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FEATURE ARTICLE

Experimental assembly of foraminiferal communities from coastal propagule banks

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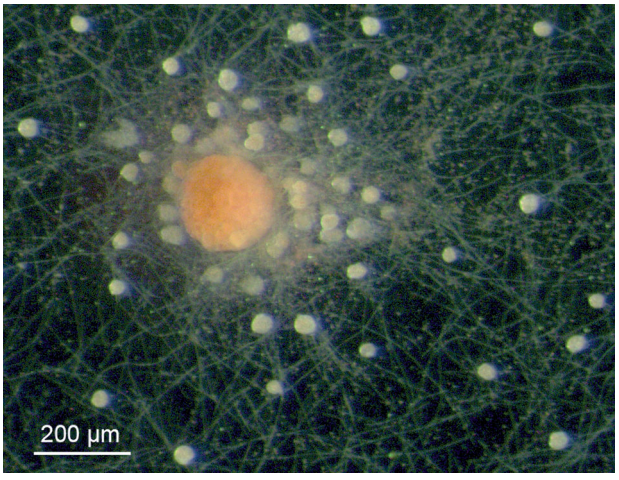
ABSTRACT: Benthic foraminifera (protists) have long been recognized as sensitive indicators in studies on natural and human-induced environmental, paleoenvironmental, and climate change. These wide-ranging applications are founded on more than a century of field-based investigations in which environmental data were related to species distributions, and have subsequently been refined by the development of chemical proxies and a variety of culture-based studies. The recent discovery of foraminiferal propagule banks that occur in the fine-sediment fraction of marine depositional settings provides a novel experimental tool for examining the ecology of benthic foraminifera, their processes of dispersal, and the responses of multi-species assemblages to changing environmental conditions. In the 'propagule method' presented here, we use experimental arrays in which foraminifera are grown from propagule banks under different controlled abiotic conditions. We examined the roles of temperature, salinity, and site (exposed vs. protected) in structuring coastal assemblages and show that, because individual species respond differently, distinct assemblages grew from the same propagule bank under different environmental regimes. Temperature was the most important factor distinguishing experimental assemblages, whereas exposure of the collection site (e.g. to waves and currents, that promote or limit species dispersal to and from each site) was most important in determining species richness. The diversity of the propagule bank therefore imparts resilience to foraminiferal associations and provides a rapid-response mechanism for changing environments. This method further provides a tool for documenting changes in coastal assemblages that potentially result from warming or cooling climates.

KEY WORDS: Dispersal · Foraminifera · Propagules · Community assembly · Sapelo Island



Parent and propagules (numerous small juveniles) of the foraminifer *Allogromia* sp. on a mat of cyanobacterial filaments.

Image: Susan T. Goldstein

INTRODUCTION

The ability of benthic foraminifera to quickly colonize new habitats and respond to changing environments hinges on their effective mechanisms of dispersal and recruitment. Benthic foraminifera disperse via several mechanisms (Alve 1999), but largely as small juveniles or propagules (Alve & Goldstein 2002, 2003). Reproduction in benthic foraminifera typically produces 100s of small offspring via either sexual or asexual reproduction (reviewed by Goldstein 1999) that can be passively transported by currents and ultimately deposited. The fine-sediment fraction of many depositional systems therefore contains a bank of abundant and diverse foraminiferal propagules that grow to maturity when exposed to the appropriate environmental conditions (Alve &

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Goldstein 2003). Propagule dispersal in benthic foraminifera is broadly analogous to larval dispersal in myriad marine invertebrates that spawn, though foraminifera differ in that their dispersal is passive and their propagules can form a 'bank' of individuals. Further, propagules disperse beyond the distribution of conspecific adults, and those of some taxa may remain viable but inactive from months to several years, then subsequently grow when exposed to suitable conditions (Alve & Goldstein 2003, 2010).

The 'propagule method' presented here is a novel experimental approach that can be used to examine the responses of some foraminifera to selected environmental parameters, as well as the effect of specific conditions on the structure of multi-species assemblages of foraminifera. In the present study, we examined the roles of temperature, salinity, and site (exposed vs. protected) in structuring communities of foraminifera from the coastal zone. To what extent does the diversity of the propagule bank permit different suites of taxa, or assemblages, to grow when exposed to different environmental conditions? Does the diversity of the propagule bank impart significant resilience to foraminiferal 'communities', and what role does it play in the assembly of foraminiferal communities in changing environments? We show that distinct assemblages develop under different conditions, and our results provide a basis for better understanding the responses of coastal foraminiferal communities to climate change.

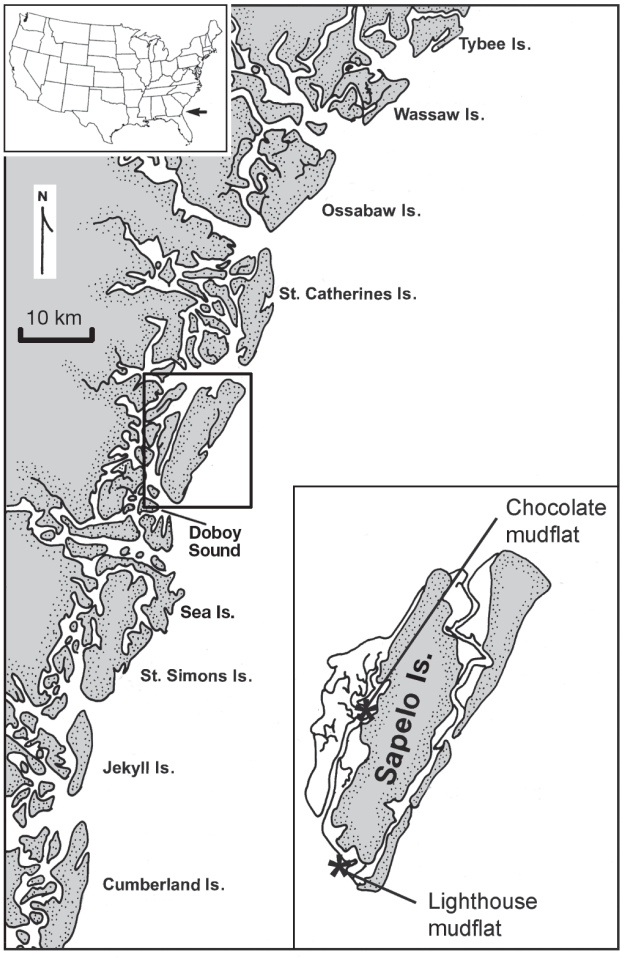

Fig. 1. Map of sampling localities on Sapelo Island, Georgia, USA. The mudflat at Chocolate is a protected backbarrier site, whereas the mudflat at the Lighthouse is an exposed site on the south end of the island

MATERIALS AND METHODS

Collection

Surface sediments (upper few millimeters) were collected over an area of ~0.5 m^2 at low tide from 2 extensive mudflats on Sapelo Island, Georgia, USA (Fig. 1) on January 22, 2005. One mudflat, referred to locally as the 'Lighthouse mudflat' (31° 23.384′ N, 81° 17.072′ W), is an exposed site located on the southern end of the barrier island near the Sapelo lighthouse. This site is adjacent to Doboy Sound, a major tidal inlet that separates Sapelo from Sea Island and St. Simons to the south. This inlet, in turn, provides fairly direct contact with the Atlantic. The Lighthouse mudflat is bordered by a large salt marsh that includes both a broad low marsh, dominated by the halophyte *Spartina alterniflora* Loisel, which is typical of Georgia salt marshes, and diverse high marsh habitats (e.g. Goldstein & Frey 1986, Goldstein & Harben 1993). The second mudflat, here referred to as the 'Chocolate mudflat' (31° 30.166′ N, 81° 15.293′ W), is located in the tidal estuary of the Mud River, and is adjacent to the historical site on Sapelo Island known as Chocolate. The Mud River, as part of the Sapelo Sound system, is one of many tidal rivers along the Georgia coast (Howard & Frey 1985), and, although deemed a 'river', the flow is tidal and the system lacks input from any major freshwater system. This is a backbarrier site with only indirect connection to the Atlantic. The Chocolate mudflat is bordered by a narrow salt marsh located on a relatively steep gradient. The low marsh is not well developed at this site, and the high marsh consists primarily of a narrow stand of the halophyte *Juncus roemerianus* Scheele.

Mudflats at both the Lighthouse and Chocolate sites are extensive and associated with large oyster bioherms. When exposed at low tide, prominent

patches of diatoms are apparent on the sediment surface, particularly during the summer months, and abundant ostracodes and mud snails *Ilyanassa obsoleta* Say are also present. Sediments at both sites are very loosely consolidated, soupy, and become black and sulfide-rich just a few millimeters below the surface. Both collecting sites are brackish, and the salinity, which can vary considerably throughout the year, measured 22 (with a refractometer) at both sites at the time of collection. The Georgia Coastal Ecosystems Long-Term Ecological Research program collects environmental data, including temperature and salinity, at a number of sites in the Sapelo Island area. Those monitoring sites closest (within 2 km) to our sampling sites used in the present study recorded daily mean temperatures and salinities of ~8 to 30°C and ~10 to 30, respectively, near the Lighthouse mudflat, and ~6 to 31°C and ~9 to 28 near the mudflat at Chocolate (see http://gce-lter.marsci.uga.edu). Environmental conditions on the adjacent continental shelf, located within the Mid-Atlantic Bight, also vary throughout the year. At depths between 10 and 200 m, temperatures and salinities are ~8 to 23°C and ~27 to 36, respectively (see http://edac-dap2.northerngulfinstitute.org/erddap/griddap/NCOM_mid_atlantic_bight_3d_agg.html).

After collection, sediments were transported to the University of Georgia Marine Institute on Sapelo Island where they were sieved using 850 and 53 µm stainless-steel sieves. The coarser sieve was used to remove larger metazoans and detritus. Material retained on the 53 µm sieve was fixed in 4% paraformaldehyde (buffered with sodium carbonate to pH > 8) in artificial seawater (salinity 35; Instant Ocean, Aquarium Systems) with 0.1% rose Bengal and used to determine the species content of the *in situ* foraminiferal assemblages at each site. This >53 µm fraction was re-sieved using a 63 µm sieve, split to obtain a workable volume (1/32 of the original), and preserved in 70% ethanol. The sample was rinsed in tap water, and foraminifera were picked wet, identified, and tallied.

Sediment that passed through the 53 µm sieve was retained in large plastic containers (1 for each site), sealed, refrigerated, transported to the University of Georgia campus in Athens, Georgia, and used for the subsequent propagule experiments, which were started on January 27 and 30, 2005, for the Chocolate and Lighthouse mudflats, respectively. The <53 µm fraction was selected based on previous results (Alve & Goldstein 2003). It excludes fully grown individuals of most foraminiferal species, but includes small juveniles including those with a relatively large proloculus (first chamber).

Experimental design

The fine-sediment fraction (<53 µm) from each site was thoroughly mixed, and 20 ml aliquots were extracted with a large pipette, measured with a graduated cylinder, and placed in a series of small, translucent containers (polypropylene; round, 118 ml) with tightly fitting lids, along with 40 ml of Instant Ocean. Containers were further sealed with parafilm (not opened over the course of the experiment) and illuminated with artificial, broad-spectrum lighting set on a 12 h cycle to promote algal growth in the containers. For the present study, we used 2 constant temperatures (12°C and room temperature, RT, ~22°C) and 3 salinity treatments. The concentration of Instant Ocean was adjusted to yield final salinities of 12, 22 (=ambient), or 36. These experimental temperatures and salinities were chosen to reflect a range of those that occur naturally at the collecting sites and on the nearby continental shelf.

Each treatment had a replicate, yielding 24 containers (2 sampling sites × 2 temperatures × 3 salinities × 2; Fig. 2), which were harvested after 6 wk. A second identical set of 24 samples was harvested after 12 wk. The 12 wk dataset, however, includes only the 12

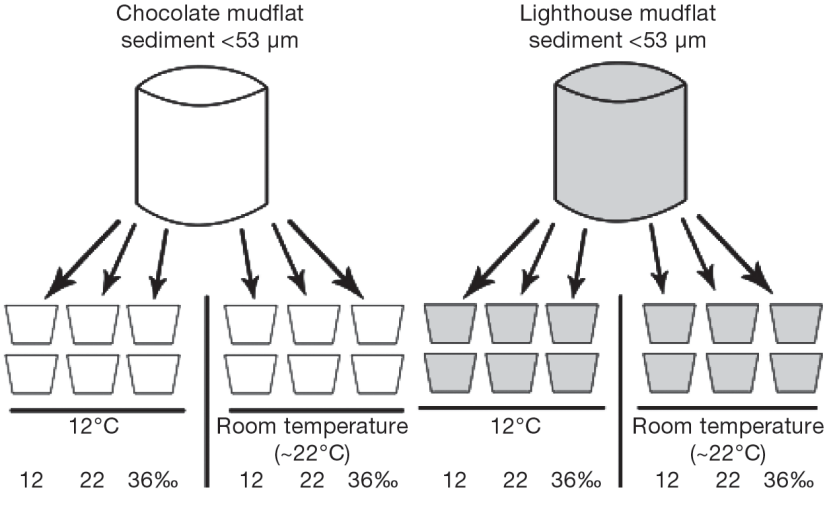

Fig. 2. Outline of the experimental design. See 'Materials and methods: Experimental design' for description

samples derived from the Lighthouse sampling site. Those from the Chocolate mudflat site were damaged after harvesting and could not be examined.

Samples were harvested by sieving over a 63 µm stainless steel sieve using artificial seawater (Instant Ocean). The 63 µm sieve is the size most commonly employed in micropaleontological analyses for foraminifera and was therefore selected for the present study. In order to be retained on this sieve, foraminiferal propagules present in the initial fine-sediment fraction would have grown by at least 10 µm. The material retained on the 63 µm sieve was fixed for 5 to 7 d in an Instant Ocean mixture that contained buffered 4% paraformaldehyde. Samples were then rinsed in tap water and stored in 70% ethanol. Samples were returned to tap water, picked wet, identified and counted. All harvested foraminifera were included in the tallies whether they were alive or not at the conclusion of the experiment because all had grown (at least 10 µm) during the study.

Data analysis

The relationship between assemblage characteristics (abundance and species richness) and salinity, temperature, and sampling site was tested using analysis of variance (ANOVA) (Systat Ver. 13; Systat Software). Cluster analysis, non-metric multi-dimensional scaling (MDS), and species diversity (species richness and Fisher's alpha; Fisher et al. 1943) calculations were performed using Primer Ver. 6.1.6 (Clarke & Gorley 2006). For all analyses, the absolute abundance values of pooled (added) replicates were used, unless otherwise stated. The MDS technique plots samples in 2-dimensional space 'such that the relative distances apart of all points are in the same rank order as the relative dissimilarities (or distances) of the samples, as measured by some appropriate resemblance matrix calculated on the (possibly transformed) data matrix' (Clarke & Gorley 2006, p. 75). For cluster analyses and MDS the 'faunal' data were square-root transformed and resemblances calculated using the Bray-Curtis method (Bray & Curtis 1957). To assess the significance of the effect of temperature, salinity, and site on the delineation of assemblage groupings, we performed an analysis of similarity (ANOSIM), also using a square-root transformation of the abundance data and Bray-Curtis resemblances. Results from the cluster analysis as well as species abundance patterns were overlain on the MDS plots using Primer Ver. 6.1.6.

RESULTS

In situ assemblages of foraminifera

The assemblages of foraminifera (individuals >63 µm) that were alive on the mudflats at each site at the time of collection (January 2005) have a similarity of 92% (Table S1 in the supplement at www.int-res.com/articles/suppl/m437p001_supp.pdf). Both assemblages were strongly dominated by *Haynesina germanica* (Ehrenberg), as were the assemblages that subsequently grew in the various treatments (see following subsection). Other common species living on these mudflats included *Elphidium excavatum* (Terquen), *Ammonia tepida* (Cushman) (=*Ammonia* 'T7' of Hayward et al. 2004), *Psammophaga* sp., and *Ovammina opaca* Dahlgren (Fig. 3). Of these, *H. germanica*, *E. excavatum*, and *A. tepida* are all rotaliids and have calcareous perforate tests. *Psammophaga* sp. and *O. opaca* are monothalamid foraminifera with flexible, agglutinated tests. These 5 most common species accounted for 97 and 95% of the *in situ* living assemblages at the Chocolate and Lighthouse mudflats sites, respectively. In addition, *Miliammina fusca* (Brady) and *Ammotium salsum* (Cushman & Brönnimann) were also present though not abundant (≤1%) in the living assemblages at the time of sampling.

The primary difference between the *in situ* assemblages at these 2 mudflat sites was that the dead assemblage (consists of empty tests) at the Light-

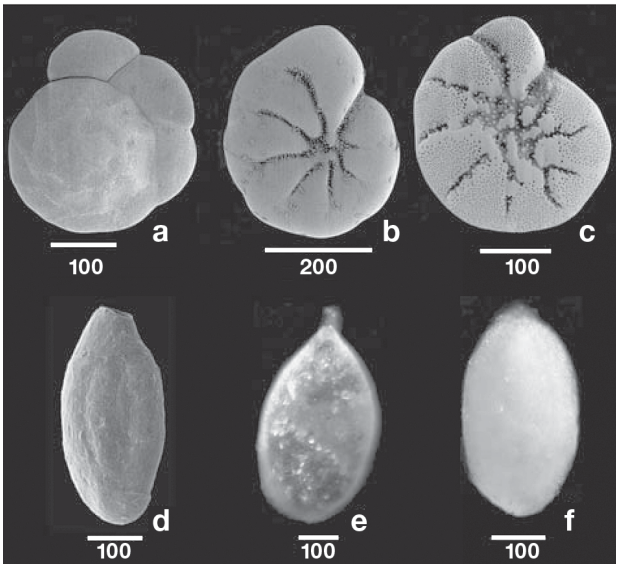

Fig. 3. The 6 species of foraminifera most commonly encountered: (a) *Ammonia tepida*, (b) *Haynesina germanica*, (c) *Elphidium excavatum*, (d) *Miliammina fusca*, (e) *Psammophaga* sp., and (f) *Ovammina opaca*. All scale bars are in µm

house site was more diverse and included a number of species that are more typical of the adjacent low marsh environments than mudflats (e.g. *Trochammina* spp., *Arenoparrella mexicana* [Kronfeld]), or the open waters of the continental shelf, sound, or beyond (e.g. *Globigerina bulloides* [d'Orbigny], *Wiesnerella auriculata* [Egger], *Guttulina laceata* [Walker & Jacob]) (e.g. Sen Gupta & Kilbourne 1976, Goldstein & Harben 1993). Overall, species richness, particularly of the dead assemblage, was higher at the exposed Lighthouse site (27 species of which 10 were alive) than at the protected mudflat at Chocolate (14 species of which 10 were alive). This pattern is also reflected in Fisher's alpha index where the assemblage from the Lighthouse mudflat consistently had higher values (Lighthouse: living assemblage = 2.2, dead assemblage = 8.3; Chocolate: living assemblage = 2.0, dead assemblage = 3.5).

Experimentally grown assemblages

At the conclusion of the experiment (6 or 12 wk), the containers showed no evidence of anoxia or fouling, and diatoms were recorded on both the sediment surface and on the sides of the containers. The sediment surface in all containers had a 'granular' appearance, which is a typical indication of foraminiferal activity in restricted fine-grained sediments. Individual benthic foraminifera commonly collect sediment and the associated microbiota around them, thus forming loosely constructed feeding cysts (e.g. Goldstein & Corliss 1994). Such cysts, whether for feeding, reproduction, morphogenesis, or other functions (Heinz et al. 2005), were common over the sediment surface in the containers. In addition, all containers yielded numerous foraminifera (see next paragraph), which further indicates the lack of fouling over the course of the experiment.

After 6 wk, individual containers, each of which contained 20 ml of the original fine-sediment fraction (<53 µm), were harvested by sieving and yielded from 323 to 1325 foraminifera >63 µm (Table S2 in the supplement at www.int-res.com/articles/suppl/m437p001_supp.pdf). Those assemblages grown over a 12 wk period yielded from 514 to 1246 foraminifera >63 µm (Table S3 in the supplement). These tallies include all foraminifera >63 µm in size that were present at the conclusion of the experiment, regardless of whether they were alive or dead, since all of these individuals were alive and growing (by at least 10 µm) over the course of the experiment.

Foraminiferal abundances varied greatly among treatments (i.e. the individual containers harvested). Those individual assemblages grown at the lowest salinity (12) yielded significantly fewer individuals (average = 516) than those grown at the higher salinities (average = 872 ind. grown at 22‰ and 763 ind. at 36‰; ANOVA F-ratio = 18.805 and 9.773, $p < 0.001$ and $p = 0.005$, respectively), whereas those grown at salinities of 22 and 36 yielded abundances that were not significantly different (F-ratio = 1.030, $p = 0.321$). With regard to site, the propagule bank from the Lighthouse mudflat overall yielded abundances that were just slightly higher than those from the Chocolate mudflat (F-ratio = 2.667, $p = 0.112$). Abundance values were comparable for assemblages grown at different temperatures (F-ratio = 0.000, $p = 0.994$) (Fig. 4).

Species richness ranged from 7 to 21 species in individual containers harvested after 6 wk and from 7 to 20 species in those grown for 12 wk. These species included calcareous, agglutinated, and organic-walled taxa. Site proved to be the only factor with a significant relationship to species richness (ANOVA: F-ratio = 15.816, $p = 0.001$). Assemblages grown from sediment collected at the exposed site (Lighthouse mudflat) collectively yielded greater species richness (49 species total) than those grown from the protected site at Chocolate (35 species total) (Fig. 5, Tables S2 & S3). The highest species richness occurred in treatments grown at the highest salinities (Fig. 5, Tables S2 & S3), but no significant correlation between species richness and either salinity or temperature was found (ANOVA: salinity, F-ratio = 0.751, $p = 0.489$; temperature, F-ratio = 1.588, $p = 0.226$). Fisher's alpha ranged from 1.1 to 2.9 (average = 1.7) for the 12 Chocolate samples and from 1.1 to 4.5 (average = 2.5) for the 24 Lighthouse samples.

The cluster and MDS analyses defined 2 main groups of assemblages distinguished by temperature: one group includes all assemblages grown at RT and the other includes those grown at 12°C (Fig. 6; ANOSIM, $R = 0.839$, $p = 0.001$). Within each of these groups, higher salinity treatments (22 and 36) grouped together, whereas those assemblages grown at the lowest salinity (12) grouped separately. The role of salinity, therefore, was not statistically significant (ANOSIM, $R = 0.129$, $p = 0.094$) than that of temperature. Overall, the greatest similarity existed among assemblages grown under the same temperature and salinity conditions, regardless of the source of propagules (i.e. site; ANOSIM, $R = 0.081$, $p = 0.186$) or duration of the experiment (6 or 12 wk).

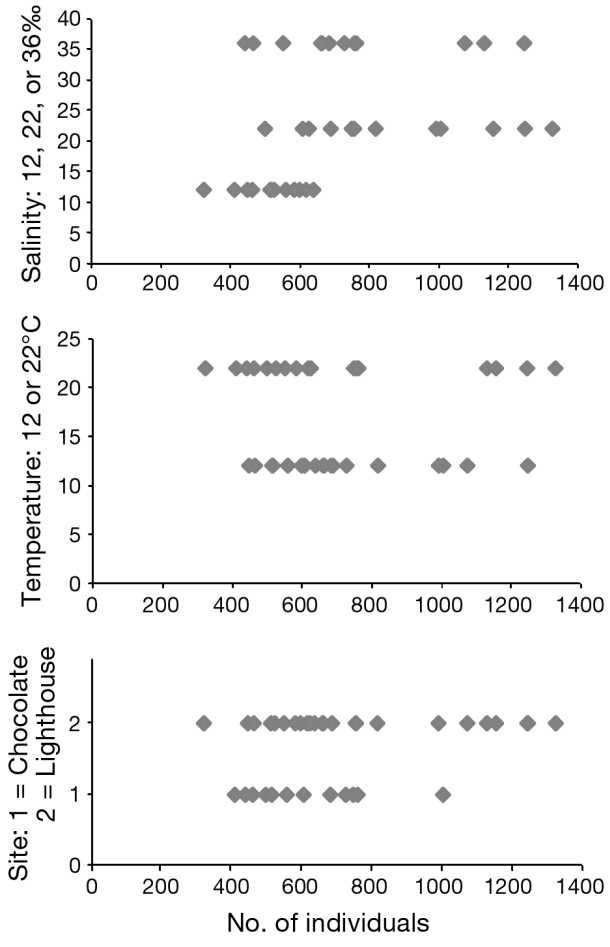

Fig. 4. Abundance of foraminifera >63 µm grown under different experimental conditions for 6 or 12 wk from propagules in 20 ml of sediment plotted against salinity (12, 22, or 36), temperature (12°C or room temperature [~22°C]), and sampling site (mudflats at either Chocolate or the Lighthouse on Sapelo Island, Georgia, USA)

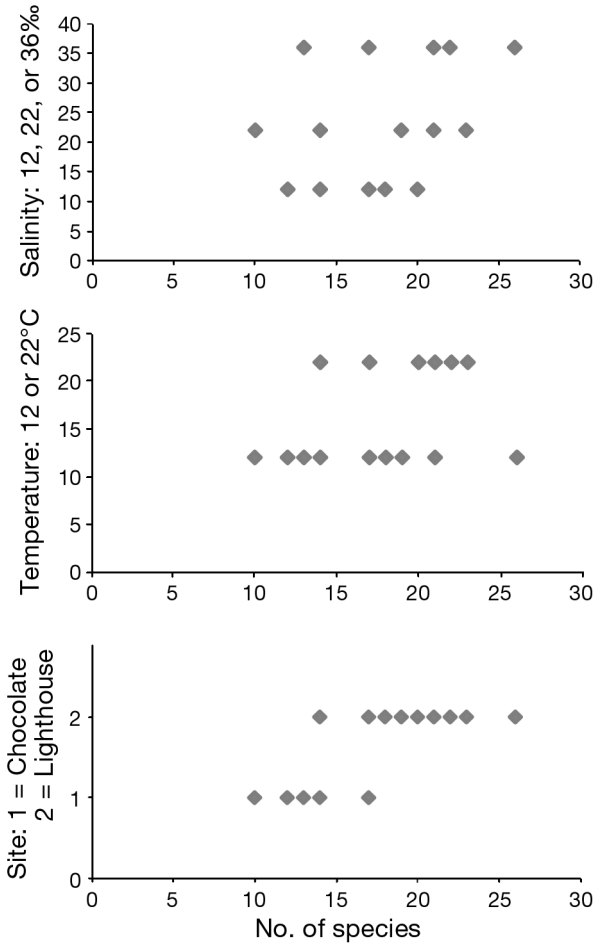

Fig. 5. Number of species in assemblages grown from 20 ml sediment under different experimental conditions for 6 and 12 wk plotted against salinity (12, 22, or 36), temperature (12°C or room temperature [~22°C]), and sampling site (mudflats at either Chocolate or the Lighthouse on Sapelo Island, Georgia, USA)

The distinction of assemblages grown under different temperature and salinity conditions reflects the different species responses to these conditions (Fig. 7). Overall, 6 species grew abundantly in multiple experimental treatments: *Haynesina germanica*, *Psammophaga* sp., *Ammonia tepida*, *Elphidium excavatum*, *Ovammina opaca*, and *Miliammina fusca* (Tables S2 & S3); all of these were also well-represented in the *in situ* assemblages. No 'exotic' (i.e. allochthonous) taxa were among these 6 most abundant ones, although several did grow over the course of the experiment (see end of this section).

Of these 6 dominant taxa, *Haynesina germanica* was by far the most common, as it was in the *in situ* assemblages from both collecting sites (Fig. 7a). It grew in all of the containers, reached a maximum abundance of 789 ind. per 20 ml sediment, and comprised 5 to 81% of the individual experimental assemblages grown for 6 wk and 32 to 88% of those grown for 12 wk. It was the dominant (most abundant) foraminiferan in 19 of the 24 assemblages that grew for 6 wk and in all of those grown for 12 wk. Both adults and juveniles were recovered at the end of the experiment (both 6 and 12 wk durations). *H. germanica* grew abundantly at both experimental temperatures and at all salinities, but the highest number of individuals occurred in the 12°C treatments.

Psammophaga sp. likewise grew abundantly (max. 442 ind. 20 ml^{-1} sediment) at both experimental temperatures and at all salinities, though it was less abundant at the lowest salinity (12) (Fig. 7b). This species comprised 1 to 43% of assemblages grown for 6 wk and 0 to 26% of those grown for 12 wk. In many cases,

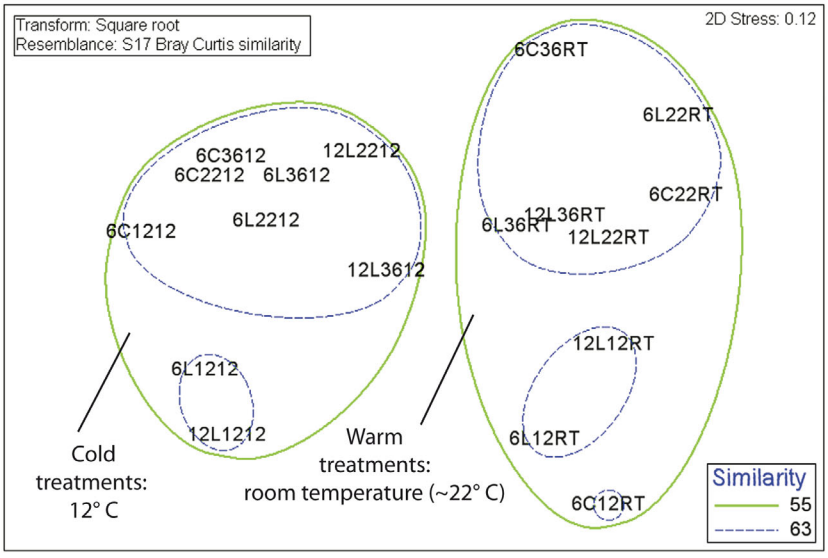

Fig. 6. Multi-dimensional scaling ordination of the sum of replicates from all treatments (both 6 and 12 wk experiments). Results of cluster analyses using 55 and 63 as threshold levels of similarity are superimposed (contour lines encircle the clusters they define). Treatment abbreviations code for duration (6 or 12 wk), site (C = Chocolate, L = Lighthouse), salinity (12, 22 or 36) and temperature (12°C or room temperature [RT, ~22°C])

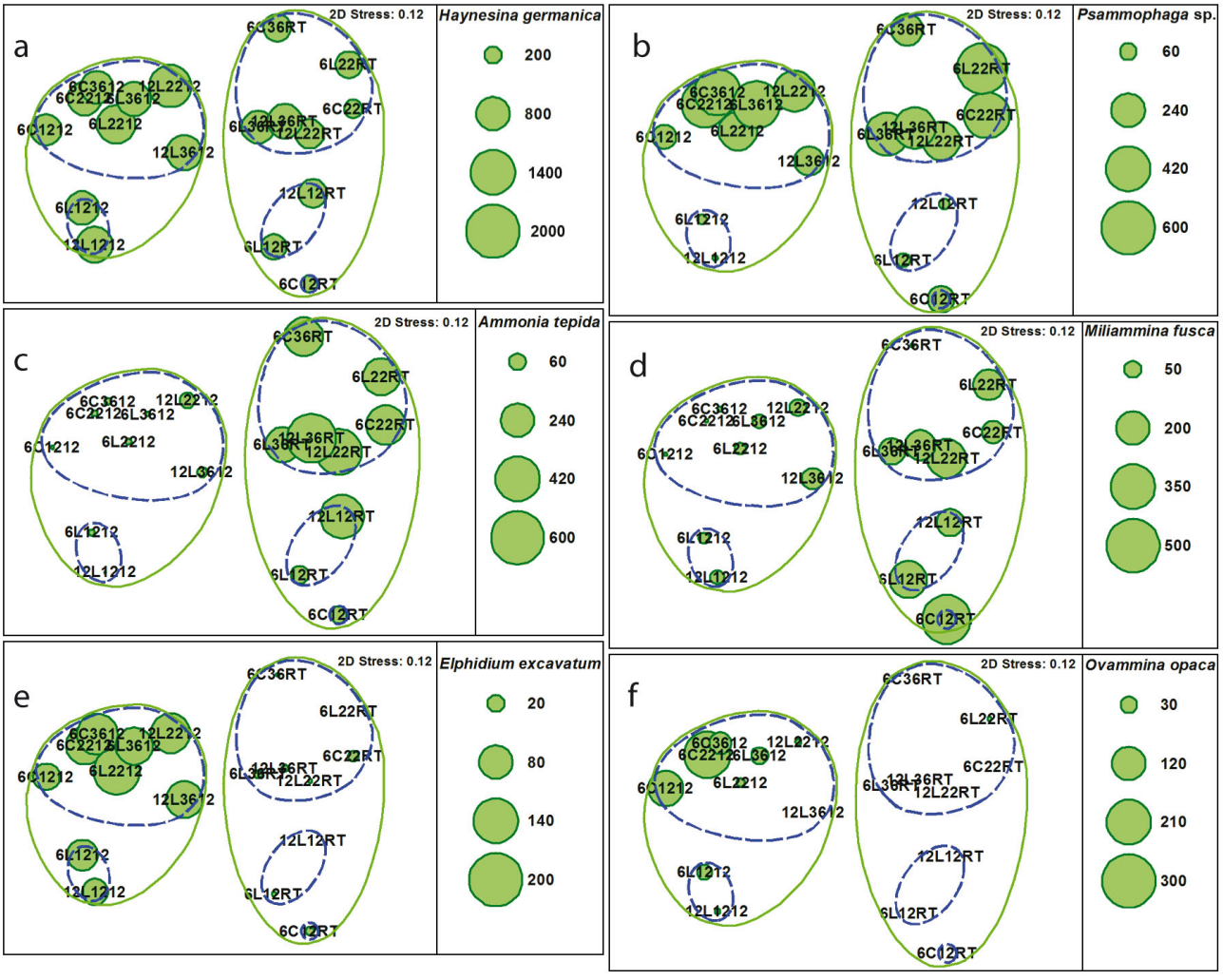

Fig. 7. Abundances of the 6 most common species grown in experimental treatments (replicates pooled; individuals per 40 ml of original sediment) overlain on the multi-dimensional scaling plot from Fig. 6. Note that the scale (bubbles) varies. (a) *Haynesina germanica*, (b) *Psammophaga* sp., (c) *Ammonia tepida*, (d) *Miliammina fusca*, (e) *Elphidium excavatum*, (f) *Ovammina opaca*

it was more abundant in the experimental treatments than at either of the collecting sites where it comprised just 7% of the living assemblage at the Lighthouse mudflat and 15% at the Chocolate mudflat.

Ammonia tepida grew abundantly (max. 348 ind. 20 ml^{-1}) at the warmer temperature (RT) (Fig. 7c). It also grew at all salinities, but generally had higher abundances at higher salinities (22 and 36). This species comprised 5 to 43% of the 6 wk assemblages grown at RT and 16 to 49% of those grown for 12 wk at this temperature. *A. tepida* was nearly absent (0 to 4%) in assemblages grown at 12°C regardless of the duration. The *in situ* assemblages at the Chocolate and Lighthouse mudflats included 8 and 3% of *A. tepida*, respectively.

Miliammina fusca likewise grew in the warmer temperature treatments (Fig. 7d). This species grew at all salinities, but was more abundant (max. 225 ind. 20 ml^{-1}) in the low salinity (12) treatments. *M. fusca* constituted 0 to 65% of the warm-water treatments grown for 6 wk and 5 to 33% of those grown for 12 wk. This species was nearly absent (0 to 3%) in the cold-water assemblages grown for 6 wk, but was somewhat more abundant (2 to 8%) in those cold-water assemblages grown for 12 wk.

Elphidium excavatum grew almost exclusively in the lower temperature (12°C) treatments but at all salinities combined with this temperature (Fig. 7e). This species showed a maximum abundance of 81 ind. 20 ml^{-1} sediment and comprised 4 to 10% of individual assemblages grown at 12°C for 6 wk and 4 to 11% of those grown for 12 wk at this temperature. It made up only 0 to 1% of those assemblages grown at RT regardless of duration. *E. excavatum* was more abundant in the *in situ* assemblages (collected during the winter) at both collecting sites, where it constituted 18% at the Chocolate and 22% at Lighthouse mudflat.

Ovammina opaca likewise grew in the lower temperature treatments, but had its highest abundances (max. 165 ind. 20 ml^{-1}) at lower salinities (12 and 22) (Fig. 7f). This species comprised 0 to 27% of the assemblages grown at the lower temperature for 6 wk. It was absent in all assemblages grown at RT. It was nearly absent (0 to 3 individuals per assemblage) from all of the assemblages grown for 12 wk regardless of temperature or salinity. *O. opaca* accounted for ~5% of the *in situ* assemblages at both collecting sites, however, it grew more abundantly from sediments collected at the Chocolate mudflat.

In addition, several species that are more typical of subtidal habitats of the continental shelf or sound (Sen Gupta & Kilbourne 1976) grew abundantly in one to several containers. These include *Textularia candeiana* d'Orbigny, *Fissurina lucida* (Williamson), and *Buliminella elegantissima* (d'Orbigny).

DISCUSSION

The bank of propagules (tiny juveniles) present in the fine-grained sediment fraction of depositional systems provides a novel tool for experimental ecological studies on foraminifera. The propagule bank can be manipulated experimentally to examine the role of selected environmental parameters on the assembly of foraminiferal communities, as well as the individual species responses to particular conditions. Here we have demonstrated that distinct assemblages can be grown from the same propagule bank by applying different combinations of environmental conditions. Further, the taxonomic makeup of these assemblages hinges on the different responses of individual species present in the propagule bank.

Past and current experimental approaches in studies on foraminiferal ecology (e.g. Talge & Hallock 2003, Pascal et al. 2008, Mojtahid et al. 2011) generally use either adults of relatively large species, which are easy to isolate and manipulate, or complete assemblages in their original sediment which are transferred from field to experimental conditions (microcosms). Separating individuals which have grown and/or reproduced from those which have not during the course of the experiment is commonly time-consuming, a logistical challenge, and may introduce errors. Here we rely instead on juveniles that grow to maturity during the experiment, thus ensuring that these individuals respond positively to the treatment. This approach has the further advantage of focusing on critical developmental stages (Olsgard 1999) while also being logistically simple.

Foraminifera grew abundantly in all treatments, and abundances varied both among treatments and between replicates. The most important factor accounting for abundance variation among treatments is reduced salinity (lowest at 12‰). The abundance variation between replicates of the same treatment most likely reflects slight variations in reproduction. In general, a single foraminiferan can produce from several to >100 offspring (e.g. Jepps 1942, Arnold 1955). Slight variations in the number of individuals undergoing reproduction, therefore, can result in large differences in assemblage abundances. In addition, the abundances of monothalamid species, particularly *Ovammina opaca*, were lower in assemblages grown for 12 wk rather than 6 wk. This may

reflect the inability of these species to reproduce effectively coupled with the post-mortem degradation of the test (e.g. Goldstein & Barker 1988).

Those species that grew most prolifically in the experimentally grown assemblages are those that were present on the mudflats at the time of collection. This suggests that most of these propagules were derived from local or *in situ* populations. Several exotic or allochthonous species grew in some treatments, but in relatively small numbers. Though capable of growing, these were not able to out-compete the resident taxa or otherwise become dominant under any of the experimental conditions employed. Their presence, particularly in assemblages grown from the exposed site, illustrates the influence of a direct physical connection to a larger regional species pool (Buzas & Culver 1994, Leibold et al. 2004).

The growth of distinct assemblages of foraminifera from the same propagule bank under different conditions demonstrates that multiple, alternative assemblage structures are possible. The diversity of the propagule bank, therefore, provides a mechanism for rapid responses to changing conditions and imparts resilience for these assemblages. The distinct foraminiferal assemblages identified by cluster and MDS analyses are delimited first by temperature and secondarily by salinity, thus demonstrating the importance of these niche-related factors. The greatest influence on species richness, however, was the location of the sampling site. Higher species richness occurred in assemblages grown from the propagule bank collected at the exposed site (Lighthouse) rather than the protected, backbarrier site (Chocolate). This trend is also reflected in the species richness of the *in situ* dead assemblages at these 2 sites. Overall, this suggests that dispersal to the protected, backbarrier site is more limited and that dispersal limitation also plays a role in structuring foraminiferal associations.

We know very little about the trophic interactions of most foraminifera and the potential roles of competition and predation in structuring foraminiferal 'communities.' Those species that grew in the experimental treatments utilize a range of nutritional resources with some degree of overlap and potential for competition. Two species use organelle retention: *Haynesina germanica* (Lopez 1979, Knight & Mantoura 1985, Austin et al. 2005) and *Elphidium excavatum* (Correia & Lee 2000), both sequester diatom chloroplasts. In addition, *H. germanica* may graze on bacteria (Mojtahid et al. 2011). *Psammophaga* sp., characterized by its avid ingestion of silt-sized sediment, is most likely a deposit feeder. *Ammonia tepida* utilizes micro-algal grazing, bacterial grazing, and deposit feeding (Goldstein & Corliss 1994, Pascal et al. 2008), but it is also a carnivore (Dupuy et al. 2010). This species appears to have the most diverse diet of those present and is the only known carnivore in any of the assemblages. *Ovammina opaca* also feeds on diatoms and other forms of micro-algae (S. T. Goldstein unpubl. data), whereas the diet of *Miliammina fusca* remains undocumented. The potential for competition among these common species exists, given both the overlap in diet and the limited space available in the growth chambers. However, if food remained abundant over the course of the experiment, then competition may have remained minimal.

Food was probably plentiful in all experimental treatments. The initial 20 ml aliquot of fine-grained sediment included both foraminiferal propagules and an associated microbiota. All assemblages were grown with exposure to light 12 h d^{-1}, and diatoms, for example, commonly grew on both the sediment surface and the sides of the containers. Initially, the food available in the treatments was probably comparable to that of the *in situ* habitats. The extent to which potential food organisms (micro-algae, bacteria) may have changed over the course of the experiment was not determined. Also, the possibility that parts of the recorded responses to different salinity and temperature treatments are due to responses in the food organisms (i.e. indirect response) cannot be ruled out. However, the result is the same if the cause is direct or indirect.

Field-based studies have been widely used to examine the relationships of foraminiferal distribution patterns and various environmental parameters (reviewed by Murray 2006), and results serve as the basis for interpreting many paleoenvironments. The results of such studies rely on demonstrating a relationship, or at least a correlation, between observed foraminiferal distributions and trends in various environmental parameters. Coastal, shallow-water systems, however, are environmentally variable over short spatial and temporal scales, and results therefore have not always been conclusive (e.g. Geslin et al. 2000). Characterizing relationships between foraminiferal occurrences and environmental parameters in these settings is therefore difficult. Results of the present study provide a better understanding of the temperature and salinity preferences of several key shallow-water species and thus build on the compilations of field-based studies by others (e.g. Murray 2006).

Haynesina germanica, the most frequently encountered species in our study, is common and broadly distributed in temperate coastal settings and is con-

sidered one of the most opportunistic brackish foraminifera of southern England (Murray 2006, p. 81). Results of the present study support this and show that it grows quickly and is capable of reproducing from at least 12 to ~22°C. This species is reportedly among those foraminifera most tolerant to environmental pollutants and is therefore a potential bioindicator species in coastal pollution studies (Armynot du Châtelet et al. 2004).

Species of *Psammophaga*, distinctive for their ingestion of numerous sediment grains, are also widely distributed biogeographically, with known occurrences in shallow waters of the Arctic, Antarctic, and temperate latitudes of both the Atlantic and Pacific (Arnold 1982, Pawlowski & Holzmann 2008, Pawlowski & Majewski 2011). These foraminiferans are probably more common than published reports would indicate. The fragile test found in these species is destroyed when sediment samples are dried, a common practice in many distribution studies on foraminifera. We know very little about the ecology of *Psammophaga* spp., though results of our study suggest that the Sapelo species is also a broadly adapted opportunist.

Four of the 6 dominant species retrieved from the experimentally grown assemblages (*Ammonia tepida*, *Miliammina fusca*, *Elphidium excavatum*, *Ovammina opaca*) illustrate different responses to temperature and salinity, and these results should serve to improve our interpretations of environmental conditions and change. For example, the results suggest that rising temperatures at southeastern United States coastal sites would be reflected in an increase in warm-adapted species such as *A. tepida* and *M. fusca*, and a corresponding decrease in the cool-adapted *E. excavatum* in foraminiferal communities. The opposite would be expected during colder periods. Previous field-based studies, however, linked the relative abundances of species of *Ammonia* (e.g. *A. parkinsoniana* or *A. beccarii*) and *E. excavatum* to hypoxia, anoxia (Sen Gupta et al. 1996), or some other form of environmental stress (Thomas et al. 2000). Here we show that temperature is also a critical factor in determining the relative abundances of these species. In the present study, *M. fusca* grew preferentially at the warmer temperature; however, populations from maritime Canada have been characterized previously as being adapted to the cold (Scott & Medioli 1980). This suggests that *M. fusca* may actually be a morphospecies complex of cryptic species with different environmental adaptations. This view is supported by the finding that *M. fusca* populations from coastal Georgia are distinct from those from maritime Canada in terms of the small subunit rDNA gene (Habura et al. 2006, A. Habura pers. comm.).

Growing assemblages of foraminifera from their propagule banks under different, controlled environmental conditions, the 'propagule method' presented here, provides a better understanding of the environmental preferences of individual taxa and how foraminiferal communities might respond to changing environments. It also provides insight into the influences of abiotic factors such as temperature and salinity on the assembly of foraminiferal associations.

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