



# Impact of zooplankton grazing on phytoplankton in north temperate coastal lakes: changes along gradients in salinity and nutrients

Inge Christensen · Lisbeth Kjæreby Pedersen · Martin Søndergaard · Torben L. Lauridsen · Sh. Tserenpil · Katherine Richardson · Cihelio A. Amorim · Juan Pablo Pacheco · Erik Jeppesen

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**Abstract** Zooplankton grazing at similar nutrient levels is generally regarded as lower in brackish than in freshwater lakes, but experimental evidence of this is lacking. Accordingly, we conducted short-term zooplankton grazing experiments in bottles with water from 12 Danish brackish lakes covering a large gradient in salinity (0.3–17.4‰) and nutrient

concentrations as well as with water from 24 mesocosms established in the same area with various salinities (0.5–12‰), two nutrient levels and low fish density. Grazing was low in 11 of the 12 lakes, even when they were dominated by edible phytoplankton and nutrient addition led to a major increase in phytoplankton biomass. By contrast, grazing was significant in most of the mesocosms, particularly at high nutrient levels and salinities of 8‰ or below where *Daphnia* dominated. Moreover, grazing decreased the biomass of most phytoplankton taxa, except for a few (e.g. *Ankya* at 0.5–2‰ and *Ochromonas* and *Chaetoceros* at 8‰). Our results provide experimental support for potentially significant grazing by zooplankton on phytoplankton in brackish lakes up to a salinity of 8‰ at low fish density; however, grazing in summer

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I. Christensen · L. K. Pedersen · M. Søndergaard · T. L. Lauridsen · E. Jeppesen (✉)  
Department of Ecoscience, Aarhus University, Århus, Denmark  
e-mail: ej@ecos.au.dk

M. Søndergaard · T. L. Lauridsen · E. Jeppesen  
Sino-Danish Centre for Education and Research, Beijing, China

S. Tserenpil  
Nuclear Research Center, National University of Mongolia, Ulaanbaatar, Mongolia

K. Richardson  
Center for Macroecology, Evolution and Climate, University of Copenhagen, Copenhagen, Denmark

C. A. Amorim · E. Jeppesen  
Limnology Laboratory, Department of Biological Sciences and Centre for Ecosystem Research and Implementation, Middle East Technical University, Ankara, Turkey

J. P. Pacheco  
Centro Universitario Regional del Este – CURE, Universidad de la República, Maldonado, Montevideo, Uruguay

E. Jeppesen  
Institute of Marine Sciences, Middle East Technical University, Mersin, Ankara, Turkey

was generally low in the majority of the lakes, which we attribute to high predation on zooplankton.

**Keywords** Brackish lakes · Saline lakes · Grazer control

## Introduction

Ecosystem structure and function are less well studied in brackish than in freshwater lakes (Leah et al., 1978; Moss et al., 1991; Jeppesen et al., 1998, 2007; Brucet et al., 2010, 2012; Jensen et al., 2010; Gutierrez et al., 2018). A few investigations comparing freshwater and brackish lakes (Moss, 1994; Jeppesen et al., 1994, 1998; Pfister et al., 2002; Sabbe et al., 2003) have shown important differences, making it difficult to transfer knowledge gained from freshwater studies directly to brackish lakes. One important difference in the north temperate zone is the occurrence of mysid crustaceans (e.g., *Neomysis integer* (Leach, 1814)) in brackish lakes (>0.5‰) (Remerie et al., 2009). Mysids increase in abundance along a nutrient gradient (Rakhesh et al., 2015) and feed efficiently on all types of zooplankton (Jeppesen et al., 1994; O'Malley & Bunnell, 2014; Devlin et al., 2017). Moreover, sticklebacks (e.g., *Gasterosteus aculeatus* Linnaeus, 1758) can be abundant and increase in dominance along a nutrient gradient in brackish lakes (Jakobsen et al. 2004; Arai et al., 2020). Sticklebacks produce offspring several times during summer and autumn (Jeppesen et al., 1998). Because of the high occurrence of mysids, and the presence of sticklebacks and their juveniles in eutrophic brackish lakes, the predation pressure on zooplankton is suggested to be much higher than in similar freshwater lakes, and the zooplankton to phytoplankton biomass ratio is accordingly lower in brackish than in freshwater lakes (Jeppesen et al., 1994; Jeppesen et al., 1998; Søndergaard et al., 2000; He et al., 2020).

Another difference is that the key grazers in freshwater lakes, particularly the large-bodied *Daphnia* spp., are rare or even lacking when salinity exceeds 4 ‰ (Jeppesen et al., 1994; Moss, 1994; Latta et al., 2012). Instead, the zooplankton community is dominated by calanoid copepods, such as *Eurytemora affinis* (Poppe, 1880) and *Acartia* spp. (Jeppesen et al., 1994; Moss, 1994; Diekmann et al., 2012; He et al., 2020), and rotifers (Heerkloss et al., 1991; He

et al., 2020). Copepods and rotifers have narrower food size spectra than cladocerans and are therefore less efficient in controlling phytoplankton (Jeppesen et al., 1994; Mayeli et al., 2005; Ger et al., 2019; Lüring 2021). Moreover, salt may affect the grazing intensity of zooplankton (e.g., Baillieul et al., 1998; Zadereev et al., 2022a, b).

The phytoplankton communities in brackish lakes differ from those of freshwater lakes because certain brackish/marine phytoplankton taxa appear or become more abundant. Examples are various diatoms, such as *Chaetoceros* and the toxic *Pseudonitzschia*, the distinctive ichthyotoxic haptophyte *Prymnesium parvum* N. Carter and the toxic dinoflagellates *Alexandrium* and *Karenia* (Moss, 1994; Paerl et al., 2018). In many eutrophic brackish lakes, as in freshwater lakes, cyanobacteria are dominant and usually form blooms, including the gelatinous colonial *Aphanothece* spp. or the N-fixing filamentous *Anabaenopsis* and *Nodularia* (e.g., Moss, 1994; Aaser et al., 1995; Paerl et al., 2018). Also, with a further increase in nutrient loading, chlorophytes, such as *Scenedesmus*, *Chlamydomonas* and *Dunaliella*, often become dominant (Wasmund & Kell, 1991; Nche-Fambo et al., 2015; Obolewski et al., 2018).

In a recent comparative study, He et al. (2020) revealed that zooplankton grazing on phytoplankton was low in a series of Danish brackish coastal lakes as judged indirectly by a low zooplankton:phytoplankton biomass ratio, this being especially low in eutrophic lakes with low cover of submerged macrophytes, high turbidity and high abundance of zooplanktivorous fish. Moreover, Jeppesen et al. (1994) showed that the calculated grazing pressure on phytoplankton in brackish lakes was as low as 0.1–9% per day, being lowest at high nutrient levels. However, experimental evidence of the low grazing pressure by zooplankton on phytoplankton is still lacking.

The aim of this study was to experimentally evaluate the impact of zooplankton grazing on phytoplankton in brackish lakes by examining changes in phytoplankton biomass and taxonomic composition in the presence and absence of zooplankton. We conducted several 4-day grazing experiments with water incubated in situ with and without zooplankton at contrasting salinities and nutrient levels. In one set of the experiment, we further added nutrients to elucidate the bottom-up control. Water was sampled

from 12 brackish lakes in the nature reserve Vejlerne, Denmark. To understand the potential role of fish for zooplankton grazing, we also conducted grazing experiments on water collected from 24 mesocosms established in the same area with various salinities and two nutrient levels at low fish density. We hypothesised that the phytoplankton in the lake water would be mainly controlled by nutrients due to high predation by fish on zooplankton, but that reduced fish predation would lead to an increasing role of zooplankton grazing, at least at low salinities where *Daphnia* can be dominant.

## Materials and methods

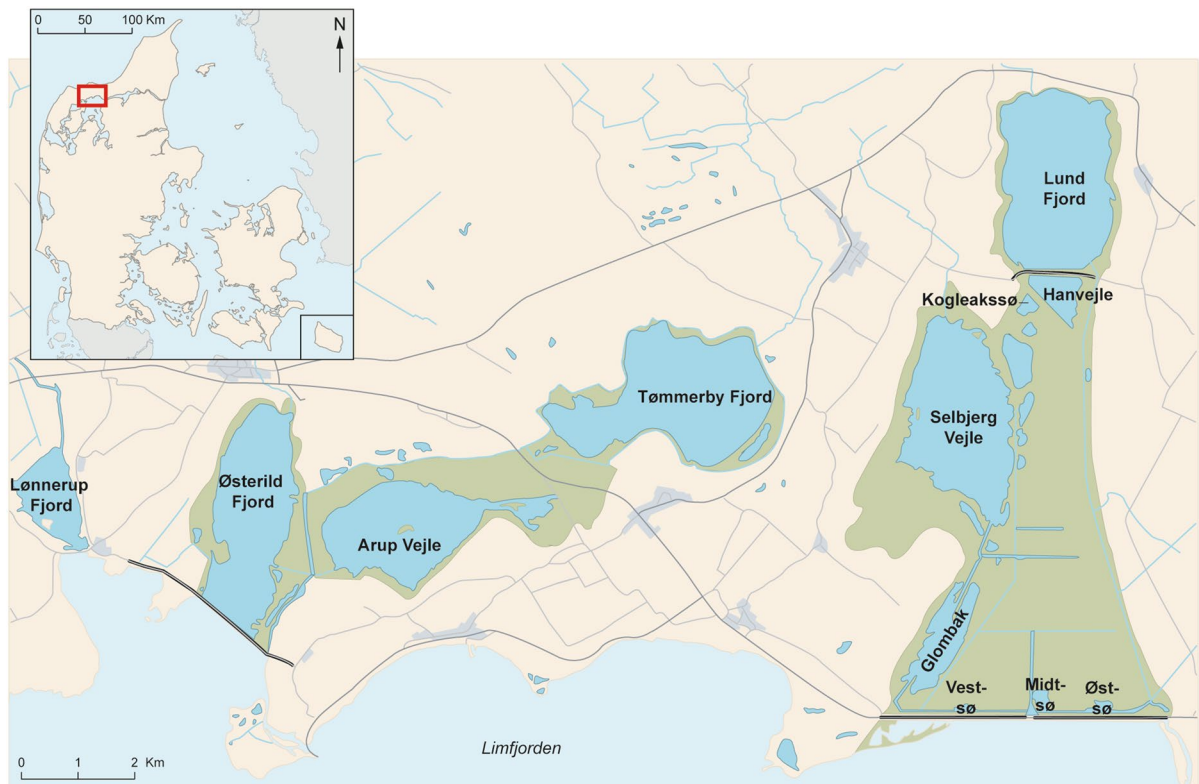
### Study area

The brackish wetland system Vejlerne is a nature reserve located in the northern part of Jutland, Denmark (Fig. 1). The wetland covers approximately

6,000 ha and consists of many shallow brackish lakes (Nielsen, 1998). There is a salinity gradient varying from 0 to 1‰ in the northern lakes most distant from the Limfjord to 5–20‰ in the most southern lakes that are partly connected to the North Sea. However, higher salinities occur during summer (Møller, 1980; Nielsen, 1998). Most of the lakes are eutrophic because of anthropogenic activities in the catchments. Yet, despite their mostly turbid state many of the shallow lakes still have submerged vegetation (Jeppesen et al., 1994; Hald-Mortensen, 1998; He et al., 2020), which is a common feature of eutrophic shallow north temperate brackish lakes (Moss, 1994; Jeppesen et al., 1994).

### Experimental set-up

During summer, three different zooplankton grazing experiments (Table 1 and detailed below) were carried out in Lake Kogleakssø (Fig. 1). We used a common set-up and experimental procedure where six



**Fig. 1** Map of Vejlerne Nature Reserve showing the 12 sample lakes and the marine area (Limfjorden)

**Table 1** Summary of the three 4-day incubation experiments conducted during summer

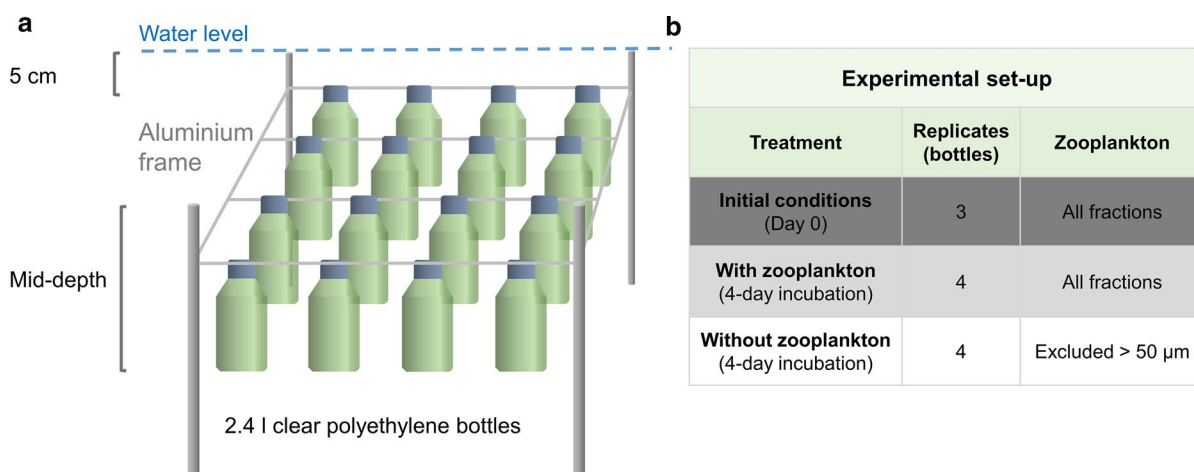
| Experiment                                   | Period | Samplings and treatments  |
|--|--------|---|
| Experiment with lake water (Exp1)            | July   | 12 Lakes: Kogleakssø, Han Vejle, Lund Fjord, Tømmerby Fjord, Glombak, Østerild Fjord, Lønnerup Fjord, Arup Vejle, Vestsø, Midtsø, Østsø |
| Lake experiment 2 (Exp2)                     | July   | 6 Lakes: Kogleakssø, Han Vejle, Lund Fjord, Tømmerby Fjord, Glombak, Østerild Fjord + addition of nutrients (N + P)                     |
| Experiments with water from mesocosms (Exp3) | June   | Mesocosms: 0.5, 2, 4, 6, 8, 12‰, low (LN) and high (HN) nutrient level  |

rectangular aluminium frames were tied to wooden sticks 5 cm under the water surface (Fig. 2a). Each frame had the capacity to hold 16 bottles at a mid-depth position. Clear polyethylene bottles (Nalgene®), with a total volume of 2.4 l, were used.

In each experiment (Table 1; Fig. 2b), we had three sets of bottles, one set ('initial conditions – Day 0') to record the starting level of phytoplankton biomass and relative contribution of various taxa on day 0, and additionally two sets 'with zooplankton' and one set 'without zooplankton' (excluding zooplankton larger than 50 µm), and these were incubated for 4 days. At the start of the experiment, water for the 'initial conditions – Day 0' and 'with zooplankton' treatments was filtered through a 50 µm filter into a funnel inserted in the incubation bottle. Just before the last portion of water had passed through the funnel, a small amount of water was held back to prevent the zooplankton on the filter from drying out. The zooplankton were then poured back into the respective

incubation bottles for recording 'initial conditions Day 0' and 'with zooplankton' after removal of mysids and larger plant material. In the 'without zooplankton' treatment, the water was sieved through a 50 µm filter to remove cladocerans, adult copepods and most nauplii and rotifers. The pre-filtration/addition procedure for the 'initial conditions Day 0' and 'with zooplankton' bottles was undertaken to obtain starting conditions similar to those of the 'without zooplankton' bottles. The with and without zooplankton bottles were then incubated for 4 days, and the samples were subsequently fixed as described below, while samples from the 'initial conditions-Day 0' were fixed at the time when the incubation started for the other bottles. Once a day, the bottles were turned manually; otherwise stirring of the incubated water occurred only by natural water body movements.

For determination of phytoplankton composition and biomass, 50 ml water (sub)samples were fixed with 1 ml of Lugol's solution, and 150 ml were used

**Fig. 2** Schematic figure of the experimental grazing set-up, showing bottles (a) and conditions (b)

for chlorophyll-*a* measurement. For determination of zooplankton composition and biomass, 2 l were filtered on a 50 µm filter and fixed with 2 ml Lugol's solution.

#### Experiments with lake water (Exp1 and Exp2)

Two experiments (Exp1 and Exp2) were conducted with water collected from a set of 12 and 6 lakes, respectively, in Vejlerne (Table 1). For that, depth-integrated water samples (30 l) from each of the lakes were collected in an open area outside the littoral zone and transported to the experimental set-up site in Lake Kogleakssø (max. 10 km away). The first experiment (Exp1) was conducted with water collected from 12 lakes (Fig. 1) that covered a large salinity (0.3 to 17.4‰) and nutrient concentration gradient (Table S1). In the second experiment (Exp2), water from six of the 12 lakes was used. These six lakes had varying nutrient concentrations (60–170 µg TP l<sup>-1</sup> and 1200–2600 µg TN l<sup>-1</sup>) and low salinities (0.3–2.4 ‰, Table S2). Before the incubation of the bottles, half of the water samples from Exp2 were enriched with nutrients (N+P) as concentrated solutions of Na<sub>2</sub>HPO<sub>4</sub> and Ca(NO<sub>3</sub>)<sub>2</sub>. The addition of nutrients increased both TP and TN by a factor of 1.6–4.4 (Table S2). Both experiments were conducted when water temperatures were 20 °C ± 0.4 and 17 °C ± 0.2, respectively.

#### Mesocosm experiments (Exp3)

For the third experiment (Exp3), we took advantage of an ongoing experiment in the shallow Lake Kogleakssø at the time of our study (for details see Jeppesen et al., 2007). This experiment consisted of 48 mesocosms with a water level of 0.8 m and a diameter of 1.2 m (volume approximately 1,000 l). The water in the mesocosms was in contact with the atmosphere at the surface and with the sediment at the bottom. A single male three-spined stickleback (*G. aculeatus*) was added to each mesocosm to obtain a moderately low fish density (1 m<sup>-3</sup>). A salt solution of NaCl, MgSO<sub>4</sub>, and NaHCO<sub>3</sub> and nutrients [nitrogen as Ca(NO<sub>3</sub>)<sub>2</sub> and phosphorus as Na<sub>2</sub>HPO<sub>4</sub>] were added to duplicate mesocosms to obtain the following salinities: 0.5, 1, 2, 4, 6, 8, 12, and 16‰, and nutrient levels: 50, 150 and 450 µg TP l<sup>-1</sup> (TN at a N:P ratio of ~10:1 by weight). To allow high salinity-adapted

species to develop in the more saline mesocosms, all the mesocosms were inoculated with an identical mixture of plankton and sediment taken from three different locations in Vejlerne (1–22.4‰) (Jeppesen et al., 2007).

In Exp3, we used water from 24 of the 48 mesocosms, including the natural low nutrient level (LN) and the highest added nutrient level (HN) crossed with the following salinities: 0.5, 2, 4, 6, 8 and 12‰. The water samples were collected with a depth-integrating tube sampler down to approximately 20 cm above the bottom to prevent interference from the sediment. Repeated water samples were taken to obtain a total water volume of approximately 15 l from each of the two duplicates and pooled; part of the pooled water was taken for the grazing experiment, which was conducted in a similar manner as in the lakes in 2.4 l bottles as described above.

#### Analyses of samples

For chlorophyll-*a*, 0.15 l were filtered onto glass microfiber filters (Whatman GF/C) immediately after sampling. The filters were frozen, and chlorophyll-*a* was determined spectrophotometrically after extraction with 96% ethanol (Riemann & Ernst, 1982). Total nitrogen (TN) was measured as nitrate + nitrite after oxidation by persulphate as described by Koroleff (1976a). Total phosphorus, TP, was measured as orthophosphate after persulphate digestion according to Koroleff (1976b). Salinity and temperature were measured in the field and in the mesocosms with a multiprobe (YSI 30).

Lugol-fixed phytoplankton samples were sedimented and counted using an inverted microscope according to Ütermöhl (1958), Lund et al. (1958) and Olrik (1991). Depending on sample concentrations, 5, 10, 25 or 50 ml sedimentation chambers were used. In addition, dilution of the samples was often necessary. Phytoplankton was generally identified to genus and, if possible, to species level at ×400 magnification, and 1–3 diagonal transects were counted. Three of the four replicates (randomly selected) of each treatment were counted. Colonial cyanobacteria were generally grouped as *Aphanothece* spp./*Aphanocapsa* spp./*Cyanodictyon* spp. as they were difficult to separate from each other. Depending on the abundance and the variation in the shape of the counted phytoplankton, the linear dimensions of up to 40



individuals of each taxon were measured (typically 10 individuals). Mean individual size ( $\mu\text{m}^3$ ) and phytoplankton biomass ( $\text{mm}^3 \text{ l}^{-1}$ ) values were obtained by fitting each taxon to simple geometric shapes according to Olrik (1991).

Phytoplankton biomass from the ‘initial’ (Day 0) treatment only was divided into three size classes using the greatest axial linear dimension (GALD) and a specific size criterion:  $\text{GALD} < 20 \mu\text{m}$ ;  $20 \mu\text{m} < \text{GALD} < 40 \mu\text{m}$ ;  $\text{GALD} > 40 \mu\text{m}$ . This classification was chosen since  $20 \mu\text{m}$  approximates the maximum size above which phytoplankton is not readily consumed by rotifers (Pourriot, 1977; Rothhaupt, 1990), whereas  $40 \mu\text{m}$  approximates the maximum size for filter-feeding zooplankton (Burns, 1968; Demott, 1982; Vyhnašek, 1983).

For zooplankton, cladocerans and adult copepods were identified to species and rotifers to genus level. Nauplii and copepodites were classified as cyclopoids or calanoids. If subsampling was conducted, it included at least 75 individuals of the most common species/genera (Hansen et al., 1992). Zooplankton biomass was calculated using standard values of biomass for each species and life stage (copepodites and nauplii) estimated from numerous length measurements in many Danish lakes (Jensen et al., 1996).

### Statistical analyses

To test for the grazing impact on phytoplankton, a one-way ANOVA was performed on the

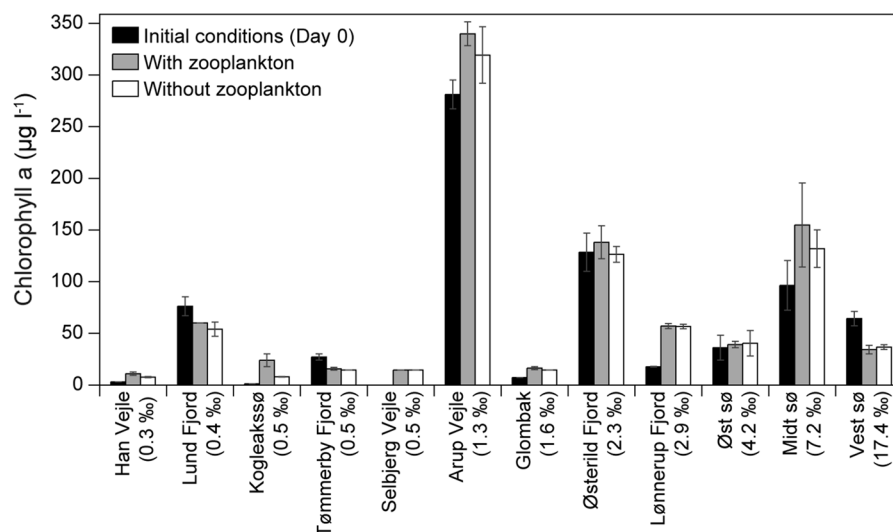
chlorophyll-*a* data from all three experiments. For Exp3 (with water from the mesocosms), a one-way ANOVA was used to compare chlorophyll-*a*, total phytoplankton biomass and several abundant taxa between the treatments. A grazing impact on phytoplankton was identified if a significant difference was observed between the treatments ‘with zooplankton’ and ‘without zooplankton’, and only if the latter was significantly higher than the former. To test for differences between nutrient levels, salinities and zooplankton treatments, a three-way ANOVA was used on chlorophyll-*a* and total biomass data. When the ANOVA analyses revealed significant differences in grazing impact, salinity or nutrients, a Student–Newman–Keuls (SNK) comparison test was applied to find group means in homogeneous subsets (Fowler et al., 1998). Before running the ANOVA analyses (SPSS version 9.0), the data were transformed (log, square root or rank) to reduce the heterogeneity of variance.

## Results

### Experiments with lake water (Exp1 and Exp2)

With one exception (Lønnerup Fjord), the changes in chlorophyll-*a* after the 4 days of incubation were small in Exp1, even in the ‘without zooplankton’ treatments (Fig. 3). In only few cases were chlorophyll-*a* after the 4 days ‘with zooplankton’ lower

**Fig. 3** Chlorophyll-*a* in 12 lakes in Vejlerne ordered along an increasing salinity gradient, showing initial conditions (Day 0) and the level after 4 days of incubation with and without zooplankton ( $> 50 \mu\text{m}$ )



**Table 2** Summary of one-way ANOVA analyses of log transformed chlorophyll-*a* data from Experiments 1 and 2 with water from the lakes. Impact of grazing (+) was identified when a comparative post hoc test (S.N.K.) revealed a signifi-cant difference ( $P < 0.01$ ) in chlorophyll-*a* between treatment 2 ('with zooplankton') and treatment 3 ('without zooplankton'), and if 3 was significantly higher than 2 (phytoplankton biomass decreased)**Experiment 1 (10 lakes)**

|                | ANOVA (treatment)              | Comparison (S.N.K. test) | Grazing impact |
|----------------|--------------------------------|--------------------------|----------------|
| Han Vejle      | $F = 144.80$ $P < 0.0001$      | $2 > 3 > 1$              | –              |
| Lund Fjord     | $F = 10.48$ $P < 0.006$        | $1 > 2, 3$               | –              |
| Tømmerby Fjord | $F = 62.52$ $P < 0.0001$       | $1 > 2, 3$               | –              |
| Kogleakssø     | $F = 248.80$ $P < 0.0001$      | $2 > 3 > 1$              | –              |
| Arup Vejle     | $F = 7.86$ $P < 0.013$         | $2, 3 > 1$               | –              |
| Østerild Fjord | $F = 0.68$ $P < 0.534$ (n.s.)  | $2, 1, 3$                | –              |
| Lønnerup Fjord | $F = 1,158.74$ $P < 0.0001$    | $2, 3 > 1$               | –              |
| Østsø          | $F = 0.185$ $P < 0.834$ (n.s.) | $2, 3, 1$                | –              |
| Midtsø         | $F = 4.05$ $P < 0.061$         | $2, 3 > 1$               | –              |
| Vestsø         | $F = 37.55$ $P < 0.0001$       | $1 > 3, 2$               | –              |

NB. Glombak and Selbjerg Vejle were excluded due to lack of replicates

**Experiment 2 (6 lakes)**

|                | Natural nutrient level         |                          |                | Added nutrient (N+P)        |                          |                |                 |
|----------------|--------------------------------|--------------------------|----------------|-----------------------------|--------------------------|----------------|-----------------|
|                | ANOVA (treatment)              | Comparison (S.N.K. test) | Grazing impact | ANOVA (treatment)           | Comparison (S.N.K. test) | Grazing impact | Nutrient impact |
| Han Vejle      | $F = 7.69$ $P < 0.014$         | $3, 2 > 1$               | –              | $F = 1,615.91$ $P < 0.0001$ | $2, 3 > 1$               | –              | +               |
| Lund Fjord     | $F = 0.834$ $P < 0.469$ (n.s.) | $2, 3, 1$                | –              | $F = 466.267$ $P < 0.0001$  | $3, 2 > 1$               | –              | +               |
| Kogleakssø     | $F = 109.28$ $P < 0.0001$      | $3 > 2 > 1$              | +              | $F = 1,004.31$ $P < 0.0001$ | $2, 3 > 1$               | –              | +               |
| Tømmerby Fjord | $F = 12.64$ $P < 0.003$        | $2, 1 > 3$               | –              | $F = 512.07$ $P < 0.0001$   | $3, 2 > 1$               | –              | +               |
| Glombak        | $F = 10.43$ $P < 0.006$        | $2, 1 > 3$               | –              | $F = 616.12$ $P < 0.0001$   | $3, 2 > 1$               | –              | +               |
| Østerild Fjord | $F = 2.23$ $P < 0.17$ (n.s.)   | $2, 1, 3$                | –              | $F = 1,537.32$ $P < 0.0001$ | $2, 3 > 1$               | –              | +               |

No grazing impact (–) was found when no significant difference occurred between 2 and 3 (phytoplankton biomass was unaffected), and if 2 was significantly higher than 3 (phytoplankton biomass increased). Impact of nutrient addition (+) was identified in Experiment 2 when treatment 1 ('Initial') was significantly lower than treatment 2 and 3

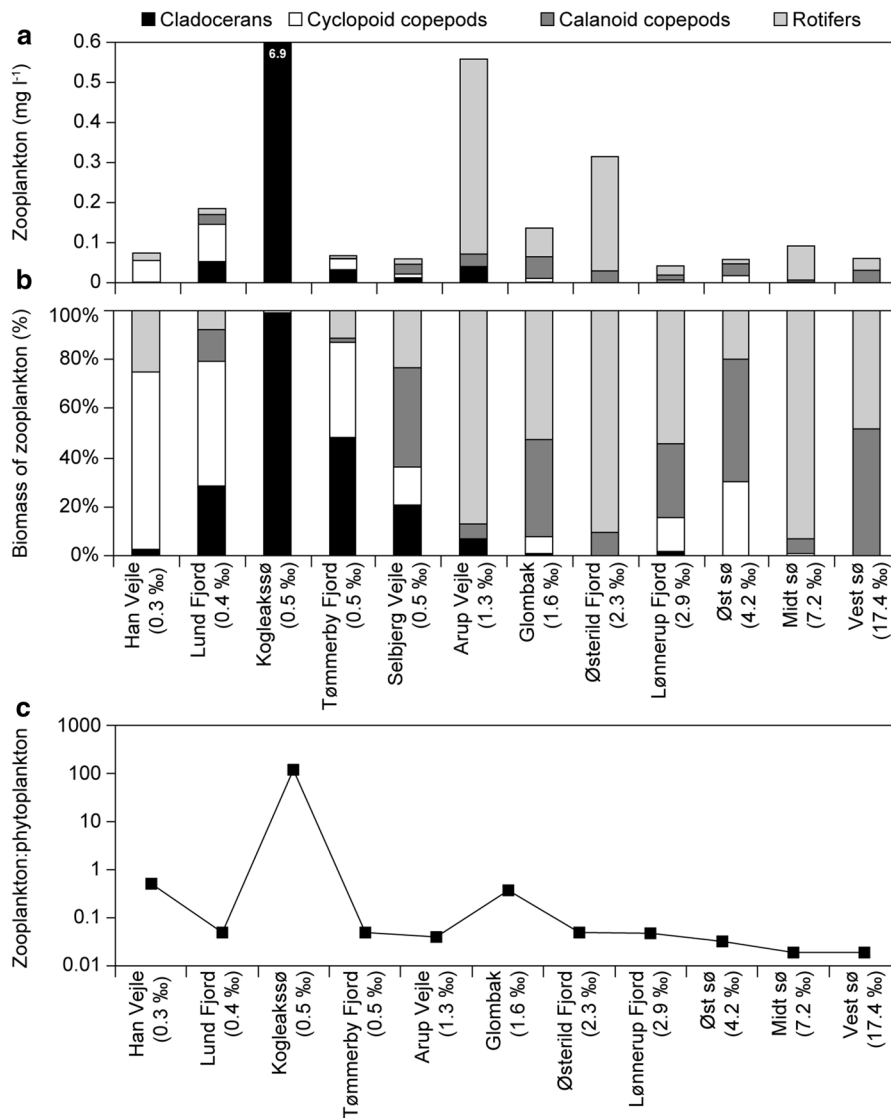
than those 'without zooplankton', but not significant (Table 2). With exception of for Lake Kogleakssø (Fig. 4a), the zooplankton biomass was low (Fig. 4b) and dominated by cladocerans and cyclopoid copepods in salinities below 6‰ and by rotifers and calanoid copepods at higher salinities. The low zooplankton biomass was also evidenced by an overall low zooplankton:phytoplankton biomass ratio of  $< 0.5$ , except for Han Vejle and Kogleakssø (Fig. 4c).

However, a highly significant increase in chlorophyll-*a* was found after nutrient enrichment in Exp2 in both treatments 'with zooplankton' and 'without zooplankton' (Fig. 5; Table 2), while no significant

differences in chlorophyll-*a* concentrations were found between these two treatments (grazing effect negative, Table 2), except for Lake Kogleakssø at natural nutrient level showing lower concentration 'with zooplankton'. In some experiments, the chlorophyll-*a* concentrations even increased in the treatments with zooplankton (Table 2).

At the time of the grazing experiments, cyanobacteria dominated the phytoplankton communities in about half of the 12 lakes (Table 3). The community structures in the two hypereutrophic lakes, Østerild Fjord and Arup Vejle, were almost identical, with exclusive dominance by filamentous cyanobacteria.

**Fig. 4** Total biomass and percentage contribution of different groups of zooplankton in 12 lakes in Vejlerne at the time of the grazing experiment ordered along an increasing salinity gradient. Also the zooplankton:phytoplankton biomass ratio is shown



In Glombak, Selbjerg Vejle and Lund Fjord, the dominant cyanobacteria were small colonies of *Aphanothece* spp./*Aphanocapsa* spp./*Cyanodictyon* spp. In Tømmerby Fjord and Han Vejle, these cyanobacteria taxa constituted approximately 50% of the total phytoplankton biomass. The phytoplankton communities in the remaining five lakes varied markedly. Cryptomonads dominated in Lake Kogleakssø, in Lønnerup Fjord and in the most saline lake, Vestsø. The second and third most saline lakes, Midtsø and Østsø, were dominated by a large dinoflagellate, *Gymnodinium* spp. (~50 µm), together with a mixed community of *Nitzschia* (pennate diatom), *Planktothrix*/*Limnothrix*

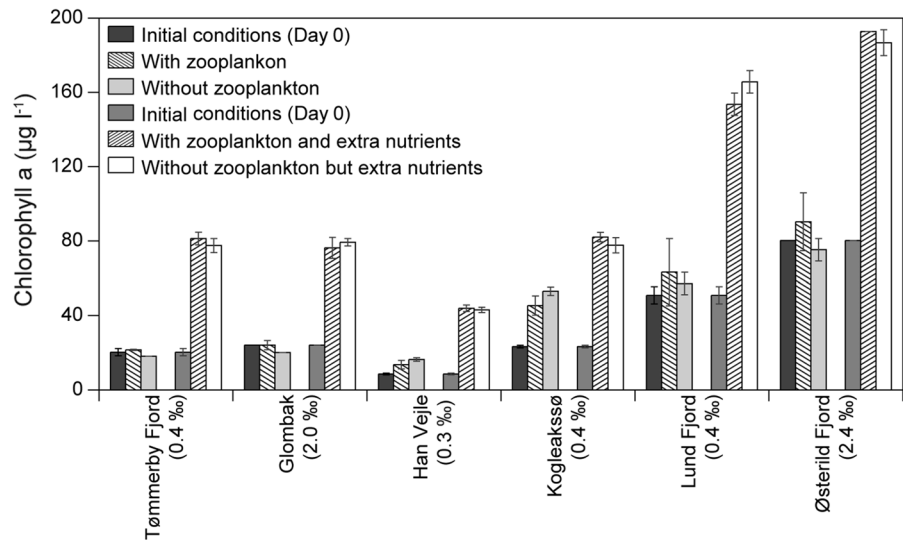
(filamentous cyanobacteria) and *Monoraphidium* (unicellular chlorophyte) (Table 3).

#### Experiments with water from the mesocosms (Exp3)

In this experiment, a shift occurred from dominance in terms of biomass of several *Daphnia* species (mainly *D. pulex* Leydig, 1860, however) at low salinities to exclusive dominance of *D. magna* Strauss, 1820 at 6–8‰ (Jeppesen et al., 2007). At higher salinities, rotifers became dominant, *Notolca* sp. at 8‰ and *Brachionus plicatilis* O.F. Müller, 1786 at 12‰ (Jeppesen et al, 2007). For the phytoplankton biomass (and also for chlorophyll-*a*, data not shown),



**Fig. 5** Chlorophyll-*a* in six lakes in Vejlerne, showing initial conditions (Day 0) and the level after 4 days of incubation with and without zooplankton (> 50 µm), without (to the left) and with (to the right) addition of nutrients (N + P)



we found a significant effect of grazing, salinity and nutrient levels, besides significant interactions between all combinations involved (Table 4). In most cases, homogeneity of variances was not met, so the three-way ANOVA analysis was performed on ranked total biomass (Table 4).

The differences in phytoplankton biomass ‘with zooplankton’ and ‘without zooplankton’ varied greatly with salinity and nutrient levels (Fig. 6; Table 5) and were the highest in HN and at salinities of 4, 6 and 8‰. The grazing impact on phytoplankton was significant over the entire salinity range (0.5–12‰), except for the 2‰, 8‰ and 12‰ experiments in LN where chlorophyll-*a* was significantly higher ‘with zooplankton’ than ‘without zooplankton’. The higher ‘initial’ chlorophyll-*a* concentration at 6‰ was an outlier due to large filamentous green algae, which could not be removed before the chlorophyll-*a* determination.

The biomass (and chlorophyll-*a*) and community composition of the phytoplankton changed greatly with salinity, nutrient level and grazing pressure (Figs. 6, 7). At low salinity (0.5–2‰) (Fig. 7a), cryptophytes, mainly *Cryptomonas reflexa* Skuja, dominated the biomass. However, the chlorophyte *Ankylra judayi* (G.M. Smith) Fott, contributed importantly to the biomass in the treatment ‘with zooplankton’ (20–100%), and it was dominant only at 2‰ salinity in LN (Fig. 7b). At 0.5‰ and 2‰, diatoms (*Nitzschia* spp.) and unidentified flagellates dominated in HN.

At intermediate salinities (4 and 6‰), different phytoplankton communities were found in LN. At 4‰, *Rhodomonas* spp. dominated (70% of total biomass), while only few cyanobacteria colonies were registered. However, at 6‰, the cyanobacteria constituted about 40% of the total biomass, mainly consisting of large-sized *Dolichospermum* spp. and *Nodularia* spp., and at salinities ≥ 6‰ the diatom *Chaetoceros* spp. dominated. Most species increased relative to their initial abundance during the incubations (Fig. 7a–f; Table S3). Two exceptions were *A. judayi* and *Mallomonas akrokomos* Ruttner in LN. Moreover, the biomass of most phytoplankton taxa was significantly ( $P < 0.01$ ) lower in the treatment ‘with zooplankton’ than ‘without zooplankton’ (Table S3 shows those taxa that were not grazed upon). However, some taxa were unaffected by, or their biomass even increased, in the presence of zooplankton (Fig. 7a–f). *Ankylra judayi* was not affected by grazers at low salinities (0.5‰ and 2‰) in both LN and HN (Fig. 7a, b). At intermediate salinities (4 and 6‰), all abundant phytoplankton taxa were significantly grazed, and biomass values in the range 0–0.04 mm l<sup>-1</sup> were found for all taxa in the ‘with zooplankton’ treatment (Fig. 7c, d). At 8‰ salinity, all abundant taxa were grazed, except the flagellate *Ochromonas* spp. and the large and dominant *Chaetoceros* spp. for which no net grazing effect was recorded (Fig. 7e). At the highest salinity (12‰), none of the phytoplankton taxa showed any significant net grazing response, except in HN, whereas the

**Table 3** Dominant phytoplankton classes as a percentage of total biomass and examples of abundant phytoplankton genera from the 12 lakes during the first grazing experiment (Exp1)

| 12 Lakes (salinity)   | The dominant phytoplankton class of total biomass (%) | Examples of abundant phytoplankton genera  |
|-----------------------|---|--|
| Østerild Fjord (2.3‰) | 95% Cyanophyceae                                      | <i>Anabaenopsis</i> , <i>Planktothrix/Limnothrix</i> , <i>Dolichospermum</i> , <i>Nodularia</i> , <i>Lyngbya</i> , <i>Aphanothece/Aphanocapsa/Cyanodictyon</i>   |
| Arup Vejle (1.3‰)     | 95% Cyanophyceae                                      | <i>Anabaenopsis</i> , <i>Planktothrix/Limnothrix</i> , <i>Dolichospermum</i> , <i>Nodularia</i> , <i>Lyngbya</i> , <i>Aphanothece/Aphanocapsa/Cyanodictyon</i>   |
| Lund Fjord (0.4‰)     | 85% Cyanophyceae                                      | <i>Anabaenopsis</i> , <i>Lyngbya</i> , <i>Planktothrix/Limnothrix</i> , <i>Aphanothece/Aphanocapsa/Cyanodictyon</i>  |
| Glombak (1.6‰)        | 85% Cyanophyceae                                      | <i>Aphanothece/Aphanocapsa/Cyanodictyon</i> , <i>Planktothrix/Limnothrix</i> , <i>Dolichospermum</i> , <i>Lyngbya</i>  |
| Selbjerg Vejle (0.5‰) | 80% Cyanophyceae                                      | <i>Monoraphidium</i> , <i>Koliella</i> , <i>Scenedesmus</i> , <i>Stephanodiscus</i> , <i>Chaetoceros</i> , <i>Gymnodinium</i> , <i>Peridinium</i>                |
| Tømmerby Fjord (0.5‰) | 50% Cyanophyceae<br>40% Chlorophyceae                 | <i>Aphanothece/Aphanocapsa/Cyanodictyon</i> , <i>Lyngbya</i>   |
| Han Vejle (0.3‰)      | 50% Cyanophyceae<br>30% Chlorophyceae                 | <i>Pediastrum</i> , <i>Scenedesmus</i> , <i>Tetraëdron</i> , <i>Nitzschia</i>  |
| Kogleakssø (0.5‰)     | 50% Cryptophyceae<br>30% Bacillariophyceae            | <i>Aphanothece/Aphanocapsa/Cyanodictyon</i>  |
| Vestsø (17.4‰)        | 50% Bacillariophyceae<br>30% Chlorophyceae            | <i>Scenedesmus</i> , <i>Monoraphidium</i> , <i>Pediastrum</i> , <i>Cosmarium</i> , <i>Cryptomonas</i> , <i>Rhodomonas</i> , <i>Peridinium</i> , <i>Nitzschia</i> |
| Østso (4.2‰)          | 70% Dinophyceae                                       | <i>Rhodomonas</i> , <i>Cryptomonas</i>   |
| Midtsø (7.2‰)         | 80% Dinophyceae                                       | <i>Nitzschia</i> , <i>Fragilaria</i>   |
| Lønnerup Fjord (2.9‰) | 70% Bacillariophyceae                                 | <i>Planktothrix/Limnothrix</i> , <i>Dolichospermum</i> , <i>Scenedesmus</i> , <i>Mallomonas</i> , <i>Gymnodinium</i> , <i>Rhabdomonas</i> , <i>Euglena</i>       |
|                       |   | <i>Stephanodiscus</i> , <i>Nitzschia</i> , <i>Fragilaria</i> , <i>Chaetoceros</i>  |
|                       |   | <i>Monoraphidium</i>   |
|                       |   | <i>Gymnodinium</i>   |
|                       |   | <i>Gymnodinium</i>   |
|                       |   | <i>Monoraphidium</i> , <i>Scenedesmus</i>  |
|                       |   | <i>Aphanothece/Aphanocapsa/Cyanodictyon</i> , <i>Dolichospermum</i>  |
|                       |   | <i>Stephanodiscus</i> , <i>Nitzschia</i> , <i>Fragilaria</i> , <i>Chaetoceros</i>  |
|                       |   | <i>Monoraphidium</i> , <i>Koliella</i> , <i>Aphanothece/Aphanocapsa/Cyanodictyon</i>   |

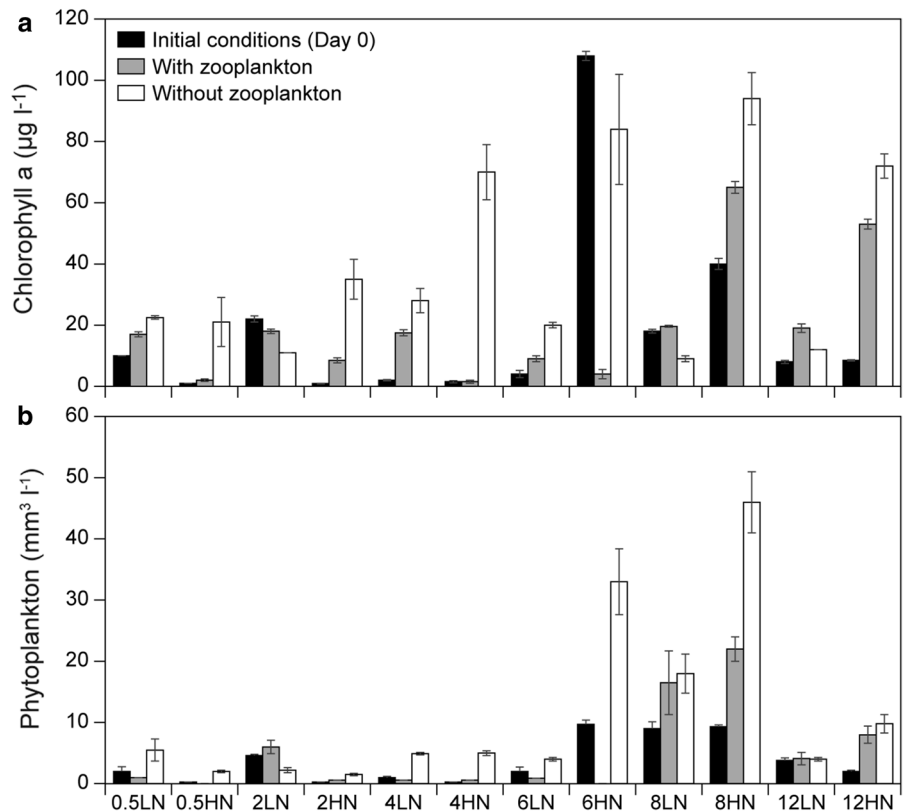
**Table 4** Three-way ANOVA on rank-transformed biomass ( $\text{mm}^3 \text{ l}^{-1}$ ). Data from mesocosm experiment

|                                 | df | F      | Significance |
|---------------------------------|----|--------|--------------|
| Nutrient                        | 1  | 25.71  | $P < 0.0001$ |
| Salinity                        | 5  | 432.00 | $P < 0.0001$ |
| Treatment                       | 2  | 338.45 | $P < 0.0001$ |
| Nutrient * Salinity             | 5  | 118.52 | $P < 0.0001$ |
| Nutrient * Treatment            | 2  | 42.75  | $P < 0.0001$ |
| Salinity * Treatment            | 10 | 84.94  | $P < 0.0001$ |
| Nutrient * Salinity * Treatment | 10 | 27.71  | $P < 0.0001$ |

biomass of *Rhodomonas* spp. declined (Fig. 7f). The species that responded positively to grazer presence were *A. judayi* at 2‰ salinity in LN and *Chaetoceros* spp. at 8‰ in HN (Fig. 7b, e; Table S3).

The percentual phytoplankton size distribution at the start of Exp3 is shown in Fig. 8, divided into three size classes. At low salinity (0.5–2‰), the biomass was dominated by phytoplankton in the intermediate size range (20–40  $\mu\text{m}$ ) due to dominance of *Cryptomonas* spp., and *A. judayi* dominated the large size class. At 4‰, the phytoplankton was completely dominated by small- and medium-sized algae. At 6‰

**Fig. 6** Biomass of dominant phytoplankton taxa in grazing experiments with water from mesocosms ordered along an increasing salinity gradient at two nutrient levels—low (LN) and high (HN), showing initial values and the level after 4 days of incubation with and without zooplankton ( $> 50 \mu\text{m}$ )



and above, large-sized algae dominated in LN (*Chaetoceros* spp., *Dolichospermum* and *Euglena*), while small-sized forms were more abundant in HN (*Rhodomonas* sp.).

## Discussion

We found that the grazing impact of zooplankton on phytoplankton in Vejlerne was low in all lakes, except in Kogleakssø (0.5‰), which had a high biomass of cladocerans and a phytoplankton community dominated by edible algae (mainly cryptophytes and various diatoms), whereas it differed substantially along a salinity gradient in the mesocosms having low fish density. We also found that nutrient addition stimulated the growth of phytoplankton in the lakes. The overall low grazing impact on phytoplankton in the lakes provides experimental support for the general hypothesis of low zooplankton grazing on phytoplankton in brackish lakes (Jeppesen et al., 1998; Brucet et al., 2010; Jensen et al., 2010; He et al., 2020).

Cyanobacteria dominated in most of the lakes. One possible explanation for the low grazing could, therefore, be the absence of edible phytoplankton as large cyanobacterial colonies are usually not easily grazed or, even if grazed, they can survive due to the presence of mucilage and even be enriched with nutrients (Sommer et al., 2006; Lüring 2021), or their nutritional quality is poor (Moustaka-Gouni & Sommer, 2020). Filamentous cyanobacteria, such as *Dolichospermum* and *Planktothrix/Limnothrix* in particular, can reduce filtering efficacy by cladocerans with implications for growth and reproduction (Lundgren et al., 2012; Motwani et al., 2018). Filamentous cyanobacteria dominated in Østerild Fjord and Arup Vejle, but edible phytoplankton such as small-sized chlorophytes, diatoms and cryptomonads were abundant and occasionally dominated the phytoplankton community in some of the other study lakes. Moreover, several studies have shown that the grazing impact on small edible cells might be high in cyanobacteria-dominated communities (Callieri et al., 2012; Motwani et al., 2018). Irvine et al. (1990) found that the commonly dominant copepod in brackish

**Table 5** Summary of one-way ANOVA on square-root transformed chlorophyll-*a* and total biomass data from the mesocosm experiment (Exp3)

|        | Chlorophyll- <i>a</i> ( $\mu\text{g l}^{-1}$ ) |                          |                | Total biomass ( $\text{mm}^3 \text{l}^{-1}$ ) |                          |                |
|--------|--|--------------------------|----------------|---|--------------------------|----------------|
|        | ANOVA (treatment)                              | Comparison (S.N.K. test) | Grazing impact | ANOVA (treatment)                             | Comparison (S.N.K. test) | Grazing impact |
| 0.5‰ L | $F=158.78$ $P<0.0001$                          | $3>2>1$                  | +              | $F=35.08$ $P<0.0001$                          | $3>1, 2$                 | +              |
| 0.5‰ H | $F=41.83$ $P<0.0001$                           | $3>2, 1$                 | +              | $F=375.81$ $P<0.0001$                         | $3>1>2$                  | +              |
| 2‰ L   | $F=106.26$ $P<0.0001$                          | $1>2>3$                  | –              | $F=26.0$ $P<0.001$                            | $2, 1>3$                 | –              |
| 2‰ H   | $F=102.45$ $P<0.0001$                          | $3>2>1$                  | +              | $F=123.73$ $P<0.0001$                         | $3>1, 2$                 | +              |
| 4‰ L   | $F=106.93$ $P<0.0001$                          | $3>2>1$                  | +              | $F=418.95$ $P<0.0001$                         | $3>1>2$                  | +              |
| 4‰ H   | $F=413.04$ $P<0.0001$                          | $3>2, 1$                 | +              | $F=643.46$ $P<0.0001$                         | $3>1, 2$                 | +              |
| 6‰ L   | $F=54.50$ $P<0.0001$                           | $3>2, 1$                 | +              | $F=37.19$ $P<0.0001$                          | $3, 1>2$                 | +              |
| 6‰ H   | $F=151.29$ $P<0.0001$                          | $1, 3>2$                 | +              | $F=199.64$ $P<0.0001$                         | $3>1>2$                  | +              |
| 8‰ L   | $F=52.79$ $P<0.0001$                           | $2>1>3$                  | –              | $F=5.87$ $P<0.039$ (n.s.)                     | $3, 2, 1$                | –              |
| 8‰ H   | $F=100.65$ $P<0.0001$                          | $3>2>1$                  | +              | $F=119.62$ $P<0.0001$                         | $3>2>1$                  | +              |
| 12‰ L  | $F=71.52$ $P<0.0001$                           | $2>3>1$                  | –              | $F=0.131$ $P<0.879$ (n.s.)                    | $2, 1, 3$                | –              |
| 12‰ H  | $F=506.89$ $P<0.0001$                          | $3>2>1$                  | +              | $F=29.395$ $P<0.001$                          | $3, 2>1$                 | –              |

Impact of grazing (+) was identified when a comparative post hoc test (S.N.K.) revealed a significant difference in chlorophyll-*a* or total biomass ( $P<0.01$ ) between treatment 2 ('with zooplankton') and treatment 3 ('without zooplankton'), and only if 3 was significantly higher than 2 (phytoplankton biomass was reduced). No grazing impact (–) was identified when no significant difference in chlorophyll-*a* or total biomass occurred between 2 and 3 (phytoplankton biomass was unaffected), and if 2 was significantly higher than 3 (phytoplankton biomass increased). Treatment 1 is initial (Day 0)

Treatment 1, L low nutrient level, H high nutrient level

lakes, *E. affinis*, was able to graze upon *Aphanothece* spp., a genus that we observed in most of the study lakes. It is therefore not likely that an adverse phytoplankton community structure can explain the overall low grazing impact in the lakes, regardless of the salinity and nutrient concentrations. A more likely explanation is a low zooplankton biomass determined by predation control. In general, high predation on zooplankton by fish and mysids is found in brackish lakes (Jeppesen et al., 1994, 1998), and this is also the case in the lakes in Vejlerne (Brucet et al., 2010; He et al., 2020). The only lake where zooplankton exhibited a significant grazing impact on phytoplankton (Lake Kogleakssø) had a higher zooplankton biomass than the other lakes and was dominated by large-bodied *Daphnia longispina*.

Although most of the lakes had salinities below 2–4‰, which several *Daphnia* species tolerate (Ortells et al., 2005; Latta et al., 2012), zooplankton grazing was low in 11 of the 12 lakes due to the absence of large cladocerans in most of them. Furthermore, the nutrient addition experiment showed that the phytoplankton in the lakes was nutrient-limited as

a fast increase in chlorophyll-*a* and biomass occurred after addition of nutrients (N+P), both in the treatments 'with' and those without 'zooplankton'. However, we cannot rule out that the increase in phytoplankton biomass in bottles with zooplankton reflects faster response of phytoplankton than of zooplankton in short-term experiment such as ours.

Cyanobacteria dominated in the lakes in the salinity range 0.4–2.3‰. Cyanobacteria have a high tolerance to salt content and salinity fluctuations (Callieri et al., 2012) and dominate in many eutrophic brackish lakes (Moss, 1994; Aaser et al., 1995; Schiewer, 1998; Paerl et al., 2018). Chlorophytes are also tolerant to salinity changes (Shetty et al., 2019) and they were found in large numbers in the most eutrophic lakes in Vejlerne. Among the chlorophytes, *Scenedesmus* and *Monoraphidium* were the most important genera like in other brackish water studies conducted in the North temperate climate zone (Schiewer et al., 1988; Wasmund & Kell, 1991). Moreover, diatoms are particularly important indicators of salinity zones as they usually increase in biomass at rising salinities

together with dinophytes (Peltomaa et al., 2019) (Table 3).

While cyanobacteria dominated in most of the eutrophic low salinity lakes, they were only occasionally in high biomass in the mesocosms. Instead, chlorophytes, diatoms and flagellates were abundant. The most marked change in phytoplankton from LN to HN was a shift to dominance of chlorophytes (50–90% out of total biomass) in HN in the salinity range 4–8‰. Previous studies in brackish lakes have shown that chlorophytes, such as *Scenedesmus* spp. and *Monoraphidium contortum* (Thuret) Komárková-Legnerová, are favored at high nutrient concentrations (Wasmund & Kell, 1991; Muylaert & Sabbe, 1999). Higher grazing by zooplankton due to the selected low fish density (manipulated by controlled low fish density condition, 1 fish m<sup>-2</sup>) may, in part, explain the lack of dominance of cyanobacteria in the mesocosms. The low fish predation allowed large-bodied cladocerans to dominate at low to intermediate salinities. Among the daphnids, a shift occurred from dominance of several species (mainly *D. pulex* Leydig, 1860, however) at low salinities to exclusive dominance of *D. magna* Straus, 1820 at 6–8‰; and among the rotifers, *Notholca* sp. dominated numerically and *B. plicatilis* Müller, 1786 at higher salinity (Jeppesen et al., 2007). Several biomanipulation experiments conducted in mesocosms and at whole-lake scale showed that removal of planktivorous fish, followed by an increase in both size and numbers of filter-feeding zooplankton (e.g., *Daphnia* spp.), led to a decline in cyanobacteria and total phytoplankton biomass (e.g., Andersson et al., 1978; Reinertsen & Olsen, 1984; Schoenberg & Carlson, 1984; Dawidowicz et al., 1988; Søndergaard et al., 1990; Moss et al., 1991). Andersson et al. (1978), for example, found that the phytoplankton composition changed from dominance of cyanobacteria in summer in fish mesocosms to *Cryptomonas* spp. in fish-free mesocosms. Similar shifts have been found in biomanipulated lakes in Denmark (Søndergaard et al., 2007; Jeppesen et al., 2012). Dominance of *Cryptomonas* spp. was, likewise, found in our mesocosm experiment at the lowest salinity (0.5‰) where this genus contributed 60–80% to the total biomass.

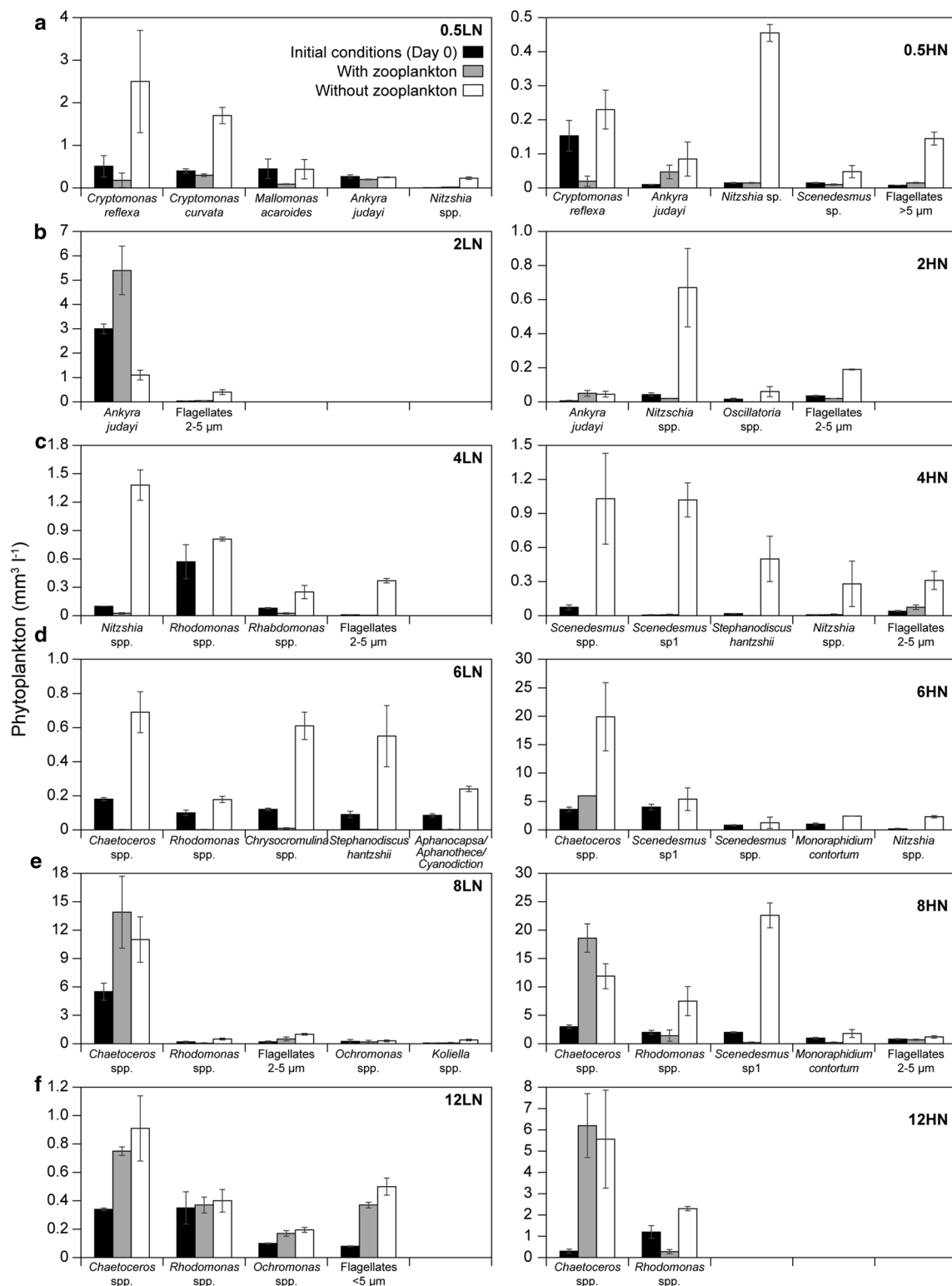
The grazing impact on phytoplankton was high over the whole salinity spectrum (0.5–12‰) in HN, while no grazing response was found in LN at 2‰, 8‰ and 12‰ salinity. This may, in part, be attributed

to differences in phytoplankton composition and, not least, in size. For instance, at low salinity (2‰) in LN where grazing was not significant, only 10% of the phytoplankton biomass consisted of algae < 40 µm compared to 85% in HN with high grazing effects. Small diatoms and unidentified flagellates dominated in HN and large grazing-tolerant *A. judayi* in LN.

A major shift occurred from large cladocerans (e.g., *D. longispina*, *D. pulex* and *D. magna*) below or equal to 6‰ to smaller zooplankton, especially rotifers (e.g., *B. plicatilis*), above 6‰ (Jeppesen et al., 2007). The grazing impact on phytoplankton was particularly high at 4 and 6‰ salinity in both HN and LN. At these salinities, the large-bodied *D. magna* dominated the zooplankton (Jeppesen et al., 2007). Grazing was significant for all phytoplankton taxa that all exhibited a low final biomass (0–0.04 mm<sup>3</sup> l<sup>-1</sup>), which concurs with other studies showing that *D. magna* potentially can control most algal taxa regardless of size (Dawidowicz, 1990). At these salinities, even the large *Chaetoceros* spp. was significantly grazed upon, unlike the situation at 8‰ and 12‰ where rotifers dominated.

At the highest salinities (8 and 12‰) where rotifers dominated, the phytoplankton > 40 µm constituted up to 80% of total biomass (e.g., *Chaetoceros* spp. and *Euglena* spp.) in LN, indicating a low grazing pressure as rotifers prefer small phytoplankton (Rothhaupt, 1990). In contrast, in HN, 60–80% of the biomass of phytoplankton had a GALD < 20 µm; e.g., *Scenedesmus* spp. and *Rhodomonas* spp. were < 20 µm, while *Chaetoceros* spp. were larger. The latter was also the only taxon not being significantly grazed upon.

An increase in phytoplankton biomass in the presence of zooplankton was found in some of the experimental treatments from both the lakes and the LN mesocosms. This indicates that the presence of zooplankton stimulated the growth of the nutrient-limited phytoplankton as seen in other short-term zooplankton removal experiments (Elser et al., 1987; Carrillo et al., 1990). The negative effects of grazing on phytoplankton may be compensated by nutrients released by zooplankton (through excrements or decay), which are rapidly sequestered by the algae and, thus, can contribute substantially to growth enhancement (Lehman, 1980; Lehman & Scavia, 1982; Sterner, 1986). Accordingly, the fact that some phytoplankton species (i.e., *A. judayi* and *Chaetoceros* spp.)

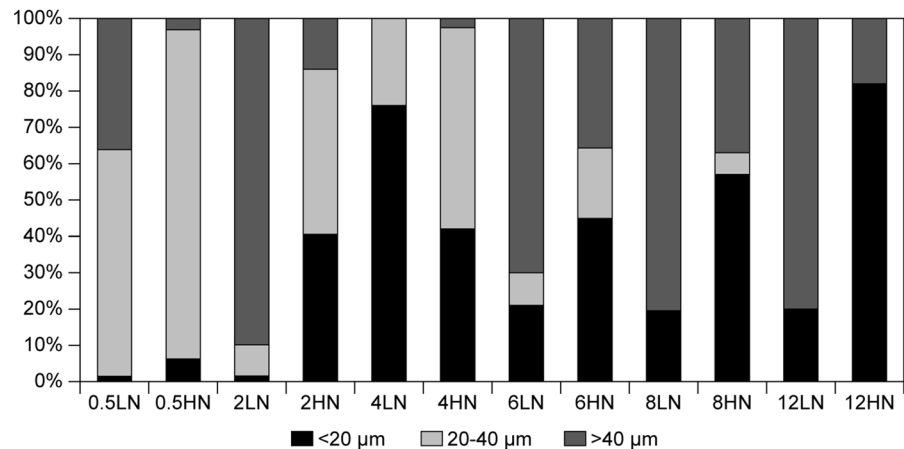


**Fig. 7** Biomass of dominant phytoplankton taxa in grazing experiments with water from mesocosms ordered along an increasing salinity gradient (a–f) at two nutrient levels—low

(LN) and high (HN), showing initial conditions (Day 0) and the level after 4 days of incubation with and without zooplankton (> 50  $\mu\text{m}$ )



**Fig. 8** Percentage contributions of different size classes (GALD) of phytoplankton at the start of the grazing experiment with water from mesocosms ordered along an increasing salinity gradient at two nutrient levels—low (LN) and high (HN)



increased significantly in the presence of zooplankton may be interpreted either as a response to nutrient regeneration by grazers or as a response to reduced competition when other algal species are reduced by grazing (Lynch & Shapiro, 1981).

## Conclusions

Our results revealed that in 11 of the 12 brackish studied lakes the low grazing on phytoplankton could be attributed mainly to low zooplankton biomass. The phytoplankton was likely mostly controlled by nutrients as indicated by an increase in phytoplankton biomass after 4 days of incubation both in the presence and absence of zooplankton ( $> 50 \mu\text{m}$ ) after addition of nutrients (N+P). Grazing experiments on water from 24 mesocosms with low fish predation showed a grazer effect at high nutrient levels at all salinities, but especially between 0.5 and 6‰ where *Daphnia* dominated. At low nutrient levels, the grazing effect was also significant at 0.5–6‰ where *D. magna* dominated, but not at the other salinities, which was attributed, in part, to different sizes of algae, which were overall larger and less palatable at low nutrient levels. Our results further support the idea that the cascading effects of reduced predation on zooplankton down to phytoplankton are strongest in eutrophic lakes (Pace et al., 1999; Jeppesen et al., 2003; He et al., 2020) at low salinities, but they also emphasise that the zooplankton grazing effect is low in the rotifer-dominated high salinity regimes (8 and 12‰). Moreover, they show low grazing effect on phytoplankton in most of the brackish lakes studied, which we attribute to high

predation on zooplankton and, at high salinity, also a salt effect.

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**Consent for publication** All the authors consent the publication of this manuscript.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** Not applicable/not required.

**Informed consent** All the authors consent to participate in this manuscript.

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