which should be cited to refer to this work.

The FOBIMO (FOraminiferal BIo-MOnitoring) initiative—Towards a standardised protocol for soft-bottom benthic foraminiferal monitoring studies

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The European Community Marine Strategy Framework Directive (MSFD) was established to provide guidelines for monitoring the quality of marine ecosystems. Monitoring the status of marine environments is traditionally based on macrofauna surveys, for which standardised methods have been established. Benthic foraminifera are also good indicators of environmental status because of their fast turnover rates, high degree of specialisation, and the preservation of dead assemblages in the fossil record. In spite of the growing interest in foraminiferal bio-monitoring during the last decades, no standardised methodology has been proposed until today. The aim of the FOraminiferal BIo-MOnitoring (FOBIMO) expert workshop, held in June 2011 at Fribourg, Switzerland, which assembled 37 scientists from 24 research groups and 13 countries, was to develop a suite of standard methods. This paper presents the main outcome of the workshop, a list of motivated recommendations with respect to sampling devices, sample storage, treatment, faunal analysis and documentation. Our recommendations fulfil the criteria imposed both by scientific rigour and by the practical limitations of routine studies. Hence, our aim is to standardise methodologies used in bio-monitoring only and not to limit the use of different methods in pure scientific studies. Unless otherwise stated, all recommendations concern living (stained) benthic foraminiferal assemblages. We have chosen to propose two types of recommendations. *Mandatory recommendations* have to be followed if a study wants to qualify as sound and compatible to the norms. The most important of these recommendations are the interval from 0 to 1 cm below the sediment surface has to be sampled, and an interface corer or box corer that keeps the sediment surface intact is to be used for offshore surveys. A grab sampler must not be deployed in soft sediments. Three replicate samples are to be taken and analysed separately. Samples are to be washed on a 63-μm screen, and the living benthic foraminiferal fauna of the >125 μm fraction is to be analysed. Splits are to be picked and counted entirely, and all counted foraminifera from at least one replicate per station have to be stored in micropalaeontological slides. Census data, supplementary laboratory data and microslides have to be archived. *Advisory recommendations* are to sample in autumn, to have a sample size of 50 cm^2 or a tube of 8 cm inner diameter, to use >70% ethanol as a preservative, rose Bengal at a concentration of 2 grams per litre for staining, and a staining time of at least 14 days. The split size should be defined by a target value of 300 specimens, heavy liquid separation should be avoided, and the 63–125 μm fraction or deeper sediment levels may be considered in some environments. We are convinced that the application of this protocol by a large number of scientists is a necessary first step to a general acceptance of benthic foraminifera as a reliable tool in bio-monitoring studies.

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1. Introduction

Legislation world-wide was implemented to protect and restore ecological quality in estuarine, coastal and marine systems, for instance Oceans Act in the USA, Australia or Canada, the Water Framework Directive (WFD, 2000/60/EC) and Marine Strategy Framework Directive (MSFD, 2008/56/EC) in Europe, and the National Water Act in South Africa (Borja et al., 2008). Since the publication of the MSFD in June 2008 (European Parliament, 2008), monitoring the quality of marine ecosystems has become an urgent priority. The MSFD states that by 2015 at the latest, "a programme of measures has to be designed to achieve or maintain good environmental status". This good status should be achieved in 2020 (European Commission, 2010). In this context, it is not only important to measure the concentrations of various pollutants in the marine environment, it is even more important to describe the possible impact these pollutants may have on the organisms living in the ecosystem. In the technical annex of the MSFD, 11 descriptors for good environmental status are defined, among which are biodiversity and food web elements (European Commission, 2010). In the chapters dealing with these two topics, the necessity to "select a set of relevant species and functional groups" is underlined. These key groups or species are characterised by fast turnover rates and specific habitats. Benthic foraminifera perfectly meet these criteria.

Monitoring the status of marine environments, and more specifically, describing the impact of pollutants on living organisms, has traditionally been based on studies of macrofauna. In the last decades, major efforts have been made by the scientific community working with macrofauna to develop easily applicable and objective descriptors, which are capable of giving a quantified assessment of the status of marine biota (Borja et al., 2009). These efforts have led to a profusion of "biotic indices", which are mostly based on measures of diversity or relative proportions of various types of benthic indicator organisms (reviews in Diaz et al., 2004; Borja et al., 2007; Dauvin, 2007; Borja and Dauer, 2008; Josefson et al., 2009). Unfortunately, these indices may give very different results, and intercalibration of the indices has become an urgent necessity (e.g., Hering et al., 2010).

As early as the 1960s, benthic foraminifera were used to describe the state of marine environments (e.g., Resig, 1960; Watkins, 1961; Bandy et al., 1964a,b, 1965a,b; Seiglie, 1968). Bio-monitoring with foraminifera has increased during the last decades, and at present more than 50 different research groups world-wide are active in this field.

In fact, foraminifera present several advantages in comparison to the more commonly used macrofaunal organisms (e.g., Alve, 1995a; Mojtahid et al., 2006; Bouchet et al., 2007; Alve et al., 2009; Jorissen et al., 2009):

- Their density in marine sediments, between 100 and 10000 living individuals >63 μm per 100 cm^2 surface area (Murray, 2006), is an order of magnitude higher than that of macrofauna. This means that much smaller sediment volumes are needed for a reliable assessment. This is especially important in deep-water settings, where macrofauna may be very scarce.
- Benthic foraminiferal faunas are highly variable, about 20 to 50 species per 300 individuals are to be expected in near-coastal environments. The various species occupy different niches at the seafloor. They live on the sediment surface or as infauna, and they show a variety of trophic strategies as suspension-, deposit-feeders, and those specialised to use recently sedimented phytodetritus.
- Foraminifera commonly have short life cycles as compared to higher organisms. The foraminiferal fauna therefore responds quickly to environmental changes.
- After reproduction or death, calcareous and many agglutinated tests of a large part of the foraminiferal assemblages are preserved in the sediment. Once the sedimentation rate has been appropriately determined at a given site, for instance from the depth gradient of ^{210}Pb concentrations or a succession of radiocarbon datings, the original, pristine faunas from pre-industrial time can be reconstructed. In this way, the fossil assemblages can serve as a reference in areas where no environmental baseline study has been carried out. These "pre-impact faunas" provide important information on the arrival of non-indigenous species, and the disappearance of pollution-sensitive, local species. This last point is particularly important. In most instances, there are no data available about the faunal composition and diversity before the onset of anthropogenic impact. Comparison with supposedly pristine "reference stations" is not a satisfactory solution, because 1) in many marine areas, such pristine conditions no longer exist and 2) of the very high spatial variability of benthic habitats, particularly in estuaries, where it is almost impossible to find sites with similar ecological characteristics (Alve et al., 2009). In this context, foraminifera offer a unique advantage because they are more evenly distributed than macroorganisms (e.g. Scoffin and Tudhope, 1985).
- A final point in favour of foraminifera is the presence of a large community of active researchers. Unlike the situation with benthic macrofauna, many students are being taught about foraminifera, and there is no lack of scientists capable of identifying the different species of foraminifera, and to use them in environmental monitoring.

As explained above, the community of macrofauna specialists has made large efforts to standardise their methods (Rumohr, 2004; ANSI, 2007; Rees et al., 2009), and especially, to develop biotic indices that give a quantitative appreciation of the state of marine environments (e.g., Grall and Glemarec, 1997; Borja et al., 2000; Rosenberg et al., 2004). Unfortunately, such efforts have never been made for foraminifera even though three books were dedicated to the topic (Martin, 2000; Scott et al., 2001; Haslett, 2002). This lack of standardised methods of sample acquisition, preparation and treatment, and data interpretation has probably been an important reason why foraminifera have remained a rather marginal monitoring tool. For instance, in spite of an abundant literature on the ecology of estuarine and salt-marsh foraminifera, they are non-existent in the European Community Water Framework Directive (European Commission, 2003), which concerns fresh water as well as coastal marine environments.

In view of the evident advantages of benthic foraminifera, we think that it is essential to give a new impetus to environmental monitoring based on this group of organisms. In order to achieve this, we organised a dedicated workshop (FOBIMO meeting, Fribourg, Switzerland, June 22–24, 2011), assembling 37 scientists from 24 research groups in 13 countries. The main aim of this first workshop was to define standardised methods for sampling and sample treatment, which should be at the same time scientifically sound and easy to apply. This paper presents the outcome of the workshop, which is a series of recommendations. The implementation of these methods as a scientific standard in bio-monitoring was agreed upon by all participants.

The proposed protocol is specifically adapted to the practical requirements of routine environmental monitoring, but also to the rigorous aspects of sound scientific standards. Other protocols may be adequate in pure scientific studies, depending on the scientific questions addressed. In the following text, recommendations will be given for sampling, sample replication, subsampling, preservation and staining, sample treatment, faunal analyses, sample and data management in bio-monitoring studies. For each of these topics, some of our recommendations are mandatory. These conditions have to be implemented in order to qualify for the future FOBIMO norms. Other recommendations are simply advisory. These recommendations are useful in most routine studies, but may not be applicable in some, very specific ecosystems (Table 1). Finally, in some cases we indicate the possibility of additional procedures, which are useful, but do not necessarily have to be part of a standard monitoring study.

We are aware that our recommendations are based on experiences gained mainly in temperate to high northern latitudes. Environmental monitoring in tropical near-shore areas and coral reefs may require different techniques (Wilson, 1998, 2008; Hallock et al., 2003).

Table 1
Summary of recommendations for methods in foraminiferal bio-monitoring.

Topic	Mandatory recommendation	Advisory recommendation
Sample acquisition	• The interval 0–1 cm should be used.	• A tube with 8 cm in diameter or a surface area of 50 cm^2 is proposed as standard device. • Sampling should preferably be done at least once a year. • Autumn samples offer best perennial persistency. Bloom periods are to be avoided.
Remotely operated sampling devices	• Soft sediments (e.g., muds, sands, and oozes): any interface corer or box corer that keeps the sediment surface intact should be used. • Grab samplers are considered as inappropriate for soft sediments and sands.	• Only one type of corer should be used in a particular sampling campaign. • Hard bottoms: grabs are only to be deployed on hard grounds.
Replication	• Three replicates are necessary to capture the variability of the system. • Each replicate is to be treated independently.	• Replicate samples should preferably come from different deployments. • In case replicate samplings come from a single deployment, they should be as far apart as possible.
Sub-sampling	• The zero level is to be defined with the midpoint of any irregular surface. • For the study of the 0–1 cm level, the whole area within the core-liner is to be sampled. • The sample volume has to be determined in a reliable way.	• When sediment layers deeper than 1 cm are sampled, the outer millimetres of sediment close to the core-liner should be removed.
Preservation and staining	• Samples should be stored in a preservative or fixative. • In order to distinguish living from dead foraminifera, a vital stain should be used. • The stain is to be added to the preservative prior to its addition to the sample. • The volume of preservative to be added should at least be equal of the volume of the sample. • Minimum time for staining with a preservative—rose Bengal solution is 14 days. • Samples have to be shaken gently until an entirely homogeneous mixture is obtained.	• Ethanol of >70% is strongly recommended as preservative. • When using rose Bengal, a concentration of 2 g per litre of preservative is recommended. • In environments with high organic matter contents, it may be necessary to add a larger volume of preservative. • In low-oxic to anoxic environments where the decay of the dead organisms is very slow, it is strongly recommended to use more critical vitality assays.
Sample preparation	• Samples are to be washed on a 63-μm screen. • Commercially available wet or dry splitters should be used for sample partitioning.	• Heavy liquid separation should be avoided as much as possible.
Faunal analysis	• Bio-monitoring studies should be based on the living fauna. • For at least one replicate, all counted foraminifera have to be picked and stored in micropalaeontological slides. • The study should be based on the faunal inventory of the >125 μm fraction. • Splits are always to be counted entirely. • Soft-shelled species are not considered in routine monitoring studies.	• In certain environments, it may be necessary to analyse the >63 μm fraction. • A target value of 300 counted specimens is recommended. • The same split-size should be used throughout the study. • Wet or dry picking are both considered as appropriate. • The colouration intensity of specimens considered as living should be assessed for every individual species. • A later re-wetting of the dry-picked specimens can help to better assess the staining. • Breaking of miliolids and agglutinants to check for cytoplasm may be necessary. • The analysis of the dead faunas may add important additional information.
Documentation	• Census data and slides with picked specimens have to be archived. • A list of all recognised species has to be archived. • All available laboratory data are to be documented and archived.	• If available, untreated sample replicates or splits are to be archived.

These schemes are not yet established and should be subject of a subsequent contribution.

2. Sampling strategy

Reliable sample acquisition is an essential prerequisite for a successful environmental monitoring study. Different sampling strategies are needed for different substrates and in different environments. For instance, undisturbed surface samples may be difficult to obtain in organic-rich, muddy sediments. Conversely, sandy bottoms may hamper adequate penetration of the corer and thus prevent sampling. Deep-sea ecosystems can only be sampled with remotely operated sampling devices from sea-going vessels, which is time-consuming and costly. Only a few deployments per working day are feasible. In coastal waters, sampling is less time-consuming and tens of samples can be collected per day.

2.1. Sample acquisition in intertidal and shallow subtidal areas

In the intertidal zone, hand-held devices are used. Quantitative samples of surface sediment are scooped out of a short, transparent plastic tube segment or ring of appropriate diameter inserted into the sediment on tidal flats. Compression of surface sediments is negligible as the tube is inserted only a few centimetres. The 1-cm sediment depth is indicated by a succession of 1-cm high varnish bands of different colours on the outside of the transparent tube. The tube is inserted up to the first colour change, and the sediment is scooped out until the next, 1-cm deeper colour change becomes visible. To obtain stacked slices, a longer tube is pushed down into the sediment, and the core is handled in the same way as that retrieved with any interface corer as described below. On salt-marsh meadows, a chamfered, sharp steel cylinder with appropriate inner diameter is turned through the vegetated surface, excavated, and the sample is cut off with a knife from the top of the core (Scott and Medioli, 1980; Horton and Edwards, 2006).

Fig. 1. The design of the Bowser-corer operated by scuba divers: 1, flexible drain; 2, valve; 3, tight cap; 4, liner; 5; rubber stopper coated with neoprene; and 6, extruder.

Shallow subtidal areas are hardly accessible to sea-going ships. Samples are taken from boats, by divers, from permanent seashore installations like jetties or platforms, and from fast ice at high latitudes. Small-sized box-corers and interface corers designed for lakes are suitable in shallow seas. These lightweight samplers are easier to be deployed with a long rod rather than using a winch and cable.

Non-cohesive, sandy sediments are common in shallow-water areas, and tend to slip from corers. The hand-held Bowser-corer, pushed into the sediment by divers, holds sediment efficiently (Fig. 1). The diver pushes the capped liner with the valve open into the sediment, closes the valve, pulls the liner vertically, simultaneously dips the other hand holding the stopper into the sediment, and inserts the stopper.

2.2. Sampling in open marine areas with remotely operated sampling devices

2.2.1. Mandatory—an interface corer or box corer, or any other device that keeps the sediment surface intact should be used for soft sediments such as muds, oozes and sands

Among a large variety of seabed samplers (Murdoch and MacKnight, 1994), corers and grabs have extensively been used in recent foraminiferal surveys (Scott et al., 2004). Conventional gravity or piston corers equipped with a core catcher may create a bow wave, which blows away the uppermost, unstable surface sediment layer when the nose cone touches the ground (McIntyre, 1971). Interface corers like Barnett multiple corer, Niemistö corer, Rumohr corer or similar devices are deployed without a core catcher, thus the bow wave is minimised (Meischner and Rumohr, 1974; Niemistö, 1974; Barnett et al., 1984), and the sediment surface remains mostly undisturbed. The core is retrieved with supernatant bottom water, which is sealed by lids or bungs during recovery. An inner core diameter of 8 cm is recommended in order to meet the requirement of 50 cm^2 surface area.

Interface corers can successfully operate in fine-grained sediments only. On sandy bottoms, a box corer facilitates a good recovery (Bouma and Marshall, 1964). The box corer should have an appropriate design that minimises the bow wave, and seals the sample with a tight spade and top lids, in order to protect against water turbulence when the corer is drawn back to the surface. The slacking speed of the winch has to be reduced before the box corer touches the seabed and some time has to be given for penetration after bottom contact.

When operated carefully, interface and box corers provide the best sediment surface preservation among all seafloor sampling devices. A further improvement of sample quality can be achieved when video-guided devices are used, for instance push-cores deployed with a Remotely Operated Vehicle (ROV). At present, ROV operations are costly and limited in time and areal extent, but ROVs will probably be used routinely in the near future.

2.2.2. Mandatory—sampling with a grab sampler is considered inappropriate for muds and sands, and should be avoided in all soft bottom sampling

Grab samplers create a strong bow wave when they touch the ground. Furthermore, grabs may only scrape the surface, distort the structure of the underlying sediments, and often do not close accurately (Wigley, 1967; Riddle, 1989). A large part of the sample is washed out when the grab is hoisted through the water column, and an intact sediment surface is rarely preserved.

2.2.3. Advisory—only one type of corer should be used in a particular sampling campaign

Systematic offsets in population densities have been observed between box corer and multicorer deployments at the same location (Bett et al., 1994; Shirayama and Fukushima, 1995). However, if different substrata are targeted, the sampling device should be changed.

2.2.4. Advisory—grabs are to be deployed on hard grounds

Van Veen or Shipek grabs are the only devices which provide samples from locations where rock outcrops, pebbles, or veneers of lag sediments are encountered (Van Veen, 1936; Shipek, 1965). These hard bottoms prevail in high-energy environments or where sediment bypassing occurs. Grabs mainly recover loose or prominent objects and large epizoans. Foraminifera prefer to live attached to stable objects under such conditions, while they are very rare on gravel or coarse sands around these prominent objects because of the frequent sediment redeposition (Schönfeld, 2002). Grab sampling therefore facilitates an assessment of the living fauna on hard grounds, although with certain limitations.

2.3. Foraminiferal sample acquisition

2.3.1. Mandatory—the 0–1 cm interval should be sampled

The surface interval chosen in different foraminiferal studies has been 0–0.25 cm to 0–2 cm, and sometimes even thicker (Moodley and Hess, 1992; Alve and Murray, 2001; Scott et al., 2001; Buzas et al., 2002). In order to obtain monitoring data, which are comparable between studies, they should evidently be based on the same sampling interval. A very thin surface interval of 0–0.5 cm or even less is difficult to collect, and the actual thickness will vary between collecting persons. Too thick an interval, on the other hand, will dilute foraminifera living at the sediment surface by a large number of empty, subfossil tests from deeper sediment layers. Furthermore, the cytoplasm of recently dead specimens degrades slowly in anoxic deeper layers, which can bias census data of rose Bengal

stained specimens (Bernhard, 1988; Murray and Bowser, 2000). The compromise is an intermediate thickness of 1 cm as was applied in many previous monitoring studies (e.g., Cearreta et al., 2000; Murray and Alve, 2000; Leorri et al., 2008). Furthermore, the diversity of living assemblages >63 μm from the 0 to 1 cm level of surface sediments along the Norwegian Skagerrak coast provided the most optimal evaluation of ecological quality at the study sites as compared to the deeper, 1–2 cm level (Bouchet et al., 2012). Therefore, we suggest to systematically use the 0–1 cm interval in foraminiferal bio-monitoring.

The recommendation to record living specimens in the 0–1 cm interval applies to sediment-dwelling foraminifera. Many foraminiferal species, however, cling to or encrust elevated substrates (Murray, 1970; Brasier, 1975; Poag, 1982; Steinker and Clem, 1984; Jones and Charnock, 1985; Kitazato, 1988). In littoral and shallow subtidal environments, elevated substrates are diverse and often have a complex three-dimensional shape, e.g. sea grass, kelp, coralline algae, mangrove roots, corals, pebbles or stones. There is yet no established practice to quantitatively monitor foraminifera dwelling on branching or elevated objects.

2.3.2. Additional possibility—in some areas the study of deeper sediment levels will yield important additional information

Vertical series in intertidal and shallow subtidal settings provide valuable information on foraminiferal ecology, in particular the habitat depth of endobenthic species and their variability, and on environmental conditions (Moodley and Hess, 1992; Alve and Murray, 2001; Hyams-Kaphzan et al., 2009). Processing of such depth-series is rather time-consuming. Additionally, organic-rich subsurface sediments in near-shore areas are often anoxic and contain free H_2S, which is lethal to many shallow-dwelling species and thus the respective levels lack living foraminifera (e.g. Lipps and Langer, 1999; Alve and Murray, 2001). For these reasons we suggest to employ only surface sediment samples for standard bio-monitoring studies. In some cases, however, the study of deeper sediment levels may be appropriate since it can yield important additional information.

2.3.3. Advisory—a sample size of 50 cm^2 (tube with 8 cm in diameter) is recommended, population density is to be standardised for 50 cm^3

Living foraminiferal population density may reach 100 specimens per 10 cm^3 (size fraction >125 μm) in near-shore areas that are rather rich in food, but values may drop to tens of specimens and even less during winter at mid to high latitudes (e.g., Lehmann, 2000; Murray, 2006). To optimise the possibility to obtain statistically significant numbers of living foraminifera per sample, we recommend a sample size of 50 cm^2 (a tube of 8 cm in diameter). If the sample size is different, population density should be normalised to 50 cm^3 of the 0–1 cm surface sediment level.

2.3.4. Advisory—sampling should be done at least once a year. Bloom periods are to be avoided, and autumn samples offer the best perennial persistency

As in many other groups of benthic organisms, foraminiferal assemblage composition and population density may vary greatly between seasons and years. Sporadic, annual or even seasonal surveys may give results that are difficult to interpret because foraminifera can show significant short-term variation in abundance, in particular they reproduce explosively during periods of abundant food (Murray, 2000; Gustafsson and Nordberg, 2001; Morvan et al., 2006). Monthly or even biweekly surveys are informative with reference to such short-term variability (Murray and Alve, 2000), but will not be feasible in most monitoring studies. To minimise year-to-year scatter in the data, periods when foraminifera reproduce, following phytoplankton blooms during spring or other seasonal blooms related to river discharge in near-coastal areas, should be avoided. At temperate latitudes, autumn samples offer the best perennial persistency (Morvan et al., 2006; Duchemin et al., 2008). Preferentially, the time window between the terrestrial vegetation season at temperate latitudes and the winter storms should be used for sampling in near-shore areas.

3. Replication

3.1. Mandatory—three replicates are necessary to describe the variability of the system

Organisms inhabiting the seafloor are seldom spread randomly. Patchiness characterises the distribution of macroorganisms and may have scales of centimetres to kilometres (Hall et al., 1994; Raffaelli et al., 2003). The population density of organisms from the meiofaunal size class changes drastically at several spatial scales (McIntyre, 1969; Soetaert et al., 1994; van Gaever et al., 2004). Benthic foraminiferal distribution is also notably patchy (Bernstein et al., 1978; Fontanier et al., 2003; Barras et al., 2010; Griveaud et al., 2010), which was observed in the 1960s already (Buzas, 1970; Schafer, 1971). The general approach to neutralise the above heterogeneity is to obtain replicate samples at each site. To prevent a 50% to 50% situation in comparison of data from replicates, an odd number of samples are normally taken, in particular 3, 5, 7 or 9. The routine in macrobenthos surveys is 7 or 5 replicates (e.g., Borja and Dauer, 2008). Foraminiferal distribution studies are often based on non-replicate samples, but these results may bring an unconstrainable variability and therefore are not considered as being representative by the bio-monitoring community. In studies where replicates were taken, five replicates per station have earlier been applied in foraminiferal research, and sometimes the number was reduced to three for practical reasons (Buzas, 1970; Suhr et al., 2003; Kemp et al., 2011). Obtaining foraminiferal census data is time-consuming and relatively expensive. In bio-monitoring projects, reports must be delivered within a few months and the investigations need to be cost-competitive. Statistical considerations of live (stained) assemblages show that three replicates are sufficient to determine reliable Ecological Quality Status (EcoQS) (Bouchet et al., 2012). Consequently, considering time and cost constraints for bio-monitoring projects, the number of replicates can be reduced to a minimum of three replicates per benthic station.

3.2. Advisory—replicate samples should preferentially come from different deployments

Replicate samples of marine macrobenthos were traditionally retrieved by a grab sampler or box-corer that was sequentially deployed from a vessel at drift. Drifting at ~1 kn, a 15-minute lapse between deployments will result in a 0.5-km distance between the individual replicate sample locations. Thus traditional macrobenthos replicate samples characterise spatial heterogeneity at a scale of tens of metres to kilometres. Smaller distances are required in coastal areas where local conditions commonly change over shorter distances than in the open sea. Surface sediment samples for foraminiferal studies are smaller, 100 cm^2 or less, and several samples can be taken from one multicorer, for instance sampling the individual cores, or from one box-core surface. In such cases, the distance between the samples will always be some tens of centimetres. Therefore, to characterise spatial heterogeneity at the same scale of tens of metres to kilometres as macrobenthos surveys do, and to avoid pseudoreplication (Hurlbert, 1984), foraminiferal replicate samples should preferentially come from separate deployments.

3.3. Advisory—in case replicate samples come from a single deployment, they should be as far apart as possible

How much time each deployment of a remote sampling device takes depends on winch speed and water depth. To this one must add the handling time on deck that is needed to recharge the interface corer or box-corer for the next deployment. Because of ship-time constraints, the possibility to obtain more than one interface corer or box-corer deployment per station is a rare option. If replicate samples come from a single deployment (i.e., pseudoreplication), they should be taken as far apart as possible. In case of a multicorer, cores from

opposite edges of the array should be used. In case of a box-corer, the samples should be taken from different corners of the box. Sampling too close to the edge should be avoided, however, as flushing of sediments often occurs in the corners and the surface layer may no longer be intact close to the corners of the box.

The issue of replicates obtained with separate or single deployments will probably become less problematic in the future. A growing number of research vessels are now equipped with advanced dynamic positioning systems, so it takes far less time to stabilise the position, and samples retrieved with different deployments on the same spot are only a few metres apart due to slope changes of the cable. Newly manufactured box-corers can be recharged almost as quickly as grabs, and multiple deployments at shallow depths become more affordable.

3.4. Mandatory—each replicate has to be treated independently

Averaging of replicate counts is the standard procedure (Buzas, 1970; Suhr et al., 2003; Kemp et al., 2011). Publications and reports often refer to these mean values only; so it is tempting to lump the replicates, and to perform only one census in order to save time on processing and counting. However, replicate counts characterise the variability of the system. This information must be preserved at all cost, therefore each replicate has to be treated independently. However, final lumping of counts may be necessary when the total numbers of living specimens obtained from individual replicates are too low for a statistically significant census (Schönfeld and Numberger, 2007a).

4. Subsampling

4.1. Mandatory—for analysis of the surface 0–1 cm level, the whole area within the core-liner is sampled

Sub-sampling has to be done immediately after retrieval of the core. The surface 0–1 cm is sliced off using *horizontal* movements (Fig. 2). The whole slice (i.e., all sediment within the graduate core-liner) is transferred to the sample container. Nothing from the margin of the core should be removed in this case. Horizontal movements are important to avoid sediment from the subsurface layer to get attached to the cutting plate and to eventually contaminate the surface sample. In coarse-grained carbonate sediments, the cutting plate may be obstructed by large shell fragments. In this case, the surface 0–1 cm should be sampled by using a spoon and pair of tweezers to remove the fragments.

4.2. Advisory—when sediment layers deeper than 1 cm are sampled, the outer millimetres of sediment close to the core-liner should be removed

The latter is done to avoid including sediment from shallower depths which may have slid or been smeared down along the inside of the core-liner. Such contamination may create irregular or artificially extended habitat depth ranges of surface dwelling species and has to be avoided.

4.3. Mandatory—the zero level is to be defined by the midpoint of an irregular surface

If the sediment surface is irregular, the zero level (i.e., the reference level for the sediment–water interface) is defined as the midpoint between the highest and the lowest point on the irregular surface. Sampling is to be done with a graduated core-liner ring and cutting plate as described above.

If the sediment surface is tilted, the .surface 0–1 cm should be sampled by using a small spoon rather than a sectioning plate.

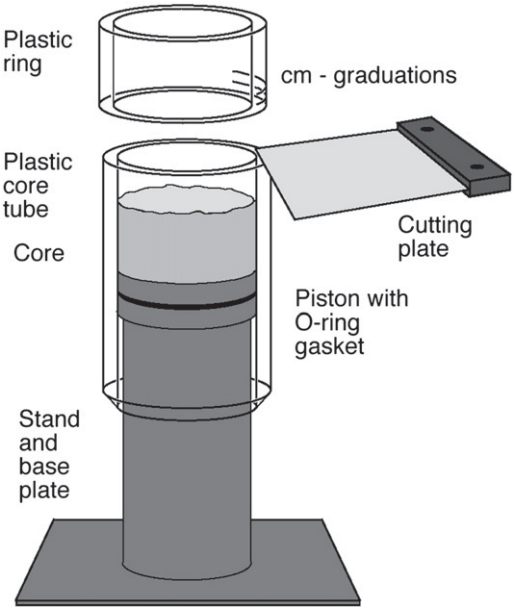

Fig. 2. Illustration showing how to subdivide a sediment core into slices (redrawn after Murray, 2006).

4.4. Mandatory—the sample volume has to be determined in a reliable way

If the sediment surface is smooth and horizontal, the volume of the sampled sediment slice is defined by the inner diameter of the core liner and the thickness of the sediment slice.

If the sediment surface is irregular, the following procedures are recommended to determine the sample volume: transfer the sediment to a transparent container, add a fixed volume of preservative (e.g., ethanol), mark the level of preservative plus sediment on the container wall, and determine the volume of sample plus preservative when the sediment has been removed from the container for processing. In particular, fill the empty container with water up to the mark, measure the volume of water in a graduate cylinder, and subtract the known preservative volume (Fig. 3).

5. Preservation and staining

5.1. Mandatory—samples should be stored in a preservative or fixative

Foraminiferal samples are normally processed weeks or months after collection. It is therefore necessary to add a preservative to the sediment samples in order to prevent decay of the foraminiferal cells by bacterial activities. Ethanol is a good preservative, but it is not a fixative. A fixative stabilises the molecular structures of the cell and should be used for cytological studies (Anderson and Bé, 1978; Hemleben et al., 1989). Formalin or glutaraldehyde are both a reliable fixative and may also be used as a preservative. If formalin is used (e.g. Kitazato et al., 2000, Hughes and Gooday, 2004), it is necessary to buffer the solution with borax in a 4% solution to avoid the dissolution of calcareous tests (Maybury and Gwynn, 1993). If glutaraldehyde is used, a sodium cacodylate buffer should be added (Gooday et al., 2000). Fixative solutions are toxic. They must be handled with protective gloves, and work should be done in a well-ventilated place, preferably in a fume chamber. Such protective measures may not be easily applied on board research vessels.

5.2. Advisory—samples should preferably be stored in >70% ethanol

In bio-monitoring studies the presence or absence of cytoplasm in the tests at the time of sampling needs to be distinguished, whereas

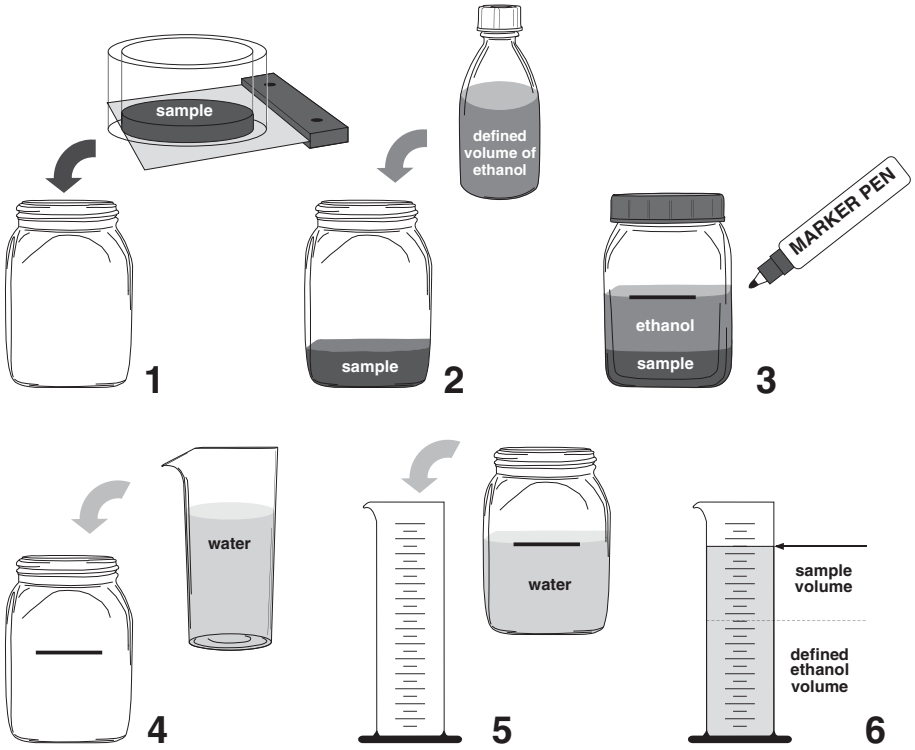

Fig. 3. Instruction how to determine the sample volume.

the quality of cytological fixation is not critical. Therefore, it is not necessary to use fixatives. Ethanol is a reliable and much more practical alternative for sample preservation and storage. In the biological literature, 70% ethanol is reported as the minimum concentration necessary for long-term preservation of specimens (Lim and Sivasothi, 2004). Therefore, we strongly recommend the use of > 70% ethanol for preservation of foraminiferal samples (Murray, 2006).

5.3. Mandatory—the volume of preservative added to the samples has to be at least equal to the sample volume

This procedure ensures that the preservative is not diluted too much by the pore water so that ethanol concentrations do not fall below 70% (Gustafsson and Nordberg, 1999; Murray, 2006). For sediment samples with high organic content and strong bacterial activity, it may be necessary to add a larger volume of preservative.

5.4. Mandatory—in order to distinguish living from dead foraminifera, cytoplasm staining should be used

Two main stains revealing the presence of cytoplasm, in the following referred to as vital stains, are reliable for foraminiferal studies: Sudan black B (Walker et al., 1974) and rose Bengal (Walton, 1952). Sudan black B is a lipophilic stain and results in a black colour of the cytoplasm. A heated solution of Sudan black B has to be applied, which is disadvantageous because it requires time and equipment. Thus, the Sudan black B method is not practical on board research vessels as with field sampling (Bernhard, 2000).

Rose Bengal adsorbs onto proteins and stains the cytoplasm in an intensive pink colour (Walton, 1952). This staining agent is largely used in routine distributional or ecological studies (Bernhard, 2000; Scott et al., 2001; Murray, 2006) because it is inexpensive and easy to use. However, this method is not strictly accurate to distinguish living from dead foraminifera (Bernhard, 2000) because rose Bengal stain is protein specific, and proteins are degraded fairly slowly under certain circumstances. Therefore, rose Bengal may stain proteins that are still in the shell after termination of metabolic activity, i.e., the death of the specimen (Bernhard, 1988; Murray and Bowser, 2000). In fact, the cytoplasm can be preserved in the test for some days to some weeks after the death of the foraminifera (Bernhard, 1988, 2000; Hannah and Rogerson, 1997; Murray and Bowser, 2000). Consequently, this method may lead to a slight overestimation of the living assemblages. In most ecological studies however, rose Bengal has been applied (Scott et al., 2001; Murray, 2006). The Sudan black B method lacks the inherent simplicity of the rose Bengal technique, and this difference may account for the preferential use of rose Bengal as a vital stain (Scott et al., 2001).

5.5. Advisory—a rose Bengal concentration of 2 g l^{-1} of preservative is recommended

For bio-monitoring studies, we recommend a concentration of 2 g of rose Bengal per litre of ethanol as described by Lutze and Altenbach (1991), which is a modification of Walton's (1952) method.

5.6. Mandatory—the stain should be added to the preservative prior to its addition to the samples

This has to be done in order to homogenise the mixture (Lutze, 1964).

5.7. Mandatory—samples have to be shaken gently until entirely homogenised

This procedure ensures that all sediment clumps are disintegrated in order to facilitate the diffusion of the preservative and dissolved vital stain into the foraminiferal cell.

5.8. Mandatory—the minimum time for staining with a preservative—rose Bengal solution is 14 days

If the samples are sieved earlier, the protoplasm in some tests of living foraminifera may not be completely impregnated, and they may show a greenish colour (Lutze and Altenbach, 1991).

5.9. Advisory—in environments where the decay of the dead organisms is very slow, it is strongly recommended to use more critical vitality assays

Rose Bengal stain is widely used in foraminiferal studies and can give a reliable temporal integrative picture of the biocoenoses, which is enough for broad-scale faunistic studies (Bernhard, 2000). In low-oxic environments, however, the decay of dead cells is slow and more accurate methods are needed to study the biocoenoses. Two main techniques (Adenosine Triphosphate analysis and fluorogenic probes) are available (Bernhard, 2000). Adenosine Triphosphate (ATP) analysis is a biochemical method to assess the vitality of cells. DeLaca (1986) adapted this protocol for foraminifera. Advantages of the ATP analysis include its accuracy and the possibility to assess large living populations in about the same time as required for traditional staining methods. A disadvantage of ATP analysis is that the samples need to be processed within a few hours after sampling, and determination of volume and wet weight of specimens is indispensable and expensive. Additionally, in samples with abundant dead tests, it is time-consuming to pick out and extract all individuals expected to be living. From a time perspective, the method is impossible to use in routine monitoring involving large numbers of samples. For these reasons, the accurate ATP method is impractical to apply on board a vessel during bio-monitoring studies.

Another technique, which allows obtaining accurate data of biocoenoses uses Cell-Tracker Green 5-chloromethylfluorescein diacetate (CTG) fluorogenic probe (Bernhard et al., 2006). Cell-Tracker Green is a non-fluorescent molecule, which is hydrolysed by non-specific esterase, producing a fluorescent compound when observed with the exact excitation wavelength. When living cells are incubated in fluorogenic probes such as Cell-Tracker Green, the probe passes through the cellular membrane, and reaches the cytoplasm where hydrolysis with nonspecific esterase causes the fluorogenic reaction. Unlike other fluorogenic substances, such as Fluorescent Diacetate, CTG does not leak out of the cell via ion channels in the cell membrane once it is incorporated in the cell (Bernhard et al., 2006). After some hours of incubation with the fluorogenic probes, samples can be fixed in 4% formalin buffered with borax and the specimens can be analysed later. The foraminifera that were living at the time of sampling appear fluorescent under an epifluorenscent stereomicroscope using the exact excitation wavelength whereas the dead foraminifera do not fluoresce and appear black. Epifluorescent microscopes are rare and CTG is expensive, so this technique is recommended for application only when conventional methods fail as with foraminifera from anoxic habitats (e.g., Pucci et al., 2009).

6. Sample preparation

6.1. Mandatory—samples are to be washed on a 63 μm screen

Although the 125 μm-fraction should be preferentially used to obtain census data of benthic foraminiferal assemblages in foraminiferal bio-monitoring as explained below, many ecological studies are based on assemblages >63 μm (Murray, 2006). In ecological investigations, a significant amount of information on species diversity and dominance may be lost when only the >125 μm size fraction is considered (Sen Gupta et al., 1987). In particular, the 63–125 μm fraction often contains small opportunistic taxa that may have a strong response to eutrophication or pollution phenomena (Mojtahid et al., 2006). The possible bias of assemblage data can be tested by comparing the proportion of dominant and common species between the small and larger size fractions by studying the >63 μm fraction for a minimum number of samples (Sen Gupta et al., 1987). We therefore recommend that the >63 μm fraction should always be retained and archived, to facilitate such tests or additional and more detailed investigations.

6.2. Mandatory—commercially available wet or dry splitters should be used for sample partitioning

The surface area of 50 cm^2 or 8 cm in diameter that we propose for sampling may contain a larger number of specimens than the target value of 300 specimens that are needed to assure statistical significance (Patterson and Fishbein, 1989). In this case and to simplify the procedures, splitting of the sample may be necessary. Commercially available wet or dry splitters must be used for sample partitioning. These devices are thoroughly tested and reliable. Wet samples may be split using an appropriate device for suspension partitioning. There are several models available and all provide an efficient method to obtain subsamples (e.g., Scott and Hermelin, 1993). Standard sediment splitters, such as the Otto Microsplitter (Scott et al., 1980) can be used for splitting residues that have been dried.

6.3. Advisory—as much as possible, heavy liquid separation should be avoided

Flotation with heavy liquids has been extensively used for the study of living foraminifera to concentrate their tests and save picking time (Semsatto and Dias-Britto, 2007). Heavy liquids (such as trichloroethylene, carbon tetrachloride, tetrabromoethane) with a density lower than that of quartz sand, cause air-filled foraminifera to float, so that they can be easily separated from mineral grains. However, Gibson and Walker (1967) demonstrated that flotation of foraminifera in certain heavy liquids is not perfect and is species-specific, thereby producing relative proportions, which are significantly different from the original assemblages. For example, they showed that a highly variable percentage of only 6–71% of all foraminiferal tests was obtained with carbon tetrachloride. Although Gibson and Walker (1967) reported a recovery of 97% when floating in bromoform, our experience with samples from the North Sea off Helgoland, which were treated with trichloroethylene (J. Schönfeld et al., unpublished data) showed that only 63% of the living (stained) assemblages floated and the agglutinated species were generally largely underrepresented in the concentrate. Therefore, the incomplete separation may result in significant errors in species abundances and diversity values.

Additionally, these heavy liquids are generally highly toxic. Tetrabromoethane (Brem et al., 1974), carbon tetrachloride and trichloroethylene are even carcinogenic (Orme and Kegley, 2004). Therefore these products have to be handled with special care e.g., fume chamber and rubber gloves (Murray, 2006), which not only reduces the efficiency of the method, but also represents a danger for human health. In some countries these chemicals are banned due to their toxicity (Gibson and Walker, 1967; Scott et al., 2004; Semsatto and Dias-Britto, 2007). For these reasons their use is not justified under any circumstances.

If flotation is absolutely necessary, for instance when working with sands having very low densities of foraminifera, alternative flotation liquids that are less harmful for the health and the environment may be used. A saturated zinc chloride solution was tested on wet samples by Gibson and Walker (1967) but the recovery was not satisfactory. A better result with this flotation agent was obtained on dry sediments by Semsatto and Dias-Britto (2007) with 91% of floated tests, whereas Gebhardt and Rupp (2008) obtained a less satisfactory percentage of 72%.

Sodium polytungstate solution is also a low-toxic heavy liquid. Although the substance is rather expensive, the solution can be reused several times (e.g., Gregory and Johnston, 1987; Husum and Hald, 2004; Abbene et al., 2006). With this heavy liquid, Semsatto and

Dias-Britto (2007) obtained a recovery of 96%. However, our experience with this substance (E. Alve, unpublished) shows that sodium polytungstate, due to its high viscosity, tends to coat foraminiferal tests. Therefore, the concentrate has to be carefully washed after floating. Furthermore, the density of the sodium polytungstate solution must be checked after every re-use. Overall, this reduces the practicability and efficiency of the method.

In summary, in environmental monitoring studies, where precise abundances of foraminifera are required, flotation, even with non-toxic heavy liquids, should be avoided as much as possible because it may create unpredictably underestimated abundances and biased species compositions (Semsatto and Dias-Britto, 2007).

7. Faunal analysis

7.1. Mandatory—studies using foraminifera as ecological indicators should be based on the living fauna

The living fauna mirrors the foraminiferal response to prevailing environmental conditions. The dead assemblage represents the successive accumulation of foraminiferal tests over time depending on species production and taphonomic factors, such as post-mortem destruction and redeposition. In most environments, empty tests are much more abundant than living individuals in sediment samples. Consequently, total assemblages including both, living and dead specimens, are purely artificial though largely resemble the dead assemblages. In order to carry out an ecological study applying foraminiferal presence, abundance and distribution as indicators of environmental influences, only the living fauna should be used. In certain environments, the dead assemblages can also provide important information but they may be biased by post-mortem processes.

7.2. Mandatory—for at least one replicate per station, all counted foraminifera have to be picked out and stored in micropalaeontological slides

By applying this archiving procedure, identification of species can be discussed with and verified by other experts. Such reference slides are essential for achieving a taxonomic consistency. Unless restricted by legal contracts, reports presenting the census data must state where the reference slides are stored and how they are accessible. The species determination should be compared with the holotype description as provided by the Ellis and Messina (since 1940) catalogue (http://www.micropress.org/em/) or other sources available. The current genus name and established synonymies should be updated by consulting the European Register of Marine Species (ERMS, http://www.marbef.org/data/erms.php), the World Register of Marine Species (WORMS, http://www.marinespecies.org/) or the Integrated Taxonomic Information System (ITIS, http://www.itis.gov/).

7.3. Mandatory—the study should be based on the faunal inventory of the >125 μm-fraction

Although most benthic foraminifera are <1 mm in size, the size varies substantially between species. Also, variations in population dynamics and different species' responses to environmental forcing cause variability in the size distribution between different communities. At present, no consensus exists concerning the size fraction on which benthic foraminiferal analyses should be based. The most commonly used fractions in ecological studies are >63, >125 and >150 μm. Some have used >50 μm, >74 μm, and, in polar regions, the size fraction >100 μm has also been used extensively. As pointed out by several authors, studying the larger fraction only, may cause underrepresentation or even the absence of some smaller species and juveniles (e.g., Schröder et al., 1987; Duchemin et al., 2007). Still, it has been shown that the diversity and assemblage composition of both the fine (63–125 μm) and the coarse (>125 μm) fraction of benthic foraminiferal assemblages are significantly correlated with two year minimum values of bottom water dissolved oxygen concentrations (Min[O_2]2 years) (Bouchet et al., 2012). Diversity indices and assemblage composition are commonly used indicators of ecological status. Hence, because both the fine and the coarse fraction of benthic foraminiferal assemblages seem to adequately reflect an important environmental parameter such as dissolved oxygen, and because analysing the >125 μm-fraction is less time consuming than analysing the >63 μm-fraction, the >125 μm-fraction seems to be an optimal choice for monitoring Ecological Quality Status (Bouchet et al., op. cit.). Finally, the >63 μm-fraction contains a higher proportion of unidentifiable juveniles and therefore the census data may have a lower statistical confidence.

7.4. Advisory—in certain environments it may be necessary to use the >63 μm fraction

Although certain benthic foraminiferal species can survive anoxia for weeks to months (e.g., Moodley et al., 1997; Piña-Ochoa et al., 2010), they cannot live and reproduce under permanently anoxic conditions (Bernhard and Reimers, 1991; Alve, 1995b). Unless excess supply of organic material has caused permanently anoxic sediment pore-waters, eutrophic areas commonly support abundant small-sized species with a high turnover rate (e.g., Phleger and Soutar, 1973; Alve, 2003). In such environments it may be necessary to analyse the entire >63 μm-fraction (i.e., not only the >125 μm). Note that in such cases the 63–125 and >125 μm-fractions should always be studied separately, to stay compatible with surveys in other areas. It is essential to analyse the same split size for each of the two size fractions (63–125 μm and >125 μm), so that the original proportions between individuals in the two fractions are maintained. A dissecting microscope with 100× magnification is necessary to reliably identify small-sized species.

7.5. Advisory—a target value of at least 300 specimens per sample is recommended

A count of 300 individuals is recommended to precisely determine the relative abundance of a species that comprises about 10% of the fauna. More specimens may be needed to assess the proportions of less abundant species with certainty (Patterson and Fishbein, 1989). For calculation of species diversity indices, 250 individuals are adequate (Bouchet et al., 2012). When the fauna consists of a few species only, for instance in marginal marine environments, it may be sufficient to count about 100 specimens in order to assess the diversity (Fatela and Taborda, 2002). We recommend that a rationale should be provided once the number of counted specimens is lower than 300. In some, particularly oligotrophic areas, it may not be possible to obtain 300 individuals from a sample with a surface area of 50 cm^2, and consequently all available specimens in the sample should be picked.

7.6. Advisory—the same split-size should preferentially be studied for all samples

To use the same size for all studied samples is essential in order to enable comparison of the number of species between different samples because the number of species is sample-size dependent. For comparative reasons, it is also desirable to analyse samples, which represent the same area. However, since the number of individuals can vary up to hundred-fold or more between samples, sub-sampling, for instance splitting into 1/2 or 1/4, may be necessary. Before starting the foraminifera count, a quick estimation of the density of all samples should be made in order to choose the adequate split size. Within the same study, the same split size should be used for all samples, if possible, to

ensure comparability and to facilitate a robust statistical treatment of faunal data.

7.7. Mandatory—splits are always to be counted entirely

The distribution of specimens in the sample studied, on the picking tray or in the petri dish is not even and homogeneous. The wrong number of specimens and a non-representative species composition will be obtained if the census is terminated when the required 250 or 300 specimens are collected and parts of the sample are left unregarded (Boltovskoy and Wright, 1976). Splits are therefore to be counted entirely.

7.8. Advisory—wet or dry picking are both considered appropriate

A highly significant positive correlation is shown between the diversity of wet-picked and dry-picked assemblages and the same seems to apply for the assemblage composition (Bouchet et al., 2012). As no evidences for a significant difference between the two methods were reported to date, they can be used as equivalent. However, reliable comparisons require consistency in the methodology used, the same method should be used consistently within the same study and, preferably, within the same geographical region. In case of wet-picking, samples are either to be screened under ethanol or tap water. Distilled water typically has a low pH of 5.5 to 6.5, it dissolves the test walls of calcareous species, and it has to be avoided.

7.9. Advisory—the colouration intensity of specimens considered as living should be assessed for every individual species

The coloration of rose Bengal stained specimens considered living at the time of sampling may vary among species. For instance in marginal marine foraminifera, *Ammonia tepida* is light rose, *Haynesina germanica* is more intense, like a raspberry, and *Elphidium excavatum* is yellowish dark red, all occurring in the same sample. Among other Rotaliids, most Polymorphinids always have a very bright stain, whereas staining is very dull in *Cancris auriculus*, *Nonion scaphum* or *Valvulineria bradyana*. *Globobulimina* species show a frothy cytoplasm that absorbs only little stain and appears brownish violet. In general, arenaceous species show a darker colour tone than calcareous tests of Rotaliids. For these reasons, staining criteria will be different for each taxon. If agglutinated tests are composed of both, large and fine particles, the stained protoplasm is often only visible through large quartz grains (Lutze and Altenbach, 1991).

7.10. Advisory—during counting and identification, re-wetting dry-picked specimens can help assess the staining

This applies particularly to species with opaque tests like thick-shelled miliolids and certain agglutinated forms.

7.11. Advisory—breaking the tests of some miliolids and agglutinants to check for cytoplasm may be necessary

For thick-shelled, opaque tests it may be necessary to break the test open, e.g., with a needle, after identification in order to check if they contain stained cytoplasm. Stain in the apertural region may indicate a live (stained) individual and warrants further investigation by wetting or breaking of the test.

7.12. Mandatory—soft-shelled, organic-walled species are not considered in routine monitoring studies

Although they represent a significant part of marine benthic communities, many allogromiid species are small and easily overlooked. They lack diagnostic features and cannot be identified consistently on a morphological basis, and hence they are often undescribed and knowledge about their distribution and ecology is scarce. Allogromiids are time-consuming to sort, and have a very low fossilisation potential (Gooday, 2002). In a comprehensive study covering environments ranging from fully oxic to anoxic bottom-water conditions, excluding the organic-walled species did not lead to a significant loss of ecological information (Bouchet et al., 2012).

7.13. Advisory—analyses of the dead assemblages may provide important additional information

In sediment accumulation areas, the empty tests constituting the dead foraminiferal assemblage can provide information about environmental and biological changes over decades, or even longer periods, whether they were natural or induced by human activities. Hence, analyses of dead assemblages in dated sediment cores can quantitatively describe potential *in situ*, temporal changes in ecological status from reference or pre-impacted conditions through to present-day environmental situations (Alve, 1991; Cearreta et al., 2002; Ruiz et al., 2004; Alve et al., 2009). This way, benthic foraminifera offer a time-perspective, which is unique, compared to traditional environmental monitoring strategies.

8. Documentation

8.1. Mandatory—census data and slides with picked specimens have to be archived

A list of all recognised species has to be archived. They are to be listed as regular taxonomic references with genus and species name, author's name and date. The original counting sheets and prints of census data tables have to be kept and archived. This allows a later re-assessment of faunal data in case inconsistencies or numerical outliers are found. An electronic submission of the data to a long-term archive, such as PANGAEA (http://www.pangaea.de), is required. In the PANGAEA database, only published data sets are available to the public whereas working data are kept confidential (e.g., Fleischer and Jannaschk, 2011).

A growing number of genetic investigations demonstrate that species concepts of many marginal marine foraminifera need to be adapted (e.g. Hayward et al., 2004; Schweizer et al., 2011). It is therefore essential that all slides with picked specimens are archived in a publicly accessible collection or reference centre. This ensures that current species concepts of the investigators are documented and later revisions are possible.

8.2. Mandatory—all available laboratory data are to be documented and archived

This includes sample volumes, weights of residues and size fractions, and weights of subsamples or splits that were made for faunal analyses as essential information. A record of laboratory procedures and preparation steps as well as chemical substances involved is required. An accurate documentation complies with the OECD (Organization for Economic Co-operation and Development) principles of Good Laboratory Practice and ensures compatibility among different laboratories. The laboratory data offer the possibility to recalculate species abundance or faunal densities if required in the future.

8.3. Advisory—if available, untreated sample replicates or splits are to be archived

This facilitates a later re-examination of different grain-size fractions, biometric studies of certain species, geochemical and stable isotope analyses. Such samples are also important for foraminiferal research in general, as they will allow creating a record of long-term

environmental change in certain areas (Schönfeld and Numberger, 2007b), and it will help to track the migrations and persistency of invasive species (e.g., McGann et al., 2000; Nikulina et al., 2008).

9. Conclusions

This paper proposes a detailed protocol for foraminiferal bio-monitoring studies, including all aspects of sample acquisition, replication, sub-sampling, sample preservation, staining, sample treatment, faunal analysis, documentation and data management. In all cases, our recommendations respect correct scientific procedures, but are also motivated by the need to be practical and time-efficient. Our recommendations fall into two categories (Table 1).

Mandatory recommendations have to be respected if the bio-monitoring study wants to concur to the future FOBIMO norms. The most important of these concern living assemblages and include the following (Table 1):

- An interface corer or box corer that keeps the sediment surface undisturbed is to be used for offshore surveys in soft sediments; grab sampler should not be used except for qualitative studies on hard grounds.
- Three replicate samples are to be taken and analysed separately.
- The interval from 0 to 1 cm below the sediment surface should be sampled.
- Samples are to be washed on a 63-μm screen.
- The living benthic foraminiferal fauna of the >125 μm fraction is to be analysed.
- Wet or dry splitters should be used for sample partitioning, and entire splits should be counted.
- Soft-shelled foraminifera should not be included in routine foraminiferal bio-monitoring studies.
- All counted foraminifera from one replicate per station have to be stored in micropalaeontological slides.
- Census data, supplementary laboratory data and microslides have to be archived.

The most important *advisory recommendations* are:

- The sample size should be 50 cm^2, which corresponds to a tube of 8 cm inner diameter.
- Ethanol with a concentration of more than 70% is recommended as a preservative.
- Rose Bengal at a concentration of 2 g per litre ethanol is advised for staining, the stain should be added to the preservative before the alcohol is poured to the sample, and a staining time of at least 14 days is necessary.
- Heavy liquid separation should be avoided.
- In some environments, particularly eutrophic ones, it may be necessary to analyse the >63 μm fraction.
- Wet and dry picking are both considered appropriate.
- The analysis of dead assemblages may yield very important additional information about pre-impacted conditions.
- The study of the living fauna of deeper sediment levels may also yield extra information.
- If possible, untreated samples should be preserved and stored for further studies.

We are convinced that the general application of the standardised methods proposed in this paper will facilitate the use of foraminifera in bio-monitoring studies, and will lead to a general acceptance of this powerful tool. This is essential in a context where monitoring of the anthropogenic impact in vulnerable marine areas and long-term observations of climatic induced environmental changes has become an absolute necessity.

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