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The role of zooplankton in controlling phytoplankton biomass in lake littoral

THE ROLE OF ZOOPLANKTON IN CONTROLLING PHYTOPLANKTON BIOMASS IN LAKE LITTORAL

ELÄINPLANKTONIN ROOLI KASVIPLANKTONBIOMASSAN SÄÄTELIJÄNÄ JÄRVILITORAALISSA

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Rehevien järvien kunnostuksen onnistuminen määritellään usein veden kirkkauden lisääntymisellä, mikä johtuu planktonlevien määrän vähenemisestä. Tämän vuoksi eläinplanktonin laidunnuksen vaikutukseen on kiinnitetty erityistä huomiota. Litoraaliyhteisön rakenne vaikuttaa olennaisesti sen kykyyn sietää häiriötekijöitä kuten ravinnekuormitusta. Järvilitoraalissa uposlehtinen vesikasvillisuus luo rakenteellisesti monimuotoisen elinympäristön ja säätelee planktonyhteisön rakennetta sekä trofiatasojen välisiä vuorovaikutussuhteita vaikuttaen mm. ravinteiden saatavuuteen. Vesikirput saattavat välttää kalojen saalistusta vesikasvillisuuden suojassa. Kasviplanktonin vähäinen määrä kasvustoissa voi olla seurausta eläinplanktonin laidunnuksesta. Vesikasvillisuudesta ja eläinplanktonlaidunnuksesta johtuvien, planktonlevien määrää säätelevien mekanismien suhteellista merkitystä on kuitenkin usein hankala erottaa, koska niihin vaikuttavat useat tekijät, kuten vesikasviyhteisön rakenne, järven trofiataso ja morfologia sekä vallitsevat ilmasto-olosuhteet. Tämän lisensiaattitutkimuksen tavoitteena oli selvittää miten kalojen saalistus, vesikasvillisuus, ravinnekuormitus ja ilmastolliset olosuhteet vaikuttavat eläinplanktonyhteisön rakenteeseen ja kykyyn säädellä kasviplanktonbiomassaa. Näitä tekijöitä tutkittiin faktorikoeasetelmaa käyttäen koealtaissa Lahden Vesijärven Kilpiäistenpohjassa sekä viidessä muussa järvilitoraalissa Euroopan eri ilmastovyöhykkeillä Espanjassa, Hollannissa, Englannissa ja Ruotsissa.

Eläinplanktonlaidunnuksella oli tärkeä rooli kasvplanktonbiomassan säätelyssä ja vesikasvit edesauttoivat vaihtelevasti, lajista riippuen, tässä säätelyssä vaikuttaen planktonlevien kasvuun ja tarjoten vesikirpuille suojapaikan kalojen saalistukselta. Kasviplanktonbiomassan säätelyyn liittyivät siis toisiaan tukien sekä laidunnus että vesikasvillisuuteen liittyvät mekanismit. Euroopan eri ilmastovyöhykkeillä toteutettujen allaskokeiden tulosten perusteella erityisesti suurikokoisten planktonäyriäisten laidunnuksella oli keskeinen merkitys kasviplanktonbiomassan säätelyssä erittäin runsasravinteisissakin olosuhteissa (jopa 1600 µg kok.P l^{-1}). Laidunnuksen merkitys heikkeni ja ravinteiden merkitys korostui korkeassa lämpötilassa (lähes 30 °C), jolloin myös yhteisön kyky vastustaa ravinnekuormitusta heikkeni. Muut maantieteelliset erot laidunnuksen merkityksessä peittyivät vuosien väliseen säätilojen vaihteluun, mikä puolestaan vaikutti litoraaliyhteisön rakenteeseen ja kykyyn vastustaa ravinnekuormitusta.

Kalojen saalistuksen vaikutukset eläinplanktonbiomassaan olivat selvempiä kuin ravinnekuormituksen. Alhainen pienikokoisten (5-10 cm) planktivorien kalojen biomassa ($\leq$ 4 g tuorepainoa, $\leq$ 2.5-4 yksilöä m^{-2}) ei vähentänyt laiduntavan eläinplanktonin biomassaa ja näiden kykyä säädellä kasviplanktonia. Kun kaloja oli tätä enemmän (20 g m^{-2}, 7-20 yksilöä m^{-2}), runsaskaan vesikasvillisuus ei kyennyt estämään kalojen vaikutuksen heijastumista ravintoverkossa planktonleviin eläinplanktonin kautta. Vaihtoehtoiset ravintokohteet kuten vesiruttokasvustoilla (*Elodea canadensis* L.) ja pohjalla elävät selkärangattomat eläimet eivät oletusten vastaisesti vähentäneet 15-17 cm kokoisten särkien (*Rutilus rutilus* L.) eläinplanktoniin kohdistamaa saalistuspainetta. Pikemminkin vaikutti siltä, että litoraaliekosysteemissä vaihtoehtoinen ravinto voi loppujen lopuksi lisätä saalistuspainetta vesikirppuihin, joka oli särkien mieluisin ravintokohde.

Eläinplanktonyhteisössä havaitut rakenteelliset muutokset viittaavat siihen, että saatavilla olevat resurssit määrittävät eläinplanktonin kokonaisbiomassan, mutta kalojen saalistus säätee eri funktionaalisten ryhmien runsautta. Kalojen saalistamat suurikokoiset vesikirput korvautuvat pienikokoisemmilla äyriäislaiduntajilla, joille vesikasvit tarjoavat suojaa ja jotka saattavat laiduntaa tehokkaasti kasviplanktonia. Litoraaliekosysteemissä on yleensä korostettu elinympäristön rakenteellista monimuotoisuutta eliöiden välisten vuorovaikutussuhteiden säätelyssä. Tämän tutkimuksen tulokset tukevat tätä käsitystä ja antavat aihetta korostaa myös yksittäisen trofiatason kuten eläinplanktonin sisäistä monimuotoisuutta ja vuosien välistä vaihtelua, mikä heijastui yhteisön kykyyn vastustaa ympäristötekijöiden vaihtelua.

Avainsanat – Nyckelord
litoraaliekosysteemi, eläinplankton, ravinnekuormitus, planktivorit kalat, ilmasto-olosuhteet

Säilytyspaikka - Förvaringställe

Muita tietoja

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ABSTRACT

The success in restoration of eutrophicated lakes is mainly judged by water clarity and algal biomass. Thus, major interest is focused on the top-down control of phytoplankton by zooplankton. In lake littoral, submerged vegetation has been shown to structure food web interactions through mediating resource availability for planktonic communities and altering trophic cascades. Littoral community composition and trophic interactions determine the resistance of ecosystem against perturbations, such as nutrient loading. Enhanced control of phytoplankton biomass in macrophyte beds may be the result of improved chances of large cladoceran grazers to avoid fish predation by using vegetation as a refuge. The relative role of different regulation mechanisms on phytoplankton in vegetation is often difficult to interpret and may depend on factors such as plant community composition, lake trophic state and morphometry as well as climate. This study aims to assess how zooplankton community and their role in controlling phytoplankton biomass are modified by predation by fish, macrophytic vegetation, nutrient enrichment and climatic conditions. A series of mesocosm experiments of factorial design were run in the littoral of Lake Vesijärvi, southern Finland and in five other lake littorals along a climatic gradient from Spain (two sites) to Holland, England and Sweden.

The top-down control of phytoplankton by zooplankton grazers was important in vegetation and was invariably, depending on species, facilitated by macrophytes, which controlled phytoplankton growth and provided zooplankton with refuge against predation by fish. Thus, complementary mechanisms by both zooplankton and macrophytes were apparently behind the control of phytoplankton biomass. The series of parallel experiments along the European climatic gradient also suggested that zooplankton, especially the functional group of large crustacean grazers was of crucial importance in controlling algal biomass even under hypereutrophic conditions (up to 1600 µg TP l^{-1}). The grazing impact of large crustaceans declined relative to resource control under high temperature conditions (close to 30°C) leading to low resistance to nutrient enrichment. Other geographical differences were probably masked by the high year-to-year variation in the prevailing weather conditions, which affected the structure of littoral communities and the resistance to nutrient enrichment and fish predation.

Small (5-10 cm) planktivorous fish had a greater role than nutrients in regulating zooplankton biomass. On the other hand, the results suggested a threshold fish biomass of 4 g FW m^{-2} or 2.5-4 ind m^{-2}, below which zooplankton grazer biomass and, subsequently, the control of phytoplankton biomass were not controlled by fish. When fish abundance was higher (20 g FW m^{-2} or 7-20 ind m^{-2}), the results did not unequivocally show that submerged vegetation hampered the trophic cascade of planktivorous fish via zooplankton grazers. Benthic and epiphytic macroinvertebrates as alternative food sources for 15-17-cm roach (*Rutilus rutilus* L.) in *Elodea canadensis* L. beds reduced predation pressure on cladocerans, the most preferred food source, less than expected. Rather, the results suggested that alternative food sources may facilitate roach populations to manage over the periods of low cladoceran abundance and thus maintain high predation pressure on zooplankton.

Compositional changes within zooplankton community suggested that available food resources determine total zooplankton biomasses but fish predation strongly regulated the relative abundances of the different functional groups of zooplankton. The biomass of large cladocerans was reduced by fish and was compensated by smaller grazers for which macrophytes appeared to provide with refuge against fish predation. In vegetated littoral, also small-sized crustaceans may be able to control algal biomass. In littoral ecosystems, the habitat structural heterogeneity has generally been emphasized causing variation among trophic interactions. The results of this work support this view and emphasize also the heterogeneity within trophic level as well as year-to-year differences in the community structure. These factors affected the resistance of the system to environmental variation.

LIST OF ORIGINAL PAPERS

This licentiate thesis is based on the following papers which in the text are referred to by their Roman numerals:

I.   Kornijów, R., Vakkilainen, K., Horppila, J., Luokkanen, E. & Kairesalo, T. 2005: Impacts of a submerged plant (*Elodea canadensis*) on interactions between roach (*Rutilus rutilus*) and its invertebrate prey communities in a lake littoral zone. *Freshw. Biol.* 50: in print.

II. Hietala, J., Vakkilainen, K. & Kairesalo, T. 2004: Community resistance and change to nutrient enrichment and fish manipulation in a vegetated lake littoral. *Freshw. Biol.* 49: 1525-1537.

III. Vakkilainen, K. & Kairesalo, T., Hietala, J., Balayla, D., Bécares, E., van de Bund, W., van Donk, E., Fernández-Aláez, M., Gyllström, M., Hansson, L.-A., Miracle, M.R., Moss, B., Romo, S., Rueda, J., Stephen, D. 2004: Response of zooplankton to nutrient enrichment and fish in shallow lakes: a pan-European mesocosm experiment. *Freshw. Biol.* 49: 1619-1632.

THE AUTHOR'S CONTRIBUTION

I.   RK and TK planned the experiment and supervised the work. RK, KV and EL set up the experiment, carried out field measurements and sampling. EL and KV planned the statistical analyses. KV analysed zooplankton and phytoplankton samples, made the data analyses, interpreted the results and draw the figures. KV wrote the paper with the co-operation by TK and RK.

II. JH and KV took charge of setting up the experiments, field sampling and measurements. KV contributed to laboratory measurements (excluding nutrient analyses), analysed zooplankton samples, wrote and interpreted the zooplankton results and contributed to discussion. TK supervised the work.

III. KV and JH collected the data from the different laboratories and planned the data analyses. KV analysed the data, interpreted the results and wrote the paper. TK supervised the work.

1. Introduction

1.1. The role of zooplankton and food web functioning

Trophic structure of an ecosystem can be defined as the partitioning of biomass into different trophic levels of food web and is basically determined by available resources and primary productivity. However, it is also regulated by dynamic feedback from higher trophic levels, which in turn affect resource availability (Carpenter *et al.* 1985, Arditi & Ginzburg 1989, Power 1992, Carpenter & Kitchell 1993, Leibold *et al.* 1997, Polis 1999, Persson *et al.* 2001). The relative importance of resource and predator control in shaping community structure is viewed as complementary, not contradictory. Thus, zooplankton community is expected to respond to changes both in available food resources and in predation pressure reflecting also the "sandwitched" role of zooplankton in lake food webs (Gliwicz 2002, Jeppesen *et al.* 2002). However, the two forces operate on different time scales. The effect of predation is immediate, while it takes more time to translate enhanced productivity into new biomass, and this time lag depends on the generation time of organisms (Gliwicz 2002, Reynolds 1994).

Notable prevalence of predation in shaping the zooplankton community structure (Hrbácek 1962, Brooks & Dodson 1965) triggered off a fruitful avenue of research in aquatic ecology. The dynamic feedback from higher trophic levels has inspired a number of studies about the way and magnitude how fish control lower trophic levels and affect also resource availability (Kerfoot & Sih 1987, Carpenter & Kitchell 1993). The trophic cascade theory (Carpenter *et al.* 1985, Polis *et al.* 1999) as well the traditional food chain theory with its modifications (Hairston *et al.* 1960, Fretwell 1977, Oksanen *et al.* 1981, Scheffer 1991) have been intensively tested in aquatic ecosystems. Evidence for the strong vertical structuring of aquatic food webs has been gained through manipulations of top trophic levels

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(Carpenter *et al.* 1985, Rask *et al.* 1996, Brett & Goldman 1997, Jeppesen *et al.* 1998c, Horppila *et al.* 1998) or primary productivity (Hansson 1992, Persson *et al.* 1992, Sarnelle 1992).

The success in restoration of eutrophicated lakes is mainly judged by water clarity and phytoplankton biomass (Benndorf *et al.* 2002). Thus, major interest is focused on the top-down control of phytoplankton by zooplankton. Direct grazing has been considered an improbable mechanism to maintain a permanent clear-water state (Benndorf *et al.* 2002). The control of phytoplankton biomass may be achieved not only via direct grazing but also via altered recycling of nutrients. Nutrient regeneration of planktonic animals in aquatic ecosystems is important (Hudson *et al.* 1999, Tarvainen *et al.* 2002, Vanni 2002), but so far there is little evidence about its role in changing phytoplankton biomass (Cyr & Curtis 1999). The relative importance of grazing and nutrient regeneration may often be difficult to interpret and depends on conditions such as time scale, lake depth and trophic state (Benndorf *et al.* 2002). In any case, low phytoplankton biomass and indirect top-down effects combined with mechanisms related to nutrient availability are decisive in controlling phytoplankton biomass.

Intense planktivory by visually foraging fish decreases the biomass of large-bodied effective grazers, especially that of *Daphnia*, leading to lowered control of phytoplankton biomass. Reduced planktivory by fish has been observed to result in positive or negligible and even negative responses of (large) zooplankton grazers (Reynolds 1994, Rask *et al.* 1996, Leibold *et al.* 1997, Horppila *et al.* 1998, Sarvala *et al.* 2000). Such heterogeneity may reflect compositional changes, which alter expectations about the regulation of trophic structure. Considerable amount of variation in planktonic community structure across productivity gradient may be due to invulnerability of prey organisms (Mazumder 1994, Abrams & Walters 1996), omnivory (Diehl & Feißel 2000), compensatory responses (Pace *et*

al. 1998) and spatial heterogeneity (Scheffer & Boer 1995). Such observations have addressed the major simplifying assumptions of most food web models and have issued the importance of taking into more profound consideration e.g. the heterogeneity within trophic levels (Leibold *et al.* 1997, Persson *et al.* 2001). Even the most complex models also tend to ignore environmental variability (Hunter & Price 1992). Habitat structural complexity is important for regulating community structure and the strength of cascades (Shurin *et al.* 2002). In shallow lake ecosystems the spatial heterogeneity, created by submerged vegetation, increases the complexity of trophic interactions within the habitat (Scheffer *et al.* 1993, Jeppesen *et al.* 1998b, Scheffer 1999) and also between habitats through spatial coupling (Schindler & Scheuerell 2002, Vadeboncoeur *et al.* 2002).

1.2. Zooplankton and littoral communities

The majority of aquatic food web research has been carried out in pelagic ecosystems (Vadeboncoeur *et al.* 2002). However, small, shallow lakes comprise the majority of the world's lakes (Wetzel 1990), thus emphasizing the fundamental role of littoral ecosystem in the flow of nutrients and energy in lake ecosystems (Schindler & Scheuerell 2002). In shallow lake ecosystems, submerged vegetation has been shown to structure food web interactions through mediating resource availability for planktonic communities and altering trophic cascades. This spatial heterogeneity works as an important buffering mechanism in shallow lakes and littoral ecosystems where the outcome of perturbation results from variable responses of organisms of different habitats that may partly or completely compensate, or strengthen, each other's effect (reviewed in Jeppesen *et al.* 1998b). The concept of alternative stable states pulls together the positive feedback mechanisms through which submerged macrophytes promote a clear-water state over a wide range of nutrient concentrations. Thus, eutrophic shallow lakes may be in either a turbid, phytoplankton-dominated

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state or a clear-water, plant-dominated state (Irvine *et al.* 1989, Scheffer *et al.* 1993). Both states are assumed to resist perturbations because complex biological interactions buffer their stability. A switch from one state to the other requires major forces. For instance, a gradual change in nutrient loading or temperature may have little effects until a threshold is reached at which a large switch occurs (Scheffer *et al.* 1993, Scheffer & Carpenter 2003).

In shallow vegetated ecosystems, zooplankton grazing has been shown to have a central role in controlling phytoplankton and maintaining the clear-water state (Schriver *et al.* 1995, Jeppesen *et al.* 1998a, 1999, Scheffer 1999). However, this mechanism has been challenged by field observations of dense submerged vegetation and low phytoplankton biomass coincident with a low abundance of zooplankton (Meijer *et al.* 1999, Blindow *et al.* 2000). Thus, other regulation mechanisms of macrophytes on planktonic communities might also be effective. Such mechanisms include shading, allelopathy, competition for nutrients, and regulation of water movements and sedimentation (Søndergaard & Moss 1998). In every case, zooplankton grazing may have a key role in affecting water clarity and, subsequently, the establishment and maintenance of submerged vegetation. Although the structuring role of submerged macrophytes is generally acknowledged, the mechanisms behind this phenomenon are still arguable (Jeppesen *et al.* 1998b). The debate stems from the ultimate complexity of these mechanisms and differences in their relative role. This controversy has been related to differences in climate, lake morphometry, plant community composition as well as trophic state of the lake (Jeppesen *et al.* 1999).

Submerged macrophytes provide with refuge for grazing zooplankton, especially daphnids, against visually predating planktivorous fish (Timms & Moss 1984, Carpenter *et al*. 1985, Carpenter & Lodge 1986, Jeppesen *et al*. 1998a, Kairesalo *et al*. 1998, 2000, Scheffer 1999). On the other hand, while zooplankton grazers may utilise the vegetation as refuge they may also be suppressed by macrophytes (Burks *et al*. 2002). A negative impact of aquatic plants on rotifers was observed already 50 years ago (Hasler & Jones 1949). Later, several studies have suggested that submerged macrophytes do not provide a favourable habitat either for cladocerans, especially *Daphnia*, due to low food supply, high daytime pH, predation or plant chemicals (Pennak 1973, Dorgelo & Heykoop 1985, Jeppesen *et al*. 1998a, Blindow *et al*. 2000, Burks *et al*. 2000, 2002). By migrating horizontally between macrophyte beds and open-water habitats, cladocerans may avoid constant susceptibility to the adverse effects of macrophytes while still benefit from the refuge (Timms & Moss 1984, Lauridsen & Lodge 1996, Burks *et al*. 2002). Littoral zooplankton may also swarm during day as well as perform microscale diel vertical migration between lake bottom and open-water as a response to predation by fish (Walls *et al*. 1990). However, in shallow lakes light may penetrate to the bottom of water and a hypolimnetic refuge may be lacking, making vertical migration less advantageous (Burks *et al*. 2002).

Vegetated littoral ecosystem may also modify fish-zooplankton interactions through sustaining a habitat with multiple alternative food sources for fish (Persson 1983, Diehl 1993, Moss *et al*. 1998, Perrow *et al*. 1999). Such generalist feeding behaviour, i.e. switching of prey preference, may reduce the predation pressure on zooplankton (Perrow *et al*. 1999) and thereby dampen their population fluctuations. On the other hand, it has been suggested that such behavioural flexibility may have only a short-term stabilizing effect depending on the predator's behaviour and food preferences (Murdoch & Bence 1987). For instance, in the littoral of Lake

Vesijärvi, southern Finland, the share of zooplankton in the diets of roach (*Rutilus rutilus* L.) decreases and the proportion of benthos and macrophytes increases with the low availability of planktonic food (Horppila & Kairesalo 1992), and with the increasing size of fish (Horppila 1994). However, the consequences of such plasticity in food preference for zooplankton-phytoplankton interactions, and on the stability of the littoral ecosystem, have been less quantified (reviewed in Jeppesen *et al.* 1998b).

1.4. Effects of climate on the functioning of plankton communities

Community structure and food web interactions in any given ecosystem are determined by a myriad of forces, both abiotic and biotic factors, often difficult to untangle when interpreting the patterns observed in natural communities (Hunter & Price 1992). Among the multiple environmental factors controlling the functioning of ecosystems, climatic aspect has been less considered in aquatic field studies, although its obvious role in affecting trophic interactions has often been suggested (Jeppesen *et al.* 1999, Petchey *et al.* 1999, Scheffer & Carpenter 2003).

All biochemical and biological processes such as population cycles, biomass turnover times and trophic interactions have rates of operation which are temperature dependent (McCauley & Murdoch 1987, Lehman 1988, Petchey *et al.* 1999). Such factors are likely to affect communities in shallow aquatic ecosystems. Production of zooplankton has been shown to increase with temperature while biomass accumulation is more dependent on resource availability (Shuter & Ing 1997). Similarly, enhanced primary productivity at higher temperatures (Petchey *et al.* 1999) becomes more controlled by nutrient turnover rate and availability. Accordingly, with rising temperature the inherently faster growth rate of algae compared to that of zooplankton may lead to reduced control of algal biomass by zooplankton. In addition, the threshold food level of zooplankton increases with water temperature

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and, for metabolic reasons, with increasing animal size (Lehman 1988). This may yield a lower share of large-bodied (efficient) grazers in the zooplankton community. Fish predation may further suppress large zooplankton since the activity and capture success of several planktivorous fish, such as roach, increase with temperature (Persson 1986). Thus, with increasing temperature, effects at the resource base may have a major role in the functioning of food web such that the cascading effects of fish via zooplankton grazers could have a minor role in controlling algal biomass.

2. Objectives of the study

This study focuses on the functioning of planktonic food web in lake littoral ecosystem and, by using factorial field mesocosm experiments, aims to assess:

- The hampering impacts of a submerged plant (*Elodea canadensis* Michx) on the cascading effects of roach via zooplankton grazers through (1) providing zooplankton with refuge against predation by roach and (2) providing omnivorous roach with a habitat rich in alternative food sources (I).

- Zooplankton community responses and, subsequently, the resistance of the littoral ecosystem to nutrient enrichment and predation by planktivorous fish during two consecutive years locally (II) and under different climatic conditions (III) assuming that the stability of the system, i.e. its resistance to nutrient enrichment, increases with increasing macrophytic vegetation but decreases with increasing temperature and fish density.

Special emphasis is paid on the role of zooplankton, primarily crustacean grazers, in controlling algal biomass with different densities of planktivorous fish. In this context, the effects of differing macrophytic

vegetation on fish-zooplankton interactions and subsequent effects on phytoplankton are addressed (Fig 1).
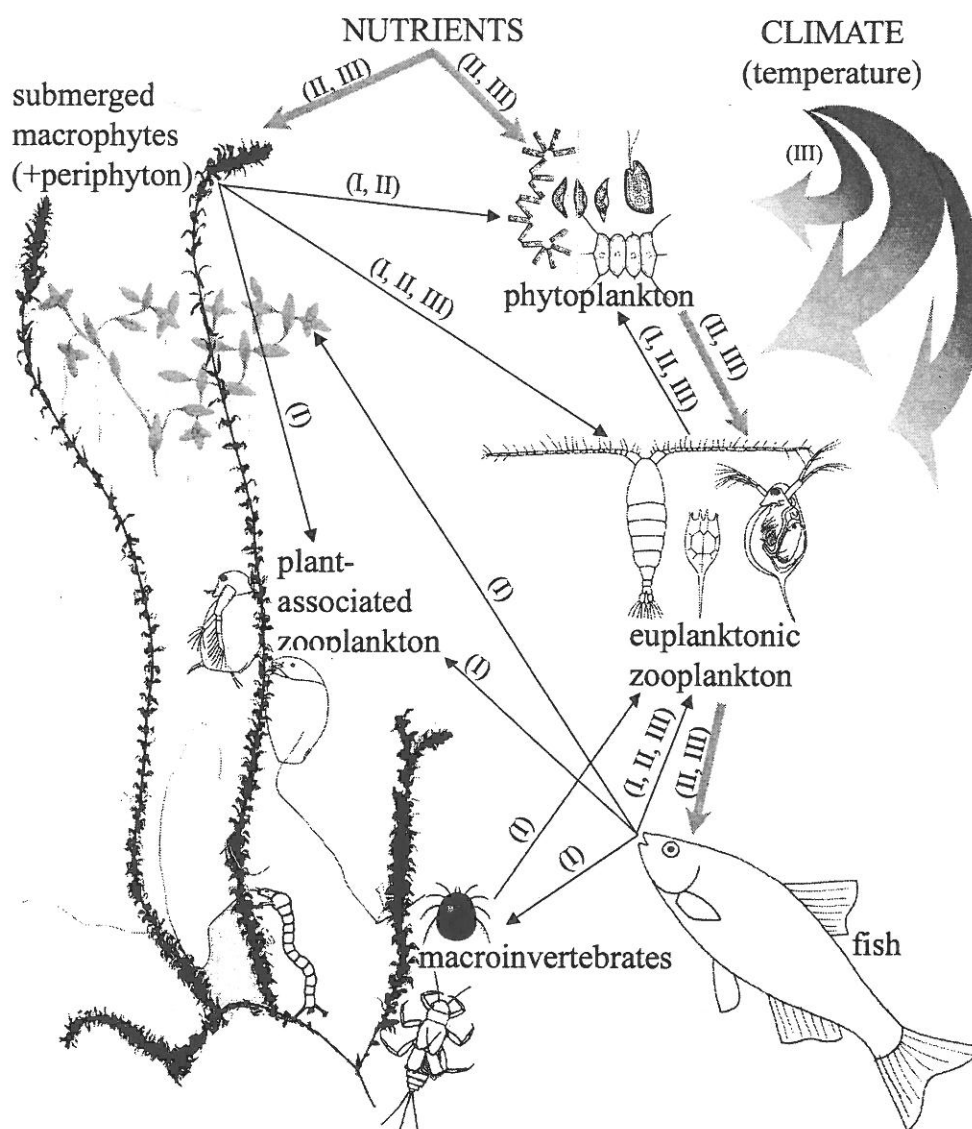
Fig. 1. A schematic picture representing the main scope of the study. The environmental variables are written in capital letters while the state variables are in regular letters. The arrows show the processes of interest. Black thin arrows illustrate consumer regulation and broader grey arrows illustrate resource regulation. Roman numerals refer to each original paper in which the processes are considered.

3. The study sites and the design of the experiments

3.1. Description of the study sites

The field experiments included in this study were performed at the 1.0-1.2 meter depth in lake littorals rich in submerged macrophytic vegetation. The Finnish study site situated at Kilpiäistenpohja Bay, which lies at the southeastern end of Lake Vesijärvi, in southern Finland (61°02' N, 25 °39' E) (I, II, III). The other study sites situated in shallow lake littorals of Lake Krankesjön (Sweden; 61°02' N, 25 °39' E), Little Mere (England; 61°02' N, 25 °39' E), Lake Naardemeer (the Netherlands; 61°02' N, 25 °39' E), Lake Chozas (Leon, Spain; 61°02' N, 25 °39' E) and Lake Xeresa (Valencia, Spain; 61°02' N, 25 °39' E) (III). More detailed descriptions of the study sites and community structure can be found in each original paper.

3.2. Design of the experiments

The experiments had fully factorial design with two factors (hereafter referred to as treatments): either different densities of *Elodea canadensis* and fish (altogether 18 enclosures) (I) or different weekly additions of phosphorus and nitrogen and different densities of fish (altogether 36 mesocosms) (II, III) (Table 1). The effects of *Elodea* and fish were studied in 2.5x2.5 meter enclosures August 1 to 29, 1994 (I). The effects of nutrient enrichment and fish predation were studied in cylindrical mesocosms of 1 m diameter and the experiments were conducted concomitantly in the different study sites along the European climatic gradient in two consecutive years: from June 8 to July 13, 1998 and from June 29 to August 10, 1999 (II, III). During the third week of the experiment in 1998 in Finland, high fish mortality occurred at the two highest nutrient levels probably due to the high pH (>9) of water. Therefore, the results of these nutrient treatments were excluded from the data analyses and the nutrient range was narrowed in

1999 (Table 1). The enclosures and mesocosms, made of clear PE-(polyethylene) plastic, were open to the atmosphere and the low ends were sealed into the lake bottom sediment. The experimental area was covered with a net to prevent the entry of birds. Fish and macrophytes used in the experiments were collected from a nearby lake area.

Table 1. Summary of the experimental designs.

Time of experiment and the size of mesocosms	Treatment 1 (levels)	Treatment 2 (levels)	No. of repli-cates	Paper
1-29 August 1994 area 5 m^2 vol. 7.5 m^3	*Elodea* (0, 250, 750 g FW m^{-2})	Roach, *Rutilus rutilus* L., of 16 cm length (0, 69 g FW m^{-2} or 0, 1.8 ind. m^{-2})	3	I
8 June – 13 July 1998 area 0.8 m^2 vol. 0.7-1.0 m^3	Nutrients (0 mg P + 0 mg N L^{-1}, 0.1 mg P + 1 mg N L^{-1}, 0.5 mg P + 5 mg N L^{-1}, 1.0 mg P +10 mg N L^{-1})	Locally appropriate planktivorous fish species of 5-10 cm length* (0, 4, 20 g FW m^{-2} or 0, 2.5-4, 7-20 ind. m^{-2})	3	II, III
29 June – 10 August 1999 area 0.8 m^2 vol. 0.7-1.0 m^3	Nutrients (0 mg P + 0 mg N L^{-1}, 0.03 mg P + 0.3 mg N L^{-1}, 0.06 mg P + 0.6 mg N L^{-1}, 0.09 mg P + 0.9 mg N L^{-1}, 0.15 mg P + 1.5 mg N L^{-1}, 0.30 mg P + 3.0 mg N L^{-1})	Locally appropriate planktivorous fish species of 5-10 cm length** 0, 4, 20 g FW m^{-2} or 0, 2.5-4, 7-20 ind. m^{-2})	2	II, III

In the Finnish experiment 6-9 cm juvenile roach (*) and 8-10 cm juvenile perch, *Perca fluviatilis* L. (**), were used.

4. Methods

4.1. Sampling procedure and sample analyses

Samples for water chemistry, chl-*a*, phytoplankton and zooplankton were collected weekly with a transparent perspex core (length 1.5 m, inner diameter 4.0 cm) from the surface to the bottom of each enclosure (I, II, III). First samples were taken just before the introduction of fish and/or first nutrient addition. Short-term impact of *Elodea* on roach-zooplankton interaction was studied by taking additional set of samples three days after the introduction of roach (I). Ten subsamples were taken from different places of the mesocosms, pooled and mixed. Concomitantly with the sampling procedure, temperature, oxygen and light penetration were measured in the field. Diel variation in zooplankton densities was studied once, on June 28-29, 1998, by taking samples at 9 to 11 a.m. and 11 p.m. to 1 a.m. The standard methods used in physical and chemical determinations as well as plankton analyses are described in Papers I, II and III.

4.2. Data analyses

Data analyses of paper I were done using the SAS package, version 6.12 (Statistical System Institute Inc., USA). Repeated-measures analyses were run using the mixed procedure. Differences between treatmens in the middle and end of the experiment were studied using the general linear model (GLM) procedure. The treatments and time were considered as fixed effect factors and the enclosure as a random effect factor in both analyses. All other statistical analyses were made using SPSS for Windows (versions 6.0 and 10.0). Intertreatment differences were calculated using the analysis of variance (I and II). In Paper III, the treatment effects were tested with MANOVA followed by univariate ANOVA. Analyses with several independent variables, i.e. total phosphorus, hereafter referred to as TP, and

macrophyte abundance, were done using linear regression. The homogeneity of variances was tested with Levene's test and the normality of the data was tested with Kolmogorov-Smirnov test and on the basis of the residual plots. When the assumptions of variance analyses and regression analyses were not met, $\log_{10}$-transformation was used to normalize the distribution of the data before the statistical analyses. More detailed description of the data analyses are given in each original paper.

5. Results and discussion

5.1. The role of the submerged plant Elodea canadensis in hampering the zooplankton-fish interactions and the trophic cascade

The biomass of euplanktonic zooplankton, especially that of the dominating cladoceran *Daphnia longispina* O.F. Müller, declined in the presence of *Elodea canadensis* (I; Fig. 1). The biomass of *Daphnia* was reduced also by roach. Thus, *Elodea* provided neither a favourable habitat nor a good refuge for *D. longispina* against predation by roach. This result is in contrast to previous observations of the refuge effect of other macrophyte species, such as *Numphar lutea* L. and *Potamogeton pectinatus* L., for several species of *Daphnia* (Schriver *et al.* 1995, Jeppesen *et al.* 1998a, Moss *et al.* 1998). Apparently, various macrophyte species have dissimilar values as refuges for cladocerans against predation by fish. Finely dissected submerged macrophytes, including *Elodea*, have been suggested to reduce predation on *Daphnia* more than for instance floating leaved macrophytes (Burks *et al.* 2002). This general hypothesis is in contrast to the negative effects of *Elodea* on *Daphnia* in our experiment.

On the other hand, the density of fish used in our study (1.9 ind. m^{-2}) was close to the threshold density (2-5 ind. m^{-2}) at which the refuge effect of macrophytes has been shown to be partially or totally lost for cladocerans

(Jeppesen *et al.* 1998a). Such threshold fish density is probably shaped by the species as well as by size and age class of fish. The ineffectiveness of the refuge was also evidenced by the equally decreasing body size of euplanktonic cladocerans in all *Elodea* treatments with fish (Fig. 2). However, *Elodea* appeared to be of value as a refuge for plant-associated cladocerans cladocerans (predominantly *Acroperus harpae* Baird, *Peracantha truncata* O.F. Müller, *Eurycercus lamellatus* O.F. Müller and *Alonella exigua* Lilljeborg), since they were even more abundant (mixed; $F_{1,12} = 9.79$, $P = 0.009$) and larger (mixed; $F_{1,12} = 6.48$, $P = 0.026$) in the presence than in the absence of fish.
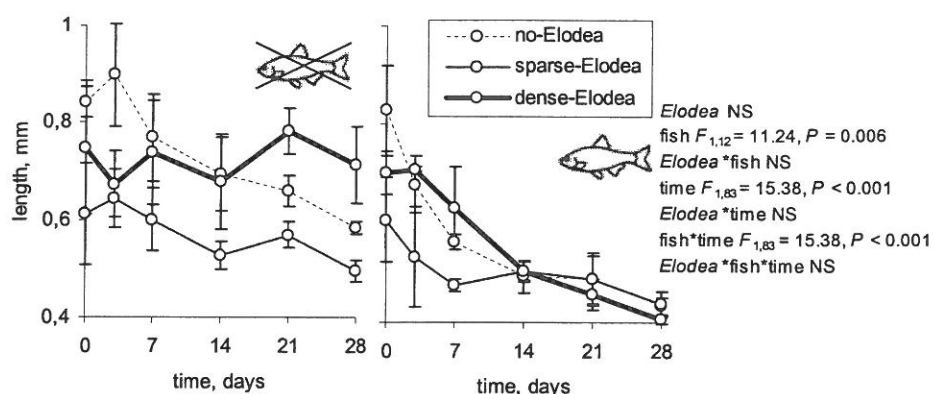
Fig. 2. Treatment means ($\pm$ 1 SE) over the course of the experiment for the body length of euplanktonic cladocerans in the *Elodea* enclosures with or without fish. The *F* ratios with significance levels for the main effects and interactions of *Elodea*, fish and time obtained in the mixed model are given for each variable.

Also the biomass of *Polyphemus pediculus* L. was positively affected by fish in all *Elodea* treatments (mixed; $F_{1,12} = 6.71$, $P = 0.024$). Large-bodied *Eudiaptomus gracilis* G.O. Sars (up to 1.6 mm in length) were not affected by fish. These prey animals have high escape ability and actively change their orientation making them suboptimal food sources for roach, despite their high abundance (Butorina 1986, Persson & Greenberg 1990). In addition, dark pigmentation may be distasteful to predators (Dodson & Frey, 1991), which may also explain why roach did not feed on *Polyphemus*.

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Fish had negligible effects on the total biomass and the clearance rate of euplanktonic zooplankton due to the compensation by the other predominating grazers (*Ceriodaphnia, Diaphanosoma Eudiaptomus* and *Bosmina*) (I; Fig. 1). Nevertheless, chl-*a* concentration was higher in all the *Elodea* treatments with fish than without fish (I; Fig. 1). Even though any differences in dissolved nutrient concentrations between the treatments could not be measured, our results suggest that phytoplankton productivity was enhanced also through nutrient regeneration by roach. Earlier experiments conducted with roach of comparable size in L. Vesijärvi suggested similar mechanisms (Horppila & Kairesalo 1992). Thus, the results did not unequivocally show that *Elodea* can hamper the trophic cascade of fish via lowered predation on grazing zooplankton.

In littoral zone (1-3 m depth) of Lake Vesijärvi, ≥15 cm roach have been shown to have a broad diet consisting of zooplankton, zoobenthos, macrophytes, epiphytic algae and detritus (Horppila 1994, Horppila *et al.* 2000). Thus, it was presumable that roach exhibit a generalist feeding behaviour, switching their food preference according to the relative abundance and availability of different prey types (*cf.* Townsend *et al.* 1986, Gliwicz 2002). During our experiment, the 15-17 cm roach faced a situation in which the relative proportion of cladocerans was diminishing but that of macroinvertebrates was increasing (I; Fig. 3). However, roach continued to pursue zooplankton and were capable of feeding on scarce, small-bodied cladocerans also in *Elodea* beds (I; Fig. 4). This is in contrast to the view that roach are weakly adapted to foraging within dense vegetation (Winfield 1986; Diehl 1988). Lowered availability of preferred cladocerans induced roach to increase consumption of less nutritious, detritus/plant food more than macroinvertebrates. The capability for using alternative, less preferred food sources may serve as life-supporting means for roach populations to manage over the periods of low cladoceran biomass. Such high preference for cladocerans is not likely to stabilize the predator-prey dynamics (Murdoch & Bence 1987, *cf.* Schindler & Scheurell 2002). Thus, roach may

ultimately maintain high predation potential on zooplankton in littoral ecosystem. Regardless of the density of *Elodea* beds, feeding of roach on zoobenthos and epiphytic macroinvertebrates decreased the predation pressure upon cladocerans less than expected. Thus, this mechanism had evidently a minor role in stabilizing zooplankton-fish interactions.

5.2. Responses of zooplankton to nutrient enrichment and fish predation

5.2.1. The Finnish experiment at Kilpiäistenpohja Bay

Nutrient enrichment resulted in the similar range of total phosphorus concentrations during both years (II; Tables 1 and 4). However, the communities differed considerably and therefore both nutrient and fish treatments resulted in contrasting results in the two experiments. The 1998 experiment began in early June when macrophytes had not started to grow probably due to the preceding cold spring. The zooplankton community was dominated by rotifers, which are highly selective grazers (Bogdan & Gilbert 1982), as well as by raptorial zooplankton (*Polyphemus pediculus* and cyclopoid copepods) (II; Fig. 2). Clearwater state with visible lake bottom occurred only in the unenriched mesocosms with negligible or low fish predation while the nutrient enriched mesocosms turned into a turbid state with very high biomass of phytoplankton (up to 200 µg chl-*a* l^{-1}) (II; Fig. 3).

Euplanktonic cladocerans were remarkably sparse in 1998 and increased only during the two last weeks of the experiments. Their time-weighted mean biomasses were not affected by the treatments (II; Table 3, Fig. 2). In littoral areas, cladocerans are suggested to stay on the bottom during day to avoid predation by fish and ascend to open water during night (e.g. Walls *et al.* 1990). The results of our experiment gave slight support to this hypothesis, since in the fish treatments higher densities of euplanktonic cladocerans (predominantly *Bosmina* spp. and *Diaphanosoma brachyurum*

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Leydig) were observed in the water column during the night than during the day (Fig. 3). However, the density of animals was low regardless of the time of the day and the differences were not significant due to a high variation between replicates.
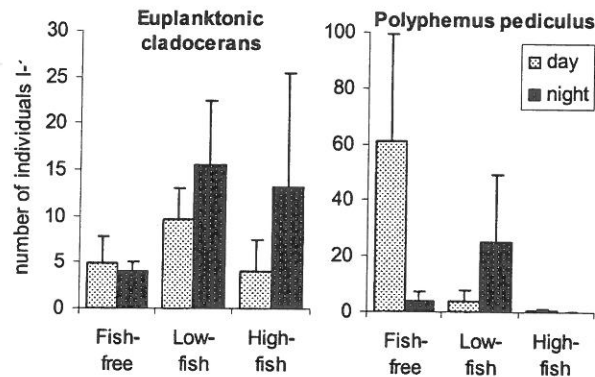

Fig. 3. The mean ($\pm$ 1 SE) density of euplanktonic cladocerans and *Polyphemus pediculus* in the different fish treatments during day and night three weeks after the start of the experiment in 1998. Each bar represents the mean of 6 replicates, i.e. the results from both nutrient treatments were combined.

Raptorial zooplankton increased in biomass with nutrients when the density of fish was low (II; Fig. 2, Table 3). The daytime numbers of the dominating cladoceran *Polyphemus pediculus* were high in the fish-free treatments, while their nocturnal numbers were remarkably lower (Fig. 3). In contrast, an opposite trend was observed in low-fish treatments and, thus, there was an interaction between fish and the time of the day (GLM; $F_{2,30} = 3.90$, $P = 0.031$). In the high-fish treatments *Polyphemus* numbers were reduced close to undetectable level, unlike in the earlier experiment, where they were affected positively by the presence of roach (I). *Polyphemus* is a day-active animal and migrates to the bottom during night (Butorina 1986). Our results suggest that such behaviour may change in the presence of planktivorous fish. Polyphemids prey on small rotifer and crustacean prey (Packard 2001, Butorina 1986). Thus, their predation may partly explain the low colonization of even the fish-free mesocosms by euplanktonic cladocerans.

In 1999, the buffer capacity of the littoral community against nutrient enrichment was high and a clear-water state was maintained even under hypereutrophic nutrient conditions (>500 µg TP l^{-1}) (II; Table 4). The biomass of macrophytes, especially that of free-floating *Lemna trisulca* L., was remarkably higher in 1999 than in 1998 (II; Fig. 1) as was also the biomass and clearance rate of cladocerans (II; Fig. 2). Cladoceran community was dominated by small-bodied *Ceriodaphnia* (0.4-0.5 mm) that increased marginally significantly with nutrients (Anova; $F_{5,18} = 2.60$, $P = 0.061$) in all fish treatments. *Ceriodaphnia* is well adapted to plant beds (Irvine *et al.* 1989, Jeppesen *et al.* 1998a) and has been shown to have higher mass-specific grazing rates than for instance *Daphnia*, *Diaphanosoma* and *Bosmina* (Mourelatos & Lacroix 1990). Thus, in our experiment, *Ceriodaphnia* appeared capable of controlling phytoplankton biomass. In addition, dense floating flocks of *Lemna trisulca* reduced the penetration of light into water and, consequently, to some extent probably suppressed the production planktonic algae (II). However, both the biomass and the coverage, as the percentage of water surface area, of *Lemna* were weakly related to chl-*a* concentration (Fig. 4). Also nutrient enrichment or fish had no effects on the biomass of *Lemna* (Anova; $P > 0.30$) (II; Fig. 1).
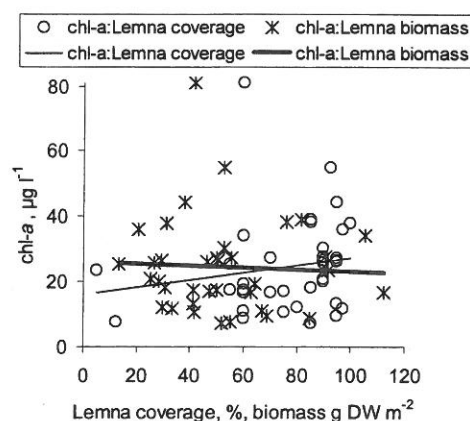
Fig. 4. The relationships between the time-weighted average chl-*a* concentration and the *Lemna trisulca* coverage (linear regression coefficient $R^2 = 0.03$) as well as the biomass ($R^2 = 0.01$) at the end of the experiment in 1999 (n = 36).

However, fish reduced the clearance rate of cladocerans (Anova; $F_{2,18}=16.94$, p<0.001) and increased chl-*a* concentration especially in high-

fish treatments (II; Fig. 1, Table 5). These results do not suggest that *Lemna* was of primary importance in controlling algal densities. Rather, zooplankton grazing together with lowered algal productivity by *Lemna* were most likely complementary mechanisms behind the control of algal biomasses. *Lemna* appeared to provide crustacean zooplankton with a refuge against predation by perch, since no effects of fish were observed in their total biomass (II Table 6). Thus, the results support the theory according to which buffers of the clear-water state are mediated by submerged macrophytes (Irvine *et al.* 1989, Scheffer *et al.* 1993). However, *Lemna* did not hamper the trophic cascade caused by the high biomass of perch (20 g FW m^{-2}).

There were striking year-to-year differences in the response of the same littoral ecosystem to nutrient enrichment and fish predation. The results showed that either clear or turbid water may result depending on the initial (enclosed) community structure. Prevailing weather conditions largely dictated the abundances of key grazers and macrophytes and thereby the resistance of the ecosystem. Thus, the results emphasize heterogeneity among trophic interactions caused by environmental variability, as was also pointed out by Hunter and Price (1992).

5.2.2. The European climatic gradient

Weather conditions in northern and central Europe differed greatly between the study years: summer 1998 was cool and windy, whereas summer 1999 was warm (III; Table 1). Zooplankton community structure and biomass also had remarkable year-to-year and geographical differences in the parallel mesocosm experiments along the European climatic gradient (III; Tables 1, 2, Fig. 1). This probably reflected the heterogeneity within the trophic level and within the littoral communities, as was observed in the Finnish experiment (II). However, some consistent patterns were evident. The multivariate effect of fish was highly significant in both study years and on

all functional groups, i.e. rotifers, both small (<0.5 mm) and large (>0.5 mm) crustacean grazers as well as raptorial zooplakton (III; Figs. 1 and 2, Table 2). The presence of macrophytes appeared to weaken the effects of fish in some of the experiments. For instance, the macrophytic refuge for zooplankton was apparent in Leon, where *Myriophyllum* was abundant (III; Table 1) and fish had negligible effects on large crustaceans (III; Fig. 2). However, in Valencia and Netherlands, large crustaceans were efficiently foraged by fish, although the study sites were colonized by *Chara*-beds, earlier considered as good refuges for zooplankton against fish predation (Diehl 1988, Jeppesen *et al*. 1998a). Strong interaction between site and fish in both study years (III; Figs. 1, 2, Table 2) may partly be explained by the hampering impacts of macrophytes.

The multivariate effect of nutrients was significant only in 1998, when biomasses of small crustaceans and rotifers were enhanced by nutrient enrichment largely independently on the presence or absence of fish (III; Tables 1, 2). The results emphasised the importance of consumer control over resource control in determining zooplankton biomasses, especially those of large zooplankton grazers (III; Table 2, Fig. 2). Time lags depending on cladoceran generation times seem an unlikely reason for the lack of nutrient response during the 5-week experiments. Assuming an average growth rate of 0.3 day^{-1} (*cf.* Brett & Goldman 1997) there would have been at least 15 population doublings. Nevertheless, these results support the general view regarding strong responses of (large) herbivores to predator manipulations within aquatic food webs (Brett and Goldman 1997, Shurin *et al*. 2002). Thus, available food resources seemed to determine total zooplankton biomasses but fish predation regulated the relative abundances of the different functional groups of zooplankton. These results emphasize the compositional changes within trophic levels. They also address the weakness of traditional food chain models to reveal such changes, as have been stated earlier by Leibold *et al*. (1997) and Persson *et al*. (2001).

Biomasses of large crustacean grazers were inversely related to chl-*a* concentrations, except at the highest temperature (close to 30 °C) in Valencia where chl-*a* was unrelated to the biomass of large crustacean grazers (III, Table 3) and the overall biomass of large cladocerans was low compared to the other study sites (III; Fig. 2). This result suggests that the role of grazing in controlling phytoplankton biomass by especially large grazers was important at all sites except in Valencia. At high temperature, nutrient enrichment apparently led to inharmoniously faster growth rate of algae compared with that of zooplankton. Probably other factors related to discrepancies between demography and behavioural time scales of organisms became determinative (Arditi & Ginzburg 1989, Power 1992). In addition, crustacean zooplankton was efficiently reduced by fish suggesting that *Chara*, the abundant macrophyte in Valencia, provided with negligible refuge for zooplankton against fish predation. Strong effects of fish on zooplankton in Valencia support the statement that with increasing temperature the consumer control will further weaken with respect to resource control if the disruption of trophic regulation is interfered by predators (Power 1992). Thus, large crustacean grazers had a minor role in controlling phytoplankton biomass at the lowest latitude. The data set, however, was insufficient to show whether there might be a threshold between temperature regimes at which the functioning of ecosystem would markedly change (*cf.* Scheffer and Carpenter 2003). Other potential geographical differences were probably masked by the high year-to-year variation in the prevailing weather conditions. Such environmental variability may greatly affect the structure of littoral communities, as was concluded also in Paper II.

5.3. Impacts of planktivorous fish, macrophytes and latitude on the role of zooplankton grazers in controlling phytoplankton biomass in lake littoral

Daphnia has been used as the keystone grazer and it's abundance as a relevant indicator for predicting the grazing impact of zooplankton on phytoplankton (Hansson 1992, Sarnelle 1992, Mazumder 1994, Cyr & Curtis 1999, Persson *et al.* 2001). Therefore, supporting evidence for the food chain theory has been gained especially from open-water systems with *Daphnia* as the key grazer (Carpenter *et al.* 1985, 1987, Jeppesen *et al.* 1998c). The capability of cladocerans other than *Daphnia* in limiting phytoplankton biomass has been considered less pronounced (e.g. Benndorf *et al.* 2002, McQueen *et al.* 1986, Carpenter *et al.* 1987). In our mesocosms where the effects of nutrient enrichment and fish predation were tested (II, III) *Daphnia* was, however, relatively sparse and virtually absent in all fish treatments. In order to compare the role of zooplankton grazers along the European climatic gradient *Daphnia* was pooled and examined together with the other large grazers, such as *Diaphanosoma*, *Sida*, *Simocephalus* and *Eudiaptomus* (III). This functional group was of crucial importance in controlling algal biomass, while smaller crustacean grazers and rotifers were of minor or negligible importance (III; Table 3, Fig. 4). In some circumstances, for instance in Finland (Chapter 5.2.1 and II), small crustaceans were able to control algal biomass contributing to a significant negative correlation between their biomass and chl-*a* (III; Table 3).

Large grazers, when having high biomass (time-weighted biomass of >80-90 µg DW l^{-1}) or proportional share (>30 %) of the grazer community, were able to control phytoplankton biomass even under hypereutrophic conditions (up to 1600 µg TP l^{-1}) (III; Fig. 4a-d). The cascading effect of fish, through planktonic grazers, is revealed by steeper slope between chl-*a* and TP concentration in systems with planktivorous fish than in fish-free systems (Hansson 1992, Mazumder 1994). Our results from high-fish treatments agree with these observations but in low-fish treatments the slope was less

steep and alike that in fish-free treatments (III; Fig. 4c,d). Changes in chl-*a* along TP concentration range were explained better by the abundance of large grazers than by the number of trophic levels, i.e. presence or absence of fish (III; Fig. 4e). The high fish biomass (20 g FW m^{-2} or 7-20 ind. m^{-2}) led to low grazer biomass and, consistently, yielded to high algal biomass with increasing potential primary productivity. However, when fish biomass was low (4 g FW m^{-2} or 2.5-4 ind. m^{-2}) high grazer biomass could coexist and control algal biomass. Macrophytic refuges may at least partly explain the weak impacts of low fish biomass. Thus, the results suggest that the threshold fish biomass, below which zooplankton biomass was not controlled by fish, appeared to be close to 4 g FW m^{-2}, which has been suggested as the desired fish biomass after biomanipulation (McQueen *et al.* 1986).

In shallow vegetated lakes, the relative importance of grazing in controlling phytoplankton biomass and macrophytes in controlling phytoplankton growth are difficult to interpret. This has been a subject of debate with the primacy of zooplankton grazing (Schriver *et al.* 1995, Jeppesen *et al.* 1998a, 1999) and macrophytes (Meijer *et al.* 1999, Blindow *et al.* 2000). The experiments conducted at the same location (Kilpiäistenpohja Bay; I, II) enabled the comparison of the magnitude and the relative role of zooplankton grazing in controlling phytoplankton biomass in different structures of macrophytic vegetation. The grazing impact on phytoplankton by euplanktonic crustaceans was compared in unenriched mesocosms without and with high biomass of fish (20, 73 g FW m^{-2}). The grazing was estimated by calculating the cladoceran clearance rates according to the equation by Knoechel and Holtby (1986), while the clearance rate of 2 ml per animal per day was used for *Eudiaptomus* (excluding nauplii) (Cyr & Pace, 1992). It is assumed that these crustaceans feed primarily on phytoplankton, despite of the fact that many cladocerans feed efficiently also on bacteria (Kankaala 1988, Jeppesen *et al.* 1998a), which are abundant in vegetation (Wetzel & Søndergaard 1998). In general, clearance rates and

chl-*a* concentrations had an inverse relationship regardless of macrophytic vegetation (Fig. 5). Perrow *et al.* (1999) suggested a threshold filtration (clearance) rate of 300-400 ml per day to maintain the clear-water phase. In our experiments, these values were exceeded especially in fish-free treatments.
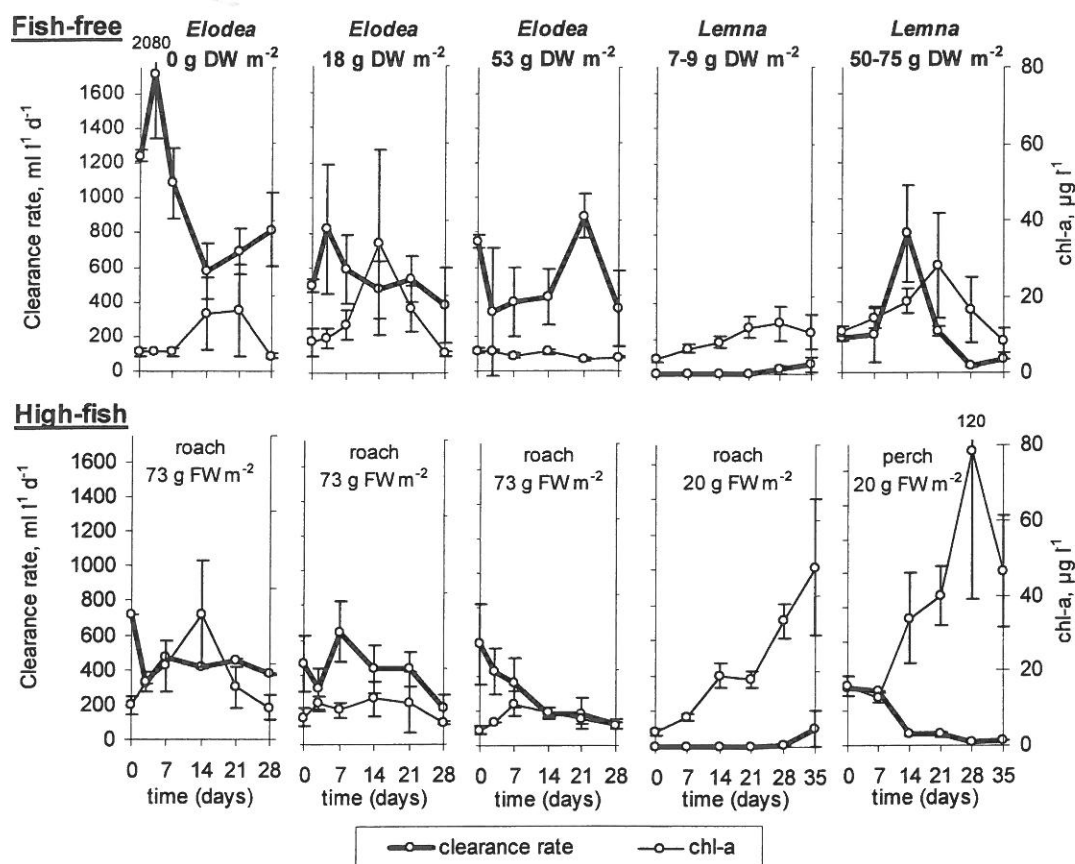
Fig. 5. Clearance rates of the euplanktonic crustacean community and chl-*a* concentrations in the mesocosms without fish (Fish-free) and with high biomass of planktivorous fish (High-fish; 73 and 20 g FW m^{-2} in experiments with *Elodea* and *Lemna*, respectively). The experiments are arranged with respect to different biomasses of *Elodea canadensis* and *Lemna trisulca* in the mesocosms. Each value represents the mean ($\pm$ 1 SE) of three replicates, except in the experiment with 50-75 g DW *Lemna* m^{-2}, where the number of replicates is two.

However, in some of the enclosures without or with sparse (18 g DW m^{-2}) *Elodea* beds, without or with roach, high algal biomass and blooms of the cyanobacterium *Anabaena lemmermannii* P. Richter occurred in the middle of the experiment (I; Fig. 1) despite of high clearance rates (Fig. 5). This result supports the view that in some circumstances selective grazing may

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shift phytoplankton community structure into dominance by filamentous cyanobacteria (e.g. Gragnani *et al.* 1999). Cyanobacterial blooms did not develop in enclosures with dense *Elodea* beds (53 g DW m^{-2}) where chl-*a* concentration was always low. This suggests that phytoplankton production were controlled also by *Elodea*.

In contrast, when the biomass of *Lemna* was low (7-9 g DW m^{-2}) relatively low chl-*a* concentrations coincided with extremely low clearance rate even in the absence of fish. Perhaps rotifers and raptorial zooplankton that dominated the zooplakton community having biomasses of up to 200-500 and 120-250 μg C l^{-1}, respectively, were able to utilise and control algal biomass. Competition for nutrients with periphytic algae was unlikely responsible for the observed low chl-*a* concentration since the biomass of periphytic algae was low, always below 1 μg cm^{-2} (II).

Highest chl-*a* concentrations were measured in fish treatments, where clearance rates were lower than in the corresponding fish-free treatments. This trophic cascade was more apparent in the systems with high biomass of *Lemna* than in those with high biomass of *Elodea* (Fig. 5). This difference is notable, as roach biomass in *Elodea* enclosures was over six times higher than perch biomass in mesocosms with high biomass of *Lemna*. Perch are superior to roach in foraging in vegetation (Winfield 1986; Diehl, 1988) and this was evidenced by markedly lower zooplankton clearance rates in mesocosms with abundant *Lemna* compared to those in enclosures with abundant *Elodea* and roach (Fig. 5).

Thus, *Elodea* appeared to facilitate the top-down control of phytoplankton biomass by zooplankton, which, in turn, operated simultaneously with the control of phytoplankton production by *Elodea*. Also Schriver *et al.* (1995) and Scheffer (1999) have suggested the importance of the synergistic effects on phytoplankton by both zooplankton and macrophytes. However, zooplankton grazing was apparently behind the control of phytoplankton

biomass more than mechanisms by *Lemna trisulca*. These results suggest considerable differences in the strength of variable macrophyte and fish assemblages in affecting zooplankton-phytoplankton interactions.

Top-down effects on phytoplankton biomass have been demonstrated in several mesocosm experiments (Schriver *et al.* 1995, Sarvala *et al.* 2000, Benndorf *et al.* 2002). Both the between-site and local year-to-year comparisons in our experiments also emphasised the general importance of zooplakton grazers, especially large crustaceans, in controlling phytoplankton biomass. In addition, depending on macrophyte species, this control may be facilitated by vegetation.

6. Conclusions

The mechanisms behind the control of algal biomass were evidently linked to the abundances of key grazers and macrophytes. The functional group of large crustacean grazers was of crucial importance in controlling phytoplankton even under hypereutrophic conditions when their proportional share or biomass was high. However, small-sized crustaceans may also be able to control algal biomass in vegetated littoral. Under high temperature conditions (close to 30 ^{o}C), the grazing impact was low in relation to resource control and this lead to low resistance of the community to nutrient enrichment. Other geographical differences were probably masked by the high year-to-year variation in the prevailing weather conditions.

Fish had a greater role than nutrients in regulating zooplankton biomass. On the other hand, the results suggested a threshold fish biomass of 4 g FW m^{-2}, at which high grazer biomass may coexist and control algal biomass. When fish biomass was higher (20 or 73 g FW m^{-2} of 5-10 or 15-17 cm fish, respectively), the results did not unequivocally show that submerged

vegetation hamper the trophic cascade of planktivorous fish via zooplankton grazers. Benthic and epiphytic macroinvertebrates as alternative food sources for roach in *Elodea* beds reduced predation pressure on cladocerans, the most preferred food source, less than expected. Rather, the results suggested that alternative food sources may facilitate roach populations to manage over the periods of low cladoceran abundance and thus maintain high predation pressure on zooplankton

Compositional changes within zooplankton community suggested that available food resources determine total zooplankton biomasses but that fish predation strongly regulated the relative abundances of the different functional groups of zooplankton. The biomass of large cladocerans was reduced by high biomass of fish and compensated by smaller grazers for which macrophytes appeared to provide with refuge against fish predation. However, variable macrophyte species established beds of dissimilar refuge value for zooplankton. In littoral ecosystems, the habitat structural heterogeneity has generally been emphasized causing high variation among trophic interactions. The results of this work support this view and emphasize also the heterogeneity within trophic level as well as year-to-year differences in the community structure, affecting the resistance of the system to environmental variation.

7. Acknowledgements

8. References

Abrams, P. A. & Walters, C. J. 1996: Invulnerable prey and the paradox of enrichment. – *Ecology* 77: 1125-1133.

Arditi, R. & Ginzburg, L.R. 1989: Coupling in predator-prey dynamics: ratio-dependence. – *J. Theor. Biol.* 139: 69-75.

Benndorf, J., Boing, W., Koop, J. & Neubauer, I. 2002: Top-down control of phytoplankton: the role of time scale, lake depth and trophic state. – *Freshw. Biol.* 47: 2282-2295.

Blindow, I., Hargeby, A., Wagner, B. M. A. & Andersson, G. 2000: How important is the crustacean plankton for the maintenance of water clarity in shallow lakes with abundant submerged vegetation? – *Freshw. Biol.* 44: 185-197.

Bogdan, K. B. & Gilbert, J. J. 1982: Seasonal patterns of feeding by natural populations of Keratella, Polyarthra and Bosmina: Clearance rates, selectivities and contributions to community grazing. – *Limnol. Oceanogr.* 27: 918-934.

Brett, M. T. & Goldman, C. R. 1997: Consumer versus resource control in freshwater pelagic food webs. – *Science* 275: 384-386.

Brooks, J. L. & Dodson, S. I. 1965: Predation, body size and composition of plankton. – *Science* 150: 28-35.

Burks, R. L., Jeppesen, E. and Lodge, D. 2000: Macrophyte and fish chemicals suppress Daphnia growth and alter life history traits. – *Oikos* 88: 139-147.

Burks, R. L., Lodge, D.M., Jeppesen, E. & Lauridsen, T. L. 2002: Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. – *Freshw. Biol.* 47: 343-365.

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

Carpenter, S. R. & Kitchell, J. F. (eds.) 1993: *The trophic cascade in lakes.* – Cambridge Studies in Ecology. 385 p.

Carpenter, S. R., Kitchell, J. F. & Hodgson, J. R. 1985: Cascading trophic interactions and lake productivity. – *BioScience* 35: 634-639.

Carpenter, S. R., Kitchell, J. F., Hodgson, J. R., Cochran, P. A., Elser, J. J., Elser, M. M., Lodge, D. M., Kretchmer, D, He, X. & von Ende, C. N. 1987: Regulation of lake primary productivity by food web structure. – *Ecology* 68: 1863-1876.

Carpenter, S. R. & Lodge, D. M. 1986: Effects of submersed macrophytes on ecosystem processes. – *Aquat. Bot.* 26: 341-370.

Cyr, H. & Curtis, J. M. 1999: Zooplankton community size structure and taxonomic composition affect size-selective grazing in natural communities. – *Oecologia* 118: 306-315.

Cyr, H. & Pace, M. L. 1992: Grazing by zooplankton and its relationship to community structure. – *Can. J. Fish. Aquat. Sci.* 49: 1455-1465.

Diehl, S. 1988: Foraging efficiency of three freshwater fishes: effects of structural complexity and light. – *Oikos* 53: 207-214.

Diehl, S. 1993: Effects of habitat structure on resource availability, diet and growth of benthivorous perch, Perca fluviatilis. – *Oikos* 67: 403-414.

Diehl, S. & Feißel, M. 2000: Effects of enrichment on three-level food chains with omnivory. – *Am. Nat.* 155: 200-218.

Dodson, S.I. & Frey, D.G. 1991: Cladocera and other Branchiopoda. – In: Thorp, J. H. & Covich. A. P. (eds.) *Ecology and classification of North American Freshwater Invertebrates*: 723-786. Academic Press, San Diego.

Dorgelo, J. & Heykoop, M. 1985: Avoidance of macrophytes by Daphnia longispina. – *Verh. Int. Verein. Limnol.* 22: 3369-3372.

Fretwell, S. D. 1977: The regulation of plant communities by food chains exploiting them. – *Perspectives in Biology and Medicine* 20: 169-185.

Gliwicz, Z. M. 2002: On the different nature of top-down and bottom-up effects in pelagic food webs. – *Fresw. Biol.* 47: 2296-2312.

Gragnani, A., Scheffer, M. & Rinaldi, S. 1999: Top-down control of cyanobacteria: a theoretical analysis. – *Am. Nat.* 153: 59-72.

Hairston, N. G., Smith, F. E. & Slobodkin, L. B. 1960: Community structure, population control and competition. – *Am. Nat.* 94: 421-425.

Hansson, L.-A. 1992: The role of food chain composition and nutrient availability in shaping algal biomass development. – *Ecology* 73: 241-247.

Hasler, A. D. & Jones, E. 1949: Demonstration of the antagonistic action of large aquatic plants on algae and rotifers. – *Ecology* 30: 359-364.

Horppila, J. 1994: The diet and growth of roach (Rutilus rutilus (L.)) in Lake Vesijärvi and possible changes in the course of biomanipulation. – *Hydrobiologia* 294: 35-41.

Horppila, J. & Kairesalo, T. 1992: Impact of bleak (Alburnus alburnus) and roach (Rutilus rutilus) on the quality, sedimentation and internal nutrient loading. – *Hydrobiologia* 243/244: 323-331.

Horppila, J., Peltonen, H., Malinen, T., Luokkanen, E. & Kairesalo, T. 1998: Top-down or bottom-up effects of fish: issues of concern in biomanipulation of lakes. – *Restoration Ecology* 6: 20-28.

Horppila, J., Ruuhijärvi, J., Rask, M., Karppinen, C., Nyberg, K. & Olin, M. 2000: Seasonal changes in the diets and relative abundances of perch and roach in the littoral and pelagic zones of a large lake. – *J. Fish Biol.* 56: 51-72.

Hrbácek, J. 1962: Species composition and the amount of zooplankton in relation to the fish stock. – *Rozpr. Cesk. Akad. Ved.* 72: 1-114.

Hudson, J. J., Taylor, W. & Schindler, D. W. 1999: Planktonic nutrient regeneration and cycling efficiency in temperate lakes. – *Nature* 400: 659-661.

Hunter, M. D. & Price, P. W. 1992: Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. – *Ecology* 73: 724-732.

Irvine, K., Moss, B. & Balls, H. 1989: The loss of submerged plants with eutrophication II. Relationships between fish and zooplankton on a set of experimental ponds, and conclusions. – *Freshw. Biol.* 22: 89-107.

Jeppesen, E., Jensen, J. P. & Søndergaard, Ma. 2002: Response of phytoplankton, zooplakton and fish to re-oligotrophication: An 11 year study of 23 Danish lakes. – *Aquatic Ecosystem Health & Management* 5: 31-42.

Jeppesen, E., Jensen, J. P., Søndergaard, Ma. & Lauridsen, T. 1999: Trophic dynamics in turbid and clearwater lakes with special emphasis on the role of zooplankton for water quality. – *Hydrobiologia* 408/409: 217-231.

Jeppesen, E., Lauridsen, T. L., Kairesalo, T. & Perrow, M. 1998a: Impact of submerged macrophytes on fish-zooplankton interactions in lakes. – In: Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. & Christoffersen, K. (eds.) *The structuring role of submerged macrophytes in lakes*: 91-114. Springer-Verlag, New York.

Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. & Christoffersen, K. (eds.) 1998b: *The structuring role of submerged macrophytes in lakes*. – Springer-Verlag, New York. 423 p.

Jeppesen, E., Søndergaard, M., Jensen, J. P., Mortensen, E., Hansen, A.-M. & Jorgensen, T. 1998c: Cascading trophic interactions from fish to bacteria and nutrients after reduced sewage loading: An 18-year study of a shallow hypertrophic lake. – *Ecosystems* 1: 250-267.

Kairesalo, T., Kornijów, R. & Luokkanen, E. 2000: Trophic cascade structuring a plankton community in a strongly vegetated lake littoral. – *Verh. Int. Verein. Limnol.* 27: 1763-1767.

Kairesalo, T., Tátrai, I. & Luokkanen, E. 1998: Impacts of water weed (Elodea canadensis Michx) on plankton-fish interactions in the lake littoral. – *Verh. Int. Verein. Limnol.* 26: 1846-1851.

Kankaala, P. 1988: The relative importance of algae and bacteria as food for Daphnia longispina (Cladocera) in a polyhumic lake. – *Freshw. Biol.* 19: 285-296.

Kerfoot, W. C. & Sih, A. (eds.) 1987: *Predation. Direct and indirect impacts on aquatic communities*. – University Press of New England. 386 p.

Knoechel, R. & Holtby, L. B. 1986: Construction and validation of a body-length-based model for the prediction of cladoceran community filtering rates. – *Limnol. Oceanogr.* 31: 1-16.

Lauridsen, T. L. & Lodge, D. M. 1996: Avoidance by Daphnia magna of fish and macrophytes: Chemical cues and predator-mediated use of macrophyte habitat. – *Limnol. Oceanogr.* 41: 794-798.

Lehman, J.T. 1988: Ecological principles affecting community structure and secondary productivity by zooplankton in marine and freshwater environments. – *Limnol. Oceanogr.* 33: 931-945.

Leibold, M. A., Chase, J. M., Shurin, J. B. & Downing, A. L. 1997: Species turnover and the regulation of trophic structure. – *Ann. Rev. Ecol. Syst.* 28: 467-494.

Mazumder, A. 1994: Patterns of algal biomass in dominant odd- vs. even-link lake ecosystems. – *Ecology* 75: 1141-1149.

McCauley, E. & Murdoch, W. W. 1987: Cyclic and stable populations: plankton as paradigm. – *Am. Nat.* 129: 97-121.

McQueen, D. J., Post, J. R. & Mills, E. L. 1986: Trophic relationships in freshwater pelagic ecosystems. – *Can. J. Fish. Aquat. Sci.* 43: 1571-1581.

Meijer, M-L., de Boois, I., Scheffer, M., Portielje, R. & Hosper, H. 1999: Biomanipulation in shallow lakes in The Netherlands: an evaluation of 18 case studies. – *Hydrobiologia* 408/409: 13-30.

Moss, B., Kornijów, R. & Measey, G. J. 1998: The effect of nymphaeid (Nuphar lutea) density and predation by perch (Perca fluviatilis) on the zooplankton communities in a shallow lake. – *Freshw. Biol.* 39: 689-697.

Mourelatos, S. & Lacroix, G. 1990: In situ filtering rates of Cladocera: effect of body length, temperature and food concentration. – *Limnol. Oceanogr.* 35: 1101-111.

Murdoch, W. W. & Bence, J. 1987: General predators and unstable prey populations. – In: Kerfoot, W. C. & Sih, A. (eds.) *Predation. Direct and indirect impacts on aquatic communities*: 17-30. University Press of New England.

Oksanen, L., Fretwell, S. D., Arruda, J. & Niemelä, P. 1981: Exploitation ecosystems in gradients of primary productivity. – *Am. Nat.* 118: 240-261.

Pace, M. L., Cole, J. J. & Carpenter, S. R. 1998: Trophic cascades and compensation: differential responses of mirozooplankton in whole-lake experiments. – *Ecology* 79: 138-152.

Packard, A. T. 2001: Clearance rates and prey selectivity of the predaceous cladoceran Polyphemus pediculus. – *Hydrobiologia* 442: 177-184.

Pennak, R. W. 1973: Some evidence for aquatic macrophytes as repellents for a limnetic species of *Daphnia*. – *Int. Revue Ges. Hydrobiol.* 60: 569-576.

Perrow, M. R., Jowitt, A. J. D., Stansfield, J. H. & Phillips, G. L. 1999: The practical importance of the interactions between fish, zooplakton and macrophytes in shallow lake reatoration. – *Hydrobiologia* 395/396: 199-210.

Persson, L. 1983: Food consumption and the significance of detritus and algae to intraspecific competition in roach Rutilus rutilus in a shallow eutrophic lake. – *Oikos* 41: 118-125.

Persson, L. 1986: Temperature-induced shift in foraging ability in two fish species, roach (Rutilus rutilus) and perch (Perca fluviatilis): implications for coexistence between poikilotherms. – *J. Anim. Ecol.* 55: 829-839.

Persson, L. & Greenberg, L.A. 1990: Juvenile competitive bottlenecks: the perch (Perca fluviatilis) – roach (Rutilus rutilus) interaction. – *Ecology* 71: 44-56.

Persson, L., Diehl, S., Johansson, L., Andersson, G., Hamrin, S. F. 1992: Trophic interactions in temperate lake ecosystems: a test of food chain theory. – *Am. Nat.* 140: 59-84.

Persson, A., Hansson, L.-A., Brönmark, C., Lundberg, P., Pettersson, L. B., Greenberg, L., Nilsson, P. A., Nyström, P., Romare, P. & Tranvik, L. 2001: Effects of enrichment on simple aquatic food webs. *Am. Nat.* 157: 654-669.

Petchey, O. L., McPhearson, T., Casey, T. M. & Morin, P. J. 1999: Environmental warming alters food-web structure and ecosystem function. – *Nature* 402: 69-72.

Polis, G. A. 1999: Why are parts of the world green? Multiple factors control productivity and the distribution of biomass. – *Oikos* 86: 3-15.

Power, M. E. 1992: Top-down and bottom-up forces in food webs: do plants have primacy? – *Ecology* 73: 733-746.

Rask, M., Järvinen, M., Kuoppamäki, K. & Pöysä, H. 1996: Limnological responses to the collapse of the perch population in a small lake. – *Ann. Zool. Fennici* 33: 517-524.

Reynolds, C. S. 1994: The ecological basis for the successful biomanipulation of aquatic communities. – *Arch. Hydrobiol.* 130: 1-33.

Sarnelle, O. 1992: Nutrient enrichment and grazer effects on phytoplankton in lakes. – *Ecology* 73: 551-560.

Sarvala, J., Ventelä, A.-M., Helminen, H., Hirvonen, A., Saarikari, V., Salonen, S., Sydänoja, A. & Vuorio, K. 2000: Restoration of the eutrophicated Köyliönjärvi (SW Finland) through fish removal: whole-lake vs. mesocosm experiences. *Boreal Env. Res.* 5: 39-52.

Scheffer, M. 1991: Fish and nutrients interplay determines algal biomass: a minimal model. – *Oikos* 62: 271-282.

Scheffer, M. 1999: The effect of aquatic vegetation on turbidity: how important are the filter feeders? – *Hydrobiologia* 408/409: 307-316.

Scheffer, M., Hosper, S. H., Meijer, M-L., Moss, B. & Jeppesen, E. 1993: Alternative equilibria in shallow lakes. – *TREE* 8: 275-279.

Scheffer, M. & de Boer, B. J.1995: Implications of spatial heterogeneity for the paradox of enrichment. – *Ecology* 76: 2270-1177.

Scheffer, M. & Carpenter, S.R. 2003: Catastrophic regime shifts in ecosystems: linking theory to observation. – *TREE* 18, 648-656.

Schindler, D. E. & Scheuerell, M. D. 2002: Habitat coupling in lake ecosystems. – *Oikos* 98: 177-189.

Schriver, P., Bøgestrand, J., Jeppesen, E. & Søndergaard, Ma. 1995: Impact of submerged macrophytes on fish-zooplankton-phytoplankton interaction: large-scale enclosure experiments in a shallow eutrophic lake. – *Freshw. Biol.* 33: 255-270.

Shurin, J. B, Borer, E. T., Seabloom, E. W., Anderson, K., Blanchette, C. A., Broitman, B, Cooper, S. D. & Halpern, B. S. 2002: A cross-ecosystem comparison of the strength of trophic cascades. – *Ecology Letters* 5: 785-791.

Shuter, B. J. & Ing, K. K. 1997: Factors affecting the production of zooplankton in lakes. – *Can. J. Fish. Aquat. Sci.* 54: 359-377.

Søndergaard & Moss, B. 1998: Impact of submerged macrophytes on phytoplankton in shallow freshwater lakes. – In: Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. & Christoffersen, K. (eds.) *The structuring role of submerged macrophytes in lakes*: 115-132. Springer-Verlag, New York.

Tarvainen, M., Sarvala, J. & Helminen, H. 2002: The role of phosphorus release by roach [Rutilus rutilus (L.)] in the water quality changes of a biomanipulated lake. *Freshw. Biol.* 47: 2325-2336.

Timms, R.M. & Moss, B. 1984: Prevention of growth of potentially dense phytoplanktonpopulations by zooplankton grazing, in the presence of zooplanktivorous fish in a shallow wetland ecosystem. – *Limnol. Oceanogr.* 29: 472-286.

Townsend, C.R., Winfield, I. J., Peirson, G. & Cryer, M. 1986: The response of young roach Rutilus rutilus to seasonal changes in abundance of microcrustacean prey: a field demonstration of switching. – *Oikos* 46: 372-378.

Vadeboncoeur, Y., Zanden, M. J. & Lodge, D. M. 2002: Putting the lake back together: reintegrating benthic pathways into lake food web models. – *BioScience* 52: 44-54.

Vanni, M. J. 2002: Nutrient cycling by animals in freshwater ecosystems. – *Ann. Rev. Ecol. Syst.* 33: 341-370.

Walls, M., Rajasilta, M., Sarvala, J. & Salo, J. 1990: Diel changes in horizontal microdistribution of littoral Cladocera. – *Limnologica* 20: 253-258.

Wetzel, R. G. 1990: Land-water interfaces: Metabolic and limnological regulators. – *Verh. Int. Verein. Limnol.* 24: 6-24.

Wetzel, R. G. & Søndergaard, Mo. 1998: Role of submerged macrophytes for the microbial community and dynamics of dissolved organic carbon in aquatic ecosystems. – In: Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. & Christoffersen, K. (eds.) *The structuring role of submerged macrophytes in lakes*: 133-148. Springer-Verlag, New York.

Winfield, I. J. 1986: The influence of simulated aquatic macrophytes on the zooplankton consumption rate of juvenile roach, Rutilus rutilus, rudd, Scardinius erythrophthalmus, and perch, Perca fluviatilis. – *J. Fish Biol.* 29: 37-48.

Impacts of a submerged plant (*Elodea canadensis*) on interactions between roach (*Rutilus rutilus*) and its invertebrate prey communities in a lake littoral zone

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SUMMARY

1. Using 5-m^2 field enclosures, we examined the effects of *Elodea canadensis* on zooplankton communities and on the trophic cascade caused by 4–5 year old (approximately 16 cm) roach. We also tested the hypothesis that roach in *Elodea* beds use variable food resources as their diet, mainly benthic and epiphytic macroinvertebrates, and feed less efficiently on zooplankton. Switching of the prey preference stabilises the zooplankton community and, in turn, also the fluctuation of algal biomass. The factorial design of the experiment included three levels of *Elodea* (no-, sparse- and dense-*Elodea*) and two levels of fish (present and absent).

2. During the 4-week experiment, the total biomass of euplanktonic zooplankton, especially that of the dominant cladoceran *Daphnia longispina*, decreased with increase in *Elodea* density. The *Daphnia* biomass was also reduced by roach in all the *Elodea* treatments. Thus, *Elodea* provided neither a favourable habitat nor a good refuge for *Daphnia* against predation by roach.

3. The electivity of roach for cladocerans was high in all the *Elodea* treatments. Roach were able to prey on cladocerans in *Elodea* beds, even when the abundance and size of these prey animals were low. In addition to cladocerans, the diet of roach consisted of macroinvertebrates and detrital/plant material. Although the biomass of macroinvertebrates increased during the experiment in all *Elodea* treatments, they were relatively unimportant in roach diets regardless of the density of *Elodea* beds.

4. Euplanktonic zooplankton species other than *Daphnia* were not affected by *Elodea* or fish and the treatments had no effects on the total clearance rate of euplanktonic zooplankton. However, the chlorophyll *a* concentration increased with fish in all the *Elodea* treatments, suggesting that fish enhanced algal growth through regeneration of nutrients. Thus, our results did not unequivocally show that *Elodea* hampered the trophic cascade of fish via lowered predation on grazing zooplankton.

5. In treatments with dense *Elodea* beds (750 g FW m^{-2}), chlorophyll *a* concentration was always low suggesting that phytoplankton production was controlled by *Elodea*. Apparently, the top-down control of phytoplankton biomass by zooplankton was facilitated by the macrophytes and operated simultaneously with control of phytoplankton production by *Elodea*.

Keywords: enclosures, fish, macroinvertebrates, submerged macrophytes, zooplankton

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Introduction

The positive feedback mechanisms, through which submerged macrophytes promote a clear-water state in lakes across a wide range of nutrient concentrations, have been crystallised in the concept of alternative stable states (Scheffer *et al.*, 1993). In shallow lakes, spatial heterogeneity and macrophyte refuges for invertebrates against fish predation hamper trophic cascades (Carpenter, Kitchell & Hodgson, 1985), thereby decreasing the strength of top-down impact of planktivorous and omnivorous fish on planktonic communities (Scheffer *et al.*, 1993; Jeppesen *et al.*, 1998a; Pace *et al.*, 1999). Indeed, one of the major regulating roles of submerged vegetation is thought to be the refuge that it provides for grazing zooplankton, especially daphnids, against visually predating planktivorous fish (Timms & Moss, 1984; Jeppesen *et al.*, 1998a; Scheffer, 1999). Lowered predation by fish on zooplankton is reflected in dampened variation in algal biomass. However, dense submerged vegetation and low phytoplankton biomass often coincide with a low abundance of grazing zooplankton (Meijer *et al.*, 1999; Blindow *et al.*, 2000), suggesting other regulatory mechanisms of macrophytes on planktonic communities, such as shading, allelopathy, competition for nutrients and influence on water movements and sedimentation (reviewed by Søndergaard & Moss, 1998).

Because of the low overall capability of roach (*Rutilus rutilus* L.) to forage efficiently in vegetation (Winfield, 1986; Diehl, 1988), macrophyte beds may provide prey populations with an important refuge against predation by roach. Submerged macrophytes may structure fish–zooplankton interactions through sustaining a habitat with multiple alternative food sources for fish. Several authors showed that in vegetation, planktivorous and omnivorous fish switch from zooplankton to other food sources, such as algae, detritus, macrophytes or macroinvertebrates (Persson, 1983; Diehl, 1993; Moss, Kornijów & Measey, 1998). Such generalist feeding behaviour has major implications for the dynamics of predator–prey interactions. Switching of prey preference may reduce the predation pressure on zooplankton (Perrow *et al.*, 1999) and thereby dampen their population fluctuations. Consequently, planktivorous fish populations are stabilised by alternative food resources in the littoral ecosystem and may become uncoupled from the

dynamics of their preferred zooplankton prey (Schindler & Scheuerell, 2002). On the other hand, depending on the predator's behaviour and food preferences, behavioural plasticity may have only a short-term stabilising effect (Murdoch & Bence, 1987). For instance, in the littoral of Lake Vesijärvi, southern Finland, the proportion of zooplankton in the diets of roach decreases and that of benthos and macrophytes increases with low availability of planktonic food (Horppila & Kairesalo, 1992) and with increasing size of fish (Horppila, 1994). In addition, in the littoral zone (at 1–3-m depths), large roach (≥15 cm in length) were shown to have a broad diet consisting of zooplankton, zoobenthos, macrophytes, epiphytic algae and detritus (Horppila, 1994; Horppila *et al.*, 2000). Yet, the consequences of such plasticity in fish food preference for zooplankton-phytoplankton interactions and for the stability of the littoral food web functioning have not been quantified experimentally taking into account all major communities (for review see Jeppesen *et al.*, 1998b).

In the present study, we set up an enclosure experiment to test two main hypotheses: (i) the cascading effect (Pace *et al.*, 1999) caused by 4–5 year old (≥15 cm) roach can be hampered by the refuge effect of *Elodea canadensis* Michx beds, primarily via reducing predation on euplanktonic filter-feeding zooplankton; and (ii) in *Elodea* beds, roach utilise variable food resources, mainly benthic and epiphytic macroinvertebrates, and thus feed less efficiently on zooplankton, while switching of prey preference stabilises the littoral plankton dynamics.

Methods

Study site and experimental design

The study was performed in Kilpiäistenpohja Bay, which lies at the south-eastern end of mesotrophic Lake Vesijärvi in southern Finland (61°02′N, 25°39′E, surface area 110 km^2, mean depth 6.0 m). Further details of the lake can be found in Kairesalo *et al.* (1999) and Keto & Sammalkorpi (1988). Kilpiäistenpohja is a shallow bay with rich emergent vegetation dominated by *Phragmites australis* (Cav.) Trin. ex Steud., submerged vegetation composed mainly of *E. canadensis*, *Myriophyllum* spp. and *Ceratophyllum demersum* L., and floating-leaved macrophytes (*Nuphar lutea* L.) together with freely floating lemnids

(*Lemna trisulca* L.). Roach and perch (*Perca fluviatilis* L.) are the most abundant fish species in the littoral area (Kairesalo *et al.*, 1999; Horppila *et al.*, 2000), while pike (*Esox lucius* L.) have decreased in abundance with eutrophication (Keto & Sammalkorpi, 1988).

On 14 July, 1994, 18 5-m^2 rectangular enclosures (2 × 2.5 m) were placed in the lake at a depth of 1.2 m. These enclosures, made of thin, clear polyethylene, were suspended from wooden frames and were open to the atmosphere. The low ends of the walls were sealed into the bottom sediment, which consisted of organic material with abundant remnants of macrophytes. After harvesting the naturally occurring macrophytes from the experimental area, six randomly chosen enclosures were planted with 250 g WW (wet weight) m^{-2} of *Elodea* (sparse-*Elodea*), another six with 750.g WW m^{-2} of *Elodea* (dense-*Elodea*) and the remaining six were left without *Elodea* addition (no-*Elodea*). Hereafter, *Elodea* treatments will be referred to by the abbreviations in parentheses. Assuming *Elodea* dry weight (DW) to be about 7% of the WW (Kairesalo, Tátrai & Luokkanen, 1998), the initial *Elodea* biomass in the sparse-*Elodea* treatment was 17.5 g DW m^{-2} and in the dense-*Elodea* treatment 52.5 g DW m^{-2}. The macrophytes were collected from the same lake, near the experimental area. In all enclosures, lemnids (*L. trisulca*) occurred at low and equal densities.

The enclosures were allowed to recover from disturbance for 2 weeks prior to the experiment, which began on 1 August (day 0), when nine randomly-selected enclosures (three for each *Elodea* treatment) were stocked with a group of 4–5 year old roach at a density of nine fish per enclosure (68.9 g or 1.8 fish m^{-2}). The mean length of the fish was 15.9 cm (SD = 1.3 cm, *n* = 62), and the mean individual weight was 38.3 g (SD = 8.6 g, *n* = 62). These fish were caught from nearby littoral area with a pound net. Three enclosures of each *Elodea* treatment were included as fish-free control systems. Thus, the experimental design was fully factorial with two factors: the density of *Elodea* (three levels) and the density of fish (two levels), with three replicates for each treatment. A net was stretched over the enclosures (2.5 m above the water surface) to prevent entry of birds. The enclosures were checked every other day and any dead roach were immediately replaced with new ones of similar size. The overall rate of fish mortality during the experiment was 3.7%. The experiment was terminated on 29 August, when all fish were removed from the enclosures with a pound net.

Sampling procedure and sample analyses

The first samples of water for chemical and plankton analyses were collected on day 0 (1 August), just before the introduction of fish. Thereafter, samples were collected on days 3, 7, 14, 21 and 28. The temperature and dissolved oxygen concentration at the water surface and near the lake bottom were measured using a YSI meter (Yellow Springs Inc., U.S.A.) prior to the sampling (at 10.00–11.00 hours). The diurnal fluctuation of water temperature and dissolved oxygen concentration was studied on days 10 and 11, with measurements taken at 10.00, 16.00, 22.00 and 04.00 hours.

Water samples were taken from 10 different places with a transparent perspex core (length 1.5 m, inner diameter 4.0 cm) from the surface to the bottom of each enclosure. The samples were then pooled and mixed. From each sample, a 100-mL subsample of water for phytoplankton was preserved immediately with acid Lugol's solution. A 1-L subsample was taken for chemical analyses, and another 1-L subsample for the analysis of chlorophyll *a* (hereafter, referred to as chl *a*). These samples were transported in a cool box to the laboratory and kept in a refrigerator prior to the analyses. For zooplankton analysis, 5-L subsamples were filtered through a 50-μm mesh. The zooplankton retained on the net were transferred to 250-mL of chilled formaldehyde-sugar solution.

In the laboratory, pH and conductivity of water samples were determined using a Radiometer ION 85 analyser and a Radiometer CDM 83 conductivity meter. Ammonium was determined according to Solórzano (1969) and orthophosphate according to Murphy & Riley (1962). Total phosphorus and total nitrogen were determined as phosphate and nitrite, respectively, after digestion with peroxodisulphate according to Koroleff (1983) and after reduction of nitrate in a Cd-Cu column according to Wood, Armstrong & Richards (1967).

Samples for chl *a* were first filtered through a 200-μm mesh net to remove larger zooplankton and then through Whatman GF/C filters. The chl *a* on the GF/C-filter was extracted with ethanol and then measured

spectrophotometrically. Phytoplankton samples taken during the middle of the experiment (14 August, day 14) were counted with an inverted microscope at 400× and 200× magnifications after 24-h sedimentation of the 10–50-mL subsample. Counting was continued up to 100 units of the two most abundant taxa from a minimum of 20 fields at both magnifications. The phytoplankton biovolumes were calculated from cell dimensions and geometric shape, according to standard geometric formulae. The phytoplankton taxa were grouped as edible and inedible forms having the greatest axial length dimensions (GALD) of <50 and ≥50 μm, respectively.

Zooplankton enumeration and length measurements were performed with an inverted microscope at 100× and 40× magnifications. At least 100 individuals of each of the most numerous species were counted. The lengths of 30 individuals of the most abundant cladoceran species in each sample were measured. Copepods, identified to genus or species level whenever possible, were separated into adults and five copepodid instars. From all samples, 20–100 individuals per instar of each copepod species were measured for length. The mean length of each instar was used for biomass calculations. The nauplii were separated to suborder level as Calanoida or Cyclopoida. The biomass of crustacean zooplankton was determined using species-specific equations for length and carbon content (A. Lehtovaara & J. Sarvala, unpubl. data; Luokkanen, 1995). The biomass of rotifers was assessed according to Bottrell *et al.* (1976); Latja & Salonen (1978) and J. Sarvala (unpubl. data). The functional group of euplanktonic crustacean grazers consisted of *Daphnia, Bosmina, Ceriodaphnia, Diaphanosoma, Eudiaptomus*, copepod nauplii and the I–III instars of cyclopoid copepods. The clearance rate of euplanktonic cladocerans was calculated using the equation $F = 11.695L^{2.480}$, where F is grazing rate in millilitre per animal per day and L is animal length in millimetre (Knoechel & Holtby, 1986). A grazing rate of 2 mL per animal per day was used for *Eudiaptomus* (excluding nauplii) (Cyr & Pace, 1992).

Samples of epiphytic fauna (macroinvertebrates living on the surface of *Elodea*) and of zoobenthos were taken on days 1, 15 and 28. The epiphytic fauna were collected using a core sampler 32 cm in length and 13 cm in diameter, with openings covered with a 180-μm mesh net (Kornijów, 1998). Three samples, each containing about 30 g WW of *Elodea*, were taken

from each vegetated enclosure; epiphytic animals were not sampled in the no-*Elodea* enclosures. The zoobenthos was sampled with a perspex core 4.0 cm in diameter. The uppermost 10-cm thick sediment layer of each core was sieved through a 350-μm mesh net and transferred to plastic containers. Ten core samples were pooled to comprise a single zoobenthos sample. Three zoobenthos samples were collected from each enclosure on each sampling occasion. Living epiphytic and benthic invertebrates were sorted visually and preserved with formaldehyde (to a final concentration of 4%). The formalin-wet biomass of the fauna was determined to a precision of 0.1 mg. Biomasses of benthic and epiphytic fauna were calculated per 1 m^2 of lake bottom. The biomass of epiphytic fauna was first calculated per 100 g WW *Elodea* and then per 1 m^2 of lake bottom when knowing the biomass of *Elodea* m^{-2}. The animals were identified to taxonomic levels, from family to species, which made it possible to pool them into trophic guilds of herbivores/detrivores and predators. This was accomplished to increase statistical power and to facilitate comparisons among systems.

Roach were sampled three times. On days 4 and 15, three fish were caught from each stocked enclosure, immediately replaced with individuals of similar size, and frozen for diet analysis. On day 29, all roach were caught and preserved for diet analyses. As roach lack a distinct stomach, the content of the anterior third of their gut area was analysed (Rask, 1989), and volume proportions of different food categories were estimated visually under a dissecting microscope. The gut contents were subsequently dried at 60 °C and weighed.

Data analyses

The analysis of zooplankton biomass and clearance rate as well as the concentrations of total nutrients, chl *a*, dissolved oxygen and conductivity were calculated with a maximum likelihood technique recommended for unbalanced designs that contain both fixed and random effects (Goldberg & Scheiner, 1993). In the repeated mixed procedure of the SAS package, version 6.12 (Statistical System Institute Inc., U.S.A.), *Elodea*, fish and time were taken as fixed effect factors, while the enclosure was used as a random effect factor. The class variables were *Elodea*, fish and enclosure. The repeated measure factor was time (six

levels: days 0, 3, 7, 14, 21 and 28). The best-fit matrix for data having heterogeneous variances was given by the ARH (1)-type covariance structure, which declines exponentially with distance (Littell *et al.*, 1996) and this was selected for analysis.

The macroinvertebrate biomass, water pH and concentrations of ammonium and orthophosphate were analysed using the general linear model (GLM) procedure of SAS. The results of the middle (day 14) and last (day 28) sampling date were tested, using the initial day (day 0) as a covariate. The water pH values were tested without the covariate. The fixed and random effects were the same as those in the mixed procedure. To assess the similarity of starting conditions in the enclosures, the initial concentrations of the main nutrients, chl *a* and invertebrate biomasses were compared using the GLM procedure of SAS.

The coefficient of variation (CV) was used to compare the variability of the chl *a* concentration and zooplankton biomass in the various treatments. To distinguish the short-term refuge effect of *Elodea* on zooplankton from predation by roach, the change in biomasses of the various taxa were compared by subtracting the biomass on day 3 from that on day 0. The biomasses of different invertebrate prey communities were calculated as DW m^{-2} assuming that DW is 10% of WW and carbon content is 50% of DW (Latja & Salonen, 1978). The volume biomasses were divided by the water depth (1.2 m) to obtain the corresponding areal values per square metre.

Inter-treatment differences of calculated CV, short-term differences between days 0 and 3 as well as phytoplankton results from day 14 were compared using the GLM univariate analysis of variance of SPSS for Windows (Version 10.0), with fish and *Elodea* as fixed factors. The Shapiro–Wilk test (SPSS) was used to inspect the normality of macroinvertebrate, phytoplankton and time-weighted means data. Logarithmic transformation was used, if necessary, to normalise the distribution of the data.

Results

Plankton communities

The total biomass of euplanktonic, crustacean grazers was significantly reduced by *Elodea* (Fig. 1), especially the biomass of the dominating cladoceran *Daphnia longispina* O.F. Müller, which decreased also with fish

(Fig. 1). The biomass of *Daphnia* was somewhat lower in the *Elodea* treatments than in no-*Elodea* treatments already when the first samples were taken on day 0 (GLM; $F_{2,12} = 3.9$, $P = 0.050$; Fig. 1). No other differences in zooplankton biomasses were found between *Elodea* treatments at the start of the experiment. The fish treatments had negligible effects on the total biomass of euplanktonic grazers because the biomasses of the other dominant taxa (*Bosmina longispina* Leydig, *Ceriodaphnia pulchella* G.O. Sars, *Diaphanosoma brachyurum* Leydig and *Eudiaptomus gracilis* G.O. Sars) were unaffected by fish presence/absence. *Elodea* also did not affect the biomasses of these taxa (mixed; $P > 0.05$), or the clearance rate of euplanktonic crustacean grazers (Fig. 1). The CV of euplanktonic crustacean grazer biomass varied widely between the replicates especially in the no-*Elodea* treatments, and did not differ between the treatments (GLM; $P > 0.1$).

Short-term differences (between days 0 and 3) between the fish treatments were observed only in the biomass of *Daphnia*. The strongest reduction in *Daphnia* biomass by fish was found in the no-*Elodea* treatments but the main effect of fish was, nevertheless, not significant (GLM; $F_{1,12} = 3.9$, $P = 0.073$). On the contrary, in the fish-free treatments the biomass of *Daphnia* decreased with the increase in *Elodea* density, resulting in a significant interaction of *Elodea* and fish (GLM; $F_{2,12} = 4.3$, $P = 0.040$).

Plant-associated and benthic cladocerans (predominantly *Acroperus harpae* Baird, *Peracantha truncata* O.F. Müller, *Eurycercus lamellatus* O.F. Müller and *Alonella exigua* Lilljeborg) were positively affected by both *Elodea* and fish (mixed; $F_{2,12} = 5.0$, $P = 0.027$ and $F_{1,12} = 9.8$, $P = 0.009$, respectively). The biomasses of these cladocerans increased strongly during the experiment, and there was a significant interaction of time and *Elodea* (mixed; $F_{2,83} = 12.6$, $P < 0.001$). Suspension-feeding rotifer densities were usually <50 individuals L^{-1}, corresponding to a biomass of <20 μg C L^{-1}. Although they increased (maximum 450 individuals L^{-1}) at the end of the experiment, no consistent pattern with respect to fish or *Elodea* was apparent.

The density-weighted mean length of euplanktonic cladocerans was significantly reduced by fish (mixed; $F_{1,12} = 11.2$, $P = 0.006$). Cladoceran length varied between 0.6 and 0.8 mm at the beginning of the experiment but declined to 0.5–0.7 mm in the fish-free treatments and to about 0.4 mm in the fish treatments
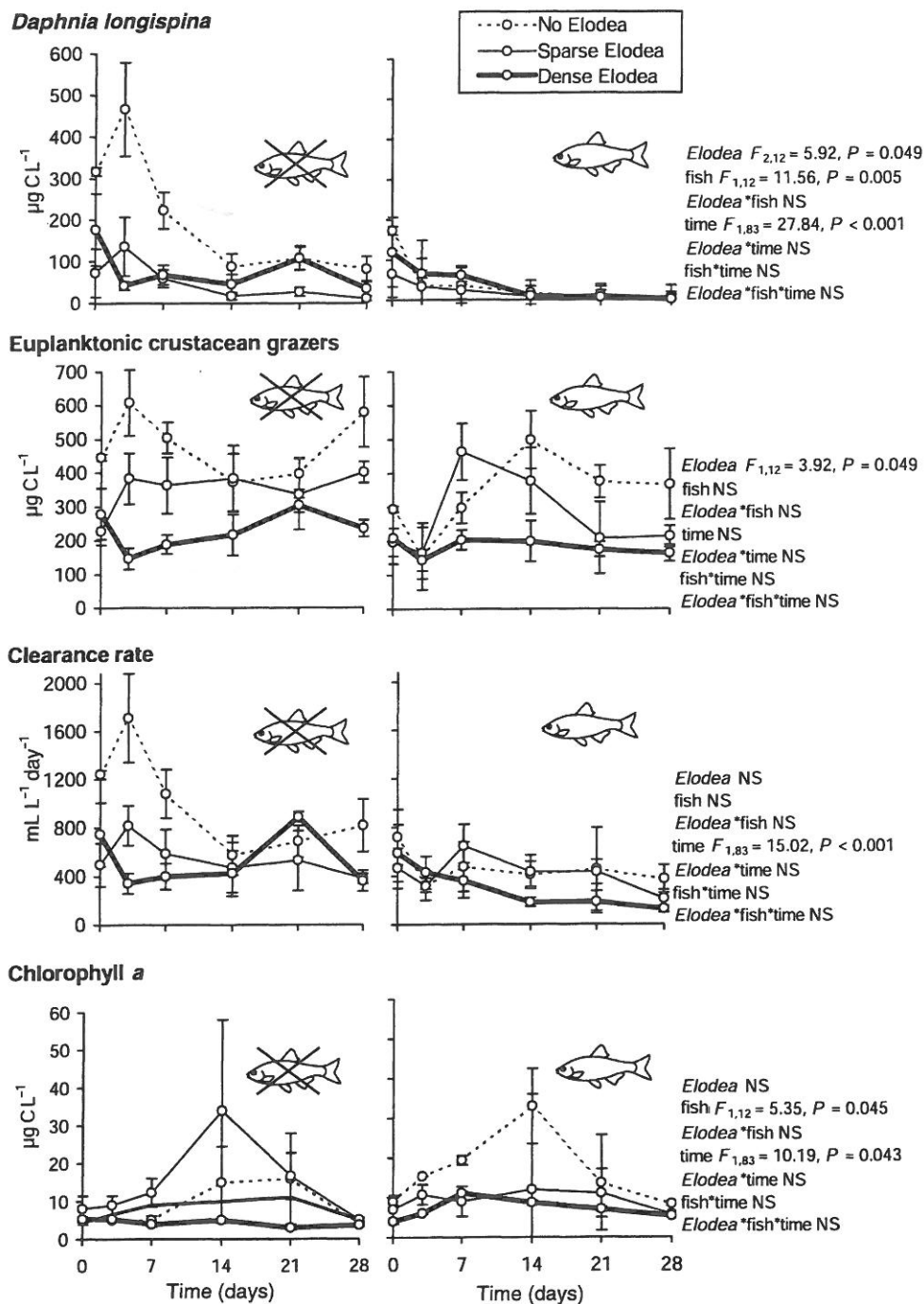
Daphnia longispina

Legend:
- ···o··· No Elodea
- —o— Sparse Elodea
- ●— Dense Elodea

$Elodea$ $F_{2,12}$ = 5.92, P = 0.049
fish $F_{1,12}$ = 11.56, P = 0.005
$Elodea$*fish NS
time $F_{1,83}$ = 27.84, P < 0.001
$Elodea$*time NS
fish*time NS
$Elodea$*fish*time NS

Euplanktonic crustacean grazers

$Elodea$ $F_{1,12}$ = 3.92, P = 0.049
fish NS
$Elodea$*fish NS
time NS
$Elodea$*time NS
fish*time NS
$Elodea$*fish*time NS

Clearance rate

$Elodea$ NS
fish NS
$Elodea$*fish NS
time $F_{1,83}$ = 15.02, P < 0.001
$Elodea$*time NS
fish*time NS
$Elodea$*fish*time NS

Chlorophyll a

$Elodea$ NS
fish $F_{1,12}$ = 5.35, P = 0.045
$Elodea$*fish NS
time $F_{1,83}$ = 10.19, P = 0.043
$Elodea$*time NS
fish*time NS
$Elodea$*fish*time NS

Time (days)   Time (days)

Fig. 1 Treatment mean values (±1 SE) over the course of the experiment for biomasses and the clearance rate of *Daphnia longispina* and other euplanktonic crustacean grazers, as well as concentration of chl *a* in the *Elodea* enclosures with or without fish. The F ratios with significance levels for the main effects and interactions of *Elodea*, fish and time obtained in the mixed model are given for each variable. The grey line in the no-fish treatment of chl *a* concentration shows the mean in the sparse-*Elodea* treatment without the *Anabaena* replicate (see text for further explanation).

Table 1 Physical and chemical properties of water (mean ± SE) in all enclosures, calculated from the data collected from all enclosures during the experiment

Variable	Overall mean ± SE	Treatment effects	Statistically different treatment mean ± SE
Water temperature (°C)	20.4 ± 1.8*	NS	
Oxygen near bottom (mg L^{-1})	6.9 ± 0.1	Elodea ($F_{2,12} = 6.41$, $P = 0.014$)	6.3 ± 0 (no-Elodea)
			6.9 ± 0 (sparse-Elodea)
			7.6 ± 0 (dense-Elodea)
pH	7.4 ± 0	NS	
Total phosphorus (µg L^{-1})	65 ± 28	Time ($F_{1,83} = 56.87$, $P < 0.001$)	74 ± 3 (day 0)
			40 ± 2 (day 28)
Orthophosphate (µg L^{-1})	22.9 ± 2.7	Elodea (on Day 28) ($F_{2,12} = 6.41$, $P = 0.014$)	6.5 ± 1.5 (no-Elodea)
			15.2 ± 2.9 (sparse-Elodea)
			5.0 ± 0 (dense-Elodea)
Total nitrogen (µg L^{-1})	790 ± 17	NS	
Ammonium (µg L^{-1})	21.3 ± 2.7	NS	
Conductivity (µS cm^{-1})	11.9 ± 0.1	NS	

Also shown are the treatment effects and, in case of statistical difference, the treatment mean values (±SE). NS refers to non-significant statistical result.
*Water temperature declined steadily from the initial 23 to <15 °C by the end of the experiment.

by the end of the experiment. Thus, there was a significant interaction of fish and time on the size of cladocerans (mixed; $F_{1,83} = 36.7$, $P < 0.001$). The opposite trend was observed for plant-associated cladocerans, as their mean length was positively related to the fish treatments (mixed; $F_{1,12} = 6.5$, $P = 0.026$).

The physical and chemical properties of the water were, in general, similar in all treatments. The bottom waters were always well oxygenated, even at night. Judging by shoot density and appearance, the planted *Elodea* managed well in the enclosures. However, their total biomass change could not been quantified because of efficient colonisation of plant roots into sediment. The daytime dissolved oxygen concentration increased with the rising density of *Elodea* (Table 1). At the end of the experiment (day 28), the phosphate concentrations were lowest in the dense-*Elodea* treatment, while no other differences in the concentrations of nutrients were observed between the *Elodea* and fish treatments (Table 1). Consequently, the chl *a* concentrations did not differ between *Elodea* treatments (Fig. 1), but remained below 15 µg L^{-1} in all dense-*Elodea* treatments, while in the other treatments two to fivefold concentrations were measured (Fig. 1). At the end of the experiment, the concentrations were similar (3–6 µg chl *a* L^{-1}) in all treatments. However, significantly higher chl *a* concentrations were measured in the fish enclosures than in the fish-free enclosures (Fig. 1), while the opposite trend was observed for the ratio of chl *a* to total phosphorus

concentration (mixed; $F_{1,12} = 5.0$, $P = 0.045$). In the middle of the experiment (day 14), small edible algae (GALD <50 µm) were slightly more abundant in treatments with fish than without fish (GLM, $F_{1,12} = 3.7$, $P = 0.077$). Positive effects of fish were found for the biovolumes of cryptophytes (*Cryptomonas* spp. and *Rhodomonas* spp; GLM, $F_{1,12} = 14.2$, $P = 0.003$), chrysophytes (mainly *Chrysamoeba* spp.; GLM, $F_{1,12} = 5.7$, $P = 0.034$) and chlorophytes (*Dictyosphaerium* spp. and *Chlorococcales* spp.; GLM, $F_{1,12} = 8.2$, $P = 0.014$). These algal groups, together with euglenophytes (mainly *Trachelomonas* spp.), dominated the phytoplankton community. The chl *a* concentration varied markedly over time but was less in the dense-*Elodea* treatments than in the no- and sparse-*Elodea* treatments (Fig. 1). The CV of chl *a* was lower in the dense-*Elodea* treatments than in the other *Elodea* treatments, but differences between treatments were not statistically significant because of high variation between the replicates. Much of the observed variation was because of visible blooms of cyanobacteria (*Anabaena lemmermannii* P. Richter) in the middle of the experiment (days 14 and 21) in four no-*Elodea* or sparse-*Elodea* treatments, both with and without fish. In these enclosures, *A. lemmermannii* constituted 55–97% of the phytoplankton biovolume on day 14. Exceptionally high biomass (up to 80 µg chl *a* L^{-1}) occurred in one sparse-*Elodea*, fish-free replicate. After excluding this replicate from the data, the mean chl *a* concentration was much lower than it was for all three replicates (Fig. 1).

Predatory zooplankton were, in general, favoured by the presence of fish. The biomass of the cladoceran *Polyphemus pediculus* L. was positively related to fish in all *Elodea* treatments (mixed; $F_{1,12} = 6.7$, $P = 0.024$). The biomass of cyclopoid copepods (predominantly *Thermocyclops oithonoides* G.O. Sars and *Mesocyclops leuckarti* Claus) was negatively affected by *Elodea* (mixed; $F_{2,12} = 4.0$, $P = 0.046$). The highest cyclopoid biomasses (>200 μg C L^{-1}) were observed in the no-*Elodea* treatments with fish, resulting in the significant interaction of *Elodea* and fish (mixed; $F_{2,12} = 5.7$, $P = 0.018$).

Epiphytic and benthic invertebrate communities

The mean biomass of all epiphytic animals (120 mg DW m^{-2}) was about one-fourth of the total zoobenthos biomass (510 mg WW m^{-2}). Chironomid larvae and gastropods predominated in the biomass of both epiphytic and benthic macroinvertebrates and, on average, constituted about 16 and 19% of the total epiphytic fauna biomass and 34 and 65% of the total benthic biomass, respectively. Other invertebrates, mainly *Asellus aquaticus* L. and hirudineans, were recorded occasionally, resulting in relatively wide spatial and temporal variations within the total biomass of the two animal communities. In the middle of the experiment, *Elodea* positively affected the total biomass of epiphytic fauna (GLM; $F_{1,12} = 11.6$, $P = 0.011$) and negatively that of the benthic fauna (GLM; $F_{2,12} = 7.3$, $P = 0.010$). A positive effect of *Elodea* was observed on the biomasses of epiphytic predators and herbivores/detrivores (GLM; $F_{1,12} = 8.1$, $P = 0.025$ and $F_{1,12} = 27.8$, $P = 0.001$, respectively) as well as on benthic herbivores/detrivores (GLM; $F_{2,12} = 4.8$, $P = 0.036$). At the end of the experiment, the biomasses of epiphytic chironomids and gastropods were even slightly higher (GLM; $F_{1,12} = 5.1$, $P = 0.058$ and $F_{1,12} = 5.3$, $P = 0.056$, respectively) in the presence than in the absence of fish, especially in the dense-*Elodea* treatments. The influence of fish on the biomasses of the most often consumed trichopteran and ephemeropteran larvae (see next section) was not significant. At the end of the experiment, only slight differences in zoobenthos biomass were found between the treatments. In the dense-*Elodea* treatments, the benthic predator biomass was higher in the fish-free enclosures than it was in enclosures with fish, resulting in a significant interaction of *Elodea* and fish (GLM; $F_{1,12} = 9.0$, $P = 0.005$).

Relative importance of invertebrate communities as food sources for roach

The food preference and switching behaviour of roach were examined assuming a density-dependent functional response. The potential availabilities of microcrustacean and macroinvertebrate prey were compared by calculating their total biomasses per square metre of lake bottom. In all *Elodea* treatments with fish, the biomass of euplanktonic cladocerans (mixed; $F_{1,84} = 18.5$, $P < 0.001$) decreased during the experiment, while that of copepods (mixed; $F_{1,84} = 9.8$, $P = 0.002$) and macroinvertebrates increased during the experiment (Figs 2 & 3). Yet, the total biomass of all invertebrates did not markedly differ between the fish and fish-free treatments (GLM; $P > 0.05$). The weight of non-empty roach gut contents (0.04–0.06 g DW individuals^{-1}) did not differ between the treatments or between sampling days (GLM; $P > 0.2$). On the contrary, the food spectrum of roach was highly variable (Fig. 2). Roach fed almost exclusively on euplanktonic cladocerans (predominantly *Bosmina* and *Ceriodaphnia*) at the start of the experiment only in the no-*Elodea* treatments (Fig. 2). Later during the experiment and in the other treatments, some roach individuals seemed to specialise on cladocerans (share in guts near 100%), while others fed on cladocerans to a minor extent or not at all (values near 0%) (Fig. 3). The mean proportion of euplanktonic cladocerans in the roach gut content exceeded considerably that in the ambient environment in the no-*Elodea* treatments at the start of the experiment and in the dense-*Elodea* treatments at the end of the experiment suggesting high electivity (Fig. 2). No identifiable remains of *Daphnia* were found in fish guts. Therefore, the actual electivity for cladocerans was probably even higher than that observed in our analyses. In the middle of the experiment, roach also favoured plant-associated and benthic cladocerans (mainly chydorids) (Fig. 2).

Macroinvertebrates constituted a minor fraction of the food items found in the roach gut, despite their relatively high ambient biomasses (Fig. 2). Only a few roach included macroinvertebrates (mainly ephemeropteran and trichopteran larvae, seldom chironomid larvae) in their diet in spite of high
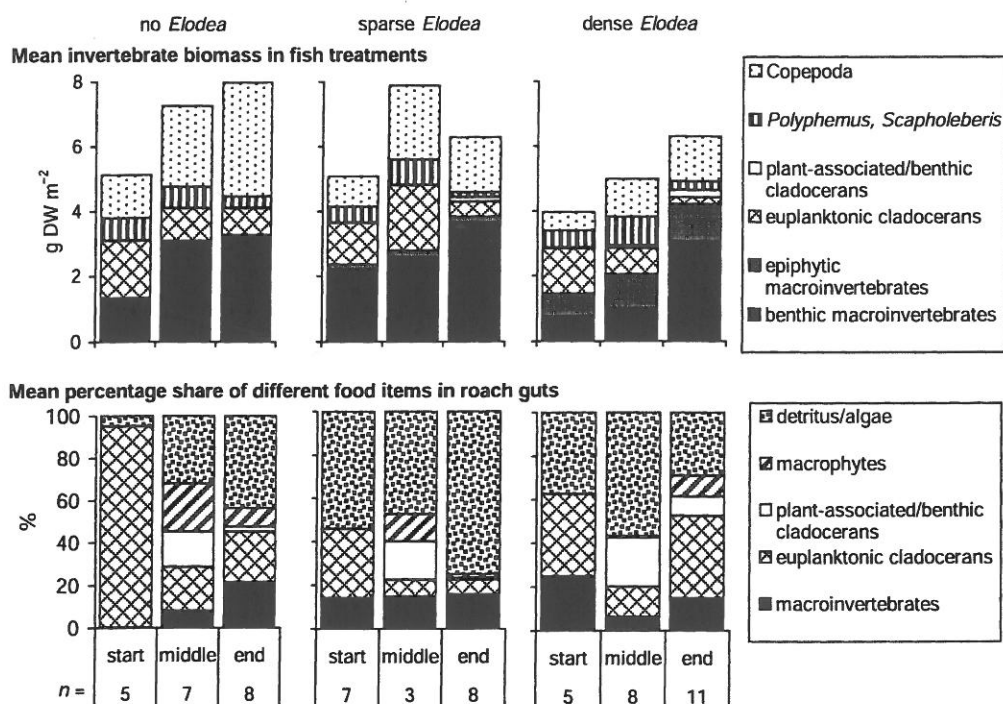
Fig. 2 Mean biomasses of animal prey communities in the *Elodea* enclosures with fish (upper) and mean percentage shares by weight of different food items found in fish guts (lower) at the start (day 0), middle (day 15) and end (day 28) of the experiment. The number of studied non-empty roach guts (*n*) is shown below the *x*-axis.

biomass of these prey (Fig. 3). The diet of roach also included non-animal food items (macrophytes, detritus and algae). The macrophytic food consisted of freely floating *L. trisulca* in the no-*Elodea* treatments, while in the other treatments it included both *Lemna* and *Elodea*. Copepods were not eaten by roach, although their proportion (predominantly *E. gracilis*) could exceed more than half of the total invertebrate biomass (Fig. 2).

Discussion

Effects of Elodea on plankton communities and trophic cascade

The total biomass of euplanktonic zooplankton, especially that of the dominant cladoceran *D. longispina*, decreased with the increase in *Elodea* density. The biomass of *Daphnia* was also reduced by roach. Thus, *Elodea* provided neither a favourable habitat nor a good refuge for *D. longispina* against predation by roach. Burks, Jeppesen & Lodge (2000) demonstrated that the growth of *Daphnia magna* Straus was strongly

suppressed when exposed to chemicals from both *E. canadensis* and roach. This phenomenon might have been pronounced in our enclosed systems, which did not allow large-scale (>2.5 m) horizontal migration of daphnids (reviewed by Burks *et al.*, 2002). Thus, the decline of *Daphnia* in our dense-*Elodea* fish treatments may partly have been affected by this chemical suppression. However, the density of macroinvertebrate predators often increases with structural complexity of the habitat (Kornijów & Kairesalo, 1994). In our experiment, the biomass of macroinvertebrate predators increased in the *Elodea* treatments. Thus, predation by macroinvertebrates, especially of water mites (Hydracarina) (Kornijów *et al.*, 2001), may also have lowered zooplankton biomasses in the *Elodea* treatments.

During the first 3 days of the experiment, fish reduced the *Daphnia* biomass more in the no-*Elodea* enclosures than in the *Elodea* enclosures. However, the strong negative impact of *Elodea* on *Daphnia* complicated estimation of the refuge effect. Thus, we found no clear evidence for even a short-term refuge effect of *Elodea* for *Daphnia* against predation by roach. The
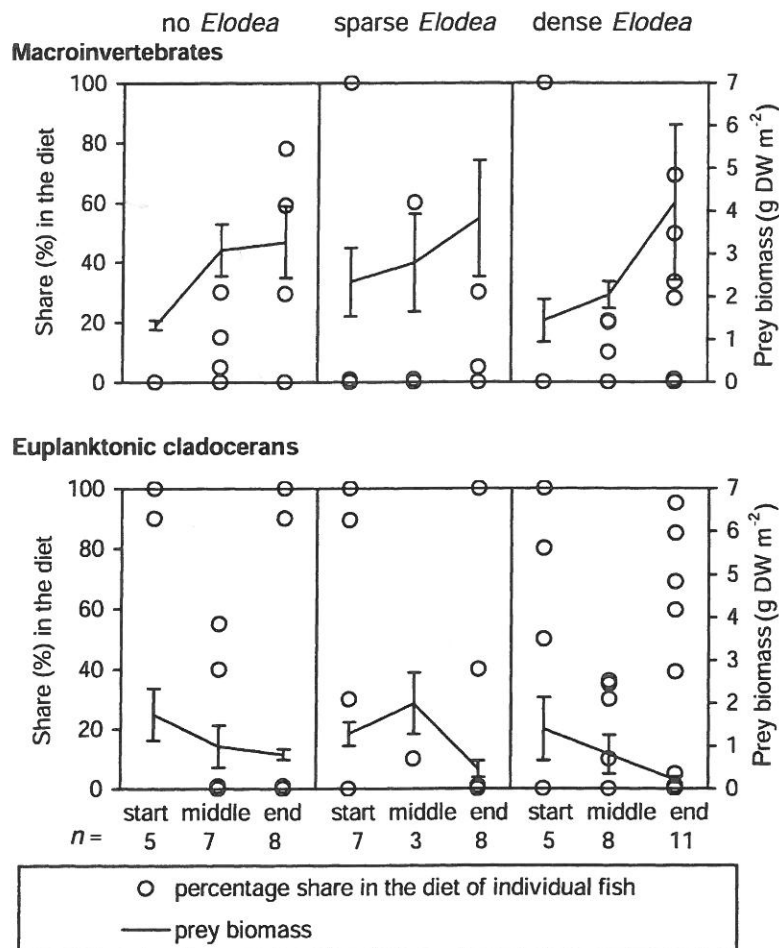
no *Elodea*   sparse *Elodea*   dense *Elodea*

Macroinvertebrates

Euplanktonic cladocerans

○ percentage share in the diet of individual fish

— prey biomass

Fig. 3 Treatment mean values (±1 SE) for biomasses of euplanktonic cladocerans and macroinvertebrates (lines) and individual preferences of roach for these prey groups (circles) in the *Elodea* treatments at the start (day 0), middle (day 15) and end (day 28) of the experiment. The number of studied non-empty roach guts (*n*) is shown below the *x*-axis.

ineffectiveness of the refuge by *Elodea* was evidenced by the equally decreasing body size of euplanktonic cladocerans in all *Elodea* treatments with fish. Many roach individuals were specialised to feed on cladocerans and continued to pursue zooplanktonic food even as it declined in abundance. This suggests that roach were capable of feeding on scarce, small-bodied cladocerans in *Elodea* beds. Among many factors affecting predator–prey dynamics, such high preference is not likely to be a stabilising effect (Murdoch & Bence, 1987; cf. Schindler & Scheuerell, 2002). The lack of stabilising effect in *Elodea* was also suggested by the similar CV of zooplankton biomass in all the fish treatments. However, the strong search image of the large roach (≥15 cm) roach for *Bosmina* is in agreement with earlier results from Lake Vesijärvi

(Horppila, 1994; Horppila *et al.*, 2000). In addition, roach also pursued the less abundant, plant-associated and benthic chydorid cladocerans. Perhaps, when foraging for these prey items, roach passively engulfed large quantities of detrital/plant material. Feeding on non-animal, low nutritional food items was clearly also an active process. An earlier diet analysis of roach food preferences in Lake Vesijärvi demonstrated that the frequent use of plant food generally indicated a low availability of animal prey (Horppila, 1994). The capability for using alternative, less-preferred food sources may serve as life-supporting means for roach populations to manage over the periods of low cladoceran abundance and thus maintain high predation pressure on zooplankton (cf. Jeppesen *et al.*, 1998a).

Macrophyte species differ in their value as refuges for zooplankton. For instance, *N. lutea* or *Potamogeton berchtoldii* Fiber were shown to provide refuge for *Daphnia hyalina* Leydig against predation by perch (Beklioglu & Moss, 1996; Moss *et al.*, 1998). Perch, moreover, is better adapted to foraging among macrophytes than roach (Winfield, 1986; Diehl, 1988). In contrast, Venugopal & Winfield (1993) showed that dense *N. lutea* stand does not have the potential to offer a refuge for zooplankton against juvenile fish. Schriver *et al.* (1995) observed a lowered impact of small (0+ and 1+) roach and three-spined stickleback (*Gasterosteus aculeatus* L.) predation on zooplankton among dense (53 g DW m^{-2}) macrophyte beds comprising of mainly *Potamogeton pectinatus* L, *P. pusillus* L. and *Callitriche hermaphroditica* L. The macrophyte biomass in our dense-*Elodea* treatment was at least as high. On the contrary, the density of fish used in our study (1.9 individuals m^{-2}) was close to the threshold density (2–5 individuals m^{-2}) at which the refuge effect of macrophytes may be partially or totally lost for *Daphnia* and small cladocerans, such as *Bosmina* and *Ceriodaphnia* (Jeppesen *et al.*, 1998a). Such threshold fish density is probably shaped by the fish species and size/age-class. For instance, *Elodea* was shown to have a refuge effect on *Bosmina* against 0+ perch at a density of three fish per square metre (Kairesalo *et al.*, 1998). In our experiment, *Bosmina* and *Ceriodaphnia* were preferred food of roach but, nevertheless, attained relatively high biomasses in the *Elodea* treatments with fish. This difference was not significant because of high variation between replicates, especially in the sparse-*Elodea* treatments. In any case, *Elodea* appeared to be of value as a refuge for plant-associated cladocerans, as these were even more abundant and larger in the presence than in the absence of fish.

Zooplankton grazing in vegetation plays a central role in controlling phytoplankton (Schriver *et al.*, 1995; Jeppesen *et al.*, 1998a, 1999). However, Meijer *et al.* (1999) and Blindow *et al.* (2000) suggested that direct interactions between macrophytes and planktonic algae, rather than mere grazing of algae by zooplankton, are probably the decisive factors in controlling algal densities in lakes with dense submerged vegetation. Although the planted *Elodea* managed well in our enclosures, they did not clearly affect the concentrations of total nutrients (dissolved and <200 μm particulate fractions) and chl *a* in the water. Thus, our results indicated that submerged plants do not reduce the availability of nutrients to planktonic algae, consistent with the results of Schriver *et al.* (1995) and Beklioglu & Moss (1996). Nevertheless, cyanobacterial blooms developed only in some replicates of the no-*Elodea* or sparse-*Elodea* treatments, but never in the dense-*Elodea* treatments. In addition, the dense-*Elodea* treatments always showed the lowest chl *a* concentrations, suggesting that phytoplankton production was constrained by *Elodea*. On the contrary, in the dense-*Elodea* treatments where the biomass of grazing cladocerans, especially large-bodied *Daphnia*, was reduced by roach, approximately twofold higher phytoplankton biomasses and lower clearance rates were observed in comparison to the corresponding fish-free enclosures. Perrow *et al.* (1999) suggested a threshold community filtration (clearance) rate of 300–400 mL per day to maintain the clear-water phase. In our experiment these high rates were observed in all treatments except the dense-*Elodea* treatment with fish. A total of 60–130% of the phytoplankton biomass was grazed per day in the *Elodea* treatments, assuming that cladocerans and copepods fed exclusively on phytoplankton in amounts corresponding to 100 and 50% of their biomass per day, respectively (Jeppesen *et al.*, 1999; Blindow *et al.*, 2000). This value is higher than that (<10%) estimated by Blindow *et al.* (2000) but lower than that (137–298%) estimated by Jeppesen *et al.* (1999) in Danish, Swedish and Latvian clear-water lakes with abundant macrophytes. In our experiment, those zooplankton grazers unaffected by *Elodea*, such as *Eudiaptomus*, *Ceriodaphnia*, *Diaphanosoma* and *Bosmina*, were apparently sufficient to control algal biomass within the *Elodea* beds. This top-down control of phytoplankton biomass was facilitated by the macrophytes and operated simultaneously with the control of phytoplankton production by *Elodea*. Thus, these results support earlier conclusions by Schriver *et al.* (1995) and Scheffer (1999) dealing with the synergistic effects on phytoplankton of both zooplankton and macrophytes.

The chl *a* concentration was two to three times higher in the no-*Elodea* treatments with fish than in the corresponding sparse- and dense-*Elodea* treatments, but there was no interaction of *Elodea* and fish with chl *a*. Fish had a negligible effect on the total biomass and clearance rate of euplanktonic grazers. Nevertheless, the chl *a* concentration and the biovolume of edible algae were higher in all the *Elodea* treatments with fish

than without fish. Although no differences in dissolved nutrient concentrations between the treatments could be measured, our results are consistent with enhancement of phytoplankton productivity through nutrient regeneration by roach (cf. Horppila & Kairesalo, 1992; Vanni, 2002). Thus, our first hypothesis that *Elodea* can hamper the trophic cascade impact of fish via lowered predation on zooplankton was not unequivocally supported; the enhancement of algal biomass was probably due not only to lowered grazing by zooplankton but also to nutrient regeneration by fish.

Macroinvertebrate communities as alternative prey for roach

Switching of prey preference occurs when different food sources are present in different microhabitats, and their abundance and availability to the predator vary. Lake littoral zone invertebrate communities include those of open water, macrophyte surfaces and bottom sediments. In principle, generalist predators such as roach focus their foraging on the most profitable prey and modify their feeding behaviour in relation to the relative and absolute abundance of prey (Townsend *et al.*, 1986; Gliwicz, 2002). At the population level, switching of prey preference is because of a changing proportion of specialists rather than to a gradual change in preferences of individual roach and this yields a bimodal distribution of percentage composition of the diet (Townsend *et al.*, 1986). During our experiment, roach faced a situation in which the relative proportion of the preferred cladocerans was diminishing (because of predation and/or negative influence by *Elodea*) but that of macroinvertebrates was increasing. Horppila & Kairesalo (1992) showed experimentally that in unvegetated environments the decreasing availability of planktonic food drives roach to forage for benthic food items. In our experiment, however, this switching did not become apparent as foraging for more abundant and much larger epiphytic/benthic macroinvertebrates did not increase. In addition, large-bodied *Eudiaptomus* (up to 1.6 mm in length), which contributed highly to the invertebrate biomass (up to 50%), were not preyed on by roach. Although roach have been shown to be capable of preying on copepods (Persson & Greenberg, 1990) these are elusive and not consistently included in the diet of

roach (Winfield *et al.*, 1983). The same probably applies to the large cladoceran *P. pediculus* (Moss *et al.*, 1998). Therefore, prey animals with an active escape reaction seem to be underexploited food sources for roach. In addition, Dodson & Frey (1991) hypothesised that dark pigmentation may be distasteful to predators which may also explain why roach did not feed on *Polyphemus* or on *Scapholeberis mucronata* O.F. Müller, the other pigmented cladoceran that were also relatively abundant (10–40 µg C L^{-1}) in our enclosures.

Our results are in agreement with those of Persson & Greenberg (1990), who showed that the overall foraging of roach for macroinvertebrates is low and that the unavailability of preferred food induces roach to increase consumption of less nutritious detritus/plant food. Utilisation of such low-energy food may, however, increase phosphorus regeneration by fish (Brabrand, Faafeng & Nilssen, 1990). Hence, roach apparently chose food items that required minimal effort to detect and capture (i.e. slowly moving cladocerans and non-animal items). In our experiment, the feeding of roach on zoobenthos and epiphytic macroinvertebrates decreased predation pressure on grazing cladocerans less than expected, which was inconsistent with our second hypothesis. On the contrary, in perch-dominated systems, benthic and epiphytic macroinvertebrates may play a more important role as alternative prey instead of cladoceran zooplankton, as perch are superior to roach in foraging for macroinvertebrates in vegetation (Persson, 1987; Diehl, 1988).

In conclusion, our results showed that the top-down control of phytoplankton by zooplankton grazers was still important in vegetation although *Elodea* beds provided only poor refuges for euplanktonic cladocerans and negligible refuges and unfavourable habitats for *Daphnia*. Although roach were able to capture cladocerans in *Elodea* beds, even when the abundance and size of these prey animals were low, the fish had negligible effects on the total biomass and the clearance rate of euplanktonic crustacean grazers. Thus, our first hypothesis that *Elodea* can hamper the trophic cascade of fish via lowered predation on grazing zooplankton was not supported. In addition, the chl *a* concentration significantly increased with fish, suggesting that fish enhanced algal growth through regeneration of nutrients. On the contrary, no cyanobacterial blooms appeared and a clear-water

state with low chl *a* concentration was maintained in the dense-*Elodea* treatments, suggesting that *Elodea* inhibited algal growth. Regardless of the density of the *Elodea* beds, macroinvertebrates were quantitatively unimportant in roach diets. Therefore, our second hypothesis that switching of food preference by roach stabilises littoral plankton dynamics was not supported either.

Acknowledgments

References

Beklioglu M. & Moss B. (1996) Mesocosm experiments on the interaction of sediment influence, fish predation and aquatic plants with the structure of phytoplankton and zooplankton communities. *Freshwater Biology*, **36**, 315–325.

Blindow I., Hargeby A., Wagner B.M.A. & Andersson G. (2000) How important is the crustacean plankton for the maintenance of water clarity in shallow lakes with abundant submerged vegetation? *Freshwater Biology*, **44**, 185–197.

Bottrell H.H, Duncan A., Gliwicz Z.M., Grygierek E., Herzig A., Hillbricht-Ilkowska A., Kurasawa H., Larsson P. & Węgleńska T. (1976) A review of some problems in zooplankton production studies. *Norwegian Journal of Zoology*, **24**, 419–456.

Brabrand A., Faafeng B.A. & Nilssen J.P.M. (1990) Relative importance of phosphorus supply to phytoplankton production: fish excretion versus external loading. *Canadian Journal of Fisheries and Aquatic Sciences*, **47**, 364–372.

Burks R.L., Jeppesen E. & Lodge D. (2000) Macrophyte and fish chemicals suppress *Daphnia* growth and alter life history traits. *Oikos*, **88**, 139–147.

Burks R. L., Lodge D.M., Jeppesen E. & Lauridsen T. L. (2002) Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. *Freshwater Biology*, **47**, 343–365.

Carpenter S.R., Kitchell J.F. & Hodgson J.R. (1985) Cascading trophic interactions and lake productivity. *Bioscience*, **35**, 634–639.

Cyr H. & Pace M.L. (1992) Grazing by zooplankton and its relationship to community structure. *Canadian Journal of Fisheries and Aquatic Sciences*, **49**, 1455–1465.

Diehl S. (1988) Foraging efficiency of three freshwater fishes: effects of structural complexity and light. *Oikos*, **53**, 207–214.

Diehl S. (1993) Effects of habitat structure on resource availability, diet and growth of benthivorous perch, *Perca fluviatilis. Oikos*, **67**, 403–414.

Dodson S.I. & Frey D.G. (1991) Cladocera and other Branchiopoda. In: *Ecology and Classification of North American Freshwater Invertebrates*. (Eds J.H. Thorp & A.P. Covich), pp. 723–786. Academic Press, San Diego.

Gliwicz Z.M. (2002) On the different nature of top-down and bottom-up effects in pelagic food webs. *Freshwater Biology*, **47**, 2296–2312.

Goldberg D.E. & Scheiner S.M. (1993) ANOVA and ANCOVA: field competition experiments. In: *Design and Analysis of Ecological Experiments* (Eds S.M. Scheiner & J. Gurewitch), pp. 69–93. Chapman & Hall, ????.

Horppila J. (1994) The diet and growth of roach (*Rutilus rutilus* (L.)) in Lake Vesijärvi and possible changes in the course of biomanipulation. *Hydrobiologia*, **294**, 35–41.

Horppila J. & Kairesalo T. (1992) Impact of bleak (Alburnus alburnus) and roach (Rutilus rutilus) on the quality, sedimentation and internal nutrient loading. *Hydrobiologia*, **243/244**, 323–331.

Horppila J., Ruuhijärvi J., Rask M., Karppinen C., Nyberg K. & Olin M. (2000) Seasonal changes in the diets and relative abundances of perch and roach in the littoral and pelagic zones of a large lake. *Journal of Fish Biology*, **56**, 51–72.

Jeppesen E., Lauridsen T.L., Kairesalo T. & Perrow M. (1998a) Impact of submerged macrophytes on fish–zooplankton interactions in lakes. In: *The Structuring Role of Submerged Macrophytes in Lakes*. (Eds E. Jeppesen, Ma. Søndergaard, Mo. Søndergaard & K. Christoffersen), pp. 91–114. Springer-Verlag, New York.

Jeppesen E., Søndergaard Ma., Søndergaard Mo. & Christoffersen K. (1998b) *The Structuring Role of Submerged Macrophytes in Lakes*. Springer-Verlag, New York, Tokyo.

Jeppesen E., Jensen J.P., Søndergaard Ma. & Lauridsen T. (1999) Trophic dynamics in turbid and clearwater lakes with special emphasis on the role of zooplankton for water quality. *Hydrobiologia*, **408/409**, 217–231.

Kairesalo T., Tátrai I. & Luokkanen E. (1998) Impacts of water weed (*Elodea canadensis* Michx) on plankton-fish interactions in the lake littoral. *Verhandlungen der*

Internationalen Vereinigung für Theoretische und Angewandte Limnologie, **26**, 1846–1851.

Kairesalo T., Laine S., Luokkanen E., Malinen T. & Keto J. (1999) Direct and indirect mechanisms behind successful biomanipulation. *Hydrobiologia*, **395/396**, 99–106.

Keto J. & Sammalkorpi I. (1988) A fading recovery: a conceptual model for Lake Vesijärvi management and fishing. *Aqua Fennica*, **18**, 193–204.

Knoechel R. & Holtby L.B. (1986) Construction and validation of a body-length-based model for the prediction of cladoceran community filtering rates. *Limnology and Oceanography*, **31**, 1–16.

Kornijów R. (1998) Quantitative sampler for collecting invertebrates associated with submersed and floating-leaved macrophytes. *Aquatic Ecology*, **32**, 241–244.

Kornijów R. & Kairesalo T. (1994) *Elodea canadensis* sustains rich environment for macroinvertebrates. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, **25**, 2270–2275.

Kornijów R, Stryjecki R., Kuoppamäki K., Horppila J., Luokkanen E. & Kairesalo T. (2001) Do water mites benefit from benthivorous fish in vegetated lake areas? *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, **27**, 3291–3294.

Koroleff F. (1983) Simultaneous oxidation of nitrogen and phosphorus compounds by persulfate. In: *Methods of Seawater Analysis*, 2nd edn. (Eds K. Grasshoff, M. Eberhardt & K. Kremling), pp. 168–169. Verlag Chemie Weinheim, Florida, Basel.

Latja R. & Salonen K. (1978) Carbon analysis for the determination of individual biomasses of planktonic animals. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, **20**, 2556–2560.

Littell R.C., Milliken G.A., Stroup W.W. & Wolfinger R.D. (1996) *SAS System for Mixed Models*. SAS Institute Inc., Cary, NC.

Luokkanen E. (1995) *The Species Composition, Biomass and Production of the Pelagic Cladoceran Community in the Enonselkä basin of Lake Vesijärvi*. University of Helsinki, Helsinki. Research and Education Center of Lahti, Report No. 25 (in Finnish with English summary).

Meijer M-L., de Boois I., Scheffer M., Portielje R. & Hosper H. (1999) Biomanipulation in shallow lakes in The Netherlands: an evaluation of 18 case studies. *Hydrobiologia*, **408/409**, 13–30.

Moss B., Kornijów R. & Measey G.J. (1998) The effect of nymphaeid (*Nuphar lutea*) density and predation by perch (*Perca fluviatilis*) on the zooplankton communities in a shallow lake. *Freshwater Biology*, **39**, 689–697.

Murdoch W.W. & Bence J. (1987) General predators and unstable prey populations. In: *Predation. Direct and Indirect Impacts on Aquatic Communities*. (Eds W.C. [8] Kerfoot & A. Sih) pp. 17–30. University Press of New England, ???.

Murphy J. & Riley J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31–36.

Pace M.L., Cole J.J., Carpenter S.R. & Kitchell J.F. (1999) Trophic cascades revealed in diverse ecosystems. *Trends in Ecology and Evolution*, **14**, 483–488.

Perrow M.R., Jowitt A.J.D., Stansfield J.H. & Phillips G.L. (1999) The practical importance of the interactions between fish, zooplankton and macrophytes in shallow lake restoration. *Hydrobiologia*, **395/396**, 199–210.

Persson L. (1983) Food consumption and the significance of detritus and algae to intraspecific competition in roach *Rutilus rutilus* in a shallow eutrophic lake. *Oikos*, **41**, 118–125.

Persson L. (1987) Effects of habitat and season on competitive interactions between roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*). *Oecologia*, **73**, 170–177.

Persson L. & Greenberg L.A. (1990) Juvenile competitive bottlenecks: the perch (*Perca fluviatilis*) – roach (*Rutilus rutilus*) interaction. *Ecology*, **71**, 44–56.

Rask M. (1989) A note on the diet of roach, *Rutilus rutilus* L. and other cyprinids at Tvärminne, northern Baltic Sea. *Aqua Fennica*, **19**, 19–27.

Søndergaard Ma. & Moss B. (1998) Impact of submerged macrophytes on phytoplankton in shallow freshwater lakes. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Eds E. Jeppesen, Ma. Søndergaard, Mo. Søndergaard & K. Christoffersen), pp. 115–132. Springer-Verlag, New York.

Scheffer M. (1999) The effect of aquatic vegetation on turbidity; How important are the filter feeders? *Hydrobiologia*, **408/409**, 307–316.

Scheffer M., Hosper S.H., Meijer M.-L., Moss B. & Jeppesen E. (1993) Alternative equilibria in shallow lakes. *Trends in Ecology and Evolution*, **8**, 275–479.

Schindler D.E. & Scheuerell M.D. (2002) Habitat coupling in lake ecosystems. *Oikos*, **98**, 177–189.

Schriver P., Bøgestrand J., Jeppesen E. & Søndergaard Ma. (1995) Impact of submerged macrophytes on fish–zooplankton–phytoplankton interaction: large-scale enclosure experiments in a shallow eutrophic lake. *Freshwater Biology*, **33**, 255–270.

Solórzano L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography*, **14**, 799–801.

Timms R.M. & Moss B. (1984) Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish in a shallow wetland ecosystem. *Limnology and Oceanography*, **29**, 472–286.

Townsend C.R., Winfield I. J., Peirson G. & Cryer M. (1986) The response of young roach *Rutilus rutilus* to seasonal changes in abundance of microcrustacean prey: a field demonstration of switching. *Oikos*, **46**, 372–378.

Vanni M.J. (2002) Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **33**, 341–370.

Venugopal M.N. & Winfield I.J. (1993) The distribution of juvenile fishes in a hypereutrophic pond: can macrophytes potentially offer a refuge for zooplankton? *Journal of Freshwater Ecology*, **8**, 389–396.

Winfield I.J. (1986) The influence of simulated aquatic macrophytes on the zooplankton consumption rate of juvenile roach, *Rutilus rutilus*, rudd, *Scardinius erythrophthalmus*, and perch, *Perca fluviatilis*. *Journal of Fish Biology*, **29**, 37–48.

Winfield I.J., Peirson G., Cryer M. & Townsend C.R. (1983) The behavioural basis of prey selection by underyearling bream (*Abramis brama* (L.)) and roach (*Rutilus rutilus* (L.)). *Freshwater Biology*, **13**, 139–149.

Wood E.D., Armstrong F.A.J. & Richards F.A. (1967) Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *Journal of Marine Biological Association*, **47**, 23–31.

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Community resistance and change to nutrient enrichment and fish manipulation in a vegetated lake littoral

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SUMMARY

1. High biomass of macrophytes is considered important in the maintenance of a clear-water state in shallow eutrophic lakes. Therefore, rehabilitation and protection of aquatic vegetation is crucial to the management of shallow lakes.

2. We conducted field mesocosm experiments in 1998 and 1999 to study community responses in the plant-dominated littoral zone of a lake to nutrient enrichment at different fish densities. We aimed to find the threshold fish biomass for the different nutrient enrichment levels below which large herbivorous zooplankton escapes control by fish. The experiments took place in the littoral of Lake Vesijärvi in southern Finland and were part of a series of parallel studies carried out jointly at six sites across Europe.

3. In 1998, when macrophyte growth was poor, a clear-water state with low phytoplankton biomass occurred only in unenriched mesocosms without fish or with low fish biomass (4 g fresh mass m^{-2}). Both nutrient enrichment and high fish biomass (20 g fresh mass m^{-2}) provoked a turbid water state with high planktonic and periphytic algal biomass. The zooplankton community was dominated by rotifers and failed to control the biomass of algae in nutrient enriched mesocosms. The littoral community thus had low buffer capacity against nutrient enrichment.

4. In 1999, macrophytes, especially free-floating *Lemna trisulca* L., grew well and the zooplankton community was dominated by filter-feeding cladocerans. The buffer capacity of the littoral community against nutrient enrichment was high; a clear-water state with low phytoplankton biomass prevailed even under the highest nutrient enrichment. High grazing rates by cladocerans, together with reduced light penetration into the water caused by *L. trisulca*, were apparently the main mechanisms behind the low algal biomass.

5. Effects of fish manipulations were less pronounced than effects of nutrient enrichment. In 1999, clearance rates of cladocerans were similar in fish-free and low-fish treatments but decreased in the high-fish treatment. This suggests that the threshold fish biomass was between the low- and high-fish treatments. In 1998, such a threshold was found only between fish-free and low-fish treatments.

6. The pronounced difference in the observed responses to nutrient enrichment and fish additions in two successive years suggests that under similar nutrient conditions and fish feeding pressure either clear or turbid water may result depending on the initial community structure and on weather.

Keywords: fish predation, littoral ecosystem, macrophytes, nutrient enrichment, plankton communities

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Introduction

Whether resource or consumer regulation is the more important force in structuring pelagic freshwater communities has been addressed often in recent

literature. The dominant view is that the total biomass is ultimately set by resources while, within a system, the biomass at a particular trophic level reflects the relative importance of resource and predator control as these drivers act simultaneously (e.g. McQueen, Post & Mills, 1986; Carpenter *et al.*, 1987; Hunter & Price, 1992; Mittelbach *et al.*, 1995). In lacustrine littoral ecosystems, submerged macrophytes structure food-web interactions through mediating resource availability for planktonic communities and altering trophic cascades (Jeppesen *et al.*, 1998).

The alternative stable states model (Irvine, Moss & Balls, 1989; Scheffer *et al.*, 1993) has been proposed to explain the functioning of shallow lake ecosystems. The model suggests that at very high phosphorus concentrations a turbid, phytoplankton-dominated state may be the only possibility whereas at low phosphorus concentrations ($<25 \mu g \ P \ L^{-1}$) a clear-water state uniquely exists. Between these extremes, both turbid, phytoplankton-dominated states and clear-water, plant-dominated states are possible (Irvine *et al.*, 1989; Scheffer *et al.*, 1993). Both states are assumed to resist perturbations, such as changes in nutrient enrichment, because complex biological interactions buffer against community changes (Scheffer *et al.*, 1993).

Grazing by zooplankton is key to determining algal biomass. Grazing efficiency is largely determined by zooplankton community structure; mean body size and taxonomic composition are the critical parameters (Cyr & Curtis, 1999). Heavy fish predation on zooplankton leads to low numbers of large-bodied zooplankton and low grazing pressure (Knoechel & Holtby, 1986). This favours the development of high algal biomass if nutrient concentrations are high (Carpenter *et al.*, 1987). This trophic cascade is thought to be stronger in deep lakes than in shallow lakes with abundant macrophytes, which modify trophic interactions by increasing habitat complexity (Jeppesen *et al.*, 1998). For instance, submerged macrophyte vegetation may provide refuges for grazing cladocerans against fish predation and therefore promote high grazing pressure on phytoplankton even in the presence of planktivorous fish (Jeppesen *et al.*, 1998; Mehner *et al.*, 2002).

A switch from one state to the other may occur often following some catastrophic event. For instance, increase in the population of large *Daphnia* after a fish kill can cause a switch from turbid state to clear-water state (Edmondson & Litt, 1982; Wright & Shapiro, 1984). Declines in macrophytes may have the opposite effect: a clear-water lake turns into the turbid state following herbicide application (Van Donk *et al.*, 1990). A switch may also occur as a result of severe nutrient enrichment (Schindler, 1977). When a threshold nutrient concentration is reached, macrophyte dominance may be replaced by phytoplankton dominance (Scheffer *et al.*, 1993).

In this study, we manipulated both nutrient loading and fish predation in order to assess the community responses in a vegetated littoral zone of a lake to these factors. Experiments were carried out in field mesocosms in 1998 and 1999. Treatments included three (1998) or five (1999) levels of nutrient additions, resulting in mesotrophic to hypereutrophic conditions that were crossed with three fish densities. The highest fish density was chosen to represent the fish stock in eutrophic, turbid lakes and the lowest one to represent the desired fish density after biomanipulation (McQueen *et al.*, 1986). We hypothesised that resistance of vegetated shallow-lake systems to nutrient enrichment decreases with the density of planktivorous fish and increases with macrophyte biomass. We aimed to find the threshold of fish biomass for the different nutrient enrichment levels below which large herbivorous zooplankters and, consequently, their grazing impact, escape fish control. This experiment was part of a series of parallel studies carried out at six sites across Europe (Stephen *et al.*, 2004a).

Methods

Study site and mesocosm design

We conducted two field mesocosm experiments in the shallow littoral zone of Lake Vesijärvi in southern Finland. Details of the lake and its eutrophication and recovery history are given in Kairesalo *et al.* (1999). The mesocosms used in the experiments, altogether 36 in each year, were 1-m diameter clear polyethylene cylinders suspended from wooden frames at the surface and weighted to the lake bottom. They were installed at one metre water depth and the walls of the mesocosms extended about 20 cm into the sediment. The mesocosms were covered with bird netting. The most abundant macrophyte species in the study area were *Elodea canadensis* Michx., *Ceratophyllum demersum* L., *Myriophyllum verticillatum* L., *Lemna trisulca* L.,

Nuphar lutea L., and *Potamogeton natans* L. The 1998 experiment began on 4 June when the natural macrophyte community had just started to grow. The distribution of macrophytes in the study area was patchy. Therefore we collected *Elodea* plants from the nearby littoral area and added 62 g fresh mass (corresponding to 27 g dry mass) to each mesocosm to ensure a similar inoculum of macrophytes in each mesocosm. In 1999, the experiment began 3 weeks later (29 June) when the natural macrophyte community had achieved good growth and no inoculum was needed.

1998 experiment

In 1998, we used a fully factorial block design with three replicates. We had three nutrient enrichment levels and three densities of juvenile (6–9 cm) roach (*Rutilus rutilus* L.). The nutrient levels were: control (N0: no nutrient addition); two-fold enrichment (N1: 100 µg P L^{-1}, 1 mg N L^{-1}); ten-fold enrichment (N2: 500 µg P L^{-1}, 5 mg N L^{-1}); and twenty-fold enrichment (N3: 1.0 mg P L^{-1}, 10 mg N L^{-1}) compared with total phosphorus and nitrogen concentrations in the lake water. Fish treatments were: fish-free treatment; low-fish treatment (4 g fresh mass m^{-2}); and high-fish treatment (20 g fresh mass m^{-2}), respectively. The nutrients were added weekly as KNO$_3$ and KH$_2$PO$_4$. Pretreatment samples of water chemistry and plankton were taken on 8 June, just before the first nutrient addition and the introduction of fish. The experiment ended after 5 weeks, on 13 July. The mesocosms were checked daily and dead fish were replaced with new ones. Fish mortality increased radically in the N2 and N3 mesocosms during the third week, probably owing to the high pH of water. Because added fish died within a few hours, we did not add any further fish to those mesocosms and excluded the results of the two highest nutrient treatments from the data analyses.

Growth of periphytic algae was measured on two 2.5 cm-wide plastic strips of wall material extending from the surface to the bottom. Two strips were placed to hang at the centre of each mesocosm in the beginning of experiment. One strip was removed and replaced weekly and the other one was removed 4 weeks after the start. Periphyton was scraped from the strips and suspended into a known volume of filtered (Whatman GF/C) lake water for chlorophyll *a* analysis.

1999 experiment

In 1999, we had five nutrient levels: (N0: no nutrient addition; N1: 30 µg P L^{-1}, 0.3 mg N L^{-1}; N2: 60 µg P L^{-1}, 0.6 mg N L^{-1}; N3: 90 µg P L^{-1}, 0.9 mg N L^{-1}; N4: 150 µg P L^{-1}, 1.5 mg N L^{-1}; N5: 300 µg P L^{-1}, 3.0 mg N L^{-1}) and three fish densities, with two replicate blocks. Fish treatments consisted of 0, 4, and 20 g fresh mass m^{-2} of juvenile (8–10 cm) perch (*Perca fluviatilis* L.). The experiment lasted for 7 weeks; pretreatment samples were taken on 29 June and the last samples on August 10. Fish were introduced and nutrients first added on 29 June after the pretreatment sampling. Nutrients were added weekly and occasional dead fish were replaced. The growth of periphytic algae was measured as in 1998.

Sampling and data analyses

Samples for chemical analyses, phytoplankton and zooplankton were taken weekly from the mesocosms and the lake. Conventional methods standardised across the whole series of experiments (Stephen *et al.*, 2004b) were used with the following additions. Water temperature and oxygen were measured weekly with a Marvet fluids AJ90 (Hyko Ltd., Kerava, Finland) oxygen-temperature meter at surface and at bottom in 10 randomly chosen mesocosms. Vertical profile of light penetration into water was measured with a LiCor LI-1400 4π sensor (Li-Cor Ltd., Lincoln, NE, U.S.A.) on the last week of the experiment in 1999. Macrophytes were dried and weighed and divided into two groups according to growth form, the free-floating *L. trisulca* and rooted submerged plants.

For every zooplankton sample, 30 individuals of each cladoceran species, five individuals of each copepodite stage and adult copepods, and 20 individuals of nauplii were measured whenever sufficient number of animals was available. Zooplankters were assigned to functional groups according to their feeding mode. Raptorial feeders included *Polyphemus pediculus* L., *Asplanchna* spp., and both copepodites and adult cyclopoids. Copepod nauplii and calanoids were classified as filter-feeding copepods, and euplanktonic cladocerans included the permanently planktonic species (Hutchinson, 1967), i.e. *Bosmina* spp., *Ceriodaphnia* spp., *Chydorus sphaericus* O. F. Müller, *Daphnia longispina* O. F. Müller and *Diaphanosoma brachyurum* Liéven. Other cladocerans, mainly

chydorids and *Simocephalus vetulus* O. F. Müller, were summed as plant-associated cladocerans, and the group of suspension-feeding rotifers included all rotifers except *Asplanchna* spp. Clearance rate of cladocerans was calculated using the equation $F = 11.695 \times L^{2.48}$ where F is filtering rate in millilitre per animal per day and L is animal length in millimetre (Knoechel & Holtby, 1986).

Because fish mortality was high in N2 and N3 treatments in 1998, only the data of the nutrient treatments N0 and N1 with all three fish densities were included in analyses. No such problems occurred in 1999 and all data were included. Differences between replicates on pretreatment sampling were tested with ANOVA. The time-weighted average concentrations of planktonic and periphytic chlorophyll *a*, and the biomasses of phytoplankton and the different zooplankton groups were used as response variables in ANOVAS (SPSS for Windows v. 6.0). Stephen *et al.* (2004a)) provide the rationale behind our choice to use time-weighted averages. Pretreatment data were not used in the calculation of the time-weighted averages. Multiple comparisons were made with *a posteriori* Tukey's tests. Heterogeneity of variances was tested with Cochran's test and normality of data was checked from the residual plots. When the assumptions for ANOVA were not met, ln-transformation was used to stabilise heterogeneous variances or to normalise the distribution of residuals. However, the non-transformed values are presented in the figures and tables.

Results

1998 experiment

In 1998, mean water temperature was 18 °C, with a range of 15–21 °C during the experiment. Water depth in the mesocosms averaged 99 cm at the start but owing to much rain the water level increased to 106 cm by the end of the experiment. No significant differences in water chemistry or plankton community were found among mesocosms before nutrient and fish addition. Chemical analyses revealed quite similar results in the lake and control mesocosms (N0) during the experiment with the exception of ammonium and nitrate concentrations, which were higher in the mesocosms than in the lake. Phosphorus concentrations were, on the contrary, slightly lower in the mesocosms (Table 1). In N1 mesocosms, pH rose to over 10 and nutrient enrichment caused also an increase in alkalinity. High roach density increased phosphorus concentrations in the mesocosms of both nutrient treatments (N0 and N1) and total phosphorus was at least twice as high in the high-fish treatment as in the control or low-fish treatments (Table 1).

The bottom sediment of the mesocosms was visible and a clear-water state occurred throughout the experiment in control and N0/low-fish treatments, whereas in high-fish treatments water turbidity increased as shown by the greater amount of total suspended solids and decreased Secchi depth (Table 1). Water transparency decreased also in N1 treatments, where the bottom was not visible 2 weeks

Table 1 Mean values (SE) in the 1998 experiment for water chemistry data, Secchi depth, and periphytic chlorophyll *a* concentration in the last experimental week, in lake water (no replicates) and mesocosms ($n = 3$). N0 and N1 are nutrient treatments and F0, F1, and F2 fish treatments. Concentrations of soluble reactive phosphorus, total soluble phosphorus, total phosphorus, and total suspended solids are abbreviated as SRP, TSP, TP and TSS, respectively.

| Parameter | Lake control | Treatment | | | | | | |
| | | N0 | | | N1 | | | |
		F0	F1	F2	F0	F1	F2
pH	6.8	7.15 (0.13)	7.34 (0.19)	7.38 (0.19)	10.36 (0.22)	10.15 (0.17)	10.30 (0.15)
Total alkalinity (meq L^{-1})	0.7	0.63 (0.00)	0.57 (0.01)	0.65 (0.02)	0.99 (0.01)	1.02 (0.10)	0.95 (0.01)
NH_4-N (μg L^{-1})	12.1	28.0 (9.7)	11.4 (3.7)	25.5 (8.5)	208 (104)	116 (27)	205 (66)
NO_3-N (mg L^{-1})	<0.01	0.08 (0.04)	0.02 (0.01)	0.07 (0.04)	0.94 (0.08)	0.90 (0.2)	0.70 (0.05)
SRP (μg L^{-1})	10.0	8.3 (2.3)	25.2 (11.2)	10.9 (1.3)	80 (48)	65 (16)	93 (18)
TSP (μg L^{-1})	34	23.2 (6.7)	26.0 (10.5)	27.2 (3.5)	134 (54)	123 (18)	190 (40)
TP (μg L^{-1})	56	44.4 (8.6)	54.4 (7.0)	107 (23)	285 (17)	330 (87)	700 (274)
TSS (mg L^{-1})	2.4	2.53 (0.5)	14.9 (6.1)	18.7 (8.6)	5.6 (1.3	4.3 (0.9)	15.7 (6.0)
Secchi depth (cm)	>100	>100 (0)	>100 (0)	77 (18)	67 (22)	60 (26)	28 (6)
Periphytic chl *a* (μg cm^{-2})		0.71 (0.13)	0.86 (0.28)	0.22 (0.54)	2.45 (0.56)	5.09 (1.47)	3.25 (1.03)

after the start. Thereafter, Secchi depth decreased even more; a depth of 67 cm was reached in the N1/fish-free treatment after 6 weeks, and in the N1/high-fish treatment it was <30 cm (Table 1). The decrease in transparency occurred simultaneously with an increase in temperature from 16 to 21 °C.

Nutrient enrichment clearly increased the biomass of phytoplankton, and the chlorophyll *a* concentration was about 10 times higher (>100 μg L^{-1}) in the N1 treatment than in the control (10 μg L^{-1}). In the N0/high-fish treatment, chlorophyll *a* concentration was twice as high as in the control. The low-fish treatment, on the contrary, did not differ from the control treatment. Such a fish effect was not seen in the nutrient-enriched mesocosms (Fig. 1). The highly significant effects of nutrients on the total biomass of phytoplankton probably masked the effects of fish (Table 2).

The phytoplankton community in the control treatment comprised equal shares of edible and inedible algae. In terms of biomass (measured as biovolume), the most abundant taxa were cyanobacteria (*Anabaena* spp.) and flagellates (*Cryptomonas* sp. and *Synura* sp.). Biomass of cyanobacteria decreased dramatically with nutrient enrichment, and cyanobacteria were almost absent in the N1 treatment. The increase of phytoplankton biomass with nutrient enrichment was because of an increase of edible algae, defined as forms with a greatest axial or linear diameter (GALD) <50 μm (mainly cryptophytes), and the inedible (GALD >50 μm) chlorophyte *Dictyosphaerium* sp. The increase in the biomass of both edible and inedible algae with nutrient enrichment was highly significant whereas the increase of edible algae with increasing fish density was only marginally significant (Table 2).

Biomass of periphytic algae (determined as chlorophyll *a*) was high, between 2.5 and 5.0 μg cm^{-2}, during the whole experiment in N1 mesocosms, whereas in N0 mesocosms the biomass of periphytic algae was always below 1 μg cm^{-2}. The difference in the biomass of periphytic algae between N0 and N1 treatments was almost ten-fold and highly significant ($F_{1,12} = 10.51$, $P = 0.007$). Biomass of periphyton was at its highest in the low-fish treatment, although neither fish addition ($F_{1,12} = 0.81$, $P = 0.47$) nor the nutrient-fish interaction ($F_{2,12} = 0.69$, $P = 0.52$) were significant. No significant correlation was found between periphytic and planktonic chlorophyll *a* concentration ($r = 0.16$, $P = 0.54$, $n = 18$).

The structure of the zooplankton community changed during the experiment. Initially, rotifers (mainly *Polyarthra* spp., *Synchaeta* spp. and *Keratella cochlearis* Gosse) contributed almost 90% of total biomass. In most mesocosms, a decrease in rotifer biomass occurred during the third and fourth week, following an increase in the biomass of the raptorial cladoceran *P. pediculus*. The cyclopoids *Mesocyclops leuckarti* Claus and *Thermocyclops* spp. were abundant in all mesocosms during the experiment. The biomasses of the filter-feeding cladocerans *Ceriodaphnia pulchella* Sars, *C. quadrangula* O. F. Müller and *D. brachyurum* started to increase during the fourth week. *Bosmina longispina* Leydig and *B. longirostris* O. F. Müller, as well as chydorids like *Alona nana* Baird and *Alonella exigua* Lilljeborg, occurred in low numbers.

The mean total biomass of zooplankton doubled with nutrient enrichment, but this increase was only marginally significant (Table 3). Raptorial feeders, especially *P. pediculus*, in N1/fish-free and N1/low-fish treatments and suspension feeding rotifers in N1/low-fish and N1/high-fish treatments, were responsible for this increase in zooplankton biomass (Fig. 2). The biomass and clearance rate of euplanktonic cladocerans increased with nutrient enrichment in the fish-free treatment but tended to decrease with nutrient enrichment in the presence of fish. However, the changes were not significant (Fig. 2; Table 3). Total biomass of zooplankton did not differ between fish treatments, although the biomasses of both raptorial feeders and euplanktonic cladocerans decreased in the high-fish treatment (Fig. 2). The biomass of *P. pediculus* increased with nutrients but decreased when fish were present, resulting in a significant joint effect of nutrients and fish on raptorial feeders (Fig. 2; Table 3). With the decrease of predators, rotifers (mainly *K. cochlearis* and *Trichocerca* sp.) built up a high biomass (up to >11 000 μg dry mass L^{-1}) in N1/low- and high-fish treatments. Tenfold rotifer biomass increases were observed in comparison with fish-free mesocosms (Fig. 2). However, the observed increase in rotifer biomass with increasing fish density was not significant (Table 3) owing to wide variation among replicates.

At the end of the experiment, the biomass of macrophytes varied between 2 and 12 g dry mass per mesocosm. Although we added *Elodea* to each mesocosm, biomass of this plant declined in all
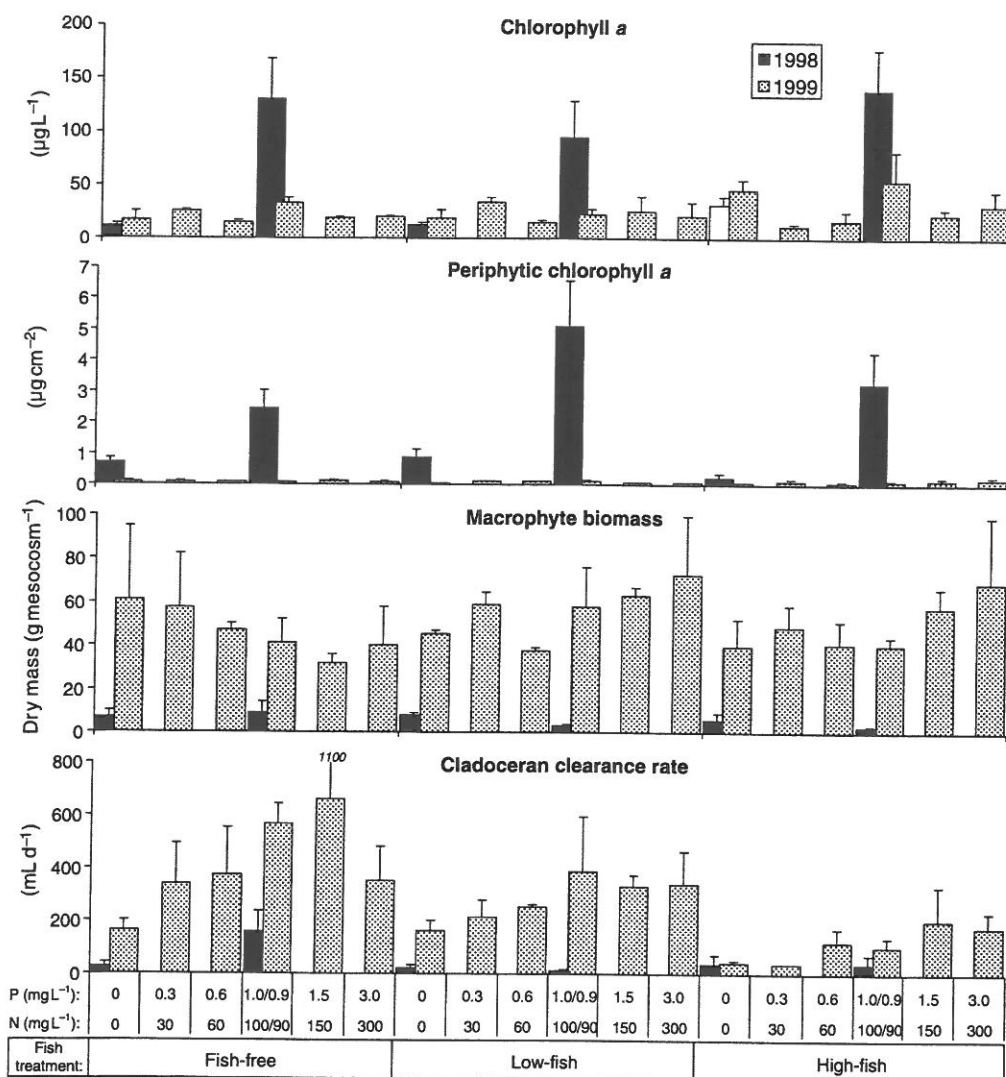
Fig. 1 Mean values (±SE) of time-weighted averages of phytoplankton chlorophyll *a* concentration, periphytic chlorophyll *a* concentration and macrophyte biomass in the last week of the experiment and time-weighted average cladoceran clearance rates in different nutrient and fish treatments in 1998 and 1999. Note that P enrichment of 1.0/0.9 mg L^{-1} and N enrichment of 100/90 mg L^{-1} refer to different nutrient additions in 1998/1999.

mesocosms and *L. trisulca* became the most abundant species. In fish treatments, the biomass of submerged plants, mainly *E. canadensis*, *M. verticillatum*, and *P. natans*, was lower in the N1 than in the N0 mesocosms. The difference in the biomass of submerged macrophytes between the two nutrient treatments was marginally significant ($F_{1,12} = 3.42$, $P = 0.089$), whereas the biomass of *L. trisulca* was not significantly affected (Nutrients, $F_{1,12} = 0.01$, $P = 0.94$; Fish, $F_{2,12} = 1.0$, $P = 0.40$; N × F, $F_{2,12} = 0.26$, $P = 0.78$).

1999 experiment

The summer in 1999 was much warmer than in 1998. Water temperatures in the mesocosms were around 21–23 °C but decreased to 17 °C at the end of the experiment. The study period was very dry and water depth in the mesocosms declined from 100 to 80 cm. Concentration of total nitrogen did not differ much between lake and N0 mesocosms at the end of the experiment, whereas total phosphorus concentration

Table 2 Results of ANOVAs on time-weighted averages of phytoplankton chlorophyll *a* concentration, biovolume of inedible algae (GALD >50 μm) and edible algae (GALD <50 μm) in 1998

Response variable	Source	DF	MS	F	P
Chlorophyll *a*	Fish (F)	2	0.72	2.46	0.13
	Nutrients (N)	1	15.7	53.4	0.001
	F × N	2	0.32	1.08	0.37
	Error	12	0.29		
Inedible algae	Fish (F)	2	0.53	0.29	0.75
	Nutrients (N)	1	11.7	12.83	0.004
	F × N	2	0.62	0.68	0.53
	Error	12	0.91		
Edible algae	Fish (F)	2	1.25	3.20	0.077
	Nutrients (N)	1	29.8	76.6	0.001
	F × N	2	0.44	1.4	0.35
	Error	12	0.39		

All variables were ln-transformed before analysis.

Table 3 Results of ANOVAs on time-weighted averages of the major zooplankton groups in 1998

Response variable	Source	DF	MS	F	P
Total biomass of zooplankton	Fish (F)	2	470	0.37	0.71
	Nutrients (N)	1	5487	4.33	0.059
	F × N	2	117	0.09	0.91
	Error	12	1266		
Suspension feeding rotifers*	Fish (F)	2	3.40	2.14	0.16
	Nutrients (N)	1	0.09	0.06	0.81
	F × N	2	0.77	0.48	0.63
	Error	12	1.59		
Euplanktonic cladocerans	Fish (F)	2	74	1.06	0.38
	Nutrients (N)	1	0.019	0.00	0.99
	F × N	2	37	0.52	0.61
	Error	12	70		
Raptorial feeders*	Fish (F)	2	1.00	3.77	0.054
	Nutrients (N)	1	0.58	2.19	0.16
	F × N	2	2.89	8.56	0.005
	Error	12	0.27		

Variables marked with an asterisk (*) were ln-transformed before analysis.

was higher in the control mesocosms than in the lake (Table 4). Nutrient enrichment caused an increase in pH of water. High values (9–10) were measured after the third week of the experiment. Concentration of ammonium increased and that of nitrate decreased with fish added. However, the water remained clear and the bottom was visible almost throughout the experiment in all mesocosms (Table 4).

Mean chlorophyll *a* concentrations were below 30 μg L^{-1} in fish-free and low-fish treatments. The highest mean chlorophyll *a* concentrations (40–60 μg L^{-1}) were measured in the high-fish treatment (Fig. 1). The increase of chlorophyll *a* concentration in the high-fish treatment was significant (Table 5). In addition, the high-fish treatment differed significantly from both the low-fish and control treatments (Tukey's test; $P < 0.05$). This difference was mainly because of a significant increase in the biomass of inedible algae when fish were present (Table 5). The biomass of edible algae also increased with fish present, although this change was not significant (Table 5). Nutrient enrichment had no significant effect on chlorophyll *a* concentration, although the biomass of inedible algae increased significantly with nutrient enrichment (Fig. 1; Table 5).

Cryptophytes were initially the dominant phytoplankters in all mesocosms, their share of the biomass being nearly 50%. Towards the end of the experiment the biomass of chlorophytes increased and *Chlamydomonas* spp. and *Scenedesmus* spp. became the most common species. However, *Volvox aureus* Ehrenberg formed a high biomass in some mesocosms, causing the observed wide variation in the biomass of inedible algae.

Periphytic chlorophyll *a* concentration was low, usually <0.10 μg cm^{-2} (Table 4). Total biomass of periphyton in the mesocosms, however, often exceeded that of phytoplankton. The biomass of periphytic algae slightly increased with nutrient enrichment in the high-fish treatment, resulting in a marginally significant fish effect ($F_{2,18} = 2.66, P = 0.097$), whereas the differences between nutrient levels ($F_{5,18} = 0.93, P = 0.49$) and the interaction between nutrients and fish ($F_{10,18} = 0.58, P = 0.81$) were not significant.

Only the biomass of suspension-feeding rotifers showed a significant increase with fish, while the other zooplankton groups responded less clearly (Fig. 2; Table 6). Biomasses of euplanktonic cladocerans showed an increasing, although not significant, trend with nutrient enrichment and a decreasing trend with fish density (Fig. 2; Table 6). However, the clearance rate of cladocerans increased significantly with nutrient enrichment ($F_{5,18} = 2.98, P = 0.04$). A more detailed analysis of the responses of cladoceran species showed that the biomass of *D. longispina* decreased significantly in fish treatments ($F_{2,18} = 11.35, P = 0.001$). The biomass of the dominating cladoceran, *Ceriodaphnia*, was reduced only in high-fish treatments (Tukey's test; $P < 0.05$). The decline of large-bodied cladocerans, *Daphnia* (0.7–1.3 mm) and
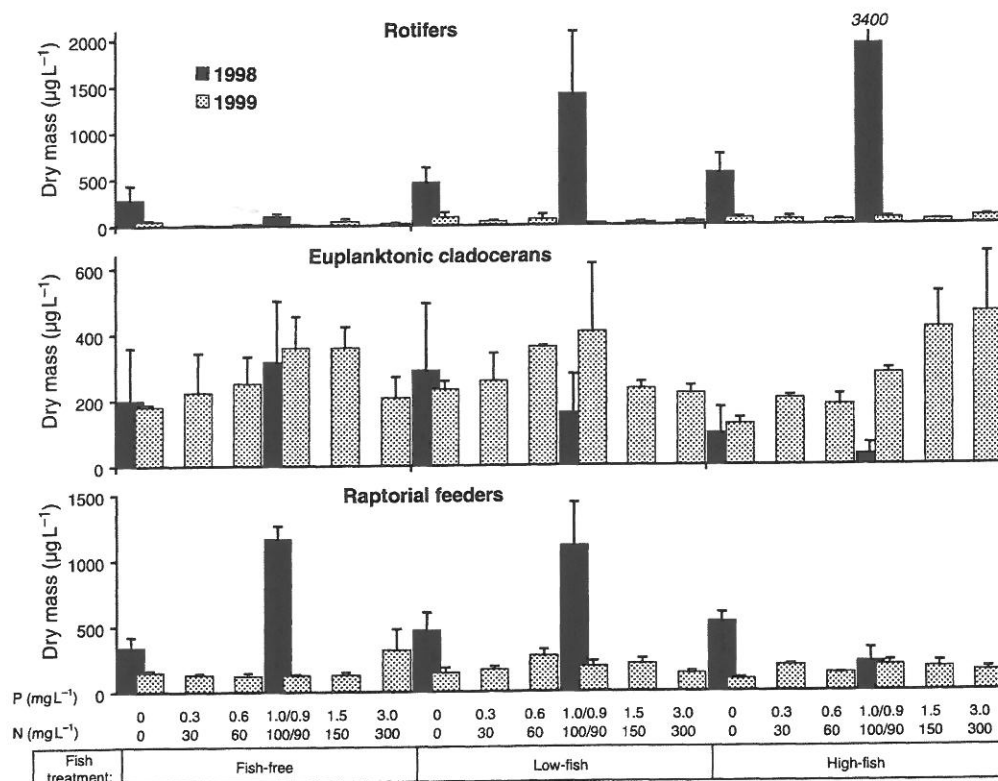
Fig. 2 Mean values (±SE) of time-weighted averages of the biomass of suspension feeding rotifers, euplanktonic cladocerans, and raptorial feeding crustaceans and rotifers in different nutrient and fish treatments in 1998 and 1999. Note that P enrichment of 1.0/0.9 mg L^{-1} and N enrichment of 100/90 mg L^{-1} refer to different nutrient additions in 1998/1999.

Diaphanosoma (0.5–0.9 mm), resulted in a significantly lower clearance rate (Fig. 1; $F_{2,18} = 16.94$, $P < 0.001$), especially in the high-fish treatment (Tukey's test; $P < 0.001$). Consequently, chlorophyll *a* concentration increased in high-fish treatments (Fig. 1; Table 5). However, in the high-fish treatments with the two highest nutrient additions, the clearance rates slightly exceeded those in the control (Fig. 1). This suggests that the threshold fish biomass for these nutrient additions was even higher than 20 g fresh mass m^{-2}.

Biomass of macrophytes varied between 30 and 70 g dry mass per mesocosm at the end of the experiment. Treatments did not explain the differences in macrophyte biomass among mesocosms (Nutrients, $F_{5,18} = 0.51$ $P = 0.77$; Fish, $F_{2,18} = 0.55$, $P = 0.59$; N × F, $F_{10,18} = 0.57$, $P = 0.82$). The share of submerged plants, *E. canadensis* and *C. demersum*, was usually <10% of the total macrophytic biomass. The most common species in all mesocosms was *L. trisulca*, which formed floating flocs in the whole

water column. *Lemna trisulca* covered 70–95% of mesocosm area and efficiently diminished light penetration into water. Light measurements showed that at 4 cm water depth the photon flux density was reduced by 90–95% as compared with macrophyte-free patches. However, at the bottom (at 80 cm depth), this reduction was only 0–40% because of scattering of light.

Comparison of 1998 and 1999 experiments

Although nutrient enrichment resulted in similar ranges of total phosphorus concentrations in both years, the response of phytoplankton to nutrient enrichment clearly differed (Fig. 3). In 1998, a clear-water state with visible lake bottom occurred only in N0/fish-free and N0/low-fish mesocosms with phosphorus concentrations <60 µg P L^{-1} (Table 1). Chlorophyll *a* concentration increased linearly with phosphorus concentration up to a maximum of

Table 4 Mean values (SE) for the 1999 experiment for water chemistry data, Secchi depth and periphytic chlorophyll *a* concentration in the last experimental week, in lake water (no replicates) and mesocosms

Parameter	Lake	N0	N1	N2	N3	N4	N5	F0	F1	F2
						Treatment				
pH	6.9	8.07 (0.06)	8.25 (0.12)	8.83 (0.32)	8.85 (0.33)	9.33 (0.25)	9.12 (0.36)	8.89 (0.17)	8.68 (0.26)	8.64 (0.22)
NH$_4$-N (µg L^{-1})	29	26.9 (6.8)	38.1 (9.1)	50.3 (11.0)	99.3 (22.6)	73.1 (20.5)	94.7 (36.4)	53.7 (8.7)	69.2 (15.9)	65.4 (20.3)
NO$_3$-N (mg L^{-1})	0.03	0.01 (0.00)	0.04 (0.02)	0.2 (0.05)	0.5 (0.1)	1.5 (0.1)	4.3 (1.07)	1.4 (0.6)	1.1 (0.6)	0.7 (0.4)
TN (mg L^{-1})	0.7	1.1 (0.07)	1.1 (0.02)	1.5 (0.1)	1.9 (0.2)	3.2 (0.3)	6.8 (1.4)	3.1 (0.8)	2.6 (0.7)	2.2 (0.5)
SRP (µg L^{-1})	3.494	3.0 (0.7)	6.3 (1.1)	30 (8.5)	61 (19)	128 (32)	301 (74)	94 (31)	77 (35)	95 (48)
TP (µg L^{-1})	44	89 (31)	64 (4.6)	111 (15)	164 (23)	255 (44)	534 (104)	212 (48)	182 (52)	214 (71)
Secchi depth (cm)	>100	>100 (0)	>100 (0)	>100 (0)	>100 (0)	>100 (0)	>100 (0)	>100 (0)	>100 (0)	>100 (0)
Periphytic chl *a* (µg cm^{-2})		0.054 (0.028)	0.10 (0.052)	0.083 (0.036)	0.098 (0.022)	0.11 (0.047)	0.11 (0.038)	0.078 (0.01)	0.078 (0.01)	0.12 (0.021)

Fish treatments were pooled across nutrient enrichment levels (*n* = 6) and nutrient levels were pooled across fish treatments (*n* = 12). Concentrations of total nitrogen, soluble reactive phosphorus and total phosphorus are abbreviated as TN, SRP and TP, respectively.

Table 5 Results of ANOVAs on time-weighted averages of phytoplankton chlorophyll *a* concentration and, and of the bio-volume of inedible algae (GALD >50 µm) and edible algae (GALD <50 µm) in 1999

Response variable	Source	DF	MS	F	P
Chlorophyll a	Fish (F)	2	1312	7.71	0.004
	Nutrients (N)	5	58	0.34	0.88
	F × N	10	133	0.78	0.64
	Error	18	170		
Inedible algae	Fish (F)	2	1183	3.88	0.040
	Nutrients (N)	5	969	3.18	0.032
	F × N	10	357	1.17	0.37
	Error	18	305		
Edible algae	Fish (F)	2	532	1.40	0.27
	Nutrients (N)	5	192	0.51	0.77
	F × N	10	439	1.15	0.38
	Error	18	380		

Table 6 Results of ANOVAs on time-weighted averages of the major zooplankton groups in 1999

Response variable	Source	DF	MS	F	P
Total biomass of zooplankton	Fish (F)	2	32	0.55	0.59
	Nutrients (N)	5	57	0.99	0.45
	F × N	10	27	0.48	0.88
	Error	18	58		
Suspension feeding rotifers	Fish (F)	2	4.39	4.85	0.021
	Nutrients (N)	5	0.98	1.08	0.40
	F × N	10	0.81	0.90	0.56
	Error	18	0.91		
Euplanktonic cladocerans	Fish (F)	2	5.53	1.24	0.18
	Nutrients (N)	5	3.59	1.91	0.33
	F × N	10	1.82	0.62	0.77
	Error	18	2.89		
Raptorial feeders	Fish (F)	2	3.09	0.70	0.51
	Nutrients (N)	5	3.17	0.72	0.61
	F × N	10	8.21	1.87	0.12
	Error	18	4.37		

approximately 200 µg L^{-1} (Fig. 3). The increase of phytoplankton biomass in N1 treatments with all fish levels resulted in a turbid state with low Secchi depth (Table 1). In 1999, on the contrary, chlorophyll *a* concentrations remained below 100 µg L^{-1}, and a clear-water state prevailed over the whole nutrient gradient (Fig. 3; Table 4).

The control treatments of the two study years behaved differently. In 1998, chlorophyll *a* concentration was 1.6 times lower and periphyton concentrations 10 times higher than in 1999. Also, the growth of macrophytes was negligible in 1998 but high in 1999. In addition, cladoceran clearance rate was seven times
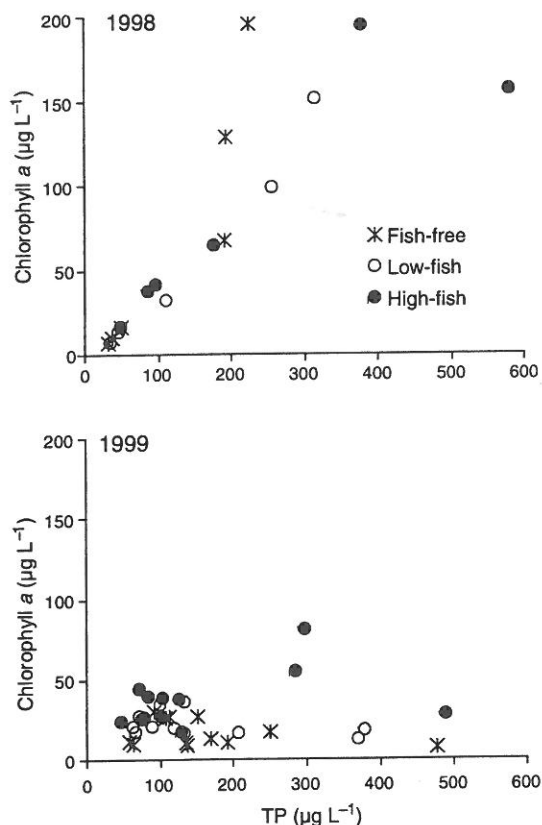
Fig. 3 Relationship between concentrations of total phosphorus and chlorophyll *a* concentration in 1998 (upper panel) and 1999 (lower panel). Data points represent the time-weighted averages from each mesocosm.

lower and rotifer biomass over five times higher in 1998 than in 1999 (Figs 1 & 2).

As the communities differed so much, both nutrient and fish treatments resulted in contrasting results in the two experiments. When the responses of the littoral communities to similar nutrient enrichment were compared, i.e. N1 treatments (100 µg P L^{-1}; 1.0 mg N L^{-1}) in 1998 and N3 treatments (90 µg P L^{-1}; 0.9 mg N L^{-1}) in 1999, clear differences are apparent. In 1998, both phytoplankton and periphyton developed high biomasses (chlorophyll *a*) compared with control treatments. In 1999, on the contrary, nutrient enrichment did not increase algal biomass, and in many nutrient treatments with different fish densities, chlorophyll *a* concentration remained even lower than in control treatments (Fig. 1). Cladoceran clearance rates were much higher in 1999 than in 1998 (Fig. 1). In 1998, the clearance rate also increased slightly in the N1/fish-free treatment but this was not reflected in the chlorophyll *a* concentration. The refuge value of macrophytes for cladocerans against fish predation was negligible in 1998, for *Daphnia* also in 1999. However, in 1999, smaller cladocerans, especially *Ceriodaphnia* (0.4–0.5 mm), increased marginally significantly with nutrients ($F_{5,18} = 2.60$, $P = 0.061$) in all fish treatments.

Discussion

The alternative stable states model suggests that a lake that initially hardly responds to continuing enrichment will eventually switch from a clear-water to a turbid state (Scheffer *et al.*, 1993). A switch is possible over a wide range of nutrient concentrations depending on buffering mechanisms, such as abundance of submerged macrophytes (Irvine *et al.*, 1989; Scheffer *et al.*, 1993). In 1998, all nutrient-enriched mesocosms rapidly reached the transition point and were turbid with very high biomass of phytoplankton, while the clear-water state was sustained in the surrounding lake littoral and in unenriched control mesocosms. In 1999, on the contrary, a clear-water state prevailed in all mesocosms over the whole nutrient gradient. Thus, resistance of the clear-water state against nutrient enrichment was very low in 1998 but high enough in 1999 to maintain a clear-water state even under nutrient conditions that normally characterise hypereutrophic lakes. What then were the principal mechanisms behind these striking differences?

The experiment in 1998 began after a cold spring in early June and water temperature remained below 20 °C during the whole experiment. The nutrient-enriched mesocosms turned into a turbid state within a few weeks and remained turbid thereafter. Under the low temperature conditions in early summer, the start of macrophyte growth was delayed, and further suppressed by strong biomass development of planktonic and periphytic algae in the enriched mesocosms. Noteworthy is that the total biomass of periphytic algae on the mesocosm walls reached or exceeded that of phytoplankton (total surface area of plastic walls in each mesocosm was about 3.2 m^2 for a total water volume 700–800 L). However, although periphyton also benefited from surplus nutrients, it was unable to prevent planktonic algae from developing high biomass and thus causing high turbidity of the water. The lack of a significant correlation between periphy-

tic and planktonic chlorophyll *a* concentration suggests that biomass of the different algal communities was not consistently controlled by the same factors such as nutrient availability. Probably the low grazing pressure on planktonic algae was a more important mechanism. Because of the cold spring, hatching of cladocerans was delayed and the suspension-feeding zooplankton community, dominated by highly selective rotifers, could not control algal biomass. Rather, they probably further enhanced the availability of nutrients to algae as small rotifers might recycle nutrients more effectively than larger cladocerans (cf. Vanni, 2002). In addition, *K. cochlearis*, one of the most abundant species in our mesocosms, probably fed effectively also on bacteria and detritus (Bogdan & Gilbert, 1982), as probably also did the small *Trichocerca* (length 50–70 μm), the other abundant rotifer species.

In the nutrient-enriched mesocosms, high pH (and low availability of CO_2) probably prevented the growth of aquatic plants, including *Lemna* (Keddy, 1976). In unenriched mesocosms with lower pH, the growth of *Lemna* was apparently limited by the availability of dissolved nutrients. However, in 1998, macrophytes grew poorly in the study area in general. This suggests that together with weather conditions in the previous summer and winter, some intrinsic mechanism like the production and use of storage material, added to the observed suppression in macrophyte growth. High pH probably prevented also the recruitment of cladocerans (Beglioglu & Moss, 1995).

In 1999, macrophytes and especially *Lemna* grew luxuriously in the mesocosms as well as in the surrounding lake area. Lemnids over-wintered successfully in the littoral zone and started to grow early, favoured by a warm and sunny spring and early summer. In 1999, the biomasses of phytoplankton in the nutrient treatments did not obviously differ from each other because cladoceran grazing increased significantly with nutrient enrichment. In addition, the significant positive effect of fish on chlorophyll *a* concentration indicates that grazers had a controlling effect on phytoplankton biomass. Indeed, the clearance rates of the cladoceran community decreased significantly in high-fish treatments, although the biomasses of most zooplankton groups did not respond significantly to the presence of fish. Only the biomass of *D. longispina* decreased and rotifers increased with high fish density. However, the biomass of *Daphnia* remained rather low and the most abundant euplanktonic zooplankton cladoceran was *Ceriodaphnia*, together with plant-associated *Simocephalus* and small chydorids, for which *Lemna* apparently provided refuge from fish predation.

The importance ascribed to zooplankton in controlling phytoplankton biomass has been criticised (De-Melo, France & McQueen, 1992; Blindow *et al.*, 2000). In general, large-bodied daphnids have been shown to be the only zooplankton species capable of limiting phytoplankton biomass (e.g. Benndorf *et al.*, 1984; McQueen *et al.*, 1986; Carpenter *et al.*, 1987; Leibold, 1989). Perrow *et al.* (1999) found a threshold filtration rate of 300–400 mL day^{-1} for cladocerans to maintain clear-water phase. In our experiment, this value was exceeded only in 1999 and followed, as predicted, by a decrease in chlorophyll *a* concentration. Our results suggest that *Ceriodaphnia* can also control phytoplankton biomass. However, the effects of zooplankton on phytoplankton observed in both our study and previous studies on small zooplankton (Vanni, 1987; Cyr & Curtis, 1999) were much less pronounced than in experiments with *Daphnia* as the key grazer.

It has been suggested that periphytic algae may buffer a clear-water state by competing with planktonic algae for nutrients (Moss, Madgwick & Phillips, 1996). This hypothesis is not supported by our results. Both algal communities developed very high and low biomasses in 1998 and 1999, respectively, in response to nutrient enrichment, with no indication of strong competitive interactions.

Buffering of the clear-water state is generally thought to be mediated mainly by macrophytes (Irvine *et al.*, 1989; Scheffer *et al.*, 1993). In 1999, the final biomass of macrophytes was ten times higher than in 1998 and water remained clear in the mesocosms despite nutrient enrichment. The most evident mechanism behind the depression of phytoplankton biomass was grazing by cladocerans, for which *Lemna* flocs provided refugia against predation by fish. In addition, dense floating flocs of *Lemna* prevented light penetration into the water and, consequently, limited the production of both planktonic and periphytic algae to some extent. The fronds of *L. trisulca* are thin and elongate and have a large surface area, suggesting that *Lemna* can efficiently take up nutrients from water. However, nutrient concentrations in the water remained high. Thus, zooplankton grazing coupled

with lowered algal productivity because of shading by *Lemna* were most likely the principal mechanisms behind the low biomasses of the algal communities.

The high-fish level (20 g FW m^{-2}), which suppressed cladoceran zooplankton in our experiment, is usually found in turbid lakes. However, daphnids were suppressed even at the low-fish level (4 g fresh mass m^{-2}), which is close to the fish biomass considered acceptable after biomanipulation (Moss *et al.*, 1996). Our results support those of Post & McQueen (1987) and Perrow *et al.* (1999) who stated that a fish biomass as low as 2–5 g fresh mass m^{-2}, or 0.2 individual m^{-2}, might be sufficient to remove *Daphnia* populations. In 1999, the clearance rates of cladocerans were similar in fish-free and low-fish treatments and decreased only in high-fish treatments. This suggests that in 1999 the threshold fish density was between low and high fish treatments, except at the two highest nutrient levels where the threshold fish biomass was even above 20 g fresh mass m^{-2}. In 1998, when we used roach rather than perch, such a threshold was found only between fish-free and low-fish treatments regardless of nutrient enrichment. Roach is an effective planktivore in open-water habitats, while the perch used in 1999 is better adapted to forage among macrophyte beds (e.g. Jeppesen *et al.*, 1998; Perrow *et al.*, 1999). Therefore, if roach had been the fish species added also in the year characterised by high biomass of *Lemna*, the fish effect might have been different. However, roach have recently been found to feed effectively on cladocerans also among macrophytes (R. Kornijów, K. Vakkilainen, J. Horppila, E. Luokkanen & T. Kairesalo, submitted), suggesting that the consequences of using different fish species in different years should not be overrated.

In conclusion, the resistance to nutrient enrichment of our mesocosm communities was very low in 1998 but exceptionally high in 1999. Striking year-to-year differences in the responses to nutrient enrichment and fish additions imply that either clear or turbid water may result in shallow-lake systems depending on the initial community structure and weather conditions. The mechanisms behind the resistance and the control of algal biomass were apparently linked directly to the abundances of key grazers and macrophytes. These, in turn, appeared to be determined largely by prevailing weather conditions, which should be taken into consideration when generalising results across studies.

References

Beglioglu M. & Moss B. (1995) The impact of pH on interactions among phytoplankton algae, zooplankton and perch (*Perca fluviatilis*) in a shallow, fertile lake. *Freshwater Biology*, **33**, 497–509.

Benndorf J., Kneschke H., Kossatz K. & Penz E. (1984) Manipulation of the pelagic food web by stocking with predacious fishes. *Internationale Revue der gesamten Hydrobiologie*, **69**, 407–428.

Blindow I., Hargeby A., Wagner B.M.A. & Andersson G. (2000) How important is the crustacean plankton for the maintenance of water clarity in shallow lakes with abundant submerged vegetation. *Freshwater Biology*, **44**, 185–197.

Bogdan K.B. & Gilbert J.J. (1982) Seasonal patterns of feeding by natural populations of *Keratella*, *Polyarthra* and *Bosmina*: Clearance rates, selectivities and contributions to community grazing. *Limnology and Oceanography*, **27**, 918–934.

Carpenter S.R., Kitchell J.F., Hodgson J.R., Cochran P.A., Elser J.J., Elser M.M., Lodge D.M., Kretchmer D., He X. & Von-Ende C.N. (1987) Regulation of lake primary productivity by food web structure. *Ecology*, **68**, 1863–1876.

Cyr H. & Curtis J.M. (1999) Zooplankton community size structure and taxonomic composition affect size-selective grazing in natural communities. *Oecologia*, **118**, 306–315.

DeMelo R., France R. & McQueen D.J. (1992) Biomanipulation: hit or myth? *Limnology and Oceanography*, **37**, 192–207.

Edmondson W.T. & Litt A.H. (1982) *Daphnia* in Lake Washington. *Limnology and Oceanography*, **27**, 272–293.

Gliwicz Z.M. (1990) Why do cladocerans fail to control algal blooms? *Hydrobiologia*, **200/201**, 83–97.

Hunter M.D. & Price P.W. (1992) Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology*, **73**, 724–732.

Hutchinson G.E. (1967) *A Treatise on Limnology. Volume II. Introduction to Lake Biology and the Limnoplankton*, pp. 1115. John Wiley & Sons, Inc, New York.

Irvine K., Moss B. & Balls H. (1989) The loss of submerged plants with eutrophication II. Relationships between fish and zooplankton on a set of experimental ponds, and conclusions. *Freshwater Biology*, **22**, 89–107.

Jeppesen E., Søndergaard Ma., Søndergaard Mo. & Christoffersen K. (Eds) (1998) *The Structuring Role of Submerged Macrophytes in Lakes*, pp. 423.Springer-Verlag, New York.

Kairesalo T., Laine S., Luokkanen E., Malinen T. & Keto J. (1999) Direct and indirect mechanisms behind successful biomanipulation. *Hydrobiologia*, **395/396**, 99–106.

Keddy P.A. (1976) Lakes as islands: the distributional ecology of two aquatic plants, *Lemna minor* L. and *Lemna trisulca* L. *Ecology*, **57**, 353–359.

Knoechel R. & Holtby L.B. (1986) Construction and validation of a body-length-based model for the prediction of cladoceran community filtering rates. *Limnology and Oceanography*, **31**, 1–16.

Leibold M.A. (1989) Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. *American Naturalist*, **134**, 922–949.

Mazumder A. (1994) Patterns of algal biomass in dominant odd- vs even-link lake ecosystems. *Ecology*, **75**, 1141–1149.

McQueen D.J., Post J.R. & Mills E.L. (1986) Trophic relationships in freshwater pelagic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences*, **43**, 1571–1581.

Mehner T., Benndorf J., Kasprzak P. & Koschel R. (2002) Biomanipulation of lake ecosystems: successful applications and expanding complexity in the underlying science. *Freshwater Biology*, **47**, 2453–2465.

Mittelbach G.G., Turner A.M., Hall D.J., Rettig J.E. & Osenberg C.W. (1995) Perturbation and resilience: a long-term, whole-lake study of predator extinction and reintroduction. *Ecology*, **76**, 2347–2360.

Moss B., Madgwick J. & Phillips G. (1996) *A Guide to the Restoration of Nutrient-Enriched Shallow Lakes*, pp. 180.Environment Agency, Broads Authority, Norwich, U.K.

Perrow M.R., Jowitt A.J.D., Stansfield J.H. & Phillips G.L. (1999) The practical importance of the interactions between fish, zooplankton and macrophytes in shallow lake restoration. *Hydrobiologia*, **395/396**, 199–210.

Post J.R. & McQueen D.J. (1987) Impact of planktivorous fish on the structure of a plankton community. *Journal of Freshwater Biology*, **21**, 79–89.

Scheffer M., Hosper S.H., Meijer M.L., Moss B. & Jeppesen E. (1993) Alternative equilibria in shallow lakes. *Trends in Ecology and Evolution*, **8**, 275–279.

Schindler D.W. (1977) Evolution of phosphorus limitation in lakes. *Science*, **195**, 260–262.

Stephen D., Balayla D., Bécares E. *et al.* (2004a) Continental-scale patterns of nutrient and fish effects on shallow lakes: introduction to a pan-European mesocosm experiment. *Freshwater Biology*, **49**, 1517–1524.

Stephen D., Balayla D.M., Collings S.E. & Moss B. (2004b) Two mesocosm experiments investigating the control of summer phytoplankton growth in a small shallow lake. *Freshwater Biology*, **49**, 1551–1564.

Van Donk E., Grimm M.P., Gulati R.D., Heuts P.G.M., De Kloet W.A. & van Liere L. (1990) First attempt to apply whole lake food-web manipulation on a large scale in the Netherlands. *Hydrobiologia*, **200/201**, 291–301.

Vanni M.J. (1987) Effects of nutrients and zooplankton size on the structure of a phytoplankton community. *Ecology*, **68**, 624–635.

Vanni M.J. (2002) Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **33**, 341–370.

Wright D.I. & Shapiro J. (1984) Nutrient reduction by biomanipulation: An unexpected phenomenon and its possible cause. *Verhandlungen, Internationale der Vereinigung Theoretische und Angewandte Limnologie*, **22**, 518–527.

Response of zooplankton to nutrient enrichment and fish in shallow lakes: a pan-European mesocosm experiment

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SUMMARY

1. Responses of zooplankton to nutrient enrichment and fish predation were studied in 1998 and 1999 by carrying out parallel mesocosm experiments in six lakes across Europe.
2. Zooplankton community structure, biomass and responses to nutrient and fish manipulation showed geographical and year-to-year differences. Fish had a greater influence than nutrients in regulating zooplankton biomass and especially the relative abundances of different functional groups of zooplankton. When fish reduced the biomass of large crustaceans, there was a complementary increase in the biomasses of smaller crustacean species and rotifers.
3. High abundance of submerged macrophytes provided refuge for zooplankton against fish predation but this refuge effect differed notably in magnitude among sites.
4. Large crustacean grazers (*Daphnia*, *Diaphanosoma*, *Sida* and *Simocephalus*) were crucial in controlling algal biomass, while smaller crustacean grazers and rotifers were of minor importance. Large grazers were able to control phytoplankton biomass even under hypereutrophic conditions (up to 1600 μg TP L^{-1}) when grazer biomass was high (>80–90 μg dry mass L^{-1}) or accounted for >30% of the grazer community.
5. The littoral zooplankton community was less resistant to change following nutrient enrichment in southern Spain, at high temperatures (close to 30 °C), than at lower temperatures (17–23 °C) characterising the other sites. This lower resistance was because of a greater importance of nutrients than zooplankton in controlling algal biomass.
6. Apart from the reduced role of large crustacean grazers at the lowest latitude, no consistent geographical patterns were observed in the responses of zooplankton communities to nutrient and fish manipulation.

Keywords: eutrophication, fish predation, littoral zooplankton, mesocosm experiments, nutrient enrichment

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Introduction

The outcomes of impacts like nutrient enrichment are reflected in community structure and interactions within the food web. The trophic structure of communities can be seen as the partitioning of biomass

into different levels. It is basically determined by available resources and primary productivity but is also regulated by feedbacks from higher trophic levels, which in turn affect resource availability (Arditi & Ginzburg, 1989; Power, 1992; Leibold *et al.*, 1997; Polis, 1999; Persson *et al.*, 2001). Theories of food-web dynamics focus on the number of trophic levels. They predict relatively discontinuous, alternating (high/low/high) changes in biomasses along the food chain and an increasing number of trophic levels with increasing productivity (Hairston, Smith & Slobodkin, 1960; Fretwell, 1977; Oksanen *et al.*, 1981; Scheffer, 1991).

Such cascading effects between trophic levels have successfully explained incongruous plant/herbivore biomass variation in terms of resource availability. Evidence for strong vertical structuring of aquatic food webs has been gained through manipulations of upper trophic levels (Carpenter, Kitchell & Hodgson, 1985; Carpenter *et al.*, 1987; Horppila *et al.*, 1998; Jeppesen *et al.*, 1998b; Kairesalo *et al.*, 1999) and nutrient availability (Hansson, 1992; Persson *et al.*, 1992; Sarnelle, 1992). However, nutrient manipulations have shown a surprisingly high variability, including positive correlations of two adjacent trophic levels and responses unrelated to the number of trophic levels in the food web (Hansson, 1992; Mazumder, 1994; Brett & Goldman, 1997; Leibold *et al.*, 1997).

Variation in the responses of aquatic plankton communities to nutrient enrichment has indicated the importance of considering not only the number of trophic levels but also the nature of the organisms within them (Leibold *et al.*, 1997; Persson *et al.*, 2001) and variation in the efficiency of consumers to exploit their prey (Power, 1992). Compensatory community responses may account for much of the observed variation, and dampen trophic cascades when species vulnerable to predation are replaced by less vulnerable species. For instance, primary producer biomass may increase with increasing nutrient availability when species composition shifts to inedible species, even in the presence of efficient grazers (Mazumder, 1994; Abrams & Walters, 1996; McCauley *et al.*, 1999). Such declines in the edibility of food with increasing primary productivity may lead to ratio-dependent interactions and proportional changes in biomass at each trophic level (Arditi & Ginzburg, 1989; Power, 1992).

In shallow lakes and the littoral zones of larger lakes, spatial heterogeneity produced by submerged vegetation, coupled with movements of organisms between open water, vegetated, and benthic environments may provide important compensatory mechanisms. Through trophic interactions, this spatial heterogeneity affects water quality and may also buffer effects of nutrient enrichment (Scheffer *et al.*, 1993; Jeppesen *et al.*, 1998c; Scheffer, 1999).

The resistance of aquatic ecosystems to perturbations, such as nutrient enrichment, are controlled by variable environmental factors that are often difficult to predict. These include climate, and its manifestations in weather, an aspect that has received little explicit consideration in field studies, which have mainly been conducted in temperate ecosystems. Most biological processes are temperature dependent (McCauley & Murdoch, 1987; Lehman, 1988; Petchey *et al.*, 1999). Production of zooplankton has been shown to increase with temperature, while biomass accumulation is more dependent on resource availability (Shuter & Ing, 1997). Primary productivity at higher temperatures may become more controlled by nutrient turnover rate. Temperature may influence demography (Arditi & Ginzburg, 1989). Thus, with rising temperature, the inherently greater growth rate of algae compared with that of zooplankton might lead to reduced control of algal biomass by zooplankton. In addition, the threshold food requirement of zooplankton increases with water temperature and, for metabolic reasons, with increasing animal size (Lehman, 1988). This may mean a lower share of large-bodied, typically more efficient grazers in the zooplankton community at higher temperatures. Thus, the resource base may have a major role in the functioning of food webs as temperature increases, such that the cascading top-down effect of fish via zooplankton grazers on phytoplankton could become less important.

In this study, the response of zooplankton communities to experimental manipulation of nutrients and fish was studied in shallow-lake systems by comparing nutrient and plankton dynamics at six field sites across Europe.

We hypothesised (i) that algal biomass is controlled by large crustacean grazers and (ii) that large grazers increase in number or biomass with nutrient enrichment, unless they are controlled by planktivorous fish or submerged macrophytes provide refuges against

fish predation (Jeppesen *et al.*, 1998a; Scheffer, 1999). We aimed to find a threshold fish density below which zooplankton escapes control by fish. Furthermore, we hypothesised (iii) that the loss of large crustaceans is compensated by an increase in the biomass of smaller zooplankton, which exert weaker control of phytoplankton biomass. As we surmised that the resistance of plankton community structure and dynamics to nutrient enrichment is temperature dependent, we hypothesised (iv) that at warmer southern sites, control of phytoplankton by large crustacean zooplankton and the cascading effects of fish via zooplankton on phytoplankton are less important than nutrient control, and vice versa at colder northern sites.

Methods

Study sites and experimental design

Similar mesocosm experiments were carried out simultaneously in 1998 and 1999 (Stephen *et al.*, 2004a). The study lakes were shallow (mean depth about 1 m over the whole lake or over extensive lake areas) and potentially rich in submerged macrophyte vegetation. Experiments were run at 1-m depth at six nearshore sites in Lake Vesijärvi (Finland), Lake Krankesjön (Sweden), Little Mere (England), Lake Naardermeer (The Netherlands), Lake Sentiz (Leon, northern Spain) and Lake Xeresa (Valencia, southern Spain). Hereafter, the study sites are referred to as Finland, Sweden, England, Netherlands, Leon and Valencia.

The mesocosms were 1-m diameter open cylinders made of clear polyethylene suspended from wooden or plastic frames at the surface and sealed into the littoral sediment. The experiments had a fully factorial block design with three levels of fish biomass (0, 4 and 20 g fresh mass m^{-2}) in both years and four levels of nutrient enrichment (0, 1, 5 and 10 mg N L^{-1} combined with 0, 0.1, 0.5 and 1.0 mg P L^{-1}) in 1998 and six levels (0, 0.3, 0.6, 0.9, 1.5 and 3.0 mg N L^{-1} combined with 0, 0.03, 0.06, 0.09, 0.15 and 0.3 mg P L^{-1}) in 1999. Hereafter, the fish treatments are referred to as fish-free, low-fish and high-fish, and nutrient treatments as N0, N1, N2, N3, N4 and N5. The fish were locally the most appropriate planktivorous species of small individual size (5–10 cm). The low-fish treatment had at least two individuals. Each treatment had three

replicates in 1998 and two replicates in 1999, i.e. altogether 36 mesocosms in each year per site. The highest nutrient additions in 1998 resulted in unrealistically hypereutrophic conditions and mortality of fish in one location (see below). We therefore narrowed the nutrient range in 1999.

Samples for zooplankton were taken weekly for 6 weeks with a plastic tube (inner diameter 4 cm) from the whole water column of the mesocosms. Pretreatment samples for zooplankton were taken just before the first nutrient addition and the introduction of fish. Separate sub-samples were taken randomly from different places in each mesocosm to obtain 10 L of water. After mixing this pooled sample, a 3-L sub-sample was filtered through a 25-μm mesh net, and a 7-L sub-sample through a 50-μm mesh net to obtain rotifer and crustacean zooplankton samples, respectively. Zooplankton samples were preserved in 70% ethanol or 4% formaldehyde (final concentration). After counting and length measurement of zooplankton, crustacean biomasses were calculated from either length-dry weight (Bottrell *et al.*, 1976) or length-carbon regressions (Vasama & Kankaala, 1990; Luokkanen, 1995; A. Lehtovaara, pers. comm.). Rotifer carbon contents were obtained from Latja & Salonen (1978) and Telesh, Rahkola & Viljanen (1998). Dry weight of zooplankton was calculated assuming that the carbon content is 40% of dry weight, the average of values reported by Wiebe, Boyd & Cox (1975) and Latja & Salonen (1978). More details of the study lakes, mesocosm experiments, fish species used, sampling protocol and analyses of the samples are available in Stephen *et al.* (2004a,b), and in individual papers describing the results of the experiments (Fernández-Aláez *et al.*, 2004; Hansson *et al.*, 2004; Hietala, Vakkilainen & Kairesalo, 2004; Romo *et al.*, 2004; Stephen *et al.*, 2004b; Van de Bund & Van Donk, 2004).

Data analysis

Zooplankton taxa were grouped into four functional groups: (i) Rotifers; (ii) small crustacean grazers (≤0.5 mm), mainly *Bosmina* spp., *Ceriodaphnia* spp., chydorid cladocerans and copepod nauplii; (iii) large crustacean grazers (>0.5 mm), mainly *Daphnia* spp., *Diaphanosoma brachyurum* Liéven, *Sida crystallina* O.F. Müller, *Simocephalus* spp. and diaptomid calanoid copepods; and (iv) raptorial feeders (*Polyphemus*

pediculus L. and cyclopoid copepods). Time-weighted averages (pretreatment data not included) for zooplankton biomass, total phosphorus (TP) and chlorophyll *a* concentrations (chl *a*) were used for each replicate mesocosm. Macrophyte abundance was classified as negligible, sparse and abundant. The corresponding numeric values used are 0, 1 and 2.

The following data were excluded from analyses: (i) results of fish treatments with the two highest nutrient additions in 1998 in Finland, owing to the complete mortality of fish in those mesocosms; (ii) results from Sweden in 1998 because heavy storms destroyed the mesocosms; (iii) the Netherlands rotifer data because rotifers were counted only in week 6 in 1998; (iv) the 1999 fish treatments in Leon because of high fish mortality and because dense growth of *Myriophyllum alterniflorum* L. made it necessary to take water samples from an artificially separated macrophyte-free area in the centre of each mesocosm; and (v) the England mesocosms in 1999, which accidentally received a 10-fold higher phosphorus enrichment than planned.

All statistical analyses were made using SPSS for Windows (version 10.0). Treatment effects were tested separately for the two study years with MANOVA followed by univariate ANOVA with type III sums of squares and Tukey's test. Time-weighted average biomasses of the four zooplankton functional groups were the response variables, study sites were treated as block effects and nutrient and fish treatments were the factorial variables. The homogeneity of variances was tested with Levene's test and the normality of the data was tested with a Kolmogorov–Smirnov test and on the basis of the residual plots. Log_{10}-transformation was used to normalise the distribution of the data before the statistical analyses. However, the criteria for homoscedasticity were not met even after log-transformation, thus increasing the risk for type I error. Pillai's trace was used in multiple comparisons as this test of significance is the most robust to violations of assumptions (Scheiner, 1993). Non-transformed values are presented in the figures and tables.

In order to study the treatments between study years and sites, the responses of similar nutrient treatments in both study years were compared, i.e. with 0.1 mg P L^{-1}; 1.0 mg N L^{-1} in 1998 and 0.09 mg P L^{-1}; 0.9 mg N L^{-1} in 1999. Between-year comparisons were not possible for the whole enrichment regime owing to the different enrichment levels. The

responses of time-weighted mean zooplankton biomasses (BIOM) to nutrient enrichment and fish density were studied by calculating biomass response factors as follows: log_{10} [(BIOM$_{treatment}$ + 1)/ (BIOM$_{control}$ + 1)]. The response factors were calculated for both fish and nutrient treatments. Using linear regression, the zooplankton biomass response factors were tested for the effects of mean water temperature and macrophyte abundance as independent variables. This approach allowed inclusion of the Netherlands results, which could not be treated in the MANOVA owing to the lack of rotifer data. The use of response factors harmonised the data and, thus enabled comparison of nutrient effects on zooplankton biomasses under different water temperature and macrophyte density (Table 1).

General trends in the relation of zooplankton functional group biomass to chl *a* were studied at individual study sites. Results of both study years were pooled and analysed after log_{10} transformation using linear regression with chl *a* as the dependent variable and zooplankton biomass as the independent variable. Further analyses focused on the relationship between chl *a* and TP at different biomasses of large crustacean grazers. Before all regression analyses, normality of the data was tested on the basis of the residual plots. The overall potential of large crustacean grazers to control phytoplankton was further analysed at different productivity levels, measured as TP concentration, by using the whole data set without separating it into different study sites. Chl *a* was compared with the large crustacean grazer biomass and their percentage of the total grazer biomass. Further analyses aimed to discover whether possible threshold biomasses and shares of large grazers might provide a buffer mechanism and an efficient control of phytoplankton biomass over the whole range of TP concentrations.

Results

Weather conditions in northern and central Europe differed greatly between the study years: summer 1998 was cool and windy, whereas summer 1999 was warm (Table 1). The southernmost study site, Valencia, had the highest water temperature (29 °C) in both years. The ranges of the time-weighted mean chl *a* and TP concentrations in all experiments and treatments were 3–870 and 15–3700 µg L^{-1}, respectively, thus covering a wide range of mesotrophic to

Table 1 Characteristics of mesocosms at six shallow-lake sites across Europe during experiments in 1998 and 1999

Site	Year	Temperature (°C)	TP (μg L⁻¹)	Chl *a* (μg L⁻¹)	Total biomass (μg dry mass L⁻¹)	Dominant zooplankton taxa	Macrophyte abundance	Dominant macrophyte taxa
Finland	1998	17.3 (15–21)	632 (40–3700)	70 (7–200)	1500 (300–4500)	Rotifers, *Mesocyclops*, *Thermocyclops*, *Polyphemus pediculus* L.	Negligible	*Lemna trisulca* L.
Finland	1999	21.3 (17–23)	140 (50–400)	25 (8–65)	670 (330–1280)	*Ceriodaphnia*, *Diaphanosoma brachyurum* Liéven, *Daphnia longispina* O.F Müller, chydorid cladocerans, cyclopoid copepods	Abundant	*Lemna trisulca*
Sweden	1999	21.3 (19–23)	70 (25–280)	50 (3–380)	1240 (430–2840)	*Daphnia*, *Bosmina*, *Ceriodaphnia*, *Eudiaptomus*	Negligible	*Myriophyllum*, *Chara*
England	1998	17.8 (17–19)	500 (120–1250)	50 (10–240)	450 (200–100)	*Daphnia hyalina* Leydig, *Eudiaptomus gracilis* G.O. Sars, *Bosmina*, *Ceriodaphnia*, cyclopoid copepods, rotifers	Sparse	*Potamogeton*, *Elodea canadensis* Michx, *Ceratophyllum demersum* L.
Netherlands	1998	18.5 (17–22)	100 (15–350)	20 (3–90)	40 (1–300)*	*Ceriodaphnia*, *Bosmina*, *Eudiaptomus*	Sparse	*Chara*
Netherlands	1999	22.4	100 (20–360)	30 (6–85)	30 (2–220)*	*Ceriodaphnia*, *Daphnia galeata* Sars, *Diaphanosoma brachyurum*	Abundant	*Chara*
Leon (northern Spain)	1998	23.2 (20–26)	430 (70–1080)	45 (5–120)	3400 (1270–8400)	*Ceriodaphnia*, chydorid cladocerans, *Daphnia longispina*, *Eucyclops*, *Macrocyclops*, *Megacyclops*	Abundant	*Myriophyllum alterniflorum* L.
Leon (northern Spain)	1999	19.7 (18–21)	65 (40–130)	20 (10–35)	760 (160–2450)	*Ceriodaphnia*, chydorid cladocerans, *Eucyclops*, *Macrocyclops*, *Megacyclops*	Abundant	*Myriophyllum alterniflorum*
Valencia (southern Spain)	1998	28.7 (28–29)	400 (20–1150)	230 (3–870)	600 (50–1800)	Rotifers, *Ceriodaphnia*, *Acanthocyclops*	Abundant	*Chara*
Valencia (southern Spain)	1999	28.7 (27–30)	80 (15–230)	50 (6–180)	555 (65–1260)	Rotifers, *Ceriodaphnia*, *Acanthocyclops*, *Thermocyclops*	Abundant	*Chara*

*Biomass available only for total crustacean zooplankton.

Mean values with ranges in parentheses are provided for temperature. Time-weighted mean values with ranges in parentheses are given for total phosphorus (TP) and chlorophyll *a* (Chl *a*) concentrations and for total zooplankton biomasses.
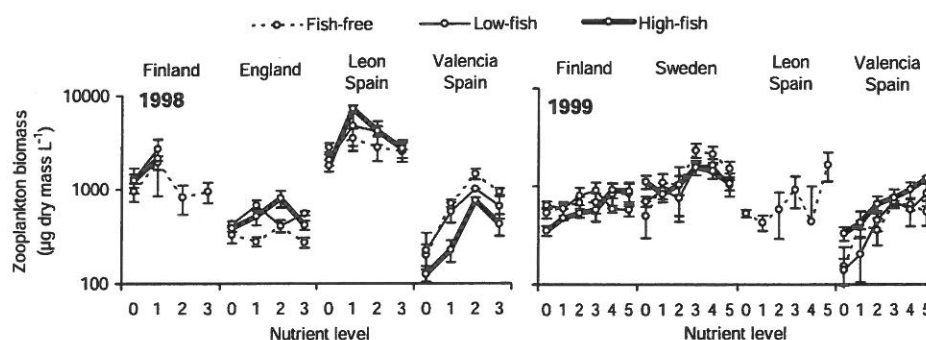
Fig. 1 Time-weighted mean (±SE) of total zooplankton biomass in different nutrient and fish treatments of a series of mesocosm experiments carried out at six shallow-lake sites across Europe in 1998 and 1999. Nutrient and fish treatments are given in sequential order according to the levels detailed in the text.

hypereutrophic conditions (Table 1). The species composition and abundance of macrophyte and zooplankton communities varied among sites and years and there was no obvious geographical pattern (Table 1). The cladoceran community was mostly characterised by small genera such as *Ceriodaphnia*, *Bosmina* and chydorid cladocerans, which often occurred together with cyclopoid copepods. *Daphnia* was found only in a few experiments, while *Diaphanosoma* was a quite common large-bodied cladoceran at many sites (Table 1). Total zooplankton biomasses reached higher values in 1998, when nutrient manipulations included greater enrichment than in 1999 (Table 1, Fig. 1). They also showed an increasing trend with nutrient enrichment in both years and in 1998, when the greatest enrichment exceeded that in 1999, the highest biomasses were often measured at intermediate nutrient levels (Fig. 1).

Nutrient enrichment had a marginally significant multivariate effect on zooplankton only in 1998, when it increased the biomass of small crustacean grazers and, in all sites except Valencia, decreased that of raptorials (Table 2; Fig. 2). The responses of large crustacean grazers to nutrient enrichment were mostly negligible in fish-free treatments, and negative in fish treatments (Fig. 2). Thus, neither the main effect of nutrients nor the joint effect of nutrients and fish were significant on large grazers.

Fish had a highly significant multivariate effect on zooplankton in both years (Table 2). In general, the biomass of rotifers increased, and those of all other functional groups decreased with increasing fish density (Table 2; Fig. 2). Differences in the biomass of almost all zooplankton groups were significant between no-fish and high-fish treatments as well as between no-fish and low-fish treatments (Tukey's test).

Table 2 MANOVA and ANOVA results of effects of treatments (nutrients and fish) and block (site) on time-weighted averages of zooplankton biomass in mesocosm experiments carried out at four shallow-lake sites across Europe in 1998 and 1999

Year	Source of effect	Pillai's trace			Anova	Rotifers		Small crustacean grazers		Large crustacean grazers		Raptorials	
		d.f.	F	P	d.f.	F	P	F	P	F	P	F	P
1998	Nutrient	12	1.8	0.052	3	1.4	0.245	2.9	0.038	1.2	0.308	3.1	0.030
	Fish	8	14.0	<0.001	2	33.1	<0.001	10.6	<0.001	44.0	<0.001	19.1	<0.001
	Nutrient × fish	24	0.8	0.743	6	1.4	0.221	0.9	0.519	1.2	0.326	0.6	0.748
	Site	12	41.8	<0.001	3	98.3	<0.001	52.0	<0.001	57.7	<0.001	28.4	<0.001
1999	Nutrient	20	0.9	0.594	5	0.7	0.652	1.2	0.322	1.4	0.242	1.3	0.271
	Fish	8	9.1	<0.001	2	34.2	<0.001	14.2	<0.001	9.6	<0.001	12.5	<0.001
	Nutrient × fish	40	0.7	0.903	10	0.4	0.922	0.2	0.992	1.9	0.057	0.3	0.985
	Site	12	27.0	<0.001	3	52.2	<0.001	90.2	<0.001	398.5	<0.001	120.4	<0.001

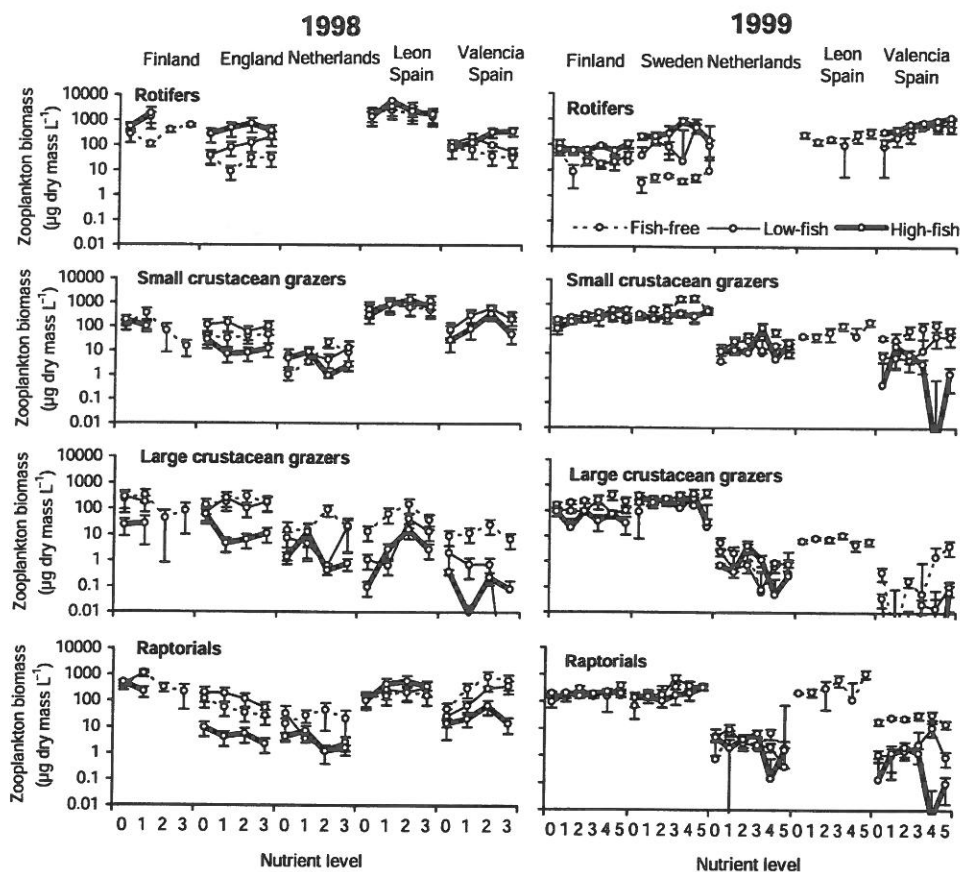Results are based on $\log_{10}$-transformed variables. The Netherlands' results were excluded from this analysis.

Fig. 2 Time-weighted mean (±SE) biomasses of four zooplankton functional groups in different nutrient and fish treatments of a series of mesocosm experiments carried out at six shallow-lake sites across Europe in 1998 and 1999. Nutrient and fish treatments are given in sequential order according to the levels detailed in the text.

Zooplankton biomass differed among study sites, resulting in a highly significant multivariate main effect (Table 2). Lowest total biomasses were found in Valencia, the southernmost site, which differed greatly from Leon, the other Spanish site, and also from almost all other sites (Figs 1 & 2). Conversely, at the two northernmost sites in Finland and Sweden, the zooplankton communities resembled each other in 1999 (Fig. 2). The response factors of total zooplankton biomass showed a consistently positive effect of nutrients, whereas the effect of fish was variable (Fig. 3). Similarly to MANOVA and ANOVA, the response factors of distinct functional groups showed strong effects of fish and less clear effects of nutrients. In the absence of fish, crustacean grazers and raptorials responded positively to nutrient enrichment, rotifers mostly negatively. In fish treatments, however, the pattern was much more variable with both positive and negative responses without clear geographical trends. In Valencia, zooplankton biomasses increased with nutrients in all fish treatments, with the exception of large crustacean grazers. The Netherlands results were generally in line with those of the other sites (Fig. 3). An exception was the positive responses of large crustacean grazers in high-fish treatments. The response factors of small crustacean grazers to nutrients were significantly related to mean water temperature (linear regression analysis; $R^2 = 0.26$; $F_{1,27} = 9.08$; $P = 0.006$). High water temperatures in 1999 (Table 1) apparently enhanced the reproduction of small crustaceans in Finland, Sweden and The Netherlands. At the high temperature in Valencia, small crustaceans increased with nutrients in both study years (Fig. 3).
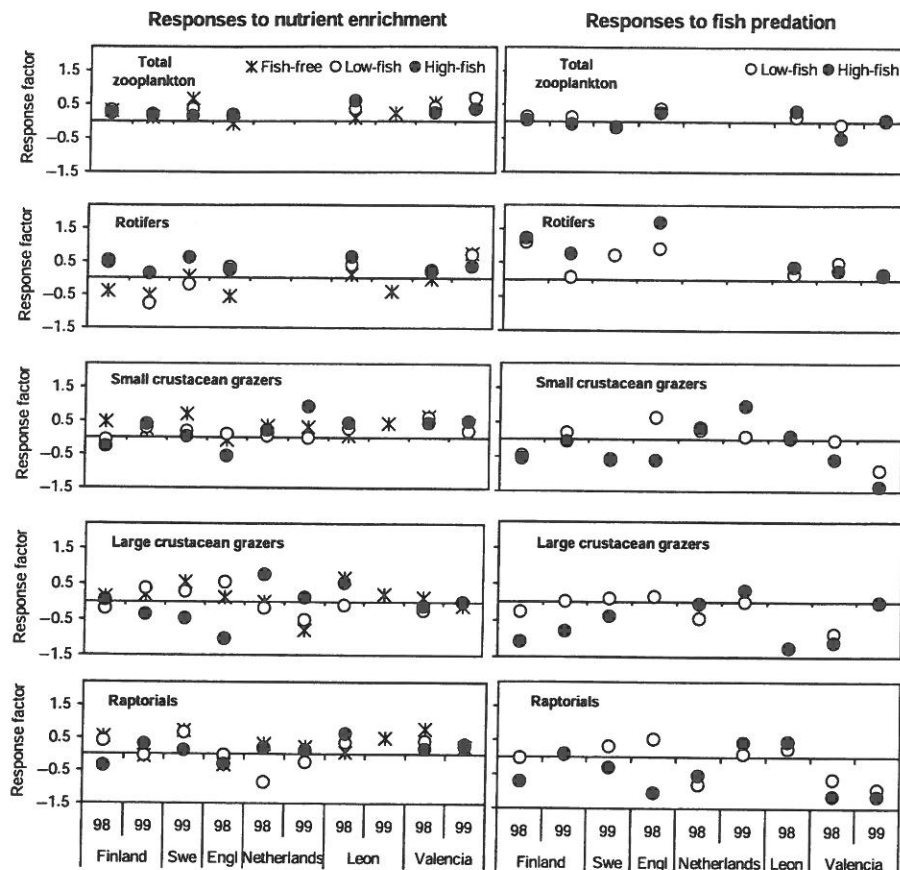
Fig. 3 Response factors of the biomasses of total zooplankton and four zooplankton functional groups for nutrient (left) and fish (right) treatments of a series of mesocosm experiments carried out at six shallow-lake sites across Europe in 1998 and 1999 with similar nutrient additions, i.e. 0.1 mg P + 1 mg N L^{-1} in 1998 and 0.09 m P + 0.9 mg N L^{-1} in 1999. Abbreviations: Swe, Sweden; Engl, England.

Interactions between zooplankton and phytoplankton

Linear regressions of log-transformed data showed in general a positive or negligible relationship between chl *a* and biomass of both rotifers and small crustacean grazers (Table 3). Chl *a* decreased with increasing biomass of small crustacean only in Finland and England. In contrast, the biomasses of large crustacean grazers were inversely related to chl *a*, except in Valencia where chl *a* was unrelated to the biomass of large crustacean grazers (Table 3). Thus, the potential of large grazers to control phytoplankton biomass was shown at all study sites except Valencia.

Extremely high chl *a* concentrations (≥190–200 µg L^{-1}) were observed when large grazers accounted for less than about 30% of total zooplankton biomass (Fig. 4a). At higher grazer biomasses,

there was only one outlying point. A similar picture was obtained when comparing chl *a* concentration with the biomass of large grazers, with only two outlying points at grazer biomasses >80–100 µg dry mass L^{-1} (Fig. 4b). At a biomass of large grazers >80 µg dry mass L^{-1}, chl *a* concentration increased only slightly with increasing TP concentration (slope = 0.22, R^2 = 0.09, P = 0.005, n = 83; linear regression analysis of log-transformed data) and the mean chl *a* concentration was 27 µg L^{-1} with a SE of ±3 (Fig. 4d). At lower grazer biomasses, there was a significant relationship between chl *a* and TP (slope = 0.56, R^2 = 0.35, P < 0.001, n = 167) and the mean chl *a* concentration was 96 µg L^{-1} with a SE of ±11.

With a >30% share of large grazers, the slope between chl *a* and TP was 0.27 (R^2 = 0.18, n = 52)

Table 3 Results of linear regressions describing relationships between zooplankton biomass and chl *a* (log$_{10}$-transformed) determined in a series of mesocosm experiments at six shallow-lake sites across Europe

Dependent variable	Site, years	d.f.	slope	R^2	F	P
Rotifers	Finland, 1998 & 1999	59	0.29	0.29	24.1	<0.001
	Sweden, 1999	35	0.17	0.07	2.6	0.12
	England, 1998	35	0.35	0.53	38.8	<0.001
	Leon, 1998 & 1999	47	0.21	0.09	4.5	0.039
	Valencia, 1998 & 1999	71	−0.13	0.01	0.6	0.43
Small crustacean grazers	Finland, 1998 & 1999	59	−0.19	0.07	4.1	0.046
	Sweden, 1999	35	0.26	0.03	0.9	0.36
	England, 1998	35	−0.25	0.18	7.6	0.009
	Netherlands, 1998 & 1999	71	0.12	0.03	1.9	0.18
	Leon, 1998 & 1999	47	0.20	0.06	3.2	0.08
	Valencia, 1998 & 1999	71	0.32	0.20	17.9	<0.001
Large crustacean grazers	Finland, 1998 & 1999	59	−0.19	0.12	7.6	0.008
	Sweden, 1999	35	−0.67	0.30	14.3	0.001
	England, 1998	35	−0.33	0.50	34.4	<0.001
	Netherlands, 1998 & 1999	71	−0.41	0.33	34.3	<0.001
	Leon, 1998 & 1999	47	−0.35	0.37	27.3	<0.001
	Valencia, 1998 & 1999	71	0.28	0.04	2.7	0.11

(Fig. 4c). Predictability of phytoplankton control was not increased further by raising the threshold biomass of large grazers from 80 to 100 μg DW L^{-1}. Moreover, the lower number of observations containing these high biomasses likely augmented the possibility of coincidence. In contrast, when the biomass thresholds were lowered, the slope increased, e.g. to 0.26 (R^2 = 0.11) with a large grazer biomass of ≥60 μg DW L^{-1}.

Similarly, when the 30% threshold of large grazers was shifted to ≥20 and ≥40%, the slopes changed to 0.34 (R^2 = 0.21, n = 71) and 0.26 (R^2 = 0.18, n = 42), respectively.

Changes in chl *a* over the TP concentration range were explained better by the abundance of large grazers than by the presence or absence of fish (Fig. 4e). There was a significant relationship between
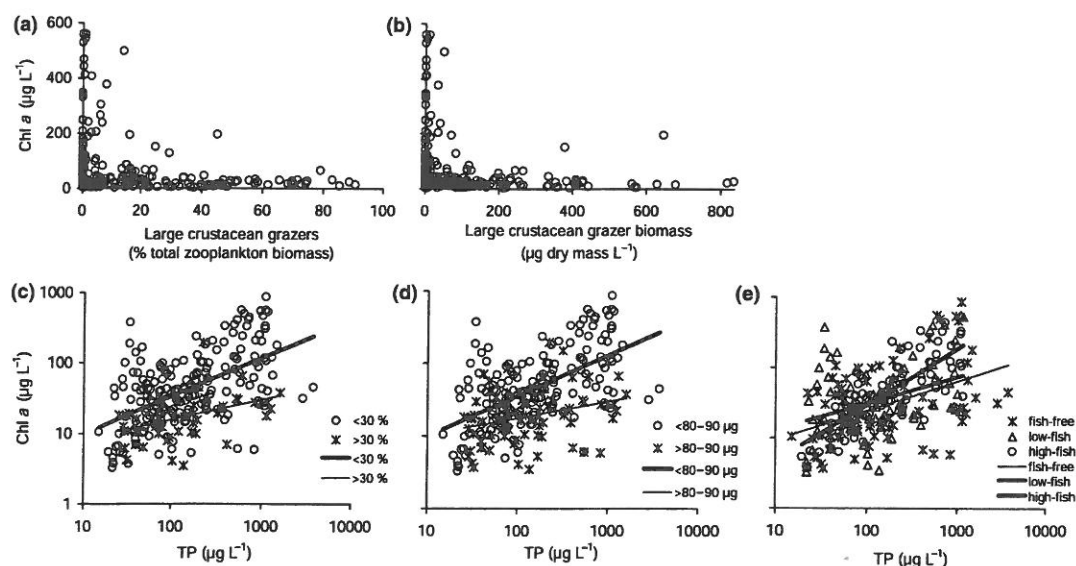
Fig. 4 Relationship between chlorophyll *a* concentration (chl *a*) and the (a) biomass share and (b) absolute biomass of large crustacean grazers; chl *a* and the total phosphorus concentration (TP) in treatments with a biomass of large grazers below and above (c) 30% of the total zooplankton biomass and (d) 80–90 μg dry mass L^{-1}; and (e) chl *a* and TP at three fish densities. Data are from mesocosm experiments carried out at six shallow-lake sites across Europe in 1998 and 1999. Note the logarithmic presentation of both axes in Fig. 4c–e.

chl *a* and TP even in fish-free treatments (slope = 0.41, $R^2 = 0.21$, $P < 0.001$, $n = 95$). This relationship did not markedly change in low-fish treatment (slope = 0.37, $R^2 = 0.13$, $P = 0.001$, $n = 77$), but a stronger response was observed in the high-fish treatment (slope = 0.78, $R^2 = 0.78$, $P < 0.001$, $n = 77$).

Discussion

The results of this pan-European mesocosm experiment emphasise the importance of consumer control over resource control in determining zooplankton biomass in shallow lake systems. Thus, our results are in agreement with the general view that (large) herbivores respond strongly to predator manipulations in aquatic food webs (Brett & Goldman, 1997; Mehner *et al.*, 2002; Shurin *et al.*, 2002). Rotifers increased with nutrient enrichment in fish treatments, while small crustacean grazers showed positive responses to nutrient enrichment largely independently of the presence or absence of fish. Small zooplankton species apparently benefited from the mostly negative responses of their larger competitors and raptorial predators to the presence of fish. Thus, within zooplankton communities different functional groups responded inconsistently to the treatments. Such compensation suggests a ratio-dependent functional response and is consistent with the view that nutrient enrichment leads to proportional increases at all trophic levels (Arditi & Ginzburg, 1989; Leibold *et al.*, 1997).

The traditional equilibrium theory of food chain dynamics and its modifications (Hairston *et al.*, 1960; Fretwell, 1977; Oksanen *et al.*, 1981; Scheffer, 1991) emphasises the control of lower trophic levels by consumers, and predicts that effects of nutrient enrichment are transferred into biomass at the top trophic level and even-numbered levels below it. Accordingly, zooplankton biomass increases with enhanced primary productivity in two-level systems (phytoplankton and herbivorous zooplankton) but does not change in three-level systems (phytoplankton, herbivorous zooplankton and planktivorous fish). In contrast to this theory, the higher zooplankton biomass we observed in fish treatments compared with fish-free treatments, especially in 1998, shows that total zooplankton biomass may increase with nutrients independently of the number of trophic links in the food chain. Several field and laboratory studies of aquatic food chains have shown similar

increases with nutrient enrichment in both two- and three-level systems (Mazumder, 1994; Leibold *et al.*, 1997; Persson *et al.*, 2001). In our study, available food resources appeared to determine total zooplankton biomass, whereas fish regulated relative abundances of different functional groups. This emphasises the importance of compositional changes within trophic levels and reveals the limitation of simple food-chain and ratio-dependent models to capture such changes, as pointed out by Leibold *et al.* (1997) and Hulot *et al.* (2000). In addition, our result that algal biomass was positively related with TP concentration even in the presence of efficient grazers has often been observed in previous studies (Hansson, 1992; Mazumder, 1994; Brett & Goldman, 1997; Leibold *et al.*, 1997; Shuter & Ing, 1997; Persson *et al.*, 2001). It reflects the positive response of adjacent trophic levels and, therefore, is not consistent with traditional food-chain theory.

The variability of responses to fish and nutrient manipulations was also influenced by substantial variation in community structure among sites. For instance, in Leon, the biomass of large crustacean grazers in 1998 increased with nutrients even when fish density was high, most probably through the refuge effect of the abundant growth of *Myriophyllum* (Timms & Moss, 1984; Jeppesen *et al.*, 1998a; Scheffer, 1999; Burks *et al.*, 2002). In shallow lakes and littoral zones, refuges provided to zooplankton by macrophytes work as an important buffering mechanism to changes in fish predation pressure (Jeppesen *et al.*, 1998c). *Chara* beds have also been considered as good refuges for zooplankton against fish predation (Diehl, 1988; Jeppesen *et al.*, 1998a), but our results do not support this view as *Chara* at the study sites in Valencia and Netherlands did not prevent efficient foraging of fish for large crustacean grazers.

There were substantial differences in zooplankton community structure and biomass among study sites, and even between study years at the same site. Nonetheless, some consistent patterns were evident. Valencia in southern Spain differed from the other sites in that zooplankton had a minor influence on phytoplankton dynamics. Nutrient enrichment apparently increased algal growth rate more than that of zooplankton and thus disrupted top-down control of algae (cf. Arditi & Ginzburg, 1989; Power, 1992). In addition, as Power (1992) pointed out, if grazers were subject to predation, the grazer control of phytoplankton would further weaken relative to resource control.

Our result supports this hypothesis because fish efficiently reduced the biomasses of large grazers in Valencia and the remaining small grazers were unable to control algal biomass.

Daphnia has been suggested as a keystone species and its abundance as a useful indicator to predict the grazing impact of zooplankton on phytoplankton (Hansson, 1992; Sarnelle, 1992; Mazumder, 1994; Persson *et al.*, 2001). Supporting evidence for food-chain theory has been gained especially from open-water systems with *Daphnia* as the key grazer (Carpenter *et al.*, 1985, 1987; Jeppesen *et al.*, 1998b). In our experiments, however, *Daphnia* was relatively sparse and virtually absent in fish treatments. Its biomass was therefore pooled with that of other large grazers. The potential of large grazers to control phytoplankton biomass was shown at all study sites except Valencia. The share of large crustacean grazers of the whole grazer biomass had a clear inverse relationship with chl *a* concentration. A consistent result was obtained when chl *a* concentration was related to the biomass of large grazers. When abundant, these large grazers efficiently controlled phytoplankton biomass even under hypertrophic conditions (up to 1600 µg TP L^{-1}). Otherwise, chl *a* concentration increased steeply with increasing TP concentration. Sarnelle (1992), moreover, found even stronger differences in the chl *a*/TP relationship in systems with both high and negligible biomass of *Daphnia*. Our empirically obtained threshold biomass (80–90 µg dry mass L^{-1}) is close to that (ca 100 µg dry mass L^{-1}) at which Hansson (1992) found an efficient control of algal biomass by grazers.

Increase in the biomass of small crustaceans and rotifers was generally associated with an increase in chl *a*, indicating the low ability of small zooplankton to control total phytoplankton biomass. In Finland and England, however, even small grazers such as *Ceriodaphnia* were apparently able to restrain algal biomass. In Finland, this control was probably enhanced by high abundance of the free-floating macrophyte, *Lemna trisulca* L. (Hietala *et al.*, 2004). Rotifers are highly specialised suspension-feeders and are not able to control total algal biomass, at least in eutrophic systems, because they feed mainly on nanoplankton such as bacteria, flagellates, ciliates, and small algae (Pourriot, 1977; Gilbert & Bogdan, 1984). In addition, rotifers probably recycle nutrients more effectively than larger crustaceans (Vanni, 2002) and therefore further enhance the availability of nutrients to algae.

Nonetheless, the food-web role of rotifers is emphasised under turbid conditions. Through providing food for young-of-the-year (YOY) fish, they may maintain a high recruitment of zooplanktivorous fish. The proliferation of small crustaceans and rotifers in our experiment was probably related to their lower susceptibility to fish predation. Additionally, their shorter generation times compared with larger crustaceans allowed the smaller zooplankton to respond quickly to nutrient enrichment. On the contrary, it is unlikely that life-history constraints affected the nutrient enrichment responses of the larger zooplankton during the 5-week experiments as with an average growth rate of 0.3 day^{-1} populations turned over at least 15 times. Suppression of large herbivores at higher water temperature (Beisner, McCauley & Wrona, 1997) and/or in enriched systems (McCauley *et al.*, 1999) has been explained by increases in inedible algae. However, no such consistent dominance of inedible algae (i.e. forms having a Greatest Axial or Linear Dimension, GALD, of ≥50 µm) was observed in our enriched or high-temperature mesocosms (Van de Bund *et al.*, 2004).

A steep slope between chl *a* and TP concentration suggests a cascading effect of fish through herbivorous zooplankton on phytoplankton in systems with three trophic levels (Hansson, 1992; Mazumder, 1994). Our results from high-fish treatments agree with these predictions, although the slope in low-fish treatments was less steep and similar to that in fish-free treatments. However, the abundance of large crustacean grazers explained the relationship between chl *a* and TP better than did the mere number of trophic levels. This can be explained by the fact that our experimental systems involved several hypereutrophic fish-free systems with low biomass of large grazers, as well as several low-fish systems with high biomass of large grazers. In addition, small crustacean grazers that were not heavily preyed upon by fish probably had at least a complementary role in controlling phytoplankton biomass. Thus, our results show that high fish biomass (20 g dry mass m^{-2}) leads to generally low grazer biomass and consistently to high algal biomass with increasing nutrient availability. However, when fish biomass is low (4 g dry mass m^{-2}) grazer biomass can be high and control algal biomass in shallow-lake systems.

In conclusion, fish had a greater role than nutrients in regulating zooplankton biomass and especially

relative abundances of zooplankton functional groups. Large crustacean grazers were of crucial importance in controlling algal biomass even under hypereutrophic conditions provided that their biomass proportion or absolute biomass was high. This result supports our first hypothesis that algal biomass is controlled by large crustacean grazers. However, our second hypothesis that large crustaceans increase in number or biomass with nutrient enrichment either in the absence of fish or when protected from fish predation by submerged macrophytes, was not supported. Although abundant submerged macrophytes often provided refuges for zooplankton against fish predation as expected, differences in the magnitude of the refuge effect occurred at different sites. These may be because of differences in macrophyte species, fish species or other factors that varied among sites. In agreement with our third hypothesis, the biomass of large crustaceans was reduced when fish were present and smaller zooplankton species with minor or complementary importance in controlling phytoplankton biomass became more abundant. Our fourth hypothesis that at southern, warmer sites control of primary producers by large crustacean zooplankton and cascading effects of fish via zooplankton grazers are less important than nutrients, and vice versa at the northern cooler sites, was supported by the Valencia results; the potential of zooplankton, especially that of large crustacean grazers, to control phytoplankton biomass was evident at all other sites. Apart from the reduced role of large crustacean grazers in Valencia, no consistent geographical patterns were observed in the responses of zooplankton communities to nutrient and fish manipulation.

Acknowledgments

References

Abrams P.A. & Walters C.J. (1996) Invulnerable prey and the paradox of enrichment. *Ecology*, **77**, 1125–1133.

Arditi R. & Ginzburg L.R. (1989) Coupling in predator-prey dynamics: ratio-dependence. *Journal of Theoretical Biology*, **139**, 69–75.

Beisner B.E., McCauley E. & Wrona F.J. (1997) The influence of temperature and food chain length on plankton predator-prey dynamics. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 586–595.

Bottrell H.H, Duncan A., Gliwicz Z.M., Grygierek E., Herzig A., Hillbricht-Ilkowska A., Kurasawa H., Larsson P. & Wieglenska T. (1976) A review of some problems in zooplankton production studies. *Norwegian Journal of Zoology*, **24**, 419–456.

Brett M.T. & Goldman C.R. (1997) Consumer versus resource control in freshwater pelagic food webs. *Science*, **275**, 384–386.

Burks R.L., Lodge D.M., Jeppesen E. & Lauridsen L.L. (2002) Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. *Freshwater Biology*, **47**, 343–365.

Carpenter S.R., Kitchell J.F. & Hodgson J.R. (1985) Cascading trophic interactions and lake productivity. *BioScience*, **35**, 634–639.

Carpenter S.R., Kitchell J.F., Hodgson J.R., Cochran P.A., Elser J.J., Elser M.M., Lodge D.M., Kretchmer D., He X. & von Ende C.N. (1987) Regulation of lake primary productivity by food web structure. *Ecology*, **68**, 1863–1876.

Diehl S. 1988. Foraging efficiency of three freshwater fishes: effects of structural complexity and light. *Oikos*, **53**, 207–214.

Fernández-Aláez M., Fernández-Aláez C., Bécares E., Valentín M., Goma J. & Castrillo P. (2004) A 2-year experimental study on nutrient and predator influences on food web constituents in a shallow lake of north-west Spain. *Freshwater Biology*, **49**, 1574–1592.

Fretwell S.D. (1977) The regulation of plant communities by food chains exploiting them. *Perspectives in Biology and Medicine*, **20**, 169–185.

Gilbert J.J. & Bogdan K.G. (1984) Rotifer grazing: *in situ* studies on selectivity and rates. In: *Trophic Interactions within Aquatic Ecosystems* (Eds D.G. Meyers & J.R. Strickler). *AAAS Selected Symposium*, **85**, 97–133.

Hairston N.G., Smith F.E. & Slobodkin L.B. (1960) Community structure, population control and competition. *American Naturalist*, **94**, 421–425.

Hansson L.-A. (1992) The role of food chain composition and nutrient availability in shaping algal biomass development. *Ecology*, **73**, 241–247.

Hansson L.-A., Gyllström M., Ståhl-Delbanco A. & Svensson M. (2004) Responses to fish predation and nutrients by plankton at different levels of taxonomic resolution. *Freshwater Biology*, **49**, 1538–1550.

Hietala J., Vakkilainen K. & Kairesalo T. (2004) Community resistance and change to nutrient enrichment and fish manipulation in a vegetated lake littoral. *Freshwater Biology*, **49**, 1525–1537.

Horppila J., Peltonen H., Malinen T., Luokkanen E. & Kairesalo T. (1998) Top-down or bottom-up effects of fish: issues of concern in biomanipulation of lakes. *Restoration Ecology*, **6**, 20–28.

Hulot F.D., Lacroix G., Lescher-Moutoué F. & Loreau M. (2000) Functional diversity governs ecosystem response to nutrient enrichment. *Nature*, **405**, 340–344.

Jeppesen E., Lauridsen T.L., Kairesalo T. & Perrow M.R. (1998a) Impact of submerged macrophytes on fish-zooplankton interactions in lakes. In: *The Structuring Role of Submerged Macrophytes in Lakes* (Eds E. Jeppesen, Ma. Søndergaard, Mo. Søndergaard & K. Christoffersen), pp. 397–406. Springer-Verlag, New York.

Jeppesen E., Søndergaard M., Jensen J.P., Mortensen E., Hansen A.-M. & Jørgensen T. (1998b) Cascading trophic interactions from fish to bacteria and nutrients after reduced sewage loading: An 18-year study of a shallow hypertrophic lake. *Ecosystems*, **1**, 250–267.

Jeppesen E., Søndergaard Ma., Søndergaard Mo. & Christoffersen K. (1998c) *The Structuring Role of Submerged Macrophytes in Lakes.* Springer-Verlag, New York.

Kairesalo T., Laine S., Luokkanen E., Malinen T. & Keto J. (1999) Direct and indirect mechanisms behind successful biomanipulation. *Hydrobiologia*, **395/396**, 99–106.

Latja R. & Salonen K. (1978) Carbon analysis for the determination of individual biomasses of planktonic animals. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, **20**, 2556–2560.

Lehman J.T. (1988) Ecological principles affecting community structure and secondary productivity by zooplankton in marine and freshwater environments. *Limnology and Oceanography*, **33**, 931–945.

Leibold M.A., Chase J.M., Shurin J.B. & Downing A.L. (1997) Species turnover and the regulation of trophic structure. *Annual Review of Ecology and Systematics*, **28**, 467–494.

Luokkanen E. (1995) *The Species Composition, Biomass and Production of the Pelagic Cladoceran Community in the Enonselkä Basin of Lake Vesijärvi.* University of Helsinki, Research and education center of Lahti, Report No. 25 (in Finnish with English summary).

Mazumder A. (1994) Patterns of algal biomass in dominant odd- vs. even-link lake ecosystems. *Ecology*, **75**, 1141–1149.

McCauley E. & Murdoch W.W. (1987) Cyclic and stable populations: plankton as paradigm. *American Naturalist*, **129**, 97–121.

McCauley E., Nisbet R.M., Murdoch W.W., Roos A.M. & Gurney W.S.C. (1999) Large-amplitude cycles of *Daphnia* and its algal prey in enriched environments. *Nature*, **402**, 653–656.

Mehner T., Benndorf J., Kasprzak P. & Koschel R. (2002) Biomanipulation of lake ecosystems: successful applications and expanding complexity in the underlying science. *Freshwater Biology*, **47**, 2453–2465.

Oksanen L., Fretwell S.D., Arruda J. & Niemelä P. (1981) Exploitation ecosystems in gradients of primary productivity. *American Naturalist*, **118**, 240–261.

Persson L., Diehl S., Johansson L., Andersson G. & Hamrin S.F. (1992) Trophic interactions in temperate lake ecosystems: a test of food chain theory. *American Naturalist*, **140**, 59–84.

Persson A., Hansson L.-A., Brönmark C., Lundberg P., Pettersson L.B., Greenberg L., Nilsson P.A., Nyström P., Romare P. & Tranvik L. (2001) Effects of enrichment on simple aquatic food webs. *American Naturalist*, **157**, 654–669.

Petchey O.L., McPhearson T., Casey T.M. & Morin P.J. (1999) Environmental warming alters food-web structure and ecosystem function. *Nature*, **402**, 69–72.

Polis G.A. (1999) Why are parts of the world green? Multiple factors control productivity and the distribution of biomass. *Oikos*, **86**, 3–15.

Pourriot R. (1977) Food and feeding habits of Rotifera. *Archiv für Hydrobiologie – Advances in Limnology*, **8**, 243–260.

Power M.E. (1992) Top-down and bottom-up forces in food webs: do plants have primacy? *Ecology*, **73**, 733–746.

Romo S., Miracle M.R., Villena M.-J., Rueda M., Ferriol C. & Vicente E. (2004) Mesocosm experiments on nutrient and fish effects on shallow lake food webs in a Mediterranean climate. *Freshwater Biology*, **49**, 1593–1607.

Sarnelle O. (1992) Nutrient enrichment and grazer effects on phytoplankton in lakes. *Ecology*, **73**, 551–560.

Scheffer M. (1991) Fish and nutrients interplay determines algal biomass: a minimal model. *Oikos*, **62**, 271–282.

Scheffer M. (1999) The effect of aquatic vegetation on turbidity: how important are the filter feeders? *Hydrobiologia*, **408/409**, 307–316.

Scheffer M., Hosper S.H., Meijer M-L., Moss B. & Jeppesen E. (1993) Alternative equilibria in shallow lakes. *Trends in Ecology and Evolution*, **8**, 275–279.

Scheiner S.M. (1993) MANOVA: Multiple response variables and multispecies interactions. In: *Design and*

Analysis of Ecological Experiments (Eds S.M. Scheiner & J. Gurevitch). pp. 94–112, Chapman & Hall, New York.

Shurin J.B, Borer E.T., Seabloom E.W., Anderson K., Blanchette C.A., Broitman B., Cooper S.D. & Halpern B.S. (2002) A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters*, **5**, 785–791.

Shuter B.J. & Ing K.K. (1997) Factors affecting the production of zooplankton in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 359–377.

Stephen D., Balayla D., Bécares E. *et al.* (2004a) Continental-scale patterns of nutrient and fish effects on shallow lakes: introduction to a pan-European mesocosm. *Freshwater Biology*, **49**, 1517–1524.

Stephen D., Balayla D.M., Collings S.E. & Moss B. (2004b) Two mesocosm experiments investigating the control of summer phytoplankton growth in a small shallow lake. *Freshwater Biology*, **49**, 1551–1564.

Telesh I.V., Rahkola M. & Viljanen M. (1998) Carbon content of some freshwater rotifers. *Hydrobiologia*, **387/388**, 355–360.

Timms R.M. & Moss B. (1984) Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. *Limnology and Oceanography*, **29**, 472–486.

Van de Bund W. & Van Donk E. (2004) Effects of fish and nutrient additions on food-web stability in a charophyte-dominated lake. *Freshwater Biology*, **49**, 1565–1573.

Van de Bund W.J., Romo S., Villena M.J. *et al.* (2004) Responses of phytoplankton to fish predation and nutrient loading in shallow lakes: a pan-European mesocosm experiment. *Freshwater Biology*, **49**, 1608–1618.

Vanni M.J. (2002) Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **33**, 341–370.

Vasama A. & Kankaala P. (1990) Carbon-length regressions of planktonic crustaceans in Lake Ala-Kitka (NE-Finland). *Aqua Fennica*, **20**, 95–102.

Wiebe P.H., Boyd S. & Cox J.L. (1975) Relationships between zooplankton displacement volume, wet weight, dry weight and carbon. *Fishery Bulletin*, **73**, 777–786.

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No. 3   2001 ONKILA HANNU: Tuhkan ja sen sisältämän kadmiumin vaikutukset metsämaan änkyrimato-
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ombrotrofiseen suokasvillisuuteen. (pro gradu)
Effects of elevanted CO2 and increased N deposition on ombrotrofic bog vegetation

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