# Spatial heterogeneity of zooplankton biomass and size structure in southern Québec lakes: variation among lakes and within lake among epi-, meta- and hypolimnion strata

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*Environmental control of zooplankton biomass size structure (53–100, 100–202, 202–500 and >500 μm) was investigated in the three limnetic strata of 25 southern Québec Shield lakes, Canada. Among-lake differences were the greatest source of variation of zooplankton biomass, whereas the strong lake–by–stratum interaction observed indicated that the vertical variations of zooplankton biomass and its size fractions were not constant from lake to lake. The analysis of spatial and local factors based on thermal strata is consistent with conceptual models of predation and nutrient control on the biomass and size structure of the zooplankton. Productivity of the aquatic systems, which was driven by lake depth, flushing rate and total phosphorus concentration, was the primary factor influencing total zooplankton biomass and size structure at among-lake scale in epilimnetic waters. The effects of the planktivorous fish on the large zooplankton biomass (>500 μm) was more clearly perceived when the effect of lake depth was removed by partial redundancy analysis. This study showed that although bottom-up and top-down forces are complementary in structuring of zooplankton communities, they can also act differently on the community attributes (e.g. biomass and size structure). Among-lake zooplankton biomass is predictable from lake trophy, but the size structure and vertical distribution of zooplankton communities appear to be controlled by lake stratification and by inference to interactions with size selective predation by fish. In metalimnetic waters, the 53–100 and 100–202 μm zooplankton biomass fractions were primarily dependent on abiotic factors, while the 202–500 and >500 μm fractions were related to planktivory and picophytoplankton concentrations. The well-oxygenated and cold hypolimnetic waters of some lakes offered a refuge from surface turbulence and planktivory to large zooplankton size fractions (202–500 and >500 μm).*

## INTRODUCTION

Since the last decades, strong debates opposed the bottom-up (BU) and top-down/trophic cascade (TD) processes as driving forces controlling the spatial structure of zooplankton community in freshwater ecosystems (Carpenter *et al.*, 1985; McQueen *et al.*, 1986; Carpenter and Kitchell, 1992, 1993; DeMelo *et al.*, 1992). However, environmental control of lake zooplankton community structure is more complex than a duality between BU and TD processes. It varies with spatial scales among lakes and within a

lake (Pinel-Alloul, 1995; Masson and Pinel-Alloul, 1998; Masson *et al.*, 2001). Regulation of plankton food web structure also depends on fish feeding guilds, either planktivores or omnivores (Pinel-Alloul *et al.*, 1998). As suggested by Carpenter (Carpenter, 1988), zooplankton community should be perceived as a spatially well-structured and dynamic system which requires a combination of abiotic and biotic factors for a better and more realistic understanding of its ecology. This statement fits the 'multiple driving forces hypothesis' (Pinel-Alloul, 1995; Pinel-Alloul *et al.*, 1995) under which control of zooplankton spatial heterogeneity among and within lakes requires the coupling of abiotic and biotic processes. Further to search for abiotic and biotic control of zooplankton community, another goal is to determine the relative role of these processes at different spatial scales. Indeed, the relative role of different ecological forces should vary among biological systems and even within the same system (Hunter and Price, 1992; Karr *et al.*, 1992). In lakes, Pinel-Alloul (Pinel-Alloul, 1995) suggests that abiotic processes have the primacy in the environmental control of zooplankton spatial heterogeneity at larger spatial scales, while biological processes have greater importance at smaller spatial scales.

In Eastern Canadian Precambrian Shield lakes, numerous studies have assessed the relative influence of abiotic (lake morphometry, water chemistry) and biotic (phytoplankton resources and fish predators) factors on limnetic zooplankton. Total phosphorus (TP) concentration was considered the best predictor of among-lake variation in zooplankton biomass (Hanson and Peters, 1984; Pace, 1986; Yan, 1986). Spatial variation in crustacean zooplankton community in Ontario lakes was related to changes in lake morphometry (mainly lake depth) and fish predation (Keller and Conlon, 1994), and to changes in lake acidity in mining regions (Locke *et al.*, 1994). Zooplankton assemblages in Québec lakes were first related to large-scale gradient in lake acidity and secondly to among-lake variation in fish and phytoplankton community (Pinel-Alloul *et al.*, 1990; Pinel-Alloul *et al.*, 1995). Fish species composition was a relatively important determinant of cladoceran abundance and size structure in the euphotic zone, when controlling for the effects of abiotic factors (Rodriguez *et al.*, 1993). From these studies, it is still difficult to draw a general pattern of environmental control of lake zooplankton community structure for several reasons. First, the number of lakes (12–132) and the scales of surveys (small to large regional areas) differed widely among studies. Secondly, zooplankton sampling design varied among studies in frequency (e.g. once a year, monthly or biweekly), time periods (spring, summer or autumn) and space (one or several vertical hauls over the whole water column or only in the epilimnetic or euphotic zone). Thirdly, explanatory variables considered in models

(biotic and/or abiotic variables, with or without fish) and zooplankton attributes (e.g. diversity, biomass, abundance and presence or absence of species) also varied among studies. Above all, there were major differences in the objectives and hypotheses tested in these studies. On this point, discrepancies among studies may arise from lake selection because, in the goal to answer to their hypotheses, researchers have eliminated, *a priori*, the influences of some lake characteristics known to influence zooplankton community structure (Shaw and Kelso, 1992).

Pinel-Alloul *et al.* (Pinel-Alloul *et al.*, 1995) suggested that factors operating at the local scale within a lake, such as spatial heterogeneity in lake environment among strata, including local effects of abiotic and biotic variables and species behaviour can exert an important influence on lake zooplankton community structure. Thus, regional variations among lakes and local variations between epi-, meta- and hypolimnion strata within a lake both may affect zooplankton community size structure and its vertical distribution. Previous studies in Precambrian Shield lakes have not simultaneously examined the potential controlling factors of zooplankton spatial heterogeneity at the regional (among-lake) and local (within-lake) scales. As most biotic and abiotic processes are scale dependent, this implies the necessity for comprehensive sampling programs that incorporate the pertinent spatial scales of physical and biological heterogeneity (Pinel-Alloul, 1995).

In this study, we examined the spatial variation in zooplankton biomass and size structure simultaneously at the regional (among-lake) and local (within-lake, among limnetic strata) scales in 25 lakes in Southern Québec. Our primary objective was to quantify the importance of regional (among lakes) and local (among-strata) sources of variation and their interactions, in controlling zooplankton biomass size structure. We tested the hypothesis that environmental differences between lakes are the main source of variability in zooplankton biomass and size structure. Our second objective was to determine which factors can explain the spatial variation in zooplankton biomass and size structure at regional (among lakes) and local (between strata) scales, using a large set of abiotic (lake morphometry and water quality) and biotic factors [chlorophyll *a* (Chl *a*) biomass and size classes and fish community] as explanatory mechanisms. Since the abiotic processes may have primacy at large scale when studying epilimnetic waters (Pinel-Alloul, 1995), we hypothesize that abiotic factors mainly regulate the among-lake variation in total zooplankton biomass and its size structure in epilimnetic waters. On the other hand, because biotic factors such as fish predation (Brooks and Dodson, 1965; Pinel-Alloul, 1995) may have more influence in vertical distribution

of zooplankton, we further tested the hypothesis that biotic factors have greater importance in structuring zooplankton biomass and size structure in the meta- and hypolimnion strata.

## METHOD

### Study lakes

The study was conducted in 25 lakes located in two contrasting regions of southern Québec, Canada: the Laurentians (19 lakes) and the Eastern Townships (6 lakes). The Laurentian lakes are located in the southeastern part of the Precambrian Shield, 80 km north of Montréal (46 N, 74 W). The bedrock in this region is mainly gneiss and granite and is covered by morainic soils and boreal forests. The Eastern Townships lakes are located 100 km south of Montréal (45 N, 72 W) in a well-buffered calcareous region containing mixed crystalline and sedimentary rocks and covered primarily by deciduous forests. The climate in both regions is temperate. Morphometric features of the lakes and their respective watershed are presented in Table I. The surface area of the lakes ranged from 0.04 to 14.4 km$^2$, and their drainage area varied between 0.1 and 1939.2 km$^2$. The maximum lake depth ranged from 3.2 to 45.7 m, and the mean lake depth, from 1.8 to 13.3 m. Lake volume varied between $3.6 \times 10^4$ and $8743 \times 10^4$ m$^3$. Most of the lakes were dimictic, but four lakes (Brome, Desjardins, Triton and Waterloo) were polymictic. The total precipitation during summer was 280 mm in 1993 in the Laurentians, 511 mm and 440 mm in 1994 in the Laurentians and Eastern Townships, respectively, and 350 mm in average for the 3-year period (1993–95) in three Laurentian lakes. Flushing rate of lakes (i.e. number of times per year the lake volume is replaced by runoff) ranged from 0.2 to 18.5 year$^{-1}$. The model of Dillon and Rigler (Dillon and Rigler, 1975) and the climatic data recorded during the last 25 years for the Laurentians and Eastern Townships area (Environment Canada, 1993) were used to evaluate the flushing rate.

The lakes under study cover a wide range of physical, chemical and biological characteristics (Table II). Water transparency ranged from 0.5 to 8.1 m, and the depth of the euphotic zone varied from 2.8 to 17 m. On average, the mixing zone was 3.9 m, but reached 9.5 m in some lakes. Water temperature, dissolved oxygen concentration, pH and dissolved organic carbon (DOC) decreased with depth, whereas alkalinity, conductivity, concentrations of nutrients, turbidity and true water colour increased. In the epilimnion, lake pH ranged from 5.5 to 9.1, whereas TP concentrations varied between 0.4 and 1.4 μM. Total Chl *a* biomass and this of most size fractions (micro-, nano- and picophytoplankton) increased with depth. Total Chl *a* biomass in the epilimnion ranged from 0.1 to 12.7 μg·L$^{-1}$.

Biomass of nano- and picophytoplankton were higher than those of microphytoplankton in all limnetic strata.

A qualitative description of fish communities was performed in this study (Table III). We classified lakes in three categories: lakes without piscivore species (five lakes), lakes with one or two piscivore species (12 lakes) and lakes with three or more piscivore species (eight lakes). The information on fish species in each lake was provided by the Ministère de l'Environnement et de la Faune du Québec and by local fishermen.

### Field sampling

Sampling was carried out once a month from June to August in 11 lakes in 1993 and another 11 lakes in 1994 (see Table I). In addition, three lakes (Corriveau, Pin Rouge and Ouimet) were monitored during three years (1993–1995).

At each sampling date, water transparency was measured using a Secchi disk at the deepest site of each lake. In order to determine the depth of epi-, meta- and hypolimnion strata, profiles of temperature and dissolved oxygen were established at 0.5 m intervals with an YSI model 51B electronic temperature/oxygen meter. Thereafter, two or three water samples were collected per stratum using a 6-L Van Dorn bottle at three stations distanced 100 m from each other. At each station and stratum, the three water samples collected in a stratum were mixed in a large plastic container to get homogeneous samples for further analyses. Water samples for Chl *a* and water chemistry (pH, alkalinity, conductivity, turbidity, true water colour and DOC) were mixed and stored in amber and brown polyethylene bottles in a cooler. Bottles were filled to the top to avoid gases exchange between the time of sampling and laboratory analysis. Water for nutrients analyses [Total nitrogen (TN), Si, and TP] was collected separately in sterilized bottles.

Because of time and cost limitations, the zooplankton sampling was not adapted to the lake size. Consequently in each lake, zooplankton samples were collected at the three stations as separate integrated samples through the epi-, meta- and hypolimnion strata, using a cantilevering towed net (53 μm mesh size) (Masson and Pinel-Alloul, 1998). After collection, organisms were immediately narcotized with carbonated water and preserved in 4% formaldehyde solution.

### Laboratory water analysis

Measurements of pH, conductivity and turbidity were made using a Fisher Acumet model 620 pH meter, a Cole Parmer model 1484 conductivity meter (standardized to 25°C) and a Nephelometer (Hach model 18 900–00) respectively. Alkalinity was determined by titrating a 200 ml water sample at a pH of 4.5 with 0.06-M HCl. True water colour was determined spectrophotometrically

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*Table I: Morphometric features of the 25 study lakes and their watershed*

| Lake (year of sampling) | Altitude (m) | Catchment area (km$^2$) | Drainage area (km$^2$) | Drainage density (km km$^{-2}$) | Lake area (km$^2$) | Shoreline (km) | Maximum depth (m) | Mean depth (m) | Lake volume (×10$^4$ m$^3$) | Epilimnion volume (×10$^4$ m$^3$) | Metalimnion volume (×10$^4$ m$^3$) | Hypolimnion volume (×10$^4$ m$^3$) | Summer precipitation (m) | Flushing rate (year$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Laurentians** | | | | | | | | | | | | | | |
| Achigan (1993) | 207 | 94.1 | 88.7 | 0.7 | 5.37 | 19.4 | 26.8 | 12.9 | 6948.1 | 2969.1 | 1214.5 | 2764.5 | 0.28 | 0.8 |
| Beaulac (1994) | 332 | 5.1 | 4.2 | 0.5 | 0.87 | 6.5 | 9.3 | 5.4 | 463.5 | 287.5 | 105.7 | 70.3 | 0.51 | 0.6 |
| Bonny (1993) | 360 | 0.3 | 0.2 | 0.0 | 0.07 | 1.0 | 10.7 | 4.7 | 32.4 | 15.9 | 5.4 | 11.1 | 0.28 | 0.5 |
| Brassard (1994) | 365 | 0.2 | 0.1 | 0.0 | 0.04 | 0.8 | 6.1 | 1.8 | 6.7 | 4.4 | 0.9 | 1.5 | 0.51 | 1.3 |
| Brûlé (1994) | 378 | 143.9 | 141.2 | 0.5 | 2.68 | 16.2 | 24.4 | 8.4 | 2259.6 | 734.7 | 538.3 | 986.6 | 0.51 | 3.7 |
| Connelly (1993) | 195 | 23.3 | 22.1 | 1.4 | 1.21 | 6.8 | 20.0 | 7.9 | 950.3 | 424.8 | 166.2 | 359.2 | 0.28 | 1.4 |
| Corriveau (1993–1995) | 325 | 13.9 | 13.9 | 1.4 | 0.06 | 1.3 | 13.7 | 7.1 | 43.5 | 16.6 | 6.7 | 20.3 | 0.35 | 18.5 |
| Cristal (1993) | 264 | 2.2 | 1.9 | 1.3 | 0.29 | 2.3 | 18.5 | 9.5 | 274.6 | 128.0 | 95.2 | 51.4 | 0.28 | 0.5 |
| Cromwell (1993) | 340 | 10.2 | 10.1 | 1.1 | 0.11 | 2.0 | 10.0 | 3.1 | 32.4 | 19.6 | 5.4 | 7.4 | 0.28 | 18.2 |
| Desjardins (1993) | 351 | 0.3 | 0.2 | 0.0 | 0.06 | 1.1 | 4.6 | 2.1 | 12.0 | 12.0 | — | — | 0.28 | 1.4 |
| Echo (1994) | 241 | 12.0 | 10.2 | 1.0 | 1.76 | 6.8 | 9.1 | 2.5 | 432.8 | 368.4 | 50.0 | 14.5 | 0.51 | 1.6 |
| Marois (1993) | 299 | 7.0 | 6.0 | 0.9 | 0.99 | 6.3 | 22.7 | 6.7 | 660.9 | 466.9 | 128.9 | 65.1 | 0.28 | 0.6 |
| Masson (1993) | 302 | 34.4 | 31.7 | 0.4 | 2.71 | 16.5 | 45.7 | 10.2 | 2763.7 | 658.7 | 488.2 | 1616.8 | 0.28 | 0.7 |
| Montagne Noire (1994) | 456 | 12.8 | 10.0 | 0.1 | 2.81 | 11.8 | 33.5 | 13.3 | 3729.9 | 1485.4 | 882.4 | 1362.1 | 0.51 | 0.2 |
| Ouimet (1993–1995) | 302 | 6.4 | 5.8 | 0.5 | 0.59 | 6.2 | 18.3 | 5.2 | 309.2 | 130.8 | 76.9 | 101.5 | 0.35 | 1.2 |
| Patrick (1993) | 347 | 63.2 | 61.7 | 0.5 | 1.51 | 7.4 | 30.5 | 10.8 | 1625.7 | 646.2 | 235.8 | 743.6 | 0.28 | 2.2 |
| Pin Rouge (1993–1995) | 332 | 6.8 | 6.6 | 1.7 | 0.15 | 2.2 | 15.2 | 5.1 | 76.7 | 37.0 | 21.9 | 17.9 | 0.35 | 5.1 |
| Tracy (1993) | 349 | 0.2 | 0.1 | 0.0 | 0.08 | 1.4 | 24.4 | 8.9 | 69.4 | 38.7 | 14.2 | 16.6 | 0.28 | 0.2 |
| Triton (1993) | 340 | 0.2 | 0.2 | 1.0 | 0.02 | 0.7 | 3.2 | 1.9 | 3.6 | 3.6 | — | — | 0.28 | 2.9 |
| **Eastern townships** | | | | | | | | | | | | | | |
| Brome (1994) | 196 | 187.3 | 172.9 | 0.6 | 14.39 | 20.8 | 12.2 | 5.9 | 8465.4 | 7650.7 | 814.7 | — | 0.44 | 1.2 |
| D'Argent (1994) | 248 | 65.0 | 64.0 | 0.6 | 1.00 | 4.2 | 15.6 | 4.5 | 446.9 | 294.2 | 100.3 | 52.4 | 0.44 | 7.7 |
| Desmarais (1994) | 279 | 1.8 | 1.6 | 0.1 | 0.24 | 4.4 | 7.0 | 2.5 | 60.9 | 51.2 | 9.2 | 0.5 | 0.44 | 1.6 |
| Lovering (1994) | 241 | 46.2 | 41.3 | 0.5 | 4.94 | 16.8 | 22.9 | 8.8 | 4351.1 | 2431.6 | 844.0 | 1075.5 | 0.44 | 0.6 |
| Magog (1994) | 193 | 1950.2 | 1939.2 | ? | 10.95 | 30.3 | 19.2 | 8.0 | 8743.0 | 5230.2 | 1616.2 | 1896.7 | 0.44 | 11.9 |
| Waterloo (1994) | 207 | 33.9 | 32.5 | 0.4 | 1.47 | 9.2 | 4.9 | 3.4 | 493.4 | 493.4 | — | — | 0.44 | 3.7 |

*Table II: Summary statistics [mean ± standard deviation (min–max)] for summer physical, chemical and biological characteristics of each water stratum of the study lakes*

| Variable | Epilimnion (25 lakes) | Metalimnion (22 lakes) | Hypolimnion (21 lakes) |
|---|---|---|---|
| Water transparency (m) | 3.3 ± 1.7 (0.5–8.1) | — | — |
| Euphotic zone (m) | 7.9 ± 3.6 (2.8–17) | — | — |
| Mixing zone (m) | 3.9 ± 1.8 (1.5–9.5) | — | — |
| Temperature (°C) | 21.7 ± 1.9 (18–27) | 14.9 ± 2.6 (10.2–23) | 6.8 ± 2.7 (4–17) |
| Oxygen (mg L$^{-1}$) | 7.5 ± 0.8 (5.9–10.7) | 6.8 ± 3.6 (0.2–13) | 4.2 ± 3.3 (0.1–10.4) |
| Alkalinity (mg CaCO3 L$^{-1}$) | 18.6 ± 12.3 (3.5–50.9) | 19 ± 13.7 (3.4–62.8) | 22 ± 18.3 (3.6–98.9) |
| pH | 7.3 ± 0.6 (5.5–9.1) | 6.9 ± 0.5 (5.6–7.9) | 6.6 ± 0.5 (5.2–8) |
| Conductivity (μS cm$^{-1}$) | 76.4 ± 45.2 (19–210) | 77.7 ± 44.9 (20–210) | 88.6 ± 49.6 (19–275) |
| Total phosphorus (μM) | 0.4 ± 0.3 (0.04–1.4) | 0.4 ± 0.3 (0.06–1.8) | 0.5 ± 0.6 (0.05–4.3) |
| Total nitrogen (μM) | 17.6 ± 8.7 (3.1–51.9) | 17.8 ± 8.4 (1.7–60.2) | 21.9 ± 9.9 (0.7–56.6) |
| Total dissolved silica (μg L$^{-1}$) | 2555.4 ± 1410.7 (12.6–6835.4) | 3495.8 ± 1755.6 (328–10 287) | 4962.2 ± 2068.3 (1048.9–10 872) |
| Turbidity (NTU) | 1.9 ± 1.8 (0.5–16.3) | 2.1 ± 2.6 (0.6–22) | 3.6 ± 3.7 (0.6–16.6) |
| True color (mg Pt. L$^{-1}$) | 44 ± 34.2 (0.5–165) | 45.9 ± 38.2 (2.5–267) | 56.8 ± 58.3 (2.5–395) |
| DOC (mg L$^{-1}$) | 5 ± 2.1 (2–12.2) | 4.4 ± 2.2 (1.6–21.8) | 4.1 ± 1.9 (1.5–12.3) |
| Chlorophyll *a* (μg L$^{-1}$) | | | |
| Total | 1.9 ± 1.9 (0.1–12.7) | 3.2 ± 5 (0.3–37.2) | 4.3 ± 11.4 (0.1–85.6) |
| Micro (>20 μm) | 0.8 ± 1.2 (0.002–9.2) | 1.3 ± 2.3 (0.02–18.6) | 1.1 ± 4.4 (0.003–58.5) |
| Nano + pico (<20 μm) | 1.1 ± 1.1 (0.05–7.9) | 1.9 ± 3.2 (0.04–19.2) | 3.1 ± 8.4 (0.02–57.6) |
| Nano (3–20 μm) | 0.6 ± 0.8 (0.01–5.8) | 1.2 ± 2.2 (0.001–15.8) | 2.2 ± 6.3 (0.01–37.6) |
| Pico (<3 μm) | 0.5 ± 0.4 (0.002–2.6) | 0.7 ± 1.3 (0.001–10.6) | 0.9 ± 2.9 (0.001–27.2) |
| Zooplankton biomass (μg L$^{-1}$) | | | |
| Total | 108.8 ± 97.6 (17.3–690) | 68.7 ± 42.2 (6.5–320.7) | 51.7 ± 75.5 (2.9–775.3) |
| >500 μm | 31.3 ± 29.6 (0.9–193.7) | 23.3 ± 19.5 (0.7–112.1) | 16.5 ± 23.1 (0.2–180.0) |
| 202–500 μm | 36.1 ± 41 (2.4–295.9) | 20.9 ± 16.1 (1.2–118.8) | 17.3 ± 25.8 (0.3–202.5) |
| 100–202 μm | 17.1 ± 18.4 (2.2–177.8) | 10.8 ± 9.1 (1.4–80) | 8.4 ± 17.5 (0.5–187.1) |
| 53–100 μm | 23.4 ± 31.4 (2.0–245.4) | 13.7 ± 15.9 (2.5–155.8) | 9.5 ± 18.7 (0.5–205.7) |

at 440 nm (Cuthbert and Del Giorgio, 1992). TP (μM) was measured using the molybdenum blue colorimetric assay after acidic persulfate digestion (Murphy and Riley, 1962). TN (μM) was analysed colorimetrically following the indophenol blue method (Armstrong *et al.*, 1967), after digestion by alkaline persulfate oxidation. Dissolved silica concentration was measured by the acidic ammonium molybdate method after reduction with SnCl$_2$ (Golterman and Clymo, 1969). The DOC concentration was determined by conductimetry after persulfate-UV oxidation (Kaplan, 1992).

Both total Chl *a* concentrations and Chl *a* concentrations in four operationally-defined size fractions (pico, <3 μm; nano, 3–20 μm, nano + pico 'NaPi', <20 μm; and micro, >20 μm) were determined by fluorometry. Procedures of filtration and fractionation are given in Masson *et al.* (Masson *et al.*, 2000).

### Laboratory zooplankton analysis

In this study, zooplankton biomass and size structure were estimated as organic carbon biomass or ash-free dry weight (AFDW) following a sieving technique (Seda and Dostalkova, 1996; Masson and Pinel-Alloul, 1998). Zooplankton samples were split in two halves with a Folsom splitter; one half was used for estimation of zooplankton biomass, and the other half served for species identification. With the second half, we established (i) the list of species in each lake without counting the individuals and thereafter (ii) the list of species found in each of the four size fractions. To estimate the total zooplankton biomass and its size structure, samples were sieved through Nitex$^{TM}$ nets of decreasing size: 500, 202, 100 and 53 μm mesh size, to obtain four size fractions: 53–100, 100–202, 202–500 and >500 μm. The material collected in each net was backwashed and transferred onto precombusted GF/C (Whatman) glass fiber filters. These filters were dried at 60°C for 24 h and weighted. Finally, they were ashed at 500°C for 24 h and weighted again using a Cahn microbalance (mean ± 0.1 μg). Zooplankton organic biomass in each

### Table III: List of fish species found in each study lake

| Fish species | | Brassard | Corriveau | Cromwell | Desjardins | Triton | Bonny | Desmarais | M.Noire | Ouimet | Pinrouge | Tracy | Beaulac | Brûlé | Cristal | D'Argent | Marois | Patrick | Echo | Masson | Waterloo | Brome | Connelly | Lovering | Achigan | Magog |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Piscivores** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Atlantic salmon | *Salmo salar* | | | | | | | | | | | | | | | | | | | | | | | X | X | |
| Brown trout | *Salmo trutta* | | | | | | | | | | | | | | | | | | | | X | | | X | X | X |
| Chain pickerel | *Esox niger* | | | | | | | | | | | | | | | X | | | | | X | X | | | | X |
| Lake charr | *Salvelinus namaycush* | | | | | | | X | X | | | | X | | X | | X | X | | X | | | | | X | X |
| Largemouth bass | *Micropterus salmoides* | | | | | | | | | | | | | | | | | | | | | | | | | |
| Muskellunge | *Esox masquinongy* | | | | | | | | | | | | | X | | | | | X | X | | | | | | X |
| Northern pike | *Esox lucius* | | | | | | | | | | | | X | | | | | | | | | | | | | X |
| Rainbow trout | *Oncorhynchus mykiss* | | | | | | | | | X | | | | | | | | | | | | X | X | X | X | X |
| Rock bass | *Ambloplites rupestris* | | | | | | | | | | | | | | | | | | | | | X | X | X | X | X |
| Smallmouth bass | *Micropterus dolomieui* | | | | | | X | | | | X | X | | | X | X | | X | X | X | | X | X | X | X | X |
| Walleye | *Stizostedion vitreum* | | | | | | | | | | | | | | | | X | | X | | | X | X | | X | X |
| **Planktivores** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brook charr | *Salvelinus fontinalis* | | | | | | X | X | X | X | X | X | X | | X | | X | X | | | | X | X | X | X | |
| Brown bullhead | *Ictalurus nebulosus* | | | | | | X | X | X | | X | X | X | | | | X | X | X | X | X | X | X | X | X | X |
| Central mudminnow | *Umbra limi* | | | | | | | | | | | | X | | | | | | | | | | X | | | |
| Common shiner | *Notropis cornutus* | | | | X | | | | | X | | | | | | | | | | | X | X | X | | X | |
| Creek chub | *Semotilus atromaculatus* | | | X | | | | X | X | | X | | | | | | | | | | | | | X | X | X |
| Cutlips | *Exoglossum maxillingua* | | | X | | | | | | | | | | | | | | | | | | | | | | |
| Fallfish | *Semotilus corpalis* | | | | | | | | | | | | X | | | | | | | | X | | | | | |
| Fathead minnow | *Pimephales promelas* | | | X | | | | | | | | | X | | | | | | | | | | | | | |
| Golden shiner | *Notemigonus crysoleucas* | | | | | | | | | | | | | | | X | | | | | X | X | | X | | X |
| Lake chub | *Couesius plumbeus* | X | X | | | | X | | X | X | | | X | X | | | X | X | | X | | | X | | | X |
| Northern redbelly dace | *Phoxinus eos* | | | X | X | X | | | | X | | | | | | | | | | X | | | | | | |
| Pumkinseed | *Lepomis gibbosus* | | | X | | X | | | | X | X | | X | X | | | X | X | | | | X | X | X | | X |
| rainbow smelt | *Osmerus mordax* | | | | | | | | | | | | | | | | | | | | | | | X | | X |
| White sucker | *Catostomus commersoni* | | | X | | | | | X | X | X | | X | | | | X | X | X | X | X | X | X | X | X | X |
| Whitefish | *Coregonus clupeaformis* | | | | | | | | | | | | | | | X | | | | | | X | | X | X | |
| Yellow perch | *Perca flavescens* | | | | | | | | X | | | | X | X | | X | X | X | X | X | X | X | X | X | X | X |
| **Total species** | | 1 | 1 | 6 | 2 | 2 | 4 | 3 | 8 | 4 | 5 | 2 | 9 | 3 | 1 | 5 | 4 | 6 | 5 | 8 | 5 | 9 | 8 | 9 | 7 | 8 |
| **Piscivore species** | | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 5 | 6 | 9 |

size fraction (organic carbon biomass: μg C) was calculated as the difference between the ash weight and the dry weight and expressed as micrograms AFDW per litre. Microscopic examination of the material and organisms collected in each net helped to determine the composition of the four size fractions.

## Statistical analyses

Although the sampled lakes were located in two different regions, geology was not considered as a source of variation in lake productivity that could indirectly have influenced zooplankton biomass and size structure. The lack of difference in the ratio of Chl *a* to TP between Laurentian and Eastern Townships lakes (Masson *et al.*, 2000) supported this assumption. Since preliminary ANOVA testing for spatial and temporal autocorrelation in the data (Dutilleul and Pinel-Alloul, 1996) had detected no temporal effect, analysis were done on summer means (months and years combined for three lakes). Thus, lake and stratum were the two factors retained as sources of spatial variation potentially influencing total zooplankton biomass and its size fractions, with the three stations within a lake as replicates. Therefore, mean values per stratum and per station within a lake were calculated for 21 lakes in which all three limnetic strata could be identified. All analyses were performed on $\log_{10}$-transformed values of zooplankton biomass.

To test if lake and stratum were significant sources of variation in total zooplankton biomass and its four size fractions, two-way repeated ANOVAs were performed on each zooplankton variable. The lake main effects represent the purely among-lake source of variation, whereas the stratum main effects represent a purely among-stratum source of variation. The lake and stratum factors being crossed, their interaction, if it exists, represents the lack of constancy of the among-stratum source of variation over lakes. For a given station within a lake, the vector of the three zooplankton biomass mean values corresponding to the three limnetic strata defines a profile vector of spatial repeated measures (Dutilleul, 1998a). This type of data requires an appropriate statistical analysis (Crowder and Hand, 1990). With lake considered as a random factor crossed with stratum as a fixed factor, the ANOVA model is a mixed two-way ANOVA with spatial repeated measures. We followed Dutilleul (Dutilleul, 1998b) for statistical analysis. In particular, the ANOVA *F*-tests were modified in order to take into account the autocorrelation and heteroscedasticity of the spatial repeated measures at the three limnetic strata (Greenhouse and Geisser, 1959). The ANOVAs were performed by using the GLM procedure, with the option REPEATED of SAS version 6 (SAS Institute Inc., 1999). ANOVAs and *t*-tests for pairwise comparisons of means

were also used to assess the differences between the mean biomass of the four zooplankton size fractions in each limnetic stratum.

To evaluate the effects of environmental factors on total zooplankton biomass and its size fractions, we chose a total of 36 potential explanatory variables grouped in three matrices. We chose explanatory variables that were usually used in limnological studies. For the morphometric matrix, we used a total of 14 variables mainly related to lake morphometric features (Table I). For the physico-chemical matrix, we also used 14 variables (Table II), whereas 8 variables (i.e. total Chl *a* and its size components and fish species composition) were retained for the biological matrix (Tables II and III). We used the Canonical Redundancy Analysis (RDA; van den Wollenberg, 1977) to identify the variables or sets of collinear variables which contribute the most to the variation in total zooplankton biomass and its size fractions. We followed three distinct approaches: (i) marginal, (ii) joint and (iii) conditional. The RDAs were performed on $\log_{10}$-transformed data for which a linear model was assumed for the relationships between the dependent variables (i.e. biomass of the four zooplankton size fractions) and the explanatory variables (i.e. morphometric, physico-chemical and biological variables) (ter Braak, 1987). In the marginal approach (i), we performed the RDA by using one of the three matrices of potential explanatory variables at a time, for the epi-, meta- and hypolimnetic strata. After the most explanatory variables in each matrix were identified, these variables were grouped in a new matrix to carry out the joint approach (ii), still by RDA, in order to identify parsimoniously the factors influencing the zooplankton biomass size structure. This analysis allowed us to evaluate the interactions between abiotic and biotic factors influencing zooplankton biomass in the three limnetic strata.

After having identified the most important variables influencing zooplankton biomass, we followed a conditional approach (iii) based on partial redundancy analysis, by performing the RDA on the variables identified in the marginal approach after removal of the effect of maximum depth. Indeed, lake maximum depth strongly influenced water physics and chemistry as well as phytoplankton and zooplankton community structure and was thus strongly correlated with some important variables that might differently explain the variations of zooplankton size structure among lakes. By removing the effect of maximum depth, the physico-chemical and biological mechanisms explaining the spatial variation in zooplankton biomass size structure could be better described in the three limnetic strata.

For all RDAs, the forward procedure of CANOCO Version 4.02 (ter Braak and Smilauer, 1997) was used, with a significance level of 0.1. The statistical significance

of each environmental variable was evaluated by Monte Carlo permutation tests. To avoid multicollinearity problems, we chose the least collinear factors by inspecting the Variance Inflation Factor.

## RESULTS

### Zooplankton composition, biomass and size structure

*Taxonomic composition of the size fractions*
The >500 μm size fraction was principally composed of large cladocerans, represented by five species of *Daphnia* (*Daphnia ambigua*, *Daphnia catawba*, *Daphnia dubia*, *Daphnia galeata* and *Daphnia pulex*), *Diaphanosoma* sp. and *Holopedium gibberum* and in less abundance by adults of five copepod species (*Cyclops scutifer*, *Epichura lacustris*, *Leptodiaptomus* spp., *Mesocyclops* sp. and *Skistodiaptomus* sp.). The 202–500 μm size fraction was composed of copepodid instars of the five copepods species identified in the >500 μm size fraction and by adults of *Tropocyclops prasinus*. This size fraction also comprised small cladocerans such as *Bosmina* spp., *Ceriodaphnia* sp., *Chydorus* spp., and immature instars of the cladoceran species identified in the >500 μm size fraction. The two small-size fractions (100–202 and 53–100 μm) were mainly composed of rotifers (*Ascomorpha* sp., *Conochilus* sp., *Kellicottia* sp., *Keratella* spp., *Lecane* sp., *Trichocerca* spp. and *Synchaeta* sp.), but the 100–202 μm size fraction also contained small copepodids and nauplii. For the small fractions (<202 μm), the material retained on the nets also included various amounts of particulate organic matter such as phytoplankton and detritus. However, algae and detritus were apparent mainly in the 53–100 μm size fraction and did not represent >20% of the sample. Other rotifers were also detected in this last fraction (*Gastropus* sp., *Keratella* spp. and *Polyarthra vulgaris*).

*Among-lake variations of the zooplankton biomass*
The mean biomass of total zooplankton and its size fractions varied strongly from lake to lake (Fig. 1). Total zooplankton biomass in the epilimnion were ranked by ascending values and ranged from 40.1 ± 17.1 μg L$^{-1}$ (Lake Lovering) to 388.6 ± 167.5 μg L$^{-1}$ (Lake Desjardins) (Fig. 1a). Strong variations were observed between lakes for the four size fractions. At the opposite of the total biomass, the biomass of the four size fractions did not increase consistently from Lake Lovering to Lake Desjardins (Fig. 1b-e).

*Among-stratum variations of the zooplankton biomass*
The zooplankton biomass of the total and the four size fractions were significantly different between the limnetic strata (ANOVA; $P < 0.001$). Although the zooplankton biomass decreased from the epilimnion to the hypolimnion in the most lakes (Fig. 1a–e), some presented the highest biomass in the metalimnion (e.g. Lovering, Montagne Noire and Ouimet), whereas others showed it in the hypolimnion (e.g. Marois, Cristal, Beaulac and Echo). Peculiarities observed with total biomass (biomass being more important in meta- or hypolimnion in some lakes) were also detected for most of the size fractions.

*Relative importance of the four size fractions*
On the basis of the summer mean, the 202–500 μm biomass (36.1 ± 41 μg L$^{-1}$) in the epilimnion was significantly ($P = 0.03$) more important than the >500 μm biomass (31.3 ± 29.6 μg L$^{-1}$) (Table II). For the small fractions, the 53–100 μm biomass (23.4 ± 31.4 μg L$^{-1}$) was significantly ($P < 0.001$) higher than the 100–202 μm biomass (17.1 ± 18.4 μg L$^{-1}$) (Table II). At the opposite, the >500 μm biomass (23.3 ± 19.5 μg L$^{-1}$) in the metalimnion was significantly ($P = 0.03$) more important than the 202–500 μm biomass (20.9 ± 16.1 μg L$^{-1}$). In the hypolimnion, the biomass of the size fractions 202–500 and >500 μm were comparable ($P = 0.426$) but significantly ($P < 0.001$) higher than those of the two small size fractions (Table II).

### Lake and stratum sources of variation

At regional scale (among lakes), the lake main effect is the greatest source of variation in total zooplankton biomass and its size fractions (36.5–55% of total variance; $P = 0.0001$; Table IV). In comparison, the strata main effect at local scale (within lake) has two-fold less importance (11.5–19.9% of total variance; $P = 0.0139$; Table IV). The lake–by–strata interaction was as important as the lake main effect for explaining spatial variations in total zooplankton biomass and the size fractions 202–500 and >500 μm (46.1 and 35.6% of total variance respectively; $P = 0.0001$; Table IV). The highly significant lake–by–strata interaction indicates that spatial variations of total zooplankton biomass and its size fractions at the local scale within each lake were not constant from lake to lake.

### Relationships between main environmental gradients and total zooplankton biomass

*Determination of the influencing variables with the RDA marginal approach*
Among the three set of environmental factors, the physicochemical factors were the most important factors explaining the spatial variation of the zooplankton biomass size fractions in the three limnetic strata (56% in the epilimnion, 35% in the metalimnion and 67% in the
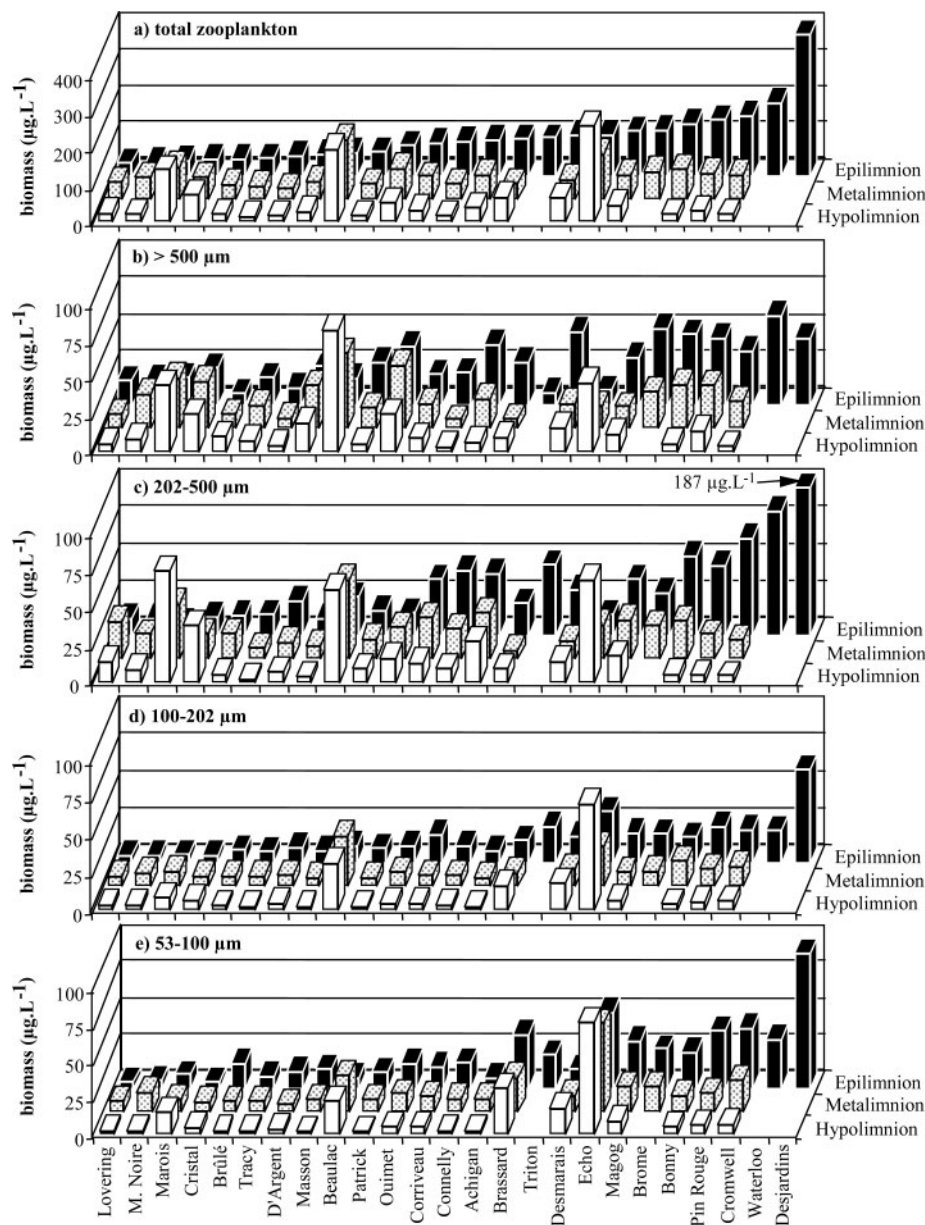
**Fig. 1.** Among-lake and among-stratum spatial variations of total zooplankton biomass and its four size fractions in the 25 lakes.

hypolimnion) (Table V). In terms of total variance explained, the morphometric factors were the less important in the three limnetic strata (30–46%), and the biotic factors' importance was intermediate (31–63%). However, when we examined the percentage of variance explained by each factor within each environmental set of variables, maximum depth was one of the most important factor influencing the zooplankton biomass size fractions among all significant abiotic and biotic factors in the epilimnion and metalimnion (Table V). In the epilimnion, the maximum depth explained 34% of the zooplankton

size structure variation, while the TP and the nanophytoplankton concentrations explained 32 and 30% respectively. In the metalimnion, the maximum depth explained 20% of the zooplankton size structure variation, compared to 14% for the water temperature and 19% for the picophytoplankton concentration. In the hypolimnion, microphytoplankton (45%) and water alkalinity (33%) were the main factors explaining variation of the zooplankton biomass. Many other factors were retained by the forward procedure of the RDA in the three matrices, but their importance in structuring zooplankton communities

*Table IV: Results of the ANOVA F-tests for among-lake and among-stratum variations of total zooplankton biomass and its size fractions*

| Source | Mean square | d.f. | F observed | P | Variation (%) |
|---|---|---|---|---|---|
| Zooplankton total | | | | | |
| Lake | 1.6537 | 20 | 63.84 | 0.0001 | 39.3 |
| Strata | 8.1758 | 2 | 10.91 | 0.0003 | 19.5 |
| Lake × strata | 0.7496 | 40 | 28.94 | 0.0001 | 35.6 |
| Epsilon = 0.9097 | | | | | |
| Zooplankton >500 µm | | | | | |
| Lake | 2.2198 | 20 | 34.75 | 0.0001 | 38.8 |
| Strata | 9.6071 | 2 | 9.43 | 0.0005 | 16.8 |
| Lake × strata | 1.0188 | 40 | 15.95 | 0.0001 | 35.6 |
| Epsilon = 0.9953 | | | | | |
| Zooplankton 202–500 µm | | | | | |
| Lake | 2.2214 | 20 | 51.79 | 0.0001 | 36.5 |
| Depth | 7.0052 | 2 | 5.00 | 0.0139 | 11.5 |
| Lake × depth | 1.4005 | 40 | 32.65 | 0.0001 | 46.1 |
| Epsilon = 0.9200 | | | | | |
| Zooplankton 100–202 µm | | | | | |
| Lake | 3.0276 | 20 | 89.61 | 0.0001 | 53.4 |
| Strata | 10.1166 | 2 | 15.17 | 0.0001 | 17.8 |
| Lake × strata | 0.6667 | 40 | 19.73 | 0.0001 | 23.5 |
| Epsilon = 0.8785 | | | | | |
| Zooplankton 53–100 µm | | | | | |
| Lake | 3.7903 | 20 | 140.55 | 0.0001 | 55.0 |
| Strata | 13.0376 | 2 | 16.82 | 0.0001 | 19.9 |
| Lake × strata | 0.7749 | 40 | 28.74 | 0.0001 | 22.5 |
| Epsilon = 0.9350 | | | | | |

The ANOVA F-tests were modified in order to take into account the autocorrelation and heteroscedasticity of the spatial repeated measures at the three strata (Greenhouse and Geisser, 1959).

in lakes was better determined in the joint and conditional approaches (Fig. 2a-f).

## Determination of the relative influence of the variables by using the RDA joint and conditional approaches

*Joint approach in the epilimnetic waters*
The maximum depth (34%), the concentration of TP (11%) and the flushing rate (4%) explained together 49% of the variation of the zooplankton biomass size fraction in the epilimnion (Fig. 2a). The 53–100, 100–202 m and 202–500 µm biomass were greater in shallow lakes of high flushing rate and high TP concentrations. The >500 µm biomass only responded to positive increase of TP concentrations.

The productivity of our system can be visualized by the relationship between TP concentration and maximum depth which is inversed but not linear (Fig. 3a). As detected with the RDA, the total zooplankton biomass and the four size fractions in the epilimnion responded positively to TP concentration which explained between 23 and 43% of their variance (Fig. 3b–f). The lake Desjardins was excluded of these models due to its strong effect on the biomass of small zooplankton probably related to the combined effects of TP level on zooplankton biomass and planktivorous fish predation on large zooplankton. It is important to note that this shallow lake was strongly affected by windy conditions that could mix sediment material into the water column (input of TP) and thus possibly contribute to high biomass fractions observed. The RDA results firstly indicated that the epilimnetic zooplankton biomass of the four size fractions increased with system productivity, secondly that productivity of systems was inversely related to the depth and thirdly that the abiotic factors were predominating in the control of epilimnetic zooplankton biomass.

*Table V: Ordination scores for zooplankton biomass size fractions in redundancy analyses (RDA) for the three limnetic strata with the significant abiotic and biotic variables as predictors*

| Variable | Epilimnion | | Metalimnion | | Hypolimnion | |
|---|---|---|---|---|---|---|
| | % of variance explained | P-value | % of variance explained | P-value | % of variance explained | P-value |
| Morphometric | | | | | | |
| Maximum depth | 0.34 | 0.001 | 0.20 | 0.001 | 0.32 | 0.002 |
| Flushing rate | 0.08 | 0.068 | | | | |
| Metalimnetic volume | | | 0.10 | 0.063 | | |
| Total volume | | | | | 0.14 | 0.031 |
| Total variance explained | 0.42 | | 0.30 | | 0.46 | |
| Physicochemical | | | | | | |
| Total phosphorus | 0.32 | 0.001 | | | | |
| Euphotic zone | 0.17 | 0.001 | | | | |
| Water transparency | | | 0.12 | 0.036 | | |
| Mixing zone | | | | | 0.20 | 0.002 |
| Temperature | 0.07 | 0.036 | 0.14 | 0.001 | | |
| Oxygen | | | | | 0.14 | 0.017 |
| Dissolved silica | | | 0.09 | 0.05 | | |
| Alkalinity | | | | | 0.33 | 0.001 |
| Total variance explained | 0.56 | | 0.35 | | 0.67 | |
| Biotic | | | | | | |
| Microphytoplankton | 0.08 | 0.064 | | | 0.45 | 0.001 |
| Nano + picophytoplankton | | | | | 0.18 | 0.001 |
| Nanophytoplankton | 0.30 | 0.001 | | | | |
| Picophytoplankton | | | 0.19 | 0.01 | | |
| No piscivore | 0.08 | 0.05 | 0.06 | 0.09 | | |
| 2 or ≥ 3 piscivores | | | 0.06 | 0.11 | | |
| Total variance explained | 0.46 | | 0.31 | | 0.63 | |

*Conditional approach in the epilimnetic waters*

After removing the maximum depth effect, the explained variance decreased from 49 (Fig. 2a) to 26% (Fig. 2b), indicating a strong influence of the lake depth on the variation of the zooplankton biomass size fractions. This later analysis allowed us to confirm the influence of the TP (11%) on the variation of the zooplankton biomass and to identify three new explanatory variables: depth of the euphotic zone (6%), water temperature (5%) and fish community (4%). Indeed, the 53–100, 100–202 and 202–500 μm biomass increased in epilimnetic waters of lakes of high temperature and shallow euphotic zone, but decreased in lakes presenting two piscivore species. The >500 μm biomass in the epilimnion increased in lakes of higher TP concentration and with more than three piscivore fish, but decreased in lakes without piscivores. These last results indicated that small zooplankton was influenced by other factors related to the productivity and that the type of fish community had

also an important impact of zooplankton size structure. On the basis of our lake fish classification (Table III), we effectively observed that the relative contribution of the large zooplankton (>500 μm) was lower than the relative biomass of the 200–500 μm fraction in the epilimnion of lakes without piscivore fish species, while it was similar or higher in lakes with piscivores (Fig. 4). Furthermore, the contribution of large zooplankton was increased two-fold in lakes with piscivores.

*Joint approach in the metalimnetic waters*

In the metalimnion (Fig. 2c), 41% of the variation of the four size fraction zooplankton biomass was explained by the lake maximum depth (20%), the water transparency (16%) and the fish community (6%). The 53–100 and 100–202 μm biomass were low in the metalimnion of deep clear lakes, whereas the 202–500 and >500 μm biomass slightly increased in the metalimnion of clearer lakes with two piscivore species. However, in lakes
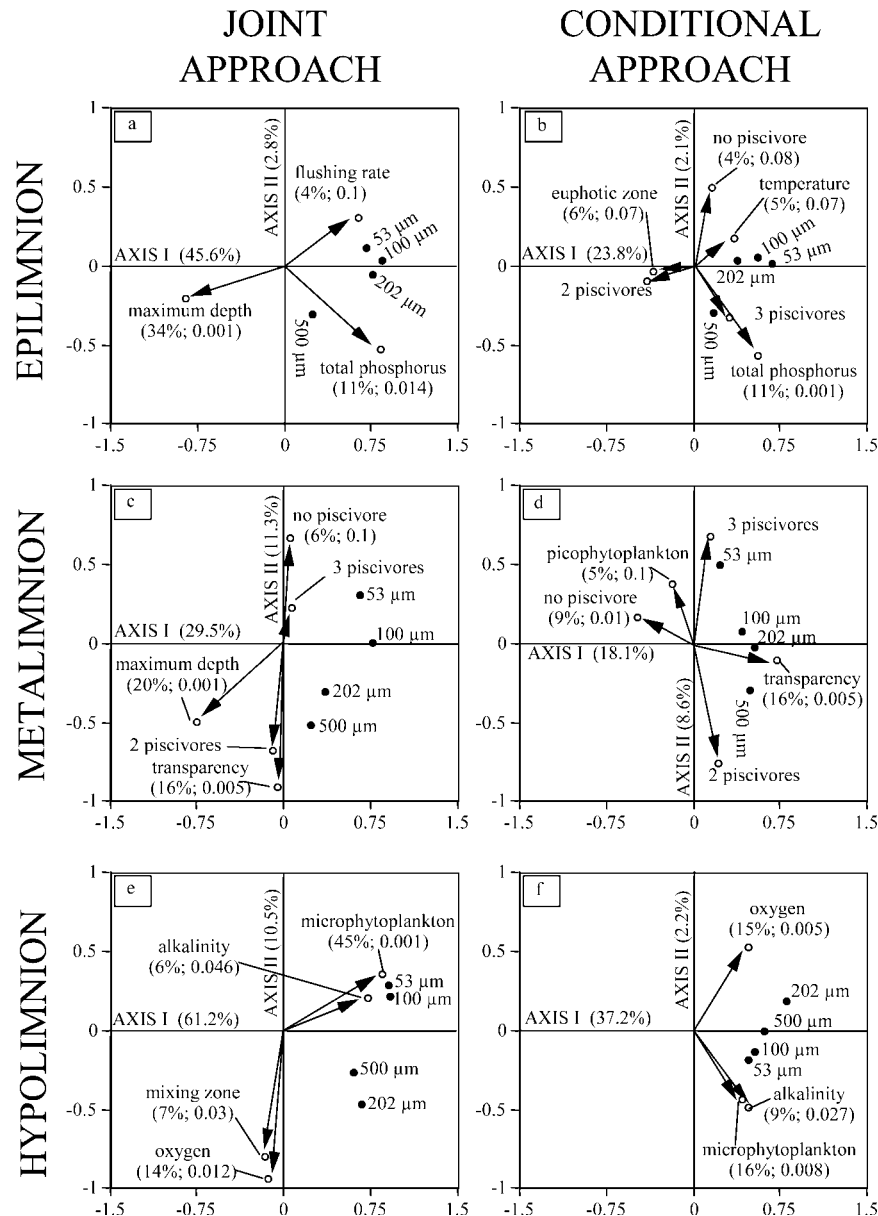
**Fig. 2.** Ordination biplots of the zooplankton biomass size fractions and environmental variables in the redundancy analysis for the epilimnetic, metalimnetic and hypolimnetic waters, in the joint (**a**, **c** and **e**) and conditional (**b**, **d** and **f**) approaches. Values in parentheses are the percentages of variation explained by each axis and some of the variables, with the associated probability for the latter. Zooplankton biomass 53 μm is identified 53–100 μm; 100 μm: 100–202 μm; 202 μm: 202–500 μm; and 500 μm: >500 μm.

without piscivores, these two fractions decreased. These results reflect the impact of planktivorous predation on zooplankton in lakes without piscivores where large zooplankton species avoid metalimnetic waters. The relative contributions of the 202–500 and 500 μm size fractions were effectively greater in metalimnion of the lakes with piscivore fish species than in metalimnion of the lakes without piscivores (Fig. 4). However, the percentage of increase was more important for the largest zooplankton size fraction (24.1–37.5% versus 26.8–30.3%).

*Conditional approach in the metalimnetic waters*

Once the maximum depth effect was removed, the explained variance decreased from 41 (Fig. 2c) to 27% (Fig. 2d). This diminution was less than the one observed in the epilimnion, suggesting that maximum depth had less influence on metalimnetic zooplankton size structure. The concentration of picophytoplankton (5%) was a new variable added to the model explaining variation of the zooplankton biomass size structure, while the water transparency (16%) and the fish community (9%) remained in
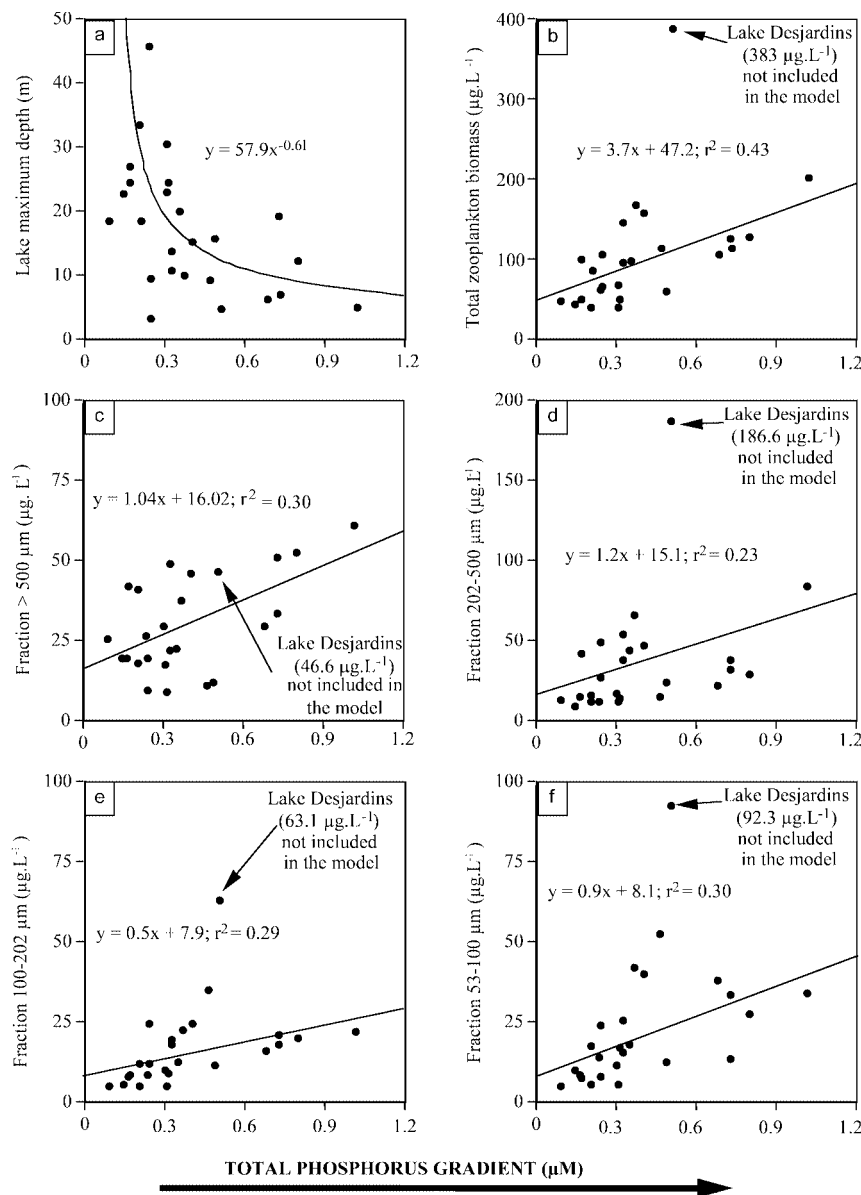
**Fig. 3.** Relationships between total phosphorus concentration and lake maximum depth (**a**), total zooplankton biomass (**b**), >500 μm biomass (**c**), 202–500 μm biomass (**d**), 100–202 μm biomass (**e**) and 53–100 μm biomass (**f**). All relationships used average raw data.

the model. The 202–500 and >500 μm biomass increased in lakes of higher transparency having two piscivore species and strongly decreased in metalimnion of lakes without piscivore species where take place higher biomass of pico-phytoplankton. This result corroborated the precedent analysis (joint approach) and might indicate that grazing by large zooplankton could control development of pico-phytoplankton (these being less abundant in clear waters) when weak predation pressure by planktivore occurs (presence of piscivores). Thus, at the opposite of epilimnetic waters where abiotic factors were dominant in structuring zooplankton biomass, biotic factors such as fish predation

and phytoplankton resources were predominant in the metalimnetic waters.

### Joint approach in the hypolimnetic waters

In the hypolimnion (Fig. 2e), >70% of the zooplankton biomass size structure variation was explained by the biomass of the microphytoplankton (45%), the oxygen concentrations (14%), the mixing zone depth (7%) and the water alkalinity (6%). The 202–500 and >500 μm biomass were more important in hypolimnion of lakes presenting a deep mixing zone and well-oxygenated waters indicating the use of this stratum as a refuge for these two zooplankton
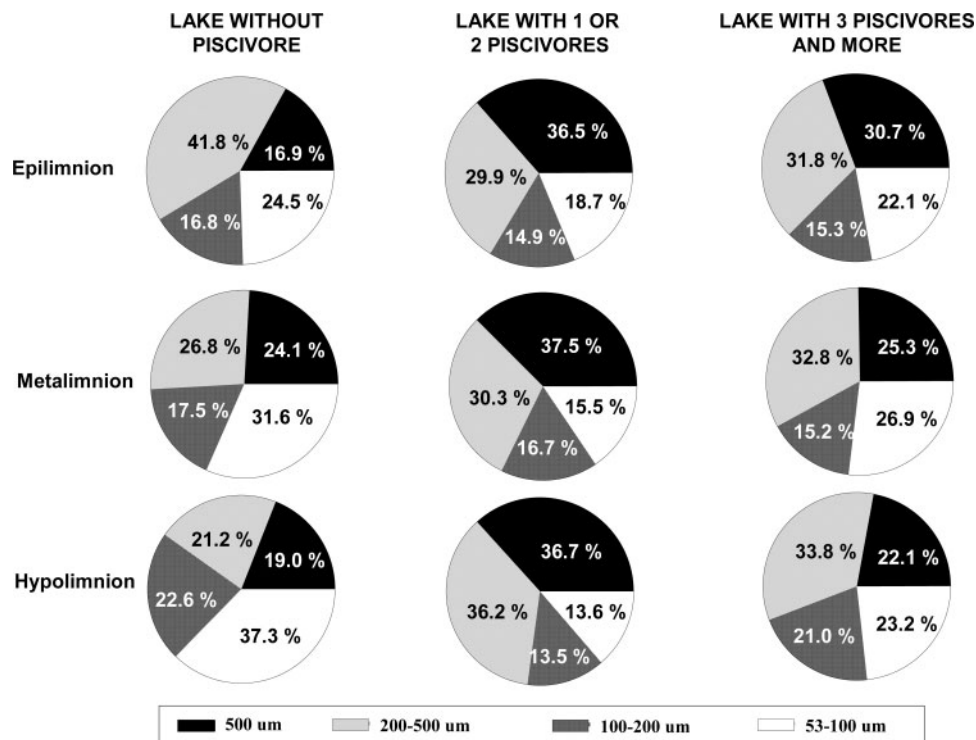
**Fig. 4.** Relative contribution of the four zooplankton size fraction in each stratum evaluated in the lakes without piscivores, with one or two piscivores and in lakes with three or more piscivore species.

fractions. The 53–100 and 100–202 μm biomass were less important in the last conditions corroboring the fact that their biomass was greater in shallow productive lakes as observed previously. The strong correlation between the fractions 53–100 and 100–202 μm with microphytoplankton concentration (inedible algae) could reflect a more important contamination by algae in these two size fractions.

*Conditional approach in the hypolimnetic waters*
After removing the effect of maximum depth, the explained variance decreased from 70 (Fig. 2e) to 40% (Fig. 2f), and the model retained the microphytoplankton (16%), the oxygen concentrations (15%) and the water alkalinity (9%). This strong diminution is related to the correlation between maximum depth and each of the three variables ($r = 0.66$; $P = 0.001$). The maximum depth did not really influence the zooplankton biomass in hypolimnion because either it was not chosen in the joint RDA or the same relationship occurred after removing its effects.

## DISCUSSION

### Source of variation of the zooplankton biomass

Like phytoplankton (Pinel-Alloul *et al.*, 1996), the greatest source of variation in our zooplankton data was observed at the among-lake scale. This result supports our first hypothesis, according to which environmental changes among lakes are the main sources of variability in total zooplankton biomass and size fractions. Our results and those of Pinel-Alloul *et al.* (Pinel-Alloul *et al.*, 1996) indicate that importance of spatial variation increases with the scale of observation (from within- to among-lake), thus following the scale-continuum concept (Haury *et al.*, 1978) for marine zooplankton. When the scale of observation (temporal and spatial) increases, the zooplankton biomass variability increases too. The environmental processes that influence zooplankton community structure are probably at the origin of this variability (Pinel-Alloul *et al.*, 1990; Tessier and Horwith, 1990; this study). The knowledge of the spatial scale at which significant variation occurs in the community facilitates the explanation of the causes of the variation in zooplankton biomass size fractions (Levin, 1992; Rundle and Jackson, 1996).

The important lake–by–stratum interaction (i.e. non-constant differences among strata from lake to lake) observed in our study reflects the local influence of physico-chemical and biological factors on the zooplankton communities. In their large-scale study on zooplankton spatial structure in Québec lakes, Pinel-Alloul *et al.* (Pinel-Alloul *et al.*, 1995) suggested that 44% of the unexplained variation could be due to factors operating at the lake scale, such as among-stratum spatial heterogeneity, local

effects of abiotic and biotic variables and species behaviour. Although not negligible (11–19%), stratum was not an important source of variation for zooplankton biomass compared to the lake effect (36–55%), but differences in variation among strata from lake to lake were very important (22–46%). Our study took this source of variation into account and attempted to identify factors influencing the variations in total zooplankton biomass and the size fractions at the among-lake and among-stratum scales.

## Primacy of the abiotic environmental control of the zooplankton biomass

As we hypothesized, abiotic environment, such as maximum depth and TP concentration, was the primary force influencing the zooplankton size structure at the among-lake scale. Our results agree with the Pinel-Alloul's model (Pinel-Alloul, 1995), which implies a predominance of abiotic factors in the environmental control of zooplankton community at large spatial scales. The primacy of the abiotic environment also confirms that physical processes *sensu lato* constitute the first step of the hierarchy of processes controlling ecosystems and influencing biological subsystems while, conversely, physical processes are merely influenced by biological systems (Allen and Starr, 1982). However, in specific situations, biological systems can influence physical and chemical processes (Pinel-Alloul *et al.*, 1998). Our findings support the 'multiple driven forces' hypothesis which states an interactive effect of abiotic factors (lake depth, residence time, phosphorus, oxygen and transparency) and biotic, BU (Chl *a* size structure) and TD (fish communities), factors on the control of heterogeneity of zooplankton within Québec Shield lakes (Rodriguez *et al.*, 1993; Pinel-Alloul *et al.*, 1995).

## Epilimnetic control of the total zooplankton biomass and its size structure

The epilimnetic zooplankton biomass was influenced positively by the lake trophic state and negatively by the lake depth. The negative correlations between depth and TP reflect the commonly observed relationship between lake productivity and lake maximum depth. The lake maximum depth (or mean depth) had a strong negative effect on the zooplankton biomass in our study, as in some other studies (Hanson and Peters, 1986; Yan, 1986; Quiros, 1991). The deepest lakes are generally recognized as being less productive systems compared to shallow lakes (Keller and Conlon, 1994), due to limitation of nutrients in the epilimnion during the stratification period.

Total zooplankton biomass and the four size fractions responded positively to TP concentration, as in previous studies (Hanson and Peters, 1986; Pace, 1986; Yan, 1986). However, strong variations were observed at low TP level in the relationship between TP and zooplankton biomass. Some morphometric, physico-chemical and biotic factors may have confounded this relationship. Once the effect of lake maximum depth had been removed, the biomass of small zooplankters (202 μm) was less important in colder and clearer lakes, while the biggest zooplankters (>500 μm) responded rather negatively to the presence of planktivorous fish (no piscivore species) and positively to higher phosphorus concentration. The combined effect of these factors on the phosphorus–zooplankton biomass relationship could thus explain the discrepancies observed in the literature about the response of zooplankton communities in terms of size structure along the phosphorus gradient.

Bays and Crisman (Bays and Crisman, 1983) argued that only small zooplankters biomass increased with lake trophy, while Quiros (Quiros, 1991) and Pace (Pace, 1986) found that both small and large zooplankters increased with lake trophy, thus refuting the hypothesis that relative biomass of small and large zooplankton changed with lake trophy. Although Pace (Pace, 1986) indicated that the disparity could be related to differences either in climate among study areas or in the methodological approach, none of those studies corrected for the confounding of morphometric, physico-chemical and biotic confounding factors, as we did in our study. The influence of abiotic factors on zooplankton biomass has already been observed in a number of studies (Yan, 1986). Fish predation was also identified as an important factor regulating the size structure of zooplankton communities (Brooks and Dodson, 1965; Rodriguez *et al.*, 1993) and their vertical distribution (Masson *et al.*, 2001). In our study lakes, predation played an important role in structuring zooplankton size. Our results support Pace's (Pace, 1986) argument that while zooplankton biomass is predictable from lake trophy, the size structure of zooplankton communities is independent of lake trophy. Quiros (Quiros, 1991) indicated that lakes with planktivorous fish showed lower macrozooplankton biomass when lake trophic state was comparable. Thus, fish predation pressure, TP concentration and thermal stratification (lake depth) are all important determinants of the size structure of zooplankton communities (Keller and Conlon, 1994; Hessen *et al.*, 1995; this study).

## Meta- and hypolimnetic environmental control of the zooplankton biomass

For the meta- and hypolimnion, we hypothesized that the biotic factors should regulate the biomass of the largest zooplankton size fractions (202–500 and >500 μm). This hypothesis was supported by our results since zooplankton

biomass decreased in the meta- and hypolimnion of lakes without piscivores and increased in lakes characterized by high transparency and deep mixing zone presenting one or two piscivore species. The larger zooplankters are more vulnerable to visual predation than smaller ones and thus were probably more influenced by zooplanktivorous fish (Brooks and Dodson, 1965; Masson *et al.*, 2001). The hypolimnetic waters, especially when well oxygenated, offer large zooplankton fractions a refuge from surface turbulence and fish predation in surface layers (Pinel-Alloul *et al.*, 1988; Tessier and Horwith, 1990; Wright and Shapiro, 1990; Masson *et al.*, 2001). The hypolimnion is a stratum with low irradiance and low temperature, which is inhabitable by herbivores but not by their predators (Shapiro, 1990). Thus, large zooplankton may subsist even if planktivores are abundant when a spatial or visual refuge exists (Wright and Shapiro, 1990; Masson *et al.*, 2001).

By removing the effect of maximum depth, we also observed that the zooplankton >500 μm was negatively correlated with the picophytoplankton concentration, suggesting a possible grazing effect on these small algae. As observed in enclosure experiments, the picophytoplankton concentration could be higher in systems dominated by planktivorous fish because the biomass of large zooplankton (>500 μm) is then reduced (Mazumder *et al.*, 1988; Mazumder *et al.*, 1990). Large zooplankters are recognized for their higher efficiency to graze within a broader range (1–50 μm) of organisms and particles.

We further hypothesized that abiotic factors should be the dominant force in structuring the 53–100 and 100–202 μm zooplankton fractions in the meta- and hypolimnetic waters. In the metalimnion, the biomass of these two size fractions still responded negatively to lake maximum depth, indicating a recurrent relationship with lake productivity. On the other hand, a strong correlation with the microphytoplankton concentration was observed in the hypolimnion. This last result does not support that small fractions are controlled by abiotic factors. However, as we mentioned in the Results section, the strong correlation of 53–100 and 100–202 μm zooplankton fraction biomass with microphytoplankton could be partly the result of the presence of algae in the samples due to our size fractionation methodology. Contamination by detritus and algae seemed to be more important in hypolimnetic samples. On the other hand, rotifer species, which are the main organisms in the 53–100 and 100–202 fractions, were also dominant in the hypolimnion of lakes characterized by anoxic waters and shallow mixing depth. These two characteristics regulate their occurrence in hypolimnetic waters (Stemberger, 1995).

# CONCLUSIONS AND FUTURE PERSPECTIVES

Our study supports the conclusion of Masson and Pinel-Alloul (Masson and Pinel-Alloul, 1998), who used the same size fractionation methodology for zooplankton and showed that the vertical distribution of the zooplankton biomass is a function of either avoidance of potential predators or occupation of the best physico-chemical environment. Thus, the analysis of spatial and local factors based on thermal strata is consistent with conceptual models of predation and nutrient control on the mass and size structure of the zooplankton. The organization of the zooplankton size communities in the water column varies according to multiple abiotic and biotic factors. Productivity is probably the most important process regulating zooplankton biomass in Shield lakes at the large scale. However, other abiotic and biotic forces, especially water temperature and transparency as abiotic factors, and planktivory and food resources as biotic variables regulate the size structure and the vertical distribution of the zooplankton community. Our study provided evidence that interaction between zooplankton and abiotic and biotic, BU and TD forces varied with scale of observation, but it did not consider microbial food web. Future work should consider bacterial and flagellate and protist communities because these have been identified as being an important component in the food webs of Shield lakes, and their abundance may be scale dependent.

Our study was conducted within a relatively narrow range of lake productivity values (mean: 3–34 μg L$^{-1}$). We suggest that wider ranges of phosphorus concentration be explored, since fish predation can become increasingly important in determining zooplankton species composition in meso- to eutrophic lakes (Hessen *et al.*, 1995). Changes can also occur in the size structure and species composition of algae and fish as eutrophication proceeds (Watson and McCauley, 1988; Masson *et al.*, 2000). These elements should be taken into account when evaluating the relative effect of biotic and abiotic factors on the biomass and size structure of the zooplankton community. We sampled three stations in each lake, but could not investigate the horizontal variability of zooplankton adequately. On a horizontal gradient, aggregation phenomena such as diel horizontal migration (DHM) and habitat heterogeneity may occur (Pinel-Alloul *et al.*, 1988; Masson and Pinel-Alloul, 1998; Masson *et al.*, 2001), increasing the complexity of the relationships between zooplankton communities, limnological factors and resource availability even further.

# ACKNOWLEDGEMENTS

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