Indirect effect of environmental factors on interactions between microbial and classical food webs in freshwater ecosystems

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Abstract – The role of environmental factors in aquatic ecosystems results from basic lake characteristics, human disturbances ('cultural eutrophication') and climate-related trends in the physical and chemical components of lakes. Although the influence of environmental factors on the abundance of aquatic animals is fairly well documented, less has been done to research their influence on food web interactions. The aim of the study was to evaluate microbial and classical food webs in lakes, with special emphasis placed on the role of environmental factors as influencing strengths. Variation partitioning, based on redundancy analysis, revealed that environmental factors played the most important role in structuring aquatic communities by accounting for 87.5% of their variation. Among all the factors measured, total solids (TS), transparency (Secchi disc) and temperature were most closely related to the variation in trophic communities. The analyses of food web interactions under low and high levels of those factors revealed that they differently influenced strengths among food web components. The strongest relations among distinct trophic levels were found under conditions of low TS, the lowest number of relations was found under conditions of low temperature. Only in low TS did bacteria correlate significantly with biogenes. Under high TS, bacteria positively influenced plenty of higher trophic levels. Top-down control was observed under conditions of high temperature. Conditions of low and high transparency did not diversify food web interactions. The obtained results can broaden our knowledge of the response of food webs to environmental factors in advanced stages of global eutrophication of water bodies and in the early stage of projected trends of global climate change.

Key words: Total solids / transparency / temperature / eutrophication / climate change

Introduction

The food web constitutes one of the most complex conceptual phenomena in modern biology (Pimm *et al.*, 1991). Food webs in aquatic ecosystems function *via* challenging the energy and flux of materials among diverse organism assemblages organized into two chains: the so-called 'microbial loop' and the classical grazing chain. They are regulated both by top-predator (top-down control) and nutrient resources (bottom-up control), and the strength of this control is dependent on trophic position and food web length (Brett and Goldman, 1997). Up until now, a countless number of possible relations between distinct levels of microbial and classical food webs in freshwater and marine ecosystems has been described (*e.g.*, Beaver and Crisman, 1982; Güde, 1986; Jack and Gilbert, 1993; Wickham, 1995a, 1995b; Jürgens and Jeppesen, 2000). Although it is well established that fish can impact lower

trophic levels through cascading trophic interactions (Carpenter *et al.*, 1985; Brett and Goldman, 1996), and that the microbial loop plays an important role in carbon cycling and nutrient remineralization, the relative strengths of interactions between the classical aquatic food web and the microbial food web are poorly understood. A poor understanding of these strengths results from the fact that natural aquatic ecosystems do not exist in a state of equilibrium but function under continuous changes induced by independent environmental factors. These environmental factors basically result from a lake's characteristics (Dawidek *et al.*, 2013), but they are also created by human disturbances (so-called 'cultural eutrophication'; Smil, 2000) and climate-related trends in the physical and chemical components of lakes (Straile *et al.*, 2003; Winder and Schindler, 2004). The influence of environmental factors is fairly well documented as cases of seasonal and spatial differences in aquatic animals' abundance and biomass. However, studies on their relations to food web interactions are scarce. The aim of the study was to evaluate microbial and classical food webs in

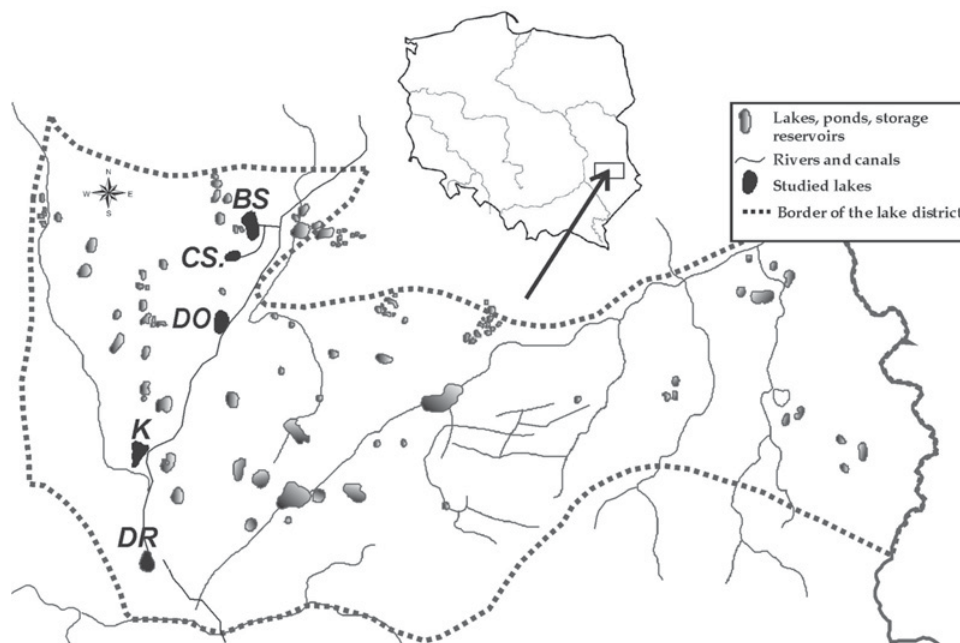*Corresponding author: malgorzata.adamczuk@up.lublin.pl

Fig. 1. Location of the study area. The studied lakes are marked with symbols: BS – Lake Białe Sosnowickie, CS – Lake Czarne Sosnowickie, DO – Lake Domaszne, K – Lake Krzczeń, DR – Lake Dratów.

lakes with special emphasis on the role of environmental factors as influencing their strengths. The general hypothesis was that interactions among distinct trophic levels of microbial and classical food webs are influenced by environmental factors. Thus, components of food webs and the values of environmental factors were identified in the study. Thereafter, ordination analysis was used to evaluate which of the environmental factors significantly influenced the food web components. In a further analysis, interactions between trophic levels were analysed under the evaluated environmental factors. Understanding and potentially forecasting the impact of environmental factors on food web interactions may help us to predict changes in aquatic ecosystems in the current era of cultural eutrophication and putative trends of climate change.

Materials and methods

The lakes selected to conduct the study are five eutrophic reservoirs situated in the area of the Łęczna-Włodawa Lake District (Eastern Poland), *i.e.,* Lake Dratów, Lake Krzczeń, Lake Domaszne, Lake Czarne Sosnowickie and Lake Białe Sosnowickie (Figure 1). The lakes are located between $51°33'92''–51°53''11''$N and $22°94'64''–23°04'75''$ W. They are shallow and small, as the mean depth does not exceed 5 m, the area reaches 1.5 km^2 at maximum, and the volume of the largest lake is 2020 m^3. In 1961, they were transformed into storage reservoirs and connected together by a man-made canal supplying high biogene-fecund waters (Dawidek *et al.,* 2004). The lakes are systematically stocked with fry.

Microbial communities (bacteria, heterotrophic nano-flagellates (HNF and ciliates), crustaceans (cladocerans and copepods) and fish were examined in the open water zone of each lake. The samples were taken monthly from April 2012 to October 2013. At each of the sites, planktonic communities were collected from the middle of the water column using a 5 L Bernatowicz sampler. The bacterial biomass was determined by means of DAPI (4′6-diamidino-2-phenylindole)-staining and epifluorescence microscopy according to the method of Porter and Feig (1980). A total of 10 ml of water was preserved with formaldehyde to a final concentration of 2% and kept in the dark at 4 °C. Four slides were made from each sample in which subsamples of 2 ml were filtered on 0.2 μm pore-size polycarbonate filters that were stained with irgalan black. The bacteria were counted in 250 randomly chosen fields of view. Biovolumes of the microbial community were estimated assuming geometric shapes and converting to carbon using the following conversion factor (Gilbert *et al.*, 1998). Each sample of HNF was fixed *in situ* with formalin (a final concentration of 2%). Subsamples of 10 ml for HNF were stained with DAPI (final concentration 1 μg.mL^{-1}) (Porter and Feig, 1980), filtered through black polycarbonate membrane filters (Millipore) with a pore size of 0.8 μm for HNF, and enumerated by means of epifluorescence microscopy. The HNF biovolume was calculated from measurements of cells and approximated to simple geometrical forms (Kalinowska, 2004). The community composition of ciliates was determined using Utermöhl's method. Five-litre samples were filtered through a plankton net of 10 μm mesh size. Samples (whole sample = 500 ml) were sedimented for 24 h in a cylinder stoppered with. In order to determine the density,

three samples were preserved with Logol's solution and stored in the dark at a temperature of 4 °C. Observation of live samples was used for taxonomic and trophic identification. Ciliates are highly perishable and their type of motility is a species-specific feature; for this reason, species determination and measurements were carried out on live material immediately after returning to the laboratory and after silver impregnation (Augustin *et al.*, 1984). Ciliate biomass was estimated by multiplying the numerical abundance by the mean cell volume (1 μm^3 = 1 pg) calculated from direct volume measurements using appropriate geometric formulas (Finlay, 1982). Obvious shrinkage of stained ciliates was noticeable after silver preparation. Therefore, the calculated cell volumes were multiplied with a correcting factor of 0.4 (Jerome *et al.*, 1993). In the case of Crustacea (Cladocera and Copepoda), double samples of the volume 0.5 dm^3 were collected and pooled to reduce heterogeneity in crustacean distribution and sampling variability, thus the final volume of a sample measured 1 dm^3. Each sample was taken in three replications. Samples were sieved through in a 40 μm mesh net and fixed with formalin–glycerine solution. In the laboratory, the classification and counts of crustaceans were made with the use of the Sedgewick–Rafter cell. Crustacean biomass was estimated on relations between the body length and body mass of a given specimen (Dumont *et al.*, 1975; Bottrell *et al.*, 1976; Culver *et al.*, 1985) by applying established mathematical formulas.

Fish were caught using the standard Norden S multimesh gillnet type (10, 60, 30, 6.25, 43, 22, 50, 33, 12.5, 25, 8, 38, 75, 16.5 mm) (Appelberg, 2000, CEN document, 2005). At each fish sampling, two nets were placed in open water in the evening for 12 h. The gillnets were placed in mid-water so as to cover the height of the water column as much as possible. Caught fish were determined to the species level and trophic group (planktivorous and predatory fish), and their body mass (in g) was measured. Fish data were converted to weight per unit effort (WPUE) as fish biomass (in grams) caught in one net after 12 h of fishing.

Simultaneously to the biological studies, a number of physical and chemical analyses were conducted. Transparency was estimated with the use of a Secchi disc (SD). Temperature, conductivity, pH and dissolved oxygen (DO) were determined *in situ* with a multiparametric probe. Total organic carbon (TOC), total suspended solids (TSS), surface active agents (SUR), chemical oxygen demand (COD) and biological oxygen demand (BOD) were determined using the PASTEL UV spectrophotometer, and the remaining factors (TS – total solids, TP – total phosphorus, P–PO_4, N–NH_4, N–NO_3 chlorophyll-a) were analysed in the laboratory (Golterman, 1969).

The differences in physical and chemical water parameters were analysed by means of one-way analysis of variance (ANOVA). Spearman's rank correlation coefficients (r) were calculated for pairs of environmental variables to recognize which of the variables were intercorrelated. Redundancy analysis (RDA) was used to explore relationships between the abundance of taxonomic groups and environmental factors (Lepš and Šmilauer, 2003). The choice of this linear ordination model was justified by the narrow range of the data (previously assessed by DCA with a length of gradient < 2 standard deviations). On the resultant plot, arrows representing the factors indicate the direction of their maximum change, and the length of each arrow is proportional to the rate of change. The proportion of variance explained by each factor was quantified using variance partitioning. RDA analysis was performed using a selection in which the Monte Carlo test of significance for all factors was assessed, and only then statistically significant factors were taken into account for further analysis. The data were log-transformed $\ln(x + 1)$ prior to the analysis (Ter Braak and Šmilauer, 2002). Ordination analyses were performed by means of CANOCO 4.5 for Windows. The next step was to check which threshold values (TV) of each factor graded the densities of distinct communities. Therefore, we tested the differences in biomass in relation to factors using one-factorial ANOVA to find the lowest value of a given factor that significantly diversified the communities' biomasses. TV were used to estimate low ($\leq$ TV) and high (> TV) values of the considered factors. Pearson's correlation coefficients were used to find interactions between distinct trophic levels under low and high values of the factors. Correlations were performed on the communities' biomasses and the data were log-transformed prior to analysis.

Results

Components of the microbial food web (bacteria, flagellata and ciliata)

The biomass of bacteria ranged between 0.91 $\pm$ 0.89 mg.dm^{-3} in Lake Dratów and 1.85 $\pm$ 1.40 mg.dm^{-3} in Lake Krzczeń, and did not differ among the studied lakes (F = 0.65, d.f. = 5, P = 0.66). Lake Krzczeń showed the lowest biomass of flagellates (0.053 $\pm$ 0.02 mg.dm^{-3}), whereas the highest biomass was found in Lake Dratów (0.062 $\pm$ 0.019 mg.dm^{-3}), but still no differences were found among the lakes (F = 0.55, d.f. = 5, P = 0.73). Ciliata displayed differences in biomass (F = 10.68, d.f. = 5, P = 0.0004) that ranged between 30.67 $\pm$ 8.50 μg.dm^{-3} in Lake Czarne Sosnowickie and 81.67 $\pm$ 16.04 μg.dm^{-3} in Lake Dratów. The most abundant ciliates were *Cinetochilum margaritaceum*, *Colpidium colpoda*, *Euplotes* sp., *Askenasia* spp., *Strombidium viride*, *Uronema* spp. and *Strombilidium* spp.

Components of the classical food web (chlorophyll-a, Cladocera, Copepoda, fish)

The chlorophyll-a concentration ranged from 18.38 $\pm$ 3.67 mg.dm^{-3} in Lake Czarne Sosnowickie to 149.74 $\pm$ 88.12 mg.dm^{-3} in Lake Krzczeń and differed

Table 1. Non-parametric correlation matrix of measured environmental variables in the studied lakes.

	Temperature	SD	pH	TS	Conductivity	DO	N-NH$_4$	N-NO$_3$	P-PO$_4$	TP	TSS	TOC	SUR	COD	BOD
Temperature (°C)	1	−0.30	0.35	−0.01	−0.25	−0.06	0.14	−0.19	0.24	0.11	0.08	0.04	0.05	0.04	0.05
SD (m)		1	−0.61*	−0.87**	−0.15	−0.11	0.55*	0.63*	−0.1	−0.33	−0.56*	−0.04	−0.56*	−0.17	−0.03
pH			1	0.79**	−0.17	0.33	−0.36	−0.32	0.13	−0.02	−0.13	−0.36	−0.35	−0.36	0.36
TS (mg.dm^{-3})				1	0.02	0.01	−0.57*	−0.66**	−0.08	0.29	0.41	−0.11	−0.53*	−0.11	0.12
Conductivity (μS.cm^{-1})					1	0.14	−0.03	0.20	−0.15	−0.67*	0.09	−0.08	0.06	−0.14	−0.10
DO (mg.dm^{-3})						1	−0.25	0.11	−0.03	−0.15	−0.26	−0.34	−0.27	−0.39	0.36
N-NH$_4$ (mg.dm^{-3})							1	0.71**	−0.46	0.24	0.84	−0.69	0.98**	0.94**	0.95**
N-NO$_3$ (mg.dm^{-3})								1	0.51	−0.19	0.20	0.39	0.64**	0.52*	0.56*
P-PO$_4$ (mg.dm^{-3})									1	0.06	0.22	0.38	0.35	0.36	0.37
TP (mg.dm^{-3})										1	0.52	0.42	0.38	0.45	0.43
TSS (mg.dm^{-3})											1	0.39	0.90**	0.94**	0.93**
TOC (mg.dm^{-3})												1	0.99**	0.99**	0.99**
SUR (mg.dm^{-3})													1	0.97**	0.97**
COD (mgO$_2$.dm^{-3})														1	0.99**
BOD (mgO$_2$.dm^{-3})															1

*Correlations significant at level $P < 0.05$; **Correlations significant at level $P < 0.01$.

among lakes ($F = 9.16$, d.f. = 5, $P = 0.0009$). The biomass of Cladocera ranged from 1.02 ± 0.98 mg.dm^{-3} in Lake Czarne Sosnowickie to 9.18 ± 12.91 mg.dm^{-3} in Lake Dratów and differed among lakes ($F = 3.94$, d.f. = 5, $P = 0.028$). *Daphnia longispina*, *Chydorus sphaericus* and *Bosmina longirostris* were more abundant in the spring and autumn samples, whereas in the summer samples the species *Diaphanosoma brachyurum*, *Daphnia cucullata*, *Bosmina coregoni* and *Ceriodaphnia quadrangula* prevailed. The biomass of Copepoda ranged from 0.06 ± 0.07 mg.dm^{-3} in Lake Krzczeń to 1.39 ± 1.05 mg.dm^{-3} in Lake Dratów and showed differences among the studied lakes ($F = 3.44$, d.f. = 5, $P = 0.029$). Samples were apparently split into two groups, namely the spring and autumn samples had dominant larvae and immature individuals and the summer samples had dominant cyclopoids *Mesocyclops leuckartii* and *Thermocyclops crassus* as well as calanoid *Eudiaptomus gracilodes*.

The fish showed differences in biomass ($F = 18.07$, d.f. = 5, $P = 0.0001$) which was from 2361 ± 964 WPUE in Lake Krzczeń to 6991 ± 2252 WPUE in Lake Białe Sosnowickie. The dominant species were roach *Rutilus rutilus*, bream *Abramis brama* and ruffe *Gymnocephalus cernuus*. No piscivorous fish were caught in Lake Czarne Sosnowickie. In the remaining lakes, the biomass of piscivorous fish ranged from 366.5 ± 324.1 WPUE in Lake Domaszne to 1488 ± 2288.1 WPUE in Lake Białe Sosnowickie and differed among lakes ($F = 18.07$, d.f. = 5, $P = 0.0001$). This group was represented by perch *Perca fluviatilis* and pike *Esox lucius*.

Environmental background

Most environmental factors showed differences among the studied lakes, including chl-a ($F = 50.71$, d.f. = 5, $P = 0.00003$), TOC, TSS, N-NH$_4$ ($F = 5.23$–55.0, d.f. = 5, $P < 0.001$), SD, TS and conductivity ($F = 3.80$–5.89, d.f. = 5, $P < 0.05$). Some pairs of factors strongly correlated. Positive values of correlation coefficients were found among N-NO$_3$, N-NH$_4$, TS, TSS, TOC, SUR, COD and BOD. Negative correlations were obtained among SD, pH, TSS and TS (Table 1). The RDA axis 1 ($\lambda = 0.587$) and axis 2 ($\lambda = 0.112$) explained 87.5% of total variance of local factors in the communities' composition. However, variables that significantly explained variance in the communities included three factors: TS ($\lambda = 0.39$, $P = 0.002$, $F = 10.13$), temperature ($\lambda = 0.23$, $P = 0.002$, $F = 9.48$) and SD ($\lambda = 0.006$, $P = 0.036$, $F = 2.48$) (Figure 2). TV of TS at a level of 9.60 mg.dm^{-3} differed for the bacteria ($F = 6.09$, $P = 0.0049$), ciliates ($F = 4.65$, $P = 0.022$) and Cladocera ($F = 3.94$, $P = 0.028$). The threshold temperature for the communities was 13.36 °C, and then it differed for the two communities, namely HNF ($F = 7.05$, $P = 0.023$) and copepods ($F = 6.19$, $P = 0.047$). SD differed only for the Cladocera ($F = 2.78$, $P = 0.037$), and the threshold level for that factor was 0.5 m. Only these factors were taken under consideration for further analyses of correlations between components of the microbial and classical food web.

Correlations between components of the microbial and classical food web in different variants of environmental factors

Under conditions of low TS, bacteria and HNF correlated positively to N-NH$_4$ and N-NO$_3$. Ciliates correlated negatively to the calanoid *Eudiaptomus graciloides*. Chlorophyll-a values correlated positively to P-PO$_4$ and negatively to Calanoidae copepodites and the cladoceran *B. longirostris*. Under high TS, bacteria correlated to the cladoceran *B. longirostris*. Positive correlations between bacteria and nauplii, Cyclopoidae copepodites as well the calanoid *E. graciloides* were also found. Negative correlations were noted between HNF and cladocerans, including *B. longirostris* and *D. cucullata*. Adult cyclopoids correlated positively to planktivorous fish (Table 2).

In low temperature, bacteria correlated positively to the cladoceran *B. longirostris*. Positive correlations were found between HNF and planktivorous fish. Under high temperature, positive correlations between bacteria and nauplii were found. HNF correlated negatively to copepodites, and ciliates correlated negatively to copepodites and adult cyclopoids, including *T. crassus* and *M. leuckartii*. The cladoceran *D. cucullata* and adult cyclopoids correlated negatively to planktivorous fish (Table 2).

Under low SD, bacteria correlated positively to Cyclopoidae copepodites and the calanoid *E. graciloides*. Negative correlations were noted between HNF and cladocerans, including *B. longirostris* and *D. cucullata*. Ciliates correlated negatively to copepodites. Under conditions of high SD, positive correlations were found between bacteria and nauplii, Cyclopoidae copepodites as well as the calanoid *E. graciloides*, and negative correlations were noted between flagellates and the cladocerans *B. longirostris* and *D. cucullata* (Table 2).

Discussion

Correlations between components of the microbial and classical food web

HNF are thought to be the main consumers of bacteria in freshwater (Riemann, 1985; Güde, 1986); ciliates consume both bacteria (Simek *et al.*, 1995) and HNF, thus the abundance of HNF often decreases as it is associated with the development of ciliates (Zöllner *et al.*, 2003). However, in the present study insignificant correlations were found among these three components. Correlation analyses showed that in the studied lakes, links between microbial and classical food webs occurred at each level of the microbial loop. Evidence for the cascading effect of crustaceans on bacteria mainly came from meso- and microcosm experiments, but the effect of crustaceans on bacteria in natural conditions was also proved (Moustaka-Gouni and Vardaka, 2006). It is generally considered that crustacean plankton affect the whole microbial food web, *i.e.*, from ciliates to larger bacteria (Jürgens and Stolpe, 1995). Filter-feeding cladocerans can directly capture bacteria,
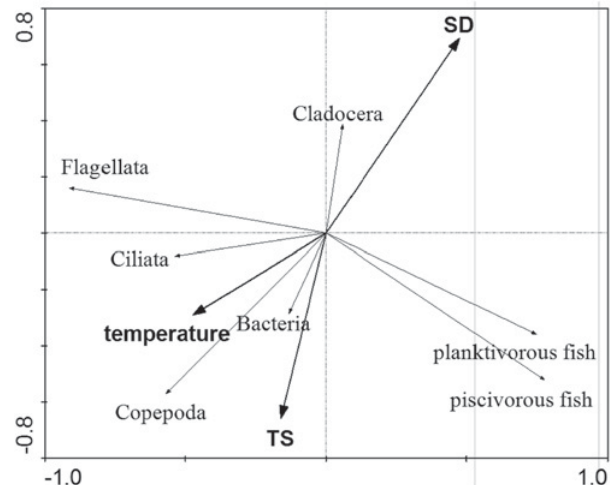
Fig. 2. Redundancy analysis (RDA) biplot showing the studied communities and environmental factors.

but can also indirectly control bacterial communities through trophic cascades (Langenheder and Jürgens, 2001), whereas copepods can selectively graze on ciliates and thereby indirectly control the bacteria (Burns and Schallenberg, 2001). In the present study, bacteria correlated positively to zooplankton, which is in accordance with many studies showing that it is impossible to show that predators diminish bacteria abundance, for bacteria are soon compensated by high production rates, thus the predators leave no visible effect (Adrian *et al.*, 2001; Zöllner *et al.*, 2003). But an inverse scenario of bacteria positively influencing higher trophic levels is highly possible. In most reports bacteria appear to be limited primarily by resources, whereas the top-down effect was never strong enough to produce negative bacterial growth rates (Billen *et al.*, 1990; Pace and Cole, 1994; Jürgens and Jeppesen, 2000). Positive correlations were found between bacteria and larvae (nauplii) as well as juvenile (copepodite) stages of Copepoda. Copepod nauplii can ingest pico- and nanophytoplankton (Uye and Kasahara, 1983), and bacterioplankton can also constitute an important food source (Turner and Tester, 1992; Roff *et al.*, 1995). Abundant and 'never-ending' sources of bacteria as food could affect the development of nauplii, of which the highest number endured to copepodite stages. Nauplii have higher rates of clearance of bacteria in the absence of alternative phytoplankton food (Turner and Tester, 1992). In the studied lakes, significant correlations between chlorophyll-a and higher trophic levels were found quite rarely, thus suggesting that phytoplankton could have a less important role in energy transfer along food webs, and phytoplankton particles were supposedly inedible by herbivorous and omnivorous species. As was mentioned above, there was a connection between bacteria and nauplii, but a high correlation between bacteria and cladoceran *B. longirostris* was also observed. HNF were preyed upon by cladocerans and copepods (in both juvenile and adult stages). The abilities of *Daphnia* and *Bosmina* to depress ciliata growth are well documented (Jürgens, 1994;

Table 2. Pearson's correlations between trophic levels of microbial and classical food webs under low and high values of local stressors.

	TS		Temperature		SD	
	L	H	L	H	L	H
Bacteria – N–NH$_4$	r = 0.49 P = 0.026	—	—	—	—	—
Bacteria – N–NO$_3$	r = 0.71 P = 0.049	—	—	—	—	—
Bacteria – *B. longirostris*	—	r = 0.71 P = 0.003	r = 0.39 P = 0.048	—	—	—
Bacteria – nauplii	—	r = 0.48 P = 0.017	—	r = 0.57 P = 0.049	—	r = 0.46 P = 0.028
Bacteria – Cyclopoidae copepodites	—	r = 0.72 P = 0.025	—	—	r = 0.91 P = 0.000019	r = 0.67 P = 0.013
Bacteria – *E. graciloides*	—	r = 0.45 P = 0.015	—	r = 0.41 P = 0.037	r = 0.41 P = 0.001	—
Flagellates – N–NH$_4$	r = 0.049 P = 0.015	—	—	—	—	—
Flagellates – N–NO$_3$	r = 0.42 P = 0.025	—	—	—	—	—
Flagellates – *B. longirostris*	—	r = −0.31 P = 0.041	—	—	r = −0.76 P = 0.023	r = −0.53 P = 0.036
Flagellates – *D. cucullata*	—	—	—	—	r = −0.44 P = 0.029	—
Flagellates – copepodites	—	—	—	r = −0.78 P = 0.031	—	—
Flagellates – planktivorous fish	—	—	r = 0.39 P = 0.046	—	—	—
Ciliates – copepodites	—	—	—	—	r = −0.39 P = 0.027	r = −0.46 P = 0.018
Ciliates – adult cyclopoids	r = −0.78 P = 0.046	r = −0.61 P = 0.040	—	r = −0.76 P = 0.021	—	—
Ciliates – *T. crassus*	—	—	r = −0.72 P = 0.014	—	—	—
Ciliates – *M. leuckartii*	—	—	—	r = −0.55 P = 0.017	—	—
Ciliates – *E. graciloides*	r = −0.37 P = 0.048	r = −0.39 P = 0.017	—	—	—	—
Chlorophyll-a – P–PO$_4$	r = 0.63 P = 0.022	—	—	—	—	—
Chlorophyll-a – Calanoidae copepodites	r = −0.39 P = 0.031	—	r = −0.59 P = 0.018	—	—	—
Chlorophyll-a – *B. longirostris*	r = −0.49 P = 0.031	—	—	—	—	—
D. cucullata – planktivorous fish	—	—	—	r = −0.82 P = 0.038	—	—
Adult cyclopoids – planktivorous Fish	—	r = 0.49 P = 0.017	—	r = 0.37 P = 0.031	—	—

L and H abbreviations mean, respectively, low and high values of environmental variables estimated empirically, '—' means insignificant correlations ($P > 0.05$).

Burns and Schallenberg, 1996), but in the studied lakes these taxa did not significantly affect ciliates that were mainly consumed by copepods. Among the crustaceans, negative correlations were found between *D. cucullata* and planktivorous fish, whereas Cyclopoidae copepods correlated to planktivorous fish, however, these relations occurred only under conditions of high TS and temperature. Although research by Müller-Solger *et al.* (1997) showed that the presence of planktivorous fish can influence significant increases in the density of ciliates, no positive fish–ciliate relations were found in the studied lakes.

Contribution of environmental factors to food web relations

Variation partitioning based on RDA revealed that local factors played the most important roles in structuring the aquatic communities by explaining 87.5% in their variation. Among all of the factors measured, total solids (TS), transparency (SD) and temperature were most closely related to variation in trophic communities.

The TS concentration contributed the most to this variation. Under that factor the largest number of

interactions was found between trophic levels. In comparison to other factors, this one influenced the strongest bacteria communities. Under high TS, significant relationships between bacteria and cladocerans and all developmental stages of Copepoda were found. Probably detrital particles constituting mostly TS created a medium for bacterial development. Some studies have reported that more than half of the bacterioplankton in aquatic ecosystems can be attached to detrital particles (Simon, 1987; Riemann *et al.*, 2000), and the abundance of those particles influences the density, biomass and production of the attached bacteria (Kepkay, 1994; Carrias *et al.*, 2002). Both cladocerans and copepods can consume particles, including the attached bacteria (Shimeta, 1993; Lemarchand *et al.*, 2006), and detrital particles with attached bacteria can often be more quantitatively important food for zooplankton than algae (Gons *et al.*, 1992). Thus, such particles could be an important non-algal food source for crustaceans; all the more that according to Marzolf (1990), phytoplankton alone do not satisfy all of the nutritional requirements of zooplankton. Only under low TS conditions are bacteria correlated to biogenes, therefore a high TS concentration seemed to autonomize the bacteria to biogenes.

Virtually all aspects of physiology, including grazing (Kiørboe *et al.*, 1982; Houde and Roman, 1987), respiration (Ikeda, 1985; Thor *et al.*, 2002), timing of reproduction and onthogenetic development (Dell *et al.*, 2011), are impacted by temperature. As temperature directly interacts in reducing or enhancing the metabolism and reproduce rates (Green, 1966; Frey, 1982; Gillooly and Dodson, 2000; de Eyto and Irvine, 2001), it has multiple indirect effects on aquatic communities' habitats by altering food resources. There have been observations that temperature can significantly influence variation in communities of microbial (Shiah and Ducklow, 1994; Lepère *et al.*, 2006; Mieczan *et al.*, 2013) and classical (Alofs *et al.*, 2014; Xin *et al.*, 2014) food webs. The effect of temperature on food web relations has been widely studied due to observed increases in global temperature (Petchey *et al.*, 1999; O'Connor *et al.* 2009; Beveridge *et al.* 2010). The present study showed that temperature influenced the food web in two ways. Low temperature weakened strengths among distinct trophic levels and impaired relations between microbial and classical food webs. High temperature, in turn, enhanced negative correlations between consecutive trophic levels, from planktivorous fish to HNF. The effect of temperature on food web interactions probably resulted from the role that factor plays in physiological processes, including the ingestion rate (Delaney, 2003). Thus, high temperature triggered the grazing mechanisms through distinct levels of the food web suggesting top-down control.

Another factor that significantly influenced aquatic communities was SD. Foraging of most planktivorous fish is directed by vision (Lazzaro, 1987), thus that factor can determine the abundance of some aquatic communities, especially crustaceans (Estlander *et al.*, 2009). Planktonic crustaceans can be protected against predation by water turbidity, for suspended particles severely disturb prey detection by fish due to their effects on light scattering (Horppila *et al.*, 2004). Because water transparency is important in order for crustaceans to avoid predators, it has less importance for their foraging since cladocerans forage unselectively (Burns, 1968), and copepods use mechanoreceptors to detect their prey (Williamson, 1986) and chemoreceptors to capture the most nutritional particles (Paffenhöfer and Van Sant, 1985). Insignificant differences in food web interactions were found between low and high transparency conditions. Although high transparency should favour planktivorous fish to seek prey, suggesting inverse correlations between fish and crustaceans, no significant interactions were found between these two communities. At lower trophic levels, both under low and high SD, crustaceans negatively influenced ciliates and HNF, while positive correlations between bacteria and crustaceans were found. The lack of expected evidence between planktivorous fish and crustaceans probably resulted from the ability of the latter community to conduct horizontal and vertical migrations to avoid predation pressure (Wissel *et al.*, 2003; Adamczuk, 2012; Adamczuk and Mieczan, 2013). Therefore, in conditions of high transparency, the active choice of crustacean plankton to avoid predatory pressure could hamper top-down control and influence interactions at lower trophic levels, thus converge them to those in conditions of low water transparency.

Conclusions

In most studies, environmental factors are considered as factors influencing the abundances of aquatic communities, whereas their regulating role in food web interactions is often underestimated. The study revealed that environmental factors influence food web interactions and play an important regulating role in bottom-up or top-down control. Taking into consideration the fact that environmental factors change not only on the perennial or seasonal scale but also in diel duration, they may trigger a variety of interaction patterns even in a short period of time. It is considered that cladocerans, especially *Daphnia*, affect whole microbial food web, but the study proved that copepods could have a key role in transferring energy from microbial to classical food web, because their grazing effect was quite resistant to local stressor and occurred at each developmental stage, including larvae, immature and mature individuals.

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