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Comparison of different sampling strategies in monitoring zoobenthos and classification of archipelago areas

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The European Water Framework Directive (EC WFD/2000/60) states that all coastal surface waters should achieve Good ecological status by the year 2015. For this purpose waterbodies have been classified, and a subsequent step is to establish monitoring and management plans for each waterbody. We assess what is an optimal, cost-effective sampling design in monitoring zoobenthos for WFD purposes in complex archipelago waters in the northern Baltic Sea. Zoobenthic grab samples were taken from three archipelago areas of different exposure, and the results of two different sampling methods were compared: the traditional replicate sampling method (3–5 replicate samples at each station) and a proposed single-sample method (no replicate samples). The choice of sampling strategy did not affect the general results on basic community parameters. The ecological status of the areas was somewhat over-estimated when the single-sample strategy was used. The replicate design turned out to be more cost-effective as it is less time-consuming in shallow archipelago areas, especially when small boats and hand-held equipment are used. Further, the replicate sample strategy was more conservative in assessing the ecological status, which may prevent too optimistic status classifications of specific waterbodies.

Introduction

The European Water Framework Directive (EC WFD/2000/60) states that all coastal surface waters should achieve Good ecological status by the year 2015. For this purpose, waterbodies have been classified all over Europe using specific biological parameters, namely phytoplankton, macrophytes and zoobenthos (Borja *et al*. 2009, Josefsson *et al*. 2009). The waterbodies are classified into five classes according to water quality: High, Good, Moderate, Poor and Bad. The class boundary between Good and Moderate is the important one, as in all water areas with a lower quality than Good, measures must be implemented to achieve a Good ecological status. A subsequent step of the WFD is to establish monitoring and management plans for each waterbody.

Eutrophication in the Baltic Sea has increased dramatically, affecting biota at all trophic levels (Elmgren 1989, HELCOM 2009a, 2009b). The coastal archipelago areas are most affected due to their morphological features (shallow areas, narrow straits, thresholds) together with high input of organic load from land (Granö *et al*. 1999). In these areas, the water turnover is low, and the archipelago zone functions as a filter between land and the open sea, leading to gradually reduced water quality in such environments (Bonsdorff *et al*. 1997, HELCOM 2009a). There are different options available for selecting the range of ecosystem components to be employed and the resources to be allocated to monitoring programmes. Zoobenthos is well suited for monitoring studies, due to the stationary lifecycle and relatively long lifespan of the animals, as well as their strategic position in the sediment-water interface (Perus and Bonsdorff 2004). The changes in environmental quality are mirrored in altered zoobenthic community parameters (Cederwall and Elmgren 1980, Diaz and Rosenberg 1995, Heip 1995, Norkko and Bonsdorff 1996, Bonsdorff *et al*. 1997) and in functional traits of the organisms (Bremner 2008, Hewitt *et al*. 2008). In stable conditions, only minor qualitative and quantitative changes over time occur, while a disturbance (e.g. hypoxia or organic enrichment) leads to changes in the benthic assemblages. Near polluted areas abundance usually increases, while biomass and species number decreases, due to increase in importance of small, tolerant, opportunistic species (Pearson and Rosenberg 1978). Zoobenthos assemblages in the Baltic Sea constantly live in a stressing environment, due to strong salinity gradient (Zettler *et al*. 2007) and problems with hypoxia (Rumohr *et al*. 1996, Karlson *et al*. 2002). The benthic assemblages in the Baltic Sea consist of marine and/or freshwater species, many of which live on their physiological tolerance limits in this brackish water environment. As a result, the number of species is low and the size of organisms small as compared with those in fully marine environments (Bonsdorff 2006, Perus *et al*. 2007, Gogina *et al*. 2010).

Traditionally in Finland, and elsewhere, zoobenthos for monitoring purposes had been sampled by taking several (usually three to five) replicate samples from a number of stations in an area (Laine *et al*. 1997, Bonsdorff *et al*. 2003, Perus *et al*. 2007, Rogers *et al*. 2008). However, lately it has been proposed that samples should be taken from a higher number of stations, using one sample (i.e. no replicate grabs) at each station to gain in areal cover (Bäck *et al*. 2006, Lax and Perus 2008). In this study, we compare the results of a traditional "replicate-sampling strategy" with those achieved using a "single-sample strategy" in three archipelago areas in the northern Baltic Sea. The aim of the study is to assess what is an optimal, cost-effective sampling design in monitoring zoobenthos for WFD purposes in the low-saline northern Baltic Sea, and to evaluate whether the chosen strategy has an impact on the overall quality-assessment and ecological classification of the area.

Material and methods

The field sampling was carried out between 14 August and 7 September 2007 in three areas of different exposure and degree of organic input to the sediments in the Åland archipelago (N Baltic Sea): inner, middle and outer archipelago areas. The inner area is sheltered from the open sea, lies in close proximity to the shoreline, and has limited water exchange. It is directly affected by human activities, mainly agriculture and food industry. The middle area is semi-exposed and moderately affected by local sources of eutrophication, and is partly influenced by the outer open coast. The outer area is directly exposed to the open sea with high water exchange rates. It is only slightly affected by local human activities, and has low nutrient levels in comparison with the other areas studied, and the sedimentary habitats are more sandy with low organic content. Detailed descriptions for the areas can be found in Aarnio *et al*. (2011). The entire region has been thoroughly studied for benthic infauna and hydrography since the early 1970s, and hence a comprehensive background on the benthic assemblages in these areas exists (e.g. Helminen 1975, Bonsdorff *et al*. 1991, Perus *et al*. 2001, Bonsdorff *et al*. 2003, Perus and Bonsdorff 2004). In all sub-areas, zoobenthic samples were taken using an Ekman-Birge grab sampler (289 cm^2) from two depth zones: ≤ 10 m (3.5–10 m, hereafter called "shallow") and > 10 m (10.5–27 m, hereafter called "deep"). All sites were located on bare sediment bottoms, with no seagrass or algae present. Five replicate samples were taken at six stations in each area; three on shallow and three on deep bottoms (replicate strategy). In addition, 20 single-grab sampling stations spread over the area; 10 on shallow and 10 on deep bottoms (single-sample strategy) were sampled randomly. However, six of the single-sample sites were the same as in the replicate-sample design (the first grab sample at the 3 shallow and 3 deep stations). Detailed information on all sampling stations is given in Appendix. All samples were sieved through a 1.0 mm screen. Fauna were identified to the lowest possible taxonomic level, counted and weighed (wwt). All grab samples were treated individually, e.g. samples were not pooled prior to analyses. An unpaired *t*-test was used to compare the number of species, abundance and biomass between the replicate and single samples in the different depth zones and areas. An overall analysis of sampling strategy effects was performed using multivariate analyses on square-root transformed abundance data (PRIMER ver. 6 statistical package). Bray-Curtis similarity was used for non-metric multidimensional scaling (MDS) ordination, and a two-way crossed PERMANOVA analysis was carried out to test differences found in the community composition between strategies (replicate *vs*. single samples) and depth strata (shallow *vs*. deep) in all areas. In addition, a PERMDISP analysis was performed to test the homogeneity of multivariate dispersions of the two factors (strategy and depth) (Anderson *et al*. 2008).

Ecological status

The ecological status *sensu* WFD was measured using the Brackish-water Benthic Index (BBI; Eq. 1) (Perus *et al*. 2007). A BBI value gives an estimate of the ecological status of an area (High, Good, Moderate, Poor, Bad), wherein the status boundaries are not the same for different archipelago zones due to different environmental conditions and fauna assemblages (*see* Perus *et al*. (2007) for detailed information). BBI is adopted and further developed from the Benthic Quality Index (BQI; Rosenberg *et al*. 2004) and adjusted for low-saline coastal areas, with low species numbers. The sensitivity values for the species have been adjusted to their actual environment (cf. Rumohr *et al*. 1996) in order to represent the local conditions of the northern Baltic Sea. BBI follows the assumption that biodiversity increases with increasing distance from a pollution source along a gradient of disturbance (Pearson and Rosenberg 1978), and can take values between 0 and roughly 1 (Perus *et al*. 2007, Leonardsson *et al*. 2009).

$$\mathrm{BBI} = \frac{\left[\left(\frac{\mathrm{BQI}}{\mathrm{BQI}_{\mathrm{max}}}\right) + \left(\frac{H'}{H'_{\mathrm{max}}}\right)\right]}{2} \times \frac{\left[\left(1 - \frac{1}{\mathrm{AB}_{\mathrm{tot}}}\right) + \left(1 - \frac{1}{S}\right)\right]}{2} \quad (1)$$

where BQI is the benthic quality index (*sensu* Rosenberg *et al*. 2004), BQI_{max} is the maximum BQI value recorded within each type (archipelago zone) after calculating all available data from the national Finnish zoobenthos database “Hertta” (Finnish version only, available after registration at www.ymparisto.fi/oiva), H' is the Shannon-Wiener diversity index ($\log_2$-transformed), H'_{max} is the maximum H' value recorded within type (archipelago zone) after calculating all available data within the national zoobenthos database, AB_{tot} is the total abundance at each station, and S is the number of species/taxa at each station.

The quality assesment for the water areas was calculated according to Leonardsson *et al*. (2009). In this “fail-safe” approach, the data from several sites in the water area are used to calculate the lower confidence limit, which is then compared with the status boundaries. The status class in which the lower limit is situated defines the ecological status of the water area. For the replicate strategy, a BBI was calculated for each station separately. Then an assesment of the water areas (for each depth intervall) based on BBI from multiple sites (here three sites/area and depth) was calculated according to Leonardsson *et al*. (2009). For the single-sample strategy, five grab samples were used to calculate a BBI value (to ensure that the size of the area sampled was the same with both designs). The assessment of the water area was then calculated using two BBI values/water area and depth.

Results

The total zoobenthic community in the studied archipelago areas consisted of 32 species/taxa (Table 1); 30 found using the single-sample strategy, and 26 the replicate-sample strategy. In the inner area, species of freshwater origin were common, while the proportion of marine species was higher in the outer archipelago (Fig. 1 and Table 1). The inner area was dominated by gastropods (*Hydrobia* sp. and *Potamopyrgus antipodarum*), *Macoma balthica* and *Chironomus plumosus*. In the middle area, the assemblage was similar to that in the inner region, but the polychaete *Marenzelleria* sp. was also abundant. The outer area was characterized by the bivalves *M. balthica* and *Mytilus edulis*, together with polychaetes (*Marenzelleria* sp. and *Pygospio elegans*) and the amphipod *Monoporeia affinis* (Fig. 1 and Table 1). The relative species compositions were similar in the inner and middle areas, while it was more dissimilar in the outer area (Fig. 1).

Univariate measures

In the inner area, 10 and 14 species were found using single and replicate samples, respectively. In the middle area, a total of 13 and 18 species

Table 1. Species of the soft-bottom communities in the three archipelago areas (inner, middle and outer) from both shallow and deep samples (+ = present, – = absent). The total number of species/taxa at shallow *vs.* deep stations are given below the table. The total number of species indicates the total number of species/taxa in each area.

Species/taxa	Inner area		Middle area		Outer area	
	Shallow	Deep	Shallow	Deep	Shallow	Deep
Cyanophthalma obscura	+	–	+	–	+	–
Halicryptus spinulosus	–	–	–	–	–	+
Oligochaeta	+	+	+	+	+	+
Bylgides sarsi	–	–	–	–	–	+
Manayunkia aestuarina	–	–	–	–	–	+
Marenzelleria sp.	+	–	+	+	+	+
Hediste diversicolor	+	–	+	+	+	+
Pygospio elegans	–	–	–	–	+	+
Cerastoderma glaucum	+	–	+	+	+	+
Macoma balthica	+	+	+	+	+	+
Mya arenaria	+	+	+	+	+	+
Mytilus edulis	+	+	+	–	+	+
Hydrobia spp.	+	+	+	+	+	+
Lymnea spp.	–	–	–	–	+	–
Limapontia capitata	–	–	–	–	–	+
Potamopyrgus antipodarum	+	+	+	+	+	–
Theodoxus fluviatilis	+	–	–	–	–	–
Ostracoda	+	–	–	–	–	–
Semibalanus improvisus	–	–	+	+	–	–
Idotea balthica	–	–	–	–	–	+
I. granulosa	–	–	–	–	–	+
I. chelipes	–	–	–	–	+	–
Jaera albifrons	–	–	–	–	+	+
Gammarus sp.	–	–	–	–	+	+
Leptocheirus pilosus	–	–	–	+	–	–
Monoporeia affinis	–	–	–	+	+	+
Corophium volutator	–	–	+	+	+	+
Saduria entomon	–	–	–	+	+	+
Neomysis integer	–	+	+	+	+	+
Mysis mixta	–	–	–	–	–	+
Chironomidae	–	+	–	+	+	+
Chironomus plumosus	–	+	–	+	+	–
Total shallow *vs.* deep	12	9	13	16	21	23
Total number of species (32)	15		18		28	

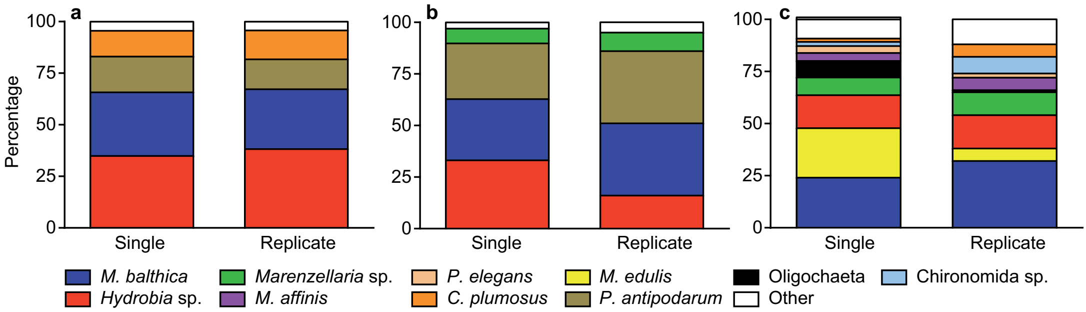

Fig. 1. Relative abundance (%) of the dominant species in single and replicate strategy samples in the (**a**) inner, (**b**) middle and (**c**) outer areas.

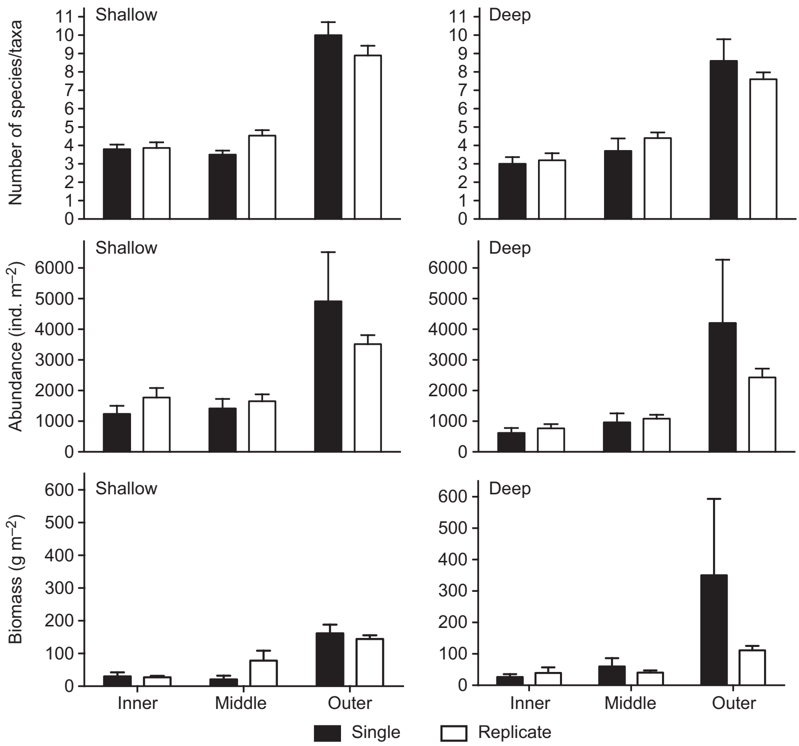

Fig. 2. Number of species, abundance and biomass (mean + SE) in single sample strategy and replicate strategy in shallow and deep bottoms in the three archipelago areas (inner, middle, outer).

were recorded using single and replicate samples, respectively. The outer area had the highest total species number: 28 found using the single-sample strategy, and 23 using the replicate-sample strategy. In shallow bottoms in the middle area, the mean number of species was significantly higher when evaluated using the replicate sampling design as compared with that evaluated using the single-sample design (unpaired t-test: t = 2.466, p = 0.0216) (Fig. 2). In the inner and outer areas, there was no difference in species numbers between sampling strategies. The total abundance and total biomass did not differ between strategies in any area, in either shallow or deep bottoms. Species number, abundance and biomass all showed a tendency (significant for species number in the middle area only) to be higher when evaluated using the replicate design in the inner and middle areas, while in the outer area the single-sample design gave the highest values (Fig. 2). The standard errors (SE) of abundance and biomass evaluated using the single-sample design were higher in the outer area, indicating high spatial heterogeneity (Fig. 2).

Multivariate measures

For the zoobenthic community composition, the MDS analysis revealed a clear grouping between shallow and deep bottoms in all archipelago areas, but no groupings between strategies could be detected (Fig. 2). The two-way crossed PERMANOVA analysis revealed no significant differences between strategies in any area, but confirmed a significant difference for depth ($p <$ 0.001 in all areas; Table 2). There was no interaction between strategy and depth in any archipelago area. The PERMDISP analysis showed a homogenous dispersion within samples from replicate and single sample strategies, while samples from different depth zones had a heterogenous dispersion in the inner and outer areas (Table 2). The average within-group similarity in the inner area was 68.9% in the shallow bottoms compared to only 48.9% in the deep bottoms ($p < 0.0001$). In the outer area, the average within-group similarity was 62.2% in shallow bottoms as compared with 54.2% in deep bottoms ($p = 0.0124$) (Table 2). Thus, the significant results that were found between depths in the inner and outer areas using PERMANOVA may have resulted (at least partly) from the significantly different dispersions in samples from shallow and deep bottoms. In the middle area the dispersion within samples from shallow and deep areas was homogenous.

Ecological status

The ecological status, as evidenced by the BBI values, was higher in the outer area than in the inner area and the single-sample strategy gave a somewhat higher estimate of ecological status as compared with the replicate strategy. In the inner and middle areas, the shallow bottoms were classified as Good by both strategies. In the outer area, the status was classified as Good by the replicate strategy and High by the single-sample strategy. The deep bottoms were classified as one status-class higher when the single sample design was used. In the inner area, the status was Poor (replicate design) and Moderate (single-sample design), in the middle and outer areas the status was Good (replicate design) *vs*. High (single sample design) (Table 3).

Discussion

The results of this study show that the choice of sampling strategy did not have significant

Table 2. Results of two-way crossed PERMANOVA analyses on effects of sampling strategy on number of species, abundance and biomass and PERMDISP analyses on homogeneity of multivariate dispersions in the inner, middle and outer areas.

Source	df	SS (Type III)	MS	Pseudo *F*	*P* (perm)	PERMDISP
Inner area						
Strategy	1	773.89	773.89	0.75938	0.593	0.8525
Depth	1	46349	46349	45.48	0.001	0.0001
Strategy × depth	1	547.12	547.12	0.53686	0.748	
Res	46	46879	1019.1			
Total	49	97246				
Middle area						
Strategy	1	686.17	686.17	0.67361	0.662	0.098
Depth	1	6131.5	6131.5	6.0193	0.001	0.143
Strategy × depth	1	486.57	486.57	0.47767	0.805	
Res	46	46858	1018.6			
Total	49	54593				
Outer area						
Strategy	1	1250.2	1250.2	1.2603	0.266	0.8433
Depth	1	14542	14542	14.66	0.001	0.0124
Strategy × depth	1	587.72	587.72	0.5925	0.774	
Res	46	45629	991.94			
Total	49	67091				

effects on basic community parameters (species number, abundance, biomass) in any of the studied areas. Only the number of species was affected in the shallow depths in the middle area, being significantly higher when the replicate sampling design was used. Also, when looking at the zoobenthic community composition, no significant differences could be found between the two sampling designs. However, the measure of ecological status (using BBI index) was somewhat higher when using the single-sample strategy than with the replicate strategy.

In general, zoobenthos in the Baltic is structured by their environment together with biotic interactions on many levels (Bonsdorff and Blomqvist 1993, Rumohr *et al*. 1996, Bonsdorff and Pearson 1999, Laine 2003, Gogina *et al*. 2010). The archipelago area is a complex system with high spatial heterogeneity. The inner sheltered areas are generally more homogeneous, while the outer areas are more diverse in terms of both sediment quality and benthic assemblage structure. The combination of sediment properties, temperature and oxygen conditions control the zoobenthic assemblages (Bonsdorff *et al*. 2003, O'Brien *et al*. 2003). The outer area is situated in a transitional zone between the coast and open sea, and these areas are generally deeper. The environment is heterogeneous, leading to more diverse zoobenthic assemblages, including species typical of both shallow and deeper areas (Bonsdorff *et al*. 1991, 2003). In our study, the outer area included more species, but the variability between samples was higher — especially when using the single-sample strategy (as shown by larger error bars in Fig. 3) — making it more difficult to interpret the results and to make temporal comparisons as well as direct numerical comparisons with the more stable inner and middle areas. In the inner and middle areas, the environmental conditions (e.g. sediment quality, salinity and temperature) are more uniform, and spatial heterogeneity in zoobenthic assemblages are generally smaller. In some deeper spots local hypoxic conditions may occur and lead to impoverished communities (Perus and Bonsdorff 2004).

Depending on the scale of interest, zoobenthos is more or less patchily distributed over an area (Hewitt *et al*. 2008). When choosing appropriate sampling sites one should consider topography, depth, exposure, sediment quality and hydrographical conditions of the area. Different habitat types, and thus probably a higher number of species, should be included into the analysis even though this will probably increase the variability between samples (Glockzin and Zettler 2008, Gogina *et al*. 2010). In our study, the variability between samples in an area was lower when the replicate design was used regarding the univariate measures species number, abundance and biomass. In the single-sample design, the variability was higher, but it probably covers several habitat types (*see* the higher total species number in Table 1). However, when analysing the zoobenthos community composition (using multivariate analyses), the dispersion within samples in an area was similar for both designs, and no significant difference could be found between the two strategies. Only samples taken from different depth zones showed different dispersion (except in the middle area), and samples taken from deep bottoms were

Table 3. Ecological status, BBI index and status class boundaries in the different archipelago areas (inner, middle and outer) and depths (shallow, deep) in single samples and replicate samples. Status: P = Poor, M = Moderate, G = Good, H = High.

		Single samples		Replicate samples	
Area	Depth	Status (BBI)	Class boundary	Status (BBI)	Class boundary
Inner	Shallow	G (0.48)	0.35–0.58	G (0.48)	0.35–0.58
	Deep	M (0.30)	0.22–0.34	P (0.21)	0.11–0.22
Middle	Shallow	G (0.44)	0.42–0.70	G (0.50)	0.42–0.70
	Deep	H (0.58)	> 0.53	G (0.45)	0.32–0.53
Outer	Shallow	H (0.77)	> 0.74	G (0.71)	0.44–0.74
	Deep	H (0.67)	> 0.62	G (0.56)	0.37–0.62

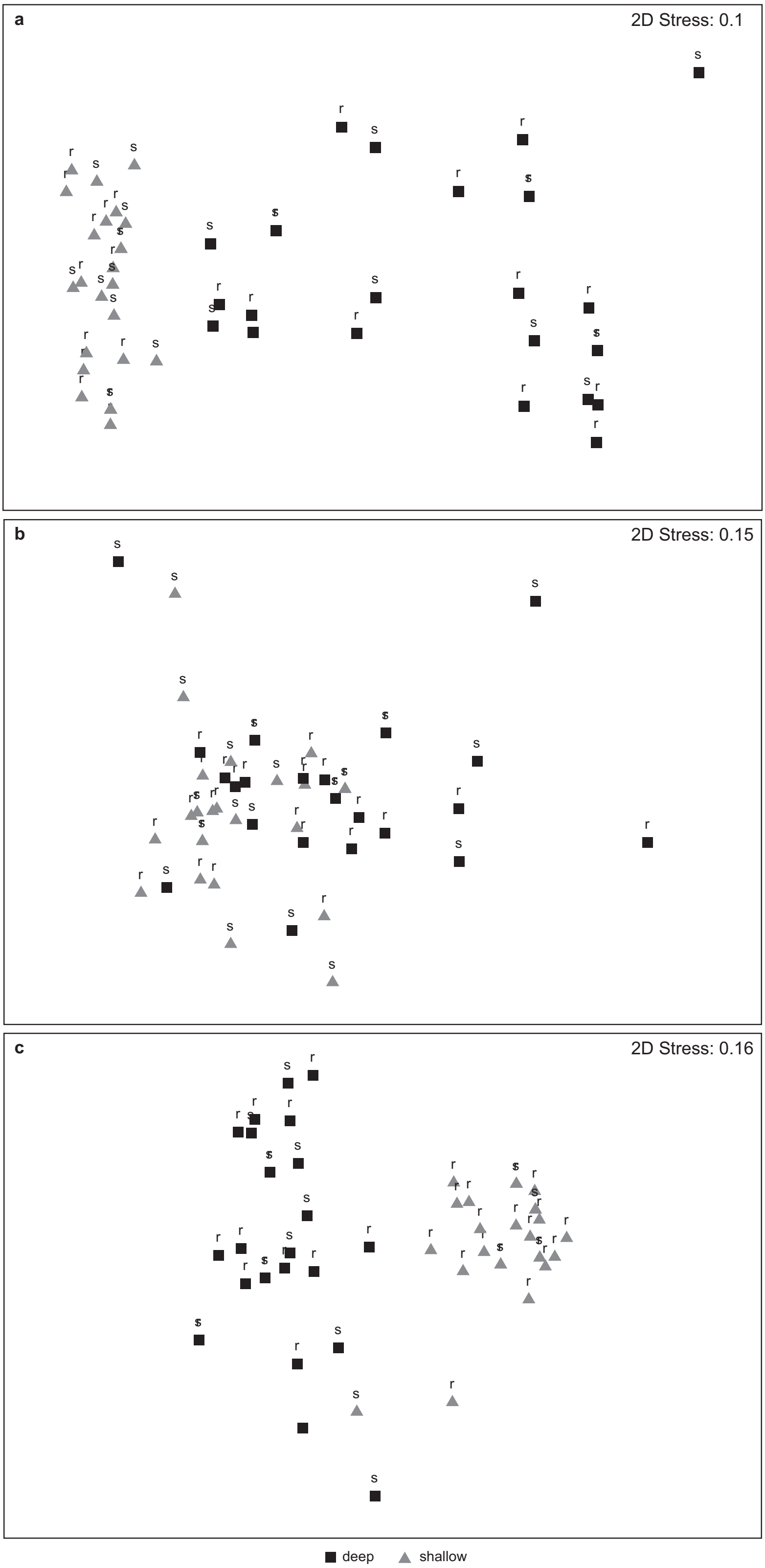

Fig. 3. MDS ordinations for single (s) and replicate (r) samples in the (**a**) inner, (**b**) middle and (**c**) outer areas (Bray-Curtis similarity based on square-root transformed abundance data for 32 species/taxa).

significantly more dispersed than samples from shallow bottoms (Fig. 3). An appropriate sampling design should also be thought of if the aim is to sample for a specific species, as patchily distributed species seem to be more effectively included in the studies when using the single-sample design (e.g. *Mytilus edulis* in Fig. 1c).

The distribution of sampling effort between many stations sampled with few or no replicates, or fewer more intensively sampled stations, depends very much on habitat variability and the objectives of the survey (Rice 2003). Single sample strategy has been proposed by K. Leonardsson (Umeå University, pers. comm.) to be used in monitoring of zoobenthos in the open coasts of the Gulf of Bothnia and in Swedish west coast areas. Within an area, variance can be reduced either by increasing the number of replicates or by increasing the number of sampling stations. In Leonardsson's investigation, it turned out that the single sample strategy would be the best and most economic strategy for monitoring studies of these rather large and uniform areas. Westberg (1978) concluded that due to spatial distribution of macrofauna in the Åland area, several replicates per station should be taken to decrease the variance at a station. Also, a study by Rogers *et al*. (2008) in offshore sand and mud sediments showed that a relatively high cost of collection was necessary to sample macroinfauna and relatively high numbers of macrofauna sample replicates were required for univariate measures such as species number, abundance and diversity. The analysis of statistical power of their surveys showed that in general, many replicate samples of fauna would be needed from each site to observe a 10% difference in univariate biological metrics and attributes 80% of the time. No single sampling regime can efficiently and adequately sample all components of the benthic community, and selecting optimal methods will require a clear understanding of the policy requirements that are to be fulfilled, and the resources available (Rogers *et al*. 2008). Also, in our study this was true when univariate measures were considered, but when the whole zoobenthic assemblages were analysed, the choice of a sampling design did not give diverging results.

In the classification of waterbodies according to the WFD, the choice of a zoobenthos sampling design may be important — too few sampling sites in specific waterbodies will give poor estimates even within the waterbodies sampled. Adequate numbers of samples in fewer areas will reduce the number of waterbody classifications based on empirical data (Leonardsson *et al*. 2009). In our study, the single sample design gave somewhat higher estimates of ecological status.

In monitoring studies the main issue is to get an overall picture of prevailing conditions and patterns of temporal change for management purposes. Generating data may require considerable investment in sampling programmes, and cost–benefit analyses have been used to optimise the practical aspects of sample collection, processing and analysis. Limited resources for assessment and monitoring programmes in the marine environment will inevitably lead to an examination of some practical issues, e.g. the optimal design of the survey station grid and levels of replication, gears used and taxonomic resolution in processing (Rogers *et al*. 2008). In our study, the replicate and single-sample designs gave very similar results. However, the replicate design turned out to be more cost-effective method, as it is less time-consuming in shallow archipelago areas, especially when small boats and hand-held equipment are used. Further, the replicate sample strategy was more conservative in assessing the ecological status. This may prevent too optimistic status classifications of specific areas in comparison to sampling with single sample strategy. Also, in these areas zoobenthos from the previous 30–40 years was sampled using the replicate design making temporal comparisons easy and suitable.

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Appendix. Replicate- and single-sampling-strategy stations with information on coordinates, depth, sediment type and loss on ignition (LOI). M = mud, C = clay, S = sand, G = gravel.

Station	Strategy	Coordinates (N, E)	Depth (m)	Sediment type	LOI (%)
Inner area					
F1	Single sample	60°15.88´, 19°59.29´	12	MC	7.2
F2	Single sample	60°15.16´, 20°00.35´	7	MC	4.5
F3	Replicate samples	60°14.87´, 20°00.29´	18	MC	7.6
F4	Single sample	60°14.46´, 20°00.81´	14	MC	6.7
F5	Single sample	60°14.01´, 20°01.99´	12.5	M	8.6
F6	Replicate samples	60°13.73´, 20°01.70´	7	MC	5.3
F7	Single sample	60°13.76´, 20°01.41´	19	MC	6.8
F8	Single sample	60°13.21´, 20°02.12´	8	C	6.2
F9	Replicate samples	60°13.28´, 20°01.12´	15.5	CM	7.2
F10	Single sample	60°12.80´, 20°01.10´	7	CM	5.1
F11	Single sample	60°12.53´, 20°01.27´	16	M	7.3
F12	Single sample	60°12.39´, 20°00.48´	5	C	4.7
F13	Single sample	60°12.39´, 20°02.09´	6	C	3.8
F14	Single sample	60°11.90´, 20°01.69´	12	MC	5.3
F15	Replicate samples	60°11.71´, 20°01.02´	6.5	C	4.4
F16	Single sample	60°11.10´, 20°02.02´	6.5	C	4.5
F17	Replicate samples	60°11.81´, 20°02.86´	14	MC	5.7
F18	Replicate samples	60°11.56´, 20°03.59´	6	CM	2.7
F19	Single sample	60°11.30´, 20°03.01´	12.5	M	6.4
F20	Single sample	60°11.00´, 20°03.35´	9	CG	2.8
Middle area					
M1	Single sample	60°16.88´, 19°46.98´	6.5	MC	7.2
M2	Single sample	60°16.17´, 19°48.58´	10.5	MC	7.0
M3	Single sample	60°17.27´, 19°48.62´	7	C	5.7
M4	Single sample	60°17.70´, 19°48.21´	27	C	8.6
M5	Replicate samples	60°18.69´, 19°48.34´	11.5	C	3.9
M6	Single sample	60°19.55´, 19°49.22´	6	M	5.9
M7	Replicate samples	60°19.52´, 19°48.24´	9	C	4.8
M8	Single sample	60°19.61´, 19°46.92´	7	C	7.7
M9	Single sample	60°19.95´, 19°46.99´	14.5	C	6.0
M10	Single sample	60°19.81´, 19°46.02´	5.5	CS	3.0
M11	Single sample	60°20.24´, 19°45.87´	11.5	CG	2.2
M12	Single sample	60°20.52´, 19°46.79´	15	C	9.2
M13	Replicate samples	60°21.13´, 19°46.79´	8.5	MC	3.9
M14	Single sample	60°21.43´, 19°47.19´	11	MC	6.6
M15	Replicate samples	60°21.79´, 19°46.13´	12	MC	8.2
M16	Single sample	60°20.79´, 19°46.10´	20	MC	8.1
M17	Single sample	60°20.98´, 19°45.35´	3.5	CS	0.9
M18	Replicate samples	60°20.66´, 19°44.47´	13	CS	2.8
M19	Single sample	60°20.31´, 19°44.76´	6	CSG	2.1
M20	Replicate samples	60°19.54´, 19°44.43´	8.5	MC	7.0

continued

Appendix. Continued.

Station	Strategy	Coordinates (N, E)	Depth (m)	Sediment type	LOI (%)
Outer area					
E6	Single sample	60°13.12´, 19°32.08´	4	SG	0.4
E11	Replicate samples	60°14.17´, 19°28.24´	19.5	S	1.0
E12	Single sample	60°13.84´, 19°28.48´	16	CS	0.7
E13	Single sample	60°13.51´, 19°29.63´	14.5	S	0.7
E14	Single sample	60°13.52´, 19°29.07´	15	S	0.7
E15	Replicate samples	60°13.14´, 19°29.33´	25	CS	3.8
E16	Single sample	60°13.12´, 19°28.97´	26	CS	2.6
E17	Single sample	60°12.59´, 19°29.59´	23	CS	3.2
E18	Replicate samples	60°12.99´, 19°30.32´	14.5	CS	1.1
E19	Single sample	60°13.48´, 19°31.06´	15	S	1.6
E20	Single sample	60°12.87´, 19°31.71´	16	CS	3.8
E23	Replicate samples	60°14.12´, 19°32.35´	4.5	CS	8.1
E24	Replicate samples	60°14.87´, 19°32.08´	7	MC	10.7
E25	Replicate samples	60°15.18´, 19°31.84´	7	MC	9.9
E26	Replicate samples	60°15.63´, 19°31.84´	6.5	SC	2.5