Associating spatial patterns of zooplankton abundance with water temperature, depth, planktivorous fish and chlorophyll

Ari Voutilainen[1)2)]*, Juha Jurvelius[3)], Juha Lilja[4)], Markku Viljanen[1)] and Minna Rahkola-Sorsa[1)]

[1)] *Department of Biology, University of Eastern Finland, Yliopistokatu 7, FI-80100 Joensuu, Finland*
[2)] *Department of Nursing Science, University of Eastern Finland, Yliopistonranta 1C, FI-70211 Kuopio, Finland (*corresponding author's e-mail: ari.voutilainen@uef.fi)*
[3)] *Natural Resources Institute Finland, Laasalantie 9, FI-58175 Enonkoski, Finland*
[4)] *Natural Resources Institute Finland, Survontie 9 A, FI-40500 Jyväskylä, Finland*

The spatial distribution of zooplankton was studied in a boreal lake system. Distribution patterns were associated with water temperature and depth, abundance of fish, and chlorophyll-*a* concentration. Principal coordinates of neighbor matrices (PCNM) were used to model spatial structures (vectors between study locations) which were in turn used in regression models to explain plankton distribution. Data were also analyzed using detrended correspondence analysis (DCA). Models based on PCNM highlighted differences between sites, whereas DCA emphasized differences between the epi-, meta- and hypolimnion. Bottom-up regulation was the primary force in determining zooplankton and fish abundance. Signs of top-down regulation were also found. The main forces driving spatial heterogeneity of zooplankton in lakes differed among thermal strata and among zooplankton size categories and species. The study stressed the need for gathering data with more than one method simultaneously and emphasized the benefits of combining results from two or more statistical techniques.

Introduction

Distribution of organisms in an aquatic ecosystem is seldom random. It is usually determined by the spatiotemporal distribution of abiotic and biotic factors (Laprise and Dodson 1994, Baranyi *et al*. 2002, Beisner *et al*. 2006). In most cases, spatiotemporal patterns of these factors are composed of a nested structure, and norms that are evident at larger scales are not necessarily valid at smaller scales. With large scales, spatial variability is often characterized by rel-atively stable patterns that are predictable over time (Beaver and Havens 1996, Romare *et al*. 2003, Viljanen *et al*. 2009). In contrast, small scale processes are likely to generate spatial distributions characterized by the ephemeral existence of discrete spots of high and low density (Romare *et al*. 2003, Seymour *et al*. 2006, Viljanen *et al*. 2009).

In general, zooplankton abundance is highest in productive warm-water areas (Colebrook 1960, McCauley and Kalff 1981, Shuter and Ing 1997, Thackeray *et al*. 2004, Masson *et al*.

2004) maintaining high phytoplankton (food for zooplankton) biomass. Abiotic factors dominate at larger and biotic factors at smaller scales (Pinel-Alloul *et al*. 1999), although productivity of the aquatic system is the primary factor determining the total plankton abundance (Masson *et al*. 2004). Smaller-scale spatiotemporal distribution of zooplankton is also related to that of planktivorous fish (Masson *et al*. 2004). To some extent, zooplankton can avoid fish predators (Lampert 1993, Huber *et al*. 2011, Lilja *et al*. 2013). The "multiple driving forces" hypothesis states that neither abiotic nor biotic processes can alone explain the spatial structure of plankton (Pinel-Alloul and Ghadouani 2007). It indicates that plankton patchiness is driven by abiotic processes interacting with biotic processes, and that the relative influence of abiotic processes varies along the scale continuum (Pinel-Alloul and Ghadouani 2007). The zooplankton size structure has very seldom been studied in relation to the spatiotemporal distribution of zooplankton.

Our aim was to study the distribution of size-grouped zooplankton in a large boreal lake system with respect to the multiple driving forces hypothesis (Pinel-Alloul and Ghadouani 2007). The spatial patterns of zooplankton abundance were associated with abiotic and biotic determinants, including water temperature, water depth, abundance of planktivorous fish, and chlorophyll-*a* concentration reflecting the abundance of phytoplankton (Pinel-Alloul *et al*. 1999, Masson *et al*. 2004). The survey was intentionally scheduled for late summer when the lakes are thermally stratified into the epi-, meta- and hypolimnion. This stable condition was thought to help to distinguish the effect of abiotic driving forces from that of biotic forces, as the main abiotic force, temperature, was "standardized".

Methods

Study area

The material was collected from nine sites in a large boreal lake system between 27 July and 2 August 2010 (Fig. 1). The sites were located in lakes in the Vuoksi watershed, which has a drainage basin area of nearly 62 000 km². The

lakes are interconnected via multiple natural channels of different widths. A high humic concentration and a low productivity are typical of the lakes. No hypoxic or anoxic conditions occur in the study sites during the summer stratification (Table 1). Biological gradients across the lakes are high enough to study the effect of biotic factors on zooplankton abundance (cf. Rahkola-Sorsa *et al*. 2014a, 2014b). From November/December to April/May, the lakes are ice-covered.

Thermal structure of the lakes

The vertical temperature profile of the lakes was monitored with a Conductivity Temperature Depth (CTD) probe (SBE 19, Sea-Bird Electronics, Inc., Bellevue, WA, USA). The rate of change in temperature was calculated for the precise determination of the thermocline i.e. metalimnion. Thermocline refers to a water layer where the change in temperature as a function of depth reaches its maximum value. Thermal stability indicates the amount of energy required for the breakdown of thermal stratification of a water body without changing the amount of a lake's internal energy. It was calculated with a macro for Microsoft Excel provided by the Finnish Environmental Institute (SYKE) and built by Petri Kiuru in 2010.

Zooplankton sampling equipment

A research vessel was used to carry out the survey. The ship, r/v *Muikku*, included sampling equipment, a laboratory, and facilities for data processing. Zooplankton samples were collected with the Laser Optical Plankton Counter (LOPC, ODIM Brooke Ocean, Dartmouth, Nova Scotia, Canada) and a towed Multi Plankton Sampler (MultiNet, Type Midi, Hydro-Bios Apparatebau GmbH, Kiel-Holtenau, Germany) with a mesh size of 100 μm. The LOPC is designed for counting the number of zooplankters and it can detect particles in the size range of 100–35 000 μm (Herman *et al*. 2004). The LOPC has also been found to be a reliable and valid tool for freshwater zooplankton (Finlay *et*
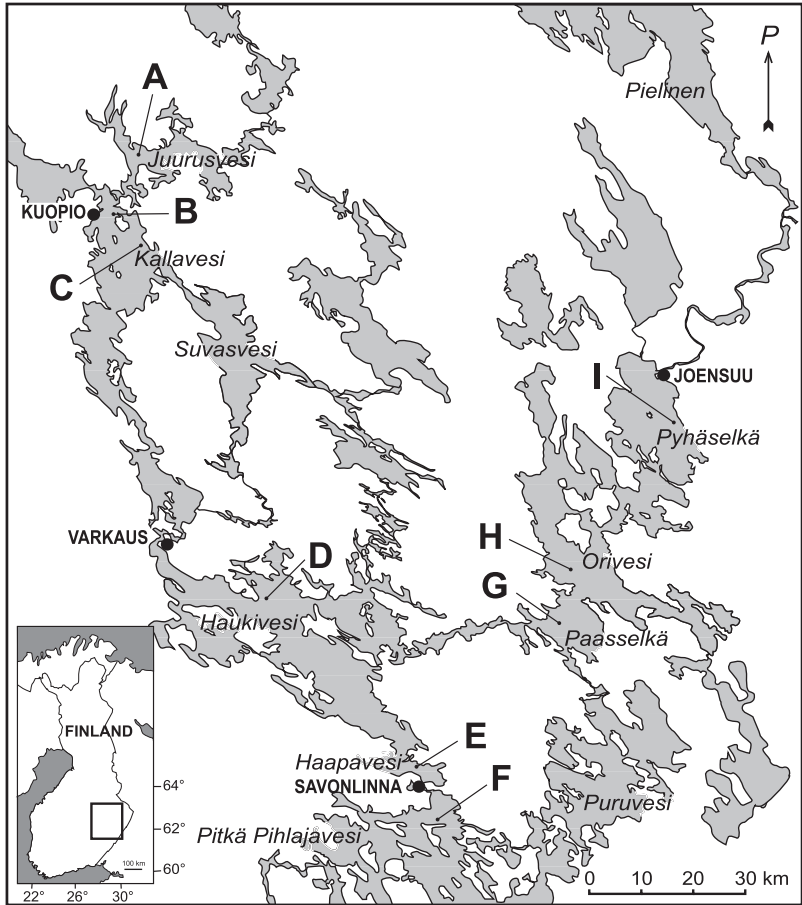
Fig. 1. The study sites in Finland indicated with capital letters: A = Juurusvesi, B = Kallavesi 1, C = Kallavesi 2, D = Haukivesi, E = Haapavesi, F = Pihlajavesi, G = Paasselkä, H = Samppaanselkä and I = Pyhäselkä.

al. 2007, Rahkola-Sorsa *et al*. 2014b). A more detailed description of the sampling equipment is given in Jurvelius *et al*. (2008) and Rahkola-Sorsa *et al*. (2014b).

Sampling procedure

Samples were collected at nine study sites from moderately deep (> 25 m) areas. In these areas, water quality samples are regularly taken by the environmental authorities. This formed a background for the present study (Table 1). Each site consisted of a 540-m-long line between two points (Fig. 2). The precise location of the collection points and the cruising distance were determined by a GPS.

Principal coordinates of neighbor matrices (PCNM) (Borcard and Legendre 2002) were

Table 1. Secchi depth, water color, concentrations of total phosphorus (P_{tot}), total nitrogen (N_{tot}) and oxygen, and the maximum depth of the study sites (OIVA database). Values of the first four parameters are means of samples from July–August 2008 (G), 2009 (E, F, and H), and 2010 (A, B, C, D, and I), and they represent the entire water column from the surface to the bottom. Oxygen is the minimum near-bottom concentration in July–August.

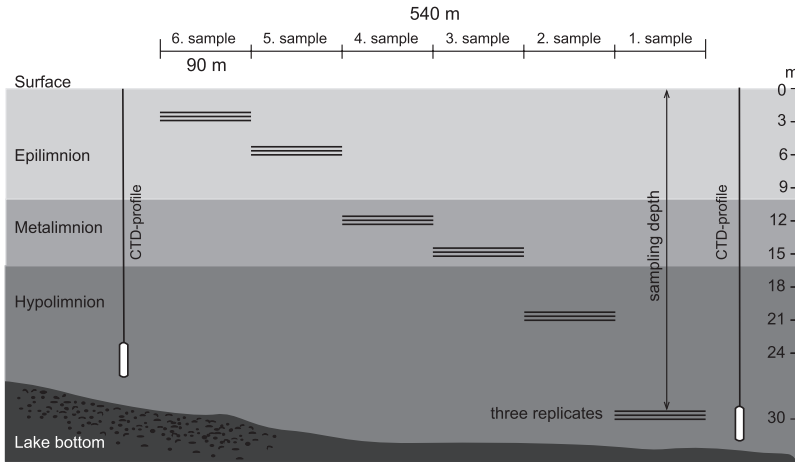| Site	Secchi (m)	Color (mg Pt l^{-1})	P_{tot} (μg l^{-1})	N_{tot} (μg l^{-1})	Oxygen (mg l^{-1})	Depth (m)
A	1.8	85	16	570	6.3	54
B	2.5	62	21	680	5.1	49
C	2.3	53	18	790	6.6	57
D	3.4	49	11	510	6.7	49
E	3.0	48	5.8	440	8.4	37
F	3.5	49	5.5	510	10.2	68
G	3.2	71	6.6	430	10.4	72
H	2.4	63	6.7	380	8.0	26
I	3.1	70	7.7	390	6.7	66

Fig. 2. Schematic representation of sampling. Horizontal lines refer to six sampling locations per site; two locations for each thermal stratum (epi-, meta- and hypolimnion). The three lines per location refer to replicates of the LOPC samples.

used to create vectors representing distances between study locations. To detect the most evident spatial processes, the PCNM vectors were interpreted as groups (Borcard and Legendre 2002, Borcard *et al.* 2004). PCNM has been used to resolve spatial patterns of communities from bacteria to fish (e.g., Beisner *et al.* 2006, Léonard *et al.* 2008, Ptacnik *et al.* 2010). The sampling procedure was planned from the viewpoint of the PCNM method. This required different 2-dimensional (horizontal) locations for each depth (Fig. 2). If all samples per site had been taken precisely at the same horizontal location, the 2-dimesional PCNM matrix based on latitudes and longitudes could not have been used to model differences between depth strata. On the other hand, a 3-dimensional PCNM matrix based on latitudes, longitudes, and depth could have led to ambiguous results.

The cruising along the sampling line was started at a speed of 2 knots (1 m s^{-1}), and the point in the deeper area was always the beginning of the line and the shallower point was considered its end. At each site, six LOPC and six MultiNet samples were taken from six different depths. In each case, two samples were collected from the epi-, meta- and hypolimnion respectively. The sampling depths were decided on the basis of vertical temperature profiles monitored with a CTD probe (Fig. 3).

The extraction of each LOPC sample lasted 90 s, which corresponded to 90 m length. The six consecutive samples corresponded to 540 m. At the end of the line, the ship turned and

cruised in the opposite direction along the same line. During this second cruise, LOPC samples were taken at the same six locations as on the first cruise. Each location referred to a precise 3-dimensional position (latitude, longitude, and depth) in the water column (Fig. 2). During the third cruise, LOPC samples were again collected at the same locations. Consequently, each LOPC sample had three replicates and altogether 3×6 = 18 LOPC samples were taken from each site.

As the aim was to associate LOPC samples with MultiNet samples, the LOPC's data recording with a 0.5-s interval was interrupted whenever the MultiNet was raised or lowered, and the LOPC values were averaged to correspond to the MultiNet samples. Four to five MultiNet samples representing the epi-, meta- and hypolimnion were taken at every site. The samples were preserved with ethanol on site. Three to six subsamples were then extracted from the original samples (total volume 210–1000 ml). Zooplankton apparent in these subsamples were identified to the species or genus level and distributed into size classes corresponding to the four LOPC size groups. A more detailed description of handling MultiNet samples is given in Rahkola-Sorsa *et al.* (2014b).

Fish densities (indiv. ha^{-1}) were estimated from acoustic data recorded by a calibrated Simrad EK60 echo-sounder (Simrad, Kongsberg Maritime AS, Horten, Norway). Its 120 kHz frequency transducer was a spherical split-beam with a 7° beam angle. The echo-sounder pulse duration was 512 μs, pulse interval 0.3 s and
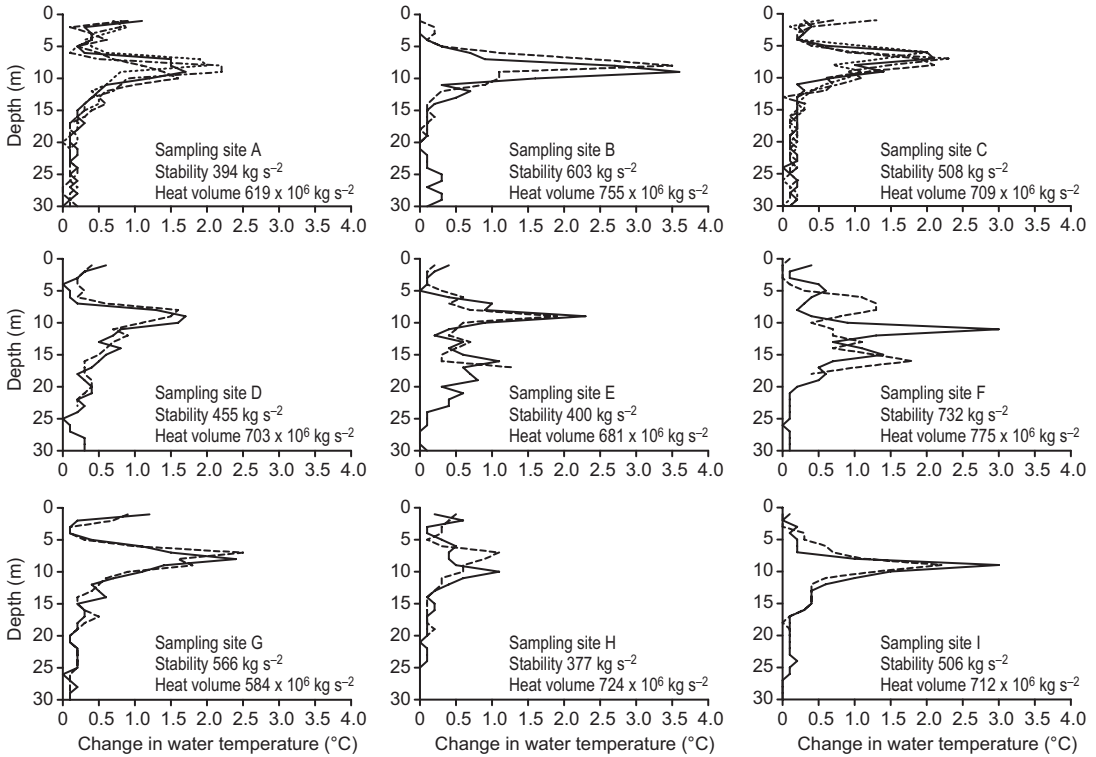
Fig. 3. Change in water temperature (°C) for every meter increase in depth in the nine study sites based on 2–4 CTD-measurements indicated with different kinds of lines. The maximum rate of change denotes the thermocline.

transmission power 100 W. The transducer was installed on the side of the ship and its acoustic beam pointed straight downwards. The minimum range from the transducer was set to 2 m and the echo energy was subsequently integrated at 1 m depth intervals. In order to remove all non-fish echoes, e.g. zooplankton, the threshold values were set to –63 dB and –60 dB for volumetric backscattering strength (S_v) and target strength (TS), respectively. The number of single echo detections (SED) was used as the estimate of fish density. In the locations where the LOPC samples were collected, the SEDs were calculated with the post-processing software Sonar5-Pro (Simrad, Kongsberg Maritime AS, Horten, Norway) (Balk and Lindem 2006). First, the acoustic data were divided into 3 m (height) × 90 m (length) "cells". Second, the SED of each "cell" was calculated. Third, the SEDs of the "cells" corresponding to LOPC/MultiNet sampling locations were extracted for later use in statistical analyses. SEDs were based on 0.8

to 1.2 relative pulse widths, a one-way beam compensation of 3 dB, and a maximum phase deviation of 0.8.

Water samples for chlorophyll-*a* concentration were taken at each sampling site at the same depths as the LOPC samples using a Limnos-type tube sampler. Water temperature was derived from CTD measurements, sampling depth from a pressure sensor attached to the MultiNet, and the depth of the sampling location from the vessel's depth meter. All LOPC, MultiNet, fish, and water samples were taken during daytime between 08:00 and 19:30 to prevent the effect of diel vertical movement (DVM) on zooplankton distribution. Typically, in the study area, zooplankton begin to ascend around sunset (Lilja *et al.* 2013). The sun rose at 04:30 and set at 22:00. The wind speed and direction were also recorded, but since they were site-specific rather than location-specific measures, it was not possible to use them as explanatory variables in the analyses. According to the ship's weather station

(Vaisala), the daily average wind speed in the study area during the study period varied from 2.6 to 5.3 m s^{-1}, which in practice meant that wind had no effect on the sampling (cf. Viljanen *et al.* 2009).

Analyzing LOPC data

For the analyses, the LOPC counts were divided into four size groups. The first group (I) represents small-sized (241–480 μm in equivalent spherical diameter, ESD), the second (II) and third groups (III) medium-sized (481–795 μm and 796–1005 μm, respectively) and the fourth group (IV) largest-sized (1006–1995 μm) zooplankton. The ESD is based on the idea that each particle (zooplankter) measured by a laser optical plankton counter can be represented with a spherical diameter equivalent to the particle's true diameter calculated from its maximum cross-section (Herman 1992).

In this study, the PCNM vectors were used to model zooplankton abundance over a range of spatial scales from approximately 100 m to 150 km. The PCNM method is based on Euclidean distances between sampling locations and it "can be applied to any set of sites providing a good coverage of a geographical sampling area" (Borcard and Legendre 2002). First, a 2-dimensional matrix of Euclidean distances (**D**) among the locations was calculated using the latitudes and longitudes of locations as initial values. The Finnish coordinate system that was used defines latitudes as distance from the equator in meters and longitudes as distance from the meridian in meters 27° east of Greenwich. For instance, site F was 6857132.932N, 3599597.662E. Second, a truncated connectivity matrix (**W**) was constructed according to the following rule: $w_{ij} = d_{ij}$ if $d_{ij} \leq t$ and $w_{ij} = 4t$ if $d_{ij} > t$, where t is a threshold value indicating the maximum distance i.e. the minimum spanning tree which maintains all sampling units being connected (Borcard and Legendre 2002, Dray *et al.* 2006). Third, eigenvectors were extracted from the centered **W**. The PCNM results in vectors corresponding to positive eigenvectors are used as explanatory factors in further analyses. A reconstruction of spatial structures is obtained by this method (Borcard

and Legendre 2002). The PCNM vectors were created using functions of the "spacemakeR" package (Dray *et al.* 2006) for the R statistical language (R 2.11.1, http://www.r-project.org/).

Four linear stepwise regressions through backward elimination were performed with IBM® SPSS® 19 for Windows to model zooplankton abundance. An average value of the LOPC count replicates ($n = 3$) representing each sampling location ($n = 9$ sites × 6 locations site^{-1} = 54 locations) and one of the four size categories (ESD 241–480, 481–795, 796–1005, or 1006–1995 μm) was used as a dependent variable in the regression. The PCNM vectors corresponding to positive eigenvectors were used as independent variables. The explanatory power of the regressions was estimated on the basis of adjusted coefficient of determination (Blanchet *et al.* 2008).

Using the PCNM vectors as independent variables in a regression model may result in an inflated coefficient of determination (r^2) due to the fact that many vectors can reflect the same spatial process (Gilbert and Bennett 2010). Therefore, it is also highly important to associate the PCMN vectors with actual explanatory variables before drawing conclusions. In the present study, associations between the actual variables and PCNM vectors were searched for by taking the actual variables as dependent variables, one by one, and explaining their variation with the PCNM vectors that best accounted for the variation in LOPC counts. To help detect the most evident spatial processes, the PCNM vectors were interpreted as groups (Borcard and Legendre 2002, Borcard *et al.* 2004).

Analyzing MultiNet data

A detrended correspondence analysis (DCA) (Hill and Gauch 1980) with PC-ORD (McCune and Mefford 1999) was conducted on zooplankton species data sampled with 40 MultiNet hauls. DCA is a development of correspondence analysis (CA) (Hirschfeld 1935). It avoids the two main faults of CA: the "horseshoe effect" and misrepresentation of ecological distances (Hill and Gauch 1980). DCA is based on rescaling the axes of CA by "cutting the axes into segments"

and then the data of each segment were normalized to zero mean (Hill and Gauch 1980). In this study, zooplankton was grouped into 21 species/groups by merging species with similar ecology, such as *Mesocyclops leuckarti* and *Thermocyclops oithonoides*, into one "functional" group. Rare species were retained in the analysis but their effect on the ordination was downweighted in accordance with their frequencies (Hill 1979).

Results

Temperature profiles

The rate of change in water temperature for every meter increase in depth reached its maximum range of 1.1–3.6 °C at 6–11 m in each lake (Fig. 3). Thermal stability (kg s^{-2}) in the lakes varied from the lowest value of 377 (site H in Fig. 1) to the highest of 732 (site F). The findings confirmed that all lakes were thermally stabilized and variation in the water temperature profile within sampling sites was negligible except for site F, where no clear temperature stratification at the shallower end of the sampling line was detected (Fig. 3).

Principal coordinates of neighbor matrices (PCNM)

The PCNM on locations of LOPC samples resulted in 27 vectors with a positive eigenvalue. The threshold value *t* was 76.5 km. The first PCNM vector corresponds to the broadest spatial scale indicating the spatial extent of the entire study area, and the last PCNM vector corresponds to the finest spatial scale. The maximum range between two sites was approximately 150 km and the minimum range between two adjacent sampling locations within a site was less than 100 m.

The first three PCNM vectors divided the study area into sub-areas (Fig. 1). The first sub-area was formed by sites A, B and C, the second by D, E and F, the third by G and H, and the fourth by I (Table 2). The next four PCNM vectors from 4 to 7 indicated variation between sites A, B and C, i.e. within the first sub-area. The

Table 2. Associations across PCNM vectors, size-grouped LOPC counts, fish density, and chlorophyll *a* concentration at different spatial scales. Variables on the same row in the table were associated with each other based on linear regression models (ns = non-significant). Letters A–I refer to sites in Fig. 1.

Spatial scales	PCNM vectors	Sites	Thermal strata	LOPC counts	Fish	Chlorophyll *a*
Sub-areas (lake groups)	1–3	ABC *vs.* DEF *vs.* HG *vs.* I	ns	241–795 μm	+	+
Lake Pyhäselkä	2	I *vs.* all other sites	ns	241–795 μm	ns	ns
Sites within sub-areas	4–7	B *vs.* C; H	ns	241–795 μm	ns	ns
Locations within sites	8–17	E; F	ns	1006–1995 μm	ns	ns
Thermal strata	18–24	A	meta *vs.* hypo	ns	ns	ns
Replicates within thermal strata	25–27	G	meta	241–795 μm	ns	ns

PCNM vectors 8–17 mostly indicated variation within single sampling sites, but also across sites within one thermal stratum. The smallest PCNM vectors indicated variation between thermal strata within one sampling site (vectors 18–24) or even within one thermal stratum represented by two sampling locations in one sampling site (vectors 25–27).

Associating LOPC-counts with PCNM vectors

The LOPC counts representing the smallest-sized zooplankton (I) (241–480 μm in ESD) were associated with the four PCNM vectors showing statistical significance ($p < 0.05$): 1 (standardized coefficient 0.670), 7 (–0.537), 27 (–0.162), and 2 (–0.146). The model explained approximately 77% of the original variation in the LOPC counts (adjusted $r^2 = 0.768$). The LOPC counts representing the smaller medium-sized zooplankton (II) (481–795 μm) associated with three of the above-mentioned PCNM vectors, i.e. 7 (standardized coefficient –0.613), 1 (0.412), and 2 (–0.228), but not with the PCNM vector 27. This model explained 57% of variation in the LOPC counts (adjusted $r^2 = 0.573$). Group I mainly comprised nauplii and copepodite stages of small cyclopoids and calanoids, such as *M. leuckarti*, *Eudiaptomus* sp., and small-sized cladocerans,

such as *Bosmina longispina*. Group II comprised *M. leuckarti*, *Daphnia cristata*, *B. longispina*, and *Diaphanosoma brachyurum* (Table 3).

The LOPC counts representing the larger medium-size (III) zooplankton (796–1005 μm) in turn did not associate with the PCNM vectors, and the LOPC counts representing the largest-sized (IV) zooplankton (1006–1995 μm) associated only with the PCNM vector 13 (standardized coefficient 0.273). The model explained about 6% of the original variation (adjusted $r^2 = 0.056$). In regard to this group (IV), one outlier with a standardized residual of 4.3 in the preliminary model was excluded from the final model. Group IV mostly comprised *Eudiaptomus* sp. and *Limnocalanus macrurus*, in that order (Table 3).

There was a weak positive autocorrelation in each model according to the Durbin-Watson statistic (1.427–1.661), but this was not considered meaningful, as residual plots showed a random pattern.

Actual variables and PCNM vectors

Water temperature, chlorophyll-*a* concentration, fish density, sampling depth, and the depth of sampling location were linearly associated with the PCNM vectors which best explained variation in the LOPC counts (vectors 1, 2, 7, 13, and

Table 3. Distribution (%) of the most abundant crustacean zooplankton species/groups sampled with MultiNet into the four LOPC size groups (μm). The sum of all values is 100%.

| Species	LOPC (241–480)	LOPC (481–795)	LOPC (796–1005)	LOPC (1006–1995)
Bosmina coregoni	1.2	1.8	0.1	0
Bosmina longispina	2.4	2.3	0.7	0
Chydorus sp.	1.3	0	0	0
Daphnia cristata	0.6	3.6	2.7	0.1
Diaphanosoma brachyurum	0	2.2	1.7	0
Limnosida frontosa	0.1	0.8	1.1	0.6
Eudiaptomus sp.	7.9	1.6	4.2	3.8
Eurytemora lacustris	0	0.1	0.4	0.5
Heterocope spp.	0	0	0.2	0.6
Limnocalanus macrurus	0	0	0.1	3.6
Cyclops spp.	1.3	0	0.2	0.2
M. leuckarti and *T. oithonoides*	20.3	16.1	13.7	0.5
Others	0.9	0.1	0.2	0.4
Total	36.0	28.6	25.3	10.3
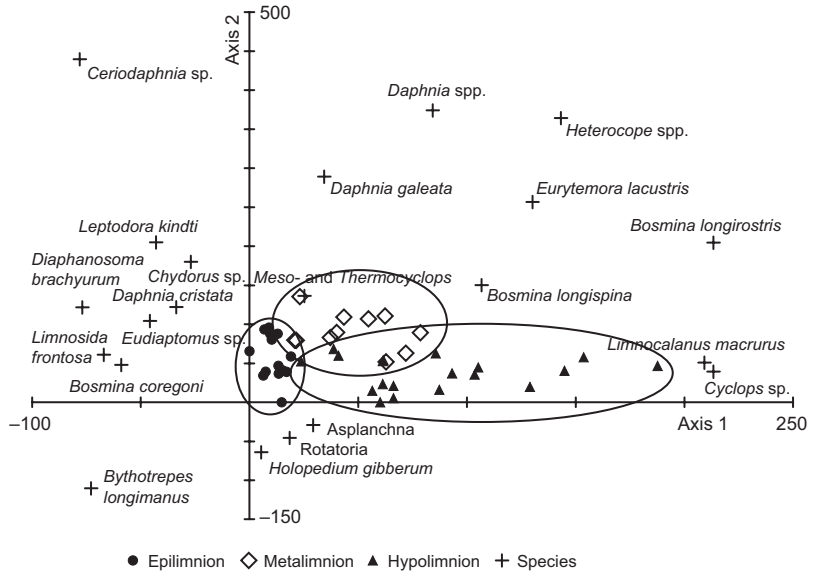
Fig. 4. The DCA ordination of 21 zooplankton species/groups from 40 MultiNet samples.

27). The only statistically significant associations were chlorophyll-*a* concentration × PCNM vector 1 ($r^2 = 0.211$, $p = 0.001$) and fish density × PCNM vector 1 ($r^2 = 0.124$, $p = 0.009$). In other words, the models based on the spatial PCNM vectors found differences in the LOPC counts between the four subgroups of lakes in the study area, and these same spatial vectors corresponded to the variation in chlorophyll-*a* concentration and fish abundance.

Detrended correspondence analysis (DCA)

The ordination arranged zooplankton by the thermal strata (epi-, meta- and hypolimnion), not by the lake or subgroup of lakes, as did the spatial vectors (Fig. 4). Samples from the epilimnion had high scores and samples from hypolimnion low scores at the first axis (eigenvalue 0.315) and samples from metalimnion were situated in the middle of the axis. The cyclopoid copepods *M. leuckarti* and *T. oithonoides*, the calanoid copepod *Eudiaptomus* sp. and the cladoceran *D. cristata* were the most abundant crustacean zooplankton species. They were found from every thermal stratum in each lake (Table 3 and Appendix). According to the DCA, the epilimnion was characterized by cladocerans (*Bosmina coregoni*,

D. cristata, *Limnosida frontosa*, *D. brachyurum*, and *Chydorus* spp.) and *Eudiaptomus* sp., whereas the calanoids *Eurytemora lacustris* and *Heterocope* spp., including *H. appendiculata* and *H. borealis*, were most abundant in the metalimnion, and the calanoid *L. macrurus* together with the cyclopoids *Cyclops* spp. were typical species in the hypolimnion. The second axis (eigenvalue 0.109) also arranged the data according to thermal strata, although much more weakly than the first axis.

Discussion

In the present study, the first finding was that models based on spatial vectors (PCNM) highlighted differences among sites and groups of sites, whereas DCA emphasized differences between thermal strata. As earlier shown, lake basins in the Vuoksi watershed have their own characteristic zooplankton community structured by the intrinsic factors of each lake such as the surface area, depth, trophic level, color of the water and, naturally, the biological community of the lake (Rahkola-Sorsa 2008). Large scale spatial variability of plankton was relatively stable and predictable while small scale processes such as variation between two adjacent sampling locations or different spatial patterns

between smaller- and larger-sized zooplankton strengthened the idea of zooplankton heterogeneity as a nested phenomenon. Also this finding emphasized that both the dependent (different zooplankton species and/or taxonomic/functional groups) and independent variables (abiotic and biotic factors causing the heterogeneity) are nested (Pinel-Alloul 1995, Pinel-Alloul and Ghadouani 2007).

The second main finding was that bottom-up regulation by phytoplankton was the primary determinant of zooplankton and further fish abundance. This holds true at least in thermally stratified boreal lakes during the summer stagnation and is underpinned by earlier findings from the same area (Rahkola-Sorsa *et al.* 2010) as well as by findings from Canada (Masson *et al.* 2004). Soon after the melting of the ice, the role of water temperature and wind may be emphasized (Rahkola-Sorsa *et al.* 2014a).

Chlorophyll-*a* concentration, zooplankton abundance, and fish density were interrelated. Abundance of smaller-sized zooplankton (I), mainly cladocerans together with nauplii and small copepodite stages of *M. leuckarti* and *Eudiaptomus* sp., related positively with scores of the PCNM vector 1. Indeed, they all had high and low values in the same areas. The conclusion is in line with previous findings concerning boreal freshwater ecosystems; abundances of phytoplankton, zooplankton and fish tend to correlate with each other, especially in late summer (Viljanen *et al.* 2009, Voutilainen and Huuskonen 2010, Voutilainen *et al.* 2012). Mid-water trawling showed that the fish detected with echo-sounding in this study are mainly zooplanktivores such as vendace (*Coregonus albula*) and smelt (*Osmerus eperlanus*) (Lilja *et al.* 2013).

Smaller-sized zooplankton (I) related with the PCNM vectors 2, 7 and 27. Associations of these vectors with the presented abiotic and biotic determinants were not obvious. This, however, does not mean that the vectors cannot be reflections of some other abiotic or biotic factors differentiating areas from each other. Vector 2 had high scores in site I and low scores in all other sites. Site I (Lake Pyhäselkä) has many unique characters mainly due to its morphology (shallow northern part, deep southern part, very

few islands) and the Pielisjoki that flows [mean runoff (MQ) = ca. 310 m^3 s^{-1}] into the lake (Korhonen 2007, Voutilainen and Huuskonen 2010, Voutilainen *et al.* 2012, 2014).

The PCNM vector 7 indicated variation between sites B and C, which were both in Lake Kallavesi. The sites resembled each other regarding the levels of phosphorus and nitrogen and chlorophyll *a*, but not with respect to higher trophic levels. The abundance of zooplankton was higher in site C, whereas the abundance of fish was higher in site B. This can be a sign of top-down regulation, as site B is a single deeper area surrounded by shallower areas and is a much more "isolated" region than site C. Another sign of top-down regulation was found in the shallowest site, H, where zooplankton abundance was the lowest and fish abundance the highest among all sites. Generally, predator control on zooplankton appears to be highest in oligo- and eutrophic shallow lakes rather than in mesotrophic lakes (Jeppesen *et al.* 2003).

The PCNM vector 27 mainly referred to small scale variation between two adjacent sampling locations, especially within sites G (between two metalimnion locations) and A (between the lower metalimnion and upper hypolimnion locations). In order to be able to explain the small scale variation indicated by the PCNM 27, the samples should have been linked to more accurate positions in the water column than was the case in the present study.

Abundance of the largest-sized zooplankton (IV), mainly *L. macrurus*, *Eudiaptomus* sp. and larger individuals of *M. leuckarti*, *T. oithonoides*, and *D. cristata*, had only weak associations with the PCNM vectors. This denotes that spatial heterogeneities of smaller- and larger-sized zooplankton obey different patterns. The PCNM vector 13, the only vector that showed a statistically significant association with abundances of this group (IV), modeled variation between the metalimnion and two other thermal strata in sites E and F, resulting in almost equal scores for the epilimnion and hypolimnion. The shape of the thermocline was variable in sites E and F (Fig. 3), deviating from other studied lakes. This may indicate a strong turbulent mixing in the metalimnion (Nõges *et al.* 2011) of sites E and F due to inflow from natural channels

(Kyrönsalmi, Hopeasalmi, and Laitaatsalmi, MQ = ca. 600 m³ s⁻¹, http://fi.wikipedia.org/wiki/Kyrönsalmi), and further affects the patchiness of plankton noted in this study. In addition to inflow, also other components, such as wind, may have an influence on thermal stratification. However, wind speed of $2.6–5.3$ m s⁻¹ detected during the present survey is assumed not to cause water currents strong enough to affect the distribution of plankton (Viljanen *et al*. 2009).

Regarding the abundance of the largest-sized zooplankton (IV), the explanatory powers of the PCNM vectors were somewhat low and thus more weight is given to the DCA results. *Limnocalanus macrurus*, and large individuals of *Eudiaptomus* sp. were dominant in the largest-sized group. The glacial relict *L. macrurus* is specialized in living in cold water i.e. in the hypolimnion in summertime, as the DCA ordination also distinctly characterized. The spatial heterogeneity of group IV appeared to be driven more by the thermal zonation within lakes than by biotic factors differentiating entire lakes from each other, in contrast to the spatial heterogeneity of smaller-sized zooplankton (I). In this study, however, the density of small zooplankters was about 10^2 times that of larger-sized plankters.

The study sites were strongly thermally stratified, apart from the shallowest site (H in Fig. 1) with the lowest thermal stability. Compared with the wintertime situation when the lakes are ice-covered, Schmidt stabilities were approximately 20 times higher (Voutilainen *et al*. 2014). A strong stratification means that zooplankton and fish must cross a barrier formed by temperature differences when moving from one stratum to another during their DVM. A rapid migration (e.g., Lilja *et al*. 2013) requires energy and an ability to adapt to changing conditions. The present finding that the main forces driving spatial heterogeneity of zooplankton in lakes may differ between thermal strata and, consequently, between zooplankton size categories and species is of special importance from the viewpoint of the ongoing climate change. Climate change might alter the thermal structure of boreal lakes so that the temperature differences between strata will increase (Voutilainen *et al*. 2014). This may hamper the DVM of plankton and fish and consequently the functioning of food webs by affecting interactions, as the species/groups will be less connected with each other due to strengthened physical borders in the environment.

The present findings demonstrated that verticality plays a significant role in the distribution of physicochemical and biological variables. The abundance of phytoplankton and small-size zooplankton was high in the epilimnion, in warm water. Planktivorous fish were most numerous in the metalimnion and large-size zooplankton in the hypolimnion (Appendix). In general, vertical distribution of zooplankton and planktivorous fish in stratified lakes is related to gradients of light and temperature as well as interactions between the zooplankton and fish (e.g., Lampert 1993, Lilja *et al*. 2013). The spatial and temporal distribution patterns may to some extent differ between lakes and across sites within lakes due to climatological and morphological factors (Karjalainen *et al*. 1999). In addition to abundance, also species composition and size distribution of zooplankton and fish communities are associated with vertical aspects (Rahkola-Sorsa 2008, Lilja *et al*. 2013).

To conclude, the present study stressed the need for gathering data by using more than one method simultaneously and emphasized the benefits of combining results from two or more statistical techniques. Large scale differences of zooplankton abundance between the sites and the groups of sites were regulated by phytoplankton as well as fish abundance whereas clear differences between thermal strata emphasized small scale differences.

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Appendix. Summary of data. T = water temperature (°C), Chl a = chlorophyll a (µg l^{-1}), Fish = planktivorous fish (fish ha^{-1}), LOPC = average count (no. m^{-3}), and average densities of the most abundant crustacean zooplankton species based on MultiNet samples (no. m^{-3}): Bc = *Bosmina coregoni*, Bl = *Bosmina longispina*, Ch = *Chydorus* sp., Dc = *Daphnia cristata*, Db = *Diaphanosoma brachyurum*, Lf = *Limnosida frontosa*, Eu = *Eudiaptomus* sp., El = *Eurytemora lacustris*, He = *Heterocope* spp., Lm = *Limnocalanus macrurus*, Cy = *Cyclops* spp., M + T = *Mesocyclops leuckarti* and *Thermocyclops oithonoides*. Copepods include nauplii, copepodites, and adults.

Site/Stratum	T	Chl a	Fish	LOPC	Bc	Bl	Ch	Dc	Db	Lf	Eu	El	He	Lm	Cy	M + T
Juurusvesi																
Epi	21.0	7.3	265	12476	0	59	30	220	15	356	3191	210	59	59	59	11723
Meta	13.0	1.8	660	11665	255	309	237	783	182	18	2494	73	91	0	128	10138
Hypo	9.2	0.9	163	281	9	0	3	6	0	3	38	9	3	86	35	146
Kallavesi 1																
Epi	22.9	10.3	103	36622	2553	689	239	1850	2867	787	7917	33	33	33	0	30862
Meta	12.9	3.1	869	12245	174	406	14	493	188	43	1652	173	130	130	230	18881
Hypo	9.4	1.3	219	2383	41	24	6	23	26	9	26	0	12	1086	343	1124
Kallavesi 2																
Epi	21.1	10.5	0	96384	4258	774	774	7355	3484	5032	31354	0	0	0	0	65419
Meta	12.8	3.8	326	6946	53	3109	0	88	53	0	422	106	334	843	18	6149
Hypo	9.7	1.6	142	1123	15	22	0	9	6	0	53	7	7	432	116	1114
Haukivesi																
Epi	21.6	7.1	0	44815	2006	1900	1982	4849	4649	602	9025	48	130	130	12	25466
Meta	15.2	1.8	699	4932	0	273	53	200	42	42	296	253	221	536	96	4564
Hypo	8.9	0.9	109	929	0	26	42	42	0	0	10	5	10	587	62	255
Haapavesi																
Epi	20.8	4.3	0	7429	39	391	183	691	235	0	1772	104	0	13	13	4837
Meta	14.9	1.3	232	1923	31	231	8	46	0	8	62	177	200	672	109	1148
Hypo	7.6	0.7	24	2252	64	234	27	206	20	0	147	15	12	863	79	679
Pihlajavesi																
Epi	21.0	3.6	0	10222	8	41	16	271	214	90	4372	230	617	8	82	6487
Meta	14.8	1.7	185	1344	4	233	9	40	0	0	102	66	40	506	61	824
Hypo	6.6	0.6	24	834	8	26	0	15	4	0	59	4	4	568	94	133
Paasselkä																
Epi	19.4	2.5	0	18841	54	2197	327	2018	54	7	5164	199	0	0	57	11453
Meta	10.4	1.2	274	3873	0	167	13	489	6	64	836	405	52	25	70	2193
Hypo	6.5	0.5	0	484	7	49	7	28	0	0	49	14	7	49	190	112
Samppaanselkä																
Epi	21.1	5.2	82	4677	9	120	9	258	0	86	1395	95	0	0	27	3228
Meta	16.5	1.5	593	2559	0	25	0	6	32	82	517	138	246	574	120	1275
Hypo	15.0	0.9	841	1763	3	38	7	22	0	0	79	49	13	823	214	1358
Pyhäselkä																
Epi	22.7	3.6	54	16656	233	1543	0	1491	0	267	2563	26	42	0	12	13812
Meta	15.5	1.0	230	1462	5	138	0	69	11	11	116	79	43	159	58	1905
Hypo	12.4	0.7	35	1302	5	49	0	7	0	0	6	7	12	330	752	571