THE BIOLOGY AND ECOLOGY OF BENTHIC FORAMINIFERA INHABITING INTERTIDAL MUDFLATS

Heather Anne Austin

A Thesis Submitted for the Degree of PhD
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University of St Andrews

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THE BIOLOGY AND ECOLOGY OF BENTHIC FORAMINIFERA INHABITING INTERTIDAL MUDFLATS

Heather Anne Austin

A thesis submitted to the University of St Andrews for the degree of Doctor of Philosophy

Division of Environmental and Evolutionary Biology

University of St Andrews

July 2003
THESIS CONTAINS

CD
To Mum and Donald

*And death shall have no dominion*

Though they go mad they shall be sane,  
Though they sink through the sea they shall rise again;  
Though lovers be lost love shall not;  
And death shall have no dominion.

By Dylan Thomas (1914-1953)
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The University of St Andrews

Declarations

I, Heather Austin, hereby certify that this thesis which is approximately 50,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Date: 13/12/03 signature of candidate.

I was admitted as a research student in October 1999 and as a candidate for the degree of Doctor of Philosophy in September 2000, the higher study for which this is a record, was carried out in the University of St Andrews between 1999 and 2004.

Date: 13/12/03 signature of candidate.

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Doctor of Philosophy in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date: 13/12/03 signature of supervisor.

In submitting this thesis to the University of St Andrews I understand that I am giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. I also understand that the title and abstract will be published and that a copy of the work may be made and supplied to any bona fide library or research worker.

Date: 13/12/03 signature of candidate.
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“It is only with the heart that one can see rightly; what is essential is invisible to the eye”

Antoine De Saint-Exupéry: The Little Prince
Abstract

Benthic foraminifera inhabiting the mudflats of the Eden Estuary, NE Scotland, exhibited a single annual peak in abundance at both high (270 individuals 10 cm^{-2}) and low shore (210 individuals 10 cm^{-2}) intertidal sampling stations during June 2000. The increased total abundance coincided with reproduction in the two dominant foraminiferal species *Elphidium williamsoni* (May) and *Haynesina germanica* (June) at both sites. Benthic diatom biomass, measured as chlorophyll c, peaked at the high intertidal site during March and April 2001 (36 and 37 mg m^{-2}, respectively) and at the low intertidal site in June 2000 (45 mg m^{-2}). The high intertidal foraminiferal populations appeared to display a lagged response to increases in chlorophyll c. Multivariate statistics suggest that abiotic factors control the abundance of foraminifera at the high intertidal site. At the low intertidal site, total foraminiferal abundance demonstrated an almost immediate response to increased chlorophyll c and multivariate statistics suggested biotic factors are more important in controlling foraminiferal species abundance here.

Ingestion rates of 13C-labelled benthic diatoms over a five-day experimental period were high (e.g. enrichment values for *Elphidium* were recorded as 687 ± 121 % after 1 day) in intertidal benthic foraminifera. Laboratory observations of *E. williamsoni* demonstrated rapid asexual reproduction (within 3 days) and growth rates of up to 14 μm day^{-1} were estimated.

Three benthic foraminiferal species *E. williamsoni*, *H. germanica* and *E. oceanensis* ingest motile benthic diatoms as a food resource on the intertidal mudflats of the Eden Estuary. Novel application of natural abundance stable isotope (δ^{13}C and δ^{15}N) techniques demonstrated the trophic position of intertidal benthic foraminifera, confirming that they largely ingest and assimilate organic matter derived from benthic diatoms. Foraminifera have largely been overlooked in meiofaunal studies, but play a significant role in the rapid transfer of autotrophic carbon to higher trophic levels within benthic marine food webs.
Chapter 1
Chapter 1

Introduction to benthic foraminifera

1.1 General

Foraminifera are eukaryotic unicellular organisms and are classified in the Kingdom Protoctista, Phylum Granuloreticulosa, Class Foraminifera (Sen Gupta 1999). There are thought to be approximately 10,000 extant foraminiferal species, the majority of these being benthic, with only 50 planktonic species known (Vickerman 1992).

The size range of adults is relatively large, from approximately 50 μm to greater than 12 cm in some tropical forms, spanning four orders of magnitude (Giere 1993). More recently, the multi-nucleated deep-sea Xenophyophores, previously placed in a separate class, have again been suggested as belonging to the Phylum Granuloreticulosa and these can measure more than 25 cm in diameter (Gooday 1999). However, the vast majority of adult foraminifera in temperate intertidal mudflats fall within the size classification for meiofauna (63 to 500 μm) (Giere 1993). One of the most conspicuous features of foraminifera is their pseudopodia, which are extruded through the aperture, forming a net (reticulum) of great complexity. Indeed their classification in the Phylum Granuloreticulosa is based upon the bi-directional streaming evident within this anastomose network. Pseudopodia are the means by which foraminifera interact with their surroundings, carrying out major functions such as motility, attachment, feeding, test formation, protection, disposal of waste products, and respiration (Goldstein 1999).

Due to their general abundance, high preservation potential in marine sediments and long geological records (benthic: Cambrian to Recent, planktonic: Jurassic to Recent) foraminifera have historically been the focus of palaeoecological studies. Although their significance in terms of numerical abundance and biomass has been demonstrated in the deep-sea (Gooday et al. 1992) and for some intertidal areas (Ellison 1984, Chandler 1989, Murray and Alve 2000, Moodley et al. 2000a), foraminifera remain a frequently overlooked group in ecological studies of coastal regions. They also have been shown to be important food items for a large variety of invertebrates (Lipps and Valentine 1970, Capriulo 1990, Murray 1991a, Berry 1994) (Figure 1.1).
Selectively preying on Foraminifera

- Isopod crustaceans *Ilyarachna bergendali*, *Echinozone arctica* and *I. Torleivi* (Gudmundsson et al. 2000)
- Eupagurus (Lipps and Valentine 1970)
- Gastropods and scaphopods (Brand and Lipps 1982)
- Retusa obtusa (Berry 1994)
- Cylichna (Lipps and Valentine 1970)
- Bittium Prosobranch (Lipps and Valentine 1970)
- Gonopla (Lipps and Valentine 1970)
- Olivella biplicata (Hickman and Lipps 1983)

Not selectively preying on Foraminifera

- From Brand and Lipps 1982 and Lipps 1983
  - Flatworms
  - Nematodes
  - Polychaetes
  - Echinoids
  - Gastropods
  - Arthropods
  - Fish
  - Bivalves
  - Asteroids
  - Tunicates
  - Crinoids
  - Nudibranchs

Figure 1.1: Schematic diagram of the major food sources for benthic foraminifera and prey species, selective and non-selective.

Foraminifera are found in a wide range of environments from marginal fresh and brackish water to the deep sea. Normally they are most common in fine-grained sediments and high standing crops (>100 "live" specimens per 10 cm²) occur in areas such as intertidal mudflats during certain months of the year (Ellison 1984, Castignetti 1996, Murray and Alve 2000).

Thus, foraminifera potentially form an important intermediate link in the lower trophic levels of benthic marine food webs, assimilating energy from primary producers (e.g. phytoplankton and microphytobenthos), dissolved organic matter (DOM), particulate organic matter (POC) and retrieving energy available in the detritus from bacteria and sediment organic matter (SOM). In turn, they are preyed upon by a variety of larger organisms.

1.2 Determining living from dead foraminifera

Studying the distribution of living foraminifera was greatly improved in 1952 by the introduction of Walton's method of staining foraminiferal protoplasm with a solution of the general protein stain rose Bengal (Walton 1952). Although rose Bengal is a non-vital stain (not detecting life itself), it does stain foraminiferal protoplasm red, indicating a living or recently dead individual (Murray 1991a, Bernhard 2000, Murray and Bowser 2000). This was followed by the use of Sudan black B (Walker et al. 1974) and both techniques are used today.

More recently, some authors have suggested the use of a luciferase-based adenosine-5'-triphosphate (ATP) assay to give a more accurate estimate of foraminiferal standing stock (Holm-Hansen and Booth 1966, Delaca 1986, Bernhard 1988, Bernhard 2000). The major drawback with this method is the high cost of reagents and specialist equipment (Photom-counter) required. All of the above methods of determining "live" foraminifera are mortal, killing the foraminifera. Bernhard et al. (1995) have investigated a vital procedure to distinguish "live" foraminifera from dead using epifluorescence microscopy and spectrofluorometry, with encouraging results. Again, the intricacy and costs of using these methods is a major limiting factor.
Chapter 1
Introduction

1.3 Morphology

Foraminiferal classification is based mainly upon the morphological features (structure and composition) of the test. Genera are defined on the basis of overall chamber arrangement and the position and appearance of the aperture(s). Distinguishing foraminiferal species is mainly carried out by assessing the number, size, shape and ornamentation (if any) of the chambers (Haynes 1981).

1.3.1 Main wall structure types

Classification of the major groups is based upon three main test wall structures.

1.3.1.1 Atestate (Allogromiida)

Atestate foraminifera are considered to be the most primitive order, in which the test wall is predominantly organic (proteinaceous). Their appearance has been described as a gelatine sac-like structure, 1-10 μm thick and some species possess a flexible test allowing them to change shape rapidly (Allogromia). Allogromiids are sometimes common in meiofaunal samples (Ellison 1984, Gooday 1986a, 1986b) but, because many are small and delicate, they are often overlooked or ignored.

1.3.1.2 Agglutinated (arenaceous)

This group of foraminifera has a test wall made up of detrital grains (e.g. fine sand) glued together with an organic cement. The strength with which these particles are bound to the test varies from one species to another. Many genera are unselective and make use of any available material, including sand grains, sponge spicules, mica flakes, coccoliths, diatoms and shell material (Haynes 1981). Others are selective of particular kinds and sizes of materials. For example, Miliammina fusca, on marshes in the Dovey Estuary, Wales show a preference for large silt grains, whereas Trochammina inflata consistently selects very fine grains averaging approximately 3 μm in diameter (Haynes 1981).
1.3.1.3 Calcareous

The test walls of this group are composed of calcium carbonate, predominantly as the mineral calcite. Two main structural types are known, porcellaneous and hyaline. The porcellaneous wall structure is made up of tiny crystallites of calcite in a randomly arranged fashion. No pores are present and the wall normally appears translucent or opaque and white or buff. In hyaline (glassy) forms, the calcite crystallites are arranged either in a radial or compound pattern. Hyaline walls always contain pores.

1.4 Growth patterns

Growth of the foraminiferal test is initially rapid, slowing down as they reach maturity (Figure 1.2).

Murray (1983) estimated growth rates from monthly size histograms of foraminiferal tests from the Exe Estuary. He reported *Nonion depressulus* growing rapidly during the first three months, with very few individuals exceeding 300 μm. Estimated increases in the average test diameter were 40 μm in the first month and 30 μm per month for the next three months. He also reported the majority of the foraminifera reproducing and then dying within four months. The rate of growth
appears to be influenced by the availability of food, temperature, salinity (Bradshaw 1961, Murray 1963), and the intensity and duration of exposure to ambient light in the case of individuals with symbionts (Ter Kuile and Erez 1984) (Table 1.1). On reaching maturity under favourable conditions, reproduction normally takes place. However, under unfavourable conditions, such as low food availability, the onset of reproduction can be delayed and the foraminifera continue to grow slowly or even cease growth.

Table 1.1: Summary of growth rates for selected species (+ indicates culture). Adapted from Murray (1991a).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Size at Maturity (μm)</th>
<th>Maturity (weeks)</th>
<th>Growth Rate (μm/week)</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amphislegina madagascariensis</em></td>
<td>700</td>
<td>~9</td>
<td>49</td>
<td>Hawaii</td>
</tr>
<tr>
<td><em>Amphislegina lobifera</em></td>
<td>1,400</td>
<td>40</td>
<td>46-81+</td>
<td>Hawaii</td>
</tr>
<tr>
<td><em>Amphislegina lobifera</em></td>
<td>1,400</td>
<td>18</td>
<td>52-81+</td>
<td>Palau</td>
</tr>
<tr>
<td><em>Amphislegina lessonii</em></td>
<td>1,200</td>
<td>14</td>
<td>58-116+</td>
<td>Hawaii</td>
</tr>
<tr>
<td><em>Amphislegina lessonii</em></td>
<td>1,400</td>
<td>14</td>
<td>40-70+</td>
<td>Palau</td>
</tr>
<tr>
<td><em>Nonion depressulus</em></td>
<td>~220</td>
<td>13</td>
<td>10-17</td>
<td>England</td>
</tr>
<tr>
<td><em>Haynesina germanica</em></td>
<td>270</td>
<td>4</td>
<td>20-70</td>
<td>Spain</td>
</tr>
</tbody>
</table>

In foraminifera, most adults and juveniles are isomorphic (superficially alike). However, some genera are heteromorphic (different forms at different times during ontogeny). For example, species of the genus *Spirulina* are planispiral when juvenile but develop a uniserial adult stage. Dimorphism (two distinct forms) is also found in association with an alternation of generations. The adult gamont (product of asexual reproduction) has a large proloculus (first chamber; megalospheric) but a small test, whereas the adult agamont (product of sexual reproduction) has a small proloculus (microspheric) and a large test.

1.5 **Morphogroups**

Many organisms show a relationship between their form and the environment they inhabit. This is true for foraminifera to a certain degree. The classification of agglutinated foraminifera into morphogroups by Jones and Charnock (1985) shows that in modern environments they have a distinctive and characteristic distribution. For example, unilocular, tubular or branching forms are
characteristic of deep-sea environments. Multilocular, planispiral/trochospiral and lenticular forms are particularly common in shelf and marginal marine environments. Conversely, elongate and quinqueloculine forms are often characteristic of marshes and lagoons.

1.6 Ecophenotypes

Within many species morphological gradients are evident. For example, in shallow water environments it is thought that populations of some species are adapted to the environmental conditions of their local habitat (ecophenotypes) and that some are morpologically distinct (morphogroups). This has generally been noticed when different names (= synonyms) are used for the same species e.g. *Elphidium excavatum* (Table 1.2). Unfortunately, distinction of certain morphotypes can be problematic because of continuous variation of morphological parameters and this has generated some confusion in the taxonomic literature relating to foraminifera.

Table 1.2: Ecophenotypes of *Elphidium excavatum* (from Murray 1991a).

<table>
<thead>
<tr>
<th>Forma</th>
<th>Environment</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>clavatum</em></td>
<td>Normal marine to slightly brackish, cold</td>
<td>Arctic</td>
</tr>
<tr>
<td><em>excavatum</em></td>
<td>Intertidal</td>
<td></td>
</tr>
<tr>
<td><em>selseyensis</em></td>
<td>Estuarine, temperate to polar (1 to 16°C)</td>
<td>Boreal</td>
</tr>
<tr>
<td><em>lidoensis</em></td>
<td>Estuarine, warm to temperate</td>
<td>Lusitanian</td>
</tr>
<tr>
<td><em>magna</em></td>
<td>Nearshore, turbulent</td>
<td></td>
</tr>
</tbody>
</table>

1.7 Stable isotopes

Much of the available literature on stable isotopes in benthic foraminifera pertains to shell chemistry; much having been written regarding the stable isotopes of oxygen and carbon recorded in benthic foraminiferal tests. Oxygen (*\(^{18}\)O and *\(^{16}\)O*) and carbon (*\(^{13}\)C and *\(^{12}\)C*) stable isotopes are commonly used in environmental studies to trace life processes, relationships with ambient water and palaeoceanographic reconstructions.

The ratios of oxygen isotopes (*\(^{18}\)O and *\(^{16}\)O*) recorded from foraminiferal tests are dependent on the isotopic composition and temperature of the seawater in
which the foraminifera were living or had been living (Epstein et al. 1953). Biological processes (vital effects) within the foraminifera will further alter the isotopic composition to some degree (Grossman 1987). The oxygen isotopic composition of seawater itself is influenced by the ratio of evaporation to precipitation and by dilution from surface runoff (Turekian 1969, Austin and Inall 2002). Vital effects, a collective term used to describe the disequilibrium in oxygen isotopes caused by life processes lead to test shell calcite depleted in $^{18}O$ relative to equilibrium conditions (Ruddiman 2001). Some of the life processes which influence the vital effect, include the uptake of metabolic CO$_2$ during calcification, growth or calcification rate and the photosynthetic activity of symbionts.

Carbon isotopes ($^{13}C$ and $^{12}C$) recorded from foraminiferal tests provide a measure of dissolved inorganic carbon (DIC) of the ambient seawater. These carbon isotopes are commonly interpreted in terms of surface water productivity, water mass origin and microhabitat effects. As with oxygen isotope measurements, carbon isotopes are influenced by physiological processes such as respiration and photosynthesis (e.g. large foraminifera with symbionts). Both processes cause fractionation in the carbon available for calcification. During photosynthesis plants incorporate $^{12}C$ into their tissue more easily than $^{13}C$, leaving a pool of $^{13}C$ for use during calcification. Similarly, respiration provides $^{13}C$-depleted CO$_2$ to the calcifying environment (Spero et al. 1997). Foraminifera therefore exhibit a greater vital effect for $\delta^{13}C$ than $\delta^{18}O$ because of the influence of this internal carbon pool.

1.7.1 Stable isotopes in the organic fraction of benthic foraminifera

Large differences are found between the $\delta^{13}C$ values for inorganic and organic carbon. These differences are mainly due to fractionation effects during photosynthesis, when plants preferentially incorporate $^{12}C$ into their tissue. Thus the $\delta^{13}C$ value of the plant becomes negative compared to its inorganic carbon source. Terrestrial vegetation has an average $\delta^{13}C$ value of $-25 \%O$ while oceanic regions have a mean $\delta^{13}C$ value of $1 \%O$ (Ruddiman 2001). To date, very few studies have measured the stable carbon isotope ratio in the organic fraction of
benthic foraminifera. Over the past 10 years there have been a few studies which used $^{13}$C-labelled food sources as a tracer in order to determine the fate of carbon (Blair et al. 1996, Levin et al. 1999, Moodley et al. 2000a, 2002). In order to follow any enrichment over time, background measurements of $\delta^{13}$C$_{org}$ are required. At the time of writing, there are no published studies that report the dual natural abundance stable isotopes of carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) to determine the trophic position of benthic foraminifera. However, this methodology is commonly applied in the study of other species (Kang et al. 1999).

1.8 Life Processes

1.8.1 Life cycles

A characteristic feature of foraminifera, also commonly found in lower plants (e.g. the algae), is the alternation of a haploid generation (gamont), which reproduces sexually, with a diploid generation that reproduces asexually (agamont). Goldstein and Moodley (1993) examined the life cycle of Ammonia beccarii, a common nearshore foraminifera, abundant in estuaries, inner shelf seas and salt marshes, with a worldwide distribution. It is well known for its repeated asexual generations in culture (Bradshaw 1957). However, Goldstein and Moodley (1993) found that fresh samples taken over spring to autumn 1990 and 1991 showed a very high incidence of gametogenesis. They concluded that the life cycle of Ammonia beccarii forma tepida includes both sexual and asexual phases and is probably best characterized as displaying a facultative alternation of generations (Figure 1.3).

The life cycle of shallow water benthic foraminifera may be strongly influenced by seasonality. Asexual reproduction should be the more common mode of reproduction in stable environments (predictable) because it produces numerous individuals adapted to that environment (Murray 1991a, b). Sexual reproduction increases genetic variability within a population, which is advantageous under variable or fluctuating environmental conditions (Hallock 1985). A polymorphic (variable) life cycle may therefore be regarded as an
advanced adaptation to spatially and temporally patchy environments (Fenchel 1987).

Figure 1.3: Foraminiferal life cycle, including an alternation between haploid, uninucleate, gamont, and a diploid, multinucleate, agamont. The schizont is produced from the agamont in some species and reproduces asexually, sometimes through numerous cycles. (redrawn from Goldstein 1999).

1.8.2 Colonization

At present, there is limited understanding of the processes by which benthic foraminifera disperse themselves and colonize new areas. Alve (1999) reviewed the principal environmental and biological processes which appear to control dispersal and colonization by modern foraminiferal assemblages. For most motile benthic foraminifera, there are generally four different modes of dispersion (three passive, one active). 1) Release to the water column of gametes, zygotes, or embryonic agamonts (diploid multinucleate products of asexual generation) or gamonts (the sexual components of the life cycle of foraminifera, which are haploid and uninucleate) following asexual and sexual reproduction; 2) adaptation to a meroplanktonic juvenile life stage with subsequent passive spread by currents;
3) self-locomotion along the sea floor; 4) passive (physiologically or biologically induced) entrainment into the water column and subsequent transport of different growth stages.

Alve (1999) reported that there is little information on the bottom current velocities required to entrain motile living benthic foraminifera, but some information exists concerning dead tests (Wang and Murray, 1983). Flume experiments have shown that the mean traction velocities for dead tests of common, free living, small (diameter 320-520 \( \mu \text{m} \)) species such as *Quinquoloculina* sp. and *Bulimina aculeata* are low: 5.1, 5.4, 6.6, and 9.2 cm.s\(^{-1}\) (Kontrovitz *et al.* 1978). Mean traction velocities of at least 10 to 15 cm.s\(^{-1}\) are probably needed to entrain living adult benthic foraminifera as long as their pseudopodia are active and "clinging" to particles outside the test. Foraminifera which are associated with loosely compacted sediments are probably more easily entrained and thereby have a higher dispersion potential.

More recently, Alve and Goldstein (2002) have suggested that foraminifera produce "propagules" which may be a resting stage. If produced in large numbers, dispersal by currents could distribute them widely. Subsequent growth and reproduction would only occur for individuals which were deposited in a suitable environment.

1.9 Food and feeding strategies

Foraminiferal food items include bacteria, small unicellular algae, plant and fungal fragments, protists, crustaceans and metazoans (Lipps and Valentine 1970, Murray 1973, Hohenegger *et al.* 1989, Rivkin and DeLaca 1990) (Table 1.3). In particular, pennate diatoms, small chlorophytes and certain bacteria are particularly favoured, whereas yeast, cyanobacteria, dinoflagellates, chrysophytes and many bacteria tend to be avoided. Studies on littoral foraminifera have shown that they feed on pennate diatoms, chlorophytes and bacteria (Lee 1980, Lee *et al.* 1966). Three major factors affect feeding: 1) the age of the food organism; 2) the age of the foraminifera; 3) the concentration of the food organism (Lee *et al.* 1966).
Table 1.3: Selected species of benthic foraminifera which have been maintained or cultured in the laboratory (Adapted from Anderson et al. 1991).

<table>
<thead>
<tr>
<th>Foraminiferal species</th>
<th>Food Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allogromina laticollaris</em></td>
<td><em>Nitzschia acicularis,</em></td>
</tr>
<tr>
<td></td>
<td><em>Cylindrotheca closterium,</em></td>
</tr>
<tr>
<td></td>
<td><em>Phaeodactylum tricornutum,</em></td>
</tr>
<tr>
<td></td>
<td><em>Chlorococcum sp.</em> and mixtures of the above</td>
</tr>
<tr>
<td><em>Allogromia sp.</em></td>
<td><em>Chlorella</em></td>
</tr>
<tr>
<td><em>Ammonia beccarii</em></td>
<td><em>Nitzschia sp.</em>, <em>Chlamydomonas sp.</em></td>
</tr>
<tr>
<td></td>
<td>Mixture of 8 species of small pennate diatoms and 3 species of cyanobacteria</td>
</tr>
<tr>
<td><em>Bolivina doniezi</em></td>
<td><em>Nitzschia angularis</em></td>
</tr>
<tr>
<td><em>Cibicides lobatulus</em></td>
<td><em>Chlamydomonas sp.</em></td>
</tr>
<tr>
<td><em>Elphidium crispum</em></td>
<td>Diatoms (vague)</td>
</tr>
<tr>
<td></td>
<td><em>Phaeodactylum tricornutum,</em></td>
</tr>
<tr>
<td></td>
<td><em>Cocconeis placentula,</em></td>
</tr>
<tr>
<td></td>
<td><em>Amphora sp.,</em></td>
</tr>
<tr>
<td></td>
<td><em>Entomoneis densistriata</em> and <em>Nitzschia subcommunis</em></td>
</tr>
<tr>
<td><em>Elphidium incertum</em></td>
<td><em>Navicula sp.,</em></td>
</tr>
<tr>
<td></td>
<td><em>Amphora sp.,</em></td>
</tr>
<tr>
<td></td>
<td><em>Cylindrotheca closterium,</em></td>
</tr>
<tr>
<td></td>
<td><em>Nitzschia acicularis</em> and <em>Amphora tennerima</em></td>
</tr>
</tbody>
</table>

Foraminifera are selective feeders at the species level, with consumption of the favoured food (organisms eaten (mg/foram/day x 10^-5)) leading to faster intrinsic rates of ingestion in at least some of the foraminifera studied (Lee and Muller 1973, Lee 1974, 1980). Experimental studies have shown that *Elphidium crispum* selects food on the basis of size (Murray 1963). Differential nutritional value of algae and bacteria can lead to faster growth when the appropriate food is available. For example, *Quinqueloculina lata* grew three times as fast on diets of *Nitzschia acicularis* and *Chlorococcum* sp. than on other species mixes (Muller and Lee 1969).

Bacteria appear to be an essential food source for maintaining reproductive activity and it has been suggested that they supply some nutritional element that is not otherwise available (Muller and Lee 1969). Low numbers of bacteria
(approximately 20,000 ml\(^{-1}\)) seem optimal for littoral species (Lee \textit{et al.} 1970). More recently, Bernhard and Bowser (1992) examined the impact of certain benthic foraminifera on bacterial biofilms. Benthic foraminifera from temperate tidal flats and shallow Antarctic waters were presented with bacterial biofilms. \textit{Allogromia} sp. harvested 80\% of the biofilm and \textit{A. laticollaris} harvested 50\%. Calcareous (\textit{Cyclopyra antarctica, Elphidium incertum, Glandulina antarctica, Pyrgo williamsoni}) and agglutinated forms (\textit{Astrammina rara, Astrorhiza} sp.) did not exhibit harvesting behaviour. This study highlighted that allogromiid foraminifera may play a previously unrecognised role in bacterial population dynamics and nutrient cycling, particularly in intertidal environments and certain areas of the deep-sea.

Most foraminifera can be classified as omnivorous, herbivorous, carnivorous or scavengers. Cannibalism, parasitism, mixotrophy and osmotrophy are less common but have also been described (DeLaca \textit{et al.} 1981, Lipps 1983, Capriulo 1990, Capriulo \textit{et al.} 1991, Nielsen \textit{et al.} 2002).

Foraminifera therefore adopt a variety of feeding strategies (Lipps 1983, Jones and Charnock 1985). Species that attach themselves to structures protruding above the substratum generally are suspension-feeders, using their pseudopodial network as a feeding net (DeLaca \textit{et al.} 1987). It is thought that some sediment-dwelling foraminifera search actively for food (Kitazato 1988). Others are sessile and gather food from their surrounding area by means of their pseudopodia. Some epibenthic species are likely to switch strategies from suspension to surface deposit feeding depending on flow conditions (Lipps 1983, Cedhagen 1988).

1.10 Symbiosis

Some foraminifera are known to have a symbiotic relationship with algae, bacteria and dinoflagellates. Foraminiferal families which are considered to have a true symbiotic relationship tend to be the large tropical species (e.g. \textit{Amphistegina} sp. Symbiont Class Bacillariophyceae) and planktonic foraminifera (e.g. \textit{Orbulina universa} symbiont, the dinoflagellate \textit{Gymnodinium bëii}) (Lee and Anderson 1991, Hallock 1999).
Other families of foraminifera (Nonionidae, Elphidiidae, Rotaliellidae) have been described as sequestering chloroplasts or showing chloroplast husbandry (Lopez 1979, Cedhagen 1991, Lee and Anderson 1991, Bernhard and Bowser 1999). That is, they remove chloroplasts when feeding on diatoms (benthic and planktonic) and keep them intact within their own protoplasm.

Hallock (1999) suggests that despite the sequestering of chloroplasts not being a true symbiosis, foraminifera could directly benefit from the products of photosynthesis. The advantages which a true symbiotic relationship would bring to the foraminifera are energy from photosynthesis, improved calcification, symbiont utilisation of host metabolites and an oxygen supply. Generally, one would consider the primary requirement for such a relationship to be light. However, Nonionella stella collected in the Santa Barbara Basin from a depth of 600 m were shown to both sequester chloroplasts and keep them intact and functioning for up to 1 year after sample collection (Grzymski et al. 2002).

Lopez (1979) reports high numbers of chloroplasts within the cytoplasm of field-collected Elphidium williamsoni ($9.7 \pm 4.9 \times 10^3$) and Haynesina germanica ($5.2 \pm 1.6 \times 10^3$) from surface sediments (0 to 6 m water depth) from the Limfjorden, Denmark. Radioactive, $^{14}$C-labelled HCO$_3^-$ was used to measure primary production in these species. At saturated light intensity, E. williamsoni takes up inorganic carbon ($2.3 \times 10^{-3}$ mg C mg$^{-1}$ hr$^{-1}$) five times faster than recorded in H. germanica ($5.0 \times 10^{-4}$ mg C mg$^{-1}$ hr$^{-1}$). Sequestered chloroplasts were retained longer in foraminifera adapted to constant dark conditions compared to those under light/dark conditions. Lopez calculated that E. williamsoni would need to consume at least 65 chloroplasts individual$^{-1}$ hr$^{-1}$ and H. germanica would have to consume 20 chloroplasts individual$^{-1}$ hr$^{-1}$ under normal light/dark conditions. This work suggested that the chloroplasts were derived from either Chrysophyceae or Bacillariophyceae, based on pigment analysis and ultrastructure studies. More recent studies have further suggested that diatoms are the major chloroplast donor to E. williamsoni and H. germanica (Knight and Mantoura 1985). To date, in smaller intertidal foraminifera it remains unclear if sequestered chloroplasts are mainly used as a food source or maintained to provide energy and oxygen from photosynthesis to the host (Bernhard and Sen Gupta 1999).
1.11 **Respiration rates**

There have been few studies investigating the respiration of benthic foraminifera (Bradshaw 1961, Lee and Muller 1973, Schwab and Hofter 1979, Hannah *et al.*, 1994, Köhler-Rink and Kühl 2000), and only one is from a temperate region (Hannah *et al.* 1994). Hannah *et al.* (1994) report respiration rates for five genera of benthic foraminifera. The rate averaged was $11.3 \times 10^{-3} \mu l O_2$ individual$^{-1}$ h$^{-1}$ at 10°C. Taking this figure into consideration along with their numbers would suggest they may contribute significantly to total microbial benthic respiration. However, the authors do point out some inconsistencies, namely foraminifera have been found apparently living at depth in the sediment (Buzas 1977, Corliss 1985, Gooday 1986a, Murray 1992) while others show that some species survive in low oxygen and anoxic environments (Bernhard 1993, Moodley and Hess 1992). Therefore, further studies of benthic foraminiferal respiration are required before their full ecological role can be highlighted.

1.12 **Distribution**

1.12.1 **Distribution studies**

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1.12.2 Vertical distribution within the sediment

As well as the lateral distribution of foraminifera in surface sediments, it is also important to understand their spatial distribution within sediments and ascertain if they are motile between depths. Many studies have shown that live foraminifera are not restricted to the surface sediment layer (Buzas 1977, Collison 1980, Corliss 1985, Gooday 1986a, Corliss & Emerson 1990, Thomsen and Altenbach 1993, Moodley et al. 2000b), but often show vertical zonation and can even occur beneath the oxic-anoxic boundary layer (Bernhard 1993, Moodley et al. 1997).

Some authors suggest that foraminifera are passively transported to different depths by bioturbation (Bernhard 1989, Moodley 1992). Corliss (1985), on the other hand, suggested that deep-sea benthic foraminifera select a depth level based on preferred physiochemical conditions within the sediment. Work by McCorkle et al. (1990, 1997) on shell and sediment pore water geochemistry supports the observation that certain foraminifera live and calcify their tests at different depths within the sediment.

1.12.3 Population density and biomass

Population density is often referred to as standing crop/stock and is the number of individuals “living” within a unit area of substratum. Units used to express the standing stock in foraminiferal literature are extremely variable. Murray (1983) recorded standing crops of *Nonion depressulus* in the Exe Estuary during 1979, observing minimum standing stock values in July (67 individuals 90 cm$^{-2}$) with two peaks recorded for April and November (536 and 584 individuals 90 cm$^{-2}$ respectively). Alve and Nagy (1986) recorded the number of living foraminifera in Sandebukta, a branch of the Oslo Fjord and reported living populations ranging from 1,000 to 11,800 specimens per 100g of sediment (it is unclear if the sediment analysed was wet or dry, and from what surface area it was collected). Ellison (1984) recorded a minimum of 46 living individuals g$^{-1}$ of wet sediment in January 1993 and a maximum of 552 individuals g$^{-1}$ wet sediment in July 1974 from an intertidal mudflat in Cornwall. Buzas (1969) reported results from a pilot study of the Choptank River, Maryland, a part of the Chesapeake Bay
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estuarine system recording 123-368, 45-599, and 18-114 individuals 20 ml⁻¹ of sediment at various sampling stations during December 1965. Casttignetti (1996) examined the previously unstudied Plym Estuary, England and found that total standing stocks varied during 1994 from a minimum in December (46 individuals 100 cm⁻³) to a maximum in May (4,125 individuals 100 cm⁻³). Haynesina germanica was found to be the most dominant species throughout the year with Elphidium williamsoni and Ammonia beccarii occurring in fewer numbers. Seasonal studies conducted over a period of at least two years reveal marked cyclicity in foraminiferal density, which has been related to variations in food supply, rate of reproduction and the survival of juveniles (Murray 1983).

Biomass is normally measured as total weight, volume, organic material or energy equivalent of organisms in a defined area. Although measuring the biomass of foraminifera is difficult due to their small size, it is not impossible.

Distribution studies using staining techniques to distinguish “living” from dead foraminifera are numerous. However, little information is available on biomass (DeLaca 1986). For example, it is known that biomass increases exponentially with increasing test diameter (Altenbach 1987). Techniques used to estimate foraminiferal biomass include the determination of a foraminiferal inner test volume, assuming that the entire space is filled with protoplasm (bio-volume). Bio-volumes are further converted to biomass values by multiplying by the protoplasmic density (Murray 1973). However, as highlighted in Gerlach et al. (1985), the test volume is rarely occupied completely by protoplasm. Hence, measurements of actual protoplasmic volumes yield a more accurate estimate of biomass. Other authors have determined wet weights (Smith et al. 1978) and dry weights (Olsson 1975) or ash free dry weights (Widbom 1984).

More recently it has been suggested that the above methods are laborious, inaccurate and may fail to distinguish viable protoplasm from total organic content (DeLaca 1986). More accurate means of measuring microbial and metazoan biomass have therefore been developed, which may clarify the importance of benthic foraminifera within the meiofauna. Determination of cell biomass can be achieved by quantitative assay for ATP (DeLaca 1986). The principle upon which this assay is based is that ATP is present in all living organisms and degrades
immediately after the death of an organism. However, this method too has its limitations. It is very time consuming and expensive in terms of equipment and reagents. Moodley et al. (2000a) carried out direct measurements of the organic carbon content of benthic foraminifera using an elemental analyser (Carlo Erba CN Analyser), reporting an average biomass of 0.75 µg C ind.\(^{-1}\) for *Elphidium* (average diameter 268 µm), 1.10 µg C ind.\(^{-1}\) for *Ammonia* (average diameter 325 µm) and 1.48 µg C ind.\(^{-1}\) for *Haynesina* (average diameter 381 µm). Results were in agreement with other studies which had used Altenbach’s wet oxidation method (Altenbach 1987, 1992, Thomsen and Altenbach 1993).

1.12.4 Relationship with the substratum

Benthic foraminifera are most common on (epifaunal) and in (infraunal) sediments, but other surfaces such as shell, rock (epilithic), and macroalgae or seagrass (epiphytic) also are utilised. Foraminifera are known to select various types of habitats in coastal environments (Boltovskoy 1966, Brooks 1967, Bernhard 1989) and in the deep sea (Corliss, 1985). Corliss (1985) presented an analysis of living benthic foraminifera in the upper 15 cm of deep-sea sediments, which revealed species-specific microhabitat preferences, with distinct morphological features within each group. For example, in response to low oxygen environments, foraminifera have a greater pore density evenly distributed over the test, and their tests tend to have rounded edges and planispiral coiling. In contrast, epifaunal species either lack pores or have large surface pores on only one side of the test, and their shape is commonly biconvex or plano-convex (Corliss and Emerson 1990). Some soft-shelled forms also inhabit empty foraminiferal tests, termed “squatter” behaviour (Moodley 1990a).

Jorissen (1999) describes four general vertical distribution patterns based upon individual taxa data and suggests these may reflect species-specific food preferences. The first has a population maximum in surface sediments (i.e. 0.5 cm or 1 cm) with none found in deeper layers, suggesting a preference for fresh labile organic matter. He suggested that this zonation pattern is found in deep ocean environments. Vertical distribution data collected by Murray and Alve (2000) suggest that this is also the case in intertidal mudflats of the Hamble Estuary. The
second distribution pattern showed similar densities with depth to approximately 3 cm, with no distinct maximum at the surface and none further down core. This may reflect the utilisation of both labile and refractory organic matter. Such distribution patterns are common in coarse grained sediment, shallow water environments where large numbers of macrofauna bioturbate the surface sediments, thus re-distributing the foraminifera and organic matter. The third pattern displays low numbers in surface sediments, but one or two down-core maxima, suggesting a preference for refractory organic matter, or labile sources provided by bacteria (Bernhard 1992). The fourth and final distribution pattern has maximum abundances at the surface and one or more deeper maxima within the sediment. Thus taxa can survive both at the surface and at depth and this alone suggests various feeding preferences.

1.12.5 Microhabitat preferences

The four distribution patterns described above highlight variable preferences and tolerances to environmental conditions among benthic foraminifera. These various conditions have been termed microhabitats (Corliss 1985). In deep water environments four factors have been proposed which could help describe the differences among species vertical distribution and between sampling sites. These are:

(1) Oxygenation of bottom water is thought to be one of the major controlling factors on foraminiferal distribution (Alve and Bernhard 1995). However, some species have been described from field studies as tolerating anoxic conditions (Sen Gupta and Machain-Castello 1993, Bernhard 1996) and laboratory experiments confirm this (Moodley and Hess 1992, Alve and Bernhard 1995, Moodley et al. 1997, 1998a, 1998b). Bernhard (1993) also demonstrated that some species tolerate elevated hydrogen sulphide. However, a combination of anoxic conditions and hydrogen sulphide production proves lethal to all foraminifera tested (Moodley et al. 1998b). Although individuals may survive short-term anoxia, such conditions may prevent successful reproduction and will ultimately lead to the disappearance of some species.
(2) Food availability is also a major factor proposed as possibly controlling microhabitat and foraminiferal depth distribution. In areas where food is less abundant (oligotrophic), oxygen penetration into the sediment is relatively deep and any food deposits are quickly consumed at the sediment water interface. In such environments it has been proposed that food is the limiting factor on foraminiferal distribution, since oxygen is available to considerable depths (Corliss and Emerson 1990, Linke and Lutze 1993).

(3) Bioturbation by macrofauna has been suggested as a passive transport mechanism accounting for the occurrence of foraminifera in deeper sediment layers (Collison 1980, Bernhard 1989, Moodley 1990b, 1992). Favourable microhabitats are also created around macrofaunal burrows (Meyers et al. 1987, 1988, Thomsen and Altenbach 1993), possibly extending the depth distribution of foraminifera. However, substantial numbers of foraminifera are often encountered below the oxygenated layer (Bernhard 1989, Moodley 1990b), beyond the depth of macrofaunal burrows.

(4) Competition and predation avoidance have been suggested as a possible explanation for deep infaunal life styles. However, there is little evidence to indicate that foraminifera are actively taking up an infaunal mode of life in response to predation or competition (Jorissen 1999).

Table 1.4: Depth distribution of living foraminifera from the Plym Estuary, southwest England during 1994. Adapted from Castignetti (1996).

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Haynesina germanica</th>
<th>Elphidium williamsoni</th>
<th>Ammonia beccarii</th>
<th>Total (100 cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>447</td>
<td>354</td>
<td>3</td>
<td>804</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>12</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>25</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>35</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>45</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Despite this range of diverse microhabitat preferences, recent studies carried out on temperate estuarine mudflats have recorded benthic foraminifera as
being concentrated within the surface centimetres (Castignetti 1996, Murray and Alve 2000) (Table 1.4). Alve and Murray (2001) report that the highest abundances of intertidal benthic foraminifera occur in the surface 0.25 cm of the Hamble Estuary, England.

1.12.6 Trox Model

Jorissen et al. (1995) summarised the ideas proposed by Corliss and Emerson (1990) that the vertical distribution of benthic foraminifera is controlled mainly by oxygen and food availability in the Trox model (Figure 1.4).

![Trox model diagram]

Figure 1.4: The Trox model, illustrating the effect of food availability and oxygen in oligotrophic, mesotrophic and eutrophic environments. Redrawn from Jorissen 1999. Stars represent deep infaunal taxa living in anoxic conditions. Inhabited benthic foraminiferal microhabitats are encompassed within the triangular area at the top of the diagram.

1.12.7 Patchiness

Population (total number of species in a defined area) spatial distributions are seldom regular or random, but are typically “clumped” (contagious: variance significantly greater than the mean) (Elliott 1977). Measures of patchiness are generally estimated from the degree of uniformity between replicate samples taken at one sampling site, compared to adjacent sampling stations. Benthic
foraminiferal populations generally display a "clumped" distribution due to local differences in environmental factors (microenvironments) and the needs of sexual reproduction (Murray 1991a). Most available data are from intertidal or shallow waters, and confirm that even the common species show patchy distribution patterns on a scale <1 m to several metres (Murray and Alve 2000, Buzas et al. 2002). See Murray (1973 and 1991a) for summary of studies carried out.

1.12.8 Motility and migration

The ability to actively migrate bestows benefits on benthic foraminifera enabling them to move away from or avoid unfavourable conditions, giving them the ability to actively seek out more favourable conditions and allowing them to maintain their preferred microhabitat position should they be re-distributed due to macrofaunal bioturbation. Several authors have examined the migratory activity of buried foraminifera (Severin et al. 1982, Moodley et al. 1998b, Gross 2000, Duijnstee et al. 2003). These studies generally suggest movement was not completely random, but displayed a tendency towards upward migration. Quinqueloculina impressa demonstrated an almost straight upward migration at speeds of approximately 5 cm in 4 days when buried under silt and silty clay. Once it reached approximately 1 cm below the sediment water interface it created a horizontal maze of burrows (Severin et al. 1982). Gross (2000) classified the migratory speeds of bathyal benthic foraminifera into slow (< 2.5 \( \mu m \) min\(^{-1}\)), fast (> 2.5 \( \mu m \) min\(^{-1}\)) and very fast (> 5.0 \( \mu m \) min\(^{-1}\)). Migration speeds for some species were greatly affected by environmental factors such as temperature, oxygen content, food concentration and substratum texture. For example, the migration speed of Allogromia spp. increased by 35% when temperature was increased from 10°C to 15°C. Species such as Quinqueloculina lamarckiana demonstrated high mean migration speeds of 24.48 \( \mu m \) min\(^{-1}\) at 4°C. Kitazato (1988) studied the locomotion of benthic foraminifera collected over a depth range of 5 to 100 m in Shimoda Bay and Otsuchi Bay, Japan. He found no relationship between velocity and temperature, the average velocity of species ranged from 8 \( \mu m \) min\(^{-1}\) to 82.3 \( \mu m \) min\(^{-1}\); vagile epifaunal species moved faster than infaunal species. Langer et al. (1989) observed elphidiids and ammoniids living on
intertidal flats of the North Sea to dig themselves into the surface sediment within 15 min using a corkscrew motion in the same direction as growth. *Ammonia catesbyana* and *Elphidium excavatum* held at room temperature (18 – 20°C) moved 2.8 cm h\(^{-1}\) on a glass substratum, but other species which extend their pseudopodia through a single aperture moved more slowly (approximately half the speed). This agrees with Kitazato (1988) who suggested a correlation between the number of pseudopodia extruded from the aperture, or supplementary apertures, and velocity.

### 1.13 Seasonality

Murray (1968a) reported a temporal sequence of successions of foraminifera genera for Christchurch Harbour. The estuary is micro-tidal and is more brackish in the winter and spring and least brackish in autumn. Foraminiferal assemblages were dominated by *Haynesina* in the winter, spring and summer, and by *Elphidium* in the autumn. Assemblages also changed seasonally. Murray (1983) showed marked cyclicity in foraminiferal density principally related to variations in food supply, rate of reproduction and survival of juveniles. However, Murray and Alve (2000) have demonstrated no annual pattern in standing crop in the Hamble Estuary, England and no significant correlation between standing stock and food availability (Chlorophyll \(a\)).

Some regions of the deep-sea experience seasonal variations in the flux of phytodetritus (planktonic remains). It is generally considered that phytodetritus is an important food supply utilised by opportunistic epifaunal foraminifera. The seasonally pulsed nature of this phytodetritus along with other inputs regulate the population dynamics and reproductive cycles of some responding species (Gooday and Turley 1990, Gooday and Rathburn 1999). This phenomenon is widespread in the mid-high latitudes and was first described for the Porcupine Seabight (in the northeast Atlantic). It is driven by large seasonal changes in mid-latitude radiation – influencing stratification and the development of phytoplankton blooms in spring and late summer.
1.14 Ecology

Estuaries are dynamic environments, being influenced by the land, the sea and atmospheric processes. Any animal or plant inhabiting such an area must be well adapted, or have life strategies which enable them to withstand fluctuations in both physical and chemical factors. Foraminiferal distribution and diversity is thought to be mainly influenced by abiotic factors such as temperature, salinity, substratum type, light, diurnal exposure to the atmosphere, turbidity, nutrients, oxygen, tidal energy and pH (Murray 1991b). Foraminiferal abundance is more influenced by biotic factors such as food availability, intraspecific, interspecific competition and predation (Boltovskoy and Wright 1976, Buzas 1978).

1.14.1 Temperature

By studying foraminifera in culture it has been possible to estimate extremes of temperature tolerance, temperature ranges over which growth occurs and temperature ranges in which reproduction occurs (Bradshaw 1961, Murray 1963) (See Table 1.5). Laboratory studies on Ammonia beccarii have suggested that a temperature range of 17-22°C is probably critical in triggering its reproduction cycle in certain geographic regions (Bradshaw 1961, Walton and Sloan 1990).

1.14.2 Salinity

As with temperature, the salinity tolerance limits for survival, growth and reproduction can be determined. The tolerance limits of benthic foraminifera are wide and quite distinct for different species. In a study of Christchurch Harbour, England, Murray (1968a) noted that an abundant living benthic foraminiferal population could flourish in waters whose salinity ranged from 0-34 although the maximum populations (representing reproduction) occurred during the warmer (higher salinity) months of summer and early autumn. During one summer in which there was unusually high dilution from stream runoff the maximum salinity reached was 4.3 and very few living specimens were found. It appears that although the specimens were able to survive a wide range of salinity values their reproduction limits were much narrower (Table 1.5). However, salinity within the
sediment is generally well buffered from overlying waters and should remain well above that of any episodic freshwater incursion into the estuary.

Table 1.5: Selected foraminifera species found in the Eden Estuary, highlighting environmental tolerances as summarised by Murray 1991a (Table 9.5) for principal associations in European lagoons and estuaries. Data show annual ranges. Salinity range is for overlying water, sediment salinity would be greater than 0.

<table>
<thead>
<tr>
<th>Species</th>
<th>salinity range</th>
<th>optimum salinity</th>
<th>temperature range (°C)</th>
<th>optimum temperature</th>
<th>substrate</th>
<th>depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia beccarii</td>
<td>0 to 35</td>
<td>&gt;10</td>
<td>0 to 20</td>
<td>17 to 20</td>
<td>muddy silt</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Elphidium williamsoni</td>
<td>2 to 35</td>
<td>0 to 32</td>
<td>0 to 20</td>
<td>17 to 20</td>
<td>sand</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>muddy sand</td>
<td></td>
</tr>
<tr>
<td>Haynesina germanica</td>
<td>0 to 35</td>
<td>0 to 20</td>
<td></td>
<td></td>
<td>mud</td>
<td>0-20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>muddy sand</td>
<td></td>
</tr>
<tr>
<td>Miliammina fusca</td>
<td>0 to 35</td>
<td>0 to 25</td>
<td></td>
<td></td>
<td>silty</td>
<td>0-2</td>
</tr>
</tbody>
</table>

1.14.3 pH

At a pH of < 7.8 calcareous tests will begin to dissolve (dissolution). Therefore, if calcareous forms are exposed to such conditions on a tidal cycle, they will spend energy re-calcifying their test. Schafer (1970) reported living specimens of *Elphidium* surviving in pH conditions of 6.7 in the Restigouche Estuary, New Brunswick. Low pH is detrimental to foraminifera and thus restricts their distribution.

1.14.4 Turbidity

Turbidity is a factor which affects the penetration of light through water as well as the availability of particulate material. Therefore, turbidity will affect species of foraminifera which have symbionts with photosynthetic properties and/or those reliant upon suspended particulate matter. It is mainly calcareous benthic and planktonic foraminifera that have symbionts and are affected by reduced light availability. Therefore, in an indirect manner increased turbidity favours the development of assemblages dominated by agglutinated forms (Boltovskoy and Wright 1976).


1.14.5 Oxygen

Oxygen is the second most abundant dissolved gas in seawater after nitrogen. Benthic foraminifera utilize the oxygen present at the sediment-water interface and in the sediment pore water for respiration. Oxygen availability can be significantly depressed where the sediment is rich in organic matter. In areas of high productivity such as mudflats, salt marshes and mangroves large amounts of organic matter accumulate in bottom sediments, leading to oxygen depletion in bottom and pore waters (Sen Gupta and Machain-Castillo 1993). Where the consumption of oxygen exceeds renewal by water exchange and primary production, anoxia results. For infaunal foraminifera burrowing several centimetres into a substratum rich in organic matter, the pore water oxygen level is generally lower than that at the sediment water interface. Microelectrode measurements demonstrate the removal of oxygen from pore waters within a few centimetres of the surface (Bernhard 1989).

Moodley and Hess (1992) demonstrated the survival of three common shallow water species, Ammonia beccarii, Elphidium excavatum and Quinqueloculina seminulum after exposure to anoxic conditions for more than 24 hours in the laboratory. This was taken as an indication that these species are capable of facultative anaerobic metabolism. Many authors consider the presence of live foraminifera at depths where oxygen is much reduced to be due to passive transport and the introduction of oxygen by macrofaunal bioturbation (Moodley, 1990b). Bernhard (1989, 1992) however, reported foraminifera living in anoxic sediment layers even in the absence of direct evidence of macrofaunal bioturbation activity. More recently, Alve and Bernhard (1995) examined the effect that dissolved oxygen content of the overlying waters would have on the distribution of infaunal foraminifera. They concluded that dissolved oxygen drives the vertical distribution of foraminifera within sediments and that foraminiferal species track and select a particular oxygen regime regardless of its position with respect to the sediment-water interface and irrespective of bioturbation activity. Moodley et al. (1996) opposed this, highlighting that Alve and Bernhard (1995) had not directly measured oxygen in the sediment. However, Moodley et al.
(1998b) carried out laboratory experiments which examined the migratory activity of some common benthic foraminifera in relation to pore water oxygen. They demonstrated that foraminifera are capable of migrating through anoxic sediment, reinforcing the idea that some common benthic foraminifera are facultative anaerobes. There was a bias for upward migration when foraminifera were buried without a subsurface oxic zone. This migration was not linked to oxygen gradients at depth, since they did not exist, but were thought to be a response to the absence of oxygen (Moodley et al. 1998b).

1.14.6 Biotic factors

Little is known of interspecific and intraspecific competition between foraminifera. Muller (1975) demonstrated that the genera Rosalina and Spiroloculina are less affected by crowding than Allogromia sp. which responds to crowding by showing reduced feeding and reproductive capacity. Buzas (1978) has demonstrated, through exclusion experiments, that the abundance of foraminifera is limited by predation.

1.15 Taphonomic pathways

As foraminifera are widely used to reconstruct past environments, it is essential not only to investigate the ecology and distribution of living assemblages, but also to determine causes of taphonomic changes during fossilization. Murray and Alve (1999) compared the living and dead assemblages in the Skagerrak-Kattegat revealing a higher relative abundance of calcareous test in living than in the dead assemblages. As the inlet is described as microtidal, there is little transport by waves and wave-induced currents. However, the major taphonomic process appears to be dissolution of the calcareous tests. This is manifest through etching and breakage of test walls. Further decalcification results in a residue rich in the organic linings of certain taxa.

Because of their small size and their hydrodynamic properties in suspension, foraminifera can be readily transported in estuarine environments (Kontrovitz et al. 1978, Wang and Murray 1983). An appreciation of sediment reworking and its impact on fossil foraminiferal assemblages can be gained by a
comparison of live and dead assemblage distribution data collected throughout a seasonal cycle.

1.16 Maintenance of benthic foraminifera in the laboratory

The key principles of foraminiferal maintenance and culture have been summarised by Muller and Lee (1969), Murray (1973), Lee (1974) and Anderson et al. (1991). However, foraminiferal life cycles and ecology are poorly understood. Anderson et al. (1991) estimated that the above knowledge is restricted to 1% of the modern living species. This is due mainly to the few species that have been successfully maintained or cultured in the laboratory. However, numerous foraminifera species have been maintained in culture (Table 1.3).

Some authors make a distinction between maintenance and culture. Maintenance is the ability to keep foraminifera alive in the laboratory for a certain period (days, weeks, months, years), with the foraminifera feeding and growing during this time and occasionally reproducing. In culture, the foraminifera go through continuous growth and reproduction resulting in multiple generations being reared in the laboratory (Anderson et al. 1991).

1.16.1 Marine aquaria

Both circulating and recirculating marine aquaria systems can support foraminifera. The water flow must be gentle and the system should be moderately illuminated, using natural or artificial light. Light should be supplied for at least 8 hours per day. Aeration must not be vigorous and temperature should be at slightly below the temperature of the sea where the organisms were collected. Anderson et al. (1991) reports that Lee, J.J. has kept *Ammonia beccarii* and *Bolivina vaughni* alive and reproducing for several years in re-circulating systems. The culture and maintenance of small batches of foraminifera can also be achieved in the laboratory using systems which are very basic and require little or no intervention.
1.16.2 Minimal intervention

Anderson et al. (1991) describe this method as being the simplest to set up and maintain. Foraminifera are selected as soon after collection as possible and transferred to deep sided petri dishes, stacking dishes or tissue culture flasks containing filtered or sterile seawater. Micro-herbivores may be present at this stage and should be removed, reducing competition for food and ensuring as little interference with the foraminiferal pseudopodia as possible. Dishes should then be placed in moderate light, the room temperature being approximately the same as their natural environment. Assuming no evaporation, this simple system requires the water to be changed every week. The salinity and room temperature should be monitored closely and checks made for evaporation and any signs of vigorous algal growth. As noted by Arnold (1974), high algal density can inhibit growth and reproduction in foraminifera. Moving the foraminifera to new vessels every month can counteract any vigorous algal growth. Bacterial growth may be stimulated by the addition of nutrients. However, this can be controlled by the addition of antibiotics or antibiotic mixtures (Muller and Lee 1969, Lee et al. 1970).

1.16.3 Intervention with feeding

There are four ways in which this type of culture can be set up. 1) Feeding live food in a seawater medium. 2) Feeding heat-killed potential food in a seawater medium. Algae can be killed by boiling them for 3 minutes. Foraminifera have been shown to consume such food items (Bradshaw 1957). 3) Inoculation of foraminifera onto lawns of pre-incubated potential food organisms. For example Bernhard and Bowser (1992) grew bacterial biofilms prior to presenting them to the foraminifera. 4) Encouraging food organisms to grow together with the foraminifera. Several authors have reported mixtures of food species to give the most vigorous growth (Muller and Lee 1969, Lee et al. 1970).

1.17 Conclusions

Foraminifera are a relatively under studied group with most significant research carried out on fossil assemblages. A number of authors suggest
foraminifera are an important component of the meiofauna, but there remains little published data on their biology and ecology. In particular, it seems that there is considerable scope for further study of intertidal foraminifera to determine their role in the transfer of autotrophic carbon to higher trophic levels within benthic marine food webs.

1.18 Aims of this study

The aims of this study were to establish, through both field studies and novel experiments, the significance of benthic foraminifera within intertidal food webs. Foraminifera as a group have been largely overlooked in meiofaunal studies, yet may well play a key role within the ecology of intertidal environments. Such intertidal environments play a significant role in sustaining biodiversity, sequestering carbon, and protecting coastal regions at all scales, from the local to global.

The principal aims can be summarised as follows:

1) To record seasonal changes in benthic foraminifera numbers and species composition along with the biomass of benthic diatoms over a 12-month period. The survey was conducted along an intertidal transect from high to low shore on the Eden Estuary, NE Scotland.

2) To determine growth rates of the dominant species found on the transect in laboratory cultures, with particular attention to growth directly after reproduction.

3) To investigate the relationship between oxygenation and food availability and its effect on the vertical distribution of benthic foraminifera within North West European intertidal sediments.

4) To determine the response of certain foraminifera taxa to simulated benthic diatom blooms.

5) To develop a methodology to measure natural abundance stable isotopes of carbon ($^{13}$C) and nitrogen ($^{15}$N) in foraminiferal protoplasm in order to determine their trophic position within the intertidal mud flat food web.
2. Materials and Methods

2.1 Study sites

2.1.1 The Eden Estuary

The Eden Estuary, NE Scotland (56° 22'N, 2° 50'W) is a small (10.41 km²) mesotidal estuary that experiences an average tidal range of 3.7 m with absolute maxima of 5 metres. The intertidal mudflats of the Eden Estuary are exposed for an average time of 6 to 7.5 hours over each tidal cycle and cover an area of approximately 9.37 km² (Clelland 1997, Davidson and Buck 1997) (Figure 2.1).

The distribution of sediments is spatially complex, with a general trend from coarse sands at the estuary mouth to mud in some inner regions. At the confluence of the River Eden and Motray Water, coarse silts dominate the sediments. A sand bar positioned across the mouth of the estuary reduces the effect of wave action, resulting in tidal currents dominating the hydrodynamics of the estuary. Clelland (1997) describes the estuary as well mixed at high tide with a uniform salinity of 28. At low tide a central channel drains the rivers (The Eden and Motray Water) and numerous rivulets drain the mudflats (Figure 2.1).

Following improvements to effluents discharged from both the Guardbridge paper mill and sewage treatment works, the chemical water quality (Chemical Classification Scheme) of the River Eden (class 1) is today considered "unpolluted". The estuary gained a class A (excellent) in 1999 under the Estuary Classification Scheme (SOAEFD, 1995). However, the Eden Estuary has extremely high levels of total oxidised nitrogen (6.92 to 8.57 mg l⁻¹) compared to other estuaries in Scotland (Mathieson and Atkins 1995). This is considered by many to be mainly due to agricultural run-off rich in nitrates (Clelland 1997).

The Nature Conservancy Council (NCC) declared the Eden Estuary a Site of Special Scientific Interest (SSSI) in 1971 because of its importance to wading and migratory bird populations. This was followed in 1978 by a district council designation of Local Nature Reserve (LNR). More recently (February 2000) the estuary has been designated a "Special Protection Area" under the EU Wild Birds Directive.
In general, little is known about the meiofaunal populations of the Eden Estuary and this is the first study to examine benthic foraminifera at this site. However, macrofaunal populations have been surveyed on a relatively regular basis, as changes in these populations will have a significant effect on bird populations (Johnstone et al. 1978, SEPA 1998, BIOPTIS 2001). Inner estuary sediments are dominated by oligochaetes, Nereis diversicolor, Pygospio elegans and the amphipod Corophium volutator. Hydrobia ulvae (gastropod) has also been described as occurring in large numbers. Numerous species of bivalve molluscs are also present in large numbers (e.g. Mytilus edulis, Scrobicularia plana).

On the intertidal mudflats, benthic diatoms are the major primary producers. In addition, seasonal formation of dense Enteromorpha spp. (macroalgae) patches takes place. Other macroalgae found in discrete but dense patches are Ulva spp. and Cladophora spp. Some saltmarsh plants are also found on the high shores in areas of stable sediment (Zostera spp. (eelgrass) and Salicornia spp. (glasswort)).

Figure 2.1: Outline map of Scotland showing the location of the Eden Estuary. Aerial photograph shows the upper reaches of the estuary with the two rivers marked. Stars on the mudflat are highlighting areas of fairly extensive Enteromorpha beds. Photograph courtesy of BIOPTIS. The dark, shaded area immediately to the north of the estuary is RAF Leuchars air base.
The study area used was located along a transect on the north bank of the estuary, relatively close to the Guardbridge paper mill (Chapter 6, Figure 6.1).

2.1.2. The Oosterschelde Estuary

The Oosterschelde Estuary (51° 32’ N, 3° 54’E) is situated in the South West of The Netherlands (Chapter 4, Figure 4.1). Although many still consider the Oosterschelde area an estuary, modifications carried out between 1979 and 1986 during the building of storm-surge barriers at the seaward end have changed its hydrodynamics. Freshwater inputs from the Scheldt, Rhine and Meuse rivers (Riera et al. 2000) were closed off during these modifications. The area has therefore changed from a turbid estuary to a tidal bay. Sampling was undertaken to the west of Yerseke (Chapter 4, Figure 4.1).

2.2 Sediment sampling

2.2.1. Sediment cores

Intact sediment cores were collected to the required depths using PVC tubes, sharpened at the bottom (Chapters 4 and 6). Details of tube diameters and depths are given in individual chapters. Short cores were also taken to a depth of 1 cm to determine sediment grain size distribution.

2.2.2. Surface scrapes

In order to collect sediment from which to isolate living benthic foraminifera and benthic diatoms ( Chapters 3, 4, 5, 7) a small spade was used to collect the upper layers of mud. The mud was returned to the laboratory and homogenised to ensure an even distribution of individuals.

2.2.3. Contact cores

Contact cores were used to collect sediment for pigment analysis, organic carbon, total nitrogen and water content. The technique required an aluminium dish (56 mm diameter, Area 2462 mm²) with a plastic collar protruding below the bottom of the dish by 2-3 mm (Anderson and Black 1980, Honeywill et al., 2002). The dish was placed on the sediment surface and pushed gently to the depth of the collar. Liquid nitrogen (LN₂) was poured into the dish and left for 15 to 30...
seconds depending on the water content of the sediment. This freezes a sediment layer to a depth of 2-3 mm. On removing the core, any sediment extraneous to the core area was removed quickly with the aid of a palette knife. The frozen core was quickly sliced from the aluminium dish into a pre-labelled square of aluminium foil, folded and placed in LN2. On returning to the laboratory, the cores were transferred to pre-weighed and labelled plastic bags, weighed, freeze-dried (-60°C, Edwards Modulyo Freeze Dryer), re-weighed, homogenised and subsampled for pigment analysis. If analysis was delayed, samples were stored in the dark at -80°C. A note of the core area was also taken and if less than 100 %, corrections were applied to the data.

2.3 Organic carbon and total nitrogen determination

2.3.1 Preparation of samples

Samples were drawn from freeze-dried contact cores and ground into a fine powder to allow for good combustion in the C/N analyser. Samples were homogenised to reduce variability between replicate samples, stored in sealed tubes and held in a dessicator to avoid moisture entering the samples.

2.3.2 Preparation of silver boats

Silver boats were bought pre-formed (Elemental Microanalysis Limited) to allow in situ acidification to remove carbonates. In order to remove any organic material, which may be contaminating the boats, they were placed in an aluminium foil container and combusted in a muffle furnace at 500°C for 3 hours prior to use.

2.3.3 Sample pre-treatment

Samples (20 - 30 mg) were weighed directly into the silver boats on a 5 figure balance and placed in a labelled glass petri dish. Carbonates were removed by in situ acidification with 1M HCl (Nieuwenhuize et al. 1994, Midwood and Boutton 1998).

Samples were wetted with 1M HCl using a micropipette, adding approximately 10 to 20 μl at a time to minimise loss of sample through bubbling over the side of the silver boat. Once effervescence had ceased the samples were
dried in an oven (60°C) overnight. These steps were repeated until no more effervescence was observed (normally 3 treatments). The silver boats were then formed into small balls with forceps and placed in a sterile 96 well tray and stored in a desiccator until analysis.

2.3.4 Analysis of samples

Analysis of samples was carried out by Dr Andrew Midwood at the MacAulay Institute, Aberdeen. The organic carbon and nitrogen content of the samples was determined using a Carlo Erba NA1500 elemental analyser (Carlo Erba Instruments, Milan, Italy).

Samples are introduced directly (via an autosampler) into an oxidation column packed with chromium trioxide (Cr₂O₃; oxidation catalyst) overlying silver cobaltous cobaltic oxide (AgCO₃O₄). Here they undergo "flash combustion" (1050°C) during a stationary phase under an atmosphere enriched in oxygen (high purity). Evolved gases (CO₂, NOₓ and H₂O) are swept through the combustion reaction during a mobile phase using helium gas (high purity). The sample then enters a reduction column containing copper wire maintained at 650°C where nitrogen oxides are reduced to N₂ and excess O₂ is removed. A trap filled with magnesium perchlorate removes excess water and the final step is to separate the mix of CO₂ and N₂ by gas chromatography (54°C) before detection by thermal conductivity (Verado et al. 1990, Nieuwenhuize et al. 1994).

2.4 Grain size analysis

Particle size analyses (0.375 μm – 2 mm) were carried out on bulk sediment, collected as 1 cm cores, using a Coulter LS230 laser granulometer. Samples were analysed both raw and after treatment to remove organics and calcium carbonate.

2.4.1 Sample preparation to remove organics and calcium carbonate

Sediment samples were homogenised and 1 to 1.2 g of wet sediment weighed out into glass beakers. In order to remove organic material hydrogen peroxide (H₂O₂ 20%) was added to the sample (10 ml) and beakers placed on a sand bath at 70-80°C. After about 2 hours another 10 ml of H₂O₂ was added, a ceramic lid placed over the beaker to slow down evaporation and left for 3-4
hours. Organic-rich samples required this step to be repeated twice. Samples were then removed from the sand bath and cooled to room temperature and placed in a fume hood. To remove calcium carbonate from the samples, hydrochloric acid (HCl 20%) was added (10 ml) and left for at least 12 hours. Fluid was decanted and the sample washed with deionised water. The sediment fluid mixture was transferred to centrifuge tubes and centrifuged (4,000 rpm for 5 minutes), this was repeated twice. Supernatant was decanted and 2 ml of a sodium hexametaphosphate solution (33g sodium hexametaphosphate, 7g sodium carbonate and 1 litre of deionised water) added. The sample was then thoroughly mixed using a stirrer apparatus (Vortex), 20 ml of deionised water added and stored for at least 24 hours in a refrigerator to ensure that the sodium hexametaphosphate solution takes effect (dispersal of clay minerals).

Samples were then washed into the mixing column of the Coulter LS230 through a 2 mm sieve and any particles trapped were retained and weighed. The range was 0.375 μm to 2,000 μm.

2.5 Water content

Sediment water content was estimated by measuring the percent weight loss of frozen (in situ) contact cores after freeze-drying (-60°C) using the following equation:

\[
\text{% Water Content} = \left( \frac{W_{t\text{wet}} - W_{t\text{dry}}}{W_{t\text{wet}}} \right) \times 100 \quad \text{(Equation 2.1)}
\]

Where \(W_{t\text{wet}}\) is the weight of a frozen contact cores and \(W_{t\text{dry}}\) is the weight of the core after freeze-drying. No salt correction was applied to the data. To avoid the breakdown of chlorophyll, freeze-drying was conducted in the dark and samples stored at -80°C in the dark.

2.6 Chlorophyll analysis

The concentration (mg m\(^{-2}\)) of chlorophyll \(a\), \(b\) and \(c\) were determined using a spectrophotometer (Cecil 3000 scanning spectrophotometer) after extraction from freeze-dried contact cores. This technique was used to track the relative changes in benthic diatom populations on the Eden Estuary and relate these to benthic foraminiferal populations (Chapter 6).
2.6.1 Chlorophyll extraction

Extraction of chlorophyll was carried out in scintillation vials by adding 2 ml of 90% dimethylformamide (DMF, analytical grade) to 0.05 g of freeze-dried sediment. Extraction was carried out in the dark at room temperature in a fume hood for at least 24 hours. The extractant was removed from the scintillation vial using a 2 ml syringe and fine needle, thus allowing removal of the sample without sediment contamination. DMF was used to extract chlorophyll following Honeywill (2001), who suggests that Chl c, a major pigment in benthic diatoms, (Table 2.1) is not extracted efficiently in acetone.

2.6.2 Analysis of sediment chlorophyll a, b, c

Although equations to calculate the concentration (mg m\(^{-2}\)) of chlorophyll a and b from absorption readings were available using DMF (Porra et al. 1989), no equations were available to calculate the concentration of chlorophyll c extracted in DMF. However, equations were available to calculate chlorophyll a, b and c concentrations (μg ml\(^{-1}\)) extracted in acetone (Jeffrey and Humphrey 1975). Since only a relative measure of benthic diatoms was required it was decided to use the acetone equations (Equations 2.2, 2.3 and 2.4).

Table 2.1: Wavelengths used to determine chlorophyll in spectrophotometer and which taxonomic group they represent (South and Whittick 1987, Jeffrey et al. 1997).

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Maximum absorbance wavelength (nm)</th>
<th>Taxonomic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>664</td>
<td>All green plants</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>647</td>
<td>Chlorophyceae, Euglenophyceae</td>
</tr>
<tr>
<td>Chlorophyll c</td>
<td>630</td>
<td>Bacillariophyceae, Phaeophyceae</td>
</tr>
</tbody>
</table>

\[
\text{Chlorophyll } a = 11.85E_{664} - 1.54E_{647} - 0.08E_{630} .............. \text{(Equation 2.2)}
\]

\[
\text{Chlorophyll } b = -5.43E_{664} + 21.03E_{647} - 2.66E_{630} .............. \text{(Equation 2.3)}
\]

\[
\text{Chlorophyll } c_1 + c_2 = -1.67E_{664} - 7.60E_{647} + 24.52E_{630} .............. \text{(Equation 2.4)}
\]
Where $E_n$ is the absorbance at the maximum wavelengths (n) for pigments (Table 2.1). Units are Chlorophyll $\mu g \text{ml}^{-1}$. A turbidity measure taken at wavelength 750 nm was subtracted from each absorbance measure. All data are finally expressed as a concentration of chlorophyll $mg \text{ m}^{-2}$ (Perkins et al. 2003).

2.7 Foraminiferal analysis

2.7.1 Extracting living foraminifera

Surface sediment scrapes were returned to the laboratory and sieved at 63 $\mu m$ to remove the fine fraction in local seawater. That part retained on the 63 $\mu m$ sieve was placed in trays in a very fine layer, covered with seawater (local), aerated and left to settle until use. Small volumes of sediment were sorted under a binocular microscope and foraminifera collected using a very fine bristle brush (0000) trimmed to a fine point. Individuals were isolated into petri dishes filled with filtered seawater. Larger volumes of sediment were also placed into glass beakers and attempts were made to isolate foraminifera from the sediment by placing glass slides into the sediment at an angle. These were left for several days and although there were generally some individuals present, it was found that searching through the sediment was a much quicker way of finding “live” individuals.

2.7.2 Extracting preserved foraminifera

The details of this method are described in Chapter 6, section 6.2.4.

2.7.3 Determining living from dead foraminifera

Non-vital staining of foraminiferal protoplasm with rose Bengal generally produces a wide spectrum of staining patterns. This could be due to several factors, for example test thickness, presence of micro-organisms and the slow decay of protoplasm after death in certain environments (Boltovskoy and Lena 1970, Bernhard, 1988, Goldstein et al. 1995, Murray and Bowser 2000). In order to minimize errors in assigning individuals to the “live” (stained) category which were in fact dead, a strict protocol was adopted to select only individuals that were stained bright pink or red (Figure 2.2). These individuals were considered to
have been alive at the time of collection. All foraminifera that were white, pale pink or stained in a patchy manner were considered dead at the time of collection.

Figure 2.2: Photographs illustrating some of the levels of staining observed in calcareous foraminiferal tests when stained with rose Bengal. Also illustrated is the protoplasm within these tests after decalcification with 2% HCl. (a) *Elphidium williamsoni* which is “live”, (b) the same individual decalcified clearly showing fresh protoplasm, (c) *Haynesina germanica* which is “live”, (d) the same individual decalcified, (e) *Haynesina germanica* which is “live” (f) the same individual decalcified, (g) *Stainforthia fusiformis* which is “dead”, test remains white after staining. Individuals (a) to (d) are approximately 350 µm, (e) and (f) - 425 µm and (g) - 250 µm.
2.7.4 Sub-sampling sediment for foraminifera

Methods used to extract foraminifera from sediments have in the past mainly involved wet sediment sieving followed by drying. Foraminiferal tests are then concentrated by the use of heavy liquids such as trichloroethylene (Murray 1979). However, some researchers have expressed concern that this process possibly destroys some of the more delicate agglutinated forms.

Gooday (1988) highlighted the fact that traditional methods of concentrating and extracting meiofauna, such as elutriation, were not suitable methods for foraminifera due to their varied morphology and the composition of their test walls. In order to count the number of living foraminifera accurately during times of high abundance, sub-sampling must be carried out in a quantitative manner. Scott and Hermelin (1993) suggested that in some environments it is difficult to obtain quantitative splits of equal size in a wet sample and describe the design for a splitter. In order to obtain quantitative sub-samples a modified version of this splitter was constructed, tested and used to process wet samples requiring sub-sampling.

2.7.4.1 Wet sample splitting

The splitter design is based on that given in Scott and Hermelin (1993) with minor modifications (Figure 2.3). Samples were first washed to remove ethanol, tap water (3,000 ml) was added to the column and, while the water was at its most turbid, the sample was added and allowed to settle for 15 to 30 min. The overlying water was then siphoned-off using a tap-driven siphon and passed over a 63 μm sieve. In this way, the supernatant was examined for foraminiferal tests, which may have been suspended and could, inadvertently have been removed from the sub-samples. Once the water level was low enough, the upper section was detached allowing access to the bottom section (Figure 2.3(b)). The bottom sub-sections (8 in total) can be drained and flushed individually through a 63 μm sieve, allowing the sub-sample to be concentrated and stored in 70 % ethanol until required for analysis.

The splitter was tested to ensure that it was splitting sediment, stained foraminifera and foraminifera size classes consistently and quantitatively. Three different sediment samples were divided in the splitter to 1/8th splits. These were
individually drawn from the base of the splitter, dried in an oven at 100°C to constant weight and the sediment weight recorded. This was also carried out for one sample to 1/64th split. Statistical analysis using the Kruskal-Wallis test revealed no significant difference between sediment weights for either of the three sets of 1/8th or 1/64th splits (H=7, Df=7, P=0.429).

Two samples were stained and processed as above and the foraminifera in each 1/8th were counted. Maximum test diameters of the two dominant species of foraminifera *Haynesina germanica* and *Elphidium williamsoni* were also measured. Kruskal-Wallis tests on the total counts in each 1/8th revealed no significant difference between counts in 1/8ths within samples (sample1: H=1.34, Df=7, P=0.987; Sample 2: H=1.98, Df=7, P=0.961). Equally, Mood’s median test reported no significant differences in size distribution of species measured between 1/8th splits within samples (*H. germanica* sample 1: $\chi^2 = 14.09$, Df=7, P=0.05; sample 2: $\chi^2 = 1.72$, Df = 7, P = 0.974; *E. williamsoni* sample 1: $\chi^2 = 14.09$, Df = 7, P = 0.050; sample 2: $\chi^2 = 6.83$, Df = 7, P = 0.446). Overall a percentage error in counts ranged from 2 to 6%.

Figure 2.3: Sediment sample splitter constructed at the University of St Andrews and modified from a design by Scott and Hermelin (1993). (a) Shows the splitter when assembled; height of column = 35 cm (b) The base unit (viewed from above) subdivided into eight equal parts, with rubber bungs in base to allow individual chambers to be drained; diameter = 15 cm.
Chapter 2 Materials and Methods

2.7.5 Biomass in benthic foraminifera

Foraminiferal test volumes (bio-volumes) from which the biomass calculations in this study derive, were limited to foraminifera with calcium carbonate tests. However, the tests of calcareous foraminifera have associated organic laminae and coverings of the inner test wall (Hansen 1999, Debenay et al. 2000, Allison and Austin 2003). The organics associated with the test are considered to be relatively refractile (DeLaca 1986). However, while the test as a whole contributes greatly to the weight of the individual, it is the sarcode (protoplasm) that contributes most to the actual biomass. Thus, biomass estimates would ideally remove any effect of the organics associated with the test, but this is difficult given the small size of some species. In addition, large variations in test thickness, both within and between individuals, make it extremely difficult to calculate actual protoplasm volume. For this reason most estimates of foraminiferal bio-volume rely on measurements of the external test wall.

2.7.5.1 Biomass estimates

Biomass estimates in this study were focused on the two dominant species of foraminifera from the Eden Estuary, *Haynesina germanica* and *Elphidium williamsoni*. The maximum test diameter of each foraminifera was measured under a binocular microscope (Wild MZ3) with the aid of an eyepiece graticule calibrated to a micrometer scale. Each subdivision of the graticule at the chosen magnification represented 18.5 μm and measurements were normally rounded to the nearest graticule subdivision. For all specimens, the percentage of the test filled with protoplasm was also noted. This allowed for a percent volume correction to be applied to test bio-volume estimates.

Sixty individuals representing a range of test diameters of both *Haynesina germanica* and *Elphidium williamsoni* were carefully measured to determine both maximum test diameter (equatorial diameter) and depth (polar diameter). From this, it was possible to regress a predictive linear model to calculate the depth of all tests subsequently measured (Equations 2.5 and 2.6).

Test depth (y, measured in μm) for both species can be described using the following equations, where x = test diameter (μm).
Elphidium williamsoni:
\[ y = 0.4631x - 8.8759, \quad R^2 = 0.9454 \] ........................................ (Equation 2.5)

Haynesina germanica:
\[ y = 0.3106x + 27.554, \quad R^2 = 0.8183 \] ....................................... (Equation 2.6)

The overall shape of both species is best described as an oblate spheroid (Murray and Alve 2000). Calculation of the bio-volume of the entire test was determined using the volume equation for an oblate spheroid (Equation 2.7).

\[ V = \frac{4}{3} \pi a^2 b \] ........................................................................ (Equation 2.7)

Where: \( a = \) equatorial radius (\( \mu m \)); \( b = \) polar radius (\( \mu m \))

Further translation from bio-volume to biomass requires a conversion factor. Two methods were compared; the first presented by Thomsen and Altenbach (1993) presents bio-volume in nano-litres (nl) and multiplies this by a correction factor of 0.06 (assuming this is a correction to wet weight, paper does not state). The second, by Murray and Alve (2000), calculates foraminiferal bio-volume by approximating test volume as an oblate spheroid and assumes a volume of 1 mm\(^3\) organic matter to be equivalent to 1 mg of organic matter. This is further converted to organic carbon assuming that 10% of the foraminiferal wet weight is organic carbon (biomass) (Gerlach et al. 1995).

Altenbach (1987) describes biomass in foraminifera as increasing exponentially with increasing in test size. The data set in this study revealed the best fit model to be a power function. However, this relationship may have differed if smaller individuals had been present for measurement (Figure 2.4 and 2.5).

Comparing the methods described by Thomsen and Altenbach (1993) and Murray and Alve (2000), there appears to be no difference in the relationship between organic carbon content and maximum test diameter. However, there are marked differences in the estimated values of organic carbon within each species depending on the method used (Figure 2.4 and Figure 2.5). Added to the graphs is a single direct measurements of organic carbon made by Moodley et al. (2000a).
Figure 2.4: Biomass estimates for *Elphidium williamsoni* collected from the Eden Estuary (various locations/dates) based on two models. □ Murray and Alve (2000), $R^2 = 0.993$. ○ Thomsen and Altenbach (1993), $R^2 = 0.993$. ▲ Moodley et al. (2000a) direct measurement of organic carbon content.

Figure 2.5: Biomass estimates for *Haynesina germanica* collected from the Eden Estuary (various locations/dates) based on two models. □ Murray and Alve (2000), $R^2 = 0.987$. ○ Thomsen and Altenbach (1993), $R^2 = 0.987$. ▲ Moodley et al. (2000a) direct measurement of organic carbon content.

These author's measurements appear to be the highest of all and may be due to the method of *in situ* acidification, which leaves organic material from test laminae and inner test lining in the sample. Murray and Alve (2000) do not take
the fact that protoplasm volume is often less than test volume into account, hence their method probably overestimates the carbon content. However, the analysis gives an idea of the possible spread of biomass within individuals (Figures 2.4 and 2.5).

For this study it was decided to use the method of Murray and Alve (2000) with an added correction factor for the percentage of test volume filled with protoplasm. Biomass was calculated for each individual and summed for each month. The number of individuals measured (maximum test diameter) each month ranged from 166 to 1104 for *E. williamsoni* and 76 to 906 for *H. germanica*.

### 2.8 Benthic foraminiferal taxonomy

Foraminifera were classified according to Loeblich and Tappan (1987). Sorting was conducted using a binocular microscope and a reflected light source. Individuals were placed onto a taxonomic slide with a black background and white numbered grid pattern. Samples were split into 1/8ths and these were counted until there were at least 300 individuals or more (Fatela and Taborda 2002).

### 2.9 Foraminiferal community structure

The quantitative count data were used to calculate community statistics such as the total number of species (N), Shannon Weiner diversity index (H'), Pielou's evenness (J') and Margalef species richness (d).

The Shannon Weiner index (Equation 2.8) has the advantage of including rare species in the calculation but not putting much weight on them. Therefore, the chance occurrence of a few individuals representing a species not previously collected will not greatly alter the index.

**Shannon Weiner Index**

\[
H' = - \sum \frac{n_i}{N} \log_2 \frac{n_i}{N}
\]

(Equation 2.8)

Where:

- \( n_i \) = the number of individuals in the species under consideration.
- \( N \) = the total number of individuals in the sample.
While the addition of a few rare species will change the index little, the evenness with which the species are distributed does change the value greatly. Consequently, when evaluating values of $H'$ some measure of evenness should be included (Equation 2.9). The maximum value of $H'$ occurs when all species are equally distributed. Any departure from complete evenness will have a value less than 1.

*Pielou's eveness*

$$J' = \frac{H'}{\log_2(S)}$$

(Equation 2.9)

Where:

- $S$ = the total number of species

The number of species counted in a sample will generally change in relation to sample size (Equation 2.10).

*Margalef Species Richness*

$$d = \frac{(S-1)}{\log_2(N)}$$

(Equation 2.10)

Where:

- $N$ = the total number of individuals in the sample
- $S$ = the total number of species

### 2.10 Isolating benthic diatoms

Benthic diatoms were isolated from field-collected sediment samples under laboratory conditions which mimic the natural semi-diurnal tidal cycle. Surface sediment was placed into large trays to a depth of 2 cm, filled with seawater and left until the next natural low tide, at which point the water was drained out of the tanks and light applied. The sediment surface was kept moist using an atomiser spray filled with seawater. As the point of low tide approached, brown patches become visible on the sediment surface. Two layers of filter paper were placed on the surface of the sediment, allowing benthic diatoms to migrate into them (Eaton and Moss 1966). After 1 hour the top layer was removed and washed in filtered seawater to release the diatoms. These were used to feed the foraminifera and carry out observations on their feeding. A similar method was used to collect motile benthic diatoms for natural abundance stable isotope ($\delta^{15}N$ and $\delta^{13}C$) analysis (Chapter 7).
2.11 Scanning Electron Microscopy (SEM)

Chosen specimens were mounted onto an S.E.M. stub, which had already been covered with double-sided tape. A sputter coater (Emscope SC 500) was used to coat the specimens with gold. A current of 15 mA at 1.2 Kvolts was applied for 30 minutes to attain a coating thickness of 15-20 nm. Pictures were then taken using a Jeol JSM-35CF SEM.

Where additional methods have been employed for specific laboratory experiments, these are described in the relevant chapter.
Chapter 3
3. Laboratory maintenance and growth observations of *Elphidium williamsoni*.

3.1 Introduction

The growth of an individual can be defined as an increase in length, volume or weight per unit of time. The most appropriate measure of growth may therefore vary between species. An accurate estimate of growth is important when studying the population dynamics of that species. In protista, such as foraminifera, their sensitivity to ambient conditions makes them susceptible to environmental change. For this reason, foraminifera exhibit a more direct relationship between growth and their environment than many higher organisms (e.g. molluscs).

Foraminifera express growth as the intermittent addition of chambers for multilocular forms, and an increase in size of a single chamber in tubular unilocular forms (Murray 1979, Goldstein 1999). Observations using light microscopy on the process of chamber formation in calcareous foraminifera are well documented (e.g. Goldstein 1999). However, knowledge of growth in unilocular foraminifera is limited for agglutinated forms and generally less well known for calcareous forms.

Foraminiferal growth takes on numerous elaborate forms. For example, planispiral foraminifera grow radially in the same plane around a single point, adding successively larger chambers as the protoplasm expands. Growth in such individuals is generally expressed by a relatively simple measure such as the maximum test diameter. By contrast, biserial foraminifera add chambers to alternate sides of the test forming two columns. In this case, growth is determined by a simple measure along the longest axis and is easier to determine than for planispiral forms (Thompson 1942).

In general, age and growth in foraminifera have been estimated by the analysis of population length-frequency data rather than direct measurement. However, several authors have examined foraminiferal growth in the laboratory (Bradshaw 1957, 1961, Freudenthal *et al.* 1963, Murray 1963, Wefer 1976, Ter Kuile and Erez 1984). Maximum test diameter and the number of chambers are the most frequently used measures of growth in foraminifera. However, these measures are discontinuous over time and are influenced by a host of
environmental variables (e.g. salinity, temperature, food quality and food concentration) (Murray 1963). In general, foraminiferal growth is described as being rapid in young individuals and slowing down towards maturity (Bradshaw 1957, Murray 1983, Murray 1991a). Once maturity has been reached the individual may reproduce if conditions are favourable or postpone reproduction if they are not. In the latter case, growth will be at an extreme minimum or cease. Cearreta (1988) reports the planispiral test diameter of *H. germanica* increasing 120 μm in the first month, 110 μm in the second month and 75 μm in the third month in a tidal pool in the Santona Estuary, Spain. The life cycle of this species is completed within three months; which was established by observing the growth of successive generations (from February to April 1985, June to August 1985, October to December 1985). Cearreta’s distinction of juveniles and adults is based upon the average test size of the species (both *H. germanica* and *A. beccarii*) after one month of growth (in this case 279 μm for both species) in accordance with Bradshaw (1957) and Boltovskoy (1964). From the growth rates reported by Cearreta, it can be inferred that both species were approximately 150 μm when first measured.

Many authors describe culturing intertidal foraminifera as easy, even in crude laboratory cultures (Lee *et al.* 1961). However, to establish axenic (grown in the absence of other micro-organisms) cultures one would require the facilities to culture axenic diatoms and maintain sterile seawater. In addition, serial washing of the foraminifera through antibiotic solutions would be necessary to rid them of bacteria and other protozoa which may contaminate the culture when the foraminifera themselves are introduced. It has also been demonstrated that bacteria are an essential part of the foraminiferal diet if reproduction is to be studied (Muller and Lee 1969). Therefore, if reproduction and subsequent growth is to be effectively studied, there are some technical difficulties which need to be overcome in establishing “reliable” laboratory cultures.

Initial investigations in this study focused on attempts to maintain the two dominant calcareous species of benthic foraminifera found in the Eden Estuary in culture, namely *Elphidium williamsoni* and *Haynesina germanica*. All attempts to establish perpetual cultures by means of attaining foraminiferal reproduction of successive generations in the laboratory failed. Instead, this chapter charts the growth and development of one asexually produced cohort of *Elphidium*
williamsoni. This was the only reproductive event seen during the culture/maintenance of foraminifera for this study. The difficulty and time required to isolate, relocate and measure foraminifera limits observations to small numbers (15-20 individuals).

3.2 Materials and Methods

3.2.1 Extraction of living foraminifera

Foraminifera were isolated from field collected surface scrapes taken from the high intertidal mudflats of the Eden Estuary, N.E. Scotland in March 2000 (Chapter 6, Figure 6.1). Individual foraminifera were picked from wet sediment using a fine bristle paintbrush (0000) trimmed to a fine point. Small groups of individuals (5 individuals) were transferred to petri dishes containing filtered seawater (< 0.2 μm) where they were washed to remove any adhering detritus. This was repeated several times in an attempt to limit the number of bacteria and other microorganisms which may be associated with the test. After washing, the foraminifera were placed into sterile petri dishes (Ø 5 cm) pre-filled with filtered (< 0.2 μm) local seawater (St Andrews Bay). All dishes were positioned in a climate-controlled room (10°C) with a 12 hours day and 12 hours night cycle. The temperature was chosen to reflect collection temperature (Table 3.1). Salinity of the water added over the experimental time period ranged from 32 to 34. The observations were conducted over a period of 179 days from 28th April 2000 to 25th October 2000.

3.2.2 Maintenance of cultures

Seawater was changed once a week to minimise bacteria and ciliates in the culture dishes. In a control experiment, ciliates and bacteria quickly dominated cultures when the seawater was not changed weekly. Food was added in the form of bulk sediment, which had previously been frozen in liquid nitrogen (-196°C) to kill all visible signs of life. This was checked by repeated examination of the sample under a binocular microscope after thawing. Three freezing and thawing cycles of approximately 3 minutes each were generally required. Bulk sediment (1 ml) was added once a week when the water was
changed. Changing the seawater would also have contributed an unknown amount of dissolved nutrients to the cultures.

Table 3.1: Summary of environmental conditions in March 2001 on the Eden Estuary, NE Scotland. Mean maximum and mean minimum air temperature data courtesy of RAF Leuchars. Mean maximum calculated as the average of the daily maximum temperature in °C. Mean minimum calculated as the average of night minimum temperature in °C.

<table>
<thead>
<tr>
<th>Tidal Height</th>
<th>High</th>
<th>Mid</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature in surface 1 cm of sediment (°C)</td>
<td>8.9</td>
<td>8.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Salinity of residual surface water</td>
<td>24</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Air temperature mean maximum (°C)</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air temperature mean minimum (°C)</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each petri dish was checked once a week for signs of foraminiferal reproduction using a binocular microscope. Finding the foraminifera generally took some time as they collect sediment and other detritus around the test, forming a "Cocoon" (Figure 3.1), which presumably provides camouflage and protection.

Figure 3.1: A benthic foraminifera encased in a cocoon of fine sediment and detritus. Length of cocoon is approximately 350 μm.
3.2.3 **Reproduction and growth**

One of the field collected individuals (Figure 3.2a) of *Elphidium williamsoni* was noted to have asexually reproduced on 28 April 2000 (Figure 3.2b). Juveniles were measured and counted where possible (initial number visible = 6). After 10 days individuals were isolated into fresh individual petri dishes as described above to allow direct measurement of a known individual, charting its increase in size and changing test morphology. At this stage of the experiment, 9 individuals were isolated. Weekly, when possible, each foraminifera was located, gently cleaned of any adhering detritus and sediment and measured using an eyepiece micrometer under an Olympus SZ60 binocular microscope. Individuals were returned to a clean petri dish with fresh filtered seawater and fresh detritus (treated as described above) and left undisturbed until the next observation seven days later.

### 3.3 Results

#### 3.3.1 Observations of the reproducing adult

Before reproduction occurred and immediately following collection, the individual had vivid cytoplasmic colouration and vigorous pseudopodial activity with no visible signs of cytoplasmic division (Figure 3.2a). On changing the water and sediment the adult readily formed a cocoon. After reproduction, the juveniles were visibly associated with the cocoon and remained so until removed 7 days later. Their maximum test diameter was approximately 99 μm with 2 to 3 chambers developed (Figure 3.2c). The proloculus diameters measured 57 μm ± 5 μm. At this stage, the adult test appeared white with no visible colouration and no pseudopodial activity (Figure 3.2b). The test wall of the terminal chamber had become very thin and a hole was apparent where juveniles may have emerged. Unfortunately, this terminal chamber collapsed when a closer examination of the specimen was performed. The adult had presumably died as a result of the reproductive event. After 7 days the juveniles were removed to individual petri dishes to facilitate charting individual growth.
3.3.2 Culturing and visual observations

A series of growth stages, following the release of the asexually reproduced juveniles were recorded (Figure 3.2). No other individuals from any of the isolated foraminifera specimens were seen to reproduce asexually. Sexual reproduction occurred within some cultures, visible by examining the protoplasm of individuals, which clearly showed a reduction and pattern as described in the literature (e.g. Goldstein and Moodley 1993). However, no juveniles were ever seen to grow in these dishes.

3.3.3 Survivorship

Given there were initially 9 individuals, survivorship has been calculated as a percentage of the initial number of individuals (Table 3.2). In general the greater proportion of individuals survived to 3 months after which point all measurements are based on one remaining individual.

<table>
<thead>
<tr>
<th>End of month</th>
<th>no.individuals</th>
<th>surviving (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
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<tr>
<td>6</td>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 3.2: Survival (%) of *E. williamsoni* individuals over the 6 months of culturing.
Figure 3.2: Photographic sequence showing an asexually reproducing *Elphidium williamsoni* and growth of juveniles. Number of chambers in the final whorl are given in parenthesis. (a) Adult collected on 17 March 2000; size 450 μm. (b) Adult has built a cocoon and reproduced asexually releasing juveniles (arrows point to juvenile specimens), 28 April 2000. (c) Juvenile 28 April 2000; size 90 μm. There are two chambers and the proloculus visible. P=Proloculus. (d) Juvenile on 17 May 2000; size 180 μm (7 chambers). (e), (f) and (g) Individuals on 25 May 2000; all approximately 250 μm (8-9 chambers). (h), (i) and (j) Individuals on 3 June 2000; sizes 250 μm, 231 μm and 346.5 μm respectively (8-9 chambers). (k) Individual on 26 June 2000; size 330 μm (10-11 chambers). (l) and (m) Individuals on 24 and 31 July 2000; size 379.5 μm and 412.5 μm (13 chambers). (n) Individual on 7 September 2000; size 503.25 μm. (o) Individual on 25 September 2000; size 528 μm (15 chambers).
3.3.4 **Relationship between maximum test diameter and age within cohort**

During the 6 months (179 days) following reproduction a total of 9 surviving specimens from the original cohort were monitored and measurements of maximum test diameters were taken every 7 days (Figure 3.3). Measurement of individuals was not always possible, due to the difficulty in finding them within individual petri dishes. Therefore, the data are discontinuous in parts. Linear regression ($R^2=0.891$, $P<0.001$) of the data suggests an average growth rate of 2.97 μm day$^{-1}$ over the experimental period (Equation 3.1). However, growth is significantly ($R^2=0.911$, $P<0.001$) better described by a polynomial, quadratic equation (Equation 3.2, Figure 3.3).

Maximum test diameter (MTD) can be described using the following linear and quadratic equations, where X = time (days):

\[
\text{MTD} = 2.97X + 110 \quad \text{(Equation 3.1)}
\]

\[
\text{MTD} = 77.19 + 4.15X - 7.80E^{-03}X^2 \quad \text{(Equation 3.2)}
\]

![Figure 3.3: Maximum test diameter (MTD) (μm) of a cohort of asexually reproduced juveniles of *Elphidium williamsoni* over 179 days. Line fitted is a polynomial, quadratic regression (Equation 3.2). Each measurement comprises between 1 and 6 individuals. After 93 days only one individual survived.](image-url)
3.3.5 *Growth expressed as number of chambers added over time*

Over the six months experimental period, the number of chambers in the final whorl of each specimen was counted (NOC). A half count (0.5) was used to describe a newly formed chamber which was not well calcified. Plotting these numbers for individuals against days of growth (Figure 3.4) reveals a steady increase with time, although plateaus (periods of no NOC increase) are apparent; notably around day 60. In order to make predictive estimates of the number of chambers (NOC) added to the test of *Elphidium williamsoni* maintained under such experimental conditions of growth, a quadratic (2nd order) polynomial regression ($R^2=0.900$) was fitted (Equation 3.3), where $X =$ time (days). This was a significantly ($P<0.001$) better fit than a linear regression ($R^2=0.840$) applied to the same data.

$$\text{NOC} = 2.72 + 0.13X - 3E^{-04}X^2$$.................................(Equation 3.3)

Figure 3.4: Total number of chambers in the final whorl versus days of growth for an asexually reproduced cohort of *Elphidium williamsoni*. Each measurement comprises between 1 and 6 individuals. After day 93 only one individual survived.
3.3.6 Mean growth rates

The mean growth rates, expressed as a change in test diameter with time (µm d\(^{-1}\)) for all individuals in the cohort are plotted in Figure 3.5. Maximum growth rates occurred during the first 3 days, assuming growth from an initial proloculus diameter of 57 µm. There was then a dramatic drop in growth rate to zero over the following 8 days, followed by less pronounced changes in the daily growth rate over the remaining period. Despite some uncertainty, the general pattern of mean growth rate appears to decreases asymptotically with time (Figure 3.5).

![Mean growth rate (µm d\(^{-1}\) ± SE) between measuring dates for a cohort of 9 asexually reproduced Elphidium williamsoni. Note error estimates are only possible up to 86 days; after 93 days only a single specimen survived.](image)

3.3.7 Growth of one individual over the 176 days

Viewing the cohort data generally fails to highlight the plateaus in growth when expressed as either maximum test diameter or the addition of chambers (Figures 3.3 and 3.4). One individual survived the whole 179 days and the rate of chamber addition in this individual clearly shows that growth is not continuous and that there are periods where growth is rapid and others when it appears to be much slower (Figure 3.6). Within the first month, this individual added 5.5 chambers from the first day of measuring, then 2, 2, 2, 1.5, 0 chambers were added into the last whorl in subsequent months. Clear plateaus, when apparently
no additional chambers were added in the final whorl, are visible (Figure 3.6). However, the overall number of chambers may have increased at these times, but are not visible as an addition in the final whorl. After the rapid addition of chambers between 0 and 27 days, no further increase in the number of chambers in the final whorl were observed over the next 8 days. A second growth phase observed between 35 and 59 days, followed by a pause of 20 days after which time the successive addition of chambers was relatively constant. The maximum number of chambers attained was greater than 15 within 149 days.

![Graph showing number of chambers in the final whorl and maximum test diameter versus days of growth for E. williamsoni](image)

**Figure 3.6:** Number of chambers in the final whorl and maximum test diameter versus days of growth for an individual of *E. williamsoni* from an asexually reproduced cohort, which survived for the 179-day experimental period.

No increase in maximum test diameter was observed over the first 11 days. Within one month of growth, maximum test diameter had reached 247.5 μm, after 2 months-363 μm, 3 months-412.5 μm, 5 months-544.5 μm, and 6 months-561 μm. Highest growth rates, expressed by chamber addition, occurred during the first and second months of growth.

As expected for a planispiral test form, the number of chambers (in the final whorl) and maximum test diameter are highly correlated ($R^2=0.978$, $P<0.001$). Both measures, for example, highlight intervals of no apparent growth.
around 27 to 35 days and 65 to 79 days (Figure 3.6). However, maximum test diameter shows a more continuous measure of growth than number of chambers in the final whorl, when the growth of one individual is examined (Figure 3.6).

3.3.8 Morphological development of individuals during growth

Juveniles were first evident as simple three chamber arrangements. The proloculus (57 μm ± 5 μm) was clearly visible as being larger than the next chamber, as expected in asexually reproduced (megalospheric) individuals (Figure 3.2c). At this stage, no septal bars were visible, and already each individual measured approximately 99 μm. After 19 days coiling was evident but the proloculus remained clearly visible. Individuals added a number of chambers (4 chambers in Figure 3.2d) and retral processes were visible, with approximately two between each chamber. Cytoplasm filled all but the most recent chamber. After 27 days, the proloculus became less evident and the outer whorl starts to cover earlier chambers. Retral processes averaged between 2-3 per chamber with those on the more recent chamber being slightly more prominent. In some individuals, at this stage, an umbilical boss was clearly visible. As individuals continued to grow the spiral form continued to cover the earlier whorls (involute), making it difficult to count the total number of chambers present under reflected light. The number of retral processes increased with chamber size and, as seen in the adult individual of 131 days (Figure 3.2n), there were five present between the final and penultimate chambers. Earlier growth in the final whorl reveals retral processes which had become relatively less prominent but still clearly visible.

3.4 Discussion

There are few specific growth rates published based on the direct measurement of small intertidal foraminifera such as Elphidium williamsoni. Bradshaw (1957) describes between 17 to 44 juveniles emerging at the two chamber stage (maximum diameter approx 78 μm) from the parental tests (250 to 350 μm) of Streblus beccarii (Linné) var. tepida (Cushman). These juveniles stayed with the adult (no mention of a cocoon) for several hours, after which time they moved away. Jepps (1956) describes reproduction in the fairly large (2 mm), shallow water foraminifera Polystomella (=Elphidium) crispum. Asexual
reproduction produced approximately 200 individuals with proloculus diameters of 60-100 μm. Within a few hours the young had moved away from the adult and calcified a second chamber. Within two days the same juveniles had calcified four chambers. Large populations drawn from the English Channel (Jepps 1956) revealed megalospheric individuals to outnumber microspheric (ratio approximately 30:1) during the winter months. From January, the proportion of megalospheric forms decreased and by March they represented less than half of the living population (Jepps 1956). In the present study of *Elphidium williamsoni*, early juveniles were observed to remain associated with the cocoon for at least 7 days, after which time the cocoon was broken in order to isolate individuals and chart their growth and development. It is possible that prior to reproduction, the adult *Elphidium williamsoni* collected food within the cocoon. Given the nature of the experiment, this cocoon structure may have provided the juveniles with a ready food source and protection. Dissolved organic matter may also be present as a food source, being leached from the dead adult or from bacteria feeding on any remnants of the adult.

Growth rates of juveniles reached a maximum over the initial 3 days of the experiment, following reproduction. No observed cytoplasmic division within the adult was observed three days prior to the emergence of juveniles. Therefore, within this 3 day period the adult had reproduced asexually to produce juveniles with proloculi of approximately 57 μm. The latter, by day 3, had also calcified one complete chamber and another chamber (third) being less well calcified (2.5 chambers). Day 0 mean maximum test diameter was taken to be that of the proloculus, as this would be the minimum size upon emergence at the earliest possible date. These rates of chamber addition after asexual reproduction are similar to those reported by Jepps (1956) for *Elphidium crispum*. One major difference between the reproductive event reported for *Elphidium crispum* and that reported here for *Elphidium williamsoni*, is the number of juveniles produced. In this study, only 9 juveniles were seen, whereas Jepps (1956) noted up to 200 juveniles emerging from a single specimen of *Elphidium crispum*. Although *E. crispum* tends to be a larger foraminifera, the size difference between these two species probably is not sufficient in its self to explain these data.

In asexual reproduction one would expect the number of juveniles
produced to be highly dependent on the biomass of the parent and there should be little loss of biomass (Hottinger 2000). In the present study, there appears to be a loss of biomass between month 0 and 1 (Figure 3.7). This is most likely due to the fact that not all of the living juveniles were initially detected and isolated. Reduced adult cytoplasm volume can be ruled out because observations made prior to reproduction described it as fully developed.

Figure 3.7: Total cohort biomass over the six months experimental period. Values in parentheses are the total number of individuals contributing to the biomass. Filled bar represents the estimated biomass (2.65 µg C) of the adult prior to reproduction.

Assuming no loss of adult biomass during asexual reproduction, the maximum number of juveniles expected can, in theory be calculated. This is simply calculated by approximating the biomass of a juvenile with a proloculus diameter 57 µm using the volume of a sphere. The maximum expected number of individuals can be calculated by dividing the reproducing adult’s biomass estimate prior to reproduction (2.65 µg C) by the biomass of a juvenile with proloculus diameter of 57 µm (0.01 µg C). Alternatively, since these calculations are derived from estimates of test volume, a simple division of adult biovolume by proloculus volume should yield the expected maximum number of juveniles. Thus the studied individual of *Elphidium williamsoni* (450 µm) could have produced up to 26 juveniles (proloculus 57 µm). This is somewhat lower than the fecundity reported for *Streblos (Ammonia) beccarii* of between 17 to 44 juveniles, depending on adult test size (250 to 350 µm, respectively) (Bradshaw 1957).

Over the first 3 months there was a rapid increase in biomass, after which
point there is only one surviving individual. These results hint at the presence of a mechanism (= rapid growth) for significant changes in biomass over short periods.

Bradshaw (1957) suggests that reproduction in *S. beccarii* does not occur below approximately 20°C. However, these foraminifera were collected from San Antonio Bay, Texas from a water depth of six feet (1.7 m). During this study, 10°C was a realistic temperature reflecting field conditions at this time of year (Table 3.1) on the Eden Estuary. However, over the six month experimental period the laboratory temperature was maintained at 10°C, while outside field conditions would, in general, be warming up to a maximum around July or August (24.4 and 25.2°C) (Chapter 6, Table 6.3). Therefore, laboratory conditions did not mimic warming in the field, and may have affected the survivorship and growth rate of individuals.

Growth rates of some foraminifera have been shown to vary according to depth, temperature, light intensity, feeding and season (Bradshaw 1957, 1961, Murray 1963, Ter Kuile and Erez 1984). However, many of these studies were carried out on the larger symbiont-bearing benthic foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii*.

The culturing/maintenance environment applied in this experiment mimicked field collection conditions (early spring, March 2000, Figure 3.1) in Scotland. During this time of year the estuary experiences low temperatures and reduced salinities due to higher rainfall, resulting in increased river flow and land runoff. Thus, due to limiting factors such as reduced food availability and fluctuating environmental conditions, growth could be expected to be at or near a minimum at this time of year. Therefore, with increasing food availability (labile organic matter) and more stable environmental conditions and higher temperatures occurring towards spring and summer, growth rates would be expected to increase.

Several seasonal studies of the intertidal area have reported a lack of small juveniles, despite sediment samples being sieved at 63 μm. This lack of juveniles has generally been thought to be due to the processing of the samples (e.g. drying of samples) (Cearetta 1988, Basson and Murray 1995, Murray and Alve 2000). However, in the present study (Chapter 6) there was a distinct lack of any juveniles <93 μm, despite the fact that sieving was carried out at 63 μm.
and all samples were processed wet.

This study demonstrates that an asexually produced *E. williamsoni* can grow 148 μm in its first month, under spring conditions, using field collected sediment as a food source. Cearetta (1988) reports from field collected population studies that *H. germanica*, on average grows 120 μm in the first month.

The data collected in this experiment and those presented by Cearetta (1988) suggest that both *H. germanica* and *E. williamsoni* were growing rapidly enough during the first 4 weeks (month) for there not to be a visible peak in small individuals (63 – 100 μm) following reproduction. However, based on the growth estimates mentioned above there should be a visible peak in the 240 μm to 300 μm size range one month after reproduction. This is apparent for the Eden Estuary (Chapter 6) where reproduction was identified as a downward shift in mean size distribution from the previous month (Chapter 6, Figure 6.17 and 6.18). However, no peaks in field-collected individuals <93 μm and > 63 μm were recorded, suggesting that the laboratory grown juveniles of this study are representative of the natural population.

Several authors have argued (for the deep-sea) that foraminifera are able to reproduce rapidly and take advantage of seasonal pulses in phytodetritus (Gooday and Turley 1990, Gooday 1993). In general, such species (*Stainforthia fusiformis*, *Epistominella exigua* and *Alabaminella weddellensis*) are described as being small, thin walled and displaying opportunistic characteristics (Gooday 1993, Alve 1994). However, Hughes *et al.* (2000) record high numbers of *Hoeglundina elegans* juveniles from the northeast Atlantic in July 1998 measuring 195 μm to 220 μm, with 91% of juveniles having 4 chambers. This species was absent or occurred in very small numbers at the sampling site during May 1998. This suggests reproduction and growth to >195 μm in <67 days. Unlike the other opportunistic deep-sea species described, *H. elegans* has a large test and thick aragonitic wall. In the Celtic Sea, *Stainforthia fusiformis* appears to reproduce and grow (test length) to >150 μm in less than 1 month following the spring phytoplankton bloom (Austin pers. comm. 2002). These data span intertidal, shelf-sea and deep-sea environments and all demonstrate comparable growth rates within the first month.
3.5 Conclusions

The high growth rates documented within the first month following asexual reproduction in *Elphidium williamsoni* suggest an ecological advantage under rapidly changing environmental conditions. High growth rates lead to rapid increases in species biomass and support the argument that intertidal benthic foraminifera are an important component within benthic food webs.

The growth data presented here are unlikely to represent maximum rates for *E. williamsoni*, given the sub-optimal experimental conditions (e.g. no tidal lab environment). It is, however, likely that growth rates increase during the warmer months, when food is more readily available (see discussion in Chapter 6). The documented reproduction at 10°C suggests that populations could be maintained by asexual reproduction during at least six months of the year (Chapter 6, Figure 6.5).

Therefore, despite the limited size data available, it appears that the selection of a 63 μm mesh size is appropriate to capture juvenile foraminifera in field collected material from intertidal environments. The size distribution of laboratory grown *E. williamsoni* closely matched the natural population, with no specimens <93 μm observed.

Care should be taken in extrapolating any of the data presented here beyond the measured environmental conditions. While the data are limited, they do provide valuable insight into the pattern and rate of growth in this species. In addition, the morphological changes charted during growth might warrant further study under differing experimental conditions in species susceptible to ecophenotypic variation. Further efforts to establish perpetual cultures and conduct ecological experiments are likely to yield important new insight into foraminiferal ecology.
4. The relative uptake of $^{13}$C-enriched benthic diatoms by intertidal benthic foraminifera and ostracoda.

4.1 Introduction

For several decades a number of authors have suggested that the meiofauna form a potentially important link in estuarine benthic food webs (Fenchel 1978, Coull and Bell 1979, Coull 1999). However, the ecology and trophic position of many meiofauna, such as benthic foraminifera, is not well defined.

Laboratory experiments with cultured foraminifera have clearly demonstrated that they readily exploit benthic diatoms as a food source (Murray 1963, Lee et al. 1966, Lee 1980). From $^{32}$P- and $^{14}$C-labelling laboratory experiments, Lee et al. (1966) reported that the diatom species *Cylindrotheca closterium* is utilised by several foraminifera genera including *Ammonia*, *Allogromia* and *Elphidium*.

However, several studies over the past decade have suggested that foraminifera show a low preference for labile carbon in the form of phytodetritus (Rudnick 1989, Widbom and Frithson, 1995). Widbom and Frithsen (1995) report that, in a $^{14}$C-labelling mesocosm experiment, most meiofaunal species showed a preference for fresh labile carbon compared to older more refractory carbon. However, foraminiferal assemblages dominated by *Ammonia beccarii* and *Elphidium* sp. were an exception to this general rule, with low uptake values (specific activity, 0.15 ±0.04 disintegrations per minute (DPM) µg C ±SE). Ostracoda, on the other hand, exhibited one of the highest uptake values among the meiofauna investigated (specific activity, 13.5 ±0.04 DPM µg C ±SE). Data concerning foraminifera from the above study are comparable with Rudnick (1989) who suggested that roughly 90% of the carbon assimilated by foraminifera was “old” carbon.

One of the few studies to examine the transfer of carbon directly from benthic microalgae to meiofauna is that of Rivkin and DeLaca (1990). They report that foraminiferal rates of ingestion (2.5 to 3.5 ng C mg$^{-1}$ wet wt$^{-1}$ d$^{-1}$) of $^{14}$C-labelled microalgae were much lower than other meiofauna examined (*Edwardsia meridionalas* (burrowing anemone), small polychaetes and clams). However, foraminifera assimilated ingested algal carbon more rapidly than the other
meiofaunal groups. In $^{14}\text{C}$-labelling laboratory experiments, Olafsson et al. (1999) demonstrated that some ostracoda species accounted for a large proportion (46%) of the labelled carbon taken up by the meiofauna.

More recently, pioneering field and laboratory experiments using the stable isotope $^{13}\text{C}$ as an in situ tracer of sedimenting phytodetritus (Blair et al. 1996, Levin et al. 1999, Moodley et al. 2000a, 2002) have presented results which contradict those of Rudnick (1989), Widbom and Frithsen (1995) and Olafsson et al. (1999). For example, Levin et al. (1999) demonstrated that continental margin ostracoda showed a limited uptake of $^{13}\text{C}$-labelled phytodetritus after 1 day, reflected in their $\delta^{13}\text{C}$ values, whereas some foraminifera (e.g. Allogromiids, Astrorhiza-like forms) exhibited $\delta^{13}\text{C}$ values indicative of high label uptake after 1 day.

In laboratory experiments, Moodley et al. (2000a) reported that intertidal foraminifera of the genus *Ammonia* responded quickly to a simulated phytodetritus event (addition of the $^{13}\text{C}$-enriched, freeze-dried fresh water green algae *Chlorella*). *Ammonia* ingested between 1 and 7% of the added carbon within 3 to 53 hours, respectively.

In the same experiments, Moodley et al. (2000a) observed $\delta^{13}\text{C}$ enrichment values (uptake) of *Haynesina* and *Elphidium* to be much reduced in comparison to *Ammonia*. This limited response from *Haynesina* and *Elphidium* may further suggest a preference for other, specific sources of carbon (e.g. living diatoms, planktonic or benthic).

Several authors have recorded in laboratory feeding experiments that the colouration of foraminiferal cytoplasm reflects that of their prey species (Murray 1963, Lopez 1979, Bernhard and Bowser 1999, Moodley et al. 2000a). This phenomenon has also been noted in individuals collected from the field at certain times of the year (Knight and Mantoura 1985, Murray and Alve 2000). *Haynesina germanica* cytoplasm has been described as varying from dark green with mottled brown to pale cream and *Elphidium williamsoni* cytoplasm as being dark to light green with brown mottling not always visible (Knight and Mantoura 1985). The mottling effect in the cytoplasm has been attributed to chloroplast retention/sequestering.

Moodley (pers. comm. 2001) has attempted to feed freeze-dried *Chlorella* and planktonic diatoms to *Haynesina*, with no immediate response observed.
However, the same genus will eventually utilise this food source if nothing else is made available. This may suggest that ‘living’ or ‘recently dead’ diatoms are their preferred food at certain times of the year.

Most stable isotope labelling experiments have been carried out using phytoplankton species (e.g. *Thalassiosira pseudonana*, *Skeletonema costatum*) and the fresh water green algae *Chlorella*. One exception to this is the study, carried out by Rivkin and DeLaca (1990), that utilised an *in situ* benthic microalgal community dominated by the diatom *Amphora antarctica*.

In estuarine intertidal environments up to 45% of gross primary production is synthesised by benthic diatoms (MacIntyre and Cullen 1996, Underwood and Kromkamp 1999) and hence their contribution to the carbon flow in coastal sediments should not be overlooked (Middelburg et al. 2000). The benthic diatom *Cylindrotheca closterium* was chosen for enrichment in this study because it is both ubiquitous in intertidal environments and widely available in laboratory cultures.

This study aims to determine whether four genera of intertidal benthic foraminifera and ostracoda ingest fresh ‘labile’ benthic diatom carbon under experimental conditions. In addition, ingestion/uptake measurements over a five-day experimental period were monitored to ascertain the form of any uptake response. Secondly, it will determine if fixation of samples in a rose Bengal and buffered formalin (4%) preparation significantly changes the isotopic signature incorporated in the protoplasm of three genera of calcareous foraminifera. This latter step was undertaken as freezing is not always a preservation option available during field studies.

### 4.2 Materials and Methods:

#### 4.2.1 Diatom culture

Axenic cultures of the benthic diatom *Cylindrotheca closterium* enriched in $^{13}$C were prepared prior to experimentation. Cultures were grown in an artificial seawater mixture with added vitamins and trace metals at 15°C (Table 4.1). Culture flasks (1,000 ml Pyrex conical flask) were exposed to fluorescent strip lights and lightly mixed daily by gentle circular motions of the culture vessel. Enrichment of the diatoms was achieved by the addition of 30% enriched $^{13}$C.
bicarbonate (Sodium Bicarbonate $^{13}$C, 99%, Cambridge Isotope Laboratories inc.) to the seawater medium at the start of culturing.

Diatoms were harvested and concentrated by gentle centrifugation after 7 days. Repeated washes with isotonic artificial seawater filtered to 2 $\mu$m were carried out in order to remove any unincorporated label. Axenic integrity of the diatom culture was checked prior to use by microscopy.

Table 4.1: Major salt and nutrients of the artificial seawater mixture in which diatoms were cultured.

(a) Major salt components

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<td>1</td>
</tr>
</tbody>
</table>

(b) Major nutrients

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Conc. medium (g l$^{-1}$)</th>
<th>Conc. Stock (g l$^{-1}$)</th>
<th>stock medium (mM l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO$_3$</td>
<td>0.34</td>
<td>34</td>
<td>10</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>0.075</td>
<td>75</td>
<td>1</td>
</tr>
<tr>
<td>NaH$_2$PO$_4$ 3H$_2$O</td>
<td>0.005</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>NaSiO$_2$ 9H$_2$O</td>
<td>0.015 – 0.030</td>
<td>15 – 30</td>
<td>1</td>
</tr>
<tr>
<td>M2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V8</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† NaHCO$_3$ For making up 1 litre: labelled 0.102g normal 0.248g

†† Trace metal mix

††† Vitamin mix
4.2.2 Field collection

Surface scrapes and intact sediment cores were collected during a late afternoon ebb tide on 17th April 2001 from an intertidal site on the Oostersheld, a marine embayment on the southwest coast of The Netherlands (Figure 4.1). Four intact 10 cm diameter (area 78.5 cm$^2$) cores of approximately 17 cm depth were collected along with surface scrapes for background isotope measurements on all foraminifera genera and ostracoda. Two further 10 cm diameter slices were taken to a depth of 1 cm for use in the measurement of any passive uptake of label by recently dead foraminifera ("living" at the time of collection). Cores were transported in an upright position to the laboratory, and immediately taken to a climate-controlled room. The temperature (9°C) had been set to reflect field conditions at the time of collection. All cores were maintained in the dark throughout the experiment. Filtered seawater (39 μm) from the site already cooled to experimental temperature was added to the cores. In order to minimise disturbance to the sediment surface, bubble wrap cut to fit the inside diameter of the core was gently placed over (not on) the sediment surface. As water is added the plastic floats up and can be easily removed once the core has been filled (Moodley et al. 1997). Cores were aerated, parafilm placed over the tops to reduce evaporation and subsequent increase in salinity, and left to stabilise overnight.

4.2.3 Experimental set up

Following core stabilisation, 6 ml (71.9 mg dry weight) of the concentrated diatom culture was added to each of four experimental cores (two day 1 cores and two day 5 cores). The same volume (6 ml) of concentrated diatom culture was reserved for freeze-drying to determine the dry weight added to the experimental cores. Average added organic enrichment was 0.92 mg dry wt cm$^{-2}$.

Addition of the 6 ml of diatoms was achieved using a long sterile Pasteur pipette and slowly moving it above the core surface without disturbing the sediment, ensuring as even a cover of diatoms as possible.
The relative uptake of $^{13}$C-enriched benthic diatoms

Figure 4.1: Location map showing the sampling area on the Oosterschelde (51°33N, 4°3E) north of Yerseke. Illustrated in relation to the rest of The Netherlands (inset).

To ensure that uptake of the label by the foraminifera was active (i.e. foraminifera choose to ingest the diatoms) and not merely being incorporated passively, the top 1 cm of an additional sediment core (diameter 10 cm) collected at the same time was added to a plastic container (500 ml) filled with 10% buffered formalin. As the diatoms were added to the experimental cores, 6 ml was also added to the container which, by this time, contained freshly dead foraminifera and ostracoda. Again this preparation was left to run over the five day experimental time period.

Cores were left un-aerated for one hour to allow the diatoms to settle, leaving a clearly visible thin green layer on the sediment surface. A perspex lid containing a stirrer was then placed on top of the core and aeration continued. All cores were incubated in the dark. After 1 day the green layer had been much reduced, either by animals grazing the diatoms or mixing of the surface sediments due to bioturbation or because of diatom migration into the sediment.

Fifty to sixty foraminifera of each of the genera *Haynesina* and *Ammonia* were extracted from surface scrapes, grouped together and added to separate petri dishes. Each petri dish was pre-filled with artificial sand (Merck) of diameter <125 μm and seawater filtered to 2 μm. During addition of the diatoms to the experimental cores, a little of the inoculum was added to each petri dish. As there
was no competition from other organisms, or removal of diatoms by bioturbation, the foraminifera had direct un-interrupted access to the diatom food source. It was intended this would reflect a possible maximum uptake over the five-day experimental period and provide additional insight into the role of competition and removal of added food.

4.2.4 Sample processing

Two cores were removed from the experimental setup after one day and two after five days. The top 1 cm of sediment was immediately sliced and washed using cooled seawater from the sampling area over a 39 µm sieve in order to remove the fine sediment fraction and any excess diatoms. The sample was then divided in two. One half was frozen at -80°C for the collection of foraminifera and ostracoda for stable isotope analysis. The remaining half was fixed and stained with a buffered formalin (4%) and rose Bengal mixture for later determination of standing stock and stable isotope analysis on ostracoda and foraminifera. The latter sample was used to further investigate the effect of rose Bengal and formalin on δ13Corg isotope signatures.

4.2.5 Picking and transferring foraminifera to silver boats

Living specimens from each foraminiferal genus (29-60 individuals) and Ostracoda (13-17 individuals) were hand-picked (0000 brush) from samples using a binocular microscope. Prior to experimentation, extensive observations were carried out on living foraminifera using both binocular and an inverted microscope to examine pseudopodial activity. This allowed strict criteria for picking living foraminifera and ostracoda to be developed prior to sample processing (Chapter 2). Living specimens were readily recognised by the collection of sediment and detritus around their aperture.

Foraminifera and ostracoda were removed to filtered seawater (2 µm) and vigorously cleaned with a 0000 brush to remove any adhering particles and diatoms. They were then washed in demi-water, concentrated and transferred to pre-combusted silver boats (4 hours at 550°C) using a sterile glass pipette, frozen (-20°C) and dried in vacuo. Decalcification, to remove the calcium carbonate (CaCO3) test, was carried out in situ (in boats) using 2.5% HCl for foraminifera
and 5% HCl for ostracoda (Moodley et al. 2000a). Samples were subsequently dried in a clean oven at 50°C overnight, folded and sent for analyses.

4.2.6 Stable isotope measurement: Methods, machines and equations

Measurements of $\delta^{13}C_{org}$ of ostracoda and foraminifera were carried out using a Carlo Erba®1106 Elemental Analyser coupled online with a Finnigan®Delta S isotope ratio mass spectrometer.

Carbon isotopes are expressed as a ratio of the heavier $^{13}$C to lighter $^{12}$C isotopes in the sample compared to a standard, in this case a marine limestone fossil Pee Dee Belemnite (PDB). The differences in ratios calculated are commonly referred to as delta notation (δ) and have units of per mil (‰) (Equation 4.1):

$$\delta^{13}C_{(organic)} = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{reference} - 1] \times 1000 .......(Equation 4.1)$$

The uptake of labelled diatoms enriched in $^{13}$C was recorded after correction for background values. This can further be expressed as specific uptake commonly referred to as “delta delta” notation ($\Delta\delta$):

$$\Delta\delta^{13}C_{(organic)} = (\delta^{13}C_{sample} - \delta^{13}C_{background}) .......(Equation 4.2)$$

4.3 Results

4.3.1 Chloroplast sequestering

Specimens of *Ammonia* were examined with the aid of a confocal microscopy (Leica TCS-NT) during this study. At this sampling site, a field collected individual of *Ammonia* contained intact chloroplasts, with the highest concentrations of chloroplasts found in the last few chambers (Figure 4.2). This demonstrates that benthic foraminifera of the genera *Ammonia* do ingest chloroplasts.
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Figure 4.2: Confocal microscope image of a decalcified field collected *Ammonia* from the Oosterschelde. The image shows numerous chloroplasts present within the protoplasm, seen here as red colouration. Note the higher concentration of chloroplasts in the last few chambers. A wavelength of 488 nm was used to excite Chl $a$ and the fluorescence signal detected at 512 nm.

4.3.2 Community structure

Enumeration of meiofauna (numbers of specimens) found in the surface 0 to 0.5 cm of sediment revealed Nematoda to represent 45% of the total assemblage, Foraminifera 44%, Copepoda 9% and Ostracoda 2% (Figure 4.3). This clearly illustrates that foraminifera are numerically as important as nematodes at this sampling site. However, this balance applies only to the surface 0 to 0.5 cm, below this depth nematodes dominate at 99%.

Percentage abundance of living foraminifera and ostracoda show the most dominant genus to be *Haynesina* (47%), followed by *Ammonia* (25%), *Elphidium* (14%), atestate foraminifera (12%) and ostracoda (2%). This remained so over the five-day experimental period. However, there was a significant (2 way ANOVA on square root transformed data; $p=0.000$) reduction in the number of “live” individuals of each genus in the experimental cores over the five-day experimental period. Although this shows experimental conditions to be sub-optimal for all.
genera examined, the average foraminiferal standing stock (number of individuals per 10 cm$^2$) over the experimental time period remained high enough to collect a sufficient number of individuals for measurement (Table 4.2).

Figure 4.3: Pie chart illustrating percentage abundance of the major meiofauna taxa found in the surface 0 to 0.5 cm of sediment at sampling site on The Oosterschelde.

Table 4.2: Average standing stock (number of “live” individuals 10 cm$^2$) of foraminifera and ostracoda ± Standard Error (SE) over the five-day experimental period.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Average standing stock (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haynesina</td>
<td>386 ± 44.9</td>
</tr>
<tr>
<td>Ammonia</td>
<td>207 ± 8.50</td>
</tr>
<tr>
<td>Elphidium</td>
<td>118 ± 14.4</td>
</tr>
<tr>
<td>Atestate foraminifera</td>
<td>96 ± 11.5</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>18 ± 1.31</td>
</tr>
</tbody>
</table>

4.3.3 Background stable isotope signature

Background measurements (samples direct from the field) of $\delta^{13}C_{\text{org}}$ were made on 3 genera of calcareous foraminifera, 1 atestate foraminifera and 1 ostracoda to provide a baseline reading for $\delta^{13}C_{\text{org}}$ (Table 4.3). In general, over both 1 and 5 days, a very marked enrichment in $\delta^{13}C_{\text{org}}$ from background values was found. All background measurements were similar to those recorded by Moodley et al. (2000a). However, slight depletions in $\delta^{13}C_{\text{org}}$ associated with Haynesina (this study: -10.22 %o, Moodley et al. 2000a: -12.93 %o) and Ammonia (this study: -12.81 %o, Moodley et al. 2000a: -16.62 %o) were noted. These differences may reflect seasonal changes in the source of $^{13}C$ in the natural diet. This experiment and sediment collection was conducted in April 2001 and
Moodley et al. (2000a) collected in October 1998. Subsequent experimental $\delta^{13}$C$_{org}$ values for each genus were compared to these background values to give the specific uptake ($\Delta \delta^{13}$C$_{org}$) (Equations 4.2 and Table 4.3).

Background measurements of $\delta^{13}$C$_{org}$ made on preserved (rose Bengal and formalin) samples showed a slight depletion of $^{13}$C (more negative $\delta^{13}$C$_{org}$ value) in Ammonia and Haynesina, whereas, in Elphidium enrichment in $^{13}$C (more positive value) was recorded (Table 4.3). However, a two way ANOVA at 5% significance shows there to be no significant difference ($p=0.744$) in background $\delta^{13}$C between the two treatments for the three calcareous genera measured. The small differences observed may therefore, reflect a measure of variability in sampling a natural population and do not suggest any systematic offset in $\delta^{13}$C$_{org}$ following sample preservation in rose Bengal and formalin.
Table 4.3: Background $\delta^{13}C_{\text{org}}$ ratios for the major foraminifera and ostracoda taxa used in an experiment to determine relative uptake ± Standard Error (n=2). Mean specific uptake ($\Delta \delta^{13}C_{\text{org}}$) for both treatments (fresh and rose Bengal formalin preserved).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Background Field Measurements</th>
<th>Background Rose Bengal &amp; Formalin Preserved Measurements</th>
<th>Passive Uptake Formalin buffered</th>
<th>Mean Specific Uptake $\Delta \delta^{13}C_{\text{org}}$ (%)</th>
<th>Mean and SE</th>
<th>Mean Specific Uptake $\Delta \delta^{13}C_{\text{org}}$ (%)</th>
<th>Mean and SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day1 Frozen Samples</td>
<td></td>
<td>Day5 Frozen Samples</td>
<td></td>
</tr>
<tr>
<td>$Ammonia$</td>
<td>-12.81±1.39</td>
<td>-16.53±0.500</td>
<td>-11.99*</td>
<td>998±192</td>
<td>765.40±55.3</td>
<td>864.8±92.7</td>
<td>1054.1±81.1</td>
</tr>
<tr>
<td>$Haynesina$</td>
<td>-10.22±0.42</td>
<td>-11.73±0.165</td>
<td>-10.07*</td>
<td>392±121</td>
<td>452.42*</td>
<td>335.2±14.4</td>
<td>1124±546</td>
</tr>
<tr>
<td>$Elphidium$</td>
<td>-14.53±0.29</td>
<td>-11.52±6.57</td>
<td>-13.57*</td>
<td>687±121</td>
<td>586.30±68.80</td>
<td>569±252</td>
<td>785.90±40.9</td>
</tr>
<tr>
<td>Atestate foraminifera</td>
<td>-12.21*</td>
<td>-11.52±6.57</td>
<td>-3.98*</td>
<td>1635±427</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ostracoda</td>
<td>-10.73±1.04</td>
<td>-6.81*</td>
<td>260±124</td>
<td>306.04±175.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Error is the range about the average of duplicate measurements (n=2), unless otherwise stated.
*Only one measurement made.
4.3.4 Mean specific uptake after day 1 and day 5 in fresh samples

All taxa examined showed a rapid response/uptake of the added diatoms after 1 day (Figure 4.4). Atestate foraminifera showed the highest specific uptake after 1 day (1,635 ± 427 ‰) and the five-day reading was reported as off scale, possibly implying that they continued to ingest the diatoms at a high rate. Alternatively, this could indicate that the foraminifera stopped ingesting the labelled diatoms and did not retain any of the label ingested after 1 day. In this study the off scale reading was considered to be the former. *Ammonia* also showed a rapid response/uptake (998 ± 192 ‰) after 1 day. Very little change is seen in the isotopic signature after 5 days for any of the taxa examined. As only 2 repeat measures were carried out on each genus, the standard errors show large variation between samples, making it difficult to determine differences between genera. In fact, a univariate analysis of variance (type IV sum of squares) at 5% significance, after removing the atestate foraminifera data, shows there to be no significant difference between uptake after 1 day and after 5 days (p=0.236). However, this test revealed that there was a significant difference between the specific uptake of the different genera (p=0.011). A further *post-hoc* test (Tukey) revealed the difference between *Ammonia*, *Elphidium* and ostracoda to be significant (P=0.002, P=0.022) and that between *Haynesina* and ostracoda to be insignificant (P=0.117). The two most similar genera were *Haynesina* and *Elphidium* (p=0.728).

![Figure 4.4: Mean specific uptake (Δδ¹³Corg (%)) of the major taxa examined in the surface 1 cm of sediment in the experimental cores after 1 day (unshaded) and 5 days (shaded). Values in parenthesis indicate mean background δ¹³Corg values for the genera.](image-url)
4.3.5 Mean specific uptake after day 1 and day 5 in rose Bengal and formalin preserved samples

The three calcareous foraminifera genera preserved in rose Bengal and formalin prior to measuring $\delta^{13}$C$_{org}$ also showed a rapid response to the added diatoms after 1 day (Figure 4.5). Therefore, the preservation had no effect on the scale of the response signal. However, large variation among individuals is again evident. A univariate analysis of variance (type IV sum of squares) at 5% significance shows there was a significant difference between mean specific uptake of individual genera ($p=0.011$). Preserving the samples in rose Bengal and formalin prior to measurement did not significantly alter the $\delta^{13}$C$_{org}$ (%o) measured for *Ammonia*, *Haynesina* and *Elphidium* (a univariate analysis of variance (type IV sum of squares) at 5% significance shows $p=0.475$).

![Figure 4.5: Mean specific uptake ($\Delta\delta^{13}$C$_{org}$ (%o)) of the 3 calcareous benthic foraminifera genera examined in the surface 1cm of sediment in the experimental cores after 1 day (white) and 5 days (shaded) with subsequent preservation in rose Bengal and formalin preparation.](image)

4.3.6 Passive uptake

Since only one measurement was made for each taxon the differences cannot be analysed statistically. It would appear that no marked enrichment of $\delta^{13}$C$_{org}$ from background occurred. However, a slight depletion was recorded for *Ammonia* and a larger depletion in the atestate foraminifera and Ostracoda. *Haynesina* showed a very slight enrichment (Table 4.3). These results suggest that
the enrichment observed in the foraminifera during experimentation were due to the ingestion/uptake of $^{13}$C-labelled benthic diatoms.

4.3.7 Direct exposure to diatoms

Individuals incubated in petri dishes, devoid of competition for food and the effects of bioturbation, gained uninterrupted access to the added food supply. Ammonia showed $\delta^{13}$C$_{org}$ values indicative of significant enrichment (3,030%). Measurements for Haynesina were returned as off scale.

4.4 Discussion

Within an estuarine environment microphytobenthos have been shown to contribute up to 45% of the gross primary production, compared to 52% by phytoplankton (MacIntyre and Cullen 1996, Asmus et al. 1998, Underwood and Kromkamp 1999). This clearly highlights the potential importance of microphytobenthos as a mediator in the carbon budget of an estuary (Middleburg et al. 2000). If benthic foraminifera respond quickly to increases in microphytobenthos biomass ("blooms"), this would make them an important link in the lower benthic food web (Lee et al. 1966), and microbial loop.

In this study, all the taxa investigated responded rapidly (within 1 day) to the simulated benthic diatom bloom. This is reflected in the $\delta^{13}$C$_{org}$ enrichment values recorded. Within one day, enrichment values had reached a steady level and remained high for the 5 experimental days. However, there were no data for days two to four, over which time there may have been fluctuations in the $\delta^{13}$C$_{org}$ values.

This response could be interpreted as (1) satiation after one day, followed by the ability to maintain a steady ingestion and assimilation rate, or (2) ingestion has stopped and the carbon retained within the foraminifera has been assimilated and few losses have yet occurred to the surrounding environment, or (3) as the added food resource becomes depleted (grazed and bioturbated), there is a dilution effect (i.e. the added food becomes mixed with sediment and becomes harder for the foraminifera to find). Foraminifera are more sedentary than most other meiofauna making them less able to forage for food. Therefore, the amount of added food the foraminifera can find in any given time period may have been much reduced by these processes of depletion. Moodley et al. (2000a) reported a
similar pattern for *Ammonia* after 53 hours when fed on freeze dried *Chlorella*. In this experiment all five taxa examined showed the same pattern to varying degrees. However, Rivkin and DeLaca (1990) demonstrate that foraminifera synthesise 2 to 5 times more protein than metazoan meiofauna. They also report the ability of foraminifera to rapidly assimilate ingested algal carbon (within hours). In order to determine any long-term patterns one would need to extend the experimental time period and measure losses due to respiration and egression.

Although $\delta^{13}$C$_{org}$ enrichment values for *Ammonia* clearly showed uptake ($1,054 \pm 81.1 \%o$), the values were much lower than those reported by Moodley *et al.* (2000a) ($>2,500 \%o$) for the uptake of freeze-dried *Chlorella*. Differences in enrichment between studies may illustrate that although *Ammonia* will respond rapidly it has a preference for freshly dead algal carbon. The atestate foraminifera showed the most rapid response ($1,635 \pm 427\%o$) after 1 day. Unfortunately, both day 5 readings were off scale, which could indicate that they continue to ingest benthic diatoms at a high rate and do not become satiated after 5 days.

Both *Haynesina* and *Elphidium* showed a very poor response to added freeze-dried *Chlorella* during experiments conducted by Moodley *et al.* (2000a). After half a day *Elphidium* exhibited specific uptake values of 40 $\%o$, falling to 10 $\%o$ after 2 days. *Haynesina* showed a reduced response (1 $\%o$) after half a day, rising to 3 $\%o$ after 2 days. In contrast to *Ammonia*, $\delta^{13}$C$_{org}$ enrichment in *Elphidium* and *Haynesina* was significantly greater in this study. While the process of freeze-drying *Chlorella* will have made the cells more accessible and easily ingested, it may be that *Haynesina* and *Elphidium* prefer "live" diatom carbon in the form of benthic diatoms.

This experiment demonstrated a more vigorous response by *Elphidium* and *Haynesina* than that reported by Moodley *et al.* (2000a). *Haynesina* had specific uptake of 392 $\%o$ after 1 day rising to 452 $\%o$ after 5 days. *Elphidium* had specific uptake of 687 $\%o$ after 1 day falling to 586 $\%o$ after 5 days. However, statistical tests revealed no significant difference in specific uptake values between the 1 and 5 day experimental time periods.

These results, when compared to Moodley *et al.* (2000a), suggest that *Elphidium* and *Haynesina* have a preference for (1) fresh "living" algal carbon and, or (2) a preference for benthic diatoms.
Rivkin and DeLaca (1990) calculated weight specific ingestion rates of microalgae by the larger foraminifera Astrammina rara and Astrorhiza sp. They found ingestion rates to be linear and no satiation occurred, further suggesting that the uniformity in $^{14}$C accumulation rates are due to foraminifera not being limited by either prey concentration or experimental conditions. Their results also suggested that foraminifera synthesised 2 to 5 times more protein and 3 to 5 times less lipid than metazoans.

Although the present study does not specifically examine food preferences, it is clear that all taxa examined will readily consume living benthic diatoms and have the ability to respond quickly to an event (within 1 day). Enrichment values suggest that they ingest large amounts of fresh algal carbon within 1 day and are readily exploiting benthic diatoms as a food source. These results contradict the conclusions of Rudnick (1989) and Widbom and Frithsen (1995). Rudnick (1989) estimated that foraminifera assimilated roughly 90% "old" carbon and that the ostracoda response varied depending on body size.

Levin et al. (1999) show that continental slope assemblages can rapidly ingest (within 1 day) and vertically mix fresh phytodetritus down into the sediment column. Agglutinated protozoa contained the greatest amount of tracer after 1 to 1.5 days. However, unlike the present study, ostracoda were shown to have a small uptake of $^{13}$C-labelled phytodetritus after 1 day. Olafsson et al. (1999) found that the uptake by ostracoda in the northern Baltic Sea of $^{14}$C-labelled Skeletonema costatum accounted for 46% of the total meiofauna uptake.

In a similar laboratory experiment focusing on nematodes, Moens et al. (2002) reported uptakes of labelled algae after 0.5 hours ($\delta^{13}$C values 80 %o) in nematodes within the top 0-1 cm of sediment. The $\delta^{13}$C values continued to rise to approximately 230%o after 3 days. In the present study, a foraminiferal response several orders of magnitude greater was recorded within 1 day and this was sustained throughout the five-day experimental time period. The algae species used by Moens et al. (2002) was lyophilised (freeze-dried) but not identified. However, the concentration of added algae was comparable to the present study (Moens et al. 2002: 0.5 mg cm$^{-2}$, this study: 0.92 mg cm$^{-2}$). The percentage composition of meiofauna at this site during the time of sampling clearly shows (Figure 4.3) foraminifera to be almost as numerically important as nematodes within the surface 0.5 cm. Comparing these two studies, it can be suggest that
foraminifera react more vigorously than nematodes to added “living” algal food. With their high percentage abundance in the surface 0 to 0.5 cm, foraminifera may be a very important link in the food web of this and perhaps other intertidal areas.

4.5 Conclusions

Clearly, intertidal benthic foraminifera and ostracoda have, to varying degrees, a strong ability to utilise sudden increases in “living” benthic diatom carbon. Specific uptake rates would more than likely vary depending on the siliceous frustules of the diatom being consumed (e.g. *Cylindrotheca closterium* is weakly silicified) and on any pre-treatment (e.g. lyophilised).

The wider significance of these results is that benthic foraminifera may play an important and perhaps overlooked role within marine intertidal ecosystems. By virtue of their often large standing stocks and rapid response to freshly available food resources (e.g. benthic diatom “blooms”), benthic foraminifera potentially form an important intermediate link in the lower trophic levels of marine food webs. Both in transferring carbon up the food chain and perhaps more importantly in making it available at the lower levels within the microbial loop.
Chapter 5
5. The effect of oxygen and food availability on the vertical distribution of intertidal benthic foraminifera and ostracoda

5.1 Introduction

In most of the diverse environments inhabited by benthic foraminifera, they reside near the surface sediment layers close to the sediment-water interface. However, in some environments, populations also show subsurface maxima at depths as great as 35 cm (Matera and Lee, 1972, Corliss 1985, Kitazato 1988, Moodley 1990b, Murray 1992, Hunt and Corliss 1993, Goldstein et al. 1995). The sediment-water interface is, on the whole, well oxygenated (Jørgensen and Revsbech 1989) and often rich in organic matter (e.g. estuarine sediments). Within intertidal muddy sediments, the oxic-anoxic interface is generally found within a few millimetres to centimetres of the sediment surface, largely depending on the grain size of the sediment and supply of organic material (Revsbech et al. 1980b, Langer et al. 1989). Within the photic zone, where light is available, benthic diatoms photosynthesise, producing an almost constant in situ fresh supply of labile carbon and oxygen (Underwood and Kromkamp 1999, Middelburg et al. 2000).

The two major environmental factors that have been suggested as being significant in controlling the vertical distribution of benthic foraminifera are oxygen concentration and food availability (organic carbon: labile versus refractory) (Bernhard 1992, Linke and Lutze 1993). However, while both are recognised as being important, there remains a debate as to which is the more important (Rathburn and Corliss 1994).

With increasing depth, sediments generally become increasingly devoid of oxygen, eventually becoming anoxic, with the possible exception of small oxic haloes around macrofaunal burrows. Localised oxic environments around such burrows (Reise 1981a,b, Aller and Aller 1986, Meyers et al. 1987, 1988, Thomsen and Altenbach 1993, Jorissen 1999) create microhabitats, within which there is enhancement of bacterial biomass and production (Fenchel and Jørgensen 1977, Alongi 1985). While these areas of increased oxygenation and nutrients may be limited, they present some organisms with the opportunity to live deeper within the sediment. However, in general, sources of organic carbon are predominantly refractory at depth, and sulphate-reducing bacteria use sulphate as
a hydrogen acceptor, producing hydrogen sulphide (H₂S). It has been shown that hydrogen sulphide is highly toxic to most meiofauna including foraminifera (Giere 1993, Bernhard 1993, Moodley et al. 1998b). This would suggest that a hydrogen sulphide layer is the lowest possible depth limit of foraminiferal distribution within the sediment.

However, many species of foraminifera have been described as being able to migrate through and survive anoxic (anaerobic) conditions for extended periods (e.g. 21 days) and have further been described as facultative anaerobes (e.g. an ability to function and survive under anaerobic conditions) (Moodley and Hess 1992, Alve and Bernhard 1995, Bernhard et al. 1996, Moodley et al. 1997, Moodley et al. 1998a, 1998b). Some foraminifera have even been described reproducing in the boundary between oxic-anoxic and the hydrogen sulphide producing layer (Alve and Bernhard 1995, Moodley et al. 1997).

The depth at which foraminifera live within the sediment of intertidal mudflats is therefore an important aspect of their ecology. More interesting perhaps is why and how they maintain their position within the upper 1 cm of sediment?

It has been shown that some foraminifera do migrate from one microhabitat to another in response to burial (Matera and Lee 1972, Severin and Erskian 1981), as environmental conditions become unfavourable, in order to optimise the acquisition of food (Linke and Lutze 1993), and in response to chemical gradients (Alve and Bernhard 1995, Moodley et al. 1998a). However, it has also been shown that some deep-sea infaunal foraminifera do not migrate in response to seasonal food inputs (Heinz et al. 2001, Heinz et al. 2002), but will migrate in response to anoxic conditions (Gooday and Turley 1990). Negative geotaxis has also been described in foraminifera and is often suggested as the best way to collect living field samples (Arnold 1974, Murray 1974, Anderson et al. 1991). However, several authors have presented data to the contrary (Alve and Bernhard 1995, Duijnstee et al. 2003), suggesting that certain species of foraminifera do not exhibit negative geotaxis, but rather that migration occurs as a response to hydrogen sulphide formation. The question as to whether there is a single trigger to these migrations has yet to be answered in full, but it seems likely that there may be more than one. To date, most experiments have been carried out to investigate migration in sub-tidal species of foraminifera.

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Intertidal areas have both lower diversity and lower foraminiferal penetration depths than shelf and deep-sea environments. In many estuarine intertidal mudflats, for example, foraminiferal maximum abundances occur in the surface 1 cm (Castignetti 1996). A more recent, higher resolution study by Alve and Murray (2001) in the Hamble Estuary, England revealed the maximum abundances of all species to be concentrated in the surface 0.25 cm. No significant seasonal change in the vertical distribution was observed. Alve and Murray (2001) concluded that seasonality observed in the position of the H$_2$S layer (often detected at 1 cm) was not the controlling factor on the vertical distribution of foraminifera in the Hamble Estuary. In addition, none of the foraminiferal species showed high subsurface abundances. Instead, Alve and Murray (2001) concluded that the high number of individuals in the surface 0.25 cm was related to microalgal food availability and the limited burrowing activity of a sparse macrofauna.

Previous laboratory experiments have reported migratory locomotion speeds for some benthic foraminifera. Severin and Erskian (1981) buried the shallow-water foraminifera Quinqueloculina impressa under 4 cm of sediment. Within 4-5 days they had returned to the surface. Gross (2000) carried out migratory laboratory experiments on subtidal benthic foraminifera, reporting speeds for a few of the foraminifera in this experiment: Ammonia beccarii was classified as “fast”, covering 4 $\mu$m min$^{-1}$; Allogromia sp. was also classified as “fast”, covering 3.86 $\mu$m min$^{-1}$; Quinqueloculina lamarckiana was “very fast”, covering 8.74 $\mu$m min$^{-1}$. These experiments were undertaken on subtidal or bathyal foraminifera and it is possible that the migration speeds of intertidal species are markedly different.

As mentioned previously, most experimentation to date has been carried out on subtidal and deep-sea species of benthic foraminifera. This chapter examines the vertical distribution of intertidal benthic foraminifera with respect to the influences of pore water oxygenation and food availability.

Indeed, the fact that most intertidal mudflats contain benthic foraminifera living in the surface 1 cm suggests foraminifera respond to a set of controlling environmental factors. From observations made in the literature it could reasonably be assumed that within the intertidal habitat foraminifera prefer
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oxygenated environments with a constant supply of food. However, this has not been fully investigated.

5.2 Materials and Methods:

Specifically, this experiment used sediment cores under conditions of fixed food availability and varying oxygen supply to examine the response of the foraminifera present. Sediments containing live foraminifera ("live layer") were buried at a depth of 3 cm under an overlying layer of sediment depauperate in foraminifera, macrofauna and sources of organic carbon. Fixed amounts of freeze-dried planktonic diatoms (Skeletonema) were mixed with the "live layer" providing a known concentration of food to all the experimental cores. Given the depauperate nature of the overlying sediments, the introduction of a plentiful food source to the "live layer" was intended to optimise food availability to the foraminifera at this depth. Oxygenation regimes at the surface and at depth were controlled in a series of experimental cores to investigate foraminiferal response.

All sediment surface scrapes and cores for this experiment were collected on ebb tides during late May (Repeat 1) and early June (Repeat 2) 2001 from an intertidal site on The Oostersheld (51° 33N, 4° 3E), a marine embayment on the southwest coast of the Netherlands (Chapter 4, Figure 5.1).

5.2.1 Depth distribution of major meiofauna taxa and sediment description

Prior to experimental set up (late May 2001), intact meiofaunal sediment cores (Ø 3.6 cm) were collected to a depth of 5 cm in order to ascertain the natural depth distribution of the major meiofauna taxa. Cores were collected and extruded using a fixed interval extrusion device, which allowed 0.5 cm slices to be horizontally sectioned from the core. Each core was divided into 10 depth slices (0 – 5 cm), preserved and stained with a mixture of buffered formalin and rose Bengal (4% buffered formalin, 0.01% rose Bengal) for later counting. Replicate cores were taken in order to describe the in situ profile of sediment.

5.2.2 Field collections of sediment

Experimental design involved an initial setup of experimental cores containing a sediment layer with living intertidal foraminifera, overlain by
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sediment depauperate of foraminifera and any other significant labile carbon source (Figure 5.1).

![Diagram](image)

Figure 5.1: General structure of artificially constructed experimental cores. Bottom of core contains a concentrated layer of living foraminifera ("live layer") and the overlying sediment has very few foraminifera and little to no organic carbon content.

5.2.3 Collection of sediment for overlying layers

Within the field area designated for sediment collection, it was found that conditions close to the sediment surface had become anoxic. This rendered deeper sediment layers unsuitable for use as the overlying sediment due to the presence of H$_2$S. Therefore, an area adjacent to the tidal pool was identified which yielded sediment depauperate in foraminifera, ostracoda, labile carbon and was free of H$_2$S. The sediment required to overlay the "live layer" was collected one day prior to starting the experiment.

Surface scrapes were collected in late May and early June 2001 on an ebb tide, avoiding any anoxic sediment. On returning to the laboratory, the sediment was immediately sieved at 200 μm to remove macrofauna, and any larger foraminifera, retaining that fraction less than 200 μm in a large plastic beaker. This was left to settle overnight in a climate controlled room set to reflect field collection conditions (16°C). Excess water was removed the following day using a syringe with silicone tubing attached.

5.2.4 Sediment collection for live foraminifera

Surface scrapes were taken from the same field area, being careful to avoid collecting any anoxic mud and returned to the laboratory where they were
immediately sieved at 500 μm to remove any macrofauna and coarse fraction, but not retaining foraminifera. A further sieve at 38 μm was used to concentrate foraminifera. This procedure also removed the very fine silt and clay fraction. The concentrate was left to settle for one hour, after which time any excess water was removed and the mixture homogenised.

5.2.5 Construction of cores

Four perspex cores of diameter 3.6 cm (Area = 10.17 cm²) were cut to the desired height (approx 16 cm). Small holes were drilled through each side of the core, just large enough to thread through a very tight fitting piece of silicon tubing (2.9 mm outer diameter and 1 mm inner diameter). This was done at a height corresponding to the depth chosen for the “live layer” of foraminifera. Diffusion through the tubing, which was gas permeable (Moodley pers. com. 2001), created a thin oxygenated layer around it. A piece of thin wire was threaded through to give the tubing rigidity and allowed it to be pulled into a horizontal z-shaped fold, thus delivering oxygen over a larger area of the core. A septum was added at the bottom of the core to facilitate extrusion of the core on completion of the experiment.

Slurries of both live and overlying sediment were prepared to the desired consistency and well mixed, to ensure an even distribution of foraminifera. Accurate division of the sediment among the experimental cores was achieved by means of a 50 ml syringe with the end cut off. This allowed the same volume of sediment to be added each time a core was built. The bottom of the core was constructed of solid perspex with an o-ring, which once inserted prevented any air permeating the base of the core. The bottom 0.5 cm was the “live layer” and then a further 2.5 cm of sediment was placed over the top (Figure 5.1). Overlying layers had to be placed carefully to minimize any mixing of sediment and foraminifera into adjacent layers due to disturbance when building. In order to quantify any mixing errors, cores were constructed from the same sediment and sliced immediately at 0.5 cm intervals. These were termed the time 0 (T=0) cores reflecting the starting distribution of foraminifera and ostracoda. Three T=0 cores were prepared at the start of each repeat experiment.
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5.2.6 Experimental setup

Four cores were built: 3 with a 0.5 cm "live layer" at the bottom and an overlying layer of sediment 2.5 cm thick. A fourth core had 2.5 cm of sediment at the bottom and a 0.5 cm "live layer" on top (Figure 5.2D). This was to test that laboratory conditions were not directly affecting foraminiferal populations. As there was plenty of room for the foraminifera to manoeuvre around the tubing, it was decided that a control for the structure was not necessary. Experimental treatments applied to the three remaining cores (Figure 5.2) were:

1. Oxygen supplied to the overlying water and to the "live layer" via the silicon tubing. Top of core sealed with parafilm to reduce evaporation and any resulting increases in salinity (O₂/O₂; Figure 5.2B).

2. No oxygen supplied to the overlying water and any traces of oxygen removed by bubbling through N₂ (flushed with high grade nitrogen, 99.99% N₂ for at least 10 min). A top was then secured ensuring no air bubbles containing oxygen were trapped. Oxygen was supplied to the "live layer" by silicone tubing (NoO₂/O₂; Figure 5.2A).

3. Oxygen bubbled into the overlying water and top sealed with parafilm as above. At the "live layer" no oxygen supplied (no holes drilled through core tube at the corresponding depth) (O₂/NoO₂; Figure 5.2C).

Two repeats of this experiment were carried out one week apart. In total, 14 experimental cores were constructed, eight of which were incubated according to the methods described here.

Within the experimental cores oxygen at top and at depth (O₂/O₂; Figure 5.2B), 3 mg of freeze-dried ¹³C-enriched planktonic diatoms (Skeletonema) were added into the "live layer" (Chapter 4). This enabled the measurement of active food uptake, thus giving an indication as to whether the foraminifera were not only surviving at the displacement depth, but were also ingesting the food (and ¹³C label) supplied. To all other cores, 3 mg of unlabelled freeze-dried Skeletonema was added. Hence food concentrations within the "live layer" were held relatively constant for each treatment. The fact that the overlying sediment layer was not rich in labile carbon meant that there would be no upward migration in the foraminifera present in the "live layer" induced by food supply.

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Figure 5.2: Schematic of core layering and experimental treatments applied, (A) No oxygen top, oxygen bottom; (B) oxygen top and bottom; (C) oxygen top, no oxygen bottom; (D) control, oxygen top.
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Figure 5.3: Experimental cores from left to right: (A) No oxygen top, oxygen bottom; (B) oxygen top and bottom; (C) oxygen top, no oxygen bottom; (D) control, oxygen top. Septum is visible as an orange layer at the bottom. The external diameter of each core is 3.6 cm.

All cores were incubated in the dark in a climate room set to 16°C (Figure 5.3) and checked daily for any changes in sediment colour or any foraminiferal movement if visible. After 6 days the cores were harvested into six 0.5 cm slices, taking careful note of any H₂S smells. While this may be a subjective approach, it has been pointed out that the nose is a good instrument for detecting even low concentrations of H₂S, sensing down to 0.1 μM H₂S (Dando et al. 1985, Giere 1993). The samples were first semi-quantitatively examined for live foraminifera by removing individuals and checking for pseudopodial movement under an inverted microscope (Leitz Labovert FS)(data presented in Appendix A). This was carried out on at least one 1/8th of a sample, which was returned after counting and the whole sample preserved in a mixture of formalin and rose Bengal. Subsequently, the stained living foraminiferal assemblage in each layer (>38 μm) was enumerated.

5.3 Data analysis

Variation in the total number of foraminifera within each core constructed for the repeated experiments was examined using a one way analysis of variance (ANOVA) on ln (x+1) transformed counts. Large variations were evident between
the repeat experiments (P=0.000). For this reason, repeats were analysed separately.

All counts were ln (x+1) transformed prior to analysis in order to improve normality and equality of variances. Further analysis was carried out on each repeat experiment using a General Linear Model (MINITAB) with nested factors of time, treatment and depth. The null hypothesis being tested was that there was no significant difference in the mean number of foraminifera at each depth interval after core treatment for 6 days.

Graphical representation of the data was produced by calculating percentage proportional abundances of the total number of foraminifera in each experimental core and plotted as a histogram beside the T=0 core data for comparison.

5.4 Results

5.4.1 Natural depth distribution of meiofauna in intertidal mudflat sediments

The natural depth distribution of meiofauna showed foraminifera to be as numerically important in the surface 0.5 cm as Nematoda (44% and 45% respectively). However, with increasing depth foraminifera become less significant, falling to 4% between 0.5 and 1 cm (Figure 5.4). One individual was found at depth (4.5-5 cm, 3%), and was most likely transported down-core by bioturbation. Below 0.5 cm nematoda are the dominant taxa, and along with the copepoda are the only two taxa observed to extend to the lowest sampling depth. At the time of collection, benthic foraminifera were largely restricted to the upper 1.5 cm, while ostracoda were restricted to the upper 1 cm.
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Figure 5.4: In situ relative vertical distribution of major intertidal meiofauna and foraminifera, expressed as a percentage of total number of individuals at each depth slice. Taken in May 2001 at the intertidal sampling site on the Oostershelde, the Netherlands (51° 33N, 4° 3E).

5.4.2 Visual observations

Visible oxygen gradients were established over short periods of time within the cores (e.g. 1 day). Cores with oxygen supplied at the surface developed an oxic layer (lightening of sediment) approximately 1 mm deep after 1 day, increasing to approximately 2 mm after 4 days. The sub-surface oxygen supply was also evident by colour changes (a slight halo around the silicone tubing) in comparison to the surrounding sediment after 1 day. This was well-developed by day 3 and by day 6 it extended 2–2.5 mm around the tubing. Surface penetration of oxygen with aerated bottom water was equal to or less than 3 mm. Around the silicon tubing the extent of oxygenation would be in the region of approximately 2.5 mm (Revesbech et al. 1980a, Revesbech et al. 1980b, Rasmussen and Jørgensen 1992, Moodley et al. 1998a, Moodley personal communication 2001).

Cores where oxygen had been removed from the overlying water or did not have oxygen supplied at depth also showed sediment colour gradients forming quickly (Figure 5.2A and 5.2C). In the nitrogen flushed core (Figure 5.2A), within 2 days a 1mm black layer was visible on the surface and black spots became visible over the complete core area. After 6 days, the top sediment was very dark and black spots were abundant along the dividing line between the “live layer”
and overlying sediment. However, the appearance of black spots was visibly reduced in the sub-surface oxic area (Figure 5.2A and 5.2B). By contrast, the core with no oxygen at depth developed a darker colouration with extensive black spotting (Figure 5.2C). A dark dividing line between the overlying layer and subsurface “live layer” developed.

Foraminifera within the “live layer” were observed to explore the sediment in all treatments. Although trails were visible, and individuals could be followed at the core edge, they inevitably moved back into the sediment. It was therefore impossible to ascertain any preferred direction of movement. No macrofaunal burrowing was evident.

5.4.3 Hydrogen sulphide formation

The experiment was terminated after six days and the cores sliced at 0.5 cm intervals observing colouration and smell (Table 5.1).

Table 5.1: Each treatment core was monitored on slicing after the six-day experimental period for any traces of Hydrogen Sulphide (H₂S). – indicates H₂S not detected by smell, + indicates the presence of H₂S detected as a very faint smell (barely noticeable), ++ indicates moderate smell, +++ indicates strong smell.

<table>
<thead>
<tr>
<th>Treatment Cores</th>
<th>Depth (cm)</th>
<th>O₂/No₂</th>
<th>O₂/O₂</th>
<th>NoO₂/O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.5</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>0.5-1</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>1-1.5</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>1.5-2</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2-2.5</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2.5-3</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Based on smell, it was evident that H₂S was formed in all cores. However, at most depths the smell of H₂S was faint, with the exception of the surface 0.5 cm of the nitrogen-flushed core (NoO₂/O₂; Figure 5.2A). It seemed that regardless of treatment, the sediment layers between 0.5 cm and 2.5 cm developed an anoxic environment (Table 5.1). It was also noted that a transition in colour occurred in this layer, from a light grey to a dark grey (beginning at 1 day), over the six days.
5.4.4 Stable isotope measurements

The planktonic diatom *Skeletonema* enriched in $^{13}$C was added to the treatment cores, oxygen at surface and oxygen at depth ($O_2/O_2$; Figure 5.2B). If the foraminifera were consuming the diatoms, their $\delta^{13}$C$_{org}$ values would become enriched compared to background values (Table 5.2).

In all cases enrichment was evident (ratios become more positive) indicating that all three foraminifera genera and the ostracoda had consumed some of the added diatoms. Unfortunately, there were not enough *Allogromia* present to make a reliable $\delta^{13}$C$_{org}$ measurement of this genus. *Haynesina* appears to have consumed the most diatom biomass over the two six day periods, exhibiting specific uptake values of 15.8 and 13.8 %o respectively. *Ammonia* exhibited a very low response in repeat1 and a greater response in repeat2, with specific uptake values of 1.9 %o and 13.5 %o respectively. *Elphidium* also recorded enrichment with specific uptakes of 11.6 and 4.9 %o in the two repeatss, although the $\delta^{13}$C$_{org}$ measured never become positive. Ostracoda showed the lowest enrichment of all genera examined with specific uptakes of 2.43 and 0.91 %o respectively.

Table 5.2: Background $\delta^{13}$C$_{org}$ values for field collected samples of all genera measured in the experimental setup. Two repeats of the experiment were undertaken (Rep1, Late May 2001 and Rep2, early June 2001). Experimental $\delta^{13}$C$_{org}$ values were taken after a six-day period for each of the major genera found (n=1 for each repeat).

<table>
<thead>
<tr>
<th>Genera</th>
<th>$\delta^{13}$C$_{org}$ background (%o)</th>
<th>$\delta^{13}$C$_{org}$ after 6 days</th>
<th>Specific uptake $\Delta\delta^{13}$C$_{org}$ (%o): Rep1</th>
<th>$\delta^{13}$C$_{org}$ after 6 days</th>
<th>Specific uptake $\Delta\delta^{13}$C$_{org}$ (%o): Rep2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ostracoda</td>
<td>-10.7</td>
<td>-8.0</td>
<td>2.7</td>
<td>-9.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Elphidium</td>
<td>-14.5</td>
<td>-2.9</td>
<td>11.6</td>
<td>-9.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Haynesina</td>
<td>-10.2</td>
<td>5.6</td>
<td>15.8</td>
<td>3.6</td>
<td>13.8</td>
</tr>
<tr>
<td>Ammonia</td>
<td>-12.8</td>
<td>-10.9</td>
<td>1.9</td>
<td>0.7</td>
<td>13.6</td>
</tr>
</tbody>
</table>

5.4.5 Live counts

Under all treatments and at all depths after the six-day experimental period, all the major genera examined revealed live individuals (Appendix A). The semi-quantitative determination of living foraminifera were found to approximate the live rose Bengal counts. It can therefore be confidently assumed
that the majority of the observed rose Bengal stained individuals were living at the end of the experimental period and not merely freshly dead. However, when examining individuals from anoxic layers, there was a consistently slower response in the time taken for the foraminifera to extend their pseudopodia.

5.4.6 Relative vertical distribution of intertidal foraminifera after 6 days

Total standing stocks (numbers of individuals) between repeats were significantly different \((P=0.000)\) with repeat 2 having more than three times as many foraminifera as repeat 1. However, within the same set of treatment cores in each repeat there was no significant difference (repeat 1 (Rep1), \(P=0.323\) and repeat 2 (Rep2), \(P=0.288\)). Raw count data are provided in the accompanying data CD.

Numbers of ostracoda in the surface 0-0.5 cm increased after 6 days for all treatments and both repeats (Figure 5.5a,b). This suggests that a migration/movement from the initial T=0 distribution has occurred regardless of treatment applied and is supported by the results (Table 5.3), which show a significant interaction between depth (time treatment); \(P=0.00\) for both repeats. A greater percentage of ostracoda in repleat 1 were found in the surface 0 - 0.5 cm after the treatment \(O_2/No_2\) (51.7%) than remained at the original "live layer" depth (2.5-3 cm, 13.31%) (Figure 5.5a). Treatments \(O_2/O_2\) and \(No_2/O_2\) also showed an increase in the percentage of ostracoda in the upper 0-0.5 cm (29.8% and 28.4% respectively). However, a higher proportion of ostracoda (39.5%) were found at the "live layer" depth within the treatment \(No_2/O_2\) (Figure 5.5a) than within the treatments \(O_2/No_2\) and \(O_2/O_2\) (13.3 % and 18.1 % respectively).

Ammonia also showed an overall significant upward movement/migration \((P=0.000\) for both rep1 and rep2). Increased numbers of Ammonia occupied the 2-2.5 cm depth layer after six days (Figure 5.5); the effect being most pronounced in treatments \(O_2/O_2\) and \(O_2/No_2\). The abundance of Ammonia after these two treatments decreased from 72% at T=0 to 41 and 42 % respectively at depth 2.5-3 cm. This was coupled with an increase from 16 % at T=0 to 49 and 37 % respectively at depth 2-2.5 cm. Treatment \(No_2/O_2\) showed little difference to the distribution sampled at T=0 (Figure 5.5), suggesting no net migration. Repeat 2 cores also showed significant differences over time, but these were not as pronounced as those in the repeat 1 cores.
Table 5.3: General Linear Model of ln (x+1) transformed counts of major foraminifera genera and ostracoda in depth slices. All individuals were retained on a 38 μm sieve. *** = Very highly significant (P<0.001), ** = Highly significant (P<0.01). * = Significant (P<0.05).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Sum of squares</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ostracoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>20</td>
<td>16.54</td>
<td>19.31</td>
<td>0.000</td>
</tr>
<tr>
<td>Rep 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>20</td>
<td>57.49</td>
<td>163.81</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Ammonia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>12</td>
<td>20.72</td>
<td>78.45</td>
<td>0.000</td>
</tr>
<tr>
<td>Rep 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>12</td>
<td>54.14</td>
<td>116.73</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Haynesina</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>12</td>
<td>25.81</td>
<td>5.21</td>
<td>0.013</td>
</tr>
<tr>
<td>Rep 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>12</td>
<td>29.21</td>
<td>7.45</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Elphidium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>8</td>
<td>8.80</td>
<td>15.79</td>
<td>0.002</td>
</tr>
<tr>
<td>Rep 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>4</td>
<td>5.31</td>
<td>7.43</td>
<td>0.039</td>
</tr>
<tr>
<td><strong>Allogromia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>4</td>
<td>12.77</td>
<td>14.39</td>
<td>0.012</td>
</tr>
<tr>
<td>Rep 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (Time treatment)</td>
<td>4</td>
<td>12.68</td>
<td>15.52</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*Haynesina* showed an overall significant movement (P=0.013 and P=0.004) in both repeats. However, in repeat 1, the relative abundance of *Haynesina* observed (44.7%) in the 2-2.5 cm depth interval in the O₂/O₂ treatment were higher than at T=0 (18.5%). This was coupled with a reduction to 28.9% under the same O₂/O₂ treatment from 67.7% at T=0 in the 2.5-3 cm depth interval. Treatment N0O₂/O₂ showed a slight increase from T=0 relative abundance at 2.5-3 cm depth (67.7% to 73.2%) with reductions at 2-2.5 cm (18.5% to 12.2%). Repeat 2 showed reduced relative abundances for all three treatments in the 2.5-3 cm depth layer when compared with the average T=0 estimates. This reduction at
2.5-3 cm was coupled with an increase in relative abundance at 2-2.5 cm. This apparent upward movement in *Haynesina* was most marked in treatment core O$_2$/NoO$_2$ (1.31% to 13.75%) (Figure 5.5b).

*Elphidium* also showed an overall significant upward movement (P=0.002 and 0.039) and a reduction in relative abundance at depth 2.5-3 cm for treatments O$_2$/O$_2$ (35.7%) and O$_2$/NoO$_2$ (50.0%) from T=0 (72%). Corresponding increases at 2-2.5 cm from 9.1% (T=0) to 40.6% (O$_2$/NoO$_2$) and 35.7% (O$_2$/O$_2$). For treatment O$_2$/O$_2$ there was also an increase at 0-2 cm (from 18.2% to 28.6%). Treatment NoO$_2$/O$_2$ showed a slight increase (but not significant) at 2.5-3 cm and 2-2.5 cm along with a decrease at 0-2 cm (from 18.2% to 3.3%). Repeat 2 showed a similar trend with a reduction in relative abundance at 2.5-3 cm and a corresponding increase at 0-2.5 cm with the exception of NoO$_2$/O$_2$ where the numbers increased at 2.5-3 and decreased at 0-2.5 cm.

*Allogromia* also showed an overall significant movement (P=0.012 and P=0.011). Movement was greatest in treatment O$_2$/O$_2$ (Figures 5.5a and 5.5b). However, NoO$_2$/O$_2$ and O$_2$/NoO$_2$ both showed increases in relative abundance at 0-2.5 cm.
Chapter 5 The effect of oxygen and food availability on the vertical distribution of foraminifera

Ammonia

Ostracoda

Haynesina

Allogromia

Elphidium

Figure 5.5a: Summary histograms of percentage proportional abundance of total counts of foraminifera and ostracoda for each treatment applied in repeat 1. T=0 ■; NO₂/O₂ ■; O₂/NO₂ ■; O₂/O₂ ■. T=0 is ± Standard Error (n=3).
Chapter 5  The effect of oxygen and food availability on the vertical distribution of foraminifera

*Ammonia*

*Ostracoda*

*Haynesina*

*Elphidium*

Figure 5.5b: Summary histograms of percentage proportional abundance of total counts of foraminifera and ostracoda for each treatment applied in repeat 2. T=0 ■ ; NoO₂/O₂ ■ ; O₂/NoO₂ □ ; O₂/O₂ □ . T=0 is ± Standard Error (n=3).
5.5 Discussion

Although the concentration of hydrogen sulphide (H$_2$S) was not measured at the termination of the experiment or throughout, it was clearly detectable as a strong H$_2$S smell within the surface layers of the nitrogen flushed cores (NoO$_2$/O$_2$, Figure 5.2A). Where oxygen was excluded at depth, H$_2$S also began to form at lower levels - smell was less strong. Obviously, 6 days is a very short time span to run the experiment. However, it was chosen in an attempt to reduce H$_2$S formation, which was successful apart from the no oxygen to surface waters (nitrogen flushed) treatment. Based on published migration speeds by Gross (2000) it was estimated that it would take approximately 5 days for both *Ammonia* and *Aliogromia* to cover 3 cm (i.e. less than the depth of burial in this experiment) if moving towards their preferred microhabitat.

*Ammonia* showed an overall significant upward movement (Table 5.3). However, the differences were not as evident in repeat 2 (June 2001) compared to repeat 1 (May 2001) (Figure 5.5b and 5.5a). It is possible that a density dependent factor may be at work but there was insufficient replication to examine this interesting possibility in any detail here.

Having reviewed the changes in vertical distribution in the experimental cores, it is clear that *Ammonia* has migrated when oxygen was available at the core surface regardless of added food. During the treatment no oxygen at the surface an increase in the proportional abundance in the "live layer" when compared to T=0 and treatment O$_2$/O$_2$ was found. Based on these results, one can hypothesise that *Ammonia* did not show an overdriving negative geotaxis on burial. However, it did show selective migration/movement when oxic conditions were provided at the surface.

*Haynesina* also showed an overall significant upward movement/migration (Table 5.3). However, from the histograms (Figure 5.5a, b) the most pronounced changes were evident in treatments O$_2$/O$_2$ (repeat 1) and O$_2$/NoO$_2$ (repeat 2). Again, repeat 2 showed reduced changes and it could be hypothesised that there is a density effect due to a four fold increase in *Haynesina* in the "live layer" of these experiments from May 2001 (repeat 1) to June 2001 (repeat 2). In the treatment NoO$_2$/O$_2$ *Haynesina* became more concentrated in the initial "live layer" (73.2 %) compared to T=0 (677%) and treatment O$_2$/O$_2$ (28.9%) (Figure 5.5 a, b).
Elphidium migrates in treatments where oxygen was supplied at the surface. However, very interestingly, a downward migration in response to the removal of oxygen at the sediment water interface took place. Concentrations at 2.5-3 cm depth were 80% with the treatment NoO₂/O₂ compared to 35.7% in the treatment O₂/O₂. This could be interpreted as Elphidium responding to the reduction in surface oxygen and migrating into the sediment in search of, or indeed following, an oxygen gradient. Elphidium therefore appears to have the ability to migrate downwards through the sediment away from unfavourable environments.

Increased numbers of Allogromia were recorded in all treatments at the 0-2.5 cm depth slice, suggesting that they exhibit an over-riding negative geotaxis and are not responding to any treatment stimuli. Ostracoda responded in a similar manner to the Allogromia, but appeared to be more mobile within the sediment. This is due of their greater locomotive abilities. For example, the increase in ostracoda in the surface layers (28.4%) of a treatment core (NoO₂/O₂) suggests that they migrated upwards, but on finding anoxic surface conditions may be returning to the sub-surface oxic layer (39.5%) where food is also available (Figure 5.5a).

No upward movement was found when comparing the depth layers 2.5–3 cm (i.e. initial “live layer”) and 2–2.5 cm for the two treatments O₂/O₂ and O₂/NoO₂. However, responses appeared to be slower in the treatment where oxygen was not supplied at depth for Elphidium, Haynesina and Allogromia, whereas, Ammonia and ostracoda showed approximately the same proportion of individuals at both depths for both treatments. Hydrogen sulphide formation may be higher in the treatment where oxygen was not supplied at depth than for the oxygen treatments (Table 5.1). This therefore suggests that H₂S formation is beginning to form a barrier to the migration of Elphidium, Haynesina and Allogromia. Although they show the ability to be facultative anaerobes, migrating through anoxic sediments, it appears that at certain critical concentrations of H₂S the migration may be slowed or halted. Higher concentrations may even induce a downward migration as seen for the nitrogen flushed core (NoO₂/O₂, Figure 5.2A).

Although specific uptake values showed ¹³C_organic enrichment from background they were extremely low when compared to those measured in the
experiments undertaken in Chapter 4 (Chapter 4, Table 4.3). For example, in this experiment, *Haynesina* appears to consume most food, exhibiting an average specific uptake of 15.8 % after six days. In earlier experiments (Chapter 4), enrichment values were as large as 335.2 % over 5 days feeding on living benthic diatoms. This may reflect a preference for living benthic diatoms as opposed to freeze-dried planktonic diatoms. Uptake values in this experiment are slightly higher than those recorded by Moodley *et al.* 2000a, who fed freeze-dried *Chlorella* to foraminifera and obtained specific uptake values for *Haynesina* of approximately 3 % after 2 days. However, the reduced uptake of this experiment may also reflect a sub-optimal habitat (infraunal=2.5-3 cm), where the foraminifera do not function as well as they would on the surface, perhaps due to sediment overburden, restricted pseudopodial activity and hence difficulty in finding food.

5.6 Conclusions

The intertidal foraminifera studied did not migrate immediately upon burial. Hence they do not exhibit a rapid negative geotaxis as an escape response to burial. However, they can survive at depth under favourable conditions and migrate away from unfavourable or sub-optimal conditions. In this experiment sediments directly above and below the oxic areas tended towards anoxic conditions. Clearly, all the foraminifera genera examined have the ability to migrate through sediments with reduced oxygen as demonstrated by other authors (Moodley and Hess 1992, Alve and Bernhard 1995, Moodley *et al.* 1998b). Because food supply was kept constant in this experiment, dissolved oxygen concentrations appear to be the most important factor in determining the vertical distribution of intertidal foraminifera.

However, further experiments are required in order to determine if \( \text{H}_2\text{S} \) can trigger negative geotaxis and an upward migration. In addition, the present experiments could be repeated, but run over a longer period of time in order to follow any migrations which were occurring over longer time periods. The foraminifera moved more slowly than expected and may therefore have required a settling period once the cores were built. One possibility is that foraminifera exhibit exploratory behaviour and then subsequently migrate or not. Measuring movement over such short distances may just be monitoring random movement.
However, if this had been the case a random distribution might have been expected, rather than one which reflected the applied experimental conditions. The genera examined seemed to respond differently to the experimental treatments, it appears that their distribution was not random and that true migratory behaviour was observed.

In conclusion, none of the three calcareous foraminifera genera examined exhibited negative geotaxis on burial with a food supply, but showed selective responses to applied oxygen treatments. When oxygen was supplied to the overlying water there was an upward movement, with the greatest movement occurring when oxygen was supplied at the surface, but was absent at depth. When no oxygen was supplied to the overlying water a marked increase in downward movement and subsequent increase in density in the oxygenated “live layer” (2.5-3 cm) took place. The Ostracoda and Allogromia, on the other hand, exhibited negative geotaxis regardless of the oxygen treatments applied. Given the distances moved from their burial depth, it is likely that foraminifera have the ability to extend their pseudopodia and “sense” the surrounding oxygen conditions and respond accordingly.
Chapter 6
6. A seasonal study of benthic foraminifera from the Eden Estuary, NE Scotland

6.1 Introduction

Coastal and estuarine environments are systems which are exposed to changing physical and chemical regimes. These changes occur on a regular basis on a variety of time scales and are generally limited by tides. These large changes result in environmental stresses for estuarine organisms. Tides in mid-latitudes are semi-diurnal in nature (two highs and two lows per lunar day), with both tides differing in height. Periods of immersion and emersion vary between spring (greatest range of high and low tides) and neap tides (minimum range of high and low water). These tidal regimes are the most important environmental factor influencing life in the intertidal area (McLusky 1989).

A secondary effect of low tides twice a day is that the intertidal area becomes sub-aerially exposed. If low tide occurs during daylight hours, organisms may be exposed to increased temperatures and light levels. At night-time they would experience extremes of low temperatures without light. Low tides leave the intertidal area susceptible to flooding by heavy rain, or runoff from the land. During such periods, both the intertidal area and the organisms living in the sediment surface are exposed to large fluctuations in salinity from marine to brackish to fresh (dependent on river flow, hydrodynamic mixing and evaporation). The very fine particle size often associated with mudflats, coupled with a shallow angle of incline means that water in/on the sediment does not drain away and is held within the substratum.

These physical and chemical ranges (abiotic) may exceed the lethal limits of many marine organisms that might otherwise exploit the intertidal zone. As a result, estuarine environments generally exhibit low diversity and individuals tend to be small when compared with those from other marine habitats.

For foraminifera to survive in such extremes, they must have the ability to grow and reproduce under almost constantly fluctuating environmental conditions (Cearreta 1988). Spatial and temporal variability in benthic foraminiferal standing stocks and species composition in intertidal mudflats have been investigated by several researchers (Table 6.1). However, the factors that determine the
Table 6.1: List of recent intertidal foraminiferal studies in temperate regions. Adapted from Murray and Alve (2000).

<table>
<thead>
<tr>
<th>Study area</th>
<th>Environment</th>
<th>Duration of study (months)</th>
<th>Time</th>
<th>Sampling frequency</th>
<th>Sediment thickness (cm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nova Scotia</td>
<td>Intertidal</td>
<td>38</td>
<td>Sep 75-Aug 78</td>
<td>irregular</td>
<td>1</td>
<td>Scott and Medioli, 1980</td>
</tr>
<tr>
<td>Hamble Estuary, England</td>
<td>Intertidal</td>
<td>27</td>
<td>Mar 94-Mar 96</td>
<td>monthly</td>
<td>1</td>
<td>Murray and Alve, 2000</td>
</tr>
<tr>
<td>Exe Estuary, England</td>
<td>Intertidal</td>
<td>31</td>
<td>Jan 79-Jul 81</td>
<td>monthly</td>
<td>1</td>
<td>Murray, 1983</td>
</tr>
<tr>
<td>Christchurch Harbour, England</td>
<td>Intertidal</td>
<td>12</td>
<td>Sep. 59-Aug 60</td>
<td>3 monthly</td>
<td>1</td>
<td>Murray, 1968a</td>
</tr>
<tr>
<td>River Tamar, England</td>
<td>Intertidal</td>
<td>20</td>
<td>Dec 72-Jul 74</td>
<td>monthly</td>
<td>1</td>
<td>Ellison, 1984</td>
</tr>
<tr>
<td>Samish Bay, Washington, USA</td>
<td>Intertidal</td>
<td>12</td>
<td>Apr. 76- Mar. 77</td>
<td>2 monthly</td>
<td>15</td>
<td>Jones and Ross, 1979</td>
</tr>
<tr>
<td>Deseado Creek, Argentina</td>
<td>Intertidal</td>
<td>26</td>
<td>Apr. 61-May 63</td>
<td>weekly</td>
<td>not stated</td>
<td>Boltovskoy, 1964</td>
</tr>
<tr>
<td>Santona Estuary, Spain</td>
<td>Intertidal</td>
<td>21</td>
<td>Apr. 84-Dec 85</td>
<td>monthly</td>
<td>1</td>
<td>Cearreta, 1988</td>
</tr>
<tr>
<td>Bahrain</td>
<td>Intertidal</td>
<td>25</td>
<td>Feb. 91-Feb. 93</td>
<td>monthly</td>
<td>1</td>
<td>Basson and Murray, 1995</td>
</tr>
</tbody>
</table>
distribution and standing stock of benthic foraminifera in estuarine environments are not well understood and interpretations vary between studies.

Benthic intertidal foraminifera are known to feed on microphytobenthos e.g. benthic diatoms (Lopez 1979, Lee 1980). Many researchers have assumed that if there is a close trophic link between meiofauna and microphytobenthos, then peak meiofaunal abundances should follow or lag peaks in diatom abundances (Montagna et al. 1983, 1995, Murray and Alve 2000). However, there have been mixed results to both field and laboratory experiments which have sought to highlight such a functional response between benthic foraminifera and microphytobenthos (in particular benthic diatoms). Such responses are clearly seen in some deep-sea foraminiferal species where seasonal pulses of phytodetritus occur (Gooday 1988, Gooday and Turley 1990, Rathburn et al. 2001). However, the deep-sea is more food limited than estuarine environments, which receive organic carbon both allochthonously (originated elsewhere) and autochthonously (formed in situ). These carbon pools vary in their lability, with autochthonous carbon likely to contain more labile material than allochthonous organic material.

Several studies from intertidal regions describe a pattern/sequence of replacement of foraminiferal genera which is thought to be seasonally controlled (Murray 1969, Jones and Ross 1979, Murray 1980, Murray and Alve 2000). Salinity and temperature have been considered the most important abiotic environmental factors governing the seasonal abundance of benthic foraminifera (Ramanathan 1970, Ellison 1984). Ellison (1984) reports the abundance of *Haynesina germanica* and foraminifera in general being strongly correlated (positively) with seawater temperature. Less emphasis has been placed on the biotic (e.g. food supply, competition) environmental factors, which may play a role in controlling seasonal abundance patterns. Increases in intertidal foraminiferal density have been related to variations in food supply (benthic diatoms and phytoplankton) (Boltovsky 1964, Ramanathan 1970, Murray 1983, Ellison 1984). However, Murray and Alve (2000) found no correlation between foraminiferal abundance and microphytobenthos biomass on the Hamble Estuary, England.

Murray (1983) reported from studies on an intertidal pool population that *Haynesina germanica* reproduces throughout the year with a peak in early
summer (May to July, 1981). *Elphidium williamsoni* was also reported in the same study as reproducing continuously at a low level with increases seen during early summer.

Distribution patterns for both microphytobenthos and meiofauna in intertidal areas are generally found to be patchy. Blanchard (1990) carried out spatial autocorrelation studies in an oyster pond (depth 40 cm) on the West Atlantic coast of France near La Rochelle. The author reported meiofaunal and microalgal patches ranging between <4 to 154 cm². Some recent studies have related the spatial distribution of meiofauna (harpacticoid copepods and nematodes) to microalgal abundance (Montagna *et al.* 1983, Blanchard 1990, Pickney and Sandulli 1990, Santos *et al.* 1995, Moens *et al.* 1999). Santos *et al.* (1995) suggested that the key factor in the correlations between certain harpacticoid copepod species abundance and chlorophyll *a* was the concentration of microalgae. Moens *et al.* (1999), on the other hand, suggest that food densities may be less important in structuring nematode communities on intertidal mudflats than the relative abundance of specific food sources. Both studies demonstrate that nematodes and *Microstiridion littorale* (harpacticoid) are attracted to microalgal patches and actively migrate towards them within a radius of 4 cm. Sandulli and Pickney (1999) found no correlation between microalgal biomass and copepod abundance. However, their study confirmed that microalgae and copepods were spatially auto-correlated and exhibited patchiness. Copepod patches ranged from 7 to 121 cm² and microalgal patches ranged from 30 to 191 cm². In summary, the spatial distribution of benthic diatoms can influence meiofaunal patchiness. However, feeding preferences of individual meiofaunal species are likely to be very important in the determination of spatial patterns.

Seasonality has been recorded in the abundance of some benthic diatoms (Admiraal *et al.* 1984, Oppenheim 1991). However, it remains unclear what factors account for these variations (Underwood and Provot 2000). Perkins (1960) describes a change in diatom species on the Eden Estuary during January and February, 1957. Changes noted were in early January, when the flora consisted largely of *Pleurosigma aestuarii* with some *P. angulatum* and *P. fasicola*, but by the end of January into February the diatom community changed to be dominated by *Suriella gemina* with some *Pleurosigma fasicola* and *P. aestuarii*. No
sampling site was declared, but sediment was characterised as 0-5% medium sand, 40-60% fine sand, 40-60% mud and silt.

Like foraminifera, a number of studies have suggested that salinity is an important factor in determining benthic diatom species composition (Admiraal 1984, Oppenheim 1988, Oppenheim 1991). Light and temperature effects combined have a regulatory effect on cell division times, thus influencing seasonal succession (Admiraal et al. 1982, Oppenheim 1991). Diatom growth is limited by nutrients (e.g. nitrate) and combinations of increased nitrate and phosphate also result in increases in the relative abundance of diatoms (Pinckney et al. 1995).

Despite several investigations of the microphytobenthos, there have been no previous investigations on recent benthic foraminifera carried out on the Eden Estuary. The seasonal sampling campaign undertaken during this study was designed to monitor benthic foraminiferal standing crops and assemblage structure, both spatially and temporally. Spatial patterns are compared from three sampling stations: at high, middle and low intertidal locations; temporal patterns are determined from monthly sampling at these stations. Foraminiferal patterns will be considered at high and low intertidal locations. These data will then be correlated to changes in the in situ microphytobenthos biomass and other environmental variables.

The main aims of the research work presented in this chapter are to:

1. Identify which benthic foraminiferal species live on the Eden Estuary.
2. Examine the spatial differences between benthic foraminiferal populations at high and low tide sampling stations on an intertidal mudflat.
3. Examine temporal changes within these sampling sites and attempt to explain these changes based on measured abiotic and biotic factors (with an emphasis on the biotic factor of diatom abundance measured as chlorophyll $a$, $c$).
4. Investigate periods of reproduction, based upon measurements of maximum test diameters.
6.2 Materials and Methods

6.2.1 The study site

The Eden Estuary, N. E. Scotland was chosen as the study site primarily for its close proximity to St Andrews and its extensive mudflats. These combined to give relatively easy access to sampling sites and the ability to return samples quickly to the laboratory.

Figure 6.1: Aerial photograph of the upper North bank of the Eden Estuary. Transect line is marked with white circles. A= upper tidal range, B= mid tidal range, C= low tidal range. Photograph courtesy of EU TIDE project.

6.2.2 Sampling transect

During early April 2000 three permanent sampling stations were levelled onto a transect on the North shore of the upper estuary, across an expansive mudflat representing upper, middle and lower tidal ranges (Figure 6.1). Each station was marked with steel poles placed 1 metre apart, hammered into the sediment (Figure 6.2). These stations were located using a Leica system SR530 - dual frequency, geodetic global positioning system (GPS) with potential accuracy 10 mm horizontal and 20 mm vertical. Accuracy of the system during the survey was 20 mm horizontal and vertically as a consequence of available satellites. An
area of 1 m$^2$ to the East of the double poles was designated as the sampling area (Figure 6.3) and disturbance of the area was kept to a minimum.

Figure 6.2: The Eden Estuary transect in April 2000. (A) High intertidal, double steel poles visible 1m apart. (B) Mid intertidal (C) Low intertidal. The view is towards the East on a low tide. The RAF Leuchars airbase is visible in the photograph above station A.

Figure 6.3: Position of the Eden Estuary sampling transect derived from a geodetic GPS survey. Open dark circles represent the transect line. Filled circles represent pole positions on the transect. Faint grey open circles represent other survey points not used in this study. Positions are OS grid coordinates in metres.
The shore profile demonstrates a gently sloping mudflat (towards the channel) of approximately 150 metres (Figure 6.4).

Figure 6.4: Mudflat profile drawn along the Eden Estuary transect, indicating height above ordinance datum (OD) and distance between sampling points. The six points, which show a sharp rise in inclination, are the shore and bank onto a track on the North side of the Eden Estuary. The fresh water channel at low water on 18th February 2003 is defined at 163 m on the x-axis of the graph.

6.2.3 Sediment sampling procedures

During each sampling event (once a month at low tide, Table 6.2) 3 foraminiferal cores, 1-5 contact cores for chlorophyll, water content, carbon and nitrogen determination, and 1 core for grain size analysis, temperature and salinity measurements were taken at each of the marked shore levels (Figure 6.4). To assess any effects caused by increased sedimentation or sediment erosion on the transect, bed-level height was measured from six fixed points on a levelled pole suspended between the paired steel poles every month, at every shore level.

Foraminiferal sediment cores were collected by hand to a depth of 5cm using PVC cores (Φ 8.1 cm). After digging out the core, a thin aluminium plate
was carefully used to sever the sediment. The core was lifted out, sealed at the bottom and top (rubber bung), placed in a core carrier ensuring the cores were retained in an upright position and returned to the laboratory for processing as quickly as possible (usually < 2 hours).

Table 6.2: List of sampling dates and heights of low tides.

<table>
<thead>
<tr>
<th>Month</th>
<th>Date</th>
<th>Time of low water (GMT)</th>
<th>Low Tide Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 2000</td>
<td>Monday 8th</td>
<td>11:24</td>
<td>0.8</td>
</tr>
<tr>
<td>June 2000</td>
<td>Thursday 1st</td>
<td>07:14</td>
<td>1.0</td>
</tr>
<tr>
<td>July 2000</td>
<td>Wednesday 5th</td>
<td>11:14</td>
<td>0.3</td>
</tr>
<tr>
<td>August 2000</td>
<td>Wednesday 2nd</td>
<td>10:12</td>
<td>0.0</td>
</tr>
<tr>
<td>September 2000</td>
<td>Friday 1st</td>
<td>10:33</td>
<td>0.2</td>
</tr>
<tr>
<td>October 2000</td>
<td>Monday 2nd</td>
<td>10:55</td>
<td>1.1</td>
</tr>
<tr>
<td>November 2000</td>
<td>Wednesday 1st</td>
<td>10:37</td>
<td>1.6</td>
</tr>
<tr>
<td>December 2000</td>
<td>Saturday 2nd</td>
<td>11:33</td>
<td>2.0</td>
</tr>
<tr>
<td>January 2001</td>
<td>Friday 12th</td>
<td>10:14</td>
<td>0.9</td>
</tr>
<tr>
<td>February 2001</td>
<td>Monday 12th</td>
<td>11:19</td>
<td>0.9</td>
</tr>
<tr>
<td>March 2001</td>
<td>Friday 9th</td>
<td>08:13</td>
<td>0.8</td>
</tr>
<tr>
<td>April 2001</td>
<td>Sunday 4th</td>
<td>17:50</td>
<td>1.2</td>
</tr>
<tr>
<td>May 2001</td>
<td>Thursday 10th</td>
<td>10:06</td>
<td>0.8</td>
</tr>
</tbody>
</table>

During sampling the cores were taken randomly but relatively close to each other to minimise disturbance of the area. This was particularly important at the high intertidal site where sediment was very soft and deep, making the sampling process potentially disruptive to the adjacent areas. Preliminary studies revealed that within each 1 m² sampling area, no significant foraminiferal species composition differences existed.

6.2.4 Processing of cores

On returning to the laboratory the cores were extruded using a piston and sliced with a sheet of aluminium (carefully cleaned after each depth slice). A collar measuring 1 cm was attached to the top of the core to act as a depth guide. Care was taken to siphon any surface water into the allocated sample pot. Once sliced, the sediment was weighed wet and then preserved with an equal volume of 80% Ethanol and rose Bengal (1g per litre) (Walton 1952). Rose Bengal stains the protoplasm allowing the differentiation between “live” and “dead” foraminifera. This was left for one day, after which time a settled sediment volume measurement was carried out. After staining, samples were washed through a 63
μm sieve to remove the clay and silt fraction present within the sediment and preserved in 70% Ethanol. Although sediment was sliced to a depth of 2 cm, time constraints dictated that only the surface sediments from the high and low intertidal were fully analysed; sub-surface samples remain archived for future study.

6.2.5 Extraction of foraminifera from sediment samples

All foraminiferal samples were processed wet. Where standing stocks were high, samples were split using the splitter described in Materials and Methods (Chapter 2). Individuals were "picked" using a fine paintbrush (0000) and transferred to taxonomic slides for later identification and biometric measurements.

6.2.6 Foraminiferal biomass

Biomass estimates in this study focused on the two dominant species *Haynesina germanica* and *Elphidium williamsoni*. The maximum diameter of each foraminifera was measured under a binocular microscope (Wild MZ3) with the aid of an eyepiece graticule calibrated to a micrometre scale. Each subdivision at the chosen magnification (x 25) represented 18.5 μm. For all specimens, it was also noted approximately what percentage of the test was filled with protoplasm, in order to make corrections to the bio-volume estimates. Sixty individuals of both *Haynesina germanica* and *Elphidium williamsoni* were further measured both for maximum test diameter and depth. From these measurements it was possible to establish a predictive model (linear regression) to estimate the depth of all tests measured. Finally, from these dimensions bio-volumes for all individuals were calculated (See chapter 2 for details).

6.2.7 Foraminiferal size frequency distributions

Maximum test diameter measurements were further used to examine variations in the monthly size frequency distribution of the two dominant species present (*Haynesina germanica* and *Elphidium williamsoni*). One of the main reasons for measuring size was to determine periods of reproduction (shift
towards smaller test sizes) and attempt to follow cohorts as they grow over the sampling period.

6.2.8 **Chlorophyll sampling**

Chlorophyll *a*, *b* and *c* concentrations (mg m\(^{-2}\)) were measured as an indication of amount and diversity of possible food sources for the benthic foraminifera (Buzas 1969). Studies into the vertical distribution of chlorophyll *a* concentration have shown that it is generally concentrated in the surface 200 μm (Kelly *et. al.* 2001). Contact cores (e.g. Honeywill *et al.* 2001) of surface area 2,462 mm\(^2\) were taken to a depth of 2-5 mm, ensuring a more accurate estimate of chlorophyll available to the foraminifera. Contact cores were taken, wrapped in aluminium foil and immediately frozen in liquid nitrogen. On returning to the laboratory, samples were stored in a -80°C freezer until freeze-drying and analysis.

Extraction of Chlorophyll *a*, *b* and *c* was carried out using HPLC grade Dimethylformanide (DMF), and quantification of pigment concentrations was carried out in a spectrophotometer (Cecil 3000 series) (Chapter 2). For the first four sampling months one contact core was taken at each sampling station. It quickly became clear that repeated samples were required to give a representative value for the sampling area. Thus, for the remaining 8 months, five contact cores were taken at each sampling station. Three were paired with the three foraminiferal cores and the other two were taken randomly within the sampling area.

6.2.9 **Grain size analysis**

Analysis was carried out using a Coulter LS230 laser particle size analyser on both raw sediment, and sediment treated to remove organics and calcium carbonate (Chapter 2).

6.2.10 **Sediment carbon, nitrogen determination**

The organic nitrogen and carbon content of the samples (30 mg) was determined by an automated Dumas combustion procedure (Pella and Colombo, 1973) using a Carlo Erba NA1500 Elemental Analyser (Carlo Erba Instruments, Milan, Italy) (Chapter 2).
6.2.11 Other environmental variables measured

Temperature was measured using a digital thermometer pushed 1 cm into the sediment. Salinity of residual surface water was measured using a refractometer. No rainfall was experienced immediately prior to or during sampling events. Therefore, it is considered that sediment salinities reflect that of the retreating high tide and any changes produced as a result of evaporation during warmer months. Air temperature and rainfall data was sourced from RAF Leuchars who have a weather monitoring station on the estuary.

6.2.12 Statistical analysis

Data analysis was carried out using a combination of univariate and multivariate tests. Canonical correspondence analysis (CCA) was used to investigate community associations between intertidal benthic foraminifera and measured environmental variables over a period of one year at high and low intertidal sampling stations. Analysis was carried out using PC-ORD for Windows version 3.01. Abundance of species (per 10 cm²) was used and any species or genus with less than 3% relative abundance in any one sample was excluded from the analysis. Absolute numbers were used for environmental variables. A Monte Carlo permutation test (999 permutations) was applied to assess the statistical significance of the correlations between species and environmental variables. The major matrix for both high and low intertidal consisted of 6 species and 39 samples. These matrices were further related to an environmental matrix of 16 measured variables and 39 samples for each tidal height. Environmental variables used in the analysis were chlorophyll \( a, b \) and \( c \) concentration, sediment temperature, salinity, water content (%), loss on ignition, total rainfall, air temperature, modal grain size (organics removed), modal grain size (raw sediment), % organic carbon present in the sediment, % nitrogen present in the sediment, C/N ration, mean monthly River Eden flow and mean monthly Motray Water flow.

Repeat measures ANOVA (Month, fixed; sampling station, fixed)(SPSS v 7.5.1) was used to analyse seasonally collected variables. In cases where significant interactions were revealed, post hoc analysis (Tukey HSD) was carried out only when Tukey’s test for non-additivitiy (Underwood 1999) revealed the differences among means to be non-multiplicative (\( P>0.05 \)) and could therefore
be attributed to the independent effect of the 2 factors. This analysis allowed for
the identification of the source of interaction and interpretation of the main effects
(Todd 2003).

Prior to analysis of seasonally collected Chlorophyll $a$, $b$ and $c$, the data
were transformed using boxcox (Minitab v 12.1) transformation, to improved
homogeneity of variance. Due to a lack of replication for all chlorophyll samples
during May, June, July and August 2000 these were removed from the data set
prior to ANOVA (Underwood 1999).

6.3 Results

6.3.1 Estuarine environmental variables

6.3.1.1 Temperature, salinity, rainfall, windspeed

Surface sediment temperatures reached maxima during July (19.2°C) at
the high intertidal sampling station and August (19.4°C) at the low intertidal
sampling station. An overall fall in temperature from these maxima to minima in
January 2001 took place for the high, middle and low intertidal sampling stations
(4, 3.8 and 3.8°C respectively). From January 2001, the temperatures fluctuated
until April, when a marked increase in sediment temperature (5 to 16°C) was
evident in May. Salinities at the high intertidal station ranged from 20 to 34, while
at the low intertidal station a higher range from 14 to 34 is recorded over the
sampling period. Salinities appeared to be within the same range from May 2000
to October 2000 after which point salinities are lower at the low intertidal
sampling station (Figure 6.5).

Sediment temperature in the surface 1 cm was closely mirrored by air
temperature for the area (Table 6.3). Rainfall data revealed September 2000 to
have been the wettest month (126.2 mm) followed by December 2000 (107.2
mm). The driest months were May 2001 (14.5 mm) and July 2000 (16 mm).
These are fairly typical seasonal patterns (Hill, RAF Leuchars pers. comm. 2001).

6.3.1.2 Sediment water content

Sediment water content was significantly different over the sampling
period of 13 months ($F_{12,72} = 38.466$, $P<0.001$) and between tidal heights ($F_{2,6} =$
365.728, P<0.001). Tukey's test for non-additivity (F_{1,95}=2.5, P=0.114) allowed for post hoc analysis (Table 6.4).

The high intertidal station had the highest water content, followed by mid intertidal and low intertidal sampling stations. At the high intertidal station water content was found to increase from May 2000 to November 2000, whilst at low tide it decreased until September 2000 and then levelled off. In general, at all tidal levels, water content was highest over the winter months January to April 2001, after which point a sharp decrease in water content for all shore levels was recorded. Highest values for the high and low intertidal stations are in April 2000 (44.9% and 42.2% respectively), whilst lows are in May 2000 (39.5%) and May 2001 (36.9%). Mid intertidal revealed a maximum water content in March 2001 (40.786%), and a low in December 2000 (38.5%) (Figure 6.6).

<table>
<thead>
<tr>
<th>Month</th>
<th>Air temperature (°C)</th>
<th>Rainfall (mm)</th>
<th>Wind speed (Kts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daytime maximum</td>
<td>Night-time minimum</td>
<td>Total</td>
</tr>
<tr>
<td>May 2000</td>
<td>18.9</td>
<td>2.4</td>
<td>75</td>
</tr>
<tr>
<td>June 2000</td>
<td>22.3</td>
<td>1.4</td>
<td>57.6</td>
</tr>
<tr>
<td>July 2000</td>
<td>24.4</td>
<td>2.8</td>
<td>16</td>
</tr>
<tr>
<td>August 2000</td>
<td>25.5</td>
<td>7.2</td>
<td>55.2</td>
</tr>
<tr>
<td>September 2000</td>
<td>21.8</td>
<td>2.9</td>
<td>126.2</td>
</tr>
<tr>
<td>October 2000</td>
<td>20.2</td>
<td>-1.0</td>
<td>81.6</td>
</tr>
<tr>
<td>November 2000</td>
<td>12.9</td>
<td>-1.4</td>
<td>95.8</td>
</tr>
<tr>
<td>December 2000</td>
<td>11.8</td>
<td>-6.5</td>
<td>107.2</td>
</tr>
<tr>
<td>January 2001</td>
<td>8.9</td>
<td>-7.6</td>
<td>34.2</td>
</tr>
<tr>
<td>February 2001</td>
<td>10.2</td>
<td>-5.1</td>
<td>88.8</td>
</tr>
<tr>
<td>March 2001</td>
<td>13.5</td>
<td>-11.7</td>
<td>55.9</td>
</tr>
<tr>
<td>April 2001</td>
<td>15.1</td>
<td>-2.3</td>
<td>43.2</td>
</tr>
<tr>
<td>May 2001</td>
<td>23.2</td>
<td>0.0</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Figure 6.6: Percent water content of surface sediments (0-0.5 cm) ± SE (n=5) from the Eden Estuary transect at high, middle and low intertidal sampling stations. Sampling period May 2000 to May 2001.
Table 6.4: Post Hoc analysis (Tukey’s HSD) results for water content at three tidal heights. Illustrating results which were not significantly different between tidal heights over the 13-month sampling period.

<table>
<thead>
<tr>
<th>Month</th>
<th>Comparison of tidal height</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 2000</td>
<td>High and Low</td>
<td>0.477</td>
</tr>
<tr>
<td></td>
<td>Low and Mid</td>
<td>0.190</td>
</tr>
<tr>
<td>June 2000</td>
<td>Low and Mid</td>
<td>0.252</td>
</tr>
<tr>
<td>September 2000</td>
<td>High and Mid</td>
<td>0.139</td>
</tr>
<tr>
<td>December 2000</td>
<td>Low and Mid</td>
<td>0.139</td>
</tr>
<tr>
<td>January 2001</td>
<td>Low and Mid</td>
<td>0.415</td>
</tr>
<tr>
<td>March 2001</td>
<td>High and Low</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td>Mid and Low</td>
<td>0.617</td>
</tr>
</tbody>
</table>

6.3.1.3 Grain size

Grain size analyses for both treated and raw sediment from the Eden Estuary transects revealed approximately the same range of sand and silt content. However, it is apparent that the raw measurements have underestimated the clay content. In Figure 6.7A (raw sediment), the clay content was consistently low (less than 5 %) while sand and silt ranged from 16 % to 54 % and 45 % to 80 % respectively. Comparing this to sediment treated to remove organics (Figure 6.7B) a similar range of silt and sand, but increased clay content up to 11 % was measured. The data clearly demonstrates the value of pre-treating sediment samples prior to grain size analysis and the increased clay content can be interpreted as the disaggregation of sediment flocs (e.g. faecal pellets). Grain size analysis of the raw (untreated) sediment failed to disaggregate these flocks. However, from a biological perspective it is apparent that the surface sediments of the Eden Estuary are typically sandy silts. The high intertidal stations sediments are finer than the low intertidal station. Both the high and low intertidal sampling stations showed a tendency towards increases in the silt fraction from December 2000 to March 2001 (Figure 6.8). However, this pattern was less well defined at the mid intertidal sampling station.

Modal peaks for both treated and untreated sediment showed varying degrees of change. At the high intertidal station there was a shift towards smaller grain sizes between January 2001 (19.8 μm, 28.7 μm) and April 2001 (14.9 μm) with modal grain size dropping from 45.8 μm in December, then increasing towards May 2001 (37.9 μm).
The mid intertidal station, both treated and untreated sediment, showed no changes in modal peaks throughout the sampling period. Some changes were recorded in the modal peaks at the low intertidal sampling station around March 2001. Treated sediment showed a pronounced change, revealing a shift to finer grain size in January (60 μm), February (60.5 μm) and March (45.8 μm) 2001, from 72.9 μm in December 2000. Untreated sediment at the low intertidal station showed a shift to lower grain size in March 2001 (45.7 μm) from February 2001 (60.5 μm). Recovery to 66.4 μm was evident from April 2001 in both sediment treatments.

![Figure 6.7: Ternary plots of intertidal sediment from the Eden Estuary. High intertidal, Mid intertidal, Low intertidal (A) Raw sediment (B) Treated sediment (organic s and calcium carbonate removed).](image-url)
6.3.2 Carbon and nitrogen determination

6.3.2.1 Organic carbon

Sediment organic carbon (%weight) content suggested a significant relationship between tidal height and month of sampling ($F_{24,72} = 6.861$, $P<0.001$).
May 2001 showed overall the lowest percent organic carbon content for all of the sampling stations (Figure 6.9A).

At the high intertidal station percent organic carbon values rose steadily from $3.12 \pm 0.7\%$ in May 2000 to maxima in January and February 2001 ($5.16 \pm 0.73\%$), after which time they declined, with the exception of one further peak in April 2001 ($4.74 \pm 1.77\%$). However, standard errors are clearly large during April 2001 (Figure 6.9) and care should be taken in the interpretation of this apparent increase.

Organic carbon content at the mid intertidal station gradually declined throughout the sampling period, with peaks in November 2000 ($3.68 \pm 0.60\%$) and March 2001 ($3.24 \pm 0.37\%$). The overall trend was decreasing values towards a low in May 2001 ($1.49 \pm 0.94\%$), falling below the value recorded in May 2000 ($3.27 \pm 0.04\%$).

One major peak in organic carbon was evident in June 2000 ($5.14 \pm 0.08\%$) at the low intertidal station, after which point it dropped steadily to a minimum in May 2001 ($1.90 \pm 0.86\%$). This minimum is below that recorded for May 2000 ($3.12 \pm 0.94\%$). Overall, the percentage organic carbon measured over the sampling periods were significantly different ($F_{12.72} = 9.618$, $P \leq 0.001$). However, further analysis using Tukey’s test for non-additivity ($F_{1.95}=0.3310$, $P=0.567$) allowed for post hoc analysis (Table 6.5).

Table 6.5: Post hoc analysis (Tukey’s HSD) results for % organic carbon content of sediment over a 13-month sampling period. Comparisons which were not significant, $P \geq 0.05$.

<table>
<thead>
<tr>
<th>Month</th>
<th>Comparison of tidal height</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 2000</td>
<td>High and Low</td>
<td>1.000</td>
</tr>
<tr>
<td>June 2000</td>
<td>High and Mid</td>
<td>0.514</td>
</tr>
<tr>
<td>July 2000</td>
<td>High and Mid</td>
<td>0.629</td>
</tr>
<tr>
<td>September 2000</td>
<td>High and Mid</td>
<td>0.638</td>
</tr>
<tr>
<td>October 2000</td>
<td>Low and Mid</td>
<td>0.819</td>
</tr>
<tr>
<td>November 2000</td>
<td>High and Mid</td>
<td>0.444</td>
</tr>
<tr>
<td></td>
<td>Low and Mid</td>
<td>0.285</td>
</tr>
<tr>
<td>December 2000</td>
<td>Mid and Low</td>
<td>0.113</td>
</tr>
<tr>
<td>January 2001</td>
<td>Low and Mid</td>
<td>0.745</td>
</tr>
<tr>
<td>February 2001</td>
<td>Low and Mid</td>
<td>0.619</td>
</tr>
<tr>
<td>March 2001</td>
<td>High and Low</td>
<td>0.624</td>
</tr>
<tr>
<td></td>
<td>High and Mid</td>
<td>0.197</td>
</tr>
<tr>
<td></td>
<td>Low and mid</td>
<td>0.580</td>
</tr>
<tr>
<td>April 2001</td>
<td>all comparisons not significantly different</td>
<td></td>
</tr>
<tr>
<td>May 2001</td>
<td>all comparisons not significantly different</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.9: Summary graphs of (A) % organic carbon at all sampling stations (B) % nitrogen at all sampling stations (C) Carbon to nitrogen (C/N) ratio for all sampling stations. All ± SE, n=3.
6.3.2.2 Nitrogen

There was an overall significant interaction between tidal height and month of sampling in the percentage of nitrogen in the sediment ($F_{24,72} = 38.880, P<0.001$). In general, the high intertidal sampling station had the highest % nitrogen values. One exception occurred in June 2000 when the low intertidal station % nitrogen value exceeded that recorded at all other tidal heights. The highest % nitrogen at the high intertidal station occurred in April 2001 (0.71%) and the lowest in June and September 2000 (0.28%) (Figure 6.9B).

Overall % nitrogen content of the sediment was significantly different over the 13-month sampling period ($F_{12,72} = 31.182, P<0.001$).

6.3.2.3 Organic carbon/nitrogen ratio

From May 2000 to February 2001 the C/N ratio was high (>8) at all intertidal sampling stations (high, mid and low), indicating depletion in nitrogen (Figure 6.9C). This is generally indicative of the dominance of refractory and or terrestrial carbon in the sediment, often with some proportion of phytoplankton present (Fenchel and Riedle 1970).

The mid intertidal station C/N ratio falls to 8 in May 2001, while C/N ratios at the high and low intertidal stations dropped below 8 from February 2001. Low C/N ratios (<8) indicate high quality, nitrogen-rich, labile material mainly derived from marine, possibly reflecting phytoplankton/microphytobenthos, sources. The high intertidal sampling station showed the lowest ratios during March and May 2001, and the low intertidal station showed a minimum during March 2001 and April 2001, rising again in May 2001 to greater than 8. There is a significant interaction between C/N ratio and tidal height ($F_{24,72} = 3.372, P<0.001$).

6.3.3 Chlorophyll a, b and c

6.3.3.1 Chlorophyll a

Chlorophyll a content of the sediment was significantly different over the 13-month sampling period ($F_{8,96} = 470.201, P<0.001$) and there was a significant interaction between shore height and month of sampling ($F_{16,96} = 255.768,$
P<0.001) (Figure 6.10A). The low intertidal sampling station had the highest concentration of chlorophyll *a* from May 2000 to August 2000, peaking in June 2000 with a concentration of 275 mg m⁻² (Figure 6.10A). Also during this period, the high intertidal sampling station had the lowest concentration of chlorophyll *a*. The clearest difference in chlorophyll *a* concentration occurred in March and April 2001 when the high intertidal sampling station exhibited a clear 3-fold increase in chlorophyll *a* (from 54 mg m⁻² in February to 194 mg m⁻² in March 2001) (Figure 6.10A).

6.3.3.2 Chlorophyll *b*

There was significant interaction between Chlorophyll *b* concentration and tidal height (F₁₆,₉₆ = 9.948, P<0.001) and it was significantly different over the 13-month sampling period (F₈,₁₆ =101.379, P<0.001). Concentration values appeared to peak during June and July 2000 at the mid and low intertidal stations, and in October 2000 at the high intertidal sampling station. Chlorophyll *b* declined to a minimum in April, rising again in May 2001 at all stations (Figure 6.10B). However, during June 2000 (low), December 2000, January 2001 (high) and March and April 2001 (high and low), values were below the limit of detection.

6.3.3.3 Chlorophyll *c*

Results showed a significant difference in Chlorophyll *c* concentration over the 13-month sampling period (F₈,₉₆ = 32.551, P<0.001). There was significant interaction between shore height and the month of sampling (F₁₆,₉₆ = 11.007, P<0.001). However, Tukey’s test for non-additivity (F₁,₁₁₁ = 0.426, P = 0.516) allowed for *post hoc* analysis, revealing the most highly significant differences to occur at the high intertidal sampling station during March and April 2001 (P<0.001) (Figure 6.10C).
Figure 6.10: Summary graphs of changes in (A) chlorophyll a (B) chlorophyll b (C) chlorophyll c at high, mid and low intertidal over the sampling period May 2000 to May 2001 on the Eden Estuary, N.E. Scotland.
6.3.4 Total counts of benthic foraminifera

Total numbers of benthic foraminifera were expressed as the mean (± SE) of three replicate cores taken at each sampling station. In general, standing stocks were higher at the high intertidal sampling station (range 31 individuals 10 cm⁻² in March 2001 – 270 individuals 10 cm⁻² in June 2000) compared to the low intertidal sampling station (range 17 individuals 10 cm⁻² in December 2000 – 412 individuals 10 cm⁻² in June 2000). With exceptions occurring during June, July 2000 and March 2001, when standing stocks at the low intertidal station exceeded those recorded at the high intertidal sampling station (Figure 6.11). However, standard errors for June 2000 were large for both stations, indicating large spatial variation (patchiness) within each sampling area. Raw count data are provided in the accompanying data CD.

Figure 6.11: Total number of stained individuals (standing stock) ± SE over the sampling period May 2000 – May 2001 at high and low intertidal sampling stations on the Eden Estuary, N.E. Scotland.

As a second year of sampling was not carried out, it is not possible to determine whether or not this seasonal pattern is repeated every year. However,
based upon the data presented, it would appear that the numbers of individuals were again increasing towards June 2001. Patterns for both stations appeared to be the same through much of the year, with those at the high intertidal station maintaining slightly higher standing stocks. From March 2001, standing stocks at the high intertidal station began to increase, while at the low intertidal station standing stocks fell very slightly, but appear to be recovering by May 2001. Simple linear regressions between total abundance of foraminifera (specimens 10 cm\(^{-2}\)) and concentration of chlorophylls \(a\), \(b\) and \(c\) revealed two weak, but significant correlations between total abundance and chlorophyll \(a\) \((R^2=0.470, P=0.003)\) and chlorophyll \(c\) \((R^2=0.486, P<0.001)\) at the low intertidal sampling station. No correlations were found in data from the high intertidal sampling station.

6.3.5 Foraminiferal species composition

A total of 12 (low intertidal station) and 15 (high intertidal station) species of living intertidal benthic foraminifera were identified (Figure 6.12). Both the high and low intertidal sampling station assemblages were dominated by two infaunal species *Haynesina germanica* and *Elphidium williamsoni* throughout the year. These two species comprised 85 to 99% of the total assemblage at the high intertidal station and 78 to 99% at the low intertidal station (Figure 6.13 and 6.14 respectively). For this reason, these two species were selected for detailed study.

At the high intertidal sampling station *Haynesina germanica* dominated the assemblage from May to November 2000 (Figure 6.13). From November 2000 *Elphidium williamsoni* dominated the assemblage until May 2001. The low intertidal assemblage showed a slightly different response, *H. germanica* dominating the assemblage between May and June 2000. In July, *H. germanica* and *E. williamsoni* were equally dominant. From July to December 2000, *H. germanica* again dominated the assemblage.
Figure 6.12: Scanning electron micrograph (SEM) images of benthic foraminifera found living in surface sediments from the Eden Estuary, NE Scotland. Scale bars 100 μm, unless otherwise stated. 1, 2 Haynesina germanica (Ehrenberg). 3, 4 Elphidium williamsoni Haynes. 5 Elphidium oceanicus (d’Orbigny). 6 Elphidium incertum (Williamson). 7 Fissurina lucida (Williamson). 8, 9 Aubignyna cf. perlocuda (in text as indeterminate trochospiral sp.). 10 Miliammina fusca (Brady)
Figure 6.13: Relative abundance (%) of the 7 dominant taxa of benthic foraminifera at the high intertidal sampling station on the Eden Estuary.

Figure 6.14: Relative abundance (%) of the 7 dominant taxa of benthic foraminifera at the low intertidal sampling station on the Eden Estuary.
6.3.6 *Species diversity*

In general, diversity was marginally higher at the high intertidal sampling station for most of the year with exceptions in July 2000, January 2001, February 2001, April 2001 and May 2001 (Figure 6.15A). This same pattern was seen in the number of species, with the high intertidal station having higher numbers of species present for most of the sampling year, with exceptions during July 2000, April 2001 and May 2001 (Figure 6.15B). A seasonal pattern is evident, with the high intertidal station exhibiting a higher diversity over winter months (Figure 6.15A).

However, averaged over the year there would appear to be little difference in diversity, evenness, richness and total number of species between the two sampling stations (Table 6.6).

Table 6.6: Species diversity, evenness and total number of species for high and low intertidal sampling stations. Values are means of three replicate cores.

<table>
<thead>
<tr>
<th></th>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Diversity (H')</td>
<td>0.43-1.21</td>
<td>0.89±0.04</td>
</tr>
<tr>
<td>Evenness (J')</td>
<td>0.31-0.71</td>
<td>0.51±0.02</td>
</tr>
<tr>
<td>Richness (d)</td>
<td>0.40-1.80</td>
<td>1.13±0.05</td>
</tr>
<tr>
<td>Number species (S)</td>
<td>3-9</td>
<td>5.87±0.20</td>
</tr>
</tbody>
</table>

6.3.7 *Monthly size distribution, reproduction*

Despite sieving at 63 μm and picking samples wet, there was a distinct lack of juveniles less than 93 μm for the two dominant species: *Haynesina germanica* and *Elphidium williamsoni*. Monthly variations in these populations at the high and low intertidal sampling stations were monitored by measuring the maximum test diameters of all individuals and plotted as % relative frequency of the total standing stock occurring each month.
Figure 6.15: (A) Species diversity ($H'$) and (B) total number of species (S) at high and low intertidal sampling stations.
6.3.8 Haynesina germanica

Within the high intertidal H. germanica population, juveniles were present in May, July, August, September and December 2000 and March and May 2001. However, at none of these times were there large peaks in juvenile abundance which could be considered indicative of a large reproductive event (Table 6.7).

The low intertidal H. germanica population exhibited a reduced frequency of juveniles compared to the high intertidal station. October is the only month with significant numbers of juveniles, but numbers remain relatively low (Table 6.7).

Possible reproductive events were, however, seen as a redistribution of size classes from May to July 2000 (Figure 6.16A-F) at both the high and low intertidal sampling stations. Despite the lack of observed juveniles at the high intertidal station, a clear shift from a unimodal population size frequency distribution skewed towards larger individuals in May 2000 (mode 333 μm) to a bimodal distribution (modes at 185 μm and 333 μm) skewed toward smaller individuals in June 2000 was recorded. Growth of the cohort recognised at 185 μm during June 2000 was charted into July 2000 and continued growth of juveniles into this size class is evident (Figure 6.16A-C).

At the low intertidal sampling station during May 2000 the size distribution was more heavily skewed towards larger individuals (mode 407 μm) with no individuals recorded smaller than 185 μm. A clear shift from a unimodal size frequency distribution during May 2000 to a bimodal size frequency (modes at 222 μm and 407 μm) distribution was evident (Figure 6.16D-F). This new cohort identified at 222 μm continued to grow resulting in a unimodal distribution in July 2000 with a size frequency distribution skewed toward smaller size classes. There was also a loss of larger individuals which could be attributed to reproduction and the introduction of individuals into smaller size classes (Figure 6.16F).
Table 6.7: Size classes (μm) and % relative frequency of the two dominant species of benthic foraminifera found on the Eden Estuary, Scotland.

<table>
<thead>
<tr>
<th>Size class</th>
<th>May'00</th>
<th>June'00</th>
<th>July'00</th>
<th>Aug'00</th>
<th>Sept'00</th>
<th>Oct'00</th>
<th>Nov'00</th>
<th>Dec'00</th>
<th>Jan'01</th>
<th>Feb'01</th>
<th>Mar'01</th>
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<td>0.00</td>
<td>0.71</td>
<td>0.50</td>
<td>2.36</td>
<td>1.88</td>
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6.3.9 Elphidium williamsoni

Within the high intertidal E. williamsoni population, juveniles were observed in March and May 2001. However, as with H. germanica these times were not represented by a large peak in the relative abundance of juveniles (<93 μm) (Table 6.7).

Juveniles were present in the low intertidal E. williamsoni population in extremely low concentrations during July, October 2000 and May 2001 (Table 6.7). Possible reproductive events were most clearly seen as a redistribution of size classes from April to May 2001 (Figure 6.17, A-D).
williamsoni population at the high intertidal station during April 2001 had a unimodal size frequency distribution (mode 315 μm). In May 2001 this had clearly shifted to a bimodal size frequency distribution, with a new cohort visible at 185-278 μm and the on growth of the existing cohort at 463-518 μm (Figure 6.17A-B).

The *E. williamsoni* population at the low intertidal sampling station shows a very similar trend. During April 2001 a unimodal peak was recorded in the size frequency distribution (mode 315 μm). By May 2001 this has clearly shifted to a bimodal distribution of size frequency, with the new cohort represented by a peak between 222 and 259 μm. Those individuals which did not reproduce in April 2001 were represented by a second peak at 463 μm (Figure 6.17C-D).

![Graphs illustrating shift in size distribution](image)

**Figure 6.17:** *Elphidium williamsoni* population size frequency at high and low intertidal. Graphs illustrating shift in size distribution at (A)-(B) High intertidal sampling station; (C)-(D) low intertidal sampling station.

### 6.3.10 Biomass

Biomass (μg C 10 cm⁻²) showed a similar trend to that of standing stock (number of individuals 10 cm⁻²) for each species (Figure 6.18(A) and (B)). Highly significant correlations (P<0.001) were found between standing stock and biomass...
for both *H. germanica* (*R^2*=0.9797, *R^2*=0.8837) and *E. williamsoni* (*R^2*=0.9683 *R^2*=0.84) at low and high intertidal stations respectively. Both species showed a large range (more than 2 orders of magnitude) of biomass over the sampling period at the high and low intertidal sampling stations (Table 6.8).

Table 6.8: Summary statistics of biomass (μg C 10 cm⁻²) for *H. germanica* and *E. williamsoni* from high and low intertidal sampling stations.

<table>
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<tr>
<th>Species</th>
<th>Range of biomass measurements</th>
<th>Mean biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
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<tr>
<td><em>H. germanica</em></td>
<td>0.64-103</td>
<td>1.99-299</td>
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<tr>
<td><em>E. williamsoni</em></td>
<td>1.45-184.9</td>
<td>1.59-147</td>
</tr>
<tr>
<td>Total</td>
<td>3.93-259</td>
<td>5.01-445</td>
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</table>

*Elphidium williamsoni* contributed the highest mean biomass at the high intertidal station, with nearly double its low intertidal station value (Figure 6.10), whereas, at the high intertidal station *H. germanica* contributed the highest mean biomass, slightly higher than that observed at the low intertidal station.

Simple linear regressions of the concentration of *H. germanica* per 10 cm² and biomass versus chlorophyll measured produced significant correlations (*P*<0.001) for the low intertidal sampling station only. Using biomass as the dependent variable improved the *R^2* of the regression considerably from 0.50 to 0.61 with chlorophyll *a*. Therefore, at the low intertidal station the biomass of *H. germanica* increased with increases in Chlorophyll *a*. Regressions with chlorophyll *c* and *H. germanica* were also significant (Table 6.9), demonstrating an increase in *H. germanica* biomass with chlorophyll *c*.

Table 6.9 Results of linear regressions between abundance and biomass of *H. germanica* (Hg) and *E. williamsoni* (Ew) against chlorophylls *a* and *c* at low intertidal.

<table>
<thead>
<tr>
<th>concentration (specimens 10 cm⁻²)</th>
<th>Biomass (μg C 10 cm⁻²)</th>
</tr>
</thead>
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<td>Hg</td>
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<tr>
<td>Chl <em>a</em></td>
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<td><em>P</em></td>
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<td>Chl <em>c</em></td>
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<td>R^2</td>
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<tr>
<td><em>P</em></td>
<td>&lt;0.001</td>
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</table>
Figure 6.18: Biomass of *Haynesina germanica* and *Elphidium williamsoni* at (A) the high intertidal sampling station (B) the low intertidal sampling stations.
6.3.11 Multivariate statistical analysis

6.3.11.1 Canonical correspondence analysis

6.3.11.2 High intertidal sampling station

Summary results of the CCA analysis for the high intertidal are presented in Table 6.10. The three axes do not explain similar amounts of the variance, with axis 2 (27.3%) explaining just over half that of axis one (49.1%). As the third canonical axis explained only a further 4.8% of the variation, only axis 1 and 2 are presented here.

These data demonstrate that the first axis is strongly influenced by sediment temperature, water content (%), mean monthly air temperature, % nitrogen, loss on ignition and salinity (Table 6.10). Axis two is largely a function of total monthly rainfall, grain size (both organics removed and raw sediment), and river flow of The Eden. None of the chlorophylls measured had a significant influence on either axis 1 or 2. However, there were weak correlations which should not be ignored.

The biplot (Figure 6.19) shows the distribution of species and samples with respect to environmental variables. The species were generally distributed in the direction of one or other of these environmental variables. Sample groupings clearly define summer (June, July, August), autumn (September, October, November), winter (December, January, February) and spring (March, April, May).

_Haynesina germanica_ and _Fissurina lucida_ were weakly positively correlated (_r_ = 0.408 and 0.419) with axis one indicating an affinity for warmer sediment temperatures, air temperature and higher salinity. _Elphidium oceanensis_ and an indeterminate trochospiral sp. demonstrated negative correlations (_r_ = -0.523, _r_ = -0.4379) with axis one, suggesting a preference for cooler, less saline conditions experienced during the winter months (Figure 6.19, Table 6.10). _Elphidium williamsoni_ also exhibited a negative correlation (_r_ = -0.4214) with axis 1, slightly less so than _Elphidium oceanensis_ indicating a preference for intermediate temperatures and salinities associated with spring (Figure 6.19, Table 6.10). _Elphidium williamsoni_ was weakly correlated to chlorophyll _c_ (_r_ = -0.379) and chlorophyll _a_ (_r_ = -0.325). Three species were negatively correlated in Axis 2:
Elphidium oceanensis, Fissurina lucida and the indeterminate trochospiral sp., indicating a strong preference for colder, less saline environments.

Table 6.10: Summary of CCA results for benthic foraminifera and environmental variables at the high intertidal sampling station on the Eden Estuary, May 2000 to May 2001. Correlations between the abundance of benthic foraminifera (specimens 10 cm⁻²) and environmental variables for Canonical axis 1 (Axis1) and 2 (Axis2) are shown. Monte Carlo species-environmental variable significance (p<0.005) is for axis 1 and 2.

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<tr>
<td>cumulative % explained</td>
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Figure 6.19: Canonical correspondence analysis biplot of foraminiferal species (Ew = *Elphidium williamsoni*, Hg = *Haynesina germanica*, Mf = *Miliammina fusca*, It = indeterminate trochospiral, Eo = *Elphidium oceanensis*, Fl = *Fissurina lucida*) and samples with environmental correlations (including those with *r*<0.500) at high intertidal sampling station.

6.3.11.3 Low intertidal sampling station

Summary results of the CCA analysis for the low intertidal sampling station are presented in Table 6.11. Axis 2 (17.7%) explains under half the variability of axis 1 (47.4%), and given that axis three described only a further 6.8% of the variance, only axis 1 and 2 are presented.

The first axis is strongly influenced by % organic carbon in the sediment, chlorophyll *a*, chlorophyll *c*, grain size (treated and untreated), % nitrogen content
of the sediment and mean monthly air temperature. The second axis is largely a function of salinity and grain size (treated).

The biplot (Figure 6.20) shows the distribution of species and samples with respect to the environmental variables described above. Overall there was a weaker seasonal pattern in the sample distribution when compared with the high intertidal sampling station (Figure 6.19). Spring (March, April, May) samples separated out, winter (December, January, February) samples were also visible, but summer (June, July, August) and autumn (October, November, December) samples were less well defined. *Haynesina germanica* was weakly correlated ($r = 0.3038$) with axis one, indicating a stronger preference for carbon rich, diatomaceous sediments than found for any of the other species present. *Elphidium oceanensis* and the indeterminate trochospiral sp. are strongly negatively correlated with axis 1 ($r = -0.8808$ and $-0.7238$ respectively) indicating a preference for lower organic carbon, less diatom rich sediments with *E. oceanensis* preferring intermediate salinities and the indeterminate trochospiral sp. preferring fresher conditions. *Elphidium williamsoni* was negatively correlated with axis 1 ($r = -0.4560$) and hence chlorophyll $a$, $c$ and % organic carbon (Table 6.11) with a weak positive correlation to chlorophyll $b$ ($r = -0.3302$: axis1).
Table 6.11: Summary of CCA results for benthic foraminifera and environmental variables at the low intertidal sampling station on the Eden Estuary, May 2000 to May 2001. Correlations between the abundance of benthic foraminifera (specimens 10 cm$^{-2}$) and environmental variables for Canonical axis 1 (Axis 1) and 2 (Axis 2) are shown. Monte Carlo species-environmental variable significance (p<0.005) is for axis 1 and 2.

<table>
<thead>
<tr>
<th>% variance explained</th>
<th>Axis1</th>
<th>Axis2</th>
</tr>
</thead>
<tbody>
<tr>
<td>cumulative % explained</td>
<td>47.5</td>
<td>65.2</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Axis1</th>
<th>Axis2</th>
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<tbody>
<tr>
<td>Haynesina germanica</td>
<td>0.3038</td>
<td>-0.0286</td>
</tr>
<tr>
<td>Elphidium williamsoni</td>
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<td>0.0944</td>
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<td>Miliammina fusca</td>
<td>-0.5418</td>
<td>-0.1603</td>
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<tr>
<td>Miliammina sp.</td>
<td>-1.5717</td>
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<td>Elphidium oceanensis</td>
<td>-0.8808</td>
<td>0.3793</td>
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<tr>
<td>indeterminate trochospiral</td>
<td>-0.7238</td>
<td>-0.7197</td>
</tr>
<tr>
<td>Chl a</td>
<td>0.7740</td>
<td>-0.0204</td>
</tr>
<tr>
<td>Chl b</td>
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<td>0.1477</td>
</tr>
<tr>
<td>Chl c</td>
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<td>0.0021</td>
</tr>
<tr>
<td>Sediment temperature</td>
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</tr>
<tr>
<td>Salinity</td>
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<td>0.3164</td>
</tr>
<tr>
<td>Water content (%)</td>
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<td>-0.2776</td>
</tr>
<tr>
<td>LOI</td>
<td>0.4377</td>
<td>0.1558</td>
</tr>
<tr>
<td>Total monthly rainfall</td>
<td>0.3524</td>
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</tr>
<tr>
<td>Mean monthly air temperature</td>
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<tr>
<td>Grain size (treated sediment)</td>
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<td>-0.3092</td>
</tr>
<tr>
<td>Grain size (raw sediment)</td>
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<tr>
<td>% Organic carbon in sediment</td>
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<td>0.0837</td>
</tr>
<tr>
<td>% Nitrogen in sediment</td>
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<tr>
<td>C/N ratio</td>
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</tr>
<tr>
<td>River Eden flow</td>
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<td>0.0277</td>
</tr>
<tr>
<td>Motray Water flow</td>
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<td>0.0278</td>
</tr>
<tr>
<td>Species-environment correlation significance</td>
<td>0.0010</td>
<td>0.0160</td>
</tr>
</tbody>
</table>
Figure 6.20: Canonical correspondence analysis biplots of (A) foraminiferal species (Ew = Elphidium williamsoni, Hg = Haynesina germanica, Mf = Miliammina fusca, It = indeterminate trochospiral, Eo = Elphidium oceanensis, Msp = Miliammina sp.) and environmental correlations (including r<0.500) and (B) samples and environmental variables at low intertidal sampling station. Drawn separately for clarity.
6.4 Discussion

6.4.1 Physical characteristics

Many of the abiotic environmental variables measured, such as sediment temperature and salinity, showed some differences at each of the high, mid and low intertidal sampling stations, revealing a slightly fresher lower shore during winter and early spring, for example. Typical seasonal patterns were evident at all stations (higher temperatures during spring and summer, decreasing towards autumn and winter). Water content was always highest at the high intertidal station and lowest at low intertidal station, as would be expected from the coarser grain size distribution of the latter (Figure 6.7). Low intertidal sediments were consistently coarser grained than the high intertidal, perhaps due to the proximity of the main channel with its higher current velocities. Percent organic carbon followed similar gradients from the high to the low intertidal sampling station, with only one exception during June 2000, when the % carbon and nitrogen values at the low intertidal station exceeded those recorded for the high intertidal station. This concentration of organic material at the high intertidal sampling station could be the result of flocculation caused by the mixing of salt and fresh water combined with slower currents.

6.4.2 Foraminiferal assemblages

Total concentrations (standing stock) of benthic foraminifera were generally highest at the high intertidal sampling station, with exceptions during June 2000 and March 2001. Only one clear peak in the benthic foraminiferal abundance was evident at both the high and the low intertidal sampling stations, during May and June 2000. Concentrations declined from June to August 2000, and numbers remained low during autumn and winter. Abundance during May and June 2000 demonstrated the highest standard errors, indicating increased patchiness during these periods (Figure 6.11). Given that the samples were collected relatively close to one another (cm rather than metres), this increase in SE and coefficient of variation reveals higher spatial variability during periods of increased standing stock. This study does not demonstrate the pattern described by Buzas (1968) who suggested that as foraminiferal abundances increase, aggregation increases, resulting in lower spatial variability. However, this could
have been due to replicate cores not encountering the same patch and therefore reflecting high spatial variability on a small scale. Coefficients of variation were generally much higher for the high intertidal sampling station (mean 32.62 % ± 6.55) than for the low (mean 24.75 % ± 6.24) intertidal station. However, the low intertidal sampling station demonstrated the highest coefficient of variation (80 %) during June 2000. In general, this suggests that at the high intertidal sampling station benthic foraminiferal distributions were less homogeneous, or indeed that the patches were smaller. Standing stocks were much lower for this study than recorded by Murray and Alve (2000) for the Hamble Estuary, England, possibly reflecting less favourable conditions for benthic foraminifera in the Eden Estuary.

Assemblages of benthic foraminifera found living at the two sampling stations (high and low) along the intertidal transect on the Eden Estuary showed little difference in species composition. Most differences were evident in the occurrences of rare species. Both sites were dominated by the infaunal calcareous species *Haynesina germanica* and *Elphidium williamsoni*, with differences being found in the third and fourth dominant species. At the high intertidal station, *Elphidium oceanensis* was third and *Miliammina fusca* the fourth dominant species, whereas at the low intertidal station *Miliammina fusca* was third and an indeterminate trochospiral sp. the fourth dominant species. Differences were seen in % relative abundance of species over the sampling period, the most notable feature being the cycling of the two dominant species. The high intertidal mudflats were dominated by *H. germanica* from late spring to late autumn and *E. williamsoni* from early winter to late spring the following year. At the low intertidal station, *H. germanica* dominated from late spring to mid winter and *E. williamsoni* from late winter to mid spring the following year. This cycling may imply that the resources (e.g. food availability) necessary for an individual species are not available throughout the year or that environmental conditions change during late autumn to early winter at high tide and late mid winter to late winter at low tide or that these two species are in competition.

### 6.4.3 Food availability

From late spring (May 2000) to winter (February 2001), the entire transect was dominated by refractory organic material derived mainly from terrestrial sources. These conditions prevailed at the mid intertidal station throughout the
year. However, the high and low intertidal sampling stations changed during spring (March, April 2001) to being dominated by organic material which was mainly marine-derived, rich in labile organics and could be attributed to the peaks measured in benthic diatoms during this period (Figure 6.10C). By May 2001, the low intertidal mudflats were once again dominated by more refractory organic material. At this time the organic material at the high intertidal station remained dominated by labile, marine derived carbon.

Significant peaks in chlorophyll \( a \) and \( c \) were measured during March, April and May 2001 (Figure 6.10A and C). These peaks in chlorophyll \( a \) and \( c \) were followed by peaks in the benthic foraminiferal populations (total abundance \( 10 \text{ cm}^{-2} \)) during May and June for both high and low intertidal sampling stations (Figure 6.11). Thus, there was a slightly lagged increase in the abundance of benthic foraminifera, partly in response to increases in the availability of certain potential food sources (e.g. benthic diatoms). This was more pronounced at the low intertidal than high intertidal sampling station, with significant linear correlations between mean chlorophyll \( a \) and \( c \) concentration in the sediment and mean total abundance of benthic foraminifera (\( R^2 = 0.8042 \) and 0.819 respectively).

Chlorophyll \( a \) is a proxy for the biomass of all photosynthetically active organisms. Chlorophyll \( c \), on the other hand, is a measure of benthic diatom biomass. As chlorophyll \( a \) increases, chlorophyll \( c \) is seen to increase proportionally at both the high (\( R^2 = 0.8672 \)) and low (\( R^2 = 0.8812 \)) intertidal sampling stations. Seasonal patterns in the concentration of chlorophyll \( c \) also mirror that of chlorophyll \( a \) (Figure 6.10A and C). Therefore, at the high and low intertidal sampling stations, benthic diatoms comprise a relatively high percentage of the total primary productivity/biomass available to the foraminifer.

6.4.4 Multivariate statistics

In an attempt to determine which environmental factors were most important in structuring the foraminiferal populations monitored, CCA was applied to the sites separately.

Results from these analyses demonstrate high seasonal separation of the samples at the high intertidal sampling station and an apparent niche separation of foraminiferal species (Figure 6.19) with regard to season and possible food
sources. This suggests that competition for food between benthic foraminifera is not an important factor in determining which species dominate at certain times of the year. Abiotic factors appeared to be the main controlling environmental variables on the distribution of benthic foraminiferal species (e.g. sediment temperature and % water content of sediment). However, there were weak correlations with chlorophyll which should not be ignored. *Haynesina germanica* and *F. lucida* demonstrated a weak positive correlation with chlorophyll *b* at high intertidal, this could be due to their utilization of chlorophyll *b* as a food source during times of low chlorophyll *c* concentration (summer). Chlorophyll *b* is indicative of chlorophyceae, euglenophyceae and more specifically perhaps the presence of *Enteromorpha* spores. Therefore, *H. germanica* and *F. lucida* may be consuming either *Enteromorpha* spores or *Euglena* as part of their diet during this time of year, when *H. germanica* dominates the assemblage. There was a weak positive correlation between *E. williamsoni* and chlorophyll *c* during the spring at the high intertidal station, when *E. williamsoni* dominated. This suggests that chlorophyll *c* (and hence benthic diatoms) is important in the diet of *E. williamsoni* during spring, when chlorophyll *c* concentration at the high intertidal station peaked. Weak negative correlations between *E. oceanensis*, the indeterminate trochospiral sp. and chlorophyll *c* suggest that they are utilising sources of food other than those containing chlorophylls.

At the low intertidal sampling station reduced seasonal differences in the samples as a whole were evident. Here the major influencing factors were biotic (% organic carbon and chlorophyll *a* and *c*). However, the benthic foraminiferal response was weak, with *H. germanica* showing the only positive correlation (with chlorophyll *a* and *c*) among the species examined at the low intertidal station. This suggests that *H. germanica* may consume benthic diatoms as part of their diet. In contrast with the high intertidal situation, *E. williamsoni* is highly negatively correlated with chlorophyll *a* and *c*, suggesting that benthic diatoms are not their preferred food at this time of year. *E. williamsoni* demonstrates a weak correlation with chlorophyll *b*, suggesting it may be utilising chlorophyceae or *Enteromorpha* as a food resource. *Elphidium oceanensis* is highly negatively correlated with chlorophyll *a* and *c*, suggesting preference for food sources which do not contain abundant chlorophyll *a* and *c*. Having observed cytoplasmic colouration in *E. oceanensis* throughout the year to be orange/red, this would also
suggest they do not consume/concentrate chlorophyll (green to brown). *Elphidium williamsoni*, on the other hand, often appears very dark greenish brown, while *H. germanica* varies from yellowish white to brownish yellow with visible spotting in the cytoplasm (Chapter 7, Figure 7.1). This characteristic spotting in the cytoplasm of *H. germanica* is known to be due to the presence of chloroplasts (Lopez 1979, Knight and Mantoura 1985).

### 6.4.5 Reproduction and presence of juveniles

This study was designed to process benthic foraminifera from wet sediment samples to avoid the loss of juveniles by drying or non-density separation (Gooday 1988, Murray and Alve 2000). However, despite the fact that all samples were sieved at 63 µm, no juveniles less than 92 µm were observed.

In future, it may be worth sieving samples at 39 µm in order to determine if juveniles less than 63 µm are present in large numbers or are in fact absent. If juveniles are indeed absent, then recruitment onto these mudflats may be taking place from adjacent areas. Alternatively, it may be that in this and other estuarine environments asexual reproduction dominates. More frequent sampling, focused at critical times of the year (e.g. April-June), would help to improve the definition of peaks in abundance related to certain size classes. Equally, such a sampling campaign would distinguish short bursts (blooms) in potential food sources. As chapter 3 of this study demonstrated, asexually produced juveniles of *Elphidium williamsoni* are typically 90 µm within days of reproduction as opposed to the microscopic flagellated zygotes produced during sexual reproduction. However, large concentrations of these smaller size classes (ca. 90 µm) were not observed although they were present in very small numbers at certain times of the year (Table 6.7). This study suggests that reproduction and growth in both *Haynesina germanica* and *Elphidium williamsoni* is so rapid that juveniles are seen as an increases in the size class 130 – 296 µm for *H. germanica* and 111 –315 µm for *E. williamsoni* (Figures 6.16 & 6.17) during May to July 2000 and April to May 2001, respectively.

Both dominant species have been described as reproducing continually at low levels with peaks at certain times of the year by previous authors (Murray 1983, Cearreta, 1988). The data collected during this study suggests that reproduction is probably continuous at extremely low levels from August 2000 to
April 2001, with one peak between May and July 2000 for *H. germanica* at both the high and low intertidal sampling stations (Figure 6.16 A-F). The *E. williamsoni* populations also showed one clear reproductive peak between April and May 2001 (Figure 6.17A-D) at both the high and low intertidal sampling stations. Reproduction during this time was clearly seen in the rapid change in size frequency distributions for both *H. germanica* and *E. williamsoni* from unimodal to bimodal. Chapter 3 of this study demonstrates that the growth in *E. williamsoni* is rapid within the first month (125 μm), and Cearreta (1988) reports *H. germanica* growing 120 μm in the first month. Thus, monthly sampling may not be frequent enough to follow the detailed pattern of population recruitment and growth.

6.4.6 Biomass

Biomass measures in this study are much lower than those given by Alve and Murray (2001). This is because of the much lower standing stocks recorded in this study. However, simple linear correlations carried out with chlorophylls *a* and *c* showed that biomass might be a more significant indicator of population change in response to biotic factors (Table 6.9).

6.5 Conclusions

Food availability is often thought to be one of the driving factors in controlling the population structure of intertidal and pelagic environments. This study, in part, supports this idea. At a very crude level it would appear that total standing stock/abundance of benthic foraminifera increases in a lagged fashion, following increases in chlorophyll *a* and *c*. However, analysis taking into consideration multiple environmental variables (both biotic and abiotic) allows the description of only very weak correlations ($R^2 < 0.500$).

This study confirms that foraminifera exploit benthic diatoms as a food resource. However, in the Eden Estuary it is unlikely that foraminifera are wholly reliant on benthic diatoms as a food resource. Little seasonality is evident in the chlorophyll *c* measured, which suggests that benthic diatoms (food) are never limiting in this environment. Future studies of this type would be better carried out where known blooms occurred in benthic diatoms, perhaps twice a year.
Given that this study was undertaken over one year, it might be that 2000/2001 was unusual in some way. Generation times for diatoms are much faster (days, Admiraal et al. 1982) than for foraminifera (months). Hence these groups interact on different spatial and temporal scales. Monthly sampling may be frequent enough to examine foraminiferal response, but it may well not be short enough to measure blooms in the benthic diatoms, which may be occurring at a higher frequency. Finally, diatom species were not identified through the seasonal cycle and foraminifera may have preferences for certain species.
Chapter 7
7. Natural stable carbon ($\delta^{13}C_{\text{org}}$) and nitrogen ($\delta^{15}N_{\text{org}}$) isotopes in benthic foraminifera

7.1 Introduction

Intertidal estuarine environments are often characterised by a lack of macrophytes. Under these circumstances, phytoplankton and microphytobenthos are the dominant primary producers and therefore represent an important source of fresh ‘labile’ autotrophic carbon. Many estuarine systems are also light limited (due to turbidity) and thus primary production by benthic diatoms during low tide becomes crucial to the estuarine ecosystem (Heip et al. 1995). Several recent studies using the stable isotope $^{13}C$ as a tracer have highlighted the importance of primary production by benthic diatoms (Bacillariophyceae) as a major source of carbon in intertidal environments (Currin et al. 1995, Barranguet et al. 1997, Herman et al. 2000, Middelburg et al. 2000).

Many authors have proposed that meiofauna are a crucial link in benthic energy fluxes (Gerlach 1971, Coull and Bell 1979, Kuipers et al. 1981, Leguerrier et al. 2003). It is generally thought that the rate of benthic diatom production, and the subsequent transfer of autotrophic carbon to the benthic marine food web via the meiofauna, controls the energy flow in an intertidal estuarine environment (Buffan-Dubau and Carmen 2000). However, there is still a lack of detailed knowledge regarding the trophic position of foraminifera, particularly since they are generally overlooked in biological studies of coastal and estuarine environments. Benthic foraminifera are known to exploit benthic diatoms as a food source (Lopez 1979, Lee 1980, Knight and Mantoura 1985, Murray 1991b, Cedhagen 1991). The rapidity with which intertidal benthic foraminifera can respond to sudden increases in benthic diatom numbers (blooms) has been demonstrated (Chapter 4). However, within the intertidal environment, it remains unclear whether or not benthic foraminifera are largely reliant on fresh labile organic material or on more refractory organic matter or both.

Estuarine and coastal areas have several carbon sources available to benthic consumers, both allochthonous and autochthonous (produced in situ). An ability to distinguish which is the more important to benthic foraminifera would help elucidate the significance of benthic diatoms (labile carbon) versus more refractory forms of carbon in their diet. Foraminifera may play a crucial role in
the transfer of carbon within marine food webs and such studies are required if the carbon cycle is to be fully understood.

Historically, carbon and nitrogen stable isotopes have been used to follow the structure of food webs in terrestrial and fresh water environments (Nadelhoffer and Fry 1994). Over the last 12 years numerous stable isotope studies have been carried out in the marine environment (Sullivan and Moncreiff 1990, Coffin et al. 1994, Curren et al. 1995, Deegan and Garritt 1997, Riera et al. 1996, Riera and Richard 1996, Riera 1998, Riera et al. 1999, 2000, 2002, Kang et al. 1999). Numerous trophic pathways and processes in field and laboratory studies have been identified and quantified by the analysis of stable isotope ratios (natural abundance and tracers) and it is becoming an increasingly important technique (Conway et al. 1989).

One drawback of existing methodology is that it generally requires sample weights in milligrams to produce detectable and reliable isotopic ratios. Hence most studies have focused on larger animals (molluscs, prawns, fish etc). However, there is now a move towards developing methodology to measure natural abundance stable isotopes in meiofauna (Riera et al. 1996, Moens et al. 2002, Carmen and Fry 2002) (Table 7.1).

Benthic foraminifera are small with relatively low individual biomass (see Chapter 2 for discussion) and many have a calcium carbonate test, which must be removed prior to analysis. Average biomass has been measured for a number of intertidal foraminifera genera, Haynesina 1.48 μg C ind\(^{-1}\), with an average diameter of 381 μm; Elphidium 0.75 μg C ind\(^{-1}\), with an average diameter of 268 μm; Ammonia 1.01 μg C ind\(^{-1}\) with an average diameter of 325 μm (Moodley et al. 2000a). This means that fairly large numbers of monospecific specimens must be collected for a single isotopic analysis, and this may have hindered researchers in the past. In addition, access to reliable isotope ratio mass spectrometer laboratories is generally limited, as is often the money and expertise to run the samples. To date, no study has been published which reports the natural abundance of dual stable isotopes (\(\delta^{13}C_{\text{org}}\) and \(\delta^{15}N_{\text{org}}\)) in the organic fraction (protoplasm) of benthic foraminifera.

The dual analysis of carbon and nitrogen natural abundance stable isotopes has become a powerful tool for identifying sources of organic matter and the
Table 7.1: Summary of stable carbon ($\delta^{13}$C$_{org}$) and nitrogen ($\delta^{15}$N$_{org}$) isotope signatures (mean ± SD) of several organic matter sources measured from several field studies of intertidal areas. Values quoted are ‰ relative to a standard.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Location</th>
<th>Date</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microphytobenthos</td>
<td>Schelde Estuary</td>
<td>June 1997</td>
<td>-16 ± 2</td>
<td>6.6</td>
<td>Middelburg et al. (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June 1997</td>
<td>-14 to -15</td>
<td></td>
<td>Herman et al. (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June 2000</td>
<td>-17.4</td>
<td>4.6 ± 0.4</td>
<td>Moens et al. (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June 2000</td>
<td>-18.2</td>
<td>5.9 ± 0.2</td>
<td>Moens et al. (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>-21.1</td>
<td>5.8</td>
<td>Moens et al. (2002)</td>
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<tr>
<td>Bay of Marinenes-Oleron</td>
<td>summer 1992</td>
<td>-15.6 ± 0.3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
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<td>4.6 ± 0.4</td>
<td></td>
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</tr>
<tr>
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<td>Winter 1993</td>
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<td>5.9 ± 0.2</td>
<td></td>
<td>Riera et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Spring 1993</td>
<td></td>
<td></td>
<td></td>
<td>Creach (1997)</td>
</tr>
<tr>
<td>French Channel</td>
<td>Salt marsh SE, USA</td>
<td>-12.76</td>
<td>3.8</td>
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<td>Couch (1989)</td>
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<td>Enteromorpha sp.</td>
<td>Bay of Marinenes-Oleron</td>
<td>summer 1992</td>
<td>-16.5 ± 0.4</td>
<td>9.2 ± 0.0</td>
<td>Riera et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Autumn 1992</td>
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<td>12.0 ± 0.5</td>
<td></td>
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<td></td>
<td>Spring 1992</td>
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<td>8.2 ± 0.3</td>
<td></td>
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</tr>
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<td>Bacteria</td>
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<td>6.4 ± 0.4</td>
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<td>Riera et al. (1999)</td>
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<td>Sediment (SOM)</td>
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<td></td>
<td>Riera et al. (1996)</td>
</tr>
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<td></td>
<td>Charente Estuary</td>
<td>-19.71</td>
<td>4.3</td>
<td></td>
<td>Couch (1989)</td>
</tr>
<tr>
<td>Nematodes</td>
<td>Schelde Estuary, Molenplaat</td>
<td>-14.69 ± 0.51</td>
<td>8.9 to 9.3</td>
<td></td>
<td>Moens et al. (2002)</td>
</tr>
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trophic position of consumers (Michener and Schell 1994). The technique is based on the measurement of predictable isotope fractionation from one trophic level to the next. The $\delta^{13}C$ composition of a predator closely reflects the composition of their prey (assimilated food), with a slight enrichment (approximately 1 %o) (DeNiro and Epstein 1978, Michener and Schell 1994). By contrast, $\delta^{15}N$ exhibits consistent enrichment (approximately 3.5 - 4 %o) between predator and prey, due to the preferential excretion of the lighter nitrogen ($^{14}N$) isotope. This can be used to predict an organism's trophic position (Minagawa and Wada 1984; Michner and Schell 1994, Post 2002). However, it should be kept in mind that in estuarine environments, the nitrogen pools utilised by primary producers (at the base of food webs) have differing $\delta^{15}N$ values both spatially and temporally (Jennings and Warr 2003). Therefore, care must be taken when interpreting results for the same species from different areas or seasonally.

The aims of this study were to develop a method for measuring the natural abundance stable isotopes of carbon and nitrogen within the organic fraction (protoplasm) of benthic foraminifera. Secondly, to identify, and measure where possible, the stable isotope composition of potential food sources available to benthic foraminifera in the intertidal mudflats of the Eden Estuary; with particular emphasis on the role of benthic diatom primary production (the dominant primary producers). The food sources considered in this study were benthic diatoms, bulk sediment and Enteromorpha (the only macrophyte occurring in large patches on the intertidal mudflats). Thirdly, to assess any differences in the natural abundance stable isotope composition ($\delta^{13}C_{org}$ and $\delta^{15}N_{org}$) between benthic foraminiferal species. Finally, observations were also made to determine how the foraminifera collect and utilise natural benthic diatom assemblages from the Eden Estuary as a food resource.
7.2 Materials and Methods

7.2.1 Cleaning of glasswear, silver weighing boats, silica gel and filter paper

When running such small samples (approximately 10 µg of carbon or less) great care must be taken to keep the samples free from contamination by other organisms (e.g. bacteria) and any other extraneous sources of carbon and nitrogen. All glass pipettes used were carefully cleaned and autoclaved (107°C for 35 min) to sterilise prior to use. Petri dishes were acid cleaned (HCl 10%) and dried in a clean muffle furnace at 500°C for at least 2 hours.

An important step in the protocol involved the acidification of the foraminifera and ostracoda in order to remove their calcium carbonate shells. Silver boats (Elemental Microanalysis Limited) were chosen over the normal tin or aluminium for analysis, as they are more resistant to hydrochloric acid (Nieuwenhuize et al. 1994). This allowed in situ acidification of foraminifera and ostracoda, reducing the chances of sample loss and chemical contamination. Prior to use, silver boats were pre-combusted at 400°C for 3 hours. Glass fibre filter papers (Whatman 47 mm diameter GF/F) and silica gel (60 to 120 µm, Fluka Chemicals, sieved at 63 µm) were also pre-combusted (500°C for 4 hours).

7.2.2 Field collection of water

Seawater was collected at high tide from the study site one day prior to sediment collection. This was returned to the laboratory where it was filtered at 63 µm and stored in the dark at 10°C. One litre of this water, required to clean the foraminifera, was further filtered at 0.2 µm to remove bacteria and stored in a dark refrigerator (4°C). Prior to using this filtered seawater for washing foraminifera, it was again filtered at 0.2 µm.

7.2.3 Sediment collection and isolation of benthic foraminifera

Surface scrapes of sediment for the isolation of living benthic foraminifera (and benthic diatoms in June 2002) were collected at low tide during October 2000, March 2002 and June 2002 from the high intertidal (71°96’76N, 34°55’79E) mudflats on the Eden Estuary (Chapter 6, Figure 1). Sediment was returned to the laboratory and sieved at 63 µm in filtered seawater (< 63 µm) from the collection site. The fraction ≥ 63 µm was transferred to containers and left to
settle for an hour. Given the time taken to isolate living benthic foraminifera from this site, sediment was divided up into small batches (50 ml) and frozen at -80°C. This enabled individual sample picking and preparation to be carried out at a later date if required, while maintaining sample integrity for stable isotope analysis.

7.2.4 Benthic foraminiferal extraction from sediment for stable isotope analysis

Small volumes of sediment (1 ml) were sorted under a binocular microscope in order to isolate foraminifera which were alive at the time of collection. Without the use of rose Bengal stain, this is determined by subtle colouration and percentage of test filled with protoplasm. Picking was performed using a brush (0000) carefully trimmed to the correct size and cleaned before use in ethanol. Individuals were isolated into sterile petri dishes (5 cm φ) pre-filled with filtered seawater (0.2 μm) from the collection site. Individuals were vigorously cleaned several times in filtered seawater, taking great care to remove all adhering detritus around the aperture and umbilical area. Once cleaned, individuals were grouped together and lifted (taking minimum amounts of water) using a sterile pipette and washed three times with demineralised water and one final wash in Milli-Q water. Final transfer of the specimens was again with a sterile pipette, taking a minimum amount of water, into pre-combusted silver boats.

Samples were then placed in clean glass petri dishes and freeze-dried at -80°C for 2 to 4 hours. Once dried, acidification was carried out in the silver boats. Foraminifera were acidified using 2.5% HCl, adding 10 μl at a time until effervescence stopped. Ostracoda were acidified using 5% HCl. Samples were then dried in a clean oven at 35-40°C overnight to evaporate any hydrochloric acid that may otherwise enter the analyser and collect in the combustion tube. Silver boats were folded (wearing latex gloves) and rolled into small balls and stored in sterile 96 well trays prior to analysis. If samples are being run separately for carbon and nitrogen, there is no need to acidify the nitrogen samples because this step is only required to remove carbon within the calcium carbonate test. Care must be taken not to include benthic foraminifera with turbillarian egg sacs attached, as this would incorporate material from the egg sac and lead to erroneous results.
Chapter 7    Natural stable carbon ($\delta^{13}\text{C}_{\text{org}}$) and nitrogen ($\delta^{15}\text{N}_{\text{org}}$) isotopes in benthic foraminifera

7.2.5 Collection of bulk sediment and Enteromorpha

A core of 1 cm depth was used to collect bulk sediment which was freeze-dried at -80°C, ground into a fine powder and acidified (1 M HCl) prior to analysis. Living Enteromorpha was collected from a stand approximately 100 m away from the sediment collection site, washed repeatedly in field collected filtered seawater (<0.2 μm), freeze-dried at -80°C, ground into a fine powder and acidified (1 M HCl) prior to analysis. Acidification was carried out to remove any carbonates that may have been associated with epiphytes not removed during washing.

7.2.6 Benthic diatom extraction from sediment for stable isotope analysis

Surface scrapes were collected during June 2002 and returned immediately to the laboratory for the extraction of motile benthic diatoms. Sediment was homogenised and placed into flat trays to a thickness of approximately 2 cm and allowed to equilibrate. Light was applied at the start of the next natural low tide period, keeping the sediment moist at all times by spraying with filtered seawater from the sampling site. By the lowest point in the natural tidal cycle, dense brown patches were visible on the sediment surface. A 63 μm nylon mesh was laid over the sediment. A mixture of silica gel and filtered seawater from the sampling site was poured over the mesh to a depth of approximately 4 mm. The sediment trays were then illuminated until the silica gel mixture changed colour as benthic diatoms migrated upwards into it. The silica gel was kept moist by spraying with filtered seawater from the site. After approximately 2 hours, the nylon mesh was lifted from the sediment surface and the upper 3 mm of silica gel was carefully removed. The sample was then washed through a 63 μm sieve to separate diatoms from the silica gel and any meiofauna which may have entered the silica gel from adjacent sediment (e.g. nematodes, Corophium). This was quickly transferred to a sterile glass filter funnel and collected on pre-combusted glass fibre filter papers, washed with 10 % HCl to remove any carbonates, briefly rinsed with Milli-Q water, freeze-dried (-80°C for 4 hours) and stored frozen (-80°C) until analysis. This method was slightly modified from Couch (1989) and followed closely that of Riera et al. (1996 and 2000).
7.2.7 Stable isotope analysis

The first set of samples was analysed at NIOO (The Netherlands) and run as separate carbon and nitrogen samples, while in the second and third sample sets run in Aberdeen (Scotland) both carbon and nitrogen were determined from a single sample. Calculations of carbon isotope ratios are the same as given in Chapter 4, Equation 4.1. Calculating the natural abundance ratio of nitrogen isotopes, expressed as $\delta^{15}N_{\text{org}}$, is given in Equation (7.1), with units of per mil (‰):

$$\delta^{15}N_{\text{org}} = \left[\frac{(^{15}N/^{14}N)_{\text{sample}}}{(^{15}N/^{14}N)_{\text{reference}}} - 1\right] \times 1000 \quad \text{Equation (7.1)}$$

The reference gas used was atmospheric nitrogen.

7.2.8 Analysis of $\delta^{15}N_{\text{org}}$ and $\delta^{13}C_{\text{org}}$

Measurements of $\delta^{13}C_{\text{org}}$ and $\delta^{15}N_{\text{org}}$ of ostracoda and foraminifera were carried out using a Carlo Erba$^\text{C}1106$ Elemental Analyser coupled online with a Finnigan$^\text{C}$Delta S isotope ratio mass spectrometer (IRMS) at the NIOO. In Aberdeen, a Carlo Erba$^\text{C}$NA1500 Elemental Analyser coupled online via an Isochrom diluter to a Micromass Optima dual inlet IRMS was used.

7.2.9 Obtaining images of foraminifera feeding on benthic diatoms

Glass cover slips were cleaned in chromic acid to remove oil and grease, thus allowing foraminifera to attain better contact with the glass surface. Foraminifera were placed onto cover slips with a seawater and benthic diatom mixture and left for several hours or overnight. In order to obtain images, two techniques were employed to preserve the foraminifera utilising diatoms.

In the first method, a metal plate was first frozen in liquid nitrogen. As much water as possible was removed from the slide using a wick of tissue paper, after which the slide was brought into contact with the frozen metal plate. Once frozen, the coverslip and sample were freeze-dried.

The second method, involved fixing the sample with a mixture of 20 ml 0.2M cocodylate buffer (pH 7.2), 10 ml of 25% gluteraldehyde, 8g sucrose, 70 mg ruthenium red all made up to 40 ml using distilled water (Bowser and Travis
2000). Water was removed from the slide as in the first method while dropping the mixture above onto the slide.

### 7.2.10 Statistical analysis

Data were tested for normality in the statistical package Minitab using the Ryan-Joiner option. Within this test the null hypothesis (data are normally distributed) is accepted when \( p > 0.05 \). Data which were normally distributed were compared using a one-way ANOVA. Data not normally distributed were compared using the Kruskal-Wallis test.

### 7.3 Results

#### 7.3.1 Picking living foraminifera

Visible colour differences were evident in the cytoplasm of the three foraminifera species collected (Figure 7.1). *Haynesina germanica* has a brownish green hue and, although not visible in the photograph (Figure 7.1A), has a granular cytoplasm visible through the test. *Elphidium williamsoni* has a very distinct green colouration, while *E. oceanensis* has a vivid red/orange colour (Figure 7.1 B&C). Colouration of the foraminifera, combined with experience gained after many hours of observations of living individuals, made it possible to distinguish live from dead (Figure 7.1).

![Figure 7.1: Digital photographs illustrating cytoplasm colouration in (A) Haynesina germanica (B) Elphidium williamsoni (C) Elphidium oceanensis after being frozen at -80°C and before final cleaning. Scale \( \sim 400 \) μm.](image)

#### 7.3.2 Sample sizes

Based on results presented in Chapter 4, it was known that samples with as few as 25 individual foraminifera (approximate diameter 350 μm, with good cytoplasm content i.e. 90%) are sufficient for stable carbon isotope analysis.
Chapter 7  Natural stable carbon ($\delta^{13}C_{org}$) and nitrogen ($\delta^{15}N_{org}$) isotopes in benthic foraminifera

Assuming a typical marine carbon to nitrogen ratio (Redfield ratio) of 6:1, it is possible to calculate the equivalent sample size required to evolve sufficient nitrogen for isotope analysis (approximately 150 individuals of 350 $\mu$m diameter).

The total number of individuals required for nitrogen analysis is highly dependent on the size range of individuals comprising the sample. Even using rough estimates of carbon content, it remains difficult to predict nitrogen evolution. Therefore, although the first samples yielded sufficient nitrogen to measure, it was decided that increasing the sample sizes would bring them into a more reliable measurement range. For the second set of samples analysed, the number and/or the average size of individuals was increased. Typically, a sample size of 5 to 6 times greater than that required for carbon was sufficient to evolve the nitrogen required for measurement. As a general rule, for all the foraminifera species studied, this was equivalent to at least 150 individuals, with at least a quarter to a half made up of large individuals (>300 $\mu$m). Ostracoda required 10 individuals for carbon and 50 individuals for nitrogen measurement.

### 7.3.3 Isotope composition of foraminifera, ostracoda and potential food sources

The $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ composition (mean ± SD) of 3 benthic foraminifera species, ostracoda and possible organic matter sources from the Eden Estuary are summarised (Table 7.2). Mean benthic diatom $\delta^{15}N_{org}$ values were 4.95 ± 1.73 ‰, *Enteromorpha* sp. $\delta^{15}N_{org}$ values were 7.40 ± 0.70 ‰ and sediment organic matter (SOM) $\delta^{15}N_{org}$ were 6.58 ± 0.18 ‰; all close to values reported for other estuarine intertidal mudflats (Table 7.1). Pooled benthic foraminifera $\delta^{15}N_{org}$ values ranged from 7.59 ± 3.13 ‰ (*Haynesina germanica*) to 10.68 ± 2.66 ‰ (*Elphidium oceanensis*). Comparison with other data cannot be made as no published data are available on the $\delta^{15}N_{org}$ values in benthic foraminifera. Ostracoda $\delta^{15}N_{org}$ values were 7.71 ± 2.83 ‰.

Bulk SOM $\delta^{13}C_{org}$ values were depleted (more negative) as compared to other end-members measured, suggesting that SOM is mainly derived from terrestrial sources (e.g. vegetation from the River Eden catchment). Differences between the $\delta^{13}C_{org}$ of SOM and foraminifera species were large (3.9 to 5.77 ‰). These differences cannot simply be explained by metabolic enrichment in $^{13}$C, since there is generally only 1 ‰ enrichment during food assimilation between
Table 7.2: Natural abundance stable isotopes of $\delta^{13}$C$_{org}$ and $\delta^{15}$N$_{org}$ ± SD in benthic foraminifera, ostracoda and possible organic matter sources. Collected from high intertidal mudflats of the Eden Estuary during October 2000, March and June 2002. October samples were analysed at NIOO, The Netherlands. May and June samples were analysed at the Zoology Department, Aberdeen University. The number of replicate samples is given in parenthesis. Values are expressed as % relative to a standard.

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<td>$\delta^{15}$N$_{org}$ ± SD</td>
<td>$\delta^{13}$C$_{org}$ ± SD</td>
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<td>10.09 ± 0.45 (2)</td>
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Chapter 7 Natural stable carbon ($\delta^{13}C_{org}$) and nitrogen ($\delta^{15}N_{org}$) isotopes in benthic foraminifera trophic levels. This may indicate that foraminifera are utilising at least one specific component of the SOM pool that is $^{13}$C-enriched compared to bulk SOM. *Enteromorpha* and benthic diatom $\delta^{13}C_{org}$ values are enriched (more positive) compared to SOM. Thus they are more influenced by the marine environment, which is to be expected since they derive carbon largely from the dissolved inorganic carbon (DIC) in seawater.

### 7.3.4 $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ comparison among co-occurring foraminiferal species

Within foraminiferal species and ostracoda, there were no significant differences in $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ values between sampling dates (Table 7.3). This allowed data to be pooled, means calculated and presented on a dual isotope plot (Figure 7.2). Overall the $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ of organic matter sources (food) analysed from the Eden Estuary were significantly different (Kruskal-Wallis, df = 2, $H = 7.87$, $P = 0.020$; df = 2, $H = 9.99$, $P = 0.007$, respectively). However, inspection of the raw data suggests a slight overlap in $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ between benthic diatoms and *Enteromorpha* sp. Comparing the $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ of all foraminiferal species and ostracoda, no significant differences were found between species (Kruskal-Wallis, df = 3, $H = 6.18$, $P = 0.103$; df = 3, $H = 2.04$, $P=0.564$, respectively).

#### Table 7.3: Comparison of $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ values within 3 benthic foraminifera species and ostracoda between 2 to 3 different sampling events. df = degrees of freedom, $F$ = test statistic, $p$ = probability at significance level 5% ($p = 0.05$)

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</tr>
<tr>
<td><em>Elphidium oceanensis</em></td>
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<td>6.92</td>
</tr>
</tbody>
</table>

### 7.3.5 Estimating the mean isotopic ratios of foraminiferal diet

As discussed above, $\delta^{15}N_{org}$ undergoes consistent enrichment (3.5 - 4 %o) during assimilation, while $\delta^{13}C_{org}$ enrichment is consistently limited to approximately 1 %o (Michener and Schell 1994, Post 2002). Therefore, to estimate the $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of any food sources for each species of foraminifera and ostracoda the above values were subtracted from each species mean isotope ratio to correct for metabolic fractionation. Consequently, the food
sources would have $\delta^{13}C_{\text{org}}$ and $\delta^{15}N_{\text{org}}$ values of approximately $-20.1\%$ and $5\%$ for *E. williamsoni*, $-18.23\%$ and $3.59\%$ for *H. germanica*, $-18.66\%$ and $6.68\%$ for *E. oceanensis* and $-13.52\%$ and $3.71\%$ for ostracoda, respectively (Figure 7.2).

7.3.6 Trophic position

Fry and Sherr (1984) have suggested that the usefulness of $\delta^{15}N_{\text{org}}$ to trace food webs is limited in estuarine environments. This is due generally to small differences in $\delta^{15}N_{\text{org}}$ measured between proposed organic matter sources (food). However, in this study there is an overall significant difference between $\delta^{15}N_{\text{org}}$ of the organic matter sources measured. Combining the $\delta^{15}N_{\text{org}}$ and $\delta^{13}C_{\text{org}}$ isotope data precludes the sole consumption of either SOM or *Enteromorpha* by the benthic foraminifera species measured. The data suggest that all three foraminifera species and ostracoda occupy a similar trophic position. From the results presented in this study, it is suggested that if foraminifera assimilate carbon and nitrogen from a single food resource, only benthic diatoms can be considered as likely candidates for that food resource (Figure 7.2).

7.3.7 How do benthic foraminifera utilise benthic diatoms?

Visual observations conducted using a binocular microscope, mainly on *Haynesina germanica*, demonstrated that individuals collect large feeding bundles of benthic diatoms. The manipulation of individual diatoms (*Pleurosigma*) was also observed. Isolated foraminifera draw the diatom towards the aperture, where it is orientated in a controlled manner, diatom frustule held with the pole towards the aperture and side on. Despite watching over several hours, observation of content removal was not seen. However, empty frustules were observed at a later time (Figure 7.3(A)).

Contact freezing of the foraminifera worked to a certain degree. However, despite removing as much water as possible, salt deposits obscured much of the image (Figure 7.3(C) and (D)). The best results were achieved following the methods of Bowser and Travis (2000) (Figure 7.3 (E)-(I)).

SEM images revealed a characteristic cracking of the *Pleurosigma* frustules (Figure 7.3 (C), (D), (H), (I)). Some individuals were seen to select mainly *Pleurosigma* when present as the dominant diatom species (Figure
7.3(H)). However, others would appear to be collecting a fairly diverse assemblage (Figure 7.3 (E)-(G)).

Slides containing benthic diatoms only were also processed and treated in the same manner as those with foraminifera as a control. Under SEM observations, no diatoms were seen to have any of the characteristic cracking features associated with the feeding bundles described above. Thus, further suggesting that the cracking had been carried out by the foraminifera in order to remove the diatom contents.

Figure 7.2: Carbon and nitrogen isotope ratios of 3 benthic foraminifera species, ostracoda and potential food sources from the Eden Estuary, NE Scotland. Filled boxes (grey) joined to species symbols by a line represent estimates of the mean isotope composition of foraminifera species and ostracod diets. Bd = benthic diatoms, Ent = Enteromorpha, SOM = bulk sediment organic matter, Ew = E. williamsoni, Ew' = estimated food source of E. williamsoni, Eo = E. oceanensis, Eo' = estimated food source of E. oceanensis, Hg = H. germanica, Hg' = estimated food source of H. germanica, O = ostracoda, O' = estimated food source of ostracoda.
7.3.8 Images of foraminifera feeding on benthic diatoms

Figure 7.3: *Haynesina germanica* with natural assemblages of benthic diatoms collected from the Eden Estuary. (A) Digital image of *H. germanica* manipulating a single diatom. (B) *H. germanica* collecting the benthic diatom *Pleurosigma* into feeding bundles. (C) and (D) SEM images of foraminiferal captured *Pleurosigma*. (E) *H. germanica* with extended pseudopodia and collected diatoms. (F) Closer view of E, showing collected diatoms around aperture. (G) Diverse diatom collection around aperture. (H) *H. germanica* with a large feeding bundle containing mainly *Pleurosigma* (I) Magnified image of (H), showing diatoms in feeding bundle. Note characteristic cracking - arrows.
7.4 Discussion

*Elphidium williamsoni, Haynesina germanica* and *Elphidium oceanensis* are considered infaunal species, with peak abundances in the surface 0.25 cm of intertidal mudflats (Murray 1991a, Murray and Alve 2000). This would put them in direct contact with benthic diatoms and other possible food sources associated with biofilms (e.g. Bacteria). However, little is known about the feeding biology of these three species in the field.

7.4.1 Identification of food sources using dual isotope analysis

Pigment-analysis of the common intertidal benthic foraminifera *E. williamsoni, H. germanica* and *E. oceanensis* has suggested that benthic microalgae are a major part of their diet (Knight and Mantoura 1985). Characteristic cytoplasmic colouration has been reported by a number of authors (Murray 1963, Knight and Mantoura 1985) and has long been though to reflect the pigments of their prey. *Elphidium williamsoni* and *H. germanica* are known to feed on benthic diatoms. However, it remains unclear if they utilise benthic diatoms as a food source or if they maintain sequestered chloroplasts in a symbiotic relationship.

*Elphidium oceanensis* does not appear to sequester chloroplasts, but may concentrate algal carotenoids – hence the red colouration (Knight and Mantoura 1985). The ratio of phaeophorbide (breakdown product of Chl a) to Chlorophyll a in *E. oceanensis* was the same as that found in *Calanus helgolandicus* (Copepoda) faecal pellets, suggesting the possibility that this species is carnivorous. Since this study reports very similar cytoplasmic colouration for all three species to those observed by Knight and Mantoura (1985) for salt marsh foraminifera in Wacker Lake, Cornwall, it might be reasonable to assume that the same feeding linkages are being observed.

This study found no significant differences in the stable isotope ratios between the benthic foraminifera species investigated. *Elphidium oceanensis* has slightly more enriched δ15N values compared to both *Elphidium williamsoni* and *Haynesina germanica*. However, this difference is not statistically significant.

A general rule states that animals have similar carbon isotopic compositions to their diets and are typically enriched in 15N by 3.5-4 (‰) between trophic levels (Fry and Sherr 1984, Peterson and Fry 1987, Carman and Fry
Chapter 7 Natural stable carbon ($\delta^{13}$C$_{org}$) and nitrogen ($\delta^{15}$N$_{org}$) isotopes in benthic foraminifera

2002). The data suggest that all three foraminifera species analysed appear to be feeding almost entirely on benthic diatoms (Figure 7.2). Ostracoda isotope values show large standard deviations but suggest that they are not wholly reliant on benthic diatoms as their main food resource. However, no alternative food resource was identified from those measured during this study.

In this study, there is no evidence from isotopic values that the dominant macroalgae of the Eden Estuary *Enteromorpha*, contributes to the diet of benthic foraminifera and ostracoda. Both $\delta^{13}$C$_{org}$ and $\delta^{15}$N$_{org}$ isotopic values were measured from living *Enteromorpha*. Decomposing plants are reported as having $\delta^{13}$C$_{org}$ values close to those of the living plants (Fenton and Ritz 1988), while $\delta^{15}$N$_{org}$ increases (Owens 1987). Therefore, any detritus containing a large proportion of decomposing macroalgal/plant material would be $\delta^{15}$N$_{org}$ enriched. If foraminifera and ostracoda had assimilated this detritus in a consistent manner, we would expect their isotopic ratios to reflect enriched $\delta^{15}$N$_{org}$ values relative to those observed in this study. Although the estuary as a whole reflects a general scarcity in macroalgae and seagrasses, dense seasonal patches of *Enteromorpha* occur and are likely to contribute to the SOM pool on a fairly local and highly seasonal basis.

The isotopic values of SOM also indicate that it does not contribute solely to the diet of benthic foraminifera and ostracoda. However, care must be taken in interpreting the data values as changes may occur seasonally depending on the organic matter input to the sediment. Thus at certain times of the year or when benthic diatoms biomass is low, a shift may be observed towards utilisation of SOM, but this requires further investigation.

### 7.4.2 Carbon isotopes from two sampling sites

Natural abundance stable isotopes of carbon measured in benthic foraminifera would appear to be depleted in the Eden Estuary compared to those measured in the Oosterschelde, The Netherlands (one way ANOVA: $F_{1,32} = 7.89$, $P = 0.008$) (Table 7.4). As a general rule slight enrichments of 1‰ are evident in the consumer relative to their prey. If benthic diatoms are a major component in the diet of foraminifera in both environments, then differences in the carbon pool utilised by the primary producers (diatoms) may be important. More negative
values in the Eden suggest greater influence from terrestrial carbon sources than those of the Oosterschelde.

Table 7.4: Mean $\delta^{13}C_{\text{org}}$ from the Oosterschelde, SW Netherlands and the Eden Estuary, NE Scotland.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean $\delta^{13}C$ ± SD</td>
<td>Mean $\delta^{13}C$ ± SD</td>
<td>Mean $\delta^{13}C$ ± SD</td>
<td>Mean $\delta^{13}C$ ± SD</td>
</tr>
<tr>
<td>Haynesina</td>
<td>-10.22 ± 0.59</td>
<td>-18.13 ± 0.72</td>
<td>-16.95 ± 0.73</td>
<td>-9.74 ± 4.06</td>
</tr>
<tr>
<td>Elphidium</td>
<td>-14.53 ± 0.40</td>
<td>-21.44 ± 2.45</td>
<td>-20.07 ± 4</td>
<td>-15.41 ± 4.0</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>-10.73 ± 1.46</td>
<td>-19.99 ± 0.62</td>
<td>-19.93 ± 2.33</td>
<td>-16.27 ± 1</td>
</tr>
<tr>
<td>Haynesina germanica</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elphidium williamsoni</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elphidium oceanensis</td>
<td></td>
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</tbody>
</table>

7.4.3 Sample sizes

Benthic foraminferal abundances at the Eden Estuary sampling site (June 2000 = 270 ± 180 specimens 10 cm$^{-2}$; October 2000 = 63 ± 4.93 specimens 10 cm$^{-2}$; March 2001 = 31.33 ± 1.35 specimens 10 cm$^{-2}$) were not as high as those found in the Oosterschelde (April 2000 = 850 specimens 10 cm$^{-2}$). However, despite these fluctuating numbers, all samples yielded sufficient specimens to carry out stable isotope analyses. One of the major drawbacks during spring and summer was the increased occurrence of turbellaria eggs, found mainly attached to $E$. williamsoni, but also on other species. It is important that attention is given to this and any other attached epizoan, as these introduce carbon and nitrogen from another source.

Large variations (SD) in the results obtained could be due to difficulties in making up samples of the same size, thus evolving different volumes of gas prior to IRMS analysis. Similarly, benthic diatoms were collected onto pre-combusted filter papers and again the distribution of numbers may not have been homogeneous between samples. While this alone should not affect the isotope ratio, it would affect the volume of gas evolved and at such small sample sizes, this may affect the precision of the analysis because of changes in linearity within the instrument.

Having used single samples for both carbon and nitrogen and separate samples for carbon and nitrogen analysis, I would suggest using separate samples
for further work. This will allow the mass spectrometer to be set up to run batches of similar sample size and reduce disturbance to the instrument when switching between carbon and nitrogen measurements. Carbon analysis requires far fewer (ca. 1/6th) individuals than for nitrogen analysis and there is therefore an immediate advantage in running separate samples. However, I would still recommend at least 6 times more sample for nitrogen analysis than that for carbon.

7.4.4 Predicted isotope ratios of foraminiferal food sources

The single predicted food source of benthic foraminifera based on trophic level enrichment in isotope ratios ($\delta^{13}C_{\text{org}}$ and $\delta^{15}N_{\text{org}}$) and the measured benthic diatom isotope ratios were very similar. It is known that benthic foraminifera are extremely efficient at collecting and ingesting fresh labile organic matter (Moodley et al. 2000a, Chapter 4). Deep-sea foraminifera have also been shown to rapidly assimilate diatom carbon (Rivkin and DeLaca 1990, Moodley et al. 2002). However, many intertidal foraminifera species sequester chloroplasts (Lopez 1979), and to date it remains unclear to what degree foraminifera are consuming diatoms as a food resource, or gaining some benefit from a symbiotic relationship with diatom chloroplasts. On these grounds it was partially expected that the intertidal foraminifera studied would carry an isotopic signature that remained close to their food source, thus diminishing the trophic level effect. However, there is a characteristic enrichment in both $\delta^{13}C_{\text{org}}$ and $\delta^{15}N_{\text{org}}$ in benthic foraminifera, suggesting that they assimilate diatoms as well as possibly using the chloroplasts symbiotically. As there is great variation in the volume of protoplasm present within individual foraminifera (pers. obs.) it is difficult to calculate the exact proportion of intact “prey” material present. Therefore, in shallow water/intertidal species there will be the added, uncertain factor of ingested material retaining a signature close to that of the original food source. These factors could potentially cause large variations between samples of the same species regardless of matching sample weight, carbon and nitrogen content.
7.4.5 Further investigations

Natural abundance stable isotope measurements of food sources and benthic invertebrates have not been investigated in the Eden Estuary prior to this study. From the experience gained in the present study, the following recommendations are suggested for future studies:

1) Laboratory studies to determine the relationship/fractionation in $\delta^{15}N_{\text{org}}$ during assimilation in benthic foraminifera. Although $^{15}N$ enrichment is generally considered to be between 3-4 $\%$, differences of 9.5 $\%$ observed between diet and a laboratory grown brine shrimp (DeNiro and Epstein 1982) and 5.5 $\%$ in laboratory grown copepods (Checkley and Entzeroth 1985) have been reported.

2) Measurements of field variations in $\delta^{13}C_{\text{org}}$ and $\delta^{15}N_{\text{org}}$ of the proposed diets; both spatially and temporally.

3) Further, replicate studies from estuarine environments where the isotopic baseline (e.g. $\delta^{13}C$ in DIC) differ from those investigated here. In this way, field observations of stable isotope enrichment can be critically tested.

7.5 Conclusions

Although limited, the technique developed here shows the potential of using dual isotope analysis to elucidate the major end-members (food sources) of benthic foraminifera. This would most definitely be reliant on improved natural abundance measurements using the mass spectrometer at the limits of the instrument detection. This in itself is highly specialised research. The results suggest that benthic foraminifera are able to preferentially ingest and assimilate benthic diatoms from a diverse range of sources within the total organic matter pool.
Chapter 8
8. General discussion

8.1 The significance of benthic foraminifera in intertidal mudflats

8.1.1 Foraminiferal reproduction and growth

In food-limited environments, such as the deep-sea, some foraminiferal species (e.g. *Alabaminella weddellensis*, *Epistominella exigua*: "phytodetritus species") are known to respond rapidly (within 4 weeks) to seasonal pulses of phytodetritus (Gooday and Turley 1990, Gooday 1993, Gooday and Rathburn 1999) and their life history (reproduction and growth) closely reflect primary productivity in the surface ocean (Gooday and Hughes 2002). These species respond to phytodetrital events by rapid reproduction, resulting in an increased abundance and colonization of the phytodetritus deposits. Gooday and Hughes (2002) report the clearest seasonal signals occurring in the size fraction 63-125 \( \mu \text{m} \) (i.e. an increase in abundance of this size class compared to previous months). *Stainforthia fusiformis*, a common benthic species found from intertidal to outer shelf and slope areas, is also described as an opportunistic species and has the ability to adapt to rapidly changing conditions (Alve 1994, Alve 2003).

By contrast with the deep-sea, intertidal estuarine environments are not generally food limited (e.g. ubiquitous benthic diatoms) and foraminiferal life histories in general do not respond in such a clear-cut fashion to changes in food availability (Murray and Alve 2000, Chapter 6). Despite clear seasonal patterns being described for shallow water populations and for individual species abundance, these have been mainly linked with substratum type, temperature, salinity and predation (Bradshaw 1961, Murray 1963, Buzas 1978, Murray 1983). However, it has also been suggested (Boltovsky 1964, Buzas 1969, Ramanathan 1970, Murray 1983, Ellison 1984, Cearreta 1988) that some shallow marine foraminifera may be directly linked in their response to primary production.

Despite this ecological uncertainty, foraminifera clearly exhibit opportunistic life history characteristics, such as the ability to both reproduce and grow rapidly to exploit environmental change and available resources (Chapter 3). Unlike the opportunistic species of the deep-sea, estuarine foraminifera do not generally possess delicate, thin walled tests but instead tend to be more robust; suggesting that more energy is used in test calcification by intertidal foraminifera.
Very little sustained emphasis has been placed on the direct measurement of intertidal foraminiferal rates of reproduction, fecundity and subsequent growth of juveniles. Most estimates of growth have come from time series population studies (Introduction to Chapter 6). If foraminiferal populations are to be modelled in order to determine their importance within ecosystems compared to other meiofaunal groups, then more detailed studies are required on their fecundity and on the growth rates of individual species, including early juveniles under natural conditions.

Asexual reproduction has very little loss in terms of biomass, as almost the whole of the adult is divided into what would appear to be equal parts, corresponding to the number of juveniles produced. Theoretically, this should produce no significant loss of biomass, at least until the juveniles are exposed to the surrounding "dangers of life". Sexual reproduction, on the other hand, is costly to the foraminifera in that it produces large numbers of small gametes, most of which have a low survival rate. Although small in relation to the total adult biomass, high gamete mortality may lead to significant loss of biomass (Hallock 1985, Hottinger 2000).

Asexual reproduction (complete in less than 3 days) and subsequent growth of juveniles was rapid in *Elphidium williamsoni* (Chapter 3). This suggests that this species is able to respond rapidly to changing environmental conditions (e.g. increased food availability) by reproducing and increasing its biomass following the growth of juveniles. Such individuals/species are generally termed r-selected and maximise their fitness by reproducing rapidly.

Sexual reproduction, on the other hand, generally results in a slower response to environmental change because gametes have to fuse before growth takes place. Thus sexual reproduction may be a better strategy for colonising new habitats where genetic variation within a population is advantageous.

### 8.1.2 The trophic position of intertidal benthic foraminifera

In general, foraminifera have been described as obtaining nutrients by herbivory, carnivory, omnivory, cannibalism, parasitism, scavenging, mixotrophy (an ability to feed and photosynthesise) and from dissolved organic matter (Capriulo *et al.* 1991).
Despite many authors suggesting that the meiofauna form an important link in benthic marine food webs (Fenchel 1978, Coull and Bell 1979, Coull 1999), benthic foraminifera remain largely overlooked in coastal and estuarine ecological studies. As a result their ecology, trophic position and significance within benthic marine food webs are generally not clearly defined. Bernhard and Bowser (1992) have, through some elegant laboratory studies, demonstrated the importance of allogromiid foraminifera (Allogromia sp. and A. laticollaris) in nutrient cycling and bacterial population dynamics, especially in intertidal areas. By contrast, the calcareous foraminifera (e.g. Elphidium incertum, Pyrgo williamsoni) examined by these authors demonstrated no harvesting of the bacterial biofilms. Intertidal calcareous species (e.g. Elphidium williamsoni, Haynsina germanica, Elphidium oceanensis) have been suggested as being mainly herbivorous, based on pigment analysis of field collected individuals and numerous laboratory-feeding experiments (Lee et al. 1966, Lopez 1979, Lee 1980, Knight and Mantoura 1985).

Organic matter available to intertidal benthic foraminifera has four main supply sources (1) production by nearshore benthic plants (diatoms, macroalgae and seagrasses), (2) oceanic phytoplankton production, (3) organic matter supplied by rivers and (4) wind blown from fields. Dissolved organic matter in seawater is also delivered from the sources stated above and is made available to the particulate pool by bacterial consumption (Loubere and Fariduddin 1999). In estuarine environments, up to 45% of gross primary production is synthesised by benthic diatoms (MacIntyre and Cullen 1996, Underwood and Kromkamp 1999). Because benthic foraminifera live in contact with these primary producers and have been shown to consume them in laboratory experiments and in field studies, one of the aims of this thesis was to determine, in nature, their reliance upon fresh labile organic matter sources.

This study has shown that benthic foraminifera will feed directly on live $^{13}$C-labelled benthic diatoms under monospecific bloom conditions (Chapter 4). However, in this study the diatom species (Cylindrotheca closterium) used is weakly silicified and may have resulted in an easy meal for the foraminifera, but more robust forms, such as Pleurosigma, are also clearly utilised (Chapter 7).
The rapidity with which intertidal benthic foraminifera can both respond to and ingest benthic diatoms has been clearly demonstrated in this study through the uptake of labelled $^{13}$C (Chapter 4).

Short-term responses measured in benthic foraminifera to a simulated benthic diatom bloom revealed high values of enrichment in $^{13}$C within 1 day ($\text{Ammonia} \ 998 \pm 192 \%, \ \text{Haynesina} \ 392 \pm 121 \%, \ \text{Elphidium} \ 687 \pm 121 \%, \ \text{Atestate} \ 1,635 \pm 427 \%$), reflecting their great efficiency in collecting and ingesting fresh labile organic matter (Chapter 4). These high levels of enrichment were maintained over a 5-day experimental period. Enrichment in the foraminifera measured in this study, compared to that seen in nematodes (230 % after 3 days; Moens et al. 2002), suggests they are extremely important in concentrating organic matter rapidly compared to some other meiofaunal organisms (Chapter 4, Moodley et al. 2002). Thus, if predation on benthic foraminifera is high, this trophic transfer pathway could be an extremely efficient means of energy transfer (Chapter 1, Figure 1.1). Coupled with their high numbers in some intertidal areas (Chapter 4, 5), this suggests benthic foraminifera are extremely important carbon links in the benthic intertidal marine foodweb.

Foraminiferal turnover time (time taken to complete a biological cycle, birth to death) is relatively rapid, with most intertidal species living for 3 to 4 months (Cearreta 1988, Murray 1983, Basson and Murray 1995, Chapter 3, 6). However, rapid reproduction and growth strategies in some taxa show the potential for variation in species turnover time depending on environmental conditions (Chapter 3, 4, 6).

Further work in this field would benefit from longer time-series sampled over shorter time intervals. The same experiments presented here (Chapter 4) could be attempted in situ, by adding $^{13}$C-labelled bicarbonate and following the $^{13}$C through the foraminiferal pathway to higher organisms (c.f. Middelberg et al. 2000). Comparative studies to look at the utilization of planktonic versus benthic diatoms would also be useful. For example, is there possibly a stronger seasonal signal in the phytoplankton within the estuary, which is not currently determined by sampling the microphytobenthos which dominate the mudflats at low tide? Laboratory feeding experiments might usefully examine the foraminiferal response to more heavily silicified diatom species, such as Pleurosigma and Gyrosigma, both common species in the Eden Estuary (pers. obs.). The high
levels of $^{13}$C enrichment recorded over five days, could be further studied over longer experimental time periods to assess if this is due to continued ingestion and rapid assimilation of carbon or due to satiation but without the processing of the ingested carbon (Chapter 4).

8.1.3. *Foraminiferal distribution and migration*

Benthic foraminifera within intertidal areas are typically concentrated in the surface 0.25 to 1 cm (Castignetti 1996, Alve and Murray 2001). In the Hamble Estuary, Alve and Murray (2001) found no clear seasonal shift in the vertical distribution of foraminifera, despite the redox depth varying to depths greater than 1 cm at certain times of the year. In intertidal environments foraminifera are not generally found at depth, unless they have been redistributed by bioturbation in a high density macrofaunal area. However, microhabitats created around macrofaunal burrows give foraminifera an opportunity to extend their depth habitat.

In this study, the vertical distribution and migration of benthic foraminifera have been examined in a series of laboratory experiments (Chapter 5). These show that both Ostracoda and *Allogromia* foraminifera displayed a negative geotactic response to burial, whereas the calcareous genera *Ammonia*, *Haynesina* and *Elphidium* displayed selective behaviour in response to oxygen supply. All these taxa demonstrate the ability to migrate downwards in response to the removal of oxygen at the surface (and possibly the formation of H$_2$S). Thus these species demonstrate an ability to select a preferred environment upon displacement. All five taxa demonstrated the ability to migrate into and through anoxic sediments.

8.1.4 *Seasonal responses*

In the Eden Estuary concentrations of chlorophyll $a$ and $c$ were highly correlated ($r^2 = 0.8672$ high intertidal mudflat, $r^2 = 0.8812$ low intertidal mudflat) suggesting that a large proportion of the primary productivity is from benthic diatoms. Two clear peaks were found in chlorophyll $a$ and $c$ concentration during March and April 2001 at the high intertidal station, and June 2000 at the low intertidal station. During the rest of the year chlorophyll concentrations remain relatively constant (Chapter 6, Figure 6.10).
Foraminifera at both high and low intertidal locations show a peak in abundance during May and June, which appear to be caused by reproductive events. At the low intertidal location, multivariate statistics revealed a weak correlation between species and chlorophyll $a$ and $c$ concentrations. It therefore appears that the major controlling factors on foraminiferal assemblages in the intertidal mudflats of the Eden Estuary are largely abiotic at the high intertidal station and more biotic at the low intertidal station. However, this may in part be due to the fact that the multivariate statistics employed do not take into account the lagged response found in the increase in foraminiferal numbers at the high intertidal location. By contrast, at the low intertidal location, there appears to be a more direct response, with the peak in total abundance of foraminifera coinciding with a peak in chlorophyll $c$ in June 2000. If one assumes adjacent years have the same annual pattern of chlorophyll $c$ and $a$, then it appears that high intertidal foraminifera species demonstrate a lagged response to diatom blooms, whereas low intertidal foraminifera respond by reproducing to coincide with peaks in diatom biomass.

Multivariate statistics show that chlorophyll concentrations in general are not a controlling factor of foraminiferal species abundance. However, at low intertidal locations, there were weak correlations with chlorophyll $a$ and $c$. If multivariate statistics are not taking into account lagged responses, then the correlations found at low tide might be indicative of a response to increases in diatom biomass (Chapter 6).

Benthic diatom biomass in the Eden Estuary expressed as chlorophyll $a$ and $c$, does not appear to be generally limiting, since only one significant change in biomass was observed over the entire year. Therefore, foraminiferal distribution is more likely to be being controlled by physical abiotic factors in the Eden Estuary. Large areas rich in *Corophium volutator* were noted during the study, especially on the high intertidal mudflats and these are known to graze benthic diatoms. The effect on the local diatom population is unknown, but may cause disturbance to foraminifera if there is direct competition for diatoms as a food resource.

Monthly size frequency data for *Elphidium williamsoni* and *Haynesina germanica* revealed live individuals within the size class 63 to 93 μm to be extremely rare (Chapter 6, Table 6.9). It might be argued that sieving at less than
63 μm might have increased numbers and may be important in future studies where the sampling interval is increased. In fact, laboratory observations of *E. williamsoni* revealed minimum juvenile test sizes following asexual reproduction to be greater than 99 μm within 3 days. It is also possible that other species have smaller juveniles or that *E. williamsoni* under different conditions produces smaller, more numerous juveniles. However, given the available information, the selection of mesh size in this study is not expected to have major consequences for the biomass estimates obtained.

8.1.5 *The trophic position of intertidal benthic foraminifera: Natural abundance stable isotope measurements and observational data*

This study is the first to use natural abundance stable isotopes to determine the trophic position of benthic foraminifera within intertidal sediments. Stable carbon (δ¹³C) and nitrogen (δ¹⁵N) results from field collected material of the Eden Estuary suggest that, if benthic foraminifera assimilate carbon and nitrogen from a single food resource, benthic diatoms are the most likely candidate (Chapter 7, Figure 7.2.). The results obtained clearly demonstrate the possibility of expanding this technique to examine a greater selection of end-members (food sources) and to examine seasonal variation more closely.

Given the stable isotope measurements made, *Elphidium williamsoni*, *Haynesina germanica* and *Elphidium oceanensis* all appear to utilise benthic diatoms as their main source of organic matter. Interestingly, Knight and Mantoura (1985) suggested that *E. oceanensis* may be feeding on harpactecoid copepods or on benthic diatoms and concentrating the carotenoids (Chapter 7, Figure 7.1). Further isotopic studies could usefully incorporate such species (i.e. copepods) and this may give a clearer indication of benthic foraminiferal food sources. The use of mixing models to determine the importance of end-members within a mixed food source diet may be problematic due to the apparently limited separation in δ¹⁵N and δ¹³C between most intertidal end-members.

SEM images clearly demonstrate that benthic foraminifera strip the contents out of larger diatoms (e.g. *Pleurosigma*) outwith their test (Banner and Culver 1978, Alexander and Banner 1984). Clear, characteristic cracks have been documented for the first time in the frustules of larger diatoms (e.g. *Pleurosigma*) where benthic foraminifera had been seen to actively gather them towards the
aperture. However, due to the dominance of _Pleurosigma_ in field collected samples, this was the dominant species in most images and the "cracking" phenomenon described here has not been recorded for any other species of diatom. Further study could make use of cultured diatoms of different sizes and degree of silicification. Palaeoecological studies of intertidal diatoms, for example, would benefit from an improved understanding of their taphonomic pathway following their utilization as a food resource by benthic foraminifera.

8.2 Summary

1. Benthic foraminifera of the species _Elphidim williamsoni_ are capable of rapid asexual reproduction and growth (monthly average growth rate 7 μm d\(^{-1}\)) within the first month. Highest growth rates were measured for the first three days of growth (14 μm d\(^{-1}\)).

2. Intertidal benthic foraminifera respond rapidly (within 1 day) to simulated benthic diatom blooms and demonstrated high ingestion rates based upon enrichment in labelled \(^{13}C\) (up to 1,635 ± 427 % after 1 day).

3. Oxygen appears to be the major controlling factor on the distribution of benthic foraminifera within intertidal sediments. However, some taxa showed an over-riding negative geotaxis regardless of treatment conditions.

4. Within the Eden Estuary, live foraminifera are present throughout the year, with one clear population peak occurring at both high and low intertidal sampling stations in June.

5. _Haynesina germanica, Elphidium williamsoni_ and _Elphidium oceanensis_ all appear to consume benthic diatoms as their major carbon source from the intertidal sediments of the Eden Estuary.

6. The novel use of natural abundance stable isotopes (\(\delta^{13}C_{org}\) and \(\delta^{15}N_{org}\)) both demonstrates the trophic position of intertidal benthic foraminifera and confirms that they largely consume and assimilate organic matter from benthic diatoms.
8.3 Conclusions

Intertidal foraminifera, by virtue of their often large standing stocks and high turnover rates, are an important food item for many invertebrates. The results of this study support the hypothesis that there is a close trophic link between microphytobenthos and foraminifera in the transfer of organic carbon towards higher trophic levels in muddy intertidal sediments. Thus foraminifera potentially play a key role in the rapid transfer of autotrophic carbon to higher trophic levels and possibly in the microbial loop after death. Ultimately, a fuller understanding of the global carbon cycle will require an improved quantitative knowledge of carbon transfer within such environments.
References
References


Appendix A: Summary tables of all live counts at each depth level in each treatment after six days (i.e. pre-rose Bengal treatment).

Table 1: T= 0, Repeat 1. Live counts made of major meiofauna taxa in 1/4th of the sample at each depth layer.
+ = Live, - = No live record and few = < 5 individuals

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Nematoda</th>
<th>Ostracoda</th>
<th>Copepoda</th>
<th>Turbellaria</th>
<th>Haynesina</th>
<th>Ammonia</th>
<th>Elphidium</th>
<th>Allogromia</th>
<th>Saccamina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>+</td>
<td>&lt; few</td>
<td>+ &lt; few</td>
<td>-</td>
<td>+ (2)</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>0.5 - 1</td>
<td>+ &lt; few</td>
<td>-</td>
<td>-</td>
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<td>+ (1)</td>
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<td>1 - 1.5</td>
<td>+ &lt; few</td>
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<td>- &lt; few</td>
<td>-</td>
<td>+ (1)</td>
<td>+</td>
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<td>1.5 - 2</td>
<td>+ &lt; few</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>&lt; + (14)</td>
<td>+ (9)</td>
<td>+</td>
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</tr>
<tr>
<td>2.5 - 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+ (14)</td>
<td>+ (9)</td>
<td>+</td>
<td>+ (8)</td>
<td>+ (1)</td>
</tr>
</tbody>
</table>

Table 2: T= 0, Repeat 2. Live counts made of major meiofauna taxa in 1/8th of the sample at each depth layer.
+ = Live, - = No live record and few = < 5 individuals

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Nematoda</th>
<th>Ostracoda</th>
<th>Copepoda</th>
<th>Turbellaria</th>
<th>Haynesina</th>
<th>Ammonia</th>
<th>Elphidium</th>
<th>Allogromia</th>
<th>Saccamina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>+ &lt; few</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5 - 1</td>
<td>+ &lt; few</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 - 1.5</td>
<td>+ &lt; few</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (2)</td>
<td>+ (2)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.5 - 2</td>
<td>+ &lt; few</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (2)</td>
<td>+ (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 - 2.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ &lt; few</td>
<td>+ (8)</td>
<td>+ (10)</td>
<td>+ (3)</td>
<td>-</td>
<td>+ (1)</td>
</tr>
<tr>
<td>2.5 - 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ &lt; few</td>
<td>+ (22)</td>
<td>+ (20)</td>
<td>+ (5)</td>
<td>+ (2)</td>
<td>+ (3)</td>
</tr>
</tbody>
</table>
Table 3: Live counts made of major meiofauna taxa in experimental core NoO₂ (Top)/O₂ (bottom), Repeat 1 in 1/4th of the sample.
+ = Live, - = No live record and few = < 5 individuals

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Nematoda</th>
<th>Ostracoda</th>
<th>Copepoda</th>
<th>Turbellaria</th>
<th>Haynesina</th>
<th>Ammonia</th>
<th>Elphidium</th>
<th>Allogromia</th>
<th>Saccamina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5 - 1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 - 1.5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.5 - 2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>(1)</td>
<td>+</td>
<td>+</td>
<td>(2)</td>
<td>-</td>
</tr>
<tr>
<td>2 - 2.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(3)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.5 - 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(11)</td>
<td>(14)</td>
<td>(4)</td>
<td>+</td>
<td>(1)</td>
</tr>
</tbody>
</table>

Table 4: Live counts made of major meiofauna taxa in experimental core NoO₂ (Top)/O₂ (bottom), Repeat 2 in 1/8th of sample.
+ = Live, - = No live record and few = < 5 individuals

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Nematoda</th>
<th>Ostracoda</th>
<th>Copepoda</th>
<th>Turbellaria</th>
<th>Haynesina</th>
<th>Ammonia</th>
<th>Elphidium</th>
<th>Allogromia</th>
<th>Saccamina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5 - 1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>(2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 - 1.5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.5 - 2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>(1)</td>
<td>+</td>
<td>(1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 - 2.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(3)</td>
<td>+</td>
<td>-</td>
<td>(2)</td>
<td>-</td>
</tr>
<tr>
<td>2.5 - 3</td>
<td>+</td>
<td>slightly more</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>(20)</td>
<td>(25)</td>
<td>(3)</td>
</tr>
</tbody>
</table>
Table 5: Live counts made of major meiofauna taxa in experimental core O₂ (Top)/NoO₂ (bottom), Repeat 1 in 1/4th of sample.
+ = Live, − = No live record and few = < 5 individuals

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Nematoda</th>
<th>Ostracoda</th>
<th>Copepoda</th>
<th>Turbellaria</th>
<th>Haynesina</th>
<th>Ammonia</th>
<th>Elphidium</th>
<th>Allogromia</th>
<th>Sacamina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>0.5 - 1</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1 - 1.5</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1.5 - 2</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2 - 2.5</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2.5 - 3</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Table 6: Live counts made of major meiofauna taxa in experimental core O₂ (Top)/NoO₂ (bottom), Repeat 2 in 1/8th of the sample.
+ = Live, − = No live record and few = < 5 individuals

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Nematoda</th>
<th>Ostracoda</th>
<th>Copepoda</th>
<th>Turbellaria</th>
<th>Haynesina</th>
<th>Ammonia</th>
<th>Elphidium</th>
<th>Allogromia</th>
<th>Sacamina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>0.5 - 1</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1 - 1.5</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1.5 - 2</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2 - 2.5</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2.5 - 3</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>
Table 7: Live counts made of major meiofauna taxa in experimental core O₂ (Top)/O₂ (bottom), Repeat 1 in 1/4th of the sample.
+ = Live, - = No live record and few = < 5 individuals

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Nematoda</th>
<th>Ostracoda</th>
<th>Copepoda</th>
<th>Turbellaria</th>
<th>Haynesia</th>
<th>Ammonia</th>
<th>Elphidium</th>
<th>Allagromia</th>
<th>Saccamina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>+</td>
<td>+ (5)</td>
<td>+</td>
<td>+ (1)</td>
<td>+</td>
<td>+ (1)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5 - 1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (1)</td>
<td>+ (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 - 1.5</td>
<td>+</td>
<td>+ (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.5 - 2</td>
<td>+</td>
<td>+ (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 - 2.5</td>
<td>+</td>
<td>+ (5)</td>
<td>+</td>
<td>-</td>
<td>+ (4)</td>
<td>+ (5)</td>
<td>+ (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.5 - 3</td>
<td>+</td>
<td>+ (4)</td>
<td>+</td>
<td>+</td>
<td>+ (3)</td>
<td>+ (7)</td>
<td>+ (2)</td>
<td>+ (5)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 8: Live counts made of major meiofauna taxa in experimental core O₂ (Top)/O₂ (bottom), Repeat 2 in 1/8th of the sample.
+ = Live, - = No live record and few = < 5 individuals

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Nematoda</th>
<th>Ostracoda</th>
<th>Copepoda</th>
<th>Turbellaria</th>
<th>Haynesia</th>
<th>Ammonia</th>
<th>Elphidium</th>
<th>Allagromia</th>
<th>Saccamina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>+</td>
<td>+ (6)</td>
<td>+</td>
<td>-</td>
<td>+ (1)</td>
<td>+ (2)</td>
<td>+ (2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5 - 1</td>
<td>+</td>
<td>+ (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 - 1.5</td>
<td>+</td>
<td>+ (2)</td>
<td>-</td>
<td>-</td>
<td>+ (1)</td>
<td>+ (1)</td>
<td>+ (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.5 - 2</td>
<td>+</td>
<td>+ (1)</td>
<td>-</td>
<td>-</td>
<td>+ (3)</td>
<td>very active</td>
<td>+ (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 - 2.5</td>
<td>+</td>
<td>+ (7)</td>
<td>+</td>
<td>-</td>
<td>+ (3)</td>
<td>+ (5)</td>
<td>-</td>
<td>-</td>
<td>+ (1)</td>
</tr>
<tr>
<td>2.5 - 3</td>
<td>+</td>
<td>+ (15)</td>
<td>+</td>
<td>-</td>
<td>+ (&gt;15)</td>
<td>+ (&gt;30)</td>
<td>+ (20)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>