (Table 2.5). At this site there was a gradual decrease in

AMBI Group III and IV species and an increase in Group II species. At the same time there was a small decrease in species richness and abundance which may account for the diversity indices decreasing in quality. IBH had lower organic carbon than the other sites studied and the best overall quality. IBH was more similar to CMT5 than to any other site and this may have been related to geographical proximity as CMT5 is the site located closest to IBH. The inconsistency of indices in detecting an increase or decrease in quality makes it difficult to interpret whether there has been a change in quality at this site and in what direction, or whether indices which detected a change were responding to natural community changes.

In KH, multivariate analysis may have indicated a trend over time from 1999 to 2005. Many indices found trends at this site with diversity indices detecting an increase in quality but other indices such as taxonomic distinctness (Delta*) and variation in taxonomic distinctness (Lambda+) detecting a decrease in quality, while BOPA and AMBI detected no change in quality. Over time the proportion of AMBI Group III and IV species increased while the proportion of Group II species decreased. However, the proportion of Group I species also increased leading to no trend in quality detected by AMBI over time. The shift in species composition was reflected by ANOSIM analysis which showed significant differences between all years. This suggests overall there may have been increasing quality at this site which was reflected in the MDS.

In KC multivariate analysis did not indicate any clear trend in a consistent direction. Most indices detected an increase in quality at this site, although ITI, BOPA, AMBI, Delta* and Delta+ all detected a decrease in quality. Overall the combined proportion of AMBI Group I and II species declined while the proportion of Group III, IV and V species increased. ITI decreased as suspension feeders decreased and surface and sub-surface detritus feeders increased. The change in trophic, ecological and taxonomic diversity detected by these indices may suggest a decrease in quality which was not detected by indices based on species richness and abundance.

RA showed a markedly different community in 1979 compared to the rest of the years analysed while no clear trend in any direction could be detected amongst other years from multivariate analysis. Most indices such as species richness and abundance detected a decrease in quality over time; although BOPA indicated an increasing quality trend. BOPA was not the most useful index at this site however, as most results were void due to a lack of both opportunistic polychaetes and amphipods, which are the key groups for the index calculation. However, there was not an overall monotonic trend in quality over the whole time period studied. For example, ITI found high quality in 1979 which at first decreased but then increased from 1991 onwards and taxonomic distinctness (Delta*) found a similar pattern.

Kröncke & Reiss (2010) found species richness and univariate diversity indices as well as multimetric indices such as IQI and m-AMBI detected a long term trend while AMBI and BOPA (which include no measure of species richness) did not. However, Frid et al. (2009) found univariate indices did not detect a temporal trend while multivariate ordination did. Most indices in this study did detect trends in quality. However, attributing changes in benthic communities and trends of quality according to indices to either disturbance or natural variability was difficult if not impossible with the data available. Several issues were apparent. Firstly, although trends were detected by indices, in most sites there were no clear directional trends according to MDS. This made distinguishing real trends from background variability difficult. Furthermore, in some cases a trend was masked or created by a particularly good or bad sample. This may suggest the timescale over which data were available for most sites was not sufficient to reliably determine trends, if present. Sites may not have shown a linear trend but changes may have occurred in other directions which may be natural or due to stress. Longer term datasets may allow the magnitude of natural changes in the community to become apparent. Additionally, in several sites indices were in disagreement about the direction of the change in quality. One overall pattern to emerge was that indices closely related to diversity and indices related to other aspects such as ecological or functional groups often detected opposing trends. This occurred in the sites LIS, IBH and KC. This is also potentially relevant to the IQI which found lower trends at most sites compared to other indices. As this index combines species richness and AMBI, the two parts of the index may have cancelled each other out resulting in no trend being detected. This combination could be considered to be masking changes in the system or it could be considered to be reducing the influence of natural variability in the system. Species richness can initially increase in response to disturbance (Connell, 1978, Pearson & Rosenberg, 1978, Odum, 1985, Dodson et al., 2000, Mittelback et al., 2001, Hooper et al., 2005). This may explain the opposing trend of increased species diversity and decreased quality according to other indices such as AMBI. High diversity may be a product of recurrent disturbances and changes in condition, which could lead to diversity increasing but quality according to AMBI decreasing. It may also explain a decrease in species richness with an increase in quality according to other indices – AMBI, BOPA, ITI, taxonomic distinctness (Delta*) – as the system tending towards equilibrium. Multivariate analysis of the benthic communities certainly supports the idea that the systems have regularly changing conditions. This would imply that species richness and indices sensitive to species richness should not be considered in a straightforward way when interpreting quality as an increase or decrease may not positively correlate with quality.

IQI, EQR, taxonomic diversity (Delta) and total biomass all found lower trends than other indices overall. If these sites are considered to be varying at a background level and not due to anthropogenic disturbance, this would suggest these indices are less sensitive to noisy data. However, this could also mean that these indices are not sensitive enough to detect trends. Detecting small trends is important so that the health of the ecosystem does not reach a point where recovery is very difficult or impossible (Tett et al., 2007). Multivariate analysis did show that changes occurred over time at many of the sites, but these variations may have been natural changes in the population rather than reflective of changes in quality. Even when using the combined knowledge of the index results and multivariate analysis it was still unclear whether changes reflected changes in quality. Benthic communities have been found to remain relatively stable for six to ten years before switching quickly to new community types (Frid et al., 2009). Data in this study spanned at most nine consecutive years and therefore the indicated state of these benthic communities is out of context of their long term natural variability. A greater time span of data may clarify the extent of natural variability within each site and whether indices are responding to this or to increasing or decreasing quality.

Although there was some evidence from multivariate analysis of the benthic communities being influenced by sediment variables e.g. CMT5 1996 data, it was difficult to draw conclusions due to the amount of missing environmental data. Studies have found strong relationships between environmental variables such as organic carbon and sediment with index quality classification (Blanchet et al., 2008, Teixeira et al., 2008a, Bouchet and Sauriau, 2008). Nickell et al. (2009) found correlation between indices and organic carbon at one site but not another. Only weak relationships were found in this study. This may be due to the sites being relatively similar environmentally; to only sites of relatively good quality being used in the analysis; to missing environmental data; or to nonlinear relationships between indices and environmental variables. Different species have been shown to respond in nonlinear ways to sediment gradients (Anderson, 2008) and indices may also reflect these nonlinear relationships. The only strong correlation found between an index and an environmental variable was between BOPA and depth. BOPA gave lower quality classification to deeper sites. However, this seems unrelated to actual quality as CMT7, the second deepest site, was one of the best quality sites.

2.2.5 Conclusion

There was greater variability between sites than within sites with each site having a distinct benthic community (Fig. 2.2) and indices showed different values between all sites. However, this was not necessarily translated to a difference in quality classification between sites (Table 2.4), although, out of five indices which assign quality categories, all but one, AMBI, showed different quality classifications between sites. These classifications included quality classifications of lower than ‘good’ quality despite sites being reference sites. The differences in sites may be due to a range of factors. Indices showed only low correlations to measured physico-chemical variables. However due to the limited environmental attribute data available it was not possible to determine which factors affected the benthic communities most strongly. Despite the similarity of many indices to each other, they nevertheless behaved differently in different analyses and indices did not all concur. Indices showed variable responses to temporal variation with different strengths of trends and opposing trends found, showing indices were responding differently to the same environmental variability. The different outcomes may be an indication of sensitivity to natural variability or to lack of sensitivity to changes in quality.

The most important criterion of an index is to distinguish impacted and unimpacted sites. None of these sites studied were impacted and yet these sites may be more representative of sites which could potentially fall between the ‘good’ and ‘moderate’ categories of the WFD. It is important to know the limitations of the indices when interpreting the assigned qualities. These results reinforce the need to use a range of methods in assessment of benthic health or risk misinterpretation of index outcomes. However, the distinction between natural and anthropogenic disturbance remains unresolved. It is likely that using reference conditions would be the most suitable method but knowledge and accessibility of reference conditions for diverse benthic communities makes this impossible in most circumstances.

2.3 Index Correlations

2.3.1 Introduction

Although many indices that can be used in the assessment of benthic community condition exist and are in use, these are based, primarily on the same raw data – species composition. Manipulations of these data differentiate the individual indices into broad groups based on diversity, evenness, ecological or functional groups, sensitivity/tolerance to pressures and taxonomic diversity. Interpretation of index results is largely based on the Pearson and Rosenberg (1978) theory on the response of benthic invertebrates to disturbance. Most authors agree that a range of indices and tools should be used in the assessment of ecosystem health, rather than relying on a single index (Albayrak et al., 2006, Salas et al., 2006, Afli et al., 2008, Bakalem et al., 2009, Borja et al., 2009, Nickell et al., 2009, Borja et al., 2011). However, studies have found indices to perform in different ways under different circumstances (e.g. Labrune et al., 2006, Blanchet et al., 2008). Some studies assign indices into groups based on underlying focus and the aspects of the community that they measure. For example, Bakalem et al. (2009) divides indices used into the groups: ecological, including AMBI and BOPA; trophic, including ITI; diversity, including Shannon Index; and combined indices, focussing on multiple aspects, including m-AMBI. These authors recommend that one index from each index group should be used in the assessment of community status. Similarly, Chaino et al. (2008) recommends, in the case of highly correlated indices, only one should be used as they are likely to contribute the same information. Different indices should be considered as complementary rather than equivalent and furthermore, indices should not be intercalibrated since they often focus on different aspects of the community and use the data in different ways (Bakalem et al., 2009). On the other hand, Blanchet et al. (2008) found using several indices which classified sites differently simply confounded matters making their interpretation and assessment of site quality more difficult. Most studies test the performance of indices by comparing impacted and unimpacted sites (e.g. Labrune et al., 2006). Thus, despite the fact that indices may be measuring different aspects of the community, in theory they should all be indicating quality and should reflect that in being somewhat correlated.

Many studies have investigated the correlations between indices (Quintino et al., 2006, Salas et al., 2006, Labrune et al., 2006, Pranovi et al., 2007, Borja et al., 2007, Dauvin et al., 2007, Bakalem et al., 2009, Bigot et al., 2008, Blanchet et al., 2008, Chainho et al., 2008, Munari and Mistri, 2008, Teixeira et al., 2008a, Teixeira et al., 2008b, Nickell et al., 2009). It is expected that indices would correlate according to quality classifications and into groups based on theoretical groups such as those outlined by Bakalem et al. (2009) (ecological, trophic, diversity and combined, as discussed above). These studies of correlations have included impacted and unimpacted sites and have attempted to identify patterns amongst the correlations. However, different studies find varying patterns of correlation amongst the indices. Most studies have found most indices to be correlated to each other to some degree. Teixeira et al. (2008b) found correlations between H', d (Margalef) and S (number of species) but AMBI was not correlated with these, while in the Mondego estuary, Salas et al. (2004) found high correlations between all indices tested (AMBI, H', Margalef, Simpson and W statistic), with the indices giving similar quality evaluations for the system. Munari & Mistri (2008) found strong correlations between the Simpson's index and H' but weak correlations between other indices tested (including AMBI, BOPA, Margalef, and taxonomic distinctness). Pranovi et al. (2007) found correlations between H' and BENTIX but did not find strong correlations between other indices (BOPA, AMBI, taxonomic distinctness and functional feeding groups). In the Indian Ocean, Bigot et al. (2008) found only weak correlations between AMBI, S and H'; and between AMBI and m-AMBI. In Portugal, Chaino et al. (2008) found H' and the Margalef's Index to be highly correlated. Strong correlations were also found between H' and EQR, Margalef and EQR. Nickell et al. (2009) calculated indices for various sample stations next to a cod farm and a salmon farm and their reference stations. They found indices to correlate more with each other in a region of restricted exchange compared to a more open area.

Out of these varied results, some patterns may be gleaned. BOPA and ITI are often found not to correlate to other indices (e.g. Pranovi et al., 2007; Dauvin et al., 2007; Blanchet et al., 2008), although, Nickell et al. (2009) did find ITI to correlate to other indices. These results indicate the high variability inherent in benthic data and the response of indices to this variability. Furthermore, it shows, that indices which could be placed into the same theoretical groups, such as ecological – AMBI and BOPA, do not necessarily correlate, depending on the dataset and this complicates the choice of a set of indices which can reflect all aspects of the benthic community.

Bustos-Baez & Frid (2003) showed that many purported 'indicator' taxa do not correspond in a consistent and predictable way to organic enrichment in different sites and studies. Of 123 taxa they identified in the literature as indicator species, only 20 of these responded consistently across several studies. Furthermore, the same authors found that no index tested, based on indicator taxa, performed better than using either species richness or abundance. Indices are mainly based on the theoretical predictable response of the benthos to disturbance but the evidence indicates that this response is not entirely predictable. This is particularly pertinent with indices, such as AMBI, which are based on the classification of species into sensitive or tolerant groups. Labrune et al. (2006) pointed out that the sedentary polychaete species *Ditrupa arietina* is classified as sensitive in AMBI but tolerant in BENTIX and that under different types of disturbance some species may become dominant while others do not. Even though indices respond to disturbance in different ways, all should give an indication of quality if they are fit for purpose. Most studies finally resort to some level of subjectivity when concluding which indices to use and which worked well and which did not. This may be due to preference (e.g. Bakalem et al., 2009) or because the index is widely used (e.g. Chaino et al., 2008).

This study will assess relationships between indices derived from a dataset which is made up of mainly undisturbed sites (depending on the index used to measure quality) and therefore should not indicate (man-made) disturbed conditions. A pollution gradient is likely to influence correlation results as some indices perform differently in response to an impact. Index correlations should therefore show relationships according to which aspects of benthic community the index is measuring. If disturbance data were included, the indices would respond to this overriding trend of disturbance and it would not be clear if they were correlated to each other or if they were only correlated in bad conditions under which all aspects of the community were degraded. In less degraded conditions different aspects of the community may respond at different rates or in different ways to disturbance and this may be important for index selection. Since indices are based on the same data and many are derived from the same ecological theories (species diversity, evenness and the Pearson-Rosenberg pollution response), it may be that many indices are effectively redundant as they are providing the same information as other indices, but this may depend on location and stress type. If the indices are not highly correlated this may suggest that these indices are measuring different aspects of the ecosystem. If correlations between indices can be found, this could be used in the subsequent choice of indices which should be used in further studies. A suite of indices which are measuring different aspects of the benthic communities could be chosen as opposed to a few arbitrarily chosen indices which are effectively measuring the same properties or discounting the performance of an index as it does not match the performance of a subjectively favoured index.

Aims

To evaluate the performance of widely used community indices by assessing their inter-relationships when applied to undisturbed conditions. In this context it will be determined if indices perform according to theoretical groups of what community aspects they measure.

Null Hypothesis

1. There will be no evident patterns of correlation of indices which could be attributed to the theoretical basis of the index.

2.3.2 Methods

Theoretical groups of indices were compiled using information from Ruellet and Dauvin (2007) (Table 2.7). Additionally, other indices were added to the appropriate groups. *Ecological groups* include those indices which assign species into groups according to sensitivity or tolerance to disturbance. *Trophic groups* (ITI only) places species into groups according to functional feeding groups. *Diversity* covers the breadth of indices which measure taxonomic richness and evenness. *Combined indices* are those which are multimetric, including more than one type of index and incorporating more than one aspect of benthic communities.

Table 2.7 Theoretical groups of associated indices based on underlying focus (adapted from Ruellet & Dauvin, 2007)

Index Type	**Index**
Ecological Groups	AMBI, BOPA
Trophic Groups	ITI
Diversity	H', BQI, S, N, Fisher, Taxonomic measures (Δ, Δ^*, Δ^+, S.Δ^+, Λ^+), A/S, d, ES(50), Brillouin, J', Simpson, N1
Combined Indices	m-AMBI, IQI, EQR

Data from NMMP sites were used to calculate index results and subsequently assess relationships, giving a total of 296 observations (Table 2.8). Pairwise correlations (Pearson product moment correlation) were carried out between each index to examine the relationship between different indices. Principal component analysis was also carried out between indices using the same data. Analyses were carried out using Minitab 15.

Table 2.8 Details of data used for index correlation. Number of replicates for each year was 5, except where indicated: *n=9, †=3; for details of data used see Table 2.2

Site	Year
CMT5	1993*, 1996, 1999-2005
CMT7	1993*, 1996, 1999-2005
LIS	1999-2005
IBH	1999-2005
KH	1999-2005
KC	2000-2005
RA	1979†, 1990-1992, 1993*†, 1994, 1994†, 1995, 1996†, 1997-1999

2.3.3 Results

Some indices were found to be highly correlated with each other (Table 2.9). Most indices correlated to all other indices to some degree, even if correlations were weak. PCA reflected results found in the correlation (Fig. 2.6). The first principal component had variance (eigenvalue) of 11.79 and accounted for 49.1% of the total variance. The second and third axes eigenvalues were 3.93 and 2.27 and accounted for 16.4% and 9.4%, respectively, of the variability with the first three axes accounting for 74.9% of the variance. Both methods showed high correlations between A/S, J' and the Simpson's Index (although A/S has an inverse relationship with these indices). These indices were also correlated to abundance (N). BOPA and AMBI were highly correlated to each other and had an inverse relationship with other indices but were correlated to the related multi-metric indices IQI, m-AMBI and EQR. These multi-metric indices were also correlated to the largest group of correlated indices which included S, H', d, Fisher, N1, Brillouin, ES (50) and sDelta+. BQI was also correlated to this group and to the other multi-metric indices, but was not strongly correlated to AMBI. Delta+ and Delta* were correlated to each other while biomass, Lambda+ and ITI showed weak correlations with all other indices.

Table 2.9 Pearson product moment correlations between all indices with percentage correlation, *r*. Darker colours indicate a stronger relationship.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

	S	N	d	J	Brillouin	Fisher	ES50	H'(ln)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta*	Delta+	sDelta+	Lambda+	AMBI	BQI	MAMBI
N	85.7																						
d	98.7	78.0																					
J'	-27.5	-50.6	-17.5																				
Brillouin	79.8	48.1	85.0	14.9																			
Fisher	87.8	61.4	92.9	3.5	79.7																		
ES50	80.9	46.9	87.4	6.7	96.1	84.4																	
H'(ln)	73.9	39.3	81.4	28.9	98.3	81.6	95.3																
Simpson	23.4	-8.8	33.6	77.2	67.7	44.9	56.9	76.8															
N1	78.5	43.6	84.3	23.8	92.6	84.8	92.6	93.3	62.6														
IQI	80.1	62.0	82.0	-13.9	77.4	74.5	77.4	73.7	36.0	69.4													
EQR	54.8	32.1	59.8	13.5	72.3	57.6	68.8	72.6	55.2	61.6	89.7												
ITI	33.4	19.6	37.7	-6.1	38.2	38.3	40.1	38.4	23.7	31.3	36.6	31.3											
BOPA	-38.1	-30.8	-39.2	11.8	-38.8	-33.1	-38.3	-36.3	-16.9	-27.9	-71.5	-73.2	-6.6										
A/S	49.7	76.5	38.0	-73.7	20.0	14.0	14.3	5.0	-37.8	6.7	36.3	11.9	8.4	-23.7									
Delta	44.1	16.0	52.9	51.8	72.9	61.4	65.9	80.1	84.3	67.1	50.1	59.1	41.6	-29.4	-17.3								
Delta*	49.5	44.2	51.5	-11.2	41.9	50.9	43.0	42.5	18.6	36.6	44.9	35.7	43.6	-34.6	24.0	67.8							
Delta+	33.6	24.0	37.1	-1.7	37.9	37.7	38.7	39.5	25.7	32.5	40.0	41.7	52.7	-25.2	8.0	60.0	75.8						
sDelta+	99.9	85.5	98.6	-26.9	79.7	88.0	80.8	74.0	23.7	78.6	80.5	55.6	35.2	-38.6	49.0	45.3	51.2	37.3					
Lambda+	6.9	4.1	8.9	-1.2	13.0	8.6	12.7	14.4	12.4	7.1	7.0	9.2	1.7	-6.8	-1.9	10.3	2.3	6.0	6.1				
AMBI	-30.0	-26.8	-29.3	16.6	-19.0	-25.0	-21.7	-16.6	2.4	-17.2	-71.6	-73.0	-9.0	78.2	-19.0	-6.5	-17.3	-19.7	-31.1	7.7			
BQI	70.1	62.7	69.3	-31.3	65.7	55.1	64.9	58.8	18.1	51.1	73.1	59.3	27.7	-58.8	51.4	39.8	50.7	38.5	70.7	15.0	-39.8		
MAMBI	77.2	56.4	79.4	-0.1	83.4	71.4	80.3	80.0	47.3	75.6	88.4	82.5	15.0	-60.9	30.2	48.3	26.9	21.4	76.6	7.6	-53.4	63.4	
Total Biomass	5.2	1.1	6.2	1.6	7.6	6.0	8.4	7.7	4.5	7.3	3.3	1.0	2.8	-1.7	-0.9	5.7	3.4	2.6	5.3	-1.5	-1.0	6.0	6.9

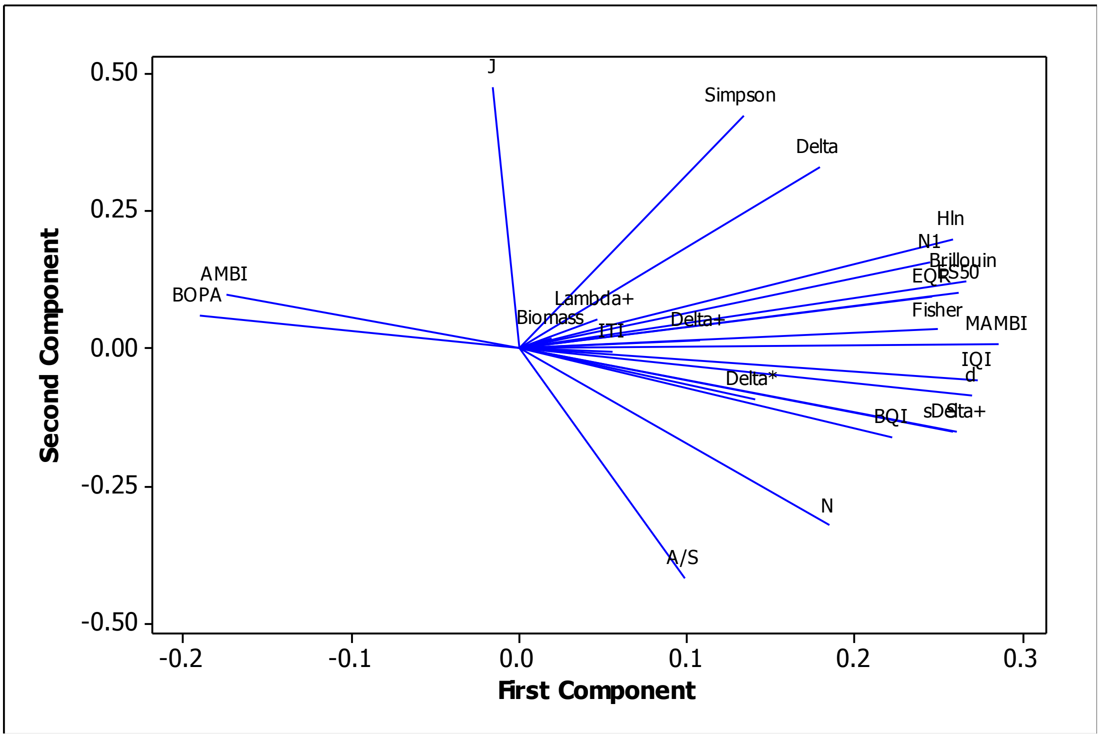


Figure 2.6 Principal component analysis of all index results

Indices could be placed into types according to strong correlations with other indices (Table 2.9 & Fig. 2.6), some of which were consistent with the theoretical groupings of indices (Table 2.7). Although, several indices overlapped more than one group since, overall, there was a gradient of similarity between all indices and while most indices were most strongly correlated to one index type they were also correlated to other index types to varying degrees. Comparing results to theoretical groups of indices (Table 2.7) indicated some apparent groups. In addition further groups were proposed based on both correlation analyses:

1. *Ecological* – AMBI and BOPA. Indices which overlapped with this group included IQI, EQR and to a lesser extent m-AMBI and BQI.
2. *Trophic Groups* – ITI only very weakly correlated to a few other indices.
3. *Diversity (Species Richness)* – The ‘diversity’ group has been subdivided into ‘species richness’ and ‘evenness’. S, d, Brillouin, Fisher, ES (50), H’, N1 and Total Taxonomic Distinctness (sDelta+) were all highly correlated measures of diversity. Other indices which showed high correlations to species richness but also fit into other groups included IQI, m-AMBI and BQI.

4. *Diversity (Evenness)* – A/S, J' and Simpson's Index were all highly correlated to each other, suggesting an additional grouping of indices which mostly measure community evenness. N and Simpson's Index (1-λ') correlated strongly to some but not all species richness indices and A/S and J' did not correlate to species richness measures.
5. *Combined Indices* – m-AMBI, IQI, EQR and BQI were all highly correlated to each other. In addition, these indices were highly correlated to other indices including species richness and the ecological group. This was expected since these indices are derived from AMBI and measures of species richness, apart from BQI, which, like AMBI integrates species sensitivities and tolerances but in a different way. The combined indices showed very low correlations to measures of evenness. This was unexpected as IQI and EQR both include Simpson's index in their calculation.
6. *Taxonomic Diversity* – Taxonomic Distinctness (Delta*) and Average Taxonomic Distinctness (Delta+) were most correlated to each other and weakly correlated to other indices.
7. *Other* – Some indices did not fit clearly into any group. Biomass and Variation in Taxonomic Distinctness (Lambda+) (along with ITI) were the only indices to have little or no correlation to other indices. Taxonomic Diversity (Delta) was correlated with some diversity measures (H' and Brillouin) but not to species richness and was most highly correlated with the Simpson's Index.

2.3.4 Discussion

Most index correlations behaved largely as expected based on the theoretical basis of the indices similar to those outlined by Ruellet and Dauvin (2007). It was expected that the indices derived from ecological groups, AMBI and BOPA, would be correlated as BOPA uses a subset of the species list used in the calculation of AMBI (Dauvin and Ruellet, 2007). This study found these indices to be highly correlated (78%). High correlation between these indices was also found by Bakalem et al. (2009). However, this disagrees with other studies which, unexpectedly, in some cases found no or only weak correlations between AMBI and BOPA (e.g. Munari & Mistri, 2008; Blanchet et al., 2008). Diversity indices could be divided into two groups, those highly correlated to species richness e.g. Shannon-Wiener Index, or those weighted by evenness e.g. Pielou's Index. The taxonomic distinctness measures mainly did not correlate with diversity suggesting they are measuring an additional aspect of the benthic community. Combined indices correlated well with both diversity and ecological groups (measures of which they incorporate). ITI did not correlate strongly with any other index. Other studies also found this to be the case (e.g. Pranovi et al., 2007, Blanchet et al., 2008, Dauvin et al., 2007), however Nickell et al. (2009) found ITI to correlate with AMBI, N, Pielou, Brillouin, H' and Simpson indices. The inconsistency of results across studies highlights the impact of the dataset used in the correlation of the indices.

Overall, according to index correlations in this study, indices correlated well with other indices which measure similar aspects of the benthic community. The proposed grouping of these aspects could be: ecological, trophic, diversity (richness), diversity (evenness) and another tentative group might include some measure of taxonomic distinctness. Multi-metric indices such as IQI, which correlated with both ecological and diversity groups, could be used together with a measure of evenness, trophic structure and taxonomic distinctness as a set of indices which measures most aspects of the benthic community structure. This is in accordance with many authors who recommend the use of several indices (e.g. Bakalem et al., 2009, Borja et al., 2009, Nickell et al., 2009, Borja et al., 2011). It simplifies the choice of indices to use in a study as one index from each group can be used which can more fully assess the different aspects of the benthic community, as opposed to choosing indices which may be highly correlated (Bakalem et al., 2009). However, these groups are relatively arbitrary as while some indices were more strongly related to others, making their concurrent use redundant, there was also a lot of overlap of groups with many indices, and almost all indices were correlated to some degree. Furthermore, as was discussed, index correlations can depend on the dataset used and while results were not unexpected, this analysis would need to be repeated using other reference type datasets or datasets not including disturbance gradients. Other studies which did not find similar patterns to this study may be due to the inclusion of disturbance data and while different indices measure different aspects of the community, all of these aspects may not respond to disturbance in a similar way. Measuring correlations excluding disturbance data shows whether indices are measuring different aspects of the benthic communities while including disturbance data would be showing the response to the disturbance gradient rather than the response to aspects of the benthic community. Indices may show high correlations to each other in disturbed conditions but the indices may not have reached this point through the same path. Nickell et al. (2009) found index performance varied at different stages of an impact. However, the same authors also suggested differences in results may also be due to physical conditions of the environment as they found differences in the correlations between indices in sites with similar levels of impact.

Using several indices could make interpretation of quality more difficult (Blanchet et al., 2008) or the different indices could be considered separately as measuring different aspects of the community (Bakalem et al., 2009). Different indices may not be calibrated against the same disturbance gradient and this leaves the question of how then to interpret several different indices which show a different response to the same disturbance. In this case, it may be that the index indicating the worst quality should be the one taken into account. However, if a station had for example, low quality according to AMBI but high taxonomic distinctness perhaps the station has bad quality but maintains the ability to recover. This station may then be better than one which has low quality according to both AMBI and taxonomic distinctness. In this case, an average quality from the different indices should perhaps be considered. A single index which is broadly applicable in all systems is unrealistic due to the diversity of benthic communities (Borja et al., 2011). It is particularly important to also consider other factors such as the physico-chemical conditions of the study site as these may explain the benthic response and prevent misinterpretation of index results (Borja et al., 2009). The sites considered in this study had similar habitats but were varying in their physical attributes (Table 2.3) and their quality (Table 2.4) although no specific disturbance gradients were apparent. Therefore, correlations were not in response to a disturbance gradient but to different aspects of the communities present under reference conditions. The results of these index correlations may indicate the response of different indices to natural variation in the environment but nevertheless show which indices are responding in the same way to this variability. However, different sites may have a range of types of natural variability and different indices may respond in varied ways to different types of variability, not necessarily in the patterns found in this study.

2.3.5 Conclusion

The correlations found between the indices were mostly found to be consistent with the theoretical groups suggested (Table 2.7), however, additional groups were proposed and some indices did not clearly fit into any group despite the theoretical derivation. Placing indices into groups is subjective although some indices were evidently more strongly correlated than others. These results show that despite the similarity in the underlying focus of many indices, different indices do measure different aspects of the benthic communities. A proposed set of indices to use would be: an ecological group index e.g. AMBI; a trophic group index e.g. ITI; diversity indices of richness e.g. species richness and evenness e.g. Simpson’s Index; and taxonomic diversity e.g. taxonomic distinctness. Alternatively, a combined index e.g. IQI could be used in this set in place of AMBI and species richness. These combinations of indices should measure most aspects of the benthic community structure, although the specific indices used within each group may be chosen as a matter of preference or other factors may determine the best specific index to use in different circumstances. As different aspects of benthic communities may be affected by disturbance in different ways, how indices respond to disturbance gradients may differ and this will be explored in subsequent chapters.

2.4 Sampling Effects

2.4.1 Introduction

Sampling protocol is a variable which can potentially affect the quality classification. Controlled factors in a sampling protocol can include the sample size, number of replicates and mesh size. 500µm and 1000µm mesh sized sieves are generally used in macrobenthic studies. Recently, Pinto et al. (2009) recommended the use of 500µm mesh in order to get a realistic representation of the infaunal benthic community. They found significantly higher densities and taxa number using 500µm mesh sieve compared to 1000µm mesh sieve. Polychaeta and Bivalvia were found to be underestimated using a 1000µm mesh sieve. Schlacher & Wooldridge (1996), sampling in estuarine environments, found 500 and 1000µm mesh size greatly underestimated density compared to 250µm mesh size. Chironomids were not retained at all using the 1000µm mesh. Ferraro & Cole (2004) found greater power to identify differences between habitats using 500µm mesh compared to 1000µm. Time and financial constraints, as well as study objectives, lead researchers and environmental managers to sample in the most efficient way. For example at SEPA, estuaries and disturbance gradients have been sampled with the 500µm mesh sieve and $0.1m^2$ Van Veen grab; NMMP sites have been sampled with 1000µm mesh and $0.1m^2$ Van Veen grab with both species abundance and biomass recorded; and fish farms have been sampled with $0.013m^2$ grab and sieved with 1000µm mesh sieve. As pointed out by Pinto et al. (2009), the 500µm mesh sized sieve collects juvenile specimens as well as small sized species which can be indicators of disturbance. For example in Scotland, *Ophryotrocha hartmanni* is a polychaete worm used as an indicator of organic pollution which would be missed by sampling with a 1000µm mesh size (Myles O'Reilly, pers. comm.). Couto et al (2010) found AMBI assigned worse quality at degraded stations using a finer mesh sieve as this sieve size captured small opportunists. Biomass, along with the 500µm mesh size, is generally considered too time consuming to measure when not completely necessary – due, for example, to a requirement for a national monitoring programme such as the NMMP. These studies indicate the importance of 500µm mesh size when the objective includes specific indicator species such as *O. hartmanni* or when sampling estuarine waters which may include important but smaller species such as Chironomidae in the infaunal assemblages. When sampling there is a trade off with cost and the traded factors may cause uncertainty in quality classifications if it is thought that the sampling protocol used does not correspond to a realistic representation of the benthic assemblage. Furthermore, implementation of the Water Framework Directive has resulted in attempts to create blanket methods for quality classification of water bodies. The UK IQI stipulates the use of $0.1m^2$ Van Veen grab and 1000µm mesh sieve. This makes comparison between water bodies and studies easier but is contrary to the reasoning behind previously used sampling protocols. However, using a 1000µm mesh sieve may be more cost effective (Ferraro and Cole, 1990, Ferraro et al., 1989). Cost effectiveness depends on the study objective and natural variability within the size categories (Ferraro and Cole, 2004). Schlacher & Wooldridge's 1996 study may in fact overestimate the relative losses of species due to sieve size as the 250µm mesh sieve is used as the reference point, but this crosses the meio- macro-fauna boundary. Warwick et al. investigated the effect of various sampling factors, including mesh size, on the results of infaunal community structure (Warwick et al., 2006). A slightly higher diversity according to H' and ES (50) was found using 500µm compared to 1000µm mesh size. However, a major change was found between the meiofauna (<500µm) and the macrofauna (>500µm) but within these categories, assemblages were comparable. They concluded that mesh size may not be such an important factor when sampling within the size classes, meio- or macro-fauna, but extrapolation between the two is not simple. Ferraro et al. (1994) found using a 1000µm mesh and a smaller than standard grab ($0.02m^2$ x 5cm) was sensitive enough to distinguish between impacted and references communities. While some information is lost by using a 1000µm mesh compared to 500µm mesh, the overall impact on a community study is low and it is more cost effective to use the larger sieve when most of the population is captured and when this size fraction behaves in the same way to pollution (Ferraro et al., 1994).

Due to natural variability, it is unlikely a single sampling procedure will be optimal in more than the study it is designed for (Ferraro and Cole, 2004). This has implications for indices such as the IQI and calibration of the IQI needs to be sensitive enough to be able to distinguish differences in many different types of coastal water bodies under different forms of stress. Pinto et al. (2009) found quality classification due to the Benthic Index of Biotic Integrity (B-IBI) differed between sieve sizes by altering the trophic weighting of larger specimens in the 1000μm mesh sieve compared to the finer 500μm mesh sieve as these species became relatively less abundant in the finer sieve. Pinto et al reached the conclusion that this was evidence of the 1000μm mesh sieve obscuring the community structure by assigning a misleading, more dominant role to larger specimens.

Since this study utilises a range of datasets which have been sampled using different mesh sizes, it was an aim to carry out an analysis of data to determine the effect this may have on interpretation of results. Additionally, a range of grab and corer sizes have been used in collecting the various datasets, however, no data existed in this study to directly compare the impact of grab or corer size. Although sampling protocol cannot be optimal for all areas or interpretation of results cannot be extrapolated between sites, the analysis may give some indication of the relative sensitivities of different indices to the mesh size. Although most indices have been developed with abundance in mind, those based on proportions and evenness should produce a comparative value when calculated using biomass. Using biomass rather than abundance may be more representative of the actual relative roles of species in the ecosystem, although the importance of both species abundance and biomass to function are unclear (Bolam et al., 2002).

Aim

The aim is to determine the effect of some aspects of sampling protocol on index results and quality classification.

Null Hypotheses

1. Index values are the same whether the benthos is sampled using 500μm or 1000μm mesh sized sieve.
2. Index values are the same whether the index is calculated using species abundance or species biomass.

2.4.2 Methods

Indices were calculated using data from two sites, a transitional water KC and a coastal water, KH (see Table 2.10 for details). Abundance and biomass data were available for both sites. Both sites were sampled using a $0.1m^2$ VanVeen grab and the benthic invertebrates were separated into two groups by sieving successively through a 1000µm and then a 500µm mesh size. Five replicates in each year were taken. For analysis, the 1000 µm sieved species abundance and biomass data were compared with the combined 500µm and 1000µm sieved species data. As data were not independent (different mesh sizes and species/biomass data came from the same sample) paired t-tests were carried out (using Minitab 15) between indices to test for differences due to mesh size and differences due to indices calculated using abundance or biomass data. Where data were not normal, log transformation was carried out. If data could not be normalised using the log transformation, a Wilcoxon Signed-Rank test was carried out (using SPSS 18).

Table 2.10 Date of sampling expedition for each site

Year	KC	KH
1999	no data	23/03/1999
2000	14/01/2000	24/01/2000
2001	15/02/2001	14/02/2001
2002	20/02/2002	19/02/2002
2003	20/02/2003	26/02/2003
2004	10/02/2004	09/02/2004
2005	01/02/2005	01/02/2005

2.4.3 Results

Graphical displays of the community structure at each site according to both sieve mesh sizes and to both abundance and biomass showed some differences (Figs 2.7-2.10). At both sites, biomass gave a much more similar picture of community structure between sieve mesh sizes while abundance showed some groups were over- or under-estimated in the 1000µm mesh sieve. For example, at KC Polychaeta were overrepresented and Bivalvia were underrepresented proportionally in the 1000µm compared to the 500µm mesh sieve. While at KH the proportional abundance of Bivalvia were again underrepresented and Phoronida were overrepresented in the larger mesh size. Nevertheless, the differences were small and the relative proportions were similar in both sieves.

However, the graphs indicated much greater differences in proportional representation when comparing abundance to biomass. Bivalves were underrepresented and polychaetes were overrepresented in both sites by measuring abundance compared to biomass.

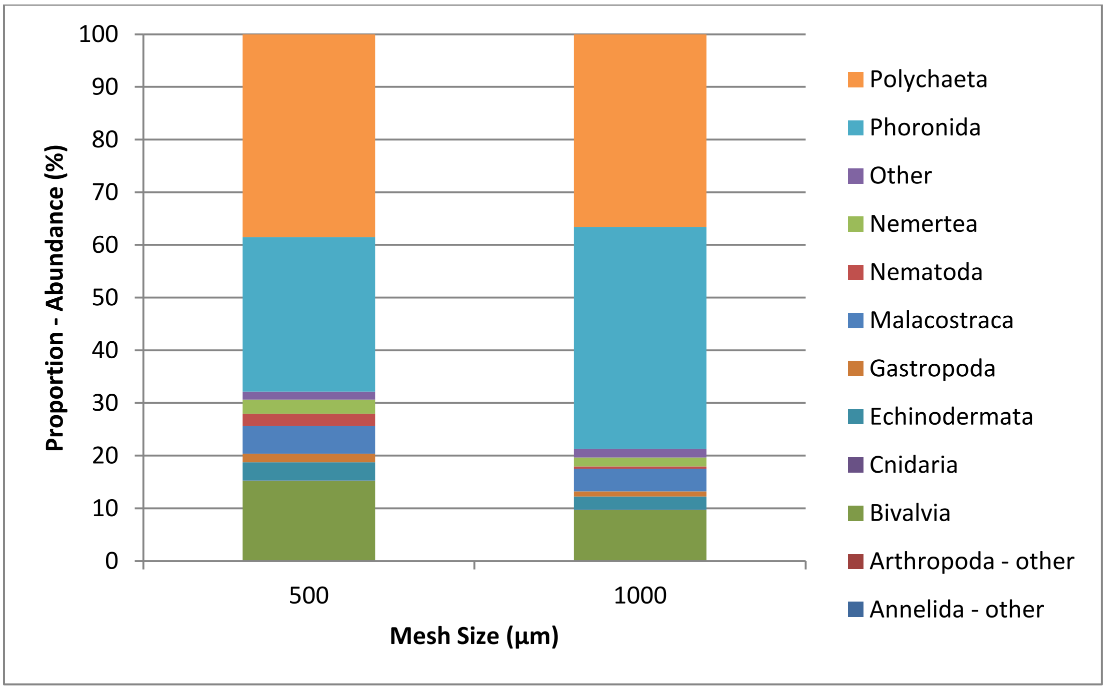


Figure 2.7 Comparison of the proportional abundance of taxonomic groups of the macrobenthic community composition in KH as captured using 500µm and 1000µm mesh sieves. (KH 2000-2004, n=25)

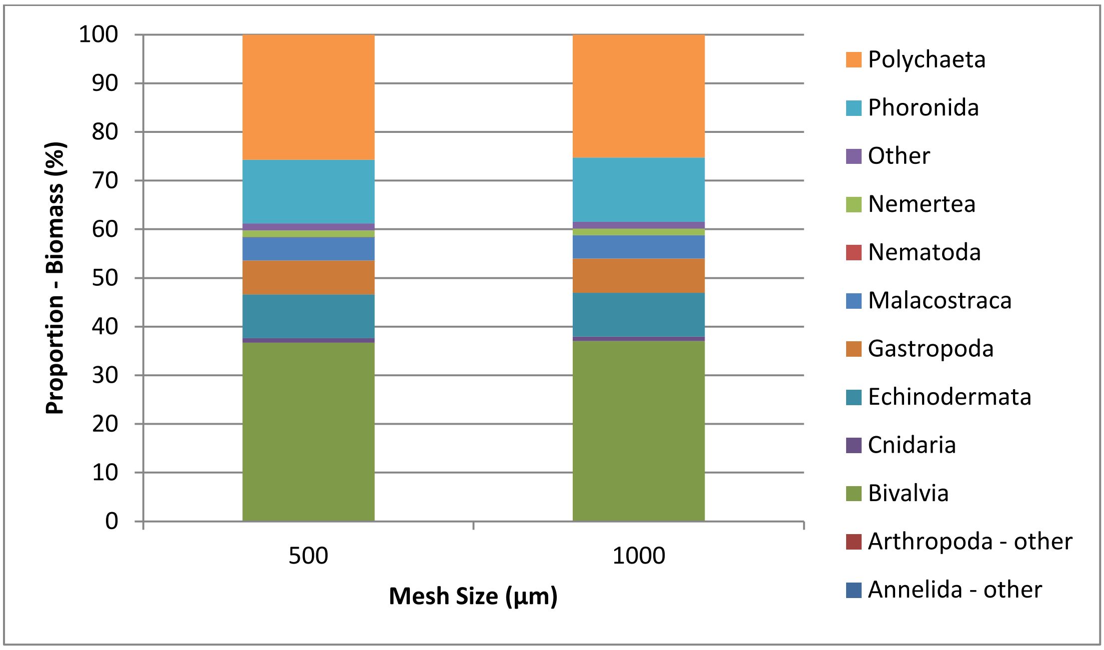


Figure 2.8 Comparison of the proportional biomass of taxonomic groups of the macrobenthic community composition in KH as captured using 500µm and 1000µm mesh sieves. (KH 2000-2004, n=25)

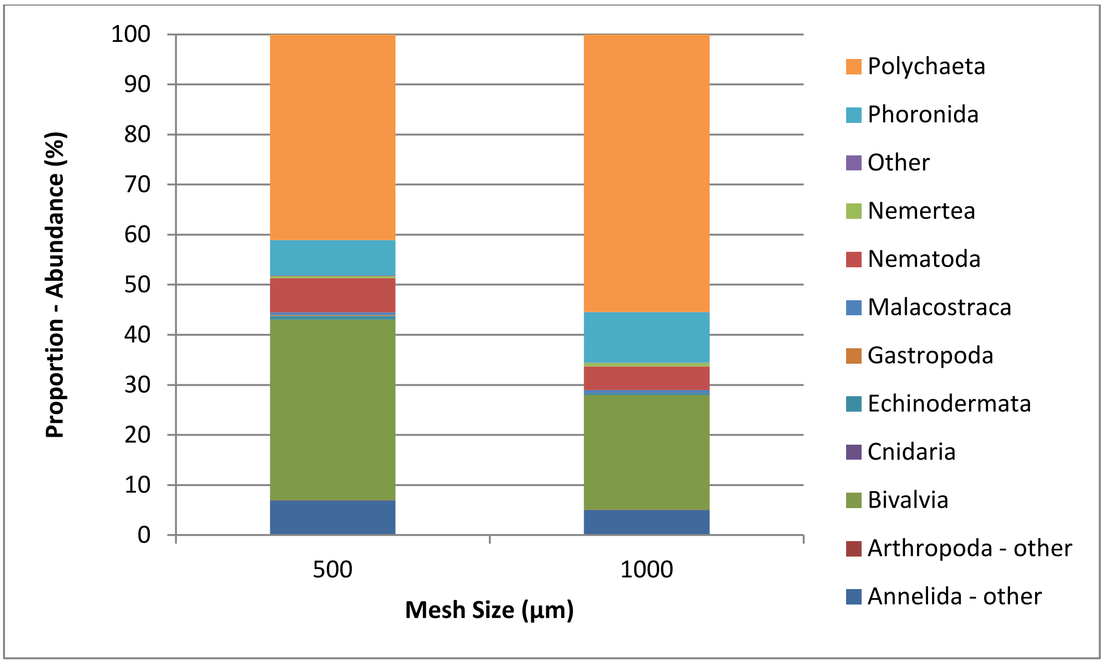


Figure 2.9 Comparison of the proportional abundance of taxonomic groups of the macrobenthic community composition in KC as captured using 500µm and 1000µm mesh sieves. (KC 2000-2005, n=30)

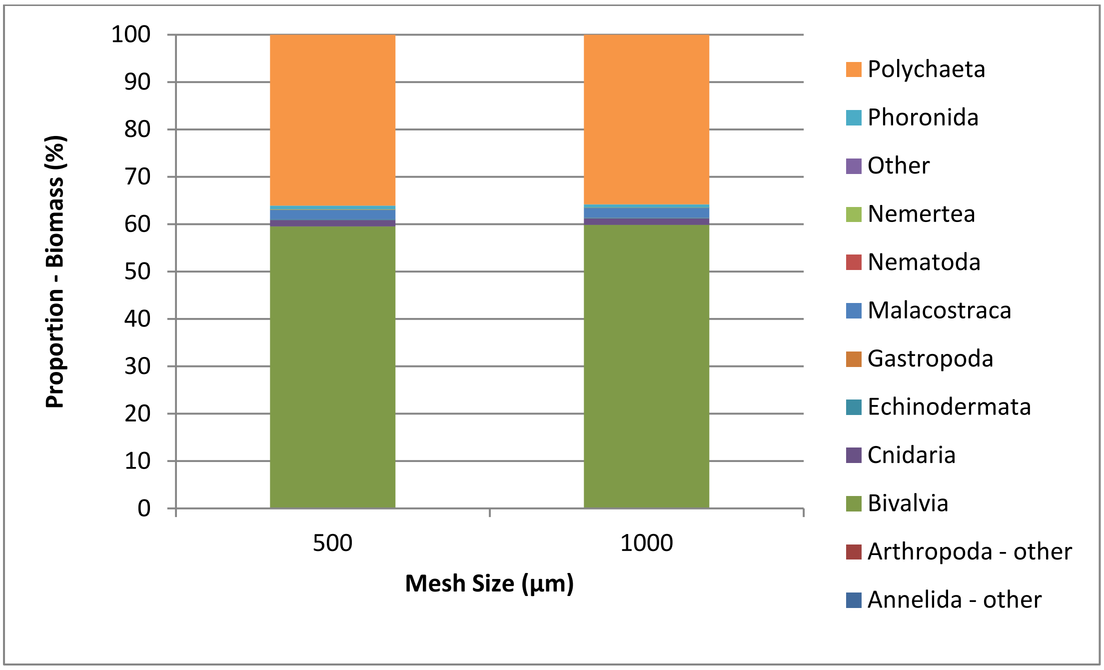


Figure 2.10 Comparison of the proportional biomass of taxonomic groups of the macrobenthic community composition in KC as captured using 500µm and 1000µm mesh sieves. (KC 2000-2005 n=30)

From this, it was expected that the indices would find some differences between mesh sizes according to abundance but not to biomass. Furthermore, we would expect to find differences between abundance and biomass within mesh sizes. Table 2.11 shows the differences between indices calculated for the two mesh sizes at the two sites. Different patterns were found at the two sites and between the indices as calculated with abundance or with biomass. When calculated with abundance, at KH, almost all indices had significantly different values between the mesh sizes while KC showed fewer differences between mesh sizes. When calculated with biomass, fewer indices showed significant differences in KH while for KC, there were slightly more differences. This may be related to biomass as KH did not show a significant difference in biomass whereas KC had significantly higher biomass in the 500µm mesh sieve. Both species richness and abundance were significantly different at both sites. In most cases a higher index value was attributed by the 500µm mesh. AMBI calculated with abundance showed greater quality classification with 1000µm in both sites.

Table 2.11 Differences between indices calculated with data captured using 500µm and 1000µm mesh sieves from two sites (KH, KC). Indices were calculated using abundance data and biomass data. Results are from a paired t-test in all cases except (^{n} data log normalised; ^{w} analysed with Wilcoxon Signed-Rank test) ***P<0.001; **P<0.01, *P<0.05, ns=not significant. Data analysed: KC 2000-2005, n=30; KH 1999-2004 n=30. Green shading indicates higher index value according to the 500µm mesh and red shading indicates higher index value according to 1000µm.

Index	Abundance 500µm vs. 1000µm		Biomass 500µm vs. 1000µm	
	KH	KC	KH	KC
S	***	***	***	***
N/Total Biomass	***^{n}	***^{w}	ns	***^{n}
d	***	***	**^{n}	***^{n}
J'	**^{w}	**^{w}	ns	**^{w}
Brillouin	***	ns^{w}	ns	ns^{w}
Fisher	***^{n}	ns		
ES50	***	ns^{w}	ns^{w}	ns^{w}
H'ln	***	ns^{w}	ns	***
Simpson	***^{w}	ns^{w}	ns^{n}	ns^{n}
N1	***^{n}	ns	ns	***
IQI	***^{w}	ns^{w}	***	**^{w}
EQR	**^{w}	**^{w}	ns	*^{w}
ITI	***	ns^{w}	ns	ns
BOPA	ns^{n}	ns^{n}	ns^{n}	*^{n}
A/S B/S	***^{n}	***^{n}	**^{n}	***^{w}
Delta	***^{w}	ns^{w}	ns^{n}	ns^{n}
Delta*	ns	***	*^{w}	ns
Delta+	***	ns^{n}	***	ns^{n}
sDelta+	***	***	***	***
Lambda+	***	**^{w}	***	ns*^{w}
AMBI	**	***	ns	**
BQI	***	***	***^{w}	***
MAMBI	**	ns^{w}	*	***

Note: A/S, B/S, BOPA and AMBI index values have inverse relationships with quality

Table 2.12 shows differences between indices as calculated with either abundance or biomass. The indices which did not show differences between abundance and biomass mainly include ITI and taxonomic distinctness while other indices mostly showed significant differences. There was a significant difference in species richness in the 500µm mesh sieve for KH. This was due to more species being counted in the abundance data. This should be due to human error as species richness should be the same. While most indices had greater index values using abundance data, there were some patterns of indices assigning higher quality with biomass data. These included Margalef's diversity, d, Simpson's Index, IQI, and AMBI. There were further differences between the sites and between the sieve sizes.

Table 2.12 Differences between indices calculated with abundance and biomass data from two sites (KH, KC). Indices were calculated using data from two sieve mesh sizes 500µm and 1000µm. Results are from a paired t-test in all cases except (^{n} data log normalised; ^{w} analysed with Wilcoxon Signed-Rank test) ***P<0.001; **P<0.01, *P<0.05, ns=not significant. Data analysed: KC 2000-2005 n=30; KH 1000µm mesh 1999-2005 n=35; KH 500µm mesh 1999 (n=3), 2000-2004 (n=5), n= 28. Green shading indicates higher index value according to the abundance and red shading indicates higher index value according to biomass.

Index	500µm Abundance vs. Biomass		1000µm Abundance vs. Biomass	
	KH	KC	KH	KC
S	** ^{w}	ns	ns	ns
N/Total Biomass	***	*** ^{w}	*** ^{n}	*** ^{n}
d	*** ^{w}	*** ^{w}	*** ^{w}	** ^{w}
J'	*** ^{w}	*** ^{w}	***	*** ^{w}
Brillouin	***	*** ^{w}	***	*** ^{w}
ES50	***	*** ^{w}	*** ^{w}	*** ^{w}
H'ln	***	*** ^{w}	***	*** ^{w}
Simpson	ns ^{w}	** ^{w}	ns	* ^{w}
N1	***	***	*** ^{n}	***
IQI	*** ^{w}	ns ^{w}	***	ns ^{w}
EQR	ns ^{w}	* ^{w}	** ^{w}	** ^{w}
ITI	ns	ns	*** ^{w}	ns ^{w}
BOPA	*** ^{w}	*** ^{n}	*** ^{n}	*** ^{n}
A/S B/S	***	*** ^{w}	*** ^{n}	*** ^{n}
Delta	ns ^{w}	ns ^{w}	* ^{w}	ns ^{w}
Delta*	ns ^{w}	**	ns ^{w}	ns
Delta+	ns	ns	ns ^{w}	ns ^{n}
sDelta+	** ^{w}	ns	ns	ns
Lambda+	ns	** ^{w}	ns	ns ^{w}
AMBI	*** ^{n}	*** ^{w}	*** ^{n}	**
BQI	*	**	*** ^{n}	**
MAMBI	*** ^{w}	*** ^{w}	***	***

Note: A/S, B/S, BOPA and AMBI index values have inverse relationships with quality

Table 2.13 shows the quality classification according to some of the indices. The quality classification mostly stayed the same between the 500 and 1000µm mesh except for M-AMBI, H', ITI and BQI which indicated some quality differences with 500µm mesh indicating greater or poorer quality according to m-AMBI in two instances; greater quality according to H' in one instance; greater according to ITI in one instance; and greater or poorer quality according to BQI in three instances. The classification using either biomass or abundance showed more differences with H', IQI, ITI, AMBI, M-AMBI and BQI finding differences in quality. H', m-AMBI and BQI indicated lower quality using biomass, while IQI, ITI and AMBI showed an increase in quality using biomass.

Table 2.13 A selection of indices showing the effects of sampling method and data type on quality classification (average quality classification KC 2000-2005, n=30; KH 2000-2004, n=25)

Index	KH				KC			
	Abundance		Biomass		Abundance		Biomass	
	500	1000	500	1000	500	1000	500	1000
H'	High	Good	Mod.	Mod.	Good	Good	Mod.	Mod.
IQI	High	High	High	High	Good	Good	High	High
ITI	Normal	Normal	Normal	Normal	Normal	Changed	Normal	Normal.
BOPA	High	High	High	High	High	High	High	High
AMBI	Good	Good	High	High	Good	Good	Good	Good
M-AMBI	High	Good	Good	Good	Good	Good	Mod.	Good
BQI	Mod.	High	Mod.	Poor	Mod.	Mod.	Mod.	Poor

2.4.4 Discussion

Most indices did show differences between mesh sizes and between biomass versus abundance. The 500µm mesh size assigned greater quality overall as has been found in previous studies (Pinto et al., 2009, Couto et al., 2010). The higher quality according to the 500µm mesh compared to the 1000µm mesh sieve partly reflects the greater number of species and abundance found in the finer sieve. Indices which are not strongly correlated to species richness and abundance such as Simpson's Index (1-lambda'), ITI, Delta and Delta+ showed fewer significant differences between sieves compared to other indices; although Lambda+ did not fit this pattern. Polychaeta, Bivalvia and Phoronida were all found to be represented proportionally differently between the two sieve sizes. Pinto et al. (2009) found Polychaeta and Bivalvia to be under-represented using a 1000µm mesh sieve compared to a 500µm mesh sieve. In this study Bivalvia were underrepresented in the 1000µm sieve but at one site Polychaeta were overrepresented in the 1000µm mesh sieve compared to the 500µm mesh sieve. A larger mesh size can determine a particular species to be rare although in reality it may be fairly abundant (Schlacher and Wooldridge, 1996). This may also lead to lower quality classifications using the larger sieve.

The indices which showed greater quality classification using biomass data compared to abundance data reflects the re-assigning of the relative importance of certain species and the evenness of biomass data compared to abundance data. Figures 2.7-2.10 showed that the relative importance of Polychaeta and Bivalvia were quite different when measures of abundance and biomass were compared. Measures of taxonomic distinctness (Delta, Delta*, Delta+, sDelta+ and Lambda+) showed little or no significant differences between abundance and biomass data.

When considering the quality classification boundaries for calibrated indices, the actual quality classification was not always affected by the sample and data type (Table 2.13). Most differences arose between comparing abundance versus biomass with fewer differences in quality classification being due to mesh size. However, it has been suggested that boundaries should be recalibrated for different types of data and this may reconcile differences found between abundance and biomass data (Warwick et al., 2010, Muxika et al., 2012). It may have been expected that more difference due to mesh size would be found with, for example, IQI and AMBI as these both showed significant differences between mesh sizes (Table 2.11). However, these relative differences were too small to show a difference in quality category and were maybe easily detected by statistical tests due to consistency within the samples. On the other hand, ITI detected no significant difference between mesh sizes at KC but there was a change in quality classification. This was due to the quality classification of both mesh sizes lying close to the quality category boundary.

Differences between mesh sizes were found between m-AMBI, H' and BQI. This may be due to these indices placing more weight with species richness compared to other indices. These quality classification changes were mainly by one category up or down. These findings partly support Warwick et al (2006) that sieve size is not a highly important factor, as quality classifications did not greatly change and populations between sieve sizes were relatively similar. Although differences did exist between sieve sizes, these were often relatively small. The exception to this was BQI which went from moderate to high in one instance, due to a higher abundance of tolerant species such as *Mediomastus fragilis* in the finer sieve sample. This reflects results found by others of smaller opportunistic species being captured in the finer mesh sieve (e.g. Pinto et al., 2009, Couto et al., 2010). The consistency between sieve sizes in this study did depend on the index used and the site sampled. Some indices, such as the IQI, could be considered to evaluate quality consistently when sampled with 500 or 1000μm mesh sieves and therefore it would be far more cost effective to sample using 1000μm mesh sieves.

This study has not included a comparison of impacted and unimpacted sites and the ability of indices to detect differences between these is arguably more important than whether there are differences between sieve sizes. Nevertheless, some indices are more sensitive to mesh size than others and this can have an impact on the quality classification. This may be particularly problematic when quality classifications are close to the moderate-good boundary for the WFD.

2.4.5 Conclusion

One type of sampling protocol is not a panacea for all sites or studies (Ferraro and Cole, 2004). This is evident in the disparity between the two sites KH and KC, and emphasises several difficulties. Firstly, different natural variability due to size classes and due to relative importance of species according to abundance or biomass in different sites makes interpretation of quality classifications difficult. Secondly, using a single index for all sites makes interpretation difficult as indices perform differently and often inconsistently according to the type of data used. Finally, if sampling protocols were to be optimised for different sites or purposes this would be both expensive and make comparison between sites and studies difficult.

2.5 General conclusion

The variable responses of indices showed the difficulty of interpreting index results against the backdrop of natural variability. Even indices which were highly correlated showed different responses in different circumstances. For example Shannon Wiener (H') was highly correlated to species richness but this index did not always perform in a consistent way relative to species richness. On the other hand, indices Margalef (d) and Total Taxonomic Distinctness (sDelta+) were very highly correlated to species richness and these always behaved in the same way as species richness.

Detecting small trends is important but extremely difficult to discern from the inconsistent results obtained from indices as changes in index results could not be attributed to background variability or genuine trends. Furthermore, the response of indices to natural disturbance may reflect the response to anthropogenic disturbance and in these cases it would be expected that indices should detect both natural and anthropogenic disturbance. Using environmental data is important in the distinction between natural and anthropogenic disturbance. However, environmental data in this study were patchy and interpreting index results was still difficult. The often opposing results of indices shows a single index is not reliable in the assessment of highly variable environments. A set of indices may add more confidence in the quality classification. A proposed set of indices based on this data alone may be: AMBI (ecological group); ITI (trophic group); species richness (diversity – richness); Simpson's Index (diversity – evenness); and taxonomic diversity, delta, (taxonomic diversity). This selection of indices may allow a more complete assessment of the benthos to be carried out as it incorporates several different aspects of the benthic community structure. AMBI classified all these sites as 'good' which may indicate a lower sensitivity to natural variation than other indices AMBI, Simpson's index, ITI and taxonomic diversity showed fewer differences in results due to sampling protocol than other indices, further indicating these indices may be robust options within different groups of indices. Combined indices such as IQI may disguise opposing trends between species richness and changes in ecological groups and therefore it is recommended to use the separate component parts of these indices rather than the combined form, thus taking a more cautious and informative approach to the assessment of benthic health.

Chapter 3
Index response to pressure

3.1 Introduction

Indices are widely used to show the response of benthic communities to anthropogenic disturbance. The benthos and benthic indices showed variable responses in sites with different levels of natural variability (chapter 2). This chapter focuses on the response of different indices to pressures – mainly human induced but also a natural pressure (salinity) which may be simultaneously associated with human induced pressures. Indices should show a stronger response to anthropogenic pressures than to natural variability.

Different types of disturbance affect the benthos in different ways. Response to organic input is described by the Pearson & Rosenberg (1978) theory which describes a succession of macrofauna from a total lack of species at the enrichment source, moving to high abundances of opportunistic species with little or no bioturbation of the sediment and succeeding gradually with time and distance from the pollution source to greater species richness, larger species, lower abundances and increasingly complex sediment burrowing structures until 'normal' conditions are reached. This response has been widely studied since and is the theoretical basis for indices such as AMBI and BQI. Physical disturbance, such as aggregate extraction or dredging by bottom fishing, has been less studied. Whomersley et al (2008) found dredge disposal sites to have lower species richness and diversity (Margalef) and that the disturbance increased the evenness at the site. Kaiser et al (2000) found chronic bottom fishing had the result of changing the community from one which contained larger, sessile species to a community with small, infaunal species. Another study also found physical disturbance to cause a decrease in species richness and abundance and an initial increase in evenness (Dernie et al., 2003). Although it has been suggested that opportunistic species may colonise after disturbance events (Quintino et al., 2006, Whomersley et al., 2008), neither the Dernie et al (2003) study nor the Whomersley et al (2008) study found evidence of this. Reduced species richness and abundance has also been found with toxic metal contamination (Lenihan et al., 2003, Mucha et al., 2005). Some species have been found to be more sensitive than others to toxic contamination and synergistic effects from other types of disturbance such as organic can add complexity to the benthic response (Lenihan et al., 2003).

Previous studies which have investigated index performance in response to pressure have often found contrasting results with different indices assigning different quality classifications (Fleischer et al., 2007, Zettler et al., 2007, Ruellet and Dauvin, 2007, Blanchet et al., 2008). These quality classifications, although different, usually exhibit similar trends corresponding to disturbance (e.g. Dauvin et al., 2007) but sometimes display opposite trends (e.g. Labrune et al., 2006). Several studies, (Labrune et al., 2006, Ruellet and Dauvin, 2007, Blanchet et al., 2008), have found AMBI and BOPA to assign higher qualities than either BQI or H'. Furthermore, studies have often found AMBI to assign most sites as 'good' quality while other indices, such as H', discriminate more between quality categories (e.g. Labrune et al., 2006; Zettler et al., 2007).

Several reasons have been identified as contributing to the differences in quality classifications. These include sensitivity to natural stress such as salinity which can result in lower quality classifications (BQI and H' but not AMBI) (Zettler et al., 2007). Reiss and Kröncke (2005) found the Shannon Index and Hurlbert index to be sensitive to seasonal variation while AMBI and BQI were less sensitive. Different classifications can also be due to a lack of sensitivity of some indices to a wide variety of disturbances. For example, AMBI has been shown to detect disturbance from different sources such as organic enrichment, hydrocarbons, anoxia and physical disturbance from dredging or engineering but has been found to be a poor detector of sand extraction and to be unsuitable for use in inner estuaries, organically poor or generally naturally stressed areas (Muxika et al., 2005) and in another study was found to miss a period of anoxia which other indices detected (Zettler et al., 2007). In addition, differences in quality classification can arise due to differences in sensitivity to species richness and species being classified as having different tolerances by different indices (Labrune et al., 2006, Zettler et al., 2007).

An important criterion of an index is to detect a trend towards disturbance thereby acting as an early warning signal. This would allow managers to take action before an ecosystem reaches a threshold and moves to an alternative stable state (Tett et al., 2007, Scheffer et al., 2009). However, studies have found indices are not discerning enough to detect gradual changes in quality (Kröncke and Reiss, 2010) and the subtle differences between 'good' and 'moderate' qualities are often undetected by indices (Puente and Diaz, 2008).

Aims

The aim of this study is to assess the performance of indices in discriminating different levels of quality and in detecting trends in quality using different data from Chapter 2 from a range of sites which have a variety of intensities and types of impacts.

Null Hypotheses

1. Indices discriminate equally well between disturbed, intermediate and undisturbed sites
2. Indices do not detect temporal or spatial trends
3. Index values do not correlate with each other
4. Index values do not correlate with environmental variables
5. Indices do not act as early warning signals

3.2 Methods

3.2.1 Study Sites

Datasets from five sites which have known impacts were obtained from SEPA. Each dataset was treated separately as sampling method and impact type differed between the sites (Table 3.1).

Table 3.1 Sampling and impact type details of SEPA datasets used in analysis

Site	Grab Size (m^2)	Mesh Size (μm)	No. Replicates	Impact Type
Barcaldine	0.1	500	1 or 2	Alginate Processing Factory
Ironrotter Point	0.1	500	2 or 3	Sewage works sea outfall
Irvine Bay	0.1	500	1, 2 or 3	Sewage outfalls and chemical factory
Fish Farms	0.015	1000	5	Fish farms stocked with various species including salmon, halibut and cod
Clyde Upper Estuary	0.025	500	5	Freshwater inputs, sewage inputs

3.2.1.1 Barcaldine

Barcaldine is located in Loch Creran, a sea loch in the west coast of Scotland. It was the site of outfall from an alginate factory which operated for about 20 years until closure in 1997 (Boyle and O'Reilly, 2001). The waste from the factory built up in the area forming a dense mat which was very rich in organic material. The waste is very slowly decomposing and monitoring has occurred since to study recovery of the benthic fauna.

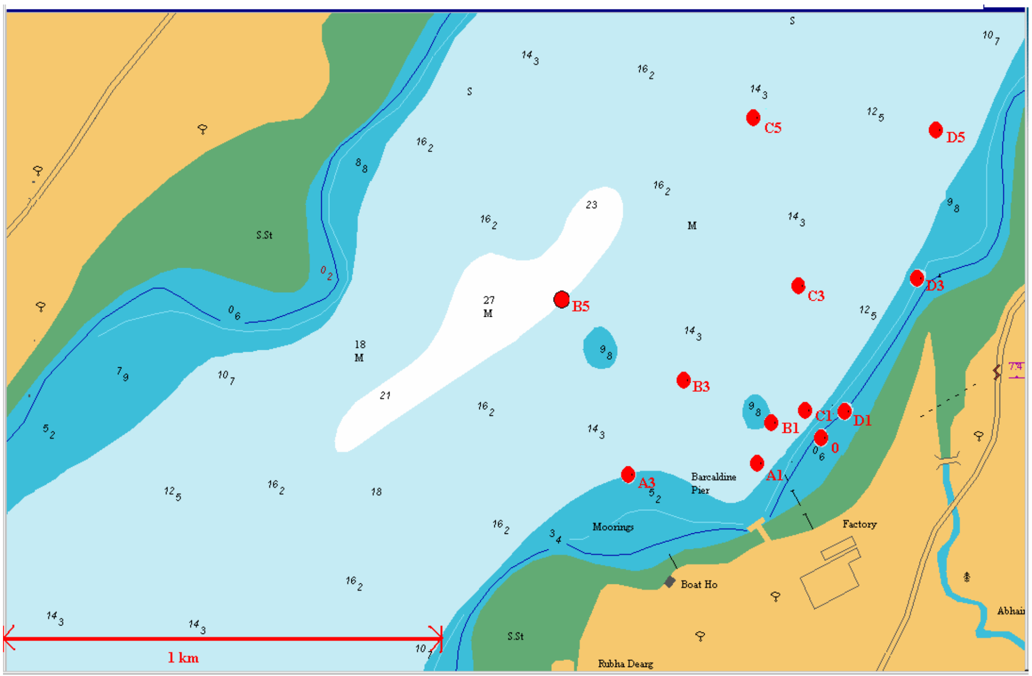


Figure 3.1 Location of sample points and transects from Barcaldine alginate processing factory outlet in Loch Creran (from SEPA summary report (Boyle and O'Reilly, 2001))

Four transects which radiated out from the discharge point were used to sample. The sample points were located 0, 150, 450 and 750m from the discharge (Fig. 3.1, Table 3.2). Data are available from 1997, 1999, 2001 and 2004. 0.1m^2 Day Grab samples were collected and sieved using 0.5mm mesh size.

Table 3.2 Details of sample points and transects at Barcaldine

Sample	Latitude/ Longitude	Depth (m) approx from map	Location	Sampled in year (with number or replicates)
0 outfall	5631.08’N 518.85’W	0.6	Outfall	1997(2); 1999 (1); 2001(1); 2004(1)
A1	5631.76’N 519.94’W	10	Transect A 150m	1997(2); 1999 (1); 2001(1); 2004(1)
A3	5631.75’N 519.33’W	5	Transect A 450m	1997(2); 1999 (1); 2001(1); 2004(1)
A5	-	-	Transect A 750m	1997(2)
B1	5631.87’N 518.95’W	9.8	Transect B 150m	1997(2); 1999 (1); 2001(1); 2004(1)
B3	5631.91’N 519.25’W	14	Transect B 450m	1997(2); 1999 (1); 2001(1); 2004(1)
B5	-	-	Transect B 750m	1997(2); 1999 (1)
C1	5631.87’N 518.85’W	12	Transect C 150m	1997(2); 1999 (1); 2001(1) ; 2004(1)
C3	5632.03’N 518.97’W	13	Transect C 450m	1997(2); 1999 (1); 2001(1); 2004(1)
C5	-	-	Transect C 750m	1997(2); 1999 (1)
D1	5631.87’N 518.76’W	0.6	Transect D 150m	1997(2); 1999 (1); 2001(1); 2004(1)
D3	5632.04’N 518.67’W	10	Transect D 450m	1997(2); 1999 (1); 2001(1); 2004(1)
D5	5632.2’N 518.49’W	12	Transect D 750m	1997(2); 2001(1); 2004(1)

3.2.1.2 Ironrotter Point

Ironrotter Point is located at Greenock in the west of Scotland (SEPA, 1996). A 14km sea outfall was commissioned for the point in 1991 which discharged waste from a primary sewage treatment plant 1.2km offshore at a depth of 25m. This pipe replaced over 30 short outfalls and received waste from a population of around 88,000 people. The baseline benthic survey was carried out in 1989, and the initial impact surveys began in 1992. The survey was cancelled in 2001 due to a redirection of resources. The point of discharge is a relatively deep and reasonably dispersive location (M. O'Reilly, Pers. comm.). The water body was classified as estuarine under the Urban Wastewater Treatment Directive so use of the pipe was stopped in 2001 as treatment was primary.

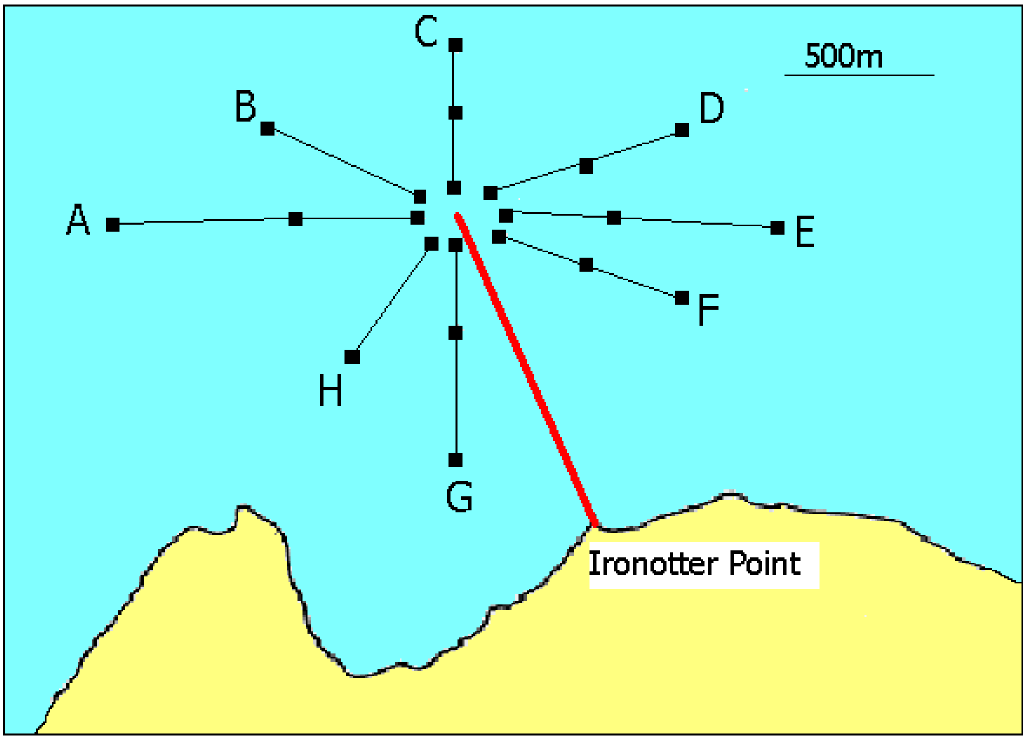


Figure 3.2 Location of transects from the sea outfall at Ironrotter Point (from SEPA summary report (SEPA, 1996)); not all sample point shown, see Table 3.3 for full details.

Eight transects which radiated out from the discharge point were used to sample. Sample points were located 100, 500, 750 and 1000m from the discharge (Fig. 3.2, Table 3.3), although the exact location of each sample point varied over years (see appendix 8.2 for latitude and longitude of all samples). Data are available from 1989, 1992, 1995 and 1998. 0.1m^2 Day Grab samples were collected and sieved using 0.5mm mesh size.

Table 3.3 Details of sample points at Ironrotter Point

Station	Distance from discharge (m)	Depth (m)	Year (and number of replicates)
A1	100	23	1989(3); 1992(3); 1995(3); 1998(3)
A2	500	30	1989(3); 1992(3); 1995(3); 1998(2)
A3	750	30	1989(3); 1992(3); 1995(3); 1998(2)
A4	1000	-	1995(3)
B1	100	23	1989(2); 1992(3); 1995(3)
B2	500	-	1995(3)
B3	750	28	1989(3); 1992(3); 1995(3)
B4	1000	-	1995(3)
C1	100	20	1989(3); 1992(3) 1995(3)
C2	500	20	1989(3); 1992(3); 1995(3)
C3	750	25	1989(3); 1992(3); 1995(3)
D1	100	18	1989(3); 1992(3); 1995(3)
D2	500	18	1989(2); 1992(3); 1995(3)
D3	750	10	1989(3); 1992(3); 1995(3)
E1	100	20	1989(3); 1992(3); 1995(3); 1998(3)
E2	500	26	1989(3); 1992(3); 1995(3); 1998(2)
E3	750	20	1989(3); 1992(3); 1995(3); 1998(2)
E4	1000	-	1995(3)
F1	100	20	1989(3); 1992(3); 1995(3)
F2	500	28	1989(3); 1992(3); 1995(3)
F3	750	31	1989(3); 1992(3); 1995(3)
G1	100	22	1989(3); 1992(3); 1995(3)
G2	500	28	1989(3); 1992(3); 1995(3)
G3	750	30	1989(3); 1992(3); 1995(3)
G4	1000	-	1995(3)
H1	100	20	1989(3); 1992(3); 1995(3)
H2	500	24	1989(3); 1992(3); 1995(3)
H3	750	-	1995(3)

3.2.1.3 Irvine Bay

The Irvine Bay survey was designed to assess the impact due to the long sea outfall from Garnock Valley Sewer and ICI Nobel Explosives Ltd (SEPA, 2000). In addition, the impact of sewage discharges at Barassie and Troon were assessed. The Ayr Bay stations act as controls for Irvine Bay though they may be impacted by sewage discharge from the local area. Discharges from Irvine Valley Sewer and Smithkline Beecham Pharmaceuticals were monitored as part of a separate survey but were also input into the bay and an extension of Irvine Valley Sewer became operational in 2003.

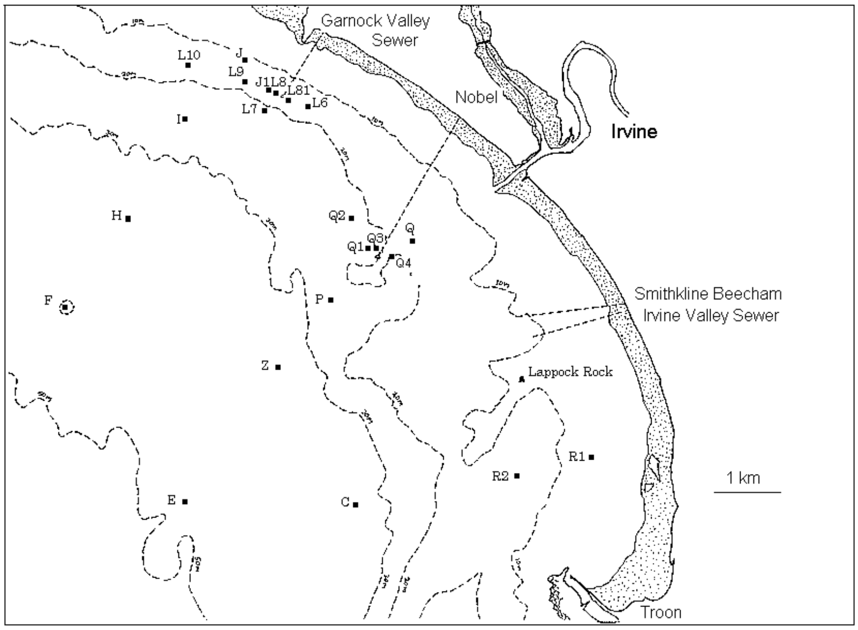


Figure 3.3 Location of sample transects and discharges in Irvine Bay (from SEPA summary report (SEPA, 2000))

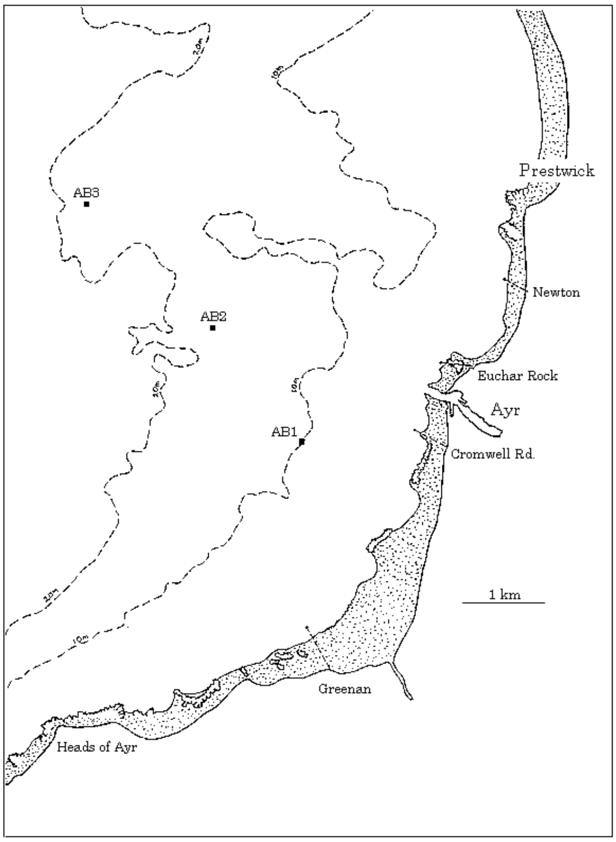


Figure 3.4 Location of sampling transect and discharges in Ayr Bay (from SEPA summary report (SEPA, 2000))

Three transects were sampled in Irvine Bay with samples located from around the discharge point to deeper waters offshore measuring the impact of the Garnock Valley Sewer (GVS), the Nobel explosives factory, and the outfalls at Barassie and Troon respectively (Fig. 3.3, Table 3.4). One transect was sampled in Ayr Bay (Fig. 3.4, Table 3.4). Data are available from 1981, 1989, 1992, 1995, 1998, 1999, 2003 and 2004. $0.1m^2$ Day Grab samples were collected and sieved using 0.5mm mesh size.

Table 3.4 Sample details for Irvine Bay and Ayr Bay

Station	Depth (m)	Latitude	Longitude	Distance and bearing from discharge	Year (and number of replicates)
C	36	55°33.60'N	04°43.95'W	4.4km off Barassie	1981(3); 1989 (2); 1992(2); 1995(2)
E	53	55°33.60'N	04°46.60'W	4.83km SW from Nobel	1981(3); 1989 (2); 1992(2); 1995(2)
F	52	55°35.15'N	04°48.25'W	4.68km SW from GVS	1981(3); 1989 (2); 1992(2); 1995(2); 1998(2)
H	38	55°35.92'N	04°47.40'W	3.06km SW from GVS	1981(3); 1989 (2); 1992(2); 1995(2); 1998(2)
I	29	55°36.72'N	04°46.55'W	1.51km SW from GVS	1981(3); 1989 (2); 1992(2); 1995(2)
J	14	55°37.25'N	04°45.75'W	0.83km NW from GVS	1981(3); 1989 (2); 1992(2); 1995(2)
J1	17	55°37.02'N	04°45.38'W	0.25km NW from GVS	1989 (2); 1992(2); 1995(2)
L6	17	55°36.90'N	04°45.00'W	0.47km SE from GVS	1989 (2); 1992(2); 1995(2); 1998(2); 2003(2)
L7	21	55°36.85'N	04°45.45'W	0.4km SW from GVS	1989 (2); 1992(2); 1995(2)
L8	17	55°36.95'N	04°45.35'W	0.14km W from GVS	1989 (2); 1992(2); 1995(2); 1998(2); 2003(2)
L81	17	55°36.92'N	04°45.18'W	0.07km SE from GVS	1989 (2); 1992(2); 1995(2); 1998(2); 2003(2)
L9	17	55°37.10'N	04°45.85'W	0.65km NW from GVS	1989 (2); 1992(2); 1995(2); 1998(2); 2003(2)
L10	17	55°37.15'N	04°46.58'W	1.3km NW from GVS	1998(2); 2003(2)
P	25	55°35.30'N	04°44.45'W	1.045km SW from Nobel	1981(3); 1989 (2); 1992(2); 1995(2); 1999(2)
Q	20	55°35.78'N	04°43.25'W	0.54km NE from Nobel	1981(3); 1989 (2); 1992(2); 1995(2); 1999(2); 2004(2)
Q1	20	55°35.72'N	04°43.80'W	0.215km NW from Nobel	1981(3); 1989 (2); 1992(2); 1995(2); 1999(2); 2004(2)
Q2	20	55°35.92'N	04°44.15'W	0.7km NW from Nobel	1981(3); 1989 (2); 1992(2); 1995(2); 1999(2); 2004(2)
Q3	20	55°35.68'N	04°43.77'W	0.15km N from Nobel	1999(2); 2004(2)
Q4	20	55°35.62'N	04°43.63'W	0.2km NE from Nobel	1999(2); 2004(2)
R1	9	55°34.05'N	04°40.55'W	0.79km off Barassie	1981(3); 1989 (2); 1992(2); 1995(2); 1998(1); 1999(2)
R2	13	55°33.88'N	04°41.65'W	1.945km off Barassie	1981(3); 1989 (2); 1992(2) ; 1995(2); 1998(1); 1999(2)
Z	40	55°34.75'N	04°45.20'W	2.305km SW from Nobel	1981(3); 1989 (2); 1992(2); 1995(2)
AB1	10	55°28.88'N	04°40.00'W	1.33km offshore Ayr Bay	1981(3); 1989 (2); 1992(2); 1995(2)
AB2	17	55°28.57'N	04°41.00'W	2.66km offshore Ayr Bay	1981(3); 1989 (2); 1992(2); 1995(2)
AB3	13	55°29.32'N	04°42.45'W	4.7km offshore Ayr Bay	1981(3); 1989 (2); 1992(2); 1995(2)

3.2.1.4 Fish Farms

Fish farm data came from a range of sites (15 in total) around Scotland (Table 3.5). Sites were of various sizes and contained different species such as salmon, halibut and cod. Sites were sampled in either 2002 or 2003. Five replicate samples were taken at the cage edge, the allowable zone of effect (25m from the cage) and at a reference point. Samples were taken with a 0.015m^2 mini-grab and sieved with 1mm mesh.

Table 3.5 Details of fish farm sampling points (AZE stands for the allowable zone of effect, located 25m from the cage edge)

Fish Farm	Location	Latitude and Longitude	Year	Depth (m)	Max consented tonnes	Tonnes at time of survey	Antifoulant used on cages
Basta Voe North (BVN)	Cage Edge	60 38.6748’ N 01 02.8076’W	2002	19.8	600	213	None
	AZE	60 38.6834’N 01 02.8262’W	2002	18.6			
	Reference	60 38.3476’N 01 01.5673’W	2002	14			
Bow of Hascosay	Cage Edge	60 36.6918’N 01 00.2352’W	2002	10.7	1250	Unknown	None
	AZE	60 36.6787’N 01 00.2383’W	2002	11			
	Reference	60 37.4593’N 01 01.0904’W	2002	10.4			
Lippie Geo	Cage Edge	60 04.482’N 01 17.681’W	2002	26.5	200	200	Copper-based
	AZE	60 04.469’N 01 17.688’W	2002	26.2			
	Reference	60 04.936’N 01 17.540’W	2002	28.3			
Aith Voe	Cage Edge	60 10.470’N 01 05.294’W	2002	6.4	400	400	Copper-based
	AZE	60 10.470’N 01 05.318’W	2002	6.1			
	Reference	60 10.581’N 01 05.423’W	2002	4.3			
Dales Voe	Cage Edge	60 11.337’N 01 11.408’W	2002	14	800	800	Copper-based
	AZE	60 11.347’N 01 11.393’W	2002	14			
	Reference	60 11.522’N 01 11.181’W	2002	15			
Hogan	Cage Edge	60 12.752’N 01 30.516’W	2002	24	1500	Unknown	Copper-based
	AZE	60 12.743’N 01 30.518’W	2002	21			
	Reference	60 12.689’N 01 31.458’W	2002	31			
Cloudin	Cage Edge	60 12.702’N 01 34.839’W	2002	18	1995	Unknown	Copper-based
	AZE	60 12.689’N 01 34.845’W	2002	17			
	Reference	60 12.509’N 01 34.332’W	2002	22			
Creran A	Cage Edge	56 31.312’N 05 22.081’W	2003	33	1500	1500	Copper-based
	AZE	56 31.301’N 05 22.102’W	2003	33			

Table 3.5 continued

Fish Farm	Location	Latitude and Longitude	Year	Depth (m)	Max consented tonnes	Tonnes at time of survey	Antifoulant used on cages
	Reference	56 31.177'N 05 21.728'W	2003	24			
Dunstaffnage	Cage Edge	56 27.062N 05 27.792'W	2003	43	700	676	Unknown
	AZE	56 27.068'N 05 27.769'W	2003	46			
	Reference	56 27.392'N 05 26.865'W	2003	46			
Charlotte Bay	Cage Edge	56 25.062'N 05 30.860'W	2003	30	600	570	Copper-based
	AZE	56 25.052'N 05 30.877'W	2003	33			
	Reference	Not noted	2003				
Castle Bay	Cage Edge	56 13.602'N 05 35.476'W	2003	23	300	199	Copper-based
	AZE	56 13.617'N 05 35.472'W	2003	25			
	Reference	56 13.256'N 05 35.360'W	2003	20			
Poll na Gille	Cage Edge	56 12.736'N 05 35.249'W	2003	30	750	669	Copper-based
	AZE	56 12.726'N 05 35.252'W	2003	32			
	Reference (same site as for Castle Bay)	56 13.256'N 05 35.360'W	2003	20			
Port a Beachan	Cage Edge	56 09.673'N 05 31.686'W	2003	18	130	122	Copper-based
	AZE	56 09.661'N 05 31.697'W	2003	19			
	Reference	56 10.065'N 05 31.157'W	2003	17			
Port na Moine	Cage Edge	56 09.308'N 05 32.147'W	2003	37	770	27	Copper-based
	AZE	56 09.296'N 05 32.160'W	2003	40			
	Reference (same site as for Port a Beachain)	56 10.065'N 05 31.157'W	2003	16			
Corry	Cage Edge	57 51.678'N 05 06.531'W	2003	25	1050	369	None
	AZE	57 51.667'N 05 06.519'W	2003	24			
	Reference	57 51.221'N 05 06.247'W	2003	23			

3.2.1.5 Upper Clyde Estuary

The upper Clyde estuary is located in the west of Scotland and begins at Glasgow with samples taken approximately every 2 miles (Boyle and O'Reilly, 2000). There are freshwater inputs from rivers and at 8 miles there is a sewage works at Dalmuir which discharges into the estuary.

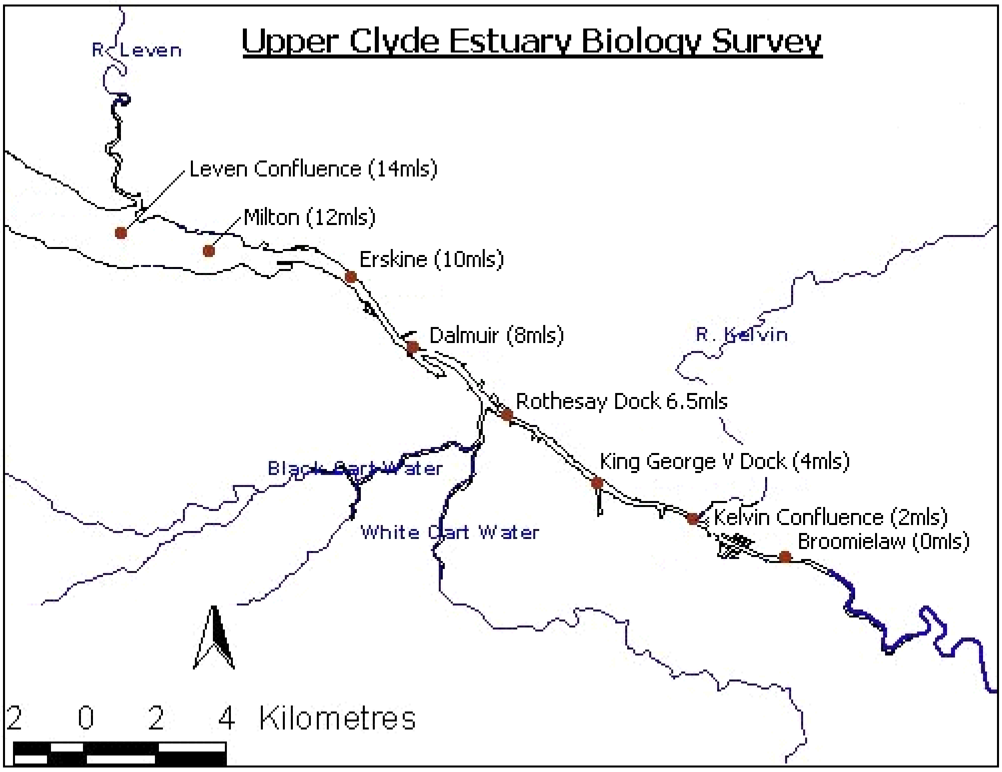


Figure 3.5 Location of sample points along the upper Clyde estuary (SEPA summary report (Boyle and O'Reilly, 2000))

Samples were taken along a transect from 0, 2, 4, 6.5, 8, 10, 12 and 14 miles in a seaward direction (Fig. 3.5, Table 3.6). Five replicate samples were taken at each sampling point using a 0.025m^2 Van Veen grab and sieved with 0.5mm mesh. Data were available from 1993 (December), 1994 (May/July), 1995 (June and September), 1996 (May and November), 1997 (May and October), 2000 (May) and 2003 (May), sites being sampled in two seasons in 1995, 1996 and 1997 as indicated. Salinity was measured at each sample point on a different occasion as part of a separate chemical survey (see Appendix 8.3 for dates of each survey).

Table 3.6 Details of upper Clyde estuary sampling points

Sample (miles)	Latitude and Longitude	Depth (m)	Notes
0	55°51.37’N 4°15.55’W	1.3	
2	55°51.90’N 4°18.51’W	6.9	Not sampled in 1993, 1994, 1995(Sep), 1996(May and Nov), 1997(Oct)
4	55°52.40’N 4°21.14’W	7.4	
6.5	55°53.43’N 4°23.62’W	7.4	
8	55°54.25’N 4°25.41’W	7.4	
10	55°55.40’N 4°27.95’W	7.6	
12	55°55.78’N 4°31.78’W	7.8	Not sampled in 1993, 1994
14	55°55.94’N 4°34.22’W	8.1	Not sampled in 1995 (Jun and Sep), 1996 (May and Nov), 1997 (May), 1997(Oct), 2000 (May), 2003(May)

3.2.2 Statistical Analysis

Data were analysed using multidimensional scaling (MDS) and ANOSIM, carried out using Primer 6, and interpreted in relation to relevant variables such as proximity to the pollution source, type and intensity of stress or pollution, transect location, year, month and depth. A suite of indices (Section 2.1.1) was calculated for each sample at each site. The mean quality classification was determined to assess consistency of quality category assignment between different indices. Pearson product moment correlation was carried out using Minitab 15 in order to assess the strength of correlation between different indices in different locations with different types of impact. Pearson product moment correlation was also used to relate index results to environmental variables (if available) to explore any relationships between indices and the physico-chemical characteristics.

3.3 Results

3.3.1 Study Site descriptions

Analyses were carried out for each site to describe the ecological status of the site and assess the performance of the indices at the different sites.

3.3.1.1 Barcaldine

Multidimensional scaling (MDS) was used to assess the similarity of samples at Barcaldine and highlight patterns according to year, transect and distance from the outfall (Fig. 3.6). 1997 showed two distinct groupings of samples while samples from other years were more evenly separated. One of 1997 clusters (to the right of the MDS) can be seen to correspond to samples near the outfall while the other group of samples are located 450m and 750m away. Differences between 1997 and 1999 were not significant while there were significant differences between all other years (One-way ANOSIM, $R=0.115$, $p<0.05$). All of the samples from 2004 are most similar to the reference samples from the other years, with the 2001 and 1999 samples being in-between. When distance from the outfall was taken into account, there were significant differences between all years, with the greatest differences found between 1997 and 2004 (Two-way ANOSIM year and distance; $R=0.539$, $p<0.01$). A trend with distance from the outfall can be clearly seen. There were significant differences found between all stations except between the outfall and 150m station (Two-way ANOSIM year and distance; $R=0.554$, $p<0.01$). There were no significant differences found between transects (One-way ANOSIM, $R=0.024$, $p>0.05$).

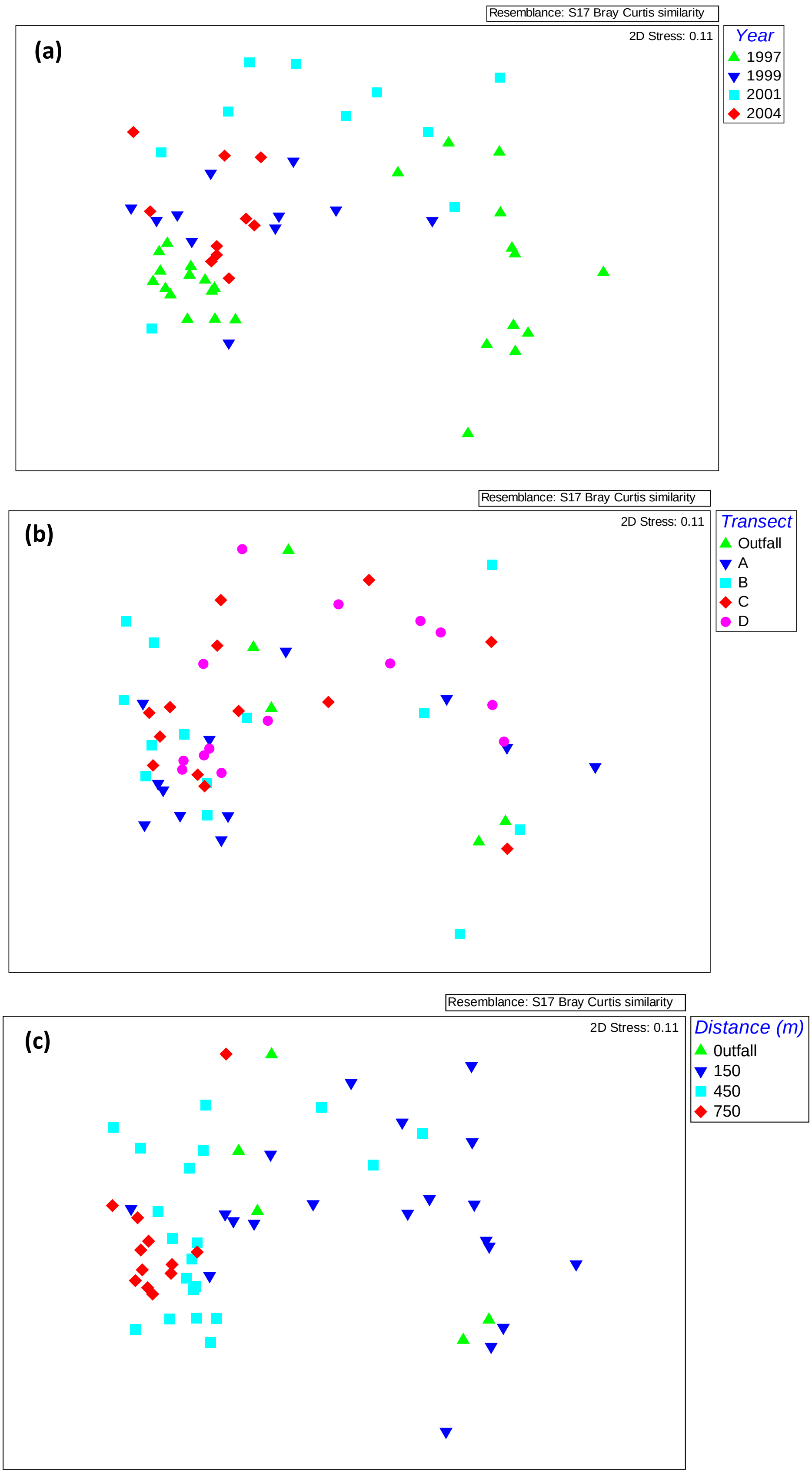


Figure 3.6 MDS plot of samples according to (a) year, (b) transect and (c) distance from outfall at Barcaldine. See Fig. 3.1 for location of transects.

Five indices were used to assess the average quality for each sample (Table 3.7). The index results largely reflect the pattern seen in the MDS graphs with 1997 samples having the worst quality and samples closest to the outfall also having the worst quality classifications. The indices are in agreement in the quality classification in 26% of the samples. Where the indices do not agree, there is often a similar trend with high and good; good and moderate; moderate and poor; and poor and bad classifications mixed (39% of samples). However in many cases (35% of samples) the classifications cross three quality categories for the same sample. The data were plotted again using MDS, this time according the level of agreement between indices (Fig. 3.7) A high level of similarity was found between samples according to the agreement of indices (One-way ANOSIM; $R=0.39$, $p<0.01$). Those indices which completely agree can be seen to correlate with the bad sites, closest to the outfall. Those assigned similar classifications are the samples most dissimilar to the bad sites and the sites where the indices disagree are mainly in-between. BQI is responsible for causing disagreement in the quality classification for 13 of the samples – more than any other index.

Table 3.7 Quality classification based on average index value at each sample point in each year for Barcaldine according to five indices (see Table 3.2 for sample details)

Year	Station	IQI	BQI	BOPA	AMBI	ITI
1997	Outfall	Bad	Bad	Bad	Bad	Degraded
1997	A1	Bad	Bad	Bad	Bad	Degraded
1997	A2	Good	Good	Moderate	Moderate	Changed
1997	A3	Good	Good	Moderate	Good	Changed
1997	B1	Bad	Bad	Bad	Bad	Degraded
1997	B2	Moderate	Moderate	Good	Moderate	Changed
1997	B3	Good	Good	Moderate	Good	Changed
1997	C1	Bad	Bad	Bad	Bad	Degraded
1997	C2	Moderate	Moderate	Good	Moderate	Changed
1997	C3	Moderate	Moderate	Moderate	Good	Changed
1997	D1	Bad	Bad	Bad	Bad	Degraded
1997	D2	Moderate	Bad	Moderate	Moderate	Changed
1997	D3	Moderate	Moderate	Good	Good	Changed
1999	Outfall	Moderate	Poor	Good	Good	Changed
1999	A1	Moderate	Poor	Moderate	Good	Changed
1999	A3	High	Good	Good	Good	Normal
1999	B1	Poor	Bad	Poor	Poor	Degraded
1999	B3	Good	Good	Good	Good	Changed
1999	B5	Good	Good	Good	Good	Normal
1999	C1	Moderate	Poor	Good	Moderate	Changed
1999	C3	Good	Poor	Good	Good	Normal
1999	C5	Good	Moderate	Good	Good	Changed
1999	D1	Moderate	Poor	Good	Moderate	Changed
1999	D3	Poor	Moderate	Poor	Moderate	Normal
2001	Outfall	Moderate	Poor	Good	Good	Changed
2001	A1	Bad	Bad	Bad	Bad	Degraded
2001	A3	Good	High	High	Good	Changed
2001	B1	Bad	Bad	Bad	Bad	Degraded
2001	B3	Good	Moderate	Good	Good	Changed
2001	C1	Moderate	Poor	Good	Good	Changed
2001	C3	Moderate	Poor	Good	Good	Normal
2001	D1	Poor	Bad	Moderate	Moderate	Degraded
2001	D3	Moderate	Poor	Moderate	Moderate	Changed
2001	D5	Moderate	Bad	Good	Good	Changed
2004	Outfall	Moderate	Poor	Good	Moderate	Changed
2004	A1	High	Good	Good	Good	Changed
2004	A3	Good	Moderate	Good	Good	Changed
2004	B1	Good	Moderate	Good	Good	Changed
2004	B3	Good	Poor	Good	Good	Changed
2004	C1	Moderate	Moderate	High	Good	Changed
2004	C3	Good	Poor	Good	Good	Changed
2004	D1	Good	Moderate	Good	Good	Changed
2004	D3	Moderate	Moderate	Good	Good	Changed
2004	D5	Moderate	Moderate	Good	Poor	Changed

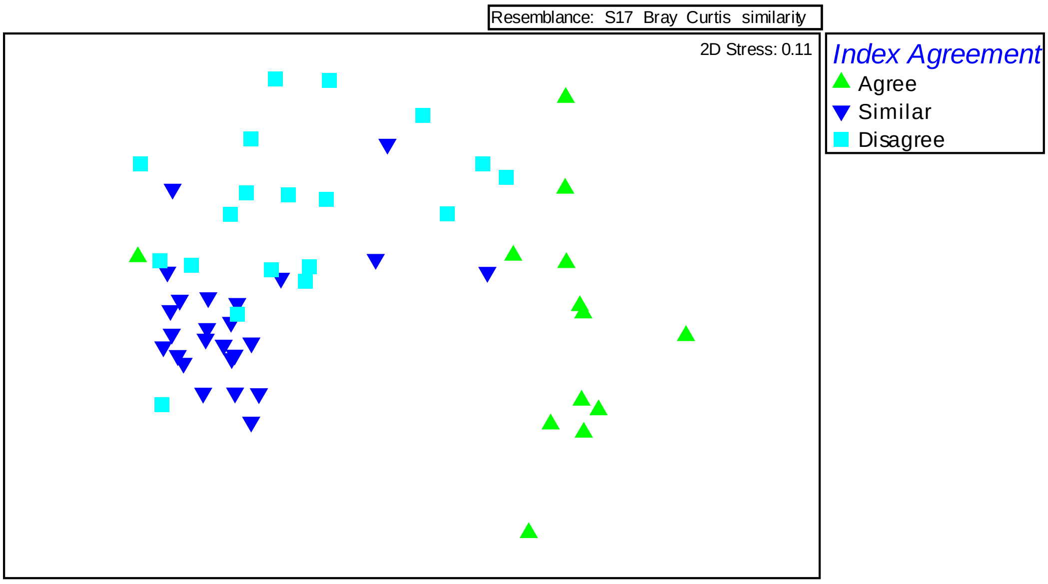


Figure 3.7 MDS plot of samples at Barcaldine according to the level of agreement in quality classification between five indices (Table 3.7). Agree = all indices agree; Similar = two quality classifications given but adjacent on the scale of quality; Disagree = three or more quality classifications given or two classifications apart on the scale of quality.

Trends over time and distance from the outfall were assessed (Table 3.8). Most indices detected an overall temporal trend, although mainly weak correlations were found. In all cases this trend indicated an increase in quality over time. A much stronger signal was detected for the spatial trend. All indices detected an increase in quality with distance from the outfall with N, J' and A/S detecting the weakest relationships between quality and distance. The strength of the relationship between indices and distance from the outfall was maintained when the effect of depth was removed, although depth was positively correlated to distance.

Table 3.8 Correlation between indices and environmental variables at Barcaldine. Pearson product moment correlations with percentage correlation, *r*. Partial correlation carried out to remove effect of confounding variable 'depth' from effect of 'distance'. Darker colours indicate a stronger relationship. Distance is from the outfall in metres; depth is in metres.

	Year	Depth	Distance	Distance (depth removed)
Depth	0.3			
Distance	-16.7	52.5		
S	0.6	3.1	53.8	53.2
N	-28.7	1.6	15.7	13.3
d	13.8	6.3	55.4	53.8
J	18.8	-13.5	9.0	20
Brillouin	19.1	9.4	57.4	51.7
Fisher	26.0	-3.0	33.3	38.6
ES(50)	29.9	14.0	50.7	43.1
H(loge)	26.5	9.4	54.2	49.9
Simpson	26.2	0.5	44.2	45.8
N1	19.5	5.2	42.2	39.5
IQI	32.4	16.6	58.0	52.1
EQR	40.1	16.8	54.3	48.2
ITI	24.7	23.8	60.4	54.4
BOPA	-48.3	-19.1	-37.3	-38.1
A/S	-18.8	0.9	-16.8	-19.4
Delta	29.7	5.4	45.1	44.3
Delta *	18.5	-1.9	29.3	33
Delta +	14.9	-3.9	30.9	34.8
sDelta +	0.5	2.8	52.8	52.9
Lambda +	30.2	19.6	49.6	46
AMBI	-36.2	-19.7	-48.5	-38.5
BQI	10.2	13.1	63.7	55.5
MAMBI	23.5	12.0	57.0	51.7

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Note: A/S, BOPA and AMBI index values have inverse relationships with quality

3.3.1.2 Ironrotter

MDS revealed a strong pattern related to the year of sampling at Ironrotter (Fig. 3.8) (One-way ANOSIM, $R=0.657$, $p<0.01$). Each year showed a distinct species assemblage and a trend over time moving from right to left of the graph. There were significant differences between all years with the greatest differences found between 1989, before the sewage pipe was implemented, and 1998. Differences between the years increased with time also with 1989 and 1992 being the most similar (ANOSIM pair wise comparison, $R=0.551$), 1992 and 1995 being more different (ANOSIM pair wise comparison, $R=0.577$) and 1995 and 1998 being more different again (ANOSIM pair wise comparison, $R=0.718$). Differences between samples according to distance were weak and only significant when year was also taken into account (Two-way ANOSIM year and distance; $R=0.062$, $p<0.01$) but the greatest differences were found between the 100m and 1000m stations. A strong pattern was also related to organic matter content (One-way ANOSIM, $R=0.394$, $p<0.01$) and this was related to the year of sampling. The strength of the relationship was weaker than that found with year but organic carbon data were not available for 1998 (Fig. 3.9).

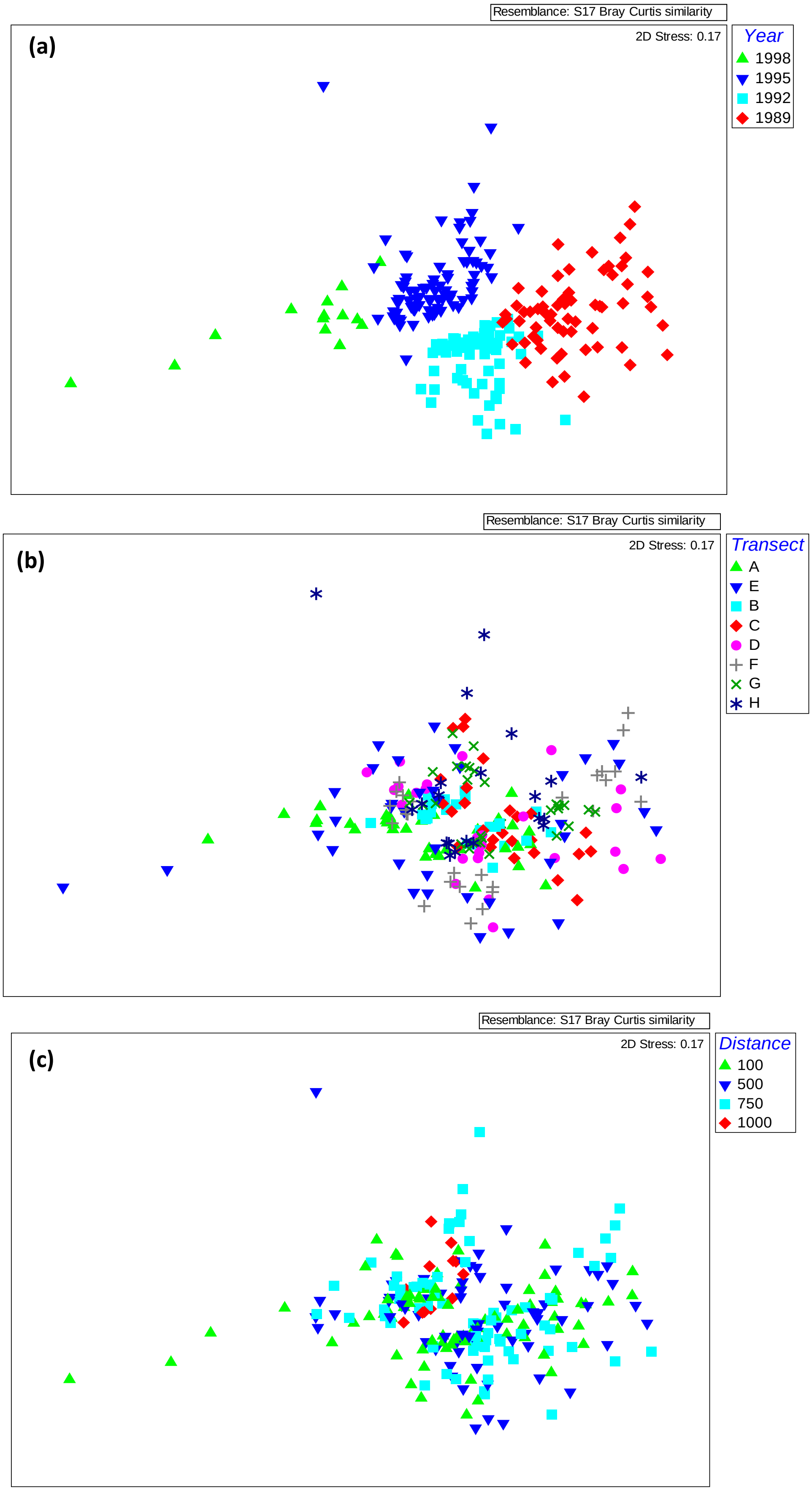


Figure 3.8 MDS plot of samples at Ironrotter Point according to (a) year, (b) transect and (c) distance from outfall. See Fig. 3.2 for location of transects. Distance in metres.

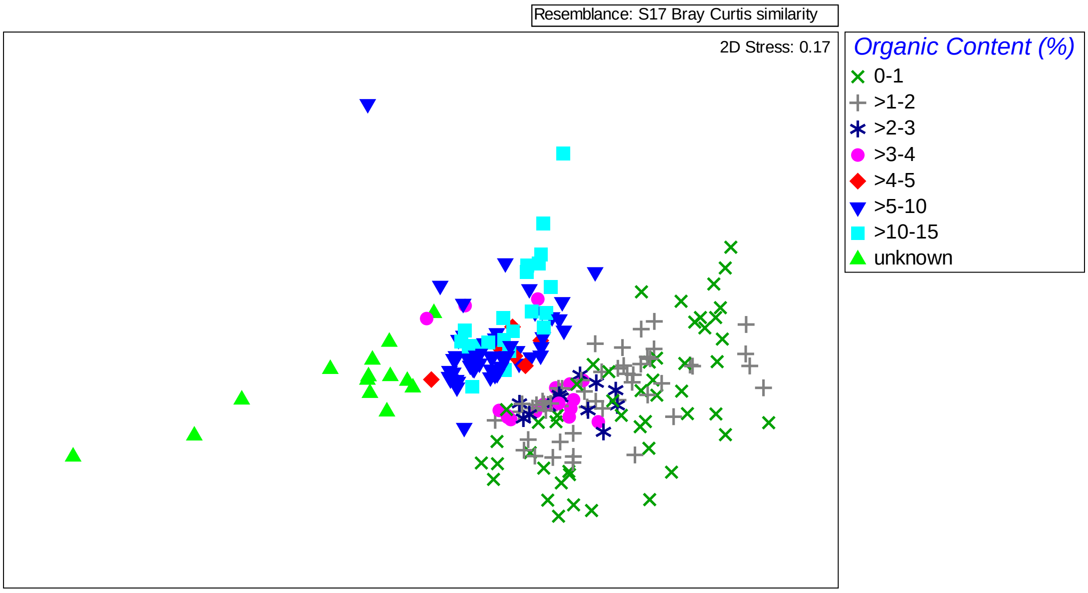


Figure 3.9 MDS plot of samples at Ironrotter Point according to organic matter content in sediment.

Most index quality classifications indicated a decrease in quality in 1998 but no decrease in quality was evident before this, apart from ITI which decreased to mainly 'changed' classification from 1995 onwards (Table 3.9). According to IQI and BQI quality appeared to increase after the sea pipe was put in place and decrease again in 1998. This does not reflect the MDS which indicated a trend in the benthic community in the direction of the lower quality sites. 8% of index classifications agreed, 45% showed a similar classification and 47% disagreed (three quality classifications given for the same sample point) and there were significant differences between the similar and disagree groups (One-way ANOSIM pairwise comparison, R=0.109, p<0.01). All the samples where the indices agreed were found in 1989 and 1992 and most of the similar classifications were also found in these two years (Fig.3.10). Most of the disagreement between indices occurred in the years 1995 and 1998. The ITI was responsible for the majority of disagreement between indices.

Table 3.9 Quality classification based on average index value at each sample point in each year for Ironrotter Point according to five indices (see Table 3.3 for sample details)

Year	Sample	IQI	BQI	BOPA	AMBI	ITI	Year	Sample	IQI	BQI	BOPA	AMBI	ITI	Year	Sample	IQI	BQI	BOPA	AMBI	ITI
1998	A100	Good	Good	Mod	Mod	Changed	1992	A100	Good	Good	Good	Good	Changed	1989	A100	Good	Good	Good	Good	Changed
1998	A500	Good	High	Mod	Mod	Changed	1992	A500	Good	Good	Good	Good	Changed	1989	A500	Good	Good	Good	Good	Changed
1998	A750	Good	Good	Good	Good	Changed	1992	A750	Good	Good	Good	Good	Changed	1989	A750	Good	Good	Good	Good	Changed
1998	E100	Mod	Mod	Poor	Mod	Degraded	1992	B100	Good	Good	Good	Good	Changed	1989	B100	High	Good	Good	Good	Changed
1998	E500	Good	Good	Mod	Mod	Changed	1992	B750	High	Good	Good	Good	Normal	1989	B750	Good	Good	Good	Good	Normal
1998	E750	High	Good	Good	Good	Changed	1992	C100	High	Good	Good	Good	Normal	1989	C100	Good	Good	Good	Good	Changed
1995	A100	High	Good	Good	Good	Changed	1992	C500	High	Good	Good	Good	Changed	1989	C500	Good	Mod	Good	Good	Normal
1995	A500	High	High	Good	Good	Changed	1992	C750	Good	Good	Good	Good	Normal	1989	C750	Good	Good	Good	Good	Normal
1995	A750	Good	High	Good	Good	Changed	1992	D100	High	Good	Good	Good	Changed	1989	D100	High	Mod	Good	Good	Changed
1995	A1000	Good	High	Good	Good	Changed	1992	D500	Good	Mod	Good	Good	Changed	1989	D500	High	Mod	Good	Good	Normal
1995	B100	Good	Good	Good	Good	Changed	1992	D750	Good	Good	Good	Good	Normal	1989	D750	Good	Mod	Good	Good	Changed
1995	B500	High	Good	Good	Good	Changed	1992	E100	Good	Good	Good	Good	Changed	1989	E100	Good	Good	Good	Good	Normal
1995	B750	Good	Good	Good	Good	Changed	1992	E500	Good	Mod	Mod	Good	Normal	1989	E500	High	Mod	Good	Good	Changed
1995	B1000	Good	High	Good	Good	Changed	1992	E750	Good	Mod	Good	Good	Changed	1989	E750	High	Mod	High	Good	Changed
1995	C100	High	Good	Good	Good	Changed	1992	F100	Good	Good	Mod	Mod	Changed	1989	F100	Good	Good	Good	Good	Changed
1995	C500	High	Good	Good	Good	Changed	1992	F500	Good	Good	Good	Good	Normal	1989	F500	High	Mod	Good	Good	Changed
1995	C750	High	Mod	Good	Good	Changed	1992	F750	Good	Mod	Good	Good	Changed	1989	F750	High	Poor	High	Good	Changed
1995	D100	Good	Mod	Good	Good	Changed	1992	G100	Good	Good	Good	Good	Changed	1989	G100	Good	Good	Good	Good	Changed
1995	D500	High	Good	Good	Good	Changed	1992	G500	High	Good	Good	Good	Normal	1989	G500	Good	Good	High	Good	Changed
1995	D750	High	Good	Good	Good	Changed	1992	G750	High	Good	Good	Good	Changed	1989	G750	Good	Good	Good	Good	Normal
1995	E100	Good	Mod	Mod	Good	Changed	1992	H100	Good	Good	Good	Good	Normal	1989	H100	High	Good	High	Good	Changed
1995	E500	High	Good	Good	Good	Changed	1992	H500	High	Good	Good	Good	Normal	1989	H500	Good	Good	High	Good	Normal
1995	E750	High	Good	Good	Good	Changed														
1995	E1000	High	Good	Good	Good	Changed														
1995	F100	Good	Good	Good	Good	Changed														
1995	F500	High	Good	Good	Good	Changed														
1995	F750	High	High	Good	Good	Changed														
1995	G100	High	Good	Good	Good	Changed														
1995	G500	High	Good	Good	Good	Changed														
1995	G750	High	High	Good	Good	Changed														
1995	G1000	High	High	Good	Good	Changed														
1995	H100	High	High	Good	Good	Changed														
1995	H500	High	Poor	Good	Good	Normal														
1995	H750	High	Mod	Good	Good	Changed														

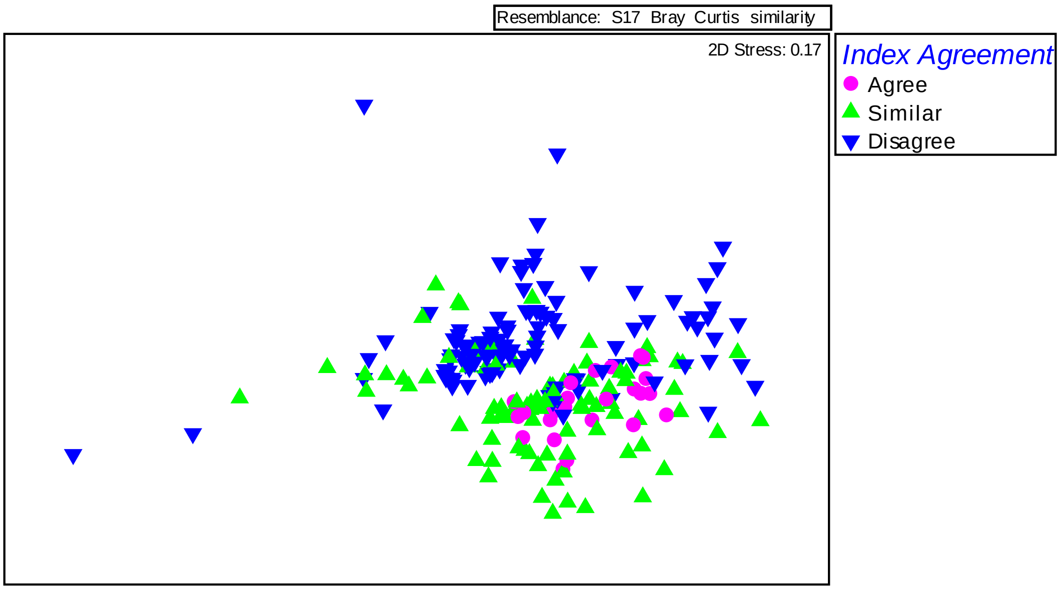


Figure 3.10 MDS plot of samples according to the level of agreement between indices (Table 3.9) at Ironrotter Point. Agree = all indices agree; Similar = two quality classifications given but adjacent on the scale of quality; Disagree = three or more quality classifications given or two classifications apart on the scale of quality.

Correlations of indices showed the strongest relationships were with organic content and year (Table 3.10) reflecting the MDS which showed a strong relationship between the benthic community with the organic content and year. The organic content was also highly correlated with the year and could be seen to increase in most stations in 1992 and in all stations in 1995 (Fig. 3.11). However, not all indices reflected the expected decrease in quality with time and with organic enrichment. Only J', EQR, ITI, A/S, BOPA, Lambda+ and AMBI indicated a decrease in quality with year and with organic enrichment. Most of the indices were not strongly correlated with depth. There were no strong correlations between indices and distance from the pollution source. This reflects the MDS (Fig.3.8) which showed strong correlations with distance only in the 1998. Only ITI showed decreasing quality when the effect of year was removed from organic matter. While J', ITI, BOPA, A/S and Lambda+ showed a decrease in quality when the effect of distance was removed from organic matter. Several indices showed a decrease in quality when the effect of organic matter was removed from the year. However, most indices found an increase in quality with year, organic matter content and distance. The strongest correlations found were between species richness, d and sDelta+ with year and organic matter.

Table 3.10 Correlation between indices and environmental variables at Ironrotter Point. Pearson product moment correlations with percentage correlation, *r*. Partial correlation carried out to remove effect of confounding variable 'year' from effect of 'organic matter' and vice versa, and to remove confounding variable 'distance' from 'organic matter'. Darker colours indicate a stronger relationship. Distance is from the outfall in metres; depth is in metres. Organic content data was not available for last year of sampling, 1998.

	Organic Matter (%)	Year	Depth	Distance	Organic Matter (Year removed)	Year (Organic Matter removed)	Organic Matter (Distance removed)
Year	80.3						81.9
Depth	16.6	1.6			29	-23.9	10
Distance	37.7	8.3	37.7		45.6	-30.7	
S	60.3	77.4	-0.6	0.9	-2.2	58.4	64.2
N	42	45.2	-1.2	-13.2	-2.4	37.9	44.1
d	53.9	61.4	-6	6.5	0	47.5	56
J	-20.1	-45.1	-1.8	11.1	10.2	-27.9	-22.1
Brillouin	42.8	28.4	3.4	11.3	0.7	34.3	44.5
Fisher	32	24.3	-17.6	12.5	5.4	18.4	30.5
ES(50)	19.3	-1.9	-10.4	13.3	6.6	6.2	18
H(loge)	32.1	10.5	-1.2	13.1	3.6	20.7	32.5
Simpson	1.6	-19.8	2.5	12.4	3.4	-3.2	0.8
N1	40.8	27.1	-4.6	10.3	6	25.7	42
IQI	40.4	3.1	6.3	24.6	34.7	-14.2	36
EQR	11.2	-36.3	6.5	24.6	40.8	-44.1	3.5
ITI	-42.7	-51.8	0.9	8.6	-17.5	-12.4	-44.8
BOPA	11	50.8	-7.8	-11.2	-39.1	53.5	13.4
A/S	31	34.7	-0.9	-12.5	-3.2	29.3	31.7
Delta	28.8	-5.4	6.1	24.7	28.5	-15.1	23.2
Delta *	40.8	16.8	8.6	29.3	40.4	-20.8	34.1
Delta +	36.5	49	18.8	16.7	0.9	28	32.4
sDelta +	61.3	78.2	0.8	2.2	-1.1	58.7	64.7
Lambda +	-15.6	-29.2	-4.5	-2	-7	-2.8	-15.1
AMBI	-4.8	38.4	-5.9	-22	-39.7	45.2	2.4
BQI	39.2	28.3	19	8.5	23.1	2	39.8
MAMBI	48.1	17.8	2.2	19.8	24.4	8.3	46.4

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Note: A/S, BOPA and AMBI index values have inverse relationships with quality

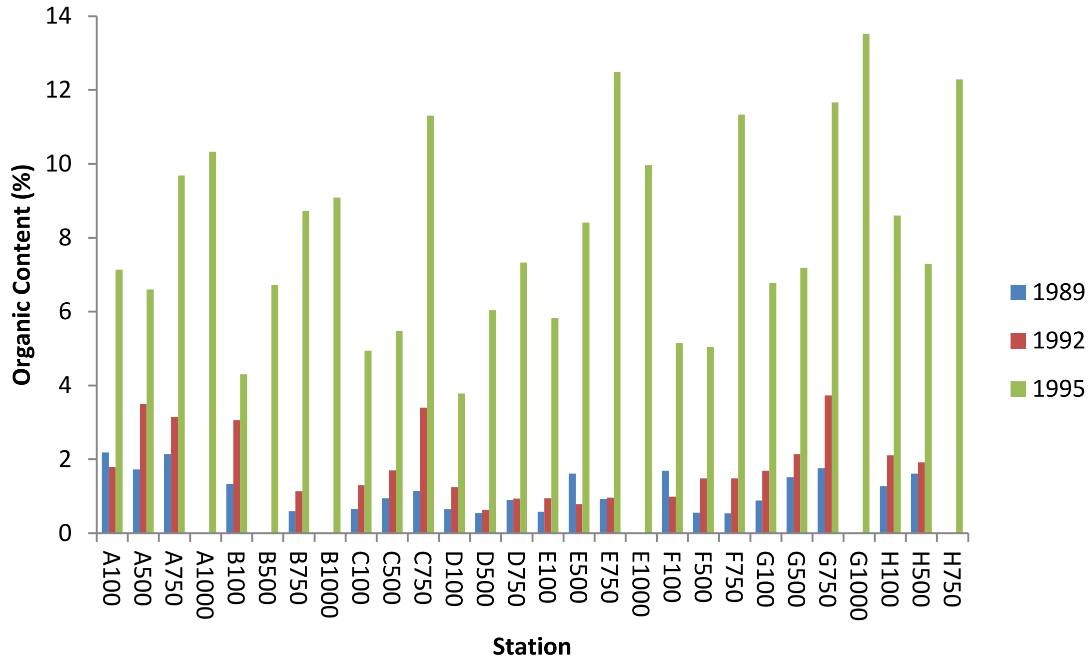


Figure 3.11 Organic matter content derived by loss on ignition at Ironrotter Point stations for three years (no data available for 1998).

3.3.1.3 Irvine Bay

MDS was carried out to assess the pattern of variation in composition between samples (Figs 3.12, 3.13). One replicate (1981 Q2.2) was excluded as species richness was zero. Analysis revealed differences between years (One-way ANOSIM, R=0.456, p<0.01), although there was no overall trend obvious as the most recent year was similar to the oldest year. The distance from the source showed a slight trend from left to right on the graph indicating a difference between the reference sites and the impacted sites (One-way ANOSIM, R=0.198, p<0.01). There was also a clear trend with depth of the sample sites (One-way ANOSIM, R=0.149, p<0.01) which was related to the trend with distance as reference sites were deeper sites. There were slight but significant differences found between stations impacted by the sewage discharge and stations impacted by chemical discharge (One-way ANOSIM pairwise comparison, R=0.073, p<0.01) but not between chemical and organic reference stations. The sites in close proximity to the sewage discharge GVS (L8, L81, J1), the chemical discharge (Q1) and the sewage discharge at Barassie (also close to pharmaceutical factory) (R1) were apart from other samples and there were significant differences between locations (One-way ANOSIM, R=0.347, p<0.01).

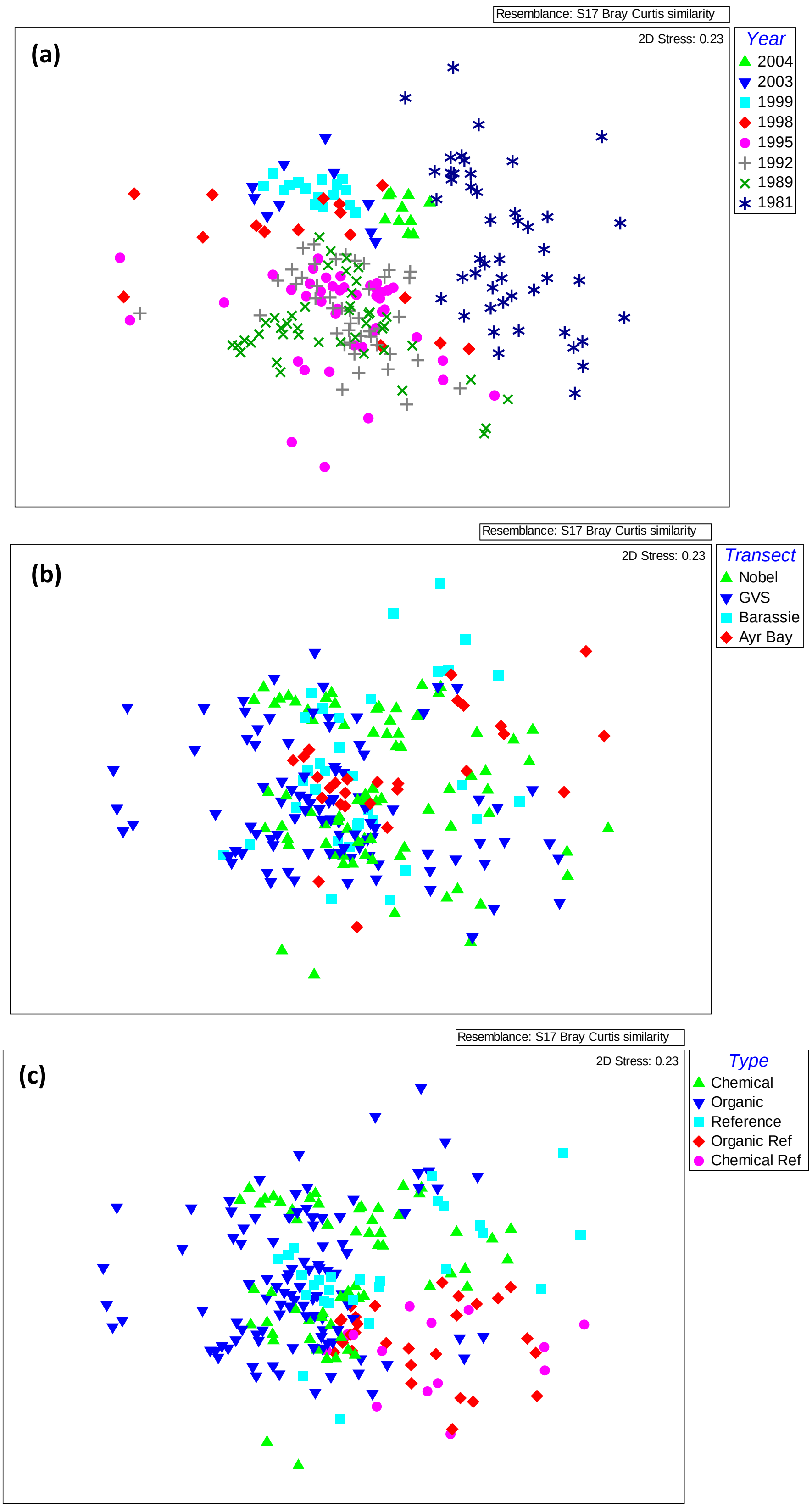


Figure 3.12 MDS plot of samples according to (a) year, (b) transect and (c) type of impact at Irvine and Ayr Bay (without sample Q2.2). See Figs 3.3 and 3.4 for location of transects. Type of impact: Organic Ref and Chemical Ref refer to reference stations along transects coming from either sewage outfall (GVS, Barassie) or chemical outfall (Nobel) and are all >2000m away from the outfall. Reference stations refer to Ayr Bay stations (Fig. 3.4).

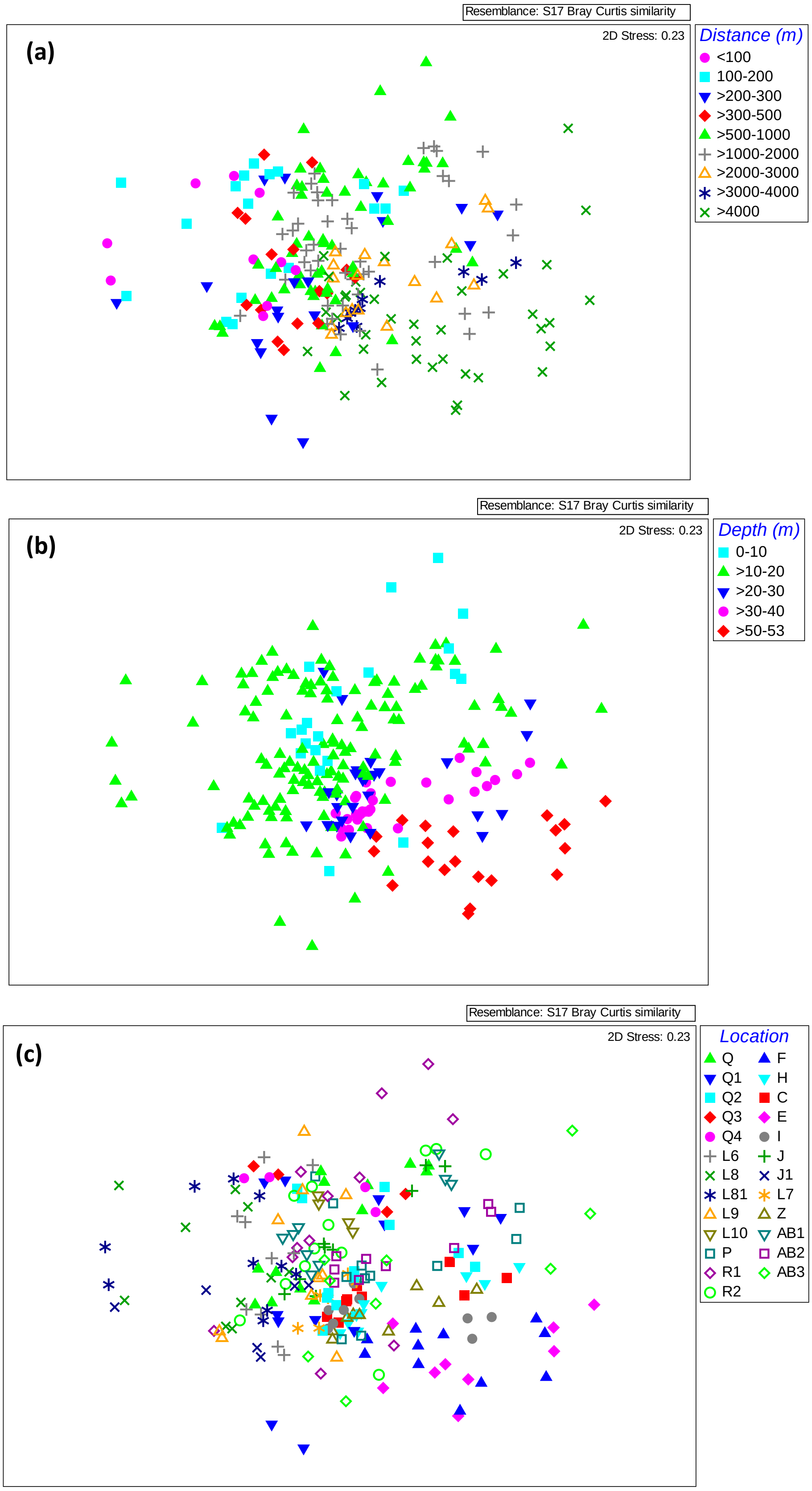


Figure 3.13 MDS plot of samples according to (a) distance, (b) depth and (c) location at Irvine and Ayr Bay (without sample Q2.2). For locations see Figs 3.3 and 3.4.

The ICI Nobel explosives factory transect was assessed separately since this site is subjected to chemical discharge while the other sites are subjected to organic waste disposal and the benthos may respond differently to different types of pollution (Fig. 3.14). Different years showed different benthic communities (One-way ANOSIM, $R=0.571$, $p<0.01$), apart from 1995 and 1992 where no significant differences were found. Significant differences were found between distance from the pollution source with the greatest differences found between those stations closest to the source from those further away (One-way ANOSIM, $R=0.255$, $p<0.01$). However, this was also related to significant differences found between stations of different depths, with the greatest differences found between the shallowest and the deepest stations (One-way ANOSIM, $R=0.216$, $p<0.01$).

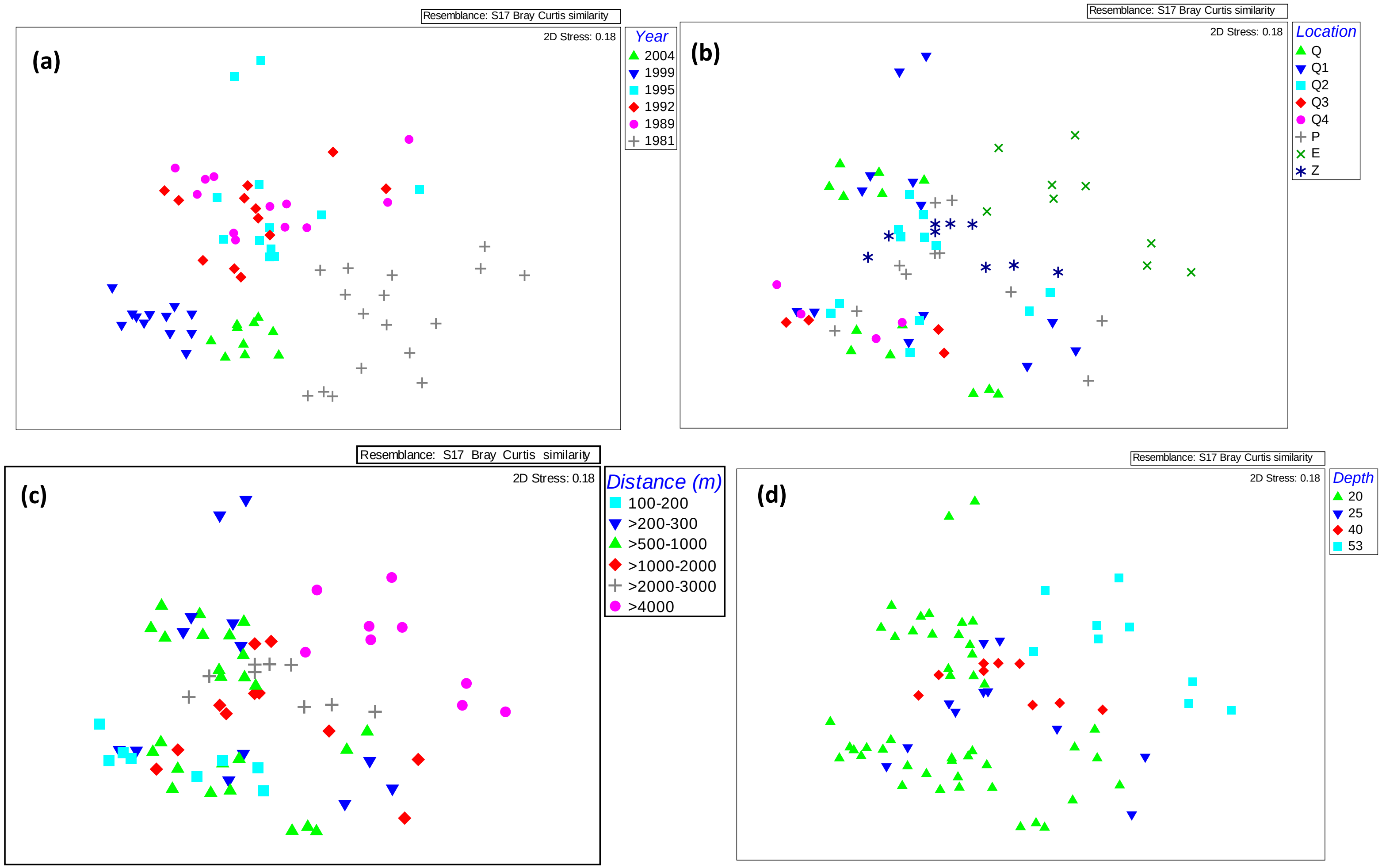


Figure 3.14 MDS plot of samples from ICI Nobel Explosives transect in Irvine Bay according to (a) year, (b) location (see Fig. 3.3 for locations), (c) distance from outfall and (d) depth (depth in metres).

The similarity of the samples taken along the Garnock Valley Sewer transect were also considered separately in order to assess the impact of the sewage pipe alone (Fig. 3.15). There was a significant effect of the year of sampling (One-way ANOSIM, $R=0.434$, $p<0.01$); the distance from the pollution source (One-way ANOSIM, $R=0.437$, $p<0.01$); the depth of the sampling station (One-way ANOSIM, $R=0.352$, $p<0.01$); and the sample location (One-way ANOSIM, $R=0.437$, $p<0.01$). The strongest differences were therefore due to distance from the outfall as well as the location of the particular sample point.

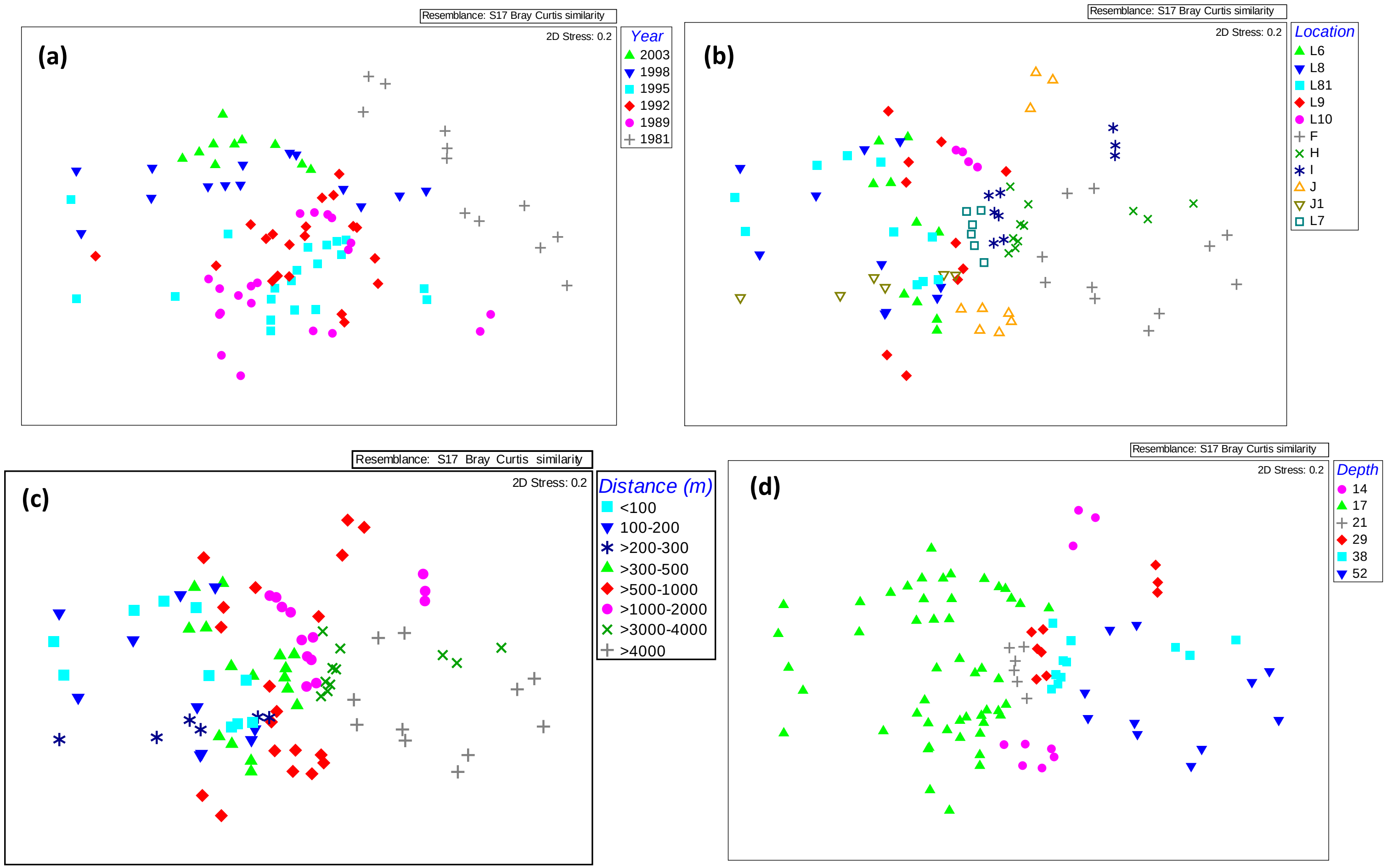


Figure 3.15 MDS plot of samples from Garnock Valley Sewer transect in Irvine Bay according to (a) year, (b) location (see Fig. 3.3 for locations), (c) distance from outfall and (d) depth (metres).

In the overall results for Irvine Bay, index quality classifications agreed in 8% of the samples; showed a similar trend in 49%; and disagreed in 43% of the samples (Table 3.11, Fig. 3.16). BQI was the only index which detected a difference in quality between 1981 and other years although MDS (Fig. 3.12) had revealed a difference between this year and other years in the benthic community. Locations which were revealed by MDS to be different from other samples were shown by some indices but not by others to have different quality from other sites. R1 (1981) was assigned bad quality only by BQI while other indices assigned good or high quality; Q1 (1995) was shown to have lower quality, by all but ITI. Indices showed lower quality in the sites L81 and L8 but BOPA did not. All indices found J1 to have worse quality. Sites which had a lower quality according to the indices were located in the left-middle section of the MDS. However, all indices did not reveal a lower quality for more locations which were found by MDS to have a high similarity to these low quality sites (left-middle section), including L7, L9, R2, Q3 and Q4.

Table 3.11 Quality classification based on average index value at each sample point in each year for Irvine Bay and Ayr Bay according to five indices (see Table 3.4 for sample details)

Year	Sample	IQI	BQI	BOPA	AMBI	ITI
2004	Q	Good	Good	Mod.	Good	Normal
2004	Q1	Good	Good	Good	Good	Normal
2004	Q2	Good	Mod.	Mod.	Good	Normal
2004	Q3	Good	Mod.	Good	Good	Normal
2004	Q4	Good	Good	Poor	Mod.	Normal
2003	L6	Good	Mod.	Good	Mod.	Changed
2003	L8	Good	Mod.	High	Mod.	Changed
2003	L81	Mod.	Mod.	High	Mod.	Changed
2003	L9	Good	Good	High	Good	Normal
2003	L10	High	High	High	Good	Normal
1999	P	Good	High	Good	Good	Normal
1999	Q	Good	Mod.	Good	Good	Normal
1999	Q1	Good	Mod.	Good	Good	Normal
1999	Q2	Good	Mod.	Good	Good	Normal
1999	Q3	Good	Good	Good	Good	Normal
1999	Q4	Good	Mod.	Good	Good	Normal
1999	R1	High	Good	Good	Good	Reference
1999	R2	Good	Good	Good	Good	Normal
1998	F	Good	Mod.	High	Good	Normal
1998	H	Good	Good	Good	Good	Changed
1998	L6	Good	Good	Good	Good	Changed
1998	L8	Poor	Bad	Good	Poor	Degraded
1998	L81	Mod.	Poor	High	Poor	Degraded
1998	L9	Good	Good	Good	Good	Normal
1998	L10	Good	High	Good	Good	Normal
1998	R1	High	Reference	Good	Good	Mod.
1998	R2	High	Normal	Good	Good	Good
1995	C	High	High	Good	Good	Normal
1995	E	Good	Mod.	Good	Good	Changed
1995	F	Good	Mod.	Good	Good	Changed
1995	H	High	Good	Good	Good	Changed
1995	I	High	High	Good	Good	Normal
1995	J	Good	Good	Good	Good	Normal
1995	J1	Poor	Poor	Poor	Poor	Degraded
1995	L6	Mod.	Mod.	Poor	Mod.	Changed
1995	L7	Good	High	Good	Good	Changed

Year	Sample	IQI	BQI	BOPA	AMBI	ITI
1995	L8	Good	Good	Poor	Mod.	Changed
1995	L81	Mod.	Poor	Mod.	Poor	Degraded
1995	L9	Good	Good	Good	Good	Changed
1995	P	High	Good	Good	Good	Normal
1995	Q	High	Good	Good	Good	Changed
1995	Q1	Mod.	Bad	Poor	Mod.	Normal
1995	Q2	Good	Good	Good	Good	Normal
1995	R1	High	Good	High	Good	Normal
1995	R2	High	Good	Good	Good	Normal
1995	Z	High	High	Good	Good	Changed
1995	AB1	High	Good	Good	Good	Normal
1995	AB2	High	Good	Good	Good	Normal
1995	AB3	High	Mod.	High	Good	Changed
1992	C	High	High	Good	Good	Normal
1992	E	Good	Mod.	High	Good	Normal
1992	F	High	Mod.	High	Good	Changed
1992	H	High	High	Good	Good	Changed
1992	I	High	High	Good	Good	Normal
1992	J	High	Good	Good	Good	Normal
1992	J1	Good	Good	Good	Good	Changed
1992	L6	Good	High	Good	Good	Changed
1992	L7	Good	High	Good	Good	Changed
1992	L8	Poor	Poor	Good	Poor	Degraded
1992	L81	Good	Good	Good	Good	Changed
1992	L9	Good	Mod.	Good	Good	Changed
1992	P	High	High	Good	Good	Normal
1992	Q	Good	Good	High	Good	Normal
1992	Q1	Good	Good	Good	Good	Normal
1992	Q2	Good	Good	Good	Good	Changed
1992	R1	Good	Mod.	Good	Good	Changed
1992	R2	Good	Good	Good	Good	Normal
1992	Z	High	Good	Good	Good	Changed
1992	AB1	Good	Good	Mod.	Good	Normal
1992	AB2	Good	Good	Good	Good	Normal
1992	AB3	High	Good	High	Good	Normal

Year	Sample	IQI	BQI	BOPA	AMBI	ITI
1989	C	High	High	Good	Good	Normal
1989	E	Good	Poor	High	Good	Normal
1989	F	Good	Bad	High	Good	Normal
1989	H	High	Good	Good	Good	Normal
1989	I	High	High	Good	Good	Normal
1989	J	Good	Good	Good	Good	Normal
1989	P	High	Good	High	Good	Normal
1989	Q	Good	Good	Good	Good	Changed
1989	Q1	Good	Good	Good	Good	Changed
1989	Q2	High	High	Good	Good	Changed
1989	R1	Good	Good	Good	Good	Changed
1989	R2	Good	Good	High	Good	Changed
1989	J1	Good	Mod.	Mod.	Mod.	Degraded
1989	L6	Good	High	Good	Good	Degraded
1989	L7	High	High	Good	Good	Changed
1989	L8	Good	Good	Good	Good	Degraded
1989	L81	Good	Good	Good	Good	Changed
1989	L9	Mod.	Good	High	Good	Degraded
1989	Z	High	Good	Good	Good	Normal
1989	AB1	High	Good	Good	Good	Normal
1989	AB2	High	High	Good	Good	Normal
1989	AB3	High	Good	Good	Good	Normal
1981	E	Good	Poor	Good	Good	Changed
1981	F	Good	Poor	Good	Good	Changed
1981	Z	High	Good	High	Good	Normal
1981	H	High	Poor	Good	Good	Changed
1981	C	Good	Mod.	High	Good	Normal
1981	I	Good	Mod.	High	Good	Normal
1981	P	Good	Mod.	Good	Good	Changed
1981	Q	Good	Poor	Good	Good	Reference
1981	Q1	Good	Poor	Good	Good	Normal
1981	Q2	Poor	No value	No value	Mod.	Degraded
1981	J	Good	Mod.	Good	Good	Normal
1981	R1	Good	Bad	High	Good	Reference
1981	R2	Good	Poor	High	Good	Normal
1981	AB1	Mod.	Mod.	Poor	Mod.	Normal
1981	AB2	Good	Mod.	Good	Good	Changed
1981	AB3	Good	Poor	High	Good	Changed

The indices mainly disagreed or were similar in most cases including those sites which were expected to have the worst quality and samples from 1981 (Fig. 3.16). The indices agreed in the middle section of the MDS where locations were mainly greater than 500m from the pollution source. No significant difference was found in the distribution of index agreement (One-way ANOSIM, R=-0.031, p>0.05).

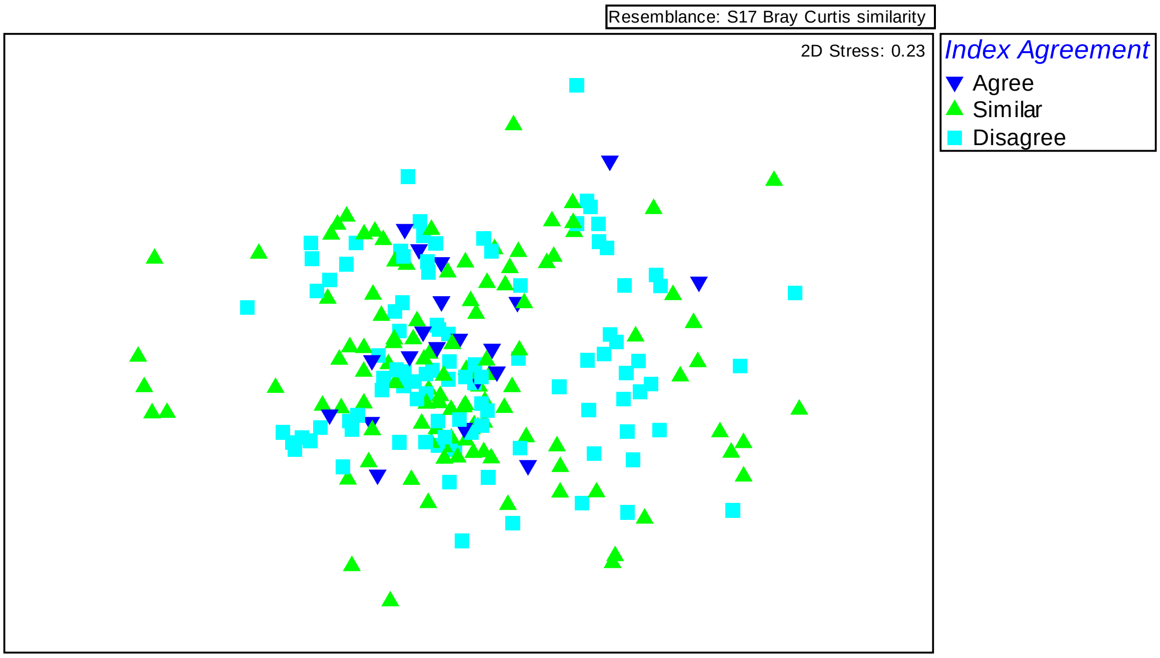


Figure 3.16 MDS plot of samples at Irvine and Ayr Bay according to level of agreement between the index classifications (Table 3.11). Agree = all indices agree; Similar trend = two quality classifications given but adjacent on the scale of quality; Disagree = three or more quality classifications given or two classifications apart on the scale of quality.

Most of the indices showed correlations with distance and with depth (Table 3.12). Although the correlations were not very strong, distance showed greater correlations than depth (Paired t-test, t=5.81, p<0.001) and when the effect of depth was removed by partial correlations the relationship with distance was maintained with most indices. Most of the indices indicated an increase in quality with distance from the pollution source but BQI showed the opposite and only taxonomic diversity (Delta) showed any considerable correlation with distance out of the measures of taxonomic distinctness and diversity. The correlation of indices with time showed mixed results with some detecting an increase and others detecting a decrease in quality. This reflects the MDS which indicated differences between years but no strong trend over time.

Table 3.12 Correlation between indices and environmental variables at Irvine and Ayr Bay. Pearson product moment correlations with percentage correlation, *r*. Partial correlation carried out to remove effect of confounding variable ‘depth’ from effect of ‘distance’. Darker colours indicate a stronger relationship. Distance is from the outfall in metres; depth is in metres.

	Year	Distance	Depth	Distance (depth removed)
Distance	-28.9			
Depth	-15.2	66.7		
S	41.7	-7.3	-10.3	-0.6
N	32.2	-31.3	-16.5	-27.6
d	28.5	7.6	-2.8	12.7
J'	-30.9	55.1	40	41.6
Brillouin	12.4	23.8	15.1	18.6
Fisher	2.3	31.1	10.6	32.4
ES(50)	-3.7	36.2	21.6	30
H'(loge)	3.6	34.4	21.9	27.3
Simpson	-7.5	40.3	29.4	29.1
N1	0.2	33.8	19.6	28.3
IQI	4.3	26.5	13.8	23.4
EQR	-20.5	45.4	28.5	36.9
ITI	-11.2	15.1	0.2	20.1
BOPA	22.6	-32.2	-23.8	-22.6
A/S	17.9	-26.2	-14.3	-22.6
Delta	3.8	33.6	28.4	20.6
Delta *	20.1	-0.3	7.3	-7
Delta +	20.1	4.6	-2.5	8.4
sDelta +	42.7	-6.7	-11	0.9
Lambda +	7.6	-7.9	1.4	-11.9
AMBI	25.7	-44	-25.6	-37.4
BQI	27.2	-14	-15	-5.3
MAMBI	6.7	31	16.7	27.1

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Note: A/S, BOPA and AMBI index values have inverse relationships with quality

The correlations along the Nobel transect showed a different pattern to the overall trend (Table 3.13). Most indices showed an increase in quality over time although some decreased. The strength of correlations between indices with depth and distance were similar. With distance and with depth, species diversity decreased while most other indices detected an increase in quality. When the effect of depth was removed from distance, species diversity and similar indices still decreased while other indices such as ITI and AMBI showed no correlation with distance.

Table 3.13 Correlation between indices and environmental variables along the Nobel transect. Pearson product moment correlations with percentage correlation, *r*. Partial correlation carried out to remove effect of confounding variable 'depth' from effect of 'distance'. Darker colours indicate a stronger relationship. Distance is from the outfall in metres; depth is in metres.

	Year	Distance	Depth	Distance (depth removed)
Distance	-28.9			
Depth	-28.4	98		
S	39.1	-30.4	-24.1	-34.9
N	36.4	-30.3	-25.4	-28.3
d	26.6	-23.4	-18.3	-28.2
J	-42.8	48.2	46.6	14.3
Brillouin	3.5	-0.8	3.4	-21
Fisher	-3.8	8.1	10.1	-9.6
ES(50)	0.3	11.5	14.2	-12.3
H(loge)	0.4	14.5	18.3	-17.3
Simpson	-25.4	34.5	35.6	-2.2
N1	-5.1	8.5	11.6	-14.9
IQI	13	9.8	13.8	-19.1
EQR	-32.4	41.1	42.8	-5.3
ITI	9.2	-19.3	-21.3	7.8
BOPA	49.1	-40.7	-39.7	-9.8
A/S	35	-35.9	-32	-23.9
Delta	-1.3	26.4	29.5	-13.3
Delta *	21.6	7.4	11	-17
Delta +	27.7	1.8	4.3	-12.3
sDelta +	41.6	-29.9	-23.8	-34.2
Lambda +	10.9	13.7	13.4	2.7
AMBI	14.8	-35.9	-38.2	8.7
BQI	30.4	-33	-29.1	-23.6
MAMBI	9.5	8.7	13.7	-24.2

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Note: A/S, BOPA and AMBI index values have inverse relationships with quality

At Garnock Valley Sewer transect, there was a decrease in quality over time according to most indices but an increase in species richness (Table 3.14). Most indices detected an increase in quality with distance from the outfall and depth, the strongest correlations being with AMBI and J'. When the effect of depth was removed from distance, ITI and AMBI showed the strongest correlations with distance while J' showed a much weaker correlation.

Table 3.14 Correlation between indices and environmental variables along the Garnock Valley Sewer transect. Pearson product moment correlations with percentage correlation, *r*. Partial correlation carried out to remove effect of confounding variable 'depth' from effect of 'distance'. Darker colours indicate a stronger relationship. Distance is from the outfall in metres; depth is in metres.

	Year	Distance	Depth	Distance (depth removed)
Distance	-27.1			
Depth	-27.5	96.9		
S	30.4	-18.3	-17.4	-5.7
N	29.1	-38.8	-31.4	-35.8
d	14.5	1	-0.8	7.3
J	-33.2	59	56.8	19.8
Brillouin	-3.6	25.4	24.3	7.6
Fisher	-10.1	23	19.7	16.3
ES(50)	-23.6	40.8	38.2	16.6
H(loge)	-12.7	35.2	33.4	12.2
Simpson	-12.4	38.2	36.4	12.5
N1	-17.4	35.9	35.9	4.8
IQI	-20	37.4	31.6	28.8
EQR	-29.9	49.5	43.7	31.9
ITI	-15.4	36.5	27	43.2
BOPA	4.3	-34	-33.5	-6.5
A/S	16.7	-30	-24.2	-27.2
Delta	-10.8	32.6	33.2	2.1
Delta *	2.4	-16.6	-10.2	-27.3
Delta +	9.6	1.4	-3.1	17.7
sDelta +	31.3	-18.3	-17.8	-4
Lambda +	1.8	-7.6	-8.1	0.8
AMBI	38.5	-52.5	-45.4	-38.7
BQI	7.1	-21	-25	13.5
MAMBI	-13	33.4	29.7	19.5

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Note: A/S, BOPA and AMBI index values have inverse relationships with quality

3.3.1.4 Fish Farms

The samples from the farm Lippie Geo (Cage edge 2 and 4) contained no species and were excluded from MDS analysis. Analysis from all other samples revealed clear differences between benthic communtities found at the cage edge, allowable zone of effect and the reference site (Fig. 3.17) (One-way ANOSIM, $R=0.388$, $p<0.01$). There were also large differences, in some cases, between different fish farm sites (One-way ANOSIM, $R=0.362$, $p<0.01$). No differences based on use of antifoulants was evident (Two-way ANOSIM location and antifoulant; $R=0.013$, $p>0.05$). A slight trend with depth was apparent when the reference sites were excluded (Fig. 3.18) (One-way ANOSIM, $R=0.186$, $p<0.01$). There was some evidence of the maximum consented tonnes also showing a pattern with a trend from the heaviest loaded sites to the least. This trend was clearer when the reference sites were excluded (One-way ANOSIM, $R=0.134$, $p<0.01$), although this could have been related to individual site differences. The actual tonnes on site at time of survey was not significant when reference sites were excluded (One-way ANOSIM, $R=0.018$, $p>0.05$).

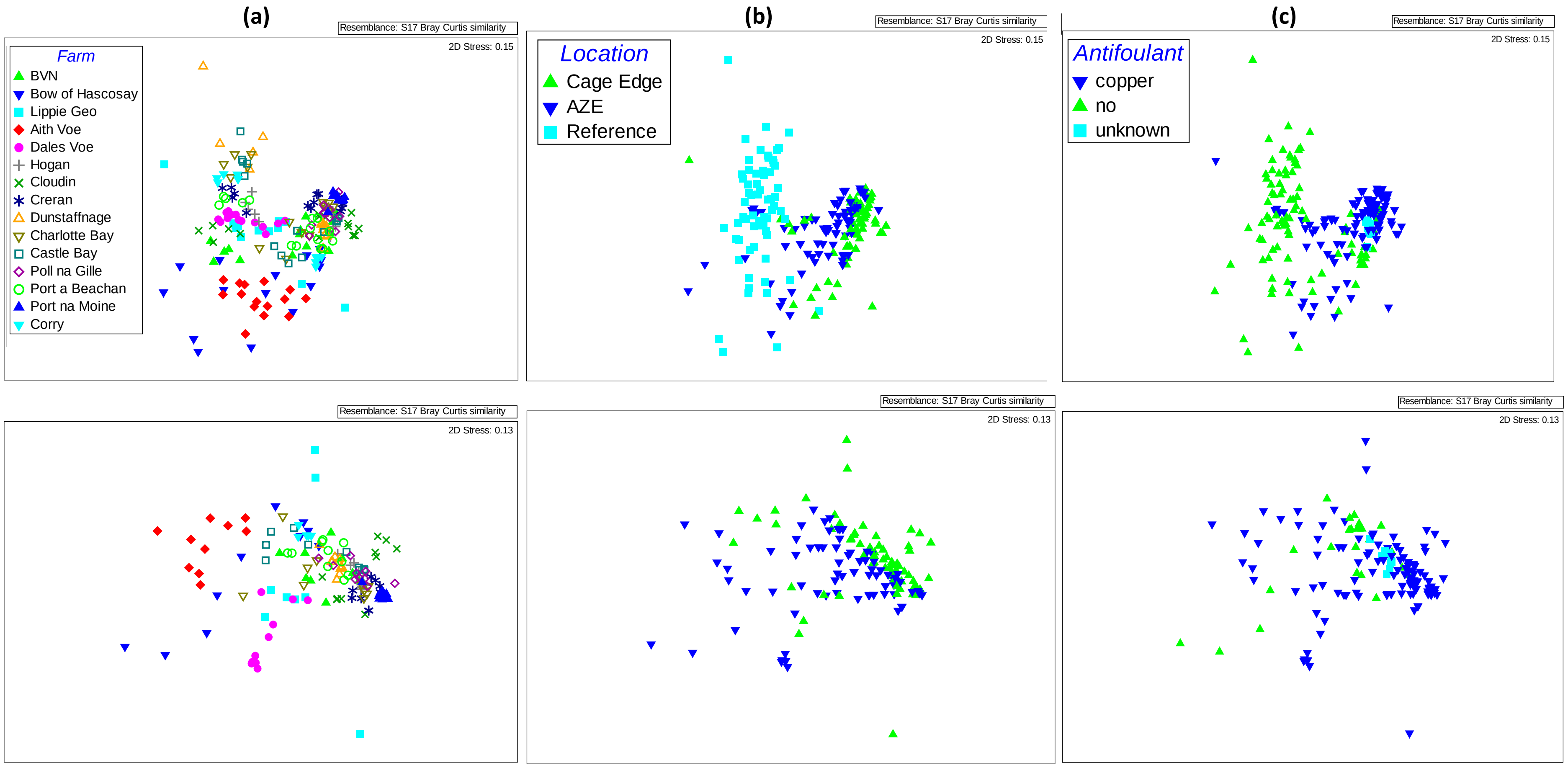


Figure 3.17 MDS plot of samples at fish farm sites according to (a) site of farm, (b) location of sample and (c) use of antifoulant on fish cages. Graphs above show all samples, graphs below exclude reference samples. Location: AZE refers to allowable zone of effect which is located 25m from the farm.

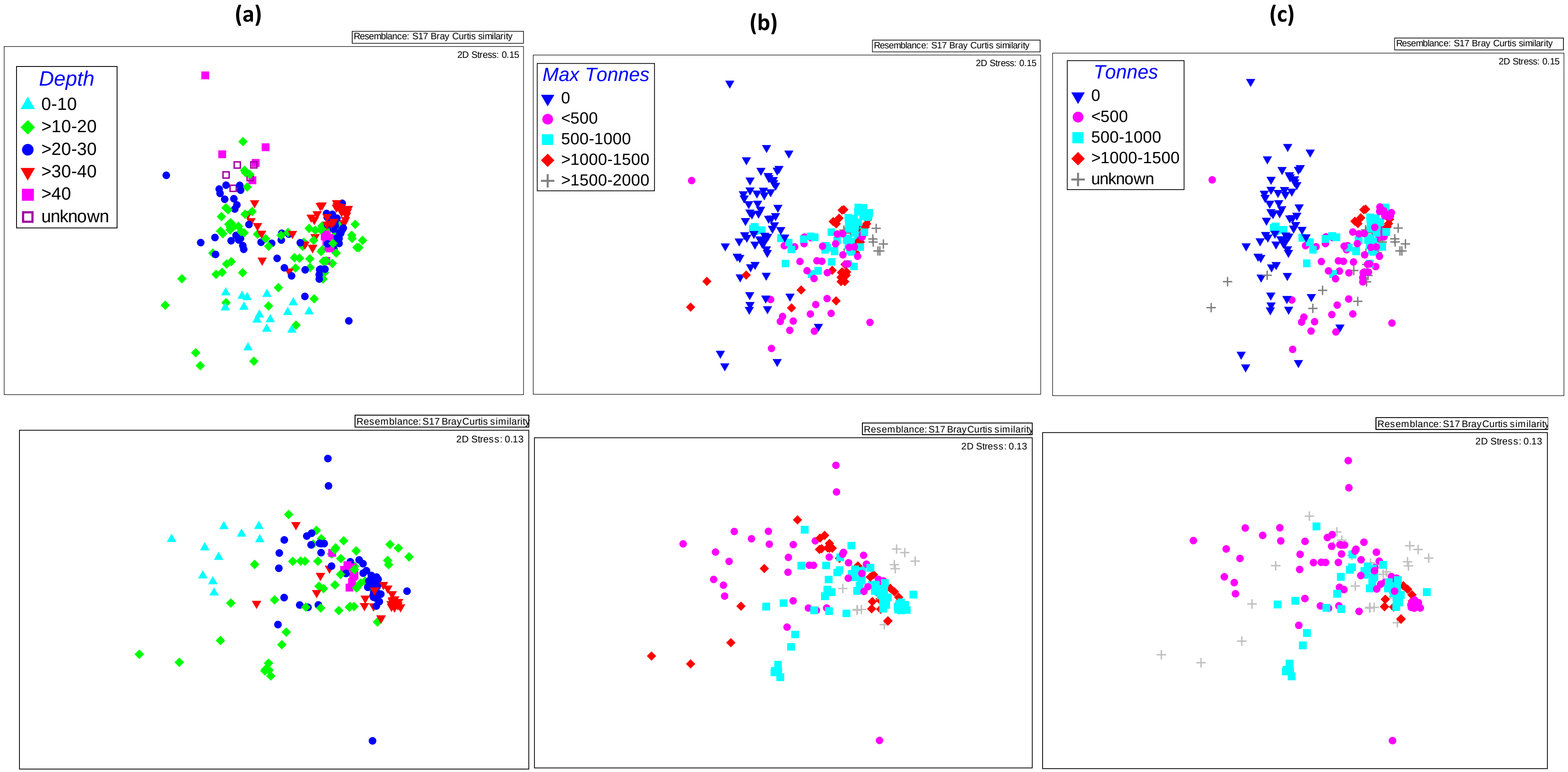


Figure 3.18 MDS plot of samples at fish farm sites according to (a) depth (metres), (b) maximum consented tonnes and (c) tonnes of fish at time of survey. Graphs above show all samples, graphs below exclude reference samples.

The index values indicated bad and poor qualities at the cage edge and allowable zone of effect sites and better quality at the reference sites (Table 3.15), reflecting the pattern shown in the MDS (Fig. 3.17). The BQI indicated much lower quality than the other indices at the reference sites. In some cases the proportion of species and abundance assigned by BQI was low indicating the species list was insufficient and this may have been a cause for particularly low values compared to the other indices. Level of agreement was high amongst the bad and degraded sites but at other sites indices disagreed or showed a similar trend (Fig. 3.19) (One-way ANOSIM, $R=0.271$, $p<0.01$). 31% of the indices agreed, 29% showed a similar classification and 40% disagreed. MDS was also carried out without BQI as these classifications may not have been valid. When BQI was excluded, most indices showed a similar trend or agreed (One-way ANOSIM, $R=0.219$, $p<0.01$).

Table 3.15 Quality classification based on average index value at each sample point for different fish farms according to five indices (see Table 3.5 for sample details; n=5 in all cases)

Site	IQI	BQI	BOPA	AMBI	ITI	Site	IQI	BQI	BOPA	AMBI	ITI
02 BVN CE	Bad	Bad	Bad	Bad	Degraded	03 Creran A CE	Bad	Bad	Bad	Bad	Degraded
02 BVN AZE	Poor	Poor	Mod.	Poor	Degraded	03 Creran A AZE	Poor	Bad	Poor	Poor	Degraded
02 BVN Ref	High	Mod.	High	High	Normal	03 Creran A Ref	High	Mod.	Good	Good	Normal
02 Bow of Hascosay CE	Bad	Bad	Bad	Bad	Degraded	03 Dunstaffnage CE	Bad	Bad	Bad	Bad	Degraded
02 Bow of Hascosay AZE	Good	Poor	High	Good	Normal	03 Dunstaffnage AZE	Bad	Bad	Bad	Bad	Degraded
02 Bow of Hascosay Ref	Good	Bad	High	Good	Normal	03 Dunstaffnage Ref	Mod.	Poor	Good	Good	Normal
02 Lippie Geo CE	Bad	No value	No value	Mod.	No value	03 Charlotte Bay CE	Bad	Bad	Bad	Bad	Degraded
02 Lippie Geo AZE	Mod.	Poor	Mod.	Mod.	Changed	03 Charlotte Bay AZE	Poor	Bad	Poor	Poor	Degraded
02 Lippie Geo Ref	Good	Poor	Good	Good	Normal	03 Charlotte Bay Ref	Good	Poor	High	Good	Normal
02 Aith Voe CE	Poor	Bad	Good	Mod.	Changed	03 Castle Bay CE	Bad	Bad	Bad	Bad	Degraded
02 Aith Voe AZE	Mod.	Bad	High	Good	Changed	03 Castle Bay AZE	Poor	Bad	Mod.	Poor	Degraded
02 Aith Voe Ref	Mod.	Bad	Good	Good	Normal	03 Castle Bay Ref	Good	Mod.	High	Good	Normal
02 Dales Voe CE	Mod.	Mod.	Good	Good	Changed	03 Poll na Gille CE	Bad	Bad	Bad	Bad	Degraded
02 Dales Voe AZE	Good	Good	Good	Good	Changed	03 Poll na Gille AZE	Poor	Bad	Bad	Bad	Degraded
02 Dales Voe Ref	Good	Mod.	High	Good	Changed	03 Port a Beachan CE	Bad	Bad	Bad	Bad	Degraded
02 Hogan CE	Bad	Bad	Bad	Bad	Degraded	03 Port a Beachan AZE	Poor	Bad	Poor	Poor	Degraded
02 Hogan AZE	Bad	Bad	Bad	Bad	Degraded	03 Port a Beachan Ref	Good	Poor	Mod.	Good	Normal
02 Hogan Ref	Mod.	Mod.	Good	Mod.	Changed	03 Port na Moine CE	Bad	Bad	Bad	Bad	Degraded
02 Cloudin CE	Bad	Poor	Good	Mod.	Degraded	03 Port na Moine AZE	Poor	Bad	Bad	Bad	Degraded
02 Cloudin AZE	Bad	Bad	Poor	Poor	Degraded	03 Corry AZE	Bad	Bad	Bad	Bad	Degraded
02 Cloudin Ref	Mod.	Poor	High	Good	Changed	03 Corry Ref	Good	Mod.	Good	Good	Normal

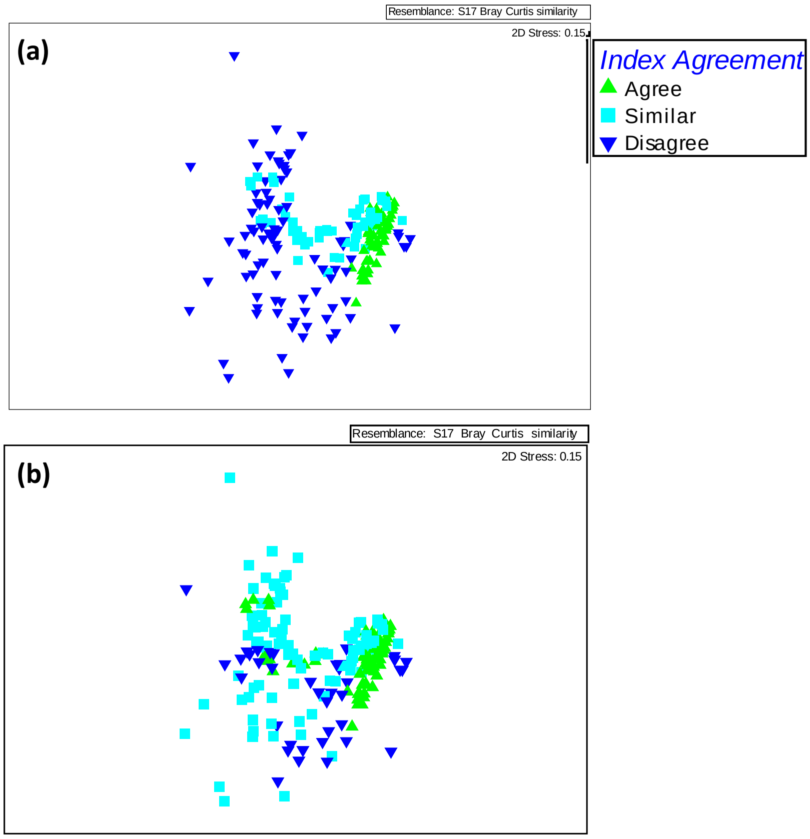


Figure 3.19 MDS plot of samples at fish farms according to level of agreement between the index classifications: (a) all indices; (b) excluding BQI (Table 3.15). Agree = all indices agree; Similar = two quality classifications given but adjacent on the scale of quality; Disagree = three or more quality classifications given or two classifications apart on the scale of quality.

A high level of correlation was found between most indices and distance from the cage as would have been expected given the clear differences in samples shown by the MDS (Table 3.16). However, some indices showed low correlations compared to other indices including taxonomic distinctness (Delta*), average taxonomic distinctness (Delta+), variation in taxonomic distinctness (Lambda+) and measures of evenness (J', A/S and Simpson's Index). Indices showed a decrease in quality with increasing depth.

Table 3.16 Correlation between indices and environmental variables at fish farms. Pearson product moment correlations with percentage correlation, *r*. Partial correlation carried out to remove effect of confounding variable ‘max tonnes’ and ‘tonnes’ from effect of ‘distance’ and to remove the effect of ‘distance’ from the effect of ‘depth’. Darker colours indicate a stronger relationship. Distance is from the fish farm in metres (Cage edge=0m, AZE=25m and reference sites were given a nominal value of 50m); depth is in metres; Max tonnes is the maximum consented tonnes of the farm; Tonnes is the amount of fish on site at the time of survey.

	Distance	Depth	Max tonnes	Tonnes	Distance (max tonnes removed)	Distance (tonnes removed)	Depth (distance removed)
Depth	-9.4				-7.2	-4.6	
Max tonnes		6.2				-38.2	1.2
Tonnes		25	86.9		3.1		19.4
S	56.9	-15.9	-38.5	-16.5	46.2	54	-12.9
N	-32.1	35.9	44.5	34.9	-9.6	-20.2	34.8
d	65.4	-22.9	-50.9	-29.5	51.4	58.6	-22.5
J	41.4	-30.9	-51	-47.8	17.4	28.9	-29.1
Brillouin	63.8	-23	-53.5	-33.1	47.9	55.2	-22.4
Fisher	61.7	-23.1	-48.7	-37.5	47.1	54.9	-22.2
ES(50)	68.5	-23.5	-55.1	-36.5	54.4	60.6	-23.5
H(loge)	67.3	-26.6	-55.2	-36.5	52.7	59.3	-27.3
Simpson	56.1	-29.3	-53.4	-38.6	36.7	44.1	-29.2
N1	60.5	-18.7	-49.8	-34.5	45.4	51.2	-16.4
IQI	72.3	-32.5	-54.3	-35.6	60.2	67.7	-36.9
EQR	72.1	-40.3	-58.1	-45.8	58.1	64.4	-48.1
ITI	70.7	-38.5	-63.4	-49.3	54.2	62.6	-44.9
BOPA	-61.4	48.3	47.7	42.5	-47.3	-54.6	53.6
A/S	-46.6	33.3	44.2	20.5	-28.8	-42.9	32.9
Delta	58.5	-35.2	-48.2	-39.5	43.3	49	-36.3
Delta *	31.5	-38.5	-8.7	-21.1	32.3	31.7	-37.5
Delta +	2.4	-14.2	5.5	2	6.7	5.4	-14.2
sDelta +	56.8	-16.5	-38.6	-16.9	46	53.8	-13.5
Lambda +	32.1	0.5	-18.7	1.2	26.6	30	4.1
AMBI	-69.4	42.5	52	64.2	-56.8	-63.5	49.4
BQI	52.6	-25.2	-30.7	-22.1	44.8	50.5	-24.1
MAMBI	71.9	-30.8	-54.2	-36.3	59.6	65.6	-34.5

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Note: A/S, BOPA and AMBI index values have inverse relationships with quality

3.3.1.5 Clyde Upper Estuary

MDS analysis was initially carried out using all data from all sample points but the 0m stations were very dissimilar from all other stations (Fig. 3.20) and the analysis was carried out without these in order to view patterns between other samples. In addition, any stations which had no individuals were removed and several other replicates which had too great differences from other samples were removed (including replicates from 1995 (June) 2 mls, 4 mls; 1996 (May) 4 mls, 8 mls; 1996 (November) 4 mls) in order to view patterns amongst the majority of samples which were clumped together in the overall MDS. The rest of the replicates, located from 2 mls and seawards, were represented in the MDS analyses (Fig. 3.21, 3.22). ANOSIM analyses were carried out using the full dataset. Significant differences were found with distance (One-way ANOSIM, R=0.219, p<0.01) and with bottom salinity (One-way ANOSIM, R=0.188, p<0.01) with the greatest differences found between the uppermost and the lowest regions of the estuary and between the most and least saline, respectively. No differences were found with top salinity (One-way ANOSIM, R=-0.08, p>0.01). There were significant differences between years (One-way ANOSIM, R=0.103, p<0.01) and month of sampling (One-way ANOSIM, R=0.246, p<0.01). The greatest differences found between months were between May and other months.

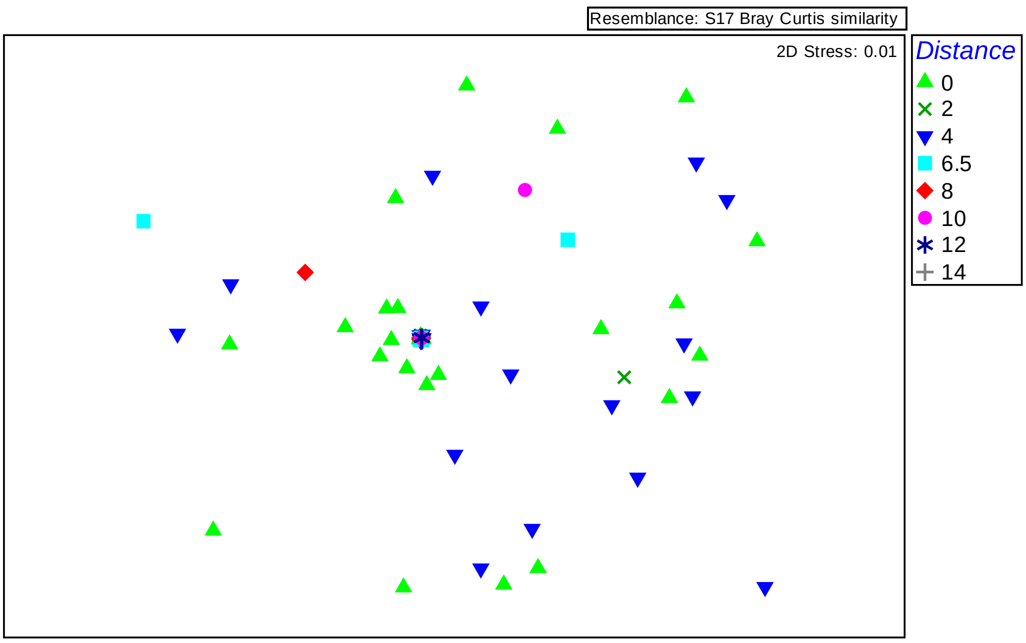


Figure 3.20 MDS plot of samples at upper Clyde estuary according to distance (miles)

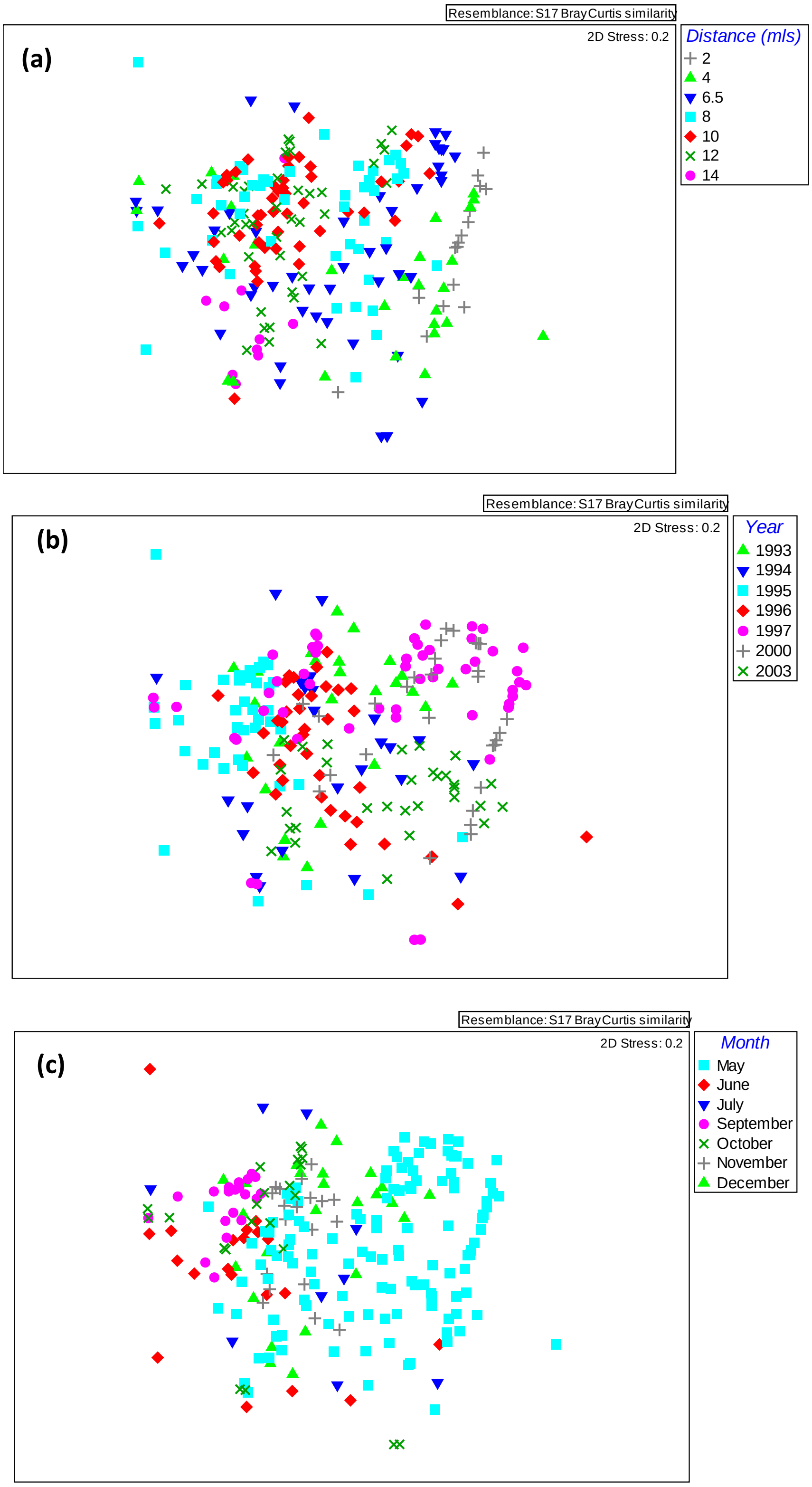


Figure 3.21 MDS plot of samples at upper Clyde estuary from 2 miles seawards according to (a) distance downstream, (b) year and (c) month.

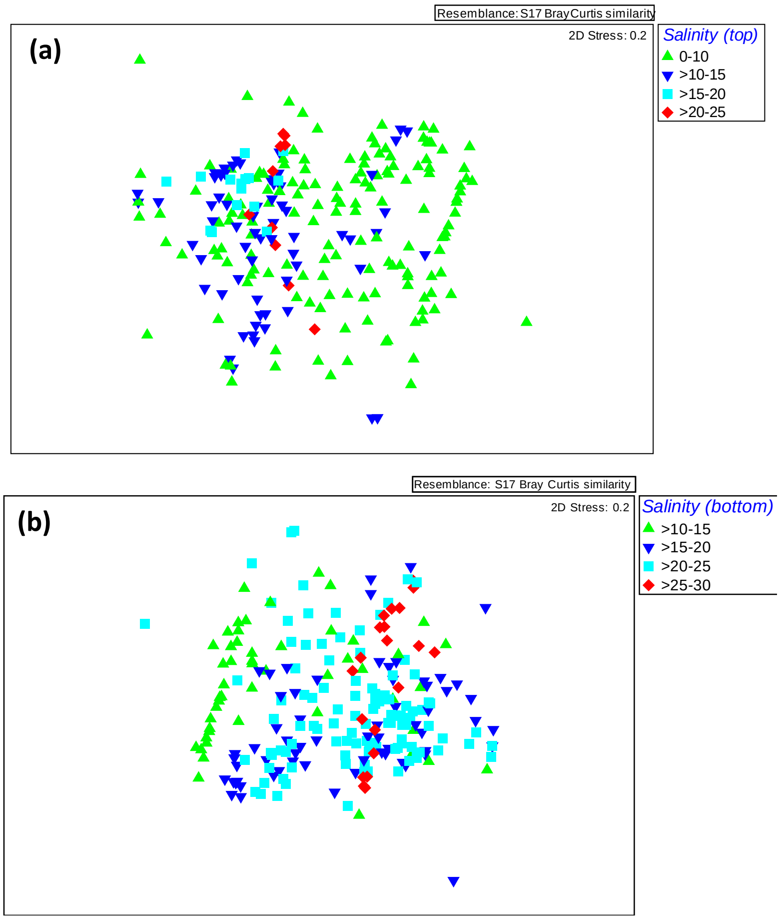


Figure 3.22 MDS plot of samples at upper Clyde estuary from 2 miles seawards according to (a) top salinity and (b) bottom salinity. Salinity was measured on separate occasion to benthos sampling (see appendix 8.3 for details).

Quality classification showed most indices classified sites as bad or poor apart from BOPA which assigned many as good and high (Table 3.17). In addition, many sites were assigned no value by BOPA. Indices agreed in 32% of locations, mainly in the 0, 2 and 4 mile stations, disagreed in 54% of locations and found a similar trend in 15% (Fig. 3.23) (One-way ANOSIM, R=0.389, p<0.01).

Table 3.17 Quality classification based on average index value at each sample point for upper Clyde estuary according to five indices (see Table 3.6 for sample details; n=5 in all cases). Distance in miles.

Year	Distance	IQI	BQI	BOPA	AMBI	ITI	Year	Distance	IQI	BQI	BOPA	AMBI	ITI
1993	0	Bad	Bad	No value	Bad	Degraded	1996(Nov)	4	Bad	No value	No value	Bad	No value
1993	4	Poor	Bad	Good	Mod.	Changed	1996(Nov)	6.5	Bad	No value	No value	Bad	No value
1993	6.5	Bad	Bad	High	Bad	Degraded	1996(Nov)	8	Poor	Bad	High	Good	Normal
1993	8	Poor	Bad	High	Poor	Changed	1996(Nov)	10	Poor	Bad	Mod.	Poor	Changed
1993	10	Poor	Bad	Good	Mod.	Changed	1996(Nov)	12	Poor	Bad	Mod.	Poor	Changed
1993	14	Poor	Bad	Mod.	Poor	Changed	1997	0	Bad	Bad	High	Bad	Degraded
1994	0	Bad	No value	No value	Bad	No value	1997	2	Bad	Bad	High	Poor	Degraded
1994	4	Bad	No value	No value	Poor	No value	1997	4	Bad	Bad	No value	Bad	Degraded
1994	6.5	Poor	Bad	Good	Poor	Degraded	1997	6.5	Bad	Bad	High	Poor	Degraded
1994	8	Bad	Bad	No value	Poor	Degraded	1997	8	Bad	Bad	High	Bad	Degraded
1994	10	Poor	Bad	High	Mod.	Changed	1997	10	Poor	Bad	Good	Poor	Degraded
1994	14	Poor	Bad	Good	Poor	Degraded	1997	12	Poor	Bad	Good	Bad	Degraded
1995(Jun)	0	Bad	No value	No value	Bad	No value	1997 (Oct)	0	Bad	No value	No value	Bad	No value
1995(Jun)	2	Bad	No value	No value	Poor	No value	1997 (Oct)	4	Bad	No value	No value	Bad	No value
1995(Jun)	4	Bad	No value	No value	Bad	No value	1997 (Oct)	6.5	Bad	No value	Mod.	Poor	No value
1995(Jun)	6.5	Bad	Bad	High	Poor	Degraded	1997 (Oct)	8	Poor	Bad	No value	Mod.	Changed
1995(Jun)	8	Bad	No value	Poor	Bad	No value	1997 (Oct)	10	Poor	Bad	Good	Mod.	Changed
1995(Jun)	10	Bad	No value	No value	Poor	No value	1997 (Oct)	12	Poor	Bad	Poor	Poor	Degraded
1995(Jun)	12	Poor	Bad	No value	Mod.	Changed	2000	0	Bad	No value	No value	Bad	No value
1995(Sep)	0	Bad	No value	No value	Bad	No value	2000	2	Bad	Bad	No value	Bad	Degraded
1995(Sep)	4	Bad	No value	No value	Bad	No value	2000	4	Bad	Bad	No value	Bad	Degraded
1995(Sep)	6.5	Poor	Bad	No value	Poor	Changed	2000	6.5	Bad	Bad	High	Poor	Degraded
1995(Sep)	8	Poor	Bad	High	Good	Normal	2000	8	Bad	Bad	High	Bad	Degraded
1995(Sep)	10	Poor	Bad	High	Good	Normal	2000	10	Poor	Bad	High	Poor	Degraded
1995(Sep)	12	Poor	Bad	High	Good	Normal	2000	12	Poor	Bad	Good	Poor	Degraded
1996(May)	0	Bad	No value	No value	Poor	No value	2003	0	Bad	Bad	No value	Bad	Degraded
1996(May)	4	Bad	No value	No value	Poor	No value	2003	2	Bad	Bad	High	Poor	Degraded
1996(May)	6.5	Bad	Bad	No value	Poor	Degraded	2003	4	Bad	Bad	High	Poor	Degraded
1996(May)	8	Bad	Bad	Good	Poor	Degraded	2003	6.5	Bad	Bad	High	Bad	Degraded
1996(May)	10	Poor	Bad	High	Mod.	Changed	2003	8	Bad	Bad	Mod.	Bad	Degraded
1996(May)	12	Poor	Bad	High	Poor	Changed	2003	10	Bad	Bad	High	Poor	Degraded
1996(Nov)	0	Bad	No value	No value	Bad	No value	2003	12	Bad	Bad	Good	Bad	Degraded

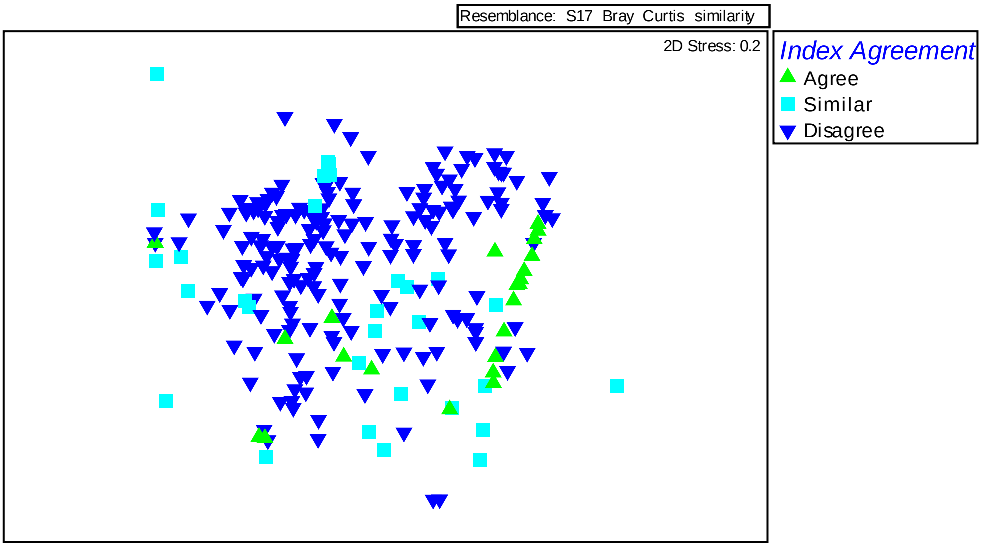


Figure 3.23 MDS plot of samples at upper Clyde estuary according to level of agreement between the index classifications (Table 3.17). Agree = all indices agree; Similar = two quality classifications given but adjacent on the scale of quality; Disagree = three or more quality classifications given or two classifications apart on the scale of quality.

Indices correlated weakly with time with some indices detecting increasing quality while other detected a decrease (Table 3.18). BOPA and ITI correlated most strongly with the month of sampling with BOPA indicating a decrease in quality while ITI indicated an increase. Most indices detected an increase in quality with distance downstream and with depth. Weaker correlations were found with salinity but an increase in quality was detected by most indices. BOPA generally indicated an opposite trend to most other indices. When depth and salinity were removed, the trend between indices and distance was maintained in most cases and overall, correlations were strongest with this variable.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 3.18 Correlation between indices and environmental variables at upper Clyde estuary. Pearson product moment correlations with percentage correlation, *r*. Partial correlations carried out to remove effect of confounding variables. Darker colours indicate a stronger relationship. Distance is seaward in miles; month assigned value between 1 and 12; depth in metres; salinity was measured on a separate occasion from benthos (see appendix 8.3 for details)

	Year	Month	Distance	Depth	Salinity (top)	Salinity (bottom)	Distance (depth removed)	Distance (salinity top removed)	Distance (salinity bottom removed)	Depth (salinity top removed)	Depth (distance removed)	Depth (salinity bottom removed)	Salinity top (distance removed)	Salinity top (depth removed)	Salinity top (salinity bottom removed)	Salinity bottom (distance removed)	Salinity bottom (depth removed)	Salinity bottom (salinity top removed)
Month	-51.1																	
Distance	-6.9	6.6																
Depth	0.3	-0.6	74.9					68.6	42.3									
Salinity (top)	8	5.9	68	43.6			59.4		23.1		-15.2	-6.8						
Salinity (bottom)	1.1	3.6	81	60.6	70.7		68	64		50.2	11.2		37.9	64.2				
S	13.8	-8.7	54.1	36.9	20.7	31.6	42.9	55.8	45	31.7	-6.4	14.6	-26.1	5.5	-7.9	-18.6	17.6	28
N	11.6	-19.8	12.3	14.7	-6.1	1.8	2	22.5	13.3	19.3	8.3	12.7	-20	-14.1	-13.9	-9.7	-6.4	11.1
d	1	-0.9	48	28	17.3	37.8	41.7	50.1	30.7	23	-11	0.4	-23.9	5.5	-18.5	-2.9	29.5	39.1
J	-1.5	-5.5	-0.9	-10.8	4.2	11.3	9.2	-4.8	-12.6	-13.7	-13.5	-17	6.4	9.7	-2.1	16.9	19.7	9.3
Brillouin	15.6	-5.9	51.4	33.5	23.9	29.7	41.7	49.2	39.6	26.3	-6.6	4.6	-17.4	10.9	-1.9	-15.4	19.7	22.9
Fisher	-6.3	1.5	31.6	13.2	12.2	26.8	31.7	31.8	13.9	8.8	-13.7	-11.7	-13	7.3	-13.3	3.1	27	27.9
ES(50)	15.7	-7.5	60.8	40.8	31.5	39.4	50	56.6	47.9	31.7	-9	13.4	-16.9	16.7	1	-15.4	25.3	28.4
H(loge)	16.5	-10.6	56.8	37.6	31.3	37.8	46.7	51.1	42	28	-9.1	9.2	-12.3	17.9	2.8	-11	26.1	26
Simpson	3.8	-6.6	26.8	15.7	12.7	26.9	22.5	24.9	8.8	11.3	-5.5	-3.8	-7.9	6.4	-10.1	8.2	23.2	26
N1	13.9	-8.3	54.7	33.8	28.3	38.4	47.1	50.3	37.9	24.8	-12.9	4.7	-14.4	16.1	-2.6	-6.8	29	29.8
IQI	-4.5	5.4	59.7	44.9	41.1	40.6	44.1	47.6	48.6	32.9	0.3	23.5	0.8	26.8	18.6	-15	21.2	18.3
EQR	-8.4	14	58.2	41.8	39	36.5	44.5	47	44.7	29.9	-1	12	-1	25.2	16.3	-15.2	21.6	16.4
ITI	-36.8	45.5	44.7	29.5	43.4	33.5	35.4	23	28.5	12.6	-4.9	6	19.7	35.2	25.9	-2.9	23.1	7.1
BOPA	-14.7	36.3	36	25.7	26.7	29.9	41.2	39.4	37	16.2	-12.7	4.8	-7	17.7	5.3	-12.5	20.9	18.2
A/S	7.2	-19.4	-6.6	9.3	-18	-13.7	-19.5	7.9	-0.7	19.4	20.4	17	-18.5	-24.6	-16.1	-7.8	-21.5	1.6
Delta	18.2	-17.7	36.6	28.3	20.7	29.1	24.3	31.5	17.7	21.9	1.4	6.4	-6.2	9.7	-2	3.6	20	22.4
Delta *	24.3	-20.1	35.8	36.7	21.7	20.2	13.5	29.4	23.9	31	16	21.7	-3.9	6.8	7	-7.4	3.3	9.6
Delta +	20.5	-14.9	42.6	39.6	25.4	24.7	21.3	35.8	29.5	32.8	12.8	20.5	-5.5	9.8	7.3	-9.3	8	12.8
sDelta +	15.5	-10.6	52.9	37.5	20.1	30.9	40.4	54.7	42.6	32.6	-3.8	14.7	-25.6	4.5	-8.5	-16.8	16.9	27.9
Lambda +	23.7	-13.9	25.7	25.4	11.7	9.3	10.4	24.3	22.6	22.7	9.7	16.4	-8.1	0.7	2.9	-13.1	-2.6	4.5
AMBI	21.9	-23.1	-45.8	-39.5	-40.7	-33.7	-26.6	-27	-30	-26.4	-8.8	-22.5	-14.6	-28.4	-23.6	3.4	-15.1	-8.9
BQI	-10.4	28.3	13.2	-11.6	9.8	16.9	31.9	8.9	15.6	-17.8	-31.2	-23.5	1.2	16.7	6.8	-2.3	28	7.3
MAMBI	8.6	-3	64.4	46.7	34.2	41.7	50.2	59.8	52	37.6	-3	20.5	-17.2	17.4	2.7	-17.5	23.9	29.2

Note: A/S, BOPA and AMBI index values have inverse relationships with quality

3.3.2 Index correlations with disturbance data

The correlations between indices at the different sites were tested using Pearson product moment correlation (Tables 3.19-3.25). Sites varied in the patterns of correlation between the indices. Indices calculated at Barcaldine showed high correlation to each other with the exception of abundance (N). In addition J' and A/S had weaker correlations than other indices. Correlations at Ironrotter Point showed a very different pattern with most indices only being weakly correlated to each other. There were stronger correlations between species diversity and evenness than were found at Barcaldine. M-AMBI, H' (ln) and ES (50) were the indices which were correlated most strongly with the largest number of other indices, with m-AMBI being more strongly related to species richness and H' and ES (50) being more strongly related to abundance. Overall correlations at Irvine and Ayr Bay showed stronger correlations than at Ironrotter and weaker than at Barcaldine and had stronger correlations to species richness and weaker correlations to abundance. When the Nobel and GVS transects were assessed separately, the main difference was a weak correlation of most indices to abundance and ITI for Nobel which were both much stronger for GVS. In addition, Taxonomic Distinctness (delta*) and Average Taxonomic Distinctness (delta+) showed very weak correlations for GVS but were stronger for Nobel. The pattern at the fish farms showed a similar pattern to Barcaldine with most indices strongly correlated to each other but with a weaker correlation to abundance. Average Taxonomic Distinctness (Delta+) and Variation in Taxonomic Distinctness (Lambda+) also showed weaker correlations than other indices. At the upper Clyde estuary there was a low level of correlation between most indices. Many were correlated with species richness but not with abundance.

Table 3.19 Pearson product moment correlations between all indices calculated for Barcaldine with percentage correlation, *r*. Darker colours indicate a stronger relationship.

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI
N	39.4																					
d	97.1	28.3																				
J	6.9	-34.6	20.2																			
Brillouin	86.9	13.8	94.1	40.8																		
Fisher	62.5	7.2	74.2	39.9	71																	
ES(50)	82.8	12.1	92.1	30.2	96	71																
H(loge)	78.9	5.1	89.9	50.8	98.1	77.7	94.9															
Simpson	52.9	-15.1	67.2	78.1	83.2	70.1	74	90.1														
N1	83.7	16.9	90.2	34.3	89.7	76.3	90.6	88.9	68.4													
IQI	75.7	6.6	86.3	38.9	92.9	69.7	90.5	94.2	84.3	75.8												
EQR	66.9	-5.6	80	42.7	90.4	69.2	87.6	94.1	89	73.3	98.7											
ITI	60.9	-0.9	72.7	37.4	80	64	78.2	84.1	78.6	65.8	91.5	91.4										
BOPA	-52.2	6.8	-65.3	-32.4	-71.2	-56.6	-73.5	-75.6	-70.2	-54.5	-87.8	-87.5	-80.1									
A/S	-17.5	79.7	-23.9	-34.3	-31.5	-22.9	-27.5	-34.6	-42.3	-21.4	-33.3	-40	-34.3	32								
Delta	55.4	-12.5	69.9	72.9	84.5	75.1	76.7	91.2	97.4	72.1	85.9	90	80.2	-73.5	-39.8							
Delta *	37.8	13.6	46.9	68.5	60.5	45	51.9	63.8	74.9	42.8	65.1	64.6	56.2	-55.1	-2.4	76.6						
Delta +	44.1	16.5	51.8	63.6	64	44.6	54.9	66	75.3	44.8	68	66.8	57.7	-54.2	-5.1	73.8	95.9					
sDelta +	99.8	39.5	96.9	7.6	86.4	63.1	82.2	78.5	52.7	84.4	75	66.2	60.4	-51.3	-17	55.6	38.3	44.7				
Lambda +	56.1	-1.7	65.5	27.1	72.9	49.7	70.8	74.6	69.4	52.7	83.2	81.7	81.4	-73.7	-33.9	69.1	54.5	63.3	55.4			
AMBI	-61.3	3.2	-74.3	-35.9	-81.8	-64.3	-82.1	-85.5	-78.2	-66.6	-94.8	-95	-91.8	89.9	33.7	-80.9	-57.4	-58.4	-60.6	-80.3		
BQI	89.4	26.2	91.6	10.9	88.5	62.1	86.1	83	61.3	74.7	86.1	78.9	77.5	-66.3	-24.2	64.6	47.7	51.7	88.4	73	-75.4	
MAMBI	87.1	14.4	95.1	34.9	97.4	74.8	94.9	96.7	81.3	87.3	96.7	93.9	86.6	-79.7	-31.5	83.5	58.4	61.8	86.6	77.2	-90.3	90.1

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 3.20 Pearson product moment correlations between all indices calculated for Ironrotter with percentage correlation, *r*. Darker colours indicate a stronger relationship.

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI
N	31.5																					
d	93.2	6.6																				
J	-29.8	-81.4	-1.5																			
Brillouin	54.1	-44.8	68.7	59																		
Fisher	58.4	-17.2	81.8	30.5	58.9																	
ES(50)	30.5	-52	59.3	74.7	80.7	82.1																
H(loge)	38.8	-57.2	61.3	75	96.4	66.8	91.9															
Simpson	-6	-78.8	16.4	91.2	77.2	31.5	72	85														
N1	60.2	-28.5	79.8	51.1	88.6	81.2	89.9	90.2	56.2													
IQI	32	-37.3	51.2	37.1	49	62	62.6	55.5	32.3	61.6												
EQR	-15.9	-68.9	10.3	71.7	41.7	39	62.8	56.4	59.4	45	85											
ITI	-26.2	-58.4	-7.7	45.1	15.1	13.1	30.6	25.8	30.3	18.7	36.4	52.3										
BOPA	32.5	51.9	14	-51.7	-11.7	-11.2	-32.9	-25	-33.3	-16.4	-65.6	-82.8	-52.6									
A/S	16.5	97.4	-6.6	-74.7	-48.6	-25.1	-54	-59.3	-74.7	-33.6	-42.1	-66	-54.9	47.5								
Delta	3.8	-65.5	24	78.6	72.6	36.5	69.7	79.1	85.4	57.4	54.5	71	19.4	-47.5	-63.2							
Delta *	15	-15.6	21.4	19.8	27.6	23.5	29.3	28.9	20.2	27.6	58.2	51.3	-4	-44	-17.7	68.1						
Delta +	46.2	12.2	42.6	-13.1	28.3	24.5	14.6	20.1	-0.7	28.8	32.2	8.3	-6.3	-1.1	5.5	22.9	43.8					
sDelta +	99.7	31.2	92.8	-29.4	54	58.2	30.6	38.8	-5.7	60.1	33.6	-14.2	-25.5	30.7	16.1	5.7	18.2	53				
Lambda +	-27.9	-13.3	-23	12.5	-9.8	-14.2	-6.8	-5.6	6.2	-10.9	-18.9	-3.4	7.2	0.9	-6.5	-13.8	-35.1	-63.1	-32.9			
AMBI	23.2	39.6	3.9	-36.3	-0.8	-25.5	-32.2	-15.5	-13.9	-17.2	-81.6	-87.7	-47.1	83.2	37.5	-35.6	-48.8	-6.6	21.3	5.7		
BQI	26.4	-8.1	18.9	11.1	43.5	-8.5	6.9	31.5	33	20.7	-2.3	-4.3	-19.5	3.5	-9.5	35.2	19.2	12.1	26.2	-5.3	27.3	
MAMBI	52.1	-42.6	73.1	52.3	80.8	77.7	85	84.5	56.3	88.2	89.5	73.6	29.6	-45.6	-48.9	68.1	49.2	34.5	53	-17.8	-52.4	15.5

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 3.21 Pearson product moment correlations between all indices calculated for Irvine and Ayr Bay with percentage correlation, *r*. Darker colours indicate a stronger relationship.

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI
N	23.8																					
d	94.3	-0.7																				
J	4.3	-58.1	30.2																			
Brillouin	72.3	-24.1	85.2	67.3																		
Fisher	66.5	-22.7	85.9	59.9	80.9																	
ES(50)	53.3	-38.6	74.5	78	90.5	87.4																
H(loge)	61.1	-32.2	78.2	80.6	97.6	84.8	95.4															
Simpson	36.1	-37.7	53.9	89.3	84.7	66.3	78.9	90.5														
N1	64	-21.4	80.9	68.8	92	88.3	93.2	92.4	74.4													
IQI	50.1	-28.4	71	67.1	83	72.8	70.8	77.3	78	59.9												
EQR	27.8	-58.9	48.7	83.6	74.5	62.6	76.9	81.7	85.3	66.8	94.3											
ITI	8.5	-57.1	22.5	36.3	30.7	27.3	36.6	34.5	33.4	24	56.4	58.7										
BOPA	-2.7	11.2	-8.9	-27.2	-16	-17.3	-19.6	-20.2	-24.1	-17.5	-44.7	-47.4	-13.2									
A/S	-5.7	89.1	-23.7	-53	-38.3	-34	-45.7	-44.9	-45.6	-30	-59.7	-64.7	-57.5	14.1								
Delta	46.1	-21.2	57.5	78.2	82.5	64.7	74.8	86.8	92.8	70.4	78.4	82.1	23.3	-34.8	-36.9							
Delta *	31.4	22.2	22.1	-5.8	15.1	12.1	18.7	26.1	7.5	15.8	60.6	13.7	-24.4	-38.3	12.9	54.6						
Delta +	26.2	2.3	27.2	3.2	21.1	18.8	26.1	32	14.6	16	73	25.2	19.7	-12	-12.9	45.5	76.6					
sDelta +	99.8	23	94.3	4.3	72	66.7	53	60.8	36.2	63.6	50.5	28.7	9.7	-3.3	-6.4	46.7	32.5	28.5				
Lambda +	-7.1	4	-20.4	-13.3	-15.3	-23.9	-2.1	-1.1	-16.7	-7.7	18.5	-21.3	-5.2	16.5	5.9	-7.4	7	15.2	-11.1			
AMBI	-13.8	59.8	-29.5	-70.4	-50.4	-46.9	-62.4	-63.3	-62.2	-47.9	-82.8	-93.4	-65.3	56	67.5	-66.1	-33.7	-43	-14.9	-0.6		
BQI	69	-2.8	64.5	5.7	55.9	38.6	35.9	45.2	31.5	40.1	54.6	35.4	14.1	-2.5	-23.4	40	32.5	31.8	69.1	-11.8	-24.7	
MAMBI	70.8	-30.2	85.2	68	94.3	84.6	89.2	94.6	81.7	85.9	87.6	86.5	45.4	-32.7	-50.2	84	37.1	41.7	71	-2.9	-74.5	57.7

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 3.22 Pearson product moment correlations between all indices calculated for Nobel transect at Irvine Bay with percentage correlation, *r*. Darker colours indicate a stronger relationship.

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI
N	63.8																					
d	93.7	36.2																				
J	-21.3	-55.9	4.3																			
Brillouin	67.3	6.9	80.6	50.6																		
Fisher	54.1	-9.7	77.8	49.8	73.1																	
ES(50)	47.7	-20.8	66.7	65.5	88.1	82.4																
H(loge)	54.9	-4.9	69.2	71.3	96	79.9	94.7															
Simpson	18.4	-16.2	35.8	86.9	76	59.1	70.8	86.7														
N1	55.8	-9.3	73.4	63	93.3	82.7	91.5	91	74.9													
IQI	46.8	15.3	72.2	49.5	85.9	72.3	64.2	74.3	76.2	47.2												
EQR	14.5	-23.4	33.8	81.8	70.1	59.1	70.6	81.4	91.4	72.1	86.6											
ITI	0.9	-16.4	8	-8	0.2	9.1	12.3	0	-12.9	1.9	5.8	0.3										
BOPA	8.4	12.1	3.2	-36.8	-20.6	-11	-18.7	-26.3	-40.6	-23.9	-46.8	-59.2	9.9									
A/S	45.6	95.6	15.2	-67.4	-12	-31.1	-46.4	-32.7	-32.6	-30.2	-8.5	-38.9	-18.1	12.5								
Delta	39.9	8	42.2	71.1	73.9	56.7	71.6	86	92.1	66.9	79.2	88.1	-16.2	-38.9	-15.5							
Delta *	42.1	27.5	33.4	9.7	35.2	24.7	45.5	58.1	37.2	30.5	90.2	41.4	-19.3	-17.1	26	80.1						
Delta +	35.1	19.3	25.8	-8.4	11.8	19.6	42.9	51.7	8	22.1	92.9	20.9	25.2	2.3	18.5	64.9	91.3					
sDelta +	99.7	64.7	93.3	-21.5	66.3	54	46.7	53.9	18.4	54.9	46.6	15.4	2.8	8.7	46.3	40.6	43.1	36.5				
Lambda +	4	-6	-22.2	-7.1	-13.1	-25.2	20.9	25	-17.8	3.9	53.3	-25.1	-12.1	-6	-10	19.4	39	45.4	-0.1			
AMBI	-15.2	14.5	-8.7	-73.1	-39.7	-42.8	-61.8	-70	-69.4	-46.6	-87.6	-90.9	-7.8	67.6	47.8	-80.9	-76.5	-77.6	-15.8	-37.5		
BQI	69	46.5	60.3	-11	54.5	24.4	26.7	38.4	22.9	36.1	51.3	21.8	-14.4	-11.6	36.8	36.8	47	33	69.3	-17.9	-2.7	
MAMBI	69.8	16.4	83.9	50.7	95.3	81.4	86.4	94.6	76.5	82.1	85.7	78.4	2.8	-30.9	-10.7	86.3	72	66.7	69.5	27.3	-76.2	53.9

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 3.23 Pearson product moment correlations between all indices calculated for GVS transect at Irvine Bay with percentage correlation, *r*. Darker colours indicate a stronger relationship.

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI
N	6.2																					
d	94.3	-18.2																				
J	15.3	-65.4	42.2																			
Brillouin	67.9	-44.5	83.6	80.5																		
Fisher	72.2	-31.9	89.6	63.2	84																	
ES(50)	51	-52.6	74.7	86.7	91.9	90.3																
H(loge)	58.5	-50.8	78.4	88	98.6	85.7	96															
Simpson	40.6	-51	60.2	91.5	90.4	68.6	83.2	93														
N1	61.1	-35.3	79.7	77.8	92.5	89.6	95.1	93.9	78.6													
IQI	53.1	-62.1	72.2	75.3	83.3	76.2	82.5	85.2	78.7	74.9												
EQR	32.7	-70.2	56.1	86.5	79.9	67.3	81.8	84.6	84.7	71.6	96.5											
ITI	19.5	-66	39.3	59.5	52.2	46.4	55.1	56.2	57.7	43.9	74.5	77.4										
BOPA	-15.8	8.9	-19.7	-20	-16.5	-21.2	-19.1	-18.7	-18.7	-13.8	-47	-44.1	-27.8									
A/S	-19	90.8	-37.9	-60.4	-53.4	-43	-55.3	-56.9	-56.1	-40.5	-70.9	-72.6	-64.1	16.2								
Delta	45.4	-43.2	61.3	84.2	87.1	66.3	78.3	88.5	94.4	74.7	80.2	83.6	51.1	-34.2	-52							
Delta *	12.7	23	1.9	-23.1	-11.7	-7.9	-16	-15	-19.1	-12.8	5.5	-2.6	-22.1	-51.2	11.7	13.8						
Delta +	13.5	-19.8	14.6	12.1	16.5	7.9	9.2	15	15.7	5.5	29.8	27.3	21.4	-30.4	-22.1	25.7	32					
sDelta +	99.8	5.2	94.1	15.5	67.8	71.7	50.6	58.4	40.8	60.2	53.9	33.6	20.4	-17.2	-19.9	46.1	14.2	19.8				
Lambda +	-8.5	7.1	-8.7	-6.7	-7.4	-5.7	-5.7	-7.3	-5.5	-4.2	-16.1	-14.5	-6.1	30.1	7.4	-17.1	-35.6	-71.3	-13.4			
AMBI	-18.6	72.8	-41.8	-73.5	-60.7	-54.8	-68.8	-66.9	-64.4	-56.1	-92	-95.1	-78.2	52.7	72.1	-65.7	-6	-29.4	-19.7	17.7		
BQI	66.8	-29.8	67	18.4	55.7	47.8	41	48.1	35.1	42.2	64.3	49.7	39.2	-11.9	-43.4	41.2	20.8	22.2	67	-4.7	-46.8	
MAMBI	67.1	-53.3	84.5	77.5	93.6	86.5	90.6	94	83.9	87.3	96.5	91.1	66.4	-36.9	-63.9	84	-0.1	24.2	67.4	-13.8	-80.6	64.1

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 3.24 Pearson product moment correlations between all indices calculated for fish farms with percentage correlation, *r*. Darker colours indicate a stronger relationship.

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI
N	-6.5																					
d	96.3	-25.2																				
J	31.6	-65.3	51.4																			
Brillouin	89.9	-30	95.7	60.2																		
Fisher	77.7	-32.5	88.6	63.5	83.7																	
ES(50)	89.9	-31.8	96.5	56.4	97	85.8																
H(loge)	84.9	-35.9	94.5	69.3	98.6	87.8	96.2															
Simpson	62.3	-43.9	77.3	84.5	84.5	79.2	77.7	90.2														
N1	89.1	-28.2	95	59.4	94.3	88.8	95.7	93.4	76.3													
IQI	66.4	-35.3	81.7	67.9	81.7	81.7	79.1	85	83.8	73.3												
EQR	66.7	-44.3	81.2	71.3	84.9	81.1	83.6	90.1	87.7	78.6	95.7											
ITI	52.2	-50.5	67.7	70	67.1	72.3	69.3	74.2	74.3	64.7	94	89.9										
BOPA	-42.2	46.4	-57.9	-62.8	-58.7	-62.3	-60.5	-65.9	-66.8	-55.5	-86.3	-88.8	-88.2									
A/S	-34.1	81.3	-45.9	-50.3	-44.3	-43.7	-46.2	-50.1	-50.6	-39.6	-55.4	-52.5	-55.4	48.6								
Delta	60.4	-43.9	75.9	83.3	81.2	80	77	88.1	96.5	75	85.1	90.5	78.5	-76.6	-52.5							
Delta *	28.2	-21.2	37	42.3	37.7	39.9	38.9	44.7	47.5	34.5	57.7	58	46.8	-61.1	-30.9	65.2						
Delta +	12.2	8.4	8.4	3.1	9.3	12.1	14.7	19.7	16.6	12.7	34.5	16.6	7.5	-11.2	7.9	34.1	76.3					
sDelta +	99.8	-6.7	96.4	32.4	90	78.4	90.2	85.2	62.6	89.7	67	67.6	53	-43.7	-33.6	61.7	30.4	15				
Lambda +	32.2	5.2	31.8	-3.2	34.6	24.8	32.4	37	29.4	24.6	36.7	30.2	19.2	-12.6	-15.6	23.9	12.1	5.7	29.5			
AMBI	-50.1	45.4	-64.9	-67.3	-64.8	-70.3	-67.9	-72.9	-71.6	-63.5	-89.4	-93.1	-94.3	96.4	50.8	-79.9	-55.5	-18.9	-51.4	-18.4		
BQI	65.9	-28.9	73.8	49.7	77.1	64.1	76.7	77.8	68.1	70.5	72.9	77.1	61.6	-66	-38.5	70.3	46.4	11.6	66.2	28.3	-67.3	
MAMBI	88.8	-32.2	96.5	62.2	95.7	88.3	95.5	96.8	84.1	92.6	89.3	92.9	81.4	-75	-50.2	85.2	47.3	18.9	89.2	33.3	-81.8	79

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 3.25 Pearson product moment correlations between all indices calculated for upper Clyde estuary with percentage correlation, *r*. Darker colours indicate a stronger relationship.

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI
N	53.5																					
d	73.2	11.5																				
J	-33.5	-37.5	22.2																			
Brillouin	75.2	17.6	74.2	26.8																		
Fisher	33.2	-6.5	72.2	39.6	45.6																	
ES(50)	86.4	19.1	82.2	-3.6	90.7	49.6																
H(loge)	72.9	13.6	81.3	48.7	97.1	59	90.3															
Simpson	23.4	-8.2	69.7	86.5	65.6	60.7	44.6	79.8														
N1	69.9	9.8	77.4	43.6	94.7	59.5	87.8	96.8	72													
IQI	55.3	10.7	40	-5.2	43.2	25.4	66.9	61	19.7	51.5												
EQR	52.5	2.7	62.7	23.8	76.5	41.8	71.7	79.7	57.4	70.7	73.1											
ITI	1.5	-20	8.6	-2.7	5.7	11.5	12.3	8.3	-1.6	7.1	84.5	51.8										
BOPA	-11.6	-25.4	22.4	46.3	22.2	26.8	9.6	30	50.2	30.6	17.1	12.1	5.5									
A/S	22.2	78.2	-15.7	-31.1	1.7	-21	-5.2	-6.9	-19.5	-9.2	-12.9	-8.4	-23	-28.7								
Delta	43.9	9	59	75.2	63	55.3	59.1	79.8	89.2	70.8	49.9	61.9	-3.4	27.4	-4.6							
Delta *	52.5	25.6	29.3	-15.2	46	12.7	59.7	62.9	32.3	46.9	61.6	59.9	1	-37.4	18.5	74.6						
Delta +	58	22.4	42.7	-3.9	55.8	22	66.9	70.3	42.9	55.4	66.5	67.7	8	-35.8	13.6	76.2	96.7					
sDelta +	98.6	54.1	72.8	-33.1	74.6	33.1	84.6	72.3	24.8	68.9	52.4	54.6	1.5	-15.9	23.7	47.7	57.6	63.6				
Lambda +	52.5	17.1	31.5	-22.5	49.9	19	61	56.3	12.8	49.1	37.2	43.1	-6.9	-12.5	3.4	36.5	52.7	52.6	50.9			
AMBI	-23.2	5.2	-2.6	9.9	0.3	-0.3	-34.8	-28.2	5.4	-21	-78.1	-50.8	-88.1	7.6	18.3	-24.9	-39.9	-42.1	-21.8	-20.6		
BQI	2.8	-8.7	10.8	18.2	8.8	25.6	8.6	10.2	9.5	14.1	9.3	1.5	-0.7	67.2	-11.1	2.7	-15.4	-13.2	0.5	-12.5	9.2	
MAMBI	88.8	31	81.5	-1.9	89.5	45.1	93.4	89	50.1	84.1	76.3	82	26.7	7	4.2	63.7	64.3	70.8	87	57.9	-51.6	5.3

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

3.4 Discussion

3.4.1 Study sites

3.4.1.1 Barcaldine

As the survey at Barcaldine was designed to assess the recovery of the benthos from the closure of an alginate processing factory, an improvement in quality over time was expected. However, the dense mat which formed during the operational time of the factory was degrading very slowly during this time and so recovery of the benthic system was limited. A trend was found with distance from the outfall and also over time, with the outfall sites in the most recent year (2004) being more similar to the reference stations from all years (Fig.3. 6). A large difference was seen between the samples taken at the outfall in 1997 compared to the reference sites, while in later years the outfall and reference sites were more similar to each other. This implied an increase in quality of the sites closest to the outfall over time. Quality classifications of the indices mostly reflected an overall increase in quality over time and with distance from the outfall (Table 3.7). However, there was a high level of disagreement in the specific quality classification between the five indices shown. Some of the samples in the good quality area of the MDS were assigned moderate and poor quality by the indices IQI, BQI, AMBI and ITI (2004 D3, D5). In addition, BQI and ITI indicated a slight decrease in quality in the most recent years, while BOPA indicated better quality than the other indices in most cases. The samples where the indices agreed were largely from the lowest quality sites and from one good quality site (Fig. 3.7). Similar trends between indices were found in the better quality sites (reference samples) while the indices disagreed in the intermediate quality sites. BQI was the cause of much of the disagreement between indices. As was discussed (p. 26; Appendix 8.1), the species list for the BQI was limited for this study. However, the level of assignment of species and abundances for Barcaldine was high when calculating the BQI so this does not explain the disagreement of this index from other indices. It may indicate however, that the $ES50_{0.05}$ values assigned to some of the species were not accurate and lead to an underestimation of the quality classifications in this case. Other studies have found

BQI to assign lower quality classifications than other indices (e.g. Labrune et al., 2006; Ruellet & Dauvin, 2007).

Although MDS and five of the indices assessed showed a change in the community and a potential increase in quality over time, only about half of the indices detected a trend over time (Table 3.8). The strength of the trend may have been dampened due to the mixture of reference and outfall sites from all years as the reference sites did not change over time. In addition, 2001 showed a slight decrease in quality from 1999, going against the apparent overall trend. However, it does show that some indices were more sensitive to the change in quality over time than others. All the indices that detected a trend detected an increase in quality. As was evident from the quality classifications, ITI and BQI did not detect a notable increase in quality. As species richness did not change over time this is likely to have determined the lack of change in many indices which are strongly correlated to species richness (Section 2.3), such as d, Brillouin, Fisher, N1, sDelta+, BQI and m-AMBI. Other indices which were weakly correlated with time included J', A/S, Delta* and Delta+. As the abundance decreased over time, it would have been expected that measures of evenness (J' and A/S) would have shown a trend.

A relatively strong trend was found with distance from the outfall with all indices apart from abundance, J' and A/S, indicating the evenness of the community did not change much along the transects. Despite indicating lower quality than the other indices overall, ITI and BQI detected the strongest trends in increasing quality with distance from the outfall. This may be due to the type of scale and calibration of the indices. Other studies have found calibration to be a reason for different quality classifications with different indices (Ruellet and Dauvin, 2007). Although depth was correlated with distance from the outfall, this factor did not seem to influence index results since indices were only weakly correlated to depth and when the effect of depth was removed from distance from the outfall, the strength of correlations was mostly retained.

Overall, the MDS, quality classifications and spatial and temporal trends, indicated that there was some improvement and recovery over time. However, the reference sites, furthest from the outfall, were not assigned consistently good or high quality by all the indices. This may imply that, while the worst sites at the outfall have shown some improvement, those further away were also impacted by the alginate waste and have shown little improvement over time. This could have led to disagreement between index classifications and weak correlations between indices and year of sampling. From the history of the site, it was expected that an improvement in quality would be seen over time and with distance from the source. However, the improvement over time was doubtful as the alginate waste had formed a peaty mat over the seabed which showed little sign of degradation over the sampling period. Recovery of the system was likely to be slow due to the nature of the waste. On the other hand, the inconsistent classifications of the reference sites may indicate that indices do not perform well in the assessment of reference sites. The greatest agreement was found in the worst quality sites suggesting indices may perform better in assessing degraded conditions.

3.4.1.2 Ironrotter Point

Ironrotter Point data included a baseline survey before implementation of a sea pipe, followed by three surveys taken one, four and seven years after the pipe was in use. The quality was expected to decrease over time with the input of organic waste. A change in species composition in each year of sampling was clearly indicated, moving away from the baseline 1989 samples (Fig. 3.8). Although organic matter data were unavailable for the last year, this trend looked to be strongly related to the level of organic matter in the sediment (Fig. 3.9). This may have implied a change in quality over time. The baseline study benthic community and the community just after implementation (1989 and 1992) were also more similar to each other than to the other two years (1995 and 1998). This change did not manifest itself in a change in quality classification according to the indices until 1998 when the community was found to have the greatest differences from other years (Table 3.9). Indeed, the IQI and BQI seemed to indicate an overall increase since the sea pipe was put in place. ITI did show some evidence of change in quality from 1995.

Distance from the outfall did not appear to influence change in the community until the final year (Fig. 3.8) and this was reflected by the quality classifications. It would be expected that those samples closest to the outfall would have lower quality than those further away. However, the organic matter increased in all samples, not just those close to the outfall, but also those that were located up to 1000m from the outfall (Fig. 3.11). This may suggest that the material was dispersed over the whole area and not concentrated next to the outfall, leading to similar quality classifications over the whole area.

The agreement between the five index classifications was also related to the year, with the baseline year and first year after implementation of the sea pipe having mainly agreement between indices or similar trends while in 1995 most indices disagreed in samples and in 1998 most indices showed a similar trend (Fig. 3.10). This could imply that disagreement between indices can occur during times of change in the environment and in this case the disagreement was mainly due to the increase in quality of IQI and to a lesser degree BQI and also to the decrease in quality by ITI. IQI and BQI therefore detected a change in the environment but in the wrong direction. This may be related to species richness, which increased over time. It may be that a moderate level of disturbance increases disagreement between indices and causes indices to act unpredictably. ITI measures the composition of the community and is not influenced by species richness which may explain the opposite trend to the other indices.

Most of the indices correlated positively with organic enrichment and year (Table 3.10). This is probably largely, though not solely, related to the increase in species richness as many indices are correlated with species richness (section 2.3). Species richness showed a strong positive, linear trend with year and showed an overall increasing, though slightly humpbacked curve with increasing organic matter content. Species richness also showed a humpbacked shaped curve response to distance from the outfall, increasing at first before decreasing. This resulted in no linear or monotonic trend being detected by correlation between species richness and distance. ITI detected a slight decreasing trend with organic enrichment, no trend with distance from the pollution source and showed a decreasing trend with year from 1992 onwards, reflecting the change in quality classifications found by this index. AMBI indicated a decrease in quality over time but showed no strong trend with increasing organic matter content. The decrease in quality detected did not manifest in a change in quality classification until the final year of sampling. BOPA performed similarly to AMBI. IQI detected an increase with organic matter and a small decrease in quality with year when the effect of organic matter was removed. This linear trend hid what was initially a slight increase in quality with year before a slight decrease again. This was reflected in the quality classifications which mostly changed from good to high to good in the final year. This also reflects the influence of species richness on this index. Species richness and AMBI overall found opposing trends at this site and this resulted in the two cancelling each other out so IQI showed little change in quality over time. This is also similar to m-AMBI, implying that multimetric indices can hide a trend of decreasing quality. A/S and J' indicated decreasing evenness over time.

Apart from ITI, the first impacts were not detected by the indices until around seven years after the pipe first started being used. This may be an indication of the resilience of the benthic community which did not show a decrease in quality despite the input of organic waste during this time. However, one of the main reasons for other indices not showing a change in quality was the increase in species richness. Species richness can respond to disturbance by initially increasing (Connell, 1978, Pearson & Rosenberg, 1978, Odum, 1985, Dodson et al., 2000, Mittelback et al., 2001, Hooper et al., 2005). Although the system may have been absorbing the enrichment, clear changes in the community were occurring and it would appear the resilience was decreasing over time. This was only detected by most indices after the community had already become degraded in 1998. This is a good indication that most of the indices are not suitable as early warning indicators of an impact. It implies that species richness was perhaps the only index which could be considered to be an early warning indicator of disturbance as this index showed the strongest response. Although no data were available before the baseline study which may have indicated an increasing trend in species richness over time, the clear shift in species composition according to the MDS suggested that the community was showing a larger response than would be expected under normal conditions and this was likely to be due to the input of organic material. ITI and AMBI also showed decreasing trends in quality which may also indicate the suitability of these indices as early warning indicators. However, ITI was the only one of these two to change quality classification before the last year of sampling. Species richness increased but this study shows any change, up or down, in species richness should be used as an indication of change in the system and this should be considered alongside other methods such as MDS, ITI, AMBI, environmental variables and other indices to interpret the change in the system.

3.4.1.3 Irvine Bay

Irvine Bay received waste from several sources for the duration of the survey. Quality was expected to improve from the inshore sites in close proximity to the outfalls to the offshore sites furthest from the outfalls. There was no obvious trend with time although there were differences between years (Fig. 3.12). There was a slight trend with distance from the pollution source but as the reference sites were also in deeper waters it was difficult to separate the effect of the depth with the effect of the distance from the pollution source. The greatest differences were found between the deepest and furthest sites from the pollution source with the shallowest and closest sites to the source. It was expected that sites impacted by sewage outfall and by chemical outfall would show differences in communities. No strong differences were found, however, there was a small but significant difference between the organic type impacted communities and the chemical type impacted communities while there was no difference between the respective reference sites. Sites L8, L81 and J1 at the GVS outfall; R1 at the Barassie outfall; and Q1 (and Q2.2) at the chemical outfall showed differences from other samples and from each other. With the exception of the R1 sample (which was assigned as ‘bad’ only by BQI but as ‘good’ or ‘high’ by other indices), these samples were assigned some of the worst quality by the five indices. This may imply that the effect of sewage or chemical waste impacts the communities in different ways but only in worst affected areas. While the sites around the sewer outfall showed characteristic, very high abundances of tolerant species, the Nobel outfall sites did not show only species tolerant of synthetic chemicals. Indeed, high abundances of some species such as *Prionospio fallax* and *Mediomastus fragilis* along with the presence of species sensitive to synthetic chemicals (including *Abra alba*, *Lumbrineris gracilis*, *Capitella capitata*, *Amphiura filiformis* and *Eteone longa* (Hiscock et al., 2004)) all suggested the sites were more influenced by an increase in nutrients than toxic chemical contamination. While most cases of multiple stressors act synergistically in the marine environment, causing the impact to be worse than may be expected, nutrients and toxins have been found to act antagonistically, the impact being less than expected (Crain et al., 2008). The effect of the nutrients can override the effect of the toxins. It may be that the Nobel sites are also impacted by nutrient enrichment and this has reduced the effect of the chemical waste and been the dominant determinant of the benthic assemblage.

The indices largely disagreed in the quality classifications (Fig. 3.16). Agreement or similar trends were found in samples which were of a better quality while samples of lower quality and samples from 1981 mainly disagreed. In 1981, this was due to BQI which found lower quality for these samples than all other indices. This may have been due to a lower proportion of species assigned when calculating BQI for 1981 data compared to other years and therefore the quality classification underestimated the quality of the samples. In other years, ITI indicated lower quality than other indices while BOPA often indicated better quality than other indices. Disagreement occurred in samples which were of a bad-poor-moderate quality while similar trends occurred in sites which were of a moderate – good or a good – high quality.

Overall, the correlations between indices and distance and depth were quite low, reflecting the slight monotonic trend indicated by MDS. The correlations with distance were greater overall than with depth and were maintained when the effect of depth was removed indicating an increase in quality from the pollution sources to the reference sites. Most of the indices detected this trend. However, species richness responded in a nonlinear way with an initial increase and then a decrease with distance and depth which resulted in no overall linear trend detected. Other indices such as BQI and taxonomic distinctness (Delta*) showed no change with distance. Along the GVS transect, most indices showed an increase in quality while taxonomic measures did not apart from Taxonomic Diversity (Delta). AMBI and ITI showed the strongest correlation with distance when the effect of depth was removed.
When the Nobel transect only was considered, most of the indices showed an increase in quality with distance, though, species richness decreased along the transect and Margalef (d), BQI and Total Taxonomic Distinctness (sDelta+) may have been sensitive to this as they also showed a decrease. However, when the effect of depth was removed, several indices showed a decrease in quality with distance, while other indices, such as ITI and AMBI, showed no trend. This indicates that

indices may have been sensitive to the effect of depth along this transect or other confounding factors. It further suggests that indices did not detect an impact due to chemical pollution which was greater than the background environmental trends. This may have been due to a lack of sensitivity by indices, such as AMBI which found no trend, to the effects of chemical pollution and the ensuing response of the benthos. However, it may also be due to the nature of the pollutant which potentially may be more easily dispersed than organic pollution. One sample at this transect was found to be devoid of life suggesting there had been an input of toxic waste. Chemical effects may be short lived and go undetected during most annual sampling events. The benthos may recover in between toxic events and indices may not be capable of detecting residual effects, particularly if complex antagonistic effects, as previously discussed, are also occurring. The lack of trends or negative trends with distance at this transect is in contrast to the trends found at the GVS transect and the overall patterns found for Irvine and Ayr Bay by most indices. This suggests that trends from organic enrichment outfall sources did reflect a change in quality with distance from the source which was greater than changes due to natural environmental gradients while the lack of or opposite trends from the chemical outfall showed low impact due to this pollution or low sensitivity by indices in detecting an impact.

The overall weak trends may suggest that other unmeasured factors were likely to have been influencing the benthic communities such as sediment variables, flow regime or biotic interactions. As some samples close to the outfalls were more impacted by others, this may suggest that the flow regime in the bay influences the distribution of waste and that distance from the outfall may not have been the best proxy for relative impact.

3.4.1.4 Fish Farms

The fish farm sites showed high similarity based on the location of the sample – cage edge, allowable zone of effect and reference site and this was the strongest trend for fish farm sites (Fig. 3.17). Apart from this, slight trends were present based on depth and the amount of tonnes consented at the sites. The indices mainly reflected the difference between these three locations with bad quality in almost all of the cage edge sites as expected (Table 3.15). The allowable zone of effect was also assigned bad or poor quality by most indices and the reference sites good, moderate or high quality. BOPA often assigned a higher quality classification and BQI a lower classification. The agreement between the indices reflected the quality in the sites with the indices agreeing mainly in the cage edge sites, disagreeing in the reference sites and showing a similar trend in the allowable zone of effect sites. When BQI was excluded from this analysis, the outcome was comparable but the indices assigned similar classifications to the reference sites rather than disagreeing. This indicated BQI was responsible for much of the disagreement in quality classification between indices. This suggests the indices are broadly good at detecting bad quality but the definition of moderate or good levels of quality differs depending on the index.

Given the difference in benthic communities highlighted by the MDS analysis, strong correlations between indices and distance from the fish farm cages were expected. In most cases this is what was found, with quality increasing from the fish farm cage (Table 3.16). However, Average Taxonomic Distinctness (Delta+) did not find a strong trend with distance and Taxonomic Distinctness (Delta*) and Variation in Taxonomic Distinctness (Lambda+) found weaker correlations than other indices along with measures of evenness, J' and A/S. Furthermore, Taxonomic Distinctness (Delta*) found a stronger correlation with depth than with distance from the cage. Sampling at the fish farm sites was carried out using a smaller grab size than usual and this may have influenced the classification assigned by some indices which require a certain sample size – such as IQI. Despite this most indices performed well in detecting the trend from the fish farm to the reference sites. Further, this does not explain the taxonomic measures which performed poorly compared to the other indices as these indices are independent of sample size (Magurran, 2004). Comparable to the MDS, most indices detected a decrease in quality with depth. Many indices also found a correlation between quality and the maximum consented tonnes of the fish farm although the relationship with distance from the farm was stronger. However, lower quality at deeper sites was unexpected given that deeper sites are recommended as optimal fish farm sites since shallower sites can be more susceptible to accumulation of waste on the sea bed (ECASA, 2011).

As the fish farm sites incorporated three distinct levels of disturbance, the performance of the indices in detecting disturbance could be tested. This revealed some indices, such as the taxonomic measures, to be less sensitive to disturbance than others.

3.4.1.5 Upper Clyde Estuary

The upper Clyde estuary is impacted by a salinity gradient but also sewage inputs, river inputs and other impacts commonly associated with estuaries such as diffuse land run-off. The greatest differences between samples indicated the upper section was most dissimilar to the lower part of the estuary. There was a trend with distance downstream and with salinity. There were no obvious monotonic trends with year or season but there were significant differences with both these variables and the month of May was found to be most dissimilar from other months.

The indices largely assigned bad and poor quality and mostly disagreed with each other apart from in the very upper part of the estuary which was mainly assigned bad quality (Table 3.17; Fig. 3.23). BOPA assigned much higher quality than other indices in many cases. No values were often assigned with BOPA due to the absence of both opportunistic polychaetes and amphipods in the sample. Confusingly, BOPA assigns a zero value to these samples although zero would result in a ‘high’ classification for BOPA. Further, high values assigned by BOPA were generally due solely to a low proportion of opportunistic polychaetes out of the total abundance as in most cases, amphipods were completely absent. None of the indices showed a clear difference in quality which could be related to the sewage works or the river input.

The factors distance, salinity and depth were all correlated. Most of the indices found an increase in quality seaward apart from BOPA which found the opposite trend. The strongest correlations between index values were with distance from the upper estuary (Table 3.18). When the effect of distance was removed from both depth and salinity (bottom), these factors showed very low correlations with indices. Salinity would have been expected to have been a strong influencing factor and has been reported to influence index results (Zettler et al., 2007, Fleischer and Zettler, 2009).

However, salinity readings were measured during a different survey to the benthic fauna collection and in some cases samples were taken more than a month away from the benthic survey (see Appendix 8.3). Analyses showed significant changes in the benthic community between months so the time of sampling is likely to be important in considering the correlation with indices and salinity results, in addition to shorter term variability in salinity which would not be detected by single event sampling. Distance, therefore, may be a better proxy for overall salinity levels in this study. Nevertheless, other factors which vary along the estuary may also contribute to determining the benthic communities, such as anthropogenic inputs or natural gradients in sediment type. Estuarine gradients including salinity, sediment type and hydrodynamics, as well as disturbance gradients, were found to influence benthic communities and index results in the Mondego Estuary (Teixeira et al., 2008b).

ITI showed the strongest correlation of any of the indices to the month sampled with greater quality in later months and also to the year of sampling, with a decrease in quality found over time. However, MDS indicated changes over time were not monotonic so the generally weak correlations between indices and time were not unexpected.

Overall, quality could not be determined reliably for the samples at this site. The site is naturally highly stressed due to the salinity gradient but the Clyde estuary is likely to be subject to multiple stresses both natural and anthropogenic. The lack of physical and chemical variables and reference values for this type of environment makes interpretation of results difficult.

3.4.2 Correlations

When tested under normal conditions, indices were found to fall into groups based on the strength of correlations to other indices (Section 2.3). These groups were ecological, trophic, diversity (richness), evenness and taxonomic, while multimetric indices were a combination of both ecological and diversity. The same indices impacted by different types of disturbance at a variety of sites showed variable behaviour and the indices did not fall into the same groups of predictable response behaviour. An exception to this was the diversity group of indices – S, d, Brillouin, Fisher, ES (50), H’ and N1 which were all fairly highly correlated to each other in most cases, although not in all cases. In addition, N, J’ and A/S were generally weakly correlated to other indices at all the sites. Other indices showed different patterns of correlation depending on the site. Barcaldine and the fish farm sites were both sites which had heavy organic loading and both these sites showed high correlation between almost all indices; even indices which were not highly correlated under normal conditions were strongly correlated at these sites. ITI, which was not strongly correlated to other indices in normal conditions, showed low correlation to other indices in all sites except for Barcaldine and the fish farm sites. In addition, ITI was moderately correlated to other indices along the GVS transect but showed very low correlations along the Nobel transect. This index is unique amongst the indices tested as it focuses on functional feeding groups and this is reflected in the normally low correlations with other indices. However, the response to heavy organic enrichment in feeding groups is comparable to the response in diversity, evenness and ecological groups. Weaker correlations than normal conditions between indices were found at Ironrotter Point and the upper Clyde estuary. BOPA and AMBI, which are expected to be highly correlated, were found to be highly correlated in all sites except for the Clyde estuary data where no correlation was found.

The correlations show a complex relationship between indices and their response to the environment. Indices performed predictably in sites which showed heavy disturbance. In other sites which were more moderately disturbed, the indices behaved differently from each other and not in line with expectations.

3.5 Conclusion

The extent of agreement of indices in the different sites showed a similar pattern, apart from in the Clyde estuary. In very bad quality areas, the indices largely agreed in the classification. This was seen in the fish farms and Barcaldine sites and was reflected in the index correlations (Tables 3.19, 3.24). In all of the sites (except the Clyde) indices disagreed or found similar classifications in intermediate quality samples. In most sites, for the best quality samples, indices agreed or assigned a similar classification. The exception to this was the fish farm sites, where indices disagreed in the classification of the reference sites. However, if the BQI was excluded, agreement or similar classifications were mostly found. This shows that the greatest level of disagreement occurred between indices in samples of intermediate quality. This is similar to findings in another study which showed indices were good at detecting bad quality sites but not at distinguishing between good and moderate quality (Puente and Diaz, 2008).

Indices showed different levels of sensitivity to temporal trends. Since indices perform less well in distinguishing intermediate disturbance, this can be important for the detection of small changes in quality and early warning signals. Indices, like AMBI, detected little change in quality at Ironrotter Point, and no change in quality classification until the last year. This may imply that these indices, and others which detected no change or an increase in quality, are unsuitable as early warning indicators. Another study has also found AMBI and BOPA to be unsuitable for detecting small changes over time (Kröncke and Reiss, 2010) and AMBI was found not to identify early symptoms of eutrophication (Salas et al., 2004).

Where there was a monotonic trend with distance from the pollution source, most indices detected this. Pielou's evenness (J') and A/S did not detect strong trends of increasing quality at Barcaldine or at the Clyde estuary. This could be an indication that these indices underestimate quality, although they did detect trends at Irvine and the fish farms. Average Taxonomic Distinctness (Delta+) found no trend with distance from the fish farm sites and Taxonomic Distinctness (Delta*) and Variation in Taxonomic Distinctness (Lambda+) found relatively weak correlations compared to other indices. Considering the differences between locations at fish farm sites was great, a high correlation with distance would have been expected to distinguish different levels of quality. This would suggest that these measures are less sensitive to disturbance than other indices. Salas et al. (2006) also found taxonomic distinctness to be less sensitive to disturbance than other indices. However, it may be that these indices are more robust against natural variability and small changes which may not be statistically significant are still important markers for change in the environment.

In this study, BOPA was frequently found to classify sites with higher quality while ITI and BQI generally assigned lower quality. AMBI was also found to classify sites as having higher quality than other indices. Similarly, several authors have noted that AMBI and BOPA often assign higher classifications than other indices such as BQI (Labrune et al., 2006, Ruellet and Dauvin, 2007, Blanchet et al., 2008).

The translation of a high correlation with species richness to a quality classification was shown to be misleading at Ironrotter where species richness increased while environmental quality was decreasing. While species richness itself could be a good indicator of change, this may not convert directly to quality. This was even the case for multimetric indices which have been recommended for being less sensitive to natural variation (Kröncke and Reiss, 2010). This reduced sensitivity to variability is due to the combination of species richness, evenness and ecological groups often incorporated in multimetric indices, which reduces the weight of any one of these components. In this study, the combination of factors was shown to cancel each other out resulting in IQI and m-AMBI not detecting a trend in decreasing quality.

Due to the variability and unpredictability of the response of indices, it is important to use a variety of methods in interpreting change in the environment. Although many indices seem to contribute the same information, this can change in different circumstances and very few indices remain highly correlated in absolutely all conditions. However, using a large number of indices can cause confusion in trying to explain trends or quality classifications. Therefore, interpretation should include species richness, a variety of different indices, multivariate analysis and physico-chemical variables in order to explain changes in benthic communities. Different types of responses to environmental gradients were found at these sites, not only monotonic, and responses were often confounded with several factors, measured and unmeasured, making interpretation of the index responses difficult. For example, Taxonomic distinctness (Delta*) showed a stronger response to depth than to distance from fish farm sites and this in turn may have been related to other unmeasured properties such as sediment type. As well as a greater amount of information benefitting interpretation of index responses, other methods may be more suitable for measuring the response of indices to environmental gradients in order to detect nonlinear trends and to try to account for confounding factors; this is discussed in a subsequent chapter. The unpredictability of responses shows the importance of not relying on a single index for quality classification. Structural properties of ecosystems can respond in variable ways to disturbance while functional properties may indicate, more reliably, the direction of changes in quality (Paul, 1997). Functional indices are explored in the next chapter.

Chapter 4

Comparison of structural and functional approaches

4.1 Introduction

Recent developments of indices have largely been driven by the Water Framework Directive (EC, 2000). This had lead to the development of multimetric indices capable of incorporating the requirements for measuring "*the level of diversity and abundance…and disturbance-sensitive taxa*" (EC, 2000). Multimetric indices such as m-AMBI (Muxika et al., 2007) or the IQI (WFD-UKTAG, 2008) incorporate these properties. However, the Marine Strategy Framework Directive (MSFD) (EC, 2008) has emphasised the "*structure, functions and processes*" of the system as well as the "*resilience to human-induced environmental change*". The indices which have been developed in the context of the WFD which are structurally focussed therefore fall short of the MSFD requirements to assess functioning of the system. Enhancement of current methods used for ecosystem health assessment could come from the measurement of functional aspects of the system such as resistance and resilience (Dolédec et al., 1999). Functional indices are potentially useful in the assessment of 'good ecological status' for the MSFD and consequently some attention has recently been given to measuring the functional diversity of the system (Bremner et al., 2003, Bremner et al., 2006c, Cooper et al., 2008, Marchini et al., 2008, Pranovi et al., 2008).

The methods proposed include the analysis of biological traits which can act as an indirect measure of function (Péru and Dolédec, 2010). The use of functional traits as a surrogate negates the need for measuring actual function such as production or energy which are difficult to measure and highly specialised. These methods can use the same data already available or monitored for most sites with the addition of known species specific information. Analysis of biological traits for ecosystem health assessment has previously been used in the freshwater and terrestrial environments (Statzner et al., 2001, Petchey and Gaston, 2002) but is only more recently being explored in the marine environment (Bremner et al., 2003, 2006, Rachello-Dolmen & Cleary, 2007, Cooper et al., 2008, Marchini et al., 2008; Pranovi et al., 2008). Potential advantages of using functional indices compared to structural indices are not only policy driven. Functional indices are potentially less variable than structural. This is partly because the functional indices do not rely on species identity. The use of biological traits allows a comparable method across geographical regions because while species identity can change over geographical gradients, traits, such as size or reproductive method, occur across regions (Statzner et al., 2001), although traits expressed will differ depending on the environmental conditions. Further, while taxonomic structure may be highly responsive to natural environmental properties, functional composition based on biological traits has been found to remain stable (Dolédec et al., 1999, Charvet et al., 2000). A less variable index may respond less to disturbance from natural environmental properties and it may be easier to distinguish between the impacts of natural and anthropogenic disturbance. It may also be possible to identify, from the traits affected, the possible causes of change in the system (Dolédec et al., 1999). Functional diversity, measured using biological traits, has been found to be affected by anthropogenic induced stress in freshwater, estuarine and marine environments (Dolédec et al., 1999, Usseglio-Polatera et al., 2000, Charvet et al., 2000, Gayraud et al., 2003, Kenchington et al., 2007, Marchini et al., 2008, Feio and Dolédec, 2012, Paganelli et al., 2012). Predictions can be made about the response of traits to various forms of disturbance, for example an increase in disturbance may lead to an increase in small-sized individuals (Dolédec and Statzner, 2008). However, these predictions need to be tested. The response of traits to disturbance can be contrasting and contradictory as species use trade-offs and different solutions to cope with different types of stress; for example while small individuals may increase in number under stress, with heavy metal contamination they may decrease owing to larger surface volume ratio (Dolédec and Statzner, 2008). Predicted response of traits has been found to perform better with some types of disturbance (organic contamination) than with others (hydrological disturbance) (Feio and Dolédec, 2012). The response of different traits to different types of disturbance is an area which is still in need of investigation.

It is now mainly accepted that an increase in species diversity does represent an increase in functional diversity and an effect on ecosystem functioning (Loreau, 2010). However, often the relationship between ecosystem functioning and species diversity can be complex (Díaz and Cabido, 2001). The significance of functional groups to the ecosystem is still in doubt as effects may be due to species richness or other factors altogether (Petchey, 2004). In addition, we may not know what the functional groups of species are or which the important traits in terms of function are (Petchey, 2004). However, there is an increasing amount of evidence that inclusion of functional traits provides a better representation of functioning than species number or biomass alone (Bolam et al., 2002, Griffin et al., 2009). Although, the traits which best represent functioning are largely unknown. The relation between biological traits and actual function has been investigated in experimental studies such as the relationship between burrowing activity and NH_4-N release (Biles et al., 2002). However experimental studies have been limited in several ways including the number of traits, species and functions investigated; the conditions of the experiment being unrealistic; and the scale of the experiments (Covich et al., 2004). Furthermore, the processes studied in these experiments such as nutrient concentrations or grazing are proxies in themselves for functioning. Using biological traits makes an assumption that behaviours and properties of species are directly linked with ecosystem functioning. This assumption may be more reliable for well studied traits such as bioturbation (Biles et al., 2002, Biles et al., 2003) but is perhaps less dependable for others about which the relation to ecosystem function can only be inferred.

Sometimes a single trait will be the most relevant for the function of a particular system. Since some traits may be fairly homogeneous while others will be highly diverse, these diverse traits might be the most relevant in assessing functional diversity as the diversity score may be meaningless if irrelevant traits are chosen

(Leps et al., 2006). If two traits which are highly correlated are used, this feature will be over-weighted in calculation of functional diversity (Leps et al., 2006). However, correlated traits can be removed but not all species will conform to the correlations (Bremner et al., 2006c). Some authors recommend the use of as many traits as possible to obtain a more complete understanding of functioning in the system for the same reason that some species may be very similar in some traits but very different in others and therefore, using a reduced set of traits may suggest homogeneity where there is variation (Bremner et al., 2006c, Marchini et al., 2008).

However, for the assessment of ecosystem health, it may not be necessary to measure all aspects of functioning of the system. For example, the indices AMBI and ITI use single functional traits, ecological groups and functional feeding groups respectively, both designed to assess the functional response to organic enrichment, and these indices generally perform well in measuring this response. This response may also be a good indicator of overall ecosystem health. Tailoring indices may allow the impacts of particular disturbances to be detected. Since species diversity does give some indication of functional diversity, for the purposes of monitoring, structural properties and indices may be adequate as indicators of overall ecosystem health and it may be a waste of resources to also measure functioning. However, a criticism for indices like AMBI is that they are geared for measuring the response to organic enrichment while chemical and physical disturbances may not be measured adequately (Quintino et al., 2006). Furthermore, most marine systems suffer from multiple sources of disturbance. Some species may respond in a similar way to organic enrichment but in different ways to other stressors or to the synergistic effects of multiple stressors and this would suggest an advantage to the further step of using multiple biological traits.

Analysis of trait data has been carried out as biological traits analysis (BTA) which includes multivariate analysis of trait data (Bremner et al., 2003, Bremner et al., 2006c, Bremner et al., 2006b, Bremner, 2008, Cooper et al., 2008, Marchini et al., 2008); an index, Rao’s Entropy Index, which measures the abundance and dissimilarity of the species according to functional traits expressed (Leps et al., 2006, Cooper et al., 2008); and using a simple index such as Hill’s Index or Shannon-Wiener Index with trait data rather than species abundance data (Cooper et al., 2008, Gamito and Furtado, 2009). These methods allow an investigation of the functional aspects of the ecosystem which can be compared to the more traditional metrics based on structural properties.

Aim

The aim of this study is to assess the performance of structural and functional indices and measures of ecosystem health assessment in two sites subjected to different levels of anthropogenic disturbance to identify suitable measures of structure and function and to identify the value of measuring both structural and functional aspects of the system.

Null Hypotheses

1. Structural and functional indices discriminate equally well between disturbed and undisturbed sites.
2. Structural and functional indices are not correlated with each other.
3. Structural and functional indices do not show temporal or spatial variation.
4. Structural and functional index quality classifications do not correlate with environmental variables.
5. Structural and functional index quality classifications do not change whether abundance or biomass data are used.
6. Environmental properties and biological traits of species do not explain variation in communities at two sites.

4.2 Methods

4.2.1 Study Sites

Data used came from the study sites Leverets Station (53^o 15.50’N 09^o 2.02’W; 8m deep) and Margaretta Station (53^o 13.50’N 09^o 6.50’W; 22m deep) both located in Galway Bay on the west coast of Ireland. The data were collected as part of a PhD thesis (Solan, 2000). Macroinvertebrates were sampled over one year 11 times using 0.1m^2 van Veen grabs, starting in December 1996 and ending in November 1997. Each sampling event was taken one month apart and consisted of a replicate being taken over each of five consecutive days with a total of 110 macroinvertebrate samples with 147 species being collected. Samples were sieved using 0.5mm mesh size sieve. In addition to macroinvertebrate samples, a number of environmental water column and sediment variables were measured (Table 4.1). Water column variables were measured once per month while three replicates of sediment properties were taken over the first three days of the macroinvertebrate sampling. Data of sediment properties were available from core samples which were measured in 1cm intervals from 1 to 10cm at Margaretta and 1 to 7cm at Leverets.

Leverets Station is considered a moderately stressed study site with several sources of pressure including freshwater input and depressed salinity, domestic sewage, river material deposition, wave exposure, occasional trawling and heavy metal contamination (Solan, 2000 and references therein). Margaretta Station is considered a pristine site and used as a reference site in this study. The site is considered unimpacted as the sediment composition and faunal communities have remained stable over time, including the consistent presence of a community of *Amphiura filiformis* which has been the focus of several studies (Solan, 2000 and references therein).

Freshwater from the River Corrib was discharged at a rate of 12.06-240.28m^{3}s^{-1} mean daily flow into Galway Bay during the study period along with untreated sewage (Solan, 2000). Leverets is situated closer to the shore and in the direct path of the River Corrib system while Margaretta is less influenced by the system as it is located further into the bay and avoids much of the incoming freshwater due to the particular circulation system within the bay. This was reflected in the salinity recorded during the sampling period which showed surface salinity commonly fell below 30 at Leverets but rarely did at Margaretta while bottom salinity always remained above 33.5 at Margaretta (range 33.61 – 35.24) but fell below 32 twice at Leverets (range 20.71 – 34.91).

The depth of Leverets is <10m while Margaretta is >25m, suggesting Leverets is more susceptible to the effects of storm, wave scour and physical disturbance (Solan, 2000). Larger sediment particle sizes at Leverets and Sediment Profile Imagery taken during the study indicated deposition from the River Corrib was occurring at Leverets but this was not detected at Margaretta. Coarser sediments at Leverets may also have been due to removal of finer sediments during high energy periods, reflecting the greater level of exposure to physical disturbance at this site. A higher sedimentation rate and higher water column nutrient levels were found at Leverets and it was suggested that nitrogenous effluent was conserved in the benthic system. However, organic enrichment effects were not evident at either station in terms of oxygen depletion. The sediment organic carbon was slightly, but not significantly, higher at Margaretta and an accumulation of organic carbon at Leverets may have been prevented by the removal of finer sediments during high energy periods. Other activities in the area which may have contributed to disturbance included sluice control of the River Corrib, shipping, and other materials such as heavy metals which may be present in sewage, of which Leverets is in closer proximity.

Table 4.1 Environmental variables measured at Leverets and Margaretta stations in Galway Bay during the PhD study (Solan, 2000).

	Symbol	Sampled from	
Water Column Properties	SPM (g/L)	Surface Bottom	Suspended particulate matter
	POC (mgC/m^3)	Surface Bottom	Particulate organic carbon
	O_2 (mg/L)	Surface Bottom	Oxygen
	salinity (ppth)	Surface Bottom	Salinity
	NH_4 (µM)	Surface Bottom	Ammonium
	NO_3 (µM)	Surface Bottom	Nitrate
	NO_2(µM)	Surface Bottom	Nitrite
	PO_4 (µM)	Surface Bottom	Phosphate
	SiO_4 (µM)	Surface Bottom	Silicate
Sediment Properties	OrgC (%)	1-10cm	Organic carbon
	Median	1-10cm	d[50]; median grain size
	SMD	1-10cm	d[3,2]; equivalent surface area mean (Sauter mean diameter)
	DBMD	1-10cm	d[4,3]; equivalent volume mean (De Brouker mean)
	Graphic mean	1-10cm	Mean grain size
	Sorting	1-10cm	Inclusive graphic standard deviation
	Skewness	1-10cm	Inclusive graphic skewness
	Sand	1-10cm	2000-63 µm
	Silt	1-10cm	62-4 µm
	Clay	1-10cm	<4 µm
	Porosity (%)	1-10cm	Porosity

4.2.2 Statistical Analysis

A number of analyses were carried out to compare the outcome of structural indices with the less used measures of functional diversity. Raw abundance data are the appropriate data to be used for structural indices such as AMBI. However, for functional studies, biomass data, $\log_{10}+1$ transformed are the recommended data. Bremner et al (2006a) found biomass to be the most appropriate quantitative measure of species as it most closely represents the resources provided by the organism to the ecosystem, such as the quantity of carbon. Therefore, in some cases, analyses were carried using both datasets for comparison purposes.

4.2.2.1 Structural Methods

Benthic indices were calculated and multivariate analysis applied to the data as was carried out in chapters 2 and 3. Multidimensional scaling (MDS) was carried out using Primer 6 to assess patterns based on site and time of sampling. Benthic indices (chapter 2 section 2.1.1) were calculated for each sample. The quality classification for each site was calculated, based on the mean index value for the five indices which have associated quality classifications across all months. Mann-Whitney U-tests were used to assess which indices detected differences in quality between sites using SPSS 18. Kruskal-Wallis tests were carried out using SPSS 18 to assess if indices detected differences in quality between months in each site. The strength of correlation between different indices at both sites and at each separately was assessed using Pearson product moment correlation with Minitab. The relationship between indices and environmental variables was also assessed using Pearson product moment correlation.

4.2.2.2 Biological Traits Analysis

The method of applying the Biological Traits Analysis (BTA) was adapted from Bremner et al. (2006a). The first stage is the selection and gathering of trait information. Selection of traits depends on three main factors including the aim of the study, the functioning of the system and the availability of information. This study aimed to find differences in the functioning of Margaretta and Leverets due to the greater level of anthropogenic disturbance which Leverets is subjected to. Traits can be described as effect traits or response traits. Effect traits are those which can indicate the functioning of the ecosystem whereas response traits are those which may indicate a functional response to a change in the system (Lavorel and Garnier, 2002, Bremner, 2008). Therefore, the general functioning of these systems was considered when choosing traits. The processes, properties and activities of functioning in marine benthic systems include the biological, chemical and structural properties of the system (Box 4.1) in addition to further properties which specifically relate to the types of anthropogenic disturbance found at Leverets (Box 4.2 and section 4.2.1).

Box 4.1 Key aspects of functioning (modified from Bremner et al. 2006a).

Process, property or activity

1. Energy and elemental cycling (carbon, nitrogen, phosphorus, sulphur, silicon, calcium carbonate)
2. Food supply/export
3. Productivity
4. Habitat/refugia provision
5. Temporal pattern (population variability, community resistance and resilience)
6. Propagule supply/export
7. Adult immigration/emigration
8. Modification of physical processes

Box 4.2 Additional aspects affecting functioning of the systems specific to Leverets station

1. Input of Freshwater
2. Wave Pressure
3. Bottom Trawling (occasional)
4. Heavy Metal Contamination (Lead)
5. River Material Deposition
6. Organic Enrichment (domestic sewage)

Once functions of the system were identified, how taxa facilitate this functioning was then investigated and this lead to the identification of the traits which were important for the functioning of the system (Tables 4.2, 4.3). Macroinvertebrates facilitate ecosystem functioning directly and indirectly through their activities, habits and life stages (Valiela, 1995, Bremner et al., 2006a). Traits serve as a proxy for these components of functions with many traits representing more than one aspect of functioning.

Table 4.2 Functions of Margaretta and Leverets ecosystems with effect traits (adapted from Bremner et al 2006a and Valiela, 1995)

Ecosystem Functions	**Components of process and facilitation by benthic macroinvertebrates**	**Traits governing facilitation**
Elemental cycling: Carbon Nitrogen Phosphorus Sulphur Silica	Transport of element from pelagos to benthos Transport of element within benthos Transport of element from benthos to pelagos Direct fixation of N Consumption Respiration Defecation and death Decomposition Reproduction Sediment processes Fixation (N only)	Feeding methods, movement, living habit, living location, palatability, reproductive method, morphology, symbiosis with bacteria, body design, tissue components, size, growth rate, longevity, defence mechanisms, exposure potential, propagule dispersal, fecundity, maturity age, migration
Food supply/ export	Consumption Food provision Recycling Resource capture Predator or prey within food chain	Feeding methods, palatability, movement, living habit, living location, reproductive method, body design
Productivity	Consumption Respiration Defecation and death Decomposition Reproduction Assimilation of organic material Growth rate Population growth rate	Feeding method, reproductive method, growth rate, size, lifespan, energy transfer efficiency, body design, defence mechanism, food type, lifespan, tissue components, attachment, living location, propagule dispersal, fecundity, maturity, migration, movement

Table 4.2 continued

Ecosystem Functions	**Components of process and facilitation by benthic macroinvertebrates**	**Traits governing facilitation**
Habitat/ refugia provision	Sediment trapping Substrate provision Habitat creation Removal of habitat	Sociability, biogenic habitat provision, body type, growth form
Temporal pattern	Population variability Community resistance and resilience Immigration Emigration Recruitment Temporal variability	Predictability, flexibility, attachment, living location, exposure potential, defence mechanisms, mobility, growth rate, recruitment success, reproductive method, migration
Propagule supply/ export	Recruitment Larval survival Reproduction	Recruitment variability, biogenic habitat provision, food type, maturity, propagule dispersal method, fecundity
Adult immigration/ emigration	Immigration Emigration Patch movements	Mobility, sociability, migration
Modification of physical processes	Modification of currents Sediment trapping	Biogenic habitat provision, bioturbator

Table 4.3 Factors at Leverets potentially affecting function with response traits

Disturbance	**Potential impact on or response of benthic macroinvertebrates**	**Response Traits**	**Reference of impacts**
Freshwater - depressed salinity	Avoid/escape Osmo-regulation Tolerance to temperature	Living location, movement, living habit, body type, tolerance to salinity	(BDC, 2008)
Physical scour - wave pressure	Abrasion	Body type, robustness, flexibility, exposure potential, wave exposure preference	(BDC, 2008)

Table 4.3 continued

Disturbance	Potential impact on or response of benthic macroinvertebrates	Response Traits	Reference of impacts
Bottom trawling	Burial, smothering; Siltation/ turbidity; Abrasion; Habitat loss; Removal of target species; Removal of non-target species; Physical damage by collision; Turbidity changes; Habitat structure changes	Burrow depth, movement, body type, flexibility, exposure potential, robustness, feeding type	(BDC, 2008)
Heavy metal contamination	Toxicity, impaired growth	Tolerance to heavy metals, size	(BDC, 2008)
River material deposition	Sedimentation, Land based pollution: Contamination – hydrocarbon, synthetic, non-synthetic, heavy metals, radionucleotides, Inputs of N and P, de-Oxygenation, nutrient and organic matter enrichment	Movement, living location, living habit, tolerance to pollution	(BDC, 2008)
Organic enrichment	De-oxygenation, Ability to utilise matter, Increased sedimentation, Formation of reduced (toxic) chemical compounds, P release from sediments, Change in biomass, productivity, species, trophic structure, Inputs of N and P	Feeding method, trophic group, tolerance to organic enrichment, movement, living habit, living location, size	(Camargo and Alonso, 2006) (BDC, 2008)

Although many traits were identified as serving as proxies for the functioning of the system and responding to disturbances in the system, some could not be used due to a lack of information available. This may cause some aspects of the functioning to be under represented. However, since many traits represent several aspects of functioning almost all processes and properties were represented by some traits. Nineteen traits were finally used in the analysis (Table 4.4). Each trait was divided into categories or modalities. Once the traits required were identified, each species in the dataset was researched using various sources; firstly previously compiled trait data were consulted, in particular, bioturbation related traits from Solan (2000) and the BIOTIC traits catalogue (MARLIN, 2006), after these, individual species were researched in the literature (see Appendix 8.4 for full reference list). Once the required information was found, this was entered into a database. Each trait for each species was assigned a total value of zero or one. Each modality within the trait was assigned a value of between zero and one, zero indicating no expression and one indicating strong expression. Fuzzy coding was used when a species exhibited more than one trait modality (Bremner et al., 2006a; Frid, pers. comm.). For example, if a species could exhibit all trait modalities equally and there were four categories, each modality would be assigned a value of 0.25 so that the total value for the trait was one. If a species exhibited one modality most of the time, or was described as doing so in most of the literature consulted, this modality would be assigned a higher proportion of one than another modality which was only sometimes expressed or mentioned in the literature. Other modalities never expressed would be given a value of zero.

Table 4.4 Biological effect and response traits representing functioning at Margaretta and Leverets in Galway Bay

Trait	Definition of trait and trait categories	Ecosystem Component Description	Reference	Abbreviation
Maximum Size	Very small (<1cm) Small (1-2cm) Small-medium (≥3-10cm) Medium (≥11-20cm) Medium-large (≥21-50cm) Large (>50cm)	Energy & elemental cycling Productivity; Food/resources	(Bremner et al., 2006a)	S.vsmall S.small S.smalmed S.med S.medlrg S.lrg
Bioturbator/ Reworking mode	Epifaunal Surficial modifier Biodiffuser Upward conveyer Downward conveyer Regenerator	Si cycling; $CaCO_3$ cycling; Energy & elemental cycling; Productivity; Temporal pattern; Food/resources	(Solan, 2000)	B.epi B.surf B.biodif B.up B.down B.regen
Burrowing Depth	Epifaunal Oxic layer Oxic & Anoxic layers Anoxic layer	Si cycling; $CaCO_3$ cycling; Energy & elemental cycling; Productivity; Temporal pattern; Food/resources	(Solan, 2000)	Bd.epi Bd.ox Bd.oxanox Bd.anox
Lifespan/Adult longevity	≤1 year 1-2 years 3-5 years 6-10 years 11-20 years >20 years	Energy & elemental cycling; Productivity	(Bremner et al., 2006a)	L.1 L.2 L.5 L.10 L.20 L.20plus
Food Type	Detritus Carrion Living material – benthic Living material - planktonic	Si cycling; Energy & elemental cycling; Productivity; Movements of propagules; Food/resources	(Bremner et al., 2006a)	Ft.det Ft.car Ft.ben Ft.plank
Feeding Method/ resource capture method	Suspension Deposit feeder Opportunistic/ scavenger Active predator	Si cycling; Energy & elemental cycling; Productivity; Movements of propagules; Food/resources	(Bremner et al., 2006a)	Fm.sus Fm.dep Fm.opp Fm.pred

Table 4.4 continued

Trait	Definition of trait and trait categories	Ecosystem Component Description	Reference	Abbreviation
Living Habit	Tube Permanent burrow Temporary burrow Crevice/hole Epizoic/epiphytic free	Si cycling; Energy & elemental cycling	(Bremner et al., 2006a)	H.tub H.pbur H.tbur H.crev H.epi H.free
Fragility	Fragile Intermediate Robust	Resistance to wave pressure, predation; Energy & elemental cycling; Temporal pattern	(MarLIN, 2006)	F.frag F.intr F.rob
Body Type	Soft Soft-protected (tube/tunic) Exoskeleton Shell	Si cycling; $CaCO_3$ cycling; Energy & elemental cycling; Productivity	(Bremner et al., 2006a)	Bo.soft Bo.softp Bo.ex Bo.sh
Sociability	Singular Occasionally gregarious Permanently gregarious Colonial	Movement of adults	(MarLIN, 2006)	So.sing So.ogre So.pgre So.col
Movement Type	None Swim Crawl/creep/climb Burrow/bore Jump	Si cycling; Energy & elemental cycling; Productivity; Food/resources; Movement of adults; Temporal pattern; Food/resources	(Bremner et al., 2006a)	Mv.no Mv.swim Mv.cr Mv.bur Mv.jump
Maturity (age at sexual maturity)	<1 year 1 year 1-2 years 3-5 years 6-10 years	Energy & elemental cycling Productivity; Food/resources; Temporal pattern;	(Bremner et al., 2006a)	Ma.0.1 Ma.1 Ma.2 Ma.5 Ma.10
Reproduction Type	Asexual Sexual-shed eggs Sexual-brood eggs	Temporal pattern	(Bremner et al., 2006a)	R.asex R.shed R.brd
Degree Attachment	None Temporary Permanent	Productivity; Temporal pattern	(Bremner et al., 2006a)	A.no A.temp A.perm

Table 4.4 continued

Trait	**Definition of trait and trait categories**	**Ecosystem Component Description**	**Reference**	**Abbreviation**
Exposure Potential	Low (infaunal or flat) Moderate (mound surface/interface dwellers) High (erect surface/interface dwellers)	Energy & elemental cycling; Temporal pattern; Food/resources	(Bremner et al., 2006a)	E.low E.mod E.high
Body Flexibility	<10 degrees 10-45 degrees >45 degrees	Temporal pattern	(Bremner et al., 2006a)	Fl.low Fl.mod Fl.high
Propagule Dispersal	Pelagic Benthic	Energy & elemental cycling; Productivity; Movements of propagules	(Bremner et al., 2006a)	P.pel P.ben
Salinity	Full salinity Variable salinity Reduced salinity Low salinity	Resistance to changes in salinity	(MarLIN, 2006)	Sl.full Sl.var Sl.red Sl.low
Tolerance	Very sensitive Sensitive Moderate Tolerant Very tolerant	Resistance to pollution/ organic enrichment	(AZTI-Tecnalia, 2011)	T.vsens T.sens T.mod T.tol T.vtol

4.2.2.3 Analysis of trait data

4.2.2.3.1 Indices

Once the trait database was created, this was the basis for functional and biological trait analyses. Functional diversity of the sites was measured in a number of ways. These included the total number of trait modalities which occur at each site; number of trait modalities multiplied by species richness at each site to give the number of times modalities occur at each site; and number of trait modalities multiplied by the abundance and the biomass (transformed $\log_{10}x+1$). These were further analysed by calculating Shannon Wiener (H'ln) and Hill's Index (N1) using the multiplied and counted trait datasets (see Appendix 8.5 for calculation of datasets). Mann-Whitney U was used to detect differences between sites and Kruskal-Wallis was used to detect differences between months at each site for each measure of functional diversity. MDS was also used to assess distribution of samples based on biological traits.

4.2.2.3.2 Rao's Entropy

Rao's Entropy is an index based on the Simpson Index for the measurement of functional diversity which measures the functional dissimilarity between species (Leps et al., 2006).

$$FD = \sum_{i=1}^{s} \sum_{j=1}^{s} d_{ij} p_i p_j$$

...where FD is the functional diversity or Rao's coefficient

s is the species richness

p_i is the proportion of the i-th species

d_{ij} is the dissimilarity of species i and j

The trait dataset created (section 4.2.2.2) was also used for the calculation of Rao's Entropy. An Excel macro file available (Macro: http://botanika.bf.jcu.cz/suspa/FunctDiv.php Leps et al 2006) was used to calculate the index for both abundance and biomass (transformed $\log_{10}$ x+1). Rao's index was calculated for each trait at each site and an average of all traits was found for each site. Mann-Whitney U was used to test for differences between sites and Kruskal-Wallis was used to test for differences between months at each site. Pearson product moment correlation (carried out using Minitab 15) was used to assess the relationship between different traits which were scaled to common range of 0-1 (see 4.2.2.3.3).

4.2.2.3.3 Comparison of structural and functional indices

Indices were scaled across both sites, using the minimum and maximum index values, to a value between 0 and 1 using the following equation from (Péru and Dolédec, 2010).

$$Y_k = \frac{x_k - \min(X)}{\max(X) - \min(X)}$$

…where Y_k is the scaled value

x_k is the value of the index at site k

X is the range of the index values before scaling

Pearson product moment correlation was then used to assess the correlation between scaled structural and functional indices as calculated using abundance and biomass data.

4.2.2.3.4 RLQ analysis

RLQ analysis is a three table ordination based on co-inertia analysis which simultaneously analyses species data, biological trait data and environmental data (Dolédec et al., 1996). R refers to the environmental variables, L the species and sampling sites and Q the functional traits. Two-tabled co-inertia analysis is an alternative multivariate analysis to the more common canonical correspondence analysis (CCA) or redundancy analysis (RDA) which are ordinations used to interpret the relationship between species and environmental variables (Dray et al., 2003). Compared with CCA and RDA, co-inertia analysis is a better alternative when using a large number of variables and when variables may be correlated (Dray et al., 2003). One drawback identified with BTA is that only biological properties are considered (Bremner et al., 2006a). RLQ may go some way to being more representative of the whole ecosystem as physical and chemical components are included. However, this also adds greater complexity to the analysis and interpretation of the analysis. RLQ analysis has not been widely used but some studies from the terrestrial environment (Barbaro and Van Halder, 2009) and the marine environment (Rachello-Dolmen and Cleary, 2007) have shown promising results for the combination of species, environmental and functional trait data.

RLQ analysis was carried out using the ADE4 package for the statistical software 'R' and biomass data (transformed ($\log_{10}$ x+1)) was used (http://pbil.univ-lyon1.fr/ade4/home.php?lang=eng). Co-inertia analysis was carried out between species and environmental variables and between species and traits and the RLQ analysis was carried out between species, environmental variables and traits. Eigenvalues for each axis were obtained and used to calculate the total percentage variance explained by the analysis. This was carried out by calculating the percentage for the first and second axes eigenvalue over the total value of all the eigenvalues. The scores of the first two axes of the RLQ analysis were correlated with the environmental variables, the biological traits and the species using Pearson product moment correlation on Minitab, to assess the properties, traits and species which had the greatest effect.

Before RLQ can be carried out each dataset must be analysed and summarised individually – species data were analysed using correspondence analysis; trait data were analysed using fuzzy correspondence analysis (fuzzy correspondence analysis is correspondence analysis using 'fuzzy' or uncertain data (Theodorou and Alevizos, 2006)) and environmental data were analysed using principal component analysis.

Since the package used for carrying out RLQ could not cope with missing data, a reduced species, environmental and trait dataset was used. There were 64 samples (32 in Margaretta and 32 in Leverets); 79 trait modalities; and 133 species.

4.3 Results

4.3.1 Spatial and temporal variation

Multidimensional scaling showed two distinct communities at Leverets and Margaretta (Figs 4.1, 4.2). Two-way crossed ANOSIM showed the difference between sites was greater than the difference between months for both abundance (Site: R=0.999, p<0.01; Month: R=0.796; p<0.01) and biomass (Site: R=0.929, p<0.01; Month: R=0.252, p<0.01). Within sites, greater similarity was found using biomass data compared to abundance data. Abundance data showed differences between months at both sites. December to May and again November showed communities which were distinct at Leverets while at Margaretta, the months April, May and September were more distinct than other months (Fig. 4.1 (b)). This pattern was not apparent with biomass data (Fig. 4.2 (b)) where a few individual replicates at Leverets stood out from the rest and the months December, January and September stood out a little; while at Margaretta no months were particularly distinctive.

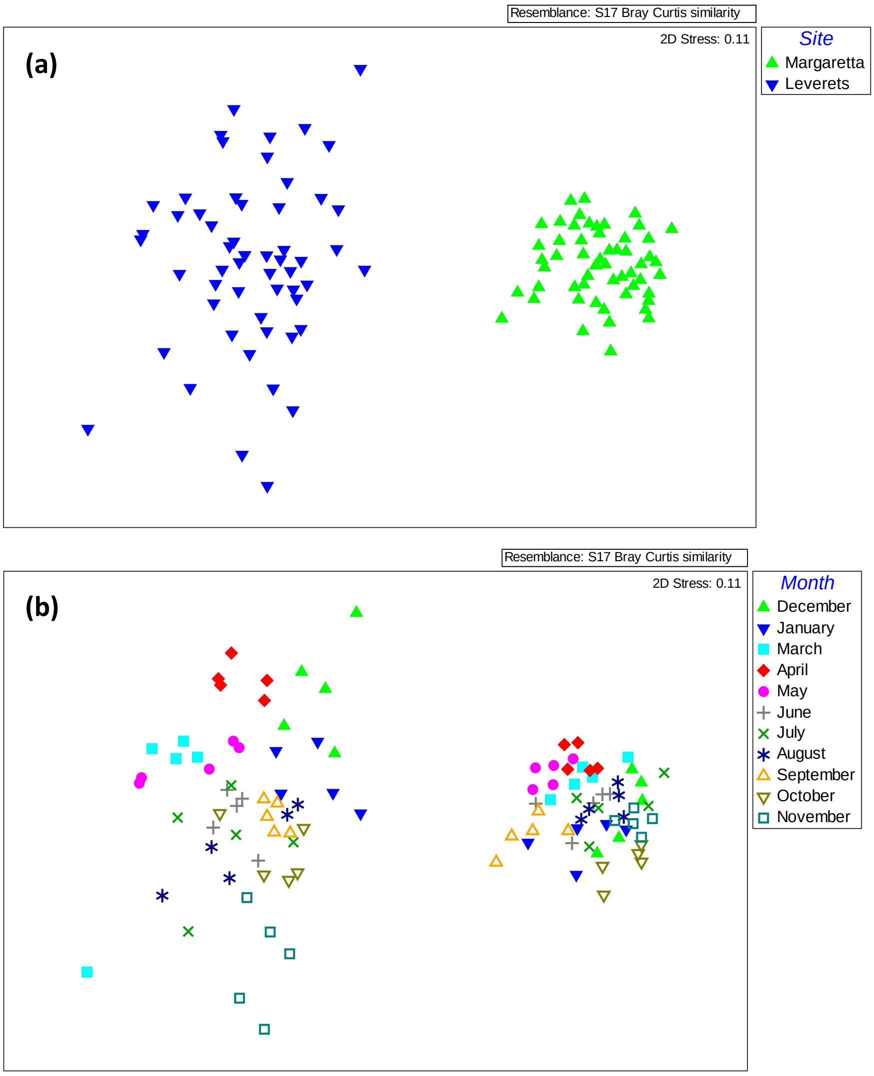


Figure 4.1 MDS plot of differences between sites (a) and months (b) at Galway Bay based on raw species abundance data

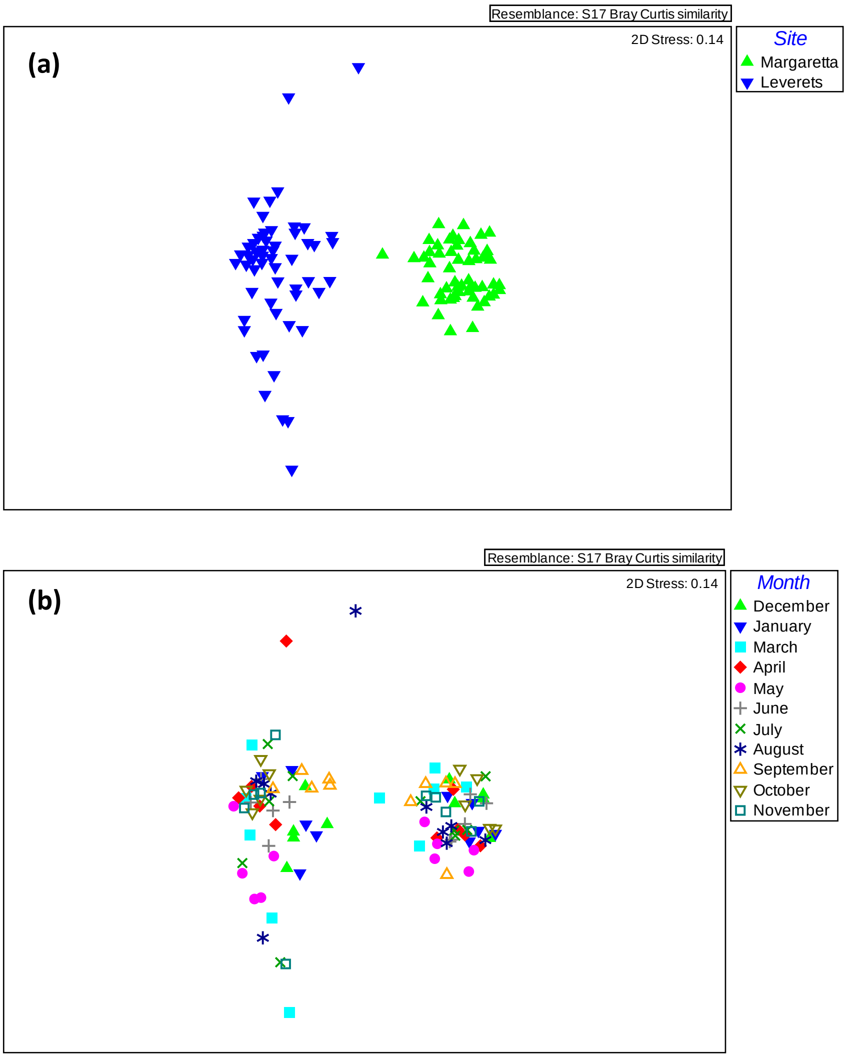


Figure 4.2 MDS plot of differences between sites (a) and months (b) at Galway Bay based on biomass data (transformed $\log_{10}$ x+1)

Quality classifications at each site differed depending on the index used. IQI and ITI both found Margaretta to have higher quality than Leverets while BOPA and AMBI found both sites had similar quality (Table 4.5). BQI assigned lower quality than other indices overall with Leverets slightly lower than Margaretta.

Table 4.5 Quality classification, based on average index value from each sample month for Galway Bay according to the five indices with associated quality classification scales (n=5 in all cases)

Site	IQI	BQI	BOPA	AMBI	ITI
Marg Dec	High	Moderate	Good	Good	Normal
Marg Jan	High	Good	Good	Good	Normal
Marg Mar	High	Moderate	Good	Good	Normal
Marg Apr	Good	Moderate	Good	Good	Normal
Marg May	High	Moderate	Good	Good	Normal
Marg Jun	High	Good	Good	Good	Normal
Marg Jul	High	Good	Good	Good	Normal
Marg Aug	High	Moderate	Good	Good	Normal
Marg Sep	High	Good	Good	Good	Normal
Marg Oct	High	Good	Good	Good	Normal
Marg Nov	High	Good	Good	Good	Normal
Lev Dec	Good	Poor	High	Good	Changed
Lev Jan	Good	Good	Good	Good	Changed
Lev Mar	Moderate	Moderate	Good	Good	Changed
Lev Apr	Moderate	Poor	Good	Good	Changed
Lev May	Good	Moderate	Good	Good	Changed
Lev Jun	Good	Moderate	Good	Good	Changed
Lev Jul	Good	Moderate	Good	Good	Changed
Lev Aug	Moderate	Moderate	Good	Good	Changed
Lev Sep	Good	Good	Good	Good	Changed
Lev Oct	Good	Moderate	Good	Good	Changed
Lev Nov	Good	Moderate	High	Good	Changed

Most other indices found Margaretta to have higher quality than Leverets (Table 4.6). However, J', Lambda+ and BOPA indicated higher quality at Leverets. Most indices found differences between months at both sites.

Table 4.6 Relationship between sites and between months at each site according to different indices as found using raw abundance data. Difference between sites tested using Mann-Whitney U (n=55). Differences between months tested using Kruskal-Wallis (n=55). ***P<0.001; **P<0.01, *P<0.05, ns=not significant. For 'site' [m]=Margaretta has greater index value; [l]=Leverets has greater index value.

Index	Site	Margaretta (Month)	Leverets (Month)
S	*** [m]	**	**
N	***[m]	**	***
D	***[m]	**	**
J'	***[l]	**	***
Brillouin	***[m]	**	**
Fisher	***[m]	**	**
ES50	***[m]	***	***
H'(ln)	***[m]	**	***
Simpson	***[m]	*	***
N1	***[m]	**	***
IQI	***[m]	*	*
EQR	***[m]	*	*
ITI	***[m]	*	**
BOPA	***[m]	***	**
A/S	***[m]	*	***
Delta	***[m]	*	Ns
Delta*	***[m]	***	***
Delta+	***[m]	**	**
sDelta+	***[m]	**	**
Lambda+	***[l]	*	**
AMBI	***[l]	**	Ns
BQI	***[m]	Ns	***
MAMBI	***[m]	*	**

Note: A/S, BOPA and AMBI index values have inverse relationships with quality

Overall (Table 4.7) and at Leverets (Table 4.9), correlations between indices followed the typical pattern of similarity with species richness (Chapter 2 section 2.3.3). However, at Margaretta, there was a low level of correlation between indices, even those which were often highly correlated to species richness (Table 4.8).

Table 4.7 Pearson product moment correlations between all indices calculated for Galway Bay based on raw species abundance data with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key).

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI	MAMBI
N	87.7																						
d	97.8	77																					
J	-30	-51.8	-16.6																				
Brillouin	84.1	62.1	87.9	21.3																			
Fisher	89.3	60.2	96.6	-0.4	85.8																		
ES(50)	67.3	34.6	78.3	39.7	89.1	86.3																	
H(loge)	75.7	48.6	83	36.7	98.1	85.6	94.3																
Simpson	50.3	27	58.8	60.9	84.2	63.9	78.5	89.8															
N1	75.2	46.6	82.5	35.2	96	85.8	95	97.8	82.9														
IQI	84.5	70.7	84	-20.3	76.9	77.3	65.7	70.4	47.6	68.3													
EQR	67.9	52.1	69.7	0	71.6	66	64.3	68.3	53.5	65.1	95.7												
ITI	82.8	80.6	77	-37.4	66.1	65	44.3	55.6	36.5	53	81.2	69.9											
BOPA	42.5	45	39.3	-6.4	41.3	33	23.6	36.6	37	33.3	20.3	10.3	41.5										
A/S	66	91.7	50.1	-63.4	39.4	28.4	5.2	22.6	3.3	21	51.9	34.3	69.8	38.8									
Delta	76.2	63.3	76.7	9.1	82	72.2	64.9	78.6	72.7	74.9	66.9	61.1	67.6	36.9	45.2								
Delta *	57.2	62.7	49.2	-49	31.4	37.6	12.2	20.7	1.7	22.5	46	31.6	58.7	15	62.1	69.8							
Delta +	64.5	65.5	57.3	-43.8	41.3	45.5	20.9	30.8	12.5	31.9	52.1	37.8	66.5	22.5	59.9	64.9	80.7						
sDelta +	99.9	88.1	97.4	-31.4	83.2	88.6	66	74.5	48.9	74.1	84.3	67.5	83.3	42.1	66.7	76.5	59.1	67.2					
Lambda +	-64.9	-67	-57.8	40.3	-41.7	-46.8	-22.6	-32	-14.9	-33.3	-46.1	-30.3	-59.5	-35.7	-59.8	-65.2	-78.8	-88.5	-67.2				
AMBI	-45.6	-38.3	-44.3	22.4	-36.1	-39.1	-33.2	-31.1	-12.4	-30.9	-84.1	-89.7	-57	11.5	-29.4	-30.7	-29.7	-31.4	-45.8	21.1			
BQI	46.8	34.5	50.1	12.1	57.2	48.7	48.4	56.9	56.7	49.8	40.6	37.3	45.9	36.8	18.5	35.5	-9.4	1.6	45.4	-3.9	-11.6		
MAMBI	91.5	73.7	92.1	-12.2	88.6	86.3	77.6	82.6	57.5	82.3	96.1	88.6	80.7	29.9	53	74.8	47.9	55	91.2	-51.1	-69.2	44.7	
Total Biomass	72.7	74.9	67.6	-40.7	48.9	58.6	30.4	40.3	24.2	37.4	57.9	42.7	61.1	29.3	62.3	53.7	51.8	56.1	73.3	-58	-30.7	27.6	58

Table 4.8 Pearson product moment correlations between all indices calculated for Margaretta in Galway Bay based on raw species abundance data with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key).

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI	MAMBI
N	45.8																						
d	94.8	15.9																					
J	-12.3	-53.9	6.3																				
Brillouin	47.2	-12.5	57.4	80.6																			
Fisher	84.8	-6.5	97.2	19.7	61.3																		
ES(50)	45	-28.5	60.7	79.1	94.7	68.3																	
H(loge)	42.9	-23.8	56.8	84.2	99.2	63.5	96.6																
Simpson	11.9	-30.5	25	90.1	87.5	33.3	75.1	88.3															
N1	43.2	-24.8	57.7	82.8	98.1	64.8	96.1	99.1	86.9														
IQI	58	11.6	60.5	12.3	43.8	58.8	49.9	42.8	16	43													
EQR	31.6	-8.1	38.3	32.8	46.5	41.2	52.1	47	29.8	46.9	94.5												
ITI	9.4	20.4	3.5	-24.7	-15.3	0.1	-9	-16.8	-26.4	-16	21.6	16.8											
BOPA	-31.4	-2.1	-33.6	0.3	-17.5	-33.3	-24.9	-17.3	2.2	-19.2	-67.4	-64.3	-33.6										
A/S	0.3	88.2	-30.8	-54.2	-37.6	-50.7	-54.1	-48.4	-41	-49.3	-14.4	-22.8	22.5	9.9									
Delta	38.6	-2.1	43.4	49.7	68.8	44.5	57.7	66.8	68	67	47.9	51.2	8	-57.7	-19.3								
Delta *	33.4	34.8	23.3	-49.1	-22.2	14.6	-20.7	-25.7	-38.6	-23.5	40.4	27.4	42.7	-75.8	26.3	41.4							
Delta +	34.6	16	30.2	-16.9	6.4	24.4	10.8	3.8	-16.3	5.1	23.5	11.6	4	-38.7	3.6	23.5	49.8						
sDelta +	99.7	45.4	94.5	-13	46.4	84.4	44.7	42.1	10.4	42.5	58.1	31.6	9.4	-33.4	0.2	39.1	35.9	41.2					
Lambda +	-27.2	-20.3	-20.3	23.5	3.1	-13	2.1	6.3	20.7	5.3	-9.8	1.8	7	25.9	-11.4	-13.3	-42.4	-94.3	-33.6				
AMBI	-25.1	2.6	-29	-9.6	-21.8	-30.3	-31.6	-22.4	-2.3	-22.7	-92.8	-96	-24.9	67.3	13	-32.7	-38.3	-15.2	-25.6	2.6			
BQI	-15.3	-11.5	-12.9	7.9	-2.2	-10.1	-13.2	-1.4	23.8	-1.3	-30.4	-24.7	18.4	17.6	-5.9	24	0.4	-20.4	-16.7	20.3	32.3		
MAMBI	66.7	0.1	74.4	43.7	76.6	75.3	80.5	76.1	49.1	75.5	88.7	84	8.3	-47.2	-32.5	58.3	12.1	18.1	66.3	-3.9	-71.8	-20.5	
Total Biomass	32	42.6	20.5	-42.8	-18.3	10.7	-24.7	-22.2	-27.2	-23.6	14.4	0.3	-1.3	-26.9	31.5	10.6	47.4	27.2	33	-28.7	-6.4	-14.7	-4.1

Table 4.9 Pearson product moment correlations between all indices calculated for Leverets in Galway Bay based on raw species abundance data with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key).

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

	S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	Simpson	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta *	Delta +	sDelta +	Lambda +	AMBI	BQI	MAMBI
N	46.5																						
d	94.6	17.4																					
J	33.5	-37.8	49.2																				
Brillouin	86.4	22	86.6	73.2																			
Fisher	82.4	-5.9	96.1	55.9	78.3																		
ES(50)	80.5	-6.3	93.1	69.2	87	95.2																	
H(loge)	82.2	6.2	88.4	80.5	98.3	84.7	92.7																
Simpson	60.1	-11.6	69.4	92.2	89.1	68.8	78	92.6															
N1	83.3	8	88.9	76.7	96.2	86.2	93.2	97.8	86.2														
IQI	60	25.7	56.7	16.9	52.8	46.9	51.4	49.6	35.6	45.7													
EQR	46.7	11.8	49.5	31.2	53.3	42.1	49.8	51.8	45.2	46	97.4												
ITI	23.8	20.3	18.1	0.1	20.4	10.6	10	16.2	14.3	11.5	55.8	56.1											
BOPA	2.5	-18.2	8.6	34.7	20.2	13.8	14.8	22.3	33.1	22.6	-19.3	-12.8	-4.3										
A/S	-3.9	84.6	-33.9	-61.3	-22.9	-52.6	-52.4	-38.9	-48.6	-35.5	-6.8	-16.9	10.7	-18.9									
Delta	44.7	5.9	47.9	62.5	63	46.1	49.6	64.2	70.8	59.3	22.3	28.4	13.8	19.4	-19.8								
Delta *	-13.9	25.5	-22	-31.6	-25.6	-23.9	-30.7	-28.8	-30.3	-26.6	-15.6	-19.7	0	-15.1	37.7	45.6							
Delta +	-14.3	16.4	-21.3	-28.5	-24.1	-24.2	-34	-27.2	-23.2	-28.1	-12.1	-14.2	16.6	-21.9	27.4	30	69.3						
sDelta +	99.6	48.4	93.5	30.9	84.8	80.9	78	80.3	58.4	81.2	59.8	49.1	25.7	0.7	-1.5	47.3	-8.2	-5.7					
Lambda +	26.6	3.9	27.3	17	28.1	23.9	30.9	27.8	21.6	26.9	34.8	34.3	20.4	-3	-13.6	-28.7	-66	-71.4	20.7				
AMBI	-12.5	-10.7	-9.1	14.7	-3.7	-3.4	-7.4	-1.2	7	1.8	-85.4	-85.2	-53.3	31.7	-3.4	9.3	5.5	2.9	-12.8	-25			
BQI	47.7	-4	52.5	45.2	56.6	48.2	56.1	58	54.2	51.9	29.9	30.7	19.4	19.8	-35.1	3.8	-64.2	-51.3	44	52.9	-0.7		
MAMBI	81.7	37.9	77.1	31.4	75.7	65.8	71.3	71.8	52	70.5	91.8	86.6	46	-12.5	-3.2	37.6	-13.6	-13.9	81.2	33.3	-61.4	36.5	
Total Biomass	25.3	-0.3	31.1	10.4	19.7	38.1	27.9	22.3	14.9	24.7	3.3	-0.6	-13.9	15.4	-10.4	4.7	-11	2.3	26	3.5	11.4	7.9	12.7

Correlation of indices with physico-chemical properties showed low correlation with water column properties (Table 4.10). However, species richness and ITI did show some correlation with salinity and the amount of oxygen was correlated with the sample month. Correlations were found between indices and median grain size, SMD, graphic mean, sorting, porosity, silt content and to a lesser degree clay (Table 4.11). However, not all indices showed these correlations. BOPA, AMBI, BQI and to some extent Delta* did not find strong correlations with these or other properties.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 4.10 Correlation between indices and water column properties at Galway Bay. Pearson product moment correlations between with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key).

Variable	Month	S	IQI	ITI	BOPA	Delta*	AMBI	BQI	MAMBI	Totalbiomass
SPM (g/L) surface	6.2	14.3	12.9	21.4	34	1.3	-1.5	21.5	20.5	2
POC (mgC/m3) surface	-32.8	-7.4	-15.3	4.4	1	-1.6	8.6	-29.1	-16.3	-0.3
O2 (mg/L) surface	-63.4	-23.9	-19.4	-14.6	8.3	5.9	2.5	-32.8	-22.5	-21.8
salinity (ppth) surface	-11.3	50.6	39.6	53.9	19.5	27.4	-17.9	47.2	41.1	47.2
NH4 (μM) surface	-16.1	-26.4	-19	-24.3	-17.8	-0.3	2.5	-46.3	-21.2	-24.5
NO3 (μM) surface	-31.2	-27.3	-17.3	-20.3	-10.5	-9.2	-1.3	-18.4	-25.1	-12.2
NO2 (μM) surface	20.7	-5.8	8.3	0.6	-25.1	3.5	-18.6	-3.3	3.1	-2.5
PO4 (μM) surface	18	-3.2	2.5	1	-18.1	22.8	-9.5	-14.7	-2.4	10.6
SiO4 (μM) surface	-8.3	7	21	15.8	4	-18.4	-19.2	28.9	19.2	-7.3
SPM (g/L) bottom	3.5	7.5	7.1	12.1	33.1	-7	1.6	12.7	13.3	-1.9
POC (mgC/m3) bottom	-48.5	-32.7	-43.7	-34	-2.3	0.5	33.5	-45.7	-41.2	-16.3
O2 (mg/L) bottom	-67	-8.4	-7.1	1.8	7.1	14.8	-2.1	-14.6	-9.6	-6
salinity (ppth)bottom	22.9	48.7	44	44.9	28.1	20.1	-14.5	48.8	49.4	34.6
NH4 (μM) bottom	27.3	1.4	14.8	1.8	-36.3	-19.6	-21	14.3	15.8	-25
NO3 (μM) bottom	1	15.6	14.1	22.1	-21.3	11.9	-17.2	5.3	7.8	23.7
NO2 (μM) bottom	38.4	2.4	16.8	7.2	-43.6	7.7	-26.1	4.5	11.1	-0.7
PO4 (μM) bottom	11.2	-5	8	4	-20.6	21.2	-18.9	-9.4	1.5	2
SiO4 (μM) bottom	-23.2	-23.2	-6.8	-14.1	-39.3	-36.2	-9.5	22.3	-15.1	-16.3

Table 4.11 Correlation between indices and sediment properties at Galway Bay. Pearson product moment correlations between with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key).

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Variable	Sample Depth	Month	S	IQI	ITI	BOPA	Delta*	AMBI	BQI	MAMBI	Totalbiomass
Median	1cm	-17.7	-26.6	-27.8	-10	10	-11.5	11.5	-26.7	-33.7	-13.6
	2cm	-33.6	-55.4	-47.7	-38.6	-26.2	-30.9	24.7	-28.7	-55	-41.9
	3cm	-33.2	-58.8	-53.1	-50.8	-30.3	-34.7	29.3	-37.3	-57.9	-40.1
	4cm	-28.4	-67.3	-59.5	-57.3	-33.8	-42.7	32.1	-40.4	-66.3	-49.6
	5cm	-25.8	-58.8	-56.4	-53.1	-32	-39.9	34	-37.2	-62.4	-45.2
	6cm	-28.8	-70.3	-62.9	-59.3	-29.3	-39.8	36.4	-45.6	-68.8	-58.2
	7cm	-35.6	-65	-62.3	-57.6	-25.8	-46.2	37.9	-43.2	-67.3	-52.7
	8cm	-3.9	-65.9	-50.9	-50	-24.1	-59.6	23.5	-19.9	-56.8	-55.8
	9cm	3.8	-67.5	-56	-53.3	-31.2	-59	32.1	-15.3	-60.6	-57.9
	10cm	-18	-50.8	-55.1	-42.3	3.3	-42.4	42.9	-18.1	-55.9	-31.9
SMD	1cm	-15.4	-56.8	-54.1	-43.6	-18.4	-30.9	32.1	-41.9	-58.7	-38
	2cm	-19.6	-67.5	-59.8	-53.9	-26	-46.7	33.8	-34.3	-66.7	-50.4
	3cm	-17.5	-73	-63.2	-63.3	-38.7	-46.2	34.5	-42.1	-69.4	-54.9
	4cm	-18.7	-77	-67	-66.8	-40.2	-50.3	36.6	-43.5	-73.3	-60.6
	5cm	-19.2	-71.2	-62.8	-63.9	-37.5	-47.6	35.2	-43.2	-69.4	-56.1
	6cm	-9.1	-76.2	-63.7	-67.2	-43.6	-46.7	35	-47.9	-69.6	-64.3
	7cm	-17.1	-76.7	-68.1	-69.4	-37.7	-56.1	39.8	-45.1	-73.8	-62.5
	8cm	7.7	-78.9	-63.8	-69.3	-36.2	-71.3	34.6	-28.2	-69.8	-66
	9cm	11	-76.7	-66	-66.6	-39.4	-67.4	39.9	-21.5	-70	-65.1
	10cm	-3.9	-70	-69	-62.9	-17.9	-60.1	50.3	-29.5	-71.8	-52.5
DBMD	1cm	-17.2	-9.3	-7.8	9.8	17.7	-2	-2.4	-9.7	-12.9	-3.1
	2cm	-6.4	-3.7	0.5	9	-12.6	-2	-4.4	9.1	-2.5	-8
	3cm	-13.1	-1.9	-0.8	-1.9	-0.2	-12.7	0.2	6.1	0.4	-2.8
	4cm	-16.7	-11.4	-8.3	-10.1	-4.6	-31.4	4.7	11	-8.5	-18.9
	5cm	-0.3	3.7	5	7	-5.6	-8	-6.6	7.3	1.9	3.9
	6cm	-28.1	-11.1	-1.4	2.9	13	-5	-7	1.4	-6.2	-13.1
	7cm	-19.5	-17.7	-14.3	-6.6	10.9	-11	7.6	-19.4	-17.2	-13.1
	8cm	-26	-19.9	-17	-4.1	23.1	-19.5	12.7	8.1	-15.7	-17.6
	9cm	-1.7	-37.3	-36.7	-23.5	-1.2	-20.7	27	1.1	-36.8	-32.1
	10cm	-34.5	-13.1	-13.9	-2.2	21.8	-3.9	8.5	-10.1	-14.2	6.5
Graphic Mean	1cm	19.1	33.2	30	14.1	0.9	12.6	-13.3	27.5	35.8	20.4
	2cm	14	48.1	38.6	33.6	31.4	32.4	-17.7	18.5	45.1	39.5
	3cm	22.3	52	44.4	45.2	27.2	37.6	-23.5	26.8	48.2	37.8
	4cm	15	63.8	54.5	55.9	36.8	48.5	-29.3	34.5	60.2	55.4
	5cm	14.3	55.9	52.2	52.6	32.8	40.5	-30.9	34.3	57.9	46.5
	6cm	17.6	65.3	54.7	55.6	28.3	40.3	-29.3	36.8	60.5	57.6
	7cm	25.9	66.7	61.7	60	25.1	52.9	-37.6	39.5	66.3	57
	8cm	-2.8	74.5	59.2	62.6	29.7	66.2	-30.9	25.6	64.5	64.8
	9cm	-10.6	68.1	59.8	57	28.8	55.3	-38.1	16	62.3	59.1
	10cm	11.9	56.6	54.9	47.7	7.6	47	-38.9	23.6	57.2	35
Sorting	1cm	-6.9	55.9	55.1	64	39.4	38.7	-41	36	54.2	42.6
	2cm	12.5	58.4	55.8	60.5	13.5	40.5	-35.9	41.8	58.9	39.8
	3cm	-5.1	53.7	48	48.8	32.4	26.1	-28.2	35.9	53.2	33.2
	4cm	1.3	67.4	60.4	59.7	34.1	26	-35.4	47.5	65.4	45.7
	5cm	10.3	74.4	64.5	70.8	35.9	43.3	-38.1	46.1	68.4	57.1
	6cm	-16	65.2	60	69.2	50.6	45.4	-40	42.5	61.4	53.2
	7cm	-20.4	62.4	48.6	63.1	56.7	37.7	-25.1	36.2	52.7	53.7
	8cm	-28	65.9	52.3	69.9	55.5	56.2	-25.3	36.6	58.6	51.1
	9cm	-16.1	38.3	30.6	46.3	39.5	44	-16.8	22.1	33	32.5
	10cm	-32.4	55.3	49.7	62.1	42.5	56.2	-36.1	20	51.4	56
Skewness	1cm	-6.3	-30.7	-30.2	-28.9	-18	-18.8	16.7	-23.9	-33.9	-22.1
	2cm	-9.6	-3.1	-4.6	-12.3	6.7	1.4	4.1	-17.9	-3.8	-0.7
	3cm	-0.6	4.6	-0.6	3.2	-3.1	17.7	2.6	-4.2	-2	29.8
	4cm	0.5	-10.2	-12.1	-7.1	-2	21.3	8.8	-23.2	-13.9	6.7
	5cm	-14.3	-34	-36	-34.9	-9.1	-15	28.4	-21.9	-34.3	-22.8
	6cm	3.8	-11.9	-24.9	-22.6	-12.4	-9.5	27.8	-10	-20.4	-4.3
	7cm	15.7	-2.7	0.8	-7.5	-33.5	16.7	-3.3	-9.9	1.5	-4.8
	8cm	14.3	-10.2	-2	-15.3	-36.8	-3.1	-8	-20.4	-7.4	-1.1
	9cm	0.2	23.6	24.4	11.7	-5.9	6.5	-16.8	-1.4	24.9	18.6
	10cm	21.3	-9.5	-11.2	-22.5	-19	-21	14.6	8.5	-7.6	-22.4
Porosity	1cm	1.8	20.8	18	14.8	11.7	6.8	-11.8	12	17.8	17.9
	2cm	16.4	65.6	61.1	55.2	26.7	40.3	-39.7	33.5	64.3	51.6
	3cm	18.7	63.6	57.9	53.7	25.8	31.7	-35.3	41.4	62.3	42.2
	4cm	13.7	72	64	62.3	37.3	34.5	-36.5	44.7	69	48.3
	5cm	-0.1	76.6	65.9	65.5	46.2	35.8	-36.9	46.3	70.9	56.2
	6cm	6.9	73.2	65.1	61.6	44.1	41	-40.4	37.7	69.4	62.6
	7cm	6.3	72.3	64.1	63	42.8	45	-39.1	42.8	67.5	56.7
	8cm	0.8	57.8	50.2	45.9	35.4	51	-29.9	19.7	52.8	45.5
	9cm	-5.1	47.9	34.5	31	26.9	52.8	-15.8	0.1	37.1	47.5
	10cm	-1.5	29	17.9	6.4	14.1	21.8	-2	-0.5	22.3	23.4

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 4.11 continued

Variable	Sample Depth	Month	S	IQI	ITI	BOPA	Delta*	AMBI	BQI	MAMBI	Totalbiomass
Organic Carbon	1cm	9.3	7.6	17.7	19.5	7.8	13.4	-21.5	6.3	13	5
	2cm	20.4	26.6	30	31.9	10.4	34.3	-26.6	0.9	27.6	18.3
	3cm	20.3	32.6	35	36.8	14	38.1	-29.1	6.9	34.7	20.7
	4cm	18.8	38	39.3	44	20.7	37.6	-31.1	11.2	38.9	22.5
	5cm	11.3	47.2	40.6	51.5	35.4	40.3	-24.7	17.3	42.7	29.6
	6cm	18.4	41.5	37.7	44	30.9	42.2	-26.5	10.3	39.3	37.7
	7cm	21	37.9	33.8	38.3	22.8	44.6	-23.5	8.6	34.1	31.8
	8cm	21.3	27.1	29.2	29.7	12.8	51.1	-25.1	-12.4	28.1	23.1
	9cm	14.7	30.8	26.6	25	17	51.8	-17.3	-16.8	29.3	31
	10cm	7.7	46.1	39.4	34.3	18	44	-21.6	18	42.5	36.2
Sand	1cm	-17.9	-16.5	-14.7	2.8	13.6	-6.2	2.3	-14.8	-20.1	-8.1
	2cm	-9.8	-17	-11.6	-2.7	-16.6	-11.3	2.9	1.3	-15.8	-17.5
	3cm	-15.5	-15	-12.2	-13.2	-7.2	-20.3	6.4	-1.9	-12.2	-12.5
	4cm	-19.4	-29.1	-23.9	-25.4	-14	-39.9	13.2	-1.2	-25.7	-31.6
	5cm	-5.8	-17.1	-13.6	-12.2	-15.5	-20.6	4.3	-6	-18.2	-12.6
	6cm	-27.4	-29	-17.2	-14.2	0.7	-16.1	2.6	-10.8	-22.9	-27.7
	7cm	-20.3	-23.8	-19.8	-12.4	7.2	-15.6	10.9	-22.7	-23	-18.1
	8cm	-20.4	-39.7	-32.9	-23.3	9.7	-37.2	20.8	-1	-33.5	-34
	9cm	0.4	-46.9	-44.5	-32.7	-8	-30.4	31.2	-2.7	-45.3	-40.2
	10cm	-31.7	-24.9	-25.5	-13.9	16.2	-14.9	17.1	-14.6	-26.3	-4.1
Silt	1cm	14.6	52.9	55.1	50.7	24.6	34.8	-41.3	31.9	55.8	32.2
	2cm	23.4	72.2	66.1	63.1	30	48.7	-41.7	32	71.1	52.8
	3cm	18.6	72.8	65.8	67.3	36.4	47.3	-39.8	36.8	70.5	52.8
	4cm	17.8	78.1	69.4	71	38.9	51.9	-41.6	41.7	74.8	59.7
	5cm	16.5	70	63.9	66.8	35.9	52.3	-39.6	36.5	69.2	56.2
	6cm	13.6	80	71.4	75.6	38.9	56.3	-45.3	41.7	75.4	65.8
	7cm	15.9	77.6	70.8	74.7	36.5	61.5	-44.8	41	74.6	63.8
	8cm	-7.3	78.8	64.9	71.3	36	69.5	-36	28.6	70	63.9
	9cm	-12.8	77.6	67.5	71.2	38.6	69.7	-41.8	21.9	70.8	64.7
	10cm	-1	75.6	71.2	70.6	29.2	67.4	-51.3	24.9	74.2	60.6
Clay	1cm	9.3	45	44.8	45.1	6.7	35.4	-32.3	29.5	44.8	29.4
	2cm	-1.1	45.1	39.9	38.4	12.3	33.6	-25.6	16.1	43.6	32.8
	3cm	7.7	57.4	46.2	48	9.6	53.2	-27.7	9.6	47	53.7
	4cm	2.9	57.3	51.9	57.8	26.5	48.1	-31.4	25.6	54.9	48.4
	5cm	2.2	21.7	19.5	25.4	6.3	40.7	-11.6	-1	21	33.8
	6cm	4.9	58	51.3	54.3	21.7	48	-30.5	29.8	52.8	47.1
	7cm	19.7	54.2	52.9	54.2	14.4	41.3	-38.2	34.8	54.6	42.1
	8cm	6.2	56.8	49.4	54.1	7.3	52.3	-32.8	22.6	49	60.6
	9cm	-14	45.8	43	51.6	26	54.9	-26.9	22.2	42.2	47.3
	10cm	-0.8	48.5	52.6	44.2	14.4	50.3	-42.3	8.7	54.2	37.2

4.3.2 Functional Diversity

Functional diversity was assessed through the number of traits expressed at each site and the number of traits as a function of the total abundance and biomass at each site. The functional diversity according to all versions of number of traits was significantly greater at Margaretta than at Leverets (Mann-Whitney U; $p<0.001$) (Fig. 4.3). Functional diversity also differed depending on the month at each site (Kruskal-Wallis; $p<0.05$) (Fig. 4.4).

When these data were used with Shannon Index and Hill's Index, the results came out differently. The number of traits was not significantly different between the sites using either index (Mann-Whitney U; Hln: U= 1558.5, n=110, p>0.05; N1: U=1558.5, n=110, p>0.05). The occurrence of traits (traits*species richness) was significantly greater at Margaretta according to both indices (Mann-Whitney U; Hln: U= 1921, n=110, p<0.05; N1: U=1921, n=110, p<0.05).The frequency of traits (abundance) was not significantly different between the sites according to both indices (Mann-Whitney U; Hln: U= 1279, n=110, p>0.05; N1: U=1279, n=110, p>0.05), while the frequency of traits (biomass) was significantly greater at Margaretta according to both indices (Mann-Whitney U; Hln: U= 2346, n=110, p<0.001; N1: U=2346, n=110, p<0.001).

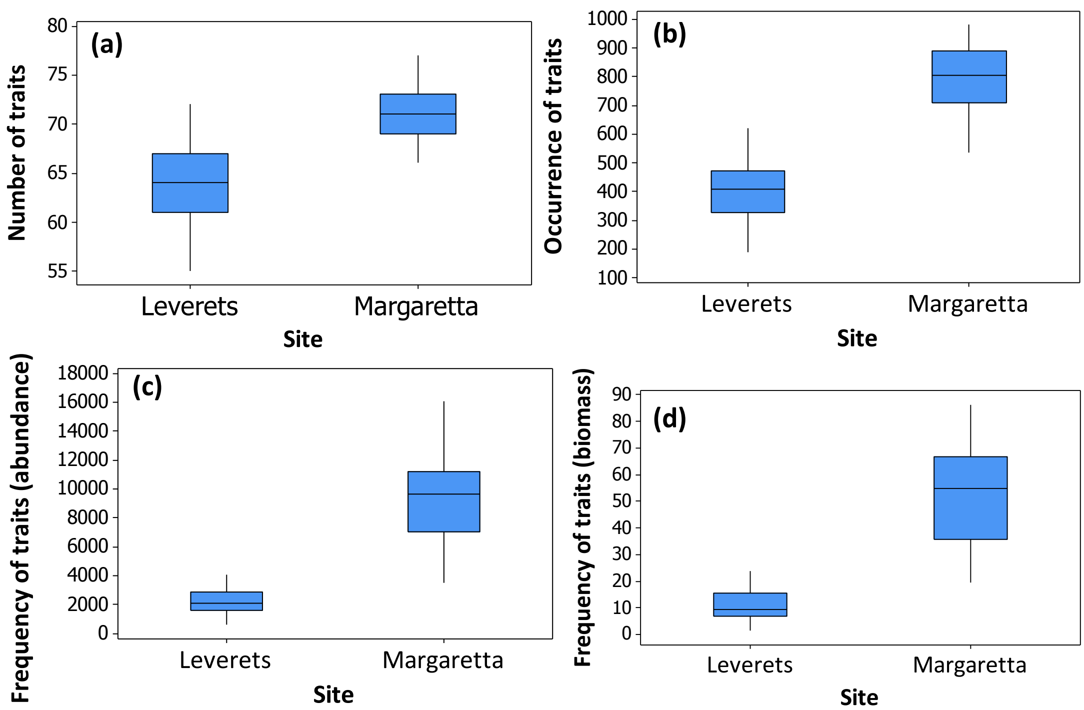


Figure 4.3 Functional diversity at two sites in Galway Bay according to (a) number of trait modalities occurring at each site (Mann-Whitney U=2798, n=110, p<0.001), (b) number of trait modalities expressed by species (i.e. species richness*trait modalities) (Mann-Whitney U=3016, n=110, p<0.001), (c) number of trait modalities expressed by individuals at each site (i.e. abundance of species*number of trait modalities) (Mann-Whitney U=3020, n=110, p<0.001), (d) number of trait modalities expressed by individual biomass at each site (i.e. biomass of species*number of trait modalities) (Mann-Whitney U=2984, n=110, p<0.001). For details of trait data see text.

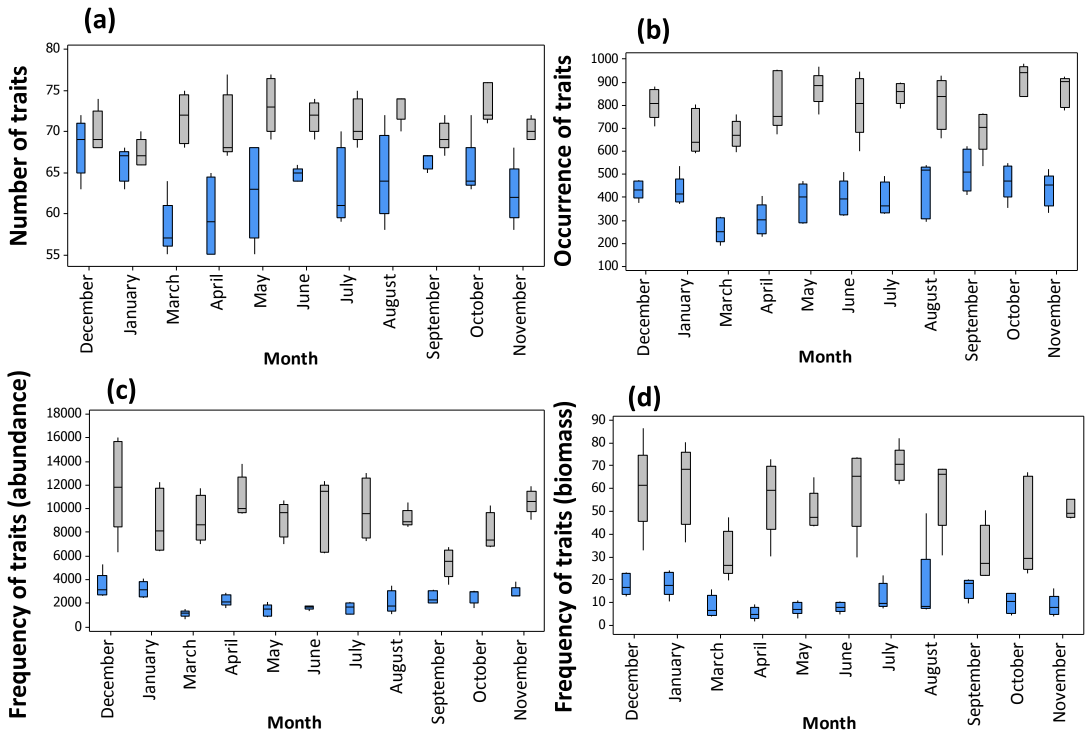


Figure 4.4 Functional diversity in each month at two sites in Galway Bay (Leverets: blue; Margaretta: grey); n=55 in all cases (a) number of trait modalities occurring (Kruskal-Wallis, Margaretta: H=20.170, $p<0.05$; Leverets: T=20.153, $p<0.05$), (b) number of trait modalities expressed by species (i.e. species richness*trait modalities) (Kruskal-Wallis, Margaretta: H=25.678, $p<0.01$; Leverets: T=25.127, $p<0.01$), (c) number of trait modalities expressed by individuals (i.e. abundance of species*number of trait modalities) (Kruskal-Wallis, Margaretta: H=19.371, $p<0.05$; Leverets: T=35.176, $p<0.001$), (d) number of trait modalities expressed by individual biomass (i.e. biomass of species*number of trait modalities) (Kruskal-Wallis, Margaretta: H=25.368, $p<0.01$; Leverets: T=27.491, $p<0.01$). For details of trait data see text.

The distribution of samples based on number of traits (Fig. 4.5) showed greater similarity between sites than similarity based on species abundance or biomass (Figs 4.7, 4.8) (Two way crossed ANOSIM; Site: R=0.617, $p<0.01$; Month R=0.302, $p<0.01$).

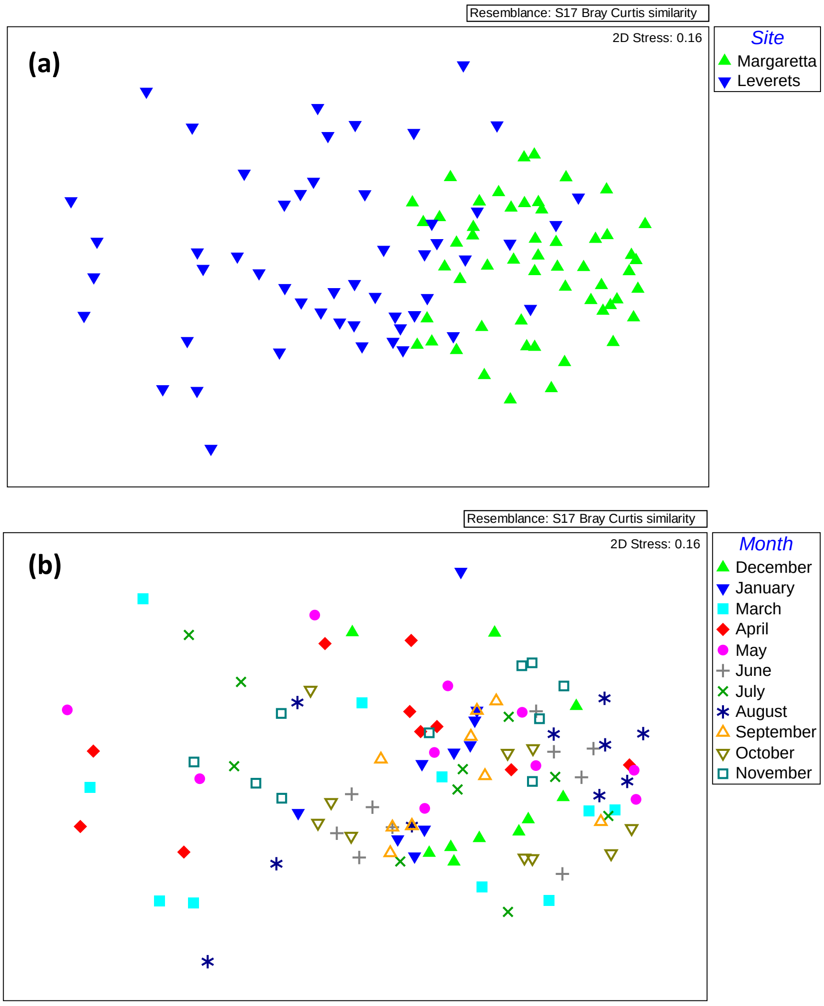


Figure 4.5 MDS plot of samples based on number of trait modalities for each (a) site and (b) month

The distribution of samples based on traits as a function of species richness, abundance and biomass were all similar with the least differences between sites detected by biomass data (Figs 4.6, 4.7, 4.8). They showed distinct functional assemblages at each site. Some differences between months were also apparent but differences between months were lower than differences between sites. March, April and May at Leverets were less similar to other months (Fig. 4.6); when abundance was considered, November was also dissimilar (Fig. 4.7). At Margaretta, September, January and March were less similar to other months (Figs 4.6, 4.7, 4.8).

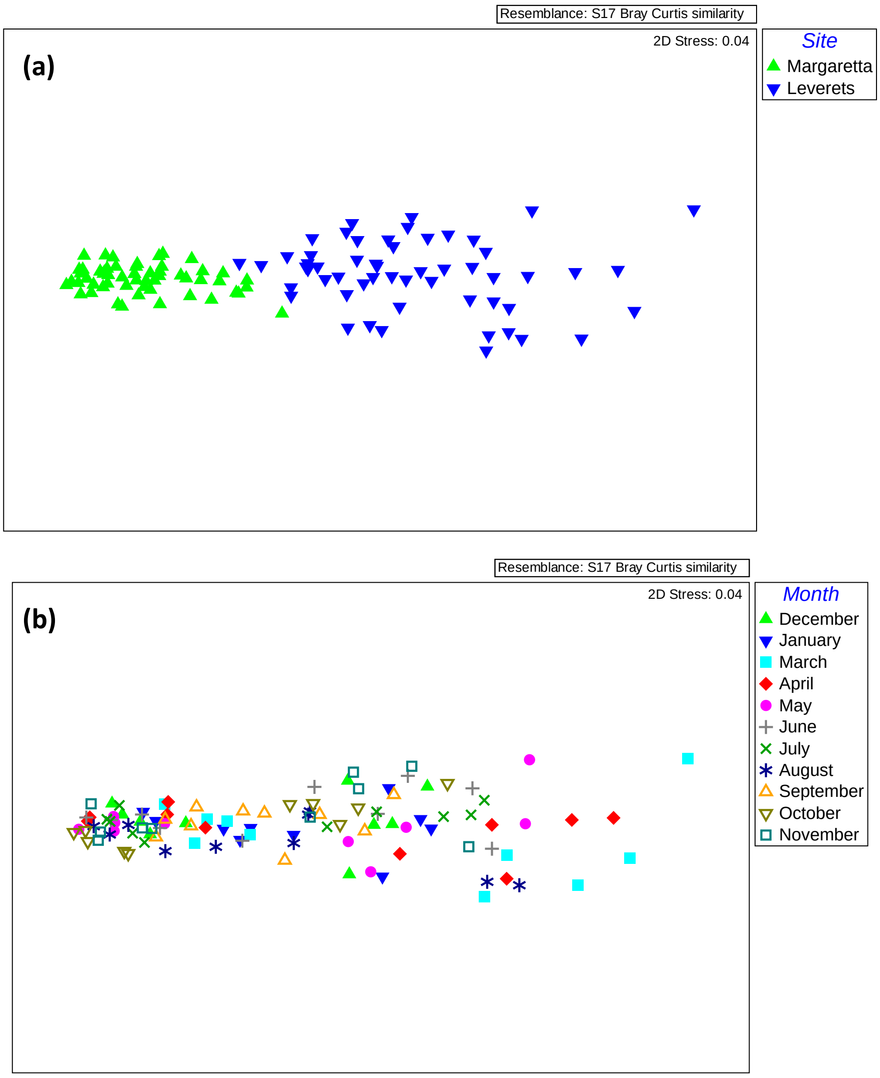


Figure 4.6 MDS plot of samples based expression of trait modalities by species for each (a) site and (b) month. Two way crossed ANOSIM; Site: R=0.915, p<0.01; Month R=0.276, p<0.01.

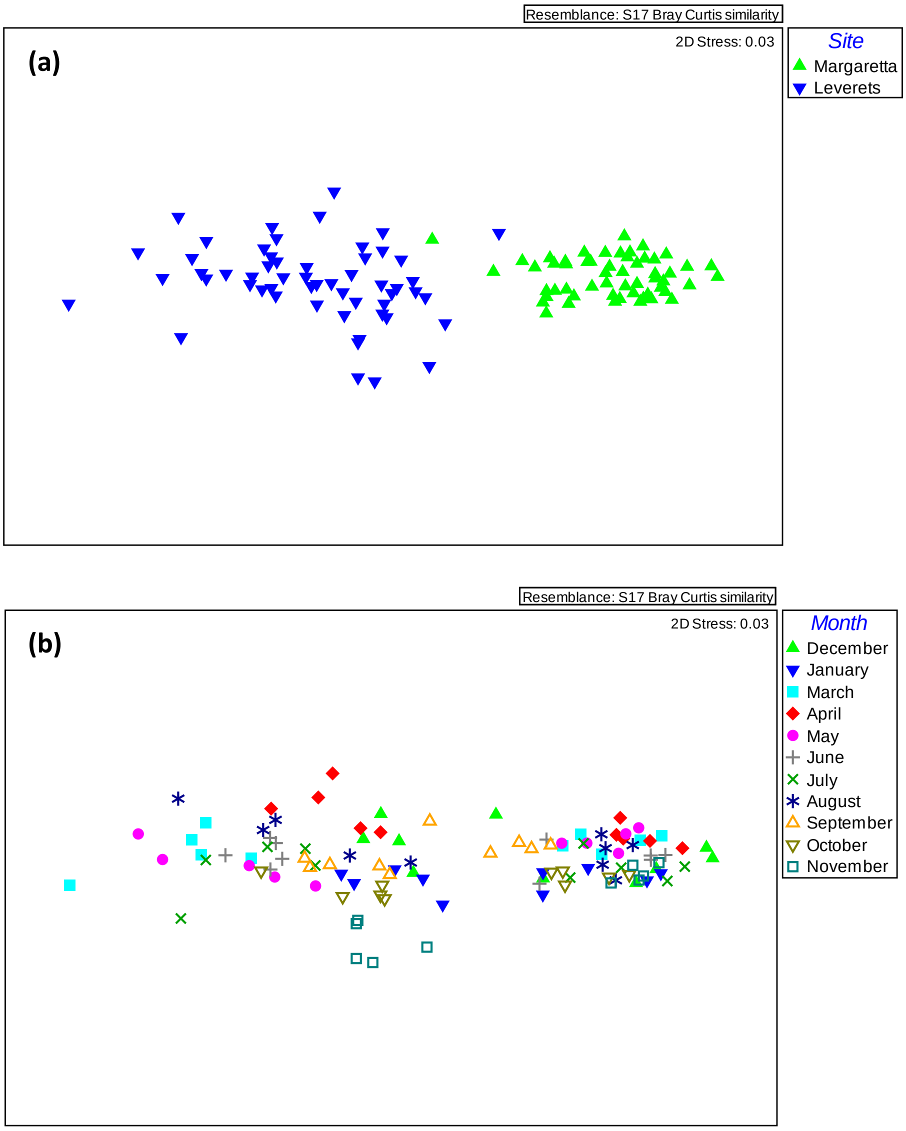


Figure 4.7 MDS plot of samples based expression of trait modalities by species abundance for each (a) site and (b) month. Two way crossed ANOSIM; Site: R=0.984, p<0.01; Month: R=0.445, p<0.01.

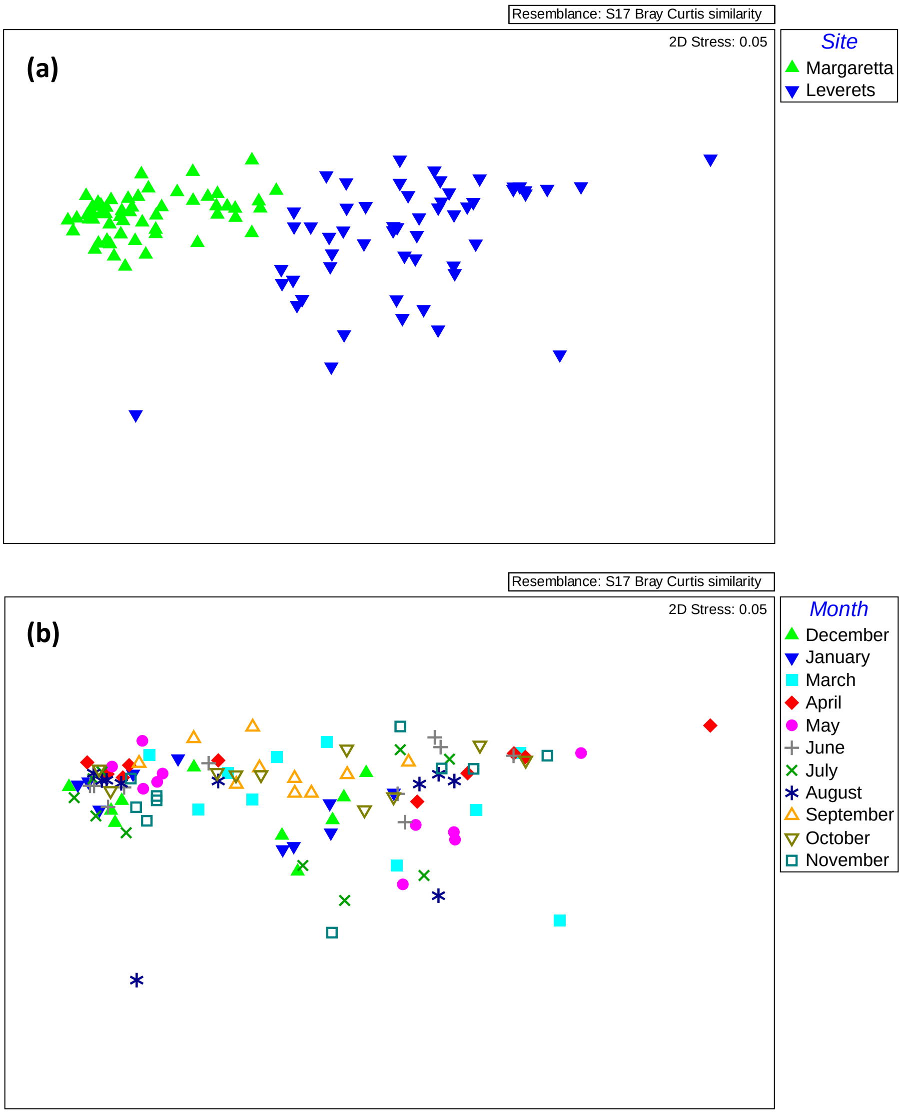


Figure 4.8 MDS plot of samples based expression of trait modalities by species biomass for each (a) site and (b) month. Two way crossed ANOSIM; Site: R=0.885, p<0.01; Month: R=0.207, p<0.01.

4.3.3 Rao's Entropy

No significant difference was found in overall average Rao's Entropy when abundance data were used but when biomass data were used Margaretta was found to have significantly greater functional diversity (Figs 4.9, 4.10) (Mann-Whitney U). Using abundance data, many individual traits were found to be significantly more diverse at Leverets while the opposite trend was found using biomass data.

Average Rao's Entropy calculated with abundance differed significantly depending on the month at Leverets (Kruskal Wallis; $H=37.627$, $n=55$, $p<0.001$) and at Margaretta (Kruskal Wallis; $H=31.124$, $n=55$, $p<0.01$) (Fig. 4.11). With biomass data, Average Rao's Entropy depended on the month at Leverets (Kruskal Wallis; $H=26.691$, $n=55$, $p<0.01$) but did not differ over months at Margaretta (Kruskal Wallis; $H=13.923$, $n=55$, $p>0.05$) (Fig. 4.12). Significant differences were found between months at both sites for all individual traits using abundance data (Kruskal-Wallis; $p<0.05$); except for movement type at Margaretta and exposure potential at Leverets which did not significantly vary over months. With biomass data only burrow depth differed significantly over different months at Margaretta (Kruskal-Wallis; $p<0.05$), while at Leverets, most traits differed depending on the month (Kruskal-Wallis; $p<0.05$) except lifespan, maturity, reproductive type, exposure potential and tolerance.

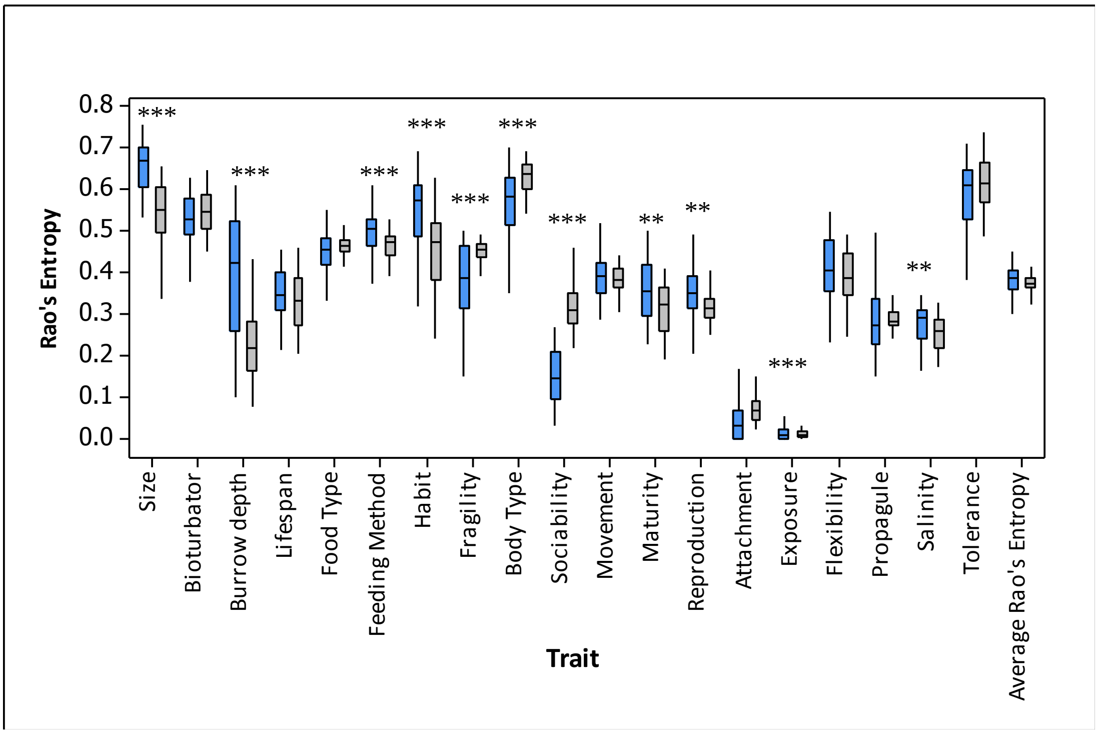


Figure 4.9 Rao's Entropy calculated using abundance data of different biological traits for two sites in Galway Bay; Leverets: blue, Margaretta: grey (Mann-Whitney U ***p<0.001; **p<0.01). For trait details see Table 4.4.

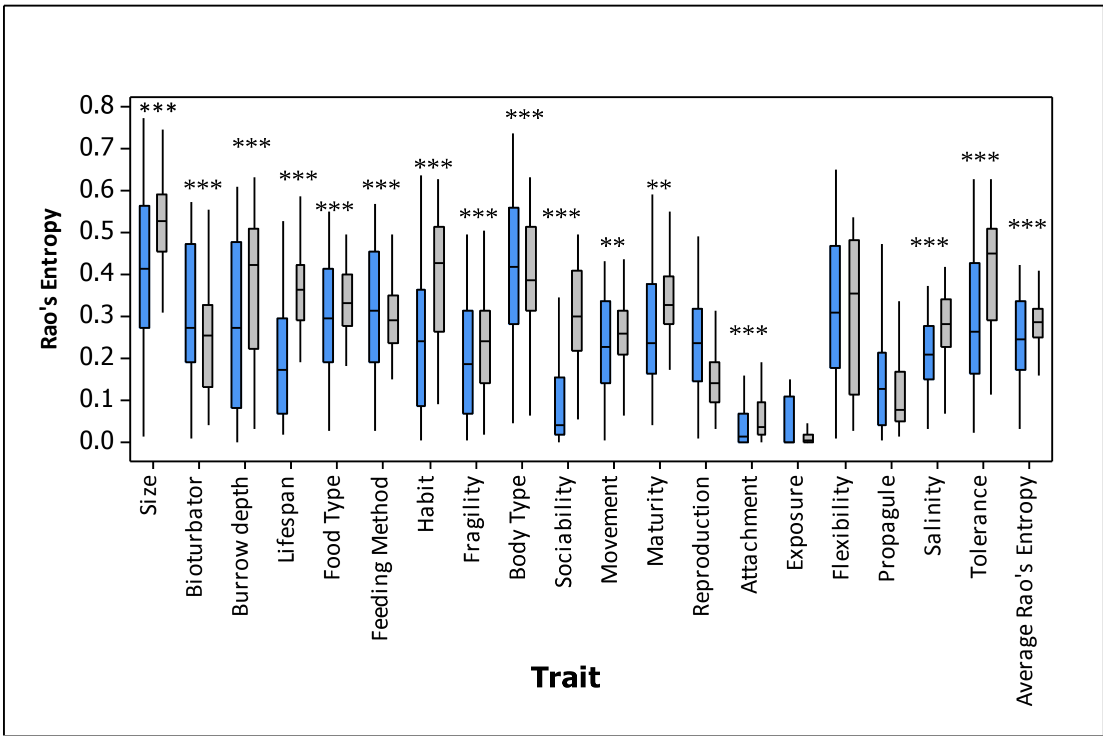


Figure 4.10 Average Rao's Entropy calculated using biomass data (transformed $\log_{10} x+1$) of different biological traits for two sites in Galway Bay (Mann-Whitney U ***p<0.001; **p<0.01). For trait details see Table 4.4. Leverets: blue, Margaretta: grey.

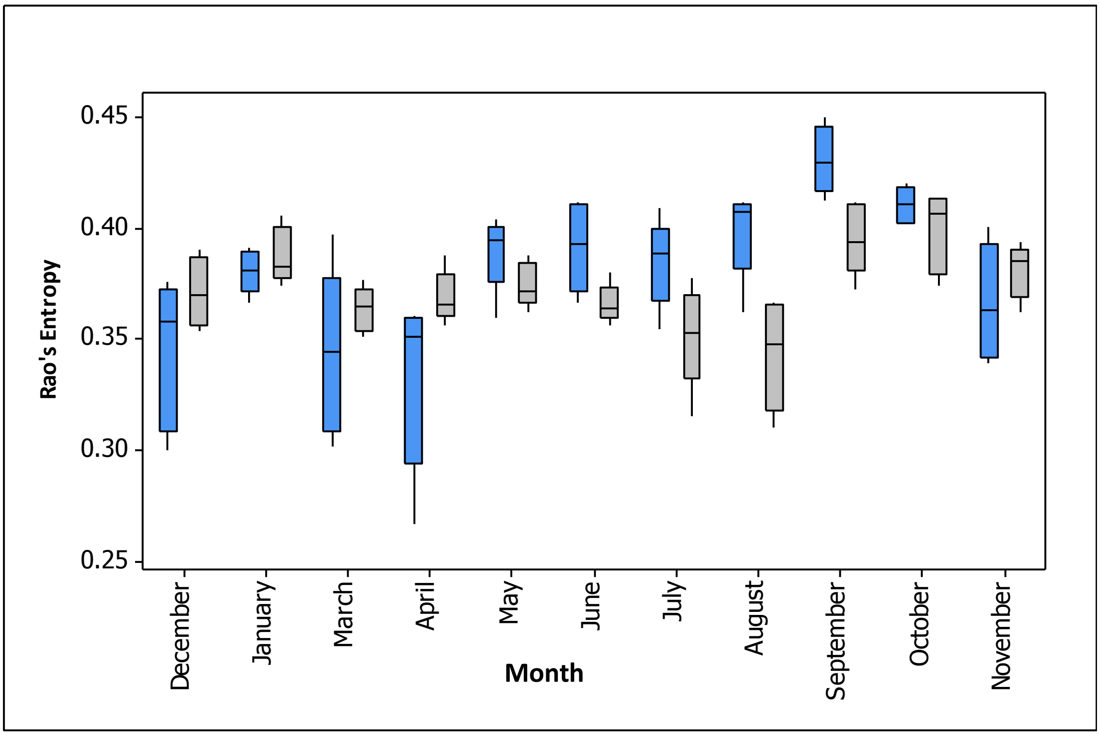


Figure 4.11 Average Rao's Entropy calculated using abundance data for two sites in Galway Bay; Leverets: blue, Margaretta: grey.

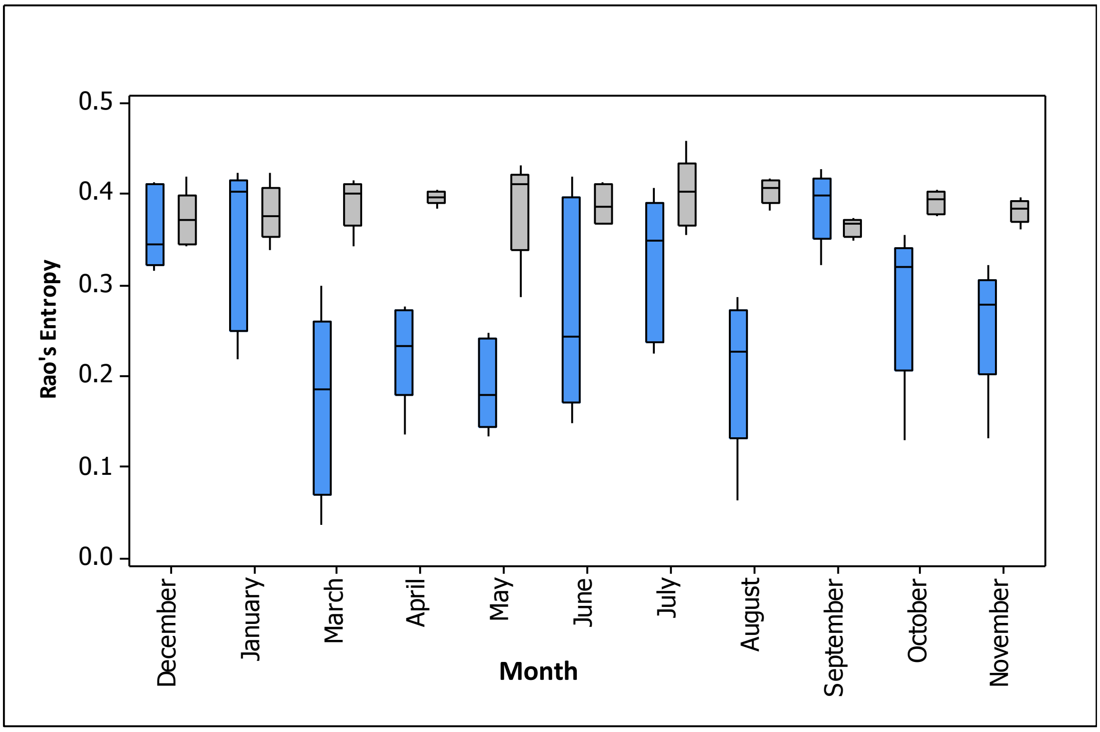


Figure 4.12 Average Rao's Entropy calculated using biomass data (transformed $\log_{10}$ x+1) for two sites in Galway Bay; Leverets: blue, Margaretta: grey.

A decrease in overall functional diversity was found at Leverets using biomass data from January to March (Fig. 4.12) and the percentage difference between these months for each individual trait was then plotted (Fig. 4.13). Degree of attachment and propagule dispersal showed the greatest percentage decrease while burrow depth, fragility and sociability also decreased.

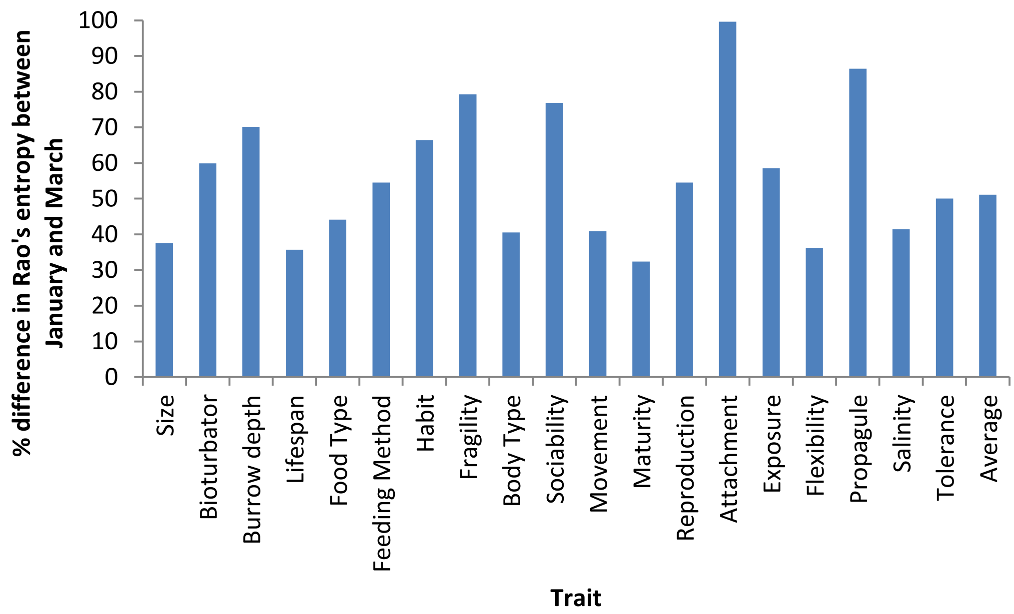


Figure 4.13 Percentage difference in Rao's Entropy between the month January and March for individual traits calculated using biomass data (transformed $\log_{10} x+1$) for Leverets. For trait details see Table 4.4.

Correlations between traits were generally low when abundance data were used (Table 4.12). The average Rao's Entropy was most strongly correlated to maximum size, food type, feeding method, living habit and tolerance. The average Rao's Entropy was also correlated to Shannon and Hill's Indices calculated using the frequency of traits (abundance) data. Stronger correlations were found overall when biomass data were used (Table 4.13) and the average Rao's Entropy was highly correlated to all traits apart from reproductive method, degree of attachment, exposure potential and flexibility. Average Rao's Entropy was also strongly correlated to the frequency of traits (biomass), in particular when this data was used with Shannon Index and Hill's Index.

Table 4.12 Pearson product moment correlations between Rao's Entropy traits and functional indices calculated for two sites in Galway Bay based on raw species abundance data with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key). All index values were scaled to a value between 0 and 1. For trait abbreviations (Rao's entropy) see Table 4.4. For details of Hln (Shannon Index) and N1 (Hill's Index) indices see section 4.2.2.3.1.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

		Si	B	Bd	L	Ft	Fm	H	F	Bo	So	Mv	Ma	R	A	E	Fl	P	Sl	T	Average	Number traits	Traits* Abundance	Traits* species richness	Hln (sum)	N1 (sum)	Hln (count)	N1 (count)	Hln (traits)
Rao's Entropy	B	11.6																											
	Bd	41.4	31.1																										
	L	16.5	-35.1	-8.5																									
	Ft	36.4	18.4	-1.5	34.1																								
	Fm	58.9	10.9	6.3	31.6	76.8																							
	H	56.8	4.5	44	25	16.3	18.7																						
	F	-0.7	39.1	-0.1	-7.9	43.6	27.6	-4																					
	Bo	3.3	1.6	-29.1	1.2	31.5	16.7	-3.6	41.2																				
	So	-47.2	5.2	-63.4	-6	3.7	-17.2	-61.3	37	55.4																			
	Mv	40.6	47	42.7	-12.8	21.4	31.8	21.7	41.3	2.1	-15.7																		
	Ma	21.4	-46.3	-5.5	90.3	29	28.4	31.5	-28	-12.1	-25.7	-29.1																	
	R	29.7	-12.8	55.1	12.7	1.6	-4.5	32.4	-19.5	6.7	-28.1	26	11.5																
	A	21.1	29.8	12.4	6.1	33	27.3	14.2	50.6	32.1	13.5	42.7	-14.5	-2.4															
	E	0.3	14.7	-1.1	-19.5	-7.4	-10	6	-1.9	4.9	5.7	9.6	-15.1	-5.9	10.5														
	Fl	23.1	-39.3	5.6	42.4	22.7	13.1	26.6	-9.3	54.2	-3.1	-26	44.2	41.1	-11.7	-3.9													
	P	1.3	11.1	53.5	-14.2	-10.5	-22.9	6.6	-2.2	9.8	0.2	22.5	-23.2	68.9	17.1	-14.9	15.8												
	Sl	35.5	-11.9	9.1	71.9	41.6	43.7	12.2	-16.2	-23.3	-17.5	6.3	74	22.8	0.4	-22.3	11.2	-5.8											
	T	6.4	14.5	31.8	35.6	64	35.5	19.1	52.5	27.9	-2.7	26.6	21.7	23.7	38.4	-17.3	35.7	29.7	21.2										
	Average	58.5	24.4	48.8	42.7	64.2	53.9	51.1	42.6	40.5	-12.7	47.7	29.5	49.2	49.8	-3.1	47.4	37	39.2	72.4									
	Number traits	-26.9	20.5	-33.5	-19	25.3	-5.1	-25	43.7	42.5	53.9	-1.2	-26.3	-16.8	24.5	10.8	-6.3	6.5	-22.1	19.7	5.1								
	Traits* Abundance	-58.8	9.4	-55.9	-21.2	11.2	-30.5	-43.2	37	42.7	71.5	-19.9	-32	-25.5	13.8	12.6	-7.6	-0.4	-34.8	14.2	-18.6	67.4							
	Traits* species richness	-44.9	18.3	-44	-14.6	26.5	-14.4	-40.3	47.4	51.6	70	-6.6	-28.9	-19.4	29	9.4	0.6	12.5	-24.2	31.6	3.6	82.6	87.8						
	Hln.(sum)	59.3	22.3	46.8	44.9	69.4	57.2	48.1	40.6	33.6	-16.2	44.2	33.6	43.7	47.3	-1.3	44.5	31.6	44.3	73.8	97	11.6	-18	6.9					
	N1.(sum)	59.7	22.2	47.6	44.8	68.7	57.2	48.2	40.2	33.9	-16.7	43.9	33.2	43.7	48.1	-2.3	44.6	31.9	44.2	73	97	10.5	-19.4	5.8	99.8				
	Hln. (count)	4.5	22.6	-4.5	-14.7	23	10.9	0.2	29	25.2	18.9	12.6	-15	0	15.9	18.7	-3.8	0.2	-7.2	7.1	15.9	77.8	26.7	41.3	22.9	22.2			
	N1(count)	4.7	22.4	-4.7	-14.9	22.6	10.9	0	28.2	24.5	18.5	12.4	-15	-0.5	15.4	18.8	-4.2	-0.4	-7.4	6.2	15.1	77.5	26.2	40.7	22.2	21.5	100		
	Hln(traits)	-2.6	6.9	-2.8	-22.7	-18.9	-14	-7.5	-3.2	19.2	11.3	-3.6	-17.3	8.8	4.2	42.3	8.4	4.7	-19.9	-20.7	-5.2	39.9	12.8	20.3	-3.9	-4.3	49.5	49.2	
	N1(traits)	-2.6	6.9	-2.9	-22.6	-18.9	-14	-7.4	-3.3	19.1	11.2	-3.7	-17.2	8.6	4.2	42.4	8.4	4.5	-19.9	-20.9	-5.3	39.7	12.6	20.1	-4	-4.4	49.3	49.1	100

Table 4.13 Pearson product moment correlations between Rao's Entropy traits and functional indices calculated for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key). All index values were scaled to a value between 0 and 1. For trait abbreviations (Rao's entropy) see Table 4.4. For details of Hln (Shannon Index) and N1 (Hill's Index) indices see section 4.2.2.3.1.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

		Si	B	Bd	L	Ft	Fm	H	F	Bo	So	Mv	Ma	R	A	E	Fl	P	Sl	T	average	Traits* biomass	Hln (sum)	N1 (sum)	Hln (traits)
Rao's Entropy	B	48.5																							
	Bd	57	52.1																						
	L	63.3	23.3	39.3																					
	Ft	75.6	46.6	50.8	69.3																				
	Fm	71.6	50.6	40.2	59.9	92.3																			
	H	64.5	46.3	55.9	61.3	64.1	63.7																		
	F	62.8	49.5	39.1	68.1	66.3	70.9	57.5																	
	Bo	85.3	54.1	44.7	59.4	72.6	70.6	49.3	76.2																
	So	76.9	35.2	43.1	84.6	68.5	57.6	64.2	65.3	68.9															
	Mv	77.5	36.1	55.7	47.5	52.2	49.7	54.5	45.8	65.2	59.6														
	Ma	58.2	29.4	50.3	76	52.6	40.7	31.3	48.8	55.9	59	52.8													
	R	31.6	48.1	42.9	-13.4	21.2	23.5	1.4	10.2	34.1	-2.8	33	22.9												
	A	42.6	26.9	22.6	28.5	28.6	34.1	42.1	33.5	38.5	37.8	61.3	17.6	7.8											
	E	-18.2	24.1	19.1	-23.8	-22.5	-27.9	-43.5	-18.8	-5.7	-27.2	0.5	32	41.8	-9.2										
	Fl	22.5	-12.9	39.7	13.9	20.9	5.5	7.4	2.5	24.2	7.5	36.8	27.4	22.8	3.2	13.5									
	P	40.7	37.3	26.7	32.8	46.5	53.2	27.7	52.9	49.4	30	32.9	14.7	25.2	36.4	-14.6	-9.8								
	Sl	77.5	42.7	76.7	68.1	68.6	54.6	62.6	57.1	67.4	69	73.9	72.8	29.9	29.9	1	46.3	25.5							
	T	66.8	41.6	46.5	70.4	78.7	74.7	73.8	54.5	60.6	66.8	50.9	40.7	3.9	46.7	-32.5	9.2	56.1	53.8						
	average	88.5	61.4	72.7	76.4	84.6	78.6	72.9	75.9	85.2	79.2	77.4	70.5	35	48.4	-3.5	30.6	51.1	86.4	78.3					
	Traits* biomass	46.7	17.2	48	71.4	47.1	29.6	49.5	44.2	41.2	72	40.7	55	-18.3	13.9	-11	28.9	6.5	59.4	54.3	58.8				
	Hln(sum)	82.6	59.4	71.7	73.3	81.9	78.3	72.9	74.7	77.5	73.4	70.6	68.6	37.2	46.9	-0.9	24	53.5	80.9	75.3	96	51.8			
	N1(sum)	82.8	58.2	71.7	73.3	80.8	77.3	72.3	74.8	76.9	74.2	71.8	68.1	36.3	48.6	-1.1	23.9	53.6	81.1	74.6	95.8	52.1	99.6		
	Hln(traits)	21.3	31.6	31.5	-3.9	8.1	9.9	10	15	30	3.2	34.6	11.6	27.6	31.7	31.6	18.4	19.7	21.3	11	27.5	11.2	28.2	27.8	
	N1(traits)	21.1	31.5	31.3	-4	7.9	9.7	9.9	14.8	29.9	3	34.5	11.6	27.5	31.6	31.6	18.4	19.6	21.1	10.8	27.3	11.1	28	27.6	100

Correlations between functional indices and environmental variables showed low correlations with water properties (Table 4.14) and mixed results with sediment properties (Tables 4.15). The number of traits, traits*abundance, traits*species richness, traits*biomass, average Rao's entropy (biomass), Shannon and Hill's index calculated with biomass all showed similar correlations with the sediment properties median grain size, graphic mean, sorting, porosity, organic carbon, silt and clay. Other indices showed no strong relationships with sediment properties.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 4.14 Correlation between functional indices and water column properties at Galway Bay. Pearson product moment correlations with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key). For details of Hln (Shannon Index) and N1 (Hill's Index) indices see section 4.2.2.3.1.

Variable	Average Rao's Entropy (abundance)	Number traits	Traits* Abundance	Traits* Species richness	Average Rao's Entropy (biomass)	Traits* biomass	Hln (sum) (abundance)	N1 (sum) (abundance)	Hln (count)	N1 (count)	Hln(sum) (biomass)	N1(sum) (biomass)	Hln(traits)	N1(traits)
SPM (g/L) surface	22.2	10	1.8	13.9	8.9	5.2	25.8	25.7	1.6	1.3	4.2	2.6	-10.6	-10.7
POC (mgC/m3) surface	-44.8	-21.8	4	-9.6	-21.1	-4.2	-48	-48.3	-24.1	-23.6	-22	-21.5	-1.4	-1.3
O2 (mg/L) surface	-25.6	-19.6	-10.3	-25.4	-23.5	-19.6	-24.3	-25	-10.5	-9.7	-26.2	-25.1	-7.6	-7.4
salinity (ppth) surface	1.9	35	46.1	51.1	34.3	51	3.6	2.1	16.6	16	31	30.4	19.9	19.7
NH4 (µM) surface	-16.1	-24.1	-22.9	-28.8	-12.4	-24.7	-18.2	-15.8	-18.4	-17.9	-9.5	-8.7	-18.9	-18.8
NO3 (µM) surface	-12.9	-26.5	-11.4	-28.6	-15	-14.2	-12.2	-11.7	-23.6	-23.2	-16.9	-14.9	-30	-29.8
NO2 (µM) surface	2.8	-10.2	9.6	-5.2	5.4	0.5	4.4	4.9	-16.6	-16.8	4.6	3.6	-6.9	-6.9
PO4 (µM) surface	-19.6	-1.4	17.1	-2.7	8.7	13.1	-17	-17.4	-6.1	-6.2	4.7	3.7	6.3	6.4
SiO4 (µM) surface	29	11.2	-0.6	7.1	25.2	0.5	30.3	31.1	8.2	7.7	26.7	27.3	-7.2	-7.3
SPM (g/L) bottom	17.7	5.2	-2.9	6.4	10.6	0.6	20.2	20.9	-1.5	-1.7	7.7	6.9	-14.9	-14.9
POC (mgC/m3) bottom	-26.5	-40.6	-24.1	-35.5	-34.5	-23.1	-30	-28.9	-29.3	-28.6	-32.2	-32.4	-11.5	-11.4
O2 (mg/L) bottom	-18	-15.9	7.7	-9.8	-16.6	-3.6	-18	-19.1	-11.9	-11.4	-18.6	-18.5	0	0.1
salinity (ppth)bottom	26.9	52.5	39.2	49.9	48.6	40	24	23.4	40.2	39.3	50.3	48.5	28.1	27.8
NH4 (µM) bottom	32.9	-0.3	-12.7	2.8	-1.5	-21.2	30.9	32.3	-4.1	-4.2	2.4	2.4	-10	-10.1
NO3 (µM) bottom	-26	3.4	24.8	15.9	5.9	20.4	-26.1	-27	-10	-10	1.3	2.1	5.9	6
NO2 (µM) bottom	0.5	2.1	11.1	4.4	10	2.6	1.3	1.2	-4.5	-4.7	10.2	8.8	12.4	12.4
PO4 (µM) bottom	-16.4	2.5	15.8	-4.3	11.3	7	-15.3	-15.8	-1.1	-1.2	7.9	7.1	2.5	2.6
SiO4 (µM) bottom	21.5	-31.3	-24.2	-22.3	-16	-17.6	20.6	21	-26.1	-26.3	-13.6	-13	-13.4	-13.4

Table 4.15 Correlation between functional indices and sediment properties at Galway Bay. Pearson product moment correlations with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key). For details of H'ln (Shannon Index) and N1 (Hill's Index) indices see section 4.2.2.3.1.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Variable	Sample Depth	Average Rao's Entropy (abundance)	Number traits	Traits* Abundance	Traits* Species richness	Average Rao's Entropy (biomass)	Traits* biomass	Hln (sum) (abundance)	N1 (sum) (abundance)	Hln (count)	N1 (count)	Hln(sum) (biomass)	N1(sum) (biomass)	Hln(traits)	N1(traits)
Median	1cm	-8	-33.6	-17.4	-27.8	-28.2	-19	-7.1	-7.4	-21.1	-21.1	-22.3	-23	-36.5	-36
	2cm	0.1	-51.9	-49.1	-55.8	-53.5	-52.9	-1.1	-1.5	-25.1	-24.2	-52.5	-51.4	-18.9	-18.7
	3cm	-0.4	-58	-56	-59.5	-56	-50.8	0.3	0.1	-29.4	-28.5	-55.3	-53.9	-13.8	-13.6
	4cm	-1.3	-68.3	-63.8	-68.1	-63.4	-62.6	-3.1	-3	-38.7	-37.8	-61.4	-60.4	-24	-23.8
	5cm	-2.4	-59	-55.9	-60	-58.8	-55.6	-5.6	-5.6	-33.7	-32.8	-56	-54	-21	-20.8
	6cm	-3.7	-55.6	-62.5	-70.2	-62.7	-67.1	-9.3	-9.2	-17.5	-16.6	-57	-55.3	-12.5	-12.4
	7cm	-5.9	-54.3	-57.5	-65	-63.4	-61.5	-7.8	-7.7	-19.9	-18.8	-58.9	-57.1	-12.4	-12.2
	8cm	21.5	-54.5	-57.8	-64.5	-56.4	-65.8	16.3	16.3	-15.3	-14.9	-51.8	-50.7	-9	-9.1
	9cm	19.8	-52.8	-66.6	-66.3	-48.5	-64.7	16.1	16.2	-9.5	-9.1	-44.2	-42.7	-2.2	-2.2
	10cm	4.4	-31.2	-44.6	-49.3	-47.2	-44.4	5.1	4.4	15.4	15.7	-46.7	-45.9	5.7	5.7
SMD	1cm	-3.2	-68.1	-46.1	-58.3	-55.8	-50.8	-10.6	-10.3	-46.8	-46.2	-50.5	-50.2	-39.8	-39.5
	2cm	0.3	-57.1	-64.9	-66.9	-56	-61.9	1	0.9	-22.2	-21.3	-53.3	-52.5	-21.3	-21.1
	3cm	2.3	-60.2	-72.1	-72.4	-62.1	-65.6	3.9	4	-23.2	-22.4	-61.5	-60.4	-13.1	-12.9
	4cm	-2.3	-72.5	-72.8	-77.3	-64.5	-72.1	-3.2	-2.8	-40.3	-39.3	-63.5	-62.8	-24.8	-24.6
	5cm	1.9	-65	-69.3	-71.6	-63.5	-66.5	-0.4	-0.1	-35.2	-34.4	-60.2	-58.1	-25.3	-25.2
	6cm	6.4	-58.3	-73.6	-75.4	-63.1	-73.4	2.4	2.7	-17.9	-17.1	-57.8	-56.2	-6.9	-6.9
	7cm	4.4	-60.4	-73.4	-76.3	-68.9	-71.5	3.7	4.2	-20.3	-19.4	-63.2	-61.8	-13.6	-13.5
	8cm	20.7	-58.6	-76.4	-77.4	-61.7	-75	17.4	17.9	-16.7	-16.2	-55.6	-54	-9.8	-9.9
	9cm	21.7	-59.6	-77.5	-75.5	-51.9	-72.7	19.8	20.2	-11.4	-11	-46	-44	-3.3	-3.3
	10cm	17.8	-48	-66.9	-68.6	-59.8	-63.5	17.2	17	6.1	6.6	-56.8	-55.3	4.5	4.5
DBMD	1cm	-6.9	-21.8	4.8	-10.4	-7.8	-6.7	-9.1	-9.4	-21.1	-21.3	-5.4	-6.2	-35.5	-35.2
	2cm	4.1	-10.7	3.6	-3.9	-9.6	-9	-1	-1.8	-12	-11.9	-9.6	-8.7	-10	-10
	3cm	0.2	-10.5	1.2	-2.6	-2.1	-1.1	1.3	0.8	-12.3	-12.3	-3.7	-3.4	3.8	3.8
	4cm	4	-16.7	-8.8	-11.4	-14.7	-19.7	2.5	1.7	-14.1	-13.6	-12.1	-11	-11.4	-11.4
	5cm	0.7	-12.8	7.9	2.1	-9.5	3.3	-3.2	-4.1	-22	-21.8	-11.6	-10.8	-11.7	-11.8
	6cm	-9	3.5	0.3	-10.5	-6.4	-12.5	-14.2	-14.7	11.2	11.2	-2.3	-0.8	-3.6	-3.7
	7cm	3.7	-3.8	-15.2	-16.7	-28.6	-16.8	6.2	5.8	15	15.5	-30.7	-29.5	4.7	4.6
	8cm	2.2	-16.8	-14	-19.7	-28.1	-26.2	-0.4	-1.3	-4.7	-5	-24.1	-23.9	-6.5	-6.6
	9cm	3.2	-23.3	-35.2	-36.9	-20.5	-33	0.1	-0.5	-1.9	-1.5	-21.1	-20.2	-8.1	-8
	10cm	-16.3	-5.1	-5.2	-12.5	-23.1	-8	-13.6	-14.4	15	15	-21.5	-21.6	3.6	3.5
Graphic Mean	1cm	4	47.1	17.9	35	36.9	28.7	9.5	9.5	38	37.9	34.7	34.8	42.4	42
	2cm	-4.9	45.2	43.6	48	44.1	48.8	-2.4	-2.1	22.5	21.9	43.1	42.5	16	15.9
	3cm	-2.3	47.8	50.2	52.2	42	44.9	-3.2	-3.1	22.6	22	40.7	39.6	4.1	4
	4cm	-2.9	58.5	61.2	63.8	57.6	64.7	-1.1	-1.3	29	28.2	53.5	52.8	20.1	20
	5cm	-0.6	56.5	53.4	56.9	53.6	54	2.6	2.4	34.3	33.6	50.3	48.4	23.1	23.1
	6cm	1.9	45.2	57.8	64.7	51.4	62.9	7.7	7.4	11.5	10.8	44.4	42.6	10.8	10.8
	7cm	0.9	51.3	60.6	66.6	61.4	64.6	2.9	2.5	16	15	54.4	52.6	14.3	14.2
	8cm	-18.9	56.8	70	73.1	59.5	73.9	-14	-14.5	17.3	16.9	52.7	51.4	10.9	11
	9cm	-17.8	49.3	69.4	66.9	41	64.1	-14.6	-14.9	7.1	6.8	35.2	33.7	4.2	4.2
	10cm	-8.9	37.3	52	55.1	53.4	51.5	-8.1	-7.6	-10.4	-10.7	48.5	47.6	-4.7	-4.6
Sorting	1cm	-4.6	45.7	63.4	55.7	47.9	50.8	-1.9	-2.8	15.8	14.9	42.4	41.4	-1.6	-1.6
	2cm	5.5	39.4	62.2	57.9	39.9	47.9	1.2	0.2	6.6	6	36.4	36.1	8.6	8.4
	3cm	-5.9	37.7	59.7	52.8	42.6	46.9	-6.1	-6.8	5.3	4.9	39.8	39.4	9.4	9.3
	4cm	0.9	53.1	65.8	67.2	48.4	55	0.7	-0.5	20.5	20	48.4	48.9	9.9	9.8
	5cm	-3.5	53.6	77.3	73.1	52.6	67.3	-4.6	-5.8	15	14.5	46.6	45.9	15	14.9
	6cm	-16.6	57.1	72.5	64.8	51.3	61.3	-16.7	-17.7	23.7	22.9	47.5	47.3	4.1	4
	7cm	-16.9	48.3	68.2	60.7	41.7	58.8	-16	-17.6	19.1	18.8	40.4	39.9	9.2	9.1
	8cm	-19.4	48.7	69.4	64.7	41	55.6	-18.5	-19.8	15.6	14.9	36.4	35.2	6.4	6.3
	9cm	-23.5	34.4	44	37.6	23.8	39.2	-25	-26.2	6.8	6.9	16.4	15.2	-5.1	-4.9
	10cm	-38.4	45.1	64	54.7	33.9	54.2	-34.8	-35.6	15.3	14.9	30.2	28.7	2.4	2.4
Skewness	1cm	0.4	-16.6	-37.5	-30.3	-32.7	-23.4	8.5	8.5	7	7.5	-30	-29.8	0.7	0.9
	2cm	-8	4.6	-5.9	-3.7	1.5	0.8	-3.8	-2.6	8.1	8.2	1.4	0.7	8.5	8.6
	3cm	3.3	-1.5	-4.8	3.2	0.6	10.5	-0.6	-0.5	3.3	3.2	3.4	3.6	3.7	3.7
	4cm	2.2	-3.6	-12.1	-10.9	-5.7	1.8	0.9	1.7	9.2	8.9	-5	-5.7	9.7	9.7
	5cm	2.9	-18	-39.2	-32.9	-21.7	-31.2	7.1	8	10.7	10.9	-15.1	-16.2	4.5	4.6
	6cm	7.5	-25.6	-17.1	-13.3	-13.9	-7.7	8.7	9.3	-19.3	-19	-14.1	-15.3	-7.6	-7.4
	7cm	3.6	-7.1	-7.4	-1.4	10.3	-4.6	0.5	1.8	-10.6	-10.8	3.7	3.5	4.6	4.6
	8cm	8.4	-16.4	-9	-10	-2.2	-3	6.7	7.6	-10.5	-9.9	-4.1	-4.3	-2.6	-2.5
	9cm	11.6	12.5	18.9	23.8	17.2	16.5	12.5	13	4.1	3.7	19.7	19.3	10.5	10.3
	10cm	30.2	-11.8	-20.2	-9.6	5.7	-15.1	25.6	25.8	-12.6	-12.3	3.4	4.1	-4.2	-4.1
Porosity	1cm	-9.6	35.1	17	21.6	30.6	24.1	-10	-10.3	29.9	29.6	35.4	37.2	26.8	26.7
	2cm	-7.2	58.1	62.7	65.3	62.1	62.7	-8.7	-8.6	24.2	23.3	61	60.9	22.5	22.3
	3cm	-2.5	56.7	62.1	64	63.7	56.2	-3.5	-3	25	24.3	63	63.3	19.2	19
	4cm	-2.9	66.4	69.8	72.3	70.1	63.4	-3.6	-3.4	33.4	32.6	69.5	69.7	20	19.8
	5cm	-13.5	71.7	74.5	76.8	73.1	71.1	-10.5	-10.9	33.9	33.2	71.1	71	18.4	18.2
	6cm	-18.1	64.7	72.4	73.2	64.1	73.5	-13.8	-14.4	26	25.2	60.8	60.1	13.5	13.3
	7cm	-14.1	66.6	72.3	72.4	65	68	-9.7	-10.4	30.7	29.9	62.5	61.9	14.7	14.5
	8cm	-24.6	60.8	56.2	57.4	54	56.3	-19.7	-20.1	32.3	31.6	54.1	52.8	18	17.9
	9cm	-38.8	53.1	45.3	47.1	38.8	50.9	-37.6	-37	30.1	29.9	39.7	38.1	15.4	15.3
	10cm	-11.4	41.2	20.8	29	26.2	22.2	-9.9	-8.9	35.8	35.7	33.1	32.1	5.7	5.7

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 4.15 continued

Variable	Sample Depth	Average Rao's Entropy (abundance)	Number traits	Traits* Abundance	Traits* Species richness	Average Rao's Entropy (biomass)	Traits* biomass	Hln (sum) (abundance)	N1 (sum) (abundance)	Hln (count)	N1 (count)	Hln(sum) (biomass)	N1(sum) (biomass)	Hln(traits)	N1(traits)
Organic Carbon	1cm	-12.3	19.4	22.7	7.9	18.5	11.7	-12.2	-12.8	9.8	9.6	18.7	18.5	15	15.1
	2cm	-16.1	29	37.9	26.3	27.8	26.3	-18.6	-18.9	7	6.6	26	26.3	6.2	6.3
	3cm	-23.1	31.1	43.7	32.5	35.4	32.6	-25.1	-25.5	6.4	5.9	33.2	33.4	9.7	9.8
	4cm	-25.3	39.1	49.1	38.4	42.3	36.9	-26.5	-27	13.5	12.9	40.6	40.7	8.6	8.6
	5cm	-26	45.2	57.8	46.8	44.9	43.7	-26.4	-27	11.6	10.9	40	39.9	6.3	6.3
	6cm	-28	37	52.2	41.3	37.3	47.4	-27	-27.7	7.6	7	32.6	31.4	6.8	6.8
	7cm	-28.4	38.9	48.2	37.8	35.9	41	-27.7	-28.4	13.3	12.8	32	31.3	10.3	10.3
	8cm	-34.2	32.6	35.7	26.3	29.9	30.5	-35.3	-35.3	6.4	6.1	26.9	25.9	13.5	13.7
	9cm	-37	31.5	37.2	30.4	33.7	38.7	-34	-34.1	10.1	10	31.8	29.8	23.2	23.3
	10cm	-15.9	42.6	43.1	45.3	33.7	42.6	-11.7	-11.8	11.5	11.2	35	33.5	2.6	2.7
Sand	1cm	-6.7	-29.4	-2.1	-17.7	-15	-13.2	-9.8	-10	-25.8	-26	-12	-12.7	-38	-37.7
	2cm	3.8	-21.2	-9.9	-17.1	-20	-20.7	-0.7	-1.5	-15.4	-15.1	-19.5	-18.6	-13.3	-13.3
	3cm	0.6	-20.8	-11.9	-15.6	-13.2	-12.9	1.9	1.4	-15.8	-15.6	-14.7	-14.2	1.2	1.2
	4cm	2.9	-32.6	-25.8	-29.2	-28.9	-35.2	1.4	0.8	-22.4	-21.7	-26.4	-25.2	-16.1	-16.1
	5cm	1.1	-29.8	-13	-18.6	-26.5	-16.1	-2.9	-3.6	-29.3	-28.8	-27.4	-26.1	-17.5	-17.5
	6cm	-6.4	-11.5	-18.1	-28.2	-21.6	-29.5	-12	-12.4	5.5	5.7	-16.6	-14.8	-4.9	-5
	7cm	3.9	-9	-21.1	-22.8	-33.6	-22.4	6.2	6	12.7	13.2	-35.2	-33.9	3.3	3.3
	8cm	7.9	-31.2	-33.9	-39.1	-41.9	-44	4.6	4	-8.8	-9	-36.8	-36.1	-8.4	-8.5
	9cm	6.7	-31.3	-45.2	-46.4	-27.6	-42.3	3.5	3.1	-3.7	-3.3	-27	-25.9	-7.8	-7.7
	10cm	-11.3	-13.7	-17.3	-24.2	-32	-19.2	-9	-9.7	14.7	14.7	-30	-29.8	4	4
Silt	1cm	-9.7	63.9	52.1	54.3	56	48.7	-3.4	-4.1	41	40.4	53	52.6	35.7	35.5
	2cm	-8	60.3	71.6	71.7	60	66.8	-7.8	-7.8	21.2	20.3	56.1	55.1	19	18.8
	3cm	-5.3	58.6	73.2	72.3	59.8	64.9	-6.1	-6.4	20.4	19.6	55.8	54.6	16.5	16.3
	4cm	-5.3	70.3	76.6	78.3	63.6	72.9	-3.2	-3.7	33.9	33.1	59.9	59.2	20.1	20
	5cm	-4.6	61.1	70.3	70.3	57.8	67.1	-1.6	-2.1	29.2	28.5	51.9	50	22.5	22.5
	6cm	-9.6	61.8	78.2	79.4	63.8	75.7	-5.7	-6.4	17.3	16.5	56	54.9	9.1	9.1
	7cm	-7.7	60.5	75.8	76.9	63.7	73.4	-6.3	-7	18.3	17.4	56.7	55.4	10	9.9
	8cm	-20.3	61.4	74.5	77.3	59	74.1	-15.9	-16.7	18.4	17.9	51.4	50.1	12.6	12.7
	9cm	-23.4	60.5	78.4	76.2	50.9	72.3	-20.6	-21.1	11.2	10.8	43	41.2	1.9	2
	10cm	-30.1	53.5	73.9	73.8	59.6	71.4	-27.4	-27.3	-2.6	-3.1	52.5	50.7	-5.2	-5.2
Clay	1cm	1.6	45.9	36.4	45.2	38.6	39.8	5.9	5.6	24.3	23.6	38.3	37.8	16.6	16.4
	2cm	2.8	33.4	36.7	44.3	32.3	38.6	5.5	5.7	10.5	9.8	29.1	29	5.3	5.2
	3cm	-21.9	48.8	58.9	57.2	40.5	59.2	-19.8	-19.6	21.7	21.4	33.2	32.1	15.8	15.7
	4cm	3.7	51.5	52.9	57.1	43.1	55	6.8	6.6	27.8	27.4	35.3	34.1	18.3	18.3
	5cm	-12.2	24.8	24.1	22.6	20.9	38.7	-7.4	-7.4	19.7	19.6	13.4	11.5	12.7	12.8
	6cm	0.4	51.5	49.8	57.9	38.4	59.1	4.7	4.4	18.6	18.2	32.7	31.6	10.1	10.1
	7cm	5.5	38.1	50.7	54.4	38.9	49.7	7.1	7	9.4	8.6	34.1	33	-4.4	-4.5
	8cm	-13.6	35.9	56	56.4	38.8	62	-12.8	-13.2	5.4	5	34	33	6.9	6.9
	9cm	-12.1	34.5	49.3	44.9	39	53.6	-10.7	-11.3	1.8	1.2	31.7	29.8	-0.4	-0.3
	10cm	-5.1	31.2	34.6	47.4	45.1	43.4	-0.3	0.2	-1.6	-2.3	40.9	39.4	2.2	2.2

4.3.4 Relationship between structural and functional indices

The correlations between functional indices and structural indices were mostly low with both abundance and biomass data (Table 4.16, 4.17) although there were some differences in the relationships between indices using different data types. Average Rao's Entropy showed no correlation to species richness and a strong correlation to measures of evenness when abundance data were used but showed an opposite trend when biomass data were used; a similar pattern was repeated for many individual traits. The number of traits and the frequency of traits (abundance and biomass) were strongly correlated to species richness.

Table 4.16 Pearson product moment correlations between functional and structural indices calculated for two sites in Galway Bay based on raw species abundance data with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key). All index values were scaled to a value between 0 and 1. For trait abbreviations (Rao's entropy) see Table 4.4. For details of Hln (Shannon Index) and N1 (Hill's Index) indices see section 4.2.2.3.1.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

		S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	1-Lambda	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta*	Delta+	sDelta+	Lambda+	AMBI	BQI	MAMBI	Biomass
Rao's Entropy	Si	-46.4	-59.6	-36.6	71	-6.8	-23.6	11.5	5.1	24	5.4	-25.9	-5.6	-51	-5.4	-60.3	-10.2	-38.4	-44.9	-47.5	43.3	10.4	-10.9	-23.7	-61.6
	B	18.5	12.1	21.5	31.4	33.3	24.5	29.3	36.1	43.8	35.3	-4.7	-8	6.4	59.2	2.7	22.1	-11.6	-1.1	17.6	-4.4	33	2.6	9.5	3.1
	Bd	-45.6	-55.6	-36.4	62.6	-12.3	-24.1	3.6	-0.8	18.6	-2.6	-55.1	-46.3	-59	-13.1	-57	-34.8	-68.8	-60	-47.2	60.5	60.2	10.4	-45.3	-48.5
	L	-16.1	-27.4	-9.5	30.6	-4	-1.2	7.3	2.7	14	2.6	4.7	17.9	1.8	-37.4	-35.1	1.4	-14.1	-16.2	-16.2	22.5	-20.3	20.6	-4.6	-12.6
	Ft	25.6	8	33.2	48.7	49.2	39.3	54.2	55.4	62.1	52.6	37.2	46.8	22.2	31.2	-11.7	47.3	3.2	-2.1	24.7	-6.3	-27.4	43.7	37	8.4
	Fm	-15.3	-31.8	-6	55.4	10	5.3	25.6	19.9	32.4	20.1	-0.6	13.4	-25.5	2.3	-43.4	17.7	-7.4	-18.4	-15.7	9.8	-6.1	-1.3	-1.3	-18.7
	H	-41.7	-45.3	-35	51.2	-12.1	-26.3	-9.7	-3.5	24	-10.3	-31.5	-16.6	-40.4	2.9	-43.9	-20.4	-53.3	-52.5	-43.2	52	26	20.6	-33.6	-33.7
	F	47.5	35.5	50.8	21.8	58	51.6	50.5	59	59	57	22.4	14.6	26.1	53.4	19.9	61.9	29.3	27.5	47.1	-43.8	17	47.7	37.7	32.6
	Bo	52.2	42	52.8	4.4	57.9	49.9	43.7	55.1	49.2	52.7	50.2	47.1	44.8	8.7	33.3	83.3	71.3	55.5	52.6	-46.8	-27	14.3	55.3	35.3
	So	71.9	70.9	65.5	-39.9	53	54.2	33.6	42.6	21.7	44.5	63.5	50.3	76.7	26.5	65.6	70	78.5	74.5	73	-71.4	-41.2	9.7	67.3	55.8
	Mv	-6.9	-19.3	1.3	65.1	27.1	11.4	34.6	36.4	52.8	34.9	-19.5	-13	-31	21.9	-28.3	16	-30.2	-22.7	-8.1	8.7	39.8	3.5	-4.4	-12.5
	Ma	-30.6	-37.8	-25.3	22.3	-22.2	-18.1	-10	-16	-3.7	-17.2	-3.7	11.2	-5.2	-42.8	-40.7	-17.6	-23.7	-23.3	-30.6	33	-22.2	21.1	-17.3	-23.4
	R	-21.5	-27.7	-15.6	49.2	9.5	-9.7	15	16.5	32.2	10.6	-6	8.2	-20.6	-36	-28.2	0.6	-32.5	-27.8	-22.6	38.1	2.7	12.6	-6	-26.2
	A	28.3	13.5	33.6	35.9	52.2	37.2	52.3	55.1	52	55.6	22	22.4	9.3	17.9	4.2	32.9	-4.9	-2.4	27.1	-6.8	1.8	30.6	33.4	5
	E	10.7	16.3	9	-0.7	9	7.1	5.4	8.3	10.7	6.3	-1.2	-5.5	12.2	19.2	13	12.1	6	17.5	11.2	-17.3	13.2	-6.1	3.1	14.7
	Fl	-0.4	-10.9	4	21.4	10.7	7.8	12.6	14.1	20.6	12.5	13.3	21.4	5.3	-37.4	-17.1	31.8	24.1	12.5	0	5.3	-17.8	13.9	8.1	-5.1
	P	11.5	-1.7	15.6	30.3	34.5	17.3	33.1	36.7	37.3	35	11.8	15.2	-0.9	-16.5	-5.9	9.3	-25.2	-15.4	10.1	25.4	2.2	22.2	19.1	-8.5
	Sl	-26.5	-39.7	-18.7	46	-5.9	-8.8	12.4	2.9	13.8	4.4	2.4	20.7	-6.1	-26.8	-47	-14.2	-36.8	-26.5	-26.7	32.6	-26	12.4	-5.4	-32.8
	T	30.7	9.7	39.3	50.3	54.7	46.2	57.1	61.4	69.6	57.4	23.8	27.2	16.3	12.3	-12.4	44.6	-8.2	-6.8	29.3	-0.4	2.4	65.7	30	20.1
	Average	1.8	-22.6	14.2	83	46.6	26.6	54.6	58.3	77.6	54.3	7.5	22.1	-7.2	3	-39.2	41	-20.5	-20.6	0.3	21.9	8.4	42.9	15.1	-17.7
	Number traits	81.2	67.2	81.7	-23.3	71.9	76.4	58.1	65.9	47.7	61.9	71.6	59.2	66.4	44.8	49.6	63.2	41.6	53.8	81.1	-51.7	-37.5	43.5	77.1	51.3
	Traits* Abundance	88.5	99.6	78.1	-49.8	63.9	61.6	36.5	50.7	29.8	48.4	72.8	55.1	82.3	44	90.4	64.7	61.7	64.7	88.8	-65.9	-40.6	40.1	75.4	74.9
	Traits* species richness	99.7	86.9	97.9	-28.4	84.7	89.8	68.7	76.6	51.3	76	84.9	68.8	82.2	41.1	64.7	75.4	55.1	62.8	99.5	-62.3	-46.2	48.3	92	71.5
	Hln.(sum)	4.6	-22	17.8	81.9	48.6	31	60.2	60.6	76.4	57.4	12	26.3	-5.5	5.7	-41.1	37.8	-24.5	-21.6	3.1	22.6	2.8	45.2	19.1	-15.1
	N1.(sum)	3.6	-23.2	17	82.1	47.9	30.4	60.2	60	75	57.4	11.1	25.4	-6.8	4.3	-41.8	36.6	-24.7	-22.2	2	23.4	3.3	43.7	18.5	-16.2
	Hln.(count)	38.1	27.4	40.5	-1.5	40.4	39.7	33.8	39.1	35.4	34.1	33.8	29.7	27.4	32.7	17.7	35	13.9	27.6	38.1	-23	-13.2	23	38.1	18.8
	N1(count)	37.5	26.9	39.8	-1.9	39.5	39.1	33.2	38.1	34.3	33.3	33.2	29	26.7	32.4	17.4	34.1	13.8	27.4	37.5	-22.9	-13	22	37.5	18.4
	Hln(traits)	18.5	15	19.5	-14.5	13.1	19.2	10.9	11.7	7.7	7.6	17.1	13.2	14	-3.6	10.9	15.6	14.8	24.7	18.9	-12.1	-8.4	-4	18.4	9.8
	N1(traits)	18.4	14.9	19.4	-14.5	12.9	19	10.7	11.5	7.5	7.4	16.9	13.1	13.9	-3.7	10.8	15.5	14.8	24.6	18.8	-12	-8.4	-4.3	18.2	9.7

Table 4.17 Pearson product moment correlations between functional indices calculated for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data with structural indices showing percentage correlation, *r*. Darker colours indicate a stronger relationship (see key). All index values were scaled to a value between 0 and 1. For trait abbreviations (Rao's entropy) see Table 4.4. For details of Hln (Shannon Index) and N1 (Hill's Index) indices see section 4.2.2.3.1.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

		S	N	d	J	Brillouin	Fisher	ES(50)	H(loge)	1-Lambda	N1	IQI	EQR	ITI	BOPA	A/S	Delta	Delta*	Delta+	sDelta+	Lambda+	AMBI	BQI	MAMBI	Biomass
	Si	60.8	55.6	58	-31.1	50.4	49.5	36.1	42.6	24.3	40.2	58	47.6	60.5	29.8	48.1	46.5	41.2	39.3	60.7	-34.4	-36.8	45.7	59.4	32.9
	B	26.9	23.6	27.5	3.3	34.6	26.1	27.6	32.9	27.9	30.7	17.3	12.2	27.9	28.3	21.7	26.1	9.6	16.1	26.6	-12.7	4	32.7	25.1	10.5
	Bd	44.4	40.4	43.2	-12.4	42.3	37.1	29.6	38.1	31.1	32.4	35.3	27.4	36.5	32.3	31.9	32.8	15.1	29.2	44.4	-25.2	-10.8	37.1	39.3	37.7
	L	78.5	70	76	-22.7	69.7	68.1	54.4	62.2	42	61.4	73.3	62.4	71.2	43.2	55.6	63.2	46.7	50.3	78.3	-47.9	-45.6	42.9	76.5	59.4
	Ft	57.9	52.8	55.6	-19.1	54.7	48.2	42.2	48.1	29.4	45.9	54.2	45	54	23.9	46.4	41.9	29.7	26	57.1	-19	-31.4	48.9	58.1	33.3
	Fm	44.8	40.2	42.6	-12.2	46.9	35.8	35	41	25.2	39.1	45.1	39.2	44	19.2	38.3	33.1	21.8	15	43.8	-5.8	-27.8	42.2	48.3	16.7
	H	60.8	58.6	56	-12.7	60.3	45.5	37.3	52.2	40.5	49.8	51.2	42.4	59	44.9	54.3	53.7	36	39.6	60.6	-39.4	-23.1	36.1	57.5	39.2
	F	52.3	40.9	53.9	3.6	59.2	51.7	47.8	56.5	47.4	53.7	48.7	44.2	41	29.8	30.8	50.4	24.5	26.5	51.6	-22.2	-23.4	39.2	54.5	35
	Bo	52.1	44	52.2	-18.9	48.3	48.3	39.5	43.5	27.6	41.5	48.9	40.6	47.9	23.8	36.7	42.7	32.6	31.2	51.7	-24	-27.5	43.3	52.4	30.6
Rao's Entropy	So	83.8	77.4	79.7	-25.9	72.9	69.2	51.9	63.9	45.1	62.6	75.1	63	81.7	45.6	63.7	71.7	56.2	59.5	84	-56.4	-44.2	51.3	79.6	57.8
	Mv	53.6	48.3	51.1	-30	42.9	43.2	30	35.9	21.5	33.3	52.3	43.6	51.9	32.3	41.5	41.9	37.6	39.5	53.7	-38.4	-34.4	27.7	52.9	26.2
	Ma	54.6	46	54.9	-24.9	44.4	51.3	39	39.9	24.2	37.2	59.3	52.6	51.3	29.3	33.1	36.9	26.2	34.9	54.5	-33.3	-45.1	36	56.2	42.9
	R	-14.2	-17.2	-12.5	3.9	-6.1	-11.9	-5.3	-5.3	-1.7	-7.7	-8.5	-5.3	-11.9	-10.1	-13.1	-14.4	-20	-9.2	-14.4	17.3	5.6	16.9	-10	-23.2
	A	36.9	28.2	36.9	-5.8	37.5	34.3	30	34.3	24.5	35.4	29.9	23.9	30.7	29.3	24.4	35.3	26.1	28.3	36.9	-33	-11.6	8.5	37.3	4.5
	E	-22.5	-23.9	-18.6	-1.3	-25.1	-12	-14.5	-21	-15.1	-23	-19.1	-16.8	-22	-10.7	-24.1	-27.4	-24.4	-13.4	-22.4	16.4	10.8	-4.2	-22.6	-9.1
	Fl	12.7	14.9	9.4	-26.3	0.1	4.6	-4.3	-4.3	-13.4	-3.6	11.6	6.4	4.2	-5.9	16.1	3.7	18.6	20.3	13.4	-13.1	-11.5	-10.5	10.1	27.4
	P	23.7	10	27.9	19.1	40.4	30.7	38.8	41.4	36.2	40.7	19.2	17.9	13	9	5.7	27.8	3.8	5.4	22.8	3.3	0.3	31.4	28.1	3.7
	Sl	62.8	54.6	61.4	-23.2	55.4	54.5	43.2	49.2	32.8	45.6	60	50.7	55.8	34	43.4	49.9	37.6	45.2	62.9	-42.3	-36.6	38.7	62.3	44
	T	67.2	59.6	64.5	-23.6	60.6	56.9	45.2	52.9	31.9	52.2	55.3	42.3	59.9	36.3	52.1	48.7	37	39.5	66.8	-36.2	-25.6	49.3	62.3	44.8
	average	69.2	60.6	67.5	-21.9	64.6	59.9	48.8	57.4	39.3	54.5	62.9	52.1	62.8	38.6	50.9	55	38.3	43.7	69	-37.6	-33.8	49.8	67.8	44.4
	Traits* biomass	83.7	84.7	77.5	-44.6	59.2	66.3	37.9	49	29.1	46.1	67.7	50.6	72.1	38.7	70.9	61.1	57.4	64.1	84.2	-64.8	-36.4	35	69.6	93
	Hln(sum)	65.2	54.5	64.2	-14.8	64.4	57.6	48.6	58.1	42	55.1	60.1	51.2	56.3	35.5	45.1	51.1	30.4	37.4	64.7	-29.5	-32.1	48.9	65.3	38.1
	N1(sum)	65.4	54.5	64.5	-14.7	64.1	58.1	48.8	57.9	41.7	55.1	59.7	50.6	55.4	34.9	44.3	51.1	30.6	37.9	64.9	-30.3	-31.6	48.2	64.9	37.7
	Hln(traits)	18.5	15	19.5	-14.5	13.1	19.2	10.9	11.7	7.7	7.6	17.1	13.2	14	-3.6	10.9	15.6	14.8	24.7	18.9	-12.1	-8.4	-4	18.4	9.8
	N1(traits)	18.4	14.9	19.4	-14.5	12.9	19	10.7	11.5	7.5	7.4	16.9	13.1	13.9	-3.7	10.8	15.5	14.8	24.6	18.8	-12	-8.4	-4.3	18.2	9.7

4.3.5 Co-inertia Analyses

4.3.5.1 Species correspondence analysis

Correspondence Analysis (CA) was carried out using the sample species biomass data transformed ($\log_{10}$ x+1). The CA of the species biomass matrix indicated that the first axis explained 12.43% of the total variation in the species data while the second axis explained a further 10.06% of the variation. Samples were arranged from left to right along axis 1 of the CA (Fig. 4.14). This was related to the site with Leverets to the left and Margaretta clustered to the right (Fig. 4.15(a)). There was also a difference among samples within Leverets along the second axis with communities dominated by *Crangon crangon*, *Chamelea gallina*, *Pagurus bernhardus* and *Phyllodoce laminosa* differing from other samples and the samples from Leverets in general having greater differences from each other than the samples from Margaretta (Figs 4.14, 4.15).

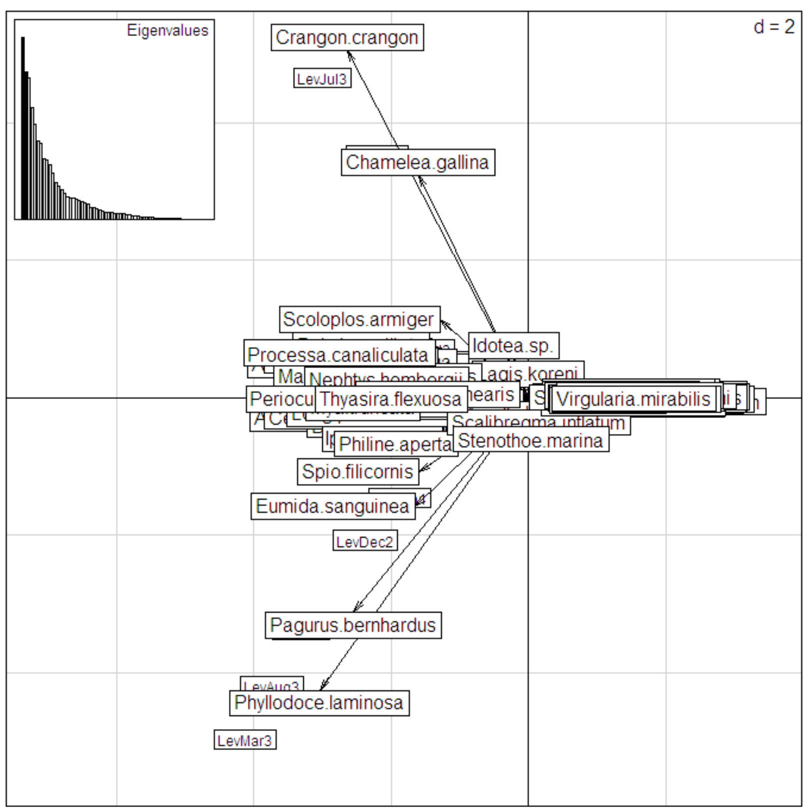


Figure 4.14 Biplot of species and sample correspondence analysis (species data used was biomass and transformed ($\log_{10}$ x+1)); number of axes selected = 2.

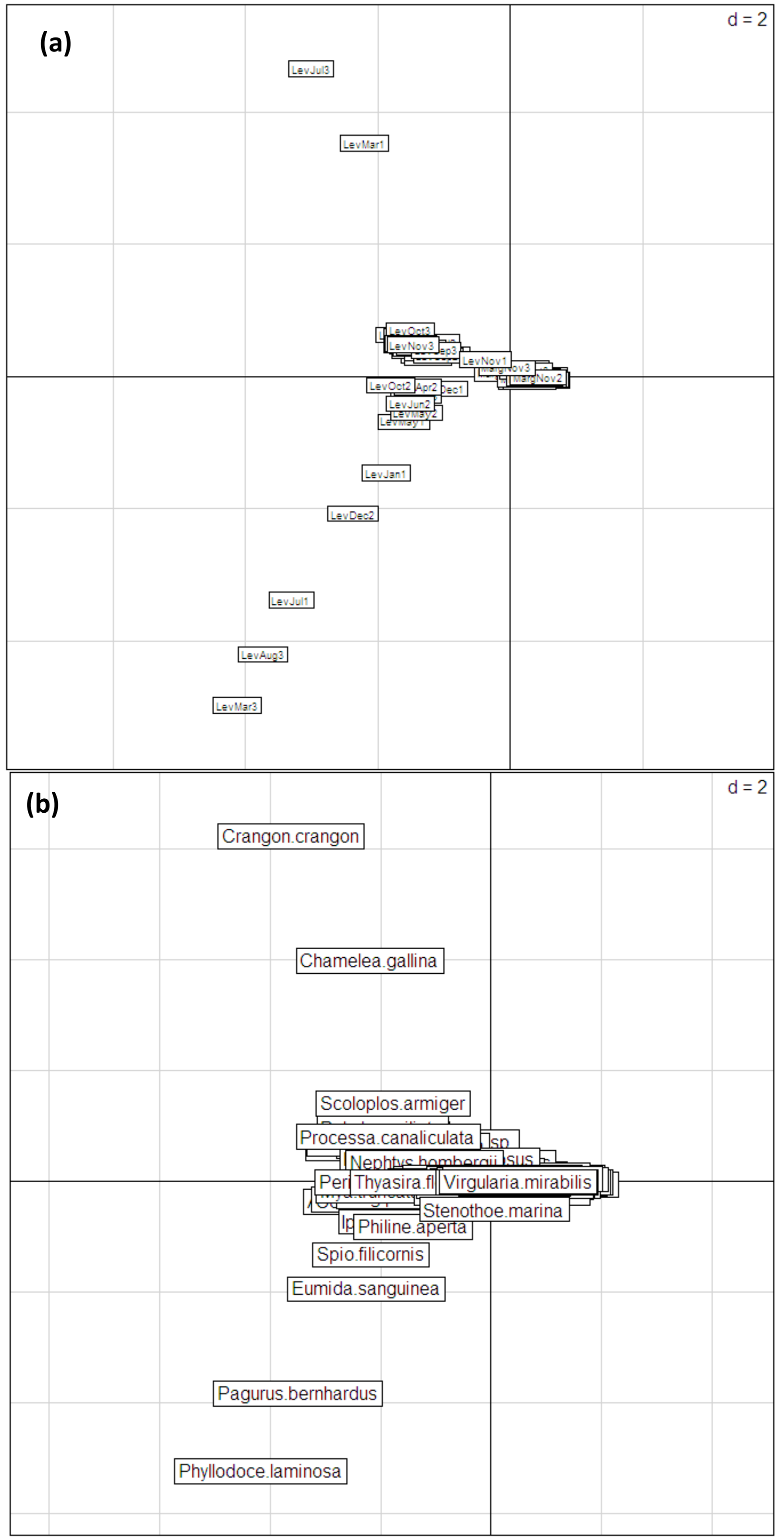


Figure 4.15 Plots of correspondence analysis of (a) samples and (b) species.

4.3.5.2. Traits Fuzzy Correspondence Analysis

Fuzzy Correspondence Analysis (FCA) was carried out using the fuzzy trait and species biomass data (transformed ($\log_{10}$ x+1)). The first axis of the FCA explained 11.28% of the variation in the trait data and the second axis explained 10.46%.

Species and traits were mainly clustered around the centre along axis 1 (Fig. 4.16, 4.17). Along axis 2 there was one big difference which was due to the species *Virgularia mirabilis* and to a lesser degree *Modiolus modiolus* and associated traits being substantially different from the other species, although this is relative as the overall variability explained by the analysis was low as indicated by the eigenvalues.

Figure 4.16 Biplot of species and traits fuzzy correspondence analysis (species data used was biomass and transformed); number of axes selected = 2. See Table 4.4 for trait abbreviations.

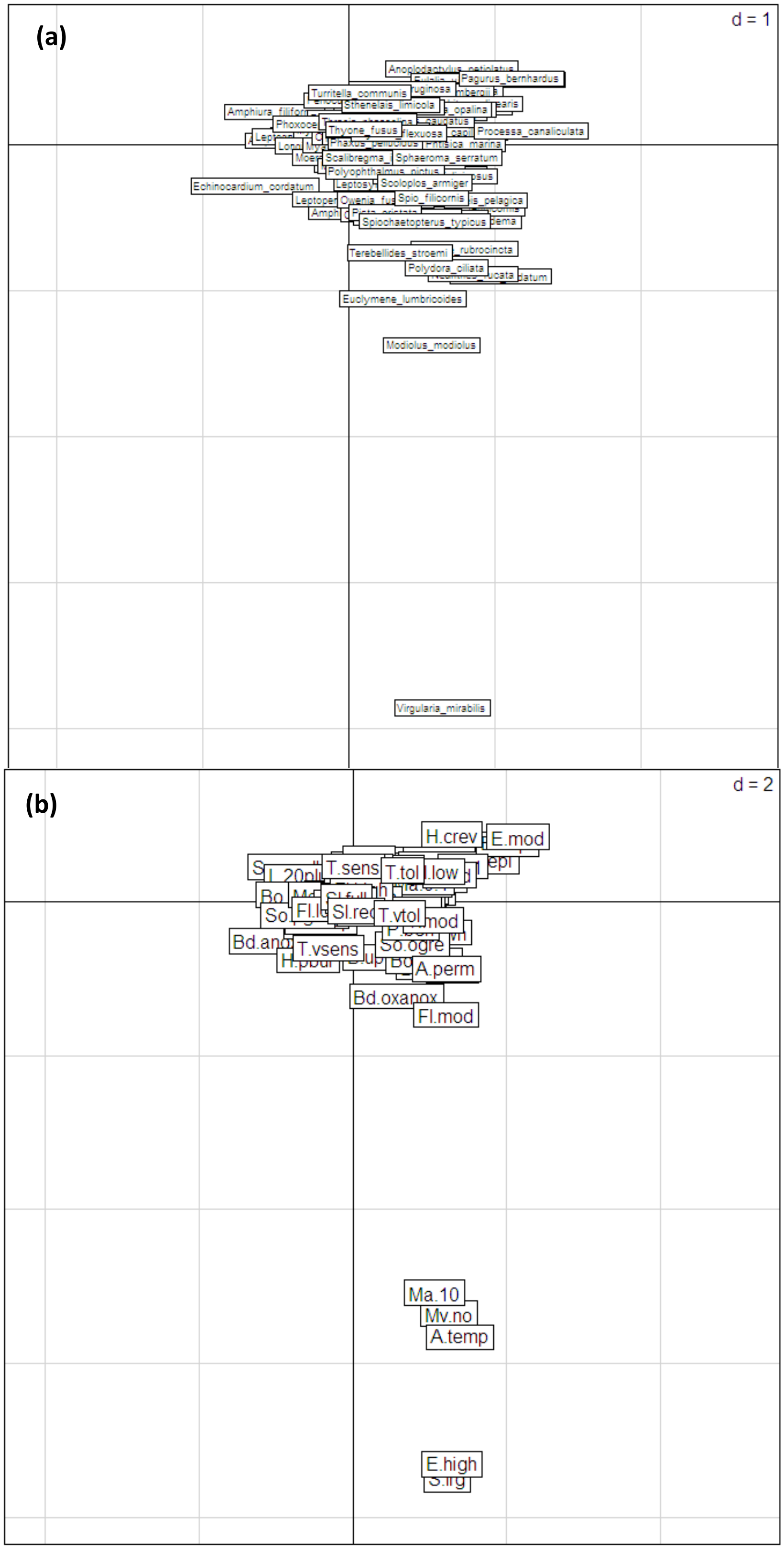


Figure 4.17 Plots of fuzzy correspondence analysis of (a) species and (b) traits. See Table 4.4 for trait abbreviations.

4.3.5.3 Environmental Variables PCA

Principal Components Analysis (PCA) was carried out for the environmental variables of the all samples. The first axis explained 26.63% of the variation and the second axis 16.59%.

Silt, clay, porosity, sorting, organic carbon and graphic mean could be seen to be negatively correlated to SMD, POC and the Median grain size along the first PCA axis (Fig. 4.18). Sand, DMBD, sorting and O_2 were negatively correlated to skewness, NH_4, SiO_4 and SPM along the second PCA axis. Margaretta samples were correlated with silt and clay, organic carbon, sorting, porosity, graphic mean, SPM, skewness, salinity, PO_4 and Nitrite while Leverets samples were negatively correlated with sorting, porosity, organic carbon, silt, PO_4 and Nitrite (Fig. 4.19).

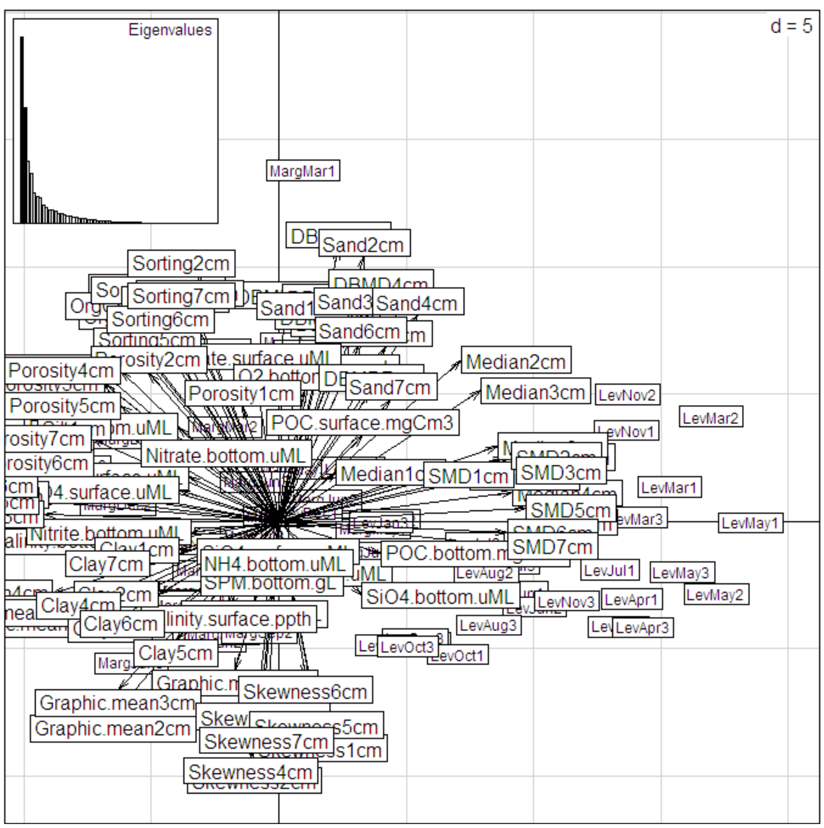


Figure 4.18 Biplot of environmental variables and samples principal components analysis; number of axes selected = 2.

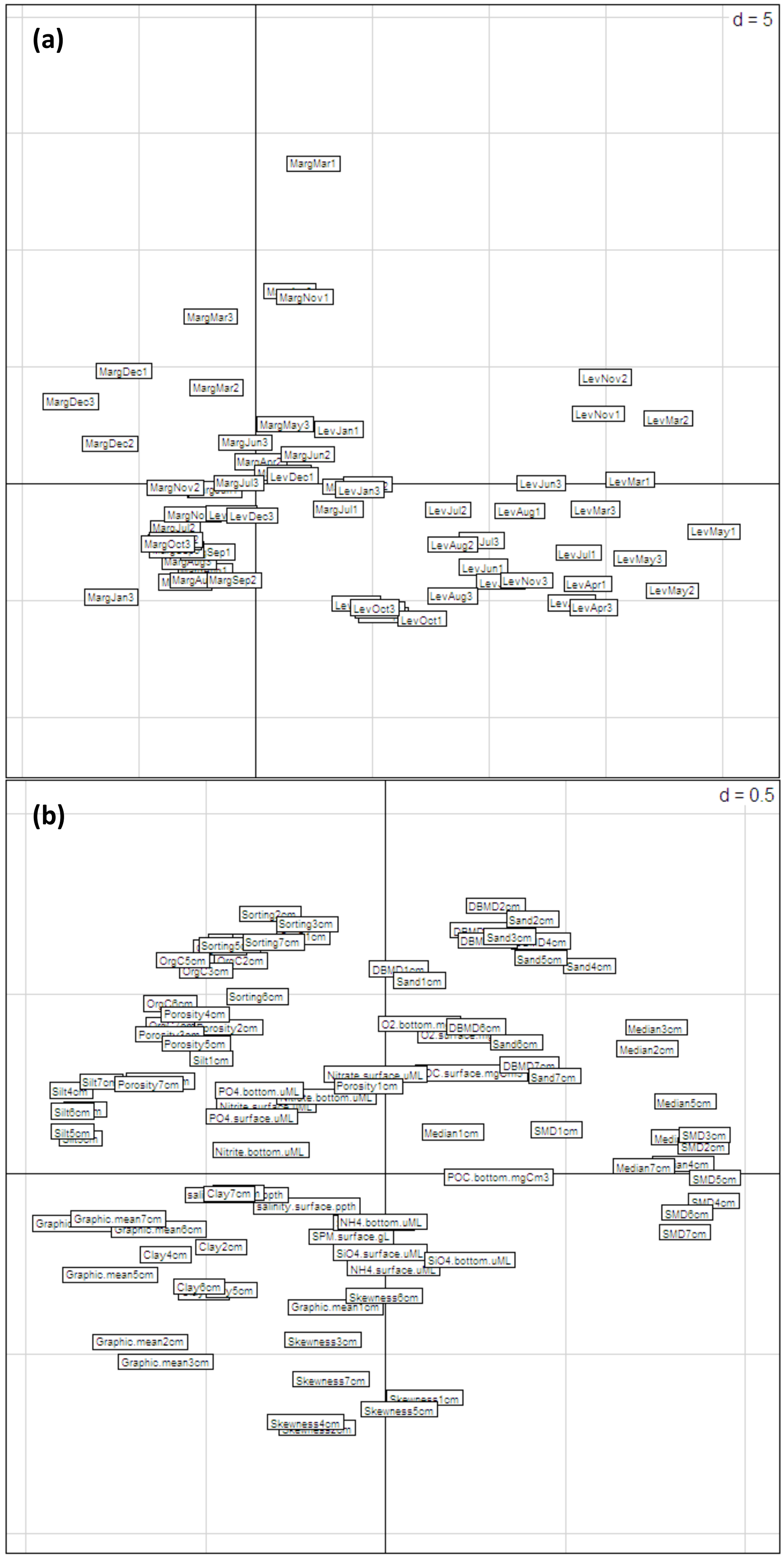


Figure 4.19 Plots of principal components analysis of (a) samples and (b) environmental variables.

4.3.5.4 Co-inertia analysis (two table ordination)

Co-inertia analysis was carried out between the species CA and the traits FCA (4.3.5.2) and between the species CA and the environmental variables PCA (4.3.5.3) for the purpose of interpretation and to compare which aspects of the community were highlighted by traits analysis and which were highlighted by environmental variables.

4.3.5.4.1 Species versus traits

Co-inertia analysis of the trait and species data tables revealed a difference between sites Leverets and Margaretta. The first axis explained 35.96% of the variation and the second axis explained 15.96%. Axis 1 was in the direction of the Margaretta sites (Fig. 4.20, 4.21 (a)). There was a greater difference between some of the Leverets samples and all other samples which fell along Axis 2. This was related in particular to the presence or absence of *Phyllodoce laminosa*, *Crangon crangon* and *Pagurus bernhardus*. The strongest trend related to traits (X axes) however, was in the direction of species tolerant to low salinity.

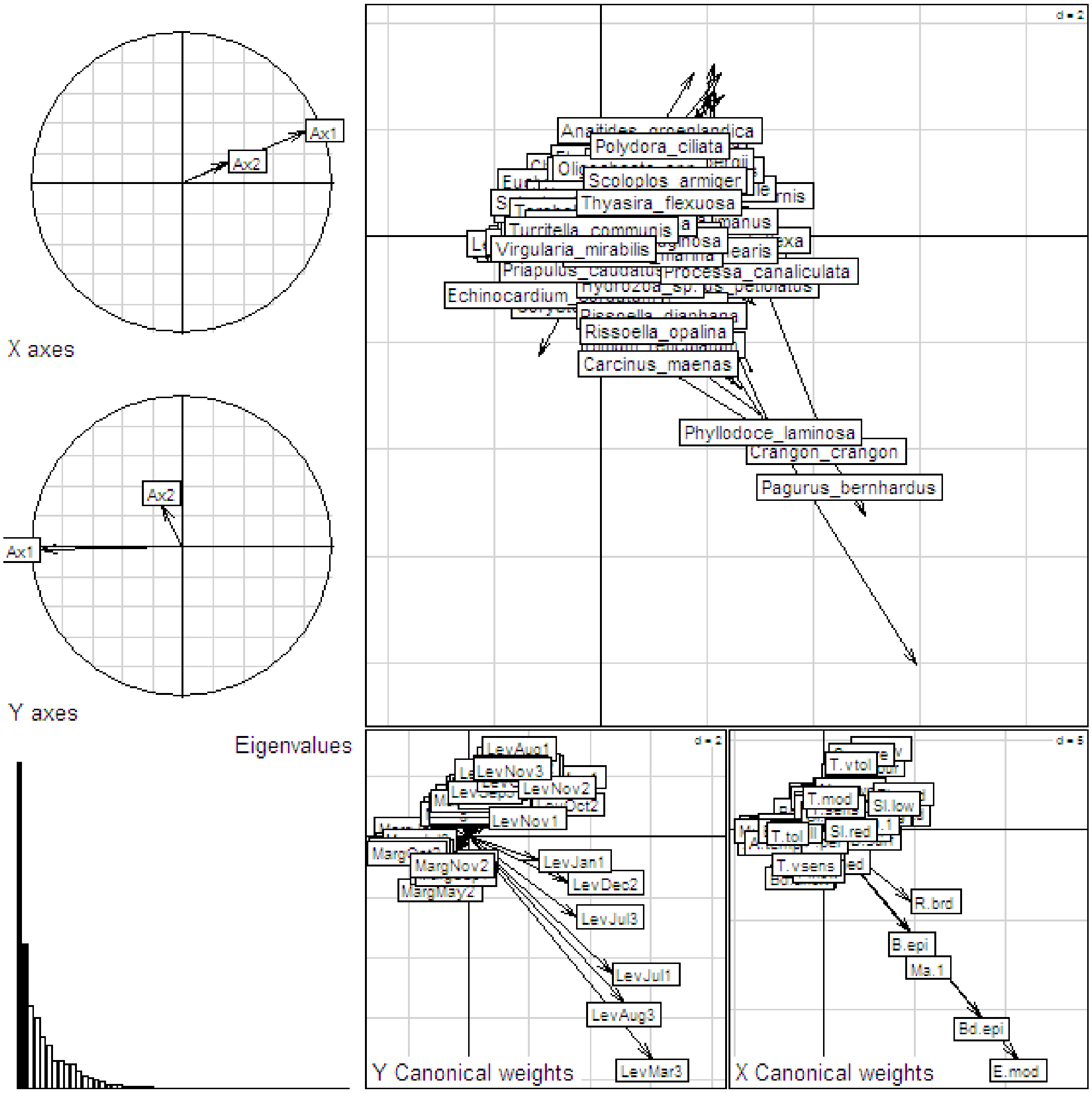


Figure 4.20 Co-inertia of species and traits; number of axes selected = 2. The main diagram represents individual species abundances with the beginning of the arrows showing the position of the species described by the traits and the end of the arrow is the position described by the sample. The X axes show the projection of the FCA axes (traits) onto the axes of the co-inertia analysis while the Y axes show the projection of the CA axes (samples). The screeplot shows the eigenvalues of the analysis. The canonical weights represent the coefficients of the combinations of the variables for species and traits to define the coinertia axes. See Table 4.4 for trait abbreviations.

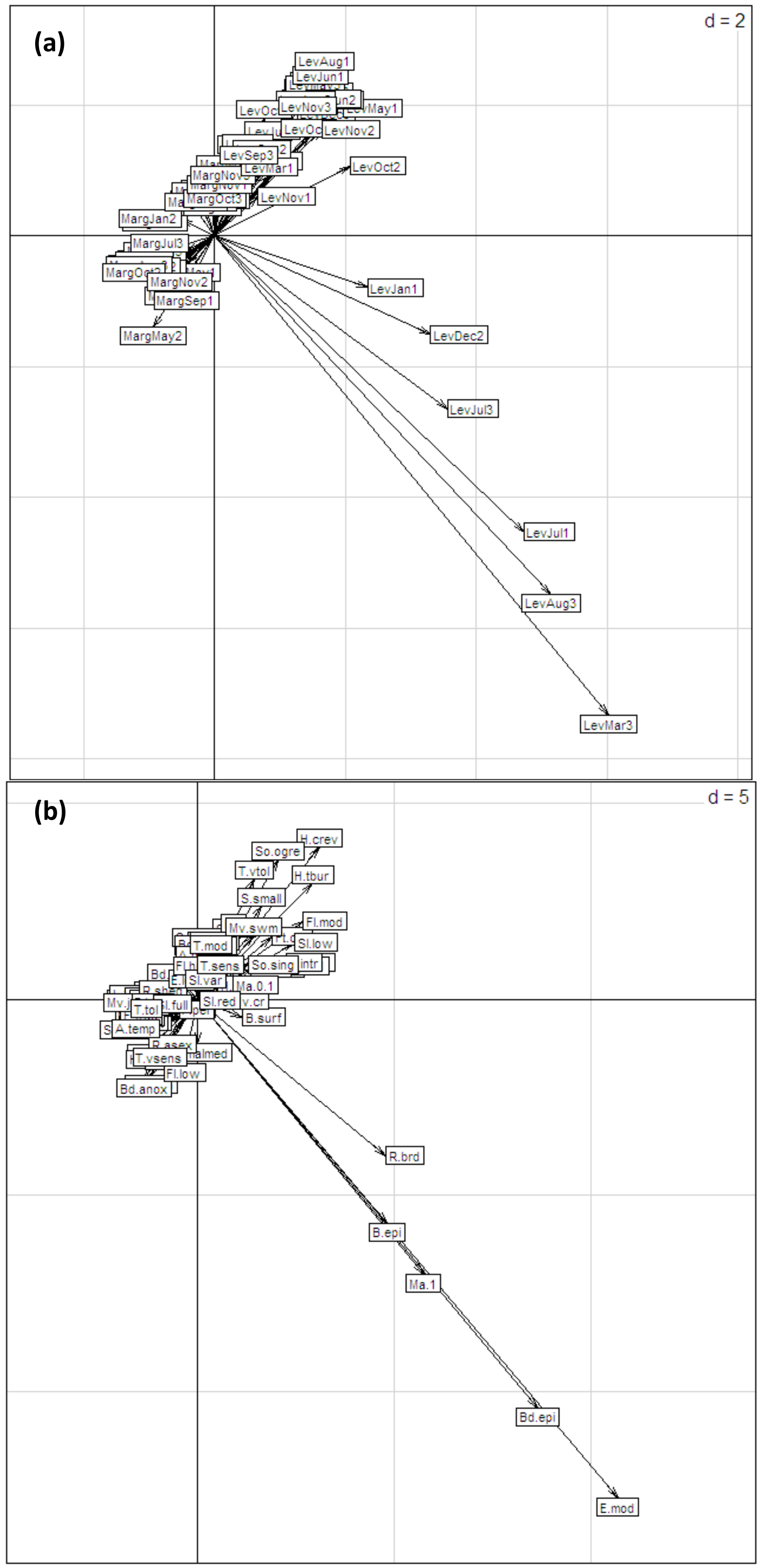


Figure 4.21 Plots of co-inertia analysis of (a) samples and (b) traits. See Table 4.4 for trait abbreviations.

4.3.5.4.2 Species versus environmental variables

The first axis of the co-inertia of species and environmental variables explained 61.07% of variation and the second axis explained 11.50%. A greater difference was found between sites than the co-inertia with functional traits (Figs 4.20, 4.22). This analysis showed Margaretta to be associated with organic carbon, silt, clay and porosity while Leverets was associated with sediment grain surface area mean (SMD) (Figs 4.22, 4.23). The strongest trends with the environmental variables (X axes) was in the direction of grain surface area mean (SMD) along axis 1 and organic carbon along axis 2. Species (Y axes) axes 1 and 2 indicated differences between the two sites.

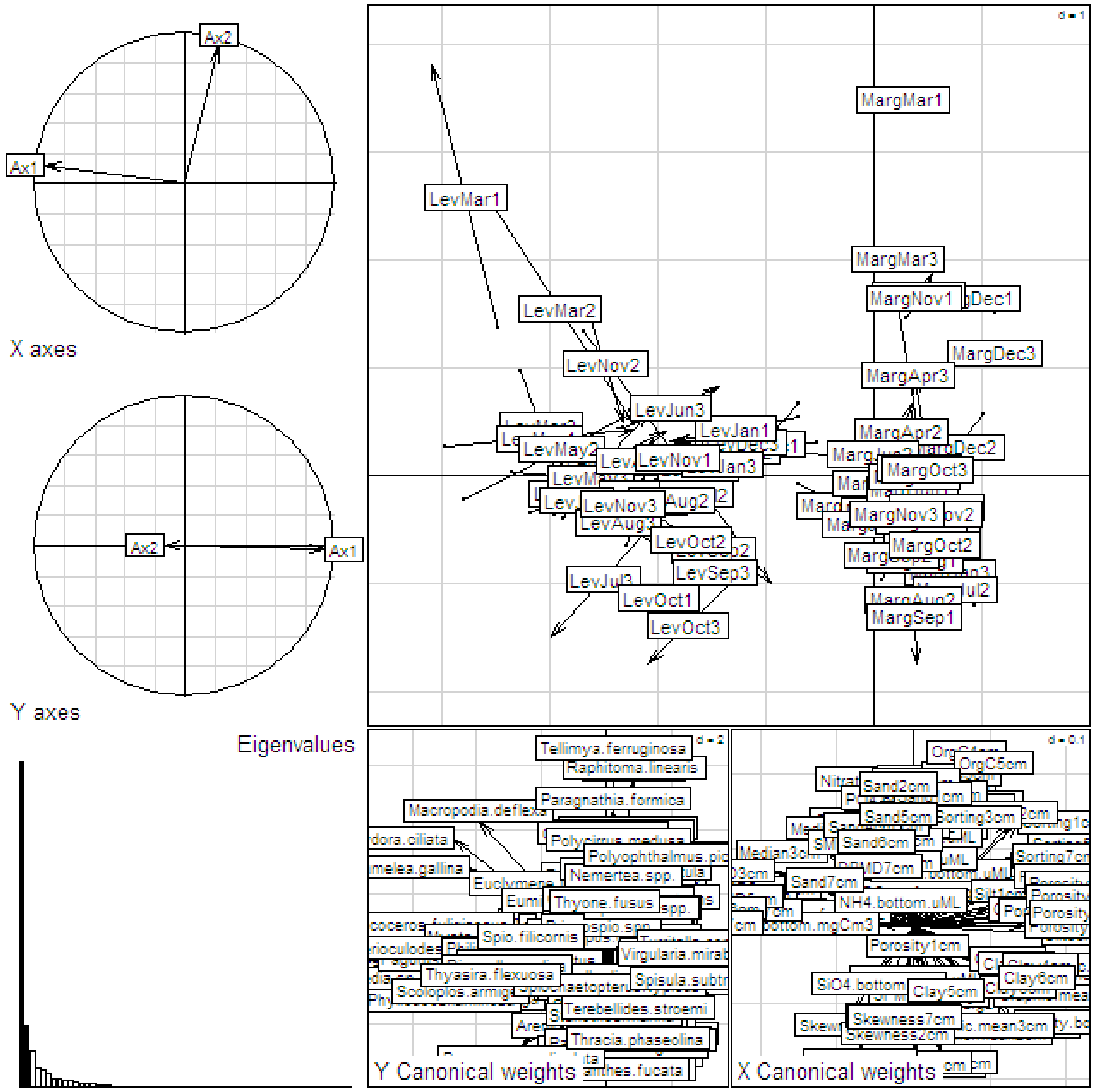


Figure 4.22 Co-inertia of species and environmental variables; number of axes selected = 2. The main diagram represents individual samples with the beginning of the arrows showing the position of the samples described by the environmental data and the end of the arrow is the position described by the species abundance. The X axes show the projection of the PCA axes (environmental variables) onto the axes of the co-inertia analysis while the Y axes show the projection of the CA axes (species abundance). The screeplot shows the eigenvalues of the analysis. The canonical weights represent the coefficients of the combinations of the variables for species and environmental variables to define the coinertia axes.

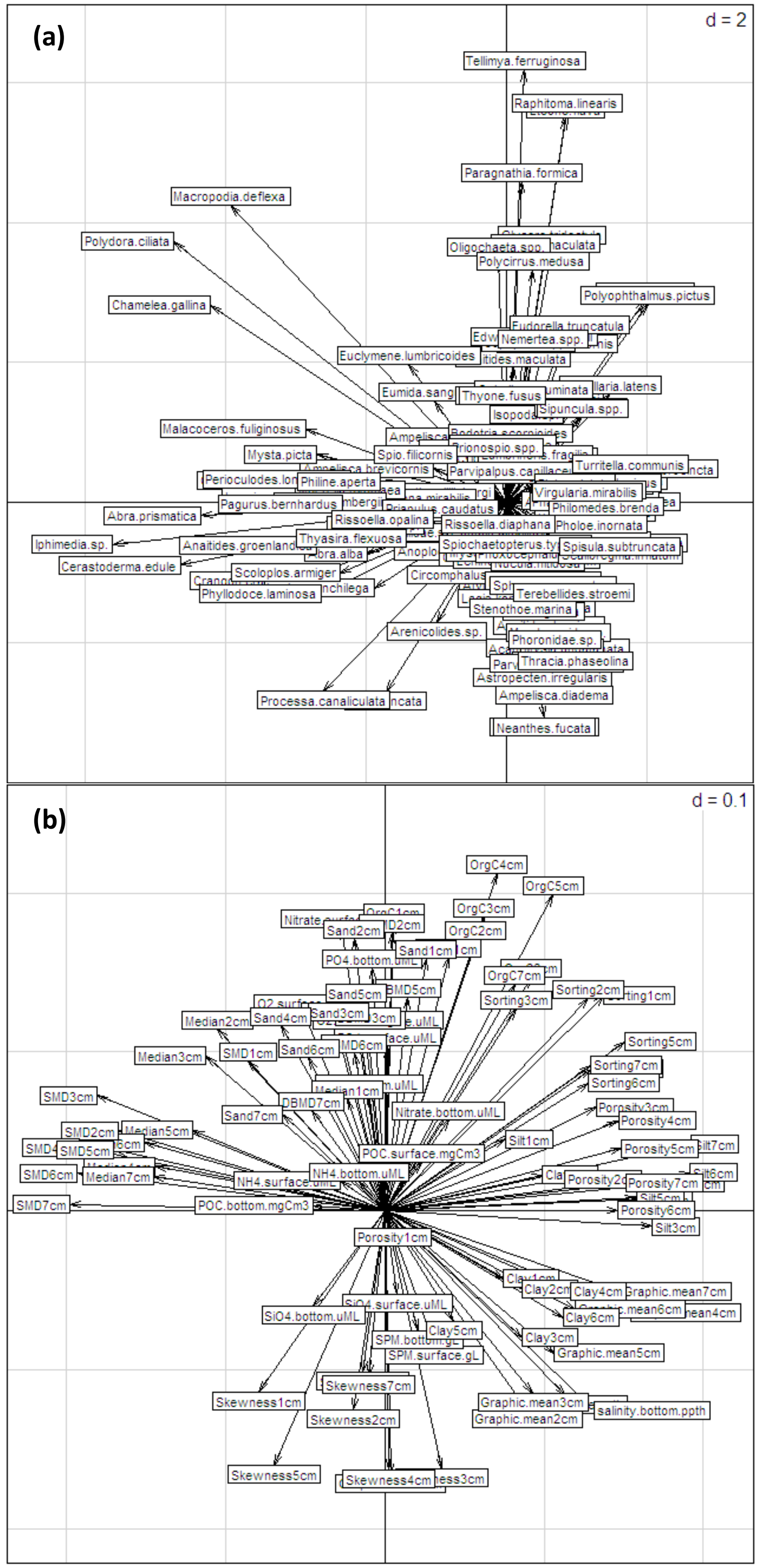


Figure 4.23 Plots of co-inertia analysis of (a) species and (b) environmental variables.

4.3.5.5 RLQ analysis (three table ordination)

RLQ analysis again showed differences between Leverets and Margaretta and within the Leverets samples (Fig. 4.24). There was significant relationship between the species traits and environmental variables (Monte Carlo test $p<0.05$). The first axis explained 78.42% of the variation and the second axis explained a further 9.13%. The analysis showed a difference between Margaretta samples. The environmental variables (R axes) showed differences in communities along the first axis characterised by sediment type with larger grain sizes - SMD (surface area mean of grain), sand fraction and median grain size to the left and finer grain sizes - clay, silt, porosity and sorting to the right (Fig. 4.25(c)). Along the second axis, the community was characterised by organic carbon in the upper right quadrant and to a lesser degree skewness of the sediment in lower left quadrant. The first two axes of the functional traits (Q axes) showed a community dominated by opportunistic and carrion feeders, singular species and soft bodied species while at the other end of this axis the community was composed of gregarious species, detritus feeders and species with exoskeletons (Fig. 4.25(d)). Along the second axis, the main characteristics were a community composed of very tolerant species, swimmers, biodiffuser type sediment modifiers and smaller species while at the other side of the axis were very sensitive species, sessile species, downward conveyer bioturbators and larger species.

The correlation between the environmental variables and the first two RLQ axes (Table 4.18) supported this. The first axis was most strongly correlated with physical properties of the sediment including SMD, median, graphic mean, silt content and porosity and to a lesser extent sorting and organic carbon. The second axis was most strongly correlated with organic carbon and some of the nutrients including PO_4, Nitrate and Nitrite. The correlations between traits and RLQ axes (Table 4.19) showed that the traits which occur in Margaretta and those which occur in December, March and November in Leverets were most strongly correlated (Fig. 4.24, Fig. 4.25 (a), (d)) to the first axis while few traits were strongly correlated to the second axis. Correlations between species and RLQ axes revealed very strong correlations between the first axis with

Amphiura filiformis, *Lumbrineris fragilis* as well as several other species while the second axis showed weaker correlations, the strongest being with *Eudorella truncatula* (Table 4.20).

Overall, the analysis revealed that Margaretta had two separate communities – March and December and another type of community which was more characteristic of the rest of the year. The March/December community was composed of species with short to moderate lifespans, robust bodies, sensitive to very tolerant species and permanently attached species. The second community was composed of long lived species, gregarious species, suspension and detrital feeders, a variety of burrowing types and very sensitive species with a high exposure potential. Leverets was also composed of two communities November/March and another with the other samples. The November/March communities were composed of opportunistic feeders and predators, species reaching sexual maturity within one year, and swimming species while the second community was composed of epifaunal species and temporary burrowers, small species, species tolerant of variable and low salinities and species with medium longevity.

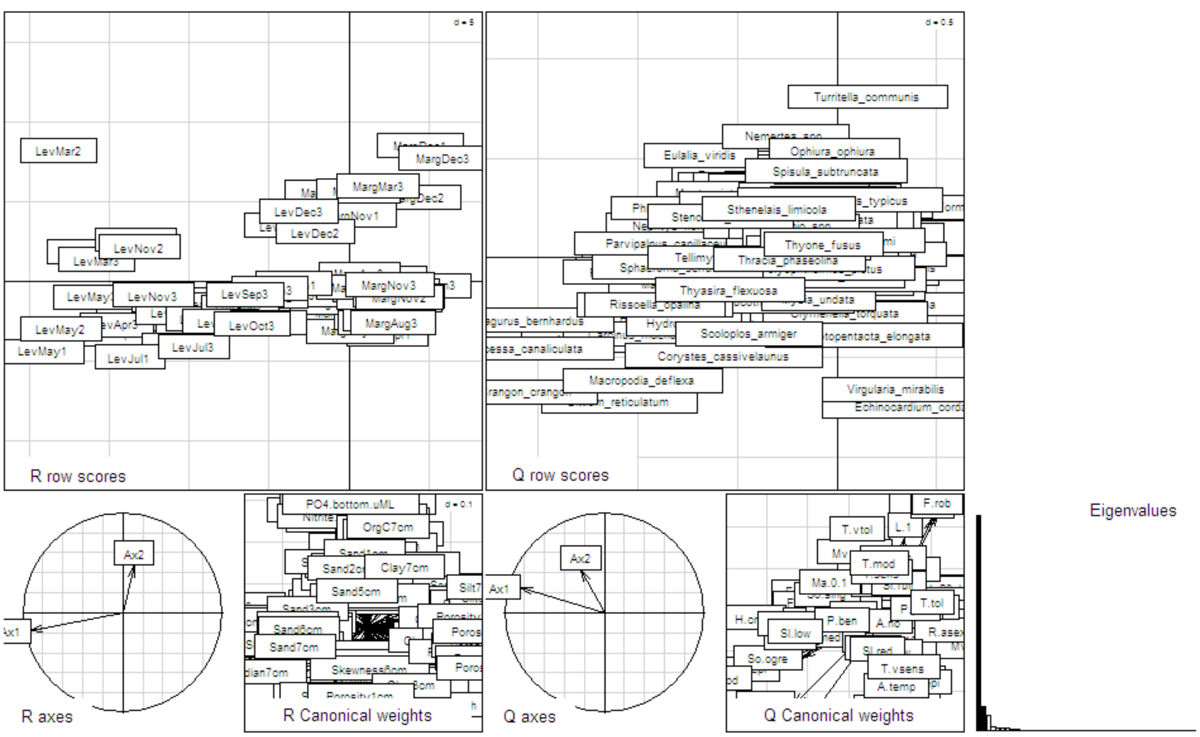


Figure 4.24 RLQ analysis of species (L), functional traits (Q) and environmental variables (R); number of axes selected = 2. See Table 4.4 for trait abbreviations.

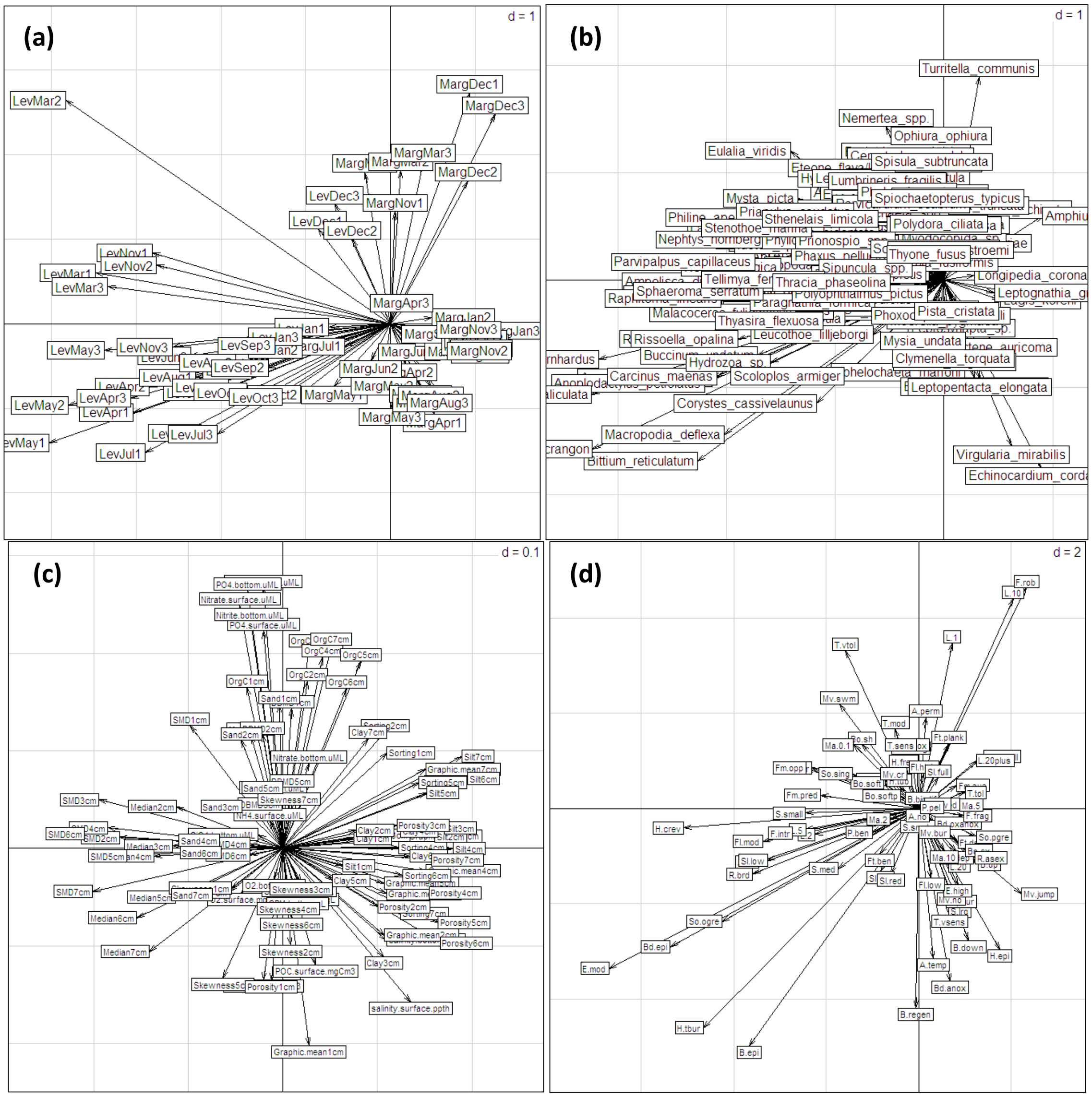


Figure 4.25 Plots of RLQ analysis of (a) sites, (b) species, (c) environmental variables (R), and (d) functional traits (Q). See Table 4.4 for trait abbreviations.

Table 4.18 Pearson product moment correlations between environmental variables and RLQ axes for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key).

Environmental Variable	Axis 1	Axis 2		Environmental Variable	Axis 1	Axis 2
SPM (g/L) surface	19.7	-19.1		Graphic.mean3cm	70.0	-1.3
POC (mgC/m3) surface	-28.4	-4.4		Graphic.mean4cm	86.8	12.1
O2 (mg/L) surface	-24.2	25.3		Graphic.mean5cm	84.2	17.0
salinity (ppth) surface	37.7	-38.0		Graphic.mean6cm	79.8	18.3
NH4 (μM) surface	-23.5	4.2		Graphic.mean7cm	85.1	27.7
NO3 (μM) surface	-17.4	62.0		Sorting1cm	57.7	52.2
NO2 (μM) surface	16.1	59.1		Sorting2cm	58.8	43.4
PO4 (μM) surface	20.3	60.7		Sorting3cm	62.6	37.0
SiO4 (μM) surface	16.4	-7.0		Sorting4cm	64.4	32.2
SPM (g/L) bottom	16.4	-8.9		Sorting5cm	74.2	36.0
POC (mgC/m3) bottom	-41.3	-28.6		Sorting6cm	68.9	33.6
O2 (mg/L) bottom	-13.9	8.6		Sorting7cm	63.7	22.4
salinity (ppth)bottom	54.3	-27.7		Skewness1cm	-36.1	-33.1
NH4 (μM) bottom	-0.7	8.3		Skewness2cm	7.2	-36.4
NO3 (μM) bottom	13.7	50.3		Skewness3cm	3.0	-26.8
NO2 (μM) bottom	22.8	48.4		Skewness4cm	2.5	-26.7
PO4 (μM) bottom	22.6	75.1		Skewness5cm	-20.4	-34.8
SiO4 (μM) bottom	-29.8	-12.1		Skewness6cm	-8.8	-14.1
OrgC1cm	33.1	62.9		Skewness7cm	0.7	1.6
OrgC2cm	48.3	71.9		Sand1cm	-22.8	56.4
OrgC3cm	58.2	71.3		Sand2cm	-36.5	30
OrgC4cm	58.8	71.5		Sand3cm	-26.4	18.7
OrgC5cm	66.1	68.2		Sand4cm	-49.5	8.4
OrgC6cm	66.9	67.0		Sand5cm	-45.0	9.2
OrgC7cm	62.8	70.7		Sand6cm	-39.0	1.0
Median1cm	-32.6	38.5		Sand7cm	-41.1	-21.3
Median2cm	-80.8	2.3		Silt1cm	67.5	11.9
Median3cm	-83.7	-5.7		Silt2cm	90.5	20.3
Median4cm	-90.1	-16.4		Silt3cm	91.9	25.3
Median5cm	-87.5	-21.7		Silt4cm	93.8	28.4
Median6cm	-90.0	-23.1		Silt5cm	91.5	34.0
Median7cm	-86.1	-27.5		Silt6cm	93.0	38.7
SMD1cm	-67.0	19.2		Silt7cm	90.8	39.8
SMD2cm	-87.8	-6.4		Clay1cm	56.0	7.2
SMD3cm	-92.6	-14.6		Clay2cm	55.6	-2.0
SMD4cm	-94.6	-17.1		Clay3cm	62.6	0.9
SMD5cm	-94.0	-22.0		Clay4cm	68.3	6.6
SMD6cm	-91.6	-28.0		Clay5cm	44.5	12.2
SMD7cm	-92.2	-30.2		Clay6cm	65.3	4.1
DBMD1cm	-14.6	58.8		Clay7cm	61.8	22.5
DBMD2cm	-20.6	34.6		Porosity1cm	31.2	-0.2
DBMD3cm	-10.2	22.7		Porosity2cm	77.2	18.9
DBMD4cm	-29.6	14.5		Porosity3cm	82.1	25.2
DBMD5cm	-20.5	17.7		Porosity4cm	81.7	18.4
DBMD6cm	-17.9	9.0		Porosity5cm	81.1	12.4
DBMD7cm	-34.3	-19.4		Porosity6cm	83.5	18.1
Graphic.mean1cm	40.7	-49.6		Porosity7cm	83.2	26.2
Graphic.mean2cm	71.2	-11.6				

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Table 4.19 Pearson product moment correlations between traits and RLQ axes for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key). For details of traits see Table 4.

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

Trait	Trait Category	Axis 1	Axis 2	Trait	Trait Category	Axis 1	Axis 2
Size	Very small(<1cm)	77.8	19.7	Body Type	Soft	61.9	18.3
	Small(1-2cm)	29.9	8.8		Soft-protected(tube/tunic)	63.2	12.6
	Small-medium(≥3-10cm)	64.3	4.1		exoskeleton	71.7	-3.2
	Medium(≥11-20 cm)	38.4	-11		shell	36.5	22.1
	Medium-large(≥21-50cm)	45.8	17.3	Sociability	Singular	50	27.1
	Large(>50cm)	25.2	-6.3		occasionally-gregarious	-9.6	-12.1
Bioturbator/ Reworking Mode	Epifauna	0.3	-14		permanently-gregarious	72.5	-2.6
	Surficial modifier	65.2	20.2	Movement Type	None	27.8	-7.3
	Biodiffuser	74	10.4		swim	28.6	34.6
	Upward conveyer	46.3	-4.1		crawl/creep/climb	70.7	19.1
	Downward conveyer	41.1	-14.4		burrow/bore	71.4	0.2
	Regenerator	22.2	-16.8		jump	21.4	-4.2
Burrowing Depth	Epifaunal	-11	-12	Maturity	<1 year	17.5	18
	Oxic layer	74.9	28.7		1 year	-8.5	-6.8
	Oxic & Anoxic layers	61.1	3.5		1-2 years	57.1	-0.2
	Anoxic layer	46.1	-24.5		3-5 years	76	5.1
Lifespan	</= 1 year	34.2	29.1		6-10 years	26.2	-4.4
	1 to 2 years	13.1	-5.1	Reproduction Type	Asexual	23.1	-3.5
	3 to 5 years	10.3	-3.9		sexual-shed eggs	74.7	6.9
	6 to 10 years	43	33		sexual-brood eggs	-11.9	-11.8
	11 to 20 years	64	-10.7	Degree of Attachment	None	76.9	9.2
	20+	78	19.9		temporary	22.2	-10.9
Food Type	Detritus	72	-0.9		permanent	55	27.7
	Carrion	35.5	31.4	Exposure Potential	Low (infauna or flat)	78	10.9
	living material-benthic	62.2	-5.6		Moderate (mound surface/interface dwellers)	-16.4	-11.7
	living material-planktonic	70.9	25.7		High (erect surface/interface dwellers)	26.2	-4.4
Feeding Method	Suspension feeder	74	14.4	Flexibility	<10	57.5	-10
	Deposit feeder	69.8	-3.4		10 to 45	3	-0.9
	opportunistic/scavenger	32	30.7		>45	80	21.8
	active predator	39	18.3	Propagule Dispersal	Pelagic	74.7	6
Living Habit	Tube	66.2	16.4		Benthic	28.6	-2.1
	permanent burrow	57.4	-11.7	Salinity	Full salinity	77.6	14.6
	temporary burrow	-7.6	-25.6		Variable salinity	47.3	-27.6
	crevice/hole	-13.7	1.6		Reduced salinity	46.5	-25.2
	epizoic/epiphytic	20.4	-6.8		Low salinity	-15.6	-16.4
	free	74.7	24.8	Tolerance	Very sensitive	57.8	-16.6
Fragility	Fragile	75.6	3.2		Sensitive	75	31.9
	Intermediate	9.3	-3.1		Moderate	53.1	29.4
	Robust	42	30.2		Tolerant	69.8	10.9
					Very Tolerant	16	23

Table 4.20 Pearson product moment correlations between species (top ten strongest correlations) and RLQ axes for two sites in Galway Bay based on transformed ($\log_{10}$ x+1) species biomass data with percentage correlation, *r*. Darker colours indicate a stronger relationship (see key).

Species	Axis 1		Species	Axis 2
Amphiura filiformis	78		*Eudorella truncatula*	53
Lumbrineris fragilis	72.4		*Glycera tridactyla*	46.8
Pholoe inornata	67.7		*Astacilla longicornis*	43.5
Owenia fusiformis	66.4		*Sipuncula spp.*	37.6
Philomedes brenda	65.6		*Polyophthalmus pictus*	33
Phoxocephalus holbolli	64.3		*Ampharete grubei*	32.8
Leptognathia gracilis	57.3		*Ophiura ophiura*	32.7
Ampharete grubei	56.3		*Melinna palmata*	31.9
Cylichna cylindracea	49.5		*Phtisica marina*	29.5
Echinocardium cordatum	45.9		*Turritella communis*	29.4

Colour	% Correlation
	<10
	≥ 10 - < 20
	≥ 20 - < 30
	≥ 30 - < 40
	≥ 40 - < 50
	≥ 50 - < 60
	≥ 60 - < 70
	≥ 70 - < 80
	≥ 80 - < 90
	≥ 90 - 100

4.4 Discussion

4.4.1 Structural Methods

Analysis of the sites revealed two distinct communities at Leverets and Margaretta (Fig. 4.1). Some temporal differences were also evident with some months being markedly different than others (Fig. 4.1 (b)). Solan (2000) attributed differences in some months to storm events which occurred in February 1997 and another in August-September 1997. Results here would support differences due to the timing of these events since at Leverets and Margaretta there were differences in species abundance composition in the months March, April and May following the February storm. At Margaretta there was also some difference in the September community abundance composition which could be explained by the August-September storm event. These differences were not evident in the species biomass composition at either site (Fig. 4.2).

All indices found significant differences between the quality at Margaretta and Leverets with most indices finding higher quality at Margaretta (Table 4.6) (Hypothesis (H) 3). However, Pielou's Index, variation in taxonomic distinctness (Lambda+) and BOPA found higher quality at Leverets. The higher quality assigned by BOPA to Leverets was mainly due to a high proportion of opportunistic polychaetes at Margaretta and a low number of both amphipods and opportunistic polychaetes at Leverets. Only BQI did not find a difference in quality between months at Margaretta and only taxonomic diversity (Delta) and AMBI found no differences between months at Leverets (H3). Actual quality classification of the sites varied depending on the index used (Table 4.5). IQI found Margaretta to be mostly of high quality over the year while Leverets had good or moderate quality. BQI found Margaretta to be good or moderate quality while Leverets was poor or moderate or good quality. Although AMBI found significantly higher quality at Margaretta this did not translate to a difference in quality classification with all months at both sites classified as good. BOPA found both sites to have good quality, except two Leverets samples which were assigned high quality. ITI found Margaretta to have normal quality and Leverets to have changed from normal conditions. IQI and BQI indicated decreased quality at Leverets in March compared to January following the February storm event and this continued at least into April according to both indices. BQI also detected a decrease in quality at this time in Margaretta, although this was not significant, and a decrease in April detected by IQI may have been related to this event.

The overall correlations between indices for Galway Bay (Table 4.7) were similar to those found at the NMMP sites (Chapter 2 Section 2.3.3). However, there were some differences. The taxonomic distinctness indices showed greater correlations to species richness and to each other. ITI also showed much greater correlation to other indices. BOPA showed only a weak correlation to AMBI which is unusual due to the relationship between AMBI and the derivation of BOPA. The total biomass showed high correlation to species richness and to other indices which was not found at other sites. When the sites were analysed separately, Margaretta showed a very different pattern from the overall pattern (Table 4.8). Most indices showed low correlations to each other and even to species richness. The strong relationships found seemed mainly related to evenness in the community with indices such as Simpson’s index, Pielou’s index and Shannon index being highly correlated to each other. At Leverets alone, the correlations were similar to those found at NMMP sites (Table 4.9).

Most indices were not highly correlated with any water column properties (Table 4.10) (H4). Despite the differences in salinity between the two sites due to the input of freshwater at Leverets (section 2.1), no relationships were found with indices and salinity except for ITI and species richness with surface salinity. Correlations were found between indices and sediment properties (Tables 4.11) (H4). S, IQI, ITI, MAMBI and Total biomass all had similar relationships with sediment properties. Strong relationships were found with these indices and the median grain size, SMD, graphic mean, sorting, porosity and the silt and clay fractions. ITI and taxonomic distinctness (Delta*) both found some correlation with organic carbon. On the other hand, BOPA, taxonomic distinctness (Delta*), AMBI and BQI were relatively independent of the sediment properties. BOPA showed some correlation with sorting. Taxonomic distinctness (Delta*) showed some correlation with silt and clay and with some other properties but only in deeper sections of the core. The sediment properties which were correlated with index results were mostly related to size of the particles suggesting different size particles at the two sites were primarily responsible for differences in the communities.

4.4.2 Biological Traits

Functional diversity based on number of traits at each site, occurrence of traits (traits*species richness) and frequency of traits based on abundance and biomass, was higher at Margaretta (Fig. 4.3) (H3). The greater number of traits at Margaretta suggests that this site had greater functional diversity than Leverets and reflects the structural indices which found higher quality at Margaretta than at Leverets. Functional diversity increased and decreased depending on the month showing that the functional diversity of the community depended on the season (Fig. 4.4) (H3). At Leverets in particular, a decrease in functional diversity could be seen following the storm event in February. This was not so apparent at Margaretta, except for frequency based on biomass. Occurrence (species richness) and frequency (abundance and biomass) of traits also decreased at Margaretta following the August-September storm event. However, results differed when these data were used with indices – Shannon and Hill’s Index. Both indices behaved similarly to each other and showed no significant difference between the sites according to the number of traits or the frequency (abundance) of traits. However, the occurrence (species richness) of traits and the frequency of traits (biomass) were found to be significantly higher at Margaretta.

The distribution of samples based on the number of trait modalities (Fig. 4.5) showed less distinct communities than had been found with species abundance data (Fig. 4.1). Although Margaretta and Leverets may have shared functional traits, they both appeared to have functionally distinct communities and this may be explained by differences in the habitat of each site. Both communities' functional diversity, in terms of species richness, abundance and biomass, showed less similarity than distribution based on the number of traits alone (Figs 4.6, 4.7, 4.8). The communities found following the storms were distinct from other communities. The September samples at Margaretta (following the August-September storm event) were most similar to Leverets samples suggesting that this was indicative of a more disturbed community and that the normal community at Leverets was one which was subject to disturbance. This would be supported since Leverets was more subject to wave pressure and abrasion in general, as well as other pressures. The samples after the February storm at Leverets were also functionally different to other samples at Leverets and Margaretta. Similar patterns were apparent when abundance frequency of traits was considered. When biomass frequency was considered, a similar pattern was only found for Margaretta but the February storm appeared not to have affected the functional diversity in terms of biomass at Leverets (Fig. 4.8).

4.4.3 Rao's Entropy

Opposing results for Rao's entropy were found depending whether abundance or biomass was used to calculate the index (H5). Using abundance (Fig. 4.9), Leverets was found to have overall higher diversity (average Rao's entropy) although this was not significant whereas when using biomass (Fig. 4.10), Margaretta had higher diversity. The biomass data support results found by structural indices while the opposing results of both sets of average Rao's Entropy reflect what was found with the functional Shannon and Hill's indices. The results could be interpreted in a number of ways. Firstly, that despite taxonomic diversity being greater at Margaretta, that the two sites have similar functional diversity and therefore finding no significant difference between the sites is accepted. This implies that Leverets maintained good resilience compared to a reference site because it had a good variety of species traits present. However, it is a known property of Rao's entropy that this index can increase with decreasing taxonomic diversity (Botta-Dukát, 2005). This is because this index incorporates both abundance and dissimilarity of species and as species richness increases this can increase the similarity between species and cause a decrease in the index. A similar result was found by Cooper et al. (2008) who found a higher value for Rao's entropy at a site which was intensively dredged compared to a site with lower intensity dredging and a reference site. Furthermore, the average Rao's entropy showed a sharp drop at Leverets station using biomass data following the February storm (Fig. 4.12), which lasted for the subsequent two months. No change at Margaretta was found and this decrease in diversity was not detected using abundance data. Overall, biomass data produced the expected results of finding greater diversity at Margaretta compared to Leverets with all types of data manipulation or index used suggesting biomass may be a more appropriate measure of ecosystem functioning in trait analysis.

When individual traits were considered, there were differences in the quality classifications between the sites; using biomass data, most traits were more diverse at Margaretta. In terms of known disturbances influencing Leverets compared to Margaretta, the interpretation of differences did not always reflect expected results. On the one hand, fragility was found to be higher at Margaretta according to both sets of data; this would be expected due to the potential for exposure to physical disturbance at Leverets. However, it may have been expected that species with a tolerance to salinity showed greater diversity at Leverets since this site is subjected to freshwater input. This was the case according to abundance data but the opposite was found for biomass data. Although, it may be that Leverets had fewer species overall which were tolerant to a range of salinities or to full salinity. Individual traits showed differences in their sensitivity to the February storm at Leverets (Fig. 4.13). Traits which were particularly affected included degree of attachment, propagule dispersal method, burrow depth, fragility and sociability. These traits, particularly degree of attachment, fragility and burrow depth, may be expected to be affected by physical disturbance such as would have been experienced during the storm event. This indicates the potential for using individual traits in identifying particular types of disturbance. However, the expression of the particular trait modalities is likely to be more important in interpreting differences due to particular disturbances than the reduced value of Rao's entropy which can only be higher or lower than a reference. A multivariate analysis may be more useful. Furthermore, the availability of trait information could be an important factor when trying to interpret individual traits and some trait information may not be complete enough to detect differences. This effect is likely to be diluted when considering all traits together in a multivariate analysis.

When average Rao's entropy was calculated using abundance, the strongest correlations were found with size, feeding type, feeding method, living habit and tolerance suggesting these traits were overall the most important factors in distinguishing the sites functionally. In addition, average Rao's entropy was correlated to functional diversity as Shannon Index and Hill's Index. Average Rao's entropy showed low or no correlation to environmental variables while Rao's entropy calculated using biomass showed strong correlation to several sediment variables, reflecting results found using structural indices. When biomass was used to calculate the Rao's entropy and functional diversity, many more traits were highly correlated to each other and to the average Rao's Entropy. This, in contrast to when abundance data was used, suggests that a whole suite of traits are important for assessing the differences in functioning of different sites. Traits which were not highly correlated to others and to the average Rao's entropy were reproduction type, degree of attachment, exposure potential and flexibility. These traits may have been similar at both sites. Overall, using biomass data resulted in expected outcomes suggesting this data is a better indicator of ecosystem functioning than abundance data. These results, therefore, would imply that several traits spanning different functional aspects of the system are important for measuring the functional diversity.

4.4.4 Comparison of structural and functional indices

The overall correlations between structural and functional indices were low although some patterns did emerge (Tables 4.16, 4.17) (H2). The number of traits, and related indices, were strongly positively correlated to species richness reflecting the relationship between species richness and functional diversity. However, this only transferred to other functional indices, such as Rao's Entropy, when biomass data were used and not when abundance data were used. Most structural and functional indices detected a difference between the sites and responded to the February storm (H1). The number of traits and related indices do not seem to be any more useful, and a lot more time consuming to calculate, than measuring species richness. However, there may be other advantages to using traits. Traits are thought to vary less over geographical areas since species identity is not used (Statzner et al., 2001). Indeed, when traits were used to distinguish sites (Figs 4.5-4.8), fewer differences were found between sites than when species abundance or biomass data were used, although the effect was only considerable when number of traits alone was used. Taxonomic structure is also thought to be more sensitive than functional properties to environmental properties (Dolédec et al., 1999, Charvet et al., 2000). This was not evident from this study as functional indices showed similar levels of correlation to environmental variables as structural indices (H4). Additional advantages of using functional indices may be the ability to determine the cause of change in systems by investigating the type of traits affected (Dolédec et al., 1999). This study found some evidence to support this since the traits affected at Leverets following the February storm were traits which would be expected to be affected by physical disturbance. This disturbance also showed that overall functional diversity as well as individual traits responded to the disturbance. However, other stressors such as the difference between the sites in salinity, nutrient enrichment and deposition of river material were not clearly distinguishable from individual traits although diversity of most traits was greater at Margaretta. The overall difference between the two sites was perhaps too great to discern subtle differences due to these disturbances.

4.4.5 RLQ analysis

For the RLQ analysis, the first axis of the correspondence analysis explained only 12.43% of the variation meaning the RLQ analysis could only explain a proportion of this 12.43% variation. This shows the great natural variability in the marine environment and the difficulty in explaining variation using any one method. In the correspondence analysis of species variation (Section 4.3.5.1), most of the Leverets and Margaretta samples were relatively similar with a few replicates from Leverets being different to other samples. The pattern of distribution reflected that found using MDS (Fig. 4.2), however, these samples came from December, January, March, July and August which was not totally consistent with MDS analysis. Some of these differences corresponded to the months before and after the February and August/September storm events, although the differences were generally individual replicates suggesting differences were more due to patchiness in the environment rather than particular environmentally driven trends.

The first two axes of the fuzzy correspondence analysis of the traits explained 22% of the variation (Section 4.3.5.2). Most species were relatively similar, with *Virgularia mirabilis* and *Modiolus modiolus* showing the greatest differences. These are both large, long lived species which explains the difference from other smaller, shorter lived, soft sediment macroinvertebrate species.

PCA of the environmental variables explained 43% of the variation with the first two axes (Section 4.3.5.3). The PCA showed two fairly distinct habitat types for Margaretta and Leverets with finer sediments, richer in organic carbon at Margaretta and Leverets associated with higher surface area mean of grains (SMD) and median grain size. These distinct communities reflect results found by MDS analyses of the structural composition of the communities which also showed distinct communities at the sites.

Co-inertia analysis of the species traits explained 52% of the variation with the first two axes (Section 4.3.5.4.1). The combined species and traits explained more variability than either component alone. The second co-inertia analysis with the environmental variables explained 73% of the variation (4.3.5.4.2). The sites were clearly separated based on environmental variables (Fig. 4.22) and the results reflect the PCA analysis (Figs 4.18, 4.19) which showed the properties of each system. The greatest trends of environmental variables were surface area mean of grains (SMD) in the direction of Leverets on the first axis and percentage organic carbon in the direction of Margaretta along the second axis suggesting that these two environmental properties characterised most the differences between the two sites.

RLQ analysis successfully identified two communities based on functional traits which were influenced by environmental variables (H6). These communities were composed, in general, of species which live for longer, are very sensitive, are active and varied bioturbators and are mainly suspension and detrital feeders at Margaretta while at Leverets, species tolerant to variable salinity, with shorter lifespan, epifaunal or temporary burrowers and opportunistic feeders and predators were found. These community types would imply a stable, undisturbed environment at Margaretta and a less stable, more impacted environment subjected to freshwater influence at Leverets. Furthermore, within these two sites, different communities were found depending on the month. In particular, samples from March were distinguishable at both sites and this would be consistent with previous analyses which have shown the impact of a storm in February on March samples. Communities at both sites showed indications of more tolerant species during this time including opportunistic species, swimmers and quickly maturing species at Leverets and robust, short lived species at Margaretta.

The environmental variables which had the greatest influence were surface area mean of grains (SMD), median, graphic mean, silt content and porosity and to a lesser extent sorting and organic carbon. The overall sediment properties were similar at both sites with both having sorting between 1.00 and 2.00 indicating moderately sorted, graphic mean between 0.00 and 3.5 indicating sand and skewness between 0.1 and 0.3 indicating fine skewed and overall indicating low energy, depositional environments at both sites (Gray and Elliott, 2009). There were differences in variables between the two sites however. SMD and median grain size were greater at Leverets (Solan, 2000). The sand fraction was similar at both sites; graphic mean, silt and clay fraction, porosity, sorting and organic carbon were greater at Margaretta. Leverets was also found to have greater levels of material deposited from the River Corrib system (Solan, 2000). This may be reflected in the functional composition of the sites since Leverets species were mainly epifaunal and surficial modifiers of sediment whereas Margaretta species were made up of deeper burrowers and species which move sediment. The impact of salinity differences between the sites, the effects of the storm and potentially the impact of deposition of river material at Leverets were therefore apparent amongst the distribution of the traits in the RLQ analysis. However, the effect of nutrient enrichment was less clear from the analysis. Higher levels of nitrogen at Leverets may have been reflected by the presence of very tolerant species although this did not seem to be typical of Leverets in general. Similarly, slightly higher levels of organic carbon at Margaretta were not associated with traits which were generally typical of Margaretta. These results may suggest that nutrient enrichment was a less important factor than others in determining the functional composition of these sites. This further supports the potential for using specific trait types to identify particular disturbances.

RLQ analysis also revealed dominant species characterising Margaretta, in particular *Amphiura filiformis* and *Lumbrineris fragilis* but also *Pholoe inornata*, *Owenia fusiformis*, *Philomedes brenda* and *Phoxocephalus holbolli.* These species consist of biodiffuser and surficial modifier type bioturbators and this indicates that conditions at Margaretta were suitable for these species and also that the presence of these species at Margaretta may influence the differences in sediment between the sites in addition to environmental drivers such as sediment deposition at Leverets. It has been suggested that the community of *Amphiura filiformis* at Margaretta strongly dominates the functioning of this system (Solan et al., 2004) and these results would support this.

In general, the most important factors in explaining the variability were those associated with sediment physical properties. All of the evidence – both environmental and functional traits – points to Leverets having a less stable environment with impacts largely due to deposition of sediments and some evidence of impacts due to freshwater input. Despite the possibility of organic enrichment due to discharge into the River Corrib, this appeared not to have an effect on Leverets and indeed, the organic carbon content was slightly higher at Margaretta. The species and functional traits represented suggest that Leverets was subject to frequent disturbances thereby not allowing the deeper bioturbators, longer lived species and larger species to establish while Margaretta contains a rich assemblage of bioturbators which may maintain sediment conditions at this site. This may have implications for the longer term stability, functioning and health of the system. The overall pattern is that of a healthier functioning ecosystem at Margaretta although the source of disturbance at Leverets seemed to come from mainly natural sources and therefore it could be suggested that this site is functioning at a healthy level for the given environmental conditions. Despite explaining a low amount of the overall variation (the three tables of the RLQ analysis explained 88% of the variation explained by species distribution (12%)), the RLQ analysis identified functional differences between the two sites and the corresponding environmental variables allowed potential correlations to be attributed to particular sources of disturbance.

4.5 Conclusion

Two distinct communities were found at the two sites and this was identified using both species abundance, biomass and species trait data. Overall, applying structural indices resulted in similar findings as applying the functional traits analysis. Sediment properties were found to be the most important environmental property separating the two sites and many indices were strongly correlated to sediment properties. This could suggest that these indices are highly responsive to natural environmental properties. However, the sediment properties are confounded with the disturbance regimes impacting the sites, mainly deposition from the River Corrib and periods of high energy at Leverets. Furthermore, these physical disturbances are natural properties of this system and not determined by anthropogenic activity however, anthropogenic activity, including the contamination of river material, is also confounded with these properties which may exacerbate any negative impacts they may have on the benthic fauna. Indices did not show strong correlations with salinity or nutrients although there was evidence of a gradient of these properties between the sites (Solan, 2000).

Most indices found a difference in quality between the two sites. The differences in quality found by indices may have been mainly due to natural disturbance – sediment deposition, rather than anthropogenic disturbance. This would imply that the indices that found a difference in quality classification such as ITI or BQI found an unacceptable level of difference between the sites and were overly responsive to natural variation. Indices such as AMBI which did find a difference in index value but not in quality classification may be more representative of the real situation.

Indices mostly showed a strong impact at Leverets due to the February storm, while Margaretta remained relatively unchanged (e.g. Fig. 4.12). This suggests resilience is much greater at Margaretta than at Leverets and would support findings of higher quality at Margaretta than at Leverets even though overall quality was good at both sites.

AMBI was one of the few indices which did not find a strong correlation between environmental variables and index value. This could suggest that this index is less sensitive to natural variation; however, since the variation may be due largely to sediment deposition it may also be that this index is not as sensitive as other indices to this sort of physical disturbance. Furthermore, AMBI was one of the only indices not to find a significant difference between months at Leverets suggesting no impact was detected by this index due to the February storm. Although it is important to be able to distinguish natural from anthropogenic disturbance, the impact of some natural disturbances such as storms may nevertheless mimic the impact of anthropogenic impacts and therefore it would be expected that indices should detect both natural and anthropogenic impacts, at least those of this magnitude. This result is consistent with Muxika et al. (2005) who found AMBI to be unreliable at detecting physical disturbance.

Applying structural and functional indices mostly resulted in the same overall outcome of quality classification. This could have several implications. Firstly, structural indices (with environmental variables and multivariate analysis as an aid to interpretation) are adequate indicators of overall ecosystem health. This suggests that measuring functional indices for general monitoring purposes would be an unnecessary extra burden for monitoring agencies. However, this study was limited in the number of study sites and the types of disturbances investigated. It is possible other sites could show more of a divergence between structural and functional components of the system. Most indices found relationships with the same environmental variables and these relationships were also reflected in the RLQ analysis. This is encouraging as it shows many of the indices detected trends which were present in the system. On the other hand, this suggests that many indices, even the functional indices are potentially sensitive to natural variation and that calibration of indices is required before they can become useful in ecosystem health assessment.

Despite similar overall results, there are further advantages to using functional traits analysis. Rao's entropy of individual traits (biomass data only) showed that traits did respond in a predictable way to storm exposure while RLQ analysis allowed the characteristic trait modalities and the main factors affecting and separating the sites to be assessed. Functional analysis was therefore particularly important in the interpretation of quality classification results which had been already indicated by structural indices.

However, BTA is still in early stages of its development and several issues surround its use which should be addressed. These issues include choosing which traits to use; the availability of biological trait information; the system of fuzzy coding data; and the use of traits as true indicators of ecosystem functioning. The nature of the traits chosen and the number of traits chosen could affect the outcome of the trait analysis (Bremner et al., 2006c). This would require further study into which traits are relevant to the functioning of the system. The number of traits used is probably most limited by the availability of trait information. A large amount of information is available for some species while very little is available for others and this is likely to be a source of bias in trait analysis. Fuzzy coding data is also a source of bias as degree of expression of traits may be poorly understood and could differ between habitats and populations. Furthermore, functional traits may not be realistic representations of ecosystem functioning and the impacts of stressors on specific traits is not yet well understood. Tests of the relationship between traits and ecosystem functioning have been limited often to a single trait, e.g. production (Tilman et al., 2001, Griffin et al., 2009), and so the effects of other traits are not well known. In some cases a single function and limited number of functional traits may describe the main properties of the system. For example, bioturbation may play a pivotal role in overall functioning and in influencing other traits (Solan et al., 2004). However, although it has not been extensively studied, the effects of multiple functions on overall functioning has been found to have a different outcome than focussing on single functions (Gamfeldt et al., 2008). Sediment properties were found to show the greatest differences between sites in this study and the presence of bioturbators reflected this, however, at the very least, the use of multiple functions aided in the interpretation of the response to physical disturbance from the storm event.

The advantage of using RLQ analysis in the assessment was the simultaneous analysis of all three tables; this added complexity to the analysis but overall aided in the interpretation of the data and identified the functional and physico-chemical characteristics of the sites more clearly than any of the other methods. There are disadvantages to RLQ analysis however, including the inability of the software to cope with missing data.

Further work which could be carried out with this analysis could be to assess differences due to use of different numbers or combinations of traits to find whether additional use of a particular number of traits would make a difference to the assessment. This could further be used to find if some traits are more important than others in the assessment of the system. A greater number of traits may be a safer approach in assessing disturbance due to multiple stressors. However, it is likely that a point would be reached after which it would not be beneficial to continue to add traits. In addition, it is likely that different results would be obtained at different sites and different habitats. Further analysis of traits and functional indices in ecosystem health assessment would complement empirical and theoretical work in biodiversity ecosystem functioning.

The importance of assessing structure and function can be considered from two perspectives, firstly the management perspective which aims to simply monitor the quality and health of the site and secondly from an investigative point of view which aims to understand the factors affecting the site. The first of these may not necessarily require a full understanding of the site and in this case structural indices seem to be a useful first step in the indication of ecosystem health while a functional study may be useful for a more investigative, exploratory study to show a fuller picture of ecosystem health. However, ignoring the functioning of the system in routine monitoring may cause important trends to be missed.

Chapter 5

Indices, variability and uncertainty in quality classification

5.1 Introduction

As previously shown and discussed, there are several sources of uncertainty in the classification of benthic ecosystem health using indices, with unexpected behaviour in some cases. Reasons for unexpected behaviour include complex interactions between species richness and evenness; response to natural disturbance such as storm and weather events; temporal and seasonal variability; and response to salinity. It is important for environmental managers to be able to detect changes statistically, despite background variability, in order to assess system condition (Johnson et al., 2008). One way to improve confidence is to increase sampling (Hering et al., 2010). Ferraro et al (1991) found greater effects due to natural disturbance than to the impact of pollution from a wastewater treatment plant and recommended sampling regimes to assess the impact of pollution should be carried out over the long term (six years or more) in order to distinguish between natural and anthropogenic disturbance. While to distinguish long term trends from short term variability, Armonies (2000) found a much greater spatial scale of sampling (180 km^2) was required in the Wadden Sea where drifting organisms and local differences due to for example, immigration and emigration of populations, patchy spatfall and re-suspension are important factors in population changes; these cause local variation leading to high variability in sampled communities over smaller scales. However, the number of samples required depends on cost and time. For management purposes the minimum number of samples is required to assess an impact and it is not necessary to completely describe a community (Ferraro et al., 1994). While there are many sources of uncertainty and therefore interpretation of the index results can be difficult, one source of uncertainty includes the metric used itself. Other studies have shown some indices to be more responsive to natural variability, influencing the confidence in the conclusions that can be drawn from these. Reiss & Kröncke (2005) found univariate indices such as the Shannon-Wiener Index to be more sensitive to seasonal variability than biotic and multimetric indices such as AMBI and BQI. In another study, univariate indices were also found to be more variable in general than multimetric indices such as IQI due to long-term variability but AMBI and BOPA were found to be the most variable indices overall of those tested (Kröncke and Reiss, 2010).

Under the WFD, environmental managers are required to indicate their level of confidence in the quality classification of water bodies and therefore they need to be aware of the level of confidence associated with the chosen tool. Furthermore, the one-out-all-out principle of the WFD requires each quality element to achieve good status in order for the water body to pass the quality classification. This principle therefore encourages high statistical certainty for each component or there is the risk of over- or under-estimating the quality of the water body (Hatton-Ellis, 2008, Borja and Rodríguez, 2010). Confidence in the quality classification and an idea of the level of uncertainty is also important for managers to be able to defend their quality classification from legal challenges (Hering et al., 2010).

Confidence in the quality classification is not only a legal issue. It is important to be able to detect trends towards disturbance from background variability before a critical threshold is reached, while the system can still recover (Tett et al., 2007). Detecting a trend towards a critical threshold could be difficult as systems can show only small changes before reaching the threshold (Scheffer et al., 2009). A high level of variability inherent in indices will make it impossible to distinguish trends. Methods which can detect trends towards a critical threshold are therefore vital (Håkanson and Duarte, 2008).

Variability in itself has been suggested as a measure of disturbance. Warwick and Clarke (1993) found an increase in variability in macrobenthic communities with increased disturbance which they suggested could be a useful indicator of disturbance impact. The coefficient of variation in populations over time has been used as a measure of variability or resistance (McCann, 2000, Ives and Carpenter, 2007) and coefficient of variation has been found to decrease with increased species richness indicating increased system stability (Worm et al., 2006). Increased variance, increased autocorrelation and slower recovery from disturbances are potential early warning indicators of a system approaching a critical tipping point or system collapse (Scheffer et al., 2009). It is therefore important to measure variability as a property of the system in itself as well as for the purposes of knowing the level of uncertainty associated with a quality classification.

Aim

The aim of this study is to assess the inherent variability of indices and determine the confidence in associated quality classifications.

Null Hypotheses

1. Different structural indices show the same level of variability using reference data.
2. Indices show the same level of variability in impacted and unimpacted sites.
3. Functional indices and structural indices show the same level of variability within and between one impacted and one pristine site.

5.2 Methods

The variability of different indices in response to natural spatial variation or patchiness in the environment was assessed by calculating the coefficient of variation (CV=standard deviation/mean) between replicates in each year for each index allowing the inherent variability of the indices to be compared in a standardised way. This was assessed across years at the NMMP sites (Ch. 2 Section 2.1.2) in order to get a replicated level of spatial variation in normal conditions. In addition, temporal variability of each index was assessed by calculating the CV between all replicates in all years across NMMP sites, although inevitably, this CV would include measures of both patchiness and temporal variability. Pressure data from fish farms (Ch. 3 Section 3.2.1.4); Ironrotter Point (Ch. 3 Section 3.2.1.2); Irvine Bay (Ch. 3 Section 3.2.1.3); and Clyde Upper Estuary (Ch. 3 Section 3.2.1.5) were used to assess how variability changed when samples were subjected to various levels and types of disturbance. Galway Bay data (Ch. 4 Section 4.2.1) were used to assess the difference in variability between structural and functional indices, spatially between replicates and temporally, between all samples in all months. Differences in CV between indices and sites were assessed using Kruskal-Wallis or Mann-Whitney U test and relationships with distance from pollution sources were assessed using Pearson correlation or Kruskal-Wallis for fish farms, carried out using SPSS 18 or Minitab 15.

The impact on sampling regime was further investigated using the UK WFD index IQI by using a sampling formula to calculate the level of error from the mean (L) at the 95% confidence level using the given number of samples collected at NMMP sites (Equation 1). The level of error from the mean, L, indicates the degree to which the measured mean is projected to vary around the expected mean (Håkanson and Duarte, 2008). For instance, a given value of L=0.2 indicates 20% error. Thus, the measured mean should lie within 20% of the expected mean at probability, *t*. This was then used to investigate the number of samples which would need to be taken for different levels of error.

Equation 1 *(from Håkonson & Duarte, 2008)*

$$n = \left(t.\frac{CV}{L}\right)^2 + 1$$

…where n=number of samples taken

t=Student's t (1.96 for 95% certainty)

CV=coefficient of variation

L=level of error from the mean

5.3 Results

5.3.1 NMMP

Spatial Variability

The coefficient of variation (CV) for all NMMP sites across all years showed significant differences between indices with some having a very high level of variability and others low (Kruskal-Wallis, $H=784$, $df=23$, $p<0.001$). The coefficient of variation across years and sites for each index shows Total biomass and BOPA had the highest variability (Fig. 5.1).

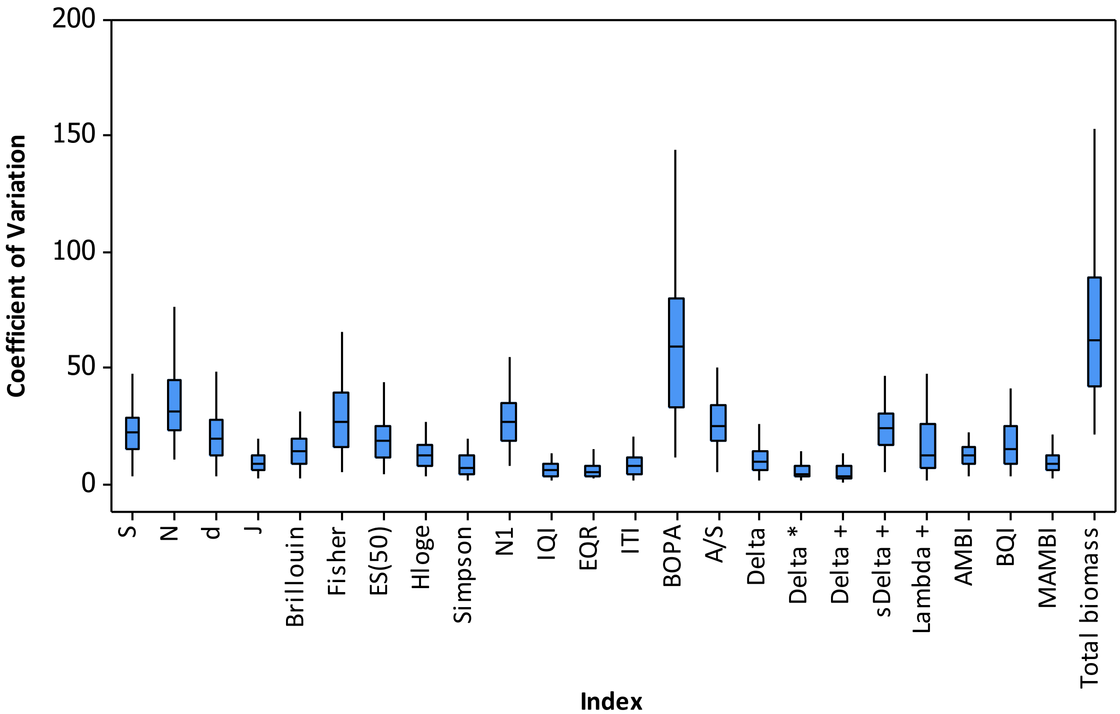


Figure 5.1 Coefficient of variation between replicates across years and sites for each index (n=58 for all indices except Total biomass where n=45).

The CV did vary amongst specific sites (Kruskal-Wallis, H=88, df=6, p<0.001) but the overall pattern remained similar with total biomass and BOPA being high while most other indices, especially Delta+ and Delta*, were much lower (Figs 5.2-5.5). There were differences amongst the sites with some sites having overall much greater levels of variation and others lower for example LIS and KC had higher variability overall and BOPA index at RA had particularly high variation.

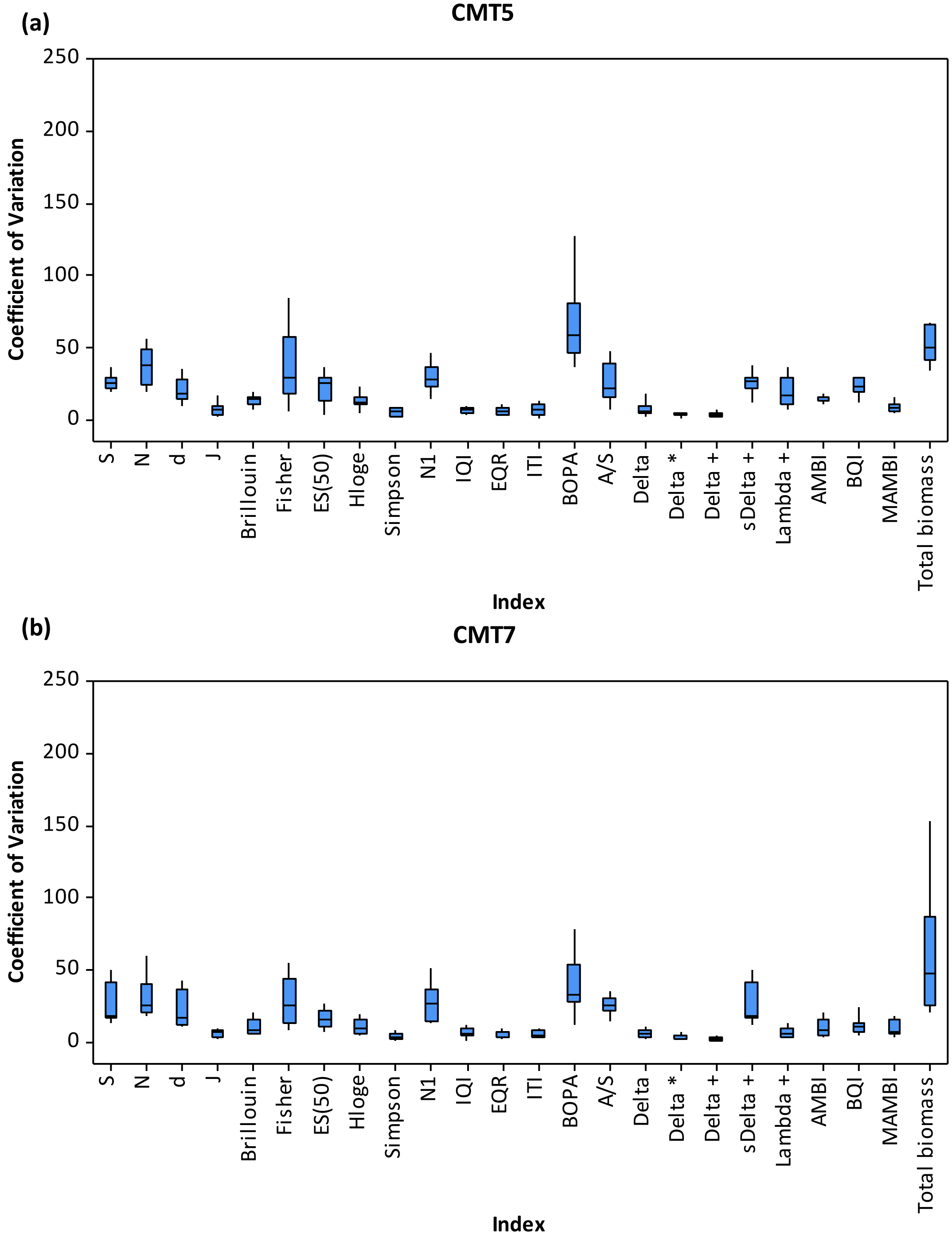


Figure 5.2 Coefficient of variation between replicates across years at CMT5 (a) and CMT7 (b) for each index (n=9 for all indices).

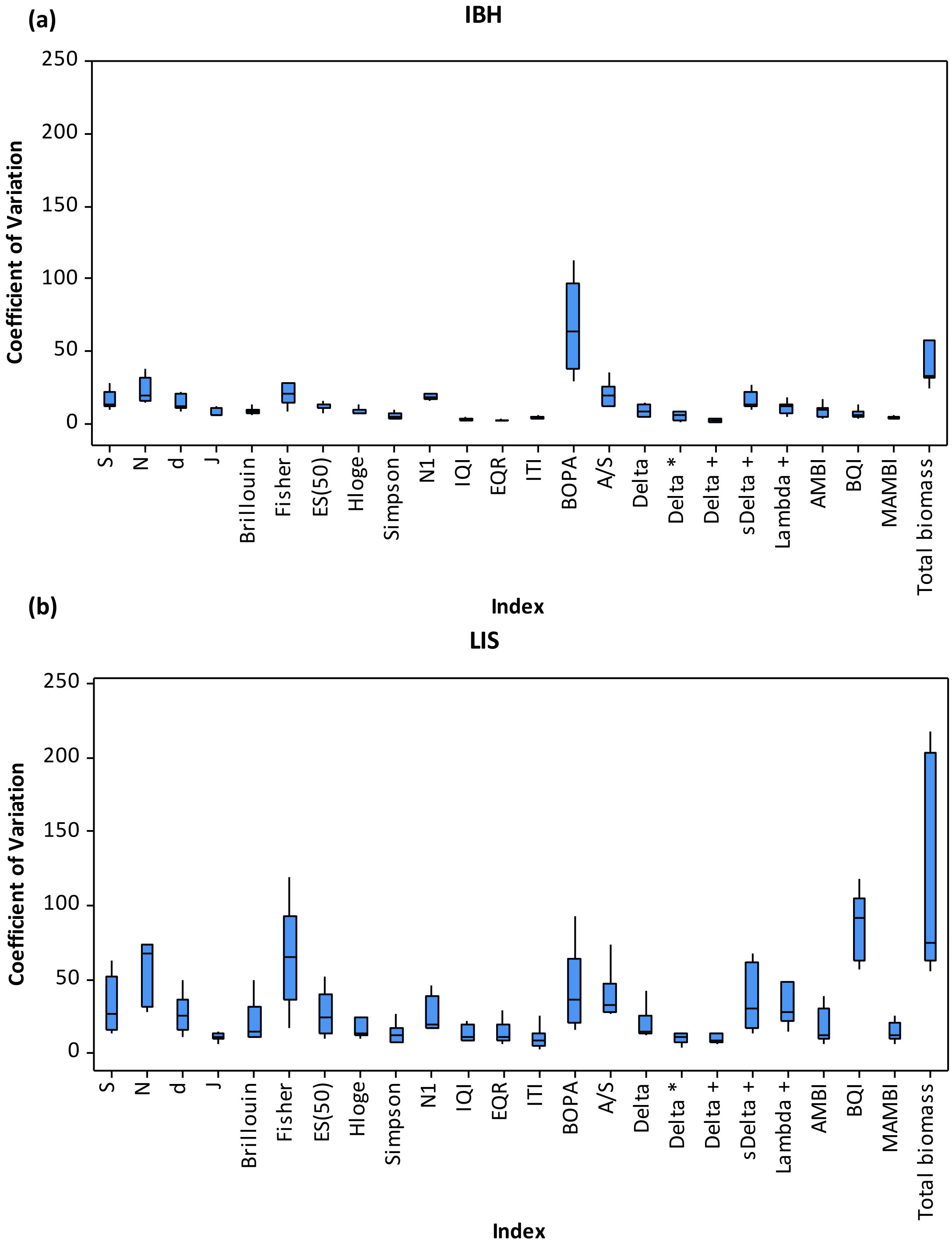


Figure 5.3 Coefficient of variation between replicates across years at IBH (a) and LIS (b) for each index (n=7 for all indices).

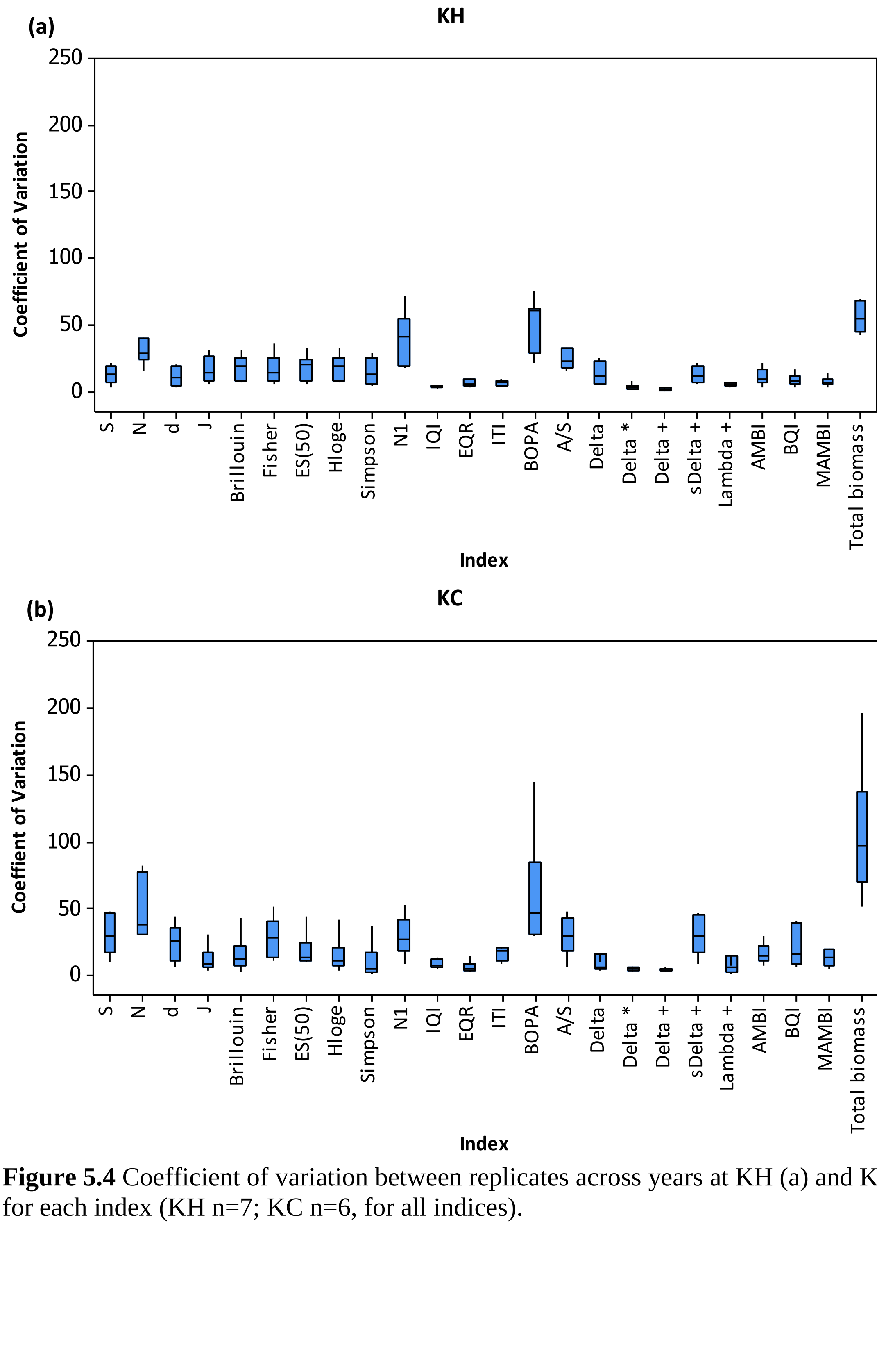


Figure 5.4 Coefficient of variation between replicates across years at KH (a) and KC (b) for each index (KH n=7; KC n=6, for all indices).

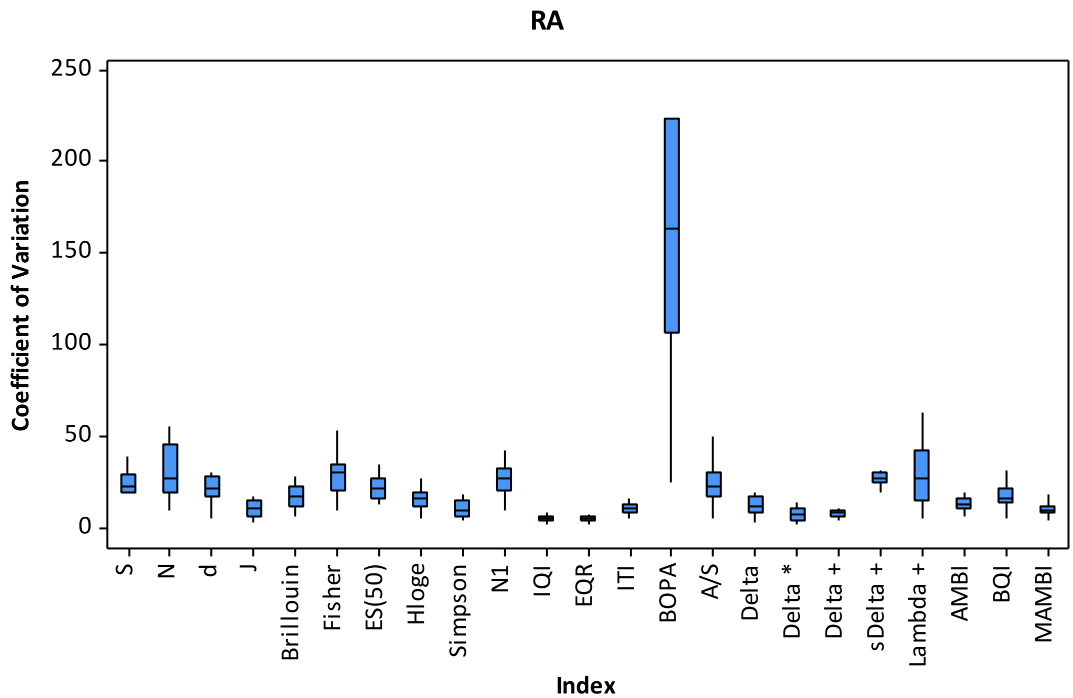


Figure 5.5 Coefficient of variation between replicates across years at RA for each index (n=13 for all indices).

Temporal Variability

Coefficient of variation was significantly different over time between indices (Kruskal-Wallis, H=136, df=23, p<0.001) (Fig. 5.6). The pattern was similar to that found for spatial variation. Total biomass had very high variation and BOPA and Abundance (N) also had high variation. However, the overall CV did not differ significantly between sites (Kruskal-Wallis, H=12, df=6, p>0.05).

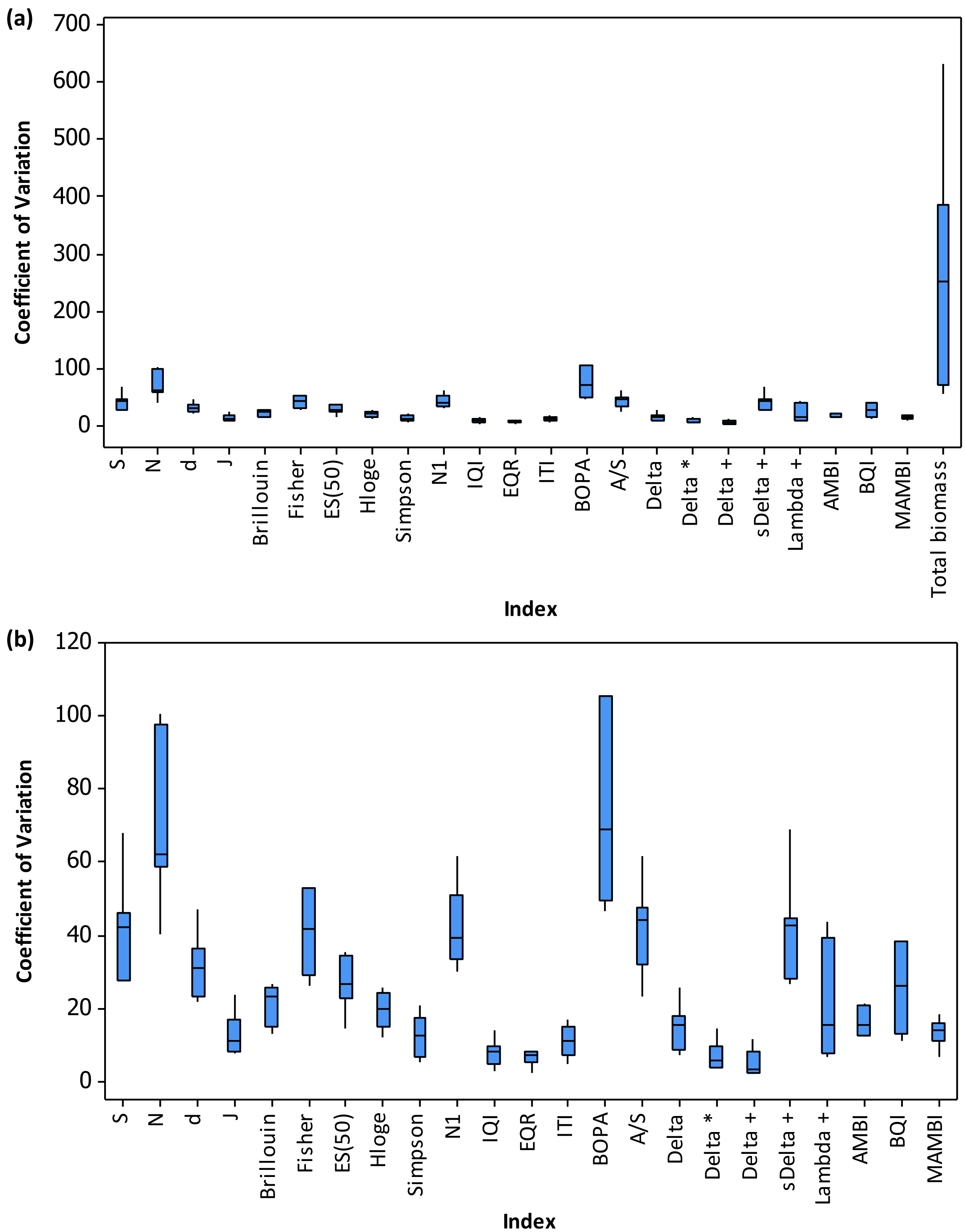


Figure 5.6 Coefficient of variation between all replicates in all years across NMMP sites for each index; including Total biomass (a) and excluding Total biomass (b); n=7 for each index except biomass where n=6

5.3.2 Pressure Data

5.3.2.1 Fish Farms

The CV varied between indices (Kruskal-Wallis, $H=294$, $df=22$, $p<0.001$) and between locations of samples (Kruskal-Wallis, $H=965$, $df=2$, $p<0.05$) (Fig. 5.7). The pattern of the reference samples was similar to that found at the NMMP sites. The pattern of the allowable zone of effect (AZE) samples also followed a similar pattern apart from ITI which showed much greater variability and BOPA which showed lower relative variability. At the cage edge, a similar pattern but slightly higher variability was found for most indices but BOPA showed lower variability again and ITI showed much higher variability compared to the reference.

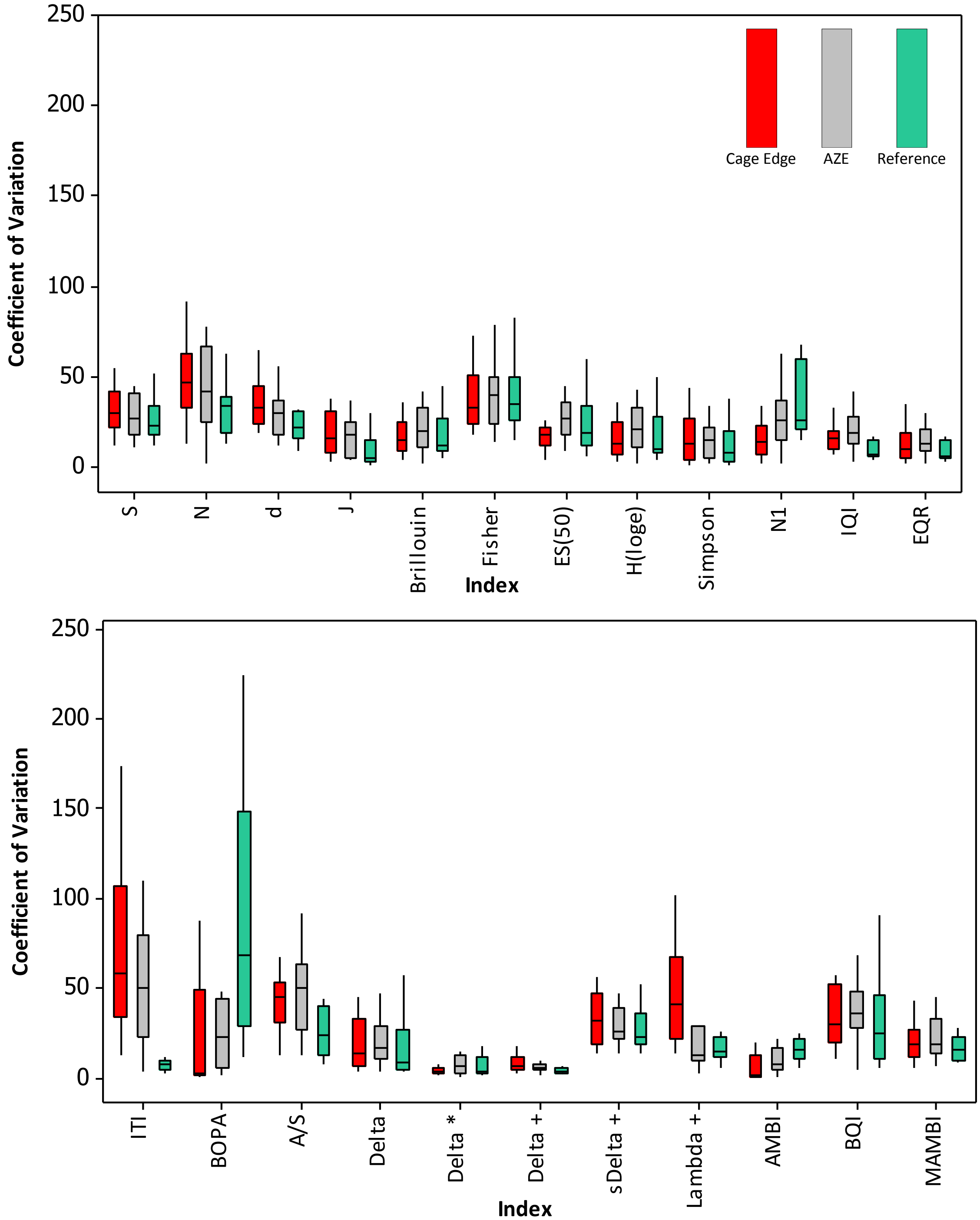
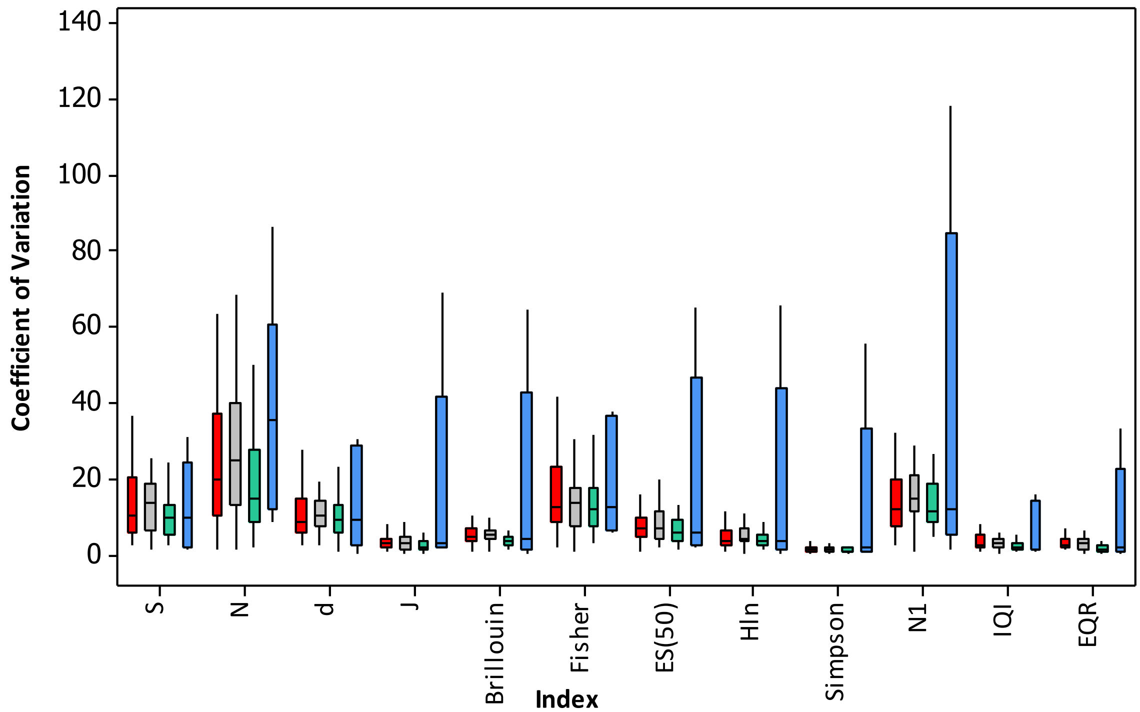


Figure 5.7 Coefficient of variation between replicates across 15 fish farm sites at each location cage edge (n=14), allowable zone of effect (AZE) (n=15) and reference (n=13) for each index.

5.3.2.2 Ironrotter

At Ironrotter Point an overall similar pattern was found as was found at other sites and there were significant differences between indices (Kruskal-Wallis, H=918, df=22, $p<0.001$) (Fig. 5.8). However, the variability did increase over time for many of the indices (Pearson Correlation, r=0.057, n=1794, $p<0.05$) and this would coincide with the increase in disturbance over time (Chapter 3). BOPA and AMBI showed the opposite trend however. Using only data from the 100m samples points (i.e. closest to the outfall), there was high variability for 1998 samples for many of the indices compared to other years (Fig. 5.9).

Variability with distance from the pollution source was calculated excluding 1989 data since this year was before the outfall was constructed (Fig. 5.10). Overall indices showed a decrease in variability with distance from the outfall (Pearson Correlation, r=-0.127, n=1288, $p<0.001$).



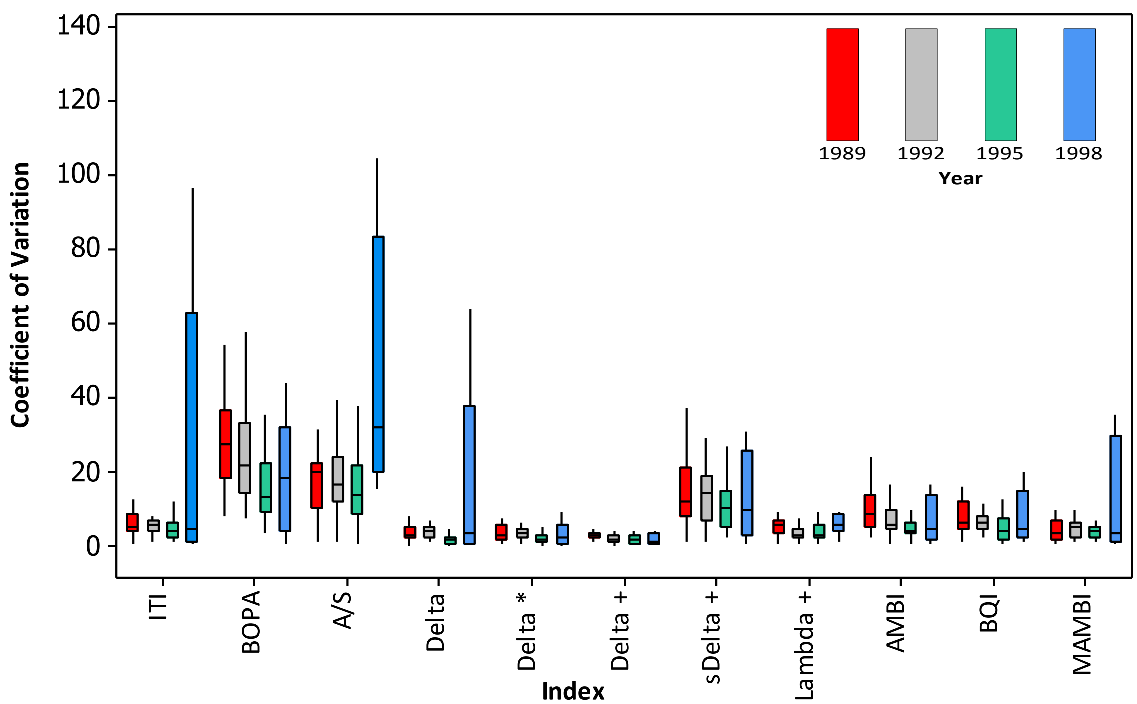


Figure 5.8 Coefficient of variation between replicates across locations for each year of sampling at Ironrotter Point, 1989 (n=22), 1992 (n=22), 1995 (n=28) and 1998 (n=6), for each index.

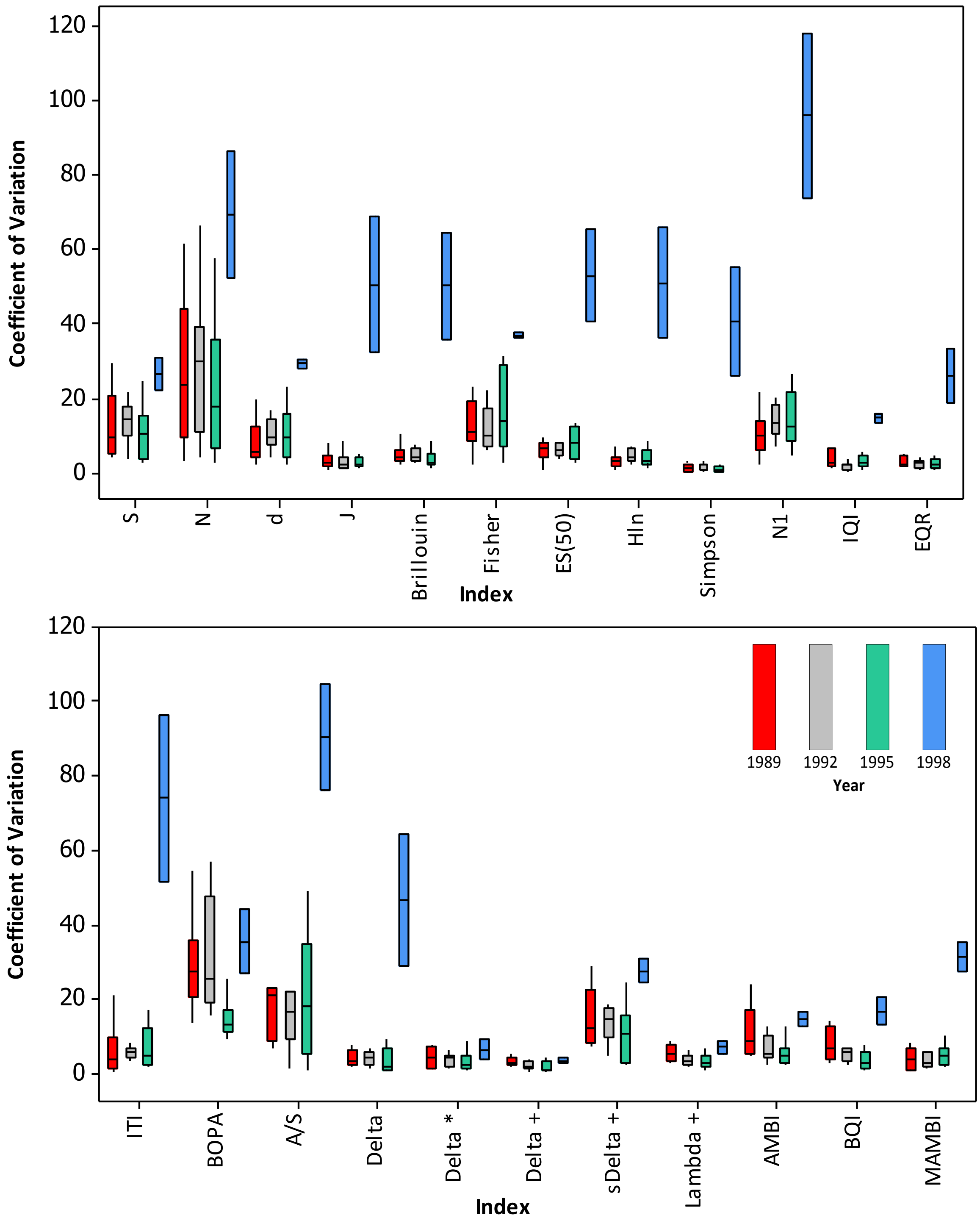


Figure 5.9 Coefficient of variation between replicates across the 100m stations for each year of sampling at Ironrotter Point, 1989 (n=8), 1992 (n=8), 1995 (n=8) and 1998 (n=2), for each index.

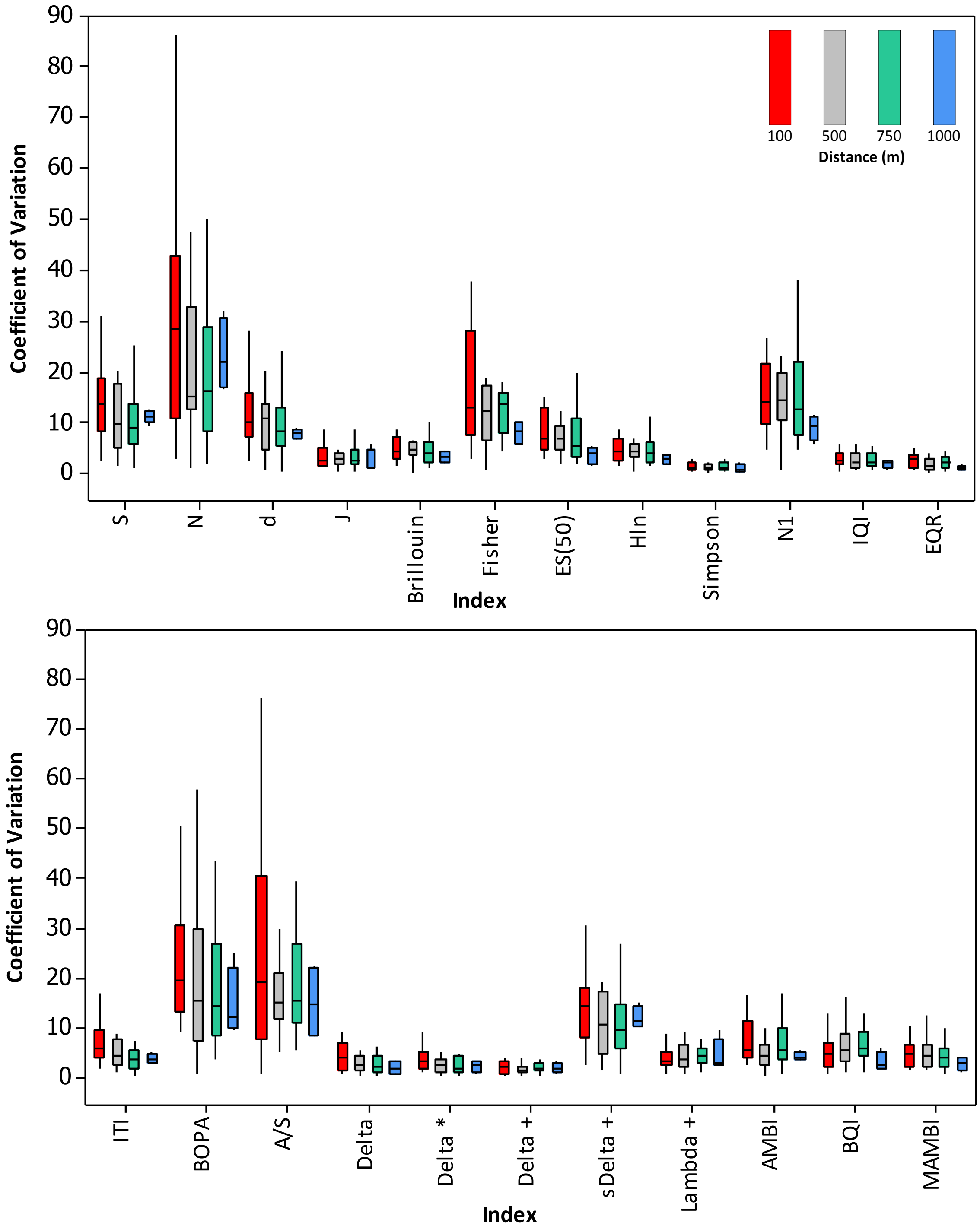
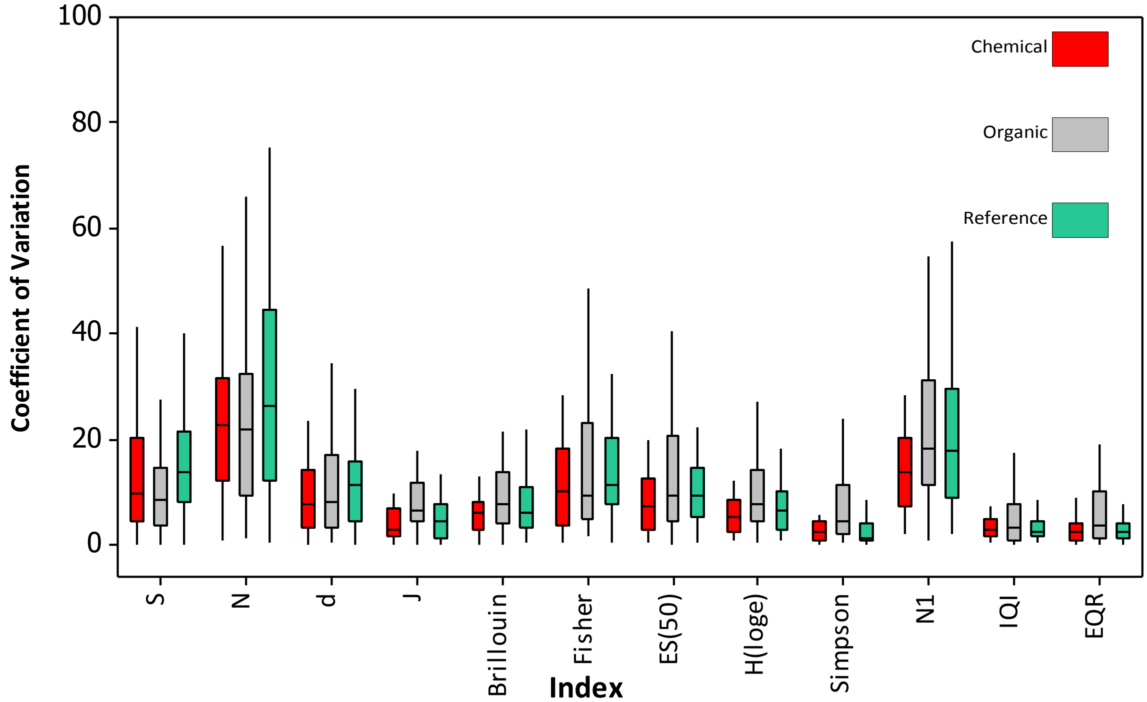


Figure 5.10 Coefficient of variation between replicates across years (without 1989) for each distance from the outfall at Ironrotter Point, 100m (n=18), 500m (n=18), 750m (n=16) and 1000 (n=4), for each index.

5.3.2.3 Irvine Bay

The variability of indices was higher overall at the sites disturbed by organic matter than those influenced by chemical pollution and compared to reference states (Kruskal-Wallis, $H=23$, $df=2$, $p<0.001$) (Fig. 5.11). This was particularly the case for ITI and BOPA. Some years showed much greater levels of variability than others across indices (Kruskal-Wallis, $HT=117$, $df=7$ $p<0.001$). 1981 and 2003 in particular showed higher variation for many indices.



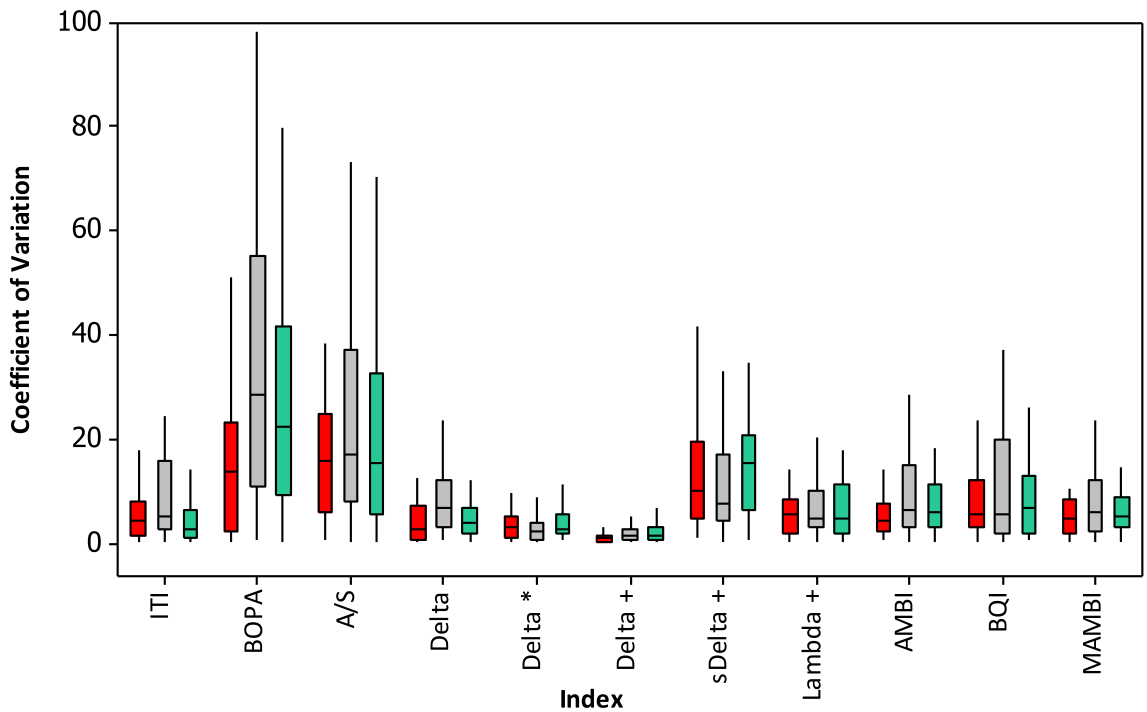


Figure 5.11 Coefficient of variation between replicates across years and transects for each type of impact at Irvine and Ayr Bays, Chemical (n=27), Organic (n=46) and Reference (n=34), for each index.

5.3.2.4 Clyde Upper Estuary

Variability was high at Clyde Upper Estuary, in particular at the very upper reaches and only decreased with distance to levels within the range of values shown at other sites at the 12 and 14 mile sample points (Pearson Correlation $r=-0.16$, $n=1330$, $p<0.001$) (Fig. 5.12). Variability also depended on the year of sampling for many indices (Kruskal-Wallis, $H=41$, $df=6$, $p<0.001$). Overall, lower variability was found in November, December and May while higher variability was found during June and October (Kruskal-Wallis, $H=31$, $df=5$, $p<0.001$).

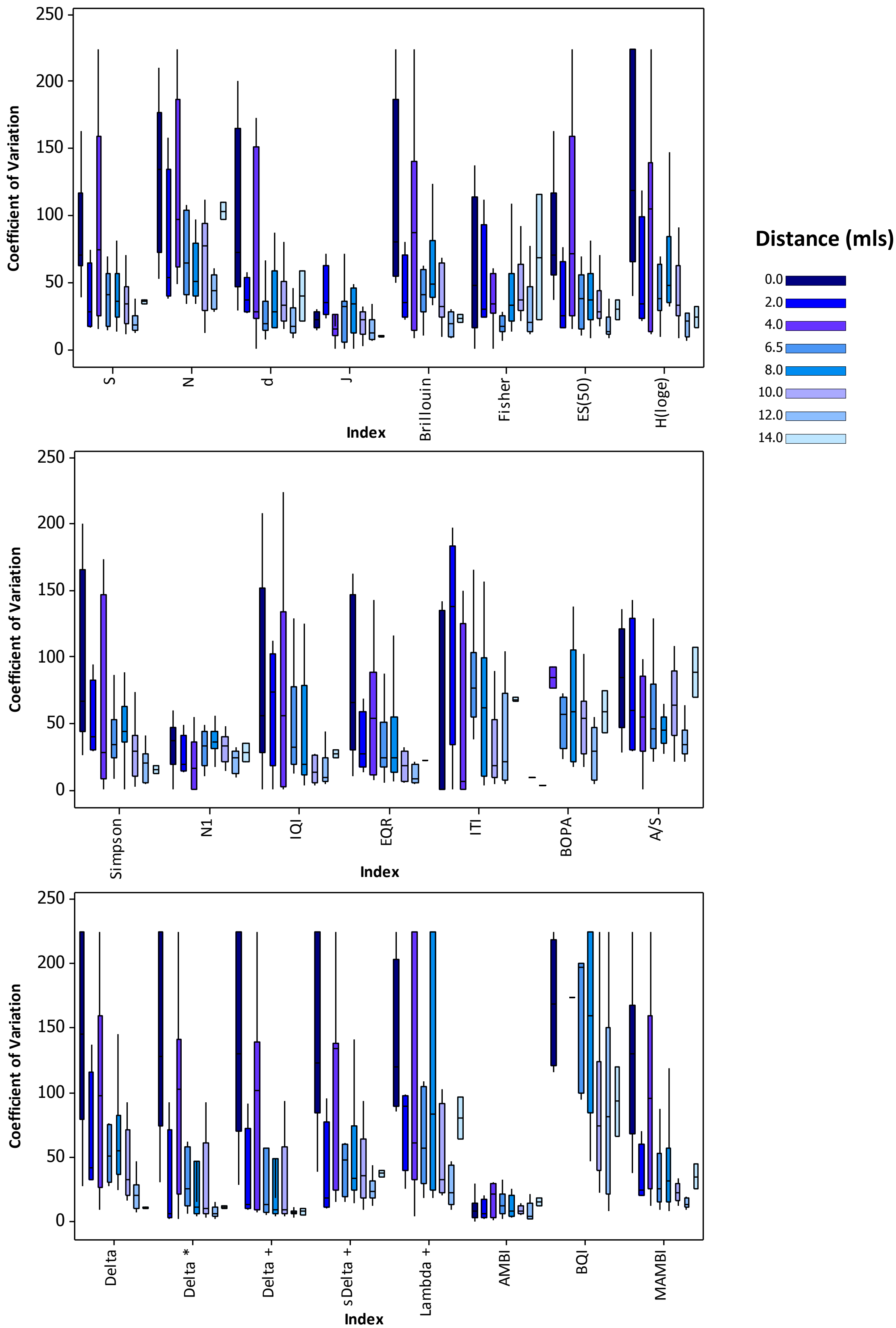


Figure 5.12 Coefficient of variation between replicates across years for each distance along the Clyde Upper Estuary for each index.

5.3.3 Galway Bay

5.3.3.1 Abundance

<u>Spatial Variability</u>

Coefficient of variation was greater for all structural indices at Leverets compared to Margaretta (Mann-Whitney U, U=80219, p<0.001) (Fig. 5.13) but there was no significant difference between sites for functional indices (Mann-Whitney U, U=13067, p>0.05) (Fig. 5.14). Of the functional indices, most showed a very low coefficient of variation but the occurrence of traits (traits*species richness) and traits*abundance were high.

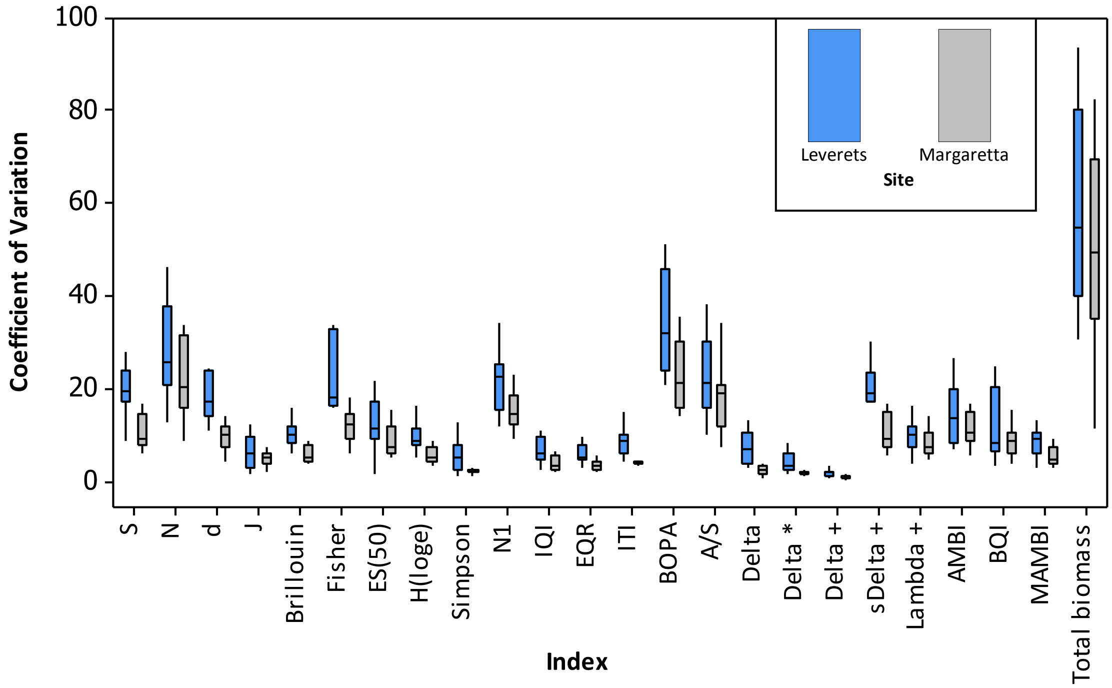


Figure 5.13 Coefficient of variation between replicates across months for two sites at Galway Bay for structural indices calculated using abundance, n=11 for all indices.

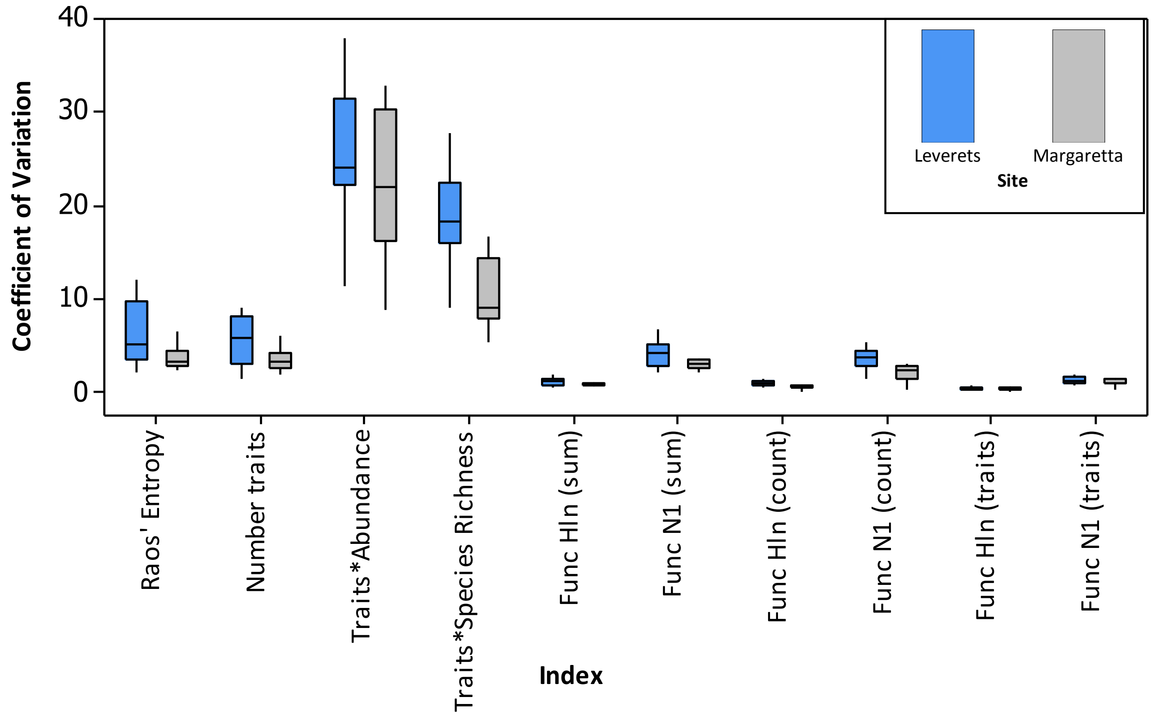


Figure 5.14 Coefficient of variation between replicates across months for two sites at Galway Bay for functional indices calculated using abundance, n=11 for all indices.

The coefficient of variation was higher overall for structural indices compared to functional indices (Mann-Whitney U, U=228004, p<0.001) (Figs 5.15, 5.16). The exception to these were the occurrence of traits and the traits*abundance.

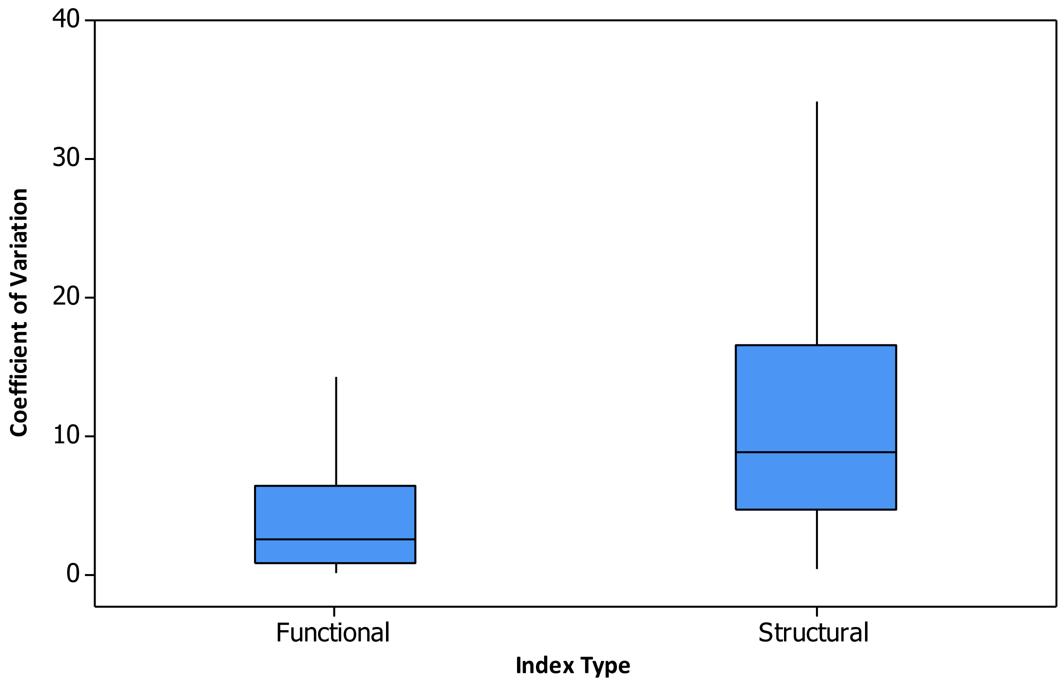


Figure 5.15 Coefficient of variation between replicates across sites at Galway Bay for two types of index calculated using abundance; n=48 for structural and n=20 for functional indices.

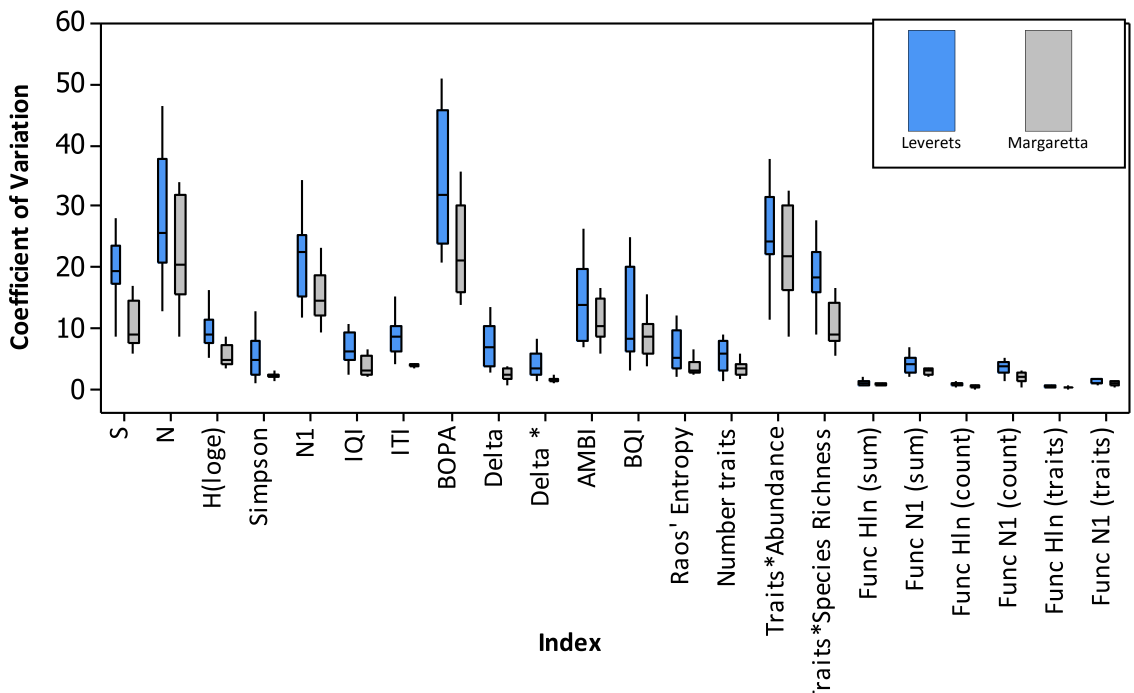


Figure 5.16 Coefficient of variation between replicates across months for two sites at Galway Bay for selected structural and functional indices calculated using abundance; n=11 for all indices.

Temporal Variability

CV varied temporally between structural indices (Kruskal Wallis H=41, df=23, $p<0.05$) (Fig. 5.17) and between functional indices (Kruskal Wallis, H=18, df=9, $p<0.05$) (Fig. 5.18). The pattern of variation reflected the pattern in the spatial variation.

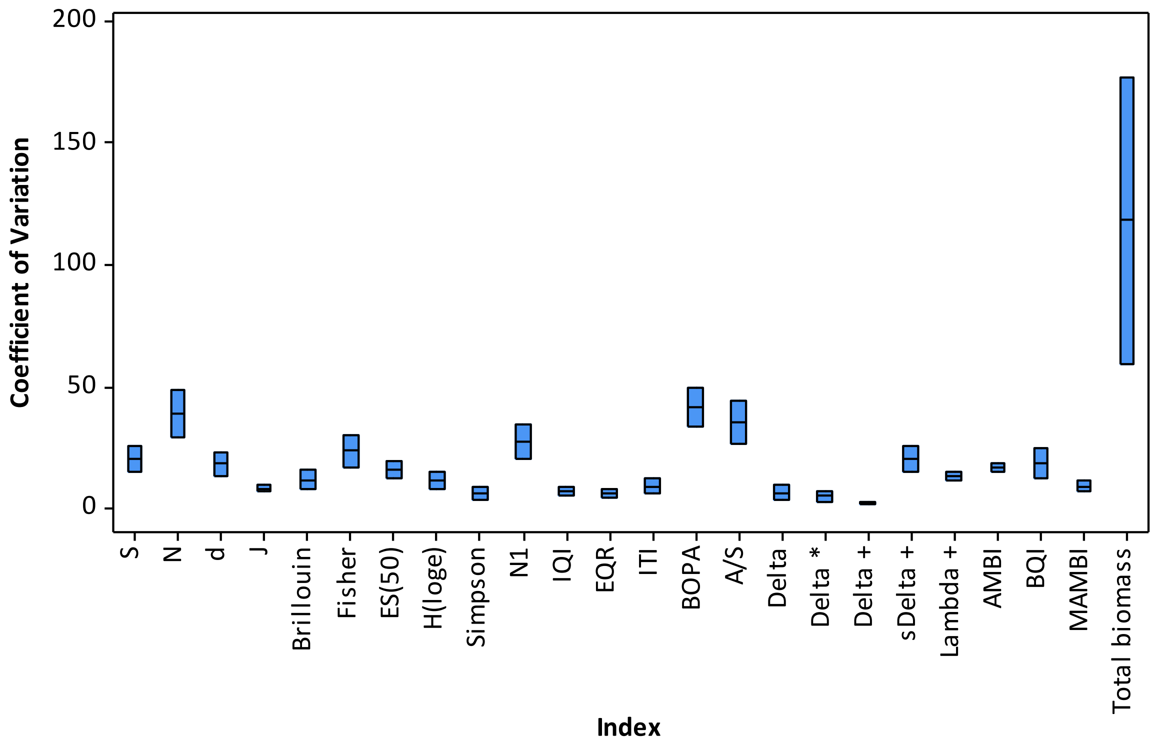


Figure 5.17 Coefficient of variation between all replicates in all months across sites at Galway Bay for each structural index, n=2 for each index

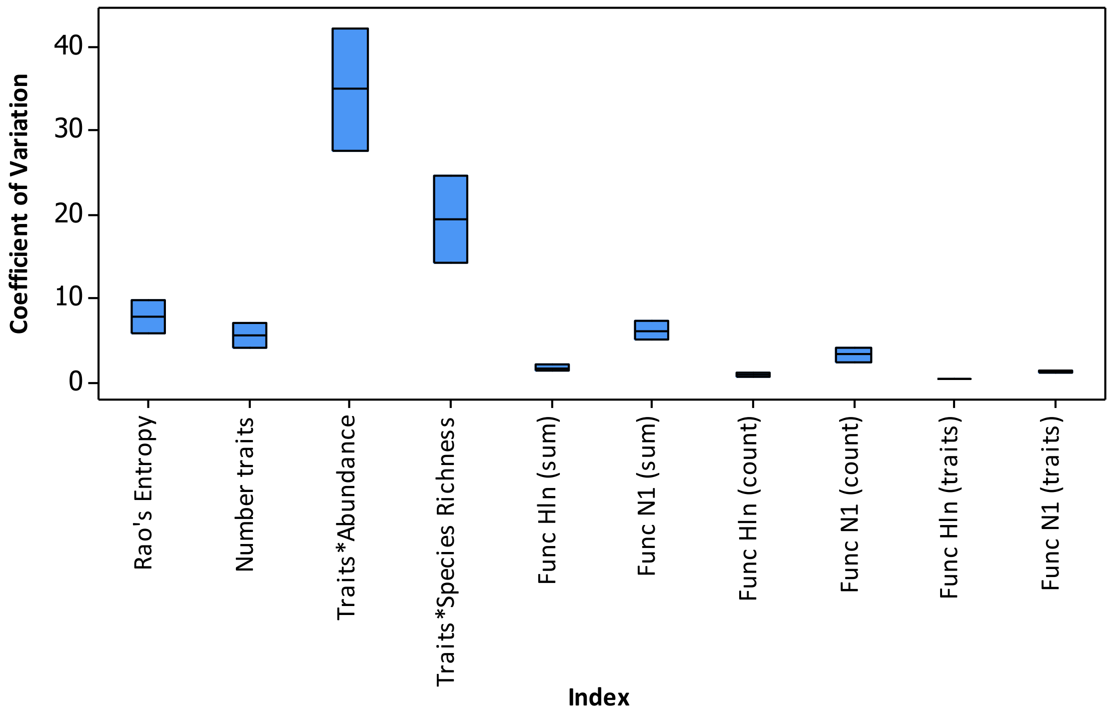


Figure 5.18 Coefficient of variation between all replicates in all months across sites at Galway Bay for each functional index, n=2 for each index

Similar to spatial variation, the coefficient of variation was significantly higher for structural indices compared to functional indices temporally (Mann-Whitney U, U=1919, p<0.001) (Fig. 5.19).

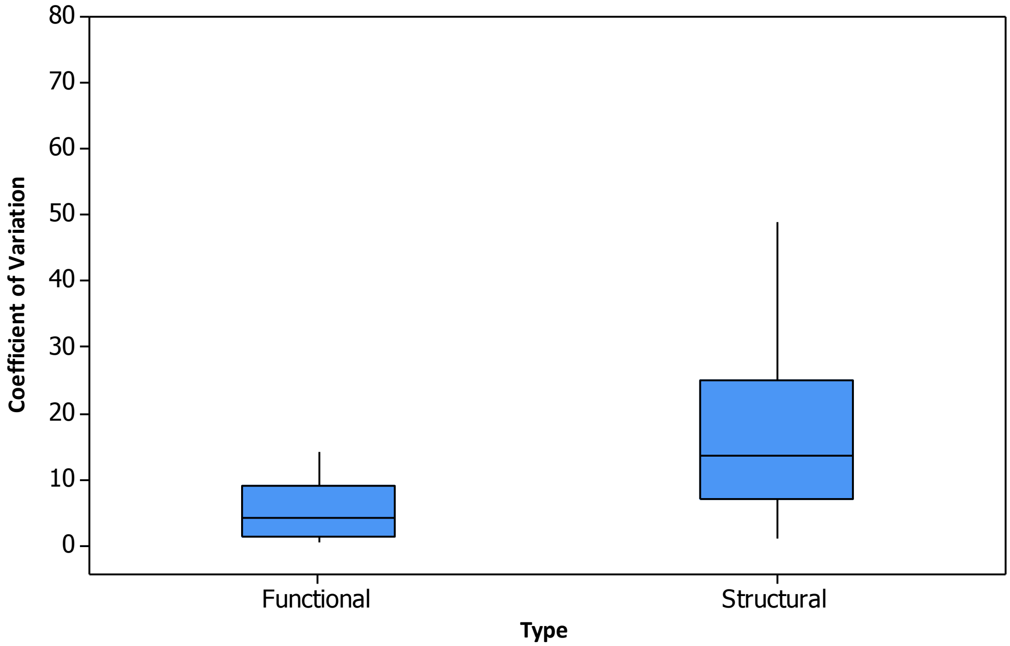


Figure 5.19 Coefficient of variation between all replicates in all months and across sites at Galway Bay for each type of index calculated using abundance data; n= 48 for structural indices and n=20 for functional indices.

The coefficient of variation was significantly higher at Leverets than at Margaretta for structural indices (Mann-Whitney U, U=696, $p<0.05$) (Fig. 5.20) but CV did not differ significantly between sites for functional indices (Mann-Whitney U, U=114, $p>0.05$) (Fig. 5.21).

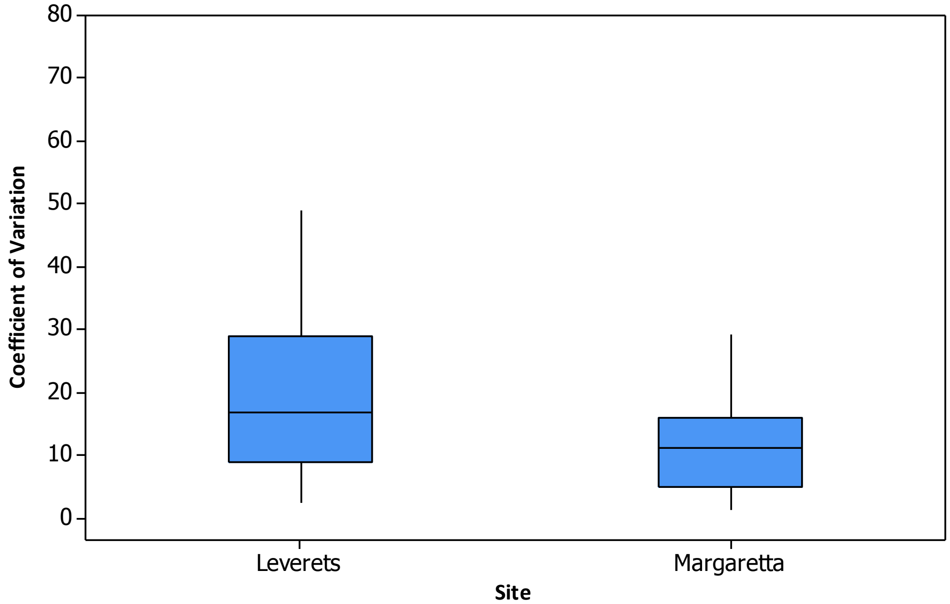


Figure 5.20 Coefficient of variation between all replicates in all months across structural indices for each site at Galway Bay, n=24 for each site

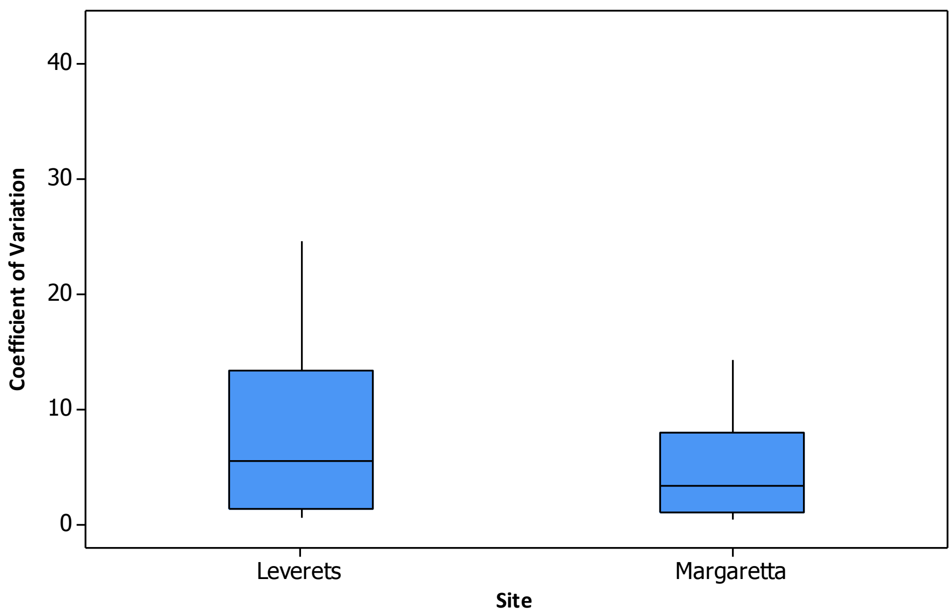


Figure 5.21 Coefficient of variation between all replicates in all months across functional indices for each site at Galway Bay, n=10 for each site.

5.3.3.2 Biomass

Spatial Variability

The coefficient of variation for indices calculated with biomass ($\log_{10}$ x+1 transformed, from Chapter 4) was higher in general than for indices calculated with abundance (Mann-Whitney U, U=398481, p<0.001) but also showed a similar pattern as for abundance based indices, being higher at Leverets for structural indices (Mann-Whitney U, U=45344, p<0.001) but also for functional indices (Mann-Whitney U, U=2414, p<0.001) (Figs 5.22, 5.23).

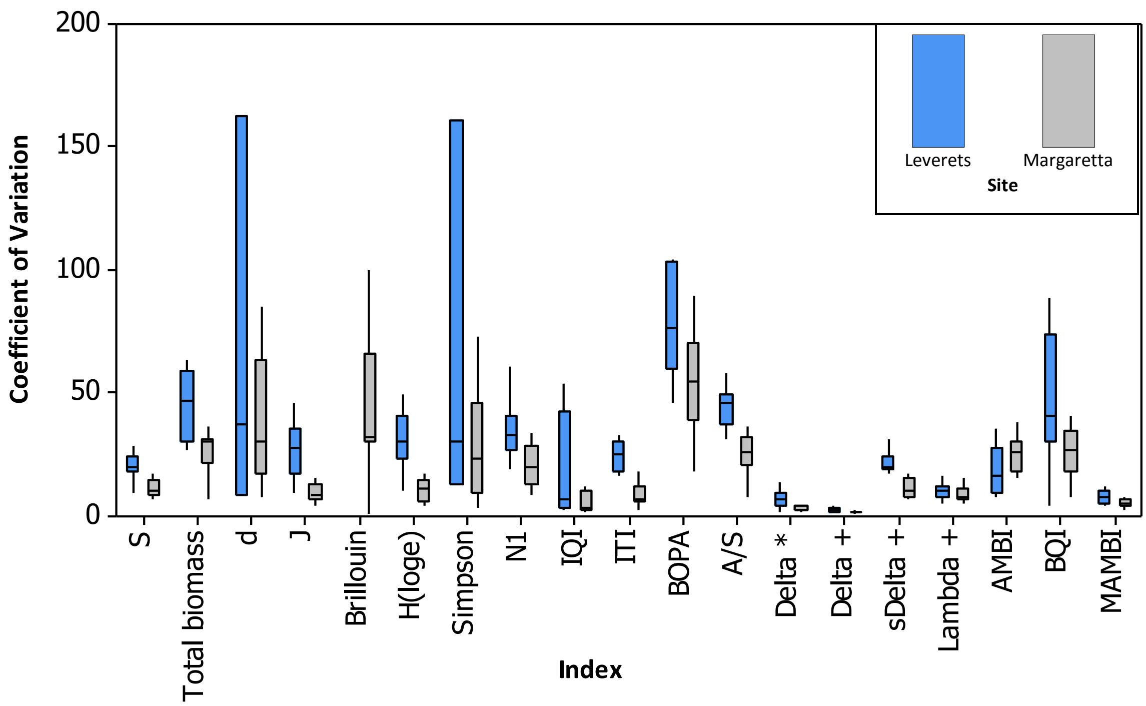


Figure 5.22 Coefficient of variation between replicates across months for two sites at Galway Bay for each index calculated using biomass ($\log_{10}$ x+1 transformed); n=11 for all indices.

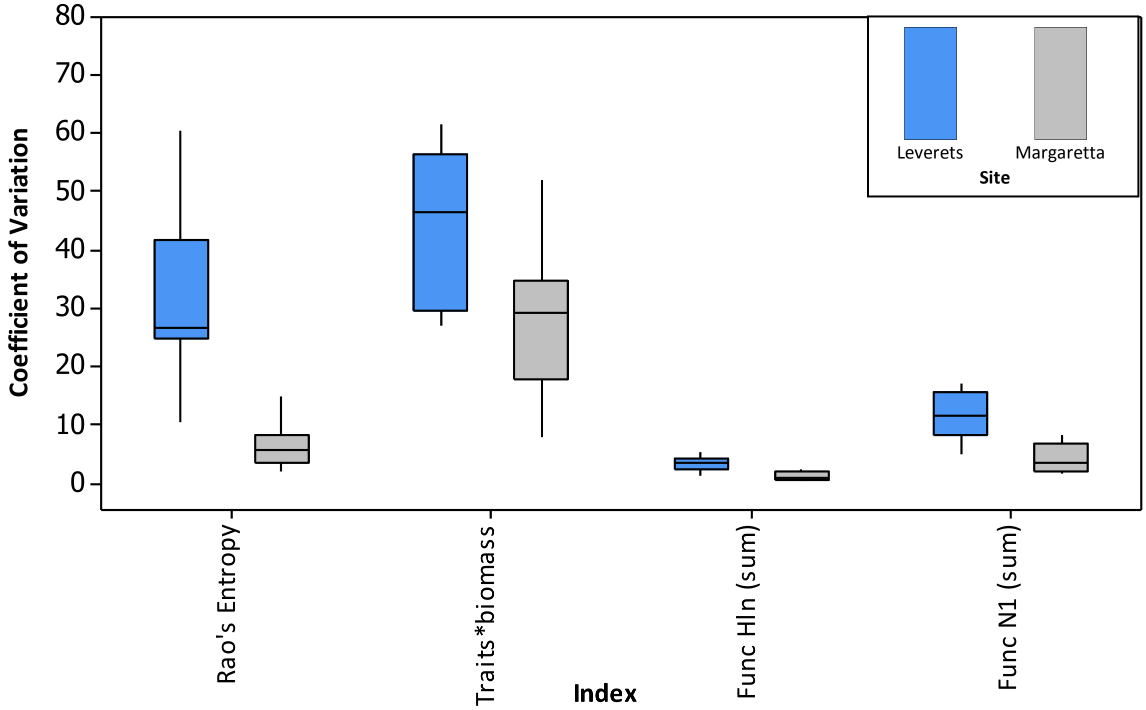


Figure 5.23 Coefficient of variation between replicates across months for two sites at Galway Bay for each index calculated using biomass ($\log_{10}$ x+1 transformed); n=11 for all indices.

Hill's and Shannon functional indices had lower coefficient of variation than all of the biomass-based structural indices while Traits*Biomass was similar to structural levels and the Rao's Entropy was only lower at Margaretta (Fig. 5.24). The variability of the functional indices was lower overall compared to structural indices (Mann-Whitney U, U=108320, $p<0.001$) (Fig. 5.25).

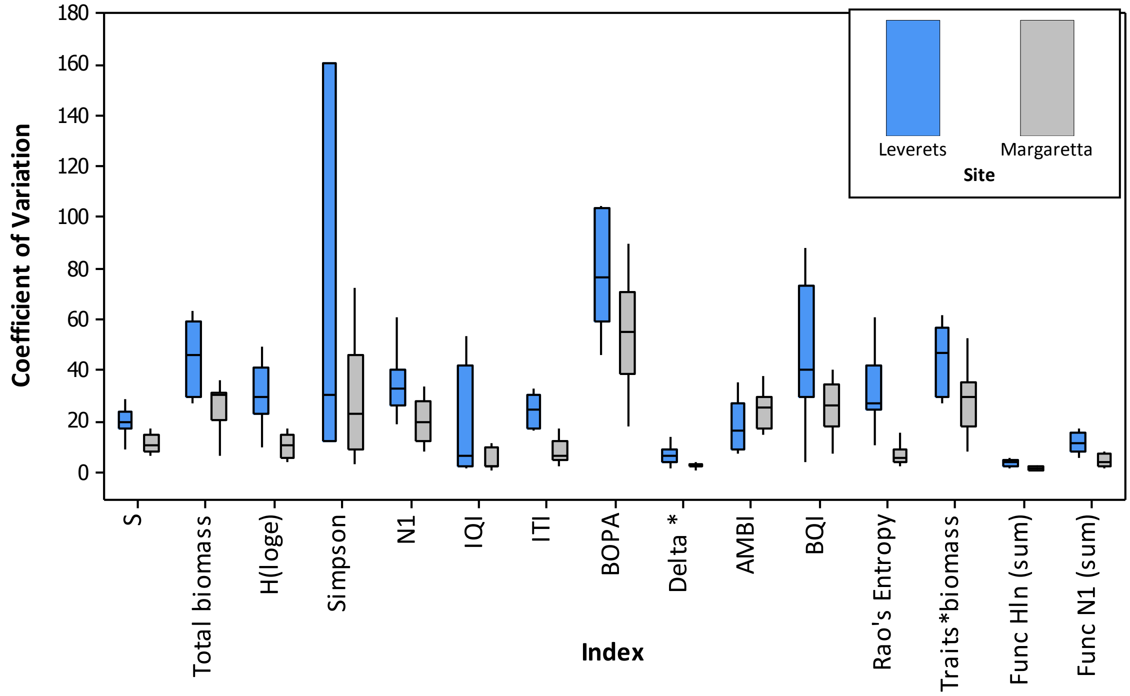


Figure 5.24 Coefficient of variation between replicates across months for two sites at Galway Bay for selected structural and functional indices calculated using biomass ($\log_{10}$ x+1transformed), n=11 for all indices.

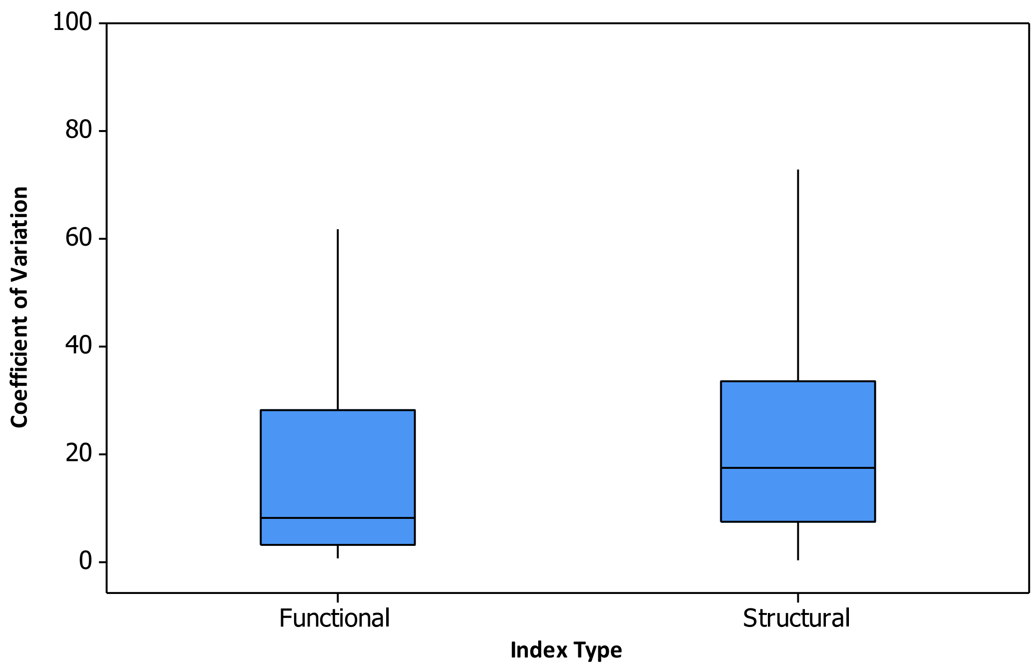


Figure 5.25 Coefficient of variation between replicates across sites at Galway Bay for two types of index calculated using biomass ($\log_{10}$ x+1 transformed), n=40 for structural and n=8 for functional indices.

Temporal Variability

Differences in coefficient of variation of indices calculated using biomass data was similar to that found using abundance data. The CV was significantly higher at Leverets for structural indices (Mann-Whitney U, $U=548$, $p<0.05$) but there was no significant differences between sites with functional indices (Mann-Whitney U, $U=22$, $p>0.05$). However, unlike when abundance data were used, there was no significant difference in the coefficient of variation between structural and functional indices (Mann-Whitney U, $U=1129$, $p>0.05$).

5.3.4 Impact on Sampling Regime

The IQI of CMT7 had an overall ‘good’ quality classification but mean values varied between ‘good’ and ‘high’ over time (Fig. 5.26). Considering also the confidence interval, the classification spanned two quality categories in 1993 and 2002 and three quality categories in 2004.

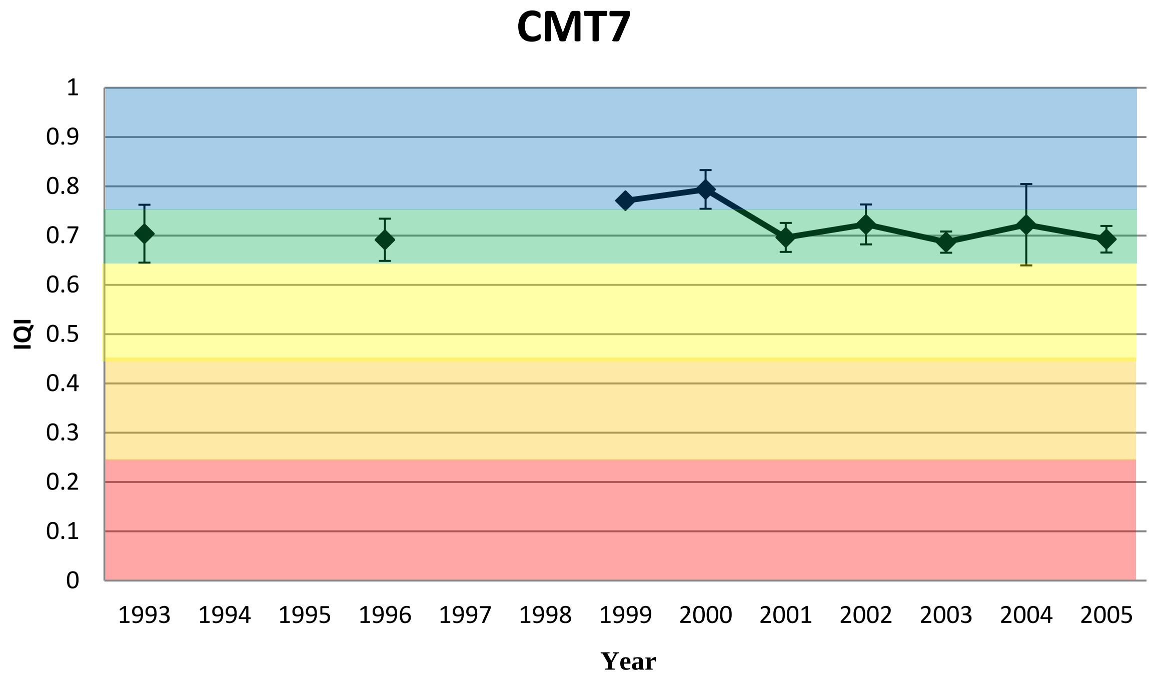


Figure 5.26 Mean IQI values for each year at NMMP site CMT7 with 95 % confidence interval (level of error from mean) (n=9 in 1993; n=5 for all other years). Colour indicates WFD quality classification: blue=high; green=good; yellow=moderate; orange=poor; red=bad (see also Ch. 2, Table 2.1).

Using equation 1, the average level of error from the mean, L for IQI was 6.54% for all NMMP sites. As different sites have different CV values (Fig. 5.27), equation 1 was used to determine the number of samples which would need to be taken for a given level of error from the mean for IQI (Table 5.1). Those sites with a higher coefficient of variation require, in some cases many more samples than would be taken as part of a normal sampling regime to reach the average error level. LIS would require 15 samples while KC would require 7 samples for the average level of error from the mean for IQI.

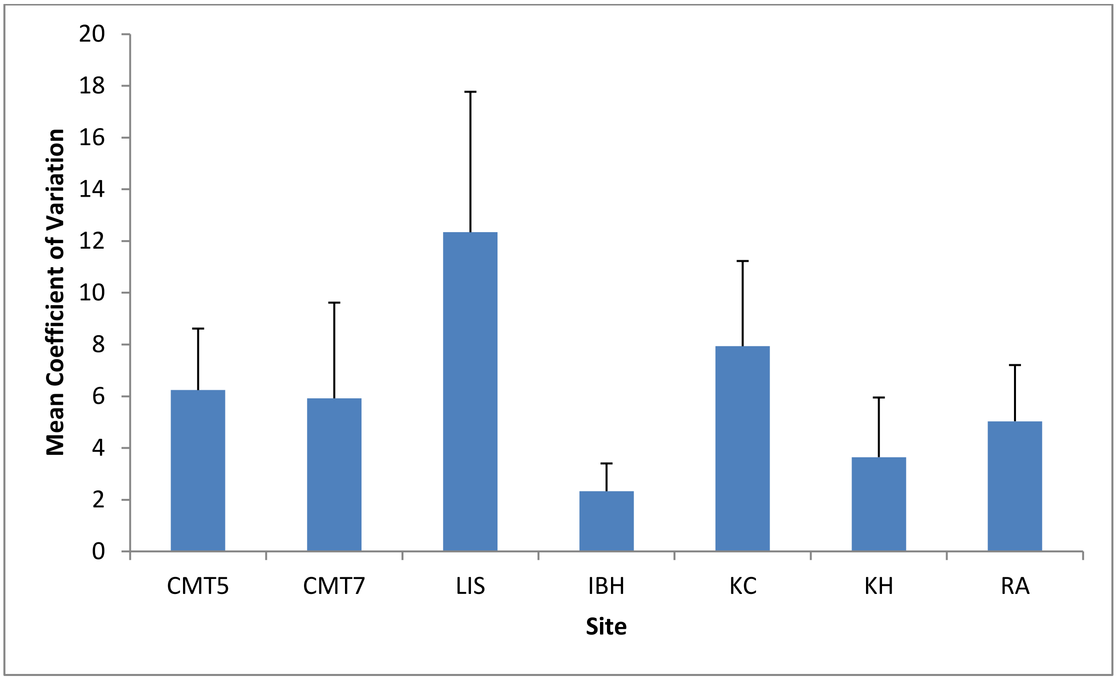


Figure 5.27 Coefficient of variation according to IQI for NMMP sites with standard deviation.

Table 5.1 Number of samples required to achieve a given level of error from the mean (L) for different sites when using IQI.

Site	L=5%	L=6.54%	L=10%
CMT5	7	4	2
CMT7	6	4	2
LIS	24	15	7
IBH	2	1	1
KC	11	7	3
KH	3	2	2
RA	5	3	2

5.4 Discussion

5.4.1 NMMP Sites

The coefficient of variation measuring spatial variation differed amongst indices with some having very high levels of variation while others had relatively low variation between replicates. This shows that the indices were responding to natural spatial variation or patchiness in the environment to different extents and we can be more confident in the classification of those indices with lower coefficient of variation than those with very high variation. While most indices are able to discriminate between different levels of disturbance (chapter 3) this is achieved with higher or lower degrees of confidence. Those indices exhibiting very high variation may be too variable to detect differences between sites statistically where they do exist, leading to Type II errors. BOPA, total biomass and abundance were all at the higher end of the scale indicating these indices are highly susceptible to small scale spatial variation (Fig. 5.1), while Taxonomic distinctness (Delta*), Average Taxonomic Distinctness (Delta+), EQR and IQI had the lowest variability indicating a low level of response to patchiness with these indices. Similar patterns were found in the index response to temporal variation, although this variation also includes patchiness. Kröncke & Reiss (2010) found similar results to this study with univariate indices being more sensitive to long term variability than multimetric indices such as IQI. In the same study it was found that BOPA had high variability, as was found in this study, although they also found AMBI to have high variability but in this study AMBI had low to moderate variability relative to other indices. It may also have been expected that measures of taxonomic distinctness would be less variable, as found here, since these indices are less sensitive to sampling effort than other indices (Magurran, 2004), and since these indices should be less sensitive to species replacements due to natural variability and more influenced by changes at higher taxonomic levels (Bevilacqua et al., 2011).

However, while we may be more confident in those indices which have a low coefficient of variation these results were derived in the absence of significant impacts. It is also important for the utility of the indices that a low response to natural variation does not indicate a low sensitivity to disturbance or change in the environment. For example taxonomic distinctness, which had a very low coefficient of variation, has been found in this study (Chapter 3) and in other studies (Salas et al., 2006, Bevilacqua et al., 2011) to have a low sensitivity to disturbance compared to other indices. This implies that although these indices have a low responsiveness to natural variation, they may also have low response to the types of environmental changes which need to be detected. Furthermore, while in one study multimetric indices, which also had low coefficients of variation, were found to absorb the effects of long term variability, such as changes in recruitment success of non- opportunistic or sensitive species (Kröncke and Reiss, 2010), these indices have been found to mask some trends due to the combination of opposing indices within them (Chapters 2, 3); this masking would also lead to low variability of these indices.

Patterns of variation between different indices were fairly consistent across sites, but there were still pronounced differences in overall levels of spatial variability across indices between sites. Classifications need to take into account the natural variability of sites and the coefficient of variation could be a potentially useful measure of this (Håkanson and Duarte, 2008). There will be lower confidence in the index classification of some sites compared to others. However, while patchiness differed between sites, there were no differences in overall temporal variation between sites. This may imply that when variation is considered on a larger temporal scale the level of variation is comparable across sites, and classification of sites may benefit from being placed in the long term context of monitoring at the site.

5.4.2 Pressure Data

Some indices performed differently in response to natural spatial variation in disturbed sites compared to reference sites. Most indices showed higher levels of variability in the most impacted sites e.g. at the fish farm cage edges. The increase in variability over time at Ironrotter Point, as the site became more impacted, and decrease with distance from the sewage outfall also supports an increase in variability with increasing disturbance. At Irvine Bay, the greatest variation was found in the sites impacted by organic pollution compared to other sites. Other studies have also found variation to increase with increasing disturbance (Warwick and Clarke, 1993) and the coefficient of variation has been used as a measure of resistance and ecosystem stability (McCann, 2000, Ives and Carpenter, 2007). An increase in variability in a site over time could potentially be monitored as an indication of increased disturbance and as an indication of ecosystem function.

Variability in the Clyde upper estuary was very high, particularly in the upper most reaches where freshwater influences were higher, reflecting the poor performance of most indices at this site (Chapter 3). Variability was particularly high at this site sincesome replicates contained no individuals, especially in the upper reaches, suggesting the substrate may have been unsuitable for sampling with grabs. Variability differed depending on the year and the month. This could be because of differences in population dynamics such as recruitment and further reflected the sensitivity of indices to natural variability.

The higher variability in more disturbed sites may reduce the confidence in the index classification at these sites. It will be particularly pertinent at the good-moderate boundary as the variability may increase as the site tends towards moderate quality but the confidence in the index classification may decrease. Quality categories should potentially be larger at the disturbed end of scale and smaller at the higher end to allow for the greater variability in disturbed sites. This is already the case with indices such as ITI and IQI which have larger categories for poorer quality but the opposite is true of AMBI and BOPA which both have very large ‘good’ categories where the variability should be lower. However, while the variability in terms of properties such as abundance may be high in lower quality sites, these sites may have the most predictable species occurring so that indices such as AMBI, which take species identity into account, may perform well despite the high variability. Indeed, it was seen that indices tended to be most in agreement with quality classifications in the worst quality sites (Chapter 3). Although, this is only true of the worst quality sites and not moderate quality sites which may have both higher variability and less predictable species.

5.4.3 Galway Bay

At Galway Bay, spatial variation was higher at Leverets when structural indices were used, reflecting what is known about Margaretta being a very stable site and indicating higher variability at the lower quality site, although differences could also be due to natural heterogeneity of Leverets. Temporal variation was also found to be higher at Leverets than at Margaretta when structural indices were used. The spatial and temporal variation of the functional indices was lower overall than the structural indices. This may be due to the high variability associated with species substitutions occurring but lower variability associated with traits which are common to all species. Other studies have also found measures of functional diversity to be stable in relation to natural variability (e.g. Statzner et al., 2001). Gamito & Furtado (2009) found the Shannon-Wiener Index to work well when used with feeding groups rather than species abundance as using functional groups reduced the variability associated with rare species making the index much more useful. This study also found Shannon-Wiener functional index to have much lower variability than its structural counterpart. Only the trait based indices which were related to structural properties – species richness, abundance and biomass, had high variability. However, function may be more resistant than structure to environmental perturbations (Odum, 1985) and therefore may not be sensitive as early warning indicators (Paul, 1997). The sensitivity of these indices to disturbance still needs to be tested as mixed results were found in this study (Chapter 4).

The coefficient of variation was higher for indices calculated using biomass, even though biomass data were transformed, but most of these indices are not designed to be used with biomass. Although, transformation did reduce the variability of raw ‘total biomass’ data (Fig. 5.13) compared to the transformed version (Fig. 5.22). The variability of functional indices was also higher with biomass. Biomass seems to be a more variable trait than species abundance. Despite the high variability of biomass, differences in quality were found with biomass compared to species abundance between these two sites (Chapter 4). However, it is not completely clear if the differences between these sites was largely due to natural disturbance and variability and therefore this would imply that biomass may not be an appropriate measure to use with indices after all, but this would require further testing in a greater number of sites with a range of quality statuses.

5.4.4 Impact on sampling regime

The variability of replicates at a given site has implications for the sampling regime. If the mean value of the index with confidence intervals crosses several quality categories, these quality classifications cannot be ruled out, reducing the confidence in the index classification. With an index such as BOPA, due to the very high coefficient of variation, there would be very low confidence in the outcome if only one or a small number of replicates were taken. The higher the coefficient of variation, the greater the number of samples required to achieve a given level of accuracy. It therefore does not make sense to use an index with a very high coefficient of variation for regular monitoring and environmental management. Even those indices with a low coefficient of variation, such as IQI, still exhibited a certain level of variability which could become of greater importance in sites which are more variable. It may be required that a given level of error from the mean is deemed acceptable in a monitoring programme but any level of error could cause the quality classification to cross into two or more quality categories. To achieve the average level of error of the IQI in LIS and KC a number of samples far greater than would be practical in a normal sampling regime would need to be taken. Therefore both the index and the site being sampled need to be considered in concert when classifying quality.

5.5 Conclusion

The indices which have low variability and high sensitivity to disturbance are most useful in determining quality classification. Functional indices showed much lower variability than structural indices although the sensitivity of functional indices to disturbance still needs to be tested. Even those indices at the lower end of the scale still had a certain amount of variability associated with them and this differed in different sites. These differences in variation need to be taken into account either through the sampling regime or through the level of confidence assigned with the index classification. The impact on indices due to variation in abundance may be reduced by pre-treating data with transformation such as carried out for AMBI by Warwick et al. (2010) and by Muxika et al. (2012). This could be explored in future studies for other indices also; there was some evidence in this study of transformation reducing the variability of total biomass at Galway Bay sites, although some indices will nevertheless have greater variability than others. As suggested previously, longer term studies (Ferraro et al., 1991) or larger scale studies (Armonies, 2000) may be more appropriate to detect real differences in sites and overcome natural variability. However, monitoring is usually constrained by time, cost and reporting limitations and ideally methods are required which can detect changes over short timescales and localised areas. There is still difficulty in interpreting the results of a one-off study due to the variability of the benthic community and tools available for their measurement. Those indices which had very high variability may not achieve statistical certainty and this could have legal, financial and environmental consequences, due to obligations to the WFD (Hatton-Ellis, 2008, Borja and Rodríguez, 2010, Hering et al., 2010), and in detecting small trends of disturbance which could lead to a critical tipping point (Scheffer et al., 2009). In order for the minimum number of samples to be required and the level of confidence to be assessed, the index used, the variability of the site, and the type of impact all need to be considered.

Chapter 6

Discussion

6.1 Introduction

This study analysed indices over several types of study sites and stressors and showed that indices vary in their behaviour in different circumstances and that the response of indices is often unpredictable and contradictory. Indices, by their very nature, reduce the amount of information imparted while methods which retain more information such as multivariate analysis showed trends which were not detected by indices. The use of a combination of indices and methods allowed a picture of the state of the ecosystem to be developed. This picture was often not very clear and could be open to interpretation. Sometimes the use of several indices or methods together confused the assessment. Indices ideally should reliably detect disturbance; discriminate between anthropogenic and natural disturbance; distinguish different levels of disturbance; and be applicable in different areas and circumstances. None of the indices tested fulfilled all these criteria. However, indices used with caution and knowledge of their limitations, can be valid tools to aid management decisions as they offer a means to simply visualise the state of the ecosystem.

6.2 Summary of index performance

Species richness and related indices: S, d, Brillouin, Fisher, ES (50), H' and N1

This group of indices were all highly correlated to species richness in most but not all situations and nevertheless produced different results on some occasions. With the exception of S, the other indices take abundance into account; sometimes the indices were influenced by this and in these cases, this lead to a greater correlation with evenness (e.g. Margaretta, Galway Bay).

Species richness was, overall, one of the most sensitive and easily understandable indices. Although the direction of change of this index was perhaps unpredictable, under the general expectation of a monotonic decrease with increasing stress, the observed responses were not beyond explanation. While species richness alone cannot tell much about the state of an ecosystem, over time, change in species richness is one of the clearest indicators of change in the system. Odum (1985) predicted that stress should first be detected at the species level. Species richness was one of the only indices to detect a trend over time at Ironrotter Point which would indicate its suitability as an early warning indicator over other indices. The coefficient of variation of S was moderately high; therefore some caution is required and background knowledge of the study site would be desirable. The functional counterpart of S, number of traits, was much less variable and warrants further exploration as an index. However, function may be more resistant than species richness (Odum, 1985) and function has been found to be maintained even when species are lost from the system until whole functional guilds are lost or almost lost (Tilman et al., 1996), suggesting measuring function may not indicate change in the system early. Any change in species richness, whether increasing or decreasing, should signal the need for further investigation. Species richness as an index is simple and effective and is highly recommended. However, species richness can respond to a number of variables and it cannot be used in isolation.

Margalef's Index, d, was always strongly correlated to species richness; changes in relative abundance patterns had a much smaller influence on this index. Nevertheless, the index did sometimes produce different results to species richness. The level of variation was similar for both. Overall, there is little advantage to using this index over species richness.

Brillouin Index was again highly correlated to species richness but also to Simpson's Index and abundance. It may be more sensitive to environmental change, finding trends where other indices did not, for example temporal trends at NMMP sites, but overall performed similarly to species richness. It also had a lower coefficient of variation than species richness which may be one advantage of using this index over species richness.

Fisher's Index was correlated to species richness and abundance. The detection of temporal trends using Fisher was weaker than other indices at NMMP sites possibly suggesting this index is less sensitive to natural variation. On the other hand, this index had a high coefficient of variation increasing the risk of Type II statistical errors.

Hurlbert's Index of Rarefaction, ES (50), although strongly correlated to species richness and less so to abundance, like other indices, detected trends occasionally which other indices did not or detected no trends when other indices did.

The widely used Shannon-Wiener Index performed very similarly overall to Brillouin, Fisher and ES50 although there were some differences. Similarly to these indices, the Shannon Index was highly correlated to species richness and less correlated to abundance. The index had a moderate to low coefficient of variation, similar to Brillouin Index.

Hill's diversity index, N1, was highly correlated to species richness and other diversity indices, H', ES50, Brillouin, Margalef and Fisher. The variability of this index was very high and much higher than its functional counterpart.

Overall, species richness would be the recommended index to use out of this group. Combining S and N can have the desirable outcome of reducing the variability of these two properties and if this is desired an index such as Shannon Index or Brillouin Index may be appropriate. However, combining these two properties can obscure what is occurring and it is always necessary to go back to species richness to find out the reasons for changes in the index. The disadvantages of using these indices are well documented (e.g. Magurran, 2004) and include being highly sensitive to sample size. However, these were useful indices when used within a site and a sampling regime rather than for comparison between sites.

Abundance (N), Total biomass

Abundance was a useful property to measure in conjunction with species richness and could be very informative. It was a highly variable property which made it not very practical on its own. It could also be unpredictable and more difficult to interpret than other indices without the context of other properties such as species richness. However, it was generally sensitive to change although overall not as useful as species richness.

Total biomass was not highly correlated to other indices generally and was a highly variable property. A lack of data meant this index could not be tested in all circumstances. It has been recommended as being more representative of the realistic state of ecosystems (Bremner et al., 2006a). Biomass data performed better when used with functional indices than abundance data but the benefit of using total biomass as an index in itself was not clear from the current analysis carried out.

Evenness: A/S, J', Simpson's Index

Pielou's Index, J', was sensitive to disturbance trends, although usually showed a weaker relationship than other indices, and seemed to be less sensitive to natural trends such as salinity in the Clyde Estuary. This index also had a low inherent variation. J' was most similar to other evenness measures, A/S and Simpson's and to a lesser degree to abundance and taxonomic diversity (Delta). Sometimes opposing trends to other indices were detected; nevertheless performance in relation to environmental trends was predictable as evenness can increase in less diverse sites. This index was also very highly correlated to Rao's entropy of functional diversity.

A/S was similar to other measures of evenness such as J' but mostly correlated to abundance and had a moderately high coefficient of variation.

Simpson's Index could sometimes respond in an opposite direction to indices which were more influenced by species richness and abundance, as with J'. This index was highly correlated to other measures of evenness – J' and also to the group of highly correlated indices Brillouin, ES50, H' and Fisher. The variation of this index was low.

Since A/S was always highly correlated to abundance, it does not seem to be more useful that measuring abundance alone. Simpson's Index and J' both performed very similarly suggesting either of these indices could be used to measure evenness.

The infaunal trophic index (ITI)

ITI was not strongly correlated to any other index except under highly degraded situations. It generally detected trends well, especially from organic sources. It had a low coefficient of variation. ITI was one of the only indices to detect an early trend of disturbance over time at Ironrotter Point, partly due to the index being independent of species richness, suggesting it may be one of the only useful indices in detecting early warning signals due to organic pollution.

AMBI and BOPA

AMBI was only highly correlated to related indices such as IQI and m-AMBI but was also correlated to BQI. Both AMBI and BQI are based on the sensitivities and tolerances of species to disturbance but the sensitivities of species for BQI are calculated in an objective way as opposed to species being assigned to a tolerance group subjectively (from literature or expert knowledge) as with AMBI. The coefficient of variation was fairly low for AMBI and this was largely consistent regardless of the state of the system i.e. this did not increase in either degraded or reference sites, unlike many other indices which performed differently in different states of disturbance. AMBI was a useful index which did not seem to be too sensitive to natural variation. However, AMBI often classified a smaller range of qualities compared to other indices; for example, AMBI classified all NMMP sites as good while other indices classified a range of qualities depending on the site. Other studies have also found AMBI to assign more sites as ‘good’ quality than other indices (e.g. Labrune et al., 2006, Zettler et al., 2007). This may be partly due to the good bracket on the AMBI scale which is larger than any other quality category. This may also be an indication that this index is less influenced by geographical location than other indices and is applicable across sites. AMBI classified the very species poor site, Lismore Deep (LIS) as ‘good’ in contrast to other indices suggesting this index may be inappropriate in situations with very low species richness. Poor performance of AMBI in species poor areas has also been found elsewhere (Muniz et al., 2005). On the other hand, as there were no known impacts causing degradation at this site and there were sensitive species present, this site may have naturally low species richness and AMBI may have assigned the appropriate quality classification. AMBI also showed lower sensitivity to physical disturbance than other indices which has also been found elsewhere (Muxika et al., 2005).

BOPA was correlated with AMBI and related indices such as IQI. It was often found to classify sites as higher quality than other indices tested, as also found in other studies (Labrune et al., 2006, Ruellet and Dauvin, 2007, Blanchet et al., 2008). BOPA also had very high coefficient of variation greatly increasing the risk of Type II statistical errors. This variability was greater in reference conditions than in degraded conditions in some cases, though not all and therefore this index may not be useful in moderate conditions due to the high degree of variability.

Multi-metric Indices: EQR, IQI, m-AMBI, BQI

EQR was strongly correlated to some of the diversity indices such as H’ and ES50 as well as to its related indices IQI and m-AMBI. It was less variable than other indices and had very low coefficient of variation.

IQI was highly correlated to species richness, diversity indices such as H', BQI and related indices such as m-AMBI. This index seemed to be less sensitive to temporal variation than other indices. However, on closer inspection, this may have been due to opposing components which make up this multi-metric index, thereby masking changes which were occurring, such as the simultaneous increase of species richness while the balance of ecological groups indicated decreasing quality. Overall this was a sensitive index in most circumstances and had a low coefficient of variation.

M-AMBI was correlated to species richness and related indices and to other similar multi-metric indices such as IQI as well as to a lower degree, AMBI. It had a low coefficient of variation but also suffered from the same problem as some other multi-metric indices by masking trends in the environment due to being made up of opposing components.

BQI was correlated to species richness and related indices and also IQI and less so to AMBI. The coefficient of variation was moderately high however for this index making this less desirable than some other indices in terms of confidence in the index classification. The quality classification was also lower with this index than several other indices suggesting that this index perhaps underestimated quality. Other studies have found BQI to assign lower classifications relative to other indices such as AMBI or BOPA (e.g. Labrune et al., 2006, Ruellet and Dauvin, 2007, Blanchet et al., 2008). Although, in this study, the reference list of species used may not have been sufficient. As discussed (pg. 26; Appendix 8.1), BQI is difficult to calculate due to the very large amount of data required to calculate the $ES50_{0.05}$ value for each species. However, if this was developed for each region, it would have a lot of potential as an index as each geographical region would have its species list with the sensitivities specific to that area. This would be an advantage over AMBI which uses a species list of sensitivities which has been developed for European waters and thus may not be more broadly applicable, although successful results have been found outside Europe (Muxika et al., 2012). The $ES50_{0.05}$ value is also an objective sensitivity/tolerance assignment whereas the assignment of sensitivity/tolerance in AMBI is subjective.

Measures of taxonomic distinctness

Taxonomic Diversity (Delta) was correlated mostly with Simpsons index but also some of the diversity indices such as H' and Brillouin, also EQR and the other measures of taxonomic distinctness. This index found only weak temporal trends at NMMP sites where other indices found trends indicating a lack of sensitivity to change or a lack of sensitivity to natural variation. This index was strongly related to the functional trait 'body type' reflecting the taxonomic component to this trait. The variability of this index was low.

Taxonomic Distinctness (Delta*) was correlated to Delta and Delta+ and not strongly correlated to any other indices. The coefficient of variation was very low for this index but in some cases this index also appeared to be less sensitive to disturbance trends than other indices, for example taxonomic distinctness did not find expected strong trends in fish farm sites.

Average Taxonomic Distinctness (Delta^{+}) was only relatively weakly correlated to Delta and Delta* and no other indices. This index also seemed to be much less sensitive to trends in quality, for example at the fish farm sites, than other indices. The coefficient of variation was the lowest out of all indices but this low variation seemed to reflect low sensitivity to change due to anthropogenic disturbance.

Total Taxonomic Distinctness (sDelta^{+}) was highly correlated to S, N and d and other related indices and performed almost identically to species richness including the coefficient of variation associated with it. There is no advantage to using this index over species richness.

Variation in Taxonomic Distinctness (Lambda^{+}) was not correlated to any other index. It seemed to be less influenced by natural properties such as sediment properties and depth than other indices but still detected some trends of quality although to a much lower degree than other indices implying it may not be a sensitive indicator of change. The coefficient of variation was low to moderate for this index.

Functional Indices

Functional indices differed in their ability to differentiate between the two sites at Galway Bay. For most of the functional indices the coefficient of variation was low. There was some evidence that the type of disturbance could be detected by these indices. These indices require further testing at a range of sites of different levels of disturbance to establish whether indices which detected a difference were responding to natural variation or anthropogenic disturbance.

6.3 Application of indices

Using individual components such as the benthos in the assessment of ecosystem health makes the assumption that these components are adequate as indicators and represent the state of the system. We have to rely on the theory that benthic invertebrates interact dynamically with their physical, chemical and biological environment and therefore may infer from their state, the state of environmental health (Reiss and Kröncke, 2005, Gray and Elliott, 2009). The benthic macroinvertebrates are both changing their environment and responding to changes and benthic indices may reflect these responses. However, benthic indices measure one component of the ecosystem in a greatly reduced form (Van Hoey et al., 2010). This brings potential problems such as missing important trends and getting a false impression of the ecosystem as a whole as the complexity of the environment is lost through condensing data into an index value.

Ecosystem health definitions include the structure, function, resistance and resilience of the ecosystem in concert with the human activities which occur (e.g. Rapport et al., 1998). Benthic indices are largely based on and are measurements of structure, in particular, species richness. However, species richness has been used as a surrogate measurement for other aspects of ecosystem health, including function (Diaz & Cabido, 2001). All aspects of the ecosystem are important but some aspects may be better indicators than others and may indeed indicate the overall environmental health. Therefore it should not be necessary to measure every aspect of ecosystem health to give an adequate assessment of the state of the ecosystem. However, if some aspects are excluded, important trends may be missed.

Structural indices showed variable performance. Diversity can respond to numerous factors, including changes in biological interactions, habitat and the environment, as well as pollution or disturbance gradients (Gray and Elliott, 2009). Indices have been found to respond to salinity (e.g. Dauvin et al., 2007), sediment type (e.g. Blanchet et al., 2008) and annual variation (e.g. Salas et al., 2004). Indices at many of the sites studied here showed responses which could be related to any of several confounding factors. At Barcaldine and Irvine Bay, pollution gradients coincided with depth gradients; at Ironrotter, the main pollution gradient coincided with time; and at the Clyde Upper Estuary, anthropogenic inputs were confounded with salinity, depth and sampling location amongst other unmeasured factors. Changes in the benthic communities may have been occurring over these spatial and temporal gradients and the effect of these changes was difficult to separate from the effect of the disturbance or impact. This makes interpretation of indices difficult and reinforces the need to use a number of approaches concurrently in the assessment of ecosystem health including multivariate analysis and information about the physico-chemical environment.

Indices responded differently depending on the type of disturbance. Most indices behaved similarly in heavy organic enrichment, as was shown at Barcaldine and at the fish farms. However, along the Nobel explosives transect in Irvine Bay where the main pollution impact was expected to be chemical in nature, results were less clear. Results suggested that indices did not detect an impact from this type of pollution except in very degraded samples. This suggested that the impact was not great or that the indices showed low sensitivity to the type of impact. Few studies have investigated the response of indices to chemical pollution impacts and this requires further investigation (Quintino et al., 2006). Physical disturbance due to a storm was detected by most indices at Galway Bay except for AMBI (at Leverets). This index has previously been found not to detect physical disturbance (Muxika et al., 2005). These results reflect the theoretical basis of many benthic indices which is the response of benthic communities to organic enrichment as described by Pearson & Rosenberg (1978). Indices performed relatively predictably when organic enrichment was high but unpredictably when disturbance was of a chemical or physical nature.

Results from data at Ironrotter Point showed that species richness increased although the system was becoming increasingly disturbed over time. Indeed species richness was one of the only indices to show a change at an early stage of anthropogenic organic input at this site. As the baseline data consisted of only one year, it is not known if the increasing trend in species richness occurred in previous years also. Despite this, multivariate analysis showed that species composition had been clearly altered after implementation of the sewage outfall pipe but most indices did not detect a change in quality until the last year of sampling, seven years after operation of the pipe began. This suggests that species richness may have been the most suitable early warning index in this case although biomass and functional indices were not tested using this dataset. Other authors have suggested that species richness may respond more quickly to stress than other aspects of the ecosystem (Odum, 1985; Paul, 1997). The direction of change of species richness however was increasing rather than decreasing. Species richness is known to respond in unpredictable ways to stress and has therefore been cited as an unreliable indicator of stress (Odum, 1985). Many studies have described the potential response of species richness initially increasing with increasing stress before subsequently decreasing (Connell, 1978, Pearson and Rosenberg, 1978, Odum, 1985, Dodson et al., 2000, Mittelbach et al., 2001, Hooper et al., 2005), although this is just one response of a number of responses which can occur including curvilinear responses, thresholds or cyclical responses (Rapport and Whitford, 1999). At Ironrotter, had the site been studied in subsequent years, it may have been found that this trend was at an increasing point of a nonlinear response and species richness may, at some point, have begun to decrease. This type of response was found to cause misleading results with some indices, for example, WFD Ecological Quality Ratios or multimetric indices.

Combining indices into a ratio has been carried out in several EU member states to satisfy the conditions of the WFD, for example the IQI in the UK and Ireland. The use of the ratio was found to be useful to a certain extent and reduced the variability of more variable individual indices like species richness while maintaining good sensitivity to disturbance overall. Nevertheless, in some instances the combination of indices used also resulted in missing important trends as the components of the multi-metric index cancelled each other out. For example, at NMMP sites, the species richness and AMBI components of IQI showed opposing trends and this resulted in no overall trend being detected by this index. This also occurred at Ironrotter Point. The multi-metric indices add further complexity to indices which can already behave unpredictably and can be difficult to interpret. Benthic indices already reduce a large amount of information into a small, individual number. By creating multi-metrics, there is a danger of losing more information and introducing more uncertainty not measurable by coefficient of variation.

These examples show that structural indices may show no response or a response in the opposite direction to the expected response and these types of responses generally occurred when sites were not heavily degraded with organic enrichment. Thus performance of indices was less predictable at more moderate levels of disturbance.

Measurement of functional aspects of ecosystems is now emphasised in the Marine Strategy Framework Directive. It is important in further testing of measures of function to establish whether these methods add to the overall assessment of the ecosystem as otherwise they may be an unnecessary burden on environmental managers. Greater species richness is largely believed to lead to greater ecosystem functioning and stability (McCann, 2000, Loreau, 2010). For the purposes of monitoring, measurement of the structure may be adequate. In this study it was found that measuring function with an index did not seem to be more useful than measuring health using structural indices alone as both methods resulted in the same outcome. Margaretta had a greater number of species than Leverets, greater quality according to most indices and a greater number of functional traits present. According to species richness and other indices, the benthic community at Margaretta responded less to a storm event than at Leverets, suggesting that this site had greater resistance. However, functioning is thought to be as important as structure (Mee et al., 2008) and it is an aspect of the environment which is currently excluded from routine monitoring. Some authors have suggested the importance of measuring the functioning of the ecosystem as this may be less variable and detect trends towards disturbance (Statzner et al., 2001). This study found that functional indices were less variable than structural indices but they may also be less sensitive than structural indices and require further testing along disturbance gradients. Ecosystem function may be more resistant than structural components and therefore functional indices may respond more slowly than structural indices to stress (Odum, 1985; Paul, 1997). This may allow measures of function to be good as indicators of the overall magnitude of disturbance but not as early warning indicators (Paul, 1997). In addition, functional indicators may indicate the direction of change more reliably (Paul, 1997). At Ironrotter Point, species richness increased as the system was becoming increasingly contaminated. In this situation, measuring function may have indicated that the system was decreasing in quality. Indeed, the ITI and AMBI did show a decreasing, although slower, change in quality concurrent with the increase in species richness; these indices may be more closely related to function than other indices. As species richness was increasing, trophic health of the system was decreasing potentially indicating a decrease in ecosystem functioning. This response of increasing species richness while ecosystem functioning is decreasing is in contrast to expected response of increased species richness leading to greater ecosystem stability and functioning. The latter has been found to occur in environments with similar conditions while under changing conditions or along a gradient other patterns may occur (Hooper et al., 2005). However, overall these types of relationships of variable species richness response to disturbance have not been reconciled in the biodiversity-ecosystem functioning debate and this would suggest species richness alone is not a reliable proxy for overall ecosystem health in the face of stress.

Analysis of individual traits may increase understanding of the system. In this study, traits affected by the storm event did indicate an impact on some functional traits which would be expected to be affected by physical disturbance. Multivariate analysis of biological traits can add to the understanding of the system although these analyses are complicated and may not be practical in routine monitoring. There is some evidence that functional traits provide a better predictor of ecosystem functioning than species richness (Bolam et al., 2002, Raffaelli et al., 2003, Griffin et al., 2009). However, the use of traits as legitimate representations of ecosystem functioning is yet to be validated

(Duffy et al., 2007). The conditions of testing relationships between traits and functioning has often been limited by the scope of experimental work carried out including the number of traits tested, the number of species used and the type of functioning assessed (Covich et al., 2004). Furthermore, if the traits which best represent functioning can be established, the response of these traits to stress or disturbance also needs to be validated. Studies have found responses of functional traits to stress to be unpredictable with certain types of stress (Dolédec and Statzner, 2008, Feio and Dolédec, 2012). Nevertheless, as discussed, structural indices may be limited in detecting the benthic response to chemical or physical stressors and functional trait analysis may be especially useful in these circumstances. As different functional types may be affected by different stressors in different ways, individual traits may be important in determining the health of the system and these traits may not have a simple relationship with species richness. In this study, many individual functional traits showed varying degrees of correlation to species richness. Whole functional groups may be lost while the corresponding change in species richness may be relatively small (Diaz and Cabido, 2001). This scenario would indicate the need to measure ecosystem functioning.

The methods currently used in ecosystem health assessment pose several problems for practical environmental management and monitoring. These issues require consideration as the current framework of index use places ecological, social and legal implications on their outcome.

Indices were found to behave differently depending on the state of the ecosystem in terms of how much in accordance they were with each other and how variable they were. Moderate disturbance increased disagreement between indices and caused indices to act unpredictably e.g. at Barcaldine indices agreed most at bad quality sites closest to the outfall and at the best sites while moderate sites showed the least agreement between indices with sites being assigned up to three different quality classifications depending on the index used. This was further illustrated when indices which were not correlated to each other generally were highly correlated under high organic loading e.g. at Barcaldine and at the fish farm sites many indices were more highly correlated than at

NMMP sites. The response of the indices in moderate conditions was complex. It seemed to be an overall trend that indices performed poorly when conditions were moderate as opposed to clearly good or poor; this lead to the problem of distinguishing moderate conditions from natural heterogeneity. Other authors have found indices to be less reliable in moderate conditions as opposed to conditions which are clearly good or poor (Quintino et al., 2006, Puente and Diaz, 2008). This may reflect succession in benthic communities which tends to be predictable in the early stages but in later stages becomes unpredictable (Gray and Elliott, 2009). In heavily degraded areas the community exhibits the clear characteristic of opportunistic species which have recolonized the area but away from the pollution source, any one of a number of species can dominate and these can change over time or space. It has also been found that only 20 of 123 tested indicator taxa responded in a predictable and consistent way to organic enrichment (Bustos-Baez and Frid, 2003).This could have implications in particular for indices which take the species identity into account as these indices may perform well in the predictable, heavily degraded areas but less well in moderately disturbed, unpredictable areas. However, higher variability in terms of the coefficient of variation was found at impacted sites although these were the sites where index quality classifications agreed the most. Although species identity may be predictable at impacted sites, other structural properties such as abundance may be highly variable. Confidence in the index classification should be considered not only through using the coefficient of variation but also through the level of agreement between indices. This property of indices showing disagreement at moderate sites and having increasing variability as disturbance increases is important in terms of the application of indices in management. Systems may only show very small changes before reaching a critical threshold (Scheffer et al., 2009) and this level of change may be undetectable with a highly variable index. It suggests that indices may be less useful for WFD surveillance monitoring purposes as trends towards disturbance may be easily missed in conditions which are still good or close to the moderate-good boundary but may be suitable for measuring the impact of known disturbances. Although the individual indices behave less variably in moderate or good conditions, the specific quality classification will greatly depend on the index used.

Indeed many of the indices which were found to be the least variable seemed to have a trade off with also being less sensitive to change or disturbance than more variable indices. Indices were sensitive to both natural and anthropogenic caused change to greater or lesser degrees. For the indices which had low variability, it is necessary to detect a much smaller change rather than looking for a bigger change in the indices which had higher variability. This change may not always result in a statistically significant difference and this is a problem for environmental managers. There may be the need to balance the sensitivity to change and the variability of the index. Nevertheless, the variability of the indices does impact upon the interpretation of statistical results. Future work could be focussed on defining natural variation which is the greatest difficulty in assessing ecosystem health at the moderate-good boundary. In addition, pre-treatment of raw data with transformations may decrease the variability and increase the utility of indices (Warwick et al., 2010, Muxika et al., 2012) and merits further investigation.

Measuring the coefficient of variation of different sites over time may allow the natural variation of individual sites to be established although this would need to be done on a site to site basis. Assessment of natural variation of sites may need to encompass the changes which can occur in benthic communities over time using long term data sets since previous evidence has suggested macroinvertebrate communities change over 6 to 10 year periods (Frid et al., 2009) or in cycles of 6 to 7 or 10 to 11 years (Gray and Christie, 1983). Furthermore, it was found that to distinguish anthropogenic impacts from natural disturbance may require longer term data sets of over six years (Ferraro et al., 1991). This could allow a greater level of error to be acceptable at highly variable sites or it could result in different sampling regimes being applied at different sites. There may however, be a trade off between properly designed studies and routine monitoring regimes. In addition, using different sampling regimes at different sites will not allow comparison across sites. An index which can detect a trend is required for routine monitoring which can then be used as an indication of the need for further investigation.

Almost none of the indices performed well in detecting early a trend towards disturbance at Ironrotter Point. In this case, multivariate analysis may have been the most important factor in assessing the site as, although it gives no indications of quality, it showed that there was a drastic shift in community composition in each year tested, much greater than would be normally expected (e.g. relative to NMMP sites). Species richness showed the strongest response of any of the indices tested suggesting it is an important aspect to consider in monitoring change in ecosystems over time. Another study also found that indices based on indicator taxa did not perform better than species richness (Bustos-Baez and Frid, 2003). However, other tools may be necessary for interpreting the change in species richness and multivariate analysis. Surveillance monitoring is only required to be carried out once every three years according to the WFD. Indices tested in this study largely showed a poor performance as early warning indicators but detecting trends towards a threshold is crucial (Carpenter et al., 2009). With the current methods for assessment of ecosystem health, three year gaps in monitoring of macroinvertebrate communities may be insufficient to detect small trends and prevent accelerating and sudden changes.

Indices represent a decision support tool which should be used in conjunction with other methods. Particularly useful in assessment of change in ecosystem quality are multivariate analyses such as MDS as these can indicate a change in species composition over time even if the overall species richness does not change much. In addition, the use of species richness and whether this shows change over time is a useful indicator of change. An index such as IQI or AMBI which can assign a quality status which is comparable to other water bodies and reference states is also very useful as they give a defined quality classification. However, it has been shown that all of these approaches in isolation do not always detect an expected change in quality depending on the context and it is beneficial to use several approaches concurrently. Relying on a single index greatly increases the risk of misclassification. Interpretation of changes in quality would benefit from having more information and site specific context such as physico-chemical variables and time series data of the study site or information about reference conditions. If change is detected and operational or investigative monitoring is required, further indices and analysis should be used to explore the potential causes of change and the impacts. Although further testing of functional indices and measurements is required, this could be used in operational and investigative monitoring programmes to give a better understanding of the system but may not give an advantage in surveillance monitoring over traditional methods.

Overall, the indices provide information to the manager who must then make an informed decision based on all the evidence. No single index attains the confidence necessary to assign a definitive quality classification which would be legally defensible in all circumstances. The use of Ecological Quality Ratios such as IQI for the WFD is highly restrictive and sometimes misleading although useful for comparative purposes. The ultimate decision and assessment of quality should be with the environmental manager while methods of assessment continue to be improved. Although this approach incorporates bias, all definitions of ecosystem health incorporate the greatly biased human value system and therefore, the end point of achieving ecosystem health is indefinite and based on value judgements (Mee et al., 2008).

Ideally, a monitoring regime will be rigorous enough to detect changes cost and time efficiently and also fulfil legal obligations for assessment. However, there is a disparity between reporting obligations and what may be the best way to assess and monitor ecosystem health.

This study and future studies would benefit from longer term data sets which have more complete physico-chemical variables which can be analysed over different spatial and temporal scales. Furthermore, simple correlations were used to test index performance in relation to environmental trends. However, diversity may change in complex ways to stress and nonlinear responses were observed in this study. Detecting nonlinear trends including thresholds has been identified as a gap in ecosystem health assessment (Carpenter et al., 2009). Attempts to overcome nonlinear responses have included classification and regression trees (De'ath and Fabricius, 2000); constrained additive ordination (Yee, 2006); multivariate adaptive regression splines (Leathwick et al., 2005); and quantile regression splines (Anderson, 2008) and these techniques could be tested in future studies. Predictive models such as MARINPACS (Gray and Elliott,

2009) may be another alternative which may better cope with complex benthic community responses. Comparing data to a reference set may be a better way to interpret change from normal conditions (Leonard et al., 2006, Lamb et al., 2009). Although obtaining suitable reference data is a major challenge. These methods may allow both nonlinear responses and confounding variables to be overcome to some extent in the interpretation of benthic responses. In addition, this study would have benefitted from data sets which were sampled using the same methods for comparison across study sites and stressors. The difference in performance of different indices at different sites highlights the problem of relying on these indices and shows conclusions drawn from a study on one site and a few indices could be severely limited. Reasons for why indices perform differently under different conditions are not clear. Combined effects of different stressors may be one factor which would be worthy of further investigation (Borja et al., 2011). Monitoring programmes are now also hoped to be useful in detecting climate change. Investigation into the types of changes which may occur in macroinvertebrate communities and whether indices can detect these changes is also important. The measurement of functional health may become more useful in expanding the scope of how and what indices can measure and this deserves further study.

6.4 Recommendations

Relying on a single index for assessing benthic ecosystem health could result in misclassification. Recommendations for environmental managers would be to use a suite of indices which measure different aspects of the system. These could be the change in species richness over time which was useful as an early warning indicator; an index which measures the sensitivity of species, AMBI being a good example; where there is the risk of organic enrichment, ITI has proved to be sensitive and useful; and a measure of evenness such as Simpson's Index. Measures of taxonomic diversity, although measuring a different aspect of ecosystem health, were found to be less sensitive to disturbance than other indices and therefore may not be a useful tool to include. Multimetric indices such as IQI, although performing well in many circumstances, do have serious risks in disguising trends and a better approach would be to interpret the multiple components of these indices alongside each other, but not combined with each other. Using a suite of indices should add confidence in the classification when indices agree but when indices disagree would be a precaution against misclassification in situations where some indices do not perform well. The set of indices should then be interpreted together with the use of multivariate analysis such as MDS and interpreted in the context of physico-chemical variables. These measures should be sufficient for general monitoring purposes while for more informative or investigative assessments, functional trait assessments could be additionally employed.

7. References

AFLI, A., AYARI, R. & ZAABI, S. 2008. Ecological quality of some Tunisian coast and lagoon locations, by using benthic community parameters and biotic indices. *Estuarine, Coastal and Shelf Science,* 80**,** 269-280.

ALBAYRAK, S., BALKIS, H., ZENETOS, A., KURUN, A. & KUBANÇ, C. 2006. Ecological quality status of coastal benthic ecosystems in the Sea of Marmara. *Marine Pollution Bulletin,* 52**,** 790-799.

ANDERSON, M. J. 2008. Animal-sediment relationships re-visited: Characterising species' distributions along an environmental gradient using canonical analysis and quantile regression splines. *Journal of Experimental Marine Biology and Ecology,* 366**,** 16-27.

ARMONIES, W. 2000. On the spatial scale needed for benthos community monitoring in the coastal North Sea. *Journal of Sea Research,* 43**,** 121-133.

AUBRY, A. & ELLIOTT, M. 2006. The use of environmental integrative indicators to assess seabed disturbance in estuaries and coasts: Application to the Humber Estuary, UK. *Marine Pollution Bulletin,* 53**,** 175-185.

AZTI-TECNALIA 2011. AMBI: AZTI Marine Biotic Index, www.azti.es. February 2010 ed.

BACHELET, G., DE MONTAUDOUIN, X. & DAUVIN, J.-C. 1996. The Quantitative Distribution of Subtidal Macrozoobenthic Assemblages in Arcachon Bay in Relation to Environmental Factors: a Multivariate Analysis. *Estuarine, Coastal and Shelf Science,* 42**,** 371-391.

BAKALEM, A., RUELLET, T. & DAUVIN, J. C. 2009. Benthic indices and ecological quality of shallow Algeria fine sand community. *Ecological Indicators,* 9**,** 395-408.

BARBARO, L. & VAN HALDER, I. 2009. Linking bird, carabid beetle and butterfly life-history traits to habitat fragmentation in mosaic landscapes. *Ecography,* 32**,** 321-333.

BDC 2008. Progress in developing a framework for biodiversity assessment and monitoring, presented by the United Kingdom. OSPAR convention for the protection of the marine environment of the North-East Atlantic. The Hague (Netherlands): Meeting of the Biodiversity Committee (BDC).

BEVILACQUA, S., FRASCHETTI, S., MUSCO, L., GUARNIERI, G. & TERLIZZI, A. 2011. Low sensitiveness of taxonomic distinctness indices to human impacts: Evidences across marine benthic organisms and habitat types. *Ecological Indicators,* 11**,** 448-455.

BIGOT, L., CONAND, C., AMOUROUX, J. M., FROUIN, P., BRUGGEMANN, H. & GRÉMARE, A. 2006. Effects of industrial outfalls on tropical macrobenthic sediment communities in Reunion Island (Southwest Indian Ocean). *Marine Pollution Bulletin,* 52**,** 865-880.

BIGOT, L., GRÉMARE, A., AMOUROUX, J.-M., FROUIN, P., MAIRE, O. & GAERTNER, J. C. 2008. Assessment of the ecological quality status of softbottoms in Reunion Island (tropical Southwest Indian Ocean) using AZTI marine biotic indices. *Marine Pollution Bulletin,* 56**,** 704-722.

BILES, C., PATERSON, D., FORD, R. B., SOLAN, M. & RAFFAELLI, D. 2002. Bioturbation, ecosystem functioning and community structure. *Hydrology and Earth System Sciences,* 6**,** 999-1005.

BILES, C. L., SOLAN, M., ISAKSSON, I., PATERSON, D. M., EMES, C. & RAFFAELLI, D. G. 2003. Flow modifies the effect of biodiversity on ecosystem functioning: an in situ study of estuarine sediments. *Journal of Experimental Marine Biology and Ecology,* 285-286**,** 165-177.

BIRK, S., BONNE, W., BORJA, A., BRUCET, S., COURRAT, A., POIKANE, S., SOLIMINI, A., VAN DE BUND, W., ZAMPOUKAS, N. & HERING, D. 2012. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. *Ecological Indicators,* 18**,** 31-41.

BLANCHET, H., DE MONTAUDOUIN, X., CHARDY, P. & BACHELET, G. 2005. Structuring factors and recent changes in subtidal macrozoobenthic communities of a coastal lagoon, Arcachon Bay (France). *Estuarine, Coastal and Shelf Science,* 64**,** 561-576.

BLANCHET, H., LAVESQUE, N., RUELLET, T., DAUVIN, J. C., SAURIAU, P. G., DESROY, N., DESCLAUX, C., LECONTE, M., BACHELET, G., JANSON, A. L., BESSINETON, C., DUHAMEL, S., JOURDE, J., MAYOT, S., SIMON, S. & DE MONTAUDOUIN, X. 2008. Use of biotic indices in semi-enclosed coastal ecosystems and transitional waters habitats--Implications for the implementation of the European Water Framework Directive. *Ecological Indicators,* 8**,** 360-372.

BOLAM, S. G., FERNANDES, T. F. & HUXHAM, M. 2002. DIVERSITY, BIOMASS, AND ECOSYSTEM PROCESSES IN THE MARINE BENTHOS. *Ecological Monographs,* 72**,** 599-615.

BORJA, A. 2004. The biotic indices and the Water Framework Directive: the required consensus in the new benthic monitoring tools. *Marine Pollution Bulletin,* 48**,** 405-408.

BORJA, A., BARBONE, E., BASSET, A., BORGERSEN, G., BRKLJACIC, M., ELLIOTT, M., GARMENDIA, J. M., MARQUES, J. C., MAZIK, K., MUXIKA, I., NETO, J. M., NORLING, K., RODRÍGUEZ, J. G., ROSATI, I., RYGG, B., TEIXEIRA, H. & TRAYANOVA, A. 2011. Response of single benthic metrics and multi-metric methods to anthropogenic pressure gradients, in five distinct European coastal and transitional ecosystems. *Marine Pollution Bulletin,* 62**,** 499-513.

BORJA, A., FRANCO, J. & PEREZ, V. 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin,* 40**,** 1100-1114.

BORJA, A., JOSEFSON, A. B., MILES, A., MUXIKA, I., OLSGARD, F., PHILLIPS, G., RODRÍGUEZ, J. G. & RYGG, B. 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. *Marine Pollution Bulletin,* 55**,** 42-52.

BORJA, Á. & RODRÍGUEZ, J. G. 2010. Problems associated with the 'one-out, all-out' principle, when using multiple ecosystem components in assessing the ecological status of marine waters. *Marine Pollution Bulletin,* 60**,** 1143-1146.

BORJA, Á., RODRÍGUEZ, J. G., BLACK, K., BODOY, A., EMBLOW, C., FERNANDES, T. F., FORTE, J., KARAKASSIS, I., MUXIKA, I., NICKELL, T. D., PAPAGEORGIOU, N., PRANOVI, F., SEVASTOU, K., TOMASSETTI, P. & ANGEL, D. 2009. Assessing the suitability of a range of benthic indices in the evaluation of environmental impact of fin and shellfish aquaculture located in sites across Europe. *Aquaculture,* 293**,** 231-240.

BOTTA-DUKÁT, Z. 2005. Rao's quadratic entropy as a measure of functional diversity based on multiple traits. *Journal of Vegetation Science,* 16**,** 533-540.

BOUCHET, V. M. P. & SAURIAU, P.-G. 2008. Influence of oyster culture practices and environmental conditions on the ecological status of intertidal mudflats in the Pertuis Charentais (SW France): A multi-index approach. *Marine Pollution Bulletin,* 56**,** 1898-1912.

BOYLE, J. & O'REILLY, M. 2000. Upper Clyde Estuary Biology Survey - May 2000. Marine Biology Summary Report N0. 00/01. Scottish Environment Protection Agency. Scotland, UK.

BOYLE, J. & O'REILLY, M. 2001. Barcaldine Survey 2001. Marine Biology Summary Report No. 01/05. Scottish Environment Protection Agency. Scotland, UK.

BREMNER, J. 2008. Species' traits and ecological functioning in marine conservation and management. *Journal of Experimental Marine Biology and Ecology,* 366**,** 37-47.

BREMNER, J., PARAMOR, O. A. L. & FRID, C. L. J. 2006a. Developing a methodology for incorporating ecological structure and functioning into designation of Special Areas of Conservation (SAC) in the 0-12 nautical mile zone. A report to English Nature. The University of Liverpool: School of Biological Sciences.

BREMNER, J., ROGERS, S. I. & FRID, C. L. J. 2003. Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. *Marine Ecology Progress Series,* 254**,** 11-25.

BREMNER, J., ROGERS, S. I. & FRID, C. L. J. 2006b. Matching biological traits to environmental conditions in marine benthic ecosystems. *Journal of Marine Systems,* 60**,** 302-316.

BREMNER, J., ROGERS, S. I. & FRID, C. L. J. 2006c. Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). *Ecological Indicators,* 6**,** 609-622.

BUCHANAN, J. B. 1984. Sediment Analysis. *In:* HOLME, N. A. & MCINTYRE, A. D. (eds.) *Methods for the Study of Marine Benthos.* Oxford: Blackwell Scientific Publications.

BUSTOS-BAEZ, S. & FRID, C. 2003. Using indicator species to assess the state of macrobenthic communities. *Hydrobiologia,* 496**,** 299-309.

BYRNES, J. E., REYNOLDS, P. L. & STACHOWICZ, J. J. 2007. Invasions and Extinctions Reshape Coastal Marine Food Webs. *PLoS ONE,* 2**,** e295.

CAMARGO, J. A. & ALONSO, Á. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International,* 32**,** 831-849.

CARPENTER, S. R., MOONEY, H. A., AGARD, J., CAPISTRANO, D., DEFRIES, R. S., DIAZ, S., DIETZ, T., DURAIAPPAH, A. K., OTENG-YEBOAH, A., PEREIRA, H. M., PERRINGS, C., REID, W. V., SARUKHAN, J., SCHOLES, R. J. & WHYTE, A. 2009. Science for managing ecosystem services: Beyond the Millenium Ecosystem Assessment. *Proceedings of The National Academy of Sciences,* 106**,** 1305-1312.

CHAINHO, P., CHAVES, M. L., COSTA, J. L., COSTA, M. J. & DAUER, D. M. 2008. Use of multimetric indices to classify estuaries with different hydromorphological characteristics and different levels of human pressure. *Marine Pollution Bulletin,* 56**,** 1128-1137.

CHAINHO, P., COSTA, J. L., CHAVES, M. L., DAUER, D. M. & COSTA, M. J. 2007. Influence of seasonal variability in benthic invertebrate community structure on the use of biotic indices to assess the ecological status of a Portuguese estuary. *Marine Pollution Bulletin,* 54**,** 1586-1597.

CHAPIN, F. S., ZAVALETA, E. S., EVINER, V. T., NAYLOR, R. L., VITOUSEK, P. M., REYNOLDS, H. L., HOOPER, D. U., LAVOREL, S., SALA, O. E., HOBBIE, S. E., MACK, M. C. & DIAZ, S. 2000. Consequences of changing biodiversity. *Nature,* 405**,** 234-242.

CHARVET, S., STATZNER, B., USSEGLIO-POLATERA, P. & DUMONT, B. 2000. Traits of benthic macroinvertebrates in semi-natural French streams: an initial application to biomonitoring in Europe. *Freshwater Biology,* 43**,** 277-296.

CLARKE, K. R. & GORLEY, R. N. 2001. PRIMER v5: User Manual/Tutorial. PRIMER-E. Plymouth, UK. 91 pp.

COGNETTI, G. & MALTAGLIATI, F. 2008. Perspectives on the ecological assessment of transitional waters. *Marine Pollution Bulletin,* 56**,** 607-608.

CONNELL, J. H. 1978. Diversity in Tropical Rain Forests and Coral Reefs. *Science,* 199**,** 1302-1310.

COOPER, K. M., BARRIO FROJÁN, C. R. S., DEFEW, E., CURTIS, M., FLEDDUM, A., BROOKS, L. & PATERSON, D. M. 2008. Assessment of ecosystem function following marine aggregate dredging. *Journal of Experimental Marine Biology and Ecology,* 366**,** 82-91.

CORBETT, C. A., DOERING, P. H., MADLEY, K. A., OTT, J. A. & TOMASKO, D. A. 2005. Using seagrass coverage as an indicator of ecosystem condition. *In:* BORTONE, S. A. (ed.) *Estuarine Indicators.* 1st ed. Florida: CRC Press.

COUTO, T., PATRÍCIO, J., NETO, J. M., CEIA, F. R., FRANCO, J. & MARQUES, J. C. 2010. The influence of mesh size in environmental quality assessment of estuarine macrobenthic communities. *Ecological Indicators,* 10**,** 1162-1173.

COVICH, A. P., AUSTEN, M. C., BÄRLOCHER, F., CHAUVET, E., CARDINALE, B. J., BILES, C. L., INCHAUSTI, P., DANGLES, O., SOLAN, M., GESSNER, M. O., STATZNER, B. & MOSS, B. 2004. The Role of Biodiversity in the Functioning of Freshwater and Marine Benthic Ecosystems. *BioScience,* 54**,** 767-775.

CRAIN, C. M., KROEKER, K. & HALPERN, B. S. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters,* 11**,** 1304-1315.

DAUVIN, J.-C., RUELLET, T., DESROY, N. & JANSON, A.-L. 2007. The ecological quality status of the Bay of Seine and the Seine estuary: Use of biotic indices. *Marine Pollution Bulletin,* 55**,** 241-257.

DAUVIN, J. C. & RUELLET, T. 2007. Polychaete/amphipod ratio revisited. *Marine Pollution Bulletin,* 55**,** 215-224.

DE'ATH, G. & FABRICIUS, K. E. 2000. CLASSIFICATION AND REGRESSION TREES: A POWERFUL YET SIMPLE TECHNIQUE FOR ECOLOGICAL DATA ANALYSIS. *Ecology,* 81**,** 3178-3192.

DEFRA 2002. Safeguarding Our Seas - a strategy for the conservation and sustainable development of our marine environment.

DERNIE, K. M., KAISER, M. J. & WARWICK, R. M. 2003. Recovery rates of benthic communities following physical disturbance. *Journal of Animal Ecology,* 72**,** 1043-1056.

DIAZ, R. J., SOLAN, M. & VALENTE, R. M. 2004. A review of approaches for classifying benthic habitats and evaluating habitat quality. *Journal of Environmental Management,* 73**,** 165-181.

DÍAZ, S. & CABIDO, M. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution,* 16**,** 646-655.

DODSON, S. I., ARNOTT, S. E. & COTTINGHAM, K. L. 2000. The Relationship in Lake Communities between Primary Productivity and Species Richness. *Ecology,* 81**,** 2662-2679.

DOLÉDEC, S., CHESSEL, D., TER BRAAK, C. & CHAMPELY, S. 1996. Matching species traits to environmental variables: a new three-table ordination method. *Environmental and Ecological Statistics,* 3**,** 143-166.

DOLÉDEC, S. & STATZNER, B. 2008. Invertebrate traits for the biomonitoring of large European rivers: an assessment of specific types of human impact. *Freshwater Biology,* 53**,** 617-634.

DOLÉDEC, S., STATZNER, B. & BOURNARD, M. 1999. Species traits for future biomonitoring across ecoregions: patterns along a human-impacted river. *Freshwater Biology,* 42**,** 737-758.

DRAY, S., CHESSEL, D. & THIOULOUSE, J. 2003. CO-INERTIA ANALYSIS AND THE LINKING OF ECOLOGICAL DATA TABLES. *Ecology,* 84**,** 3078-3089.

DUFFY, J. E. 2009. Why biodiversity is important to the functioning of real-world ecosystems. *Frontiers in Ecology and the Environment,* 7**,** 437-444.

DUFFY, J. E., CARDINALE, B. J., FRANCE, K. E., MCINTYRE, P. B., THÉBAULT, E. & LOREAU, M. 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters,* 10**,** 522-538.

EA. 2011. *Environmental Quality Standards* [Online]. Environment Agency. Available: http://www.environment-agency.gov.uk/research/planning/40295.aspx [Accessed December 2011].

EC 1992. On the conservation of natural habitats and of wild fauna and flora. Council directive 92/43/EEC. *Official Journal of the European Communities,* L206**,** 7-50.

EC 2000. Establishing a framework for Community action in the field of water policy. Directive 2000/60/EC of the European Parliament and of the Council. *Official Journal of the European Communities* L327**,** 1-72.

EC 2008. Establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Directive

2008/56/EC of the European Parliament and of the Council. *Official Journal of the European Communities,* L164**,** 19-40.

ECASA. 2011. *Fin-fish farming: finding a site* [Online]. Available: http://www.ecasatoolbox.org.uk/the-toolbox/informative/matrix-files/fin-fish-farming-finding-a-site [Accessed 1 June 2011].

ELLIOTT, M. 2002. The role of the DPSIR approach and conceptual models in marine environmental management: an example for offshore wind power. *Marine Pollution Bulletin,* 44**,** iii-vii.

ELLIOTT, M. & QUINTINO, V. 2007. The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin,* 54**,** 640-645.

FEIO, M. J. & DOLÉDEC, S. 2012. Integration of invertebrate traits into predictive models for indirect assessment of stream functional integrity: A case study in Portugal. *Ecological Indicators,* 15**,** 236-247.

FERRARO, S. P. & COLE, F. A. 1990. Taxonomic level and sample size sufficient for assessing pollution impacts on the Southern California Bight macrobenthos. *Marine Ecology Progress Series,* 67**,** 251-262.

FERRARO, S. P. & COLE, F. A. 2004. Optimal benthic macrofaunal sampling protocol for detecting differences among four habitats in Willapa Bay, Washington, USA. *Estuaries,* 27**,** 1014-1025.

FERRARO, S. P., COLE, F. A., DEBEN, W. A. & SWARTZ, R. C. 1989. Power-cost efficiency of eight macrobenthic sampling schemes in Puget Sound, Washington, USA. *Canadian Journal of Fisheries and Aquatic Sciences,* 46**,** 2157-2165.

FERRARO, S. P., SWARTZ, R. C., COLE, F. A. & DEBEN, W. A. 1994. Optimum macrobenthic sampling protocol for detecting pollution impacts in the Southern California Bight. *Environmental Monitoring and Assessment,* 29**,** 127-153.

FERRARO, S. P., SWARTZ, R. C., COLE, F. A. & SCHULTS, D. W. 1991. Temporal changes in the benthos along a pollution gradient: Discriminating the effects of natural phenomena from sewage-industrial wastewater effects. *Estuarine, Coastal and Shelf Science,* 33**,** 383-407.

FLEISCHER, D., GREMARE, A., LABRUNE, C., RUMOHR, H., VANDEN BERGHE, E. & ZETTLER, M. L. 2007. Performance comparison of two biotic indices measuring the ecological status of water bodies in the Southern Baltic and Gulf of Lions. *Marine Pollution Bulletin,* 54**,** 1598-1606.

FLEISCHER, D. & ZETTLER, M. L. 2009. An adjustment of benthic ecological quality assessment to effects of salinity. *Marine Pollution Bulletin,* 58**,** 351-357.

FRID, C. L. J., GARWOOD, P. R. & ROBINSON, L. A. 2009. Observing change in a North Sea benthic system: A 33 year time series. *Journal of Marine Systems,* 77**,** 227-236.

GAMFELDT, L., HILLEBRAND, H. & JONSSON, P. R. 2008. MULTIPLE FUNCTIONS INCREASE THE IMPORTANCE OF BIODIVERSITY FOR OVERALL ECOSYSTEM FUNCTIONING. *Ecology,* 89**,** 1223-1231.

GAMITO, S. & FURTADO, R. 2009. Feeding diversity in macroinvertebrate communities: A contribution to estimate the ecological status in shallow waters. *Ecological Indicators,* 9**,** 1009-1019.

GAYRAUD, S., STATZNER, B., BADY, P., HAYBACHP, A., SCHÖLL, F., USSEGLIO-POLATERA, P. & BACCHI, M. 2003. Invertebrate traits for the

biomonitoring of large European rivers: an initial assessment of alternative metrics. *Freshwater Biology,* 48**,** 2045-2064.
GESAMP 2001. (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection) and Advisory Committee on Protection of the Sea. A sea of troubles. Rep. Stud. GESAMP No. 70, 35 pp.
GRAY, J. S. & CHRISTIE, H. 1983. Predicting long-term changes in marine benthic communities. *Marine Ecology Progress Series,* 13**,** 87-94.
GRAY, J. S. & ELLIOTT, M. 2009. *Ecology of Marine Sediments,* Oxford, Oxford University Press.
GRIFFIN, J. N., MÉNDEZ, V., JOHNSON, A. F., JENKINS, S. R. & FOGGO, A. 2009. Functional diversity predicts overyielding effect of species combination on primary productivity. *Oikos,* 118**,** 37-44.
HÅKANSON, L. & DUARTE, C. M. 2008. Data variability and uncertainty limits the capacity to identify and predict critical changes in coastal systems - A review of key concepts. *Ocean & Coastal Management,* 51**,** 671-688.
HATTON-ELLIS, T. 2008. The Hitchhiker's Guide to the Water Framework Directive. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 18**,** 111-116.
HERING, D., BORJA, A., CARSTENSEN, J., CARVALHO, L., ELLIOTT, M., FELD, C. K., HEISKANEN, A.-S., JOHNSON, R. K., MOE, J., PONT, D., SOLHEIM, A. L. & DE BUND, W. V. 2010. The European Water Framework Directive at the age of 10: A critical review of the achievements with recommendations for the future. *Science of the Total Environment,* 408**,** 4007-4019.
HILLEBRAND, H. & MATTHIESSEN, B. 2009. Biodiversity in a complex world: consolidation and progress in functional biodiversity research. *Ecology Letters,* 12**,** 1405-1419.
HISCOCK, K., LANGMEAD, O. & WARWICK, R. 2004. Identification of seabed indicator species from time-series and other studies to support implementation of the EU Habitats and Water Framework Directives. Report to the Joint Nature Conservation Committee and the Environment Agency from the Marine Biological Association. Plymouth: Marine Biological Association. JNCC Contract F90-01-705.
HOOPER, D. U., CHAPIN, F. S., EWEL, J. J., HECTOR, A., INCHAUSTI, P., LAVOREL, S., LAWTON, J. H., LODGE, D. M., LOREAU, M., NAEEM, S., SCHMID, B., SETÄLÄ, H., SYMSTAD, A. J., VANDERMEER, J. & WARDLE, D. A. 2005. EFFECTS OF BIODIVERSITY ON ECOSYSTEM FUNCTIONING: A CONSENSUS OF CURRENT KNOWLEDGE. *Ecological Monographs,* 75**,** 3-35.
HYLAND, J., BALTHIS, L., KARAKASSIS, I., MAGNI, P., PETROV, A., SHINE, J., VESTERGAARD, O. & WARWICK, R. 2005. Organic carbon content of sediments as an indicator of stress in the marine benthos. *Marine Ecology Progress Series,* 295**,** 91-103.
IMPRESS 2002. Guidance for the analysis of pressures and impacts in accordance with the Water Framework Directive. Common Implementation Strategy Working Group 2.1. Office for Official Publications of the European Communities.
IVES, A. R. & CARPENTER, S. R. 2007. Stability and diversity of ecosystems. *Science,* 317**,** 58-62.

JOHNSON, R. L., PEREZ, K. T., ROCHA, K. J., DAVEY, E. W. & CARDIN, J. A. 2008. Detecting benthic community differences: Influence of statistical index and season. *Ecological Indicators,* 8**,** 582-587.

KAISER, M. J., RAMSAY, K., RICHARDSON, C. A., SPENCE, F. E. & BRAND, A. R. 2000. Chronic Fishing Disturbance Has Changed Shelf Sea Benthic Community Structure. *Journal of Animal Ecology,* 69**,** 494-503.

KARR, J. R. 1999. Defining and measuring river health. *Freshwater Biology,* 41**,** 221-234.

KENCHINGTON, E. L., KENCHINGTON, T. J., HENRY, L.-A., FULLER, S. & GONZALEZ, P. 2007. Multi-decadal changes in the megabenthos of the Bay of Fundy: The effects of fishing. *Journal of Sea Research,* 58**,** 220-240.

KRÖNCKE, I. & REISS, H. 2010. Influence of macrofauna long-term natural variability on benthic indices used in ecological quality assessment. *Marine Pollution Bulletin,* 60**,** 58-68.

LABRUNE, C., AMOUROUX, J. M., SARDA, R., DUTRIEUX, E., THORIN, S., ROSENBERG, R. & GREMARE, A. 2006. Characterisation of the ecological quality of the coastal Gulf of Lions (NW Mediterranean). A comparative approach based on three biotic indices. *Marine Pollution Bulletin,* 52**,** 34-47.

LAMB, E. G., BAYNE, E., HOLLOWAY, G., SCHIECK, J., BOUTIN, S., HERBERS, J. & HAUGHLAND, D. L. 2009. Indices for monitoring biodiversity change: Are some more effective than others? *Ecological Indicators,* 9**,** 432-444.

LAVOREL, S. & GARNIER, E. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology,* 16**,** 545-556.

LEATHWICK, J. R., ROWE, D., RICHARDSON, J., ELITH, J. & HASTIE, T. 2005. Using multivariate adaptive regression splines to predict the distributions of New Zealand's freshwater diadromous fish. *Freshwater Biology,* 50**,** 2034-2052.

LENIHAN, H. S., PETERSON, C. H., KIM, S. L., CONLAN, K. E., FAIREY, R., MCDONALD, C., GRABOWSKI, J. H. & OLIVER, J. S. 2003. Variation in marine benthic community composition allows discrimination of multiple stressors. *Marine Ecology Progress Series,* 261**,** 63-73.

LEONARD, D. R. P., ROBERT CLARKE, K., SOMERFIELD, P. J. & WARWICK, R. M. 2006. The application of an indicator based on taxonomic distinctness for UK marine biodiversity assessments. *Journal of Environmental Management,* 78**,** 52-62.

LEPS, J., DE BELLO, F., LAVOREL, S. & BERMAN, S. 2006. Quantifying and interpreting functional diversity of natural communities: practical considerations matter. *Preslia,* 78**,** 481-501.

LOREAU, M. 2010. Linking biodiversity and ecosystems: towards a unifying ecological theory. *Philosophical transactions of the Royal Society B,* 365**,** 49-60.

MAGURRAN, A. E. 2004. *Measuring Biological Diversity,* Oxford, Blackwell Publishing.

MARCHINI, A., MUNARI, C. & MISTRI, M. 2008. Functions and ecological status of eight Italian lagoons examined using biological traits analysis (BTA). *Marine Pollution Bulletin,* 56**,** 1076-1085.

MARLIN. 2006. *BIOTIC - Biological Traits Information Catalogue. Marine Life Information Network* [Online]. Plymouth: Marine Biological Association of the

United Kingdom. Available: www.marlin.ac.uk/biotic [Accessed 4 August 2010].
MARLIN. 2011. *The Marine Life Information Network* [Online]. Available: www.marlin.ac.uk [Accessed February 2011].
MAY, R. M. 1973. Time-Delay Versus Stability in Population Models with Two and Three Trophic Levels. *Ecology,* 54**,** 315-325.
MCCANN, K. S. 2000. The diversity-stability debate. *Nature,* 405**,** 228-233.
MCNAUGHTON, S. J. 1977. Diversity and Stability of Ecological Communities: A Comment on the Role of Empiricism in Ecology. *The American Naturalist,* 111**,** 515-525.
MEE, L. D., JEFFERSON, R. L., LAFFOLEY, D. D. A. & ELLIOTT, M. 2008. How good is good? Human values and Europe's proposed Marine Strategy Directive. *Marine Pollution Bulletin,* 56**,** 187-204.
MILBRANDT, E. C. 2005. Bacterial communities as indicators of estuarine and sediment conditions. *In:* BORTONE, S. A. (ed.) *Estuarine Indicators.* 1st ed. Florida: CRC Press.
MITTELBACH, G. G., STEINER, C. F., SCHEINER, S. M., GROSS, K. L., REYNOLDS, H. L., WAIDE, R. B., WILLIG, M. R., DODSON, S. I. & GOUGH, L. 2001. What Is the Observed Relationship between Species Richness and Productivity? *Ecology,* 82**,** 2381-2396.
MRAG & UNEP-WCMC 2007. Defining concepts of ecosystem structure and function for UK marine monitoring. Joint Nature Conservation Committee, Peterborough. 65 pages. JNCC report No. 397.
MUCHA, A. P., VASCONCELOS, M. T. S. D. & BORDALO, A. A. 2005. Spatial and seasonal variations of the macrobenthic community and metal contamination in the Douro estuary (Portugal). *Marine Environmental Research,* 60**,** 531-550.
MUNARI, C. & MISTRI, M. 2008. The performance of benthic indicators of ecological change in Adriatic coastal lagoons: Throwing the baby with the water. *Marine Pollution Bulletin,* 56**,** 95-105.
MUNIZ, P., VENTURINI, N., PIRES-VANIN, A. M. S., TOMMASI, L. R. & BORJA, Á. 2005. Testing the applicability of a Marine Biotic Index (AMBI) to assessing the ecological quality of soft-bottom benthic communities, in the South America Atlantic region. *Marine Pollution Bulletin,* 50**,** 624-637.
MUXIKA, I., BORJA, Á. & BALD, J. 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. *Marine Pollution Bulletin,* 55**,** 16-29.
MUXIKA, I., BORJA, A. & BONNE, W. 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. *Ecological Indicators,* 5**,** 19-31.
MUXIKA, I., SOMERFIELD, P. J., BORJA, Á. & WARWICK, R. M. 2012. Assessing proposed modifications to the AZTI marine biotic index (AMBI), using biomass and production. *Ecological Indicators,* 12**,** 96-104.
NAEEM, S., THOMPSON, L. J., LAWLER, S. P., LAWTON, J. H. & WOODFIN, R. M. 1994. Declining biodiversity can alter the performance of ecosystems. *Nature,* 368**,** 734-737.

NICKELL, T. D., CROMEY, C. J., BORJA, Á. & BLACK, K. D. 2009. The benthic impacts of a large cod farm -- Are there indicators for environmental sustainability? *Aquaculture,* 295**,** 226-237.

ODUM, E. P. 1985. Trends expected in stressed ecosystems. *Bioscience,* 35**,** 419-422.

OSPAR 2009. EcoQO Handbook: Handbook for the application of Ecological Quality Objectives in the North Sea. Second ed.

PAGANELLI, D., MARCHINI, A. & OCCHIPINTI-AMBROGI, A. 2012. Functional structure of marine benthic assemblages using Biological Traits Analysis (BTA): A study along the Emilia-Romagna coastline (Italy, North-West Adriatic Sea). *Estuarine, Coastal and Shelf Science,* 96**,** 245-256.

PAUL, M. J. 1997. Back to Odum: Using ecosystem functional measures in stream ecosystem management. *In:* HATCHER, K. J. (ed.) *Proceedings of the 1997 Georgia Water Resources Conference, held March 20-22, 1997, at the University of Georgia.* Athens, Georgia: Institute of Ecology, The University of Georgia.

PEARSON, T. H. & ROSENBERG, R. 1976, 1978. Changes in fauna and sediment structure along a gradient of organic enrichment. *http://pubs.usgs.gov/of/1998/of98-502/chapt4/figure%2011.htm*.

PEARSON, T. H. & ROSENBERG, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology Annual Review,* 16**,** 229-311.

PÉRU, N. & DOLÉDEC, S. 2010. From compositional to functional biodiversity metrics in bioassessment: A case study using stream macroinvertebrate communities. *Ecological Indicators,* 10**,** 1025-1036.

PETCHEY, O. L. 2004. On the statistical significance of functional diversity effects. *Functional Ecology,* 18**,** 297-303.

PETCHEY, O. L. & GASTON, K. J. 2002. Functional diversity (FD), species richness and community composition. *Ecology Letters,* 5**,** 402-411.

PINTO, R., PATRÍCIO, J., BAETA, A., FATH, B. D., NETO, J. M. & MARQUES, J. C. 2009. Review and evaluation of estuarine biotic indices to assess benthic condition. *Ecological Indicators,* 9**,** 1-25.

PRANOVI, F., DA PONTE, F. & TORRICELLI, P. 2007. Application of biotic indices and relationship with structural and functional features of macrobenthic community in the lagoon of Venice: an example over a long time series of data. *Marine Pollution Bulletin,* 54**,** 1607-1618.

PRANOVI, F., DA PONTE, F. & TORRICELLI, P. 2008. Historical changes in the structure and functioning of the benthic community in the lagoon of Venice. *Estuarine, Coastal and Shelf Science,* 76**,** 753-764.

PUENTE, A. & DIAZ, R. J. 2008. Is it possible to assess the ecological status of highly stressed natural estuarine environments using macroinvertebrates indices? *Marine Pollution Bulletin,* 56**,** 1880-1889.

QUINTINO, V., ELLIOTT, M. & RODRIGUES, A. M. 2006. The derivation, performance and role of univariate and multivariate indicators of benthic change: Case studies at differing spatial scales. *Journal of Experimental Marine Biology and Ecology,* 330**,** 368-382.

RACHELLO-DOLMEN, P. G. & CLEARY, D. F. R. 2007. Relating coral species traits to environmental conditions in the Jakarta Bay/Pulau Seribu reef system, Indonesia. *Estuarine, Coastal and Shelf Science,* 73**,** 816-826.
RAFFAELLI, D., EMMERSON, M., SOLAN, M., BILES, C. & PATERSON, D. 2003. Biodiversity and ecosystem processes in shallow coastal waters: an experimental approach. *Journal of Sea Research,* 49**,** 133-141.
RAFFAELLI, D. G. & FRID, C. L. J. 2010. *Ecosystem Ecology: A New Synthesis,* New York, USA, Cambridge University Press.
RAPPORT, D. J., COSTANZA, R. & MCMICHAEL, A. J. 1998. Assessing ecosystem health. *TREE,* 13**,** 397-402.
RAPPORT, D. J. & WHITFORD, W. G. 1999. How Ecosystems Respond to Stress. *BioScience,* 49**,** 193-203.
REISS, H. & KRÖNCKE, I. 2005. Seasonal variability of benthic indices: An approach to test the applicability of different indices for ecosystem quality assessment. *Marine Pollution Bulletin,* 50**,** 1490-1499.
REISS, J., BRIDLE, J. R., MONTOYA, J. M. & WOODWARD, G. 2009. Emerging horizons in biodiversity and ecosystem functioning research. *Trends in ecology & evolution (Personal edition),* 24**,** 505-514.
ROGERS, S. I. & GREENAWAY, B. 2005. A UK perspective on the development of marine ecosystem indicators. *Marine Pollution Bulletin,* 50**,** 9-19.
ROSENBERG, R., BLOMQVIST, M., NILSSON, H. C., CEDERWALL, H. & DIMMING, A. 2004. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. *Marine Pollution Bulletin,* 49**,** 728-739.
RUELLET, T. & DAUVIN, J.-C. 2007. Benthic indicators: Analysis of the threshold values of ecological quality classifications for transitional waters. *Marine Pollution Bulletin,* 54**,** 1707-1714.
SALAS, F., MARCOS, C., NETO, J. M., PATRÍCIO, J., PÉREZ-RUZAFA, A. & MARQUES, J. C. 2006. User-friendly guide for using benthic ecological indicators in coastal and marine quality assessment. *Ocean & Coastal Management,* 49**,** 308-331.
SALAS, F., NETO, J. M., BORJA, A. & MARQUES, J. C. 2004. Evaluation of the applicability of a marine biotic index to characterize the status of estuarine ecosystems: the case of Mondego estuary (Portugal). *Ecological Indicators,* 4**,** 215-225.
SANDIN, L. & SOLIMINI, A. G. 2009. Freshwater ecosystem structure–function relationships: from theory to application. *Freshwater Biology,* 54**,** 2017-2024.
SCHEFFER, M., BASCOMPTE, J., BROCK, W. A., BROVKIN, V., CARPENTER, S. R., DAKOS, V., HELD, H., VAN NES, E. H., RIETKERK, M. & SUGIHARA, G. 2009. Early-warning signals for critical transitions. *Nature,* 461**,** 53-59.
SCHLACHER, T. A. & WOOLDRIDGE, T. H. 1996. How sieve mesh size affects sample estimates of estuarine benthic macrofauna. *Journal of Experimental Marine Biology and Ecology,* 201**,** 159-171.
SEPA 1996. Biological quality at Ironrotter Point, Greenock - 1989-1998. Scottish Environment Protection Agency. Scotland, UK.
SEPA 2000. Benthic biological quality - Irvine and Ayr Bays, 1979 - 1998. Scottish Environment Protection Agency. Scotland, UK.

SOLAN, M. 2000. *The concerted use of 'traditional' and sediment profile imagery (SPI) methodologies in marine benthic characterisation and monitoring.* PhD, National University of Ireland, Galway.

SOLAN, M., CARDINALE, B. J., DOWNING, A. L., ENGELHARDT, K. A. M., RUESINK, J. L. & SRIVASTAVA, D. S. 2004. Extinction and Ecosystem Function in the Marine Benthos. *Science,* 306**,** 1177-1180.

SOLIMINI, A. G., PTACNIK, R. & CARDOSO, A. C. 2009. Towards holistic assessment of the functioning of ecosystems under the Water Framework Directive. *TrAC Trends in Analytical Chemistry,* 28**,** 143-149.

STACHOWICZ, J. J., BRUNO, J. F. & DUFFY, J. E. 2007. Understanding the effects of marine biodiversity on communities and ecosystems. *Annual Review of Ecology, Evolution, and Systematics,* 38**,** 739-766.

STATZNER, B., BADY, P., DOLEDEC, S. & SCHOLI, F. 2005. Invertebrate traits for the biomonitoring of large European rivers: an initial assessment of trait patterns in least impacted river reaches. *Freshwater Biology,* 50**,** 2136-2161.

STATZNER, B., BIS, B., DOLÉDEC, S. & USSEGLIO-POLATERA, P. 2001. Perspectives for biomonitoring at large spatial scales: a unified measure for the functional composition of invertebrate communities in European running waters. *Basic and Applied Ecology,* 2**,** 73-85.

SZAVA-KOVATS, R. C. 2008. Grain-size normalization as a tool to assess contamination in marine sediments: Is the <63 µm fraction fine enough? *Marine Pollution Bulletin,* 56**,** 629-632.

TEIXEIRA, H., SALAS, F., BORJA, Á., NETO, J. M. & MARQUES, J. C. 2008a. A benthic perspective in assessing the ecological status of estuaries: The case of the Mondego estuary (Portugal). *Ecological Indicators,* 8**,** 404-416.

TEIXEIRA, H., SALAS, F., NETO, J. M., PATRÍCIO, J., PINTO, R., VERÍSSIMO, H., GARCÍA-CHARTON, J. A., MARCOS, C., PÉREZ-RUZAFA, A. & MARQUES, J. C. 2008b. Ecological indices tracking distinct impacts along disturbance-recovery gradients in a temperate NE Atlantic Estuary – Guidance on reference values. *Estuarine, Coastal and Shelf Science,* 80**,** 130-140.

TETT, P., GILPIN, L., SVENDSEN, H., ERLANDSSON, C. P., LARSSON, U., KRATZER, S., FOUILLAND, E., JANZEN, C., LEE, J.-Y., GRENZ, C., NEWTON, A., GOMES FERREIRA, J., FERNANDES, T. & SCORY, S. 2003. Eutrophication and some European waters of restricted exchange. *Continental Shelf Research* 23**,** 1635-1671.

TETT, P., GOWEN, R., MILLS, D., FERNANDES, T., GILPIN, L., HUXHAM, M., KENNINGTON, K., READ, P., SERVICE, M., WILKINSON, M. & MALCOLM, S. 2007. Defining and detecting undesirable disturbance in the context of marine eutrophication. *Marine Pollution Bulletin,* 55**,** 282-297.

THEODOROU, Y. & ALEVIZOS, P. 2006. The fuzzy eigenvalue problem of fuzzy correspondence analysis. *Journal of Interdisciplinary Mathematics,* 9**,** 115-137.

TILLIN, H. M., ROGERS, S. I. & FRID, C. L. J. 2008. Approaches to classifying benthic habitat quality. *Marine Policy,* 32**,** 455-464.

TILMAN, D. 1999. The ecological consequences of changes in biodiversity: A search for general principles. *Ecology,* 80**,** 1455-1474.

TILMAN, D., REICH, P. B., KNOPS, J., WEDIN, D., MIELKE, T. & LEHMAN, C. 2001. Diversity and Productivity in a Long-Term Grassland Experiment. *Science,* 294**,** 843-845.

TILMAN, D., WEDIN, D. & KNOPS, J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature,* 379**,** 718-720.

UKMMAS 2007. UKMMAS: A Strategy for UK Marine Monitoring and Assessment.

USSEGLIO-POLATERA, P., BOURNAUD, M., RICHOUX, P. & TACHET, H. 2000. Biomonitoring through biological traits of benthic macroinvertebrates: how to use species trait databases? *Hydrobiologia,* 422-423**,** 153-162.

VALIELA, I. 1995. *Marine Ecological Processes,* USA, Springer.

VAN HOEY, G., BORJA, A., BIRCHENOUGH, S., BUHL-MORTENSEN, L., DEGRAER, S., FLEISCHER, D., KERCKHOF, F., MAGNI, P., MUXIKA, I., REISS, H., SCHRÖDER, A. & ZETTLER, M. L. 2010. The use of benthic indicators in Europe: From the Water Framework Directive to the Marine Strategy Framework Directive. *Marine Pollution Bulletin,* 60**,** 2187-2196.

VASSALLO, P., FABIANO, M., VEZZULLI, L., SANDULLI, R., MARQUES, J. C. & JORGENSEN, S. E. 2006. Assessing the health of coastal marine ecosystems: A holistic approach based on sediment micro and meio-benthic measures. *Ecological Indicators,* 6**,** 525-542.

WALKER, B., KINZIG, A. & LANGRIDGE, J. 1999. Original Articles: Plant Attribute Diversity, Resilience, and Ecosystem Function: The Nature and Significance of Dominant and Minor Species. *Ecosystems,* 2**,** 95-113.

WAN HUSSIN, W. M. R., COOPER, K. M., FROJÁN, C. R. S. B., DEFEW, E. C. & PATERSON, D. M. 2012. Impacts of physical disturbance on the recovery of a macrofaunal community: A comparative analysis using traditional and novel approaches. *Ecological Indicators,* 12**,** 37-45.

WARWICK, R. M. & CLARKE, K. R. 1993. Increased variability as a symptom of stress in marine communities. *Journal of Experimental Marine Biology and Ecology,* 172**,** 215-226.

WARWICK, R. M., DASHFIELD, S. L. & SOMERFIELD, P. J. 2006. The integral structure of a benthic infaunal assemblage. *Journal of Experimental Marine Biology and Ecology,* 330**,** 12-18.

WARWICK, R. M., ROBERT CLARKE, K. & SOMERFIELD, P. J. 2010. Exploring the marine biotic index (AMBI): variations on a theme by Ángel Borja. *Marine Pollution Bulletin,* 60**,** 554-559.

WFD-UKTAG, W. F. D.-U. K. T. A. G. 2008. UKTAG coastal water assessment method benthic invertebrate fauna. Invertebrates in soft sediments (Infaunal quality Index (IQI)). Edinburgh, Scotland.

WHOMERSLEY, P., WARE, S., REES, H. L., MASON, C., BOLAM, T., HUXHAM, M. & BATES, H. 2008. Biological indicators of disturbance at a dredged-material disposal site in Liverpool Bay, UK: an assessment using time-series data. *ICES Journal of Marine Science: Journal du Conseil,* 65**,** 1414-1420.

WORD, J. Q. 1979. The Infaunal Trophic Index. *In:* BASCOM, W. (ed.) *Southern California Coastal Water Research Project, Annual Report 1978,* . El Segundo, California.

WORM, B., BARBIER, E. B., BEAUMONT, N., DUFFY, J. E., FOLKE, C., HALPERN, B. S., JACKSON, J. B. C., LOTZE, H. K., MICHELI, F.,

PALUMBI, S. R., SALA, E., SELKOE, K. A., STACHOWICZ, J. J. & WATSON, R. 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science,* 314**,** 787-790.

YEE, T. W. 2006. CONSTRAINED ADDITIVE ORDINATION. *Ecology*, 87**,** 203-213.

ZETTLER, M. L., SCHIEDEK, D. & BOBERTZ, B. 2007. Benthic biodiversity indices versus salinity gradient in the southern Baltic Sea. *Marine Pollution Bulletin,* 55**,** 258-270.

8. Appendix

Appendix 8.1 BQI

Calculation of the BQI, in particular the $ES50_{0.05}$, appears to have caused some confusion in the literature with the initial paper (Rosenberg et al, 2004) being vague on the details and criteria of calculation. This was highlighted by Leonardsson et al (2009) who identified that some studies had calculated BQI incorrectly (e.g. Fleischer et al, 2007) and went on to clarify the method. The requirements for calculating BQI which have been unclear in the past are summarised in Box 8.1.1. This mainly describes the calculation of the sensitivity for each species which should be carried out using a large, uniformly sampled and independent data set which includes a range of samples types from unimpacted to impacted. Sensitivity values for different species may vary in different areas so ideally they should be calculated for different sea areas and for similar habitats.

Box 8.1.1 Criteria for calculating the Rosenberg Benthic Quality Index (BQI) from Leonardsson et al (2009)

1. Gradient data, including several samples from heavily disturbed to undisturbed.
2. A large data set
3. Only calculate $ES50_{0.05}$ for each species occurring in circa 20 or more grabs
4. Sensitivity values should be calculated for each sea area
5. Uniform grab and sieve sizes
6. Similar habitats and environmental conditions
7. Must be an independent dataset from the dataset used to assess ecological status

Aim

To calculate a range of sensitivity ($ES50_{0.05}$) values for species found in Scottish and Irish sub-tidal, soft sediment habitats.

Methods

A large dataset was compiled using samples from several of the available datasets. Since the samples should be uniform in size and collection, only samples collected using a $0.1m^2$ grab and sieved using a 0.5mm mesh were used. Samples chosen ranged from 'High' to 'Bad' quality, as classified by M-AMBI (Table 8.1.1). The samples were taken from a range of sites in the east and west coast of Scotland and from the west coast of Ireland in order to create a species list which would be representative of the overall area. A total of 123 grabs were used in the dataset. Of these, 11 stations were classified as 'High' status; 70 were classified as 'Good'; 26 were 'Moderate'; 6 were 'Poor'; and 10 were classified as 'Bad'. However, there were some instances where m-AMBI results were not reliable due to the very low number of species in the sample and these resulted in a 'Good' classification where it should have been lower quality. This would have resulted in at least seven additional samples being classified as 'Bad' or 'Poor'.

Table 8.1.1 Characteristics of grab samples used in the calculation of BQI $ES50_{0.05}$ values

No.	Site	Stations	M-AMBI	Status	No.	Site	Stations	M-AMBI	Status
1	Barcaldine	01 A11	0.77	Good	63	Ironrotter	98 E100.3	0.74	Good
2	Barcaldine	01 A11	0.77	Good	64	Ironrotter	98 E100.3	0.74	Good
3	Barcaldine	01 B11	0.06	Bad	65	Ironrotter	98 E500.1	0.88	High
4	Barcaldine	01 B11	0.06	Bad	66	Ironrotter	98 E500.1	0.88	High
5	Barcaldine	01 D51	0.43	Mod.	67	Ironrotter	98 E500.2	0.63	Good
6	Barcaldine	04 A3.1	0.50	Mod.	68	Ironrotter	98 E500.2	0.63	Good
7	Barcaldine	04 B1.1	0.53	Mod.	69	Irvine Bay	04 Q1.2	0.66	Good
8	Barcaldine	04 D3.1	0.45	Mod.	70	Irvine Bay	04 Q2.1	0.58	Good
9	Barcaldine	97 0.1	0.08	Bad	71	Irvine Bay	89 AB1	0.61	Good
10	Barcaldine	97 0.2	0.40	Mod.	72	Irvine Bay	89 AB32	0.68	Good
11	Barcaldine	97 0.2	0.40	Mod.	73	Irvine Bay	89 C2	0.83	Good
12	Barcaldine	97 A1.1	0.06	Bad	74	Irvine Bay	89 H2	0.78	Good
13	Barcaldine	97 A1.1	0.06	Bad	75	Irvine Bay	89 I1	0.74	Good
14	Barcaldine	97 A1.2	0.30	Poor	76	Irvine Bay	89 J11	0.43	Mod.
15	Barcaldine	97 A1.2	0.30	Poor	77	Irvine Bay	89 L7	0.71	Good
16	Barcaldine	97 A3.1	0.53	Mod.	78	Irvine Bay	89 L81	0.46	Mod.
17	Barcaldine	97 A5.2	0.56	Good	79	Irvine Bay	89 Q11	0.57	Good
18	Barcaldine	97 B1.1	0.02	Bad	80	Irvine Bay	89 Q2	0.55	Good
19	Barcaldine	97 B1.2	0.30	Poor	81	Irvine Bay	89 R22	0.54	Mod.
20	Barcaldine	97 B1.2	0.30	Poor	82	Irvine Bay	89 Z2	0.75	Good
21	Barcaldine	97 C1.1	0.06	Bad	83	Irvine Bay	95 C.2	0.79	Good
22	Barcaldine	97 C1.1	0.06	Bad	84	Irvine Bay	95 H.1	0.70	Good
23	Barcaldine	97 C3.2	0.44	Mod.	85	Irvine Bay	95 J 1.1	0.29	Poor
24	Barcaldine	97 D1.1	0.43	Mod.	86	Irvine Bay	95 L 81.2	0.17	Bad
25	Barcaldine	97 D1.1	0.43	Mod.	87	Irvine Bay	95 Q 1.2	0.38	Poor
26	Barcaldine	97 D5.2	0.52	Mod.	88	Irvine Bay	95 R 1.2	0.79	Good
27	Barcaldine	99 A1.1	0.39	Mod.	89	KC	2000 A	0.47	Mod.
28	Barcaldine	99 A3.1	0.79	Good	90	KC	2000 B	0.65	Good
29	Barcaldine	99 B1.1	1.00	High	91	KC	2001 E	0.67	Good
30	Barcaldine	99 B1.1	1.00	High	92	KC	2002 A	0.62	Good
31	Barcaldine	99 B5.1	0.53	Mod.	93	KC	2004 D	0.60	Good
32	Barcaldine	99 C1.1	0.41	Mod.	94	KC	2004 E	0.55	Good
33	Cromarty	05 Cromarty	0.86	High	95	KC	2005 A	0.73	Good
34	Cromarty	07 Cromarty	0.71	Good	96	KC	2005 E	0.59	Good
35	Cromarty	08 Cromarty	0.87	High	97	KC	2006 A	0.09	Bad
36	Cromarty	7 Cromarty	0.92	High	98	KH	1999 A	0.58	Good
37	Firth of Forth	07 FOF	0.75	Good	99	KH	1999 B	0.58	Good
38	Ironrotter	89 A1.1	0.64	Good	100	KH	2000 E	0.78	Good
39	Ironrotter	89 B2.3	0.67	Good	101	KH	2001 A	0.65	Good
40	Ironrotter	89 C2.3	0.56	Good	102	KH	2002 D	0.82	Good
41	Ironrotter	89 D2.2	0.68	Good	103	KH	2002 E	0.80	Good
42	Ironrotter	89 E2.3	0.60	Good	104	KH	2003 A	0.93	High

Table 8.1.1 continued

No.	Site	Stations	M-AMBI	Status	No.	Site	Stations	M-AMBI	Status
43	Ironrotter	89 G1.1	0.65	Good	105	KH	2004 B	0.94	High
44	Ironrotter	89 G3.3	0.63	Good	106	Leverets	LevApr1	0.51	Mod.
45	Ironrotter	92 A3	0.71	Good	107	Leverets	LevAug1	0.51	Mod.
46	Ironrotter	92 B1	0.68	Good	108	Leverets	LevDec5	0.49	Mod.
47	Ironrotter	92 D1	0.71	Good	109	Leverets	LevJan1	0.59	Good
48	Ironrotter	92 F1	0.54	Mod.	110	Leverets	LevJul5	0.48	Mod.
49	Ironrotter	92 G3	0.68	Good	111	Leverets	LevJun3	0.57	Good
50	Ironrotter	92 H1	0.69	Good	112	Leverets	LevMar5	0.52	Mod.
51	Ironrotter	95 A100	0.73	Good	113	Leverets	LevNov3	0.58	Good
52	Ironrotter	95 A1000	0.65	Good	114	Leverets	LevSep4	0.65	Good
53	Ironrotter	95 A750	0.65	Good	115	Margaretta	MargDec1	0.68	Good
54	Ironrotter	95 B500	0.78	Good	116	Margaretta	MargJan5	0.65	Good
55	Ironrotter	95 B750	0.66	Good	117	Margaretta	MargJul4	0.64	Good
56	Ironrotter	95 H750	0.76	Good	118	Margaretta	MargJun1	0.65	Good
57	Ironrotter	98 A100.1	0.63	Good	119	Margaretta	MargMar1	0.65	Good
58	Ironrotter	98 A100.1	0.63	Good	120	Margaretta	MargMay5	0.69	Good
59	Ironrotter	98 A100.2	0.95	High	121	Margaretta	MargNov1	0.74	Good
60	Ironrotter	98 A100.2	0.95	High	122	Margaretta	MargOct5	0.72	Good
61	Ironrotter	98 E100.1	0.52	Mod.	123	Margaretta	MargSep2	0.70	Good
62	Ironrotter	98 E100.1	0.52	Mod.					

The ES50 was calculated for each site using Primer. A script was written in Matlab in order to calculate the ES50 at the 5th percentile (end of this section). The ES50 value is associated with the frequency of each species and the 5th percentile value of the ES50 is found for each species (where the species has 5% of its total abundance). The input data for Matlab should be in a text file format (.txt). The sites should be in rows. The first column should be the ES50 values. The second and subsequent columns should be the species (e.g. Table 8.1.2). Data should be sorted according to ES50 from the lowest to highest values. The names of species and sites should be deleted before being imported to Matlab.

Table 8.1.2 Example set up of data set for $ES50_{0.05}$ calculation

Site	ES50	*Abra alba*	*Capitella capitata*	*Mediomastus fragilis*	*Thyasira flexuosa*
97 B1.1	1.66	0	2692	0	0
97 0.1	2.16	1	193	0	0
95 L 81.2	4.09	70	3271	21	0
97 D5.2	13.32	0	0	166	0
2000 E	16.56	0	0	18	5
08 Cromarty	17.05	158	0	10	17
95 R 1.2	21.69	0	0	4	0
89 A1.1	21.86	17	0	10	16
04 Q1.2	22.35	6	0	1	10
2003 A	22.44	6	0	3	18
89 Z2	22.45	0	0	30	5
2004 B	27.42	0	0	67	9
89 C2	28.35	0	0	38	35

The $Es50_{0.05}$ values were then used to calculate the BQI using this dataset and these values were used to set a scale for BQI quality classification from Bad to Good.

Results

The ES50 0.05 was calculated for every species which occurred in 5 or more grabs (Table 8.1.3).

Table 8.1.3 Sensitivity value for each species found in 5 or more grabs. Blue $ES50_{0.05}$ value represents the corresponding values found by Rosenberg et al. (2004) for the west coast of Sweden.

Taxa	$ES50_{0.05}$	No. of grabs	Taxa	$ES50_{0.05}$	No. of grabs
Abra sp.	9.09	18	*Magelona minuta*	11.31 12.06	9
Abra alba	9.81 3.96	48	*Magelona mirabilis*	13.99 12.49	15
Abra nitida	11.72 9.26	26	*Malacoceros fuliginosus*	1.66 2.16	13
Abra prismatica	10.83	11	*Maldanidae sp.*	6.82	6
Acanthocardia echinata	6.08 9.58	7	*Mediomastus fragilis*	6.08 5.39	80
Ampelisca brevicornis	9.09 12.49	35	*Melinna cristata*	20.84 8.58	5

Table 8.1.3 continued

Taxa	$ES50_{0.05}$	No. of grabs	Taxa	$ES50_{0.05}$	No. of grabs
Ampelisca diadema	15.15 10.73	14	*Melinna palmata*	9.81	52
Ampelisca sp.	19.97	5	*Minuspio cirrifera*	11.31	7
Ampelisca spinipes	10.90	6	*Minuspio multibranchiata*	18.51	9
Ampelisca tenuicornis	11.31 9.99	38	*Moerella pygmaea*	10.98	9
Ampharete baltica	17.28 8.21	23	*Mya arenaria*	6.08 3.48	10
Ampharete grubei	14.78	8	*Mya sp.*	8.53	5
Ampharete lindstroemi	9.81 10.15	32	*Mya truncata*	12.63 6.24	8
Ampharete sp.	7.23	10	*Myriochele oculata*	18.51 9.39	11
Ampharetidae sp.	15.91	14	*Mysella bidentata*	12.90 6.83	86
Amphictene auricoma	18.51	14	*Mysia undata*	14.82 9.37	10
Amphitrite cirrata	17.87	11	*Mysta picta*	7.40	8
Amphiura chiajei	14.50 7.80	9	*Mytilus edulis*	14.50 7.05	19
Amphiura filiformis	13.39 7.80	53	*Nematoda*	11.72	32
Amphiura sp.	12.63	22	*Nemertea sp.*	6.08 7.99	46
Amphiuridae sp.	18.99	6	*Nephtys caeca*	14.50 6.01	9
Anobothrus gracilis	16.06 10.67	40	*Nephtys hombergii*	9.81 5.04	56
Anoplodactylus petiolatus	5.91 9.39	9	*Nephtys incisa*	5.00 7.99	14
Aoridae sp.	9.01	10	*Nephtys kersivalensis*	6.08	33
Aphelochaeta marioni	10.96	27	*Nephtys sp.*	12.87	52
Aphelochaeta sp.	4.50	5	*Nereimyra punctata*	6.23 8.73	8
Aphrodite aculeata	12.63 9.91	24	*Nereis longissima*	2.57	21
Apistobranchus tullbergi	15.15 9.17	13	*Notomastus latericeus*	12.63 9.79	33
Arctica islandica	14.11 5.92	15	*Nucula nitidosa*	14.50 8.12	56
Arenicolides sp.	9.16	5	*Nucula tenuis*	9.81	9
Aricidea catherinae	5.68	6	*Nuculoma tenuis*	18.51	18
Aricidea minuta	13.11	13	*Oligochaeta sp.*	10.83 5.10	15
Astacilla longicornis	13.39	7	*Ophelina acuminata*	14.50 9.44	33

Table 8.1.3 continued

Taxa	$ES50_{0.05}$	No. of grabs	Taxa	$ES50_{0.05}$	No. of grabs
Atylus vedlomensis	6.08 12.76	7	*Ophiodromus flexuosus*	9.81 7.49	34
Bodotria scorpioides	13.39	8	*Ophiura affinis*	10.13	5
Calanoida sp.	13.11	9	*Ophiura albida*	6.08 7.49	19
Capitella capitata	1.66 1.10	36	*Ophiura sp.*	6.08	11
Capitellidae spp.	13.39	17	*Ophiuroidea*	14.31	14
Caulleriella killariensis	12.90 11.83	8	*Ophryotrocha hartmanni*	6.08	21
Caulleriella zetlandica	6.08	29	*Owenia fusiformis*	12.63 7.70	54
Cerebratulus fuscus	11.94	9	*Paradoneis lyra*	15.49 11.73	38
Cerebratulus sp.	9.98	23	*Paranais litoralis*	6.08	5
Cerianthus llyodii	9.01 8.68	14	*Pariambus typicus*	13.70 6.53	22
Chaetoderma nitidulum	12.63 9.66	15	*Parougia caeca*	6.82	8
Chaetozone christiei	14.50	8	*Peresiella clymenoides*	12.63	5
Chaetozone setosa	9.81 10.23	61	*Perioculoides longimanus*	10.83 11.74	23
Chaetozone sp.	9.01	7	*Phascolion strombus*	8.07 9.35	6
Chamelea gallina	12.63 10.79	15	*Phaxus pellucidus*	12.63 5.92	33
Chamellia striatula	14.50 9.01	19	*Philine aperta*	5.98 6.76	7
Circomphalus casina	9.10	5	*Philine sp.*	14.50	10
Cirratulidae sp.	6.08	19	*Philomedes brenda*	13.39	10
Cirratulus cirratus	6.08 9.76	27	*Pholoe baltica*	10.37 9.41	27
Cirriformia tentaculata	4.09	15	*Pholoe inornata (incl. synophthalmica)*	11.46 9.66	54
Corbula gibba	14.11 4.58	40	*Pholoe minuta*	18.51 9.55	12
Cossura longocirrata	4.00 10.79	8	*Phoronis muelleri*	11.94 8.34	39
Cryptocelides loveni	6.82	7	*Phoronis sp.*	12.63	23
Cucumariidae	19.26	5	*Photis longicaudata*	11.53	8
Cylichna cylindracea	11.46 9.53	49	*Phoxocephalus holbolli*	14.78	9
Cylindroleberis mariae	13.39	5	*Phyllodoce (Anaitides) groenlandica*	20.56 6.05	5

Table 8.1.3 continued

Taxa	ES50$_{0.05}$	No. of grabs	Taxa	ES50$_{0.05}$	No. of grabs
Diastylis bradyi	4.09 9.54	17	*Phyllodoce (Anaitides) longipes*	7.20 10.68	10
Diastylis rugosa	7.20	5	*Phyllodoce (Anaitides) maculata*	5.68 6.75	5
Diplocirrus glaucus	14.11 10.49	53	*Phyllodoce (Anaitides) mucosa*	4.09 6.10	18
Dosinia sp.	14.50	15	*Phyllodoce (Anaitides) rosea*	6.08 13.03	24
Dosinia exoleta	10.00	5	*Pista cristata*	8.45 10.61	7
Dosinia lupinus	12.63	7	*Pleurogonium rubicundum*	6.08	11
Echinocardium	13.11	9	*Podarkeopsis capensis*	11.13	24
Echinocardium cordatum	7.20 8.80	9	*Poecilochaetus serpens*	11.62	19
Edwardsia claperedii	13.01	18	*Polinices pulchellus*	14.50	11
Eteone longa	4.09 4.58	51	*POLYCHAETA*	6.82	5
Euchone rubrocincta	18.71	9	*Polycirrus sp.*	6.82	8
Euclymene lumbricoides	12.29	9	*Polycirrus medusa*	10.72	8
Euclymene oerstedii	14.71	18	*Polycirrus norvegicus*	6.08	12
Eudorella truncatula	13.01 10.52	34	*Polycirrus plumosus*	10.39	33
Eulalia viridis	10.83	11	*Polydora caeca*	12.63 8.13	7
Eulima glabra	5.91	6	*Polydora caulleryi*	3.54	7
Eumida bahusiensis	4.09 10.67	23	*Polydora ciliata*	6.00 4.99	6
Eumida sanguinea	6.08 10.85	8	*Polydora flava*	5.00	7
Eumida sp.	4.09	19	*Pomatoceros triqueter*	9.01	5
Exogone hebes	11.62 12.43	24	*Praxillella sp.*	12.63	6
Exogone naidina	6.08	23	*Praxillella affinis*	11.72	16
Fabulina fabulina	13.11	29	*Priapulus caudatus*	9.81 7.96	15
Galathowenia oculata	15.15	26	*Prionospio fallax*	9.81 11.03	56
Gammaropsis palmata	9.01	10	*Prionospio malmgreni*	21.47	8
Gari fervensis	8.39	8	*Prionospio sp.*	10.90	16
GASTROPODA sp.	12.63	8	*Protodorvillea kefersteini*	12.16	8
Gattyana cirrosa	16.61 8.04	21	*Pseudocuma longicornis*	6.82	6
Glycera alba	6.08 6.73	43	*Pseudopolydora antennata*	12.16 4.19	15

Table 8.1.3 continued

Taxa	ES50$_{0.05}$	No. of grabs	Taxa	ES50$_{0.05}$	No. of grabs
Glycera rouxii	13.76 10.92	10	*Pseudopolydora paucibranchiata*	6.08	19
Glycera sp.	6.08	23	*Pseudopolydora pulchra*	9.77 8.01	13
Glycera tridactyla	13.01	20	*Rhodine gracilior*	12.63 10.41	13
Glycinde nordmanni	12.63 11.64	10	*Sabellaria spinulosa*	10.37	14
Gnathia sp.	8.15	5	*SABELLIDA sp.*	5.41	5
Golfingia sp.	6.82	5	*Scalibregma inflatum*	8.50 6.65	70
Goniada maculata	9.01 9.27	48	*Scoloplos armiger*	11.72 6.24	49
Harmothoe impar	9.43 6.74	10	*Sipuncula sp.*	14.78	6
Harmothoe marphysae	17.27	17	*Sipunculidae sp.*	3.50	10
Harmothoe sp.	10.83	29	*Sphaerodorum gracilis*	15.15 7.49	12
Harpinia antennaria	12.63 11.74	26	*Sphaerosyllis taylori*	6.08	18
Harpinia crenulata	7.56 11.74	6	*Sphenia binghami*	11.72	7
Heteromastus filiformis	12.63 8.95	12	*Spio decorata*	11.72	30
Hiatella arctica	10.37 3.95	11	*Spio filicornis*	14.99 9.37	6
Hydroides norvegicus	5.00	6	*Spiophanes bombyx*	12.63 11.68	37
Hydrozoa sp.	13.39	5	*Spiophanes kroyerii*	15.60 12.03	42
Jasmineira caudata	6.08	14	*Spisula subtruncata*	12.08 6.43	12
Kefersteinia cirrata	8.00 7.51	11	*Sthenelais limicola*	6.82 6.97	11
Labidoplax buski	13.11 10.66	6	*Syllis sp.*	6.82	8
Lagis koreni	9.09	30	*Synchelidium maculatum*	14.90	9
Lanice conchilega	12.90 11.68	37	*Tanaopsis graciloides*	11.72	37
Laonome kroyeri	8.78 8.29	6	*Tellimya ferruginosa*	16.56	6
Leitoscoloplos mammosus	9.81	27	*Terebellides stroemi*	11.22 8.29	38
Lembos sp.	8.78	5	*Tharyx killariensis*	14.71	8
Lembos longipes	9.01	8	*Tharyx marioni*	18.30	10
Leptognathia gracilis	13.39	9	*Thracia sp.*	15.04	19

Table 8.1.3 continued

Taxa	ES50$_{0.05}$	No. of grabs	Taxa	ES50$_{0.05}$	No. of grabs
Leptopentacta elongata	6.82 8.78	11	*Thracia phaseolina*	13.11	10
Leptosynapta inhaerens	15.38	8	*Thyasira flexuosa*	9.81 4.53	82
Leucothoe lilljeborgi	13.39 10.44	10	*Trachythyone elongata*	9.75	5
Levinsenia gracilis	11.31 9.23	57	*Trichobranchus roseus*	15.60 10.65	18
Longipedia coronata	10.83	12	*Tubificoides amplivastus*	11.56	18
Lucinoma borealis	15.15 6.92	16	*Tubificoides benedii*	4.09	7
Lumbricillus sp.	6.08	5	*Tubulanus polymorphus*	6.08	64
Lumbrineris fragilis	13.39	13	*Tubulanus sp.*	14.11	41
Lumbrineris gracilis	12.63 14.71	45	*Turritella communis*	12.63 7.80	10
Lunatia poliana	6.58	5	*Virgularia mirabilis*	5.00 9.66	12
Magelona alleni	13.91 11.55	22	*Westwoodilla caecula*	6.27 11.06	8
Magelona filiformis	13.11	12			

The BQI was calculated for all sites in the dataset using the equation:

$$BQI = \left(\sum_{i=0}^{n}\left(\frac{A_i}{totA} \times ES50_{0.05i}\right)\right) \times log_{10}(S+1)$$

...where Ai is the abundance of species I, totA is the total abundance

ES50 $_{0.05}$ is the ES50 at 5% of the population of species i

S is the total species richness

Only species which were found in 15 grabs or more were used in the calculation in order to increase the reliability of the evaluation. The minimum and maximum values could then be used to create a scale from ‘Bad’ to ‘High’ by dividing into 5 equal parts. The minimum value was 0 and the maximum value was 21.58 (Table 8.1.4).

Table 8.1.4 BQI quality classification for each station; ≤ 4.32 = 'Bad'; > 4.32 ≤ 8.64 = 'Poor'; > 8.64 ≤ 12.96 = 'Moderate'; > 12.96 ≤ 17.28 = 'Good'; >17.28 = 'High'.

Site	Sample	BQI	Quality	Site	Sample	BQI	Quality
Barcaldine	01 A11	1.67	Bad	Ironrotter	98 A100.1	11.84	Mod
Barcaldine	01 B11	0.50	Bad	Ironrotter	98 A100.2	15.07	Good
Barcaldine	01 D51	3.93	Bad	Ironrotter	98 E100.1	10.33	Mod
Barcaldine	04 A3.1	11.13	Mod	Ironrotter	98 E100.3	11.79	Mod
Barcaldine	04 B1.1	11.00	Mod	Ironrotter	98 E500.1	13.05	Good
Barcaldine	04 D3.1	10.95	Mod	Ironrotter	98 E500.2	13.59	Good
Barcaldine	97 0.1	0.63	Bad	Irvine Bay	04 Q1.2	13.11	Good
Barcaldine	97 0.2	0.55	Bad	Irvine Bay	04 Q2.1	14.69	Good
Barcaldine	97 A1.1	0.00	Bad	Irvine Bay	89 AB1	17.22	Good
Barcaldine	97 A1.2	0.40	Bad	Irvine Bay	89 AB32	15.48	Good
Barcaldine	97 A3.1	14.95	Good	Irvine Bay	89 C2	18.01	High
Barcaldine	97 A5.2	14.45	Good	Irvine Bay	89 H2	17.48	High
Barcaldine	97 B1.1	0.98	Bad	Irvine Bay	89 I1	19.84	High
Barcaldine	97 B1.2	0.33	Bad	Irvine Bay	89 J11	8.58	Poor
Barcaldine	97 C1.1	0.50	Bad	Irvine Bay	89 L7	18.90	High
Barcaldine	97 C3.2	11.83	Mod	Irvine Bay	89 L81	15.13	Good
Barcaldine	97 D1.1	0.53	Bad	Irvine Bay	89 Q11	15.23	Good
Barcaldine	97 D5.2	12.86	Mod	Irvine Bay	89 Q2	17.51	High
Barcaldine	99 A1.1	5.88	Poor	Irvine Bay	89 R22	18.74	High
Barcaldine	99 A3.1	16.81	Good	Irvine Bay	89 Z2	14.75	Good
Barcaldine	99 B1.1	1.98	Bad	Irvine Bay	95 C.2	17.36	High
Barcaldine	99 B5.1	15.10	Good	Irvine Bay	95 H.1	15.51	Good
Barcaldine	99 C1.1	6.22	Poor	Irvine Bay	95 J 1.1	6.12	Poor
Cromarty	05 Cromarty	19.29	High	Irvine Bay	95 L 81.2	1.56	Bad
Cromarty	05 Cromarty	19.51	High	Irvine Bay	95 Q 1.2	2.41	Bad
Cromarty	05 Cromarty	19.62	High	Irvine Bay	95 R 1.2	16.35	Good
Cromarty	05 Cromarty	19.85	High	KC	2000 A	8.26	Poor
Cromarty	05 Cromarty	20.57	High	KC	2000 B	10.55	Mod
Cromarty	07 Cromarty	19.08	High	KC	2001 E	10.25	Mod
Cromarty	07 Cromarty	19.92	High	KC	2002 A	13.63	Good
Cromarty	08 Cromarty	15.27	Good	KC	2004 D	11.51	Mod
Cromarty	08 Cromarty	16.91	Good	KC	2004 E	11.88	Mod
Cromarty	08 Cromarty	17.58	High	KC	2005 A	12.31	Mod

Table 8.1.4 continued

Site	Sample	BQI	Quality	Site	Sample	BQI	Quality
Cromarty	08 Cromarty	17.65	High	KC	2005 E	15.70	Good
Cromarty	08 Cromarty	21.18	High	KC	2006 A	0.40	Bad
Cromarty	7 Cromarty	16.29	Good	KH	1999 A	13.63	Good
Cromarty	7 Cromarty	19.07	High	KH	1999 B	10.12	Mod
Cromarty	7 Cromarty	20.76	High	KH	2000 E	18.82	High
Firth of Forth	07 FOF	3.56	Bad	KH	2001 A	15.81	Good
Firth of Forth	07 FOF	4.50	Poor	KH	2002 D	21.58	High
Firth of Forth	07 FOF	6.34	Poor	KH	2002 E	21.23	High
Firth of Forth	07 FOF	10.50	Mod	KH	2003 A	17.67	High
Firth of Forth	07 FOF	13.79	Good	KH	2004 B	15.62	Good
Ironrotter	89 A1.1	15.79	Good	Leverets	LevApr1	6.64	Poor
Ironrotter	89 B2.3	14.78	Good	Leverets	LevAug1	11.42	Mod
Ironrotter	89 C2.3	12.64	Mod	Leverets	LevDec5	6.74	Poor
Ironrotter	89 D2.2	10.74	Mod	Leverets	LevJan1	14.37	Good
Ironrotter	89 E2.3	12.92	Mod	Leverets	LevJul5	13.50	Good
Ironrotter	89 G1.1	15.41	Good	Leverets	LevJun3	12.50	Mod
Ironrotter	89 G3.3	16.88	Good	Leverets	LevMar5	11.97	Mod
Ironrotter	92 A3	16.43	Good	Leverets	LevNov3	14.34	Good
Ironrotter	92 B1	16.17	Good	Leverets	LevSep4	13.07	Good
Ironrotter	92 D1	13.83	Good	Margaretta	MargDec1	13.23	Good
Ironrotter	92 F1	12.90	Mod	Margaretta	MargJan5	14.02	Good
Ironrotter	92 G3	15.23	Good	Margaretta	MargJul4	10.74	Mod
Ironrotter	92 H1	14.80	Good	Margaretta	MargJun1	12.86	Mod
Ironrotter	95 A100	17.30	High	Margaretta	MargMar1	12.82	Mod
Ironrotter	95 A1000	19.26	High	Margaretta	MargMay5	11.54	Mod
Ironrotter	95 A750	17.81	High	Margaretta	MargNov1	13.27	Good
Ironrotter	95 B500	16.76	Good	Margaretta	MargOct5	12.98	Good
Ironrotter	95 B750	17.59	High	Margaretta	MargSep2	12.80	Mod
Ironrotter	95 H750	6.69	Poor				

Discussion

Many species in this study have been found to have sensitivity values in line with those Rosenberg et al (2004) have found, for example *Capitella capitata* was found to have a sensitivity value of 1.66 in this study and 1.10 in the Rosenberg list. In other cases, the values are very different. This may be due to the species being found in a low number of grabs in this study and therefore making the result unreliable, for example *Phyllodoce groenlandica* was found in only 5 grabs in the dataset used in this study and the sensitivity value was found to be 20.56 whereas Rosenberg found the value 6.05. However, some $ES50_{0.05}$ values disagreed even when the species was found in a large number of grabs, for example, *Mysella bidentata* was found in 86 grabs and assigned a sensitivity value of 12.90 while in the Rosenberg list this species has a value of 6.83. This may be due to a difference in sensitivity of this species in different sea areas; however it may also be due to the data set used in this study being too small. Rosenberg et al (2004) calculated $ES50_{0.05}$ values based on 4676 grabs and the 123 grabs in this study is nowhere near that magnitude. Despite this, many of the species do have values corresponding to what would be expected and the values may still be reliable for the most common species.

A balance of samples including both very impacted and unimpacted is required to prevent the sensitivity value becoming skewed in one direction, in particular disturbed sites increase the reliability of the sensitivity value (Leonardsson et al., 2009). In the dataset used here there are more good sites than bad. The sensitivity values found are mainly higher than those found by Rosenberg et al (2004) implying species are classified as more sensitive than they actually are. This may be due to the relatively low number of impacted sites included in the dataset.

The species list found in this dataset contains many species not found in the Rosenberg list. This suggests it is useful to create a list for Scottish sea areas. Rosenberg et al (2004) created different lists for both the east and west coasts of Sweden implying the whole geographic region included in the dataset used here is probably too large to include together as there are likely to be differences in which species occur and the sensitivities of species between areas.
Using the sensitivity value of species found in around 20 or more grabs is recommended by Rosenberg et al (2004). The occurrence of many species is below 20 (Table 8.1.3). This means only very widespread species will be included. When calculating the index for individual sites, there will be many species not accounted for and the evaluation will be based on only a few species which often occur. It is conceivable that there will be samples where no species will be accounted for and

the index cannot be applied. On the other hand, the sensitivity of the common species will be the most reliable and therefore applying the index using these species would be more consistent. The minimum BQI classification of all these sites was zero. This site achieved a zero classification as only one species was found and this was not included in the species sensitivity list. This site clearly has bad quality so this is not such a problem. However, at Margaretta, many of the samples are classified as moderate when it was expected that these sites would be good or high quality. This may be due to a different suite of species occurring at this site which occurred overall in less than 15 grabs and therefore were not included in the species sensitivity list. This would suggest that this site is of a different sea area and would require a fitting species list. It also implies that the percentage of species not classified at a site should also be taken into account and caution used where this is high.

The BQI, using this species sensitivity list, needs to be tested against other indices in different sites. However, in this study, this list will not meet all the criteria required (Box 8.1.1). For the datasets used to create the list, the list is not independent. For this reason, only a few grabs from each site were included, however this is likely to have an impact on results. In particular, due to an overall lack of disturbed data, most of the bad quality samples were used in creating the sensitivity list. The other datasets used in this study have been sampled in a variety of ways including different grab sizes and sieved using different mesh sizes from the data used to create the sensitivity list. This is likely to have an impact on results as some species may be more prevalent sampled with a larger grab and smaller mesh than smaller grabs and larger mesh sizes. The 0.5mm mesh size is likely to have captured more juveniles than may be captured with 1mm mesh sieve and the juvenile specimens may have a different sensitivity to adults. Rosenberg et al (2004) used samples which were sieved with 1mm mesh so this may further explain differences in the sensitivity values.

This $ES50_{0.05}$ list represents a beginning stage for further development for Scottish waters. In addition, testing the index with other datasets in this study may reveal whether it is worthwhile pursuing the development of species sensitivity lists for Scottish waters. BQI could represent a useful alternative to an index such as AMBI because the sensitivity of species is assigned in an objective way based on the frequency of occurrence of species and provided there is data available, sensitivity of species can be specific for different geographical areas without having to rely on studies or expert knowledge of particular species.

References

Fleischer, D., Gremare, A., Labrune, C., Rumohr, H., Vanden Berghe, E. and Zettler, M. L. (2007) 'Performance comparison of two biotic indices measuring the ecological status of water bodies in the Southern Baltic and Gulf of Lions', *Marine Pollution Bulletin*, 54, 1598-1606.

Leonardsson, K., Blomqvist, M. and Rosenberg, R. (2009) 'Theoretical and practical aspects on benthic quality assessment according to the EU-Water Framework Directive - Examples from Swedish waters', *Marine Pollution Bulletin*, 58(9), 1286-1296.

Rosenberg, R., Blomqvist, M., Nilsson, H. C., Cederwall, H. and Dimming, A. (2004) 'Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive', *Marine Pollution Bulletin*, 49, 728-739.

Matlab Script

```
%Code to calculate BQI Test
%Started by Fiona Culhane
%Improved on the 31 Jan 2011 by Ana Brito

%load data
load bqi.txt;
M=[bqi];
[a,b]=size(M);

%Select data, sort and calculations
A=M(:,2:b);
ES50=M(:,1:b);
[ES50,Index]=sort(ES50);
NumSamples=sum(A);
Per5Ind=.05*(NumSamples+1);
CumFreq=cumsum(A);

%To obtain CumFreq>=Per5Ind -> using floor
[l,c]=size(A);
[ll,cc]=size(Per5Ind);
resultado = zeros(ll,cc);
for i=1:c;

   for j=1:l;
```

```
    if CumFreq(j,i)>= floor(Per5Ind(1,i));

        resultado(j,i) = 1;
    else
        resultado(j,i) = 0;
    end

    end
end

% To obtain Per5BinFloor for each column
Per5BinFloor=zeros(ll,cc);
for i=1:c;

    for j=1:l;

    Per5BinFloor(1,i)=find(resultado(:,i),1,'first');

    end
end
% To obtain CumFreq>=Per5Ind -> using ceil
[l,c]=size(A);
[ll,cc]=size(Per5Ind);
resultado2 = zeros(ll,cc);
for i=1:c;

    for j=1:l;

    if CumFreq(j,i)>= ceil(Per5Ind(1,i));

        resultado2(j,i) = 1;
    else
        resultado2(j,i) = 0;
    end

    end
end

% To obtain Per5BinCeil for each column

Per5BinCeil=zeros(ll,cc);
for i=1:c;

    for j=1:l;

    Per5BinCeil(1,i)=find(resultado2(:,i),1);

    end
end

%And finally ... Per5.
Per5=(ES50(Per5BinFloor)+ES50(Per5BinCeil))/2;
```

'Per5' gives you the ES50 0.05 (5th percentile) for each species in the same order they were in your excel spreadsheet.

Appendix 8.2

Table 8.2.1 Ironrotter Point sample point locations for each year

Sample Point	Latitude	Longitude	Sample Point	Latitude	Longitude	Sample Point	Latitude	Longitude	Sample Pc	Latitude	Longitude
1989			1992			1995			1998		
A1	55 58.33’ N	04 48.40' W	A1	55 58.33’ N	04 48.40' W	A100	55 58.33’ N	04 48.40' W	A100	55 58.33’ N	04 48.40' W
A2	55 58.33’ N	04 48.70' W	A2	55 58.33’ N	04 48.70' W	A500	55 58.33’ N	04 48.78' W	A500	55 58.33’ N	04 48.78' W
A3	55 58.33’ N	04 49.31' W	A3	55 58.33’ N	04 49.31' W	A750	55 58.33’ N	04 49.02' W	A750	55 58.33’ N	04 49.02' W
B1	55 58.37’ N	04 48.42' W	B1	55 58.37’ N	04 48.42' W	A1000	55 58.35’ N	04 49.39' W	E100	55 58.32’ N	04 48.20' W
B2	55 58.50’ N	04 48.81' W	B2	55 58.50’ N	04 48.81' W	B100	55 58.37’ N	04 48.42' W	E500	55 58.32’ N	04 47.81' W
C1	55 58.38’ N	04 48.27' W	C1	55 58.38’ N	04 48.27' W	B500	55 58.41’ N	04 48.81' W	E750	55 58.32’ N	04 47.57' W
C2	55 58.51’ N	04 48.27' W	C2	55 58.51’ N	04 48.27' W	B750	55 58.46’ N	04 48.99' W			
C3	55 58.65’ N	04 48.27' W	C3	55 58.65’ N	04 48.27' W	B1000	55 58.50’ N	04 49.21' W			
D1	55 58.38’ N	04 48.16' W	D1	55 58.38’ N	04 48.16' W	C100	55 58.38’ N	04 48.30' W			
D2	55 58.45’ N	04 47.88' W	D2	55 58.45’ N	04 47.88' W	C500	55 58.60’ N	04 48.30' W			
D3	55 58.51’ N	04 47.57' W	D3	55 58.51’ N	04 47.57' W	C750	55 58.73’ N	04 48.30' W			
E1	55 58.32’ N	04 48.11' W	E1	55 58.32’ N	04 48.11' W	D100	55 58.35’ N	04 48.20' W			
E2	55 58.31’ N	04 47.75' W	E2	55 58.31’ N	04 47.75' W	D500	55 58.42’ N	04 47.84' W			
E3	55 58.30’ N	04 47.23' W	E3	55 58.30’ N	04 47.23' W	D750	55 58.47’ N	04 47.62' W			
F1	55 58.30’ N	04 48.13' W	F1	55 58.30’ N	04 48.13' W	E100	55 58.32’ N	04 48.20' W			
F2	55 58.25’ N	04 47.85' W	F2	55 58.25’ N	04 47.85' W	E500	55 58.32’ N	04 47.81' W			
F3	55 58.15’ N	04 47.50' W	F3	55 58.15’ N	04 47.50' W	E750	55 58.32’ N	04 47.57' W			
G1	55 58.28’ N	04 48.26' W	G1	55 58.28’ N	04 48.26' W	E1000	55 58.32’ N	04 47.33' W			
G2	55 58.10’ N	04 48.28' W	G2	55 58.10’ N	04 48.28' W	F100	55 58.31’ N	04 48.20' W			
G3	55 57.88’ N	04 48.28' W	G3	55 57.88’ N	04 48.28' W	F500	55 58.24’ N	04 47.84' W			
H1	55 58.26’ N	04 48.37' W	H1	55 58.26’ N	04 48.37' W	F750	55 58.20’ N	04 47.61' W			
H2	55 58.06’ N	04 48.60' W	H2	55 58.06’ N	04 48.60' W	G100	55 58.27’ N	04 48.29' W			
						G500	55 58.06’ N	04 48.29' W			
						G750	55 57.92’ N	04 48.29' W			
						G1000	55 57.79’ N	04 48.29' W			
						H100	55 58.28’ N	04 48.35' W			
						H500	55 58.11’ N	04 48.57' W			
						H750	55 57.99’ N	04 48.71' W			

Appendix 8.3

Table 8.3.1 Dates of biological and chemical sampling surveys at Clyde Upper Estuary

Station (miles)	Location	Biology survey date	Date of chemistry (salinity) survey
0	Broomielaw	02/12/1993	22/10/1993
2	Kelvin confluence	No Sampling	22/10/1993
4	King George V Dock	02/12/1993	22/10/1993
6.5	Rothesay Dock	02/12/1993	22/10/1993
8	Dalmuir	02/12/1993	22/10/1993
10	Erskine	02/12/1993	22/10/1993
12	Milton	No sampling	22/10/1993
14	Leven Confluence	02/12/1993	22/10/1993
0	Broomielaw	01/07/1994	14/07/1994
2	Kelvin confluence	No sampling	-
4	King George V Dock	01/07/1994	14/07/1994
6.5	Rothesay Dock	01/07/1994	14/07/1994
8	Dalmuir	04/05/1994	16/05/1994
10	Erskine	04/05/1994	16/05/1994
12	Milton	No sampling	-
14	Leven Confluence	04/05/1994	16/05/1994
0	Broomielaw	07/06/1995	05/06/1995
2	Kelvin confluence	07/06/1995	05/06/1995
4	King George V Dock	07/06/1995	05/06/1995
6.5	Rothesay Dock	06/06/1995	05/06/1995
8	Dalmuir	06/06/1995	05/06/1995
10	Erskine	06/06/1995	05/06/1995
12	Milton	06/06/1995	05/06/1995
14	Leven Confluence	21/09/1995	
0	Broomielaw	No sampling	01/09/1995
2	Kelvin confluence	21/09/1995	-
4	King George V Dock	21/09/1995	01/09/1995
6.5	Rothesay Dock	21/09/1995	01/09/1995
8	Dalmuir	21/09/1995	01/09/1995
10	Erskine	21/09/1995	01/09/1995
12	Milton	No sampling	01/09/1995
14	Leven Confluence		-
0	Broomielaw	17/05/1996	29/05/1996
2	Kelvin confluence	No sampling	-
4	King George V Dock	16/05/1996	29/05/1996
6.5	Rothesay Dock	16/05/1996	29/05/1996
8	Dalmuir	16/05/1996	29/05/1996
10	Erskine	16/05/1996	29/05/1996
12	Milton	16/05/1996	29/05/1996
14	Leven Confluence	No sampling	-
0	Broomielaw	01/11/1996	21/10/1996
2	Kelvin confluence	No sampling	-
4	King George V Dock	01/11/1996	21/10/1996
6.5	Rothesay Dock	01/11/1996	21/10/1996
8	Dalmuir	01/11/1996	21/10/1996
10	Erskine	01/11/1996	21/10/1996
12	Milton	01/11/1996	21/10/1996
14	Leven Confluence	No sampling	-
0	Broomielaw	30/05/1997	12/05/1997
2	Kelvin confluence	30/05/1997	12/05/1997
4	King George V Dock	30/05/1997	12/05/1997
6.5	Rothesay Dock	29/05/1997	12/05/1997
8	Dalmuir	29/05/1997	12/05/1997
10	Erskine	29/05/1997	12/05/1997
12	Milton	29/05/1997	12/05/1997
14	Leven Confluence	No sampling	-
0	Broomielaw	06/10/1997	30/09/1997
2	Kelvin confluence	No sampling	-
4	King George V Dock	06/10/1997	30/09/1997
6.5	Rothesay Dock	06/10/1997	30/09/1997
8	Dalmuir	06/10/1997	30/09/1997
10	Erskine	06/10/1997	30/09/1997
12	Milton	06/10/1997	30/09/1997
14	Leven Confluence	No sampling	-
0	Broomielaw	22/05/2000	23/05/2000
2	Kelvin confluence	22/05/2000	23/05/2000
4	King George V Dock	22/05/2000	23/05/2000
6.5	Rothesay Dock	22/05/2000	23/05/2000
8	Dalmuir	22/05/2000	23/05/2000
10	Erskine	22/05/2000	23/05/2000
12	Milton	22/05/2000	23/05/2000
14	Leven Confluence	No sampling	-
0	Broomielaw	08/05/2003	23/05/2000
2	Kelvin confluence	08/05/2003	23/05/2000
4	King George V Dock	08/05/2003	23/05/2000
6.5	Rothesay Dock	08/05/2003	23/05/2000
8	Dalmuir	08/05/2003	23/05/2000
10	Erskine	08/05/2003	23/05/2000
12	Milton	08/05/2003	23/05/2000
14	Leven Confluence	No sampling	-

Appendix 8.4

Reference List for Biological Traits Database

ALLEN, J. A. 1961. The British species of Thracia (Eulamellibranchia). *Journal of the Marine Biological Association of the UK,* 41**,** 723-735.

ALLEN, J. A. 1967. *The Fauna of the Clyde Sea Area Crustacea: Euphausiacea and Decapoda,* Millport, Scottish Marine Biological Association.

ANSELL, A. D. 1974. Seasonal changes in biochemical composition of the Bivalve *Abra alba* from the Clyde Sea area. *Marine Biology,* 25**,** 13-20.

APPELTANS, W., BOUCHET, P., BOXSHALL, G. A., FAUCHALD, K., GORDON, D. P., HOEKSEMA, B. W., POORE, G. C. B., VAN SOEST, R. W. M., STOHR, S., WALTER, T. C. & COSTELLO, M. J. E. 2011. *World Register of Marine Species* [Online]. Available: http://www.marinespecies.org [Accessed 16 July 2010].

AZTI-TECNALIA 2011. AMBI: AZTI Marine Biotic Index, www.azti.es. February 2010 ed.

BEARE, D. J. & MOORE, P. G. 1998. Aspects of the life histories of *Perioculodes longimanus*, *Pontocrates arcticus* and *Synchelidium maculatum* (Crustacea: Amphipoda) at Millport, Scotland. *Journal of the Marine Biological Association of the UK,* 78**,** 193-209.

BIRKETT, L. & WOOD, R. J. 1959. Shell and siphon regeneration in *Mactra stultorum* Linne (Lamellibranchiata). *Nature,* 184**,** 1075-1076.

BLAKE, J. A. 1993. Life history analysis of five dominant infaunal polychaete species from the continental slope off North Carolina. *Journal of the Marine Biological Association of the UK,* 73**,** 123-141.

BODDEKE, R. 1976. The seasonal migration of the brown shrimp *Crangon crangon*. *Netherlands Journal of Sea Research,* 10**,** 103-130.

BODDEKE, R. 1982. The occurrence of winter and summer eggs in the brown shrimp (*Crangon crangon*) and the pattern of recruitment. *Netherlands Journal of Sea Research,* 16**,** 151-162.

BOWMER, T. & KEEGAN, B. F. 1983. Field survey of the occurrence and significance of regeneration in *Amphiura filiformis* (Echinodermata: Ophiuroidea) from Galway Bay, west coast of Ireland. *Marine Biology,* 74**,** 65-71.

BREMNER, J., ROGERS, S. I. & FRID, C. L. J. 2006. Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). *Ecological Indicators,* 6**,** 609-622.

BUCHANAN, J. B. 1964. A comparative study of some features of the biology of *Amphiura filiformis* and *Amphiura chiajei* (Ophiuroidea) considered in relation to their distribution. *Journal of the Marine Biological Association of the UK,* 44**,** 565-576.

BUCHANAN, J. B. 1966. The biology of Echinocardium cordatum [Echinodermata: Spatangoidea] from different habitats. *Journal of the Marine Biological Association of the UK,* 46**,** 97-114.

CALLAWAY, R. 2006. Tube worms promote community change. *Marine Ecology Progress Series,* 308**,** 49-60.

CHAPMAN, G. 1965. The egg cocoons of *Scoloplos armige*r O. F. Muller. *Biological Bulletin,* 128**,** 189-197.

CHARMANTIER, G. & CHARMANTIER-DAURES, M. 1994. Ontogeny of osmoregulation and salinity tolerance in the isopod crustacean *Sphaeroma serratum*. *Marine Ecology Progress Series,* 114**,** 93-102.

CHINTIROGLOU, C. & KOUKOURAS, A. 1992. The feeding habits of three Mediterranean sea anemone species, *Anemonia viridis* (Forskal), *Actinia equina* (Linnaeus) and *Cereus peduncalatus* (Pennant). *Helgol Meeresunters,* 46**,** 53-68.

CORBARI, L., CARBONEL, P. & MASSABUAU, J.-C. 2005. The early life history of tissue oxygenation in crustaceans: the strategy of the myodocopid ostracod *Cylindroleberis mariae*. *The Journal of Experimental Biology,* 208**,** 661-670.

DALES, R. P. 1957. The feeding mechanism and structure of the gut of *Owenia fusiformis* Delle Chiaje. *Journal of the Marine Biological Association of the UK*, 36**,** 81-89.

DAY, J. H. 1967. *A Monograph on the Polychaeta of Southern Africa,* London, The British Museum (Natural History).

DIXON, A. Y. 1944. Notes on certain aspects of the biology of *Cumopsis goodsiri* (van Beneden) and some other Cumaceans in relation to their environment. *Journal of the Marine Biological Association of the UK*, 26**,** 61-71.

DORSETT, D. A. 1961. The reproduction and maintenance of *Polydora ciliata* (Johnst.) at Whitstable. *Journal of the Marine Biological Association of the UK,* 41**,** 383-396.

FANKBONER, P. V. 1981. A re-examination of mucus feeding by the sea cucumber *Leptopentacta (=Cucumaria) elongata*. *Journal of the Marine Biological Association of the UK,* 61**,** 679-683.

FISH, J. D. & FISH, S. 1989. *A Student's Guide to the Seashore,* London, Unwin Hyman.

FREEMAN, S. M., RICHARDSON, C. A. & SEED, R. 2001. Seasonal abundance, spatial distribution, spawning and growth of *Astropecten irregularis* (Echinodermata: Asteroidea). *Estuarine, Coastal and Shelf Science*, 53**,** 39-49.

FRETTER, V. 1948. The structure and life history of some minute prosobranchs of rock pools: *Skeneopsis planorbis* (Frabicius), *Omalogyra atomus* (Philippi), *Rissoella diaphana* (Alder) and *Rissoella opalina* (Jeffreys). *Journal of the Marine Biological Association of the UK*, 27**,** 597-632.

GRAHAM, A. 1988. *Molluscs: Prosobranch and Pyramidellid Gastropods,* The Bath Press, Avon, Published for The Linnean Society of London and The Estuarine and Brackish-water Sciences Association by E. J. Brill/Dr W. Backhuys.

GREMARE, A., DUCHENE, J. C., ROSENBERG, R., DAVID, E. & DESMALADES, M. 2004. Feeding behaviour and functional response of *Abra ovata* and *A. nitida* compared by image analysis. *Marine Ecology Progress Series*, 267**,** 195-208.

GUDMUNDSSON, H. 1985. Life history patterns of Polychaete species of the family Spionidae. *Journal of the Marine Biological Association of the UK,* 65**,** 93-111.

GUILLOU, M. & HILY, C. 1983. Dynamics and biological cycle of a *Melinna palmata* (Ampharetidae) population during the recolonisation of a dredged area in the vicinity of the harbour of Brest (France). *Marine Biology,* 73**,** 43-50.

HANSEN, B. & OCKELMANN, K. W. 1991. Feeding behaviour in larvae of the opisthobranch *Philine aperta*. *Marine Biology,* 111**,** 255-261.

HARTNOLL, R. G. 1968. Reproduction in the burrowing crab, *Corystes cassivelaunus* (Pennant, 1977) (Decapoda, Brachyura). *Crustaceana,* 15**,** 165-170.

HARVEY, C. E. 1969. Breeding and distribution of *Sphaeroma* (Crustacea: Isopoda) in Britain. *Journal of Animal Ecology,* 38**,** 399-406.

HASTINGS, M. H. 1981. The life cycle and productivity of an intertidal population of the Amphipod *Ampelisca brevicornis*. *Estuarine, Coastal and Shelf Science,* 12**,** 665-677.

HAYWARD , P. J. & RYLAND, J. S. 1990. *The Marine Fauna of the British Isles and North-West Europe,* Oxford, Clarendon Press.

HIGHSMITH, R. C. & COYLE, K. O. 1991. Amphipod life histories: Community structure, impact of temperature on decoupled growth and maturation rates, productivity, and P:B ratios. *American Zoologist,* 31**,** 861-873.

HIMMELMAN, J. H. & HAMEL, J.-R. 1993. Diet, behaviour and reproduction of the whelk *Buccinum undatum* in the northern Gulf of St. Lawrence, eastern Canada. *Marine Biology,* 116**,** 423-430.

INGLE, R. W. 1996. *Shallow-Water Crabs,* Shrewsbury, Published for The Linnean Society of London and The Estuarine and Coastal Sciences Association by Field Studies Council.

JONES, N. S. 1976. *British Cumaceans Arthropoda: Crustacea*, London, Published for The Linnean Society of London by Academic Press.

KRUSE, I., STRASSER, M. & THIERMANN, F. 2004. The role of ecological divergance in speciation between intertidal and subtidal *Scoloplos armiger* (Polychaeta, Orbiniidae). *Journal of Sea Research,* 51**,** 53-62.

LANCASTER, I. 1988. *Pagurus bernhardus* (L.) - An introduction to the natural history of hermit crabs. *Field Studies,* 7**,** 189-238.

LINCOLN, R. J. 1979. *British Marine Amphipoda: Gammaridea,* London, British Museum (Natural History).

MANUEL, R. L. 1988. *British Anthozoa (Coelenterata: Octocorallia & Hexacorallia),* The Bath Press, Avon, Published for The Linnean Society of London and The Estuarine and Brackish-water Sciences Association by E. J. Brill/Dr W. Backhuys.

MARLIN. 2006. *BIOTIC - Biological Traits Information Catalogue. Marine Life Information Network* [Online]. Plymouth: Marine Biological Association of the United Kingdom. Available: www.marlin.ac.uk/biotic [Accessed 4 August 2010].

MARTEL, A., LARRIVEE, D. H., KLEIN, K. R. & HIMMELMAN, J. H. 1986. Reproductive cycle and seasonal feeding activity of the neogastropod *Buccinum undatum*. *Marine Biology,* 92**,** 211-221.

MAUCHLINE, J. 1971. *The Fauna of the Clyde Sea Area Crustacea: Mysidacea,* Dunstaffnage Marine Research Laboratory, Oban, Argyll, Scottish Marine Biological Association.

METTAM, C. 1980. On the feeding habits of *Aphrodita aculeata* and commensal polynoids. *Journal of the Marine Biological Association of the UK,* 60**,** 833-834.

MICHEL, C., BHAUD, M., BOUMATI, P. & HALPERN, S. 1984. Physiology of the digestive tract of the sedentary polychaete *Terebellides stroemi*. *Marine Biology,* 83**,** 17-31.

MILLAR, R. H. 1970. *British Ascidians Tunicata: Ascidiacea,* London, Published for the Linnean Society of London by Academic Press.

NAYLOR, E. 1972. *British Marine Isopods,* London, Published for The Linnean Society of London by Academic Press.

NEWELL, G. E. 1951. The life-history of *Clymenella torquata* (Leidy). (Polychaeta). *Proceedings of the Zoological Society of London,* 121**,** 561-586.

NICKELL, L. A. & ATKINSON, R. J. A. 1994. Observations on the behaviour of *Thalassema thalassemum* (Echiura: Echiuridae). *Journal of the Marine Biological Association of the UK,* 74**,** 963-966.

NICOL, J. A. C. 1958. Observations on the luminescence of *Pennatula phosphorea*, with a note on the luminescence of *Virgularia mirabilis*. *Journal of the Marine Biological Association of the UK,* 37**,** 551-563.

NICOLAIDOU, A. 1983. Life history and productivity of *Pectinaria koreni* Malmgren (Polychaeta). *Estuarine, Coastal and Shelf Science,* 17**,** 31-43.

O'FOIGHIL, D., MCGRATH, D., CONNEELY, M. E., KEEGAN, B. F. & COSTELLOE, M. 1984. Population dynamics and reproduction of *Mysella bidentata* (Bivalvia: Galeommatacea) in Galway Bay, Irish west coast. *Marine Biology,* 81**,** 283-291.

OLIVE, P. J. W. & GARWOOD, P. R. 1981. Gametogenic cycle and population structure of *Nereis (Hediste) diversicolor* and *Nereis (Nereis) pelagica* from north-east England. *Journal of the Marine Biological Association of the UK,* 61**,** 193-213.

OLIVE, P. J. W. 1975. Reproductive biology of *Eulalia viridis* (Muller) (Polychaeta: Phyllodocida) in the north eastern U.K. *Journal of the Marine Biological Association of the UK,* 55**,** 313-326.

OLIVE, P. J. W. 1977. The life-history and population structure of the Polychaetes *Nephtys caeca* and *Nephtys hombergii* with special reference to the growth rings in the teeth. *Journal of the Marine Biological Association of the UK,* 57**,** 133-150.

PICTON, B. E. & MORROW, C. C. 2010. *Encyclopedia of marine life of Britain and Ireland* [Online]. Available: http://www.habitats.org.uk/marinelife/index.html [Accessed 4 August 2010].

RICHARDS, S. 2008. *Thalassema thalassemum. A spoon worm. Marine Life Information Network: Biology and Sensitivity Key Information Sub-programme.* [Online]. Plymouth: Marine Biological Association of the United Kingdom.

Available: http://www.marlin.ac.uk/speciesinformation.php?speciesID=4455 [Accessed 18 November 2010].

S. BENITEZ, J. 2000. *Cerianthus lloydii (On-line), Animal Diversity Web* [Online]. Available: http://animaldiversity.ummz.umich.edu/site/accounts/information/Cerianthus_lloydii.html [Accessed 26 October 2010].

SALISBURY, A. E. 1932. On *Lepton squamosum* (Montagu) and *Montacuta ferruginosa* (Montagu) and some other Molluscs observed in the Salcombe Estuary, Devon. *Proceedings of the Malacological Society,* 20**,** 100-103.

SCHOTTLER, U. & GRIESHABER, M. 1988. Adaptation of the polychaete worm *Scoloplos armiger* to hypoxic conditions. *Marine Biology,* 99**,** 215-222.

SHEADER, M. 1977. Production and population dynamics of *Ampelisca tenuicornis* (Amphipoda) with notes on the biology of its parasite *Sphaeronella longipes* (Copepoda). *Journal of the Marine Biological Association of the UK,* 57**,** 955-968.

SHIRLEY, T. C. 1990. Ecology of *Priapulus caudatus* Lamarck, 1816 (Priapulida) in an Alaskan subarctic ecosystem. *Bulletin of Marine Science,* 47**,** 149-158.

SOLAN, M. 2000. *The concerted use of 'traditional' and sediment profile imagery (SPI) methodologies in marine benthic characterisation and monitoring.* PhD, National University of Ireland, Galway.

TEBBLE, N. 1966. *British Bivalve Seashells: A handbook for identification,* London, Trustees of the British Museum (Natural History).

THOMPSON, T. E. & BROWN, G. H. 1976. *British Opisthobranch Molluscs. Mollusca: Gastropoda. Keys and Notes for the Identification of the Species,* London, Published for The Linnean Society of London by Academic Press.

UPTON, N. P. D. 1987. Asynchronous male and female life cycles in the sexually dimorphic, harem-forming isopod *Paragnathia formica* (Crustacea: Isopoda). *Journal of Zoology (London),* 212**,** 677-690.

WELCH, H. E. & MARTIN-BERGMANN, K. 1990. Does the clam *Mya truncata* regenerate its siphon after predation by walrus? An experimental approach. *Arctic,* 43**,** 157-158.

WILSON, W. H. 1991. Sexual reproductive modes in Polychaetes: Classification and diversity. *Bulletin of Marine Science,* 48**,** 500-516.

WOOD, C. 2005. *Seasearch guide to Sea Anemones and Corals of Britain and Ireland,* Ross-on-Wye, Marine Conservation Society.

YONGE, C. M. 1946. On the habits and adaptations of *Aloidis (Corbula) gibba*. *Journal of the Marine Biological Association of the UK,* 26**,** 358-376.

YONGE, C. M. 1946. On the habits of *Turritella communis* Risso. *Journal of the Marine Biological Association of the UK,* 26**,** 377-380.

Appendix 8.5

Creating trait datasets

The trait database was created with the expression of trait modalities for each species for each trait recorded, each whole trait adding up to 0 or 1 (Table 8.5.1). The trait database was then multiplied by the species abundance (Table 8.5.2) or biomass data to give the degree of trait expression for each site (Table 8.5.3). The multiplied values for each species were then summed for each modality to give a total value for each site which was then used as a dataset for analysis (Table 8.5.4) (traits*abundance or traits*biomass). The counted value (Table 8.5.3) was also used to create a dataset of the number of times a species occurs which expresses that trait modality (traits*species richness).

Table 8.5.1 Example of trait database with expression of modalities

	Trait 1		
Species	**Modality 1**	**Modality 2**	**Modality 3**
Species 1	0	1	0
Species 2	1	0	0
Species 3	0.5	0.5	0

Table 8.5.2 Example of species abundance data for each site

Species	Site 1	Site 2
Species 1	10	0
Species 2	5	2
Species 3	3	1

Table 8.5.3 Example of output of traits*abundance; the data for each modality at each site was then summed or counted

	Site 1			Site 2		
Species	**Modality 1**	**Modality 2**	**Modality 3**	**Modality 1**	**Modality 2**	**Modality 3**
Species 1	0	10	0	0	0	0
Species 2	5	0	0	2	0	0
Species 3	1.5	1.5	0	0.5	0.5	0
Sum	**6.5**	**11.5**	**0**	**2.5**	**0.5**	**0**
Count	**2**	**2**	**0**	**2**	**1**	**0**

Table 8.5.4 Example of dataset used for analysis with traits*abundance for each site

Trait	Modality	Site 1	Site 2
Trait 1	Modality 1	6.5	2.5
	Modality 2	11.5	0.5
	Modality 3	0	0