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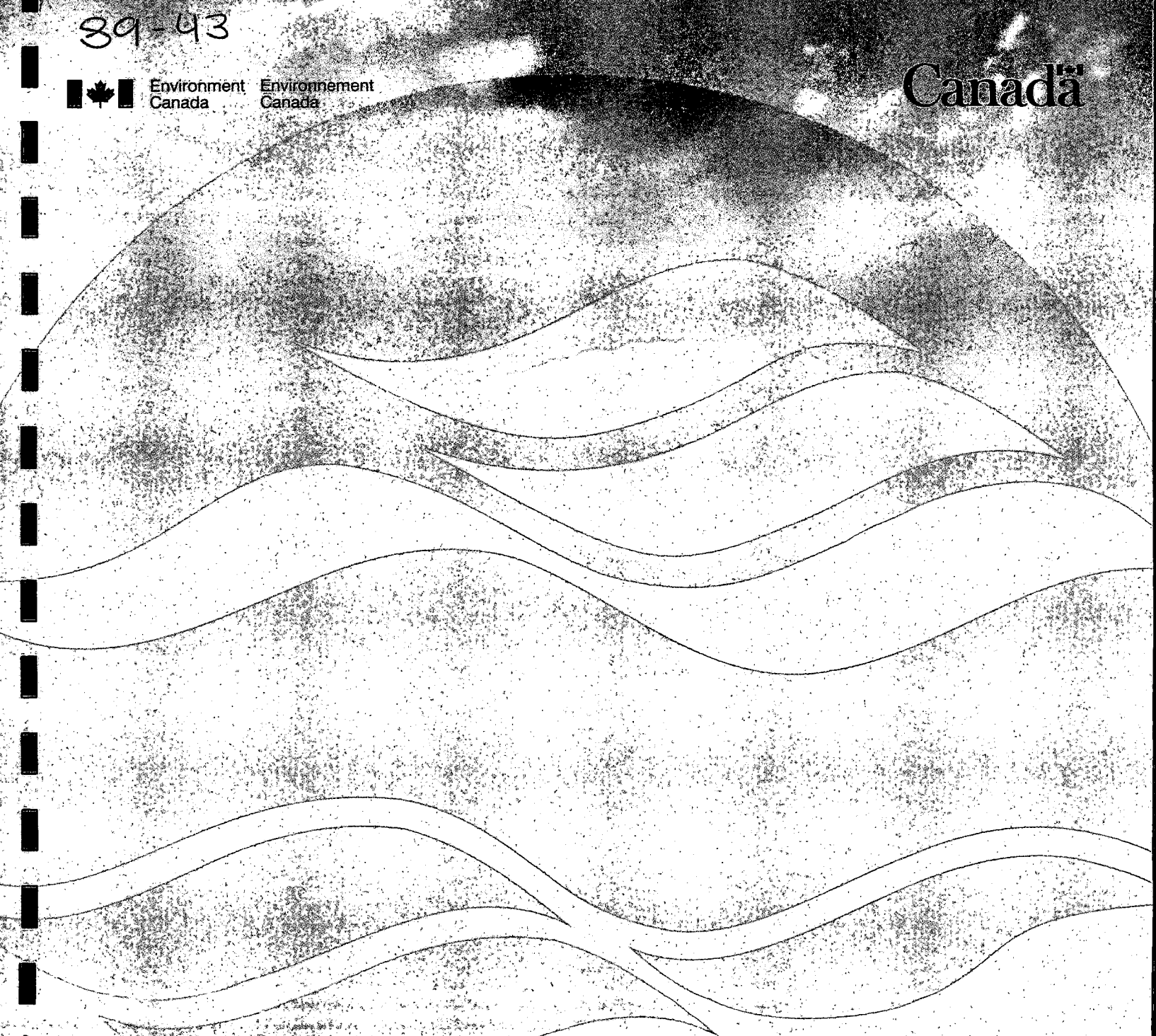

Micro- and mesozooplankton grazing on Natural pico- and nano-plankton in contrasting plankton communities produced by planktivore manipulation and fertilization

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EXECUTIVE SUMMARY

The transport of material from phytoplankton and bacteria through micro- (41-200 um) and mesozooplankton (>200 um) to planktivorous fish is a significant processes in lake ecosystems. We measured in situ grazing by these two classes of zooplankton in large enclosures over two summer periods. Nutrients (nitrate and phosphate) and planktivorous fish (1+ yellow perch) were added in a 2x2 factorial design. Rates in enclosures without yellow perch were always higher without fish. Here, twenty to 90% of the entire epilimnion was filtered by zooplankton each day. In the enclosures with fish the rates were 5-25 % per day and the contribution due to smaller zooplankton was greater. (Midsummer rates in Lakes Ontario and Erie are 5-25 % per day). Of particular interest is the comparison between bottom up control of phytoplankton abundance through nutrient enrichment compared to top-down control through zooplankton grazing. These factors are of particular interest when chlorophyll concentration in lakes such as Lake Ontario fail to respond to reduced phosphorus loading. Changes in planktivorous fish abundance in lakes can totally mask any change in nutrient concentration.

RÉSUMÉ EXPLICATIF

Le transfert de matières depuis le phytoplancton et les bactéries jusqu'aux poissons planctonophages par l'intermédiaire d'organismes microzooplanctoniques (41-200 um) et mésozooplanctoniques (sup. à 200 um) constitue un processus d'importance au sein des écosystèmes lacustres. On a mesuré l'activité in situ de ces deux catégories de zooplancton brouteur dans de grandes enceintes pendant deux périodes estivales. Des éléments nutritifs (nitrate et phosphate) et des poissons planctonophages (perchaude 1+) ont été ajoutés aux enceintes selon un plan d'expérience factoriel 2x2. Les taux relevés dans les enceintes sans perchaude étaient invariablement plus élevés. Dans ces enceintes, entre 20 et 90 % de l'ensemble de l'épilimnion étaient filtrés par le zooplancton tous les jours. Dans les enceintes où des poissons avaient été ajoutés, les taux étaient de 5 à 25 % par jour et le taux de filtration dû aux organismes zooplanctoniques de plus petite taille était plus élevé. (Au milieu de l'été, les taux dans les lacs Ontario et Érié sont de 5 à 25 % par jour.) La comparaison de l'effet ascendant de l'addition d'éléments nutritifs sur l'abondance du phytoplancton et de l'effet descendant du broutage par le zooplancton revêt un intérêt spécial. Ces facteurs sont particulièrement intéressants quand la réduction des rejets de phosphore dans les lacs, comme le lac Ontario, n'influe pas sur la teneur en chlorophylle. Les changements de l'abondance des poissons planctonophages dans les lacs peuvent totalement masquer tout changement de concentration des éléments nutritifs.

ABSTRACT

We conducted in situ grazing experiments over two years in large enclosures to determine how zooplankton regulate the biomass of pico- and nanoplankton. Size fractionated natural pico- (0.2 - 1 and 1 - 3 μm) and nanoplankton (3 - 20 μm) and cultured food organisms (Enterobacter aerogenes and Rhodotorula glutinis) were offered to contrasting micro- (41 - 200 μm) and mesozooplankton (> 200 μm) communities produced by fertilization and addition of planktivorous fish (yellow perch). Community filtering rates of mesozooplankton were always higher in the enclosures without fish. This was largely due to the higher individual filtering rates of mesozooplankton. Microzooplankton had higher community filtering rates than mesozooplankton in the enclosures with fish, and sometimes were as great as mesozooplankton community filtering rates even in the enclosures without fish. Biomass-specific filtering ($ml \cdot \mu g\ zooplankton^{-1} \cdot d^{-1}$) and feeding ($\mu g\ P \cdot \mu g\ zooplankton^{-1} \cdot d^{-1}$) rates of both micro- and mesozooplankton were higher in the enclosures without fish, especially for mesozooplankton, but were similar for the two groups of zooplankton within treatments. Although community filtering rates for both micro- and mesozooplankton changed with size and type of food, microzooplankton had high filtering only on a narrow range of nanoplankton. Experiments in which natural food (0.2 - 20 μm) was exchanged between enclosures suggested that microzooplankton were more selective in their grazing within the nanoplankton fraction. High biomass and community filtering rates of mesozooplankton in the enclosures without fish were always associated with lower biomass of nanoplankton. Mesozooplankton more effectively reduced nanoplankton because they exerted similar grazing pressure on all nanoplankton particles.

Résumé

Des essais de broutage in situ ont été réalisés pendant deux années dans de grandes enceintes afin d'examiner les effets de diverses communautés de zooplancton sur le picoplancton et le nanoplancton indigènes. On a donné du picoplancton (0,2-1 et 1-3 um) et du nanoplancton (3-20 um) classés selon leur taille et des organismes cultivés (Enterobacter aerogenes et Rhodotorula glutinis) à diverses communautés de microzooplancton (41-200 um) et de mésozooplancton (sup. à 200 um) produites par l'addition de nitrate et de phosphate et de poissons planctonophages (perchaude). Les taux de filtration des communautés de mésozooplancton étaient invariablement plus élevés dans les enceintes sans poisson, phénomène dû, dans une large mesure, au taux de filtration plus élevé de chaque organisme mésozooplanctonique. Les taux de filtration des communautés de microzooplancton étaient plus élevés que ceux des communautés de mésozooplanton dans les enceintes avec poissons et étaient parfois même aussi élevés que ceux des communautés de mésozooplancton dans les enceintes sans poisson. Les taux de filtration (ml.ug zooplancton^{-1}.j^{-1}) et d'alimentation (ug P.ug zooplancton^{-1}.j^{-1}) étaient plus élevés tant pour le microzooplancton que le mésozooplancton dans les enceintes sans poisson, et ces taux étaient davantage marqués chez le mésozooplancton; toutefois, ils étaient similaires chez les deux groupes de zooplancton faisant l'objet de traitement. Bien que les taux de filtration des communautés de microzooplancton et de mésozooplancton aient varié en fonction de la taille et du type d'aliment, le taux de filtration de la communauté microzooplanctonique n'était élevé que pour une étroite plage de taille de nanoplancton. Les essais au cours desquels du phytoplancton indigène (0,2-20 um) a été transféré d'une enceinte à l'autre ont révélé que le microzooplancton est plus sélectif dans la fraction nanoplanctonique. Chez le mésozooplancton dans les enceintes sans poisson, les valeurs élevées de biomasse et de

filtration étaient invariablement associées à une plus faible biomasse nanoplanctonique. Le mésozooplancton a réduit de façon plus marquée la communauté de nanoplancton, car ces organismes broutaient indifféremment les particules nanoplanctoniques de toute taille.

INTRODUCTION

The importance of nutrients in regulating phytoplankton biomass and water clarity has been recognized by many early studies (Sakamoto, 1966; Vollenweider, 1969; Schindler et al., 1971; Dillon and Rigler, 1974), but more recent studies have found that pelagic food web interactions are also important (Shapiro et al., 1975; Benndorf et al., 1985, 1988; Carpenter et al., 1985, McQueen et al., 1986; Vanni, 1987b). Mazumder et al. (1988) demonstrated that the responses of phytoplankton communities to changes in zooplankton size-distribution caused by fish predation were mainly in nanoplankton biomass rather than total phytoplankton biomass, and that predation can mediate the impact of nutrient loading.

Although the factors regulating phytoplankton biomass in lakes have received considerable attention, the mechanisms involved in phytoplankton responses to food web changes are still not clear. Some authors suggest that the increase in phytoplankton biomass following a shift from a large to small zooplankton dominated community is mainly determined by more active nutrient regeneration by the abundant microzooplankton (Peters, 1975; Bartell and Kitchell, 1978; Lehman, 1980; Bartell, 1981), and it has been suggested that nutrient regeneration by fish also enhances phytoplankton biomass (Lamarra, 1975; Drenner et al., 1986; Threlkeld, 1988). Nakashima and Leggett (1980) found that nutrient regeneration by fish is far too small to account for nutrient requirements for phytoplankton.

A large literature suggests that zooplankton grazing is an important mediator of phytoplankton response to fish abundance (Lynch and Shapiro, 1981; Bergquist et al., 1985; Lampert et al., 1986; Benndorf et al., 1988). A few studies suggested that the impacts of predation are not significant at phytoplankton level (McQueen et al., 1986, 1989), based on a poor relationship between zooplankton biomass and chlorophyll a

concentration. This poor relationship may be caused by compensatory contributions of small vs large zooplankton and of small vs large phytoplankton to total zooplankton and phytoplankton biomass under contrasting planktivore predation and/or nutrient loading. Pace (1984) found that it is the zooplankton community structure, not biomass, that influences phytoplankton biomass.

Bergquist et al. (1985) suggested that experiments should be done to distinguish the effects of nutrient cycling, sedimentation, and grazing on natural phytoplankton assemblages. Although fish less enclosures and lakes with low planktivore abundances show reduced phytoplankton biomass and this observation has been attributed to grazing (e.g., Shapiro et al., 1975; Scavia and Fahnensteil, 1987; Lampert et al. 1986; Mazumder et al., 1988, 1990; Benndorf et al. 1988), this idea has not been supported by direct measurements of grazing. Also, although high community biomass (Makarewicz and Likens, 1979; Pace and Orcutt, 1981; Pace, 1986; Vanni, 1987a) and filtering rate (Bogdan and Gilbert, 1982; Riemann, 1985; Geertz-Hensen et al., 1987) of microzooplankton have been documented, it appears that only a high biomass of mesozooplankton produces clear water by reducing nanoplankton biomass (Benndorf et al., 1988; Lampert, 1988; Mazumder et al., 1988).

Zooplankton grazing pressure is usually expressed as the percent or volume of water cleared by the zooplankton community per unit time. A very large literature documents zooplankton filtering rates from laboratory and field studies (Peters and Downing, 1984; McCauley and Downing, 1985; reviewed in Lampert, 1988). One might argue that if the abundance and size distribution of zooplankton are known, it may be possible to estimate community filtering or grazing rates using the relationship of individual filtering or clearance rate with body length (Knoechel and Holtby 1986a). However, zooplankton filtering rates are known to vary with food quality and quantity. As we are

interested in the edible size-classes of natural particles in enclosures where they are likely to be qualitatively and quantitatively different, actual measurements of community filtering rates of both micro- and mesozooplankton on different sizes of natural food particles are required.

McCauley and Downing (1985) have emphasized the paucity of grazing rate estimates for zooplankton consuming phytoplankton from natural assemblages, and have suggested that these data will be required before we will be able to understand the dynamics of the interaction between zooplankton and phytoplankton. The importance of using natural plankton assemblages as food for zooplankton in grazing studies also has been emphasized by others (Okamoto, 1984; Bergquist et al., 1985; Haney and Trout 1985; Riemann, 1985; Geertz-Hensen et al., 1987), and although a few studies have used natural plankton as food, they have only considered either a few zooplankton taxa grazing on natural assemblages of phytoplankton and bacteria (Peterson et al., 1978; Okamoto, 1984; Riemann and Søndergaard 1986; Bern, 1987) or major size classes of the zooplankton community grazing on a fraction of the edible plankton assemblage, such as bacteria (Riemann, 1985).

The purpose of this paper is to determine the importance of zooplankton grazing and associated mechanisms in mediating the responses of natural pico- and nanoplankton to changes in zooplankton community structure produced by nutrient and/or planktivorous fish additions to large lake enclosures. We also examine the relative contribution of micro- (41 - 200 μm) and mesozooplankton (> 200 μm) to total zooplankton biomass and community filtering rates, and determine why only a high abundance mesozooplankton, but not that of microzooplankton should be associated with a reduced biomass of nanoplankton, and improved water clarity. We have developed a new method to label food for grazing experiments that allowed us to measure filtering rates of both size-classes of

zooplankton on different size-classes of ambient picoplankton (0.2 - 1, and 1 - 3 μm) and nanoplankton (3 - 8, 8 - 20, and 3 - 20 μm). Although the species composition of both phytoplankton and zooplankton are important in controlling zooplankton grazing rates (Bogdan and Gilbert, 1982, 1984, 1987; Lehman and Sandgren, 1985), there is a rapidly growing literature that suggests that processes regulating the biomass and structure of plankton communities are size-dependent (e.g., Brooks and Dodson, 1965; Peters and Downing, 1984; Chow-Fraser and Knoechel, 1985; Knoechel and Holtby, 1986a,b; Dickie et al., 1987; Mazumder et al., 1988, 1989; Stein et al., 1988). We, therefore, chose to adopt a size-based approach as a means of summarizing particle availability and consumption. But because our grazing estimates ignore species-specific interactions among different species of zooplankton and phytoplankton, we switched food between contrasting enclosures to examine the effect of food quality on grazing rates and we calculated biomass-specific feeding and filtering rates to examine the differences in functional response of zooplankton among treatments. We also used two types of cultured food (Enterobacter aerogenes and Rhodotorula glutinis), to make comparisons across communities that were independent of the ambient particles, and to see how our grazing rates using natural particles compared from those using these two widely used foods.

MATERIALS AND METHODS

Experimental setup and treatments

Data were collected from eight large enclosures (8 m in diameter, 15 m deep and open at the sediment) in Lake St. George, Toronto, Ontario, during the summers of 1986 and 1987. Nutrients (N and P) and planktivorous fish (1+ yellow perch) were added in a 2x2 factorial design. Treatments were control (Control), fish (+F), nutrients (+N), and

nutrients and fish (+NF). Two replicate enclosures were used for each treatment (see Mazumder et al., 1988 for details).

Zooplankton sampling and enumeration

The biomasses of micro- and mesoplankton for 1986 are described elsewhere (Mazumder et al., 1988). In 1987, samples were collected separately for each meter depth from 0 to 12 m using a 35 liter Schindler-Patalus trap with a 41 μm mesh screen, and preserved in 4% sugar - formalin solution (Haney and Hall, 1975). Only the samples collected from the epilimnion (0, 1, 2, 3, and 4 m) are reported here. Details of the counting procedures are described in Post and McQueen (1987). Abundance and biomass of total zooplankton were divided into micro- (including rotifers, nauplii, Bosmina, cyclopoids, and chydorids) and mesozooplankton (Daphnia, Ceriodaphnia, Diaphanosoma, calanoids, and Asplanchna) for convenience of illustration. Biomasses of zooplankton from fractionated grazing apparatus samples were estimated using length-weight relationships (McCauley, 1984). Collection and analysis of pico- and nanoplankton are described in Mazumder et al. (1988).

Description of the in situ grazing apparatus

A three-chambered grazing apparatus (Fig. 1) equipped with a filter fractionating device was used for in situ grazing estimates. This three-chambered device is simple to operate and it saves time. A syringe injects radio-labelled food particles into each chamber, promoting rapid mixing, and a removable food holder minimizes the likelihood of spilling radioactive materials. Although this chamber can be used for three different types of food simultaneously, we used it to collect triplicate estimates of community filtering rates. Grazing measurements were conducted between 09:00 and 18:00 hrs. On each date, when

5 to 7 different food types were used, a total of 120 to 186 grazing estimates were obtained in 6 to 8 hours. This allowed us to obtain all the measurements in one day. Comparison of our design with the original one-chambered design (Haney 1971) indicates that the mean community filtering rates are similar using both designs (Table 1; and Mazumder and Dickman, 1989).

The apparatus (Fig.1a) consists of three transparent plexiglass cylinders (4.13 liters) mounted on a light stainless steel frame (74 cm wide and 70 cm high). Each cylinder (CY) has a plexiglass holder (SH) at the top for a 10-mL hypodermic syringe (S). Upper and lower closures (U and L) made of 2 mm stainless steel with silicon-rubber lining (3 mm thick) were mounted to the frame with two brass hinges. Each lid is closed by 2 straps of 1 cm diameter elastic surgical tubing (EL). These are attached to the outer edge of each lid and to the frame near the base of the cylinders. The elastic tubing can be replaced quickly as required for changes in chamber volume (e.g., stronger tubing for larger cylinders). Two wires (UW and LW), one from each lid, are used to keep the cylinders open. A messenger-triggered release unit (MRS) made of brass is mounted on the back of the frame (F; Fig.1c). This is used to close the chambers under water at the desired depth. The MRS unit consists of a trigger-head (TH), the trigger-rod (TR), and a rotating disc (LD) with two knobs (WL). The trigger-head is connected with the trigger-rod, which engages a hole on the rotating disc.

A rope (R) is passed through the center of the trigger-head and is tied at the lower end of the trigger-head holder (Fig. 1c). When the trigger-head is pulled up and the trigger-rod is engaged with the hole on the disc, the rotating disc is locked with both knobs positioned diagonally (Fig.1c). Both lids are opened, with their wires passing over the wire retainers (WR) and kept open by locking the loops of the wires around the knobs. The hypodermic syringes are then removed from the syringe holders, the desired volume

of food is drawn into each syringe, and they are put back into the holders. The trap is then ready to be lowered. A messenger is dropped, simultaneously pushing down the trigger-head (1), unlocking the rotating disc (2) and releasing the wires holding lids open (3). As soon as the lids are closed (4), the piston-heads of the syringes are pushed down (5) and the radiotracer food is injected immediately into the closed grazing chambers (6). The efficiency of the food ejection was tested by observing the dispersion of colored dye. Injected dye was found to disperse in less than 5 sec.

Description of the filter fractionation device

We have designed a filter fractionation device that can be used for immediate screening or size-fractionating of zooplankton in one to several size-categories (Fig. 1d). This filter fractionation device was used for separating mesozooplankton (> 200 μm) from microzooplankton (41 - 200 μm), and produced little overlap in taxonomic or functional groups, suggesting that this separation is of heuristic value (Table 2). It is also practical, in terms of the amount of time required, compared to where individual zooplankters are measured and counted microscopically.

One filtration unit (containing 1 to 3 filter holders) remains connected to the outlet (Tygon tubing) of each grazing chamber. The filtration units are made of glass (Pyrex) funnels and cylinders (42 mm internal diameter) with threaded glass ends. Funnels and cylinders are held together with 42 mm couplings (C, SVL coupling, J. Bibby Science Products Ltd, Staffordshire, U.S.A). These couplings consist of two separately threaded sections, which are made rigid by a collar so that both sections can be used for screw connection to threaded glass ends independently of each other. A silicon rubber o-ring is placed between the two glass ends, which makes the joint water tight and prevents the glass ends from breaking. For our use, we modified the o-ring into a filter holder (H) by

mounting a plastic screen (1.5 mm mesh) on the ring. The filter holder supports a nylon (Nitex™) screen (SCR) used to filter the zooplankton.

The grazing experiments followed Haney (1971) with some modifications, such as the use of natural plankton assemblages instead of cultured food and assay of the radioactivity collected on a nylon screens without using preservatives. Grazing experiments were conducted on eight occasions over two years. On the first two dates (19 June and 6 July, 1986), experiments were conducted only in the control and +F enclosures, and community filtering rates were estimated for mesozooplankton feeding on one size category of natural plankton (0.2 - 20 μm). Similar experiments were conducted on 24 July, 9 August, 1986 and 25 June, 1987, but were run in all four treatments. Both natural and cultured food were used on 25 June. On the other dates (20 August, 1986, 17 July and 24 August, 1987), experiments were conducted in all treatments, and community filtering rates were estimated for both micro- and mesozooplankton feeding on different size fractions of natural pico- (0.2 - 1, 1 - 3 μm) and nanoplankton (3 - 8 or 3 - 20 μm), and for laboratory cultured bacteria (Enterobactor aerogenes) and yeast (Rhodotorula glutinis). Bacteria and yeast concentrations in the culture were ~6 - 8 x 10^6 cells · ml^{-1} and ~2 - 3 x 10^5 cells · ml^{-1}, respectively. Final concentrations of these organisms in the grazing chamber were 0.75 - 1.0 x 10^4 cells · ml^{-1} and 250 - 375 cells · ml^{-1}.

Plankton were labelled with $^{32}PO_4^{3-}$. A potential problem associated with using carrier free $^{32}PO_4^{3-}$ to label the natural assemblage of plankton is that most (> 80%) of the isotope is assimilated by bacteria-sized particles (0.2 - 1 μm), and very little is taken up by the larger plankton (Mazumder et al., 1988). This is critical for our method of using ^{32}P-labelled natural pico- and nanoplankton as food for zooplankton, because grazing estimates on may be biased for size-selective grazers when the proportion of isotope assimilated by these fractions is different from their contribution to biomass. This difficulty

can be reduced by adding unlabelled phosphate ($^{31}PO_4^{3-}$) to plankton samples prior to addition of $^{32}PO_4^{3-}$, which promotes the uptake of more isotope by the larger plankton (Taylor and Lean, 1981).

Lake water (0 - 4 m integrated samples) collected from each enclosure was filtered through a 20-μm screen to remove the grazers and was spiked with $^{31}PO_4^{3-}$ (5 μg P · liter^{-1} final concentration) 30 seconds prior to addition of 7.4 MBq of carrier free $^{32}PO_4^{3-}$ (final concentration 0.037 MBq · ml^{-1} or 1.8 x 10^6 dpm · ml^{-1} of food, or 2250 dpm · ml^{-1} in the grazing chamber). After 12 hours of incubation, 20 ml of labelled water was filtered through each of 0.2, 1, 3, or 8 μm Nuclepore™ filters. The 1, 3, and 8 μm filtrates, and the < 20 μm screened lake water, were used as food. Aliquots of 1 ml from each filtrate were diluted 100 times, and 1 ml subsamples were placed in glass scintillation vials. Radioactivity in these samples was estimated to determine the proportion of $^{32}PO_4^{3-}$ assimilated into different fractions (0.2 - 1, 1 - 3, 3 - 20 or 8 - 20 μm). Virtually all of the added isotope was assimilated by the plankton (> 95% of the ^{32}P was retained by the 0.2 μm filter).

As the concentrations of particulate phosphorus (PP) in these fractions were determined for the same dates (Mazumder et al., 1988; and this study), it was possible to calculate the specific activity, or the ratio of assimilated $^{32}PO_4^{3-}$ to PP, in each size fraction. These ratios for different size fractions were close to 1, indicating that the specific activity of phosphorus in the size classes was approximately equal (Table 3). We also labelled the plankton sample long enough (12 h) so that even phagotrophic organisms (flagellates and ciliates) would be at least partly labelled via grazing. Pure cultures of bacteria and yeast were maintained in phosphorus deficient culture medium. They were labelled in 50 ml aliquots with 3.7 MBq of $^{32}PO_4^{3-}$ for 12 hrs.

A single run of a grazing experiment involved loading each of the three food holder syringes with 5 ml of labelled food, lowering the feeding chamber to 2 m depth (middle of the epilimnion), and simultaneously closing the chambers and injecting the labelled food using the messenger. After 6 to 8 min of feeding, the trap was retrieved and the control valve to the fractionating device was opened to collect the zooplankton. The fractionating device had removable 200 µm nylon mesh at the top and 41 µm nylon mesh at the bottom. Zooplankton retained on the nylon screens were washed several times with filtered lake water, and each screen with zooplankton was placed in a scintillation vial. To estimate the radioactivity in the chamber, one 5 ml aliquot of filtrate was collected from each chamber. On a few occasions, both micro- and mesozooplankton were screened out immediately (0 exposure time) after the labelled food was injected. No radioactivity was detected in the zooplankton.This suggests that the screens were not retaining uningested labelled food.

Liquid scintillation fluor (10 ml of PCS, Amersham) was added to the samples within 24 hrs of collection. After about 2 hrs, 5 ml of water (~30%) was added to the fluor to form a transparent gel, so that the zooplankton did not settle to the bottom of vial thereby reducing counting efficiency. Quenching by the nylon screen was assessed by putting a known amount of isotope, fluor (10 ml), and water (5 ml) in three sets of five vials. Nylon screens, 41 µm or 200 µm, were added to two of these sets. There was no observable difference in counting efficiency among the three sets of vials. Community filtering rates (FR, $\%\cdot d^{-1}$) for each size category of zooplankton (41 - 200 µm or > 200 µm) on each prepared food(< 1, < 3, < 8, or < 20 µm), bacteria, and yeast were calculated using the following equation:

$$FR = \frac{\text{radioactivity in zooplankton x 60 min x 24 h x 100}}{\text{radioactivity in chamber x feeding time (min)}}. \qquad (1)$$

Community filtering rate (%·d^{-1}) on any specific size fraction (0.2 - 1, 1 - 3, 3 - 8, or 8 - 20 μm) was calculated using the following equation:

$$FR_{ij} = \frac{FR_j \times DPM_j - FR_i \times DPM_i}{DPM_j - DPM_i}. \quad (2)$$

DPM_i, DPM_j = proportion of total $^{32}PO_4^{3-}$ assimilated by food size categories <i, <j

FR_i, FR_j = community filtering rate on food size categories <i, <j, as calculated by equation (1)

FR_{ij} = community filtering rate on food size category i to j

i, j = 0.2, 1, 3, 8, or 20 μm, i < j.

Statistical analysis

The design of the experiment was a Model I two-way analysis of variance (ANOVA) with two replicate enclosures per treatment and six repeated measurements of community filtering rates per enclosure for micro- and mesozooplankton feeding on each size and type of food particle. Effects of fertilization, predation, and their interaction were estimated for community filtering rates of micro-, or meso- or total zooplankton on each size fraction and type of food (ANOVA, error degrees of freedom (edf) = 45). The effects of food size or food type on community filtering rates were estimated separately for micro- and mesozooplankton. Comparison of community filtering rates on bacteria and yeast with natural size fractions (0.2 - 1 and 1 - 20 μm) was done using paired t-tests. Effects of switching of food (0.2 - 20 μm) on micro- or mesozooplankton were also assessed using paired t-test. Prior to conducting statistical analysis, variance ratio tests were done to examine whether the variances were different between replicate enclosures or over all. Wilkinson's (1986) SYSTAT statistical package was used for all statistical analyses.

RESULTS

Biomasses of micro- and mesozooplankton

On all dates in 1987, abundance and biomass of microzooplankton were higher in the enclosures with fish ($P < 0.001$), while abundance and biomass of mesozooplankton were higher in the enclosures without fish ($P < 0.001$; Fig. 2). Biomass of total zooplankton (micro- + mesozooplankton) was lower with fish on 13 July and 12 August ($0.009 < P < 0.031$). On three of four dates, biomass of microzooplankton was as high as the biomass of mesozooplankton, even in the enclosures without fish. Fertilized enclosures had higher microzooplankton biomass on 23 June and 12 August ($0.021 < P < 0.038$), and increased mesozooplankton biomass only 12 August in the enclosures without fish. The data for abundance and biomass of micro- and mesozooplankton for 1986 are not presented here because the mesh-size (80 μm) of net used in 1986 underestimated microzooplankton. Comparable data for micro- and mesoplankton in1986 are presented in Mazumder et al. (1988).

Concentration of pico- and nanoplankton

The concentration of total PP in pico- and nanoplankton was consistently higher in the enclosures with fish and was largely attributable to an increase in nanoplankton. Concentrations of pico- and nanoplankton in different treatments for 1986 were presented in Mazumder et al. (1988). In 1987, a similar response was observed. The small picoplankton (0.2 - 1 μm) fraction was higher with fish on two dates ($0.039 < P < 0.058$; Fig. 3), and the large picoplankton (1 - 3 μm) fraction was higher in the enclosures with fish on all three dates ($0.006 < P < 0.019$). Fertilization increased the concentration of large picoplankton only in the enclosures with fish. Nanoplankton (3 - 20 μm) showed the greatest response to fertilization and addition of fish. Concentrations were with fish on all

dates ($P < 0.001$), and higher with fertilization on two out of three dates (13 July and 12 August; $0.021 < P < 0.047$). Maximum concentration of nanoplankton was observed in the fertilized enclosures with fish (Fig. 3).

Zooplankton community grazing

Community filtering rates (Figs. 4 and 5) of mesozooplankton on natural 0.2 - 20 μm particles were higher without fish (control and +N) ($0.001 < P < 0.02$) on all 8 dates in 1986 and 1987, and higher with fertilization (+N and +NF) on only two of eight dates ($0.021 < P < 0.058$). The community filtering rates of mesozooplankton were highest in the fertilized enclosures without fish on seven of eight dates (exception 17 July, 1987). Total (micro- + meso-zooplankton) filtering rates were higher without fish on two of three dates (17 July and 24 August, 1987 (Fig. 5). On 20 August, 1986, total filtering rates were not significantly different in different treatments because of higher community filtering rates for the microzooplankton in the enclosures with fish where meso-zooplankton community filtering rates were lower. In the enclosures with fish, the community filtering rates of microzooplankton were often as great as or greater than the community filtering rates by the mesozooplankton. On all three dates, there was no effect of treatments, either addition of fish or fertilization, on the community filtering rates of microzooplankton feeding on 0.2 - 20 μm natural plankton assemblages (Fig. 5). However, community filtering rates of micro-zooplankton on larger fractions (1 - 20 μm on 17 July, and 8 - 20 μm on 24 August) were higher with fish addition (Fig. 6; $0.011 < P < 0.025$), and sometimes higher than mesozooplankton even in the enclosures without fish (17 July, 1987).

Effect of food size on filtering rates (Fig. 6) was significant for both size classes of zooplankton on all three dates ($0.009 < P < 0.021$). Mesozooplankton community filtering

rates increased with increasing size of food particles in the enclosures without fish (control and +N), while they decreased or changed little in the enclosures with fish (+F and +NF), especially on nanoplankton fractions. Community filtering rates of microzooplankton also usually increased with increasing size of food particles (Fig. 6), but the magnitude of the increase was much lower than for mesozooplankton in the enclosures without fish. The community filtering rates of mesozooplankton were also consistently high on all size-classes of large pico- and nanoplankton.

Individual and biomass specific filtering rates and feeding rates

On one date (24 August, 1987), when community filtering rates among treatments and between the two groups of zooplankton were significantly different (Fig. 7a), we measured the abundance and biomass of micro-, meso-, and total zooplankton from samples collected and fractionated using the grazing apparatus (Table 2 and Fig. 7b, c). Concentration of P in pico- and nanoplankton fractions were also measured (Fig. 7d). These estimates were used to calculate the individual and biomass specific filtering (Fig. 7e, g) and feeding rates (Fig.f, h) of micro- and mesozooplankton. Higher community filtering rates of micro- and mesozooplankton were associated with higher individual and biomass specific filtering rates. Individual filtering rates for both sizes classes of zooplankton were two to five times higher in the enclosures without fish and three to eight times higher for mesozooplankton than microzooplankton within any treatment. However, filtering rates per unit biomass of zooplankton were similar for micro- and mesozooplankton within treatments, and much higher in the enclosures without fish (Fig. 7g). Biomass specific feeding rates for micro- and mesozooplankton were higher in the enclosures without fish, even though nanoplankton biomass was lower, but similar for both groups within treatments with the exception of higher rate for microzooplankton in the +NF enclosures (Fig. 7h).

Micro- and mesozooplankton filtering on cultured food and natural particles

Community filtering rates were similar for total zooplankton feeding on cultured bacteria and on natural bacteria-sized particles (Fig. 8). However, when micro- and mesozooplankton are considered separately, compensating differences emerged. Community filtering rates based on cultured bacteria produced different treatment effects than those based on natural bacteria-sized particles. Microzooplankton had slightly lower filtering rates on natural bacteria sized particles than on cultured bacteria in the enclosures with fish. The reverse was observed for mesozooplankton (Fig. 8). In the control enclosures, microzooplankton had higher filtering rates on natural bacteria than on cultured bacteria, and again, the reverse was observed for mesozooplankton.

Community filtering rates based on cultured yeast and natural nano-plankton produced similar treatment effects, but filtering rates on yeast were several fold lower than those based on natural nanoplankton in all treatments (Fig. 8). Microzooplankton community filtering rates were three to ten times higher on ambient 1 - 20 μm plankton than on yeast, while mesozooplankton feeding was similar or slightly lower for natural nanoplankton.

Micro- and mesozooplankton filtering on ambient and exchanged natural particles

Estimates of community filtering rates of microzooplankton from enclosures with fish (+F and +NF) were higher ($P < 0.001$) when they were fed on plankton from the enclosures without fish (control and +N) (Fig. 9). On the other hand, microzooplankton in the enclosures without fish (control and +N) had lower filtering rates on plankton from enclosures with fish (+F and +NF).

Mesozooplankton community filtering rates changed slightly or remained the same following these exchanges of similar sizes of plankton among treatments (Fig. 9). The mesozooplankton in the enclosures without fish (control and +N) had similar or slightly higher filtering rates on food from the enclosures with fish (+F and +NF). In the fertilized enclosures with fish (+NF), mesozooplankton community filtering rates were higher when they were presented with food from enclosures without fish (+N).

DISCUSSION

The addition of planktivorous fish to the enclosures had the expected effects on zooplankton; biomass of mesozooplankton (mostly Daphnia) was reduced, and the zooplankton community was dominated by microzooplankton (rotifers, Bosmina, nauplii, and cyclopoids). Total zooplankton biomass was reduced in most cases. These changes in zooplankton community structure were associated with increased biomass of nanoplankton (this study; Mazumder et al., 1988). In the fish free enclosures, both unfertilized and fertilized, abundant mesozooplankton (mostly Daphnia) reduced the biomass of pico- and nanoplankton and improved water clarity.

Community filtering rates of mesozooplankton on 0.2 - 20 μm food were always highest in without fish. Microzooplankton had higher filtering rates than mesozooplankton in the enclosures with fish, but the sum of micro- and meso-zooplankton community filtering rates was higher on 2 of 4 dates for enclosures without fish. Riemann (1985) reported higher grazing of mesozooplankton (> 140 μm) on natural bacteria (^{3}H-thymidine labelled particles) in enclosures without fish and higher grazing of microzooplankton (50 - 140 μm) in enclosures with fish. He speculated that the grazing rate of heterotrophic flagellates may be an order of magnitude higher than for mesozoo-plankton in the enclosures with fish. Although we did not have data on the abundance of

heterotrophic flagellates, we found that ciliates, some of which graze on bacteria, were more abundant in the enclosures with fish (unpublished data). Therefore, although filtering rate was often reduced in the enclosures with fish compared to the enclosures without fish, protozoan micro- and nanoplankton grazers might compensate for this difference.

Our estimates of individual filtering rates for both groups of zooplankton indicate that the higher community filtering rates are largely due to higher individual filtering rates of micro-, meso-, and total zooplankton in the mesozooplankton dominated communities of enclosures without fish. In these enclosures, abundant mesozooplankton filtered two to five times faster per individual than those in the enclosures with fish. Zooplankton filtering rate is often higher below limiting food concentrations (Rigler, 1961; Lampert, 1977, 1978). In our enclosures with fish, pico- and nanoplankton biomass were at least two times greater than those in the enclosures without fish (Mazumder et al., 1988; Figs. 3 and 7d, this study). It is possible that these high concentrations of food particles in the enclosures with fish were above limiting food concen-trations. However, that differences in food concentration underlie the treatment differences in filtering rate is contradicted by the biomass specific feeding rates (Fig. 7h). The biomass specific filtering rates of both micro- and mesozoo-plankton were similar within treatments, but were much lower in the enclosures with fish. This indicates that zooplankton, especially mesozooplankton, in fishless enclosures are ingesting more 0.2 - 20 μm food per unit weight. This probably reflects a difference in species composition, rather than in the activity of the same species. Some of the dominant zooplankton taxa in the enclosures with fish, for example cyclopoids and their nauplii, may contribute more to biomass than to community filtering rate. Therefore, the conclusion that micro-zooplankton had higher biomass-specific feeding rates may not contradict the known allometric relationships between body-size and metabolism (e.g., Hall et al., 1976; Dickie et al., 1987).

Within the size-spectrum of food we used (0.2 - 20 μm), there was a strong effect of particle-size on filtering rates. Nanoplankton were removed at higher rates than picoplankton, especially in the enclosures without fish. This is consistent with the low observed biomass of nanoplankton in the enclosures without fish (this study; Mazumder et al., 1988). Small picoplankton (0.2 - 1 μm) were usually removed at lower rates, especially by the mesozooplankton, and treatment effects on community filtering rates for this fraction were weak; this fraction differed least between enclosures with and without fish. However, we must emphasize that this analysis does not include protozoan grazers.

Similar observations of food-size effects on filtering rate were reported for Daphnia feeding on different size classes from natural plankton assem-blages (Okamoto, 1984). Knoechel and Holtby (1986b) obtained higher slopes for the relationship of filtering rate to body length when they used larger laboratory food. This may have been due to relatively high filtering rates for larger zooplankters on larger food. Small zooplankton also prefer larger food (Bleiwas and Stokes, 1985; Bogdan and Gilbert, 1982, 1987).

The "exchanged food" experiments (Fig. 9), in which labelled food from enclosures without fish was used in enclosures with fish, and vice verse, suggest another important difference between these enclosures in addition to total community filtering rate. These results suggest that pico- and nanoplankton food from microzooplankton dominated systems (+F and +NF enclosures) was not filtered efficiently by microzooplankton in the mesozooplankton dominated systems (control and +N enclosures). Similar sized food from mesozooplankton dominated systems produced higher filtering rates by microzooplankton in microzooplankton dominated systems. This implies that the favored food particles for microzooplankton were depleted in microzooplankton dominated enclosures, but not in mesozooplankton dominated ones, despite the fact that pico- and nanoplankton were most abundant in microzooplankton dominated enclosures. This is consistent with the

hypothesis that microzooplankton are more selective in their feeding, and therefore may have a greater effect on species composition within the pico- and nanoplankton size classes. Mesozooplankton community filtering rates changed slightly or remained unchanged on food from contrasting systems. This suggests that larger zooplankton exert less selection within these size classes. We suggest that high grazing pressure from microzooplankton may tends to favor grazing resistant or inedible plankton within the nanoplankton size-class (this study), while high grazing pressure from mesozooplankton tend to favor grazing resistant or inedible microplankton (Bergquist et al., 1985; Mazumder et al., 1988). The qualitative difference in the nanoplankton of the two systems should be verified by more detailed analysis of grazing on individual species of pico- and nanoplankton.

Our results also suggest that knowing only the community filtering rates of zooplankton on standard foods is not enough to predict the impact of grazing on phytoplankton community structure and biomass. Filtering rates on cultured yeast were lower than on natural nanoplankton, especially for microzoo-plankton, and less different among treatments. Cultured bacteria produced similar filtering rates when compared with natural bacteria sized particles, but treatment effects on filtering rate were different. The response of microzoo-plankton to exchanged food, of course, could not have been discovered with cultured foods.

Although grazing by zooplankton reduces nanoplankton directly, it may also influence the biomass of plankton in different size-categories and total biomass by mediating the competition for phosphate between small and large plankton . Turnover-time for phosphate was longer in the mesozooplankton dominated enclosures without fish, indicating less severe nutrient limitation (Mazumder et al., 1988). Large inedible phytoplankton, which usually cannot compete with small cells, may take advantage of the

available nutrients and increase in abundance following reduction in nanoplankton abundance by the large grazers. Therefore total algal biomass will be reduced less than nano-plankton biomass, and the effect of the mesozooplankton grazing on water clarity will be greater than might be expected from changes in phytoplankton biomass or chlorophyll *a* concentration alone. The large microplankton in enclosures without fish also contribute more to sedimentation (Mazumder et al., 1989). Lastly, the larger fraction of P sequestered by zooplankton is not available to phytoplankton.

Our study supports the hypothesis that nanoplankton experience greater filtering rates in mesozooplankton dominated communities in the enclosures without fish. Zooplankton biomass is higher in these enclosures, but the higher grazing rates are largely due to higher individual and biomass specific filtering rates. The feeding selectivity of microzooplankton within the nanoplankton size-category may be an important factor in allowing nano-plankton to become abundant in the enclosures with fish. This selectivity may explain how high community filtering rates of microzooplankton in the enclosures with fish can be associated with high biomasses of nanoplankton and how changes in the biomass of mesozooplankton, but not in micro-zooplankton, produce the manipulation effects of reduced biomass of nanoplankton and improved water clarity.

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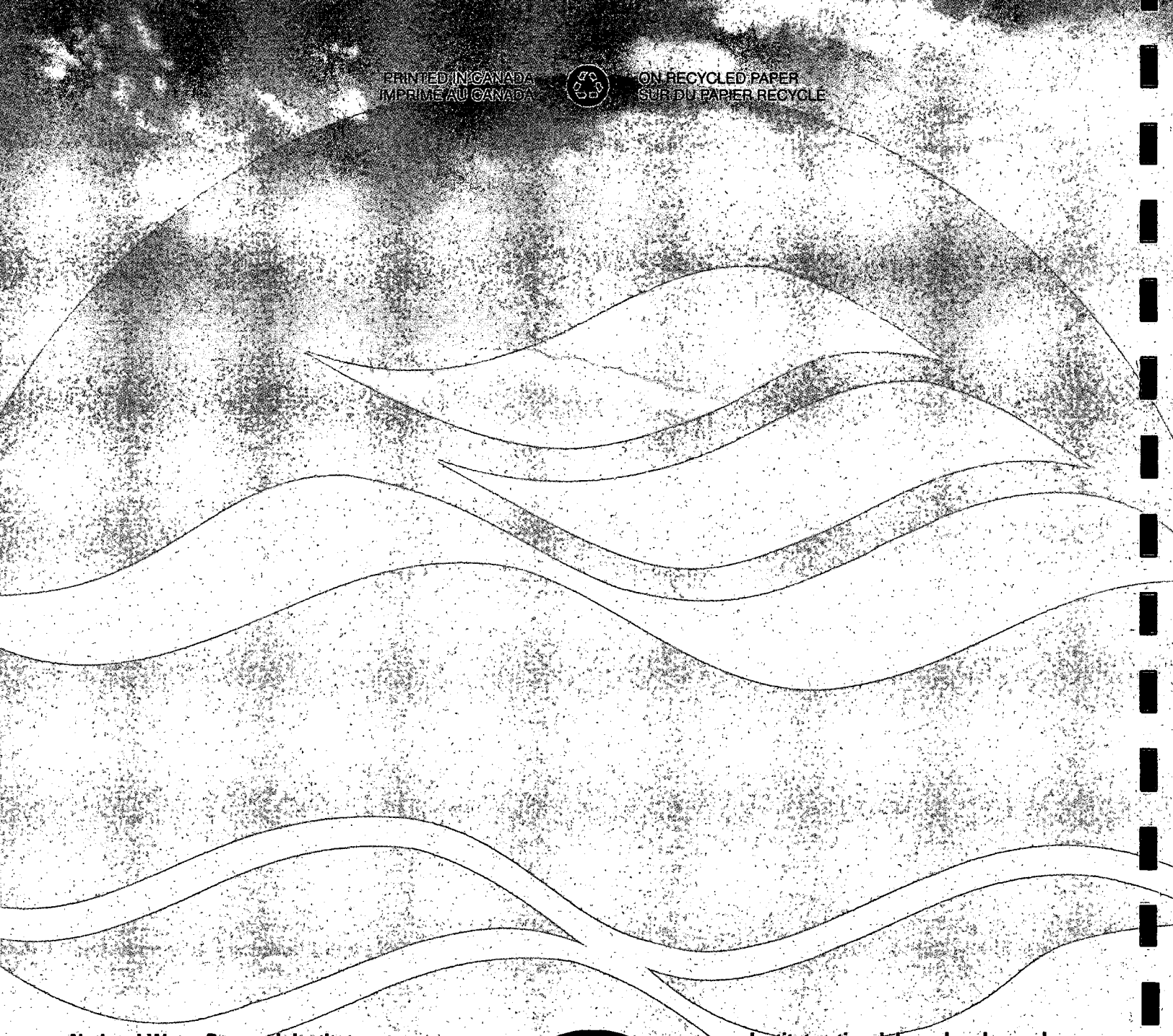

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