Defining reference conditions for coastal areas in the Baltic Sea

*Elinor Andrén, Annemarie Clarke, Richard Telford, Kaarina
Weckström, Sirje Vilbaste, Juris Aigars, Daniel Conley, Torbjørn
Johnsen, Steve Juggins and Atte Korhola*

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Store Strandstræde 18

DK-1255 Copenhagen K

Phone (+45) 3396 0200

Fax (+45) 3396 0202

Nordic Council

Store Strandstræde 18

DK-1255 Copenhagen K

Phone (+45) 3396 0400

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Preface

The project DEFINE – “Defining reference conditions for coastal areas in the Baltic Sea for the Water Framework Directive” is a research project funded by the Nordic Council of Ministers 2004-2006. The project involves partners from Sweden, Finland, Estonia, Latvia, Denmark, Norway and the United Kingdom. The objectives of the project are:

- To define reference conditions for nutrient concentrations in coastal areas of the Baltic Sea in order for the national authorities surrounding the Baltic Sea to implement the Water Framework Directive (WFD).
- Create transfer functions for defining background TN and/or TP concentrations in estuaries and coastal areas for the entire Baltic Sea as well as for the Kattegat/North Sea.
- Separate through variance partitioning the effects of climate from anthropogenic influences on nutrient concentrations.
- Make transfer functions and supporting documentation (e.g. taxonomic identification guide for sediment diatoms) publicly available and accessible via the WWW especially for use by all national authorities and environmental decision-makers in the Baltic Sea.

The DEFINE project is a continuation of the EU-funded project MOLTEN – “Monitoring long-term trends in eutrophication and nutrients in the coastal zone: Creations of guidelines for the evaluation of background conditions, anthropogenic influence and recovery” (EVK3-CT-2000-00031) running between January 2001 to January 2004. The results from these projects and a third project DETECT, financed by the Finnish Academy of Science, can be found on the web page <http://craticula.ncl.ac.uk/Molten/jsp>

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The Nordic Council of Ministers, The Air and Sea working group together with the Monitoring and Data working group for financial support. The monitoring authorities in Denmark, Sweden, Finland, Estonia, Latvia, Germany and Norway for providing water chemistry data. The Danish counties for helping with fieldwork for the Danish training set sediment samples. Per Jonsson for providing the sediment core from Gårdsfjärden. Peter Frenzell for providing the German surface sediment samples. Siim Veski and Atko Heinsalu for coring the Saunja Bay sediment cores. The sediment cores from the Arkona Basin and Oder Rinne were cored within the Project ODER (Oder Discharge Environmental Response), which was initiated during 1993 as a component of the EC Environment programme (PL 910398).

Executive summary

Background

A historical perspective is important for managing impacted marine ecosystems as it can indicate trajectories of change that more traditional local ecological studies cannot (e.g. Hughes *et al.* 2005). Changes in phytoplankton composition may reflect structural and functional shifts in the ecosystem (Wasmund & Uhlig 2003), but living phytoplankton concentrations are extremely variable in time and space and consequently have a very patchy distribution (Kononen 2001). Palaeoecological studies of fossil diatom assemblages can present a regional historical depiction without the patchiness of living phytoplankton data. In this study we have used a palaeoecological approach to try to enhance the knowledge of historical nutrient concentrations in different parts of the Baltic Sea coastal waters.

Humans have a long history in the Baltic drainage area and recent studies show that they have influenced the environment over thousands of years, both locally via different land-use (Bradshaw *et al.* 2005) and regionally as an effect of e.g. metal industry (Brännvall *et al.* 1999). Although the period of human occupation covers a long time span, it is mainly after the industrialization and introduction of artificial fertilizers following World War II that the main changes in nutrient loads occurred (Jansson & Dahlberg 1999) and effects of eutrophication are recorded in Baltic Sea coastal waters (e.g. Elmgren 1989). Systematic monitoring of water quality started in the late 1960s to early 1970s (Cederwall & Elmgren 1990) and the background nutrient levels prior to the increased discharge are not known (Larsson *et al.* 1985). This makes the present study an important contribution to the quantification of background nutrient conditions, as needed when identifying a good ecological status of coastal waters within the European Water Framework Directive (Anonymous 2000).

Objectives

The primary objective of the DEFINE project is to create diatom-based transfer functions for the entire coastal zone of the Baltic Sea that can be used by all national authorities to define reference conditions for nutrient concentrations using a sound scientific basis. This will be a significant step in implementing the European Water Framework Directive (WFD) and provide support for all the monitoring programs surrounding the Baltic Sea. In addition the project will try to separate the effect of a changing

climate during the last century (IPCC 2007), from the effect of anthropogenic nutrient enrichment causing eutrophication, by using those diatoms that respond to ice cover.

Resulting training set

Transfer functions describe the relationship between organisms and their environment with the ultimate goal to use the present-day ecology of organisms to infer past conditions from fossil assemblages. Transfer functions are established using the recent organism assemblages (e.g. diatoms) and measured environmental variables from a training set consisting of a wide range of sites. Work undertaken during the DEFINE project added 124 new sites from areas not previously studied in the Gulf of Bothnia (both Finnish and Swedish sites), Estonia, Latvia and Germany and water chemistry data from these sites has been collated, harmonised and added to the MOLTEN database (chapter 2). Data for each site is publicly available and accessible via the WWW from the database-driven MOLTEN/DEFINE website at <http://craticula.ncl.ac.uk/Molten/-jsp/>. The new sites span a wide environment range, with water depths between 2 and 101 m, salinities between <0.1 and 24 psu, and with nutrient status between oligotrophic and eutrophic. A principal components analysis of the environmental data shows that total nitrogen and total phosphorus variables are correlated which will make it difficult to create independent transfer functions for these two nutrients, but they are independent of salinity. While a total of 1081 diatom taxa were identified in the DEFINE surface samples, most were rare (chapter 3). Benthic forms are more common than planktonic taxa, which is expected due to the predominantly shallow nature and coastal position of the samples analysed. The project website is referred to for further details including distribution maps and abundance plots against key environmental variables.

Transfer function development

Constrained Correspondence Analysis (CCA; ter Braak, 1986) showed that total nitrogen (TN) can explain a small but significant and independent proportion of the variance in the diatom data, even when allowing for autocorrelation (chapter 4).

The transfer function technique weighted averaging partial least square (WAPLS) is the model used by DEFINE. The MOLTEN/DEFINE dataset is very heterogeneous, with salinity, exposure, and water depth all explaining more of the variance than TN. This is not ideal, and a solution is to divide the training set up into smaller, more homogeneous training sets, which resulted in 6 transfer functions splitting the sites into either exposed

or sheltered, and saline (>8 psu), intermediate (8-12 psu) and brackish (<8 psu). For all sites except the exposed intermediate and exposed brackish models, splitting the training set has improved the transfer functions.

To conclude, the environmental variables have a statistically significant independent effect on the diatom assemblages and a robust transfer function can be built to reconstruct TN in at least some of the coastal water types included in the database.

Background conditions

The resulting transfer functions were applied on sediment cores to reconstruct background TN concentrations from Baltic Sea coastal sites. In total we present diatom stratigraphies and diatom inferred total nitrogen reconstructions from four sites located from the Bothnian Sea to the southwestern Baltic Sea (chapter 5).

A background condition was defined in the Gårdsfjärden estuary (Bothnian Sea), using the diatom-inferred TN value of c. $270 \mu\text{g L}^{-1}$ recorded until 1920. In Oder Rinne (southern Baltic proper), background condition was possibly met until 1920 with a reconstructed TN value of c. $400 \mu\text{g L}^{-1}$. Essential for the performance of the inference model is that the training set contains the same species composition in similar quantities as found in the stratigraphy i.e. that we have good analogues of the fossil assemblages in the present data. This condition was not met in the sediment cores from the Arkona Basin (southern Baltic proper), and Saunja Bay (western Estonia), where background conditions consequently could not be defined.

A more complete interpretation of the diatom-inferred nutrient concentrations could be attained if they are supported by other sediment proxies, e.g. lithology, organic carbon content and diatom community indices such as species richness and ratio planktonic/periphytic diatoms. Some overall shifts in the stratigraphical data could be interpreted in terms of increased nutrient availability but all these changes are not occurring concurrently at all investigated sites, which indicate that marine eutrophication works on both local and regional scales.

Nutrients versus climate change

The most intense nutrient discharge to the Baltic Sea has occurred during the last century, concurrently with a warming trend of about 0.08°C per decade between 1860 and 2000 (HELCOM 2007). Climate variability acts on centennial and decadal time scales and overlaps with increased human impact in the Baltic drainage area, which makes it difficult to separate natural climate variability and anthropogenic climate change

from other human influences such as increased nutrient discharge (BACC Lead Author Group 2006). The future warming expected for the next century (IPCC 2007) with an altered meteorological setting will create changed conditions for the Baltic Sea ecosystem at all scales. Knowledge on how global warming contributes to the effect of nutrient enrichment will be valuable for the management of preventive measures to counteract eutrophication. Unfortunately the intention of DEFINE to separate the effects of climate on the diatom community from the effects of increased nutrient discharge by the use of variance partitioning was not successful. The method depended on the presence of taxa that respond to ice cover in the training set, but the abundance of ice-associated taxa was too low to be useful.

Application and monitoring

While aimed at national authorities and environmental decision-makers in the Baltic Sea area, all transfer functions and necessary supporting documentation will be publicly available as a coherent management tool (see chapter 6), and accessible via the MOLTEN/DEFINE web page (<http://craticula.ncl.ac.uk/Molten/jsp>).

Diatoms are not currently a quality element for the Water Framework Directive in coastal waters, except as a component of the phytoplankton. Phytoplankton is one of the biological quality elements used where the composition and abundance of taxa together with the intensity and frequency of blooms must be consistent with undisturbed conditions for a system to achieve a high ecological status. Many diatom taxa can not be identified to species level in live plankton counts and consequently diatom training sets like that in MOLTEN/DEFINE can offer great potential for biomonitoring schemes. In addition to water chemistry sampling at coastal monitoring stations, surface sediment samples for analysing present-day diatom assemblages could also be collected. The sampling frequency of once a year is sufficient as such a sample would be both temporally and spatially integrative incorporating all diatom habitats over one to a few years. Optima for the individual diatom taxa found at each monitoring site can be obtained from the MOLTEN/DEFINE data set: the abundance-weighted average of all species' optima gives a good estimate of the nutrient (TN) status of a specific site with statistically reliable errors of prediction. The approach is essentially the same as when reconstructing TN concentrations from fossil down-core samples, only here the transfer functions are applied to the surface sediment sample for calculating present TN concentrations. Diatoms could therefore be incorporated into the WFD as biological quality elements and applied using these techniques for water quality monitoring purposes in the coastal waters of the Baltic Sea area.

1. Introduction

The overall aim of DEFINE is to provide a methodology to define reference conditions for nutrient concentrations in the coastal zone of the Baltic Sea. This will aid the national authorities that surround the Baltic basin in implementing the EU's Water Framework Directive (WFD) by providing decision-makers with a methodology to assess reference conditions and the degree of past and present departure from this state, such that appropriate policy and management measures can be taken at national and European levels. DEFINE adopts a palaeoecological approach grounded on diatom-based transfer functions, which can then be applied to define background total nitrogen (TN) or total phosphorus (TP) concentrations in estuaries and coastal areas over the entire Baltic Sea. This method has been applied in Roskilde Fjord, Denmark (Andersen *et al.* 2004) using a Danish transfer function for TN (Clarke *et al.* 2003), as well as in Finnish waters where the history of eutrophication in embayments impacted by urban (Weckström *et al.* 2004) and agricultural pollution (Weckström 2005) has been successfully determined. While aimed at national authorities and environmental decision-makers in the Baltic Sea, these transfer functions and all necessary supporting documentation will be publicly available as a coherent management tool (chapter 6), and accessible via the WWW (<http://craticula.ncl.ac.uk/Molten/jsp/>).

1.1 Requirements of the EU Water Framework Directive

The primary objective of the WFD is to protect the structure and function of the aquatic environment in its entirety, by ensuring ecological coherency and a minimum chemical standard (Anonymous 2000). Environmental standards and criteria are needed to allow the classification of water bodies, set appropriate management objectives, and monitor progress towards these objectives. To provide this, two elements of 'good ecological status' and 'good chemical status' have been introduced.

Ecological status is defined by the quality of the biological community, together with the hydrological and chemical characteristics. Five classes ranging from 'high' which is equivalent to reference conditions, through 'good', 'moderate', 'poor' to 'bad' where a large part of the expected biological community would be missing will be defined. By 2015 all European waters are supposed to meet the 'good' ecological status criteria of only slight departure from conditions expected under minimal anthropogenic influence. While the legislation defines these conditions to be pristine with no, or very minor, deviations from undisturbed condi-

tions, practically they are being defined as conditions prior to the intensification of agriculture 100-150 years ago. Current status criteria will be used to determine present-day departure from reference conditions via an Ecological Quality Ratio (EQR). The definition of these classes, and the borders between them have yet to be clearly determined. Current research by the relevant authorities, supported by scientists, is working towards this goal, which will have a large impact on the effectiveness of the WFD in protecting aquatic resources. The science behind management is important, it needs to be able to supply realistic errors and uncertainties in measurement and prediction, while still being able to credibly demonstrate environmental damage (Gray 1999).

1.2 Nutrient reference conditions in the marine environment and palaeoecology

Although coastal waters and estuaries are naturally fertile ecosystems, receiving nutrient inputs from a variety of sources (Nixon *et al.* 1986), they are believed to be increasingly at risk from eutrophication as the magnitude of anthropogenic impact increases. However, determining the actual rate and long-term effect of anthropogenic eutrophication is severely hampered by the limited time span of contemporary monitoring programmes, which at the most extend for about 30 years. Our ability to manage and protect coastal resources is ultimately constrained by such limitations, as without knowledge of past conditions it is difficult to set appropriate targets and monitor the effectiveness of policies. Therefore alternative methods must be used to obtain pertinent and reliable information on baseline conditions. Several potential methods exist including data mining from historical literature in combination with expert judgement to estimate reference conditions, development of predictive computer models (e.g. Billen & Garnier 1997) and palaeoecological reconstructions based on relationships between fossil remains and the modern environment to infer past conditions (Birks 1995).

Paleoecology, the examination of past environments based on the biological and chemical indicators preserved in sediments, is being increasingly used as a source of information on how environmental variables have changed through time. While many biological indicators can be used as proxies for environmental change, diatoms are amongst the most frequently used as they can be identified to the species level, and are usually present in diverse, numerically abundant assemblages that are typically well preserved (Charles & Smol 1994). There is a long history of diatoms being used as indicators in marine systems (see review in Stoermer & Smol 1999), including qualitative reconstructions of eutrophication (e.g. Andrn 1999). Now there is a move towards a quantitative approach to

marine environmental reconstructions, pioneered by the transfer function approach used in DEFINE.

Statistically robust methods, based on weighted-averaging, can be used to quantify the distribution of modern diatoms, in terms of optima and tolerances with respect to key environmental gradients. These can then be used in turn to infer historical changes in water chemistry from fossil assemblages. The work reported here from the Baltic Sea is the first large-scale attempt (together with the EU-funded MOLTEN project) at developing transfer functions for nutrients in the coastal zone, and the calibration data set contains over 340 sites (chapter 2). At each site there is diatom assemblage data, and associated environmental variables relating to water quality. Multivariate statistics are used to determine suitable variables for transfer functions, as only variables that explain a unique and significant portion of the diatom data can be reconstructed, and the models themselves are cross-validated prior to use (chapter 4). This allows prediction errors to be provided for individual levels of the reconstruction, which is important as reliability may vary between fossil samples depending on factors such as preservation. Finally the developed transfer functions are used on sediment cores to reconstruct background TN conditions from four Baltic Sea coastal sites (chapter 5).

1.3 The Baltic Sea and eutrophication

Situated in a large, semi-enclosed basin draining a watershed some four times larger than itself, the Baltic Sea is one of the most intensively studied ecosystems in the world. Over 85 million people in nine highly industrialised countries inhabit this watershed (and additional five countries without Baltic Sea coastline), and many of the large rivers draining into the Baltic have historically carried a large pollution load. The hydrography of the Baltic is strongly influenced by its' topography. The narrow nature of the connection to the North Sea through the Danish straits and a threshold limits the intrusion of saline water (e.g. Voipio 1981). As a result the Baltic Sea is brackish, with a strong salinity gradient running south-west (7 – 13 ‰ in the Baltic Proper) to north-east (2 – 4 ‰ in the Gulf of Bothnia). The Baltic is also vertically stratified on a nearly permanent basis, mainly due to salinity-dependent density differences, with the saline bottom water low in oxygen as a result. Pulses of oxygen-rich water are occasionally added to the bottom layer when meteorological conditions permit (e.g. Lass & Matthäus 1996). This situation makes the Baltic Sea sensitive to nutrient enrichment. For example, Jansson & Dahlberg (1999) suggest that the Baltic of the 1940s was nutrient poor, with clear water, dense growths of the brown seaweed *Fucus vesiculosus* on rocky shores and sufficiently high oxygen concentrations for cod to breed in the deeper areas of the Baltic proper. This is no longer the case.

It is believed that the major increase in nutrient loads started in the 1950s (Rosenberg *et al.* 1990) as agricultural development and industrialisation increased after World War II. Estimates of the degree and timing of nutrient enrichment vary significantly: e.g. Jansson & Dahlberg (1990) suggest a three-fold increase in nitrogen load since the 1940s, with a doubling of nitrogen concentration since 1950, while Elmgren (1989) suggests a three-fold increase in winter nitrate concentrations since the 1960s.

As public demand for action over the pollution of the Baltic Sea increased, the countries surrounding the Baltic agreed to reduce nutrient loading by 50%. While this is a significant commitment, questions remain over whether this is enough to improve water quality. Provision of robust quantitative estimates of past nutrient conditions will be a significant step forward in assessing the impact of reductions in nutrient load on achieving better water quality.

1.4 Outline of the report

The introduction gives the background and rationale behind the DEFINE project and the need of past nutrient conditions essential for implementation of the EU Water Framework Directive. This chapter has been written by Annemarie Clarke.

Chapter 2 deals with the water chemistry data collected from the national monitoring programmes. This chapter has been written by Richard Telford.

Chapter 3 focuses on the methodology for surface sediment diatom analysis and has been written by Annemarie Clarke.

Chapter 4 describes the techniques behind transfer function development. This chapter has been written by Richard Telford.

Chapter 5 focuses on the long cores nutrient reconstructions. This chapter has been written by Elinor Andrén, with contribution from Sirje Vilbaste on the site description of Saunja Bay.

Chapter 6 gives a review of the palaeolimnological approach with a step-by-step flow chart for a coastal palaeoecological study. This chapter has been written by Kaarina Weckström, Steve Juggins and Atte Korhola.

The overall editing of the report has been carried out by Elinor Andrén as well as writing the preface and compiling the executive summary.

2. Collation and processing of environmental data

High quality environmental data play three essential roles in quantitative palaeoecology. A training set of paired environmental and biotic observations is used both to determine which environmental variables have a significant impact on the biotic assemblages and to develop transfer functions. Both these aspects are covered in the chapter on transfer function development. The third role of environmental data is to validate down-core reconstructions, an aspect covered in the reconstructions chapter. This chapter discusses the acquisition, processing and analysis of the environmental data used in the DEFINE project.

2.1 Site selection

Because of the cost and time requirements of collecting environmental data, especially mean water chemistry data, for many sites in a large geographic region, DEFINE depends entirely on data collected by existing monitoring programmes. This pragmatic decision has many implications. First, the sites monitored by the different agencies may not be the ideal sites for inclusion in a training set as they have been selected according to other criteria, in particular there is preponderance of deep open sites, rather than more enclosed sites. Secondly, the different agencies have adopted different working practices, measuring different environmental variables, at different frequencies. Solutions to these problems are developed below.

There are many more monitoring stations than are needed for the development of a training set (at least with available resources), so site selection criteria are needed. The absolute criteria are that the site must have a minimal set of environmental variables available and must be at, or close to, a location where sediment is accumulating. Transfer functions training sets can be designed to maximise the amount of variability explained by the environmental variable of interest, by selecting sites that have different values for this variable, but are similar in other respects (Birks 1995). In a lacustrine training set, this is, for example, achieved by selecting similar sized lakes. Strict accordance to this guideline is difficult to achieve for coastal training sets as coastal morphology varies greatly between regions, with, for example, the coastlines of Estonian, Finland and Sweden respectively having exposed coastlines, many shallow lake-like bays, and deeper fjords. DEFINE solved this problem, and

problems arising from the large salinity difference between sites on the Norwegian coast and the Bothnian Sea, by developing several training sets, each covering a portion of the salinity and exposure gradient (see chapter 4). To maximise the probability of having good biotic analogues for the fossil samples, training set sites should be selected from similar environments as the long cores. This guideline has been difficult to follow, as the types of sites most suitable for the collection of long cores e.g. sheltered and anoxic to minimise sediment disturbance by waves and benthic animals, are often atypical.

Some of the most widely used statistical methods for transfer function development perform best when sites are evenly distributed along the environmental gradient (Birks 1995). With weighted-averaging, and related methods, reconstructed trends may be little affected by uneven sampling, but the absolute values will be biased towards the most common environment in the training set. This is a serious problem if the aim is to estimate reference conditions. The problem can be minimised by preferentially selecting sites from undersampled parts of the environmental gradient.

2.2 Data processing

Raw water chemistry data was collected from the responsible agency in each region. The data are stored in a wide variety of formats by the different agencies, so the first step of processing the data is to collate it into a common format. We chose to design and build a database to store the data, using MS Access. Databases have several advantages over spreadsheets for storing the large amounts of water chemistry data DEFINE collated. The most important of these are the ease of manipulating the data using SQL (Structured Query Language), and the separation of data storage from data manipulation, minimising the risk of corrupting the data. This database also includes the data collected during MOLTEN and earlier projects.

The different agencies have report their data using different units. For example, total nitrogen (TN) is reported in $\mu\text{g/l}$, mg/l , and $\mu\text{mol/l}$, but it is not always obvious which units are used from meta-data. Harmonising the data to common units is essential. In DEFINE we used $\mu\text{g/l}$ for all nutrients. The units are harmonised using a query in the database. The advantage of storing the raw data, then running the query each time to harmonise the data, is that it is easy to check that the transformation is being done correctly.

The data were quality controlled to check for implausible values. Problems discovered include the representation of missing values with a zero, and data with the decimal point omitted.

Water chemistry varies on all time scales, with seasonality and weather having large impacts. To reduce this variability, and to make the water chemistry more comparable with the 1cm sediment slices analysed for diatoms, the mean of the chemistry data over the five years prior to diatom sampling was calculated, where this much data was available. Monitoring had recently commenced at a few Finnish sites, and there was insufficient pre-sampling data. For these sites, post-collection data is used, on the assumption that the environment has not changed substantially over the last few years.

Some of the deeper fjords have a pronounced stratification, often driven by a salinity contrast, with much higher nutrient concentrations in the bottom water than at the surface. Since diatoms are photosynthetic organisms and grow within the photic zone, they do not experience these deep waters, so rather than calculating the mean chemistry for the entire water column, only data from the top ten metres were used.

In most sites, nutrient concentrations in surface waters are lowest in the summer, when much of the nutrients have been taken up by algae and then either sedimented out, or eaten and moved up the food web. Nutrient concentrations are typically highest in winter, when they have been regenerated, but not yet reused. This seasonal cycle in nutrient availability means that water chemistry measurements from all seasons are required. Unfortunately, either because of logistical reasons, for example extensive ice cover, or because of the focus of the monitoring programme, such as summer anoxia or cyanobacterial blooms, there is a bias towards summer sampling. A simple mean of all available values would bias estimates of nutrient concentration downwards towards the summer state. The solution adopted by DEFINE is to first calculate the mean chemistry for each season, and then calculate the mean of these as the annual mean.

Ideally, we would have a full range of environmental variables recorded from each site. Unfortunately, the variables available vary substantially between agencies and between sites. Water depth and exposure were available from all sites. Depth was measured at the time the sediment sample was collected. Exposure was estimated by measuring the fetch on maps of each site, and subjectively expressing this as a categorical variable – open/enclosed. Previous work in MOLTEN (Clarke *et al.* 2006) had shown that these variables, together with salinity, are important in determining diatom community composition. Salinity was available from all sites, except for some where conductivity was recorded, and we estimated the salinity from this. Since the aim of DEFINE was to reconstruct nutrient conditions, sites without TN or TP were omitted. Several other nutrients, and other important environmental variables, were recorded from at least some sites, including nitrate, nitrite, ammonium, phosphate, silicate and turbidity. We had the choice of either omitting the sites that lacked these variables, or omitting these variables. Since there were many sites lacking several of these variables, and for

turbidity, measuring it with incompatible methods, all these variables were excluded from subsequent analyses. This is unfortunate as diatoms may respond to silica availability, as it is essential for construction of their frustules. All the data are retained in the database, and a subset of sites could be extracted to test the importance of particular variables.

The mean annual chemistry data is skewed. This non-Gaussian distribution has the potential to adversely affect the analyses, so the data were transformed, by taking logs or square roots as appropriate.

Validation data was processed in an identical manner to the training set data, except that means were calculated for each year, rather than for five year blocks.

The mean data for each site is available from the database-driven MOLTEN/DEFINE website at <http://craticula.ncl.ac.uk/Molten/jsp>

2.3 Results

Work undertaken during the DEFINE project added 124 sites to the MOLTEN database (Figure 2.1; Table 2.1). These sites were all from areas not previously studied. The new sites span a wide environment range, with water depths between 2 and 101 m, salinities between <0.1 and 24 psu, and with nutrient status between oligotrophic and eutrophic. These values are comparable with the sites already in the database (Figure 2.2; Table 2.1).

In a biplot of a principal components analysis of the environmental data, sites from Norway plot together with sites from the Swedish west coast, showing that they have similar environments (Figure 2.3). Similarly, Estonian and Latvian sites plot together, and Finnish and most Swedish sites plot together. German sites lie in an intermediate position between the inner Baltic and the Danish sites. The arrows on this plot show that salinity is almost orthogonal to depth and nutrient concentrations; that depth and nutrient concentrations are inversely correlated; and that TN and TP are correlated. The correlation between TP and TN will make it difficult to create independent transfer functions for these two nutrients.

Water chemistry data from 124 sites has been collated, harmonised and added to the MOLTEN database. PCA shows that nutrient variables are correlated, but are independent of salinity.

Table 2.1. Summary of the MOLTEN/DEFINE database. Environmental variables are expressed as minimum – (medium) – maximum. * Includes some samples from Russia.

Country	No. DEFINE Samples	Total No. Samples	Salinity (psu)	Depth (m)	TP (µg/L)	TN (µg/L)	Enclosed / Open
De	30	30	1.1 - (7.5) - 14.0	2.0 - (7.3) - 40	24.3 - (59.7) - 217	265 - (678) - 2180	18 / 12
Dk	0	91	2.5 - (18.3) - 31.3	1.1 - (10.1) - 40	16.4 - (45.4) - 476	232 - (539) - 2930	54 / 37
Ee	23	23	4.2 - (5.4) - 7.0	5.0 - (25) - 101	21.1 - (25.1) - 60.8	194 - (279) - 595	0 / 23
Fi*	33	102	0.1 - (4.3) - 6.3	0.7 - (4.2) - 29.8	9.4 - (32.3) - 176	317 - (590) - 3100	102 / 0
Ho	0	22	14.9 - (29) - 31.7	3.0 - (13.9) - 44.8	56 - (117) - 433	595 - (1190) - 3890	2 / 20
La	11	11	5.1 - (5.9) - 6.8	8.0 - (13.6) - 54.3	15.7 - (28.1) - 32.5	315 - (383) - 550	0 / 11
No	7	7	16.0 - (23.4) - 24.3	18.7 - (26.2) - 100	14.4 - (19.2) - 22	242 - (299) - 339	0 / 7
Sw	20	55	0.1 - (5.4) - 26.3	0.5 - (15.6) - 83	5.7 - (24.7) - 112	150 - (365) - 1340	53 / 2
Total	124	341	0.1 - (6.1) - 31.7	0.5 - (9) - 101	5.7 - (33.2) - 476	150 - (518) - 3890	229 / 112

Figure 2.1 Map of sites with both water chemistry and diatom counts in the MOLTEN/DEFINE database. Sites added by DEFINE are marked in red.

Figure 2.2 Boxplots of the four environmental variables in the MOLTEN/DEFINE database.

Figure 2.3 Principal components analysis of the environmental data, colour-coded by country.

3. Diatoms in surface sediments

Here we report on surface sediment diatom assemblages in the Baltic Sea area. Methods for sample collection, preparation and identification are provided. Details are also provided of the taxonomic harmonisation work that was performed to allow the Baltic-wide transfer functions to be constructed. We do not report in detail on the diatom species found in these samples as this information is considerably large and is available on the project website; <http://craticula.ncl.ac.uk/Molten/jsp>.

3.1 Collection of sediment samples

Surface sediment samples were collected at the sampling stations between 1996 and 2005. The majority of these sites were shallow (< 3 m) enclosed areas such as lagoons, estuaries and embayments, but more exposed and deeper areas (> 10 m) were also sampled. All sampling sites apart from those of the Finnish south coast correspond to sampling stations used in national monitoring programmes. Sediment cores, collected using Kajak-type gravity corers (Renberg 1981), were extruded at 1 cm intervals as soon after collection as possible. Subsamples of wet sediment from the top 1 cm were taken for diatom analysis, with the remaining sediment freeze-dried for storage. Some of the more exposed sites had substrates dominated by sand, in such location Eckman-type grabs were used instead of corers to collect the surface sediments. Subsamples of the top 1 cm from such grabs were collected in the field and returned to the laboratory for analysis.

3.2 Preparation of diatom slides

The methodology of either Battarbee (1986) or Renberg (1990) was followed in preparing the diatom samples for analysis. In short, organic material was digested using hot 30% hydrogen peroxide (H_2O_2) for a minimum of three hours, and any carbonates were removed with the addition of a few drops of concentrated hydrochloric acid (HCl). Samples were then washed repeatedly with distilled water to remove all traces of acidity. Methanol caps (Hinchey and Green 1994) were added to the H_2O_2 digestions were necessary to prevent any organic-rich samples from foaming over. Diluted samples were strewn onto coverslips, and left to dry out overnight, before being mounted onto slides with Naphrax™ (refractive index 1.72).

3.3 Diatom identification

Diatoms were identified at 1000× magnification on light microscopes using either differential interference contrast or phase contrast illumination. Diatom identification was based on the volumes of: Cleve-Euler (1951-1955), Hasle & Syvertsen (1996), Hendey (1964), Hustedt (1930, 1985), Krammer & Lange-Bertalot (1986, 1988 & 1991a & b, Pankow (1976), Snoeijs *et al.* (1993, 1994, 1995, 1996 & 1998), Witkowski (1994) and Witkowski *et al.* (2000). Further reference was also made to the following papers: Cooper (1995), Fryxell & Hasle (1972), Harris *et al.* (1995), Hasle (1973, 1978, 1979 & 1980), Hasle & Lange (1989), Hasle & Syvertsen (1990), Håkansson (1996), Håkansson *et al.* (1993), Laws (1988), Muylaert & Sabbe (1996), Mölder (1962), Mölder & Tynni (1967-1973), Sabbe & Vyverman (1995), Snoeijs (1992) and Tynni (1975-1980).

There are several different schemes as to the naming of diatoms currently present in the literature, but no clear consensus or preference is given to any one approach. This can cause some confusion, as to the identity of a given taxa. Within the DEFINE project we have tended to follow the advances in nomenclature as proposed by Round *et al.* (1990). We have also attempted to clarify the taxa we mean by providing on the project website for each taxa used in the models: the authority, the reference to the literature we used to identify the taxa and where possible an image taken by one or more of the DEFINE diatomists.

Harmonisation between the project diatomists was accomplished through workshops, slide exchanges and cross-counting exercises, using the nomogram of Maher (1972) to check the similarity of results within 95% confidence limits. Harmonisation involved the adoption of six different aggregates, details of which are shown in Table 3.1. These aggregates incorporate taxa where the demarcation of individual species is difficult, and accordingly ideas of the species concept can vary widely between diatomists, leading to blurred borders between species. While the use of aggregates does blur borders, it does so uniformly across the dataset. The merges to create the aggregates were performed using a relational database, leaving each diatomist free to enumerate samples at the taxonomic level they prefer, but ensuring all samples are then harmonised according to the agreed taxonomy.

Table 3.1. Diatom species within MOLTEN and DEFINE aggregates.

Aggregate	Included species
AcnMinAG	<i>Achnanthyidium microcephalum</i> Kützing 1844 <i>A. minutissimum</i> (Kützing) Czarnecki 1994
CocScuAG	<i>Cocconeis costata</i> Gregory 1855 <i>C. scutellum</i> v. <i>scutellum</i> Ehrenberg 1838 <i>C. scutellum</i> v. <i>parva</i> (Grunow in Van Heurck) Cleve 1896
FalFenAG	<i>Fallacia escorialis</i> (Simonsen) Sabbe & Vyverman 1995 <i>Navicula fenestrella</i> Hustedt 1959
FraEllAG	<i>Fragilaria neoelliptica</i> Witkowski 1994 <i>F. sopotensis</i> Witkowski & Lange-Bertalot 1993 <i>F. elliptica</i> Schumann 1867 <i>Opephora guenter-grassii</i> (Witkowski & Lange-Bertalot) Sabbe & Vyverman 1995 <i>Opephora krumbeinii</i> Witkowski, Witak and Stachura 1999 <i>Pseudostaurosira perminuta</i> Sabbe & Vyverman 1995 <i>P. zeilleri</i> (Héribaud) Williams & Round 1987 <i>Staurosira elliptica</i> (Schumann) Williams & Round 1987 <i>S. punctiformis</i> Witkowski, Metzeltin & Lange-Bertalot 2000
NitFruAG	<i>Nitzschia amphibia</i> v. <i>amphibia</i> Grunow 1862 <i>N. amphibia</i> f. <i>rostrata</i> Hustedt 1959 <i>N. frustulum</i> (Kützing) Grunow in Cleve & Grunow 1880 <i>N. inconspicua</i> Grunow 1862 <i>N. liebetruithii</i> Rabenhorst 1864 <i>N. liebetruithii</i> v. <i>major</i> (Grunow in Cleve & Möller 1879)
PlanDelG	<i>Planothidium delicatulum</i> (Kützing) Round & Bukhtiyarova 1996 <i>P. engelbrechtii</i> (Cholnoky) Round & Bukhtiyarova 1996 <i>P. haukianum</i> (Grunow) Round & Bukhtiyarova 1996 <i>P. septentrionalis</i> (Ostrup) Round & Bukhtiyarova 1996
PlanLanG	<i>P. dubium</i> (Grunow) Round & Bukhtiyarova 1996 <i>P. frequentissimum</i> (Lange-Bertalot) Round & Bukhtiyarova 1996 <i>P. lanceolatum</i> (Brébisson) Round & Bukhtiyarova 1996 <i>P. robustius</i> (Hustedt) Lange-Bertalot 1999 <i>P. rostratum</i> (Ostrup) Round & Bukhtiyarova 1996
TabFasAG	<i>Tabularia affinis</i> (Kützing) Snoeijs 1992 <i>T. fasciculata</i> (C. A. Agardh) Williams & Round 1986 <i>T. cf. laevis</i> <i>T. tabulata</i> (C. A. Agardh) Snoeijs 1992 <i>T. waernii</i> Snoeijs 1991

3.4 Diatom enumeration protocol

At least 500 valves were counted for each sample, and once enumerated all taxa expressed as relative percentage abundance. *Chaetoceros* species resting spores, *Chaetoceros* spp. vegetative valves, *Skeletonema* spp., *Rhizosolenia* spp. and *Pseudosolenia calcar-avis* were excluded from this sum, because, with the exception of *Chaetoceros* spp. resting spores, the weak silicification and quick dissolution of these cells means the distribution of these taxa are probably not accurately recorded in sediments. *Chaetoceros* spp. resting spores were excluded because spores of different species were lumped together. Occurrences of these taxa were recorded as the number encountered during a count of 500 other valves.

3.5 Summary of the diatom data

While a total of 1081 diatom taxa were identified in the DEFINE samples, most were rare, and only 279 taxa were present at 1% abundance or

greater at 2 or more sites, the cut-off used to define those taxa whose distribution is sufficiently well defined in the data-set to contribute to model development. Benthic forms are more common than planktonic taxa, which is expected due to the predominantly shallow nature and coastal position of the samples analysed. Most taxa occur with a maximum abundance below 10% at relatively few sites (< 40), only 11 taxa occur with a maximum abundance greater than 30 %, of which the most abundant are: *Cyclotella choctawhatcheeana* (at 76% the most abundant taxa), *Diatoma moniliformis* (in the Bothnian Bay) and *Pauliella taeniata* (in the Estonian samples). The most frequently occurring taxa included *Cocconeis placentula*, *Navicula perminuta*, and the aggregates of *Nitzschia frustulum*, *Fragilaria elliptica*, *Planothidium delicatulum* and *Tabularia fasciculata*. Interested readers are referred to the project website (<http://craticula.ncl.ac.uk/Molten/jsp>) for further details including distribution maps and abundance plots against key environmental variables.

4. Transfer function development

Multivariate statistical analyses can be used to investigate patterns in the environmental data and the diatom assemblages described in chapters 2 and 3. We need to address three questions: what are the main patterns in the diatom assemblage data; which environmental variables can explain these patterns; and can a robust diatom-nutrient transfer function be generated? If possible, this transfer function will be used to reconstruct nutrient histories from the long core sites.

4.1 Techniques for uncovering the main patterns in the diatom data

There are two multivariate statistical techniques that can be used to reveal the patterns in the diatom data: cluster analysis and ordination. Cluster analysis partitions the data into subsets of similar sites, and is most useful if the aim is to assign names to, or map ecological communities, but can become unstable if there are intermediate sites. Ordination is an attempt to represent the high-dimensional biotic data in, typically, two or three dimensions, ordering the sites so that biotically-similar sites are near each other and biotically-dissimilar sites are further from each other. The two techniques are complementary and both are valuable, but ordination is often more useful as an exploratory tool.

A great variety of ordination techniques exist, but only some perform well with the particular characteristics of biological data (many zeros, much noise). There is an important distinction between techniques that consider only the biotic data, using any environmental variables in a second interpretative stage (unconstrained ordination or indirect gradient analysis), and techniques that use environmental data in addition to the biotic data (constrained ordination or direct gradient analysis). The former finds the major gradients in the biotic dataset, the latter is the best method of determining if the environmental variables influence the biotic data. The VEGAN library (Oksanen *et al.* 2007) in R (R Development Core Team 2006) was used to ordinate the diatom data.

4.2 Indirect gradient analysis

We are first interested in using indirect gradient analysis to find the main gradients in the biotic data. Suitable methods include Detrended Corre-

spondence Analysis (DCA) and Non-Metric Multidimensional Scaling (NMDS). DCA is a modification of Correspondence Analysis (CA; also known as reciprocal averaging) to remove the arch effect which afflicts CA and rescale the axes so that they are in units of beta diversity. If the length of an axis is less than about 2, most species have a linear relationship with that axis, if it is greater, many species have a unimodal relationship. The length of the first axis of a DCA of the diatom data is 3.3 SD units (Figure 4.1), indicating that statistical models that assume a unimodal species environment relationship are appropriate.

Figure 4.1 Plot of sites scores on axes one and two from a Detrended Correspondence Analysis (DCA), colour coded by country. The analysis, like all others in this chapter, included all species found in two or more sites and having a maximum abundance greater or equal to 1%.

Principal Co-ordinates Analysis (PCoA) attempts to represent the distances between sites by maximising the linear correlation between the distances in the distance matrix, and the distances in a space of low dimension (typically, 2 or 3 axes). Like CA, PCoA suffers from the arch effect, in part, because PCoA maximizes the linear correlation. NMDS (Minchin 1987) instead maximises the rank order correlation and avoids these problems. To perform an NMDS, the number of dimensions (N) required is selected and the distance matrix calculated with an appropriate distance metric. Then the measure of stress (the mismatch between the

rank order of distances in the data and the rank order of distances in the ordination) is calculated for an initial configuration that can be random, but it is more efficient to derive it from another ordination method. The sites are then moved slightly in a direction that decreases the stress. This step is repeated until stress is minimised. This algorithm is not guaranteed to find the optimal solution, as it may become trapped in a local minima. To avoid this, the computer intensive algorithm is typically run several times from different starting positions.

The configuration is dependent on the number of dimensions selected: the first two axes of a 3-dimensional solution do not necessarily resemble the 2-dimensional solution. This suggests that some care is required to select the optimal number of axes. The negative relation between stress and the number of axes can aid this selection. For the diatom data, the stress is 33.7, 19.6, 14.0, 11.3, and 9.2 for solutions with one to five axes, using Bray Curtis distances. The large drop between the axis one and two solutions, and the smaller declines for subsequent axes, suggests that a second axis is required, but others are not necessary.

The two axes NMDS solution (Figure 4.2) is similar to the DCA solution, but does not have the triangular configuration of sites, which can be an artefact of the detrending. The order of the NMDS axes is arbitrary, the first axis is not necessarily more important than the second axis. Conveniently, in this case the DCA and NMDS solutions have the same axis order, with the axes in the same orientation, and only the latter will be discussed further. Each countries' sites tend to cluster together in the NMDS, with Danish and Dutch sites on the right and Baltic sites on the left, suggesting that the first axis is a salinity gradient. Sites at the top of the plot tend to be exposed sites.

The relationship between the gradients uncovered by NMDS and the environmental variables can be explored by adding a smooth surface representing the environment data to the ordination. Figure 4.3 shows contours for salinity, depth, and nutrients added to the NMDS plot. Salinity has a linear relationship with axis one. Depth has a unimodal relationship with axis two. The relationship between nutrient concentration and the diatom composition is more complex.

A more powerful approach for exploring the relationship between the species assemblage data and the environmental data is to use constrained ordination.

Figure 4.2 Plot of the site scores, colour coded by country, ordinated with Non-Metric Multidimensional Scaling (NMDS) using the Bray-Curtis distance metric on two axes.

Figure 4.3 Contours of salinity, depth, TN, and TP fitted to the NMDS. Environmental variables have been transformed to make them more Gaussian.

4.3 Constrained ordination

Robust transfer functions can only be developed for environmental variables that have a statistically significant, independent effect on the biotic data. This effect can be tested for using direct gradient analysis or constrained ordination, which directly relates the assemblage data to the measured environmental factors, and the site scores are constrained to be linear combinations of explanatory variables.

Since the length of the DCA axis suggests that the diatoms have a unimodal species environment relationship, the most appropriate constrained ordination techniques is Constrained (or Canonical) Correspondence Analysis (CCA; ter Braak 1986), the constrained form of CA.

The statistical significance of a single variable can be tested by comparing the observed eigenvalue length, with the distribution of eigenvalues generated from analyses with permuted environmental data, keeping the biotic data intact. If the observed statistic is greater or equal to 95% of the statistics from the permuted data, the null hypothesis, that this environmental variable has no effect on the species assemblages, can be rejected. Since the environmental variables are all correlated to some extent, it is important to test if the environmental variables have a statistically significant effect after removing (partialling out; ter Braak 1988) the effect of the other variables. Variables that are significant in partial-CCA have an independent effect on the assemblages (at least amongst the variables tested). All five environmental variables used by DEFINE have a significant independent effect with $p < 0.01$.

Figure 4.4a shows the site scores on the first two axes of a CCA of the diatom data; the species data are shown in Figure 4.4b. The length of the arrows representing the environmental variables is proportional to the importance of that environmental variable. Sites that are close together have similar assemblages and biota, and taxa that are close together occupy similar sites.

Variance partitioning (Borcard *et al.* 1992; Table 4.1) allocates the variance in the biotic data to environmental variables, and covariance between the environmental variables, with a remaining unexplained portion. In total, the environmental variables explain 14.4% of the variance, with only salinity explaining more than 5%. Although low, the amount of variance explained is not atypical for diatom transfer functions.

The hypothesis testing undertaken has made the assumption that the observations are independent. Lack of independence between observations inflates the risk of Type I errors (erroneously rejecting the null hypothesis). One important cause of lack of independence is spatial autocorrelation, a common phenomenon in ecological data where nearby observations are more similar than expected by chance. To determine which variables have a significant influence on assemblage composition, the test

of the null hypothesis needs to be modified to allow for spatial auto-correlation.

Figure 4.4 Plot of a) site scores, and b) species scores from a Constrained Correspondence Analysis (CCA).

Table 4.1. Variance partitioning of the MOLTEN/DEFINE data set using CCA.

Variable	Percent variance explained
Salinity	5.2
Exposed	1.8
Depth	1.6
TN	1.3
TP	1.2
Covariances	3.3
Sum explained	14.4
Unexplained	85.6

All taxa occurring in two or more sites, with a maximum abundance greater than 1% are included in the analysis. Species data are square root transformed to balance the variances.

Fortin & Jacquez (2000) suggest two randomisation procedures for testing a null hypothesis when the data are spatially autocorrelated. The first is to map the species and environmental data separately, and then slide the environmental map over the species map into a random position and recalculate the test statistic. This is only readily applicable if the observations are on a grid: few, if any, training sets have such an arrangement. The second technique, which Fortin & Jacquez (2000) recommend, is to simulate environmental variables with the same autocorrelation structure as the measured data and recalculate the test statistic.

There are three steps in this procedure. The first is to find the spatial structure in the environmental variable of interest using an empirical variogram. An empirical variogram is a plot of half the squared difference between two observations against their distance in space, averaged for a

series of distance classes. The second is to select and fit a theoretical variogram model. Finally, unconditional Gaussian simulation (Wackernagel 2003) is used to generate a spatially structured random variable with the same spatial structure as the original data. We used the gstat package (Pebesma 2004) in R (R Development Core Team 2004) for most of these calculations.

Empirical variograms for the environmental variables are shown in Figure 4.5, each fitted with a circular variogram model. The range of the variogram, the distance at which the semi-variance stops increasing, is largest for salinity (469 km). This, and the small nugget variance (the variability at short distances), is expected from the large salinity trends from the Bothnian Sea to the North Sea. Variograms for depth, TP, and TN all have a range of about 100 km, and for each the nugget is about half the variability of the circular model. This suggests that there is a tendency for spatially close sites to have similar values for depth and nutrient concentrations, but over a smaller distance and with more noise than for salinity.

Figure 4.5 Empirical semi-variograms with fitted variogram models for four environmental variables. The nugget is the semi-variance at distance zero, the range is the distance at which the variogram model levels off, and the sill is the semi-variance at this point.

When used as a single explanatory variable, all the observed continuous environmental variables explain more of the variance in the diatom data than any of the 999 simulated environment variables with the same auto-correlate structure. If salinity, exposure, and depth are partialled out, both nutrients explain a significant proportion of the remaining variance at the $p=0.001$ level.

4.4 Transfer function development

CCA showed that TN can explain a small but significant and independent proportion of the variance in the diatom data, even when allowing for autocorrelation. We can now build and test a transfer function model to use this relationship in the modern data to reconstruct past nutrient concentrations from the fossil assemblage data from the long cores.

There are a bewildering array of transfer functions methods, each with many options. Choosing which model to use is a non trivial task. Traditionally, this was guided by training set performance statistics, especially the root mean square error (RMSE: the square root of the mean of the squared differences between observed and predicted value). As the true RMSE is invariably under-estimated when based solely on the training set (Birks 1995), some form of cross validation with an independent test set is required to derive a more reliable and realistic estimate of prediction error (RMSEP) and hence to evaluate the predictive abilities of the transfer function model (Birks *et al.* 1990). However recent work (Telford & Birks 2005) has shown that these statistics are biased if the environment is spatially structured (as is common), and that the more complex the model is, the greater the bias is. This greatly complicates model selection, instead, we need to consider the theoretical and empirical support for each method.

Gaussian logit maximum likelihood regression and calibration (ter Braak & Looman 1986; ML) fits a unimodal (or sigmoidal if there is insufficient support for a unimodal model) response curve to each species. The likelihood for each value on the environmental gradient can be calculated for a fossil observation, and the maximum likelihood selected. ML has strong support from ecological theory, but this computer intensive technique often fails to outperform a simpler approximation, weighted-averaging.

Weighted averaging (WA) assumes a unimodal species environment relationship. It finds the optimum of each taxon as the mean of the environmental values at sites where it is present, weighted by the abundance of the taxon at these sites. Reconstructions are calculated as the average of the optima of the taxa in the observation, weighted by their abundance. Since means are taken twice, there is a tendency for values to shrink towards the mean. Several deshrinking procedures are available to correct this, including WAPLS, an extension to WA that uses additional components to extract information from patterns in the residuals to improve performance. WAPLS always performs at least as well as WA.

The modern analogue technique (MAT) assumes no species response model. It is based on the premise that assemblages that resemble one another are derived from similar environments (Prell 1985). This is quantified by selecting the k -nearest neighbours in the modelling set, using an appropriate distance or dissimilarity metric, and calculating the mean (or

a dissimilarity-weighted mean) of the environmental parameter of interest. There are different criteria for choosing k . The most common is to use the same value of k for each sample, with k chosen to minimize the RMSEP in the optimization set. MAT is perhaps the most widely used transfer function for reconstructing ocean SSTs, as it gives the lowest RMSEP, but this performance is probably due to the spatial structure in the marine training sets, which tends to make models with too few analogues appear to perform well. For training sets with no autocorrelation, MAT does not reliably outperform unimodal models.

Artificial neural networks (ANN) are algorithms that, by mimicking biological neural networks, have the ability to learn by example. They learn by iteratively adjusting a large set of parameters, which are initially set at random values, to minimize the error between the predicted and actual output. They can approximate any continuous function (Hornik *et al.* 1989) and provide a flexible way to generalize a linear regression function (Venables & Ripley 2002). If trained for too long, ANNs can over-fit the data, learning particular features of the modelling set rather than the general rules. This is normally controlled by using a second data set and stopping the training when the model stops reducing the RMSEP of this data set. Typically many ANN models are generated from different random initial conditions and network configurations and the best model used. ANNs assume no explicit species-environment response model, and have been heavily promoted as a new transfer function methodology. However, for training sets with no spatial structure, provided overfitting is avoided, they do not reliably outperform more traditional methods (Telford *et al.* In prep).

Of the four transfer function techniques described here, WAPLS has the advantage that it has a good theoretical basis, is computationally simple, and has few metaparameters to determine. This is the model that will be used by DEFINE, with the constraint that only models with three or less components will be used, to avoid problems with overfitting.

A two component WAPLS model (Figure 4.6) has a leave-one-out RMSEP of $0.38 \log \text{TN } \mu\text{g l}^{-1}$, about 10% of the log TN range in the dataset. This performance is similar to many other transfer functions, and is excellent considering the heterogeneity of the dataset. However, we do need to be cautious about the effects of autocorrelation, which could have “improved” this statistic. Autocorrelation is a problem during cross-validation as the sites in the test set are not independent of the training set sites. One simple test of the importance of autocorrelation is to exclude neighbouring sites from the training set, and test how much the RMSEP increases by. With an exclusion zone of 20 km, the RMSEP increases to $0.41 \log \text{TN } \mu\text{g l}^{-1}$, and the r^2 decreases from 0.62 to 0.55. This relatively small drop in model performance after excluding neighbouring sites indicates that the transfer function predictive power is not an artefact of autocorrelation. An alternative test is to compare the performance of transfer

function models using the measured environmental data with models using the simulated environmental data developed above. The r^2 of the model using the measured data exceeds that of all those simulated data.

Figure 4.6 Plot of leave-one-out predicted against measured TN for a two component Weighted-Averaging Partial Least Squares model for the entire dataset.

The MOLTEN/DEFINE dataset is very heterogeneous, with salinity, exposure, and depth all explaining more of the variance than TN. This is not ideal, and may be contributing to the scatter in Figure 4.6. One solution is to divide the training set up into smaller, more homogeneous training sets, and use these instead. Figure 4.7 shows the results of 6 transfer functions, splitting the sites into either exposed or sheltered, and saline (>8 psu), intermediate (8-12 psu) and brackish (<8 psu). Only the exposed intermediate and brackish models have a worse r^2 than the combined model. This suggests that for these sites, there may be no useful transfer function (this is partly due to the small TN gradient between these sites). For other sites, splitting the training set has improved the transfer functions.

Identifying and removing outliers can improve transfer function performance. The difficulty is deciding when to stop, as removing more outliers will generally yield even more improvements in model performance. The approach used in DEFINE has been to be cautious, and to only remove gross outliers. Although several sites have distinctive assemblage

compositions, there are no gross outliers in the WAPLS models examined.

Environmental variables have a statistically significant independent effect on the diatom assemblages. A robust transfer function can be built to reconstruct TN in at least some of the coastal water types included in the database.

Figure 4.7 WAPLS models for six subsets of the DEFINE data, split by salinity and exposure.

5. Long core nutrient reconstruction

5.1 Introduction

The analysed diatom assemblages in the surface sediments (i.e. the training set) were used together with water chemistry data to infer past total nitrogen concentrations in the coastal waters by using a diatom-based transfer function developed for the Baltic Sea area. Two new sediment cores, one from Gårdsfjärden situated on the Swedish side of the central Bothnian Sea and the other from Saunja Bay located in the Moonsund area at the Estonian west coast, have been enumerated for diatoms in this study. Diatom analyses and dates of eight previously published cores from the Oder estuary and the southwestern Baltic Sea were available (Andrén 1999; Andrén *et al.* 1999). Only two of these cores were considered suitable for diatom-inferred total nitrogen reconstructions in this study, 18017 from the Arkona Basin and 18025 from Oder Rinne.

In total we present diatom stratigraphies and diatom inferred total nitrogen reconstructions from four sites located from the Bothnian Sea to the southwestern Baltic Sea (figure 5.1, Table 5.1). The sites chosen are situated in different parts with varying basic conditions both regarding the surrounding natural setting (e.g. bedrock and soils) and the nutrient limitation for biological growth. Some estuarine systems display seasonal switches in nutrient limitation (Conley 2000), but the Bothnian Bay is phosphorus-limited (Andersson *et al.* 1996) and the open Baltic Proper is nitrogen-limited (Granéli *et al.* 1990), both throughout the year. Further, the studied sites have a diverging history of human impact with the sites from the southern Baltic Sea and the Estonian estuary exposed to nutrient discharge from the surrounding agricultural region whilst the Bothnian Sea site receives discharge from an industrial point-source.

Table 5.1. Sediment cores used for diatom inferred total nitrogen reconstruction.

Area	Core label	Water depth (m)	Latitude	Longitude	Recovery (cm)
Saunja Bay	S-001	1	58°59'37"N	23°37'39"E	40
Gårdsfjärden	IGG 2	12	61°37.25'N	17°09.39'E	55
Arkona Basin	18017-1	45	54°48.71'N	13°40.33'E	60
Oder Rinne	18025-2	20	54°29.53'N	13°43.02'E	60

Figure 5.1 Map of the Baltic Sea with location of the sites investigated in the present study marked in red. The black dots show other sites where reconstruction of total nitrogen has been carried out using the same transfer function technique (Clarke *et al.* 2006; Ellegaard *et al.* 2006; Weckström 2006; <http://craticula.ncl.ac.uk/Molten/jsp/>).

5.2 Methods

Diatoms were prepared, identified and enumerated in the same way as the surface sediment samples described in chapter 3.

Detrended correspondence analysis (DCA), a unimodal indirect ordination method (Hill & Gauch 1980) was used to summarise the diatom compositional changes over time and performed with CANOCO for Windows, version 4.0 (Ter Braak & Smilauer 1998). Species richness of the fossil diatom assemblages was calculated using rarefaction analysis (Birks & Line 1992). Rate of change was calculated from the squared chord distance between adjacent samples. The dissimilarity between each fossil assemblage and its closest modern analogue was estimated using the squared chord distance (Overpeck *et al.* 1985) in the programme C2 (Juggins 2004). A good analogue was defined as a fossil sample having a squared chord distance less than 0.55, which is the 5th percentile of the distribution of all distances among modern samples in the training set. A

squared chord distance less than the 10th percentile, 0.69, was considered as fairly good.

5.3 Saunja Bay

5.3.1 Site description

Saunja Bay, situated in western Estonia, is the eastern innermost part of Haapsalu Bay situated in the Väinameri (Moonsund) area, which can be regarded as a connecting link between the Gulf of Riga and the open Baltic Sea (figure 5.2). The mean depth of the bay is less than 1m. Three small streams, Salajõgi (15 km; 93 km²), Taebla (32 km; 107 km²) and Võnnu (12 km; 69 km²), bring nutrient-rich freshwater into the bay. The mean concentration of TN in the Taebla stream was measured in August 1985 and 1989 and reached 2800 µg L⁻¹, with TP at 180 µg L⁻¹ (Porgasaar 1993).

Figure 5.2 Map of the Estonian study area (A) with location of the coring site in Saunja Bay (B) (58°59'37''N; 23°37'39''E). Isolines show the present land uplift of the area which indicates c. 2.75 mm/year at the studied site

The water exchange between Haapsalu Bay and Saunja Bay is limited due to the shallow water depth in the narrow straits between the basins. However, westerly storms can raise the water level considerably causing inflow of brackish Baltic Sea water to the easternmost part of the Saunja

Bay. The mean water salinity in Saunja Bay about 2 km south of the coring site was 2.8 PSU, measured six times during the vegetation period in 1985 (Porgasaar 1993) and surface water salinity ranged between 2.5-4.2 PSU measured in a similar way in year 2000 (Jaanus 2003).

The central part of Haapsalu Bay situated west of Saunja Bay receives municipal and industrial sewage waters from Haapsalu, a town with 13 000 inhabitants (figure 5.2). A mechanical wastewater treatment plant was first installed in 1981 and a modification started in 1995 and was completed in 1997. As a result the pollution load decreased notably, especially BOD and phosphorus. Nitrogen concentrations were still at a very high level and in January 2001 the construction for nitrogen removal in the treatment plant was finalised (HELCOM 2001).

There is no regular monitoring of water quality carried out either in Saunja Bay or in Haapsalu Bay and only scattered data about the P and N concentrations can be found. In 1985, the mean concentrations were 780 (370-1100 $\mu\text{g L}^{-1}$) and 43 (33-49 $\mu\text{g L}^{-1}$), for TN and TP, respectively (Porgasaar 1993). In 2000 during the vegetation season, there were some measurements of TN from Saunja Bay and in most cases the concentration was above 100 $\mu\text{mol L}^{-1}$ (1400 $\mu\text{g L}^{-1}$) (Jaanus 2003). According to the nitrogen concentration Haapsalu Bay can be regarded the most nutrient enriched part of Estonian coastal waters (Saat & Jaanus 2001).

5.3.2. Sampling and dating

A sampling site was selected in the central part of the Saunja Bay where the probability to recover an undisturbed core was considered best (figure 5.2). Sediment coring was performed in May 2005 from an inflatable boat at a water depth of 1 m with a Willner gravity corer (inner Ø 5.5 cm) resulting in the recovery of a 40 cm long sediment core. The same station was also sampled with a Russian peat corer, but the uppermost 20 cm was unconsolidated and not possible to sample with this type of equipment. The cores were transported to the laboratory and the gravity core was subsampled every cm for diatom analyses and dating. The lithology of the gravity core was; 0-20 cm dark greenish grey silty-gyttja with sulphide stains and shells, 20-40 cm dark grey gyttja-clay/silt.

Every cm of the core was analysed for ^{210}Pb at the Radioisotope Research Laboratory in Vilnius, Lithuania (figure 5.3). The unsupported ^{210}Pb concentrations varied between 380 and 28 Bq/kg and reached the supported value 38 Bq/kg at 24 cm sediment depth. An age model was constructed and the mean linear sediment accumulation rate in the core is estimated to 0.15 cm/year, which indicates an age of c. 100 years at 15 cm depth.

Figure 5.3 Graph showing the down core variations in total ^{210}Pb measured in the Saunja Bay sediment core. The supported ^{210}Pb in the core 38 Bq/kg is approached around 24 cm sediment depth.

5.3.3 Diatom analyses

Altogether 40 levels were counted for their content of diatoms and a total of 153 different taxa were identified. Diatoms with an abundance of more than 4% in at least one stratigraphical level are displayed in figure 5.4 and diatom life forms are displayed in figure 5.5. There are some clear trends in the diatom stratigraphy visible as shifts in life forms and salinity requirements and it can be divided into 3 local diatom assemblage zones using visual examination of changes in the data as well as a cluster analysis (CONISS in the Tilia program). The core is dominated by brackish-freshwater taxa (50-75%) and the most common life form is periphytic (c. 80-95%), however, the core includes a fluctuating amount of epiphytes (5-20%) and a minor occurrence of plankton in the uppermost part.

The lowermost zone S-1 (40-29.5 cm) is dominated, as all zones in the core, by the *Fragilaria elliptica* aggregate and reaches 35% in the zone. This zone has a maximum occurrence of brackish-marine taxa consisting mainly of the taxon cf. *Brachysira estoniarum*. Other taxa with a maximum occurrence in this zone are *Cocconeis neothumensis* and *Cocconeis placentula*. The zone has a high amount of epiphytes reaching between 15 and 20%. Species richness varies between 62-51, which are the highest values in the core.

Figure 5.4 Diatom stratigraphy and lithology of the core from Saunja Bay located in western Estonia, showing relative abundance of species occurring at 4% in at least one level. Taxa are grouped into salinity requirements and sorted in order of first appearance. An age model has been constructed from ^{210}Pb measurements.

In zone S-2 (29.5-14.5 cm) there is a maximum occurrence of *Amphora pediculus*, *Navicula jaernefeldtii* and *Mastogloia braunii* and further *Nitzschia elegantula* has its minimum occurrence. Freshwater taxa reach their maximum abundance in this zone at the same time as brackish taxa reach their minimum. There is an increase of periphytic taxa at the expense of epiphytes, which reach minimum levels of <5%. Species richness is decreasing and varies between 56-43.

In zone S-3 (14.5-0 cm) the *Fragilaria elliptica* aggregate reaches its maximum abundance of 50%. Other taxa with a maximum abundance in this zone are *Staurosira construens* var. *subsalina*, *Diatoma tenuis* and the freshwater taxa *Cymbella cf. subaequalis*, *Staurosira construens* and *Encyonopsis microcephala*. In this zone there is a slight increase in planktonic taxa and a clear increase of epiphytic taxa in the topmost part of the core. The lowest species richness is attained in this zone and it varies between 56 and 37.

Figure 5.5 Graphs from the Saunja Bay core plotted on an age-scale showing diatom life-forms (benthic, epiphytic and planktonic taxa), diatom DCA axis 1 scores, diatom species richness, rate of change in the diatom data, reconstructed diatom-inferred TN and analogue quality (pair wise squared chord distance in the training set). Zonation of the diatom species data is shown as diatom assemblage zones (DAZ) to the right.

5.3.4 Nutrient reconstruction and environmental interpretation

The reconstructed TN ranges between 424 and 762 $\mu\text{g L}^{-1}$ showing high values in the lowermost diatom assemblage zone S-1, decreasing to a minimum value in S-2 and increasing towards present time in the uppermost zone S-3 (figure 5.5). There are no long time series of monitoring data from the Saunja Bay to validate the model and only the measured yearly mean from 1985 of 780 $\mu\text{g L}^{-1}$ for TN (Porgasaar 1993) could be compared with a reconstructed value of ca 700 $\mu\text{g L}^{-1}$ from the same time according to the core age model. To get a measurement of the analogue quality the dissimilarity (squared chord distance) between the fossil and the modern assemblages were calculated for all stratigraphical levels. The squared chord distances of the fossil assemblages were ranging between 0.87 and 1.26 throughout the core, increasing upwards, which are much higher values than the delimitation of the 10th percentile (0.69) indicating not even fairly good analogue quality (figure 5.5). It is obvious that the bay has a naturally high nutrient background level (the lowermost part of core has the best analogue quality) which could be expected in an enclosed, shallow basin surrounded by arable land with limited water exchange and inflow from 3 rivers. The reconstruction is mainly driven by the *Fragilaria elliptica* aggregate giving a similar shape to the reconstructed TN curve as the relative percentage of this aggregate. Several taxa showing distinct changes in the core like *Amphora pediculus*, *Navicula jaernefeltii*, *Mastogloia braunii* and *Nitzschia elegantula* are present in a much lower percentage in the training set than in the sediment core. Consequently there is a lack of modern analogues from the surrounding areas of several important fossil species found in the core and the recon-

structed TN values of Saunja Bay have to be considered as tentative at the best.

Nevertheless the diatom stratigraphy displays some interesting changes that could be interpreted both in terms of changed nutrient discharge and water exchange with the open Baltic Sea. Most of the changes in the stratigraphy occurred in the mid nineteenth century visible as a distinct increase in DCA axis 1 scores up core. Simultaneously there is an increase of freshwater taxa and brackish-marine taxa more or less disappear. This coincides with a change to more organic production evident in the lithological change in the core from gyttja-clay/silt to silty gyttja. The species richness is steadily decreasing and there is a clear decrease in epiphytic taxa from the beginning of the nineteenth century together with a minor occurrence of planktonic taxa from ca. 1900 onward. All these changes could be interpreted as the effect of decreased water exchange between the Saunja Bay and the Baltic Sea and possibly also increased discharge of freshwater from the rivers. This could result in an increased amount of nutrients from land trapped in the bay and increased biological production causing deteriorated water transparency with decreased coverage of benthic macroalgae. Only a slight change in the threshold area is needed to alter the water exchange, which would influence the environment in such a shallow, enclosed, coastal site. The land uplift rate at the site is ca. 2.75 mm/year (figure 5.2) resulting in a 0.55 m decrease in the threshold in 200 years, in addition to the sediment accumulated, making the bay shallower over time. Taking into account that the bay has a mean water depth of less than 1 m at present this shallowing would probably result in significant changes in the water exchange and a natural trapping of nutrients in the bay.

In 1981 a sewage treatment plant was installed in the bay, which later expanded to include also nitrogen removal in 2001 (HELCOM). It should be possible to observe such an improvement of the quality of discharged water in the diatom stratigraphy and further in the TN reconstruction. The time resolution of the sediment core is unfortunately low. A sediment accumulation rate of about 1.5 mm a year in Saunja Bay would result in only 3.5 cm sediment for the time span since the sewage treatment plant was installed. There are apparent changes in the three topmost samples visible as an increase in epiphytic taxa, which could indicate improved light conditions and an expanded phytal zone. The *Fragilaria elliptica* aggregate decreases and some freshwater and brackish-freshwater taxa increase (figure 5.4). In spite of this there is no clear decreasing trend in the reconstructed TN indicating a better water quality, however the analogue quality of this section is bad and hence the reconstruction should be viewed with caution.

5.4 Gårdsfjärden

5.4.1 Site description

Gårdsfjärden is a small almost enclosed estuary covering an area of 4 km² situated at the east coast of central Sweden. The estuary is connected to the Bothnian Sea through the narrow Dukarsundet and a threshold of 8.7 m, which limits the exchange of water (figure 5.6). The mean water depth in Gårdsfjärden is around 6 m and the deepest part is 18 m close to the threshold area in the eastern part.

Figure 5.6 Map of the Gårdsfjärden area located at the Swedish east coast of central Bothnian Sea showing the coring site IGG 2 (61°37.25'N; 17°09.39'E). The bathymetric isolines of 3 and 6 m and the discharge from the Iggesund paper mill are indicated. The water exchange between Gårdsfjärden and the open Bothnian Sea is limited by the narrow Dukarsundet.

The estuary was dredged 1991, 1992 and 1994 to facilitate entrance of large ships into the inner part. The extensive dredging in 1994 removed 240,000 m³ of sediment from the threshold area (Jonsson 2002). The mean surface water salinity in the Bothnian Sea is about 5 PSU and the stratified Gårdsfjärden estuary has a yearly mean salinity of 2.4 in the surface water and 4.7 PSU at a water depth of 10 metres (measured between 1990 and 2001). Gårdsfjärden receives discharge from the regulated River Delångersån with an annual mean runoff of 10 m³s⁻¹. The estuary also receives a discharge of about 1 m³s⁻¹ from the pulp and paper mill Iggesund, which has an industrial history dating back to AD 1685. The industry started out as an ironworks, an activity that continued until the end of the 19th century when it expanded to include a sawmill, and after the World War II it turned strictly into timber industry. The water

discharge from the mill supplies the estuary with three times the annual natural input of phosphorus and nitrogen (Nilsson & Jansson, 2002). The maximum discharge from the mill occurred in the 1960s and has since then drastically been reduced as an effect of technical improvements in the industry (Nilsson *et al.* 2003). Water discharge data from the paper mill is available from 1940 up to the present day and the water quality in Gårdsfjärden has been monitored since 1969. From the start water monitoring concentrated on oxygen content, turbidity, colour and COD (chemical oxygen demand). Time series of nutrient concentrations date back to 1980 with 6 yearly measurements. The yearly mean nutrient concentrations between 1990 and 2001 calculated from the integrated water depth between the surface and ten metres was $23.2 \mu\text{gL}^{-1}$ for total phosphorus ($17.3\text{--}34.3 \mu\text{gL}^{-1}$) and $337 \mu\text{gL}^{-1}$ for total nitrogen ($253\text{--}428 \mu\text{gL}^{-1}$).

5.4.2 Sampling and dating

A sampling site was selected in Gårdsfjärden, Bothnian Sea after thorough investigation of the bottoms by a low frequency eco sounder (14 kHz) and side scan sonar (500 kHz) from R/V Sunbeam in September 2002. There was accumulation of sediment on c. 56% of the bottoms in Gårdsfjärden and c. 28% of the sediment was gas charged (Jonsson 2002). The bathymetry surrounding the sample site was relative smooth and flat and only the areas close to the shore were relatively steep (figure 5.6).

Sediment coring was performed at a water depth of 12 m in the centre of the embayment with a Gemini gravity corer ($\varnothing$ 8 cm), resulting in the recovery of two parallel 55 cm long sediment cores, with the top 35 cm to a large extent laminated. One core was sub sampled directly in the field (uppermost 10 cm every cm, 10–20 cm every other cm, 20–55 cm every 5th cm) and put in a refrigerator, and the other transported to the laboratory for description. The lithology of the core was; 0–14.1 cm laminated olivegreen/darkgrey clay gyttja, 14.1–19.1 cm diffuse laminated clay gyttja, 19.1–23.9 cm dark laminated clay-gyttja, 23.9–26.8 cm dark homogeneous clay-gyttja, 26.8–30.7 cm laminated clay-gyttja, 30.7–35.5 cm diffuse laminated clay gyttja, 35.5–39 cm homogeneous clay-gyttja, 39–54.5 cm light homogeneous gyttja-clay. Dredging in the threshold area 1991, 1992 and 1994 resulted in light clayey layers in the sediment core, which can be used as marker horizons (Jonsson 2002).

Measurements of ^{137}Cs were carried out and used as a marker horizon indicating the outburst of radioactivity from the Chernobyl power plant in 1986 (figure 5.7). The investigated area is situated in the direct plume of the radioactivity fallout and there was no delay in the signal recorded in the sediment as seen in other parts of the Baltic Sea. The laminations in the top 35.5 cm of the sediment core were counted and the thickness varied between 10–23 mm in the uppermost part to 2–3 mm in the lowermost.

The ^{137}Cs indicated that the laminations were annually formed and could be used to construct an age model. Assuming that the sediment accumulation rate in the lowermost laminated part, where laminae displayed a thickness of 3 mm, had been similar from the bottom of the core, the age model could be extended back to approximately 1870.

Figure 5.7 Graph showing measurements of ^{137}Cs in the sediment core from Gårdsfjärden. The peak between 18-17 cm indicates the radioactive fallout from the Chernobyl power plant in 1986.

5.4.3 Diatom analyses

Twelve levels were counted for their content of diatoms and a total of 206 different taxa were identified. The diatom assemblage is a mixture of brackish, brackish-freshwater and freshwater taxa with both planktonic and periphytic life-forms throughout the core. Diatoms with an abundance of more than 2% in at least one stratigraphical level are displayed in figure 5.8 and diatom life forms are displayed in figure 5.9.

Figure 5.8 Diatom stratigraphy and lithology of core IGG 2 from Gårdsfjärden, Bothnian Sea showing relative abundance of species occurring at 2% in at least one level. Taxa are grouped into salinity requirements and sorted in order of first appearance. An age model has been constructed from laminae counting and ^{137}Cs measurements.

The diatom stratigraphy can be divided into 3 local diatom assemblage zones using visual examination of changes in the data as well as a cluster analysis (CONISS in the Tilia program). There are some clear trends in the diatom stratigraphy visible as shifts in life forms and salinity requirements.

Zone I-1 (54.5-32 cm) is dominated by brackish water taxa e.g. *Rhoicosphenia curvata*, *Martynia atomus*, *M. schultzei* and the *Fragilaria elliptica* aggregate as well as some brackish-freshwater taxa e.g. *Mastogloia smithii*, *Amphora pediculus* and *Epithemia sorex*. All these taxa are typically found all over Baltic Sea coastal waters. The zone has a high proportion of periphytic taxa (more than 80% in the lowermost 4 samples) of which around 20% are epiphytic. The zone also attains the highest species richness with 84 taxa.

In zone I-2 (32-12.5 cm) the proportion of planktonic taxa increases to a maximum value of 54% and it is dominated by 60-70% of freshwater taxa e.g. *Aulacoseira subarctica*, *A. ambigua*, *A. granulata*, *Cyclotella radiosa* and *Tabellaria flocculosa*. There is a low abundance of periphytic taxa in the zone, especially epiphytic taxa, which occur at less than 10 % at some levels. Species richness attains lower numbers than in the previous zone, but is still fairly high compared to the other sites in this study.

In the uppermost zone I-3 (12.5-2 cm) the abundance of freshwater taxa decreases and some new brackish water taxa e.g. *Thalassiosira levanteri*, *Pauliella taeniata*, *Diatoma moniliformis* and brackish-freshwater taxa e.g. *Thalassiosira baltica*, *Cyclotella meneghiniana* appear.

Figure 5.9 Graphs from Gårdsfjärden estuary (core IGG 2) plotted on an age-scale showing diatom life-forms (benthic, epiphytic and planktonic taxa), diatom DCA axis 1 scores, diatom species richness, rate of change in the diatom data, reconstructed diatom-inferred TN, measured TN in the Gårdsfjärden estuary and analogue quality (pair wise squared chord distance in the training set). Zonation of the diatom species data is shown as diatom assemblage zones (DAZ) to the right.

5.4.4 Nutrient reconstruction and environmental interpretation

The diatom inferred total nitrogen fluctuates between 306 and 264 $\mu\text{g L}^{-1}$ in the lowermost part of the core (figure 5.9). Between 1935 and 1950 TN starts to increase and reaches ca 350–365 $\mu\text{g L}^{-1}$ between 1950 and 1980. A maximum value of ca 400 $\mu\text{g L}^{-1}$ is reached in 1990 and from here on there is a decreasing trend visible down to 357 $\mu\text{g L}^{-1}$ in the uppermost sample representing 2001. This value is comparable with 342 $\mu\text{g L}^{-1}$, which is the monitored yearly mean TN of Gårdsfjärden (calculated as a mean of 5 years between 1997 and 2001).

The fossil diatom assemblages found in the Gårdsfjärden sediment core displays fairly good analogues with the training set from the surrounding areas. To get a measurement of the analogue quality the dissimilarity (squared chord distance) between the fossil and the modern assemblages were calculated for all stratigraphical levels. A 5th percentile of distance is conventionally taken as the delimitation of a good analogue and this condition was met in the lowermost part of the core (figure 5.9). The upper part of the core was with one exception within the 10th percentile of distance indicating fairly good analogues.

The trend in the reconstructed TN-values agrees with the history of the water discharge from the paper and pulp mill reaching maximum impact during the high discharge between 1945 and 1990 (Nilsson *et al.* 2003). The decrease in the reconstructed total nitrogen during the last decade agrees both with the measured TN in Gårdsfjärden and the fact that technical improvements of the paper industry have decreased the discharge from the mill (Nilsson *et al.* 2003).

The reconstructed TN-values in the lowermost part of the core could be considered as a background level for the Gårdsfjärden estuary even though one should keep in mind the very long industrial history of the paper mill Iggesund, which dates back to AD 1685. Several stratigraphical evidences points towards a good ecological status before 1920 with an assemblage totally dominated by benthic and epiphytic taxa indicating excellent water transparency, high species richness and less organic sedimentation resulting in homogeneous well oxygenated sediments. A change in the diatom assemblage starts between 1920 and 1935 where the species richness declines and the proportion of planktonic taxa increases clearly from < 20% to > 50 % at the expense of benthic and epiphytic taxa. This change occurs slightly before the increase in the reconstructed TN-values and can be interpreted in terms of less water exchange between the Bothnian Bay and Gårdsfjärden estuary visible as a decrease in brackish taxa and an increase in freshwater taxa (figure 5.8). Increased organic carbon sedimentation after 1920 lead to deteriorated oxygenation of the bottom water, which resulted in laminated clay-gyttja sediments. This supports an interpretation of a weakened ecological status up core with an assemblage dominated by fewer and mainly planktonic species. The extensive dredging of the threshold area in 1994 (Jonsson 2002) seems to have had a direct impact on the diatom composition in the estuary visible as an increase in brackish water taxa, especially the planktonic *Thalassiosira levanderi*, but also as minimal species richness.

Monitoring data provide an opportunity to validate the DI-TN reconstruction in Gårdsfjärden. When comparing the diatom inferred TN values with the measured TN from the monitoring program it is clear that the model has difficulties to reconstruct the high values measured in the 1980s. In this part of the core, however, there is a decrease in the analogue quality.

5.5 Arkona Basin and Oder Rinne

5.5.1 Site description

The area investigated is situated in the southwestern Baltic Sea and comprises the Arkona Basin and Oder Rinne (figure 5.10). The surface water in the Baltic proper is separated from the deep water by a halocline, which varies between 30 and 40 m in the Arkona Basin (Voipio 1981). The surface water in the investigated area has a salinity of c. 8 PSU. The Oder Rinne station is situated in the direct plume of the outflowing water from the River Oder, which carries most of the sediment load accumulating in the Arkona Basin (Neumann *et al.* 1996). Satellite observations show that the Oder outflow depends on the wind direction and is directed northwest towards the Arkona Basin during springtime when the river

load is highest (Siegel *et al.* 1994). Outside the Oder estuary no organic sediments are deposited and the sea bottom between the Arkona and Bornholm Basins consists of sand (Emelyanov *et al.* 1995).

5.5.2 Sampling and dating

Sediment cores from the area investigated were retrieved from R/V *Alkor* in March 1993 at a water depth of 45 m in the Arkona Basin (18017) and 20 m in Oder Rinne (18025). The sampling device used was a Rumohrrot, a 1-m long gravity corer (Ø 74 mm). The sediment cores obtained were 60 cm long. The cores were sub sampled with an interval varying between 1 and 5 cm, the younger top parts of the cores more closely than further down. The sediments collected consisted of silty-clay, gyttja-clay and clay-gyttja with an organic carbon content ranging between 1 to 8 % (Andrén *et al.* 1999). Core 18017 from the Arkona Basin shows homogeneous very fine-grained sediment with an organic carbon content of 4-7.5 %. The sediment in core 18025 from the Oder Rinne has an organic carbon content ranging from 1 to 3 % in the lower part and 3 to 7.5 % in the upper 7 cm. The sediment was homogeneous with a high silt content.

Dating of the sediments was carried out on parallel cores, using ^{210}Pb at the University of Edinburgh, UK. The methodology and results are presented in Brand & Shimmield (1991) and Andrén *et al.* (1999), respectively.

Figure 5.10 Map of the southern Baltic Sea with the coring sites in the Arkona Basin 18017 (54°48.71'N; 13°40.33'E) and Oder Rinne 18025 (54°29.53'N; 13°43.02'E). Bathymetric isolines of 10, 15, 20, 25 and 40 m are indicated. The arrow shows the major outflow from the River Oder.

5.5.3 Diatom analyses

Fifteen levels were counted in core 18017 from the Arkona Basin and 21 levels in the Oder Rinne core 18025. The complete diatom stratigraphies can be found in Andrén *et al.* (1999) and only the top part covering the last ca. 130 years and taxa with an abundance of more than 3% in at least one level are presented in this report. Both cores are totally dominated by brackish-marine, brackish and brackish-freshwater taxa with only a minor freshwater taxa component.

The Arkona Basin core is divided into two local diatom assemblage zones (figures 5.11 and 5.12) described as follows:

AB-1 (20-12 cm) is dominated by the tychoplanktonic species *Paralia sulcata* and has a maximum abundance of the benthic taxa *Diploneis didyma* and *Dimeregramma minor*. The planktonic species *Actinocyclus octonarius* attains over 10% in this zone. The zone is totally dominated by periphytic taxa, which reach a maximum abundance of ca 80%.

The uppermost zone AB-2 (12-0 cm) shows an increased abundance of planktonic taxa reaching ca 50% with a maximum of *Actinocyclus octonarius*, *Thalassiosira hyperborea* var. *lacunosa*, *Thalassiosira* spp., *Cyclotella choctawhatcheeana* and *Coscinodiscus granii*. The brackish-freshwater taxon *Pseudostaurosira brevistriata* and the freshwater taxon *Amphora copulata* also increase in this zone whilst *Paralia sulcata* decreases towards the top of core. Species richness varies between 58 and 65 throughout the core.

Figure 5.11 Diatom stratigraphy and lithology of core 18017 from Arkona Basin, south-western Baltic Sea showing relative abundance of species occurring at 3% in at least one level. Taxa are grouped into salinity requirements and sorted in order of first appearance. An age model has been constructed from ^{210}Pb measurements (Andrén *et al.* 1999).

Figure 5.12 Graphs from the Arkona Basin (core 18017) plotted on an age-scale showing diatom life-forms (benthic, epiphytic and planktonic taxa), diatom DCA axis 1 scores, diatom species richness, rate of change in the diatom data, reconstructed diatom-inferred TN and analogue quality (pair wise squared chord distance in the training set). Zonation of the diatom species data is shown as diatom assemblage zones (DAZ) to the right.

The Oder Rinne core is separated into two local diatom assemblage zones (figures 5.13 and 5.14) described as follows:

The lowermost zone OR-1 (14-10.5 cm) is dominated by the planktonic taxa *Thalassiosira baltica* and also has a maximum abundance of the planktonic *Pauliella taeniata* together with the benthic *Diploneis didyma* and the freshwater taxon *Amphora copulata*. The lowermost sample has a high abundance of periphytic taxa (ca 60%) shifting to a dominance of planktonic taxa further up in this zone. Species richness varies between 59 and 46 taxa and reaches the highest value simultaneously with the maximum abundance of periphytic taxa.

The topmost zone OR-2 (10.5-0 cm) is totally dominated by the planktonic *Cyclotella choctawhatcheeana* together with *Thalassiosira levanteri*. Some of the other planktonic taxa also have their maximum occurrence in this zone; *Thalassiosira* cf. *angulata* and *Coscinodiscus granii*. The abundance of periphytic taxa is low reaching values between 15 and 30%. Species richness is slightly lower in this upper zone and reaches a minimum value of 33 concurrent with a peak in planktonic taxa.

Figure 5.13 Diatom stratigraphy and lithology of core 18025 from Oder Rinne, south-western Baltic Sea showing relative abundance of species occurring at 3% in at least one level. Taxa are grouped into salinity requirements and sorted in order of first appearance. An age model has been constructed from ^{210}Pb measurements (Andrén et al. 1999).

Figure 5.14 Graphs from Oder Rinne (core 18025) plotted on an age-scale showing diatom life-forms (benthic, epiphytic and planktonic taxa), diatom DCA axis 1 scores, diatom species richness, rate of change in the diatom data, reconstructed diatom-inferred TN and analogue quality (pair wise squared chord distance in the training set). Zonation of the diatom species data is shown as diatom assemblage zones (DAZ) to the right.

5.5.4 Nutrient reconstruction and environmental interpretation

In the core from Arkona Basin the diatom-inferred total nitrogen fluctuates between 325 and 450 $\mu\text{g L}^{-1}$, but judging from the analogue quality (figure 5.12) no level in the core is even close to a chord distance less than the 10th percentile and the reconstruction could not be considered reliable. This is probably due to the lack of high percentages of the domi-

nating core taxa *Paralia sulcata*, *Actinocyclus octonarius* and *Coscinodiscus granii* in the training set.

In the sediment core from Oder Rinne the diatom inferred total nitrogen starts with a value around 400 to 420 $\mu\text{g L}^{-1}$ in the lowermost 3 levels between ca 1860 and 1920 (figure 5.14). Thereafter an increasing trend to a maximum value of 500 $\mu\text{g L}^{-1}$ in 1960 is visible. Following this maximum a first slight decrease to c. 485 $\mu\text{g L}^{-1}$ around 1975 is detected and followed by a rapid decrease in 1980 back to about the same values of 400 to 420 $\mu\text{g L}^{-1}$ as in the lowermost part of the core.

The historical trend of the diatom-inferred total nitrogen cannot be validated with monitoring data, but the uppermost reconstructed TN-value of 415 $\mu\text{g L}^{-1}$ representing 1991, can be compared with 304 $\mu\text{g L}^{-1}$, which is the present yearly mean TN of a monitoring station situated close to the Oder Rinne sediment core.

The analogue quality of the taxa found in the core can with the exception of the two lowermost samples and the uppermost sample, be considered as good with a majority of the samples having a chord distance less than the 5th percentile. The reconstruction seems to be driven mainly by *Cyclotella choctawhatcheeana* and *Thalassiosira levanderi* (figure 5.13). The dominating taxa in the two lowermost levels are *Thalassiosira baltica* attaining more than 40% in the core but only found at abundances up to 22% in the training set, *Diploneis didyma* (15% in the core, max 2% in the training set), and *Amphora copulata* (10% in the core, max 2.8% in the training set) leading to an unreliable TN reconstruction before 1920. The main changes in the diatom stratigraphy are captured in the DCA axis 1 scores and occur between diatom assemblage zone OR-1 and OR-2 which dates to around 1900. The overall changes in the diatom stratigraphy are more thoroughly discussed in Andrén *et al.* (1999).

5.6 Discussion

5.6.1 Background conditions contra overall trends in the stratigraphic data

The aim of the DEFINE project was to define background conditions for nutrients in the Baltic Sea coastal zone, which could be used within the EU Water Framework Directive (WFD) to characterize a good ecological status of coastal waters. With this scope there is no actual need to identify the causes of deterioration and departure from the background conditions, but a discussion on this subject will benefit the interpretation and clarify whether undisturbed conditions actually are met. A changed nutrient supply could have various natural causes as for example land uplift, which affects the water exchange over a shallow threshold, or climatological changes with increased temperature and runoff that create a faster rate of

nutrient turnover in the system (e.g. HELCOM 2007). Eutrophication in the coastal zone is, however, most commonly associated with human population growth in the Baltic Sea drainage area, changed agricultural practice (e.g. use of artificial fertilizers and ditching of wetlands), airborne deposition of especially nitrogen due to fossil fuel combustion and industrial point sources, which increase the amount of nutrients added to the system (e.g. Bonsdorff *et al.* 1997; Jansson & Dahlberg 1999; Elmgren 2001).

According to the WFD background levels are achieved in undisturbed conditions equalling a high ecological status (Anonymous 2000). To classify the ecological status the WFD uses biological-, hydromorphological- and physico-chemical quality elements. Phytoplankton is one of the biological quality elements used: the composition and abundance of taxa together with the intensity and frequency of blooms must be consistent with undisturbed conditions for a system to achieve a high ecological status. To be classified as having a good ecological status, a system is allowed to show slight signs of disturbances. In this study we are not using the species composition itself to define the background conditions, but the reconstructed total nitrogen concentrations defined in the WFD physico-chemical quality elements, where nutrient concentrations should remain within the range normally associated with undisturbed conditions (Anonymous 2000). We also use some diatom community indices like the percent of planktonics and species richness to strengthen our interpretation.

Background conditions could be defined in the Gårdsfjärden estuary using the diatom-inferred total nitrogen value of c. $270 \mu\text{g L}^{-1}$ recorded until 1920. This could be considered a physico-chemical quality element associated with a pristine environment, which is supported by biological quality elements of phytoplankton showing low biomass (low organic sedimentation) and a species composition consistent with undisturbed conditions, giving a classification of high ecological status. In the Oder Rinne in the southern Baltic proper, background conditions are possibly met until 1920 with a reconstructed total nitrogen value of c. $400 \mu\text{g L}^{-1}$. It is, however, uncertain if the reconstructed TN would have been even lower in the two lowermost samples if the analogue quality of the training set had been better. A physico-chemical quality element classifying this value as a good ecological status is supported by biological quality elements of phytoplankton showing low biomass but slight signs of disturbances in species composition. In the Saunja Bay and Arkona Basin background conditions could not be defined.

Even if not all of the sediment cores in this study turned out to be suitable for reconstructions of background concentrations of total nitrogen in the Baltic Sea coastal zone due to a lack of good analogues, there are still some overall useful shifts in the stratigraphical data visible in the sediment cores. There is a clear shift from a total dominance of periphytic

taxa in the lowermost part of the cores to an assemblage more or less dominated by plankton (except in the very shallow Saunja Bay where a slight increase of plankton occurs). Similar results have been seen in diatom stratigraphies from Chesapeake Bay (Cooper 1993), the south-western Baltic Sea (Andrén *et al.* 1999) and Finnish coastal waters (Weckström *et al.* 2004; Weckström 2006) and are interpreted as a result of increased nutrient input. In particular, epiphytic taxa, which depend upon submerged vegetation, decrease in the shallowest sites in Saunja Bay and Gårdsfjärden, a trend also recorded in the Danish estuary Mariager fjord (Ellegaard *et al.* 2006). This result could be compared with a study of the phytal zone in the Greifswald Bay, which decreased from 90% in 1938 to about 10% in 1985 and was interpreted as a deterioration of light conditions due to increased plankton production caused by eutrophication (Messner & von Oertzen 1990). Similar results have also been recorded from the Bothnian Bay with decreased depth penetration of bladder wrack (Kautsky *et al.* 1986) and are considered to be a widespread consequence of coastal eutrophication (Bonsdorff *et al.* 1997). Further, a decreasing trend in species richness is recorded in Saunja Bay, Gårdsfjärden estuary and Oder Rinne, which also could be seen in Danish (Clarke *et al.* 2006; Ellegaard *et al.* 2006) and Finnish coastal waters (Weckström 2006). Increased organic carbon sedimentation is recorded in Saunja Bay, Gårdsfjärden estuary and Oder Rinne indicating a primary production growth resulting in anoxia and laminated sediments in the Gårdsfjärden estuary. This phenomenon has previously been recorded in Swedish coastal waters (Persson & Jonsson 2000) and in the open Baltic Sea as an effect of increased primary production (Jonsson & Carman 1994).

All these changes recorded as increased production and organic sedimentation as well as species shifts are not occurring concurrently at all investigated sites, which indicates that marine eutrophication works on both local and regional scales. Furthermore, the morphology of the coastal basins affecting the water exchange will influence their susceptibility to changes even if the pollution pressures were equal.

5.6.2 Methodological strengths and weaknesses

The advantage of using the transfer function approach is that it provides an independent robust signal of the environmental development linked to species compositions instead of using interpretations of indicator species and, primarily, it is a quantitative method, which gives an actual number of the reconstructed parameter. A more complete interpretation of the diatom-inferred nutrient concentrations could be attained if they are supported by other sediment proxies, e.g. lithology, organic carbon content, stable isotope data, fossil pigments, and diatom community indices such as diversity and ratio planktonic/periphytic diatoms.

Paleoecological studies have drawbacks. These include the difficulty in locating a suitable sediment archive and problems with dating giving erroneous age models (see chapter 6). Other methodological weaknesses could be resuspension leading to transportation and re-accumulation of sediment, bioturbation (both disturbing the historical archive as well as the chronology), and differential dissolution of the diatom valves. A laminated stratigraphy consisting of annually formed varves gives the most exact chronology compared to ^{210}Pb -dating, especially when using extrapolated mean accumulation rates.

Essential for the performance of the inference model is that the training set contains the same species composition in similar quantities as found in the stratigraphy i.e. that we have good analogues of the fossil assemblages in our present data. This condition was not met in the sediment cores from the Arkona Basin and Saunja Bay. Several cores from the Oder estuary and Greifswalder Bodden considered for this study (Andrén 1999; Andrén *et al.* 1999) could not be used for nutrient reconstruction as their diatom stratigraphies were dominated by taxa that were not present in the training set with sufficient frequency to constrain their optima. In the nutrient reconstruction from Gårdsfjärden the transfer function underestimates the TN-values around 1980 when comparing with monitoring data from the same period. This could be considered an analogue problem as systematic underestimates of actual TN concentrations most probably are due to lack of very nutrient rich sites in the training set comparable to the most apparent eutrophic periods in the past (Weckström 2006). The reconstructed TN in Saunja Bay indicates a decrease towards a minimum value around the mid 19th century, which contradicts the stratigraphical evidence of increased biological production and altered diatom structure indicative of increased nutrient discharge (figure 5.4). This illustrates the significance of reliable modern analogues and the importance to use knowledge about the environmental setting and diatom community indices to interpret diatom-inferred nutrient reconstructions (Weckström 2006). Further, Saunja Bay is dominated by benthic *Fragilaria* taxa, opportunistic species with a wide ecological range, which have proved to be poor as indicators of environmental change but possibly could be used as a first indication of anthropogenic disturbance (e.g. Weckström 2006). Mass abundances of *Fragilaria* are according to Denys (1990) associated with environmental instability, a useful quality when studying shoreline displacement as they can adjust to rapid shifts in salinity following a transgression/regression (Stabell 1985; Yu *et al.* 2004).

5.6.3 Climate impact and future changes

A statistical evaluation of a 21-year long time-series of phytoplankton monitoring data from the open Baltic Sea within HELCOM reveals some

significant changes such as a reduction in the diatom spring blooms and an increase in diatom biomass in the autumn (Wasmund & Uhlig 2003). These changes could be attributed to an increased water temperature during the last decades (HELCOM 2007) as during mild winters the water column remains stratified replacing diatom spring blooms with dinoflagellates (Fennel 1999; Wasmund & Uhlig 2003). In addition to climatic reasons, the reduction in diatom spring blooms could also be caused by increased silica limitation (due to eutrophication) in the Baltic Sea. The most intense nutrient discharge to the Baltic Sea has occurred during the last century, simultaneously with a warming trend of about 0.08°C per decade between 1860 and 2000 (HELCOM 2007). Climate variability acts on centennial and decadal time scales and overlaps with increased human impact in the Baltic drainage basin, which makes it difficult to separate natural climate variability and anthropogenic climate change from other human influences such as increased nutrient discharge (BACC Lead Author Group 2006). The biostratigraphical changes seen in the Saunja Bay and Gårdsfjärden estuary which indicate an alteration in the nutrient discharge and the exchange of sea water might have been enhanced by climatic effects of increased precipitation and temperature, as well as influenced by increased anthropogenic nutrient load.

A secondary objective of this study was to try to separate the effects of climate on the diatom community from the effects caused by anthropogenic nutrient enrichment. The methodology for this approach was to use variance partitioning to partial out the climate signal in the diatom data from the variance explained by nutrients, and was depending on the appearance of taxa responding to ice cover in the training set. Unfortunately the abundance of ice-associated taxa (e.g. *Pauliella taeniata*, *Fragilariopsis cylindrus* and *Melosira arctica*) was far too low. In Oder Rinne, apart from the increasing amount of planktonic taxa up core, which could be interpreted in terms of increased nutrient discharge, there are also changes in ice-related taxa (*Pauliella taeniata*, *Thalassiosira baltica*). This is possibly reflecting climatically driven changes in the assemblage caused by a decrease in ice cover following the warming after the Little Ice Age (Andrén *et al.* 1999). The length of the ice season in the Baltic area has decreased by 14 to 44 days over the past century (HELCOM 2007). In the Pärnu Bay, southern Estonia, the variance in annual phytoplankton development was better explained by large-scale climatic pattern (exemplified by the North Atlantic Oscillation index) than by local environmental variables such as river discharge, salinity and temperature (Kotta *et al.* 2004).

A strengthening of the zonal circulation in the North Atlantic area is suggested to happen in the next 100 years as an effect of global warming. This would lead to a decreased salinity of the Baltic Sea as a result of increased runoff and to increased oxygen concentrations due to decreased stratification (Zorita & Laine 2000). A divergent development between

different parts of the Baltic Sea shows a clearer change in the northern part, which would receive most of the precipitation and increased river flow compared to the southern Baltic, which will receive a decreased river discharge; almost no change in salinity and oxygen is modelled for the Gotland Basin (Graham 2004; Zorita & Laine 2000). This scenario would change the nutrient balance of the Baltic Sea as most of the rural landscapes are situated in the southern part of the drainage basin leading to a decrease in anthropogenic input of nutrients in the southern Baltic proper. However, increased winter runoff and warmer winter temperatures could have a negative effect on the nutrient balance of the Baltic Sea as a result of increased discharge of latent nutrients in agricultural areas (Graham 2004). There is little doubt that the future warming expected for the next century (IPCC 2007) with an altered meteorological setting will create changed conditions for the Baltic Sea ecosystem at all scales, but it is hitherto not feasible to isolate the predominant process.

6. Application – a management tool

6.1 The palaeolimnological approach

In palaeolimnology, transfer functions describe the relationship between organisms and their environment with the ultimate goal to use the present-day ecology of organisms to infer past conditions. Transfer functions are established using the recent organism assemblages and measured environmental variables from a *calibration data set*. These calibration sets involve sampling a range of sites (e.g. lakes, estuaries) for a suite of environmental variables, which are then related to the biological indicators (e.g. diatoms) preserved in the surface sediments of the systems concerned using numerical techniques. The data collected in monitoring programs provide an ideal opportunity to construct calibration data sets.

The mathematical methods for establishing a transfer function are based on non-linear regression techniques. The most popular approaches in palaeolimnology are the weighted averaging (WA) and the weighted average partial least squares (WA-PLS) methods. After estimating environmental optima and tolerances of indicator taxa using the most appropriate modeling scheme, the transfer function is applied to organism data from a sediment core to quantitatively reconstruct the parameter values of the key environmental variable(s) affecting the distribution of the organisms in the modern reference data set.

Palaeolimnological approaches are now being applied to a wide array of eutrophication-related problems. Experiences from lake environments are encouraging. Although nutrient transfer functions are not as strong as those originally developed for lakes, i.e. lake-water pH, they are sufficiently robust to infer long-term trends in the directions and magnitude of eutrophication. In a few cases where long-term monitoring data have been available, comparisons between diatom-inferred and lake-water nutrient levels have shown relatively good correspondences (e.g. Bennion *et al.* 1996; Lotter *et al.* 1998; Bradshaw & Anderson 2001).

Palaeolimnological studies on estuaries have much more rarely been attempted, as they typically have complex and variable sedimentation patterns in space and time, and resuspension of sediments can be a serious problem. According to the results achieved during the MOLTEN project, transfer function approaches based primarily on diatom compositional data can be used to track cultural eutrophication patterns in sites where reliable sediment deposition zones have been identified (Clarke *et*

al. 2003; Weckström *et al.* 2004; Kauppila *et al.* 2005; Clarke *et al.* 2006; Ellegaard *et al.* 2006; Weckström 2006).

The following “management tool” should serve as a guide on how to use palaeolimnological methodology, in particular diatom-based transfer functions, to determine reference conditions in coastal waters. The guide starts with a flow chart that summarizes and outlines the steps needed to carry out a palaeolimnological study in an estuary and is followed by in-depth information regarding each step in the process (figure 6.1). The first section summarizes the palaeolimnological approach and then detailed methodologies are presented for site selection, sediment coring and dating. The next section is a step-by-step guide to hind-casting total nitrogen concentrations. This section includes information on taxonomic harmonization using the MOLTEN/DEFINE diatom database. The report ends with methods to evaluate the reconstruction of nutrient concentrations and suggestions on how to use calibration data sets in contemporary monitoring programmes.

6.2 Site selection

Level 1 – Study Area

A successful project requires careful evaluation of all possible sites prior to the selection of the final study site. After the definition of the project aims and problem formulation, the proposed study site should be chosen after an evaluation of all available data both in terms of previous palaeolimnological studies, water quality monitoring data and land-use data (historical and contemporary classifications) where relevant (i.e. restricted embayments or estuarine sites with extensive land-water interfaces). Where there is a range of monitoring and documentary information available for a given location, preference should be given to this location as these data can facilitate interpretation of the sediment core data. These requirements may have to be modified by considerations about the sedimentation environment (see below).

Level 2 – Core Location

After the location has been selected, possible coring sites should be identified on the basis of available bathymetric charts. It is generally advisable to core in the deepest part of the basin as sediment accumulation rates are often highest there, wind-induced resuspension will be minimized and seasonal anoxia (if the basin is deep enough to stratify) can reduce bioturbation by benthic invertebrates. Both resuspension and bioturbation can cause substantial problems for ^{210}Pb dating (see below).

Figure 6.1 Flow chart for a coastal palaeoecological study.

6.3 Sediment coring

There are a variety of sediment coring devices available for use in shallow coastal waters, most of which were developed for lakes. Most of these corers (e.g. Haps corer, Kajak, etc.) will readily recover sufficient sediment for the majority of projects that are undertaken as part of a contemporary water quality management program. There are, however, sites where sediment accumulation rates are so high (> 2 cm/yr) that a 1-m sediment core will not reach pre-disturbance conditions (i.e. pre-20th century). Unfortunately, this is generally not known until preliminary dating is completed, hence the importance of checking for previous studies and

available data. In these situations alternative techniques will be required (e.g. large Kullenberg corers), many of which will destroy (blow away) the uppermost, more flocculent layer of sediment during penetration. In these situations a surface core that overlaps with the underlying core is required; this can often be correlated by means of sedimentary carbon profiles. It is vitally important that the uppermost sediment layer is retrieved, both to provide a sediment record that covers the whole time span of interest (i.e. up to the present) but also because its loss can lead to errors in the ^{210}Pb chronology (due to an incomplete ^{210}Pb inventory – see below).

After retrieval, cores should be stored in an upright fashion (where appropriate), kept cool (ca. 4°C) and in the dark. Cores should be sectioned and sub-sampled as soon as possible. It is standard practice to section sediment cores at 1-cm intervals, although, as the chronology is rarely known in advance (unless the site is being re-visited), this can prove to be either too coarse or too fine, depending on the sediment accumulation rate. After the core has been sub-sectioned, the individual core slices should be divided into three subsamples for lithostratigraphic analysis, diatom analysis and dating.

Basic parameters such as bulk density (percentage dry weight) and loss-on-ignition at 550°C (or carbon and nitrogen content via an elemental analyzer) are important and should be undertaken on every sample. These data are important for core correlation, both of a long core to a surface core, and of replicate cores. The sediment section for dating should then be dried (preferably freeze-dried) while the sub-samples for diatom analysis should be kept cool and dark. ^{210}Pb (by gamma counting) is non-destructive and so some analyses can be undertaken on this sediment sample after dating analyses are complete. Hence these samples should be retained for archive use or re-analysis.

6.4 Dating

Dating forms an integral part of any palaeolimnological study. Although a variety of techniques can be used (e.g. pollen, carbonaceous particles – see below) the most widely used tool to date recent sediments (i.e. the last ~100-150 years) is ^{210}Pb . ^{210}Pb is a naturally occurring radioisotope from the ^{238}U decay chain with a half-life of 22.26 years. This effectively means that it can, under optimal conditions date sediments up to 150 years old, although beyond 100 years the error associated with a given age is often substantial. It is important to realize that ^{210}Pb chronologies are dependent on the dating model that is chosen. The underlying assumptions can result in large differences in the final chronology. Where the ^{210}Pb distribution in the sediment is optimal (a log-linear decline with increasing sediment depth) the models will give essentially the same re-

sult. Where the ^{210}Pb distribution is non-uniform, as in many estuarine and near-coastal environments, the choice of model/approach is critical. In these situations, supplementary information from other dating methods is extremely useful. The main causes of non-uniform distributions are sediment mixing and variable sediment accumulation rates. ^{210}Pb is normally undertaken by specialist agencies/consultancies, which will provide help with deriving the ^{210}Pb chronology.

6.4.1 Problems with ^{210}Pb and dating estuarine and coastal marine sediments

The primary problem with the dating of estuarine and coastal marine sediments is that the energy input to the system (as wind, currents and tides) is considerably greater than that to lakes, with the result that resuspension of sediments is considerable. Resuspension results in non-uniform sediment distribution, erosion and mixing of the uppermost, unconsolidated sediments. The former can create hiatuses while the latter temporally smooths and destroys the resolution of the palaeolimnological record as well as affecting the ^{210}Pb distribution.

Mixing by resuspension or benthic invertebrates is a major problem affecting ^{210}Pb in marine environments, but a secondary problem is that of non-uniform ^{210}Pb distribution due to variable sediment accumulation rates. With the substantial land-cover changes that have affected coastal margins and estuarine catchments, sediment inputs to coastal marine embayments have increased substantially. The constant rate of supply (CRS) model, developed for lakes (Appleby 2001), is relevant in these circumstances but has not been widely adopted by marine sedimentologists.

Dating is critical for the success of the project and given the problems associated with ^{210}Pb chronologies from cores derived from suboptimal locations, considerable effort should be spent on carefully selecting coring sites, both at the regional and within-basin scales.

6.4.2 Other dating techniques

There are a variety of other techniques that can be used to supplement ^{210}Pb dating of recent sediments. Most relevant is ^{137}Cs , which can be important in providing independent corroboration of the ^{210}Pb chronology. Spheroidal carbonaceous particles (SCPs), produced by oil and coal combustion can also be useful although, as with ^{137}Cs , they only provide a selected number of dates (for unambiguous features in the profile). Pollen can provide dates for selected horizons, e.g. the introduction of exotic plants and extensive conifer plantations in estuarine catchments. Where sedimentation rates are very low it may be useful to obtain ^{14}C dates for selected horizons beyond the range of ^{210}Pb . AMS dates are best under-

taken on terrestrial macrofossils, where available, to counter problems associated with marine reservoir effects.

6.5 A step-by-step guide

6.5.1 Hindcasting total nitrogen (TN) concentrations using the MOLTEN/DEFINE diatom-based transfer functions

This section gives a step-by-step guide to hindcasting TN concentrations from sediment-core diatom assemblages using transfer functions. The main project aim of DEFINE was to create transfer function(s) for defining background TN and/or TP concentrations in coastal areas of the Baltic Sea. Of these two nutrients, only TN had a significant relationship to the diatom assemblages, which is a key requirement for transfer function development. Hence we did not create transfer functions for TP.

The transfer function approach is a 3-step process. Firstly, the taxonomy of the sediment core diatom counts must be harmonized with that of the training set. Secondly, an appropriate transfer function is chosen and applied to the fossil diatom assemblages. Finally, the results of the hindcasting procedure must be carefully evaluated to assess the reliability of the nutrient reconstructions. A user guide that gives details on the practical application of the MOLTEN/DEFINE transfer functions is also available from the project website <http://craticula.ncl.ac.uk/Molten/jsp/index.jsp>. A review of the transfer function approach to hind-casting can be found in Birks (1995).

6.5.2 Taxonomic harmonization

The MOLTEN/DEFINE transfer functions model the relationships between the distribution and abundance of diatom taxa and the nutrient concentration of overlying waters. These models are generated using a training set of modern surface sediment samples and associated water chemistry data. Given these relationships, expressed as a series of model coefficients, the transfer function can be applied to sediment core assemblages to predict the TN concentration under which the fossil assemblage was deposited. Since the transfer functions rely on taxon-specific responses, it is crucial that exactly the same diatom nomenclature and identification criteria are used for both the modern training set and fossil diatom counts.

In practice the matching of training set and sediment core taxonomy is achieved by using the same taxon codes in both datasets. A complete list of MOLTEN/DEFINE diatom taxa and their codes is listed in the user guide and on the web site <http://craticula.ncl.ac.uk/Molten/jsp/index.jsp>. The website also contains taxonomic and distributional information for

all diatom taxa together with images to help with the correct identification of problematic species. Once harmonized and coded in this way the taxonomy of a sediment core dataset can be verified using the MOL-TEN/DEFINE transfer function software. This procedure compares species codes and abundances between sediment core and training datasets and reports any discrepancies.

6.5.3 The MOL-TEN/DEFINE diatom-based transfer functions

The transfer function approach is based on the implicit assumption that the modern training sets encapsulate the range of chemical and other environmental conditions likely to be represented by the sediment core material. To fulfill this need the MOL-TEN/DEFINE training sets have been chosen to cover a range of coastal environments and span the TN gradient from less than 200 to over 3000 $\mu\text{g l}^{-1}$. Statistical analysis of the training sets indicate that there are essentially two separate groups of samples showing regionally distinct species/environment relationships: a Western Baltic group comprising samples from Norway, Denmark, Germany and western Sweden and an Eastern Baltic group comprising samples from Latvia, Estonia, Finland, eastern Sweden and some sites from Germany. To this end we have merged the MOL-TEN/DEFINE regional datasets into the new Western and Eastern Baltic training sets and derived transfer functions for these training sets using the numerical technique of weighted-averaging partial least squares (WAPLS: ter Braak & Juggins 1993). Analysis also indicates that these new transfer functions perform better than original regional or a single Baltic combined transfer function. We therefore recommend that the Western Baltic training set be applied to cores collected from the Western Baltic and *visa versa*.

Once the core data taxonomy has been coded in accordance with the training sets the transfer function can be applied using MOL-TEN/DEFINE software. The output will be reconstructed TN concentrations for each core sample, sample-specific prediction errors, and diagnostic statistics to aid in the evaluation of the reconstruction.

6.6 Evaluation of the reconstruction

All MOL-TEN/DEFINE transfer functions will provide a quantitative TN reconstruction. An important part of the transfer function approach to hind-casting TN concentrations is a thorough evaluation of the reliability of the reconstructed values. This can be done in three ways:

1. Closest analogue analysis

Implicit in the transfer function approach is that the training set samples provide good analogues for the sediment diatom assemblages. This assumption can be tested first using the verification function described above to highlight fossil samples that contain significant numbers of taxa that are absent from the training set. A second more quantitative assessment can also be made using the MOLTEN/DEFINE software to find the closest modern analogues for each fossil sample based on the modern analogue technique (MAT: Birks 1995). MAT quantifies the dissimilarity between modern and fossil samples using dissimilarity measures such as squared chi-square distance.

2. Compare reconstructions using different training sets / transfer functions

In addition to the Western and Eastern Baltic WAPLS transfer functions we also provide WAPLS and locally-weighted weighted averaging (LWWA) transfer functions developed using the total combined Baltic dataset. We recommend that reconstructions also be performed using these transfer functions and the results compared. Given that some taxa have apparently different TN preferences in the different datasets we would expect the different transfer functions to produce different reconstructions. However, if down-core changes in diatom taxa are primarily driven by changes in TN concentrations then we would expect the three reconstructions to follow similar trajectories, even if they differed in the absolute values of the hind-cast TN concentrations. If the three methods produce widely divergent reconstructions this would suggest that the core contains key taxa whose distribution is not primarily related to TN, and as such, the reconstructions should be treated with caution.

3. Validation against historical TN concentration measurements

The most robust evaluation of hind-cast TN concentrations is to compare reconstructed values with historical time-series where these exist. Close agreement between hind-cast and measured TN increases confidence in the reconstruction whereas divergence between the two sets of data warrants caution. However, close agreement during the monitoring period does not necessarily imply accurate reconstructions for earlier periods, especially if assemblages have very different composition. Conversely, divergence for the monitoring period does not necessarily imply reconstructions for earlier periods are in error.

6.7 Use of the calibration data set in contemporary monitoring

Diatoms are presently not part of the quality elements of the Water Framework Directive in coastal waters, which may be due to a lack of in-

formation in management. Although they are indirectly included in the WFD as part of the phytoplankton, many diatom taxa can not be identified to species level in live plankton counts.

Diatom-nutrient calibration data sets like the MOLTEN/DEFINE data set can offer great potential for biomonitoring schemes. In addition to water chemistry sampling at coastal monitoring stations, surface sediment samples for analysing present-day diatom assemblages could also be collected. The sampling frequency of once a year is sufficient as such a sample would be both temporally and spatially integrative incorporating all diatom habitats over one to a few years (depending on the sediment accumulation rate of the site). Due to its integrative nature this approach would be particularly suitable for stations, which are sampled only 1-2 times a year (or less-frequently) for water quality parameters.

Optima for the individual diatom taxa found at each monitoring site can be obtained from the MOLTEN/DEFINE data set: the abundance-weighted average of all species' optima gives a good estimate of the nutrient (TN) status of a site with statistically reliable errors of prediction. The approach is essentially the same as when reconstructing TN concentrations from fossil down-core samples, only here the transfer functions are applied to the surface sediment sample for calculating present TN concentrations.

Diatoms could therefore be incorporated into the WFD as biological quality elements and applied using these techniques for water quality monitoring purposes in the coastal waters of the Baltic Sea area.

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Svensk sammanfattning

Förändringar i Östersjöns ekosystem, t.ex. omfattande blomningar av cyanobakterier, syrefria bottenar, minskad ljusgenomsläpplighet och en förändrad artsammansättning anses bero på ökande utsläpp av olika näringsämnen. Även om vi människor har levt i och påverkat Östersjöområdet under tusentals år är det främst sedan en markant förändring av markanvändningen och en ökad förbränning av fossila bränslen startade efter andra världskriget som effekterna av eutrofiering har blivit påtagliga. När mätningar av kustområdenas vattenkvalité började på 1970-talet var ekosystemet redan påverkat och de naturliga bakgrundsvärdena av näringsämnen är därför okända. Alla europeiska vatten inkluderade i den Europeiska unionen ska enligt dess vattendirektiv ha uppnått god ekologisk status med liten eller ingen avvikelse från ostörda förhållanden senast år 2015.

DEFINE-projektets målsättning är att konstruera transferfunktioner baserade på kiselalger (diatoméer) som kan användas av nationella myndigheter för att definiera referensvärden för koncentrationer av olika näringsämnen i Östersjöns och Skagerraks kustområden. En transferfunktion beskriver förhållandet mellan organismer och den omgivande miljön med syftet att använda organismernas nuvarande ekologi för att förstå tidigare miljöförhållanden.

I DEFINE-projektet används uppmätta miljövariabler från de nationella miljöövervakningsprogrammets vattenstationer tillsammans med analyser av kiselalger från ytsediment. Dessa data utgör ett kalibreringsdataset där 124 nya kustnära stationer från Bottenhavet (den svenska och finska kusten), Estland, Lettland, Tyskland och Norge undersökts. De nya stationerna täcker in en mycket lång miljögradient med vattendjup på 2 till 101 m, en salthaltsgradient från <0.1 till 24 psu och näringsstatus från oligotroft till eutroft. En "Principal Component Analysis" (PCA) av dessa miljödata visar att totalkväve och totalfosfor är korrelerade med varandra, vilket medför svårigheter att konstruera oberoende transferfunktioner för dessa två näringsämnen. Totalt 1081 kiselalgstaxa identifierades i ytsedimentproverna och majoriteten var bentiska. Totalkväve förklarar en liten, men statistiskt signifikant del av variationen i kiselalgsdata. Som transferfunktionsteknik valdes "Weighted Averaging Partial Least Square" och till följd av kalibreringsdatasetets heterogena sammansättning med avseende på salthalt, exponering och vattendjup delades det upp i mindre, mer homogena delar. Transferfunktionerna applicerades på fyra sedimentkärnor från olika delar av Östersjöns kust för att rekonstruera bakgrundsvärden för totalkväve. Grundläggande för modellens prestanda är att kalibreringsdatasetet innehåller samma arter som återfinns i sedi-

mentkärnornas stratigrafi, dvs. att det finns bra analogier hos den fossila artsammansättningen i dagens ytprover. Denna förutsättning uppfyllades inte i två av sedimentstratigrafierna och där kunde således bakgrundsvärden inte rekonstrueras. I Gårdsfjärden, Bottenhavet, rekonstruerades totalkvävehalten till $270 \mu\text{g L}^{-1}$ runt 1920, vilket bör kunna betraktas som platsens bakgrundsvärde. I Oder Rinne, sydvästra Östersjön rekonstruerades totalkvävehalten till $400 \mu\text{g L}^{-1}$ från samma tidpunkt.

Alla transferfunktioner och den understödjande dokumentationen är tillgänglig, som ett förvaltningsverktyg som kan användas i arbetet med EU:s vattendirektiv, och åtkomligt via DEFINE-projektets hemsida: <http://craticula.ncl.ac.uk/Molten/jsp>. På hemsidan hänvisas även till mer ingående detaljer om kalibreringsdatasetet som t.ex. kiselalgernas areella utbredning samt enskilda arter plottade mot olika miljövariabler. Kiselalger är idag som fytoplankton endast en indirekt del av de kvalitétskomponenter som används i EU:s vattendirektiv, men borde i framtiden inkluderas och användas för övervakning av Östersjöområdets vattenmiljö.

List of DEFINE partners in alphabetical order

Juris Aigars
Department of Marine Monitoring
Latvian Institute of Aquatic Ecology
Daugavgrīvas, 6, Rīga LV-1007, Latvia
E-mail: juris@monit.lu.lv

Elinor Andrén
School of Life Sciences
Södertörn University College
SE-141 89 Huddinge, Sweden
E-mail: elinor.andren@sh.se

Annemarie Clarke
APEM Ltd
Riverview
A17 Embankment Business Park
Heaton Mersey, Stockport, GB-SK4 3GN
UK
E-mail: a.clarke@apemltd.co.uk

Former affiliated with:
National Environmental Research Institute
Department of Marine Ecology
P.O. Box 358, DK-4000 Roskilde
Denmark

Daniel Conley
GeoBiosphere Centre, Department of Geology
Lund University
Sölvegatan 12, SE-223 62 Lund, Sweden
E-mail: daniel.conley@geol.lu.se

Former affiliated with:
National Environmental Research Institute
Department of Marine Ecology
P.O. Box 358, DK-4000 Roskilde
Denmark

Torbjørn Johnsen
Norwegian Institute for Water Research
Postboks 2026
NO-5817 Bergen, Norway
E-mail: torbjorn.johnsen@niva.no

Atte Korhola
Dep. of Biological and Environmental Sciences
Division of Aquatic Sciences
P.O. Box 65
FI-00014 University of Helsinki, Finland
E-mail: atte.korhola@helsinki.fi

Sirje Vilbaste
Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences
181 Riia Str., EE-51014 Tartu, Estonia
E-mail: sirje.vilbaste@emu.ee

Richard Telford
Ecological and Environmental Change Research Group
Department of Biology
University of Bergen
Allégaten 41, NO-5007 Bergen, Norway
E-mail: richard.telford@bjerkes.uib.no or richard.telford@bio.uib.no

Former affiliated with:
Bjerkes Centre for Climate Research
Allégaten 55, NO-5007, Bergen, Norway

Kaarina Weckström
Institute for Limnology
Austrian Academy of Sciences
Mondseestrasse 9, AT-5310 Mondsee
Austria
E-mail: kaarina.weckstrom@helsinki.fi

Former affiliated with:
Dep. of Biological and Environmental Sciences
Division of Aquatic Sciences
P.O. Box 65
FI-00014 University of Helsinki, Finland

Steve Juggins
School of Geography, Politics & Sociology
University of Newcastle
Newcastle upon Tyne, GB-NE1 7RU, UK
E-mail: stephen.juggins@newcastle.ac.uk