

THE EFFECT OF SOME ENVIRONMENTAL FACTORS ON PROTOZOA POPULATIONS OF THE RIVER DANUBE

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Abstract. The simultaneous effect of nine environmental factors (water discharge, water temperature, dissolved oxygen content, ammonium content, pH, chemical oxygen demand (by KMnO_4), total dissolved solids, Coliform and psychrophyl bacterial counts) were investigated on the population size of 30 planktonic Protozoa species.

Data series of four years with different hydrological regimes were evaluated by correlation and path analyses.

Regarding the direct effects higher than 5%, 57% of the species were influenced by ammonium content, 40% by pH, 33% by dissolved oxygen content, by chemical oxygen demand and by total dissolved solids each, 27% by Coliform, 23% by psychrophilic bacterial count, 17% by water discharge and 7% by water temperature.

Key words: planktonic Protozoa populations, running water, path-analysis, environmental factors, River Danube.

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Introduction

One of the classic and even today most important fields in ecology is the investigation of the effects of various biotic and abiotic environmental factors on the living organisms.

For the classic and evergreen nature of this topic, even a concise and selective survey of the references is impossible. The majority of the studies, however deals with the effect of one, or a few factors. Papers dealing with the joint effects of several factors there are in much smaller number (e.g. Nosek and Bereczky, 1981; Pratt et al., 1987; Reed, 1978; Samuels et al., 1979; Say and Whitton, 1983; Stewart et al., 1986; Wehr and Whitton, 1983; etc.)

There are basically two possibilities for the investigation of the effects of various environmental factors: laboratory experiments and/or field studies. Under laboratory conditions the range of a factor can be adjusted, and the experiment can be

reproduced several times. The factors however are not independent from each other, so the effects of their combinations may not be neglected. The adjusting of the factors and their combinations can be solved relatively simply only for a few ones, and the interpretation of the results may be difficult for natural conditions.

In the case of field studies the ranges of each factor and their combinations are determined by nature. The effect of a single factor itself is hardly to be determined (apart from very extreme biotopes). The responses of living organisms reflect the effect of all relevant factors. There are difficulties in the separation of the effects and the determination of their relative importance. The use of the path analysis may, however, overcome these difficulties (Anders, 1986; Lee, 1955; Le Roy, 1956).

This paper describes the complex effect of certain environmental factors on the size of some free living Protozoa populations of River Danube.

Methods

The sampling site is in the main channel of the Danube at Göd, about twenty kilometers above Budapest, at river km. mark 1669. The Danube is here of midland character, with a mean annual water discharge of $2200 \text{ m}^3 \text{ s}^{-1}$. The substrate type is river ballast.

Samples have been taken for more than two decades from this site with weekly frequency, filtering 100 l water through a plankton net of $10 \mu\text{m}$ mesh size at each occasion. Samples have been analysed partly living and partly fixed by the Pargol method modified by Wilbert (1974) as well as by Bereczky's staining method (Bereczky, 1985).

As in running waters the water discharge is regarded to be the most important abiotic environmental factor, for detailed analysis from the long-term data series data of four years (1981, 1985, 1986 and 1987) were selected so, that the range of water discharge values be the widest possible. The total number of samples analysed was 140. Nine environmental factors were selected to reflect the natural conditions and the anthropogenic effects, too. The factors and their extreme values are listed in Table I.

Dissolved oxygen content was determined by Winkler's method, the ammonium content by the Nessler's method and the chemical oxygen demand by acid potassium permanganate (KMnO_4). Total dissolved solids means the evaporation residue of the filtered water. Bacterial counts were determined after incubation at 20°C and 37°C by counting the colonies developed.

Chemical data originate from the chemical laboratory of the Danube Research Station. The bacteriological analyses were carried out in the Institute for Public Health and Epidemiology of the City of Budapest.

Of the more than 500 Protozoa species occurring in the plankton of the Danube, species having

relative frequency of 10% or more were included in the study (Table 2.).

The effect of the factors studied was evaluated by path analysis. The path analysis takes the factors simultaneously into consideration. It is also possible to separate the effects of the single factors, to determine their relative importance, to make distinction between the direct and indirect effects as well as to assess the sum of effects of other factors not investigated (this is the so called error path).

To make the results comparable, species were investigated one by one, using the same path diagram (Fig. 1). The nine environmental parameters represent the independent variables and the number of individuals (the population size) of the given species the dependent one. The correlation between variables was estimated by Bravais correlation coefficient. Correlation between two independent variables was accepted in the diagram only if the sign of the correlation coefficient computed for the single years each and for the pooled data was the same, and its value was higher than 0.2 for the pooled data.

A bidirectional connection was accepted if a mutual influence could be supposed between two variables. This fact is represented in the diagram by dotted lines, and the degree of the correlation was estimated by the correlation coefficient. If only a "one way" effect (unidirectional connection) could be accepted, an arrow with one head, pointing to the direction of the effect was set in the diagram and the degree of correlation was estimated by the value of an internal path coefficient. E.g. in the case of water discharge - total dissolved solids, changes in water discharge may affect the amount of total dissolved solids, but a vice-versa effect is unimaginable. From each independent variable a direct path was ordered to the dependent one. In the diagram the thick, short arrows represent these direct effects of the separate independent variables. (To avoid the confusion they have not been drawn to the Y variable.) The indirect effect of one factor

Table I. Values of the environmental parameters

parameter	code	minimum	mean values	maximum
ammonium content (mg l^{-1})	NH	0.00	0.47	2.68
chemical oxygen content (mg l^{-1})	OD	5.00	7.20	17.30
dissolved oxygen demand (mg l^{-1})	DO	6.59	10.36	20.00
number of Coli-form bacteria (ind ml^{-1})	CB	0.00	222.40	931.00
number of psychrophil bacteria (10 ind ml^{-1})	PB	10.00	6.05	21.00
pH	PH	7.24	8.15	9.68
total dissolved solids (mg l^{-1})	TS	230.00	322.40	466.00
water discharge ($\text{m}^3 \text{ sec}^{-1}$)	WD	986.00	2375.00	6100.00
water temperature ($^\circ\text{C}$)	WT	0.40	12.72	22.00

Table 2. Relative frequency values and indicator character of the species

species	code	relative frequency	indicator character
<i>Carchesium polypinum</i> (Linnaeus,1758).	CAPO	24.3	a
<i>Codonella cratera</i> (Leidy,1877)	COCR	49.3	ob
<i>Coleps hirtus</i> (O.F.Mueller,1786)	COHI	66.4	ba
<i>Coleps hirtus</i> var. <i>lacustris</i> (Faure-Fremiet,1924)	COLA	22.9	*
<i>Colpidium campylum</i> (Stokes,1886)	CPCA	21.4	p
<i>Colpidium colpoda</i> (Losana,1829)	CPCO	16.4	p
<i>Epistylis plicatilis</i> (Ehrenberg,1831)	EPPL	15.7	a
<i>Epistylis pyriformis</i> Perty	EPY	33.6	*
<i>Glaucoma scintillans</i> Ehrenberg,1830	GLSC	22.9	p
<i>Paramecium caudatum</i> Ehrenberg,1833	PACA	39.3	a
<i>Paramecium putrinum</i> Claparede & Lachmann,1859	PAPU	27.9	p
<i>Phascolodon vorticella</i> Stein,1859	PHVO	80.0	b
<i>Prorodon teres</i> Ehrenberg,1833	PRTE	23.6	a
<i>Pseudovorticella margaritata</i> (Fromentel,1876)	PVMA	65.7	b
<i>Stauriphrya elegans</i> Zacharias,1893	SPEL	50.7	b
<i>Stentor polymorphus</i> (O.F.Mueller,1773)	STPO	55.0	b
<i>Stokesia vernalis</i> Wenrich,1929	SKVE	45.0	b
<i>Strobilidium caudatum</i> (Fromentel,1876)	SRCA	7.9	ob
<i>Strobilidium viride</i> Stein,1867	SBVI	21.4	b
<i>Tintinnidium fluviale</i> (Stein,1863)	TIFL	75.0	ob
<i>Trithigmotoma cucullulus</i> (O.F.Mueller,1786)	TRCU	27.1	a
<i>Urotricha farcta</i> Claparede & Lachmann,1859	URFA	32.9	a
<i>Vorticella campanula</i> Ehrenberg,1831	VOCA	70.0	b
<i>Vorticella convallaria</i> (Linnaeus,1758)	VOCO	37.1	a
<i>Vorticella incisa</i> Stiller	VOIN	52.9	*
<i>Vorticella microstoma</i> Ehrenberg,1830	VOMI	37.9	p
<i>Vorticella nebulifera</i> O.F.Muller,1773	VONE	79.3	*
<i>Vorticella similis</i> Stokes,1887	VOSI	80.0	*
<i>Zoothamnium minimum</i> Stiller	ZOMI	30.7	*
<i>Zoothamnium varians</i> Stiller	ZOVA	44.3	*

(ob - oligo-betameso-, b - betameso-, ba - beta-alfameso-, a - alfa-, p - polysaprob indicator species,* - species without indicator character)

means the effect of the factor caused by the modification of one or more another factor. E.g. chemical oxygen demand may affect the population size of a species direct and by modifying the ammonium content, or the dissolved oxygen content, etc., too (cf. Fig. 1).

Results

The environmental factors investigated were generally independent from each other, a moderate ($0.4 < r < 0.7$) or strong ($0.7 < r < 0.9$) correlation could be established only in a few cases (Table 3). The correlation between the dissolved oxygen content and pH was already registered in the main channel (Nosek and Berezky, 1981), but its value was then much lower.

Between the number of individuals of the species and the separate environmental factors moderate positive and/or negative correlation was found only in the case of eight species (*Epistylis plicatilis*-water temperature, $r=0.427^*$; *Epistylis*

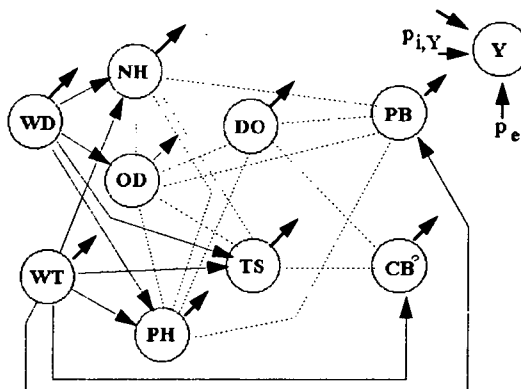


Fig. 1. The path diagram applied to the species. Thin solid lines represent the inner paths, short thick solid lines the direct paths to the dependent variable and dotted lines the bidirectional connections. p_e is the error path.

plicatilis-ammonium content, $r=0.571^{**}$; *Epistylis plicatilis*-pH, $r=0.452^*$; *Urotricha farcta*-ammonium content, $r=-0.632^{***}$; *Urotricha farcta*-chemical oxygen demand, $r=0.521^{***}$; *Glaucoma scintillans*-water temperature, $r=0.462^{**}$; *Parame-*

cium putrinum-water temperature, $r=-0.434^{**}$; *Strobilidium caudatum*-water discharge, $r=0.636^{*}$; *Vorticella microstoma*-ammonium content, $r=0.659^{***}$; *Vorticella microstoma*-number of psychrophil bacteria, $r=0.609^{***}$; *Vorticella similis*-chemical oxygen demand, $r=0.678^{***}$; *Zoothamnium minimum*-ammonium content, $r=0.647^{***}$; *Zoothamnium varians*-total dissolved solids, $r=0.491^{***}$, where $^{*}=p<0.05$, $^{**}=p<0.01$ and $^{***}=p<0.001$).

Table 3. The values of the correlation coefficients between the environmental factors

	WT	NH	DO	OD	TS	PH	PB	CB
WD	.125	.290	-.092	-.239	-.506	-.257	.066	-.100
WT		-.415	.014	.064	-.610	.378	-.223	-.282
NH			.039	-.423	-.174	-.500	.135	-.068
DO				.315	.080	.434	-.190	-.159
OD					.174	.205	-.140	-.064
TS						.016	.003	.239
PH							-.302	-.098
PB								.315

[critical values for correlation coefficients (n=140): P.05 = 0.167, p.01 = 0.217]

The numerical results of the path analyses are summarized in Tables 4., 5., 6. and 7. (Environmental factors and species are presented by their codes in the tables). The negative sign of a direct, or indirect path coefficient means, that the factor influences the variance of the dependent variable (the population size of species) inversely, that is by increasing value of the factor the population size decreases. The value of R^2 (multiple determination coefficient) shows the proportion of the variance of the dependent variable attributed to the independent variables (all direct and indirect effects). Since p_e^2 equals $1-R^2$, the last column in Table 5. reflects the effect of all unknown factors.

Most of the environmental factors (8) produced a direct effect on *Urotricha farcta*. None of them affected directly the species *Vorticella campanula* and *Tintinnidium fluviatile*. Five species were affected by only one factor (*Coleps hirtus*, *Colpidium campylum*, *Epistylis pyriformis*, *Vorticella margaritata* and *Vorticella nebulifera*). Two factors were effective on seven species (*Coleps hirtus* var. *lacustris*, *Paramecium caudatum*, *Phascolodon vorticella*, *Prorodon teres*, *Strombidium viride*, *Staurophrya elegans* and *Vorticella incisa*). The other species were affected

by three or four factors.

Among the 29 species (the path analysis failed in the case of *Vorticella microstoma*), considering direct effects as high as and higher than 5% (Table 6.), water temperature had an effect on two species, water discharge on five species, psychrophilic bacterial count on seven species, Coliform bacterial count on eight species, dissolved oxygen content, chemical oxygen demand and total dissolved solids on ten species, pH on twelve species and ammonium content on seventeen species.

Table 5. contains the sum of indirect effects, too. Most of the indirect effects were also small, of them generally those combinations reached higher values, where the corresponding direct effects were also greater. Regarding the two-step indirect effects ($x_i \rightarrow x_j \rightarrow Y$, $i \neq j$) greater than 5%, in the majority of them one member of the combination were ammonium content, or total dissolved solids, or chemical oxygen demand or pH. Species in general were negatively affected by the indirect effects. Table 7. contains the number of species affected by two-step indirect effects greater than 5%. (Three-step indirect effects - as e.g. the $WT \rightarrow PH \rightarrow DO \rightarrow Y$, or $WD \rightarrow OD \rightarrow TS \rightarrow Y$ path - were all below 0.01%).

Discussion

The effect of environmental factor on the organisms may be investigated and/or evaluated by different manner. One of the possibilities is the investigation of the ecological valence, revealing whether the range of occurrence or the frequency distribution (whithin this range) of a species along a single factor. Such investigation may be carried out under laboratory conditions (e.g. Bick, 1968) or based on field observations (e.g. Bereczky and Nosek, 1993). Another possibility is the application of path analysis, demonstrated in this paper.

Although the aim of these approaches is the same, their results cannot be compared directly, or explained mutual, because of the differences in their theoretical foundations. In the case of an ecological valence study the question is to establish the ecological demand of a species that is the extreme values of the factor - lower (t_l) and upper (t_u) - are to be found whithin those the species occurs (cf. Fig. 2).

Table 4. Percentage values of the direct path coefficients ($p^2_{i,Y}$)

species	parameters								
	WD	WT	NH	DO	OD	TS	PH	PB	CB
CAPO	(-)5.34	(-)0.24	23.17	(-)9.27	3.85	5.79	7.86	(-)0.66	4.36
COCR	(-)0.62	0.02	(-)8.67	5.54	(-)4.05	(-)4.18	0.92	8.22	1.26
COHI	(-)2.02	(-)0.08	(-)4.46	(-)0.03	(-)2.56	14.23	(-)0.26	(-)4.80	(-)1.46
COLA	.19	(-)0.43	(-)10.93	8.26	(-)3.08	2.36	(-)0.42	(-)3.28	(-)0.01
CPCA	1.56	(-)0.07	1.73	(-)0.01	(-)3.47	17.17	1.24	3.49	(-)0.05
CPCO	4.20	0.02	14.50	0.76	(-)9.18	13.10	24.51	0.27	(-)0.26
EPPL	0.02	0.10	(-)0.10	(-)51.72	15.15	(-)0.17	40.09	(-)1.27	(-)14.39
EPPY	(-)2.10	(-)0.42	(-)0.06	11.60	(-)0.58	(-)0.49	0.01	(-)1.98	0.06
GLSC	0.06	(-)0.13	22.45	(-)4.73	8.06	7.35	2.88	1.61	16.25
PACA	(-) 10.50	0.32	(-) 0.24	(-) 1.47	(-) 2.78	3.32	1.17	(-) 4.24	(-) 13.56
PAPU	(-)0.87	(-)4.58	(-)9.71	0.99	(-)12.02	14.17	(-)19.55	(-)3.91	(-)1.51
PHVO	4.86	(-)0.90	(-)15.96	7.60	(-)3.64	0.73	0.52	(-)0.01	(-)3.18
PRTE	0.01	(-)2.46	(-)5.18	(-)0.61	5.67	0.66	(-)2.24	0.69	0.42
PVMA	(-)4.25	(-)0.19	(-)1.25	(-)0.01	(-)0.69	0.88	(-)1.98	(-)1.01	8.76
SBVI	4.32	0.74	9.49	0.25	(-)2.11	(-)0.96	(-)0.88	(-)8.78	(-)2.36
SKVE	0.93	(-)0.01	0.24	0.40	(-)0.01	1.74	9.63	9.27	(-)9.11
SPEL	0.06	(-)5.78	(-)10.41	3.25	(-)0.49	(-)0.12	(-)0.33	0.87	(-)0.01
SRCA	37.78	(-)0.22	(-)7.88	11.38	(-)0.61	(-)0.02	(-)26.38	(-)0.83	0.75
STPO	(-)5.83	(-)0.92	(-)1.66	0.41	(-)0.14	2.82	(-)11.31	(-)1.44	11.90
TIFL	0.67	0.19	(-)0.24	0.98	(-) 2.40	0.01	(-) 1.51	(-) 3.63	(-) 0.10
TRCU	(-)3.19	0.18	8.08	(-)1.44	(-)0.96	15.19	12.10	7.08	(-)0.74
URFA	(-)0.17	(-)5.59	(-)6.01	(-)10.62	17.83	13.07	13.62	8.59	8.34
VOCA	(-) 4.13	(-) 3.88	(-) 0.83	(-) 0.01	(-) 0.63	1.77	(-) 0.20	0.06	2.18
VOCO	(-)6.73	(-)2.45	(-)5.16	0.05	(-)6.89	4.23	(-)13.37	0.02	0.87
VOIN	0.01	(-)1.06	(-)3.08	(-)0.35	8.34	(-)2.59	0.24	25.01	(-)4.58
VOMI	(-)0.13	1.10	217.83	(-)33.30	74.18	22.30	97.23	82.54	(-)36.21
VONE	(-)1.80	0.20	1.35	(-)0.02	(-)0.98	5.78	(-)0.11	1.69	(-)0.01
VOSI	0.79	(-)1.89	(-)16.26	13.88	(-)5.62	2.42	(-)0.40	(-)0.04	(-)0.20
ZOMI	(-)0.21	(-)2.67	(-)75.01	1.55	(-)5.35	12.29	(-)66.93	(-)6.61	1.57
ZOVA	(-)0.78	(-)4.87	(-)36.17	5.76	(-)3.57	4.53	(-)37.93	(-)0.63	9.27
IND76		(-) 4.90		(-) 3.50	(-) 0.30				
IND87	(-)0.07	(-)0.58	(-)2.67	2.28	(-)3.83	5.58	(-)0.01	.09	(-)0.01

(Parameters and species corresponding to the codes are listed in Tables 1 and 2. IND76 and IND87 represent the results of the analyses carried out on the total number of individuals of the previous and present study. Negative sign in parenthesis indicates a negative effect of the factor in question.)

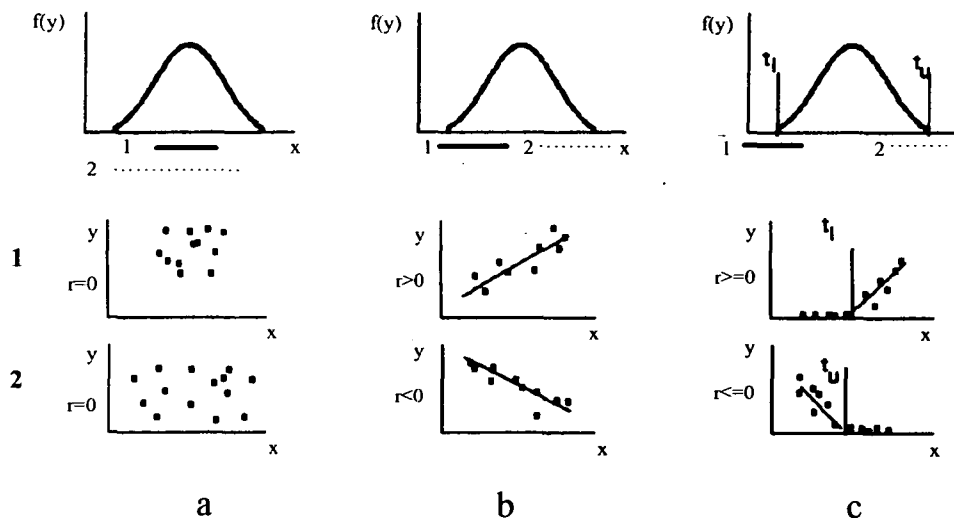


Fig. 2. The relationship between ecological valence and path analysis. (Explanation see in the text.)

The path analysis relies on the behaviour of the variances, that is on the relation between the variances of environmental factors and the variance of the population size of a species. The question is to what extent the size of the population is influenced by the different factors.

The analysis of ecological valences is a univariate method, the factors are considered one by one, independent from each other; even if they are numerous. Path analysis whereas is essentially a multivariate method, revealing the relative importance not only the factors incorporated in the system, but also the total significance of the factors considered (the error path).

Regarding only one factor the relation of these approaches is the following.

If the range of the factor values overlaps with the middle part of the occurrence interval of the species, or involves the total interval, no effect can be detected, because factor values both increasing and decreasing the population size are present. The population size appears to be independent of the changes of the factor. The value of the path coefficient will be very low or zero (Fig. 2a).

If the range of the factor coincides only with the lower or upper peius area (suboptimal range) of the species, negative or positive effect can be established (Fig. 2b). The path coefficient will be negative or positive and of different, but not negligible value.

Finally, if the range of the factor is about or exceeds the lower or upper pessimum value of the population, species will be absent, or present only in small and fluctuating degree (Fig. 2c). The value of the path coefficient will be very low or zero (as in case 2a), nevertheless there is an effect, since below (or beyond) a given value (the treshhold value) of the factor the species does not occur. This effect is, however, a treshhold effect. With other words the treshhold effect is a digital sign, while the effect reflected in the path coefficient is an analog sign.

The rank order of the factors, their relative importance, the degree of their effects may be detailed for species to species, the separate species may be compared to one another, but these conclusions are involved in the tables (Tables 4. and 5.), so a textual repetitions seems to be redundant. Therefore instead of this enumeration, we try to draw some generalized conclusions.

Dividing all species into two groups, of which the one (A) contains the oligosaprobic, oligo-beta mesosaprobic and beta mesosaprobic species, indicating a better water quality, and the other (B) contains the beta-alpha mesosaprobic, the alpha mesosaprobic and polysaprobic ones, indicating a

worse water quality, we can establish the followings on the basis of direct path coefficients higher than 1%.

Table 5. Percentage values of the direct total, indirect total, R² and error path coefficients

species	direct % total	indirect % total	R ² %	error %
CAPO	60.58	-22.38	38.21	61.79
COCR	33.53	-9.26	24.27	75.73
COHI	29.94	2.51	32.46	67.54
COLA	29.01	-10.77	18.23	81.77
CPCA	28.81	-6.81	22.01	77.99
CPCO	66.84	-29.37	37.48	62.52
EPPL	123.06	-44.94	78.13	21.87
EPY	17.33	-1.54	15.79	84.21
GLSC	63.55	-12.26	51.30	48.70
PACA	37.64	.99	38.63	61.37
PAPU	67.35	-16.94	50.41	49.59
PHVO	37.43	-14.15	23.28	76.72
PRTE	18.01	-3.93	14.08	85.92
PVMA	19.06	.96	20.03	79.94
SBVI	29.91	14.56	44.48	55.52
SKVE	31.36	-14.81	16.56	83.44
SPEL	21.35	-12.71	8.65	91.35
SRCA	85.89	-30.98	54.91	45.09
STPO	36.48	.62	37.10	62.90
TIFL	9.78	-2.85	6.92	93.08
TRCU	49.01	-19.58	29.43	70.57
URFA	83.90	-7.87	76.03	23.97
VOCA	13.73	8.31	22.05	77.95
VOCO	39.80	-1.03	38.78	61.22
VOIN	45.30	-13.91	31.39	68.61
VOMI	564.85	-398.39	166.47	-
VONE	11.96	.16	11.81	88.19
VOSI	41.54	-21.05	20.49	79.51
ZOMI	172.22	-83.43	88.79	11.21
ZOVA	103.55	-45.98	57.58	42.42
IND76	8.70	2.30	10.90	89.10
IND87	15.12	-3.28	11.85	88.14

(Species corresponding to the codes are listed in Table 2. IND76 and IND87 represent the results of the analyses carried out on the total number of individuals of the previous and present study.)

Ammonium content has a negative effect on all species of group A. Dissolved oxygen content influences positively the population size of the species in group A and negatively the majority of the species in group B. Chemical oxygen demand and the population size of species in group A are inversely related. Total dissolved solids affect positively the number of individuals of all species in group B.

In the case of most species the change in the size of their population were not or hardly determined by the factors investigated (cf. error path values). That is the important factors are not abiotic (physical and chemical), but biotic ones, such as competition, predation, human impacts, etc.

Among the factors studied, water temperature

and water discharge affected the fewest of species. In running water in continental climate this statement might seem a paradox, since seasonal fluctuations in temperature and the flow would be the most important abiotic factors. We should keep in mind, however, that the species investigated are the dominant ones of the Danube. Species with a wide range of temperature-tolerance are present during fast the whole year - if other conditions are suitable -, and species with a narrower tolerance-range are present during some seasons. In the period of their occurrence, however the effect of temperature cannot be important, since as dominant species, they must tolerate the changes in temperature.

Investigating the effect of flow, a very important question is the body size of the organisms. The unicellulars are very small compared to the size of loops produced by the turbulent flow occurring in running waters. Therefore these loops cannot cling to these organisms and damage them mechanically. They pass with the water body, performing revolving motion along the turbulent flows. The effect of water discharge in the case of some species can be explained first of all by the direct mechanical effect of the suspend inorganic particles (e.g. *Carchesium polypinum*), or with the drift above a certain water level from the side arms.

Table 6. Direct effects greater than 5%

species	WD	WT	NH	DO	parameters					effective parameters			
					OD	TS	PH	PB	CB	-	+	Σ	
CAPO	-		+	-		+	+				2	3	5
COCR			-	+				+			1	2	3
COHI						+					-	1	1
COLA			-	+							1	1	2
CPCA						+					-	1	1
CPCO			+	-	-	+	+				1	3	4
EPPL			-	+	+		+			-	2	2	4
EPPY			+								-	1	1
GLSC			+		+	+				+	-	4	4
PACA	-									+	1	1	2
PAPU			-		-	+	-				3	1	4
PHVO			-	+							1	1	2
PRTE			-		+						1	1	2
PVMA										-	-	1	1
SBVI			+								1	1	2
SKVE							+	+			1	2	3
SPEL		-	-								2	-	2
SRCA	+		-	+			-				2	2	4
STPO	-						-			+	2	1	3
TIFL													
TRCU			+			+	+	+			-	4	4
URFA		-	-	-	+	+	+	+		-	4	4	8
VOCA													
VOCO	-		-		-		-				4	-	4
VOIN					+			+			-	2	2
VONE						+					-	1	1
VOSI			-	+	-						2	1	3
ZOMI			-		-	+	-	-			4	1	5
ZOVA			-	+			-			+	2	2	4
+	1	-	5	7	5	10	6	5	5			44	
-	4	2	12	3	5	-	6	2	3	37			
Σ affected species	5	2	17	10	10	10	12	7	8				81

(Parameters and species corresponding to the codes are listed in Tables 1 and 2. Negative sign indicates a negative, positive sign a positive effect of the factor in question.)

Table 7. Number of species affected by indirect effects greater than 5%

parameter	WD	WT	NH	DO	OD	TS	PH	PB	CB
WD	5					5	1		
WT		5				4	4		
NH	3	5	5		2	1	1	2	
DO				5			1		
OD			10	4	5	2	5		
TS	2	1	1			5	1		
PH	1	1	10	7	1		5		
PB					1		6	5	
CB				2		2		5	5

(Parameters corresponding to the codes are listed in Table I. Upper right semimatrix contains the number of positively affected species, lower left one contains the number of negatively affected species. Selecting the number of positively affected species = row by column, negatively affected species = column by row. First link of the path is the initial factor, second is the modified. E.g. effect of water discharge via total dissolved solids: positively affected species - intercept of WD row and TS column = 5, negatively affected species - intercept of WD column and TS row = 2.)

Regarding the other factors, it cannot be said, however, that the ranges of their fluctuations correspond to the natural conditions devoid of anthropogenic effects. The human effects, especially the various pollutions have decreased (e.g. dissolved oxygen content) or increased (e.g. ammonium content, chemical oxygen demand, pH) the range of the factors. These changes also extended over the lower or upper suboptimal range of the dominant species adapted to the former 'natural' conditions, and cause appreciable changes in the population size.

In our former study (Nosek and Berezky, 1981) the effects of some physical and chemical factors were investigated on the total number of planktonic Protozoa species. As the species investigated in this study are the dominant ones of the plankton, forming at least 90% of the total individual number of all species, so the results could be compared.

In the first study water temperature showed the greatest effect, dissolved oxygen content, chemical oxygen demand and pH played a more subordinated role (4.9%, 3.5%, 0.3% and less than 0.1% respectively). In the present situation the relative importance of chemical oxygen demand and dissolved oxygen content is greater than that of the water temperature (OD > DO > WT > PH).

The values of the error path is the same in both study. This indicates, that the total effect of the abiotic factors did not increase, but their relative importance altered.

This rearrangement in the relative importance and the effect of ammonium content, pH and total dissolved solids on a considerable number of the dominant species suggests that a gradual change has been started in the Danube compared its earlier state and this will involve the rearrangement of the species composition of the protozoan fauna.

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