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Significance of replicates: Environmental and paleoenvironmental studies on benthic foraminifera and testate amoebae

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ABSTRACT: Foraminifera (Rhizaria) and testate amoebae (Rhizaria and Amoebozoa) are single-celled organisms with marine and continental affinities. They are regarded as valuable bioindicators in transitional areas such as tidal and salt marshes and have been widely used for (paleo)-environmental characterization. A long-lasting debate regularly occurs on the use of living *vs.* dead fauna to accurately represent modern environments. Moreover, environmentally based benthic foraminiferal and testate amoebaean studies need a reliable sampling strategy to capture the spatial variability, particularly in transitional environments where patchiness complicates the data interpretations. The objective of the present study is therefore to define the minimum required number of replicates for capturing the variability of either living (environment) or dead (paleoenvironment) benthic foraminifera and testate amoebae.

To address this question, 49 samples (i.e., replicates) were selected from a square meter zone in the tidal flat-salt marsh transitional zone along the Canche Estuary (Northern France). The range of faunal spatial variability was measured using geostatistical tools. The minimal number of samples for capturing the patchiness was determined using bootstrap resampling procedure.

We provide evidence that for both living and dead fauna more than 26 samples (and even higher for some species) are needed to correctly evaluate the patchiness. Indeed, the living and dead fauna do not follow spatial homogenous trends, and this might bias paleoenvironmental interpretations.

The commonly-suggested number of three replicates might not be enough to characterize the fauna in such heterogeneous environments.

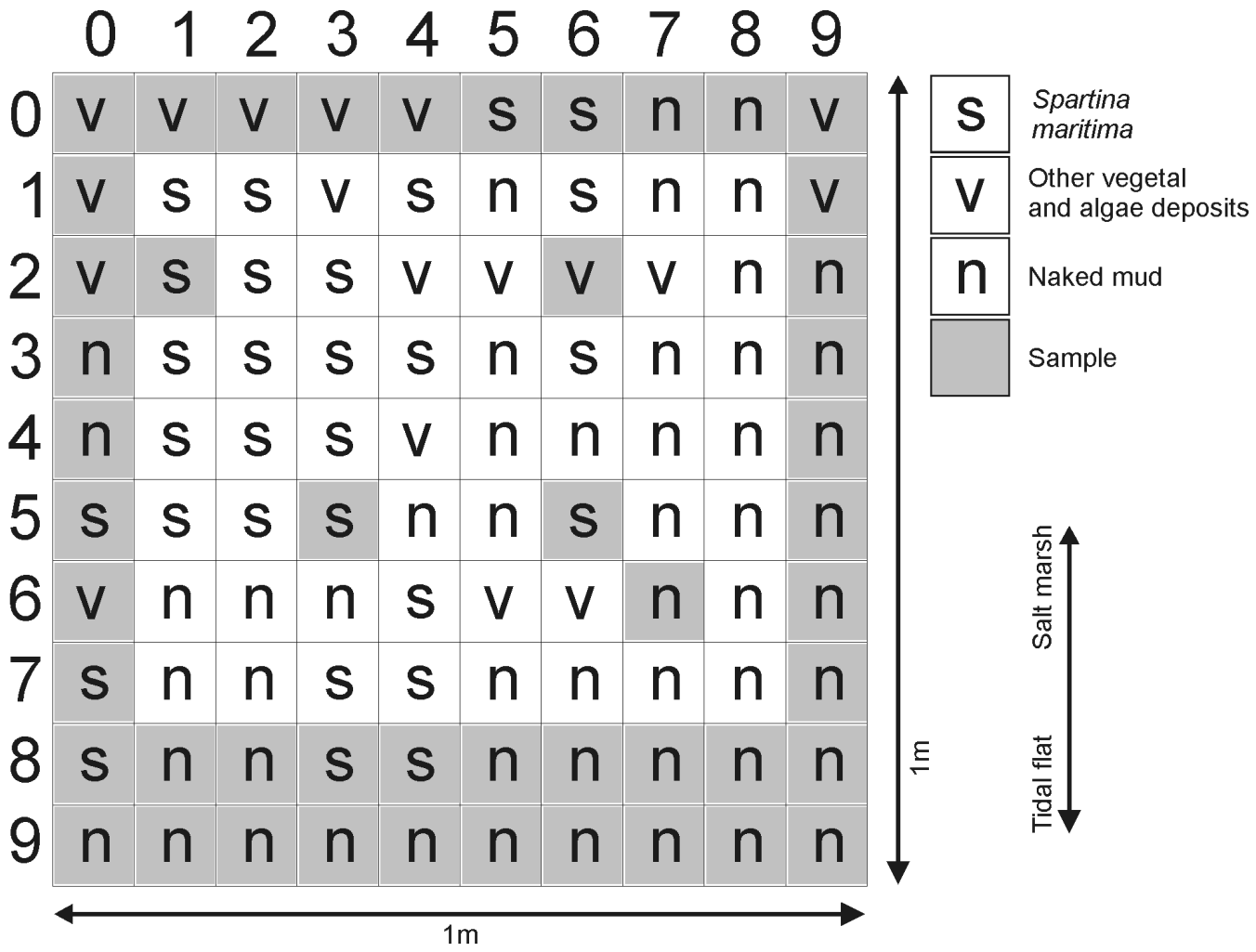
Keywords: Replicates; environmental monitoring; foraminifera; sampling strategy; paleoenvironmental reconstruction

INTRODUCTION

Foraminifera (Rhizaria) and testate amoebae (Rhizaria and Amoebozoa) are unicellular eukaryotes (Adl et al. 2012, Pawlowski et al. 1994, Pawlowski et al. 2013). Foraminifera primarily occur in marine environments (Murray 2006), whereas testate amoebae are commonly found in freshwater settings (Charman 2001). In transitional environments, the groups co-occur, even if only a few and specialized species can survive in brackish waters (Vazquez Riveiros et al. 2007, van Hengstum et al. 2008). It is well known that in transitional temperate areas benthic foraminifera show similar distributions and the same dominant species can be found along the Atlantic Regions (e.g., Darling et al. 2016, Leipnitz et al. 2014), in China (e.g., Wu et al. 2015), Australia (e.g., Berkeley et al. 2008) and New Zealand (e.g., Hayward et al. 2014), and in Chile (e.g., Jennings et al. 1995). Although the literature focuses commonly on local problems and areas, this study is not only of regional importance but rather its conclusions can be applied to all coastal environments. Due to their ecological characteristics and ability to fossilize, benthic foraminifera and testate amoebae are widely used to characterize modern environments (e.g., Leipnitz et al. 2014) and to reconstruct past environmental conditions (e.g., Hayward et al. 2004, Delaine et al. 2015, Alve 1991, Francescangeli et al. 2016). Their marine and continental habitats make them valuable indicators of changes in transitional areas, such as within tidal flats and salt marshes. However, under a hypertidal regime leading to dynamic and small-scale heterogeneous environments, some methodological considerations for the reliability and comparability of data must be considered. The understanding of the representativeness of the living *vs.* dead fauna and the number of replicates represent key points for a correct application of foraminiferal and testate amoeba assemblages in paleoenvironmental studies. Frequently, the rationale behind the methodological choices, particularly in these environments, has not been thoroughly and objectively evaluated.

What is the representativeness of dead *vs*. living benthic foraminifera and testate amoebae?

A long-standing debate regularly reappears in the use of living fauna for ecological interpretation (Murray 1982, Murray 2000)



TEXT-FIGURE 1
Systematically and randomly selected (gray-colored) samples (10 cm x 10 cm) within 1 m^2. Substrate characteristics are also reported.

and opens the question of the reliability of the dead counterpart for paleoenvironmental reconstructions (e.g., Goineau et al. 2015, Murray 1976, Avnaim-Katav et al. 2015, Martins et al. 2016). Living fauna are a snapshot of the ecological conditions at the time of sampling. Dead assemblages represent the addition of organisms over time from a succession of previously living assemblages, denoting time-averaged assemblages modified by taphonomic processes: post-mortem transport, reworked specimens, early diagenetic processes (Murray 2000, Hawkes et al. 2010). For environmental studies, it is evident than living fauna are preferable, but for the paleoenvironment, only dead fauna are available. The first aim of the present paper is to investigate both foraminifera and testate amoebae in a salt marsh environment under a hypertidal regime and to define the representativeness of dead fauna compared to that of the living one for paleoenvironmental studies.

What is the number of replicates needed to account for benthic foraminiferal and testate amoebae spatial variability?

Because of patchiness, environmental studies based on benthic foraminifera and testate amoebae need a reliable sampling strategy (Boltovskoy and Lena 1969, Buzas 1970, Schafer 1971, Buzas et al. 2002a, Mitchell et al. 2000). The results of large-scale surveys can be biased if the sampling design does not account for this phenomenon.

The patchiness might cause heterogeneity of sediment characteristics like the occurrence of ripples, mud cracks, or bioturbation (Debenay et al. 2015) and the difference in altitude, flora (Mitchell et al. 2000), water depth, exposure rate, seagrass, and benthonic algae (Hohenegger et al. 1989). All these physical, chemical, and biological variabilities might promote small-scale patchiness in the distribution of the meiobenthos, including foraminiferal and testate amoebae assemblages.

For a long time, it has been known that patchiness affects the distribution of organisms; as a consequence, in ecological studies, an appropriate number of replicates (commonly three to six) would be considered to mitigate the effects of patchiness (Seuront and Spilmont 2002). However, the minimal number of replicates is barely discussed. For foraminifera, the most accurate method to examine spatial distribution is the use of contigu-

ous cells (Buzas et al. 2015). Only three studies have considered such methods (Buzas 1968, Olsson and Eriksson 1974) in (Buzas et al. 2015) and (Hohenegger et al. 1989). It is concluded that patchiness in benthic foraminiferal distribution may occur on a scale from 10 to 100 cm. For testate amoebae, this question was addressed only once with a similar contiguous cell sampling procedure, although it was in *Sphagnum* peatlands, not in sediments (Mitchell et al. 2000).

As soon as the patchiness is evaluated, the appropriate number of replicates to adopt in sampling can be defined and can reliably account for such small-scale heterogeneity. However, only a very limited number of papers have correctly addressed it. Commonly, two (Buzas et al. 1993), three (e.g. Avnaim-Katav et al. 2016, Swallow 2000) or four (Milker et al. 2015) replicates are considered. Schafer (1971) proposed the use of mean or modal values of replicates calculated from three to six samples. Swallow (2000) suggested three replicates as a sufficient number to capture the patchy nature of benthic foraminifera. Similarly, three replicates were considered sufficient to reliably determine the Ecological Quality Status of basins along the Norwegian Skagerrak coast (Bouchet et al. 2012). Following previous findings and considering time and cost constraints, three replicates were recommended by Schönfeld et al. (2012) to standardize the sampling and treatment procedures on foraminifera for environmental monitoring. In the same paper, it was suggested that three replicates from independent deployments should be used and considered separately. On the other hand, Brooks (1967) mentioned that seven to ten replicates per sample are needed to ensure an accurate mean value. For all these studies, a real validation of the minimum number of replicates to capture the spatial variability of the fauna has never been tested, and therefore, the reliable applicability of that fauna compared to the requested number of replicated samples must be tested. Because of this, the second aim of the present study is to objectively assess the minimum number of replicates required for an environmental study based on benthic foraminifera and testate amoebae in a transitional environment.

MATERIAL AND METHODS

Study area and sampling strategy

The study was carried out at the transition between the tidal marsh and salt marsh (50.5256°N, 1.6156°E, WGS84 datum) within the Canche estuary (Northern France). This area experiences a megatidal regime , with a tidal range exceeding 9 m during the highest astronomical tides.

In a square meter grid composed of 10 x 10 cells, 49 sediment samples (100 cm^2 and 1 cm in thickness) were scraped off at low tide (text-fig. 1). Samples were both systematically and randomly selected that allowed all scale distances among the samples for statistical purpose. Selected samples were located all along the outline and in several places in the center. Halophilic herbs (like *Spartina maritima)* and algae were sporadically present (text-fig. 1).

The sediment was placed in plastic boxes, immediately treated with a Rose Bengal-ethanol (2 g/l) solution and used for faunal purposes (Walton 1952, Murray and Bowser 2000).

Biotic parameters: Foraminifera and testate amoebae

In the laboratory, after four weeks of staining, wet samples were washed through 63 and 315 µm mesh sieves. Before observation, the foraminiferal and testate amoebae specimens of the intermediate fraction (63–315 µm) were concentrated by flotation on trichloroethylene. This separation was necessary because of the very high detritic content in these sediments. After separation, we checked that no specimens were left within the residue. All the living fauna were counted, whereas a micropaleontological splitter was used for dead fauna to obtain at least 200 foraminiferal and testate amoebae specimens. This allows species to be considered as significant when recorded proportions were as low as 1.6% with a probability of failure to detect their presence of 5% (Fatela and Taborda 2002). For observation of cytoplasm, specimens were counted slightly wet. For each sample, the density, the number of species, and the Shannon–Wiener diversity index (H') were determined for both the living (stained) and dead fauna. All observations and the taxonomical identifications were carried out under a binocular Olympus SZX16.

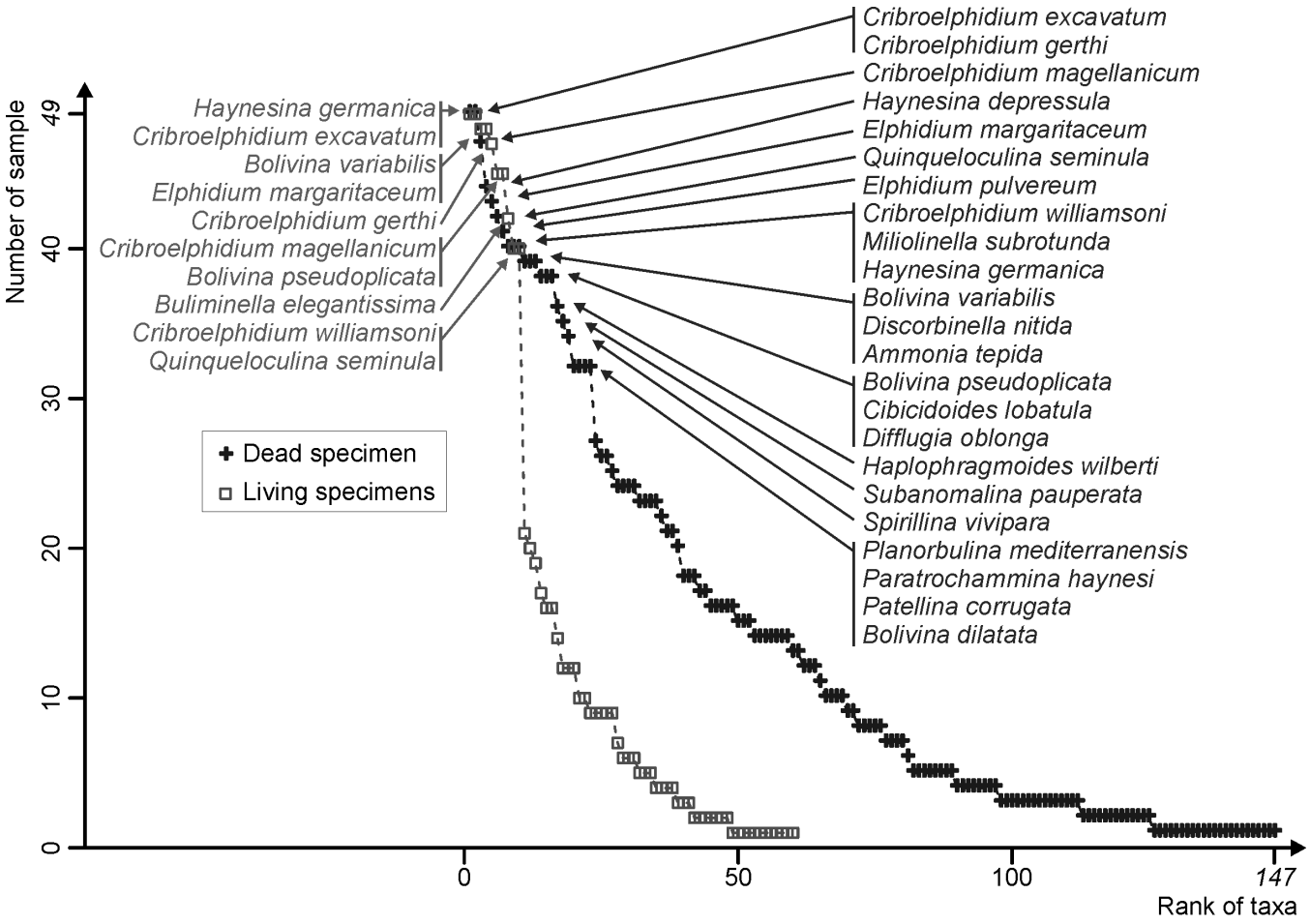
Descriptive analysis and calculation tools

As an initial approach, basic descriptive statistics were used to quantify the distributions (standard deviation, skewness, and normality, as estimated by the Shapiro-Wilk test). The possible occurrence of patchiness within the analyzed surface was estimated using a bootstrapping technique by generating 10,000 random sets of samples composed of five to seven replicates, whose differences (i.e., the patchiness) were tested by means of a Kruskal-Wallis H test. A non-parametric test was necessary, as patchiness may occur within the random sample and may skew the data. The homogeneity of variance (homoscedasticity) was therefore tested by means of the non-parametric Fligner-Killeen test.

The ranges of variability of diversity, density, and proportion of the fauna are illustrated by boxplot calculated from 1,000 randomly selected groups of replicates from 1 to 49 samples. The confidence interval around the mean was estimated for living and dead faunal diversity and density. It was estimated using classical t-test after randomly sampling n replicate ranging from 3 to 49 with a 95 percent confidence interval. Then, the range and spatial dependency of the species densities were analyzed using geostatistical techniques. The semivariance was calculated to measure the dissimilarity between samples infused by the spatial structure, namely, the position within the quadrat. The semivariance for foraminiferal density was defined as half the expected squared difference between values at places x and x + h. Accordingly, the variograms were calculated with Eq. 1

$$\gamma(h) = \frac{1}{2n_h} \sum_{i=1}^{n_h} \left[Z(x_i + h) - Z(x_i) \right]^2 \qquad \text{Eq. 1}$$

where γ(h) is the semivariance at lag distance h; n(h) is the number of observation pairs separated by h; and $Z(x_i)$ and $Z(x_i + h)$ represent the paired values of the variable Z at two locations separated by h. The semivariance for species is formalized by theoretical models: nugget (Oliver and Webster 2014) in association with exponential, spherical, or Matérn Stein's parameterization (Minasny and McBratney 2005, Stein 1999). The Matérn Stein parameterization is a more flexible model for modeling local spatial random processes than the classical exponential and spherical model (Webster and Oliver 2001), as the shape of local variation can be adapted to different parts of a field (Minasny and McBratney 2005, Stein 1999). These models consider the sill and the practical range. The sill value is the



TEXT-FIGURE 2
Rank frequency diagram for living (red square) and dead (blue crosses) species. The most frequent species are indicated.

upper limit of the fitted variogram model. The nugget-sill ratio indicates the spatial dependency of a given variable (Webster and Oliver 2001, Webster and Oliver 1990). The ratios were categorized as low (<25%), moderate (25 to 75%), or strong (>75%). A low ratio means that a large part of the semivariance is introduced spatially, implying the strong spatial dependency of the analyzed variable (i.e., species). A high ratio commonly indicates a weak spatial dependency. The range of the variogram represents the average distance through which the species semivariance reaches its peak value (Weindorf and Zhu 2010). A small effective range implies a distribution pattern composed of small patches. The exponential model suggests a sharp increase of the semivariance at short distances, and suggests greater increases at longer distances. Spherical models put forward a gradual increase of the semivariance as a function of distance up to the range distance from which the spatial variance no longer evolves with increasing distance. The Matérn Stein model exhibits a large range of spatial behavior that spans from the highly continuous pattern at short distances (similar to the classical Gaussian model) to trends similar to exponential (Minasny and McBratney 2005).

To determine the number of samples needed for an accurate estimation of the average species density, a procedure similar to that of Spilmont et al. (2011), based on Bartoli et al. (2003), was considered. A bootstrap technique was used to randomly resample 1,000 sets of n subsamples, with n ranging from three to the maximum of 49. For each set, the mean density (mD) was calculated and the difference from the true average density (tD) (calculated as the mean of all the samples from the quadrate) was estimated as error d (Bartoli et al. 2003) (Eq. 2):

$$d = \frac{|tD - mD|}{tD} \quad \text{(Eq. 2)}$$

The number of samples required to estimate the average density was determined as the lowest n where the 95% confidence interval reaches a value $d \leq 0.05$, meaning that the considered average within the interval is the 'true average ±5%'.

All statistical analyses were implemented in R software v3.3.2 using the packages base, e1071 (Meyer et al. 2017), ggtern (Hamilton 2016), and ggplot2 (Wickham 2009); basic descriptive statistics, pvclust (Suzuki and Shimodaira 2015); work on clustering and bootstrapping, gstat (Pebesma 2004) and automap (Hiemstra et al. 2009); and work on semivariogram calculations.

TABLE 1
Descriptive statistics for the density of the most frequent living and dead species (normality, skewness, SD, minimum number (*n*) of the sample for estimating average density, number of sample occurrences, and average density per cm^3). Normality was tested using the Shapiro-Wilk test α

	Living specimen						Dead specimen					
	Normality	Skewness	SD	n	Number sample occurrence	Average density (/cm^3)	Normality	Skewness	SD	n	Number sample occurrence	Average density (/cm^3)
Diversity	Yes	0.24	0.28	8	-	-	Yes	-0.29	0.27	1	-	-
Density	No	1.39	1.72	19	-	4	Yes	1.11	199.46	23	-	423
Bolivina dilatata	-	-	-	-	-	-	No	4.51	538.21	46	32	244
Bolivina pseudoplicata	No	4.04	3.31	42	45	2.76	No	1.32	366.48	42	38	350
Bolivina variabilis	Yes	0.01	2.15	26	48	4.26	No	1.91	477.13	43	39	415
Cribroelphidium excavatum	No	1.67	19.94	31	49	34.2	Yes	1.31	1119.73	34	49	1617
Cribroelphidium gerthi	No	1.62	3.47	40	47	3.74	Yes	1.47	1271.85	36	49	1618
Cribroelphidium gunteri	No	2.19	1.10	46	17	0.6	-	-	-	-	-	-
Cribroelphidium magellanicum	Yes	0.30	2.69	32	45	4.26	Yes	0.41	2579.37	30	47	4474
Elphidium margaritaceum	Yes	1.28	3.06	34	48	4.16	No	1.57	1772.66	39	43	2009
Elphidium pereirum	-	-	-	-	-	-	No	2.55	311.21	46	26	172
Cribroelphidium williamsoni	No	1.70	3.13	40	40	3.22	Yes	1.01	1273.11	38	40	1499
Difflugia oblonga	-	-	-	-	-	-	No	2.34	596.34	46	38	368
Haynesina depressula	-	-	-	-	-	-	No	1.81	438.66	42	44	392
Haynesina germanica	No	1.53	68.90	26	49	135.64	Yes	1.09	667.35	40	40	694
Cibicidoides lobatulus	-	-	-	-	-	-	No	2.46	331.62	42	38	287
Miliolinella subrotunda	-	-	-	-	-	-	No	3.65	997.57	43	40	775
Quinqueloculina seminula	-	-	-	-	-	-	No	1.16	320.56	41	42	329
Spirillina vivipara	-	-	-	-	-	-	No	3.51	383.65	45	34	223
Triloculina trigonula	-	-	-	-	-	-	No	3.75	125.30	47	14	53

RESULTS

Comparison of dead *vs*. living assemblages

A total of 58 (four testate amoebae and 54 foraminifera) and 145 taxa (seven testate amoebae and 138 foraminifera) were identified in the living and dead assemblages, respectively. Some planktonic foraminifera from watershed Cretaceous outcrops were also observed in the residue, even in large proportions but were ignored in the study. The diversity in the dead assemblages is two times greater than that of the living ones (mean 2.89 vs 1.29) (Appendix 1). No relationships (linear or non-linear) have been determined between them. All the living species were recognized in the dead assemblages (except *Centropyxis constricta* in sample 70) within one out of 49 samples. The dead assemblage species richness is commonly 2 to 4 times higher than that of the living counterpart (Appendix 1). The only exception is sample 53, where 24 living species are observed *vs*. 16 dead species.

The rank frequency diagram provides evidence of the wide occurrence of two living species, namely, *Haynesina germanica* and *Cribroelphidium excavatum* (text-fig. 2, Appendix 1). These taxa are the only two species identified in all the samples (Appendix 1). The other frequent species (present in most samples) occurring in more than 40 samples are *Cribroelphidium magellanicum*, *Bolivina variabilis*, *Cribroelphidium margaritaceum*, *Cribroelphidium gerthi*, *Cribroelphidium williamsoni*, *Bolivina pseudoplicata*, *Buliminella elegantissima*, and *Quinqueloculina seminula*. All the other species were identified in no more than 21 samples.

This high-frequency/low-frequency dichotomy did not occur within the dead assemblages (text-fig. 2). For comparison, 36 species occurred in more than 21 samples. In the dead assemblages, *C. excavatum* and *C. gerthi* were the only two species occurring in all the samples. *Haynesina germanica* occurred in 40 samples. The five species with the highest number of specimens were *C. magellanicum*, *E. margaritaceum*, *C. williamsoni*, *C. excavatum,* and *C. gerthi* followed by *Miliolinella subrotunda*. Among the dead assemblage, numerous species originating from a deeper environment than the sampling zone were found (i.e., *Pseudononion japonicum*, *Uvigerina canariensis*, and *Textularia truncata*). Some others were more widespread in the dead assemblage than in the living one (i.e., *Stainforthia fusiformis* was observed in nine and 16 samples in the living and dead assemblages, respectively). Specimens belonging to epiphytic species were more numerous in dead than in living samples (i.e., *Quinqueloculina seminula* density = 93 specimens/cm^3 in the living assemblages *vs*. 134 in the dead assemblages).

Minimum number of replicates for analyzing fauna

The species densities were characterized by a significantly positive skewness implying a combination of a wide range of low-density patches with a few denser ones (Table 1). This asymmetry is underlined by the frequent non-normality of the distribution (Table 1). Only dead diversity is negatively skewed with a small asymmetry.

After 10,000 trials, considering the number of replicates from three to seven, non-identical density (Kruskal-Wallis test, *p-value* < 0.05), and heterogeneous variance (Fligner-Killeen test, *p*-value < 0.05) were documented (Table 2).

This heterogeneity of fauna distribution is illustrated with the randomly sampled group of replicates (text-fig. 3). Even when no outliers are present, the ranges of estimated density, diver-

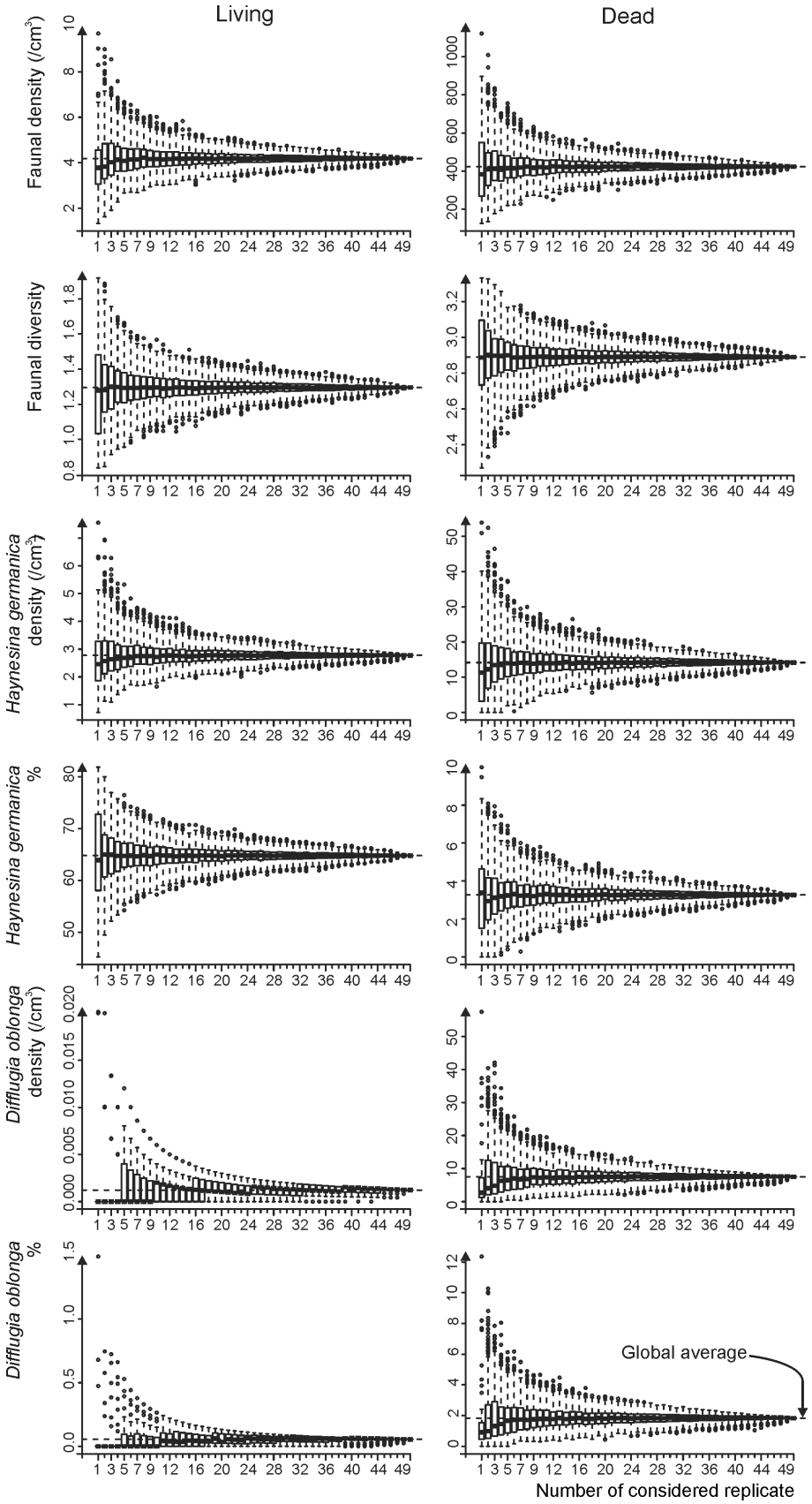
TABLE 2
Occurrence after 10,000 trials of non-equivalent average density and variance between groups of samples randomly formed with five to seven replicates. *N*1 is the number of times the density between groups of replicates can be considered non-equal (Kruskal Wallis test with α 0.05). N2 is the number of times the variances between groups of replicates are heterogeneous (Fligner-Killeen test with $\alpha < 0.05$).

	Living density						Dead density					
	N1	N2	N1	N2	N1	N2	N1	N2	N1	N2	N1	N2
Number of replicate	5		6		7		5		6		7	
Bolivina dilatata							391	88	362	1869	412	536
Bolivina pseudoplicata	339	28	375	822	370	163	356	11	386	853	405	126
Bolivina variabilis	340	3	427	192	377	13	341	9	403	1057	395	180
Cibicidoides lobatulus							337	6	391	731	395	81
Cribroelphidium excavatum	330	21	383	1130	405	127	312	6	390	650	389	83
Cribroelphidium gerthi	314	70	350	1509	384	295	316	8	367	947	402	150
Cribroelphidium gunteri	358	222	369	2034	367	417						
Cribroelphidium magellanicum	323	4	376	235	398	35	319	1	390	238	389	18
Cribroelphidium williamsoni	342	11	386	496	413	130	367	1	381	369	416	58
Difflugia oblonga							326	45	392	2953	410	568
Elphidium margaritaceum	368	16	365	546	374	93	341	5	363	527	389	122
Elphidium pereirum							354	269	386	2766	380	832
Haynesina depressula							303	11	374	1534	393	232
Haynesina germanica	355	7	379	812	407	127	345	7	360	587	416	78
Miliolinella subrotunda							352	13	407	600	411	115
Quinqueloculina seminula							392	5	392	595	382	97
Spirillina vivipara							351	46	353	1817	407	371
Triloculina trigonula							345	476	347	3069	355	854

sity, and proportion – whatever the rank of the species, such as *H. germanica* (dominant) or *Difflugia oblonga* (minor) – are high (text-fig. 3). Some of these estimations are far from the global average (calculated from all 49 samples and considered the correct value) with a lower number of replicates (approximately < 10–15). To estimate the diversity and density for dead and living fauna, the dispersion measurement relative to the confidence level estimated around the mean is always high for sampling 3 or 4 replicates (Appendix 2). For example, the error reaches 100% for estimating the dead density with 3 replicates. For the living fauna, density, errors are lower. At best, the error is less than 5% for estimating the dead fauna diversity using 17 replicates.

All species' spatial distributions are fitted with exponential, spherical, or Matérn Stein models (Table 3). The ranges for most of the species are within the quadrat (except for *B. pseudoplicata* for living species and *E. margaritaceum* and *H. depressula* for dead species). As the range represents the distance from which two samples no longer resemble each other, on average, the faunal densities could be considered structured as patchiness. The patchiness trend in the living assemblages is amplified for species that have large intrinsic semivariance compared to the nugget (semivariance at the shortest distance). *Haynesina germanica* and *C. excavatum* constitute the largest number of living specimens and are spatially completely structured (strong spatial dependency and a null nugget partial sill). The other living species' distribution should be considered to be structured by more random spatial processes (e.g., *C. gunteri* and *C. williamsoni*). When Matérn Stein's (Ste) model was used, the smoothed species (highest Kappa) is *E. margaritaceum*. These 'other' species, with more random distribution and weak rise of the semivariance with an increase of distance, are poorly represented taxa (no more than 22 specimens per cm^3, whereas *H. germanica* and *C. excavatum* are 5 to 20 times denser). For the dead assemblages, the highest densities were, in decreasing order, *C. magellanicum*, *E. margaritaceum*, *M. subrotunda*, *C. gerthi*, *C. williamsoni*, and *C. excavatum*. The Ste models were used for all these species and have a slow increase of semivariance with increasing distance. Nevertheless, due to a strong nugget, the spatial dependency was moderate or strong.

Using the bootstrapping procedure, the number of samples required to estimate the average species density with a confidence of 95% ranged from 26 to 30 for the main living species and 30 to 47 for the dead species (Table 1). Only a single sample is enough to estimate the dead diversity, whereas eight replicates are necessary for the living counterpart (Table 1). Global density is well estimated with 19 and 23 replicates for living and dead, respectively.



TEXT-FIGURE 3
Example for biotic parameters (density, diversity (*H'*), and relative abundance) and estimation of living and dead fauna and, in greater detail, *Haynesina germanica* and *Difflugia oblonga* after 1,000 random samples from 1 to 49 replicates.

TABLE 3
Semivariogram models for the living and dead dominant species. The gray boxes correspond to the species whose ranges are inferior to the larger theoretical distance within the quadrat (141 cm) and for species whose spatial dependency is strong. The nugget and species partial sill are provided as well as Kappa (smoothing parameter) in the case of the use of Ste model.

	Dominant species	Nugget partial sill (C0)	Model	Species partial sill (C)	Range	Kappa	Spacial dependency C/(C+C0)	
Living	*Bolivina pseudoplicata*	8.64	Exp	26.4	359	0.00	75	Strong
	Bolivina variabilis	3.40	Ste	1.5	3	0.20	31	Moderate
	Criboelphidium magellanicum	1.80	Sph	5.8	20	0.00	76	Strong
	Cribroelphidium excavatum	0.00	Sph	397	15	0.00	100	Strong
	Cribroelphidium gerthi	8.80	Sph	2.1	10	0.00	19	Weak
	Cribroelphidium gunteri	0.99	Ste	0.0	13	1.40	0	Weak
	Cribroelphidium williamsoni	9.87	Ste	0.0	38	1.20	0	Weak
	Elphidium margaritaceum	7.06	Ste	46.9	126	10.00	87	Strong
	Haynesina germanica	0.00	Ste	5985	22	0.40	100	Strong
					0			
Dead	*Bolivina dilatata*	0	Ste	530508	29	10.00	100	Strong
	Bolivina pseudoplicata	55750	Sph	100713	14	0.00	64	Moderate
	Bolivina variabilis	0	Ste	258960	7	10.00	100	Strong
	Cibicidoides lobatulus	107003	Ste	90964	117	10.00	46	Moderate
	Criboelphidium magellanicum	4389754	Ste	5072001	34	5.00	54	Moderate
	Cribroelphidium excavatum	1051083	Ste	859764	75	1.00	45	Moderate
	Cribroelphidium gerthi	1179252	Ste	2018683	57	5.00	63	Moderate
	Cribroelphidium williamsoni	0	Ste	1734110	8	10.00	100	Strong
	Difflugia oblonga	133611	Ste	391503	31	5.00	75	Strong
	Elphidium margaritaceum	2643785	Ste	1020105	1687	0.05	28	Moderate
	Elphidium pereirum	73804	Ste	17963	19	10.00	20	Weak
	Haynesina depressula	181714	Ste	592100	205	1.80	77	Strong
	Haynesina germanica	297334	Sph	115684	5	0.00	28	Moderate
	Miliolinella subrotunda	216826	Ste	7646152	85	10.00	97	Strong
	Quinqueloculina seminula	47475	Exp	62859	14	0.00	57	Moderate
	Spirillina vivipara	11182	Ste	211642	24	10.00	95	Strong
	Triloculina trigonula	12551	Ste	11851	129	5.00	49	Moderate

DISCUSSION

Foraminifera and testate amoebae species occurrence in a transitional environment

Foraminifera and testate amoebae are commonly found in transitional environments in a wide range of changing parameters. For example, foraminifera have been investigated in response to salinity (Frontalini et al. 2011) and water oxygen content gradients (Frontalini et al. 2013); along cross-shore profile (elevation) (Kemp et al. 2009, Culver et al. 2015) and associated to sea-level rise (Gehrels et al. 2001). Both groups are, too rarely, also studied together with particularly sensitive gradients in transitional environments such as salinity (van Hengstum et al. 2008, Vazquez Riveiros et al. 2007) or organic matter from natural and anthropogenic origin (Laut et al. 2016). Within these gradients' contexts, Arcelacean (*Centropyxis* and *Difflugia)* are typical in the 'floating marsh zone' (lower marsh) (Scott et al. 1991). Vazquez Riveiros (2007) observed centropyxid dominating the brackish assemblage associated with difflugiids. In our study area, a tidal flat-low marsh transitional zone, the same genera were observed but with a lower species diversity in association with foraminifera. The main species *D. oblonga* and *C. aculeata* are probably more tolerant of salty waters. The dominant living foraminiferal species are *H. germanica* and *C. excavatum*. Their tolerance to manifold stress is amply described in the literature (e.g., Martins et al. 2016). Even if *H. germanica* is more tolerant to lower salinity variations than *C. excavatum* (Armynot du Châtelet et al. 2005), it seems that this species is well adapted to live in the study area. *Cribroelphidium gerthi*, *Elphidium margaritaceum*, and *C. williamsoni* occurred among the dominant species in both the dead and living assemblages. These species are commonly associated in transitional environments (Cearreta et al. 2002, Camacho et al. 2015), though singularity exists between them with their relationships to sediment grain size (Martins et al. 2016), as epiphytic species *Cibicidoides lobatulus* and

Quinqueloculina seminula were predominantly recognized in large numbers among the dead assemblages. In this area, along the tidal gradient, *Q. seminula* commonly inhabits the higher salt marshes, whereas *C. lobatula* comes from deeper environments. Consequently, their presence, at the same location, demonstrates that the sediment (and the faunal content) in the salt marsh/tidal flat transition must have been transported from large areas that are either from more marine or more continental environments. Moreover, testate amoebae species of the *Cyclopyxis* genus that are commonly identified in the transition from the salt marsh and freshwater environment (Vazquez Riveiros et al. 2007) were not observed; which would indicate that the primary influence on the studied quadrat is oceanic and the influence of the continental is limited.

Representativeness of dead *vs.* living benthic foraminifera and testate amoebae for environmental and paleoenvironmental studies

Significant differences in terms of diversity, density and species composition between living and dead assemblages were observed. Living fauna represents the instantaneous picture of the biota at the time of the sampling, whereas dead fauna represents the test accumulations over a long time (Murray 1991). The 1-cm sediment accumulation calculated for a nearby estuary might account for seven months to 2.5 years (Marion 2007) or one single event in case of a storm accumulation (Dalrymple and Choi 2007). It is known that population dynamics and predation (biological effects), post-deposition and transport (physical effects), and taphonomy (physical and chemical processes) may introduce discrepancies between living and dead assemblage structures in the deep sea (Rathburn and Miao 1995, Duros et al. 2014, Gooday and Hughes 2002). In coastal environments, all these processes are concurrently involved and enhanced, but sediment dynamics triggered by tide and waves represent the key parameter for introducing differences between living and dead assemblages (Murray 2006, Martins et al. 2016, Armynot du Châtelet et al. 2009). As tides follow a hypertidal regime (tidal range over 6.5 to 9 m twice per day), sediment dynamics are particularly important in our study area and triggered by strong ebb and flood currents (up to five knots ~10 km/h) in the middle of the main channel of the river.

In a salt marsh area, Milker et al. (2015) observed higher dissimilarities among living populations than among dead counterparts. Similarly, Avnaim-Katav et al. (2016) showed that the species present in living and dead assemblages are not the same and that numbers of inland fresh and brackish water species, transported by the floods, are mixed with inner to mid-shelf benthic and planktonic foraminifera, ostracods, and molluscs. Our study reproduces these observations but with a more smoothed spatial distribution for the dead assemblages. We also observed more transported dead specimens from a deeper environment and Cretaceous planktonic foraminifera transported from inland outcrops. According to Martins et al. (2016), this leads to a higher number of dominating species in dead assemblages than in living ones but, in our case, with no ecological-environmental significance.

In order to address the representativeness of the dead assemblages for paleoenvironmental studies, a careful surface living study should be carried out to precisely define the autochthonous species. In most cases, because of the biological effect, seasonal and regional studies should be preventively performed. Similar to Martins et al. (2016), the core for the paleoenvironmental study should be selected in the area where the differences between the living and dead surface assemblages are as low as possible. On the other hand, we can never perfectly reconstruct paleoenvironmental conditions without a multiproxy study (Francescangeli et al. 2016). The broad range of proxies (physical, biological, or chemical measurements) and historical documents may help in the past environment characterization (Delaine et al. 2015, Avnaim-Katav et al. 2015).

Significance of replicates and the number of replicates to account for benthic foraminiferal and testate amoebae spatial variability

True replication is required since the degree of independence at a given level cannot be deduced solely on the basis of spatiotemporal proximity, but can only be tested after the experiment is done (Koehnle and Schank 2009). Berkeley et al. (2008) explained that multiple cores are necessary to distinguish variations due to 'true' seasonality or simply study spatial patchiness. On a field experiment on taphonomy of molluscs and foraminifera, Walker and Goldstein (1999) expressed their disappointment in the lack of replication. Field experimental studies are, most of the time, carried out with replicates (Pascal et al. 2008, Ernst et al. 2006, Sherman and Coull 1980), and using replicates has become particularly common since the publication of the Foraminifera BIoMonitoring (FOBIMO) protocol (Schönfeld et al. 2012), which recommended three replicates.

The minimum number of replicates is very hard to determine, particularly for protists. In a highly dynamic environment, they show complex biological and ecological behaviors under numerous forcing parameters. The more heterogeneous the distribution is, the more replicated samples are needed (Debenay et al. 2015). Our calculation shows that the average species density should be well estimated by randomly sampling 26 to 47 replicated sampled in 1 m^2. These figures correspond with those reported by Spilmont et al. (2011), where, to give a correct estimate of the microphytobenthos biomass, between 15 and 115 samples should be analyzed because of the high spatial variability. The microphytobenthos is known to follow a very structured pattern, displaying a few dense patches over a wide range of low-density patches (Seuront and Spilmont 2002). It means that the distribution of microphytobenthos is structured by environmental parameters that might be identified. For foraminifera and testate amoebae, it should not be different.

Our calculation reveals that the confidence level around the mean is always high; a confidence level about the mean of plus or minus 5% is rarely reached.

In the end, with a single sample, only the global diversity could be addressed in the paleoenvironment and three replicates are insufficient in a transitional environment to reliably infer environmental processes based on foraminifera and testate amoebae.

Here our findings are in accordance with important and pioneering works of Buzas et al. (2002b) who stated that foraminifers are spatially distributed as a heterogeneous continuum forming patches with different densities that are only meters apart. For Buzas et al. (2002b) the main reason is asynchronous reproduction causing pulsating patches that vary in space and time. To detect any patchiness, we suggest sampling as many replicated samples as possible and processing them independently. Patchiness could then be managed using average and standard deviation after having correctly removed outliers. Similarly, when dealing with paleoenvironmental interpretation, some replicated

cores must be considered in transitional environments. However, collecting and processing more than 26 samples at a single station are clearly not possible when multiple stations are involved. Three is a small number for statistical comparisons; 4 or 5 is much better. Even with a large number of replicates, a confidence level around the mean density of plus or minus 5% remains unrealistic.

CONCLUSIONS

Dead specimens are more affected by transport, even at a scale of 1 m², unlike the living specimens that have more limited dispersion ability. Dead specimens then provide a more diffuse signal than living ones. In paleoenvironmental reconstruction changes, they should be used with caution after a local distributional survey.

The minimal number of replicates for equivalent average density is large (>26). For studies based on the living foraminifera and testate amoebae, the common consideration of three replicates is therefore insufficient in transitional environments.

Some consensus on the number of replicates used in foraminiferal studies is desirable. Reliability (number of replicates) as well as the cost (field and laboratory) must simultaneously be taken into account.

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Appendix 1

Appendix 1 — Foraminifera counting table and parameters

Faunal parameters	Sample	S00	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S19	S20	S21	S26	S29	S30	S39	S40	S49	S50	S53	S56	S59	S60	S67	S69	S70	S79	S80	S81	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95	S96	S97	S98	S99
	Dead Counted Thecamoebians (TA)	2	3	2	13	2	3	10	3	2	3	2	3	0	2	8	2	5	1	4	1	2	4	6	2	4	4	4	0	1	2	0	8	2	3	0	0	2	0	7	6	5	4	1	1	3	5	2	2	10
	Dead Counted Foraminifera (F)	189	192	178	76	183	183	100	204	194	66	209	219	207	226	58	205	111	181	203	218	207	79	56	206	72	199	200	204	199	201	211	203	203	220	211	206	211	196	206	110	191	206	202	215	211	193	210	217	69
	Dead Counted Fauna	191	195	180	89	185	186	110	207	196	69	211	222	207	228	66	207	116	182	207	219	209	83	62	208	76	203	204	204	200	203	211	211	205	223	211	206	213	196	213	116	196	210	203	216	214	198	212	219	79
	Counted Fossil	32	56	44	37	68	36	23	77	72	52	32	30	8	10	61	58	27	9	36	14	19	38	32	10	49	76	21	15	35	119	28	95	34	21	14	20	38	17	32	28	48	19	24	15	37	66	28	44	51
	Living Counted Thecamoebians	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Living Counted Foraminifera	227	484	202	188	347	217	153	133	166	225	242	352	257	160	167	194	215	152	124	263	194	306	415	189	174	242	147	210	162	153	134	160	166	451	333	124	151	183	178	66	96	154	149	227	205	176	144	264	228
	Living Counted Fauna	228	484	202	189	347	217	153	133	166	225	242	352	257	160	167	194	215	152	124	264	194	306	415	189	174	242	148	211	163	153	134	160	166	451	333	124	151	183	178	67	96	154	149	227	205	176	144	264	228
	Dead thecamoebiens species	2	3	1	3	1	2	2	1	1	1	1	2	0	1	3	1	2	1	3	1	2	3	2	2	2	2	3	0	1	2	0	3	2	2	0	0	2	0	3	1	3	2	1	1	2	3	1	1	4
	Dead foraminifera species	45	39	41	20	47	42	21	46	45	23	41	43	39	42	21	44	22	36	41	39	34	16	20	40	20	38	42	44	38	50	41	45	41	43	35	38	44	44	36	23	45	42	42	36	42	45	39	44	23
	%Fossiles / dead	14	22	20	29	27	16	17	27	27	43	13	12	4	4	48	22	19	5	15	6	8	31	34	5	39	27	9	7	15	37	12	31	14	9	6	9	15	8	13	19	20	8	11	6	15	25	12	17	6
	Living thecamoebiens species	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Living foraminifera species	14	13	14	10	16	11	14	12	20	11	15	13	11	12	13	18	14	15	13	27	15	24	19	15	15	14	22	22	20	18	13	15	18	15	15	14	15	17	11	10	17	12	13	14	15	16	12	18	10
	Dead diversity TA +F	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	2	2	2	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Living diversity TA+F	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	2	1	2	2	2	2	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1
	Living density (/cm3)	5	10	4	4	7	4	3	3	3	5	5	7	5	3	3	4	4	3	2	5	4	6	8	4	3	5	3	4	3	3	3	3	3	9	7	2	3	4	4	1	2	3	3	5	4	4	3	5	5
	Dead density (/ 50cm3)	25472	7104	11776	15104	9728	30080	19200	15232	10752	12992	18240	21632	15872	23168	37888	9344	25400	27520	26240	18272	12432	15104	17664	56064	29568	6336	17664	10112	44800	29312	25984	24832	16640	20224	16896	32512	30848	13440	13360	12032	26240	38272	29312	19712	16448	17408	27776	11776	23296
	Dead density (/cm3)	509	142	236	302	195	602	384	305	215	260	365	433	317	463	758	187	508	550	525	365	249	302	353	1121	591	127	353	202	896	586	520	497	333	404	338	650	617	269	267	241	525	765	586	394	329	348	556	236	466

Living fauna / counting table		S00	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S19	S20	S21	S26	S29	S30	S39	S40	S49	S50	S53	S56	S59	S60	S67	S69	S70	S79	S80	S81	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95	S96	S97	S98	S99
All foraminifera per 50cm3	*Centropyxis aculeata discoides*																				1																														
	Centropyxis aculeata																																																		
	Centropyxis constricta																													1																				
	Difflugia sp																																																	
	Difflugia oblonga																											1	1													1								
	Difflugia urceolata	1			1																																													
	Difflugia urens																																																	
	Difflugia cf bacillifera																																																
	Adelosina dubia																																																
	Adelosina longirostra							1																						1																			
	Adelosina pulchella																																																
	Adelosina sp																																																
	Affinetrina planciana																																																
	Ammonia beccarii																																																
	Ammonia tepida	1				2				1						1					5		4	1					1		1							1				1		2	1			1	
	Amphicoryna sp																																																
	Arenoparrella mexicana		1	1		1		2	3						1	3			3		1		1					3	1	1	1		1	1	1			1	1			1						1	
	Astacolus crepidulus																																																
	Asterigerinata mamilla	1		1						1								1				1				2																							1	
	Bathysiphon sp																																																
	Bolivina difformis																																																
	Bolivina dilatata									1		1						1	1	1	2		1	1	1		1	1	1	1				1		1				1			1		2		1			
	Bolivina pseudoplicata	3		1	2	1	1		3	3	1	4	1	3		2	2	2	4	3	22	2	1	1	1	3	1	2	6	2	2	5	6	3	2	2	1	5	8	1	2	2	4	2	1	5	2		6	2
	Bolivina sp																										1		1					1				1		4									1	
	Bolivina spathulata			1	2							1																	1	2				1												1	1		1	
	Bolivina striatula																				1										1															2	2				
	Bolivina variabilis	5	1		1	6	3	8	6	3	6	8	6	1	3	3	2	6	4	5	6	7	6	3	4	5	6	5	6	5	7	2	3	6	1	3	5	4	2	4	2	5	2	3	4	6	8	2	9	5
	Bolivinita quadrilatera																																																
	Buccella frigida																				1																													
	Bulimina elegans											1					1		1		1		1							1					1														
	Bulimina marginata																																																
	Buliminella elegantissima	6		2		4	7		3	3	2	2	2	3	4	2	3	2	3	1	7	4	3	3	3	4	2	2		2	7	6	1	2	2	2	4	2	3		3	4	1	3		1	3	4	1
	Calcituba polymorpha																																																
	Cassidulina laevigata																																																
	Cibicides refulgens																															1					1			1			1	2						
	Cibicides sp																																																
	Cibicidoides lobatulus																		1		1			2							1																		
	Cibicidoides pseudoungeriana																																																
	Cornuspira involens																						1					1															1							
	Cribroelphidium excavatum	70	65	25	22	46	52	17	25	34	38	34	107	33	26	40	12	19	21	17	18	20	62	58	23	29	16	16	46	21	32	25	31	34	95	49	30	35	26	31	12	23	26	22	36	44	26	20	65	36
	Cribroelphidium gerthi	3	15	2		12	1	3	1	2	2	3	3		3	4	13	2	2	2	4	12	5	7	3	10	3	1	6	2	2	3	5	3	8	7	1	3	1	2	3	3	4	1	7	1	1	3	3	1
	Cribroelphidium gunteri					2			3	2	2				1		1			1	1	4				1			2						1		1				5			1	1			1	
	Cribroelphidium magellanicum	8	3	3	4	7	7	5	1	2	7	6	5	3	10	6		5	6	5	6	5	9	7	2	11		7	4	5		2	5	1	9	3	3	4	4	3		4	6	1	4	2	2	1	6	4
	Cribroelphidium poeyanum																																																
	Cribroelphidium williamsoni	2	3	4	6	1	3			3		6	6	16	2	3	2	2		2	11	2	4	3	5	2	6	3	5	7	4	5	3	4	9	4		4			1		1	1	2		2	1	3	8
	Cushmanina feildeniana																																																
	Cycloforina granulocostata																																																
	Cycloforina villafranca																																																
	Discorbinella nitida															1												1																						
	Discorbis vilardeboanus		1									1		1				1															1	1	1			1	1			1			1					
	Edentostomina rupertiana																																																
	Ehrenbergina trigona																																																
	Elphidium aculeatum																																																
	Elphidium cuvillieri																																																
	Elphidium macellum																																																
	Elphidium maregitaceum	1	7	3	4	2	2	6	1	10	1	9	3	7	7	2	3	4	2	8	12	6	15	5	4	8	1	7	2	6	2	2	5	5	2	5	2	2	5	1	2	3	1		5	4	3	3	4	4
	Elphidium pereirum			1		2	1	2	1				2						1					1					1	1				1		1		2							1	2		
	Elphidium pulvereum									1													1										1									1			1				
	Entzia macrescens	1		1													2				1			1						1	1					1			1										
	Epistominella sp																																																
	Favulina hexagona																																																
	Favulina melo																																																
	Favulina squamosa																																																
	Fijinonion fijiense																																																
	Fissurina cf foliformis																																																
	Fissurina lucida																																																
	Fissurina orbignyana																																																
	Fissurina sp.																																																
	Gavelinopsis praegeri																																																
	Glabratella milletti																																																
	Globocassidulina crassa																				8				1				1									1	1											
	Haplophragmoides wilberti																						1																											
	Haynesina depressula					1	2			2					2				2				1					2	1						1									1						
	Haynesina germanica	123	378	156	144	257	136	96	85	91	163	162	212	187	96	97	134	168	100	77	143	124	175	316	131	92	198	82	119	95	84	78	93	99	314	251	72	85	120	123	36	43	101	111	157	132	121	106	153	166

Appendix 1. *continued*

Heronallenia lingulata																																																	
Homalohedra williamsoni																																																	
Hopkinsina atlantica					2		1						1											1		1	1	2	1					1												1			
Hopkinsina sp																																																	
Laevidentalina subsoluta																																																	
Lagena aspera																																																	
Lagena inaequilateralis																																																	
Lagena sp																																																	
Lagena sulcata																																																	
Lagenosolenia cf radiata																																																	
Lagenosolenia lagenoides																																																	
Lamarckina haliotidea																																																	
Lenticulina rotulata																																																	
Lepidodeuterammina eddystonensis		2							1	1		3						1		2					2		5								1	1								2				1	
Lepidodeuterammina ochracea							2															2					2			1																			
Massilina secans																																																	
Melonis barleeanus																																																	
Miliammina fusca	1	1							1							1					1							1		1									1							1			
Miliolina (Triloculina) sp																																																	
Miliolina schlumbergeri																																																	
Miliolinella elongata																																																	
Miliolinella sp																																																	
Miliolinella subrotunda																					1																											1	
Neoconorbina terquemi																																																	
Palliolatella bradii																																																	
Palliolatella fasciata subsp. Carinata																																																	
Paraffisurina sp																							1																										
Parafissurina inaequilateralis																																																	
Paratrochammina (Lepidoparatrochammina) haynesi							6									1						1		1			1	1	1	1	1							2							1	1			
Patellina corrugata				1												4	1			1				2		1																							
Patellina sp																1																																	
Planispirillina inaequalis																																																	
Planorbulina mediterranensis																			1																														
Polymorphina sp																																																	
Portatrochammina murrayi																3										2																							
Procerolagena clavata																																																	
Pseudonodosaria sp																																																	
Pseudononion japonicum																																																	
Quinqueloculina dunkerquiana																																																	
Quinqueloculina duthiersi																																																	
Quinqueloculina jugosa																																																	
Quinqueloculina lata																																																	
Quinqueloculina seminula	2	5	1	1			2	1	2		2	1	2	5	3	7		1			4	5	1	4	2	3	1	1	1	1	3	3	1	4	2	1	2		8		1	2	2	1	1		2	1	1
Quinqueloculina sp																																																	
Quinqueloculina venusta																																																	
Remaneica plicata																				1																													
Reophax nana																																																	
Rosalina anglica																																																	
Rosalina bradyi																							1																										
Rosalina globularis																				1																													
Rosalina sp																			1			1	1																										
Rotaliella sp																																																	
Rubratella intermedia																				1								1																					
Sigmoilina sp																																																	
Siphonaperta aspera																																																	
Siphouvigerina sp																																																	
Spirillina obconica																																																	
Spirillina sp																																																	
Spirillina tuberculata		2																											4																				
Spirillina vivipara									1	1						1	1			1		1													1					1	1								
Spiroloculina sp																																																	
Spirophthalmidium sp																																																	
Spirosigmoilina sp																																																	
Stainforthia fusiformis																						1			1		1		1	1		1					1		2		1								
Stilostomella sp																																																	
Subanomalina pauperata																				1							1																	1		1			
Textularia earlandi																											1																						
Textularia truncata																																																	
Trifarina angulosa																																																	
Trifarina reussi																																																	
Triloculina trigonula																																																	
Trochammina inflata					1		1		2		1	1								1	1	3	1		1				1				1	1				1								3	1		
Uvigerina canariensis																																																	
Juvenil Impossible to identify				1		2	1			1	1					1				3		1	1	3	1		1		2	3								1	1		1			1	2			1	

Appendix 1. *continued*

Dead fauna / counting table	Sample	S00	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S19	S20	S21	S26	S29	S30	S39	S40	S49	S50	S53	S56	S59	S60	S67	S69	S70	S79	S80	S81	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95	S96	S97	S98	S99
Foraminifera per splitted fraction	*Centropyxis aculeata discoides*	1	1										2					1	1	1	1		1		1	1	2			1			1	2			1		2		1	1			1				1
	Centropyxis aculeata																						1				1																		3			
	Centropyxis constricta																																																
	Difflugia sp						1																							1																		1		2
	Difflugia oblonga	1	1	2	11	2	2	9	3	2	3	2	1		2	5	2	4		2		1	2	5	1	3	3			1		5	1	1				4	6	3	3	1	1	2	1	2	2	6
	Difflugia urceolata				1											2				1								1				1																
	Difflugia urens		1		1			1								1						1		1			1				2							1										1
	Difflugia cf bacillifera																												1		1																
	Adelosina dubia	3											1	1				1	1	2			2	2				1		1	1	1						1	1			1	1			1				
	Adelosina longirostra	1							1		1				2	1						1		1			1						1		1				1			1				1	1		
	Adelosina pulchella											1			1									1														1											
	Adelosina sp					1							1			1						1				1									1	1									1			
	Affinetrina planciana																									1																						
	Ammonia beccarii		1																												1																	
	Ammonia tepida	2	4	2	1	6	1	1	2	3	2	1	2		4				1	1	2	3	1	1	1	2	1	4	1	3			1	1	2		5	3	4	6	2		2	2	2			1	1	1
	Amphicoryna sp					1																																										
	Arenoparrella mexicana	3	4	1			3			1		3	1		2		4		4			1			5			4	1		1	3	3	2				2	2					2		1	3	
	Astacolus crepidulus										1																																			1			
	Asterigerinata mamilla	2	3	1		2			7	2	1				1		1		1				1					2	1	1	2	1	1			1		4			2	3	1			2	1	2	
	Bathysiphon sp	1	1						1									1																															
	Bolivina difformis									1							1																							1									
	Bolivina dilatata		1	4	1	2		1	4		3	1	3		1	6	1		1		2		2	5	1	1		3		3	4	2	1	1	1		2	2	1		1		1				3
	Bolivina pseudoplicata	1	9	5		10	2		7	10		5	8	1	3		7			2	5	2			1	2	9	8	2	1	8	7	10		3		2	11	3	4	2	3	5	2	4	1	12	4	1	
	Bolivina sp																									1																							
	Bolivina spathulata			1		1	1					1	1				1													1	1		2																
	Bolivina striatula																		1					1																										1
	Bolivina variabilis	5	3	3		3	4		4	8		4	4	2	2		10		4	3	6	1			4		5	9	5	1	8		13	3	6	1	4	3	3	2	4	3	13	4	7	3	16	10	6	
	Bolivinita quadrilatera	1									2		1		1		1	1								1		1			2	2	2					2	2	1	1	1		1		1		
	Buccella frigida		2	3		1	5			6		1			3				1	1	2				3				1		1	1			3	1	1	4			1	1	1	2	2	1			
	Bulimina elegans					3	1					1	1				2			1	1					1						1	1					1						1	1	1			
	Bulimina marginata																																													1				
	Buliminella elegantissima						1			1		1				1	3			1	3				2		1	1	1	1	2		1		1			2	2			2	2			1	3			
	Calcituba polymorpha																							1																									
	Cassidulina laevigata								1			1	1		1												2	1				2						1		1				2		1	1		
	Cibicides refulgens								1	1													1		1			3		1	4			2				1			1			1	2	2		2	
	Cibicides sp	1																1																															
	Cibicidoides lobatulus	1	5	3		6	4		4	1		3	2	2		1	10	4		1	1				3	2	4	7	5	2	13	2	4	7	5	2	1	1	2	5		3	3	2	3	3	4	1	
	Cibicidoides pseudoungeriana												1					1		1										1			1		1	3	1					1	2	1					
	Cornuspira involens							1	3					1	1										1			1	1	1			2		1										1		1		
	Cribroelphidium excavatum	9	14	10	15	11	18	12	22	13	7	16	6	12	19	8	11	11	4	6	20	6	11	5	20	11	13	8	10	13	10	11	11	11	11	16	20	14	16	10	22	16	14	20	13	13	11	15	26	9
	Cribroelphidium gerthi	19	8	7	16	17	17	11	5	17	9	15	15	12	20	9	13	12	8	24	12	7	21	1	23	12	11	14	23	9	11	11	12	10	15	11	13	18	4	12	21	9	9	15	31	22	13	11	17	12
	Cribroelphidium gunteri				1				2					1											2		2					1		3		1					1						1		
	Cribroelphidium magellanicum	23	38	33	16	30	36	26	23	25	12	40	66	69	50	12	43	30	62	59	65	85	18		42		45	32	57	45	32	56	47	63	51	72	53	48	55	68	19	40	43	53	61	48	25	52	46	12
	Cribroelphidium poeyanum	1		1																																													
	Cribroelphidium williamsoni	26	15	17		12	9	16	20	13		18	21	22	25		11		19	13	21	24			11		15	13	16	26	12	16	9	18	17	21	18	26	18	11		13	24	23	18	23	7	21	23	
	Cushmanina feildeniana																																								1								
	Cycloforina granulocostata					1																																											
	Cycloforina villafranca																																													1			
	Discorbinella nitida		5	5		7	6	2	4	6		4	2	3	2	1	4		4	7	3				1		9	8	5	1	9	5	7	4	5		1	4	1		1	4	1	2	2	4	8	3	3	1
	Discorbis vilardeboanus	1	2	2		1	2			2		3	1	1			1			2	2	2						1	2		1		1		1	1			1	1		1	1	1		1	1			
	Edentostomina rupertiana	2																2																															1
	Ehrenbergina trigona										1																														3							
	Elphidium aculeatum																																1															
	Elphidium cuvillieri	1			1						1					1	2	3		2		2	1	1	1					1		1		3	1	1		1	3				1	1		2	3	2
	Elphidium macellum								1																		1				5		1	1										1	1	1			
	Elphidium maragitaceum	24	10	24	4	19	19		26	15		20	20	17	30		25	27	18	19	19	11	10		15	20	28	17	14	36	22	30	11	15	21	25	19	9	19	15		24	23	25	20	26	26	18	21	
	Elphidium pereirum	3	6	1		1			6	1			1	1			1		1											5	2	3	1	3	2	4		2	3	3	9	6	1	1			1			5
	Elphidium pulvereum	3	1	1	3	3	1		3	1	2	4	4	4	8		5		1	3	3	2			3		6	4	5	2	3	6	3	3	3	7	6		2	6		3	5	3	2	4	2	3	3	1
	Entzia macrescens	1	1	1								2	1	5	1		1	2		1		1						1	2	3	1	1			4	3	1	1	3				1		1		1		
	Epistominella sp			1																																													
	Favulina hexagona				2											1	1		1			1				1										1		1				1							
	Favulina melo				1			1												1											1							1			1						1		
	Favulina squamosa																							1																									1
	Fijinonion fijiense		2																											1																		
	Fissurina cf foliformis									1					1																																1		
	Fissurina lucida			1		2			1			1					2								1			1	1		1	1	1	2	1	1						2	1			3		1	
	Fissurina orbignyana						1																						1				1											1	1		1	
	Fissurina sp.																												1																			
	Gavelinopsis praegeri	1	2				2		3	4		1					2			1					3		1		2		2		3	1	2		3	2	1		2	2			1	1	2	1	
	Glabratella millettii					1				1	1																				1								1								
	Globocassidulina crassa		1									3	2		2					1		1			2							1	2	5	2	2		1	1	1		2	2		1	1	2	2	
	Haplophragmoides wilberti	1	1	3		1	1		4	2		2	1	2	1		1		3	1	1	1			4		1	1	2	2	1	1	1		3	1		2	3	3		1	4	3	3	2	3	3	1	
	Haynesina depressula	3	6	4		1	2	7	3	4	4	1	1	2	2		2		1	13	2	2	3	2	5	5	5	7	4	2		3	4	1	6	5	7	1	2	6		4	1	9	5	5	3	1	4	1
	Haynesina germanica	19	9	5		3	10		1	7	3	20	7	12	13		7		9	7	6	17			10		3	7	10	4	8	8	5	9	10	5	9	9	2	12		3	11		8	6	3	13	5	3
	Heronallenia lingulata		1						1								1											1				1										1	1			1			
	Homalohedra williamsoni				3	1		2								2	1	4					4				1				1					1	1		1	3	2			1				2
	Hopkinsina atlantica	2													1	1									2		1														1	1							
	Hopkinsina sp	1				1																																											
	Laevidentalina subsoluta							1																																									
	Lagena aspera				1																																			1								
	Lagena inaequilateralis																										1																1	1	1					
	Lagena sp																	1																															
	Lagena sulcata																	1				1												1															
	Lagenosolenia cf radiata																											1																	1			
	Lagenosolenia lagenoides																									1																						
	Lamarckina haliotidea			1		2	4		5	3		1		1					1		1						8	2	1		1	1	3		4	1	1		1		1			2	3			
	Lenticulina rotulata											1																						1						1								
	Lepidodeuterammina eddystonensis		8	2					2	3									2	1	1						1	7		1	1	1			2			5		2				1				
	Lepidodeuterammina ochracea	1										4	1	1	2		2			3	1				1		1	1	2			2	4	2	3			1		3		2		1	2		3	1	
	Massilina secans	2			1		1	2												1	2			1		1																					1	
	Melonis barleeanus		3						3							2										1	1									1		1										
	Miliammina fusca	1		1																		1			1																							1

Appendix 1. *continued*

Miliolina (Triloculina) sp						1											1																										1						
Miliolina schlumbergeri	1				1		2		1						1	1		1	1	1						1							2					1	1		1		1	1		1			
Miliolinella elongata		1	1		2	1		3	1	1	1	1		1				1											2	2							1		1				2		1				
Miliolinella sp						1																																											
Miliolinella subrotunda	8	7	13		6	5		13	10		7	10	10	3		5		15	6	10	11		22	8		2	4	6	6	1	6	4	10	7	5	10	15	10	11		6	7	6	8	15	8	12	9	
Neoconorbina terquemi			1		1	1								1		1						1																				1		1					
Palliolatella bradii																										1																							
Palliolatella fasciata subsp. Carinata								1													1																									1			
Paraffisurina sp			2		1	1		1	1					1		1											1			1				1				1									1		
Parafissurina inaequilateralis								1		1																																						1	
Paratrochammina (Lepidoparatrochammina) haynesi	1		2			1		1	5		2	8	2	3		4		1	2	1	2			3				2	1	1	3	7		4	1		1	1	3		1	2	1		1		3	2	1
Patellina corrugata	1	5			4	1		1	2		1		1	4		2		3	2	4		1		4	3	3	2		2	3		2		2	2	1		3	1	4	1	1	2		1	1			
Patellina sp																																																	
Planispirillina inaequalis										2				1														1						1														1	
Planorbulina mediterranensis		1	1	1	2	1		1			4	7	2	1		2			4	3	4			4				4	4	1	2	2	4	2	3	3	1	4	2		3	4		4			3	4	
Polymorphina sp											1	1	1	1		1			1	1	1						1			1	1	1			1	1					1	1	3					1	
Portatrochammina murrayi																																							1										
Procerolagena clavata	2			1		1			1			1												1							1							1	1									1	
Pseudonodosaria sp					1																																												
Pseudononion japonicum														1																	2						1											1	
Quinqueloculina dunkerquiana	1																	1										1																				1	
Quinqueloculina duthiersi												1	1																				1																
Quinqueloculina jugosa																												1	1		2				1	2													
Quinqueloculina lata	1			3	2	1	3	2	1				2	2				1		1		1			2	1		1				1		2			1	1			1	3		2					
Quinqueloculina seminula	2	2	1		1				3	5	6	5	2	5		2	2	1	1	5	3			5	3	3	6	1	2	2	5	1	3	5	5	5	6	1	4	3	4	3	2	2	1	6	4	3	3
Quinqueloculina sp	1	1			1	3	4	1	1			1	1					2	1		1					1			1			1														1			
Quinqueloculina venusta									1			1																																1			1		
Remaneica plicata	1	3	3		1	5		2	4				2			2		3	2	4						3	3	1	4	3	1	1	3					4		1						2		1	
Reophax nana									1																		1																1						
Rosalina anglica		1				1																		1						1				1															
Rosalina bradyi					2	1						1				1										1	2			1	1	4		1		1		2			1		1					1	
Rosalina globularis		2	1		1								1	1							1				1			2					1						1		2					1	3	3	
Rosalina sp	1							1	1																			1		1			1	1															
Rotaliella sp					1			1	4							2		1										1					1	1								1			2	3	1		
Rubratella intermedia																											1														1								
Sigmoilina sp					1					1	1																													1									
Siphonaperta aspera															1						1																1												
Siphouvigerina sp																																																	1
Spirillina obconica																							1														1						1		1				1
Spirillina sp															1																																		
Spirillina tuberculata	1		1	3			1								1		2						3					1		1	2						1		1	1									
Spirillina vivipara		2	2		4	4	3		3		1	1	3		4	3		1	2	1	1		3	2	1		2	1			2		2	2	2	2		3		1	7	1	1		1		2	2	3
Spiroloculina sp				1																			1																										
Spirophthalmidium sp										1					1																																		
Spirosigmoilina sp																	1																																
Stainforthia fusiformis							2	1	1				1		2						2		1			2							2			2	1					1		1	2			1	2
Stilistomella sp																									1																								
Subanomalina pauperata	2		5		1	2		3			5	2	2	1		2		1	1	1				5		2	6	2	1	7		4	2	2	1	1	3	1	2		1	3	1	2	1	2		7	1
Textularia earlandi											1	1																																	1				
Textularia truncata											1				1	1			1					2				1			3		3	1		1							1	1	2	1		1	
Trifarina angulosa								1				1								1	1									2	1	1	1			2		2	2					1		4	1	1	
Trifarina reussi									1								1																											1					
Triloculina trigonula						1	1			4							1	1		1	1		1								1		1				1		3						2	1			
Trochammina inflata				1			1						1	1					1	1	1	1					1	1	2									1				1						1	
Uvigerina canariensis			1							1																																							
Juvenil Impossible to identify	1	1	1									1					1				5		1			2			1	2										2	1						1		1

Appendix 2

Confidence level around the mean

Replicate number	Dead_diversity	Dead_density	Living_diversity	Living_density
3	21.20	100.53	47.73	76.37
4	14.02	65.01	32.07	54.08
5	11.35	52.29	25.40	43.99
6	9.54	44.83	22.00	38.23
7	8.58	41.16	19.14	35.48
8	7.75	36.80	17.69	31.93
9	7.14	34.38	16.34	29.28
10	6.71	31.70	15.10	27.52
11	6.28	30.20	14.29	25.88
12	5.95	29.13	13.63	24.93
13	5.68	27.44	12.83	23.67
14	5.44	26.46	12.43	22.99
15	5.21	25.38	11.78	21.97
16	5.04	24.50	11.36	21.35
17	4.87	23.73	11.02	20.56
18	4.70	22.95	10.65	19.99
19	4.56	22.25	10.34	19.39
20	4.42	21.61	10.04	18.78
21	4.32	21.13	9.72	18.43
22	4.19	20.62	9.50	17.86
23	4.09	20.04	9.26	17.46
24	3.99	19.57	9.07	17.03
25	3.90	19.20	8.87	16.64
26	3.83	18.65	8.62	16.51
27	3.74	18.48	8.46	16.19
28	3.67	18.04	8.30	15.85
29	3.61	17.84	8.19	15.50
30	3.53	17.44	7.98	15.28
31	3.48	17.17	7.87	14.92
32	3.43	16.91	7.72	14.68
33	3.36	16.65	7.62	14.39
34	3.31	16.33	7.49	14.27
35	3.25	16.09	7.38	14.05
36	3.21	15.76	7.28	13.87
37	3.17	15.61	7.16	13.67
38	3.12	15.38	7.05	13.49
39	3.08	15.19	6.96	13.32
40	3.04	14.99	6.86	13.16
41	3.00	14.79	6.77	12.99
42	2.96	14.67	6.71	12.80
43	2.93	14.48	6.60	12.66
44	2.89	14.30	6.54	12.51
45	2.85	14.11	6.45	12.36
46	2.82	14.00	6.37	12.21
47	2.79	13.84	6.31	12.08
48	2.76	13.69	6.24	11.96
49	2.73	13.53	6.17	11.83