Foraminifera from the sea grass communities of the proposed marine conservation zone in Tor Bay

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Modern foraminifera living of plant substrates (epiphytic) have been studied in a number of different areas but those from southern England have not been described in any detail. This investigation concerns the diversity and distribution of the benthic foraminifera in two of the sea grass meadows in Tor Bay, Devon. Both contain an assemblage typical of in-shore waters in Southern England with a dominance of species such as *Elphidium crispum* (Linné), *Ammonia beccarii batavus* (Hofker), *Cibicides lobatulus* (Walker and Jacob) and *Quinqueloculina seminulum* (Linné). Of particular note is the presence of large numbers of living *E. crispum* on the fronds of the sea grass in the spring sampling campaign which confirms the phototropic behaviour suggested by previous research.

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Introduction

Sea grass meadows support quite extensive assemblages of foraminifera (single-celled protists) and these have been documented in various parts of the world (e.g. Langer, 1993; Langer *et al.*, 1998; Semeniuk, 2000; Richardson, 2006; Michael *et al.*, 2008; Nesti *et al.*, 2009; Debenay and Payri, 2010; Mateu-Vicens *et al.*, 2010). The foraminifera associated with the sea grass meadows of southern England, however, have hardly been described and Tor Bay's sea grass community provides an ideal location for their study. In Tor Bay there are a number of protected sea grass meadows that are being examined on a continuing basis by the Torbay Seagrass Project as part of the Local Biodiversity Action Plan. These meadows are formed by the eel grass *Zostera marina* (Linné, 1753) and form an important marine habitat. This study is being undertaken in collaboration with Natural England and the Torbay Coast and Countryside Trust. The total area of the sea grass meadows that have been mapped in Tor Bay was 81.6 hectares in 2006, representing about 80% of the known sub-tidal *Zostera marina* meadows in Devon with most of the others mainly based in estuaries (e.g. Salcombe). *Zostera marina* meadows support abundant species and recent studies of the partially inter-tidal sea grass meadows in Tor Bay have shown that, regardless of their size, all the sea grass patches that were examined supported a higher level of biodiversity (e.g. sea horses, cuttlefish, etc.) than the surrounding bare sand (Hirst and Attrill, 2008). With the planned introduction of Marine Conservation Zones (MCZ) around the United Kingdom, it is important that data on the many groups of organisms associated with sea grass meadows are gathered and included in the formal definitions of the individual MCZs.

Marine conservation zones

The Marine and Costal Areas Act (2009) has created a new type of Marine Protected Area (MPA) that will be known as a Marine Conservation Zone (MCZ). MCZs will protect nationally important marine wildlife, marine habitats, geology and geomorphology. The Marine Conservation Zone Project is involved in the selection of MCZs in English in-shore waters and off-shore waters next to England, Wales and Northern Ireland. The sites are to be selected for a wide range of reasons and are not being defined only to protect the rare or endangered, but the complete range of marine wildlife and submarine features (including areas in which dynamic processes are known to operate). The management measures required to ensure the protection of MCZs will be decided on a site-by-site basis and reflect the items or features covered by the individual designations. In a similar way to protected areas on land (e.g. Sites of Special Scientific Interest – SSSI), there will be sites where some activities are not allowed but others can occur, or where there are seasonal restrictions on activities rather than a complete ban. Not all sites will require the same management measures and there is no presumption that any specific type of activity will be restricted. There will, however, be a number of delicate and nationally important sites in which there will be a large range of restrictions.

The Marine Conservation Zone Project has been established by the Department for the Environment, Food and Rural Affairs (Defra), Natural England and the Joint Nature Conservation Committee to identify and recommend MCZs to the Government. The Marine Conservation Zone Project is being delivered through four Regional Projects covering the South-West, Irish Sea, North Sea and Eastern Channel. In the

South-West, users and interest groups have come together under the organisation of "Finding Sanctuary" to select and propose the management of a number of MCZs. Final proposals (following consultation and discussion of 3 interim reports) were delivered to the National Steering Group of the Marine Conservation Zone Project in June 2011 (Lieberknecht *et al.*, 2011), with final approval and designation of the selected MCZs in 2012. One of the proposed MCZs in the South-West region covers the sea grass meadows in Tor Bay.

The Tor Bay proposed MCZ has been laid out in the report to the National Steering Group (Lieberknecht *et al.*, 2011, pp. 503-527). While the potential boundaries of the site have been identified there may be further modifications during the final designation process. While a full range of sea floor features are covered by the proposed MCZ (sub-tidal mud, infra-littoral rock, circa-littoral rock, inter-tidal coarse sediments, inter-tidal mixed sediments, muddy sand, etc.) the main features of interest are the sea grass meadows. Within these meadows are important populations of the short-snouted sea horse *Hippocampus hippocampus* (Linné, 1758) and the long-snouted sea horse *H. guttulatus* Cuvier, 1829. While the scientific case for designation as an MCZ appears to have been made, there are a number of potential conflicts (harbour and boat usage, pleasure boat activities, water skiing and fishing).

Tor Bay sea grass meadows

About 50 species of marine angiosperms, commonly known as sea grasses, live in today's inner continental shelves (den Hartog, 1977). Sea grass ecosystems are recognised to be amongst the richest and most productive coastal ecosystems where a sizeable part of the primary production is being carried out by a variety of epiphytic algae, which directly provide food to a range of meiofauna and microfauna (Sen Gupta, 2002).

The presence of sea grass in sub-tidal and inter-tidal habitats can have substantial effects on these habitats and, as a result, cause pronounced changes in the benthic communities. Such sea grass meadows are considered to be amongst the most productive of shallow water sedimentary environments. For instance, prior to the evolution of sea grass there were none of the associated habitats and – by default – no sea grass related foraminiferal assemblages (Murray, 2006). Sea grass meadows have also been reported to enrich the dissolved nutrient concentration of their surrounding marine environments by absorbing from the sediment ammonia and phosphates generated by bacterial activities and/or organic matter accumulation and releasing it to the environment (Lee and Anderson, 1991).

Under the European Water Framework Directive angiosperms (flowering plants), phytoplankton, macroalgae, benthic invertebrate fauna and fish are considered as important bioindicators and helpful in defining the ecological status of coastal waters. They are referred to as biological quality elements (WFD, 2000). Sea grasses are the only truly marine flowering plants and can be used for monitoring purposes because they are sensitive to human disturbance (Foden and Brazier, 2007). Five sea grass species are found around the British Isles: two species of 'tassel weed' (*Ruppia maritima* Linné, 1753 and *R. cirrhosa* (Petagna) Grande, 1918) and three species of 'eelgrass' (*Zostera* spp.).

In the United Kingdom, *Zostera marina* is the dominant species and, similar to those of the brackish waters along the Atlantic coast, displays an annual growth closely linked to water temperature with the optimum range being between 5°C and 30°C (UKSACS, 2010). Living *Zostera marina* leaves are the favourite substratum for many epiphytic algae and there have been reports of other algae living between the sea grass shoots and within the surface layers of the sediment below. Whelan and Cullinane (1985) identified 60 algal species in a *Zostera marina bed* in Ventry Bay, Ireland. Dense meadows of *Zostera marina* leaves slow down the water flow and increase rates of sedimentation. Its rhizome and root networks bind the substrate together, resulting in reduced erosion and stabilisation of the sediment (Garcia and Duarte, 2001). The penetration of *Zostera* roots into the sediment ventilates the upper layers, allowing for a deeper penetration of oxygen into the sediment layer and providing a more favourable habitat for burrowing animals (UKSACS, 2010). De Boer (2007) highlights the importance of light on sea grass growth and identifies the process through which the reduction of turbidity results in a decrease of the sediment load and the resulting improved light conditions as the most significant positive feedback in sea grass systems.

Tor Bay is located on the south coast of Devon in south west England (see Figure 1). Recent studies by the Torbay Coast and Countryside Trust's (TCCT) Torbay Seagrass Project (*personal communication*: Dominic Flint, Seagrass Project Officer, 2009) has shown that there are at least 80 ha of sea grass meadows in Tor Bay representing nearly 31% of the total reported area of all sea grass species in Devon. Samples were collected at 3 geographical sites: two from within the sea grass meadows at Fishcombe Cove (050° 24.16' N, 003° 31.3' W) and Millstones Bay (050° 27.34' N, 003° 31.3' W) and one from the bare sediments in the middle of Tor Bay's coastal waters (050° 26.06' N, 003° 30' W). The sea grass meadows are within the normal salinity range (25–34 ‰), sub-tidal and appear to be mainly comprised of *Zostera marina*. The sea grass bed depth in Fishcombe Cove ranges from 1.0–3.5 m and 0.1–4.3 m in Millstones Bay. The sea grass patches in Fishcombe Cove are quite dense and cover a large area whereas, in Millstones Bay, they are sparse and cover smaller areas. However, the overall area of the Millstones Bay meadow is almost 3 times larger than those at Fishcombe Cove with the former reported at 1.5 hectares and the latter 0.41 hectares. The weather event statistics acquired by the Torbay Coast and Countryside Trust (TCCT) during the sea grass survey project in 2006 has shown that, as a result of their geographical locations, these sea grass meadows are subject to different levels of storm exposure with Fishcombe Cove being very sheltered and mainly subject to north-easterly winds while Millstones Bay is less sheltered with a medium risk from (often stronger) south and south-easterly winds.

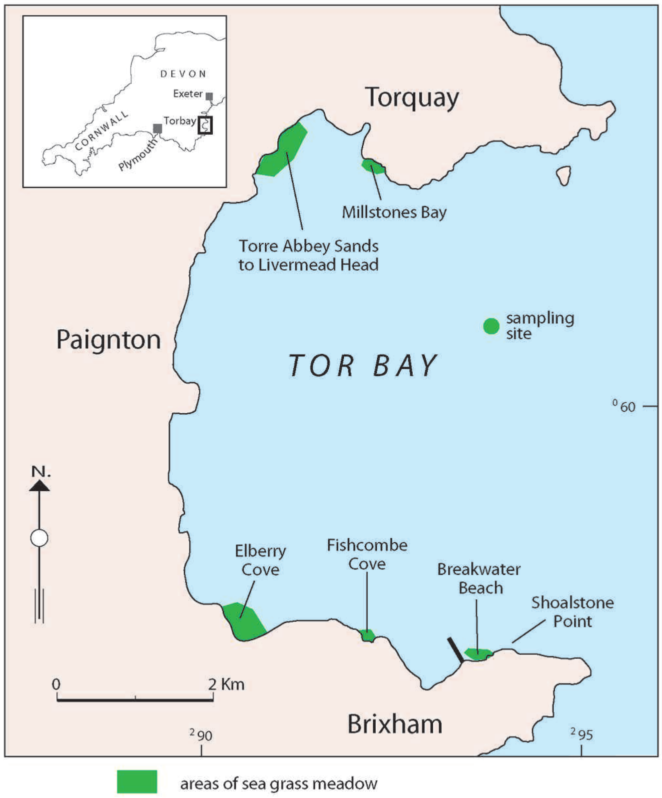

Figure 1. *Location map of the sea grass meadows in Tor Bay, South Devon. The open marine sediment site in the outer part of Tor Bay was sampled as a control.*

Investigation of Foraminifera

With no published works on the foraminifera of sea grass meadows in the UK there is no published methodology available with which to design both sampling and investigation strategies. As the main phase of the investigation had to be completed during 2010, a sampling strategy was devised – in collaboration with the Tor Bay Project Officer, Dominic Flint – that would gather the samples and the associated physical and chemical data at three times during the year. Samples were collected during winter (17th March 2010), spring (28th May 2010) and summer (13th August 2010) by the Sea Search volunteer scuba divers aboard RV *Jennifer Ann*. The first set of winter samples were collected in Millstones Bay at around 14:30 hrs and followed by the Tor Bay Coastal waters about one hour later during the early stages of the flood tide. Fishcombe Cove was sampled last during the time of high water. The spring and summer samples were collected in the same order but during different tidal phases. The exact sample locations were marked using in-house fabricated stainless steel "screw-markers" equipped with light and temperature sensors and an attached, floating, bright colour underwater buoy to facilitate the finding of the exact substrate locations during the subsequent seasonal sampling periods. A YSI Sonde device was used to record water quality data (e.g. depth, temperature, salinity, pH, turbidity and dissolved oxygen; see Table 1). These data indicate that all of the sample locations have a normal temperature, salinity and pH profile for South West England coastal waters. All sample bottles and bags were labelled with the name of the sample site and the substrate and assigned an abbreviated short name for convenient use during subsequent data analysis and presentations (see Table 2). Original and replicate samples were suffixed by digits 1 and 2 respectively. In total, 18 samples were collected with 8 samples from each of the sea grass meadows and 2 samples from the Tor Bay coastal waters as follows: (a) 4 replicate samples from the sea grassleaves; (b) 4 replicate samples from the sea grass rhizome/roots; (c) 4 replicate samples from the bare sediments near the sea grass patch; (d) 4 replicate samples from the bare sediment at the edge of the sea grass meadow; plus (e) 2 replicate samples from the sediments of the Tor Bay coastal waters.

	Millstones Bay			Fishcombe Cove			Torbay Waters		
	Winter	Spring	Summer	Winter	Spring	Summer	Winter	Spring	Summer
Time	14:38	9:34	9:45	16:23	11:34	11:40	15:27	12:40	12:55
Depth (m)	3.5	3.5	3.5	4.5	4.5	4.5	12	12	12
Temperature (°C)	7.26	13.5	16.2	7.3	14.43	15.9	6.94	14.12	15.5
Salinity (‰)	34.82	35.13	35.8	34.9	35.19	35.4	35.1	35.21	35.6
pH	8.12	8.33	8.5	8.19	8.25	8.4	8.18	8.29	8.6
Turbidity (ntu)	0	0	0	0	0	0	1.9	0	0
DO (mg/L)	8.74	0.23	0.52	9.12	0.46	0.72	9.06	1.83	0.92

Table 1. *CTD seasonal data for the sampling sites. Note that DO is the level of dissolved oxygen (in mg/L) and, in the single turbidity measurement, ntu is the nephelometric turbidity unit.*

Sample ID	Sample description
MBSS	Millstones Bay in-between Seagrass Sediment
MBSE	Millstones Bay Seagrass Edge Sediment
MBSR	Millstones Bay Seagrass Root
MBSL	Millstones Bay Seagrass Leaf
FCSS	Fishcombe Bay in-between Seagrass Sediment
FCSE	Fishcombe Cove Seagrass Edge Sediment
FCSR	Fishcombe Cove Seagrass Root
FCSL	Fishcombe Cove Seagrass Leaf
TORS	Torbay Coastal Waters Sediment

Table 2. *Sample identification codes for the various locations.*

Sea grass leaves and root samples of approximately 20 cm in length were taken from randomly selected patches and placed in Ziplock plastic bags and sealed under the water (Figure 2). Sediment samples were taken from a 10 cm circular area using a plastic ring sampler similar to the one used by Murray in his 1983 sampling of the Exe Estuary (Murray, 1983), removing the top 1 cm of the sediment and placing it in the plastic sampling bottles (Figure 3). All samples were kept in a cool box during the sampling trip and once onshore (approximately 3 hours later) were preserved using buffered formalin to prevent decay of the protoplasm within the tests of the foraminifera. Samples were gently stirred to ensure the full exposure of the samples to the preserving chemical agent.

The rose Bengal staining technique is often used to stain the protoplasm of foraminifera that were alive at the time of collection. While there are inherent problems with this methodology (Murray and Bowser, 2000) it is important to separate the "living assemblage" from empty tests that may be transported or may be representative of several years standing crop. In order to identify the precise composition and numbers of the living assemblage, staining was employed but every precaution was taken in how the results were interpreted. The wet-picking technique, though time-consuming, was found to be the most reliable way in which to separate the rose Bengal stained individuals.

Samples were delivered to the laboratory the following day to be prepared for examination. It was decided that "wet picking" of the foraminifera would be employed as the main method for separation of the individual specimens. The sediment samples were prepared as follows. (1) A 10 cc sub-sample was taken from the original sample using a small plastic spoon and placed in a marked glass beaker. (2) The 10 cc sub-sample was placed into a 63 μm sieve and gently washed with tap water to remove any clay and silt. (3) The residue was poured into another container and mixed with an equal amount of rose Bengal (1 gram rose Bengal to 1 litre distilled water) and allowed to stand for 3 hours before being gently washed through the 63 μm sieve again to remove excess stain. (4) The washed residue was split into two 5 cc sub-samples using a small plastic spoon, a plastic pipette and marked glass beakers and preserved with distilled water in cleanly washed and labelled small glass jars. (5) Using a plastic pipette, samples were thinly spread into the channels of the "wet picking" tray and the foraminifera and ostracods were separated using a fine 0.5 sable brush under a stereoscopic binocular microscope. Each 5cc split of the sub-sample was picked for at least 250 foraminifera specimens and, if necessary, the second 5cc split was also picked. (6) The remaining picked and unpicked samples were labelled accordingly and kept in small plastic bottles for any future examination. The original sediment samples were also labelled to indicate that they had been sub-sampled by 10 cc. (7) Specimens were mounted on gridded cardboard slides and ordered according to the species. All slides were labelled with the sample name, date, method of picking, picker name and final count.

The sea grass leaf and root samples were processed as follows. (1) The whole sample was emptied into a 63 μm sieve and gently washed with tap water to remove any clay and silt. (2) The sea-grass fronds and the roots were thoroughly washed into the sieve under running water to remove any epiphytic specimens. (3) The residue was poured in another container and was processed as above in steps 3 to 7. (4) The sea grass fronds and the roots were preserved in distilled water separately and examined under the microscope for any foraminifera that might still be attached. The use of a Petri dish, instead of "wet picking" tray proved to be more practical.

Samples were analysed under a binocular microscope and a representative index slide was arranged and its constituent were examined and identified according to the work of Murray (1973, 1979) and living and dead populations were counted. Living individuals were recognised by their cytoplasmic colouration. Only specimens with one or more red-stained chamber were considered as having been alive at the time of sampling.

Figure 2. *Diver collecting a sample of sea grass fronds within the Millstones Bay sea grass meadow, Tor Bay.*

Figure 3. *Diver collecting a sample of sediment within the Millstones Bay sea grass meadow, Tor Bay.*

A JEOL-JSM 5600 Scanning Electron Microscope (SEM) was used to produce images of the foraminifera.

Results

A full analysis of the foraminiferal assemblages is being prepared for publication in a specialised journal and only the highlights of the research are presented below. The dominant species recorded in Tor Bay are *Ammonia beccarii batavus, Bulimina gibba, Cibicides lobatulus, Elphidium crispum, E. williamsoni, Quinqueloculina lata, Q. seminulum* and *Rosalina globularis*. This assemblage (Figure 4) is not unusual and is typical of UK coastal waters in general (Murray, 2006).

In each of the seasonal sampling campaigns the total count of foraminifera (living and empty [= dead]) was documented, the results being presented in Figure 5. These graphs show the five most abundant species in each of the seasonal counts with the living (stained) and empty (un-stained = dead) presented. The over-wintering assemblage contains few living individuals and this is in line with most previous research in the UK (e.g. Murray, 1983 and Castignetti, 1997). In the spring samples *E. crispum* becomes the most abundant species and remains dominant in the summer samples while other taxa are already returning to figures approaching the over-wintering levels. The most distinctive, and un-predicted, feature of the sample counts was the abundance of living *E. crispum* on the fronds of the sea grass (Figure 6). While this species is abundant in many UK coastal waters, the specific link to a life on sea grass fronds has not been mentioned (Murray, 1986, 2006; Castignetti, 1997). This species has a bi-convex, planispiral test and – unlike plano-convex forms such as *Cibicides lobatulus* – does not look adapted to an adherent mode of life.

Species of *Elphidium* are known for their ability to move both within and on a variety of surfaces. This was first noted by Dujardin (1841) and his observations have been confirmed by later workers (Arnold, 1953, 1974; Banner and Williams, 1973; Severin, 1987; Kitazato, 1981, 1986, 1988; Weinberg, 1991; Murray, 2006, p.37). Catherine Manley (reported in Manley and Shaw, 1997) undertook a series of experiments to investigate the movement of *E. crispum* both within, and on the surface of sediment samples collected from Plymouth Sound in 1994/1995. She was testing the species' response to (1) geotaxis – the upward (-ve) or downward (+ve) movement in or on sediment; and (2) phototaxis – the movement towards (+ve) or away from (-ve) light.

In her experiments there was a variable response to geotaxis but an overwhelming positive response to phototaxis. This response to light allows *E. crispum* to remain epifaunal or epiphytal (living above the substrate on fronds of sea grass or algae) as indicated by the results of both the spring and summer sampling campaigns. This is almost certainly a function of the presence of algal chloroplasts that are hosted by this species through ontogeny (Fenchel, 1987; Lee and Anderson, 1991; Murray, 1991). The presence of these chloroplasts are obvious in the green colouration shown by living individuals, which often removes the need for staining this species with rose Bengal. This +ve response to light indicates that *E. crispum* can be regarded as a phototropic taxon and the presence of this species on the *Zostera marina* fronds confirms it as epifaunal and epiphytic in habit.

Epiphytic Foraminifera

Epiphytic foraminifera live on sea grass or algae in a range of marine, largely near-shore, environments. Langer (1988) describes the foraminifera from the Mediterranean island of Vulcano living on: (1) the Neptune Grass (or Mediterranean tapeweed) *Posidonia oceanica* (Linné, 1753); (2) the brown alga *Ectocarpus* sp.; and (3) the green alga *Udotea petiolata* (Turra) Børgesen, 1926.

In this work, Langer also investigated the rhizomes (root system) of *P. oceanica*, much as we investigated the root systems of *Zostera marina* in Tor Bay. Langer (1988) records that the fronds of *P. oceanica* are colonised by a large number of foraminifera (e.g. *Cibicides lobatulus, Cyclocibicides vermiculatus* (d'Orbigny), *Planorbulina mediterranensis* (d'Orbigny) and *P. acervalis* Brady but *Elphidium* spp. is relatively rare. The brown alga *Ectocarpus* sp. also supported

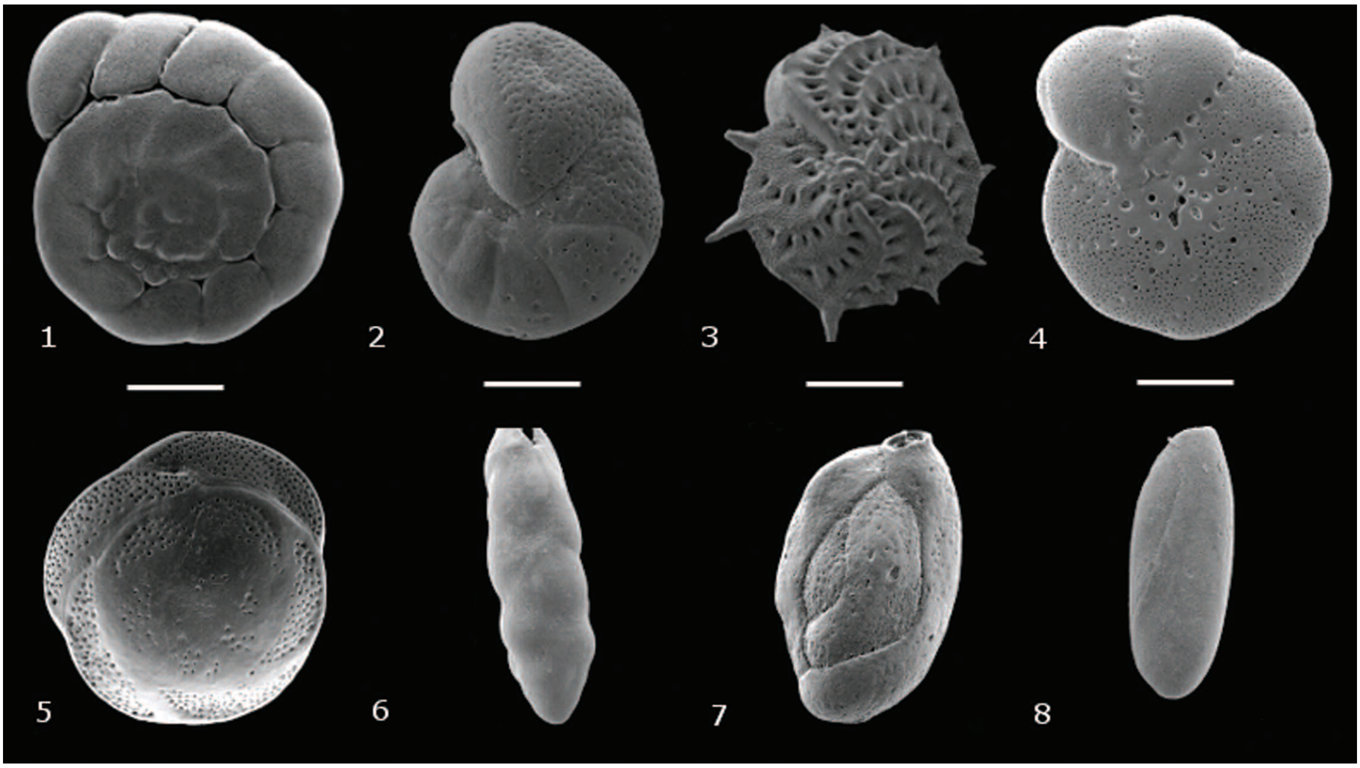

***Figure 4.** Representative foraminifera from Tor Bay. All scale bars = 100 µm. (1)* Ammonia beccarii batavus *(Linné). (2)* Cibicides lobatulus *(d' Orbigny). (3)* Elphidium crispum *(Linné). (4)* Elphidium williamsoni *(d' Orbigny). (5)* Rosalina globularis *(d' Orbigny). (6)* Bulimina gibba *(Fornasini). (7)* Quinqueloculina seminulum *(Linné). (8)* Quinqueloculina lata *(Terquem).*

Figure 5. *Graphs of living (= stained) and dead (= un-stained) taxa present in the winter, spring and summer surveys.*

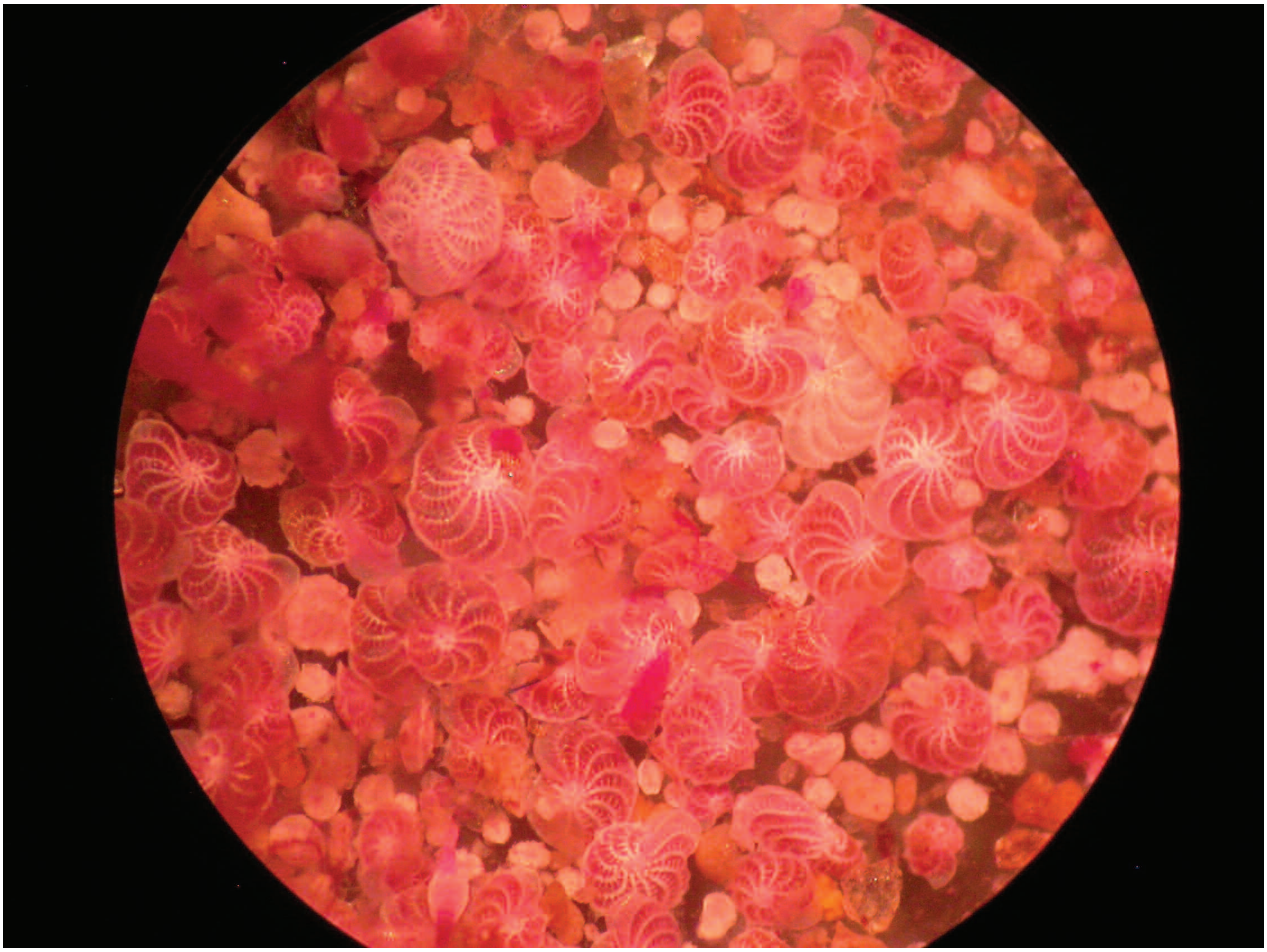

Figure 6. *Assemblage of stained* Elphidium crispum *collected from sea grass fronds in Fishcombe Cove (spring sampling). Circular field of view is 5 mm.*

large numbers of foraminifera with ~ 60% of the assemblage composed on *Elphidium aculeatum* (d'Orbigny). On the green alga *U. petiolata* elphidiids were only <10% of the recorded assemblage.

Langer (1988) identified *Elphidium* spp. as an important constituent of his "morphotype C": forms that are permanently mobile. The canal system of *Elphidium* (visible in Figure 4(3) and Figure 6) allows the whole surface of the test to act as an apertural face with rhizopods extruding over the whole test surface. This forms a network of pseudopodia that holds the test in place and assists in the rapid movement displayed by this genus (Lister, 1895; Murray, 1963, 2006; Kitazato, 1986). Epiphytal elphidiids must be grazing on a food source in order to survive but we have not been able to observe this in the samples from Tor Bay. Lipps (1975, 1983) claims they feed on detritus while Reiss and Hottinger (1984) suggest that it may be diatoms (or the bacteria associated with them).

Conclusions

The foraminifera associated with two of the sea grass meadows in Tor Bay have been described and their numbers determined over the winter – spring – summer period. Using samples from the sediment, sea grass fronds and sea grass roots it has been possible to identify the environmental preferences of some of the taxa. The over-wintering assemblage of living individuals is very low (as expected) but the most striking discovery has been the abundance of living *Elphidium crispum* on the fronds of *Zostera marina* in the post-bloom spring samples. This confirms the positive phototropic and negatively geotropic behaviour of this species (and genus). We confirm that the sea grass meadows of Tor Bay contain interesting assemblages of foraminifera and that further research in this proposed Marine Conservation Zone should be undertaken.

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TAXONOMIC NOTES ON FORAMINIFERA

The species mentioned in the text are well-known from UK near-shore marine environments and a full taxonomy is not presented. In alphabetical order, the key species are as follows.

Ammonia beccarii batavus (Hofker)
Streblus batavus Hofker, 1951, pp. 340, 341, 498.
[see Hayward *et al*. (2004) for a comprehensive review of this genus]

Bulimina gibba Fornasini
Bulimina gibba Fornasini, 1902, p. 378, pl. O, figs 32, 34.

Cibicides lobatulus (Walker & Jacob)
Nautilus lobatulus Walker & Jacob, 1798, p. 642, pl. 14, fig. 36.

Cyclocibicides vermiculatus (d'Orbigny)
Planorbulina vermiculata d'Orbigny, 1826, p. 280, no. 3.

Elphidium aculeatum (d'Orbigny)
Polystomella aculeata d'Orbigny, 1846, p. 131, pl. 6, figs 27, 28.

Elphidium crispum (Linné)
Nautilus crispus Linné, 1758, vol. 1, p. 709.

Elphidium williamsoni Haynes
Elphidium williamsoni Haynes, 1973, p. 207, pl. 24, fig. 7, pl. 25, figs 6-9, pl. 27, figs 1-3.

Planorbulina acervalis Brady
Planorbulina acervalis Brady, 1884, p. 657, pl. 92, fig. 4.

Planorbulina mediterranensis d'Orbigny
Planorbulina mediterranensis d'Orbigny, 1826, p. 280, no. 2.

Quinqueloculina lata Terquem
Quinqueloculina lata Terquem, 1876, p. 82, pl. 11, fig. 8a-c.

Quinqueloculina seminulum (Linné)
Serpula seminulum Linné, 1758, vol. 1, p. 786.

Rosalina globularis d' Orbigny
Rosalina globularis d'Orbigny, 1826, p. 271, pl. 13, figs 1, 2.