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PRIMARY RESEARCH PAPER

# Dynamic adaptation of phytoplankton vertical migration to changing grazing and nutrient conditions

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**Abstract** Despite the ubiquitous occurrence of vertical migration of phytoplankton its quantitative significance remains poorly known. We eliminated a dense *Daphnia* population in a pond by introducing whitefish fingerlings, and assessed the effects on the vertical migration of dominating motile phytoplankton. At the highest abundance of *Daphnia*, cryptophytes reduced grazing losses by staying in the hypolimnion day and night, but *Mallomonas* species armoured by silica bristles remained in the epilimnion. After the fish introduction, phytoplankton was released from *Daphnia* grazing pressure, allowing cryptophytes to occur in the epilimnion also at noon. At the same time, increased phytoplankton biomass exacerbated the nutrient depletion. Cryptophytes compensated for that by migrating into the anoxic hypolimnion, whereupon their growth rates increased. The collapse of *Daphnia* was also associated with a temporary increase in nutrient regeneration by enzyme activities and decreases in total nutrient concentration and bacterial biomass in the whole water column. Our results show that cryptophytes can dynamically modify their vertical migration to balance between the exploitation of various nutrient resources and the risk of becoming eaten. Hypolimnetic nutrient resources can be quantitatively more important for phytoplankton than previously assumed.

**Keywords** Anoxic hypolimnion · Enzyme activity · Fish manipulation · Grazing · Nutrients · Zooplankton

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## Introduction

Occurrence of diel vertical migration (DVM) in aquatic organisms from bacteria to fish (Pedrós-Alió & Sala, 1990; Mehner, 2012; Bandara et al., 2021) suggests its fundamental role in aquatic ecosystems. Despite numerous studies (e.g., Heaney & Talling, 1980; Salonen & Rosenberg, 2000; Clegg

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et al., 2004), the quantitative and qualitative significance of factors explaining phytoplankton DVM are only emerging. Predation avoidance has been long accepted as the ultimate reason for the vertical migration of zooplankton (Hays, 2003). Theoretically, the same applies to phytoplankton, but experimental and observational evidence is scarce. Bollens et al. (2012) found experimentally that a marine dinoflagellate *Akashiwo sanguinea* (K. Hirasaka) Hansen & Moestrup responded to copepod grazing by increasing its DVM amplitude. However, the data (two tank experiments and one copepod species) were so limited that the results are only indicative.

Because phytoplankton depends on photosynthesis, their DVM is generally synchronized with the diel light cycle, but it can be modified by contemporary changes in weather, nutrient availability, and grazing. Consequently, the day and night vertical distributions of flagellated phytoplankton differ from time to time. Along with mixed layer nutrients which typically limit phytoplankton in lakes (Sommer, 1988) as well as oceans (Howarth, 1988), grazing, and sedimentation losses also influence phytoplankton biomass. During thermal stratification, sedimentation of nutrients from the epilimnion often exceeds their inflow, and reflux from the hypolimnion is limited by slow diffusion and mixing processes. Consequently, in summer, epilimnetic inorganic nutrient concentrations often decrease to a limiting level (Lewis & Wurtsbaugh, 2008), which has led to the evolution of a striking number of nutrient acquisition strategies in phytoplankton. These include storage of nutrients (e.g., Fitzgerald, 1966; Lewis et al., 1999), induction of extracellular enzymes (Vrba et al., 1993; Stoecker & Gustafson, 2003), the substitution of non-phosphorus (P) membrane lipids for phospholipids (Van Mooy et al., 2009), dormancy (McQuoid & Hobson, 1996), bacterivory (Urabe et al., 2000; Medina-Sánchez et al., 2004), and migration between nutrient-depleted and replete water layers (e.g., Salonen et al., 1984; Cullen, 1985; Watanabe et al., 1991; Salonen & Rosenberg, 2000). Thus, the nutrient uptake of phytoplankton under limiting conditions can be highly complex and variable (Cullen, 1985) which under natural conditions has never been addressed in all its dimensions.

Vertically migrating phytoplankton can harvest resources apart from each other in space or time. In the daytime, light is available in the uppermost water layer, while richer nutrient resources are typically available in the dark metalimnion or hypolimnion. Phytoplankton vertical migration has been observed in waters ranging from small ponds (e.g., Salonen et al., 1984; Gasol et al., 1992) to oceans (Cullen, 1985; Watanabe et al., 1991). One of the highest DVM amplitudes of flagellated phytoplankton has been found in Lake Cahora Bassa, Mozambique (Sommer & Gliwicz, 1986), where the *Volvox* green algae migrated 20 m deeper at night probably to reach higher concentrations of phosphorus (P). In small lakes and ponds, high nutrient concentrations can be found so near the surface that a DVM amplitude of less than 1 m may be needed to reach the adequate light intensity and nutrient concentration. Some phytoplankton species can even penetrate through the thermocline into the anoxic hypolimnion typically with high nutrient concentration (e.g., Salonen et al., 1984; Gervais, 1997a,b).

The energy consumption of phytoplankton motility has been estimated to be so low (Raven & Richardson, 1984; Inoue & Iseri, 2012; Raven & Lavoie, 2021) that DVM remains profitable. Through motility, phytoplankton may balance between the availability of light and nutrients, as well as the presence of competing species and grazers, to find optimal growth conditions. We hypothesized that under natural lake conditions flagellated phytoplankton can adapt to a combination of simultaneous environmental factors by dynamic modification of their vertical migration behavior. We took advantage of a highly coloured and strongly stratified small pond where physical conditions and simple plankton food web lend themselves to precise and practical sampling and experimental studies with reasonable effort.

## Study pond, methods and materials

A fish introduction experiment was made in a shallow (maximum depth 4 m, mean depth 3 m) pond, Mekkojärvi, located at 61° 13′ N, 25° 3′ E in southern Finland (Northern Europe). Because of the brown water colour (absorption at 260 nm 0.7–1.0 $cm^{-1}$) and small size (25 m wide, 130 m long), the pond is atelomictic, i.e., the thermocline could reach the surface during the afternoon of warm days. Nevertheless, we use ‘epilimnion’ to describe the uppermost water layer, which is generally mixed by night convection. In Mekkojärvi, the epilimnion is always oxic but the

hypolimnion is anoxic. A more detailed description of the pond can be found in Salonen et al. (2005).

The only fish in Mekkojärvi are pike (*Esox lucius* Linnaeus, 1758), which in their juvenile stage feed on zooplankton. Invertebrates, mainly *Notonecta* sp. (Heteroptera) and *Chaoborus* (Diptera) larvae, are the main predators of zooplankton. The large cladoceran *Daphnia longispina* (O.F. Müller, 1776) almost alone dominates the zooplankton biomass in summer (Salonen & Lehtovaara, 1992). As an efficient filter feeder, *D. longispina* exerts intense grazing pressure on phytoplankton. Similar to many small ponds, the phytoplankton assemblage of Mekkojärvi is simple and predominantly composed of flagellated species (Salonen et al., 1992a,b).

## Experimental approach

One thousand whitefish [*Coregonus lavaretus* (Linnaeus, 1758) fingerlings (age 0+)] were introduced to Mekkojärvi on July 14, 1994, to eradicate the *Daphnia longispina* population. Before that, a 2 m diameter fish-free reference enclosure made of transparent polyethylene film was installed in the middle of the pond. Its upper support ring was kept 0.1 m above the water level by polystyrene floats at the corners of a square wooden frame. To avoid movement of the enclosure, the corners of the frame were cross-anchored to trees near the shoreline. 2 h after the installation of the enclosure frame, the water column disturbance was considered settled and a boat was slowly attached to the side of the frame. A polyethylene tube was lowered to enclose an undisturbed water column. The lower end of the enclosure was anchored against the bottom at 3 m depth with an iron ring welded inside the polyethylene film.

## Sampling

Samples for chemical determinations, phytoplankton and bacterioplankton were taken with a 0.3 m long Limnos tube sampler 2–3 h before solar noon. Water samples in 1 l polyethylene bottles were transported to the laboratory in a polystyrene foam box filled with crushed ice. Vertical bacterioplankton and phytoplankton samples, and samples for the determination of enzyme activities were collected weekly. In the laboratory, enzyme activity assays were done in acid-washed polycarbonate bottles about 1 h after the sampling. Plankton samples were preserved with acid Lugol's iodine (final concentration ~ 0.2%) solution.

To study DVM of phytoplankton, samples were taken every 6 h from the 0–2 m depth zone with a horizontal box sampler (Salonen & Lehtovaara, 1992) with four 0.1 m wide superimposed square tubes. Each 24 h sampling period started and finished at solar noon. To guarantee undisturbed samples the sampler was kept ~ 1 m in front of a slowly moving rowing boat. Successive samples were taken along three lanes with a similar depth parallel to the longitudinal axis of the pond and marked by white buoys visible also at night. When entering the same lane again during the sampling occasion, we used the opposite side than in the previous sampling. There was also at least a 1.2 m depth difference compared with the previous sampling. Thus, the boat (draft ~ 0.2 m) did not disturb the vertical distribution of phytoplankton. After closing the sampler, two successive 0.1 m samples were combined to get a composite sample of a 0.2 m deep water layer. The 0–2 m water column was sampled twice on each sampling occasion, and the replicates were mixed in buckets by moving water gently with a paddle. Phytoplankton sample bottles were filled by immersion under water and preserved with acid Lugol's iodine solution. Due to the limited water volume of the reference enclosure, and to minimize mixing, we took samples from its centre with a Limnos tube sampler at noon and midnight only during the last two sampling periods of the experiment. The lowest 0.1 m water layer of the Limnos sampler was drained away to have samples comparable with those obtained with the horizontal box sampler.

Zooplankton samples were collected from the 0–1 m and 1–2 m water layers in the marginal and central zones of the pond every 3–4 days with a 1 m long tube sampler (volume 6.3 l). Each sample consisted of five replicates taken from positions selected randomly in advance over the zone exceeding 2 m depth and filtered through a 50 μm net.

## Analyses

Temperature and oxygen profiles were measured before or after each DVM sampling with a YSI 58 probe equipped with a stirrer (Yellow Springs Instruments, Ohio, USA) starting from the highest depth. This way the depth of the oxycline could be estimated precisely (±0.1 m). Solar radiation was measured at

10 min intervals at Lammi Biological Station ~ 20 km away from Mekkojärvi with a Kipp and Zonen solarimeter and data logger. Air temperature and precipitation were measured by the observation station of the Finnish Meteorological Institute located at Lammi Biological Station.

Inorganic nutrient samples were filtered through 47 mm diameter Whatman GF/F glass fibre filters within 3 h after sampling, and frozen at − 20 °C. Next autumn, after melting, inorganic P (Murphy & Riley, 1963), nitrite ($NO_2$-N), and nitrate ($NO_3$-N) nitrogen (Wood et al., 1967), and ammonium nitrogen ($NH_4$-N) (Solorzano, 1969) were determined in duplicate with a segmented flow AKEA autoanalyzer (Datex, Finland). Total organic P and N concentrations were determined after alkaline persulfate digestion (Koroleff, 1983). Particulate nutrients were determined using similar methods from material retained by the Whatman GF/F filter placed in deionized water. Chlorophyll filtered on Whatman GF/C filters was extracted overnight in ethanol at room temperature and chlorophyll *a* concentration was calculated from absorption at 665 nm against the background at 750 nm with Shimadzu UV2100 spectrophotometer. Water colour of the sample filtrate was determined with a spectrophotometer at 420 nm against platinum standard solutions.

Bacterioplankton (including autotrophic bacteria) samples (with an added drop of 0.1 N sodium thiosulfate to remove the colour of the iodine) were filtered onto black polycarbonate membranes (Millipore, pore size 0.2 µm), stained with acriflavine (Bergström et al., 1986), and counted with an epifluorescence microscope at 1250×magnification using blue excitation. During counting, a proprietary computer program (Salonen, unpublished) provided the standard error of the counted cell numbers in real time. At least 35 random fields in each sample were counted. For bacterial cell volume, the length and width of 100 randomly chosen cells were measured against the calibrated dots of a Patterson Globe and Circle eyepiece graticule. The standard error of the mean biomass of bacterioplankton was typically ~ 30%. Cell volumes were converted to carbon (C) using a factor of 0.36 pg C $\mu m^{-3}$ (Tulonen, 1993) which also compensates for the shrinkage of bacteria by drying during sample preparation.

Phytoplankton (including protozoans) samples were settled (Utermöhl, 1958) in the laboratory for at least 24 h in 50 ml tubes or, when phytoplankton abundance was high, 16 h in 10 ml tubes. Samples were counted at 300× and 600× magnification with a Wild M40 inverted microscope with phase-contrast optics. At least 25 randomly selected fields were counted. When the depth-integrated total abundance of cells was markedly higher at noon than at midnight, we used the total abundance observed at noon in the estimation of the median depth occurrence of the algal populations. Total phytoplankton biomass was calculated as a sum of flagellated taxa (0–2 m) counted from vertical migration samples and the other taxa (0–1 m) counted from samples taken by the tube sampler from 0 m, 0.5 m, and 1 m depths. Because Mekkojärvi was anoxic below ~ 1 m, the results from the uppermost 1 m were assumed to satisfactorily represent the biomass of non-flagellated autotrophic phytoplankton. Cell volumes of different phytoplankton taxa were taken from the list used by the Finnish Environment Institute and conversion to carbon biomass was done by assuming 10% C in wet mass. Specific increase rates (r) of taxa were calculated according to the formula

$$r = \ln\left(N_t/N_0\right)/\Delta t$$

where $N_0$ and $N_t$ are the initial and final abundances of cells, respectively, and $\Delta t$ is the time interval.

The final zooplankton abundances were the averages of the central and marginal zones (10 samples). *Daphnia longispina* was preserved in ethanol (ca. 50% final concentration by volume) and counted under a dissection microscope. Rotifers were preserved and counted at 100× magnification as described for phytoplankton. For the determination of the individual carbon contents of *D. longispina*, portions of separate samples frozen in 1–4% formaldehyde (Salonen & Sarvala, 1985) were melted and filtered on glass fibre filters. On average 162 individuals per date (range 21–237, after the fish introduction 137–237) were randomly picked by forceps under a dissection microscope and washed twice in deionized water (to remove formaldehyde) before the determination of carbon by the high-temperature combustion method of Salonen (1979). For rotifers, the carbon content of 0.024 µg C $ind.^{-1}$ for *Keratella cochlearis* (Gosse, 1851) was used to calculate biomass (Latja & Salonen, 1978).

Acid phosphomonoesterase (AcPME) enzyme activity was measured to estimate the cleavage of inorganic P from organic matter. 4-methylumbelliferyl phosphate (4-MUF-$PO_4$) was used as a model substrate according to Hoppe (1983) with modifications of Münster et al. (1989) and Hoppe et al. (1990). Leucine aminopeptidase (LAP) activity was measured to indicate cleavage of inorganic N using a similar protocol except that leucine-7-amino-4-methylcoumarin (Leu-AMC) served as a model substrate (Hoppe, 1983). Substrate saturation levels of 500 µM for AcPME and 100–250 µM for LAP were determined before each assay. For assays, sample water was filtered through a 100 µm mesh net to remove *Daphnia*. Each assay consisted of 1.3 ml of pond water and 15 µl of freshly prepared 4-MUF-$PO_4$ or Leu-AMC stock solution, respectively, which was mixed in a 10 mm thick 1.5 ml quartz cuvette to reach saturating substrate concentrations. An increase in fluorescence was measured for 10 min at 15 °C at 330 nm excitation and 455 nm emission wavelengths with a Hitachi F-4000 spectrofluorometer. Enzyme hydrolysis rates were calculated from 4-MUF and 7-amino-4-methylcoumarin standard curves prepared for the same water sample to correct for quenching effects of pH and humic substances (Münster et al., 1989).

### Data analyses

The precision of the phytoplankton and bacterioplankton results was controlled during microscopy by a computer program (K. Salonen, in preparation) which followed in real time the development of the taxon-specific variability to reach a uniform standard error for each of the main taxa (< 20% of the mean for phytoplankton, and < 6% for bacterioplankton). Higher standard error was accepted for the lowest cell abundances because the highest abundances primarily affect the interpretation of the results.

Statistical analyses were performed in Microsoft Excel 2016. Temporal changes in water chemistry were examined with linear regression. Differences in the vertical distributions of phytoplankton taxa between the times of day and night were tested with the Kolmogorov–Smirnov test. Because the sample sizes were large, most of the differences were statistically significant, except the distributions at successive noons, which usually did not differ significantly. In some cases, medians were used to summarize the temporal changes in the distributions of the phytoplankton taxa.

## Results

### Physical and chemical conditions

The time between sunset and sunrise increased from 4 h 40 min in the beginning of the experiment in late June to 8 h 15 min at its end in the middle of August, but otherwise the variation in light energy was largely masked by cloudiness. Therefore, the differences in light energy between the sampling periods were modest (Supplementary Fig. S1). The only exception was in the beginning of the experiment when light energy was about one-third compared to the next two sampling periods. The radiation fluxes around noon in the beginning and at the end of each 24 h sampling period were generally rather balanced. The largest difference was observed 4 weeks after the fish introduction when the solar radiation at the end of the sampling period was less than half that in the beginning.

The daily maximum air temperature steadily rose from ~ 15 °C in late June to 30 °C soon after the fish introduction, and even the diel minimum reached 25 °C (Supplementary Fig. S2). About one week later temperature decreased for a few days, but two weeks after the fish introduction very high air temperatures again continued for 5 days before temperature consistently decreased to 12–17 °C at the end of the experiment.

Due to the small size and high water colour of Mekkojärvi, the epilimnion was shallow with a minimum depth of < 0.5 m at the time of the fish introduction (Supplementary Fig. S3). Epilimnetic temperature varied between 12.8 and 26.7 °C. The highest water temperature recorded at the time of the fish introduction could have been lethal for the introduced fish, but the population could survive in the cooler metalimnion between 0.3 and 0.5 m depths with oxic conditions. The low volume of the epilimnion led up to 6 °C cooling during the night, accompanied by nocturnal convection. In the metalimnion (1 m depth), solar radiation slowly increased the water temperature from 8 to 15 °C during the experiment. At 3 m depth, the temperature was constantly around ~ 5 °C. Temperature and oxygen results

indicated stable stratification (Supplementary Fig. S3). Oxygen saturation at the surface was rather stable 50–70% (4–6 g $m^{-3}$), but the hypolimnion was always anoxic.

The only marked precipitation episodes occurred during the last 10 days of the experiment (Supplementary Fig. S2), but due to the preceding dry period, the small inflow brooks to Mekkojärvi remained dry.

In the beginning of the experiment, the water colour was very high (358–418 g Pt $m^{-3}$) at all depths. During the study, it decreased by 38% ($R^2 = 0.904$, $p < 0.001$, $n = 27$) in the epilimnion suggesting that dissolved organic carbon (DOC) loading from the catchment could not compensate for DOC losses due to biological and photochemical decomposition. At 1.5–2 m depth, where light penetration was negligible, the respective decrease in the water colour was low (Supplementary Fig. S4).

The concentrations of dissolved inorganic P and $NO_2 + NO_3$-N were below the detection limit of the methods, but the concentration of $NH_4$-N was detectable (5–9 mg m − 3) in the whole water column one week after the fish introduction. The $NH_4$-N concentration was highest at 2 m and lowest at 1 m, where the phytoplankton biomass was typically highest. Total nutrient concentrations at each depth were rather stable until the fish introduction (Fig. 1). During the following five weeks, hypolimnetic total P concentrations decreased, but the change was significant ($R^2 = 0.93$) only at 2 m where the concentration was reduced by 33 mg $m^{-3}$. Total N concentration decreased by 80 mg $m^{-3}$ at 1 m and 220 mg $m^{-3}$ at 2 m, but the changes were significant only at the surface and 0.5 m depth ($R^2 = 0.94$ and 0.86, respectively). From the beginning of the experiment to the fish introduction, the molar N:P ratio of particulate matter in the 0–1 m water layer decreased from ~ 40 to ~ 20 (Fig. 1). Thereafter it remained constant at 1 m depth until the end of the experiment. Instead, in the epilimnion the ratio increased up to 70 within 2 weeks, indicating P limitation. Toward the end of the experiment, the epilimnetic N:P ratio decreased again, suggesting a balanced P and N availability at all depths.

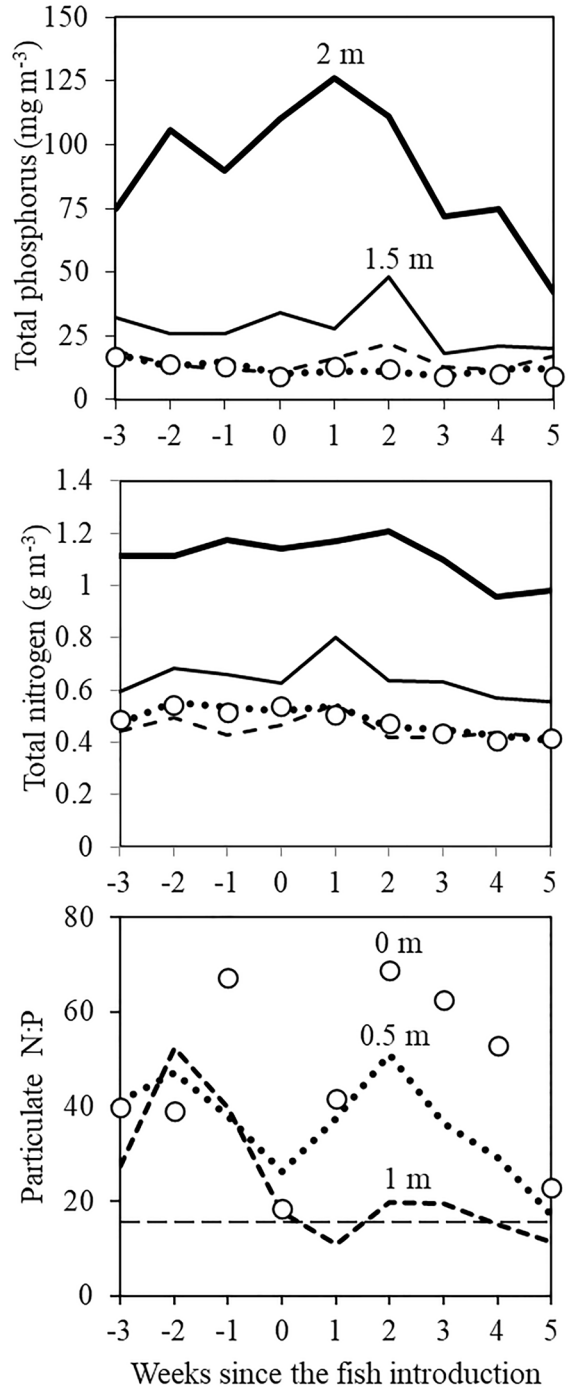



**Fig. 1** Total phosphorus and total nitrogen concentrations, and the molar N:P ratio of particulate matter at different depths of Mekkojärvi; the horizontal broken line in the bottom panel denotes the molar Redfield N:P ratio)

## Development of zooplankton

When zooplankton samples taken from the middle and marginal zones of the pond were treated as

replicates, the average coefficient of variation (CV) of the abundance of *D. longispina*, was 18% of the mean, which provides a reasonable basis to evaluate the impacts of zooplankton grazing on phytoplankton. Before the fish introduction, *D. longispina* practically alone formed the metazooplankton biomass, and its abundance reached ~ 30 ind. $l^{-1}$ in the uppermost 1 m water layer. During the first week of the experiment, ~ 25% of the population was found below 1 m depth before noon, but after the decrease in the thickness of the epilimnion the proportion was reduced to 1–7% at the time of the fish introduction. Despite the quite linear decrease of *D. longispina* abundance after the fish introduction, its increasing mean size maintained the zooplankton total biomass stable for 2 weeks (Fig. 2), but during the following 2 weeks the species disappeared.

After the fish introduction, protozoan biomass started to increase and reached a maximum four weeks later (Fig. 2). Rotifers, mainly *Keratella cochlearis*, which on average composed 95% of total rotifer biomass, started to increase rapidly a couple of weeks later at a high rate of up to 0.29 $d^{-1}$ and reached similar maximum biomass almost at the same time as the protozoans. The combined protozoan and rotifer biomass was higher than the maximum zooplankton biomass during *Daphnia longispina* dominance before the fish introduction. During the early phase of the growth of the rotifer population, only 3–8% of individuals occurred below 1 m depth, but at the end of the experiment, the proportion increased to 29%.

In the isolated control enclosure, the results for the *D. longispina* abundance were inconsistent: four weeks after the fish introduction, the abundance was only 3.6 ind. $l^{-1}$ in the uppermost 1 m, whereas one week later it was an order of magnitude higher, 37 ind. $l^{-1}$, which closely corresponded to the maximum abundance observed in the pond before the fish introduction. Such abundance change within a week is possible but unlikely, and we consider the higher value more realistic estimate. The discrepancy between the abundances in the enclosure might result from the patchy distribution of *D. longispina* due to the shading of the frame of the enclosure. A decimal error in the original abundance data cannot either be ruled out. The results for rotifers were consistent and their total abundance was only 2–3% of the maximum observed at the same time in the pond.

## Phytoplankton abundance and biomass

Prior to fish introduction, the chlorophyll *a* concentration varied between 3.5 and 5.4 mg $m^{-3}$ in the

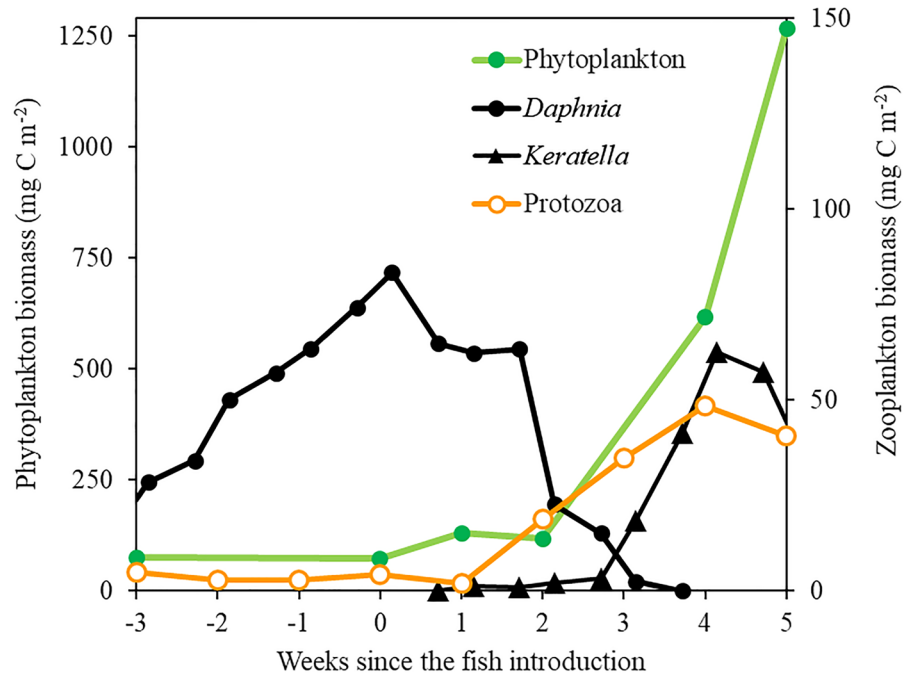



**Fig. 2** Biomass of phytoplankton, protozoans, *Daphnia longispina* and *Keratella cochlearis* in the 0–1 m depth zone

uppermost 0.5 m layer. Thereafter it increased rather smoothly and tripled (13.9 mg $m^{-3}$) by the end of the experiment (Supplementary Fig. S5). At 1 m depth, the chlorophyll concentration was higher and started to increase already a week before the fish introduction and 2 weeks later it reached 54 mg $m^{-3}$. Thereafter chlorophyll concentration rapidly decreased at 1 m depth and at the end of the experiment it was at the same level (15–20 mg $m^{-3}$) as in the epilimnion. In the hypolimnion, the chlorophyll concentration was one (at 1.5 m) to two orders (at 2 m) of magnitude higher than in the epilimnion throughout the experiment. The high hypolimnetic concentrations (up to 1140 mg $m^{-3}$ three weeks after the fish introduction) are attributed to bacteriochlorophyll *d* of green sulphur bacteria (Kuuppo-Leinikki & Salonen, 1992).

When the peak abundance of flagellated phytoplankton taxa was > 20 cells $ml^{-1}$, their vertical distribution curves were typically smooth (Figs. 4, 5, 6), indicating good reliability of the count results. The same was shown by the depth-integrated abundances of *Mallomonas* taxa in the 0–2 m samples taken five times during each 24 h sampling period. The mean CV for *Mallomonas* sp. and *M. akrokomos* Ruttner, which stayed within the sampling zone throughout the experiment, was 23% (range 15–31%, SD 21%, $n = 8$). The mean difference between noon median depths in the beginning and at the end of each 24 h sampling period ($n = 5$) was 0.14 m (SD 0.17) for *Cryptomonas* spp. and 0.05 m (SD 0.05 m) for *Mallomonas* spp. Thus, the differences in vertical position of dominant phytoplankton taxa could be interpreted with about a 0.2 m resolution.

Despite the increase in *Daphnia longispina* biomass, phytoplankton biomass remained stable (Fig. 2) from the beginning of the experiment to the fish introduction in mid-July, but later it started to increase. In the beginning of the experiment, flagellated phytoplankton contributed half of the total phytoplankton biomass, but already at the time of the fish introduction their proportion was > 90% and this continued until the end of the experiment. The share of cryptophytes in the total biomass increased from 26 to 66% before the fish introduction to 96% by the end of the experiment. The abundances of small and large cryptophytes were initially similar, but already at the time of the fish introduction, large individuals contributed 70% to cryptophyte abundance, increasing to 78% (> 90% of biomass) by the end of the experiment. The share of *Mallomonas* species, particularly *M. akrokomos*, in phytoplankton biomass increased markedly after the fish introduction. The rates of increase of *Mallomonas caudata* Iwanoff and *M. akrokomos* were in July 4–5 times higher than those of cryptophytes, but toward the end of the experiment in August, the situation reversed (Supplementary Table S1). After the fish introduction the rapid growth of *Mallomonas* spp. started a couple of weeks earlier than that of cryptophytes (Fig. 3).

The small-sized flagellated phytoplankton species, *Chlamydomonas* sp. and *Scourfieldia* sp., together always contributed < 5% to the phytoplankton biomass. *Scourfieldia* sp. rapidly declined after the fish introduction and practically disappeared during the following 4 weeks.

## Vertical distribution and migration of phytoplankton

*Mallomonas* species remained in the epilimnion throughout the experiment (Figs. 4, 7). In the beginning, *Mallomonas* sp. performed a distinct DVM of about 0.5 m amplitude, with similar morning and noon, and evening and night distributions. After the fish introduction, the highest contrast in the median depth was between the morning and evening distributions, but in late August, the DVM amplitude of *Mallomonas* was negligible. At the time of the fish introduction, *M. akrokomos* was present in only about a 0.2 m thick layer at around 0.3 m depth at noon, and at night the species concentrated around 0.5 m depth (Fig. 4). Later, the vertical distributions of *M. akrokomos* at noon and night resembled those of *Mallomonas* sp. and there were only faint indications of DVM, although vertical distributions of noon and midnight differed significantly. The position of the median depth of *M. caudata* was deeper (mean 0.7 m, SD 0.1 m) at night than at noon (mean 0.3 m, SD 0.1 m). The mean percentage (105%, range 70–142%, SD 28, $n = 6$) of the midnight abundances of the *Mallomonas* taxa in the 0–2 m water layer compared with the respective noon abundances indicated no migration below 2 m depth.

Cryptophytes and the chlorophyte *Chlamydomonas* sp. performed more extensive DVM than *Mallomonas* (Figs. 5, 6, 7). In the beginning of the experiment, small cryptophytes were mostly distributed in the epilimnion at noon with a maximum abundance near the surface. At night they descended

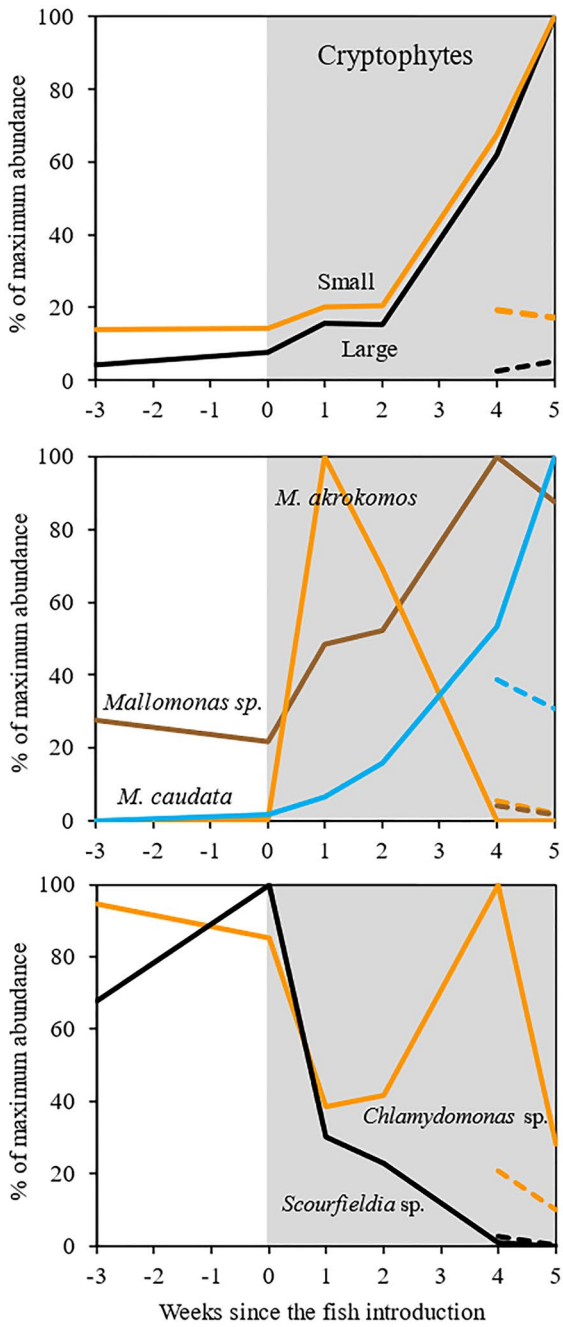



**Fig. 3** Seasonal development of dominant flagellated phytoplankton taxa in the pond (solid lines) and the enclosure (dashed lines) in relation to their maximum abundance in the 0–2 m water column in the pond

about 1 m deeper into the anoxic hypolimnion, but a small proportion remained in the epilimnion. At the time of the highest *Daphnia longispina* abundance just before the fish introduction, they consistently stayed around the oxycline at noon, in the morning, and the evening, migrating into deeper water for the night. High water temperature at the time of fish introduction hardly explained the absence of small cryptophytes from the uppermost water layer, since later they did not avoid that layer at similar water temperatures. Although the vertical resolution of the routine samples taken with the tube sampler was > 0.5 m, their results suggested daytime surface avoidance already one week before the fish introduction.

After the fish introduction, the vertical distribution of small cryptophytes did not change at night, but at noon most of the cells occurred in the upper part of the epilimnion (Figs. 5, 7), and the same pattern continued to the end of the experiment. Just before the fish introduction, the highest median depth of the population occurred at midnight, during the following 2 weeks 6 h earlier. After week 2, ~ 75% of small cryptophytes migrated below the 2 m depth for night.

Large cryptophytes were generally located in deeper water than the small ones (Figs. 5, 7). From the beginning of the study until 2 weeks from the fish introduction, they were mostly within a ~ 0.5 m thick water layer around the oxycline at noon but later they increasingly appeared in small numbers in the epilimnion. For the night they initially migrated 0.6–0.7 m deeper into the anoxic hypolimnion, but already 2 weeks after the fish introduction part of the population started to migrate below the 2 m depth. Finally, only 12–16% of the population was present in the 0–2 m sampling zone at midnight.

*Chlamydomonas* sp. was mostly concentrated in the upper part of the anoxic hypolimnion (Figs. 6, 7) with no detectable DVM before the fish introduction, but one week after that the species started to appear in the epilimnion. Its DVM amplitude was about 0.5 m, which later seemed to increase. At successive noons, the highest abundance of *Chlamydomonas* sp. was found either at the surface or near the metalimnion. Because *Chlamydomonas* sp. did not seem to migrate below 2 m at night, the variable midnight abundances indicated patchy distribution (Fig. 6).

*Scourfieldia* sp. generally remained in the anoxic water layers with a maximum abundance slightly below 1 m (Figs. 6, 7). The species generally displayed no detectable DVM, but during its marked decrease at the end of the experiment, it migrated

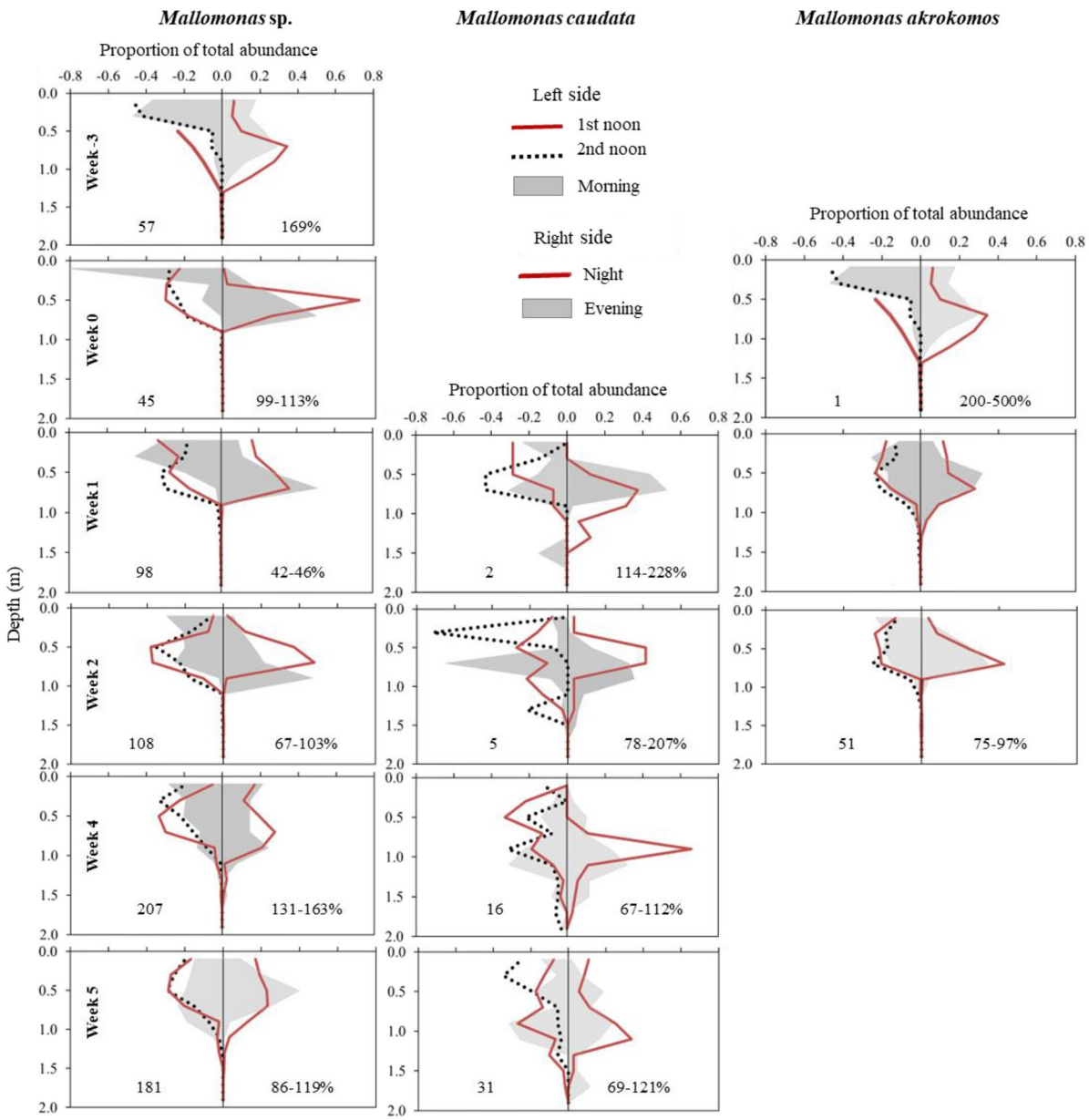



**Fig. 4** Vertical distributions during the experiment of the chrysophytes *Mallomonas* sp., *M. caudata*, and *M. akrokomos* in Mekkojärvi, from noon to next noon at 6 h intervals (solar time) (values on the left bottom of each panel = mean abundance (cells $ml^{-1}$) in the 0–2 m water column, values on the right bottom of each panel = proportion of mean abundance at night from respective abundances at noon)

even up to a depth of 0.5 m in the morning and evening.

### Phytoplankton in the enclosure

In the enclosure, the abundances of cryptophytes remained roughly at the same level as in the beginning of the experiment, while those of *Mallomonas* sp. and particularly *Chlamydomonas* sp. and *Scourfieldia* sp. were lower (Fig. 3). Only *M. caudata* increased markedly but did not reach as high abundance as in the pond at the end of the experiment. In the enclosure data, according to the Kolmogorov–Smirnov test, all differences in vertical distributions between day and night were significant. *Mallomonas* sp. and *M. caudata* were mainly located in the epilimnion but showed a more distinct DVM than in the pond. At the end of the experiment, vertical distribution and DVM of small and large cryptophytes in the enclosure were at noon rather similar to those found in the pond in the beginning of the experiment without clear penetration below 2 m at night. The vertical distribution and DVM of *Chlamydomonas* sp. were similar to those in the pond but, in contrast, *Scourfieldia* sp. showed a distinct DVM with 0.2–0.3 m amplitude (Fig. 8).

### Enzyme activity

AcPME activities typically decreased from the surface to the bottom (Fig. 9). However, 1 week after the beginning of the experiment the lowest value was found at 0.5 m depth, while it was an order of magnitude higher at 3 m. At the time of the fish introduction, AcPME activity was very low throughout the water column, but two weeks later high values were observed in the 1–1.5 m water layer corresponding to the median depth distribution of the large cryptophytes at noon, and the time of their increasing DVM amplitude (Fig. 5). The high AcPME activity lasted roughly one week and thereafter activities at all depths approached the level prevailing at the time of the fish introduction.

LAP activity varied less between depths, but there were two distinct maxima during the experiment (Fig. 9). One week before the fish introduction, LAP activity slightly increased down to the 3 m depth at the same time as there was a less distinct increase in epilimnetic AcPME activity. Thereafter activity in the epilimnion decreased to a very low level, but in the hypolimnion, the change was smaller. After the fish introduction LAP activity started to increase in parallel with AcPME, but with slightly delayed timing. The maximum LAP activity also faded rapidly and one week after the maximum only ~ 20% of that was left.

### Bacterioplankton

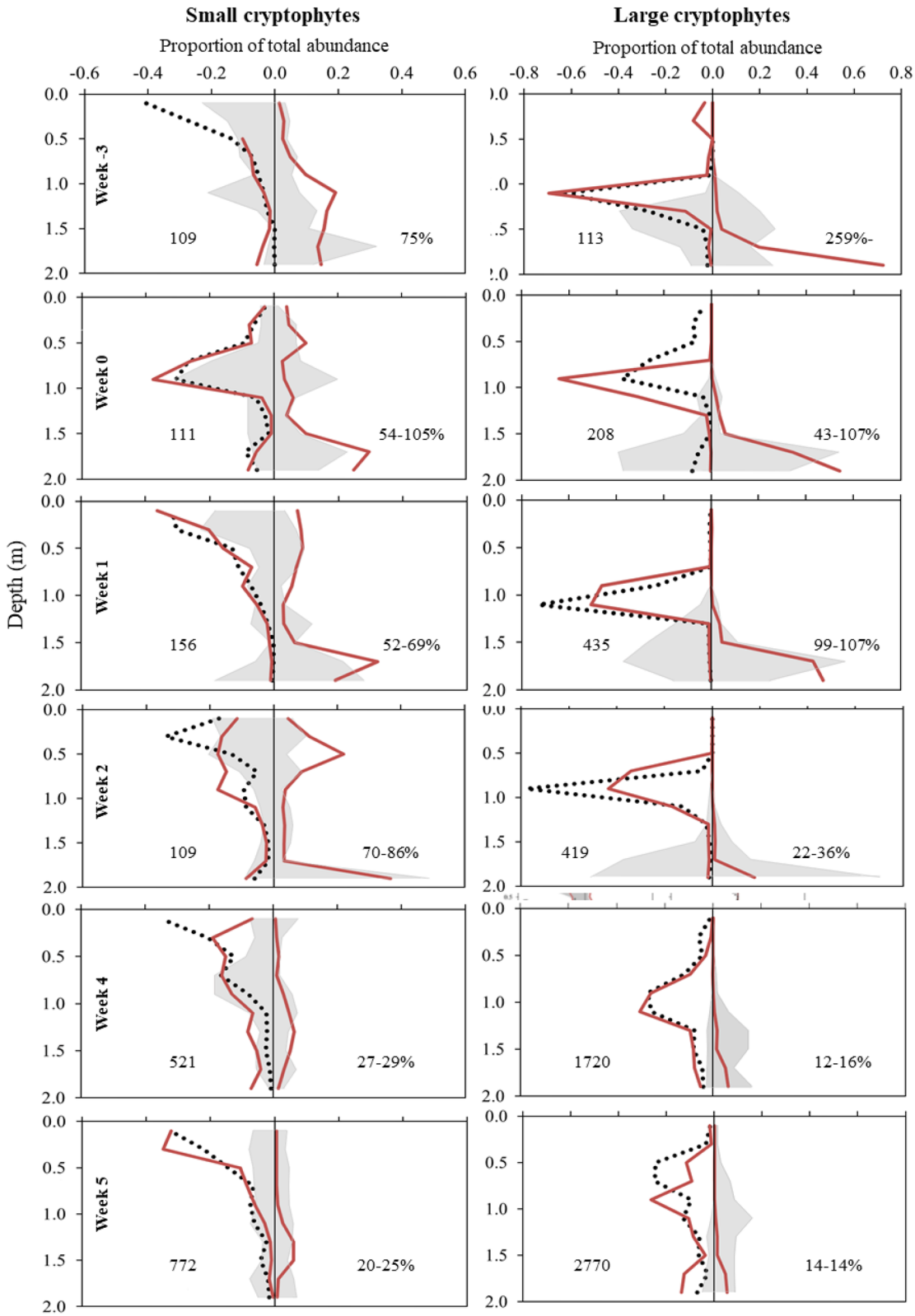
Bacterioplankton biomass was strongly stratified (Fig. 10) following the vertical distribution of temperature and oxygen. In the epilimnion the abundance of bacteria was 0.9–2.8 $10^6$ cells $ml^{-1}$, while in the anoxic hypolimnion at 1.5–2 m depth it was much higher, 3.9–15.2 $10^6$ cells $ml^{-1}$. The mean cell volume of bacteria in the hypolimnion was also 2–3 times larger than in the epilimnion. Therefore, biomasses at 1.5 m and 2 m depth were about 5 and 20 times, respectively, higher than in the epilimnion at the time of the fish introduction. Thereafter, bacterioplankton biomass rapidly decreased at all depths. In absolute terms, the decrease was higher towards the deeper water layers, but the relative change was highest in the surface layer. There was some recovery of bacterioplankton biomass at the end of the experiment.

## Discussion

During the whole pond experiment of Mekkojärvi, phytoplankton responses to grazing and increasing nutrient shortage could be studied under realistic, natural conditions. Because the changes in weather conditions were modest, the results were neither masked by hydrological fluctuations.

### Phytoplankton DVM and zooplankton

Large *Daphnia* cladocerans are effective filter feeders with a wide food-size spectrum (Lampert, 1974). In the absence of efficient predators, *D. longispina* can control phytoplankton biomass keeping epilimnetic chlorophyll concentration typically < 3 mg $m^{-3}$ (Kuuppo-Leinikki & Salonen, 1992; Münster et al., 1992; Salonen et al., 2005). The distinct increase in the mean size of *D. longispina* after the fish introduction suggests that small individuals typically


Small cryptophytes
Large cryptophytes
Proportion of total abundance
Proportion of total abundance
Depth (m)
Week -3
Week 0
Week 1
Week 2
Week 4
Week 5
109
75%
113
259%-
111
54-105%
208
43-107%
156
52-69%
435
99-107%
109
70-86%
419
22-36%
521
27-29%
1720
12-16%
772
20-25%
2770
14-14%

◂**Fig. 5** Vertical distributions during the experiment of small and large cryptophytes in Mekkojärvi from noon to next noon (other explanations as in Fig. 4)

dominating in the epilimnion (Salonen & Lehtovaara, 1992) were more heavily exposed to predation by the introduced fish than large haemoglobin-rich individuals able to live around the oxic-anoxic interface.

After the collapse of *D. longispina* in Mekkojärvi, protozoans and rotifers were released from the food competition or predation, and rotifers became dominant in the metazoan zooplankton. In line with Jack and Thorp (2002) the rapid increase in *Keratella cochlearis* suggests that it did not suffer much from whitefish fingerling predation. Although the maximum population biomass of *K. cochlearis* was comparable to that of *D. longispina* before the fish introduction (Fig. 2), the two orders of magnitude smaller body size of *Keratella* probably restricted its grazing on the dominant phytoplankton species. Ronneberger (1998) found 1–2 µm as the optimum size of food items for *K. cochlearis*, but the species can also feed on larger particles.

According to Bogdan and Gilbert (1982) the seasonal median clearance rate of *Keratella cochlearis* was ~ 2 µl ind.$^{-1}$ h$^{-1}$ which means that it might have filtered 14% of the epilimnetic water of Mekkojärvi in a day. Because the clearance rate of *Daphnia longispina* is much higher, ~ 0.5 ml ind.$^{-1}$ h$^{-1}$ (Kankaala, 1988), the grazing pressure on phytoplankton by *K. cochlearis* may have been about one-third of that by *Daphnia longispina*. This might explain why the grazing by rotifers had no clear effect on the biomass (Fig. 2) and DVM behavior of flagellated phytoplankton.

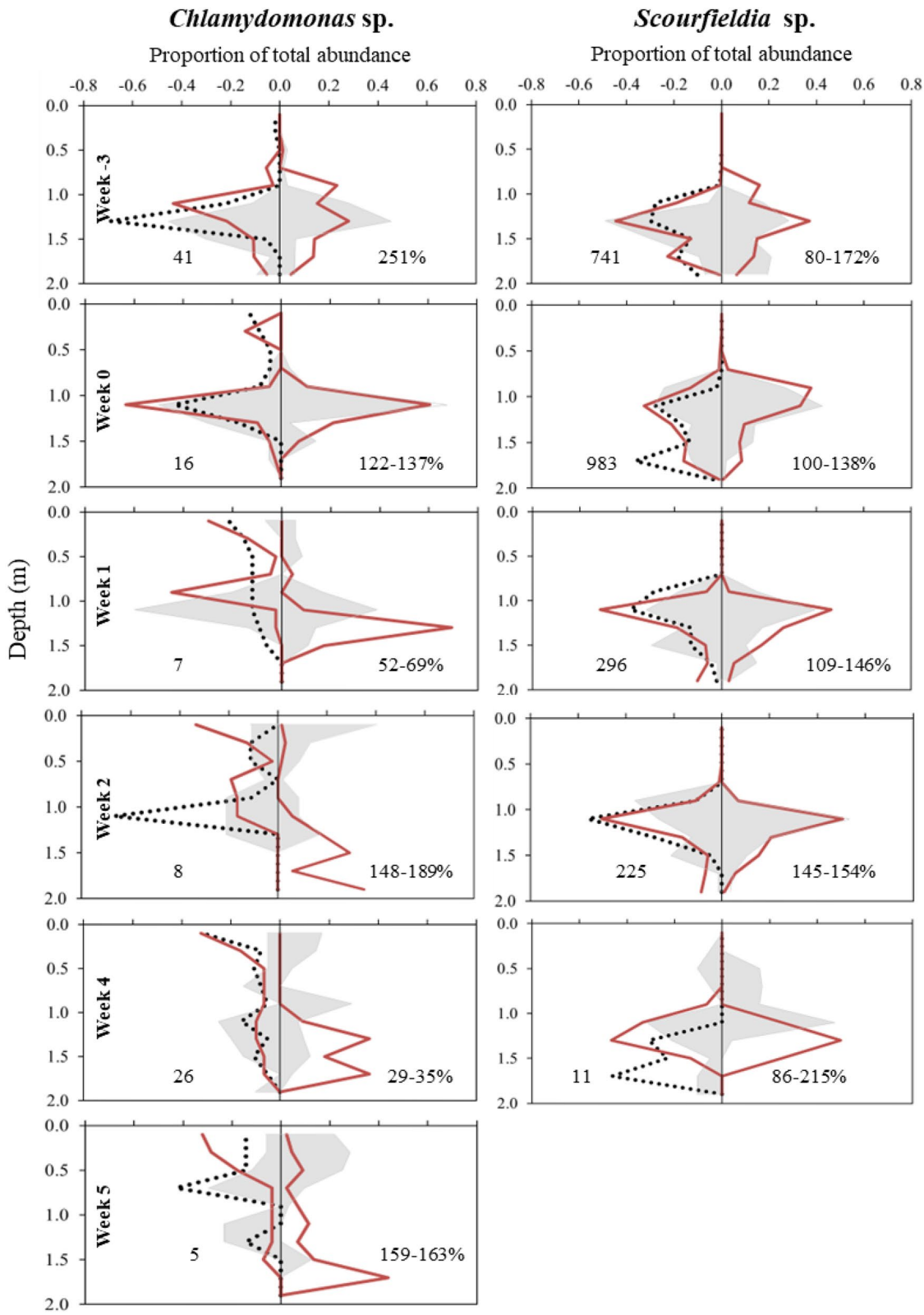
Our present results and earlier studies at Mekkojärvi (e.g., Arvola et al., 1992) suggest that the coexistence of *Mallomonas* species in the epilimnion in the presence of abundant *Daphnia longispina* is largely explained by silica bristles, which make the cells difficult to ingest. Morphological defence was also supported by field experiments where about four times higher proportion of radiocarbon fixed by phytoplankton was found in zooplankton in the *Cryptomonas* than *Mallomonas*-dominated samples (Salonen and Arvola, 1988).

In contrast to *Mallomonas*, the naked cryptophytes and *Chlamydomonas* sp. seemed to capitalize on their motility (Figs. 5, 6, 7) to reduce the risk of grazing. The later beginning of the intense population growth of cryptophytes compared to *Mallomonas* spp. after the fish introduction also corroborates a higher sensitivity to grazing. At the time of the highest *Daphnia* biomass, the migration of cryptophytes out of the epilimnion at noon was hardly due to the high light intensity because in almost similar light conditions one week later (Supplementary Fig. S1), small cryptophytes and *Chlamydomonas* sp. were in the surface layer at noon (Fig. 2). The low light compensation point (see below) of the large cryptophytes could explain why their movement towards the surface was less pronounced after the decrease in *Daphnia longispina* abundance.

According to earlier studies (e.g., Salonen & Lehtovaara, 1992) photosynthetically active radiation in Mekkojärvi was about 10 µmol m$^{-2}$ s$^{-1}$ at 1 m depth at noon. Gervais (1997a) found a compensation point of 5–7 µmol m$^{-2}$ s$^{-1}$ for *Cryptomonas ovata* Ehrenberg, which is of similar size to large cryptophytes of Mekkojärvi. Regular avoidance of the epilimnion by large cryptophytes has often been reported in lakes with a steep oxycline (e.g., Gasol et al., 1993; Gervais, 1997a, b, 1998; Knapp et al., 2003). The deep occurrence of *Scourfieldia* sp. below the oxycline and the appearance of the species in the epilimnion at the end of the experiment probably indicated grazing avoidance. In the tank experiment of Salonen et al. (1992a), *Scourfieldia* sp. could grow near the surface at 16 °C when *Daphnia longispina* was absent and grazing avoidance was not needed.

On the other hand, in summer 1986 in Mekkojärvi, the vertical distributions and DVM behavior of *Mallomonas* spp., *Chlamydomonas* sp., and *Scourfieldia* sp. (Arvola et al., 1992) were similar to those in the present study, although the maximum abundance of *Daphnia longispina* was about two times higher (Salonen & Lehtovaara, 1992). However, despite very similar thermal stratification, in 1986 cryptophytes in the 0–1.75 m water layer performed only slight DVM with no indication of grazing avoidance.

Further, in a brown water pond Nimetön in Finland, with ca.1 m deep epilimnion, Smolander and Arvola (1988) reported continued DVM by small cryptophytes after the rapid decline of the biomass of the dominant *Bosmina longispina* Leydig, 1960 and *Daphnia longispina* cladocerans from 274 to 10 mg C m$^{-2}$ in 2 weeks (A. Lehtovaara, unpublished), likewise suggesting a negligible role of


Chlamydomonas sp.
Scourfieldia sp.
Proportion of total abundance
Proportion of total abundance
-0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8
Depth (m)
0.0 0.5 1.0 1.5 2.0
Week -3
41
251%
741
80-172%
Week 0
16
122-137%
983
100-138%
Week 1
7
52-69%
296
109-146%
Week 2
8
148-189%
225
145-154%
Week 4
26
29-35%
11
86-215%
Week 5
5
159-163%

◂**Fig. 6** Vertical distributions during the experiment of *Chlamydomonas* sp. and *Scourfieldia* sp. in the 0–2 m water column of Mekkojärvi at 6 h intervals from noon to next noon (solar time) (other explanations as in Fig. 4)

grazing on DVM behavior. However, in Nimetön cryptophytes migrated at night deeper than necessary to reach water layers with richer nutrient concentrations, which suggests probable zooplankton grazing avoidance.

## Nutrients and vertical migration of phytoplankton

In headwater ponds, rain episodes typically cause distinct pulses of nutrient loading. Due to the preceding dry period, however, the inflowing brooks of Mekkojärvi remained dry until the end of our experiment. In summer, the mixing of the epilimnion of ponds is largely governed by nocturnal convection which keeps epilimnetic nutrient concentrations rather uniform. However, as shown by the narrow vertical distributions of *Mallomonas* species in the epilimnion at night (Fig. 4), cooling induced convective mixing (Supplementary Fig. S3) was too weak to govern the vertical distribution of flagellated phytoplankton.

In agreement with Andersen and Hessen (1991), the molar N:P ratio of *Daphnia longispina* in Mekkojärvi was low, 2.7:1, while for *K. cochlearis* it was an order of magnitude higher (Järvinen & Salonen, 1998). Assuming the latter N:P ratio for phytoplankton and protozoans and half of that for bacterioplankton, it may be estimated from the Redfield C:N:P ratio that at the time of the fish introduction the share of *D. longispina* was 86% of the of the total amount of P in phytoplankton, bacterioplankton, and zooplankton. This roughly agrees with an earlier 50–70% estimate of the P share of *D. longispina* based on chemical determinations (Salonen et al., 1994). Two weeks after the fish introduction, the proportion of P in zooplankton fell to ~ 10% and nutrient regeneration was low which together with the increasing phytoplankton biomass led to a shortage of nutrients, and a high N:P ratio of 50–70 indicated P limitation. When cryptophytes started to migrate deeper into the hypolimnion, the N:P ratio of particulate matter in the epilimnion again approached the Redfield ratio suggesting that N and P retrieved from the hypolimnion gradually balanced the nutrient availability for phytoplankton.

The N:P ratio of particulate matter close to the Redfield ratio in the upper water layers (Fig. 1) and the low AcPME and LAP enzyme activities (Fig. 9) just before the fish introduction suggest that despite undetectable inorganic nutrient concentrations phytoplankton dominated by cryptophytes was neither N nor P depleted. This conclusion is supported by the results of a nutrient enrichment experiment done along with this study in Mekkojärvi (Järvinen & Salonen, 1998). Before the fish introduction, the excretion of *D. longispina* (James & Salonen 1991) probably satisfied the relatively high nutrient requirement (Cloern, 1977) of cryptophytes. The introduced fish likely further improved the nutrient availability for a short period, as indicated by the momentary small increase in $NH_4$-N concentration. The rapid depletion of increased $NH_4$ -N at 2 m depth also suggests significant uptake of nutrients by cryptophytes in the hypolimnion. After week one the increase in phytoplankton biomass again led to a high N:P ratio of particulate matter, indicating intensifying P depletion which likely forced cryptophytes to shift to complementary nutrient sequestration strategies. They benefited from the nutrients in the anoxic hypolimnion, as has also been reported elsewhere (e.g., Salonen et al., 1984; Gasol et al., 1992; Salonen & Rosenberg, 2000). The elevated increase rate of cryptophytes (Supplementary Table S1) and almost total dominance of cryptophytes in the phytoplankton biomass at the end of the experiment corroborate the crucial role of deep water nutrient retrieval in Mekkojärvi.

The different timings in the development of the DVM of small and large cryptophytes suggest complex responses to the availability of nutrients in the water column. Judging from the long stay (at least 12 h) of large cryptophytes at depths below 1.5 m since the day of the fish introduction (Fig. 7), they probably needed a longer time than small cryptophytes to satisfy nutrient requirements. This conclusion is supported by the systematically earlier ascent of small cryptophytes in the morning (Fig. 7).

*Mallomonas* species have a high phosphate affinity (Sandgren, 1988), which can explain why they occurred in the Mekkojärvi epilimnion despite undetectable concentrations of inorganic nutrients. *Mallomonas* spp. may also have been able to utilize short-living nutrient micropatches that *Daphnia longispina* and fish excreted into the water. Although

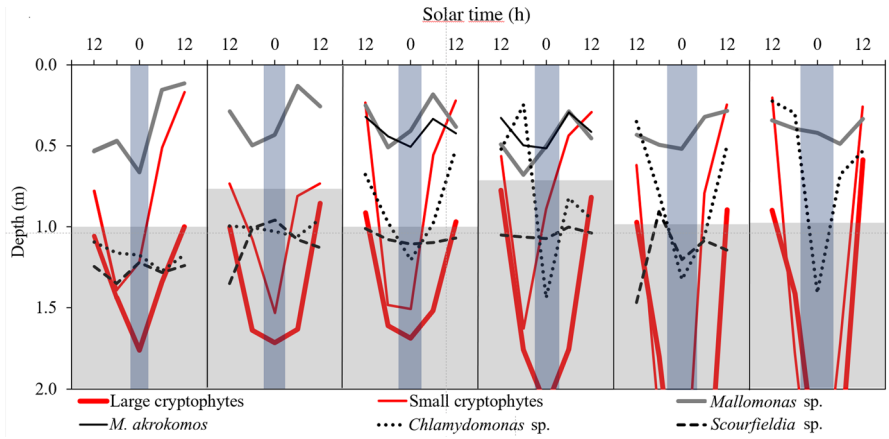



**Fig. 7** The median diel vertical positions of motile phytoplankton species at 6 h (solar time) intervals on the sampling days. Bottom grey area—anoxic zone; vertical dark shaded bars—periods between sunset and sunrise; because the two uppermost samples of the first sampling day were lost, the broken lines (except for *Scourfieldia* sp.) underestimate the height of the median position at the first noon

Currie (1984) concluded that, due to their short lifetime, nutrient patches probably have little importance for phytoplankton, the paucity of turbulence in the epilimnion of the small and wind protected Mekkojärvi probably makes a distinct difference. Small-scale patchiness can also generally be a more significant feature of open-water ecosystems than previously anticipated (Basterretxea et al., 2020).

The more distinct DVM of *Mallomonas* spp. in the control enclosure of Mekkojärvi and the migration of the species into the anoxic hypolimnion might result from the depletion of epilimnetic nutrients through sedimentation in the absence of contact with the littoral zone. Jones (1988) found a similar DVM pattern of *Mallomonas* spp. in Pond Nimetön with a shallow oxic epilimnion. The contrast between the results of Mekkojärvi and the enclosure can hardly be explained by differences in stratification because the temperature and oxygen profiles in the enclosure and pond typically closely follow each other (Ojala et al., 1995; Arvola & Salonen, 2001).

The unusually late development of *Daphnia longispina* population in 1994, possibly because of successful pike reproduction, allowed phytoplankton to develop high biomass which depleted inorganic nutrients in the whole water column before the fish introduction. In earlier studies at Mekkojärvi, when abundant *D. longispina* controlled phytoplankton biomass, inorganic P concentration was always detectable in the epilimnion and hypolimnetic concentration exceeded 50 mg $m^{-3}$ (Arvola et al., 1992; Salonen et al., 1994, 2005). Then, in contrast to our results, grazing by *D. longispina* only marginally modified the DVM of cryptophytes (Arvola et al., 1992).

Anoxia in the hypolimnion of lakes and ponds is generally associated with increased nutrient concentrations. However, in Mekkojärvi, the anoxic hypolimnion became depleted of nutrients, most likely due to the uptake by migrating cryptophytes. Similar to that, Gasol et al. (1993) found an exceptional depletion of inorganic P of the anoxic hypolimnion in Cisó where metalimnetic population of cryptophytes was extremely high (up to $10^6$ cells $ml^{-1}$). In another pond in Finland, Valkea-Kotinen, intensive growth of a raphidophycean flagellate, *Gonyostomum semen* (Ehrenberg) Diesing, rapidly exhausted inorganic P in the anoxic hypolimnion (Salonen & Rosenberg, 2000). Rohrlack (2020) also found a marked decrease in hypolimnetic $NH_4$-N concentration when the abundance of *G. semen* increased in a small Norwegian lake. In Grosser Vätersee ($Z_{max}$ 11.5 m) in Germany (Gervais et al., 2003), inorganic P in the hypolimnion was gradually depleted during the summer which suggests that migrating phytoplankton was responsible although the authors did not think so. Salonen et al. (1984) experimentally verified that inorganic radiophosphorus injected into the anoxic deep water in a small pond, was transported to the epilimnion by vertically migrating cryptophytes. We conclude that hypolimnetic depletion of nutrients by migrating phytoplankton is likely more common than anticipated. It has probably remained unnoticed because in larger lakes the swimming capability of motile phytoplankton probably limits nutrient retrieval by vertical migration to the upper part of the hypolimnion.

### Compensation of nutrient limitation by enzyme activity and bacterivory

Under nutrient-limiting conditions, phytoplankton can harvest P from organic substrates using phosphatases (Cembella et al., 1984; Reinl et al., 2022) and there is growing evidence that organic N can also be a major N source for phytoplankton, including cryptophytes (Fagerbakke et al., 1996; Bronk et al., 2007; Berges & Mulholland, 2008; Hernández-Ruiz et al., 2020; Volponi et al., 2023). Activation of enzymes is possible both under oxic (Hoppe, 2003; Duhamel et al., 2010) and anoxic (Bañeras et al., 1999) conditions. In Cisó, Bañeras et al. (1999) linked high phosphatase activity in anoxic hypolimnion to photosynthetic bacteria. In Mekkojärvi, photosynthetic bacteria are also regularly abundant between 1.5 and 2 m depths, but their photosynthesis is so low (Kuuppo-Leinikki & Salonen, 1992) that they cannot prevent the increase in hypolimnetic nutrient concentration during summer stratification. Although photosynthetic bacteria may occasionally face phosphate limitation (Bañeras et al., 2010) and enzyme activity may change rapidly (Dyhrman & Palenik, 2003), it is unlikely that photosynthetic bacteria in the stable and cold hypolimnion of Mekkojärvi could have changed their enzyme activity back and forth in so short time. Because the enzyme activities increased in the whole water column, cryptophytes extending their DVM deeper into the hypolimnion to compensate for the low nutrient regeneration by zooplankton, are the only plausible explanation for the appearance of the high enzyme activity. The short duration of high enzyme activities suggests that cryptophytes rapidly overcame the most acute nutrient shortage.

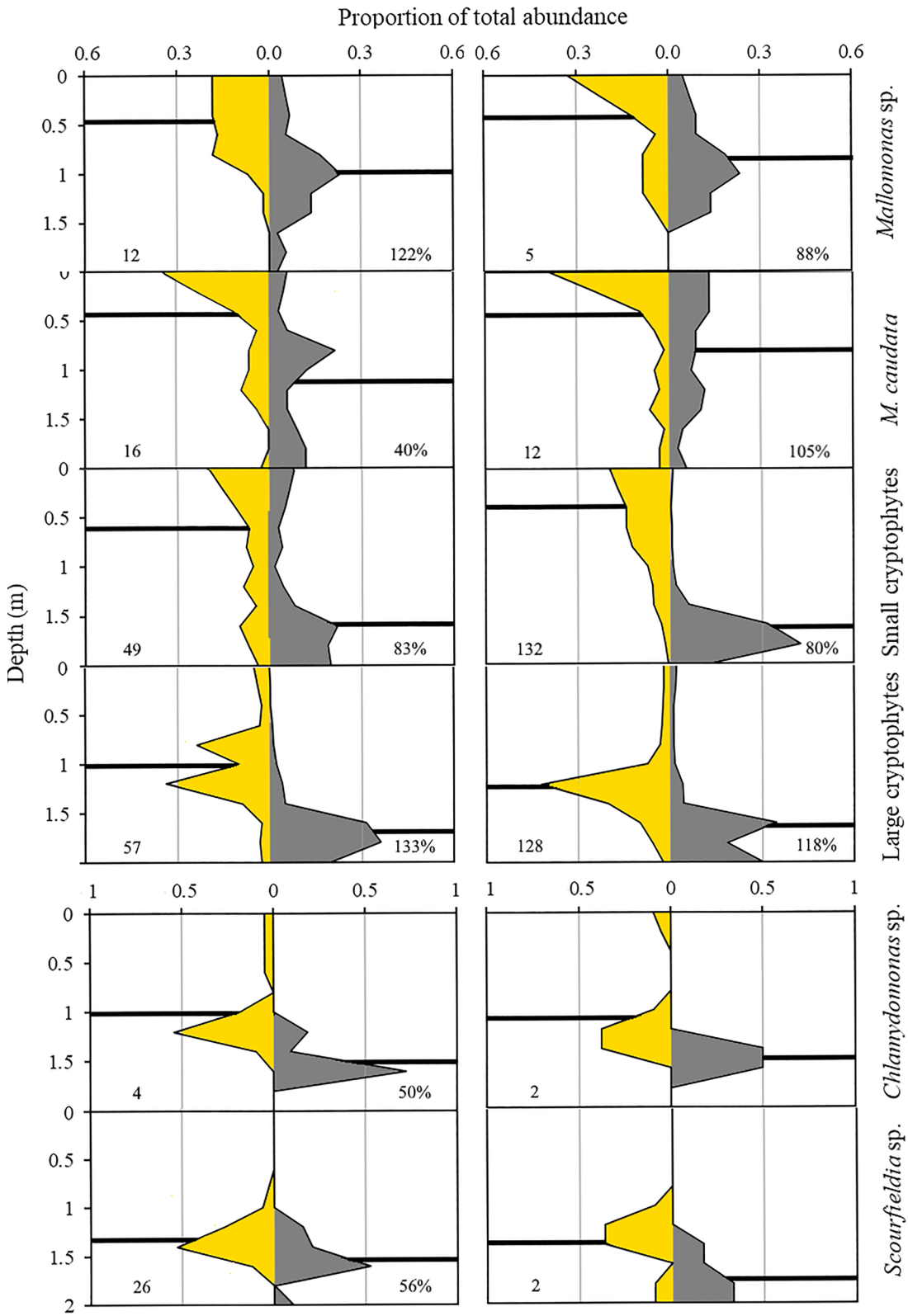
Because the cryptophytes in Mekkojärvi were at daytime mostly located in the uppermost 1.5 m, the enzymes found in the hypolimnion before noon must have been excreted during the previous night(s). This presumption is in line with the results of Münster (1992) who found 60–80% of AcPME and 45% of LAP enzyme activity of Mekkojärvi in dissolved form. According to Steen & Arnosti (2011), extracellular phosphatase and LAP enzymes can persist in cold seawater for days, implying that in Mekkojärvi dissolved enzymes probably contributed to nutrient cleavage from organic matter in the hypolimnion also during daytime when cryptophytes were in the epilimnion.

The decreases in the hypolimnetic dissolved total P and N concentrations after the fish introduction corroborate that enzymes played a marked role in the nutrition of cryptophytes. When the decrease in TP concentration (33 mg $m^{-2}$) between 1 and 2 m depths during 5 weeks after the fish introduction is converted to respective C concentration (assuming the molar Redfield C:N:P ratio of 106:16:1) the resulting 1.4 g $Cm^{-2}$ closely corresponds to the phytoplankton biomass at the end of the experiment supporting the significance of hypolimnetic organic nutrients for phytoplankton.

In the enrichment bioassays made near noon in parallel with this study, phytoplankton representing 0–0.5 m water layer of Mekkojärvi started to respond positively to the N additions, but not to the P additions, two weeks after the fish introduction (Järvinen & Salonen, 1998). The response faded toward the end of the summer along with the N:P ratio of particulate matter approaching the Redfield ratio. Thus, the changes in the AcPME and LAP enzyme activity, cryptophyte DVM amplitude, and bioassays consistently show that the time 2–3 weeks after the fish introduction was a turning point toward the relaxation of the nutrient limitation of phytoplankton.

The temporary N-limitation after the fish introduction in the bioassays, and enzyme results of this study contradict the N:P ratios of particulate matter indicating P limitation. The discrepancy may be explained by the sampling depth and incubation time. Particulate N:P reflected the integral for the period of a few days, while the bioassays reflected the instantaneous nutrient demand of phytoplankton. After the fish introduction, roughly 75% of phytoplankton, and even more of cryptophytes, located in water deeper than 0.5 m. One-day incubation time in the enrichment experiments may have been too long to yield a proper estimate of nutrient limitation. Because the particulate N:P ratio at 1 m, at which depth cryptophytes were typically most abundant at noon, indicated a balanced availability of P and N at the time of the fish introduction, these cryptophytes may have been less nutrient depleted than the cryptophytes occurring at 0–0.5 m depth.

It seems that after the fish introduction the increase in the DVM amplitude of cryptophytes largely supplemented the nutrient availability for phytoplankton. The contrast in the vertical distribution and migration pattern between *Cryptomonas* and *Mallomonas*


Proportion of total abundance
Depth (m)
Mallomonas sp.
M. caudata
Small cryptophytes
Large cryptophytes
Chlamydomonas sp.
Scourfieldia sp.
12
122%
5
88%
16
40%
12
105%
49
83%
132
80%
57
133%
128
118%
4
50%
2
26
56%
2

◂**Fig. 8** Vertical distributions of *Mallomonas* sp., *M. caudata*, small and large cryptophytes, *Chlamydomonas* sp., and *Scourfieldia* sp. in the 0–2 m water column of the enclosure at noon (yellow area) and midnight (grey area) on week 4 (left panels) and week 5 (right panels) (horizontal bars—median depth of the population; other explanations as in Fig. 4)

species in Mekkojärvi as well as the linkage between vertical migration and enzyme activity highlight combined physiological and behavioral solutions to nutrient depletion. Further, the decline of bacterioplankton after the fish introduction indirectly suggests that nutrient-depleted photosynthetic cryptophytes might switch to harvesting the abundant bacterioplankton of anoxic hypolimnion.

Bacterioplankton may contain relatively high concentration of phosphorus (Vadstein 2000), and hence it is a potential nutrient source for mixotrophic phytoplankton (Stibor & Sommer 2003). Several studies have suggested bacterivory of *Cryptomonas* (e.g., Porter, 1988; Tranvik et al., 1989; Roberts & Laybourn-Parry, 1999). However, Salonen and Jokinen (1988), Gasol et al. (1993) and Gervais (1998) found no bacterivory by *Cryptomonas* in the epilimnion. In the experiments of Salonen and Jokinen (1988), inorganic nutrient concentrations of Mekkojärvi were so high that probably mixotrophy was infeasible. Šimek et al. (2023) observed abundant heterotrophic cryptophytes in the whole water column of 14 European and Japanese lakes but larger autotrophic taxa did not utilize bacteria and were also absent in the hypolimnion. Similarly, Urabe et al. (2000) found little grazing on bacteria in the metalimnion of Lake Biwa. The combined results of Mekkojärvi suggest that the seemingly contradictory results in previous studies may be explained by different responses of cryptophytes to differing nutrient availability and grazing.

Our study shows that bacterivores can play a versatile role in the lake ecosystem corresponding to mixoplankton in the sea (Flynn et al., 2019). Mixotrophy provides nutritional flexibility that enhances the transfer of biomass to larger size classes in the food chain (Mitra et al., 2014; Stoecker et al., 2016; Ward & Follows, 2016).

### Role of asynchronous vertical migration

In addition to DVM, flagellated phytoplankton can do more complex asynchronous vertical migration (AVM) (Pearre 1979, 2003), where the migration cycle is not fixed to the diel course of solar radiation. By extending their stay in deep water longer than one night, phytoplankton can retrieve nutrients from greater depths and during a longer time interval. Due to methodological challenges, AVM is poorly known but phytoplankton can store enough nutrients for the whole life cycle or even several generations (Pearre 2003), so the duration of the AVM cycle may only be limited by the available energy reserves.

In this study, the bimodal vertical distributions of small cryptophytes found in the early phase of the experiment could have resulted from AVM or different behaviors of subpopulations. Bimodal vertical distribution seems to be common in small ponds (e.g., Smolander & Arvola, 1988; Arvola et al., 1992). Salonen and Rosenberg (2000) found a strong indication of AVM of the raphidophyte flagellate *Gonyostomum semen*, which partly stayed in the hypolimnion over noon. Only when the *Gonyostomum* abundance was low, one night in the hypolimnion seemed to meet the nutrient demand for the following day. Due to the migration of large cryptophytes below our sampling zone of Mekkojärvi, it was not possible to estimate whether they partly stayed in deep water over a day.

## Conclusions

Phytoplankton can quickly respond to changing conditions through behavioral and physiological adaptations (MacIntyre et al., 1997; Pearre, 2003; Mellard et al., 2012; this study). Vertical space partitioning, niches, and the ecological role of lake phytoplankton species can hardly be properly understood without sufficient knowledge of their migration behavior.

Our results show that cryptophytes can perform marked vertical migrations to balance their acquisition of light as well as nutrients and to reduce grazing losses. Vertical migration enabled *Cryptomonas* to reach an overwhelming dominance in phytoplankton biomass and Salonen and Rosenberg (2000) described the same for another flagellate, *Gonyostomum semen*, in a larger pond with anoxic hypolimnion. These examples, leading to hypolimnetic nutrient depletion, may be extreme, but they illustrate the potential of hypolimnetic nutrients to support phytoplankton primary productivity still awaiting quantification. In

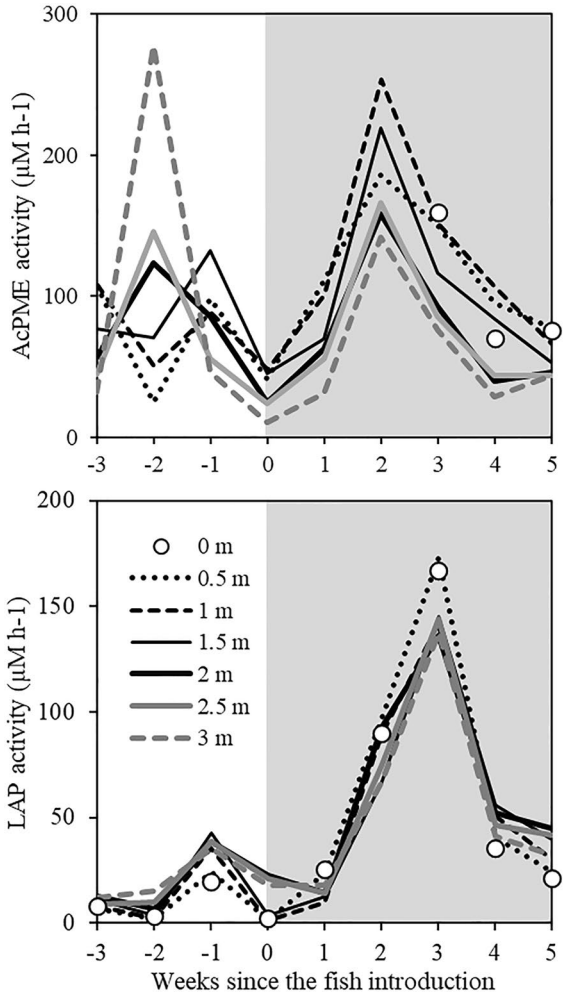



**Fig. 9** Acid phosphomonoesterase (AcPME) and leucine aminopeptidase (LAP) enzyme activities in the water column during the experiment

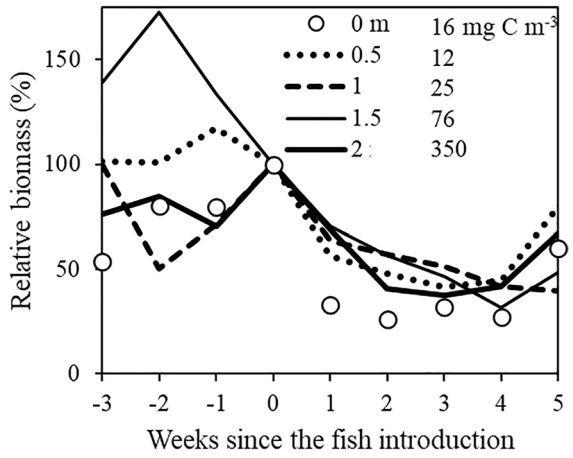



**Fig. 10** Bacterioplankton biomass at different depths during the experiment relative to the biomass observed at the time of the fish introduction (shown on the right side of the legends)

marine waters, field studies combined with modelling have suggested substantial increases of primary productivity due to phytoplankton vertical migration (Ault, 2000; Kowalewski, 2015; Wirtz & Smith, 2020; Wirtz et al., 2022). It seems justified to conclude that vertical migrations should not be overlooked in aquatic productivity studies. This will be a big challenge where modelling solutions are also needed.

Although vertical migrations have a fundamental role at all trophic levels of pelagic ecosystems, we are only starting to understand the complexity related to the fine-scale temporal and spatial impacts on the physiology and behavior of plankton, as well as the interplay with biological, physical, and chemical factors. Our results on phytoplankton emphasize that small ponds with stable summer stratification as well as simple hydrodynamics and food web are unique "natural laboratories" where complicated ecological phenomena can be studied under realistic (Carpenter, 1996) but still practical conditions.

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**Author contributions** All authors have contributed substantially to this study. K.S. designed the study, participated in sampling, analysed data, and wrote the manuscript; M.J. took and counted routine phytoplankton samples, and assisted with writing; T.A. collected zooplankton data and made carbon determinations of *Daphnia*; M.L. collected and counted bacterioplankton samples; V.L. collected phytoplankton vertical migration samples and analysed data; U.M. assessed the enzyme activities and contributed to writing the manuscript; J.S. contributed to study design and writing of the manuscript. All authors commented on the final manuscript.

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**Data availability** The datasets generated during and/or analysed during the current study are available from the authors on reasonable request.

**Declarations**

**Conflict of interest** The authors have no competing interests to declare that are relevant to the content of this article.



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