Is the sampling strategy interfering with the study of spatial variability of zooplankton communities?

Carol Avois, Pierre Legendre, Stéphane Masson, and Bernadette Pinel-Alloul

Abstract: Surveys at the whole-lake scale take some time to carry out: several hours or several days. For logistic reasons, the sites are not sampled simultaneously or in a random sequence. Traditional limnological sampling methods require an appreciable amount of time at each site. Any sampling strategy that is not random or simultaneous introduces dependencies among the observations, which must be taken into account during the analysis and interpretation of the data. What is the real nature of the variation measured using a given sampling design? This question is approached using sites sampled by two boat teams during two consecutive days. Statistical modelling was used to partition the variation of zooplankton size-class data into environmental and spatial components. The conclusions reached after an analysis that did not control for the sampling design are erroneous and quite different from those reached when the effect of the sampling design (factors Day, Boat, and Hour) was taken into account. Clearly, when a significant effect of the sampling design is found, one must control for it during the analysis and interpretation of ecological variation.

Résumé : Réaliser un relevé à l'échelle panlacustre peut prendre du temps : plusieurs heures ou même plusieurs jours. Pour des raisons de logistique, les stations ne sont pas échantillonnées simultanément ni en ordre aléatoire Les méthodes classiques d'échantillonnage limnologique imposent de consacrer un temps appréciable à chaque station. Une stratégie d'échantillonnage qui n'est ni simultanée ni aléatoire introduit des dépendances entre les observations, ce qui doit être pris en compte dans l'analyse et l'interprétation des données. Quelle est la vraie nature de la variation mesurée avec un plan d'échantillonnage donné? Nous avons abordé la question en examinant des stations échantillonnées par deux équipes embarquées pendant deux journées consécutives. La modélisation statistique a servi à répartir la variation des données sur les classes de taille du zooplancton en composantes environnementales et spatiales. Les conclusions atteintes après une analyse qui ne tenait pas compte du plan d'échantillonnage sont erronées et très différentes de celles que l'on obtient quand on prend en compte l'effet du plan d'échantillonnage (les facteurs jour, bateau et heure). Il apparaît clairement que, si l'on observe un effet significatif du plan d'échantillonnage, il faut le prendre en compte dans l'analyse et l'interprétation de la variation écologique.

[Traduit par la Rédaction]

Introduction

Studies on the spatial variation of ecological communities began at the turn of century. Perception of the importance of spatial structures has changed with time. Whereas spatial variation was considered a statistical nuisance some years back (e.g., Steele 1976), it is now recognised as an ecologically important feature of ecosystems (Platt and Harrison 1985; Legendre 1993). Concomitant with this new approach is the notion that the patterns of spatial variation to be observed may depend on the scale of observation (e.g., Levin 1992; Legendre et al. 1997). Methods have been developed for analysing spatial variation as an ecological quantity, at a single scale or as a function of scales (e.g., Dutilleul 1998; Gardner 1998). The success of such analyses depends, however, on hypotheses and on sampling strategies; in a great many cases, sampling strategies are decided a priori without studying their possible effects on the analysis of the ecological phenomenon.

Many studies of freshwater zooplankton have considered the spatial heterogeneity of community composition along the vertical axis. The sampling strategies used in these studies assumed that the habitat was unstructured horizontally, the distribution of zooplankton being homogeneous or with negligible variance compared with the vertical axis (Keller and Yan 1991; Pinel-Alloul et al. 1995). So sampling was often conducted at a single site in the central portions of water bodies. This view is justified by the work of Richerson et al. (1978), who noted that thermal stratification represents the most important physical characteristic in lakes; it is at the origin of the physical, and hence the biological, heterogeneity among strata. The inadequacy of this sampling strategy to allow for the analysis of the spatial structure of communities was noted (e.g., Malone and McQueen 1983) when limnologists became interested in horizontal spatial variation of zooplankton at different scales of observation (e.g., Pinel-Alloul and Pont 1991; Lacroix and Lescher-Moutoué 1995). Few authors have simultaneously studied the spatial variation of zooplankton along the horizontal and vertical axes as well as the interaction between these axes (e.g., Pinel-Alloul and Pont 1991; Masson and Pinel-Alloul 1998). Many investigations pointed out the importance of the sampling design when studying the spatial structure of

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communities and the environmental variables that may influence them (e.g., Pinel-Alloul and Pont 1991). The spatial distribution of zooplankton depends to a large extent on the observation scale; spatial heterogeneity increases rapidly with the extent of the sampling area, and communities have different types of spatial distribution at different scales (e.g., Pinel-Alloul and Pont 1991; Lacroix and Lescher-Moutoué 1995). Patalas and Salki (1993) insisted on the fact that the type of spatial structure that can be detected in lake communities depends on the size of the lake (i.e., the "extent" of the sampling area) and on the number of sampling sites (which, together with the extent, determines the sampling interval; the components of a scale are described by Legendre and Legendre 1998).

Zooplankton patches have been detected on scales ranging from centimetres (1–100 cm; Byron et al. 1983; Butorina 1986) to kilometres (1–100 km; Richerson et al. 1978; Patalas and Salki 1992), but the greatest spatial heterogeneity was observed along the vertical rather than the horizontal axis. Spatial variation in plankton distributions in whole lakes has been attributed to a wide variety of environmental factors (Lens et al. 1986). The environmental variables that explain the spatial structure of zooplankton communities also depend on the scale of the observations. Malone and McQueen (1983) recognised four categories of spatial structures representing: (*i*) large patterns (>1 km in diameter) induced by vectorial forces (Patalas 1969) or by seasonal (Urabe 1989) and morphometric variables (Urabe and Murano 1986), (*ii*) coarse- and fine-scaled patterns (approximately 10–1000 m in diameter) caused by wind-induced currents (e.g., Riley 1976) or by the combined effects of shore avoidance and vertical migrations (Ringelberg 1991), (*iii*) Langmuir circulation patterns caused by behavioural interactions (De Nie et al. 1980) and a combination of water movement, light orientation, and active swimming, and (*iv*) swarming patterns (from a few centimetres to several metres in diameter), potentially caused by biotic factors. Zooplankton patchiness over broad and fine scales is the result of many physical and chemical processes interacting with several biological processes, as described by the "multiple driving force hypothesis" (for a review, see Pinel-Alloul 1995), with the predominance of abiotic factors at large spatial scales and that of biotic factors at smaller scales.

Sampling strategies for analysing the horizontal and vertical structures of zooplankton communities have focused, in most cases, on spatially limited lake areas (e.g., transects or quadrats; e.g., Pinel-Alloul and Pont 1991; Visman et al. 1994). Ecologists now wish to maximize the extent of the study area (Masson and Pinel-Alloul 1998) and the number of sites sampled in order to expand the investigations to a scale of observation covering whole lakes (Pinel-Alloul et al. 1999). By doing so, they hope to understand the spatial structuring of communities at that scale and be able to compare the results with the spatial structures determined for spatially limited lake areas (Gaudy et al. 1995; Lacroix and Lescher-Moutoué 1995). However, this may change the nature of the variation measured, since, generally, all sites are not sampled simultaneously. In many studies where sampling covered a few hours or days, the temporal effects were not taken into account during the analysis of spatial variation because daily and short-term variations were considered negligible (e.g., Gaudy et al. 1995; Lacroix and Lescher-Moutoué 1995). Was this assumption justified? The effects of the abiotic and biotic factors on the spatial variation of zooplankton change not only with the spatial scale but also with the temporal scale over days or seasons (e.g., Pinel-Alloul and Pont 1991). Gaston and McArdle (1994) recognised the importance of incorporating the temporal dependency of spatial sampling in their analysis.

Temporal and spatiotemporal short-term variations in community structure are well established. They are inherent to the zooplankton community, caused by migrations of organisms which may be vertical and (or) horizontal (Angeli et al. 1995; Lauridsen and Buenk 1996) and linked or not to diurnal variations (e.g., Lauridsen and Buenk 1996). Light (e.g., Ringelberg 1991; Richards et al. 1996), food (Haney and Hall 1975), temperature of the upper water layers (Manca et al. 1986), competition (e.g., Wright et al. 1980), and visual or nonvisual predation (Bollens and Frost 1989; Herwig and Schindler 1996) are all important in explaining migration movements. Some of these factors, like the physical stratification of water columns (Richerson et al. 1978; Pinel-Alloul et al. 1988), predation and competition (e.g., Visman et al. 1994), and sources of food (Tessier 1986), are also considered important in determining the spatial distributions of zooplankton communities (Pinel-Alloul 1995).

An artifactual spatial structure may be generated in the data by the sampling design, caused by the route followed by the boats visiting the sampling stations in sequence, if the duration of the trip is important (Ibanez 1973). In such a case, the sampling design does not guarantee that spatial variation is the only type of variation found in the data, and we must ask the question: what is the real nature of the variation measured using the sampling design?

The original purpose of the sampling program reported in this paper was to verify hypotheses put forward in previous studies at the within-lake scale ($H(1.x)$ designates an alternative hypothesis whereas $H(0.x)$ stands for the corresponding null hypothesis): $H(1.2)$: "the abiotic factors play a dominant role in determining the horizontal spatial variation of zooplankton" and $H(1.3)$: "a combination of abiotic and biotic factors is necessary to explain the vertical spatial variation of zooplankton"; the sampling extent was the whole lake.

Actually, the sampling strategy used in the field generated variation among sites that may look like spatial variation,while in fact, it may reflect other aspects of the sampling design. To check this, the following questions must be asked. How large is the short-term temporal variation found among sites during sampling compared with the spatial variation? How does the collinearity between short-term temporal variation and spatial variation affect the relationships identified between the zooplankton and the abiotic and biotic factors? Accordingly, the objectives of this paper are (*i*) to test the null hypothesis $H(0.1)$ that "there is no significant effect of the sampling design on the detected spatial structure of the zooplankton data" and (*ii*) if a significant effect is identified ($H(1.1)$) to determine its impact on the analysis of the spatial structure of the zooplankton data, before proceeding with hypotheses $H(1.2)$ and $H(1.3)$ above. On the other hand, if a significant effect of the sampling design is not identified, one can proceed directly with hypotheses $H(1.2)$ and $H(1.3)$.

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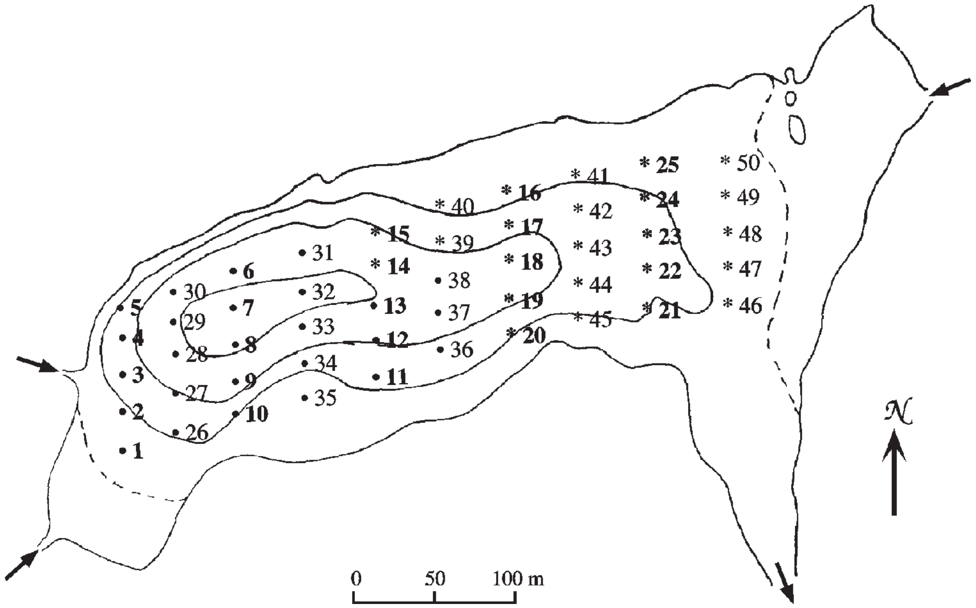
Fig. 1. Map of Lake Cromwell (74°00′W, 45°49′N) showing the 50 sampling sites in Québec, Qué. Day 1 (bold): sites 1–25; day 2: sites 26–50. The sites sampled from boats 1 and 2 are identified by a bullet and an asterisk, respectively. Solid lines are the isobaths (3, 6, and 9 m), broken lines delimit the macrophyte zones, and arrows represent water inputs and output.



The statistical methods used to test these hypotheses are appropriate for lakes; they may also prove useful in studies of marine plankton. The description of the spatial distribution of zooplankton and its interpretation using explanatory abiotic and biotic factors (hypotheses *H*(1.2) and *H*(1.3) above) will be the subject of another paper.

Materials and methods

Sampling strategy

Lake Cromwell (74°00′W, 45°49′N) is located on the territory of the Station de biologie des Laurentides (Université de Montréal), approximately 80 km north of Montréal, Québec. This small Canadian Shield mesotrophic lake (surface area 9.2 ha) is shallow (mean and maximum depths of 3 and 9.1 m, respectively), humic, lightly acidic, and dimictic (Pinel-Alloul and Pont 1991). Lake Cromwell is also characterised by high planktivorous and benthivorous fish abundances and the absence of piscivorous fish species.

Considering the purposes of this study as well as the results of previous investigations on the spatial structure of zooplankton, particularly those conducted previously in Lake Cromwell (e.g., Pinel-Alloul and Pont 1991), a sampling strategy was developed to cover the whole lake while minimising the variance due to the temporal variation inherent to zooplankton communities. Sampling, conducted in the summer, was diurnal (from 07:00 to 19:00) to eliminate possible effects of nycthemeral vertical migrations of zooplankton. The lake area, diurnal period, and allowable sampling effort were considered in determining the elements of the sampling scale (grain size, extent, and sampling interval). Systematic sampling of 50 sites, in a staggered arrangement on the surface of the lake, was conducted during 2 consecutive days (factor Day below); 25 sites were sampled on 3 August (sites 1–25) and 25 more on 4 August 1994 (sites 26–50). Two teams (hereafter called "boats") were at work each day, conducting sampling at the first 13 sites (out of 25) in parallel with the last 12 sites in order to reduce possible daily effects (Fig. 1). The time of sampling was recorded for each site (factor Hour below). The spatial sampling interval was 35 m east–west and 22 m north–south; so the sampling grid allowed the study of coarse-scale spatial phenomena (type II variation in Pinel-Alloul and Pont 1991). Due to lake morphology, the limnetic strata contained 50 sampling points in the epilimnion (0–2.5 m), 36 in the metalimnion (2.5–5 m), and 13 in the hypolimnion (5 m-bottom). The sampling dates were chosen to correspond to the period of maximum summer stratification (Jordan et al. 1988).

At each site, 11 physical and chemical variables were measured: pH, turbidity (NTU), temperature (degrees Celsius), alkalinity (milligrams per litre), maximum depth (metres), conductivity (microsiemens per centimetre), dissolved oxygen (DO, milligrams per litre), dissolved silica (DSI, micrograms per litre), total dissolved nitrogen (TDN, micrograms per litre), dissolved organic carbon (DOC, milligrams per litre), and total dissolved phosphorus (TDP, micrograms per litre). Four biological variables were also measured: chlorophyll *a* concentration (micrograms per litre) of phytoplankton <20 and >20 μm, density (individuals per litre) and mean length (millimetres) of Chaoboridae. The biomass of limnoplankton (micrograms per litre) (i.e., all zooplankton and organic matter collected in the water samples) was the response variable of the study; it was divided into four size-classes (>500, 202–500, 100–202, and 53–100 μm). The field and laboratory methods are fully described in Masson and Pinel-Alloul (1998).

Statistical analyses

Is there a significant effect of the sampling design on the detected spatial structure of the zooplankton size-class data?

Multifactorial analysis of covariance (ANCOVA) was used to test the hypothesis *H*(0.1) of no significant effect of the sampling design on the spatial structure of the zooplankton size-class data. Multifactorial analyses of variance (ANOVA) and covariance (ANCOVA) are tools which provide insights into the nature of variation of natural events, considering simultaneously the effects of several factors. Three factors were used to characterise the sampling design: Day (two levels: fixed and qualitative; this factor is considered fixed because the sampling days were chosen to be consecutive), Boat (two levels: fixed and qualitative), and Hour (covariable). The factors Day and Boat are crossed. The design of

Table 1. Three-way ANCOVA design with two fixed factors and one random factor (mixed model) used to test the hypothesis *H*(0.1) of no significant effect of the sampling design on the spatial structure of the zooplankton size-class data. MS = mean square.

Source of variation	MS	*F* statistic
Main effects		
Day (d), fixed, qualitative, balanced	MS_d	MS_d/MS_{hd}
Boat (b), fixed, qualitative, unbalanced	MS_b	MS_b/MS_{hb}
Hour (h), covariable	MS_h	MS_h/MS_{res}
First-order interactions		
Day × Boat (db)	MS_{db}	MS_{db}/MS_{dbh}
Hour × Day (hd)	MS_{hd}	MS_{hd}/MS_{res}
Hour × Boat (hb)	MS_{hb}	MS_{hb}/MS_{res}
Second-order interaction		
Day × Boat × Hour (dbh)	MS_{dbh}	MS_{dbh}/MS_{res}
Residual (res)	MS_{res}	

the analysis was unbalanced, since the factor Boat had unequal sample sizes (i.e., level 1, $n = 13$; level 2, $n = 12$), so type III sums of squares (SS) were used in the analyses (Shaw and Mitchell-Olds 1993). Type III SS are designed to quantify the effects of particular factors adjusted for all the other factors in the model; they have the property that the various SS resulting from the partition do not add up to the total SS. The hypotheses tested using type III SS are constructed as if each combination of factors in the model had an equal number of observations, giving each combination of factors an equal weight. Thus, three-way ANCOVA with mixed model, unbalanced, has been used to test the null hypotheses about the main effects Day, Boat, and Hour, the first-order interactions Day × Boat, Hour × Day, and Hour × Boat, and the second-order interaction Day × Boat × Hour in the model (Table 1), starting with the latter: $H_{0(1)}$: there is no interaction between factors Day, Boat, and Hour.

If the second-order interaction was not significant, it was eliminated from the model and the first-order interactions were tested: $H_{0(2)}$: there is no interaction between factors Day and Boat, $H_{0(3)}$: there is no interaction between factors Day and Hour, and $H_{0(4)}$: there is no interaction between factors Boat and Hour.

If there is independence between any effects of Day, Boat, and Hour, the main effects can be tested: $H_{0(5)}$: there are no differences among the levels of factor Day, $H_{0(6)}$: there are no differences among the levels of factor Boat, and $H_{0(7)}$: there are no differences among the levels of factor Hour.

The procedure was stopped when at least one high-order interaction was found to be significant, considering the hierarchy of the analysis. A significant interaction tells us that the size and (or) nature of a main effect changes across the levels of the other factor (and vice versa). Thus, one always examines the highest order interaction first, i.e., Day × Boat × Hour. If this interaction is significant, there is no point in examining the first-order interactions or the main effects on their own. The SS component Day SS measured the unevenness between days, independent of boat and hour (if the interaction terms are not significant). If a difference between days was found, it could be attributed either to temporal variation inherent to the zooplankton size-class data or to the sampling design, since the sites sampled on the two days were neither the same nor paired into strata. The same reasoning applied to the component Hour SS. For the component Boat SS associated with differences between boats, independent of day and hour (if the interaction terms are not significant), the observed variation may have been caused by the routes of the boats, which may have generated a spatial artefact; it may depend on the generation time or the ability of the organisms to move, relative to the duration of the sampling campaign.

It is not possible to determine exactly the cause of the variation explained by the interaction terms. This variation may be caused by factors influencing the migration of organisms (i.e., temporal and (or) spatiotemporal variations of community) or it may be an artefact due to the sampling design. For this reason, we prefer to talk about an "effect of the sampling design," which includes both.

All hypotheses were tested in a single ANCOVA for each size-class of zooplankton. This analysis was done separately for the epi-, meta-, and hypolimnion. The analysis was repeated while considering the three limnetic strata as an additional factor.

We were interested in describing in some detail the effect of the sampling design on the observed patterns of distribution of the zooplankton size-class data in order to optimise the sampling strategy for future studies. A general model was established for the zooplankton community (all four size-classes) in each water stratum and another one for all strata. To obtain a general model, particular models were first computed for each size-class of zooplankton. For each source of variation, the SS from the particular models were added; we recomputed the mean squares, the *F* ratio (which is then a "stacked" univariate *F* statistic, as in Verdonschot and ter Braak 1994), and the associated *p* value, reconstructing the ANCOVA table. The resulting probabilities were approximate, since the covariances among size-classes of zooplankton were not taken into account when summing the SS to create the stacked *F* statistics; this should not generate any important effect, since the covariances among size-classes were low. Results of the stacked *F* analysis were verified using redundancy analysis (see below), which is a multivariate method. Therefore, there were as many general models as there were particular models. The general model selected for the zooplankton size-class data in each stratum and for all strata was the one that had the highest-order significant interaction term, provided that the model was significant; this model also had the highest amount of explained variation. If there was no significant interaction term, we selected the model where the significant main effects explained the largest fraction of the total variance. The SS explained by the model represented the variation generated by the "sampling design"; the residual SS was that unexplained by the design.

No significant effect of the sampling design on the detected zooplankton spatial structure

When the null hypothesis *H*(0.1) could not be rejected, the sampling design was assumed to have no significant effect on the spatial structure of zooplankton and hypotheses *H*(0.2) and *H*(0.3) could now be tested. The method of partition of ecological variation between environmental and spatial components (Borcard et al. 1992; Borcard and Legendre 1994) was used for this purpose. The analysis produced four independent and additive fractions of the variation: (E), a fraction attributed to the nonspatially structured component of the environmental variables (abiotic and (or) biotic); (SE), a fraction explained by the spatially structured portion of these explanatory variables; (S), a pure broad-scale spatial component that remains unexplained by the current environmental variables and may reflect processes generating spatial heterogeneity that have not been explicitly included in the analysis; and (Un), a fraction of the ecological variation that remains unexplained by the spatial and environmental variables (Fig. 2*a*). This method produces measures of the importance, for the zooplankton data, of the environmental variables and the broad-scale spatial structure. This analysis was carried out using redundancy analysis (RDA) (Rao 1964, 1973; van den Wollenberg 1977) and partial RDA (ter Braak 1988).

RDA combines the properties of two families of methods: regression and ordination. The steps of RDA are as follows: (1) regress each variable in the matrix of response variables on all the explanatory variables and compute the fitted values and (2) carry

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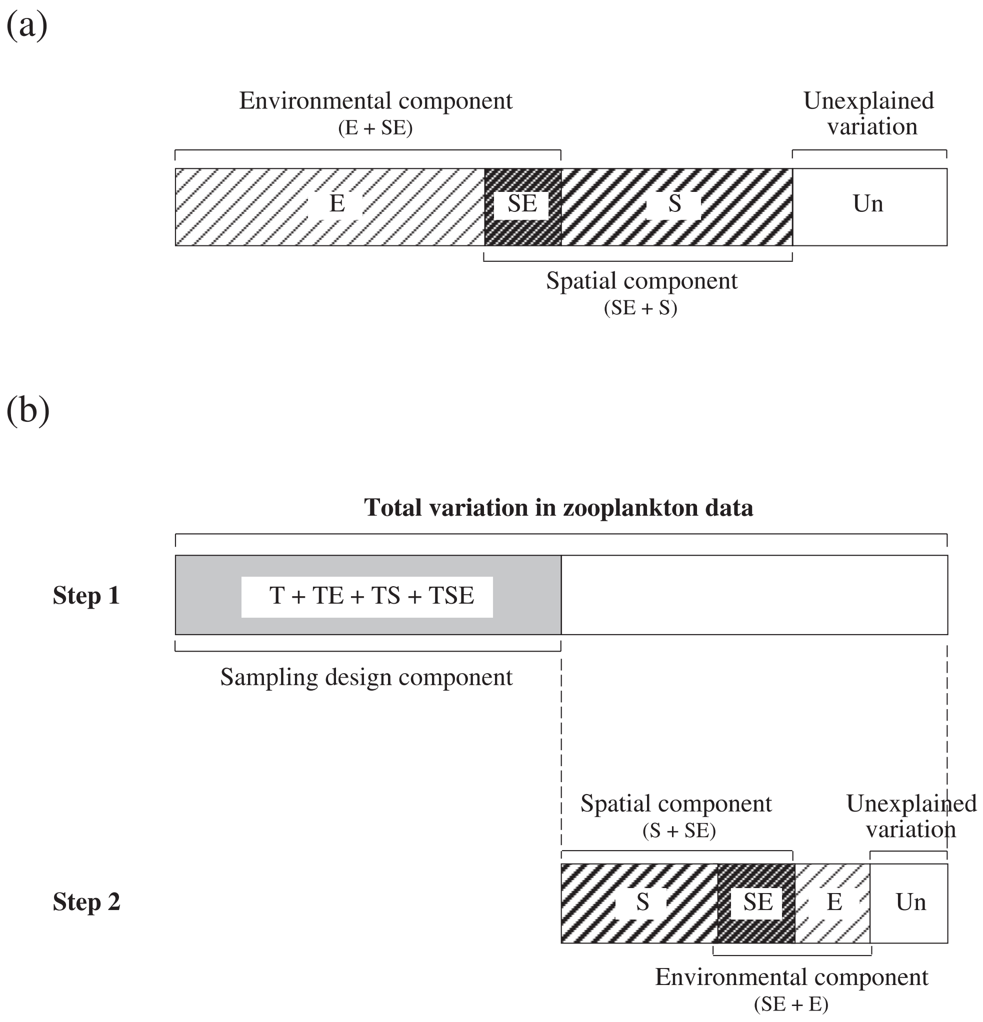
Fig. 2. (*a*) Partition of the ecological variation of the zooplankton data following Borcard et al. (1992). The total rectangle represents the ecological variation. It is divided into the following: (E), pure environmental variation; (SE), spatially structured environmental variation; (S), pure spatial variation; (Un), unexplained variation. (*b*) Partition of total variation of the zooplankton data, including the sampling design. Step 1: quantification and elimination of the variation explained by the sampling design, which includes the following: (T), pure sampling design variation; (TE), environmental variation explained by the sampling design; (TS), spatial variation explained by the sampling design; (TSE), spatially structured environmental variation explained by the sampling design. Step 2: partition of the residual variation among the environmental and spatial components.



out a principal components analysis of the matrix of fitted values to obtain the eigenvalues and eigenvectors representing the variation explained by the explanatory variables. Partial RDA is a direct extension of partial regression (for details, see Legendre and Legendre 1998). Using RDA, fractions (S + SE + E), (S + SE), and (SE + E) were determined; fractions (S) and (E) were obtained by partial RDA, whereas fraction (SE) was obtained by difference, since (SE) = (S + SE) + (SE + E) – (S + SE + E) (Fig. 2*a*).

Significant effect of the sampling design on the detected zooplankton spatial structure

Rejecting hypothesis *H*(0.1) meant that the sampling design had a significant effect on the spatial structure of the zooplankton variables. When this was the case, the variation of the zooplankton size-class data was analysed in more detail, considering all the deterministic components of the variation for which information was available (Fig. 2*b*). (*i*) The sampling design component (T) characterises the variation generated by the sampling program. This component contains the factors Day, Boat, and Hour as well as their interaction terms and includes (T), (TE), (TS), and (TSE) as defined in the caption to Fig. 2. (*ii*) The spatial component (S) characterises the spatial structure (i.e., the spatial distribution of organisms in their habitat) and is modelled using the geographic positions of the sites, as described below. Fractions (S), (SE), (TS), and (TSE) of variation contain this component. (*iii*) The environmental component (E) includes the effects of abiotic and biotic variables. This component characterises the organisational (functional) structure of populations or communities (reproduction, growth, density, mortality, etc.). Fractions (E), (SE), (TE), and (TSE) of variation contain parts of this component.

Although we cannot be certain whether the components (TE), (TS), and (TSE) should be attributed to the sampling design or to spatial or environmental effects, conservative interpretation of the results requires that we remove all the variance that can be attributed to the sampling design, i.e., fractions (T), (TE), (TS), and (TSE), before testing ecological hypotheses *H*(1.2) and *H*(1.3) (Fig. 2*b*). By doing this, we place ourselves in a situation less likely to promote our hypotheses. This is in agreement with the principle of parsimony (Ockham's razor): we must first try to attribute the observed variance to the most simple source of varia-

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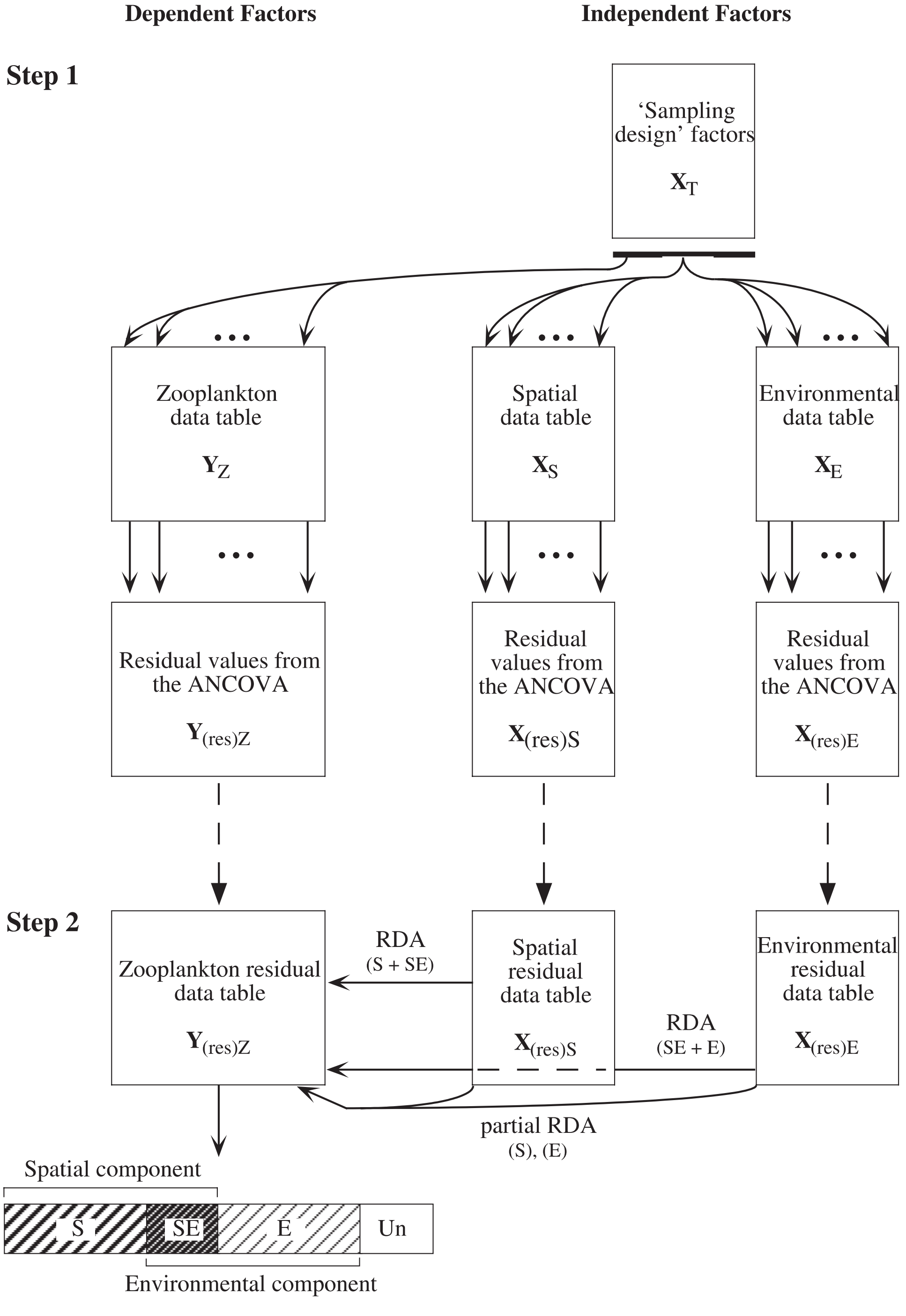
Fig. 3. Summary of the method for partitioning the measured variation among the spatial and environmental components while controlling for the effect of the sampling design (method 1). Step 1: ANCOVA for each variable y_Z, x_S, and x_E with the sampling design factors of the general model $\mathbf{X}_T$. Compute the residual values $y_{(res)Z}$, $x_{(res)S}$, and $x_{(res)E}$ and construct the residual tables $\mathbf{Y}_{(res)Z}$, $\mathbf{X}_{(res)S}$, and $\mathbf{X}_{(res)E}$ in view of the second step. Step 2: RDA and partial RDA to partition the residual variation of zooplankton among the spatial and environmental variables.



tion, which is the sampling design in the present study. In the most extreme case, it would account for all the variation of the response variables, leaving nothing to be explained by our ecological hypotheses.

The first step of the analysis was to find the ANCOVA residuals representing the variation unexplained by the factors of the sampling design for each zooplankton size-class variable, thus eliminating the fraction (T + TE + TS + TSE) of Fig. 2*b*. The table of residuals was constructed (Fig. 3) and became the new matrix of response variables for the second step of the analysis. Before proceeding with the variance partitioning, it was necessary to also eliminate the effect of the sampling design from the deterministic components. An ANCOVA against the sampling design factors was carried out for each explanatory variable, and residual values were computed; they were assembled into tables of residuals of the environmental and spatial variables. In the second step of the anal-

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Table 2. Summary of analyses for partitioning the measured variation to obtain fractions (T), (S), (E), (SE), (TE), (TS), and (TSE) where (T), (S), and (E) are sampling design, spatial, and environmental components, respectively.

Type of analysis	Matrix of explanatory variables	Matrix of covariables	Measured fraction
RDA (1)	Sampling design		(T + TE + TS + TSE)
Partial RDA (2)	Environmental (forward selection)	Sampling design	(E + SE)
Partial RDA (3)	Spatial (forward selection)	Sampling design	(S + SE)
Partial RDA	Environmental and spatial	Sampling design	(S + SE + E)
Partial RDA	Sampling design and spatial	Environmental	(T + TS + S)
Partial RDA	Sampling design and environmental	Spatial	(T + TE + E)
Partial RDA	Sampling design	Environmental and spatial	(T)
Partial RDA	Environmental	Sampling design and spatial	(E)
Partial RDA	Spatial	Sampling design and environmental	(S)

Note: Fractions (T), (S), and (E) are obtained directly from the analyses, whereas fractions (SE), (TE), (TS), and (TSE) are calculated by difference: (SE) = (S + SE) + (E + SE) – (S + SE + E), (TE) = (T + TE + E) – (T) – (E), (TS) = (T + TS + S) – (T) – (S), and (TSE) = (T + TE + TS + TSE) – (T) – (TE) – (TS) or (T + TE + TS + TSE) – (T + TE + E) – (T + TS + S) + (T) + (E) + (S). The first three steps are carried out in the order indicated (1, 2, 3), including a forward-selection procedure for steps 2 and 3. The sampling design, environmental, and spatial factors are used as explanatory variables and covariables, respectively, in the RDA and partial RDA runs.

ysis (Fig. 3), these new matrices were used as “environmental variables” in RDA and as “covariables” in partial RDA, allowing partitioning of the residual variation of zooplankton among the environmental and spatial components. Steps 1 and 2, as described here and in Fig. 3, form method 1.

RDA was used to verify the results obtained by ANCOVA and the stacked univariate *F* statistic. The factors describing the sampling design in the ANCOVA were encoded and formed the matrix of explanatory variables in RDA. The qualitative factors Day and Boat were binary coded (–1, 1); the number of dummy variables necessary to code for a factor is equal to the number of levels minus 1. The quantitative factor Hour (i.e., the covariable of the ANCOVA) was not recoded. The interaction terms were obtained by multiplying the dummy variables coding for the factors involved in the interaction. The number of dummy variables necessary to code for a factor or an interaction term is equal to the number of its degrees of freedom. We obtained the fraction (T + TE + TS + TSE) of the variation explained by the sampling design factors and compared it with the results of the combination of ANCOVA and the stacked univariate *F* statistic. We also used the matrix of sampling design factors as the matrix of covariables in partial RDA runs, in which the environmental and spatial variables, in turn, formed the matrices of explanatory variables. This procedure, called method 2, allowed us to verify the selection of environmental and spatial variables made by the variation partitioning method after controlling for the effect of the sampling design and to quantify the variation generated exclusively by the sampling design (i.e., fraction (T)). Using these results, all the fractions of variation for the available variables could now be determined: (T), (TE), (TS), (TSE), (S), (SE), and (E) (see Table 2). Tests of equality of variances (Sokal and Rohlf 1995) were computed to compare the variances among the four size-classes in each stratum, the variances among the three strata for the total biomass of zooplankton, and the variances among size-classes for the three strata using stacked univariate *F* statistics. The probabilities were examined after Bonferroni correction (Legendre and Legendre 1998).

Practical aspects of the statistical analyses

Before undertaking the analyses, histograms of frequency distributions were produced for all measured variables; a suitable normalisation was calculated for all variables that were not normally or symmetrically distributed using the Kolmogorov–Smirnov test of normality (using the tables corrected by Lilliefors 1967). All phytoplankton and zooplankton variables were normalised by logarithmic transformation, log(100*x*); the constant 100 was used in order to prevent the appearance of negative transformed values. The transformed data were used in all subsequent statistical analyses.

The matrix of response variables contained the transformed biomasses of the four zooplankton size-classes. The matrix of spatial variables was constructed using a polynomial of the Cartesian coordinates (*X*, *Y*) of the sites, as suggested by Legendre (1990). The forward-selection procedure of program CANOCO was used to select the terms of the geographic polynomial that significantly contributed to the explanation of the zooplankton size-classes. In all analyses, the polynomial of degree 4, made of 14 spatial monomials, was used as the starting point of the backward-selection procedure.

Before selecting the environmental variables (abiotic and biotic) to be used in RDA and partial RDA, it was necessary to establish the type of relationship found between the response and explanatory variables. Pearson correlation coefficients combined with scatter diagrams allowed us to determine if the relationships between variables of the two groups were linear; in the case of nonlinear relationships, a polynomial of the environmental variables (containing the centred variables to the powers 1 and 2) was used. Following the forward selection of variables, using in each case the power(s) explaining the largest amount of variation, 20 physical and chemical variables and two morphometric variables were retained to form the matrix of abiotic variables.

While depth is designated as a morphometric variable, it may also be an indicator (proxy variable) for the spatial distribution of fish in the lake; this biological variable is pertinent when studying the spatial distribution of zooplankton size-classes. Fish echolocation data obtained by echosounder in Lake Cromwell during the summer of 1994 (Gaudreau and Boisclair 1998) showed a spatial distribution of fish with low abundances of fish in the pelagic zone and high abundances in the littoral zone.

The matrix of biotic variables contained two size-classes of phytoplankton as well as the density and mean length of *Chaoborus* larvae. The size fraction <20 µm represents the edible algae, an important food resource for zooplankton. Depletion of edible algae biomass is generally related to strong zooplankton grazing (e.g., Angeli et al. 1995). The size fraction >20 µm represents the inedible algae; in this fraction, filamentous cyanobacteria may inhibit zooplankton grazing by mechanical interference or toxic effect (Ghadouani et al. 1998). Invertebrate predation by chaoborid larvae zooplankton is also an important factor controlling the zooplankton biomass, especially in humid or fishless lakes (Masson and Pinel-Alloul 1998). *Chaoborus* predation on zoo-

Table 3. ANCOVA tables to test the hypothesis *H*(0.1) of no significant effect of the sampling design on the zooplankton spatial structure for all dependent variables (i.e., the four size-classes of zooplankton), defining a general model for each stratum. MS = mean square.

General model of zooplankton per stratum	Source of variation	df	Type III SS	MS	*F* statistic
Epilimnion	Day	1	1.3416	1.3416	0.7544 ns
	Boat	1	2.3996	2.3996	1.1221 ns
	Hour	1	0.0768	0.0768	0.4717 ns
	Hour × Day	1	1.7783	1.7783	10.9232**
	Hour × Boat	1	2.1384	2.1384	13.1351***
	Model	5	5.3745	1.0749	6.6026***
	Residual	44	7.1646	0.1628	
	Total	49	12.5391		
Metalimnion	Boat	1	5.7073	5.7073	20.0820***
	Model	1	5.7073	5.7073	20.0820***
	Residual	34	9.6637	0.2842	
	Total	35	15.3709		

Note: Significant *F* statistics are coded as follows: $***p \le 0.001$; $**p \le 0.01$; ns, not significant.

plankton appears to be controlled by both density and size of predators (e.g., Pinel-Alloul 1995).

The limnetic strata were coded in the form of two (–1, 1) dummy variables; these variables, as well as their interactions with the *X* and *Y* spatial coordinates, were included among the spatial variables in the study of the vertical spatial distribution of zooplankton. These dummy variables were considered to represent a physical forcing variable, since stratification of the water column is generated by temperature and may induce spatial heterogeneity of the zooplankton (Richerson et al. 1978); if this was the case, we expected a strong correlation between temperature and the dummy "strata" variables. The limnetic strata may also been considered as representing spatial variables, since they code for the coordinates of the sampling points along the vertical axis. We also included in the analysis the interactions between the spatial monomials describing the horizontal distribution of the sites and the vertical limnetic strata.

To select the explanatory variables (environmental and spatial) that significantly explained the ecological variation of zooplankton and to partition this variation, we used the forward-selection procedure available in the program CANOCO for RDA. Environmental variables were selected independently of the spatial variables. The selected variables determined the fractions (E + SE) and (S + SE), respectively. We then analysed all selected variables together to determine the fraction (S + SE + E). Using the three quantities (E + SE), (S + SE), and (S + SE + E), the fractions (S), (SE), and (E) were obtained; note that fractions (E) and (S) could also have been obtained directly using partial RDA (Borcard and Legendre 1994). The contribution of each selected variable and that of the whole set of variables were tested by permutation under the full model (ter Braak 1990). The statistical analyses were first carried out along the horizontal axes of the lake (i.e., for the epi-, meta-, and hypolimnion separately) and then along the vertical axis.

ANOVAs and ANCOVAs were carried out using Super-ANOVA™ version 1.11. The program CANOCO™ version 3.11 (ter Braak 1990) was used for the RDA and partial RDA.

Results

Is there a significant effect of the sampling design on the detected spatial structure of the zooplankton size-class data?

ANCOVA was carried out to test the null hypothesis *H*(0.1) of no significant effect of the sampling design on the spatial structure of the zooplankton size-class data along the horizontal axis, i.e., in the epi-, meta-, and hypolimnion separately, and vertically, i.e., across the three strata.

Horizontal spatial structure

The null hypothesis was rejected for the epi- and metalimnion but not for the hypolimnion. As the null hypothesis *H*(0.1) could not be rejected for the hypolimnion, the null hypothesis *H*(0.2) corresponding to hypothesis *H*(1.2) that the abiotic factors play a dominant role in determining the horizontal spatial variation of zooplankton was tested using the method of partition of ecological variation of Borcard et al. (1992) (see below).

As a significant effect of the sampling design is identified for the epi- and metalimnion, the variation of the zooplankton size-class data was analysed considering the sampling design component. For these two strata, the general models established after ANCOVA and the stacked univariate *F* statistics show a significant variation generated by the sampling design variables; the coefficients of determination (R^2) were 0.4286 ($p < 0.001$) for the epilimnion and 0.3713 ($p < 0.001$) for the metalimnion (Table 3).

In the epilimnion, the variation generated by the sampling design variables was attributed in great part to the interaction terms Hour × Day and Hour × Boat ($R^2 = 0.1418$, $p < 0.01$ and $R^2 = 0.1705$, $p < 0.001$, respectively). This indicated that factors Hour and Day on the one hand and Hour and Boat on the other hand were not independent and that the biomass of zooplankton changed as a function of these effects. For each first-order interaction term, two different effects were found: Hour × Day 1 and Hour × Day 2 for the interaction Hour × Day and Hour × Boat 1 and Hour × Boat 2 for the interaction Hour × Boat; for each effect, the zooplankton biomass was significantly different. The factor Boat is the only factor that generates significant variation in the metalimnion; it amounts to 37.13% ($p < 0.001$) of the total variation measured in this stratum.

Each size-class of epi- and metalimnetic zooplankton is significantly influenced by the sampling design variables (Tables 4 and 5). In the epilimnion, the particular models of ANCOVA are similar to the general ANCOVA model, ex-

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Table 4. Particular models (ANCOVA tables) for each size-class of epilimnetic zooplankton to test the hypothesis *H*(0.1) of no significant effect of the sampling design on the zooplankton spatial structure. MS = mean square.

Dependent variable	Source of variation	df	Type III SS	MS	*F* statistic
Zooplanton >500 µm	Day	1	0.1116	0.1116	0.5018 ns
	Boat	1	0.5657	0.5657	1.2419 ns
	Hour	1	0.0001	0.0001	0.0026 ns
	Hour × Day	1	0.2224	0.2224	5.7172*
	Hour × Boat	1	0.4555	0.4555	11.7095***
	Model	5	1.1021	0.2204	5.6658***
	Residual	44	1.7129	0.0389	
	Total	49	2.8150		
Zooplanton 202–500 µm	Day	1	0.8043	0.8043	0.8336 ns
	Boat	1	1.4002	1.4002	1.0168 ns
	Hour	1	0.0004	0.0004	0.0056 ns
	Hour × Day	1	0.9648	0.9648	13.5887***
	Hour × Boat	1	1.3771	1.3771	19.3958***
	Model	5	2.7231	0.5446	7.6704***
	Residual	44	3.1242	0.0710	
	Total	49	5.8474		
Zooplanton 100–202 µm	Day	1	0.3637	0.3637	0.8126 ns
	Boat	1	0.2221	0.2221	7.2238**
	Hour	1	0.0100	0.0100	0.3249 ns
	Hour × Day	1	0.4476	0.4476	14.5602***
	Model	4	0.7071	0.1768	5.7508***
	Residual	45	1.3833	0.0307	
	Total	49	2.0904		
Zooplanton 53–100 µm	Day	1	0.0980	0.0980	0.5054 ns
	Boat	1	0.2563	0.2563	1.1971 ns
	Hour	1	0.0526	0.0526	2.2383 ns
	Hour × Day	1	0.1939	0.1939	8.2511**
	Hour × Boat	1	0.2141	0.2141	9.1106**
	Model	5	0.7504	0.1501	6.3872***
	Residual	44	1.0359	0.0235	
	Total	49	1.7863		

Note: Significant *F* statistics are coded as follows: ***$p \le 0.001$; **$p \le 0.01$; *$p \le 0.05$; ns, not significant.

Table 5. Particular models (ANCOVA tables) for the size classes of metalimnetic zooplankton.

Dependent variable	Source of variation	df	Type III SS	MS	*F* statistic
Zooplanton >500 µm	Boat	1	0.9545	0.9545	20.9780***
	Residual	34	1.5480	0.0455	
	Total	35	2.5025		
Zooplanton 202–500 µm	Boat	1	1.7877	1.7877	22.6291***
	Residual	34	2.6844	0.0790	
	Total	35	4.4721		
Zooplanton 100–202 µm	Boat	1	1.5769	1.5769	16.2567***
	Residual	34	3.2985	0.0970	
	Total	35	4.8754		
Zooplanton 53–100 µm	Boat	1	1.3882	1.3882	22.1404***
	Residual	34	2.1328	0.0627	
	Total	35	3.5209		

Note: Significant *F* statistics are coded as follows: ***$p \le 0.001$; ns, not significant.

cept for size-class 100–202 µm for which the variation is mostly due to the Hour × Day interaction and to the factor Boat ($R^2 = 0.2141$, $p < 0.001$ and $R^2 = 0.1062$, $p < 0.01$, respectively). For the other three size-classes, the first-order interaction Hour × Boat explains the largest fraction of the variation (from 11.99% for size-class 53–100 µm to 23.55% for size-class 202–500 µm) followed by the interaction Hour × Day (from 7.90% for size-class >500 µm to 16.50% for size-class 202–500 µm). The sampling design variation explains from 33.83% of the variance for size-class 100–

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Table 6. Partitioning of the variation measured for each stratum and vertically across the three strata.

	Epilimnion		Metalimnion			
Partition of the variation	Method 1	Method 2	Method 1	Method 2	Hypolimnion	Vertical axis
Total variance	0.2559	0.2559	0.4392	0.4393	0.2834	0.3615
% explained	69.15	69.04	71.55	71.55	75.84	73.36
	0.001	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*
(T + TE + TS + TSE)	42.86	42.85	37.13	37.14	na	na
	0.001	*0.001*	*0.001*	*0.001*		
(T)	na	33.39	na	0.42	na	na
		0.001		*0.650*		
(E)	21.89	21.79	28.32	28.32	49.58	8.77
	0.001	*0.001*	*0.001*	*0.001*	*0.001*	*0.121*
(S)	0.43	0.40	3.85	3.84	6.84	11.39
	0.560	*0.560*	*0.046*	*0.027*	*0.119*	*0.049*
(SE)	3.97	4.00	2.25	2.25	19.42	53.20
	0.002	na	*0.005*	na	*0.001*	*0.001*
(TE)	na	3.73	na	11.51	na	na
(TS)	na	12.24	na	–0.04	na	na
(TSE)	na	–6.51	na	25.25	na	na
% unexplained	30.85	30.96	28.45	28.45	24.16	26.64

Note: The variation explained by the fractions (expressed as a percentage; probabilities obtained after 999 permutations are in italics) is indicated for methods 1 and 2 for the epi- and metalimnion. The fractions of variation of the hypolimnion and the vertical axis are obtained directly using the variation partitioning method (Borcard et al. 1992). na, not applicable.

202 μm to 46.57% for size-class 202–500 μm; these fractions are very highly significant ($p < 0.001$) (Table 4). Note that the total variance of size-class 202–500 μm is much higher than that of the other three size-classes (tests of equality of variances, $p < 0.006$). The model for each size-class of metalimnetic zooplankton is the same as the general ANCOVA model. The factor Boat explains from 32.34% of the variance for size-class 100–202 μm to 39.97% for size-class 202–500 μm (Table 5); neither the total variances nor the sampling design variances differ significantly among size-classes. The analyses show that more than 37% of the total measured variation in the epi- and metalimnion is explained by the sampling design. The differences between the total variances and between the sampling design variances of the two strata are not significant.

Vertical spatial structure

The hypothesis $H(0.1)$ of no significant effect of the sampling design on the spatial structure of the zooplankton size-class data was not rejected for the vertical axis, so the null hypothesis $H(0.3)$ corresponding to hypothesis $H(1.3)$ that a combination of abiotic and biotic factors is necessary to explain the vertical spatial variation of zooplankton was tested using the method of partition of ecological variation of Borcard et al. (1992) (see below).

Impact of the sampling design on the analysis of the zooplankton spatial structure

Hypothesis $H(0.1)$ was rejected for the epi- and metalimnion; the zooplankton data variation was then analysed after controlling for the effect of the sampling design variables. Hypothesis $H(0.2)$ corresponding to hypothesis $H(1.2)$ that the abiotic factors play a dominant role in determining the horizontal spatial variation of zooplankton was tested for both strata using method 1, which combines the ANCOVA, stacked univariate F statistic, and the variation partitioning method (Table 6).

The small differences between the results obtained from methods 1 and 2 for the epi- and metalimnion are caused by the stacked univariate F statistic. It does not take into account the covariances between the size-classes of zooplankton, whereas RDA and partial RDA used in method 2 take these covariances into account. However, the spatial and environmental variables selected by the two methods are the same as well as the fractions (T + TE + TS + TSE), (E), (S), (SE), and (Un) and their probabilities.

In the epi- and metalimnion, the variation after controlling for the sampling design effect (i.e., elimination of fraction (T + TE + TS + TSE)) is explained mainly by the spatially unstructured environmental fraction (E), indicating that the influence of these variables remains the same independently of the positions of the sites. Whereas the unexplained spatial variation, fraction (S), is not significant in the epilimnion and is slight in the metalimnion, a small significant fraction of variation is explained by the spatially structured component of the environmental variables, fraction (SE) (Fig. 4). Therefore, in both strata, the spatial component of the zooplankton variation is slight (epilimnion: (S + SE) = 4.40%, $p = 0.03$; metalimnion: (S + SE) = 6.10%, $p = 0.012$).

If we consider the partitioning of (T + TE + TS + TSE) into fractions (T), (TE), (TS), and (TSE) obtained by method 2, we observe that the partitioning of this fraction is not the same in the two strata (Table 6). In the epilimnion, fraction (T) explains 33.39% ($p = 0.001$) of the total variation, whereas this fraction is 0.42% and not significant in the metalimnion; therefore, the variation generated exclusively by the sampling design (i.e., the factors Day, Boat, and Hour and theirs interactions) is important in the upper layer and nonexistent in the other strata. However, fraction (TSE) is high in the metalimnion (25.25%) as well as fraction (TE), which explains 11.51% of the total variation; this means that

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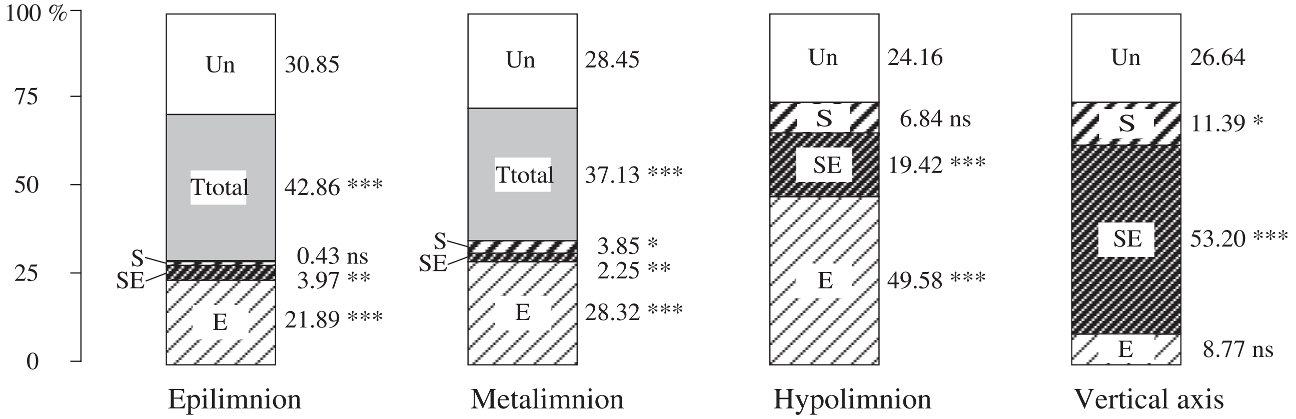
Fig. 4. Partitioning of the variation in the epi-, meta-, and hypolimnion and along the vertical axis. Ttotal represents all components of the sampling design, i.e., (T + TE + TS + TSE). Significant fractions are coded as follows: ***$p \le 0.001$; **$p \le 0.01$; *$p \le 0.05$; ns, not significant.



part of the observed environmental variation explained by the environmental variables is influenced by the factor Boat, which dominates the ANCOVA in this stratum (Table 3). These fractions are small in the epilimnion, even negative in the case of (TSE); fraction (TS) explains 12.24% of the total variation, meaning that part of the spatial heterogeneity of the zooplankton is explained by the sampling design factors. Fractions (TS) in the metalimnion and (TSE) in the epilimnion, which are obtained by subtraction (see above) and not by estimating an explicit parameter, are negative. A negative fraction, e.g., (TS) in the metalimnion, indicates that the spatial and sampling design variables, taken together, explain the response variables (i.e., the four size-classes of zooplankton) better than the sum of the individual effects of these factors. This is due to the fact that one or the other (or both) of these groups of explanatory variables has a positive as well as a negative effect on the response variables, one of them being direct and the other indirect (Legendre and Legendre 1998).

These results show that the sampling design generated a high amount of variation in the epilimnion zooplankton, the other important part of the variation being explained exclusively by the environmental variables. In the metalimnion, the measured variation was largely environmental, but the factor Boat generated some environmental and spatially structured environmental variation.

Spatial structure of the zooplankton size-class data

The results obtained from method 1 were verified using method 2; both sets of results are presented in Table 6. For the horizontal and vertical axes, the total variation explained by each model is higher than 69% and is always highly significant ($p < 0.001$).

Horizontal spatial structure

Tests of equality of variances showed that the “total variances” of the three horizontal layers are not significantly different. The “ecological variance” (i.e., fractions (E) and (SE)) was, however, smaller in the epilimnion (mean square (MS) = 0.0670) than in the other analyses (metalimnion: MS = 0.1512, comparison with the epilimnion: $p < 0.01$; hypolimnion: MS = 0.2149, comparison with the epilimnion: $p < 0.01$; results not shown in the tables). However, the difference between the meta- and hypolimnion is not significant.

The spatial heterogeneity of the zooplankton (i.e., fraction (S + SE)) is slight in the horizontal strata, except in the hypolimnion (Fig. 4). The epilimnetic spatial variance (fraction (S + SE): MS = 0.0113 for the epilimnion) is smaller than in the metalimnion (MS = 0.0268, comparison with the epilimnion: $p < 0.01$) and hypolimnion (MS = 0.0744, comparison with the epilimnion: $p < 0.001$), whereas the meta- and hypolimnion show no significant difference. The “pure” spatial variation is small and not significant in the epilimnion and small and marginally significant in the metalimnion; this may indicate that the processes that generate spatial heterogeneity are well captured by the other variables included in the analysis for these two strata. The environmental variables (E) provide the main explanation for the zooplankton variation; they explain 49.58% ($p < 0.001$) of the hypolimnetic variation. In the upper strata, the environmental variation (E) is higher than 20% ($p < 0.001$).

Vertical spatial structure

The spatial heterogeneity of the zooplankton is highest (fraction (S + SE) = 64.59%, $p < 0.001$) in the vertical analysis across the three strata (Fig. 4); the dummy variables coding for strata, as well as their interactions with the X and Y spatial coordinates, were included among the spatial variables coding for this analysis. Part of the spatial heterogeneity, brought out by the spatial polynomial of the geographic (X, Y) coordinates, is significantly explained by the environmental variables (fraction (SE) = 53.20%, $p < 0.001$). The pure environmental variation (E) is not significant and the pure spatial variation (S) is 11.39% ($p = 0.049$), indicating that some variables generating spatial heterogeneity have not been included in the analysis. Along the vertical axis, the total variance of size-class 202–500 µm (MS = 0.1521) is significantly higher than that of size-classes >500 µm (MS = 0.0680, comparison with 202–500 µm: $p < 0.01$) and 53–100 µm (MS = 0.0676, comparison with 202–500 µm: $p < 0.01$).

Role of the environmental factors in determining the spatial variation of the zooplankton

Hypotheses $H(0.2)$ and $H(0.3)$, corresponding to hypothesis *H*(1.2) that the abiotic factors play a dominant role in determining the horizontal spatial variation of zooplankton, and hypothesis *H*(1.3) that a combination of abiotic and biotic factors is necessary to explain the vertical spatial variation of zooplankton, have been tested as a byproduct of the variation partitioning (Table 6). The significant spatial and environmental variables selected during forward selection are presented in Table 7, together with the percentage of the ecological variation that they explain. The order of the variables for each deterministic component in the table is that of their selection by the forward-selection procedure, which was done independently in each group of variables (Table 2).

The description of the zooplankton distribution and the detailed interpretation of the environmental variables explaining zooplankton variation will be the subject of another paper.

Were the abiotic factors playing a dominant role in determining the horizontal spatial variation of zooplankton?

The horizontal spatial heterogeneity of the zooplankton is characterised only by the Cartesian coordinate X of the sites; this variable describes the zooplankton distribution along the east–west axis of the lake. The relationships are hyperbolic in the epi- and hypolimnion (X^4 and X^2, respectively) and sinusoidal in the metalimnion (X^3) (Table 7). We know from Table 6, however, that in each stratum, zooplankton variation is explained mainly by the nonspatially structured component (E) of the environmental variables, and we observe that the abiotic variables are those that explain the highest amount of environmental variation: (Depth)^2 in the epilimnion (considering maximum depth of the sampling stations as a physical variable), (Temperature)^2 in the metalimnion, and (DSI)^2 in the hypolimnion. Some biotic variables also influence the structure of the zooplankton: chlorophyll *a* of phytoplankton >20 µm in the epilimnion, Depth and (Depth)^2 in the epi- and metalimnion (now considering these variables as indirect indicators of the spatial distribution of fish), and the density of *Chaoborus* larvae in the deepest stratum. The abiotic variables play a predominant role in explaining the spatial variation of the zooplankton, but zooplankton is influenced by a combination of abiotic and biotic variables.

Was a combination of abiotic and biotic necessary to explain the vertical spatial variation of zooplankton?

The vertical spatial heterogeneity of zooplankton is explained by the dummy variables describing the strata and by various multiplicative terms between the Cartesian coordinate X and the hypolimnetic stratum (H) characterising simultaneously the vertical and horizontal axes (Table 7). No biotic variables were selected during the forward-selection procedure. This implies that the biotic factors measured during our study do not significantly explain the vertical heterogeneity of zooplankton. So no biotic variable was accepted in the model. On the other hand, a great part of the zooplankton heterogeneity is explained by the abiotic variables. Therefore, hypothesis *H*(0.3) that a combination of abiotic and biotic factors is not necessary to explain the vertical spatial variation of zooplankton cannot be rejected. The vertical spatial variation of the zooplankton is influenced only by the vertical gradient in temperature, oxygen, turbidity, pH, and (DOC)^2.

Table 7. Sampling design, spatial, and environmental variables contributing significantly to the explanation of the variation measured in the epi-, meta-, and hypolimnion and along the vertical axis.

Deterministic component	Explanatory variable	Explained variation
Epilimnion		
Sampling design	ANCOVA model	0.43***
Spatial	X^4	0.04**
Environmental	(Depth)^2	0.10***
	pH	0.05**
	(Alkalinity)^2	0.05**
	Chloro. >20 µm	0.03*
	Conductivity	0.03*
Metalimnion		
Sampling design	ANCOVA model	0.37***
Spatial	X^3	0.06*
Environmental	(Temperature)^2	0.11**
	Depth	0.05*
	DOC	0.07**
	(Depth)^2	0.08**
Hypolimnion		
Spatial	X^2	0.26*
Environmental	(DSI)^2	0.45**
	Chaoborus density	0.24***
Vertical axis		
Spatial	Stratum E	0.03***
	X^4H	0.14**
	XH	0.08*
	X^2H	0.07*
Environmental	Oxygen	0.28**
	Turbidity	0.18***
	pH	0.05*
	(DOC)^2	0.05*
	Temperature	0.06*

Note: ANCOVA model refers to the general models in Table 5. X^a represents site coordinates to the power *a*; Chloro. >20 µm, chlorophyll *a* concentration of phytoplankton >20 µm; E, epilimnion; H, hypolimnion. Probabilities were obtained after 999 permutations; significant fractions are coded as follows: ***$p \le 0.001$; **$p \le 0.01$; *$p \le 0.05$; ns, not significant. The percent explained variation is the conditional effect, i.e., after considering the other variables selected in the model.

Discussion

Variation generated by the sampling design

The variation generated by the sampling design in the epi- and metalimnion may be related to the number of sites; 50 and 36 sites were sampled in the epi- and metalimnion, respectively, whereas there were only 13 sites in the hypolimnion and along the vertical axis. Since the sites were not sampled simultaneously, increasing the number of sites may have caused temporal variation characterised by the factors Day and Hour. The factor Boat may have generated a further spatial artefact caused by the route of the boats visiting the sampling sites in the given sequence (Ibanez 1973). If this is the case, the factor Boat is more like a spatial sampling de-

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sign factor than temporal, and the greater the number of sampled sites, the greater the spatial artefact should be. Patalas and Salki (1993) indicated that the spatial structure of organisms depended on the number of sites sampled in a lake; indeed, more sites sampled in the same lake involves a more accurate measure of the variability. Other authors add that spatial heterogeneity increases rapidly with the extent of the sampling programme (e.g., Patalas 1990); this supports the idea that the design variation generated during sampling may depend on the number of sites. If one cannot reduce the design variation through the sampling strategy, it must be taken into account during the statistical analysis.

The sampling design variation in the metalimnion is explained only by the factor Boat. This may indicate that this part of the variation is a spatial artefact which can explain the importance of fractions (TE), (TS), and (TSE) and the small importance of the pure sampling design fraction. The Boat effect is confounded with the spatial structure described by the spatial variables (fractions (TS) and (TSE)), and it explains environmental variation that is spatially unstructured (i.e., unexplained by the spatial variables), but it remains correlated with the spatial artefact generated by this factor (TE). Moreover, the factor Boat has a positive as well as a negative effect on the response variables; since the correlation is positive with dummy variables Boat 1 and negative with Boat 2 (result not shown in the tables), this might explain the negative fraction (TS). Taking depth-integrated hauls has eliminated vertical heterogeneity, which can vary over short time periods as a result of vertical migrations (Colebrook 1960); this could explain in part the fact that the metalimnetic zooplankton did not show significant temporal variation, so that fraction (T) is small and not significant. The same sampling method has been used in the epilimnion; however, the total sampling design variation is explained by the interaction terms Hour × Day and Hour × Boat that may characterise not only the spatial artefact but also a temporal variation that could explain the importance of fraction (T) compared with the other fractions. The horizontal temporal variation may be explained by the predation pressure from the fish and (or) the *Chaoborus* larvae and (or) by the source of food. However, only chlorophyll *a* of phytoplankton >20 μm is correlated with the sampling design factors (i.e., interaction term Hour × Boat: $r = 0.594$, $p < 0.0001$). This may explain fraction (TE), but it would indicate, in this case, that the horizontal temporal variation is generated by other variables that have not been included in the study.

Zooplankters are able to accomplish diurnal horizontal migrations (Davies 1985), although they have little directional movements horizontally beyond a few metres, whereas they can move by more than 10 m vertically in response to diel changes in light levels or food. The wind-induced currents may also generate temporal variation; Pinel-Alloul and Pont (1991) indicated a correlation between wind direction and the spatial distribution of macrozooplankton in Lake Cromwell. To affect the spatial heterogeneity of zooplankton, the windward must be regular. The dominant wind was mostly from the southwest during the 10 days prior to sampling, but the day before sampling, the wind was from the northeast (9 km·h^{-1}). During the sampling days, it turned southwest again. This could explain the greater concentration of zooplankton in the central part of the lake when the wind was from the north-northeast. Together with the swimming capabilities of the species (Steele 1978), the wind direction change may explain the temporal variation in the epilimnion (i.e., fraction (T)) as well as fraction (TS), which characterised the spatiotemporal variation of the zooplankton. However, since Lake Cromwell is small and sheltered by forest, the changes in wind direction possibly had negligible effects on the distribution of zooplankton. In that case, the sampling design variation would be a complete artefact, since the sampling sites were not the same during the two days and the number of sites sampled in the epilimnion was higher than in the metalimnion. This may have generated a temporal artefact, which is not found in the metalimnion because of the smaller number of sites visited. So the effect of the factor Boat would increase more rapidly with the number of sites sampled than the effects of the factors Day and (or) Hour.

Some studies on the distribution of zooplankton have been carried out at broad temporal and spatial scales (e.g., Gaudy et al. 1995; Masson and Pinel-Alloul 1998). However, in many studies on the spatial structure of organisms where sampling was carried out over a few hours or days, spatial variation analysis has not taken the temporal effect into account because daily and short-term variations were considered negligible (e.g., Gaudy et al. 1995; Lacroix and Lescher-Moutoué 1995). Previous studies in Lake Cromwell showed that the sampling date was not a significant predictor of the spatial heterogeneity of zooplankton, suggesting no short-term effect. On the contrary, the spatial heterogeneity along the vertical axis was found to be related to the hour of sampling (Pinel-Alloul and Pont 1991). However, none of these studies took into account the route of the boat or the interaction that could exist between the factors of the sampling design, and the sampling did not cover the whole lake, since it was carried out in a delimited rectangular area of the lake or along a transect (e.g., Pinel-Alloul and Pont 1991).

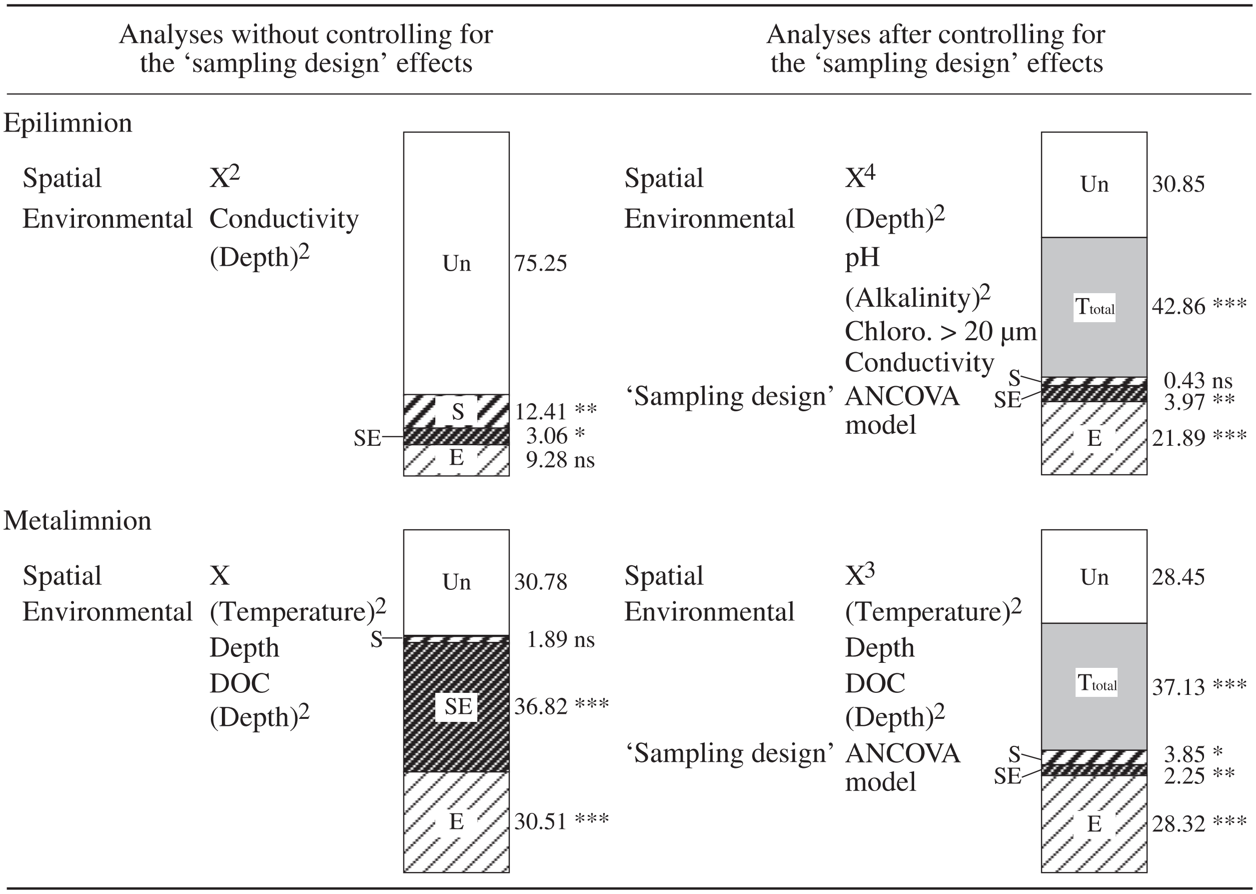
Impact of the sampling design on the detected zooplankton spatial structure

If we compare the analyses without and with control for the sampling design effect (Fig. 5), we find that the partition of the measured variation is not the same in the two analyses; nor are the same explanatory variables selected by the forward-selection procedure.

Epilimnetic stratum

If we had studied the epilimnetic zooplankton variation without controlling for the sampling design, we would have concluded that 75.25% of the measured variation remains unexplained by the environmental and spatial variables, which would indicate that some important processes generating ecological variation have not been included in the study. However, considering the effect of the sampling design, 69.15% of the measured variation is explained by the variables selected. The decrease in unexplained variation demonstrates the importance of the variation generated exclusively by the sampling design (i.e., fraction (T)), showing that fraction (S + SE + E), which represents the variation explained by the spatial and environmental variables, does not change in any drastic way (fraction (S + SE + E) = 24.75 and 26.29%, without and after controlling for the sampling

Fig. 5. Sampling design affecting the analysis of the zooplankton variation. Sampling design, spatial, and environmental factors are the significant variables selected in the analyses. The measured variation represented by the fractions is expressed as a percentage. X^a represents site coordinates to the power a; Chloro. >20 µm, chlorophyll a concentration of phytoplankton >20 µm. Significant fractions are coded as follows: $^{***}p \leq 0.001$; $^{**}p \leq 0.01$; $^{*}p \leq 0.05$; ns, not significant.



design, respectively). This is not the case for the metalimnion (see below).

The pure environmental variation characterising the functional structure of the zooplankton (E) in the epilimnion, which is not significant in the analysis that did not control for the spatial design, now represents 21.89% of the total variation and is significant. The spatial structure of the zooplankton remains unchanged (significant spatial factor X^2 in the first analysis and X^4 in the second) but the environmental variables are not the same; without controlling for the sampling design, conductivity is the main variable explaining the measured variation. In the second model, on the contrary, the environmental variables corroborate most of the conclusions of the previous studies in Lake Cromwell (e.g., Pinel-Alloul and Pont 1991, see below).

Metalimnetic stratum

Contrary to the epilimnion, the total explained variance remains largely unchanged in the metalimnion, without or after controlling for the sampling design (69.22 and 71.55%, respectively). The pure spatial variation (S) as well as the unexplained (Un) and spatially unstructured (E) environmental variations are unchanged in the two analyses, whereas the ecological spatial variation ((SE): 2.25%, $p < 0.01$) is less important than in the analysis without controlling for the sampling design (36.82%, $p < 0.01$). It corroborates the spatial artefact caused by the factor Boat, which is confounded by the spatial structure of the zooplankton and the nonexistence of a pure sampling design variation (i.e., fraction (T)).

In both strata, controlling for the sampling design decreased the estimated amount of the variation which was attributed to the spatial structure of the zooplankton (SE + S), although the effects of the sampling design remain different in the two strata.

Variation of the zooplankton size-class data

Horizontal variation

In the horizontal strata, there are no significant differences in total variation (after controlling for the sampling design) among the four size-classes of zooplankton, except for size-class 202–500 µm (MS = 0.0638) in the epilimnion, which shows a total variation significantly higher than that of size-classes 100–202 µm (MS = 0.0282, comparison with 202–500 µm: $p < 0.01$) and 53–100 µm (MS = 0.0211, comparison with 202–500 µm: $p < 0.001$). The heterogeneity among size-classes of zooplankton for the three strata (using stacked univariate F statistics) differs in a significant way for size-class 202–500 µm (MS = 0.0687); its variation is higher than that of size-classes >500 µm (MS = 0.0409, comparison

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with 202–500 μm: $p < 0.01$) and 53–100 μm (MS = 0.0407, comparison with 202–500 μm: $p < 0.01$). The differences could be related to the trophic requirements and swimming capabilities of the species (Steele 1978) and to their patterns of vertical migration (Pinel-Alloul and Pont 1991). Body size has also been considered a specific component of plankton heterogeneity in Lake Cromwell when all zooplankton groups, from small rotifers to large copepods, are included in the analysis (Pinel-Alloul et al. 1988). This could also explain the importance of the sampling design variation of size-class 202–500 μm in the epilimnion, which is significantly higher than that of the other size-classes and would suggest that part of the sampling design variation is temporal variation inherent to this size-class rather than an artefact caused by the sampling.

Otherwise, how can one explain the fact that the sampling design effect on this size-class is greater than on the others size-classes? Adaptation specifically associated with horizontal spatial variation may be a major feature in some zooplankton species but not in others (Lewis 1978). Pinel-Alloul and Pont (1991) indicated that the heterogeneity among the macrozooplankton species of Lake Cromwell (which corresponded to sizes 430–1430 μm in their study) showed no significant differences on the horizontal axes and with depth over the horizontal axes, whereas a strong species effect was noted along the vertical axis. The differences between the two studies could be due to the range of body sizes of the organisms. In the same study, the authors pointed out that the maximum spatial heterogeneity in relation to population abundanceswas observed along the vertical and not the horizontal axis. However, a stronger aggregation was also observed in the hypolimnion for the total zooplankton whose spatial heterogeneity increased with depth (Pinel-Alloul et al. 1988). Surface turbulence and advective processes in surface waters may have had a randomising effect, whereas thermal stratification may have induced high spatial heterogeneity (Pinel-Alloul and Pont 1991). Moreover, the predation pressure by *Chaoborus* larvae may be higher in deeper waters, yielding aggregation as a predation-avoidance behaviour (e.g., Masson and Pinel-Alloul 1998).

The abiotic variables play an important role in the horizontal spatial variation of zooplankton, except in the epilimnion where the spatial distribution of the zooplankton is also influenced by phytoplankton >20 μm (e.g., Richerson et al. 1978). The ecological variation along the horizontal axes is generated by a combination of the abiotic and biotic variables. Planktivorous fish (*Notropis cornutus*, *Lepomis gibbosus*, and *Exoglossum maxillingua*) occur in greatest concentration in the littoral zone of Lake Cromwell (Pinel-Alloul and Pont 1991). Fish echo-location data obtained by echosounder in Lake Cromwell during the summer of 1994 showed a spatial distribution of fish from the littoral to the pelagic zone with low abundance of fish in the pelagic zone and high abundance in the littoral zone (Gaudreau and Boisclair 1998). The presence of planktivorous fish would explain the smaller spatial heterogeneity in the epilimnion, since the predation pressure seems to be an essential variable in the maintenance of horizontal patterns in zooplankton distributions (e.g., Gliwicz and Rybowska 1992). Two species of *Chaoborus* are present in Lake Cromwell: *Chaoborus flavicans* is more or less restricted to the littoral zone, whereas *Chaoborus punctipennis* inhabits the pelagic zone, where its highest density is 3374 individuals$\cdot$m^{-2} (Harper and Cloutier 1986). The *Chaoborus* larvae move deeper with age (Fedorenko and Swift 1972); that may explain the influence of *Chaoborus* larvae density on the zooplankton community in the hypolimnion.

Vertical variation

The vertical heterogeneity of the zooplankton data is explained exclusively by the abiotic variables and not by a combination of the abiotic and biotic variables, as was assumed by our hypothesis $H(1.3)$. All sites analysed along the vertical axis are pelagic and their number is small (13 sites) due to the morphology of the lake. The 26.64% unexplained vertical variation indicates that others biotic factors that were not considered during the study could explained the vertical heterogeneity of zooplankton. However, the environmental variables inducing environmental and spatial heterogeneity of the zooplankton are in agreement with the results of previous studies conducted in Lake Cromwell (Pinel-Alloul et al. 1988; Pinel-Alloul and Pont 1991). Our results support the idea that physical stratification is at the origin of the biological heterogeneity among strata (Richerson et al. 1978).

In conclusion, in ecological field studies, visiting prelocated sites in some random order (Ibanez 1973) or sampling all sites simultaneously are certainly the best sampling strategies, but they are utterly unrealistic for field studies. Any other sampling strategy is not random and introduces dependencies among the observations, which must be taken into account during the following phases of analysis and interpretation of the data. The magnitude of this effect depends, however, on the generation time or the ability of the organisms to move relative to the duration of the sampling campaign.

Most sampling designs through geographic space cannot guarantee a correct estimation of the spatial variation of a set of variables. We must always ask what is the real nature of the variation measured using our sampling design. The design may modify in a significant way the study of the spatial variation of a community and of the contribution of the environmental variables sampled concomitantly. The first step is to take into account a possible effect of the sampling design and thus not consider a priori the daily or short-term temporal variation as well as the route of the boats as negligible artefacts without testing this hypothesis. If a significant effect of the sampling design is found, one must control for it during the following analysis and interpretation of ecological variation.

If the effect of the sampling design is identified during a pilot study (Amanieu et al. 1989; Fortin et al. 1989), an alternative solution is to change the sampling strategy before the full-size study is undertaken. The measured variation depends, however, on the number of sites sampled as well as the duration of the sampling programme. In some research, a large sampling area may be required, so we may not want to limit the sites visited to some small number.

The traditional methods of sampling (Wisconsin plankton net, Clarke–Bumpus sampler, Schindler–Patalas trap) do not permit the filtration of large volumes of water and require an appreciable amount of time to sample a site. In the last two decades, four advanced technologies have been developed: acoustic devices, optical plankton counters, video systems, and laser fluorosensors. Although they have limitations, these sampling methods would permit researchers to rapidly cover a large lake surface area (for reviews, see Williamson et al. 1992; Pinel-Alloul 1995). Besides these high-technology methods, motorised pumps seem to be the best sampling gear for volumetric sampling. They permit the filtration of large, controlled volumes of water rapidly and can be manipulated easily in order to obtain integrated water samples covering the scale of a lake (e.g., Rahkola et al. 1994; Lacroix and Lescher-Moutoué 1995).

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References

Amanieu, M., Legendre, P., Troussellier, M., and Frisoni, G.-F. 1989. Le programme Écothau: théorie écologique et base de la modélisation. Oceanol. Acta, **12**: 189–199.

Angeli, N., Pinel-Alloul, B., Balvay, G., and Ménard, I. 1995. Diel patterns of feeding and vertical migration in daphnids and diaptomids during the clear water phase in Lake Geneva (France). Hydrobiologia, **300/301**: 163–184.

Bollens, S.M., and Frost, B.W. 1989. Predator-induced diel vertical migration in a planktonic copepod. J. Plankton Res. **11**: 1047–1065.

Borcard, D., and Legendre, P. 1994. Environmental control and spatial structure in ecological communities: an example using oribatid mites (Acari, Oribatei). Environ. Ecol. Stat. **1**: 37–61.

Borcard, D., Legendre, P., and Drapeau, P. 1992. Partialling out the spatial component of ecological variation. Ecology, **73**: 1045–1055.

Butorina, L.G. 1986. On the problem of aggregations of planktonic crustaceans [*Polyphemus pediculus* (L.), Cladocera]. Arch. Hydrobiol. **105**: 355–386.

Byron, E.R., Whitman, P.T., and Goldman, C.R. 1983. Observation on copepod swarms in Lake Takoe. Limnol. Oceanogr. **28**: 378–382.

Colebrook, J.M. 1960. Plankton and water movements in Lake Windermere. J. Anim. Ecol. **29**: 217–240.

Davies, J. 1985. Evidence for a diurnal horizontal migration in *Daphnia hyalina lacustris* Sars. Hydrobiologia, **120**: 103–105.

De Nie, H.W., Bromley, H.J., and Vijverberg, J. 1980. Distribution patterns of zooplankton in Tjeukemeer, The Netherlands. J. Plankton Res. **2**: 317–334.

Dutilleul, P. 1998. Incorporating scale in ecological experiments — data analysis. *In* Ecological scale. *Edited by* D.L. Peterson and V.T. Parker. Columbia University Press, New York. pp. 387–425.

Fedorenko, A.Y., and Swift, M.C. 1972. Comparative biology of *Chaoborus americanus* and *Chaoborus trivittatus* in Eunice Lake, British Columbia. Limnol. Oceanogr. **7**: 721–730.

Fortin, M.-J., Drapeau, P., and Legendre, P. 1989. Spatial autocorrelation and sampling design in plant ecology. Vegetatio, **83**: 209–222.

Gardner, R.H. 1998. Pattern, process, and the analysis of spatial scales. *In* Ecological scale. *Edited by* D.L. Peterson and V.T. Parker. Columbia University Press, New York. pp. 17–34.

Gaston, K.J., and McArdle, B.H. 1994. The temporal variability of animal abundances: measures, methods and patterns. Philos. Trans. R. Soc. Lond. Ser. B, Biol. Sci. **345**: 335–358.

Gaudreau, N., and Boisclair, D. 1998. The influence of spatial heterogeneity on the study of fish horizontal daily migration. Fish. Res. **35**: 65–73.

Gaudy, R., Verriopoulos, G., and Cervetto, G. 1995. Space and time distribution of zooplankton in a Mediterranean lagoon (Étang de Berre). Hydrobiologia, **300/301**: 219–236.

Ghadouani, A., Pinel-Alloul, B., Zhang, Y., and Prepas, E. 1998. Relationships between zooplankton community structure and phytoplankton in two lime-treated eutrophic hardwater lakes. Freshwater Biol. **39**: 775–790.

Gliwicz, Z.M., and Rybowska, A. 1992. ‘Shore avoidance’ in zooplankton: a predator-induced behavior or predator-induced mortality? J. Plankton Res. **14**: 1331–1342.

Haney, J.K., and Hall, D.J. 1975. Diel vertical migration and filter-feeding activities of *Daphnia*. Arch. Hydrobiol. **75**: 413–441.

Harper, B.P., and Cloutier, L. 1986. Spatial structure of the insect community of a small dimictic lake in the Laurentians (Québec). Int. Rev. Gesamten Hydrobiol. **71**: 655–685.

Herwig, B.R., and Schindler, D.E. 1996. Effects of aquatic insect predators on zooplankton in fishless ponds. Hydrobiologia, **324**: 141–147.

Ibanez, F. 1973. Méthode d’analyse spatio-temporelle du processus d’échantillonnage en planctonologie, son influence dans l’interprétation des données par l’analyse des composantes principales. Ann. Inst. Océanogr. **49**: 83–111.

Jordan, I.T., Ross, P.E., and Pinel-Alloul, B. 1988. Seasonal variations in phytoplankton size structure in Lake Cromwell (Laurentian Shield), Quebec. Hydrobiologia, **169**: 167–182.

Keller, W., and Yan, N.D. 1991. Recovery of crustacean zooplankton species richness in Sudbury area lakes following water quality improvements. Can. J. Fish. Aquat. Sci. **48**: 1635–1644.

Lacroix, G., and Lescher-Moutoué, F. 1995. Spatial patterns of planktonic microcrustaceans in a small shallow lake. Hydrobiologia, **300/301**: 205–217.

Lauridsen, T.L., and Buenk, I. 1996. Diel changes in the horizontal distribution of zooplankton in the littoral zone of two shallow eutrophic lakes. Arch. Hydrobiol. **137**: 161–176.

Legendre, P. 1990. Quantitative methods and biogeographical analysis. *In* Evolutionary biogeography of the marine algae of the North Atlantic. *Edited by* D.J.Garbary and R.G. South. NATO ASI Series. Vol. G 22. Springer-Verlag, Berlin. pp. 9–34.

Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology, **74**: 1659–1673.

Legendre, P., and Legendre, L. 1998. Numerical ecology. 2nd English ed. Elsevier Science Publishers BV, Amsterdam, The Netherlands.

Legendre, P., Thrush, S.F., Cummings, V.J., Dayton, P.K., Grant, J., Hewitt, J.E., Hines, A.H., McArdle, B.H., Pridmore, R.D., Schneider, D.C., Turner, S.J., Whitlatch, R.B., and Wilkinson, M.R. 1997. Spatial structure of bivalves in a sandflat: scale and generating processes. J. Exp. Mar. Biol. Ecol. **216**: 99–128.

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Lens, P.H., Cooper, S.D., Melack, J.M., and Winkler, D.W. 1986. Spatial and temporal patterns of three trophic levels in a saline lake. J. Plankton Res. **8**: 1051–1064.

Levin, S.A. 1992. The problem of pattern and scale in ecology. Ecology, **73**: 1943–1967.

Lewis, W.M., Jr. 1978. Comparison of temporal and spatial variation in the zooplankton of a lake by means of variance components. Ecology, **59**: 666–671.

Lilliefors, H.W. 1967. The Kolmogorov–Smirnov test for normality with mean and variance unknown. J. Am. Stat. Assoc. **62**: 399–402.

Malone, B.J., and McQueen, D.J. 1983. Horizontal patchiness in zooplankton populations in two Ontario kettle lakes. Hydrobiologia, **99**: 101–124.

Manca, M., de Bernandi, R., and Scavia, A. 1986. Light and temperature fluctuations on *Daphnia* dynamics. Mem. Ist. Ital. Idrobiol. **44**: 177–202.

Masson, S., and Pinel-Alloul, B. 1998. Spatial distribution of zooplankton biomass size fractions in a bog lake — abiotic and (or) biotic regulation. Can. J. Zool. **76**: 805–823.

Patalas, K. 1969. Composition and horizontal distribution of crustacean plankton in Lake Ontario. J. Fish. Res. Board Can. **26**: 2135–2164.

Patalas, K. 1990. Diversity of the zooplankton communities in Canadian lakes as a function of climate. Verh. Int. Ver. Limnol. **24**: 360–368.

Patalas, K., and Salki, A. 1992. Crustacean plankton in Lake Winnipeg: variation in space and time as a function of lake morphology, geology, and climate. Can. J. Fish. Aquat. Sci. **49**: 1035–1059.

Patalas, K., and Salki, A. 1993. Spatial variation of crustacean plankton in lakes of different size. Can. J. Fish. Aquat. Sci. **50**: 2626–2640.

Pinel-Alloul, B. 1995. Spatial heterogeneity as a multiscale characteristic of zooplankton community. Hydrobiologia, **300/301**: 17–42.

Pinel-Alloul, B., and Pont, D. 1991. Spatial distribution patterns in freshwater macrozooplankton: variation with scale. Can. J. Zool. **69**: 1557–1570.

Pinel-Alloul, B., Downing, J.A., Pérusse, M., and Codin-Blumer, G. 1988. Spatial heterogeneity in freshwater zooplankton: systematic variation with body size, depth and sampling scale. Ecology, **69**: 1393–1400.

Pinel-Alloul, B., Niyonsenga, T., and Legendre, P. 1995. Spatial and environmental components of freshwater zooplankton structure. Écoscience, **2**: 1–19.

Pinel-Alloul, B., Guay, C., Angeli, N., Legendre, P., Dutilleul, P., Balvay, G., Gerdeaux, D., and Guillard, J. 1999. Large-scale spatial heterogeneity of macrozooplankton in Lake of Geneva. Can. J. Fish. Aquat. Sci. **56**: 1437–1451.

Platt, T., and Harrison, W.G. 1985. Biogenic fluxes of carbon and oxygen in the ocean. Nature (Lond.), **318**: 55–58.

Rahkola, M., Karjalainen, J., and Viljanen, M. 1994. Evaluation of a pumping system for sampling zooplankton. J. Plankton Res. **16**: 905–910.

Rao, C.R. 1964. The use and interpretation of principal component analysis in applied research. Sankhya Ser. A, **26**: 329–358.

Rao, C.R. 1973. Linear statistical inference and its applications. 2nd ed. John Wiley & Sons, New York.

Richards, S.A., Possingham, H.P., and Noye, J. 1996. Diel vertical migration — modeling light-mediated mechanisms. J. Plankton Res. **18**: 2199–2222.

Richerson, P.J., Powell, T.M., Leigh-Abbott, M.R., and Coil, J.A. 1978. Spatial heterogeneity in closed basins. Mar. Sci. **3**: 239–276.

Riley, G.A. 1976. A model of plankton patchiness. Limnol. Oceanogr. **21**: 873–880.

Ringelberg, J. 1991. A mechanism of predator-mediated induction of diel vertical migration in *Daphnia hyalina*. J. Plankton Res. **13**: 83–89.

Shaw, R.G., and Mitchell-Olds, T. 1993. ANOVA for unbalanced data: an overview. Ecology, **74**: 1638–1645.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry — the principles and practice of statistics in biological research. 3rd ed. W.H. Freeman, New York.

Steele, J.H. 1976. Patchiness. *In* Ecology of the sea. *Edited by* D.H. Cushing and J.J. Walsh. Blackwell, London, U.K. pp. 98–115.

Steele, J.H. 1978. Some comments on plankton patches. *In* Spatial pattern in plankton communities. *Edited by* J.H. Steele. NATO Conf. Ser. 4. Mar. Sci. **3**: 1–20.

ter Braak, C.J.F. 1988. Partial canonical correspondence analysis. *In* Classification and related methods of data analysis. *Edited by* H.H. Bock. North-Holland, Amsterdam, The Netherlands. pp. 551–558.

ter Braak, C.J.F. 1990. Update notes: CANOCO version 3.10. Agricultural Mathematics Group, Wageningen, The Netherlands.

Tessier, A.J. 1986. Comparative population regulation of two planktonic Cladocera (*Holopedium gibberum* and *Daphnia catawaba*). Ecology, **67**: 285–302.

Urabe, J. 1989. Relative importance of temporal and spatial heterogeneity in the zooplankton community of an artificial reservoir. Hydrobiologia, **184**: 1–6.

Urabe, J., and Murano, M. 1986. Seasonal and horizontal variations in the zooplankton community of Ogochi reservoir, Tokyo. Bull. Plankton Soc. Jpn. **33**: 101–112.

van den Wollenberg, A.L. 1977. Redundancy analysis. An alternative for canonical correlation analysis. Psychometrika, **42**: 207–219.

Verdonschot, P.F.M., and ter Braak, C.J.F. 1994. An experimental manipulation of oligochaete communities in mesocosms treated with chlorpyrifos or nutrient additions: multivariate analyses with Monte Carlo permutation tests. Hydrobiologia, **278**: 251–266.

Visman, V., McQueen, D.J., and Demers, E. 1994. Zooplankton spatial patterns in two lakes with contrasting fish community structure. Hydrobiologia, **284**: 177–191.

Williamson, C.E., Schulze, P.C., and Sprules, W.G. 1992. Opening remarks: the need for advanced techniques for in situ studies of zooplankton. Arch. Hydrobiol. Beih. Ergebn. Limnol. **36**: 135–140.

Wright, D., O'Brien, W.S., and Vinyard, G.L. 1980. Adaptative value of vertical migration: a simulation model argument for the predation hypothesis. Am. Soc. Limnol. Oceanogr. Spec. Symp. **3**: 138–148.