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Patterns of psammophilous ciliate community structure along the salinity gradient in the Chernaya River estuary (the Kandalaksha Gulf, the White Sea)

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Summary

Species composition patterns and mechanisms of formation of ciliate community structure in the Chernaya river estuary (the Kandalaksha Gulf, the White Sea) were studied in 1996-2000 at stations distributed along the salinity gradient. From the marine part of the estuary riverwards, salinity decreases and oxidative-reductive properties of the sediments become more reduced due to organic accumulation. The greatest variability of salinity, acidity and redox-potential is observed in the brackish water part of the estuary. All other factors have a more complex distribution in the intertidal zone, being both gradient and mosaic. During the period of the investigation altogether 123 species of psammophilous ciliates were found. Psammophilous ciliate community in the estuary may be considered as rather unified, continual and two-polar (with marine and brackish-water pool of species). A relative and fuzzy border between the two poles is situated in brackish water part of the estuary, where the greatest variability of salinity and redox-potential as well as critical salinity level (3-8‰) are observed. In the estuary riverwards the complexity of ciliate community in terms of ciliate density, biomass and species richness decreases significantly. Decrease in species richness results from congeners. It probably reflects a simplification of niche structure. Seasonal changes of ciliate community structure in marine zone may be characterized as rather trended and stable. Changes in community structure in the brackish water zone of the estuary, on the contrary, are more variable and stochastic. Community in the intermediate zone of the estuary is both trended and highly heterogeneous. Community biomass, abundance and species richness are positively connected with average salinity and redox-potential levels. Variability of integral community characteristics is related predominately to sediment parameters (amount of organic matter and relative ability of large size fraction of sand grains). Variability of saline regime affects that of the total abundance and biomass of the community but not the variability of species richness and diversity. Analysis of the role of salinity in ciliate community formation reveals trended changes of species composition and non-linear modifications of integral community characteristics. All community variants are divided into two groups with equal complexity but different species composition: marine and brackish water. Ability of complex marine community to sustain its own structure under the influence of lower salinity is the first mechanism of community formation in brackish waters. It predominates at a relatively higher salinity level as well as during terminal succession stage. Ability of oligohaline ciliates to immigrate from marine to freshwater estuarine zone is the second mechanism. It determines the community structure in the initial stages of succession at lower salinity level.

Key words: ciliates, community structure, estuary, salinity gradient, interstitial

Introduction

Estuaries are specific areas with a very non-stable environment and a pronounced salinity gradient. One of the most important constraints of the estuarine communities is the salinity of 3-8‰, which is a physiological barrier for many marine and freshwater species (Khlebovich, 1974, 1981, 1986). It prevents their distribution and demarcates corresponding communities. Critical interval is filled by characteristic or opportunistic species, which form highly variable groups with low complexity (Burkovsky, 1984; Pogrebov, 1988; Pogrebov and Goryanina, 1988; Guiral, 1992; Burkovsky and Stolyarov, 1995; Jiang, 1996; Hamels et al., 1998; Underwood et al., 1998; Burkovsky and Mazei, 2001). However, the mechanisms of formation and sustenance of community structure in a greatly fluctuating environment within a strong salinity gradient including critical salinity level are not yet fully known.

In this study the following questions are discussed. What are the main environmental characteristics that determine heterogeneity of the estuarine sandy littoral biotope? How many species constitute the psammophilous ciliate community in the estuary? Is ciliate community in oligohaline zone of the estuary constituted by specific brackish water species or by ubiquitous ones? What species determine the differences between local communities in different estuarine zones? Are there any spatial and temporal trends of ciliate community transformation in the estuary? Are they gradual or discrete? What factors determine community features in different parts of the estuary? What is the role of salinity in formation of ciliate community structure? Can more complex communities, for example, those of marine biotopes, normally exist or sustain their structure for a long time in brackish water conditions?

Material and Methods

Investigations were conducted in the summers of 1993, 1996, 1998, 1999 and 2000 in the Chernaya River estuary (the Kandalaksha Gulf, the White Sea). Aquatoria of the estuary is divided into a system of three connected water basins with different salinity (Fig. 1). Material was collected at 5 stations. The distance between station 1 and station 5 was about 2.5 km. The stations were located on the middle horizon of the intertidal zone along the estuary on the borders dividing relatively homogenous zones, which had been previously defined according to the data on the heterogeneity of the environment and benthos composition (Stolyarov, 1994; Burkovsky and Stolyarov, 1995). The sampling was carried out at an interval of 5-7 days during May-August. At every station 14 quantitative samples were collected per year. Each sample was a series of 15 subsamples (1 cm square, 3 cm in height), collected from a strictly fixed area 50 × 50 cm. A random sampling, corresponding to 1/15 of the total sample, was examined (i.e., one mean statistical square centimetre). Extraction and counting of ciliates was conducted on live material according to Uhlig (1964, 1965). Identification of ciliates was performed using silver impregnation methods (Foissner, 1991).

In order to reveal the influence of salinity on formation of the species community structure, experiments were conducted with other environmental factors levelled by creating physically homogeneous microbiotopes (isolates of sediment with the same granulometric parameters, organic matter content, position as to the zero depth, etc. placed along the estuary between stations 2-5). To minimize the influence of mechanical and physical-chemical parameters of the sediment upon the community structure, trays with an area of 20 × 15 cm and the height of the wall 10 cm were placed at every station at the

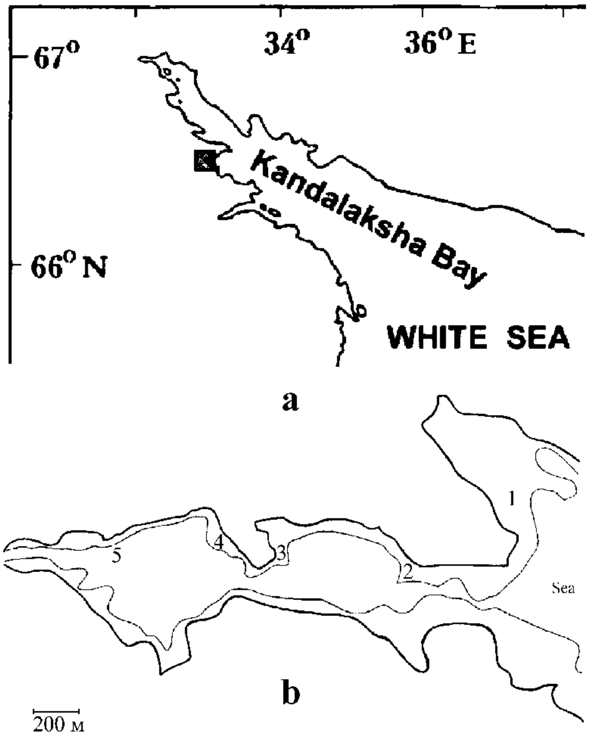


Fig. 1. Area of investigation (a) and a scheme of location of stations in the estuary (b). Thick line, coastline; thin line, zero of depth.

middle horizon of the intertidal zone. The trays were filled with dried sterile sand with the same granulometric composition. Experiments started on May 31. Samples were taken fortnightly from July 14 to August 15 (in total, 6 samples at every station per season). Each sample was a series of 5 subsamples (1 cm^2 area, 3 cm high) taken at the site studied. A random sampling corresponding to 1/15 of the total sample (i.e., 1/3 of statistically average square centimetre) was examined.

In order to reveal mechanisms of sustenance of ciliate communities in the brackish zone, experiments on the transfer of the sediment, inhabited by marine organisms, into the brackish water part of the estuary were performed. The investigation was carried out in the May-August of 1998. Material for experiments was chosen from a sandy area of the middle intertidal zone in the marine part of the estuary (station 1). Bars of sediment were cut by metal cylinders of stainless steel (25 cm in diameter and 17 cm high) from the area of 2 × 2 m, represented by middle-grainy heterogenous and oligosaprobic sand. Then, the bars were transferred, without taking them out of the cylinders, to the brackish water part of the estuary (at stations 2-4 located at a distance of 300 - 2000 m from station 1) and placed there instead of a corresponding amount of local sediment. After that, the cylinders were covered by a synthetic net (mesh 100-150 µm), which ensured the passing of light (2/3-1/2 of total light flux), water exchange with the water column (during high tide), did not prevent active migration of small organisms, but prevented abundant detritus sedimentation and disruption of the vertical structure of the sediment. Earlier it was shown that decrease in light intensity up to 1/3 did not tell negatively on the microbenthos population (Burkovsky et al., 1989). Experiments were conducted twice during the summer: in the initial (May 25) and final (July 12) stages of the seasonal succession. They are characterized by different degrees of sensitivity of species structure of psammophilous community to environmental factors (Burkovsky, 1992). Duration of experiments in each series was 6 weeks. The isolated biotope (E1) obtained by the method described above but left in close vicinity (20-30 cm) of the donor (natural) area (N1) was the control for all three experiments (E2, E3, E4), situated in the estuary at stations 2-4. For different experiments, their surrounding biocenoses were the natural background.

For each station, changes of total abundance and biomass were calculated, as well as the Margalef index of species richness and Shannon diversity index basing on the biomass. To reveal general components of environmental and community variability in the estuary, Principal Component analysis (PCA) was used. Existence of general trends of successional changes of community structure was estimated with the aid of linear correlation coefficients "temporal distance between samples vs. Czekanowsky similarity index". Higher negative values of this proof indicate clear trend in ciliate community changes. Heterogeneity of the succession was estimated using average Czekanowsky similarity index for all pairs of samples. All calculations were performed with the aid of STATISTICA 5.5A and ECOS 1.3 Software (StatSoft, Inc., 1999; Azovsky, 1993).

Results

Biotope characteristics. The results of the measuring of different environmental parameters (Table 1) show that the Chernaya river estuary is a spatially heterogeneous and temporally unstable environment. Stations 1-5 differ significantly by the salinity regime, acidity, oxidative-reductive properties of the environment as well as by the granulometric structure of the sediment and organic matter content. Of the major environmental factors influencing the structure of psammophilous ciliate community, only salinity and oxidative-reductive properties of the sediment are characterized by a strongly pronounced longitudinal gradient (Table 1, Fig. 2). Besides, the greatest variability of salinity and redox-potential is observed in the brackish water

Table 1. Environmental parameters of the estuarine biotope.

Factor	Stations				
	1	2	3	4	5
Water salinity, ‰					
average for 2000	14.6	11.6	7.6	5.6	2.0
standard deviation	3.61	5.34	5.46	4.13	2.80
Redox potential (Eh) on the surface of the sediment, mV					
average for 2000	170	129	140	19	-6
standard deviation	50.2	65.6	83.4	98.8	49.5
pH on the surface of the sediment					
average for 2000	7.2	6.4	7.1	6.2	7.4
standard deviation	0.52	0.21	0.24	0.76	0.51
Amount of debris in the sediment, % from sediment weight	2.2	3	2	3.7	8.4
Amount of suspended organic matter in the sediment, % from sediment weight	0.07	0.24	0.11	0.13	0.4
Granulometric composition of sediment					
Fractions:					
>1000 µm	1.94	12.4	3.65	3.67	4.86
500-1000 µm	10.15	18.69	26.34	25.14	18.47
250-500 µm	40.46	37.57	53.46	51.93	41.01
100-250 µm	31.16	18.16	11.45	10.86	19.05
<100 µm	16.29	13.18	5.1	8.4	16.61
mean size of the sand grains	318	450	460	447	391
standard deviation of the sand grains size	245	104.5	430.6	382.7	171.3

2). Altogether they describe 74.5 % of the total environmental variability. The first component (40.5 % of variability) affects the differences between marine (stations 1-3) and brackish (stations 4 and 5) zones of the estuary. It may be verified as a combination of mean salinity and redox-potential (Eh) level (these factors reach their highest levels in the marine part) as well as debris and dissolved organic content and pH variability. The latter factors reach their highest levels in the riverine part. Such patterns of abiotic parameters reflect general trends of environmental variability in the estuary: from the marine part riverwards, the salinity decreases and oxidative-reductive properties of the sediments become more reduced due to organic accumulation. Terrigenous flow and rich development of higher plant tidal marsh vegetation may explain higher organic content in brackish water part of the estuary. The second component (34.0 %) includes the differences between brackish water stations (station 2 on the one hand, stations 3 and 4 on the other hand) and is connected with sand characteristics (granulometric structure and organic content) and pH/Eh variability. This component reflects mosaic changes of sediment characteristics along the estuary, which may be caused by complex hydrological regime and coast geomorphology. In addition, pH and Eh regime (on spatial and temporal scale) at stations 3 and 4 is more variable than at station 2. It may be considered that the central part of the estuary is characterized by both the most variable conditions (salinity, pH, Eh) and critical salinity level.

part of the estuary (stations 2-4). All other factors have a more complex distribution in the intertidal zone, being both gradient and mosaic.

With the aid of PCA two general components of environmental variability were detected (Fig. 3, Table 2).

Table 2. Results of PCA of environmental characteristics.

Factor Loadings		
Station	PC1	PC2
1	**0.71**	-0.02
2	0.41	**0.72**
3	0.51	**-0.69**
4	-0.58	**-0.73**
5	**-0.87**	0.40
Total variability explained, %	40.5	34.0
Factor Scores		
Average salinity	**1.41**	0.35
SD salinity	0.41	-0.29
Average Eh-value	**2.47**	0.10
SD Eh-value	-0.24	**-0.97**
Average pH-value	-0.01	-0.09
SD pH-value	**-1.10**	**-0.87**
Amount of debris in the sediment, %	**-1.59**	0.70
Amount of suspended organic matter in the sediment, %	**-1.22**	**1.36**
Mean size of the sand grains	-0.12	-0.28
SD the sand grains size	-0.04	**-1.96**
Fractions:		
>1000 µm	0.18	**1.75**
500-1000 µm	-0.35	-0.84
250-500 µm	-0.12	-0.69
100-250 µm	0.49	0.69
<100 µm	-0.18	**1.04**

Notes: Significant values (P<0.05) are highlighted, SD – standard deviation, Bold – highest values.

So, PCA allows us to divide abiotic factors into two groups. It is important that hydrochemical (salinity, Eh) and granulometric factors may be considered as independent, which reflect general hydrochemical trends in the estuary on the one hand and mosaic pattern of sediments on the other hand. Moreover, in the brackish water part of the estuary the greatest spatial and temporal environmental variability is detected.

Spatial and temporal patterns of psammophilous ciliate community structure. Altogether, in the studied zones of the Chernaya river estuary 123 species of ciliates were found: station 1 - 100, station 2 - 94, station 3 - 87, station 4 - 72, station 5 - 59 species. As salinity decreases, the number of species falls significantly. Annually 23 dominating species compose the basis of the ciliate community in the estuary. They are: at station 1 - *Trachelocerca incaudata, Remanella margaritifera,*

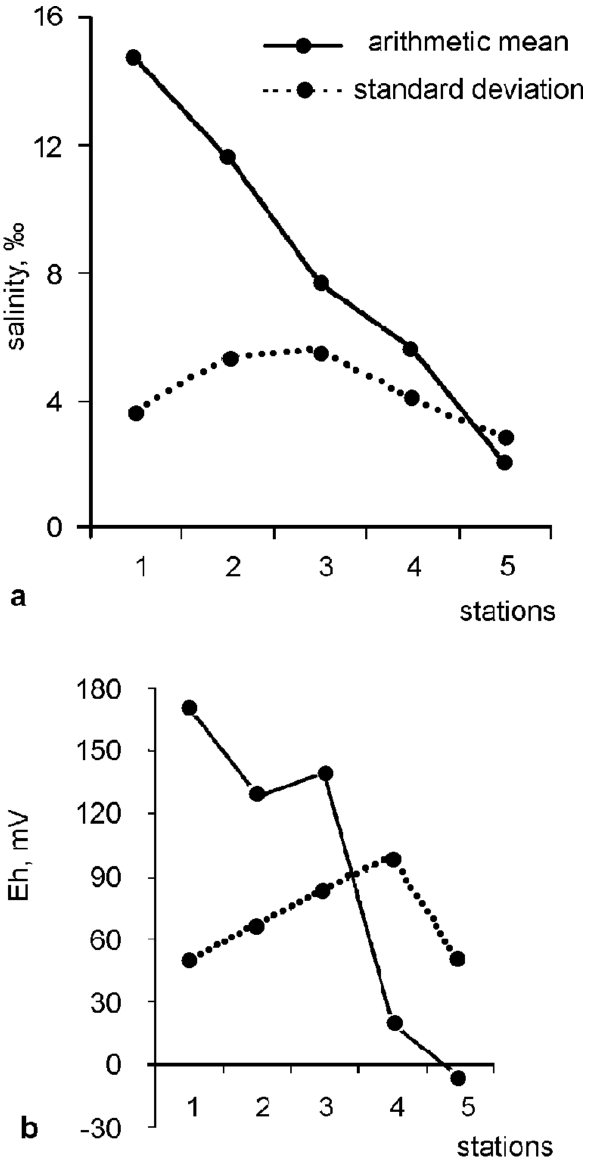


Fig. 2. Salinity (a) and redox-potential-Eh (b) of the upper 1 cm sediment layer in the Chernaya river estuary in May-August 2000.

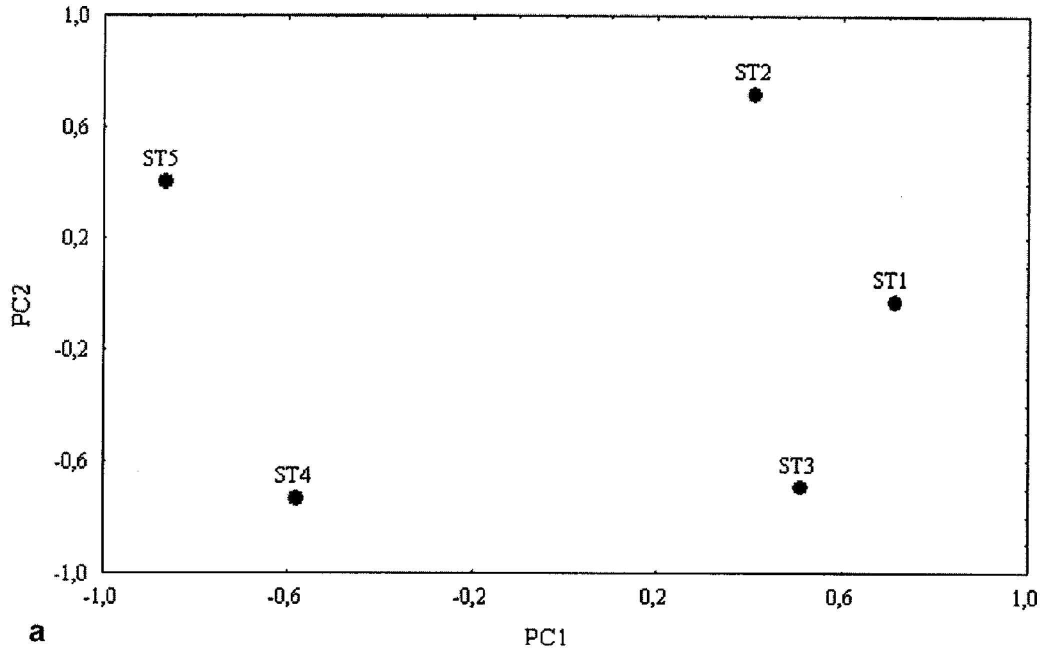
Cardiostomatella vermiforme, Histiobalantium marinum; at station 2 - *R. margaritifera, H. marinum*, different species of Trachelocercidae, *C. vermiforme*, at station 3 - *C. vermiforme, Frontonia tchibisovae, Diophrys scutum*; at station 4 - *Pleuronema crassa, Prorodon* sp., *F. tchibisovae, Cyclidium fuscum, Prorodon morgani*, unidentified species from the family Enchelydae; at station 5 - *P. crassa, C. fuscum, Prorodon morgani*, unidentified species from the family Enchelydae. Neighbouring stations have common dominating species, which indicates a considerable smoothness of the change of structure along the estuary.

In order to reveal general patterns of species structure of the ciliate community, the PCA based on average seasonal abundances of each species in each station was performed (Fig. 4). PCA allows us to divide biotic variable into 2 components/groups, which explain 80 % of the total species structure diversity. They are associated with the differences between stations 1-3 (from the marine part of the estuary) and stations 4, 5 (from the riverline zone). Moreover, from Fig. 4 it is obvious that the basic differences between communities are due to 6 species. *T. incaudata, H. marinum, R. margaritifera* and *C. vermiforme* formed specificity of marine variants of the community, whereas *P. crassa* and *P. morgani*, of brackish water ones.

Thus we may consider psammophilous ciliate community in the estuary as rather continualand two-polar (with marine and brackish-water pool of species). A relative and fuzzy border between the two poles lies between stations 3 and 4, where the greatest variability of salinity and redox-potential as well as the critical salinity level (3-8‰) are observed (see Fig. 2).

In the estuary towards the river complexity of the ciliate community in terms of average ciliate density, biomass and species richness significantly decreases (Fig. 5). The decrease resulted from congeners. Species/genera ratio decreases from 1.75 in the marine part to 1.48 in the brackish part of the estuary. In other words, the higher number of congeners that are closely related as to their biological properties and ecological preferences composes ciliate community in the marine part of the estuary. They coexist due to fine niche partitioning (Burkovsky, 1992). On the contrary, community in the brackish water part of the estuary is constituted by species with different biology, which makes niche structure more fragile. However, the maximal value of Shannon's species diversity index is noted at stations 4 and 5 due to higher evenness of species abundances distribution, in spite of lower species richness. Community in the brackish zone of the estuary is composed by a few ciliate species, and each of them may reach higher abundances of species populations independently.

The seasonal trends in the community structure are manifested in succession of dominant species and regular changes in major integral characteristics of the community (e.g., species richness and diversity, average abundance and biomass of the community etc.). However, direction of changes and their significance vary in different stations as well as in the same stations in different seasons (for details see Mazei and Burkovsky, 2002). Existence of general trends of changes of community structure may be estimated with the aid of linear correlation coefficients "temporal distance between samples vs. Czekanowsky similarity index". Higher negative values of this proof indicate a clear



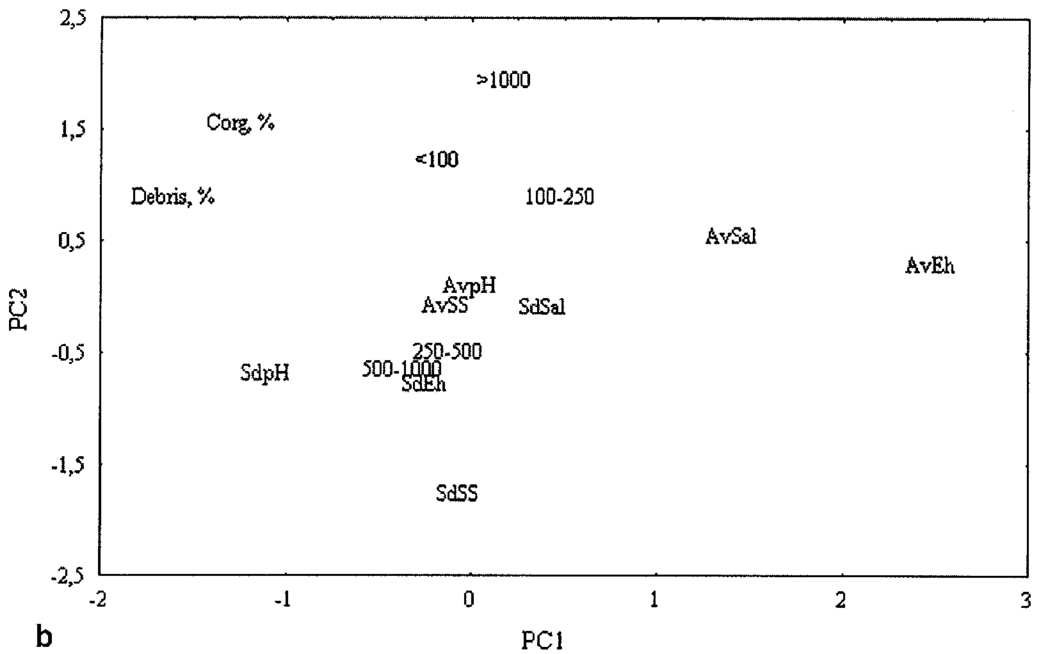


Fig. 3. Environmental variability in the estuary (scatter plots of principal component analysis). a - factor loadings, b - factor scores. PC1, PC2 - first and second principal components. ST1-ST5 - stations 1-5. AvSal - average salinity, SdSal - standard deviation of salinity, AvEh - average Eh-value, SdEh - standard deviation of Eh-value, AvpH - average pH-value, SdpH - standard deviation of pH-value, Debris, % - amount of debris in the sediment, Corg, % - amount of suspended organic matter in the sediment, AvSS - average size of the sand grains, SdSS - standard deviation of the sand grains size, >1000, 500-1000, 250-500, 100-250, <100 - amount of corresponding sand size fractions.

trend in ciliate community changes during the summer season at stations 1-3, whereas community variability in the brackish zone of the estuary is more stochastic (Fig. 6). Moreover, average heterogeneity of the succession estimated with the aid of average Czekanowsky similarity index for all pairs of samples (Fig. 6) became more pronounced at stations 3-5 compared with stations 1 and 2.

The main external cause of trended changes of ciliate community structure might be an increase of

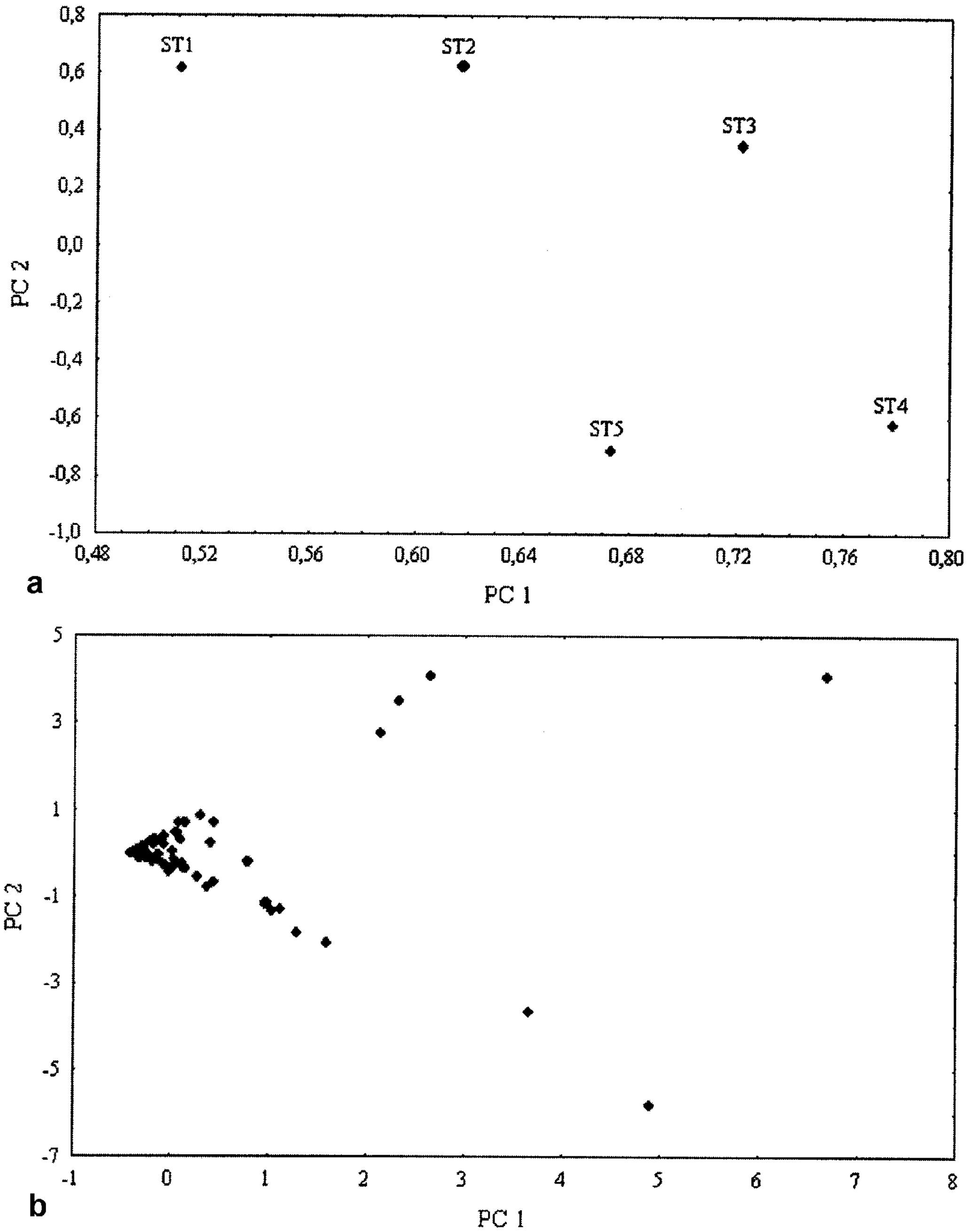


Fig. 4. Community variability in the estuary. Scatter plots of principal component analysis. a - factor loadings, b - factor scores. PC1, PC2 - first and second principal components. ST1-ST5 - stations 1-5. 1 - *Cardiostomatella vermiforme*, 2 - *Trachelocerca incaudata*, 3 - *Remanella margaritifera*, 4 - *Histiobalantium marinum*, 5 - *Prorodon morgani*, 6 - *Pleuronema crassa.*

water salinity in summer. However, strong spatial and temporal environmental fluctuations and critical salinity level predominately registered in the brackish part of the estuary modify general trends and affect the stochastic character of changes and decreasing stability of species structure. So, communities in the marine zone of the estuary (stations 1 and 2) may be characterized as rather trended and stable in terms of their seasonal changes. Changes of community structure in the brackish zone of the estuary (stations 4 and 5), on the contrary, are more variable and stochastic. Community in the intermediate zone of the estuary (station 3) is rather trended as well as highly heterogeneous. We come to the conclusion that the main breaking of ciliate community structure occurs at the border between stations 3 and 4. In the marine part of

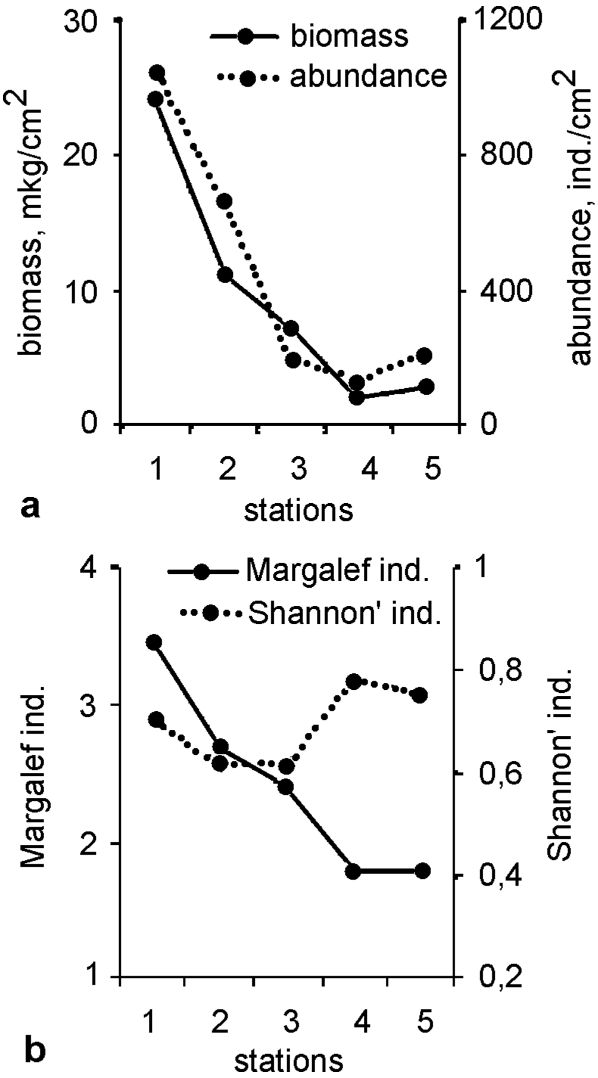


Fig. 5. Integral characteristics of psammophilous ciliate community structure in the estuary. a - biomass, mkg/cm2 and abundance, ind./cm2, b - Margalef species richness index and Shannon species diversity index.

the estuary communities are characterized by generalized seasonal succession trends whereas in the brackish zone seasonal changes are rather stochastic.

Factors and mechanisms of ciliate community formation. Correlation between integral characteristics of ciliate community structure and abiotic factors (Table 3) shows that biomass, abundance and species richness are positively connected with the first principal component average salinity and Eh levels. At the same time species diversity estimated by Shannon diversity index is positively related with variability of pH value and negatively, with the average size of sand grains in the sediment. On the other hand, variability of integral community characteristics is related predominately to sediment parameters (amount of organic matter and relative availability of large size fraction of sand grains). It is interesting that variability of the saline regime affects variability of the total abundance and biomass of the community but not the variability of species richness and diversity.

In order to reveal the role of salinity as a basic specificity-forming estuarine factor, we conducted a series of experiments. During the first series the influence of salinity on formation of the species community structure was revealed in colonization experiments where other environmental factors were levelled by creating physically homogeneous microbiotopes. Distinct changes in species composition of the ciliate community are observed throughout the estuary along the salinity gradient. Results of ordination of dominant species by the PCA are given in Fig. 7. In the space of the first two principal components, which explain 57.9 % of species structure variance, no distinct groups of species can be distinguished that react similarly to changes in environmental conditions (first of all, to the salinity regime - groups are distinguished along the first principal component). However, we may distinguish the species that tend to be associated with the marine estuary part. They are *Trachelocerca incaudata, Histiobalantium marinum, Cardiostomatella vermiforme, Diophrys scutum, Tracheloraphis kahli, Frontonia tchibisovae*, and *Gastrostyla pulchra*. On the

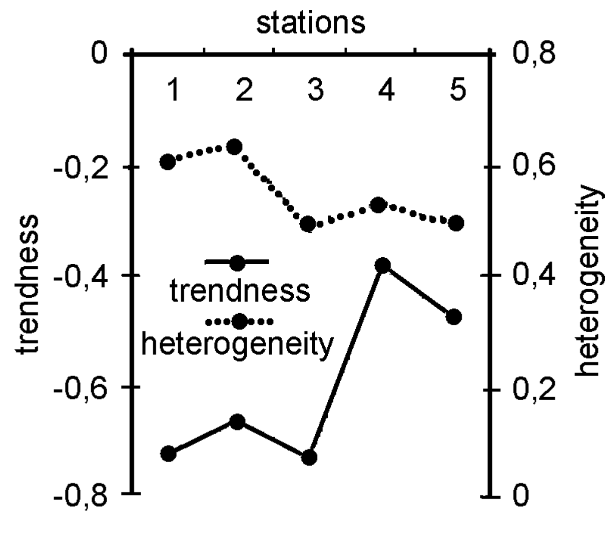


Fig. 6. Integral characteristics of seasonal succession of ciliate community in the estuary. Value of trendness is the linear correlation coefficient "temporal distance between samples vs. Czekanowsky similarity index"; value of heterogeneity is the average Czekanowsky similarity index for all pairs of samples.

Table 3. Spearman rank order correlation coefficients between abiotic parameters and integral community characteristics.

Factor	Community characteristics							
	Biomass	Abundance	Margalef index	Shannon index	CV of biomass	CV of abundance	CV of Margalef index	CV of Shannon index
PC1	**0.8**	**0.8**	**0.8**	0.1	-0.7	-0.7	**-0.9**	-0.7
PC2	0.5	0.5	0.5	-0.2	0.0	0.0	0.5	0.6
Average salinity	**0.9**	**0.9**	**0.9**	0.2	-0.6	-0.6	-0.7	-0.6
SD salinity	0.1	0.1	0.1	-0.7	**-0.9**	**-0.9**	-0.3	-0.1
Average Eh-value	**0.8**	**0.8**	**0.8**	0.1	-0.7	-0.7	**-0.9**	-0.7
SD Eh-value	-0.4	-0.4	-0.4	-0.2	-0.4	-0.4	-0.3	-0.4
Average pH-value	0.3	0.3	0.3	0.1	0.3	0.3	0.1	0.3
SD pH-value	-0.4	-0.4	-0.4	**0.8**	0.6	0.6	-0.3	-0.6
Amount of debris in the sediment	-0.6	-0.6	-0.6	0.3	**0.9**	**0.9**	**0.8**	0.5
Amount of suspended organic matter in the sediment	-0.5	-0.5	-0.5	-0.3	0.5	0.5	**1.0**	**1.0**
Average size of the sand grains	-0.2	-0.2	-0.2	**-0.9**	-0.7	-0.7	0.1	0.3
SD sand grains size	-0.3	-0.3	-0.3	-0.1	-0.3	-0.3	-0.6	-0.5
Fractions:								
>1000 μm	-0.3	-0.3	-0.3	-0.4	0.2	0.2	**0.9**	**0.9**
500-1000 μm	-0.5	-0.5	-0.5	-0.7	-0.5	-0.5	0.0	0.1
250-500 μm	-0.6	-0.6	-0.6	-0.3	-0.1	-0.1	-0.2	-0.1
100-250 μm	0.7	0.7	0.7	0.4	0.2	0.2	-0.1	0.0
<100 μm	0.2	0.2	0.2	0.5	0.7	0.7	0.4	0.3

Notes: Significant coefficients (P<0.1) are highlighted, PC1 and PC2 – principal components (see Table 2), CV- variation coefficients, SD – standard deviation, Bold – significant (p<0.05) values.

other hand, Helicostoma notata, non-identified Trachelocercidae gen. sp., *Prorodon morgani, Anigsteinia clarissima, Strombidium* sp., *Trachelostyla pediculiforme, Spirostomum teres, Pleuronema crassa* and *Urosoma caudata* tend to be associated with the brackish part of the estuary.

Analysis of quantitative parameters (biomass, abundance, species richness and species diversity) reveals that community at station 5 differs significantly from the others (Fig. 8). It is characterized by high abundance and biomass with the lower values of species richness and diversity. Only one species (*Pleuronema crassa*) develops there in large numbers (55% of abundance and 44% of biomass of the whole community). Probably, this community variant may be considered as a border between marine and brackish variants of ciliate community with different species composition but approximately equal integral community characteristics. A slightly pronounced tendency towards decreasing of the total number of species in the brackish part of the estuary is observed (Fig. 8).

The second series of experiments was carried out in order to understand whether complex ciliate communities from marine biotopes can normally exist and sustain their structure for a long time in specific brackish water conditions (critical salinity level and highly variable environment) or they are converted to brackish water community with distinct structural features. To do so, we transferred a marine community to the brackish zone of the estuary.

Initial species structure of the transferred communities transforms significantly when they are placed into a lower salinity environment. The changes observed take place under the influence of local environmental factors affecting elimination (death) of marine organisms during the transition of salinity tolerance threshold and active immigration of brackish water species during the levelling of experimental and natural conditions. As water freshening increases (from station 1 to station 4), the density (and relative abundance) of oligohaline species in transferred communities considerably increases and the density of marine euryhaline species decreases (Fig. 9). In the first half of summer, when average water salinity is lower at all stations (see Table 1), immigration of oligohaline species is much more intensive than in the second half of summer, and it influences significantly the structure of these communities.

The similarity of transferred and control communities decreases significantly in the direction towards

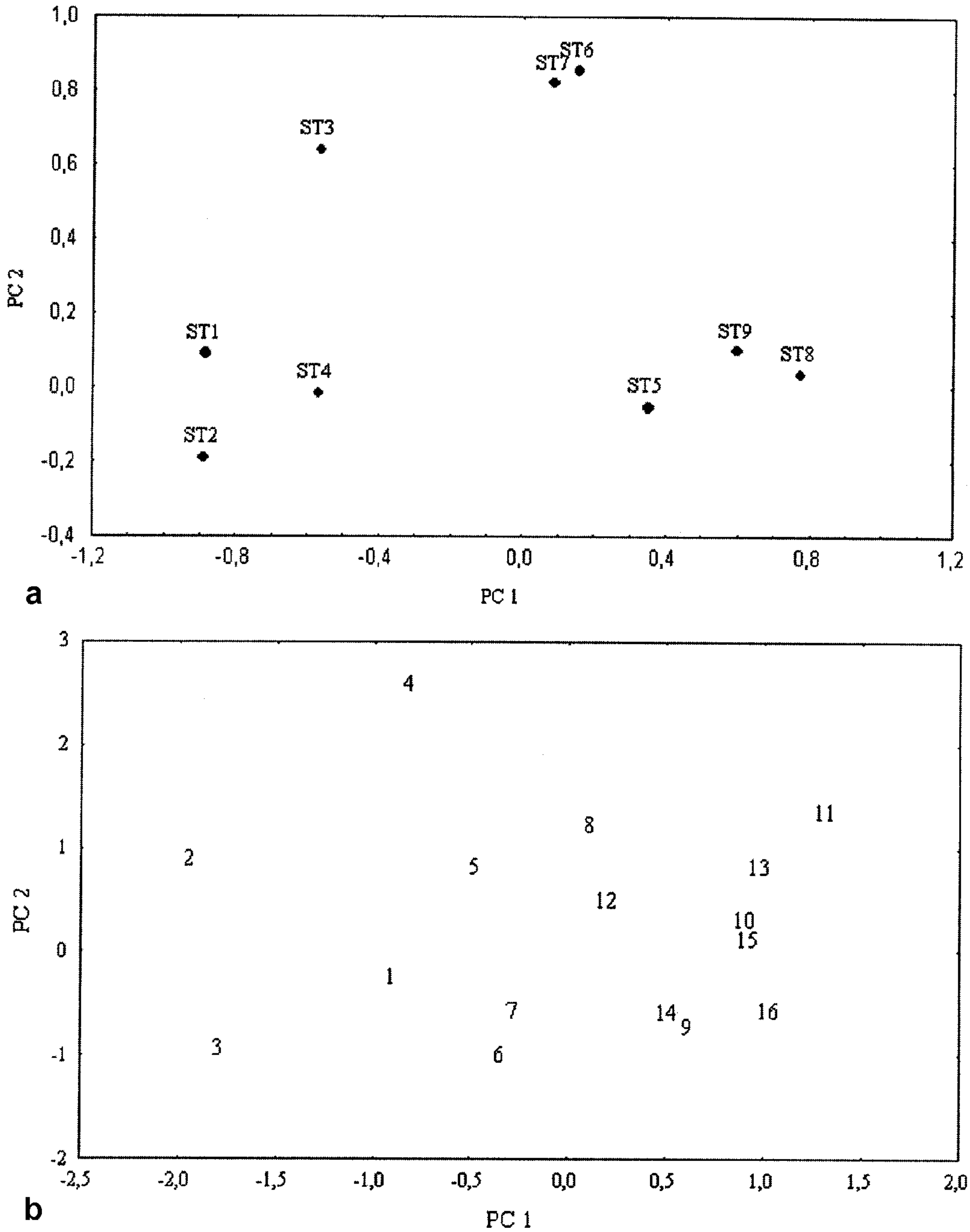


Fig. 7. Ordination of ciliate species (1-16) in the space of the first two principal components (PC) during colonization experiments: a - factor loadings, b - factor scores. ST1-ST9 - stations 1-9. 1 - *Trachelocerca incaudata*, 2 - *Histiobalantium marinum*, 3 - *Cardiostomatella vermiforme*, 4 - *Diophrys scutum*, 5 - *Tracheloraphis kahli*, 6 - *Frontonia tchibisovae*, 7 - *Gastrostyla pulchra*, 8 - *Helicostoma notata*, 9 - *Urosoma caudate*, 10 - *Pleuronema crassa*, 11 - P*rorodon morgani*, 12 - Trachelocercidae gen. sp., 13 - *Anigsteinia clarissima*, 14 - *Strombidium* sp., 15 - *Trachelostyla pediculiforme*, 16 - *Spirostomum teres.*

the river (Fig. 10). In all cases in the first half of the summer (at a greater water freshening and low balance of initial composition of transferred communities, corresponding to the initial stage of seasonal succession) it is lower than in the second. Similarity between the transferred communities and the control decreases significantly by the end of the experiments (Fig. 11), reflecting growing specificity of species structure of the isolates, which combines donor (N1) qualities and that of the surrounding natural community (N2-N4). However, in the first half of summer species structure of the isolates turns to the control at the end of

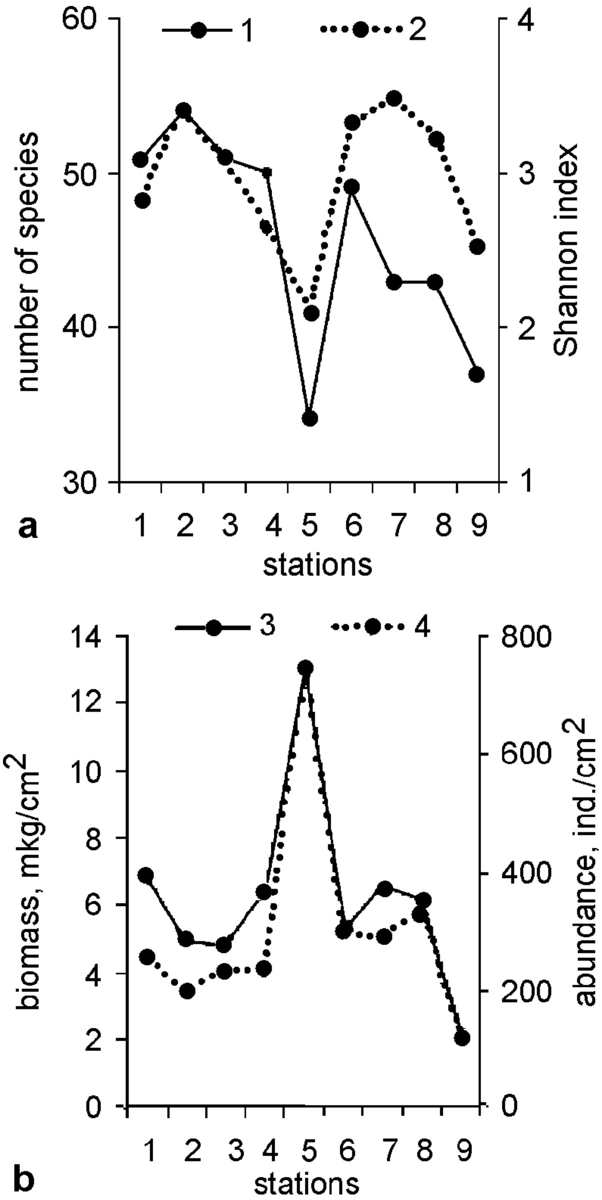


Fig. 8. Integral characteristics of ciliate community structure during colonization experiments. a - number of species (1) and Shannon diversity index (2), b - biomass, mkg/cm^2 (3) and abundance, ind./cm^2 (4).

experiments, which reflect reconstruction of their community structure.

Another important result of the experiments is that under the influence of the environment and immigration of brackish-water species the species structure of the transferred community is transformed into a corresponding natural community. At stations 2-4 the similarity between the experiment and the natural background is relatively lower (Fig. 12). In the experiments in the first half of the summer (Fig. 13), at low average water salinity values, a rapid change of the isolate's structure is observed, which approaches the structure of the natural community, and deviates, also rapidly, from the initial state. However, isolates at station 2 (E2) are characterized by lower similarity with the natural background (N2) at the end of the experiment that corresponds to reconstruction phase of community changes. In the second half of the summer, at higher average salinity values, the similarity with the local natural community tends to increase during the experiment at stations 2 and 3 and is preserved at the initial lower level at station 4.

It may be concluded that the specificity of brackish water community variants is affected both by the ability of complex marine community to sustain its own structure under the influence of environmental conditions and by the ability of oligohaline ciliates to immigrate into the disturbed (by the influence of the lower salinity) isolates. The former mechanism predominates at a relatively higher salinity level as well as during terminal succession stage; the latter determines community structure in the initial stages of succession within lower salinity level.

Discussion

Within the diversity of modern opinions on the community structure in the zone of mixing of sea and river waters, two main approaches may be singled out. According to one of them, such communities are discrete and contain species complexes, which are stable in time and space (Petukhov et al., 1991; Salzwedel et al., 1993). According to the other one, these communities are represented by a continual shift of species not connected closely with each other (Pogrebov, 1988; Porgebov and Goryanina, 1988). Our study of estuarine ciliate community demonstrates the combination of discrete and continual characteristics of species distribution: the complexity of the continuum observed is increased by the presence of stable complexes of species, their composition being determined by local conditions. Estuary ecosystem can be treated as a gradient series of communities, which often transit into each other.

Ciliate abundances in the estuary appear to be largely determined by physical constraints, namely, the amount of interstitial space and hydrodynamic disturbances, whereas ciliate community composition is affected by salinity regime (Hamels et al., 2004). Moreover, the main species of ciliates in brackish waters were euryhaline marine rather than estuarine (Gaughan and Potter, 1995). Our data confirm these conclusions. Colonization experiments along the salinity gradient reveal a constancy of species complexity in terms of species diversity within the estuary as well as clear

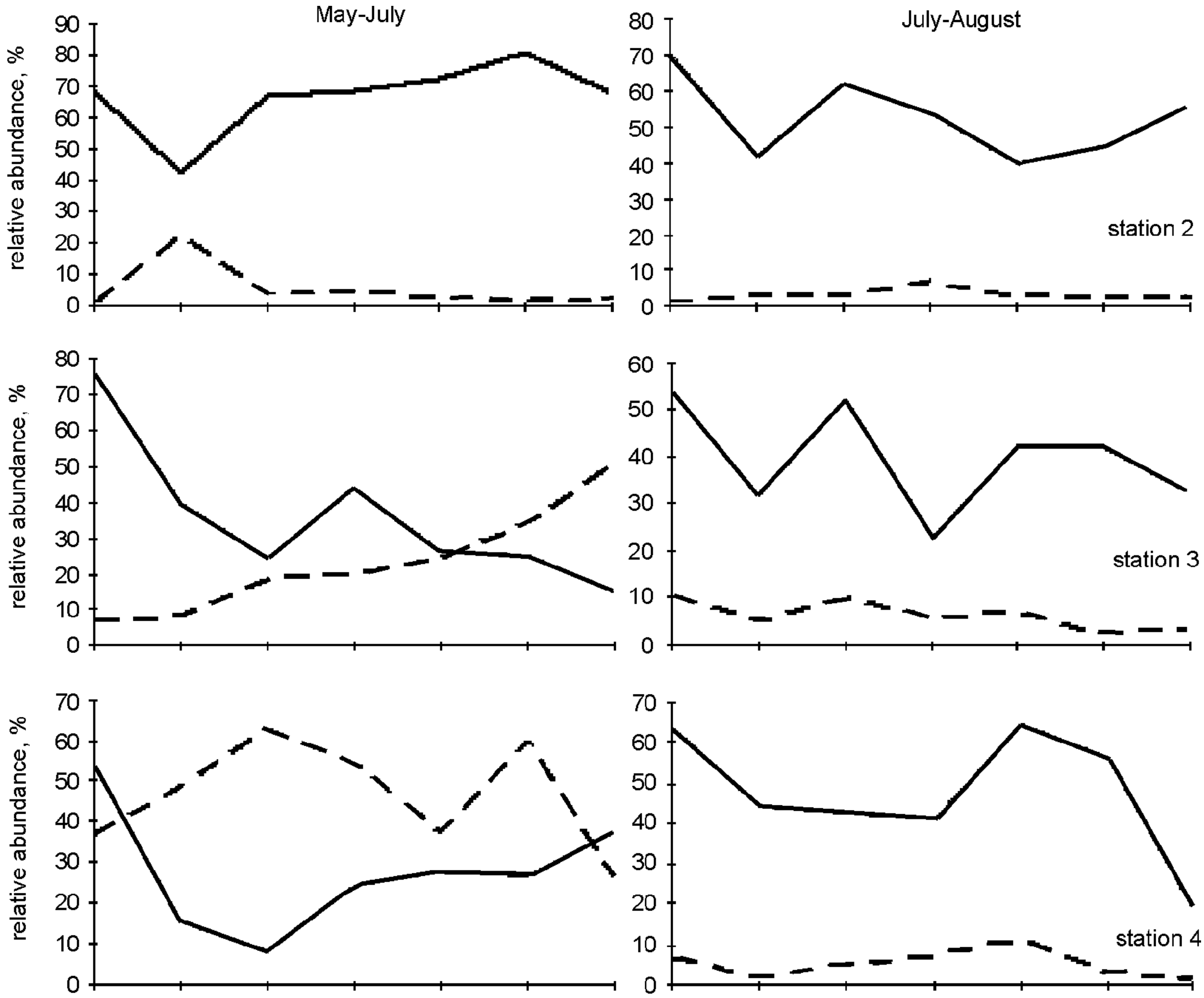


Fig. 9. Abundance changes of marine (continuous line) and brackish-water (dotted line) ciliates in experimental communities (from Burkovsky and Mazei, 2001).

differences in species composition. On the contrary, sediment characteristics affect both complexity and abundance of ciliate community. This is a common characteristic of coastal microzoobenthic communities (Hansen and Alongi, 1991; Fernandez-Leborans et al., 2001). Planktonic ciliate community in the estuary also shows clear reduction of abundance with decreasing salinity (Elserehy and Sleigh, 1993). However, planktonic heterotrophic flagellate community is characterized by the opposite tendency. Colourless nanoflagellate concentrations decrease from freshwater to the marine estuarine zone (Lovejoy et al., 1993). Thus protozoan community in the estuary may be considered as environmentally constrained and the relative role of factors influencing species diversity may change rapidly in space and time (Dolan and Gallegos, 2001).

Another important set of factors determining ciliate community structure is trophic interactions. Ciliates are active grazers of flagellate, algal and bacterial production, the amount of which is positively correlated with organic matter content (Epstein and Shiaris, 1992a, 1992b; Vaque et al., 1992; Jonsson and Johansson, 1997; Zimmermann, 1997; Garstecki and Wickham, 2001; Mazei et al., 2001). In summer the important influence of protozoans on the microbial food web functioning was evident, which indicated positive feedbacks in the trophic structure (Delorenzo et al., 2001). Ciliates and heterotrophic nanoflagellates showed predator-prey-like coupled oscillations (Kuuppoleinikki et al., 1994). On the other hand, a significant reduction in ciliate numbers in the presence of the predatory nematode in the intertidal zone was shown (Hamels et al., 2001). Moreover, ciliates are considered as a trophic link between picoplankters and filter-feeding bivalves, oysters, for example (Legall et al., 1997).

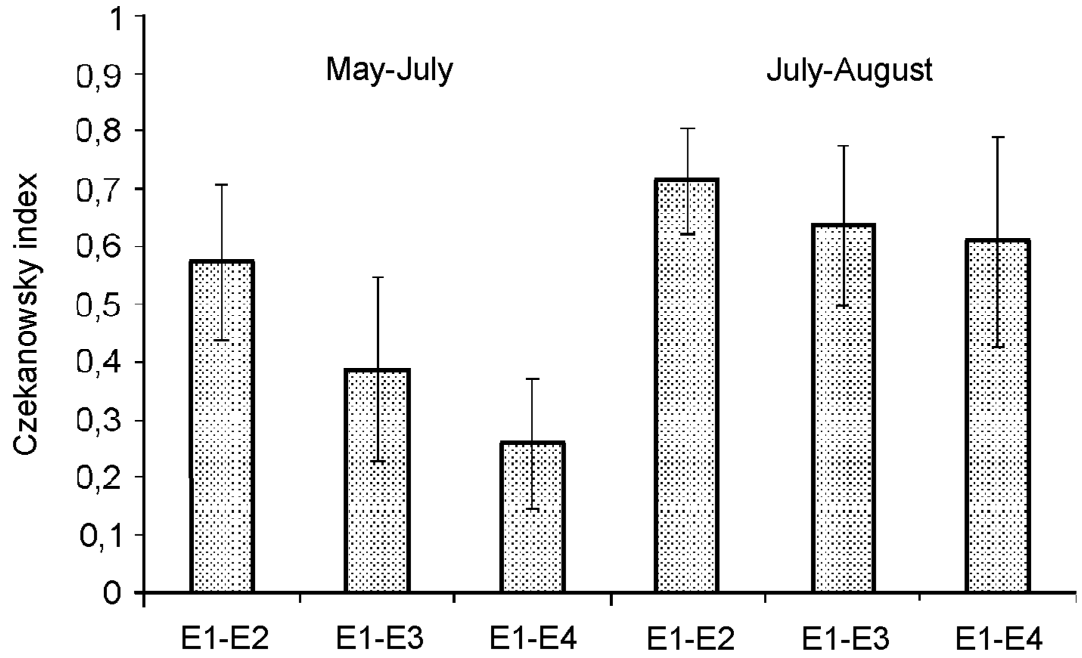


Fig. 10. Average structural similarity (Czekanowsky indices) of control (E1) and experimental (E2-E4) communities. Whiskers are standard deviations.

Comparative study of different groups of benthic organisms may clarify the role of trophic interactions in structuring the community composition and abundance. Analysis of distribution of different size classes of benthic organisms in the Chernaya river estuary was conducted (Burkovsky et al., 1994; Udalov et al., 2004). Bacteria are most abundant in fine-grained sediments with a great share of organic matter. Therewith, salinity itself does not affect the total density of this group. Total biomass of diatoms increases in the brackish water part of the estuary in coarse-grain sediments. Distribution of autotrophic flagellates (dinoflagellates) is inverse with respect to diatoms, showing the maximum abundance and biomass in the seaward part of the estuary (see Saburova et al., 2001 for details). The abundance of nematodes is independent of salinity and directly related to the grain-size, namely, higher densities are observed on silts and fine-grained sands. The abundance of ostracods increases with salinity decrease. The abundance of harpacticoid copepods is related, to an equal extend, to salinity and sediment properties (see Udalov et al., 2004 for details). In macrobenthos community, as the mean values of salinity decrease, a gradual decrease in the total biomass and mean size of the organisms as well as increase of the total abundance is observed. With the salinity decrease, large forms of macrobenthos (mostly molluscs and polychaetes) disappear and small ones (mostly chironomids and oligochaetes) being to occupy the dominant position (see Burkovsky et al., 1995 and Stolyarov et al., 2002 for details).

Thus, biomasses of micro- and macrobenthos decrease towards the river, whereas biomass of the meiobenthos shows an opposite tendency. Probably this is associated with spatial differentiation of neighbouring size classes that play an important role in reducing trophic intensity of the estuarine benthic community (Udalov et al., 2004). On the other hand, the trophic structure of micro- and meiobenthos changes in a mosaic way on a scale of tens of centimetres to a few meters and does not depend on the estuarine salinity gradient. At the same time, it is directly related to sediment properties. Bacterio- and detritophages prevail on silt sediments, while algophages, on sandy ones. In contrast, the changes in the trophic structure of the macrobenthos occur on a scale of hundred of meters to a few kilometers and follow the estuarine salinity gradient reflecting the content of organic matter in the ecosystem and the features of hydrodynamics. On the whole, ciliates, as well as macrobenthos, form a community, which is unified, continual and two-polar (with marine and brackish-water pool of species), with the border between the two poles being much closer to the river (between stations 3 and 4, with the average salinity 7-9‰) than in macrobenthos community (there it is further to the sea than station 2, with the average salinity 14-16‰). Moreover, the border between marine and brackish water community variants

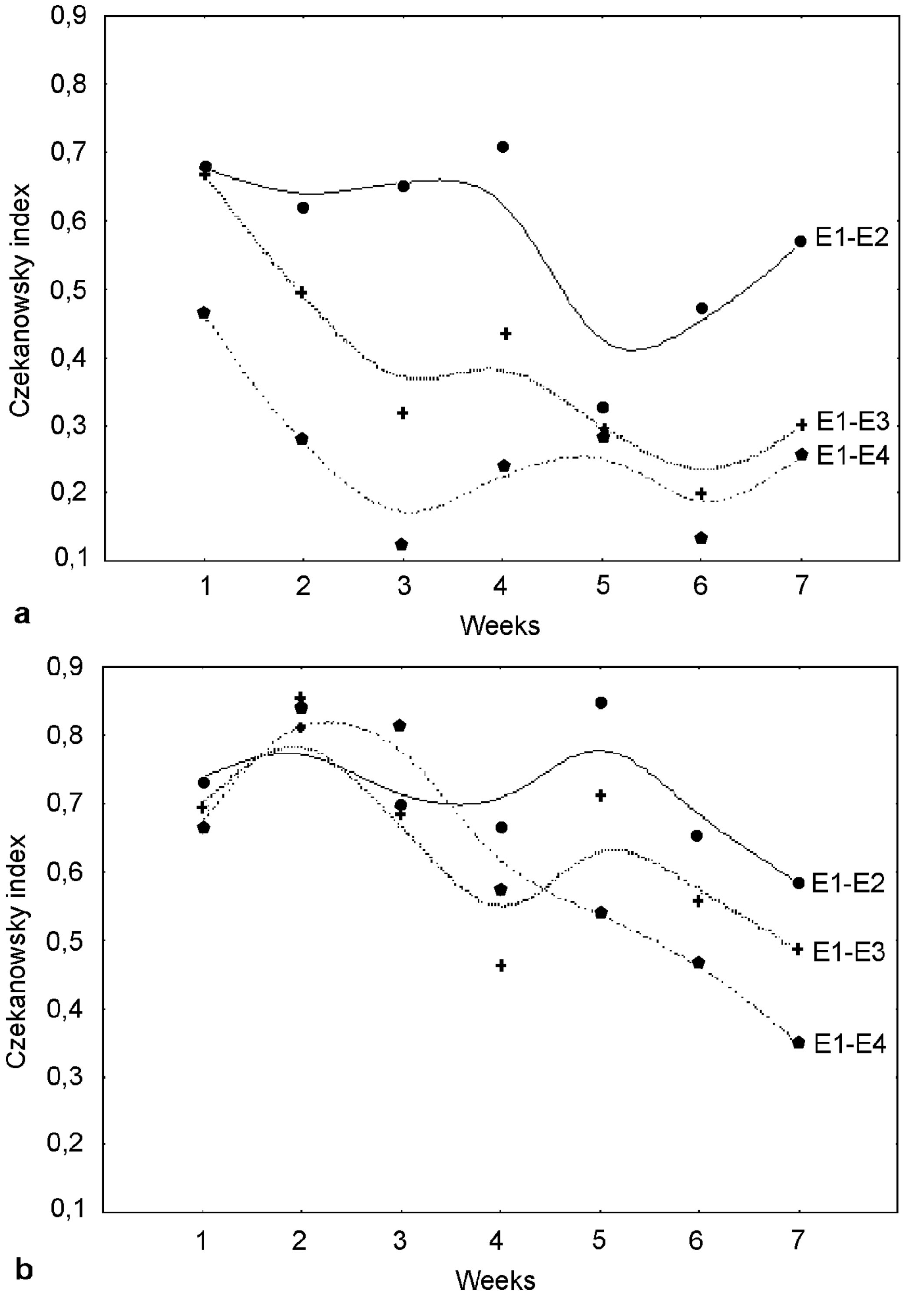


Fig. 11. Dynamics of structural similarity (Czekanowsky indices) of control (E1) and experimental (E2-E4) communities during May-July (a) and July-August (b). Curves constructed with the aid of distance-weighted least squares fitting.

is affected not only by the salinity regime but also by the total environmental variability. This fact indicates that organisms from different size classes "perceive" the environment on a different scale: for larger organisms

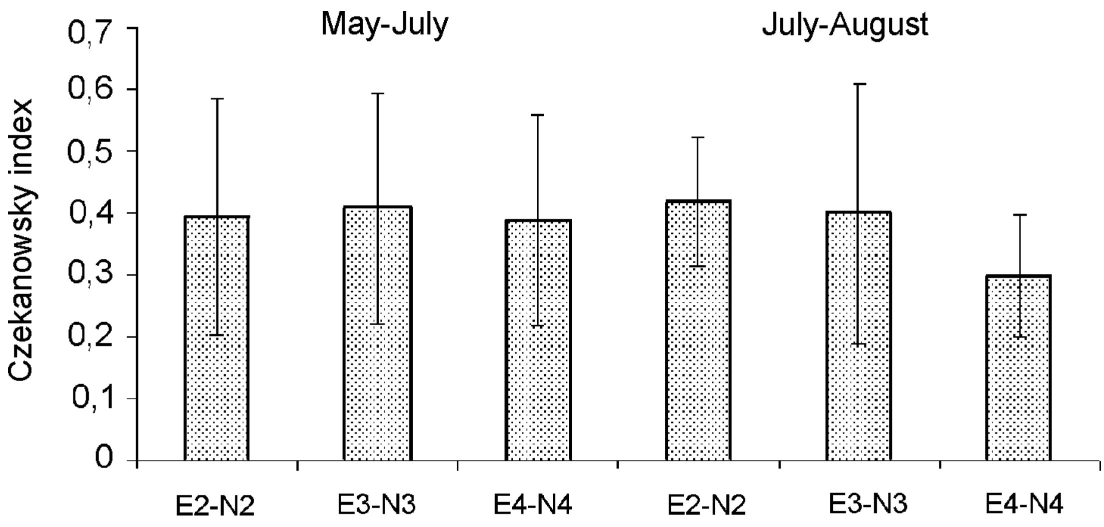


Fig. 12. Average structural similarity (Czekanowsky indices) of experimental (E2-E4) and corresponding natural (N2-N4) communities. Whiskers are standard deviations.

the average values of factors are more important, whereas for smaller ones environmental variability is more significant.

Conclusions

1. From the marine part of the estuary riverwards salinity decreases and oxidative-reductive properties of the sediment become more reduced due to organic accumulation. The greatest variability of salinity, acidity and redox-potential is observed in the brackish water part of the estuary. All other factors have a more complex distribution in the intertidal zone, being both gradient and mosaic.

2. During the period of the investigation altogether 123 species of psammophilous ciliates were found.

3. Psammophilous ciliate community in the estuary may be considered as a rather unified, continual and two-polar (with marine and brackish-water pool of species). A relative and fuzzy border between the two poles is situated in the brackish water part of the estuary, where the greatest variability of salinity and redox-potential as well as critical salinity level (3-8‰) are observed.

4. In the estuary towards the river the complexity of ciliate community in terms of average ciliate density, biomass and species richness significantly decreases. Decrease in species richness results from congeners. This probably reflects a simplification of the niche structure.

5. Seasonal changes of ciliate community structure in the marine zone may be characterized as rather trended and stable. Changes of community structure in the freshened zone of the estuary, on the contrary, are more variable and stochastic. Community in the intermediate zone of the estuary is both trended and highly heterogeneous.

6. Community biomass, abundance and species richness are positively connected with average salinity and redox-potential levels. Variability of integral community characteristics is related predominately to sediment parameters (amount of organic matter and relative availability of large size fraction of sand grains). Variability of saline regime affects that of the total abundance and biomass of the community but not the variability of species richness and diversity.

7. Analysis of the role of salinity in ciliate community formation reveals the trended changes of species composition and non-linear modifications of integral community characteristics. All community variants are divided into two groups with equal complexity but different species composition: marine and brackish water.

8. Specificity of brackish water community variants is affected both by the ability of complex marine community to sustain its own structure under the influence of environmental conditions and by the ability of oligohaline ciliates to immigrate into the disturbed (by the influence of the lower salinity) isolates. The former mechanism predominates at a relatively higher salinity level as well as during terminal succession stage, whereas the latter determines community structure in the initial stages of succession within lower salinity level.

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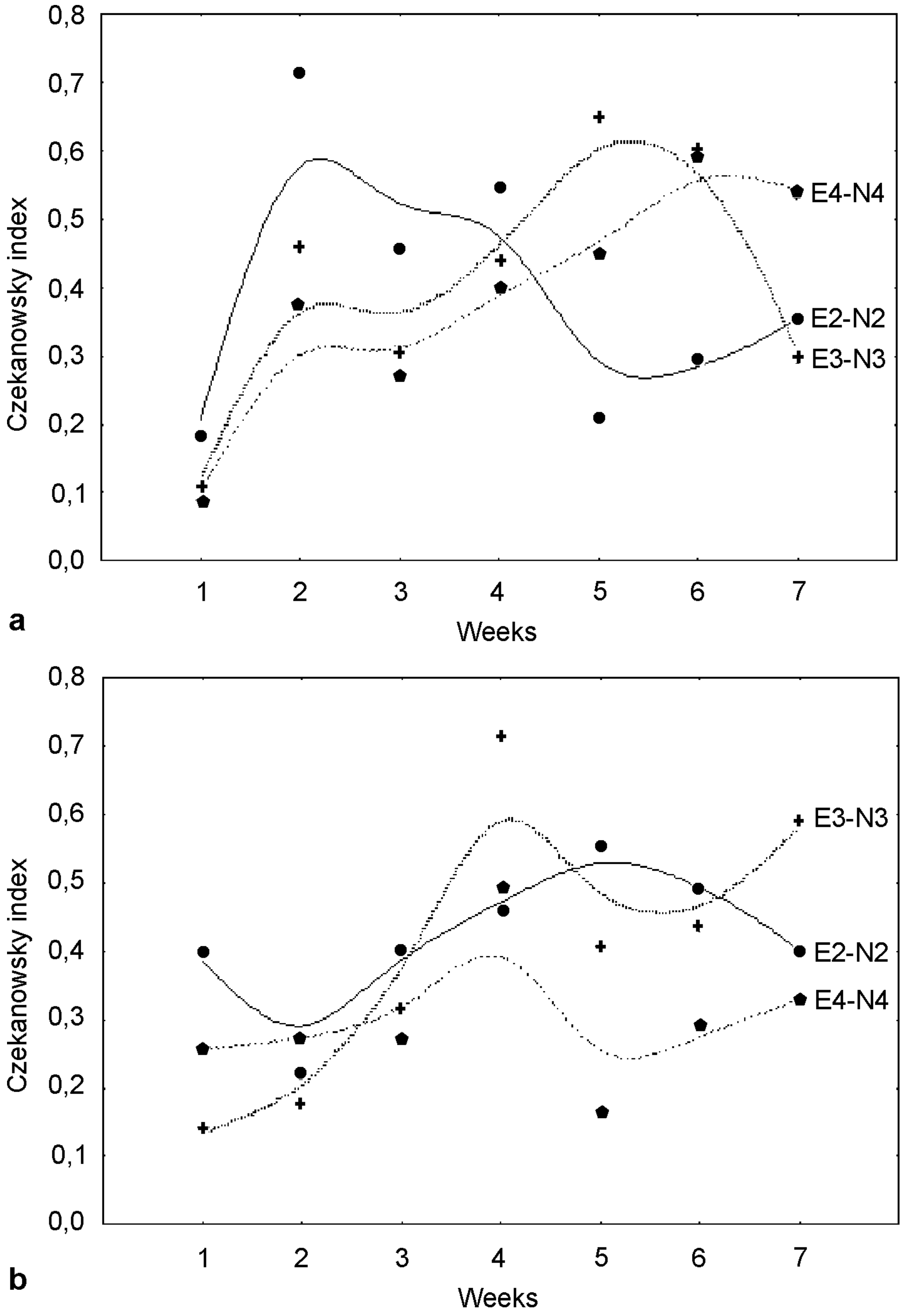

Fig. 13. Dynamics of structural similarity (Czekanowsky indices) of experimental (E2-E4) and corresponding natural (N2-N4) communities during May-July (a) and July-August (b). Curves constructed with the aid of distance-weighted least squares fitting.

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References

Azovsky A.I. 1993. ECOS - a problem-oriented software on community ecology. Version 1.3.

Burkovsky I.V. 1984. Ecology of free-living ciliates. Moscow State Univ. Publ., Moscow (in Russian).

Burkovsky I.V. 1992. Structural-functional organization and stability of marine bottom communities. Moscow State Univ. Publ., Moscow (in Russian).

Burkovsky I.V., Azovsky A.I. and Mokiyevsky V.O. 1994. Scaling in benthos: from microfauna to macrofauna. Arch. Hydrobiol. Suppl. 99, 517-535.

Burkovsky I.V., Azovsky, A.I., Stolyarov, A.P. and Obridko S.V. 1995. Marcobenthos community structure of the White Sea intertidal zone in conditions of a pronounced gradient of environmental factors. Zhurn. Obsch. Biol. 56, 59-70 (in Russian with English summary).

Burkovsky I.V. and Mazei Yu.A. 2001. Ciliate community structure in the zone of mixing of sea and river waters. Entomol. Rev. 81 (Suppl.), 7-16.

Burkovsky I.V. and Stolyarov A.P. 1995. Characteristic features of the structural organization of macrobenthos in a biotope with a pronounced salinity gradient. Zool. Zhurn. 74, 32-46 (in Russian with English summary).

Burkovsky I.V., Zubkov M.V. and Kol'tsova T.V. 1989. The response of marine psammon to a decrease in light intensity. Ecologiya. 1, 35-42 (in Russian with English summary).

Delorenzo M.E., Lewitus A.J., Scott G.I. and Ross P.E. 2001. Use of metabolic inhibitors to characterize ecological interactions in an estuarine microbial food-web. Microb. Ecol. 42, 317-327.

Dolan J.R. and Gallegos C.L. 2001. Estuarine diversity of tintinnids (planktonic ciliates). J. Plankt. Res. 23, 1009-1027.

Elserehy H.A.H. and Sleigh M.A. 1993. Ciliates in the plankton of the river Itchen estuary, England. Acta Protozool. 32, 183-190.

Epstein S.S. and Shiaris M.P. 1992a. Rates of microbenthic and meiobenthic bacterivory in a temperate muddy tidal flat community. Appl. Envir. Microbiol. 58, 2426-2431.

Epstein S.S. and Shiaris M.P. 1992b. Size-selective grazing of coastal bacterioplankton by natural assemblages of pigmented flagellates, colorless flagellates, and ciliates. Microb. Ecol. 23, 211-225.

Fernandez-Leborans G., Valganon B. and Perez E. 2001. Characterization of the protistan communities inhabiting the benthic area of an inner estuary. Bull. Mar. Sci. 68, 451-467.

Foissner W. 1991. Basic light and scanning electron microscopic methods for taxonomic studies of ciliated Protozoa. Eur. J. Protistol. 27, 210-223.

Garstecki T. and Wickham S.A. 2001. Effects of resuspension and mixing on population dynamics and trophic interactions in a model benthic microbial food-web. Aquat. Microb. Ecol. 25, 281-292.

Gaughan D.J. and Potter I.C. 1995. Composition, distribution and seasonal abundance of zooplankton in a shallow, seasonally closed estuary in temperate Australia. Estation Coastation Shelf Sci. 41, 117-135.

Guiral D. 1992. L'instabilite physique, facteur d'organisation et de structuration d'un ecosysteme tropical saumatre peu profond: la Lagune Ebrie. Vie et Milieu. 42, 73-92.

Jiang H. 1996. Diatoms from the surface sediments of the Skagerrak and the Kattegat and their relationship to the spatial changes of environmental variables. J. Biogeogr. 23, 129-137.

Jonsson P.R. and Johansson M. 1997. Swimming behavior, patch exploitation and dispersal capacity of a marine benthic ciliate in flume flow. J. Exp. Mar. Biol. Ecol. 215, 135-153.

Hamels I., Sabbe K., Muylaert K., Barranguet C., Lucas C., Herman P. and Wyverman W. 1998. Organization of microbenthic communities in intertidal estuarine flats, a case-study from the Molenplaat (Westerschelde estuary, the Netherlands). Eur. J. Protistol. 34, 308-320.

Hamels I., Moens T., Mutylaert K. and Vyverman W. 2001. Trophic interactions between ciliates and nematodes from an intertidal flat. Aquat. Microb. Ecol. 26, 61-72.

Hamels I., Sabbe K., Muylaert K. and Vyverman W. 2004. Quantitative importance, composition, and seasonal dynamics of protozoan communities in polyhaline versus freshwater intertidal sediments. Microb. Ecol. 47, 18-29.

Hansen J.A. and Alongi D.M. 1991. Bacterial productivity and benthic standing stocks in a tropical coastal embayment. Mar. Ecol. Progr. Ser. 68, 301-310.

Khlebovich V.V. 1974. Critical salinity of biological processes. Nauka Publ., Leningrad (in Russian).

Khlebovich V.V. 1981. Acclimation of animal organisms. Nauka Publ., Leningrad (in Russian).

Khlebovich V.V. 1986. On biological typology of estuaries of the Soviet Union. Proc. Zool. Inst. AS USSR. 141, 5-16 (in Russian).

Kuuppoleinikki P., Autio R., Hallfors S., Kuosa H., Kuparinen J. and Pajuniemi R. 1994. Trophic interactions and carbon flow between picoplankton and protozoa in pelagic enclosures manipulated with nutrients and a top predator. Mar. Ecol. Progr. Ser. 107, 89-102.

Legall S., Hassen M.B. and Legall P. 1997. Ingestion of a bacterivorous ciliate by the oyster *Crassostrea gigas* - Protozoa as a trophic link between picoplankton and benthic suspension-feeders. Mar. Ecol. Progr. Ser.. 152, 301-306.

Lovejoy C., Vincent W.F., Frenette J.J. and Dodson J.J. 1993. Microbial gradients in a turbid estuary - application of a new method for protozoan community analysis. Limnol. Oceanogr. 38, 1295-1300.

Mazei Yu.A. and Burkovsky I.V. 2002. Spatial and temporal changes of psammophilous ciliate community in the White Sea estuary. Devel. Curr. biol. 122, 183-189 (in Russian with English summary).

Mazei Yu.A., Burkovsky I.V., Saburova M.A., Polikarpov I.G. and Stoljarov A.P. 2001. Trophic structure of psammophilous ciliate community in the Chernaya River estuary. Entomol. Rev. 81, Suppl. 1, 26-35.

Petukhov Yu.M., Shalovenkov N.N., Revkov N.K. and Petrov A.N. 1991. Analysis of spatial distribution of macrozoobenthos in the Black Sea Bay Laspi with the use of multivariate statistics methods. Okeanologiya. 31, 780-786 (in Russian with English summary).

Pogrebov V.B. 1988. Intertidal zone of a half-closed aquatory of the White Sea in conditions of water freshening, III. Species ordination and gradient analysis. Vestn. Leningr. Univ. 3, 8-17 (in Russian).

Pogrebov V.B. and Goryanina O.O. 1988. Intertidal zone of a half-closed aquatory of the White Sea in conditions of water freshening. I. Major ecological factors, composition and spatial structure of settlements of hydrobionts. Vestn. Leningr. Univ. 3, 10-17 (in Russian with English summary).

Saburova M.A., Polikarpov I.G., Burkovsky I.V. and Mazei Yu.A. 2001. Macroscale distribution of interstitial microphytobenthos in the Chernaya River estuary (Kandalaksha Bay, the White Sea). Ekologiya Morya. 58, 7-12 (in Russian with English summary).

Salzwedel H., Mora J., Garmendia J.P. and Lastra M. 1993. Estructuro trofica del macrozoobentos submareal de la Ria de Ares-Betanzos. I. Composicion y distribucion. Instation Esp. Oceanogr. 11, 33-40.

StatSoft, Inc. 1999. STATISTICA for Windows - Computer program manual (http://www.statsoft.com).

Stolyarov A.P. 1994. Zonal character of macrobenthos distribution in the River Chernaya estuary (the Kandalaksha Bay, the White Sea). Zool. Zhurn. 73, 65-71 (in Russian with English summary).

Stolyarov A.P., Burkovsky I.V., Chertoprud M.V. and Udalov A.A. 2002. Satial-temporal structure of littoral macrobenthos community in the Chernaya River estuary (Kandalaksha Bay, the White Sea). Dev. Curr. Biol. 122, 537-547 (in Russian with English summary).

Udalov A.A., Burkovsky I.V., Mokievsky V.O., Stoljarov A.P., Mazei Y.A., Chertoprood M.V., Chertoprood E.S., Saburova M.A., Kolobov M.J. and Ponomarev S.A. 2004. Changes in the general characteristics of micro-, meio- and macrobenthos along the salinity gradient in the White Sea estuary. Okeanology. 44, 549-560.

Uhlig G. 1964. Eine einfache Methode zur Extraktion der vagilen mesopsammalen Mikrofauna. Helgoll. Wiss. Meeresuntersuch. 11, 178-185.

Uhlig G. 1965. Untersuchungen zur Extraktion der vagilen Mikrofauna aus marinen Sedimenten. Verh. Dtsch. Zool. Ges. 151-157.

Underwood G.J.C., Phillips J. and Saunders K. 1998. Distribution of estuarine benthic diatom species along salinity and nutrient gradients. Eur. J. Phycol. 33, 173-183.

Vaque D., Pace M.L., Findlay S. and Lints D. 1992. Fate of bacterial production in a heterotrophic ecosystem - grazing by protists and metazoans in the Hudson estuary. Mar. Ecol. Progr. Ser. 89, 155-163.

Zimmermann H. 1997. The microbial community on aggregates in the Elbe estuary, Germany. Aquat. Microb. Ecol. 13, 37-46.

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