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# Unravelling heterogeneity of soft bottom communities in littoral zone using cluster analysis: Methodical recommendations

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ABSTRACT

Study of spatial-temporal heterogeneity of the marine benthos is often done using the classification methods, cluster analysis in particular. To date, numerous procedures for determining the number of groups, present in a data set, have been proposed. As a rule, these methods are based on averaged characteristics of the structure of benthic communities, not taking into account the variability of biota abundance or biomass among individual samples. But the number of replicates can strongly affect similarity measures, especially ones, that have often been favored in field studies. The main aim of the present research was to study the effect of different number of replicates on the results of cluster analysis of the benthic community structures. Objects of investigations were the typical soft-bottom associations in the intertidal zone of the White Sea, occupying 9 locations and studied since 2008. The repeated comparisons of community structures described by different number of replicates (10 to 1) in each location were performed. The study showed that the weaker the differences between compared communities, the greater amount of samples were needed to obtain a reliable result. When comparing community structures from different locations at the same year as few as 5 samples were sufficient to obtain relatively reliable community descriptions. However, if the task was analysis of long-term changes in the community structure in the same site, the number of samples should be increased to 11. The reliability of the cluster analysis results depended on 1) similarity level, at which all stations merged into a single group, 2) similarity level, at which clusters were identified, and 3) ratio of these similarity levels.

## 1. Introduction

Study of the macrobenthos distribution is of great interest because benthic organisms are seen as key elements of marine ecosystems in relation to their role in maintaining and regulating ecological processes (Del-Pilar-Ruso et al., 2014).

Analysis of the spatial heterogeneity of macrobenthos often involve sampling at widely-spaced locations (for example, kilometers or tens of kilometers apart) (Morrisey et al., 1992). A common practice in these surveys is to collect a number of replicate samples (or, in some cases, only one sample) at each of several locations or stations (Ingólfsson, 1999; Solyanko et al., 2011; Aneiros et al., 2014 and others).

The question about the number of samples required to describe adequately the community structure, has been raised repeatedly in scientific publications (Andrew and Mapstone, 1987; Norén and Lindegarth, 2005; Eleftheriou, 2013 and others). But none of these studies has given an unambiguous recommendation about quantity of replicates, buttressed by the serious statistical analysis. The number of replicates varied from 1 to 50 per station (50 samples were taken along transects of 1 km) and sampling of 3 replicates has been the most common procedure. Sampling of 20 replicates and more has been carried out for reliable assessment of the individual species abundance (Möller, 1986; Beukema and Essink, 1986). In some cases, it was recommended to conduct pilot studies and estimate the number of replicates needed through the standard error or the coefficient of variation of individual species abundance (Elliott, 1977; Andrew and Mapstone, 1987; Olabarria and Chapman, 2001). However, the number of replicates per station required to provide reliable estimates of density of different species varied considerably (Hartley, 1982; Andrew and Mapstone, 1987). Then the only adequate recommendation is to design sampling so that minimal probability of error in analyses was achieved (Eleftheriou, 2013).

The study of spatial-temporal heterogeneity of benthic communities is often done using the classification methods, the cluster analysis in particular. Clustering is a common first step in the exploratory analysis of experimental data. It is used to find structure in the data, most often

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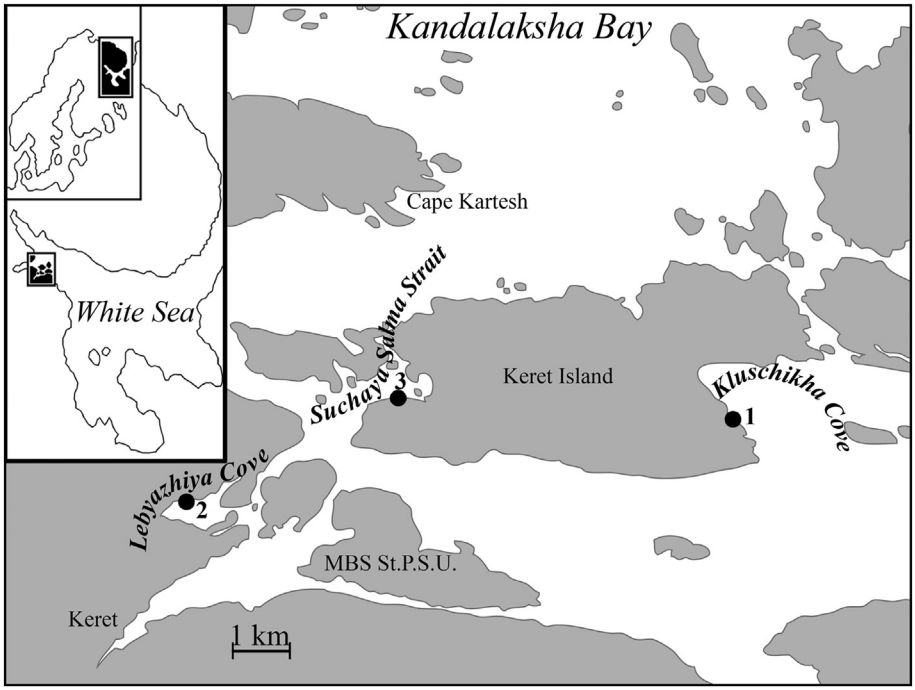


**Fig. 1.** Map of the studied area showing the sampling sites (1–3).

by identifying groups of similar objects (Liu et al., 2008). Nevertheless, many studies have repeatedly discussed the question of the reliability of the selected groups (Tichy et al., 2011; Hennig, 2007; Cao et al., 1997). To date, numerous procedures for determining the number of clusters, present in a data set, has been proposed, for example, bootstrap technique (Jain and Moreau, 1987; Hammer et al., 2001; Hennig, 2007), or the similarity profile routine (Clarke et al., 2008). As a rule, these methods are based on averaged characteristics of the structure of benthic communities, not taking into account the variability of biota abundance or biomass between individual samples. It is also noteworthy, that bootstrap technique and similarity profile routine gave different results. Until now, the impact of sampling design on the results of cluster analysis has hardly been investigated. While it is known that the number of replicates can strongly affect similarity measures, especially ones that have often been favored in field studies (Cao et al., 1997).

The study of the White Sea intertidal benthic communities has been held for decades (Babkov and Golikov, 1984; Beklemishev et al., 1975; Burkovsky, 1992; Burkovsky et al., 1997; Naumov, 2013; Naumov and Fedyakov, 1993; Stolyarov and Burkovsky, 1996; Sukhotin and Berger, 2013; Varfolomeeva and Naumov, 2013 and others). As a result typical benthic associations in the gradients of abiotic environmental variables (sediment composition, tidal stress, thermohaline characteristics, etc.) were described. Since 1980, students and staff of the Ichthyology and Hydrobiology Department, Biological Faculty, St. Petersburg State University, have been performing a long-term monitoring of the structure of several intertidal soft bottom communities at the vicinity of Marine Biological Station (educational and research base “Belomorskaya”) (Kandalaksha Bay, the White Sea) (Gerasimova and Maximovich, 2013; Gerasimova et al., 2006; Maximovich, 1989; Maximovich and Guerassimova, 2003). During 1983–2003 the material was collected by different researchers (including students) and using different sampling gear. Using the data on the species composition, It has been studied how the number of replicates affected the results of the similarity analysis (using a cluster analysis) of the structure of the bottom community at one site in different years of observation (Filippova and Maximovich, 2008). It was shown that 5 samples did not guarantee the absolute repeatability of the classification results. The uneven distribution of macrobenthic organisms in habitats and the human factor (participation of different researchers in collecting and processing the material in different years) have been identified as the main reasons of this result (Filippova and Maximovich, 2008).

In 2008 the number of replicates per station was deliberately increased to 10 and both collecting and processing the data was made by one person. A comparative analysis of benthic community structures at different sites showed that there were differences in the number of replicates (samples) required to obtain reliable classification results depending on the approach to distinguish the communities (Filippova et al., 2015b). When the community structure was described by dominant taxa only 3 replicates were quite sufficient to separate the communities, while using the data on species composition - 5 and more samples are needed, on species biomass - 4 and more samples, on species abundance – 6 and more samples.

These results were obtained by comparing the structure of communities of very different species composition at several different sites, collected in only one year of observations. But it is often necessary to compare benthic associations from very similar habitats, or to study long-term changes in community structure in the same location. Hence the idea of this work was to continue previous research, collecting material at the same sites, by one person with 10 samples per stations. The aim of the investigations was to study the effect of different number of replicates on the result of similarity analysis of the bottom community structure. In particular the work addressed following issues: a) comparative analysis of community structure in different habitats in the same year of observation; b) analysis of interannual changes in community structure in the same habitat; c) the effects of the number of samples and different parameters of cluster analysis (such as standardization and transformation of data) on the results of community structure comparison. As a result of the research it is supposed to identify factors that affect the number of samples, sufficient to reliably describe the macrobenthos spatial-temporal heterogeneity.

## 2. Materials and methods

### 2.1. Study area

The sampling was carried out at three intertidal locations of the Keret archipelago (sites) (Fig. 1). Study sites (1 – Kluschicha Cove (lat. 66°18′N; long. 33°46′E), 2 – Lebyazhiya Cove (lat. 66°17′N; long. 33°35′E), 3 – Suchaya Salma Strait (lat. 66°31′N; long. 33°65′E)) are located relatively close to each other, have approximately the same bottom slope (5–6°), but differ from each other in respect of exposure to wave action, thermohaline regime and sediment composition (Gerasimova et al., 2006; Maximovich, 1989; Maximovich and Gerasimova, 2004; Maximovich and Guerassimova, 2003). The site 1 is a sand beach (with 98% of fractions 0.5–0.1 mm) about 60 m long, subjected to moderate levels of wave activity (see Fig. 1, 1). The site 2 is clay-sand tidal zone (average grain diameter is 0.13 mm) 80 m length, separated from the open sea by the system of narrow straits (see Fig. 1, 2). The site 3 is muddy-sand beach (average grain diameter - 0.12 mm) about 100 m length, moderately sheltered from wave activity. During the hydrological summer (July–August) the water temperature at all the sites is 12–22 °C, salinity varies from 11 to 20‰ (site 2) to 23–28‰ (site 1).

### 2.2. Sampling

The sampling was undertaken in late June - early July in the middle (station A) and low (station B) intertidal zone and in subtidal zone at a depth of 0–0.5 m (station C) at each site. At site 1, the surveys were conducted every year from 2008 to 2013, on site 2 - in 2008 and 2011–2013, at site 3 - in 2008, 2012 and 2013. 10 samples (using frames of 0.1 catching square) were taken randomly at each station every sampling time. Sediments were excavated from up to 8–10 cm depth and washed out through a sieve of 1 mm mesh size. The invertebrate and plants retained on the mesh were removed from any remaining sediment, identified to species level where possible, with exception of the chironomides, amphipodes, oligochaetes, nemertines and filamentous algae, counted and weighed. At the site 2 bivalve *Mya arenaria* dominated by biomass in each station. Because of the different burrowing depth of *M.arenaria* of different age (and size), the sampling was done separately for specimens < 20 mm and > 20 mm in shell length (Maximovich and Guerassimova, 2003). Specimens smaller than 20 mm were collected according to the procedure described earlier (using frames of 0.1 $m^2$ catching square). Frames of 0.25 $m^2$catching square were used to sample larger-size clams (> 20 mm). Sediments were excavated by a spade from up to 30 cm depth. Frame of 0.1 $m^2$ was taken inside a frame of 0.25 $m^2$. At the site 1 all individuals of polychaeta *Arenicola marina* were collected using frame 1 $m^2$. Each frame of 0.1 $m^2$ was taken inside a frame of 1 $m^2$, from which *A. marina* were then scooped.

### 2.3. Data processing

#### 2.3.1. Cluster analysis

Comparative analysis of the community structure was conducted in two ways: comparison of stations from different habitats sampled in the same year (data from 2008, 2012 and 2013–3 sets of stations) and comparison of stations from the same habitats sampled in different years (data from site 1, 2 and 3–3 sets of stations). The comparison of stations was made using classification procedure. Hierarchical cluster analysis with Bray-Curtis similarity measure and Unweighted pair group average method was used to group stations on the basis of biota similarities. It is recommended to restrict attention to a single similarity coefficient but allow a choice of prior transformation of the data (Clarke and Warwick, 2001). Therefore, it was decided to use presence-absence data, transformed to the fourth root and standardized by ranging (Y′ = (Y-Ymin)/(Ymax-Ymin), Clarke and Warwick, 2001) biomass and densities of species and then compare the results. So for each set of stations 5 dendrograms were obtained: 1) from presence-absence data, 2) from species biomass transformed to the fourth root, 3) from species densities transformed to the fourth root, 4) from standardized biomass of species and 5) from standardized densities of species. When identifying clusters on the dendrogram we took into account the possibility of interpreting the results and the relative level of similarity.

To evaluate how the number of replicates affected the results of cluster analysis, new descriptions of community structure were made from the original data, but on the basis of fewer samples (from one to nine). For this purpose 20 (or 10 in case of one and nine samples) variants were selected randomly from all the possible combination of 1–9 samples. Then procedures of classification were repeatedly performed (6 sets of stations, 5 original dendrograms for each station, 160 new variants for each dendrogram – a total of 4805 comparisons were made). The dendrogram based on all 10 samples was considered as an objective result and used as the standard. When dendrogram, based on some number of samples, did not differ from standard, this number of samples was considered sufficient to obtain reliable results discribing the community structure. If dendrogram, based even on 9 replicates, was different from standard, approximate sufficient number of samples was calculated using linear regression analysis.

#### 2.3.2. Factors affecting sampling effort

Obtained values of the sufficient number of samples (*sns*) were examined using one-way Kruskal-Wallis tests and Mann-Whitney pairwise post-hoc tests. To identify the reasons for the *sns* variability the type of data (biomass, abundance or presence-absence), data treatment (Transformations or Standardizations) and the type of the monitoring (one-year observations at different sites or several-year observations at the same site) was considered as factors. We also assumed that some properties of the studied communities influenced *sns*, so the correlation was examined between *sns* and all available community characteristics and their derivates, such as: similarity level, at which all stations merged into a single group (a); similarity level, at which standard clusters were identified (b); ratio of similarity of these levels (a/b); total number of taxa in the station; average, minimal and maximal standard error of mean biomass or abundance of taxa in the station; average, minimal and maximal occurrence of taxa in the station; average, minimal and maximal standard error of mean biomass or abundance of dominant taxa in the station; number of taxa with 100, 70 and 50% occurrence in the station; percentage of taxa with 100, 70 and 50% occurrence in the station. Since the distribution of the variables in question did not as a rule correspond to the normal distribution, Spearman's rank correlation coefficients (at significance level $\alpha \leq 0{,}05$) between *sns* and the variables was calculated. In order to predict a theoretical *sns* depending on the values of the above variables, linear regression analysis was performed. In this case the dependence of *sns* on the variables was assessed using Pearson's correlation coefficient (at significance level $\alpha \leq 0{,}05$).

All statistical analysis was carried out using PAST 3 software (Hammer et al., 2001).

## 3. Results

### 3.1. Community structure

A total of 55 species and higher taxa were found in benthic samples, but only two of them (*Limecola balthica* and *Hydrobia* spp.) were common for all 9 stations. Minimum taxonomic diversity was observed at site 2 (23 taxa), maximum – at site 1 (47 taxa).

Some changes in the structure of benthic communities were observed during the study period (Tables S1–S3). At site 1 in 2008 *L.balthica* and *Arenicola marina* were dominating by biomass (48–71% and 21–24% of the total macrobenthos biomass respectively). In 2009 eelgrass *Zostera marina* became subdominant component (by

biomass) and dominated in low intertidal zone since 2010 and in upper subtidal zone since 2012 (up to 74% of total macrobenthos biomass, Table S1). *L. balthica* was mostly dominating by abundance (20–82% of the total number of zoobenthos, Table S1). At site 3 eelgrass also showed considerable temporal variation by biomass: it dominated in 2008 at all stations, but in 2012 and 2013 - only in upper subtidal zone. In the middle intertidal zone *Fucus vesiculosus*, *L. balthica*, *Mytilus edulis* and *Hydrobia* spp. were dominating by biomass. *Hydrobia* spp. was mostly dominating by abundance (49–92% of total number of zoobenthos, Table S3). Only at site 2 the community structure was stable: *Mytilus edulis* and *Mya arenaria* prevaled by biomass (12–79% and 15–75% of the total macrobenthos biomass respectively, Table S2), *Hydrobia* spp. mostly dominanted by abundance (37–91% of the total number of zoobenthos, Table S2).

### 3.2. Comparative analysis of community structure at different sites in the same year of observations (data from 2008, 2012 and 2013)

*Community descriptions on the basis of 10 replicates.* Analysis of station similarity by standardized biomass and densities of species showed that the stations grouped according to the distribution of dominant taxa. But when station classification was based on transformed biomass and densities of species, in 75% of cases the constructed dendrograms were identical to those, obtained from presence-absence data (Fig. S1).

*Community descriptions on the basis of a different number of replicates.* Analysis of station similarity obtained using a different number of replicates showed that the *sns* (sufficient number of samples) was the lowest for the data from 2012 (1 to 5 replicates, Fig. S2). In most cases (80%) *sns* did not exceed 6 replicates. Only when comparing stations in 2013, the *sns* was > 10 samples (see Fig. S2). The average *sns* was 5 replicates. Thus, in most cases when comparing the structure of communities in different habitats, 1 to 6 replicates (on average, 5) were sufficient for a relatively reliable description of macrobenthos in the station (Fig. 2).

### 3.3. Analysis of interannual changes in community structure at the same site

*Community descriptions on the basis of 10 replicates.* Analysis of station similarity at the same site by standardized biomass and densities of species in different years of observations also showed that the stations grouped according to the distribution of dominant taxa. And when classification was based on transformed biomass and densities of species, in 75% of cases the constructed dendrograms were identical to those, obtained from presence-absence data (Fig. S3).

*Community descriptions on the basis of a different number of replicates.* Analysis of station similarity at the same site in different years of observations by macrobenthos characteristics showed that only in 27% of cases the dendrograms were similar to standard one. In this case, descriptions of benthos at the stations were obtained using at least 9 replicates. The greatest *sns* was characteristic for site 2 (11 to 35 samples, Fig. S4). The average *sns* was 16 replicates. But since the distribution of *sns* was non-symmetrical (positively skewed), it can be assumed that the best estimate of this parameter is determined from the median, which was equal 11 (Fig. 3).

### 3.4. The causes of sns (sufficient number of samples) variability

One-way Kruskal-Wallis tests showed that the type of data and the data treatment did not have significant influence on *sns* (Table 1a). But type of monitoring showed significant impact on *sns* ($p = 0.018$). Mann-Whitney pairwise comparison of the *sns* at different sites showed its significant differences (Table 1b). So we assumed that some characteristics of the communities influenced the *sns*. Therefore, a correlation was studied between *sns* and 19 community characteristics (Table 2). Correlation analysis was conducted separately for various data treatment (Transformations or Standardizations), and for

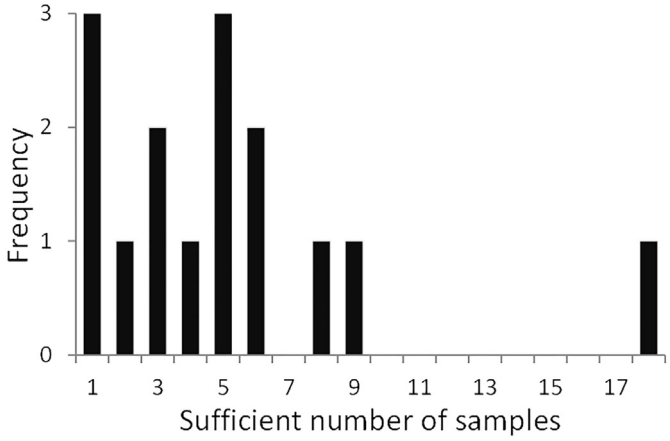


**Fig. 2.** Distribution of sample number, sufficient to obtain reliable results about the community structure on the basis of one-year observation at different sites.

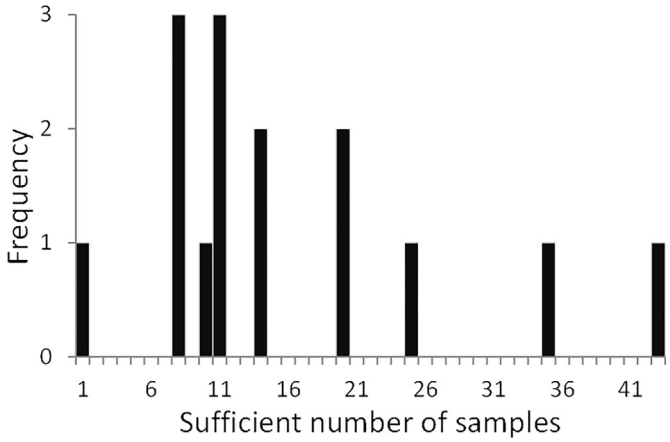


**Fig. 3.** Distribution of sample number, sufficient to obtain reliable results about the community structure on the basis of classification of stations from several years observation at one site.

**Table 1**
Analysis of factors that affect the number of samples, sufficient to obtain reliable description of the community structure: a) Results of one-way Kruskal-Wallis tests (factors: B – biomass, N – abundance, PA – presence-absence data, $\sqrt[4]{}$ - forth root transformation, RT – standardization by ranging, sites 1–2-3 – comparing of the station from different sites for one year of observation, site 1–3 – comparing station from one sites for several years of observation); b) Bonferroni corrected *p* values of Mann-Whitney pairwise comparison of number of samples, required for different sites.

a)

| Factors: | Kruskal-Wallis *p* |
|---|---|
| Type of data (B, N, PA) | 0,4719 |
| Data treatment ($\sqrt[4]{}$, RT) | 0,5305 |
| Type of the monitoring (sites 1–2-3, site 1, site 2, site 3) | 0,0018 |

b)

| | Site 1 | Site 2 | Site 3 |
|---|---|---|---|
| Site 2 | 1 | | |
| Site 3 | 1 | 0,847 | |
| Site 1–2-3 | 0,026 | 0,017 | 0,743 |

**Table 2**
Correlation coefficients (Pearson *r* and Spearman *r*) between sufficient number of samples and 19 community characteristics (also minimal and maximal values of these characteristics). Significant correlations are highlighted in bold. Correlations based on: all data, $^4\sqrt{}$ - forth root transformed data only, $^4\sqrt{}$ + PA - forth root transformed and presence-absence data, RT – standardized by ranging data.

| Community characteristics: | min | max | All data | | $^4\sqrt{}$ | | $^4\sqrt{}$ + PA | | RT | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pearson | Spearman | Pearson | Spearman | Pearson | Spearman | Pearson | Spearman |
| Similarity level, at which all stations merged into a single group (a), % | 5 | 76 | **0,59** | **0,72** | **0,78** | **0,87** | **0,71** | **0,88** | **0,63** | **0,86** |
| Similarity level, at which standard clusters were determined (b), % | 35 | 85 | **0,50** | **0,58** | **0,67** | **0,59** | **0,65** | **0,65** | 0,30 | 0,43 |
| Ratio of similarity levels (a/b) | 0,09 | 0,95 | **0,52** | **0,68** | **0,67** | **0,81** | **0,59** | **0,78** | **0,70** | **0,90** |
| Number of taxa | 22 | 47 | −0,23 | −0,18 | −0,46 | −0,32 | −0,32 | −0,25 | −0,06 | −0,17 |
| Average standard error of mean biomass or abundance of taxa, % | 45,6 | 61,0 | −0,26 | −0,05 | −0,01 | 0,14 | −0,23 | −0,14 | 0,02 | 0,07 |
| Minimal standard error of mean biomass or abundance of taxa, % | 0 | 10,1 | −0,16 | −0,06 | 0,13 | 0,13 | −0,13 | −0,10 | −0,06 | 0,06 |
| Maximal standard error of mean biomass or abundance of taxa, % | 100 | 100 | | | | | | | | |
| Average occurrence of taxa, % | 50,9 | 59,4 | −0,07 | −0,17 | −0,10 | −0,23 | 0,00 | −0,16 | −0,25 | −0,16 |
| Minimal occurrence of taxa, % | 10 | 10 | | | | | | | | |
| Maximal occurrence of taxa, % | 100 | 100 | | | | | | | | |
| Average standard error of mean biomass or abundance of dominant taxa, % | 13,5 | 37,6 | −0,14 | −0,03 | 0,15 | 0,22 | −0,13 | −0,10 | 0,03 | 0,17 |
| Maximal standard error of mean biomass or abundance of dominant taxa, % | 19,7 | 55,0 | −0,12 | 0,10 | 0,20 | 0,35 | −0,12 | −0,05 | 0,14 | 0,44 |
| Minimal standard error of mean biomass or abundance of dominant taxa, % | 4,10 | 16,6 | −0,11 | 0,00 | 0,46 | 0,44 | −0,03 | 0,00 | −0,15 | 0,02 |
| Number of taxa with 100% occurrence | 10 | 16 | −0,27 | **−0,39** | **−0,73** | **−0,62** | −0,29 | −0,39 | −0,25 | −0,42 |
| Number of taxa with > 50% occurrence | 20 | 27 | −0,27 | **−0,69** | **−0,83** | **−0,81** | **−0,60** | **−0,78** | −0,38 | **−0,58** |
| Number of taxa with > 70% occurrence | 17 | 23 | −0,27 | **−0,75** | **−0,89** | **−0,94** | **−0,58** | **−0,83** | −0,42 | **−0,64** |
| % of taxa with 100% occurrence | 27,9 | 61,5 | −0,27 | 0,03 | 0,01 | 0,04 | 0,18 | 0,09 | −0,18 | 0,01 |
| % of taxa with > 50% occurrence | 53,2 | 92,3 | −0,27 | 0,06 | 0,30 | 0,22 | 0,19 | 0,14 | −0,12 | 0,01 |
| % of taxa with > 70% occurrence | 44,7 | 80,8 | −0,27 | 0,05 | 0,28 | 0,16 | 0,20 | 0,12 | -0,13 | 0,02 |

combined data (see Table 2).

Significant correlation of the *sns* was found with 6 considered characteristics (Table 2): (1) similarity level, at which all stations merged into a single group; (2) similarity level, at which standard clusters were identified; (3) ratio of this similarity levels; (4) number of taxa with > 50% occurrence, (5) number of taxa with > 75% occurrence and (6) number of taxa with 100% occurrence at station. The high values of the correlation coefficients in the last three cases related primarily to the objectives of the cluster analysis: comparing the community structure at different sites or analyzing the interannual changes in the community structure at one site, and only indirectly to the percentage of species with different occurrence. When comparing the structure of communities at different areas, the total number of species included in the analysis and, accordingly, the number of species with high occurrence increased. As a rule, in that case, the requirements for sampling (the *sns* value) were the lowest - sometimes as low as one replicate. When analyzing the interannual changes, the total number of species (only for one site) and the number of species with high occurrence were much smaller, and the value of *sns* was usually high (16 on average). Therefore, there is no reason to take into account the revealed correlations between *sns* and the number of taxa with > 50% occurrence, > 75% occurrence and 100% occurrence at station. There was no correlation between *sns* and standard errors of mean biomass or density of species. Thus, only correlations of interest were those between *sns* and similarity levels, and one of them (similarity level, at which clusters were identified) was determined subjectively - by the researcher himself. These correlations were positive and mostly significant (Table 2, Fig. 4), i.e. the weaker the differences between compared communities, the greater amount of samples were needed to obtain a reliable result.

At the Fig. 4 plots, which show the sufficient number of samples for a different similarity levels, the dots fall into two groups (Fig. 4a, c). One of them consists of values, based on the standardized biomass and densities of species; the other includes values, based on transformed biomass and densities of species and presence-absence data. As it was mentioned above, the results of station classification on the basis of transformed biomass and densities of species and presence-absence data coincided in most cases, so we decided to treat this group of values separately. According to the linear regression analysis reliable classification results based on 5 (for example) samples can be obtained if: a) the similarity level, at which all stations merge into a single group is no > 18% for standardized data and 45% for transformed data (Fig. 4a); b) similarity level, at which standard clusters are determined, is no > 60% for transformed data (Fig. 4b); c) ratio of these similarity levels is no > 0,3 for standardized data and 0,7 for transformed data (Fig. 4c). It is noteworthy that when we consider the same similarity level, use of transformed and presence-absence data required fewer samples comparing to standardized data (see Fig. 4a, c). For example, in cluster analysis, researchers often use a 50% level of similarity to separate clusters. In this case, 5–6 replicates will be sufficient to obtain relatively reliable results of cluster analysis based on transformed data, only if the level of similarity, at which all objects merge into one group, will be no higher than 35% (if $n = 5$ than a/b = 0,7; and if b = 50% than a = 0,7*50%, Fig. 4c). Under similar conditions (similar similarity levels, 50 and 35% respectively) and on the basis of standardized data, *sns* would be about 10 (Fig. 4c). In this case, reliable results of cluster analysis with 5–6 replications will be obtained if the level of similarity, at which all stations merge into one group, would not exceed 15%.

## 4. Discussion

In the modern practice of nature management, measures for the development of biological and mineral resources of the sea shelf are impossible without environmental monitoring. One of the principal goals of monitoring is to analyze the structure and dynamics of biosystems in natural and anthropogenically influenced environmental gradients. The traditional object of such observations is marine benthos. The change in marine benthos structure should be recognized as one of the most reliable evidence of changes in natural biotopes. Changes in the structure of the benthos are traditionally detected on benthic communities. The results of such studies are very often amount to the mapping of the most characteristic communities. (Britayev et al., 2010; Denisenko et al., 2003; Pogrebov et al., 1997; Vedenin et al., 2015). Meanwhile, usually, it is necessary to compare obtained results with the ones from previous studies. There are often situations when the previously described biosystems are not detected during repeated studies or new communities are identified. This situation can involve both the environmental changes, as well as errors in the collection of material. In

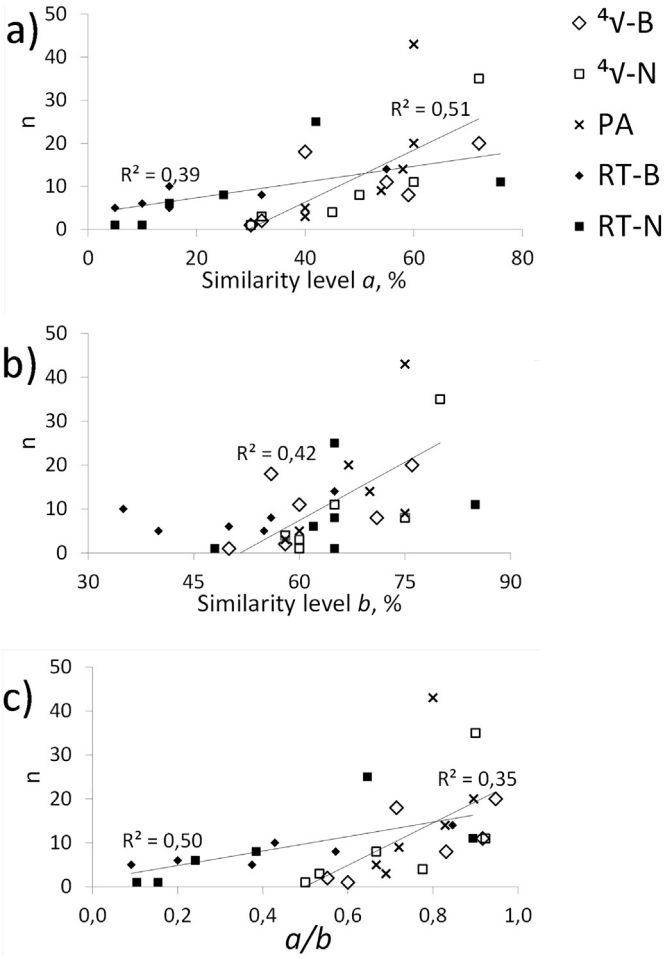


**Fig. 4.** The relationship between sufficient number of samples (n) and a) similarity level, at which all stations merged into a single group (*a*); b) similarity level, at which standard clusters were determined (*b*) and c) ratio of these similarity levels (*a*/*b*). On the plots (a) and (c) coefficients of determination are provided for two groups of values: one consists of standardized by ranging biomass and densities of species and the other consist of forth root transformed biomass and densities of species and presence-absence data. On the plot (b) coefficient of determination is provided only for forth root transformed biomass and densities of species and presence-absence data. B – biomass, N – abundance, PA – presence-absence data, $^4\sqrt{}$ – forth root transformation, RT – standardization by ranging.

comparative analysis of community structure, the classification procedures in particular cluster analysis, are widely used (Quinn and Keough, 2002). The presented study attempted to assess which sampling design could lead to the false results of cluster analysis.

The structural characteristics of the studied intertidal soft-bottom communities, such as species composition, abundance and biomass, were consistent with those revealed in other parts of the White Sea (Babkov and Golikov, 1984; Berger, 1995; Burkovsky et al., 1997; Gerasimova et al., 2016; Golikov et al., 1985; Naumov, 2013; Varfolomeeva and Naumov, 2013). Moreover, similar in terms of structure and dominant taxa communities were described in soft bottom areas of the Barents Sea, Wadden Sea and North Sea (Beukema, 1976; 1989; Beukema et al., 1993; Naumov, 1991; Zenkevitch, 1963; Kulakov et al., 2004; Sakshaug et al., 2009). Even benthos of the upper shelf of Azov Sea and Far Eastern seas have resemblance with that of the northern European seas (Kafanov et al., 2004; Naumov, 1991). Thus, even a cursory review of the literature allows us to consider analyzed sites (sand and silty-sandy beaches) as usual intertidal habitats in the temperate zone, and macrobenthic communities developed there - as expected biosystems in such circumstances. So we can assume that the results of this work can be applied not only to the White Sea intertidal zone, but to the other areas with similar environmental conditions.

### 4.1. The aim of cluster analysis and data treatment

The aim of classification procedures is to make a large amount of data more perceptible and to reveal the latent structure of the material. There are numerous similarity coefficients and clustering methods, which can be used in classification. As it was mentioned above, one convenient way of providing a choice of suitable procedures, is to restrict attention to a single similarity coefficient but allow a choice of prior transformation of the data (Clarke and Warwick, 2001). So Bray-Curtis similarity measure and Unweighted pair group average method was used to group stations on the basis of biota similarities. While, it was decided to use several options of the data pretreatment: presence-absence data, transformed to the fourth root and standardized by ranging biomass and densities of species. All these methods are fairly common and recommended for studying community structure (Clarke and Warwick, 2001; Quinn and Keough, 2002).

It turned out that classifications of benthic communities, based on transformed biomass and density values, in most cases (75%) were identical to those, based on the presence-absence data, and did not always coincide with those based on standardized biomass and densities of species. In the latter case, the stations were grouped into clusters according to the distribution of the dominant taxa. In classical Hydrobiology biocenosis is usually considered as the smallest unit of macrobenthos organization. The structure of the biocenosis is characterized by a set of dominant and subdominant species by biomass (Golikov et al., 1990). Obviously the classification on the basis of standardized biomass is most suitable for identifying biocenosis because its results are influenced by the variables with high values (e.g. very abundant species). Presence-absence data (or transformed biomass and densities of species to the fourth root) can be also used for this purpose, but only if stations are very different in species composition. As a rule, the presence-absence data is used in biogeographic investigations aimed at studying the patterns of distribution of the biota qualitative composition (Golikov et al., 1990). When it is needed to compare the stations with the same dominant species, or when the object of interest is the whole community including small and rare species, standardization could give false ideas about a single set of data, but transformation will reveal more subtle differences.

### 4.2. The number of replicates and reliability of the cluster analysis results

Cluster analysis is a method for combining similar objects into groups or clusters based on their attributes or variables. Thus it is necessary to take into account that these attributes (in our case - the species composition, abundance or biomass) should be described most exhaustively. So far there is no definitive recommendation about the number of replicates required to obtain reliable (objective) description of the benthos structure.

This study has shown that there are high requirements for the number of replicates necessary to obtain relatively reliable results of cluster analysis of the littoral soft bottom communities: on average *sns* was no < 5 samples. The main reason of this result is irregular distribution of macrobenthos within the studied habitats. If benthic organisms were distributed regularly, a relatively reliable description of the community structure could be obtained even by one - two samples. Such distribution pattern characterized some benthic organisms. For example, it is known that often dominant species on the intertidal soft sediment in the temperate zone of the Northern Hemisphere, such as *Arenicola marina* and *Limecola balthica*, show a significant degree of uniform dispersion (Holme, 1950; Kalyakina, 1971; Flach and Beukema, 1994). Similar benthic organism distribution were noted on a

sandy beach at Klyuschiha Cove (site 1), where biocenosis *Zostera marina* + *L. balthica* + *A. marina* was presented. But the whole community does not consist only of the dominant forms, and majority of macrobenthic organisms rarely exhibit an even distribution. As rule the distribution of intertidal benthic species is aggregated or random, which was observed in various seas, including the White Sea (Ardisson et al., 1990; Burkovsky et al., 1994; Azovsky, 1996; Burkovsky et al., 1997; Reise, 1979; Tufail et al., 1989; Volckaert, 1987), and was shown in a course of long-term monitoring at the same sites (Gerasimova et al., 2004; Gerasimova et al., 2006).

The choice of data type (biomass or density of species) and data treatment (standardization or transformation) did not influence the reliability of cluster analysis results. The choice of data treatment, as previously stated, should only be defined by the aim of the research.

It was shown that the weaker the differences between compared communities, the greater amount of samples were needed to obtain a reliable result. As mentioned before, our work was concerned with two issues: (1) comparing the structure of communities in several sites, significantly different in abiotic conditions and consequently in the structure of biota, and (2) analyzing the interannual changes in the structure of the macrobenthic community in the same site. Concerning the first task, it turned out that on average, 5 replicates were sufficient to obtain reliable (unshifted) results of the community structure analysis. Concerning the second task, the number of samples should be increased to 11 (on average). This increase in replicate number can be explained by relatively weak interannual variations in the structure of macrobenthic communities in the same site in a relatively stable environment. For example, at one of the considered site - a silty-sandy beach in Lebyazhya Bay (Site 2), bivalve *Mytilus edulis* and *Mya arenaria* were dominant by biomass during all years of observations. As a result, the reliability of the cluster analysis results could be achieved only with 11–35 samples at the station. Long-term observations (1980–2015) of the benthic community structure in this site (Gerasimova et al., 2015; Maximovich and Gerasimova, 2004; Maximovich and Guerassimova, 2003) also did not reveal significant changes, despite the fluctuations (sometimes very significant) of absolute abundance and biomass of the dominant species *Mytilus edulis* and *Mya arenaria*. To the contrary, at the two remaining studied areas (sites 1 and 3), significant changes in the structure of benthic communities were noted during the study period, accompanied by the change of the dominant species. At both cases they were induced by the fluctuation of eelgrass biomass and affected the whole community structure. It should be noted that shift in the structure of macrobenthic communities also occurred here against the background of seemingly unchanged environmental conditions. It is noteworthy that *sns* for these sites was minimal (in some cases 1–8 samples).

The question arises why under the same conditions significant interannual changes in the structure of communities were observed. Perhaps the reason should be sought in the intensity of the influence of the dominant taxa on both the structure of benthic communities, and on the distribution of minor species.

When there is no strong interaction between macrobenthic organisms (Filippova et al., 2015a), interannual changes in community structure are not likely to happen in stable environmental conditions even if there is significant fluctuations in the abundance of dominant species. Independence of species distribution in soft bottom intertidal zone of the White Sea, especially in *Mya arenaria* communities, and temporal stability of these communities are well known (Gerasimova et al., 2006). Perhaps, this can be explained by almost complete absence of macrophytes at the studied area, such as *Zostera marina* or *Fucus vesiculosus*, which provide living space and shelter for a variety of species (Constable, 1999; Mattila et al., 1999). Due to the lack of species interaction the community structures in Lebyazhya Cove (site 2), were very similar in different years of observations, as was confirmed by cluster analysis. *Sns* at this site were maximal because the data had no internal structure that could be revealed by classification. If there is an interaction between species (as in *Z. marina* community), changes in the community structure from year to year can be quite significant when the dominant taxa changes, as we observed in the community structure at sites 1 and 3. As a result, fewer replicates were required to reliably describe the structure of communities. Nevertheless, even if there were significant interannual changes in the community structure, the characteristics of macrobenthos at one site in different years of observation were more similar than those at different sites. Therefore the *sns* in the first case was much greater than in the second.

The dependence of *sns* on the degree of heterogeneity of the compared communities was confirmed by correlation analysis, which showed positive correlation between the *sns* and similarity level, at which all stations merged into a single group. Correlation between sufficient number of samples and ratio of similarity levels, at which all stations merged into a single group and at which standard clusters were determined, is of particular interest, because the latter is usually defined by the researcher. Although factor analysis did not show influence of the data treatment on the sns, using of transformed and presence-absence data required fewer samples comparing to standardized data. Similar results were observed in studies of macroinvertebrate assemblages in tropical streams (Schneck and Melo, 2010): fewer sampling units were necessary for adequate estimations of resemblance using presence-absence data compared to relative abundance data.

## 5. Conclusion

The characteristics of the studied soft bottom littoral communities in the White Sea were close to those of similar biosystems in the temperate zone of the Northern Hemisphere. In addition this study showed that the number of replicates necessary to obtain reliable results of cluster analysis in a comparative study of the structure of the soft bottom littoral communities do not depends on the characteristic of studied communities (such as standard errors of biomass or densities of species, number of taxa and occurrence of taxa). Consequently, it can be assumed that the result of this work can be applied in study of the wide range of communities and not only to the intertidal zone of the White Sea.

## 6. When studying soft-bottom communities the following recommendations can be done

- When comparing communities on the basis of one-year observations at different sites 5 samples can be sufficient to obtain reliable results using cluster analysis. But when classification is based on interannual observations at one site, even 10 samples can be not enough to obtain reliable result about community structure.
- The reliability of determining the number of clusters depends on 1) similarity level, at which all stations merged into a single group, 2) similarity level, at which standard clusters were determined, and 3) ratio of this similarity levels. At the same similarity level to identify clusters, using of transformed and presence-absence data required fewer samples comparing to standardized data.
- The choice of the certain type of data (biomass or density of species), or a method of data treatment (transformation or standardization) depends on the aim of the research and do not influence the reliability of the result. It is noteworthy that when classification is based on fourth root transformed biomass and densities of species, in most cases (75%) dendrograms are identical to those, obtained from presence-absence data.

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