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PRIMARY RESEARCH PAPER

Could artificial plant beds favour microcrustaceans during biomanipulation of eutrophic shallow lakes?

David Balayla · Thomas Boll · Carolina Trochine · Erik Jeppesen

Abstract Introduction of artificial plants may facilitate the transition from a turbid to a clear-water state in shallow lakes, particularly when plant establishment is delayed. We investigated the usefulness of artificial plants as a restoration tool in an experimental setup mimicking open submerged plant beds with high plant density [80%, HPD] and low plant density [20%, LPD] in shallow Lake Vaeng, Denmark, having undergone biomanipulation in the form of extensive fish removal. Biological measures of the fish, and of both free-swimming (FSM) and plant-attached microcrustaceans (PAM) within the experimental beds and in the lake, were obtained from before, during and after biomanipulation. We found that microcrustacean measures (density, biomass and Cladocera:FSM) were significantly larger in the HPD beds, before and during fish removal, while the effect of plants was not significant after biomanipulation, with low fish biomass. On PAM, these effects were less pronounced and only significant after biomanipulation. Microcrustaceans were larger-bodied at HPD in all years, for both FSM and PAM. In conclusion, artificial plant beds acted as an effective microcrustacea refuge against fish, particularly for the FSM at HPD and in the years with high fish densities, providing further evidence that artificial plant beds could assist lake restoration efforts.

Keywords Microcrustacea · Fish predation · Cladocerans · Plant-associated microcrustaceans · Body size

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D. Balayla (✉) · T. Boll · C. Trochine ·
E. Jeppesen (✉)
Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark
e-mail: balayla@liverbula.com

E. Jeppesen
e-mail: ej@bios.au.dk

T. Boll
Institute of Biology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark

E. Jeppesen
Arctic Research Centre, Aarhus University, Building 1540, Aarhus C, Denmark

E. Jeppesen
Sino-Danish Centre for Education and Research (SDC), Beijing, China

C. Trochine
Laboratorio de Limnología, Instituto de Investigaciones en Biodiversidad y Medioambiente-Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional del Comahue, Bariloche, Argentina

T. Boll
Water and Nature, COWI A/S, Jens Chr. Skous Vej 9, 8000 Aarhus C, Denmark

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Introduction

Aquatic plant beds are essential for a suite of mechanisms and help stabilise the clear-water state of shallow temperate lake ecosystems (Moss, 1990; Scheffer et al., 1993; Beklioğlu & Moss, 1996; Jeppesen et al., 1997). Plant beds can provide a daytime refuge for microcrustaceans against fish predation as the former migrate out of plant beds at night and thereby enhance the overall grazing pressure on phytoplankton (Lauridsen et al., 1996; Burks et al., 2002; Balayla & Moss, 2004). The refuge effectiveness of plant beds is known to depend on plant cover or on the plant bed size-to-edge ratio (Lauridsen & Buenk, 1996), plant density (Schriver et al., 1995), plant types dominating the lake (Carpenter et al., 1987, Balayla & Moss, 2003), lake depth (Hessen et al., 1995) and lake trophy (Jeppesen et al., 1997). The importance of even small plant beds was demonstrated by Lauridsen et al. (1996), who found that small dense patches of plants (ca. 3% coverage of the lake surface area) doubled night-time microcrustacean density by allowing the microcrustacea to avoid daytime predation by seeking refuge in the vegetation. The refuge effect was plant density-dependent and was most pronounced at high plant density (Lauridsen et al., 1996). Schriver et al. (1995) interpreted refuge effectiveness as the outcome of the balance between fish predation pressure and submerged plant density. However, based on experiments with artificial plants among which animal densities (prey and predator) were manipulated to co-vary with plant densities, Scheinin et al. (2011) suggested that this relationship is complex and perhaps influenced by factors acting on predator and prey abundances, such as increased food resources and reduced competition in the plant beds.

When lakes are restored by reducing the external nutrient loading, lake recovery is typically delayed for multiple reasons (Moss, 1990), and additional tools may therefore be used to precipitate recovery (Hansson et al., 1998). One such method is biomanipulation, where massive removal of fish is conducted to enhance water clarity and, potentially, to pave the way for establishment of submerged plants and stabilising the clear-water state (Jeppesen et al., 2012). However, following fish removal, plant establishment may occasionally be slow despite improvements of the light climate (Lauridsen et al., 2003; Jeppesen et al., 2005; Hilt et al., 2006). Schou et al. (2009) proposed that the introduction of artificial plants could help accelerate recovery of eutrophic lakes by their acting as daytime refuge for large microcrustaceans. They showed that artificial plant beds were particularly effective when turbidity was low. However, how fish feeding inside and outside the artificial plant beds interfere with their role as a refuge has never been specifically addressed (Schou et al., 2009).

When lakes are restored by fish biomanipulation, a progressively improved refuge effect of artificial plants may be expected because the lake gradually becomes clearer, as suggested by Schou et al. (2009). However, removal of large, planktivorous and benthivorous fish may reduce their competition for food with small fish, thus increasing the small fish density. The small fish may use the plant beds as foraging areas, taking advantage of the clearer water to enhance their foraging efficiency (Diehl, 1988), and to avoid predation by piscivores. On the other hand, the predation pressure on small fish by piscivores within the plant beds may *increase*, because the predation effectiveness of visually hunting fish species (e.g., perch) could be greater with the improved light climate (Sagrario et al., 2009). The net outcome of these opposing effects remains unknown.

In this study, our working hypothesis was that the artificial plants would act as an effective refuge for microcrustacea, particularly for free-swimming cladocerans, and that this refuge would potentially aid lake recovery efforts. Specifically, our predictions were that

- the refuge effect would be strong for free-swimming microcrustaceans (i.e., large differences in microcrustacean measures of biomass, size and community structure between plant beds and the lake and between plant density levels, regardless of food conditions) immediately upon biomanipulation;
- the refuge effect for free-swimming microcrustaceans would gradually abate because of increasing within-bed fish planktivory by small fish (i.e., a higher proportion of small fish and a gradually increasing number of predatory fish driving these into the plant beds);
- the refuge effect would be less strong for plant-associated microcrustaceans, which are presumably less exposed to fish predation than free-swimming species such as *Daphnia* spp. because

they feed and live in close association with plant surfaces (Nurminen & Horppila, 2006). The abatement with time in refuge effect would accordingly also be lower for PAM than for FSM.

By crossing plant densities and fish data in our experimental design, we were able to identify the conditions under which plant refuge effectiveness is maximal. We used an experimental setup that mimicked submerged plant beds, colonised by the natural biota of the lake, of different densities (two levels of water column volume occupied, 80 and 20%) in shallow Lake Vaeng. A substantial proportion of the fish community of the lake (mainly roach (*Rutilus rutilus* Linnaeus, 1758) and bream (*Abramis brama* Linnaeus, 1758)) was fished out from the lake during winter–spring 2007–2008 and 2008–2009 with the aim to increase water transparency. The experiment ran for three years and covered the period before, during and after biomanipulation. We monitored microcrustaceans and fish within the artificial plant beds and in the lake during the three growing seasons (2007–2009). A parallel study of the macroinvertebrates on the artificial plants is reported in Boll et al. (2012).

Methods

Study site

Lake Vaeng is a small, shallow eutrophic lake (15.7 ha, Z_{mean} = 1.2 m, Z_{max} = 1.9 m) located in Central Jutland (Denmark). The lake has a number of small inflows that drain patches of forest, but the main input is from groundwater. The lake was biomanipulated in 1986–1988 by removing a large proportion of the benthivorous–planktivorous fish population (mainly roach and bream; Jeppesen et al., 1990) and was soon covered almost entirely by submerged vegetation (mainly *Elodea canadensis* Michaux; Lauridsen et al., 1994). The lake returned to the turbid state after the macrophytes disappeared in 1996/1997 (Lauridsen et al., 2003; Søndergaard et al., 2007, 2008). Internal nutrient loading has decreased progressively since then. However, the lake has remained largely in a turbid state since 1996 (Søndergaard et al., 2007). To increase the lake's water transparency, a substantial proportion of the

benthivorous and omnivorous fish were removed, which led to clear-water conditions and submerged macrophyte establishment (Jeppesen et al., 2012; Søndergaard et al., 2017). During the present study, the fish community of the lake was dominated by bream (benthi-planktivore), roach (plankti-benthivore) and perch (planktivore; larger individuals are piscivores). Northern pike (*Esox lucius* L., piscivore), eel (*Anguila anguila* L., benthi-piscivore), rudd (*Scardinius erythrophthalmus* L., omnivore), ruffe (*Gymnocephalus cernuus* L., benthivore) and tench (*Tinca tinca* L., omnivore) were also found in variable numbers.

Experimental design

Six square aluminium frames (1.5 × 1.5 × 0.9 m) were used to simulate small plant beds by attaching strips of plastic plants to strings drawn across the top face of each frame. Because the artificial plant beds had no walls, free exchange with the lake of water and animals was allowed. Each artificial plant bed contained 16 sampling plants and additional plants were attached to the strings at the top and bottom of the aluminium frame to simulate two levels of plant density (low plant density, LPD: 20% or high plant density, HPD: 80%).

The plants to be sampled were attached to an outer ring of strings within each frame so that each sampling plant was placed 30 cm from the outer edge of the artificial plant bed. The ten artificial plant beds were placed so that they formed a line parallel to the western shore (Fig. 1) in a region of the littoral zone of Lake Vaeng with a relatively flat bottom. Water depth in this area was approximately 1.1 m, so the top of each plant bed was positioned about 20 cm below the water surface. The location of plant beds of each plant density was randomised along the line using a random number generator. The artificial plant beds were removed from the lake each winter.

Each sampling plant consisted of a wire stem covered by green plastic with about 51 double or triple plastic leaves of four different sizes (total plant surface on each stem: 2,110 cm^2). The plant was attached around a string tied to a bottom cup and loosely attached to a top string for easy release. Each bottom cup consisted of a round lid of plastic supported by a ring of stainless steel. The lid was filled with well-mixed sediment collected from the lake, which acted

Fig. 1 Map of Lake Væng (author: Thorkild Høj) showing the line of artificial plant beds (see text for details) and depth isolines (m). The artificial plant beds were placed on a line in the western part of the lake. Sites for microcrustacean sampling are also shown
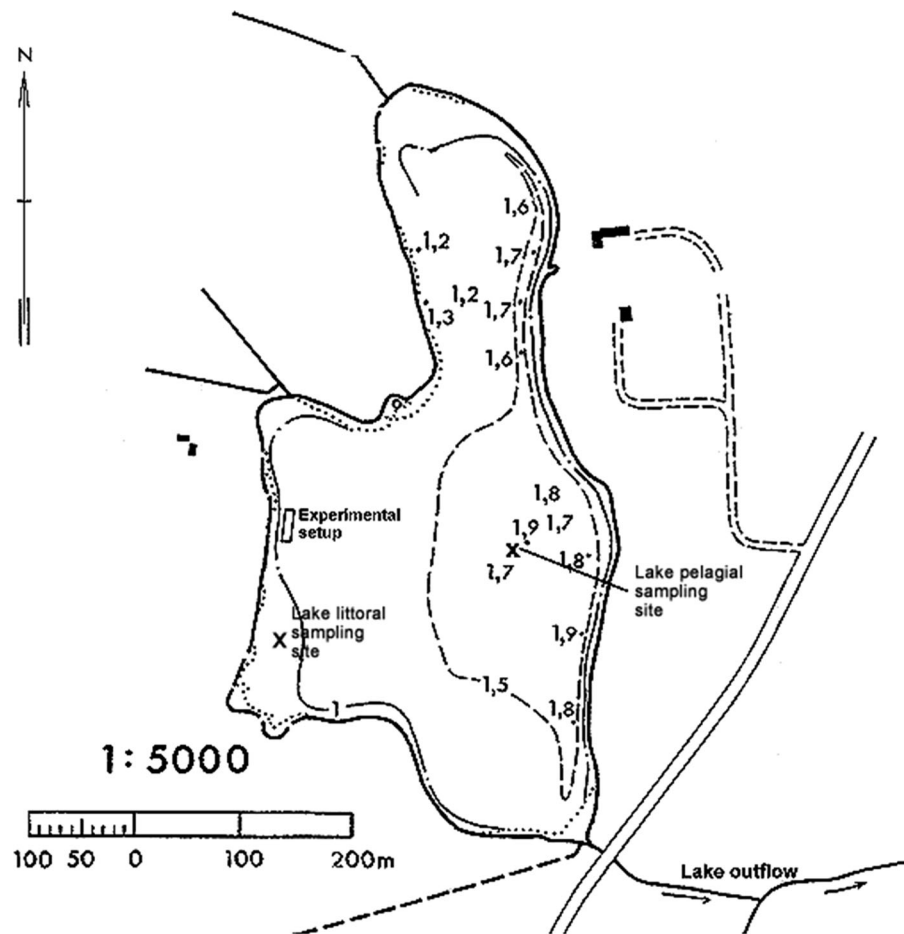
as inoculum for macroinvertebrates and other fauna and secured similar conditions for all plants. The lid had been designed to fit snugly onto a clear plastic 1.2 m, 12.2 cm Ø tube sampler surrounding the plant to be sampled. The tube sampler could rapidly be lowered over the plant so that the plant plus the surrounding water and the sediment-filled lid could be lifted to the boat by pulling up the plant and the bottom cup from the string (for further details on the sampling method, see Fig. 1c in Boll et al., 2012).

Microcrustaceans

On each sampling occasion, a plant sample was taken from three randomly chosen plant beds at each level of plant density (3 samples × 2 levels). To sample the

artificial plants, the clear plastic tube was used for enclosing the sample plant. Water and plant samples were separately processed for macroinvertebrates and microcrustaceans, while sediment was discarded. The plants were returned to their original positions after being processed.

Samples of the free-swimming microcrustaceans were taken from each of the ten artificial plant beds during July–August each year (2007–2009). Sampling began around midday and was completed in about 1–2 h. The depth-integrated samples of free-swimming microcrustaceans were taken by submerging a thin tube (10 cm Ø) three times into the centre of each artificial plant bed to about 10 cm from the lake bottom to avoid disturbing the sediment and then collecting the tube contents into a bucket. Six well-

mixed litres were then taken, filtered through a 50-μm mesh and preserved in 70% ethanol. On each sampling occasion, we also took depth-integrated samples of the lake zooplankton from a site located in the deepest area of the lake and from a littoral site (Fig. 1). In addition, lake transparency (Secchi depth), depth-integrated chlorophyll a concentration and total nitrogen and total phosphorus concentrations were determined for both sites (for analytical methods, see Søndergaard et al., 2017) (Fig. 2).

All animal samples were counted under a stereomicroscope at 30X. Before subsampling, the >2 mm size fraction was separated and counted in full, and body sizes were measured under a magnifying lens. The <2 mm fraction was subsampled and at least 60 individuals per species were counted. For calculating the biomass of the microcrustacea, size measurements were conducted on 25 individuals per species, when possible, at each sampling date. Microcrustacean biomass was calculated using the size–weight regressions in Bottrell et al. (1976), McCauley (1984) and Hansen et al. (1992). For nauplii, we used a standard biomass of 1.175 μg DW ind^{-1} (Kankaala et al., 1990). The density and biomass of plant-associated

animals were expressed as numbers per plant and μg DW m^{-2} of plant, respectively.

Fish

Fish surveys were conducted every 4–5 weeks during two growing seasons (July–October 2007 and May–October 2008) and once in August 2009. We used multiple-sized gill nets to determine fish density and community composition in the littoral region near the experimental setup. Two survey gill nets (multi-mesh-size length: 42 m, height: 1.5 m, 14 sections—order of mesh sizes: 10, 60, 30, 43, 22, 50, 33, 12.5, 25, 38, 75, 16.5, 8, 6.25 mm) were placed on the shoreward and lakeward sides of the littoral line of the artificial plant beds (Fig. 1). Nets were placed one day after ending the invertebrate sampling to allow the fish populations to recover from potential disturbance from sampling. Nets were placed at ca. 6 p.m. and retrieved the following morning at ca. 8 a.m. All fish in the nets were counted, and individual wet weights (g) were measured.

We also used specific small-fish gill netting (6.25, 8 and 12.5 mm mesh sizes) to obtain estimates of the

Fig. 2 Lake water transparency (Secchi depth, m) and chlorophyll a (Chl-a), total phosphorus (TP) and total nitrogen (TN) concentrations shown for the same period (littoral Secchi depth until February 2009). Z$_s$ is Secchi depth; TN, TP (all years) and Chl-a (since 2009) are from Søndergaard et al. (2017)
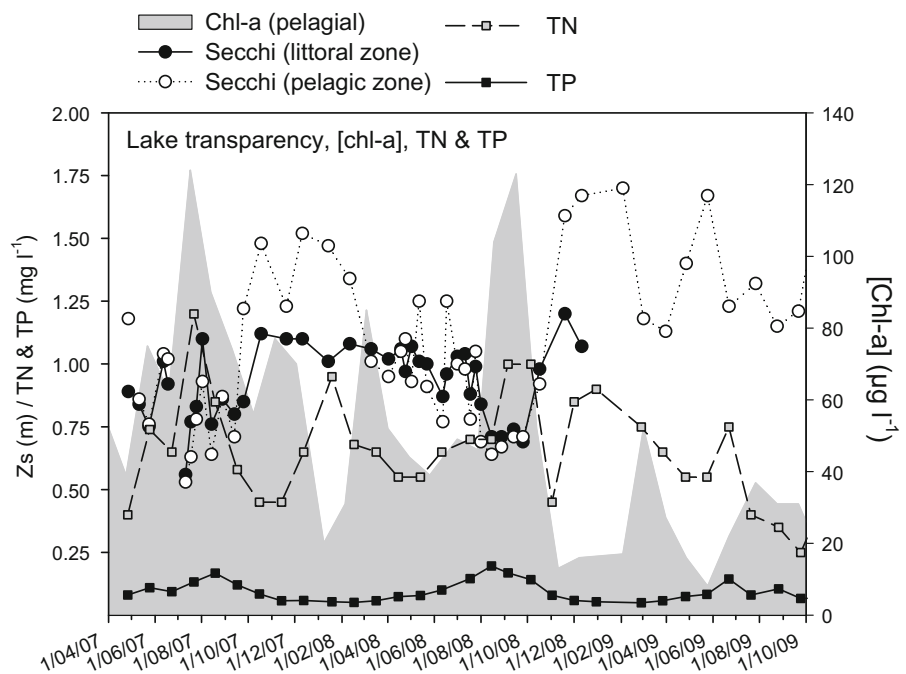
number of small fish swimming in and out of the artificial plant beds. Nets were attached with poles and cable ties onto the open-water side of each artificial plant bed and left for approx. 14 h as described above for the survey nets. Nets were placed and removed on three consecutive days immediately after each of the above fish surveys. Fish densities are expressed as the total catch of fish/net/night.

Data analyses

We used two-way ANOVAs to compare means of biomass and density and mean individual biomass and the free-swimming microcrustacean:phytoplankton biomass ratios of a number of microcrustacean groups, i.e., all microcrustaceans and all cladocerans, across plant treatments and years (July–August 2007–2009). Data on free-swimming (samples taken from water among plants) and plant-associated microcrustaceans (plant samples) were analysed separately. Post hoc

tests (Tukey–Kramer LSM differences) were then carried out for multiple comparisons.

We also used two-way ANOVAs to compare mean counts and mean wet weights of fish from nets attached onto the artificial plant beds across plant treatments (high and low plant density) and across years (2007–2009). Year was included as a factor in the analyses as a proxy for the expected levels of fish predation on the microcrustacea as a result of biomanipulation. An analogous two-way ANOVA was used for comparing means of data derived from fish survey nets.

We calculated community structure ratios based on the biomass of selected taxa, i.e., total cladocerans/total microcrustaceans and large cladocerans/total cladocerans. Ratios were calculated for mid-summer data (July–August) and separately for water samples and plant samples. Two-way ANOVAs (factors: year and plant density) were used, followed by post hoc t tests for Tukey–Kramer-corrected LSM differences in
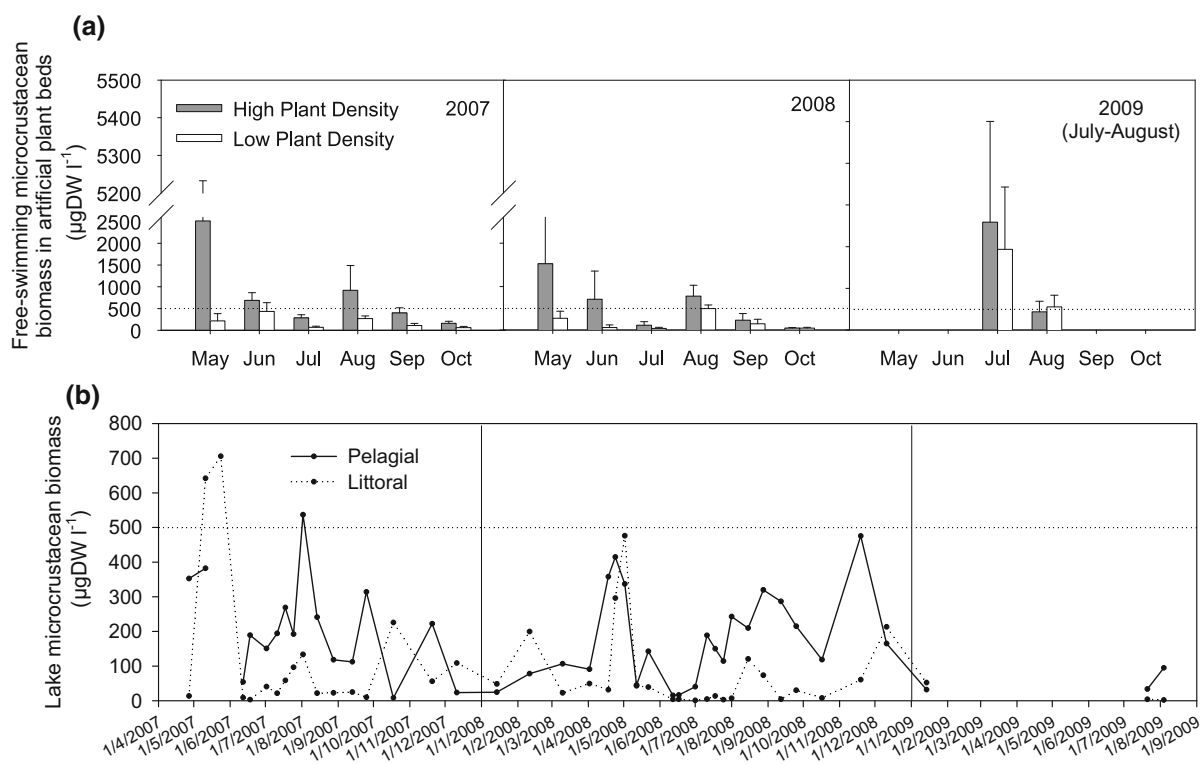

Fig. 3 a Microcrustacean biomass in high-plant density (HPD) and low-plant density (LPD) beds (20 and 80%) during the growing season (May–October) in 2007–2009. Error bars on plots indicate one standard deviation. Biomass was consistently larger in the HPD beds. **b** Total free-swimming microcrustacean biomass (μgDW l^{-1}) in the pelagic and littoral areas of the lake during the period from April 2007 to August 2009. The horizontal dotted line shows 500 μgDW l^{-1} in both plots to aid comparison

multiple pairwise comparisons. To meet the assumptions of ANOVA of approximate normality of underlying distributions and stability of variance, main response variables were $\log_{10}(x + 1)$ transformed. All tests were performed in SAS v. 9.2 (SAS Institute Inc.).

Results

Microcrustaceans in the lake and in artificial plant beds

Microcrustacean biomass was higher in the artificial plant beds than in the lake during most of the growing season, particularly in the high-plant density beds (vs lake littoral zone $t_{13} = -3.59$, $P < 0,01$; vs lake pelagic zone $t_{13} = -3.011$, $P = 0.01$). On several occasions during mid-summer, microcrustacean biomass was 5–10 times higher in the high-density plant beds than in the lake (Fig. 3). By contrast, microcrustacean mean individual biomass was often half or less in the plant beds than in the lake, in both the littoral and the pelagic areas, particularly in 2007 and 2008 (Fig. 4).

In the artificial plant beds, mid-summer (July and August) free-swimming microcrustacean (FSM) density was lower in 2009 than in August 2007. In contrast, FSM biomass did not differ between 2009 and 2007 ($F = 6.06$, $P = 0.0025$; Tukey $t = 1.16$, n.s.), indicating that microcrustaceans were generally larger in mid-summer 2009 than in 2007. Mid-summer FSM biomass was significantly lower in 2008 than in the other two years (Tukey 2007 vs. 2008, $t = 4.78$, $P < 0.0001$; 2009 vs. 2008, $t = 3.53$, $P = 0.0004$). FSM biomass was particularly low in the experimental plant beds in July 2008 (Fig. 3). Both density and biomass of all FSM groups, except copepods, were higher throughout the growing season in the HPD than in the LPD beds in 2007 and 2008 (Tables 1, 2). Plant density effects on FSM density/biomass were comparable in 2007 and 2008, with a significantly larger microcrustacean biomass in the HPD than in the LPD beds in both years ($F = 17.58$, $P < 0.0001$; Tukey 2007, $t = 4.19$, $P < 0.0001$; 2008, $t = 3.67$, $P = 0.003$), while no significant difference was found in 2009, the year with less fish (Tukey 2009, $t = 0.7931$, n.s.). In contrast, no significant effects of year or plant treatment on microcrustacean biomass
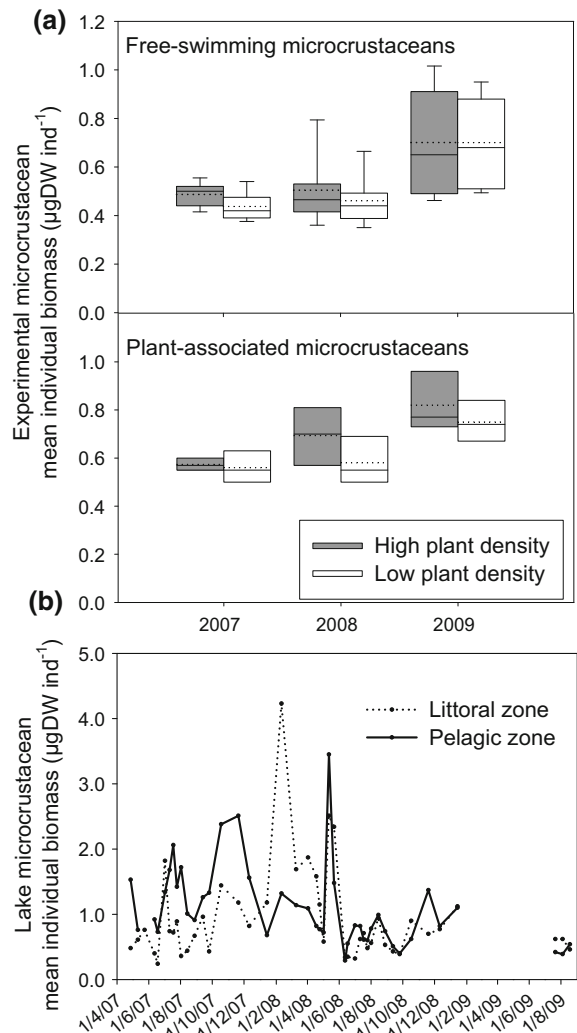

Fig. 4 a Microcrustacean mean individual biomass (µgDW ind^{-1}) in HPD and LPD beds in July–August (2007–2009). *Top panel* free-swimming microcrustaceans (from water samples); *bottom panel* plant-associated microcrustaceans (from plant samples, see the "Methods" section for details). The dotted lines in boxplots indicate the mean, while the continuous lines show the median. **b** Lake microcrustacean mean individual biomass (µgDW ind^{-1}) during the period from April 2007 to September 2009

and density were identified for any of the groups of the plant-associated microcrustaceans (PAM) during 2007–2009 (Tables 1 and 2).

Mean individual biomasses (i.e., individual size) of both free-swimming and plant-associated cladocerans were significantly larger in mid-summer 2009 than in the other two years in both the HPD and the LPD beds (Tukey–Kramer HPD2009 vs. 2007, $t = -0.99$, n.s.;

Table 1 Results of two-way ANOVAs comparing biomass or density (top) in mid-summer (July–August) between years (2007, 2008, 2009) and plant density (HPD, LPD) for all microcrustaceans, all cladocerans and large cladocerans in artificial plant beds

Biomass/density	Year	Plant density	Year*Plant density
Free-swimming microcrustaceans			
All microcrustaceans	****/**	****/**	**/*
All cladocerans	****/****	****/***	****/*
Large cladocerans[a]	****/***	*/ns	ns/ns
Plant-associated microcrustaceans			
All microcrustaceans	*/ns	ns/ns	ns/ns
All cladocerans	ns/ns	ns/ns	ns/ns
Large cladocerans	ns/ns	ns/ns	ns/ns

Results are shown separately for free-swimming microcrustaceans (FSM) and for plant-associated microcrustaceans (PAM). See the "Methods" section for details on sampling FSM and PAM

[a] Large cladocerans are *Daphnia* spp., *Diaphanosoma brachyurum* Liévin 1848, *Eurycercus lamellatus* O.F. Müller, 1776, *Sida crystallina* O.F. Müller, 1776 and *Simocephalus vetulus* O.F. Müller, 1776

**** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; *ns* not significant

Table 2 As for Table 1, but for microcrustacean mean individual biomass and the microcrustacea:phytoplankton biomass ratio

Microcrustacean mean individual biomass/Microcrustacea:phytoplankton	Year	Plant density	Year*Plant density
Free-swimming microcrustaceans			
All microcrustaceans	****/****	ns/***	ns/***
All cladocerans	ns/****	ns/****	ns/****
Large cladocerans[a]	*/****	*/**	ns/*
Plant-associated microcrustaceans			
All microcrustaceans	**/****	ns/ns	ns/ns
All cladocerans	ns/**	ns/ns	ns/ns
Large cladocerans	ns/ns	*/ns	ns/ns

LPD2007 vs. LPD2009, $t = -2.56$, $P < 0.05$; HPD 2008 vs. HPD2009, $t = -2.13$, $P < 0.05$; LPD2008 vs. LPD2009, $t = -4.36$, $P < 0.0001$; Fig. 4). However, the refuge effect of plants on larger individual sizes in the HPD beds was only significant in 2008 when fish densities were highest (Tukey $P < 0.05$).

The proportion of large-bodied cladoceran species of the FSM community was generally higher in the HPD beds than in the LDP beds (Tukey, $t = 3.89$, $P < 0.001$; Fig. 5; Table 3). However, in 2009, the year with less fish, no plant effect on FSM community structure was detected. By contrast, an effect of plants on PAM community structure was only observed in 2009 (Table 3).

In both 2008 and 2009, plant-associated cladocerans consisted mainly of small-bodied species, which stands in contrast to a higher proportion of larger-bodied species in 2007 (Table 3). Contrary to FSM, PAM community composition did not vary significantly between plant densities in any year (Table 3). The microcrustaceans were dominated by cladocerans in 2007 and 2008, but copepods were clearly dominant in 2009.

Fish

Within the artificial plant beds, the catch of fish in small nets (see the "Methods" section for details) was significantly lower regarding both number and biomass (wet weight) in 2009 than in 2007 and 2008. Both density and biomass of fish were significantly higher in 2008 than in the other two years (density: year $F = 125.4$, $P < 0.0001$; plant density $F = 14.6$, $P = 0.0008$; Tukey's 2008 versus 2007, $t = -5.27$,
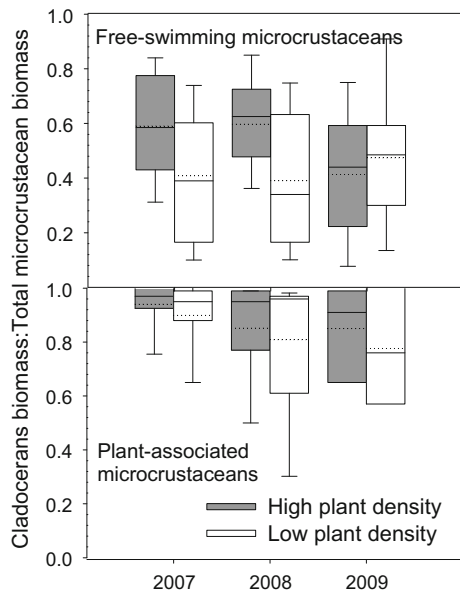
Fig. 5 Microcrustacean community structure and plant refuge. *Top panel* free-swimming microcrustaceans (water samples); *Bottom panel* plant-attached microcrustaceans (plant samples, see the "Methods" section for details). Community structure is indicated by the cladocerans:total free-swimming microcrustacean biomass ratio in the HPD and LPD beds (2007–2009). The dotted lines in boxplots indicate the mean, while the continuous lines show the median

$P < 0.0001$; 2008 versus 2009, $t = 15.57$, $P < 0.0001$; biomass: Tukey's 2008 versus 2007, $t = 2.17$, $P < 0,05$; 2008 versus 2009, $t = 10.36$, $P < 0.0001$; 2007 versus 2009, $t = 8.19$, $P < 0.0001$). Moreover, small fish were significantly more abundant in the low- than in the high-plant density beds only in 2008, the year with the highest fish numbers (Tukey HPD versus LPD, $t = -2.21$, $P < 0.05$).

Discussion

We found a consistent and highly significant plant refuge effect for the free-swimming microcrustacean groups, which exhibited higher biomasses and body sizes in the high-plant density treatment on most sampling occasions. In the high-density plant beds the free-swimming microcrustacean community was mainly composed of larger-bodied species, as indicated by the biomass ratios of differently sized taxa. These results, when combined, support our general

hypothesis that artificial plant beds would favour microcrustaceans and potentially aid in lake recovery efforts.

In our comparison of refuge role between years, we found that the differences between plant treatment levels were large and particularly the refuge effect diminished in the year with relatively low fish biomass (2009). This supports our specific prediction that artificial plant beds would be particularly effective in high-fish biomass scenarios.

Mean individual biomass, but not density or community structure, of the plant-associated microcrustaceans was significantly larger in the high-density plant beds than in low-density plant beds, fulfilling our specific prediction that these microcrustaceans would be influenced to a lesser extent by the refuge role of plants. It is well established that submerged plants provide refuge against fish predation for microcrustaceans (e.g., Leah et al., 1980; Timms & Moss, 1984; Lauridsen et al., 1996). This effect is thought to favour large-bodied zooplankton species and their larger individuals (Brooks & Dodson, 1965; Hall et al., 1976), which are more vulnerable to visual predators, for instance perch and roach, foraging among the plants (Winfield, 1986). In our experiment, the artificial plants apparently provided a strong refuge for both large- and small-bodied cladocerans, including more tightly plant-associated species such as chydorids and *Sida sp.*

A threshold fish density beyond which plants may lose a large part of their effectiveness as refuge for the microcrustacea has often been used to explain instances where submerged plant beds harbour small numbers of microcrustaceans, and fish densities, are high (Schriver et al., 1995; Stansfield et al., 1997; Perrow et al., 1999). In our study, however, the levels of microcrustacean biomass were much higher within the artificial plant beds than in the vegetated littoral zone of the lake during most of the growing season (Fig. 3), despite seasonal changes in fish biomass. Moreover, fish CPUE in the lake was larger in 2008 than in 2007 (Fig. 6), but this was not reflected in the seasonality of microcrustacean biomass in the artificial plant beds, which showed the usual peaks at the beginning of the growing season (May) and in midsummer (August) in both years. Thus, the plants are a resilient refuge in the face of a wide range of fish densities in the lake. A refuge effect may also explain the comparable densities of the plant-associated

Table 3 Results of two-way ANOVAs (*F* values) with year and plant density as factors and of post hoc *t* tests for Tukey–Kramer-corrected least square mean (LSM) differences used in the analysis of the following community structure biomass ratios (August, 2007–2009): large cladocerans:total cladocerans, total cladocerans:total microcrustaceans, see text for details)

	Year df = 54		Plant density df = 54		Plant*Year	
	ns	n/a	ns	n/a	ns	n/a
Free-swimming microcrustaceans (*water samples*) **Large cladocerans:Total clad.**						
Total cladocerans:Total microcrustacea	3.32*	2007 vs. 2008 $t = 2.45$* 2007 vs. 2009 ns 2008 vs. 2009 ns	15.17***	HPD > LPD, $t = 3.89$***	7.69**	2007: HPD > LPD, $t = 4.73$**** 2008: HPD > LPD, $t = 2.76$** 2009: HPD $\approx$ LPD, ns
Plant-associated microcrustaceans (*plant samples*)	df = 12		df = 12			
Large cladocerans:Total clad.	5.14*	2007 vs. 2008 $t = 2.97$* 2007 vs. 2009 $t = 2.53$* 2008 vs. 2009 ns	ns	n/a	3.80, $P = 0.0526$	2007: HPD $\approx$ LPD, ns 2008: HPD $\approx$ LPD, ns 2009: HPD > LPD, $t = 2.28$*
Total cladocerans:Total microcrustaceans	4.60*	2007 vs. 2008 ns 2007 vs. 2009 $t = 2.74$* 2008 vs. 2009 $t = 2.50$*	ns	n/a	ns	2007: HPD $\approx$ LPD, ns 2008: HPD $\approx$ LPD, ns 2009: HPD $\approx$ LPD, ns

Results are shown separately for free-swimming microcrustaceans and plant-associated microcrustaceans. See the "Methods" section for details. High plant density (HPD): 80%; low plant density (LPD): 20%

**** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, *ns* - not significant, n/a - not applicable
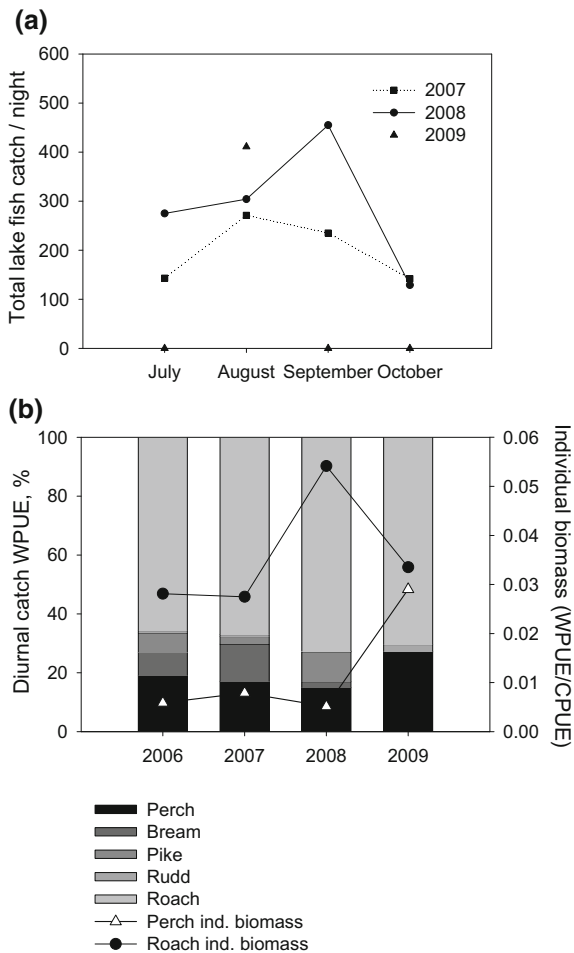
(a)

(b)

Fig. 6 **a** Total fish catch (summed catch of two large gill nets, see the "Methods" section for details) per night during summer in 2007–2009. **b** Fish community species composition (% WPUE). The community was largely dominated by roach and perch. The lines indicate the changes in fish individual biomass (WPUE/CPUE) for these two species over the experimental period

microcrustacean between the high- and low-density plant beds, both before and after biomanipulation, despite the potentially higher food availability for these microcrustaceans under the higher light levels in the low-density plant beds.

The lack of plant refuge effect in 2009, the year with relatively low fish biomass, is consistent with our prediction that the refuge role of plants would be stronger with high fish biomass. We cannot discard the possibility that it could, at least in part, reflect larger plant growth in the lake this year (Søndergaard et al., 2017), resulting in a less intensive use by the

microcrustacea of the artificial plant beds as a refuge. Nevertheless, the lack of a refuge effect for all microcrustacean groups in 2009 coincided with relatively large mean individual biomass (i.e., calculated as species biomass/species density) this year than in the other two years, suggesting that the microcrustacean might have suffered lower levels of fish predation.

We predicted that the refuge effect would decline in intensity as the planktivorous fish sought refuge in the plants against piscivorous fish after biomanipulation, implying a higher predation pressure on the plant-associated microcrustaceans than under the more turbid, pre-biomanipulation conditions. We did not, however, observe such an increased predation pressure. A possible explanation of the lower than predicted fish predation in 2009 may be that young-of-the-year fish exhibited rapid growth immediately upon biomanipulation because of lower competition for food from the larger size classes of fish that had partly shifted to feeding on macroinvertebrates by August (Fig. 6b). Thus, the effectiveness of artificial plant refugia is not only the result of a balance between plant density and fish density; it may also depend on shifts in feeding habits of the fish within the growing season, from predation on smaller (microcrustacean) to larger (macroinvertebrates, small fish) items. Indeed, the effectiveness may be enhanced in the later stages of lake restoration when clearer water may lead to faster fish growth and earlier shifts to feeding on larger food items such as macroinvertebrates living in the artificial plant beds (Boll et al., 2012).

An alternative explanation for the low number of planktivores within the artificial plant beds in 2009 may be that the predation risk for small fish was higher within the plant beds as the larger, piscivorous fish foraged here (Sagrario et al., 2009). This hypothesis is supported by the low fish catches within the artificial plant beds in 2009 when the lake was clearer than in the previous years, and a higher foraging efficiency of the large fish could therefore be expected.

In conclusion, our results suggest that plant beds have a direct, protective effect against fish predation for both free-swimming and plant-attached microcrustacean species. In our experiment, artificial plants enhanced microcrustacean survival as shown by the higher and sustained densities of large microcrustaceans in the artificial plant beds than in the lake itself. From a management perspective, our results

support those of Schou et al. (2009), indicating that artificial plant beds aid lake restoration efforts by providing refugia for the microcrustaceans against predation by fish in those cases where the development of the natural plant community is delayed.

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