

# Fish induced macrophyte loss in shallow lakes: top–down and bottom–up processes in mesocosm experiments

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## SUMMARY

1. Macrophyte loss from Sites of Special Scientific Interest in England has become widespread over the last 20 years. One reason for this may be changing trends in angling, a multimillion pound industry that has an enormous impact on aquatic ecosystems. Stocking with cyprinid fish is a common angling management practice but the particular fish species and distribution of their biomass may be crucial to the ecosystem.

2. Carp (*Cyprinus carpio*), roach (*Rutilus rutilus*), bream (*Abramis brama*) and tench (*Tinca tinca*) at biomasses ranging from 0 to 800 kg ha<sup>-1</sup> and at various sizes were placed into experimental mesocosms in Little Mere, a shallow, fertile lake in Cheshire, U.K. The effects these treatments had on the aquatic ecosystem were studied over two summers.

Specifically the effects of the treatments on macrophyte growth, benthic and macrophytic macro-invertebrate populations, water chemistry, epiphyton production and plankton survival were investigated.

3. Carp had a greater detrimental effect on the macrophytes than bream, tench and in particular roach. A biomass of fish > 200 kg ha<sup>-1</sup> adversely affected the extent of macrophyte growth.

4. The decline in macrophyte growth was most likely as a result of increased epiphyton growth that probably reduced the amount of light and carbon dioxide available to the plant. There were no observed direct fish impacts on macrophytes.

5. The chemical data suggested that inorganic nitrogen levels were low and it is possible that release of nitrogen, from fish excreta, followed by immediate uptake, could have been a major factor stimulating epiphyton growth and subsequently macrophyte loss.

Phosphorus concentrations increased even in the controls and substantial amounts were available. Phosphorus stimulation can therefore be discounted. Macrophyte-associated macro-invertebrates were positively correlated with epiphyton load but had no impact on the extent of epiphytic growth. Shading from disturbed sediment or phytoplankton was also unimportant.

*Keywords:* bottom–up, carp, epiphyton, macrophyte loss, top–down

## Introduction

Coarse angling is a changing sport (NRA, 1970). Stocking of waters with particular species, sizes and

biomasses of fish is now a common practice but there is an emerging conflict between nature conservation interests and the requirements of competitive anglers. Stocking of certain fish species may lead to undesirable effects. In the past, stocking with species such as roach and pike has apparently had little detrimental effect on lake ecosystems. However, with the changing attitudes of anglers and their market power, fishery managers

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have been forced to change their stocking programmes. Therefore the stocking of waterbodies with benthivorous fish such as carp and bream has become prevalent, at the possible expense of the aquatic ecosystem.

King & Hunt (1967) showed that carp were associated with the destruction of aquatic vegetation. Conversely the removal of fish, such as carp and bream, from lakes has often seen submerged macrophytes flourish (Ozimek, Gulati & Van Donk, 1990; Wright & Phillips, 1992). Indeed Hill, Wright & Street (1987) found that submerged vegetation abundance was inversely proportional to fish biomass in ponds containing a mixture of roach and bream. The mechanisms behind these findings are complex and not fully understood.

Benthic-feeding fish suck up sediment and food organisms, filter out the organisms and eject the sediment, clouding the water. Bream and carp, in particular, have been shown to increase sediment re-suspension (Meijer *et al.*, 1990; Breukelaar *et al.*, 1994). Disturbed sediment can release nutrients into the water column and re-settlement of winnowed sediment can smother young plants (Wright & Phillips, 1992). It is also possible that shading from disturbed sediment (Meijer *et al.*, 1990) or phytoplankton (Moss, 1976) reduces light penetration and adversely affects macrophytes. The direct movement of fish can disturb macrophyte propagules and further disturb sediment and another indirect pathway for nutrient release is via fish excretion (Andersson, Graneli & Stenson, 1988). Increased nutrient loading has been shown to increase crops of phytoplankton and epiphytic algae, both of which compete against macrophytes for light and CO<sub>2</sub> (Phillips, Eminson & Moss, 1978; Sand-Jensen, 1983). Attached epiphyton can also create high O<sub>2</sub> and pH regimes unfavourable to macrophytes (Simpson & Eaton, 1986). Furthermore, zooplankton and macro-invertebrate epiphyton grazers may be removed by fish, again aiding the competition of phytoplankton and epiphyton over macrophytes (Bronmark & Weisner, 1992). In addition, the presence of fish in a lake, especially where no macrophytes are present and acting as zooplankton refugia (Timms & Moss, 1984), invariably reduces large-bodied zooplankton, typically resulting in increased phytoplankton populations (Andersson *et al.*, 1978; Duncan, 1997). Alternatively, fish may remove macrophytes by directly feeding on them which in itself can increase the light availability to any

remaining epiphyton which may then flourish, or indeed some other factor such as allelopathic chemical release or a specific physico-chemical reaction may be involved (Van Vierssen & Prins, 1985). The decline of macrophytes could be because of a combination of any of these factors. All the fish effects described above favour the growth of algae and competitively disadvantage macrophytes, although the relative importance of each mechanism is debatable.

The decline of macrophytes and dominance by phytoplankton is highly undesirable in most lakes, but especially shallow Sites of Special Scientific Interest (SSSIs) where SSSI status is often assigned because of the specific macrophytes present. It is imperative therefore to understand the complex processes involved with macrophyte loss. These experiments were designed to understand how macrophyte decline might be affected by:

- top-down effects of fish in removing zooplankton (Timms & Moss, 1984) and macro-invertebrate (Bronmark & Weisner, 1992) grazers that may lead to macrophyte shading through reduced phytoplankton and epiphyte removal as well as direct macrophyte ingestion by fish.
- bottom-up effects of fish that release nutrients from disturbed sediment (Hilsenhoff, 1965) or via fish excretion (Andersson *et al.*, 1988) which aids the growth of phytoplankton (Breukelaar *et al.*, 1994) and epiphytes (Phillips *et al.*, 1978) and along with disturbed sediment can shade out macrophytes (Meijer *et al.*, 1990).

## Methods

### *Experimental Design*

Mesocosms constructed from white wood and white cotton netting (mesh size about 200 µm), open at the top and bottom, with a 4-m<sup>2</sup> surface area (2 × 2 m) were placed in Little Mere, a shallow eutrophic SSSI lake in Cheshire, U.K. with a maximum depth of 1.5 m.

The mesocosms were established randomly in an area, at the time bare but anticipated from previous experience to develop a relatively uniform stand of submerged vegetation, largely *Potamogeton berchtoldii* (Fieber) but with *Callitriche hermaphroditica* (L.) also present.

Experiments were run over two consecutive summers between May and August. In 1996, a fully

factorial three-block random design experiment was devised in which four different species of fish: carp, roach, bream and tench were investigated. Fish were of equal size (40 g) but were stocked in mesocosms as single species additions at three stocking biomasses: 0, 200 (two fish) and 700 kg ha<sup>-1</sup> (seven fish) making 12 treatments. These were replicated three times giving 36 mesocosms and studied for a period of 6 weeks. During 1997, a fully factorial four-block random design experiment was chosen. Two species of fish: carp and roach at two stocking biomasses: 0 and 800 kg ha<sup>-1</sup> were added as single species additions. The 800 kg ha<sup>-1</sup> biomass existed as two alternatives: 4 × 80 or 1 × 320 g fish resulting in six treatments. This investigation concerned not merely fish species and biomass effects but also size. The subsequent six treatments were replicated four times thus 24 mesocosms were used and investigated for a period of 9 weeks.

#### *Fish used*

Benthivorous carp and bream were used as the effects of these species have been well documented as a cause of macrophytic decline and sediment disturbance (Meijer *et al.*, 1990; Breukelaar *et al.*, 1994), although carp tend to be more omnivorous than bream. Tench were used as a less well studied, but nonetheless relatively common benthivorous fish, which may consume at least small quantities of plants. Roach were used as although large specimens will feed benthically they are, on the whole, more generalised planktivores (Hartley, 1940) and are not considered to disturb sediment or bring about macrophyte decline.

#### *Biomass used*

Over the 2 years, fish were added at either 700 or 800 kg ha<sup>-1</sup> as a high but feasible stocking biomass found in many shallow lakes, for instance Lake Bleiswijkse Zoom, the Netherlands (Meijer *et al.*, 1990) and Bay Pond, U.K. (Tenner, 1996). The 200 kg ha<sup>-1</sup> treatment mimicked a lower stocking biomass, suggested as 'safe' by the English Nature and the Environment Agency (Giles, 1998) and 0 kg ha<sup>-1</sup> was used for control. It was anticipated that the fish would grow, however, this was considered acceptable as long as the final fish biomass did not exceed the likely maximum carrying capacity of

the system at around 1000 kg ha<sup>-1</sup>. The lake itself has a fairly low fish biomass of about 140 kg ha<sup>-1</sup> of which >120 kg ha<sup>-1</sup> consists of piscivorous pike.

#### *Sizes used*

It is important to discover whether size or biomass of a given fish species is of greater significance in determining macrophyte decline. Very few experiments have dealt with fish size; total fish biomass is usually the focal point (Andersson *et al.*, 1978; Crivelli, 1983; Bronmark & Weisner, 1992; Wright & Phillips, 1992). Experiments that have dealt with size have used carp, bream or roach (Meijer *et al.*, 1990; Breukelaar *et al.*, 1994). In the 1997 mesocosm experiment, carp and roach from two distinct size groups were used.

#### *Sampling*

After placement in the lake, the mesocosms were electro-fished, to remove any native fish, and left for 1 week to allow the disturbed system to settle. For both years the first week of sampling (week 1) was a pretreatment week, designed to check that the mesocosms were similar before the different treatments were applied. As soon as possible, after the pretreatment samples had been taken, the treatments were added. The experiments were then sampled weekly for a further 5 weeks (weeks 2–6) in 1996 and fortnightly for a further 6 weeks (weeks 3–9) in 1997. The final week (weeks 6 and 9, respectively, for 1996 and 1997) consisted of regular sampling, but in addition terminal sampling took place in which samples were destructively removed and the experiments terminated. Variables sampled therefore fell into one of two categories depending upon how often they were measured: (1) Pretreatment and weekly (1996)/fortnightly (1997) sampling. (2) Pretreatment and terminal sampling.

#### *Pretreatment and weekly/fortnightly sampling and analysis*

Phytoplankton (whole water), zooplankton (63 µm mesh) and an integrated water sample were taken using a tube sampler. Phytoplankton were preserved on site with Lugol's Iodine (Vollenweider, 1969). Under an inverted light microscope, phytoplankton

were, where possible, identified to species and counted. The most numerous algal species were assayed to  $\pm 20\%$  with other common species counted to a precision of  $\pm 50\%$  (Lund, Kipling & LeCren, 1958). Phytoplankton were grouped into either edible ( $<30 \mu\text{m}$ ) or inedible ( $>30 \mu\text{m}$ , filamentous or largely mucilaginous) forms.

Zooplankton were preserved as recommended by Ganon & Ganon (1975) and De Bernardi (1984). Zooplankton subsamples were removed from the preserved samples and, where possible, at least 100 individuals of the most abundant species were counted from each sample (Bottrell *et al.*, 1976). All zooplankters were identified to species and counted. Individuals were recorded as being either small ( $<1.0 \text{ mm}$ ) or large ( $>1.0 \text{ mm}$ ) at the time of counting. Individual biomass was calculated from linear regressions relating length (mm) to dry weight ( $\mu\text{g}$ ):  $\ln w = \ln a + b \ln L$ , where  $w$  is the dry weight estimate,  $a$  is the intercept,  $b$  is the slope,  $L$  is the total length of the zooplankter and  $\ln$  is logarithm to base  $e$ .

Water was analysed for total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) and ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) using standard methods (Stephen, Moss & Phillips, 1998). As a surrogate measurement of disturbed sediment Total Suspended Solids (TSS) were measured. Water was filtered through predried and weighed GF/C filters and then re-dried and weighed. TSS were calculated by subtraction. Zooplankton biomass estimates were calculated for each filter, as described above and subtracted from the initial TSS result to give a better indication of total fine particulate suspended solids. Chlorophyll  $a$  was extracted in acetone from algae collected on GF/C filter papers and concentrations calculated spectrophotometrically using the formula presented by Talling & Driver (1961).

#### *Pretreatment and terminal sampling and analysis*

Benthic macro-invertebrates were collected from each mesocosm using a  $25 \times 25 \text{ cm}$  Ekman grab. Benthic samples were washed and filtered through a  $0.8\text{-mm}$  sieve and the remaining fibrous material preserved in a 4% formaldehyde solution. Macro-invertebrates were removed by eye and identified, where possible, to species. The number of individuals  $\text{m}^{-2}$  was calculated.

In 1997, macrophyte-associated macro-invertebrate samples were collected. Sampling involved the careful random removal of two *P. berchtoldii* specimens from each mesocosm. Each specimen was then preserved separately with 4% formaldehyde solution. Each plant was washed in water and any remaining attached macro-invertebrates removed under a dissecting stereo microscope with forceps. Macro-invertebrates were, where possible, identified to species. The plant material was dried and weighed, and macrophyte-associated macro-invertebrate number  $\text{g}^{-1}$  dry macrophyte calculated. There were no macrophytes present in the mesocosms during the pretreatment week, so only samples from the terminal week were collected.

For both years, three random whole *P. berchtoldii* plants were carefully removed, to avoid epiphyton disturbance, from each mesocosm and placed in small polythene bags. Epiphyton chlorophyll  $a$  and species abundance on each of the *P. berchtoldii* specimens was found. Each plant was placed in water and mechanically shaken vigorously. All plant material was then removed, dried and weighed. An aliquot of the plant-free water sample was analysed for chlorophyll  $a$  concentration as described above. A further aliquot was preserved with Lugol's iodide and, as described above, epiphyton species composition determined. Again, as there were no macrophytes present in the mesocosms during the pretreatment week, only samples from the terminal week were collected.

At the conclusion of both experiments, all remaining macrophytes were removed from the mesocosms with a rake. Macrophytes were washed, sorted into species, dried at  $60 \text{ }^\circ\text{C}$  for a week and dry weight calculated for each mesocosm. Fish were removed by electro-fishing, anaesthetised with MS222, killed and fork length and wet weight measured. Guts were also removed and contents examined under a dissecting microscope.

#### *Statistical analysis*

Normal distribution of data was checked by using the Kolmogorov-Smirnov goodness of fit procedure and where necessary data were transformed by  $\log_{10}$ ,  $\log_{10}(X + 1)$  or arcsine. All data were then analysed using General Linear Models (GLMs). Three-way Repeated Measures ANCOVA models were used to analyse all time series data. A two-way ANCOVA was employed if only a before- and after-time factor was present such

as with the benthic macro-invertebrate data. In all cases the pretreatment week (week 1) was used in the model as a covariate to test whether any initial differences within the mesocosms could explain between-treatment variation over time. None of the pretreatment covariate data for any of the dependent variables, or the interactions between them, showed significant differences between the mesocosms, and as such all mesocosms were considered similar at the start of both experiments. Overall, these models look for significant differences between treatments, time and their interactions. So as to avoid pseudo-replication, the two macrophyte-associated macro-invertebrate and three epiphyton chlorophyll *a* results from each mesocosm were grouped and means found for each mesocosm on each sampling occasion. These data along with the macrophyte dry-weight data were then investigated using two-way ANOVA models. The

ANOVA model is similar to the ANCOVA model described above but does not take into account time or a pretreatment covariate. When an ANCOVA or ANOVA identified significant differences between means, at the  $P = 0.05$  level, a Least Significant Difference (LSD) test was used to determine which factors were significantly different from one another.

## Results

### Experiment A – 1996

Throughout the experiment, *P. berchtoldii* was the dominant and typically the only macrophyte present in the mesocosms. The total dry weight of macrophytes within each mesocosm varied from 9 to 460 g. There was a strong effect of fish biomass ( $P = 0.001$ ) on the abundance of macrophytes (Table 1b, Fig. 1).

**Table 1** Summary of the statistically significant effects of the main treatments (a and b) and two-way interaction (c) of fish species and biomass ( $\text{kg ha}^{-1}$ ) against various variables as identified by GLMs and subsequent LSD testing in experimental mesocosms in Little Mere, 1996

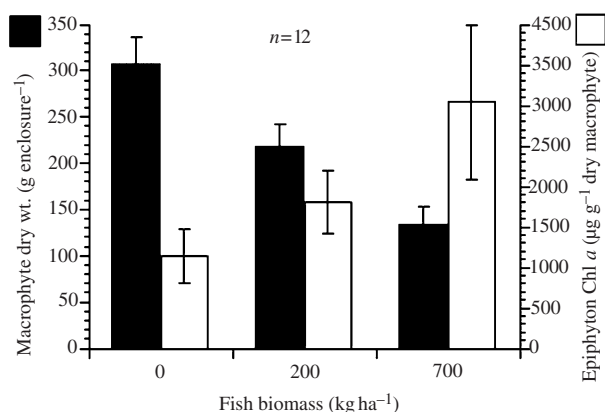
Treatment		Variable							
Fish species	Fish biomass ( $\text{kg ha}^{-1}$ )	Macrophytes	Epiphyton	TP	DIN	TSS	Small zooplankton	Large zooplankton	Edible phytoplankton
(a)									
Carp							-		-
Bream							+		+
Tench							-		-
Roach									-
(b)									
	0	+	-			-	-	+	-
	200	-				-	-		-
	700	--	+			+	+	-	+
(c)									
Carp	0								
Carp	200			+	-				
Carp	700			--			-		-
Bream	0						-		-
Bream	200				-		-		-
Bream	700			-			+		+
Tench	0			-					
Tench	200			--	-				
Tench	700			+			-		-
Roach	0				-				
Roach	200			-	+				
Roach	700				-		-		-

'-' Indicates that the treatment has significantly less ( $P < 0.05$ ) of the variable than treatments marked '+'.

'+' Indicates that the treatment has significantly more ( $P < 0.05$ ) of the variable than treatments marked '-'.

A double '--' indicates significantly less ( $P < 0.05$ ) than a single '-'.

All missing variables showed no differences between treatments.



**Fig. 1** Means (fish species and weeks 2–6 combined) for macrophyte dry-weight biomass (g enclosure<sup>-1</sup>) and epiphyton chlorophyll *a* concentration (µg g<sup>-1</sup> dry macrophyte) against fish biomass (kg ha<sup>-1</sup>) in experimental mesocosms in Little Mere, 1996 (±SEM).

Comparison of the means using the LSD test revealed that there was significantly lower macrophyte biomass in mesocosms with 700 kg ha<sup>-1</sup> of fish than in those with 200 kg ha<sup>-1</sup> which in turn had significantly lower macrophyte biomass than the control mesocosms. Neither fish species nor the two-way fish species–biomass interaction were significant in determining the macrophyte biomass (Table 1a,c).

The abundance of epiphytic algae on the macrophytes, measured as chlorophyll *a*, ranged from 120 to 19000 µg g<sup>-1</sup> dry macrophyte. Fish biomass was significant in determining the amount of epiphyton present ( $P = 0.019$ ; Table 1b, Fig. 1). Comparison of the means using the LSD test indicated that mesocosms stocked with 700 kg ha<sup>-1</sup> of fish had significantly greater amounts of epiphyton than mesocosms containing no fish. The main factor of fish species and the two-way fish species–biomass interaction did not significantly alter the abundance of epiphyton. Cyanophyta dominated the epiphyton with 92% of all cells counted being *Anabaena* spp.

Oligochaeta and Chironomidae dominated the sedimentary benthic macro-invertebrate community with overall means of 45 and 24 individuals m<sup>-2</sup>, respectively, in week 1 rising to 110 and 88 individuals m<sup>-2</sup>, respectively, in week 6. This corresponded to a rise in the total number of macro-invertebrates m<sup>-2</sup> from 77 to 210 during the experiment. However, neither of the independent fish factors or their interactions were significant in determining population structure.

Time was a significant factor in determining TP ( $P = 0.001$ ). From week 2–4 the overall mean TP was about 84 µg L<sup>-1</sup>. By week 5 it had dropped significantly to 61 µg L<sup>-1</sup> and remained at a similar level in week 6. Neither of the main fish factors had a significant effect. However TP was significantly altered by two two-way interactions between fish species and biomass and fish species and time ( $P = 0.034$  and 0.015, respectively). Further investigation revealed that mesocosms containing 200 kg ha<sup>-1</sup> of carp had significantly higher concentrations of TP than the equivalent tench- and roach-containing mesocosms and higher TP values than mesocosms with 700 kg ha<sup>-1</sup> of carp. Mesocosms containing 700 kg ha<sup>-1</sup> of tench had significantly increased TP values than those containing carp or bream at the same biomass and higher concentrations of TP than in either the 200- or 0-kg ha<sup>-1</sup> tench-mesocosms (Table 1c). TP concentrations in weeks 2, 3 and 4 were higher than in week 6 for bream and roach mesocosms, whilst for tench TP concentrations were only significantly higher in week 3. In addition, TP values in weeks 2 and 3, within bream, roach and tench mesocosms, were significantly higher than those in week 5, as were those bream and tench mesocosms in week 4. TP concentration in mesocosms containing carp did not fall with time and remained constant at around 78 µg L<sup>-1</sup>. Therefore, there were significantly greater concentrations of TP in carp mesocosms in week 5 than mesocosms containing any other fish species. Likewise, TP values were higher in mesocosms with carp than in those with bream or roach in week 6. Tench mesocosms, in week 6, also had higher TP concentrations than roach mesocosms.

Soluble reactive phosphorus levels were not significantly affected by any of the main effects of fish species or biomass or the interactions between them. Time however, was significant ( $P = 0.001$ ). The overall average SRP concentration in week 2 was 43 µg L<sup>-1</sup>. SRP then fell significantly to 19 µg L<sup>-1</sup> in weeks 5 and 6. Dissolved Inorganic Nitrogen (DIN), the summation of NH<sub>4</sub>-N and NO<sub>3</sub>-N, fell in concentration over time, although not significantly, from an overall average of 44 µg L<sup>-1</sup> in week 2 to 30 µg L<sup>-1</sup> by week 6. Neither of the main factors of fish species or biomass were significant however, there was a significant interaction between them ( $P = 0.038$ , Table 1c). Analysis found that DIN was significantly higher in 200 kg ha<sup>-1</sup> of roach-stocked mesocosms

than those mesocosms stocked with either 700, or 0 kg ha<sup>-1</sup> of roach or 200 kg ha<sup>-1</sup> of any other fish species.

After an estimate of the dry weight of zooplankton had been removed from the TSS weights, the main effect of fish biomass was significant ( $P = 0.049$ ; Table 1b). LSD analysis revealed that mesocosms with 700 kg ha<sup>-1</sup> of fish had higher values of TSS, overall mean 6.5 mg L<sup>-1</sup>, than mesocosms with 200 or 0 kg ha<sup>-1</sup> of fish, with overall means of 3.8 and 3.6 mg L<sup>-1</sup>, respectively. Time was also significant ( $P = 0.011$ ). The weekly average in week 2 was 2.7 mg L<sup>-1</sup>; it then rose significantly to an overall average of 5.8 mg L<sup>-1</sup> by week 6. Fish species was not significant but there was a two-way fish biomass–time interaction ( $P = 0.007$ ). Further analysis revealed that mesocosms stocked with 700 kg ha<sup>-1</sup> fish had significantly higher TSS in week 4 than week 2. These mesocosms also had higher values in week 6 than they did in the other weeks and either of the other biomass treatments of week 6. In week 4, mesocosms with 200 kg ha<sup>-1</sup> of fish had significantly greater TSS values than they did in week 2.

*Bosmina longirostris* (O.F. Muller) s. str., and small *Daphnia hyalina* (Leydig) were the most numerous zooplankters with overall weekly averages of 34 and 30 individuals L<sup>-1</sup>, respectively. The total numbers of small and large zooplankton were altered significantly by fish biomass ( $P = 0.003$  and  $0.047$ , respectively; Table 1b). The total number of small zooplankton was significantly greater when 700 kg ha<sup>-1</sup> of fish were present than when either 200 or 0 kg ha<sup>-1</sup> were present. This was especially true for *B. longirostris* but small *D. hyalina* were an exception and showed the same pattern as the large zooplankton in that the number of these decreased significantly as fish biomass rose from 0 to 700 kg ha<sup>-1</sup>. The two-way interaction between fish species and biomass significantly affected, as a whole, small zooplankters but not large zooplankters ( $P = 0.044$ , Table 1c, Fig. 2). LSD analysis found that small zooplankters were more numerous in mesocosms containing 700 kg ha<sup>-1</sup> of bream than in any of the other mesocosms.

Fish species had a significant effect on the density of small zooplankters and in particular *B. longirostris*. Mesocosms stocked with bream had greater densities of small zooplankters than mesocosms stocked with carp or tench ( $P = 0.047$ ). For *B. longirostris*

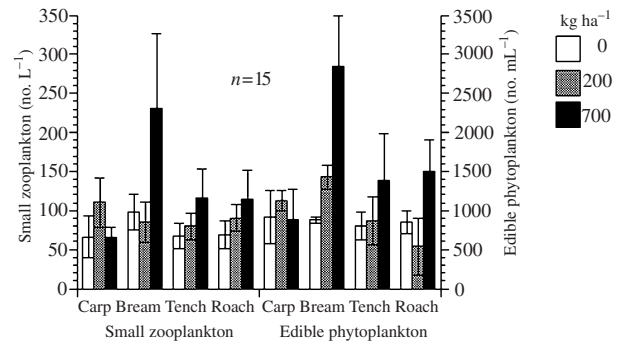


Fig. 2 Means (weeks 2–6 combined) for small zooplankton density (no. L<sup>-1</sup>) and edible (<30 μm) phytoplankton density (no. mL<sup>-1</sup>) against a two-way interaction between fish species and biomass (kg ha<sup>-1</sup>) in experimental mesocosms in Little Mere, 1996 (±SEM).

roach-mesocosms also had lower densities ( $P = 0.014$ , Table 1a).

Phytoplankton chlorophyll *a* concentrations ranged from undetectable to 64 μg L<sup>-1</sup> during the course of the experiment although weekly averages for all mesocosms ranged from 5 μg L<sup>-1</sup> in week 3 to 12 μg L<sup>-1</sup> in week 6. Neither of the main independent factors or their interactions had any significant affect on chlorophyll *a* concentrations.

As a total overall average for weeks 2–6 there were 2000 phytoplankton cells mL<sup>-1</sup>. Of these the most numerous were the Chrysophyta with 510 cells mL<sup>-1</sup> and the Cryptophyta with 980 cells mL<sup>-1</sup>. *Rhodomonas minuta* (Skuja) was the most abundant species with 610 cells mL<sup>-1</sup>. The overall total number of phytoplankton cells were unaffected by any of the main fish treatments or their interactions. When phytoplankters were divided into edible (< 30 μm) and inedible (> 30 μm or filamentous or largely mucilaginous) forms, several of the main and interactional factors were significant in determining the density of these phytoplankton forms. Time was the only significant factor to affect the density of less palatable or larger phytoplankters ( $P = 0.001$ ). There were more inedible phytoplankters in week 2 (overall mean = 1800 cells mL<sup>-1</sup>) than during any of the other weeks, which all had a similar density (overall mean = 540 cells mL<sup>-1</sup>). However, time, fish species, biomass and a two-way fish species–biomass interaction all had significant effects on the density of edible phytoplankton ( $P = 0.031$ ,  $0.009$ ,  $0.001$  and  $0.029$ , respectively; Table 1). Further analysis found that in weeks 3 and 4 there was a greater density of

edible phytoplankton (overall mean for both = 1500 cells mL<sup>-1</sup>) than during weeks 2 and 5 (overall mean = 770 and 840 cells mL<sup>-1</sup>, respectively). In mesocosms that were stocked with bream there were significantly more numerous small edible phytoplankton than mesocosms stocked with the other fish species (Table 1a). Within mesocosms stocked with 700 kg ha<sup>-1</sup> of fish there was a greater density of edible phytoplankton than in mesocosms stocked with 200 or 0 kg ha<sup>-1</sup> (Table 1b). Mesocosms stocked with 700 kg ha<sup>-1</sup> of bream had a significantly greater abundance of edible phytoplankton than any of the other mesocosms (Table 1c, Fig. 2).

Fish grew during the experiment, however, none of the mesocosms stocked with 200 kg ha<sup>-1</sup> of fish increased beyond 260 kg ha<sup>-1</sup> and none stocked with 700 kg ha<sup>-1</sup> of fish increased beyond 900 kg ha<sup>-1</sup>. Dead fish were rarely seen but when they were observed they were replaced immediately with fish from a mesocosm, located in Little Mere, containing spare stock.

#### Experiment B – 1997

Where 800 (1) kg ha<sup>-1</sup> is written this refers to mesocosms with 800 kg ha<sup>-1</sup> and one large fish whilst

800 (4) kg ha<sup>-1</sup> refers to mesocosms with 800 kg ha<sup>-1</sup> and four small fish.

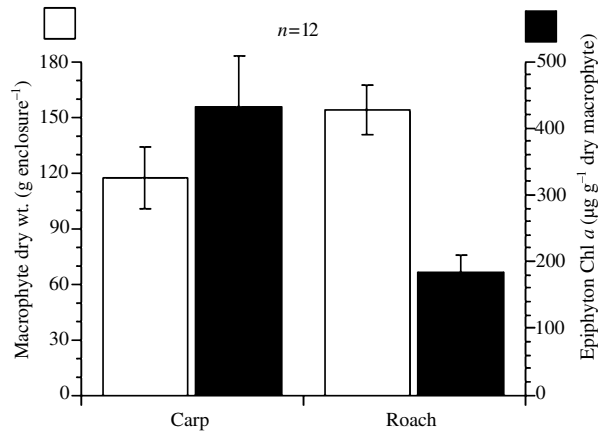
At the start of the experiment, no macrophytes were present in the mesocosms. Mid-way through, *P. berchtoldii* was the dominant macrophyte but by the end of the experiment the macrophyte community consisted of *Ceratophyllum demersum* (L) (61%), *P. berchtoldii* (24%) and *C. hermaphroditica* (12%). However, two-way ANOVA revealed that there were no significant differences in macrophyte community structure between any of the different fish treatments or their interactions at the end of the experiment.

The total dry weight of macrophytes, within the mesocosms ranged from 25 g in a mesocosm containing four small carp to 230 g present in a control mesocosm. The main effect of fish biomass/size was insignificant in relation to macrophyte biomass. However, both fish species and a two-way interaction between fish species and biomass/size were significant in determining the abundance of macrophytes ( $P = 0.023$  and  $0.031$ , respectively). Comparison of the means indicated that mesocosms containing carp had a significantly lower macrophyte abundance than mesocosms stocked with roach (Table 2a, Fig. 3). The two-way interaction failed to reveal where the differences lay when an LSD analysis was performed.

**Table 2** Summary of the statistically significant effects of the main treatments (a and b) and two-way interaction (c) of fish species and biomass/size (kg ha<sup>-1</sup>) against various variables as identified by GLMs and subsequent LSD testing in experimental mesocosms in Little Mere, 1997

Treatment		Variable						
Fish species	Fish biomass/size (kg ha <sup>-1</sup> )	Macrophytes	Epiphyton	Benthic macro-invertebrates	Plant-associated macro-invertebrates	TSS	Small zooplankton	Chlorophyll <i>a</i>
(a)								
Carp		-	+		+			
Roach		+	-		-			
(b)								
	0			+	-	-	-	
	800 (1)			-	+	+	+	
	800 (4)			-	+	-	-	
(c)								
Carp	0					-		-
Carp	800 (1)					+		+
Carp	800 (4)					-		-
Roach	0							
Roach	800 (1)					-		-
Roach	800 (4)							

'-' Indicates that the treatment has significantly less ( $P < 0.05$ ) of the variable than treatments marked '+'.  
 '+' Indicates that the treatment has significantly more ( $P < 0.05$ ) of the variable than treatments marked '-'.  
 All missing variables showed no differences between treatments.

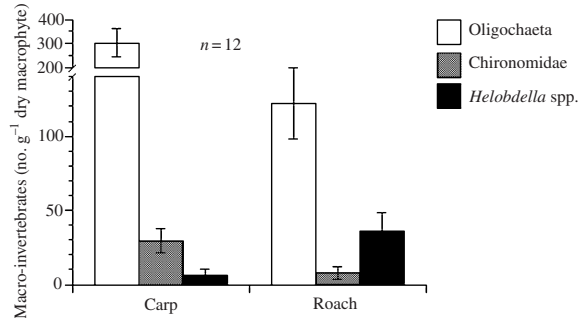


**Fig. 3** Means (fish biomass and weeks 3–9 combined) for macrophyte dry-weight (g enclosure<sup>-1</sup>) and epiphyton chlorophyll *a* concentration (µg g<sup>-1</sup> dry macrophyte) against fish species in experimental mesocosms in Little Mere, 1997 (±SEM).

The amount of attached epiphyton, measured as chlorophyll *a*, ranged from undetectable in a control mesocosm to 1070 µg g<sup>-1</sup> dry macrophyte in a mesocosm containing four carp. Fish species was the only main factor to control epiphytic abundance ( $P = 0.006$ ). Further analysis revealed that mesocosms with carp had a higher quantity of epiphyton than those containing roach (Table 2a, Fig. 3). There was no significant two-way interaction between fish species and biomass/size. Cyanophyta dominated the epiphyton with 35% of all cells being counted being *Anabaena* spp.

The sedimentary benthic macro-invertebrate community was dominated by *Asellus aquaticus* (L.) and Chironomidae with overall average weekly means of 7 and 21 individuals m<sup>-2</sup>, respectively, in week 1 rising to 44 and 25 individuals m<sup>-2</sup> by week 9. Correspondingly there was a rise in the total number of benthic macro-invertebrates m<sup>-2</sup> from 41 in week 1 to 95 in week 9.

Total numbers of benthic macro-invertebrates as well as *A. aquaticus* were significantly influenced by fish biomass/size ( $P = 0.010$  and  $0.001$ , respectively, Table 2b). Further examination revealed that in week 9, mesocosms with no fish had significantly greater densities of macro-invertebrates (overall mean = 164 m<sup>-2</sup>) compared with those containing 800 kg ha<sup>-1</sup> of fish (overall mean = 61 m<sup>-2</sup>). The main effect of fish species and its interaction with biomass/size were ineffective in determining the benthic macro-invertebrate community.



**Fig. 4** Means (fish biomass and weeks 3–9 combined) for macrophyte-associated macro-invertebrate density (no. g<sup>-1</sup> dry macrophyte) against fish species in experimental mesocosms in Little Mere, 1997 (±SEM).

Several macro-invertebrate species were found associated with macrophytes. The more numerous species belonged to Chironomidae, Oligochaeta and Hydracarina. Fish species often had a significant effect on the numbers of macro-invertebrates present ( $P \leq 0.036$ ; Table 2a). For the dominant Oligochaeta as well as Chironomidae LSD analysis found that macro-invertebrates were more numerous in carp-mesocosms than in roach-mesocosms. Only in the case of *Helobdella* spp. was the opposite scenario observed (Fig. 4). Chironomidae density was influenced by fish biomass/size ( $P = 0.006$ ). Mesocosms with 800 kg ha<sup>-1</sup> of fish were more abundant with chironomids (overall mean = 28 g<sup>-1</sup> dry macrophyte) than control mesocosms (overall mean = 1 g<sup>-1</sup> dry macrophyte) (Table 2b). There were no significant two-way fish species–biomass/size interactions.

Concentrations of TP and SRP changed significantly with time ( $P = 0.001$  in both cases). Overall average TP concentrations started at 99 µg L<sup>-1</sup> in week 3, but rose to 210 µg L<sup>-1</sup> by week 9. SRP started at 58 µg L<sup>-1</sup> and rose to an overall average of 200 µg L<sup>-1</sup> by week 9. No other main factor or interaction was significant for TP or SRP.

The concentration of DIN was significantly affected by time ( $P = 0.001$ ). LSD analysis revealed that, from an overall average of 140 µg L<sup>-1</sup> in week 3, the DIN concentration fell significantly to 100 µg L<sup>-1</sup> by week 5. It continued to fall, and by week 7 DIN concentration was 70 µg L<sup>-1</sup> whereupon it rose significantly to 110 µg L<sup>-1</sup> by week 9. There were no main or interactional effects of fish species or biomass/size.

Total Suspended Solids were significantly affected by the main factors of time ( $P = 0.001$ ) and fish

biomass/size ( $P = 0.047$ ; Table 2b). In week 3 the overall average was  $2.2 \text{ mg L}^{-1}$  but it rose significantly with time reaching  $6.6 \text{ mg L}^{-1}$  by week 7 where it remained until the experiment ended. In mesocosms stocked with one large fish the TSS was significantly higher, with an overall mean of  $1.9 \text{ mg L}^{-1}$ , than in mesocosms with either four small fish or no fish, with overall means of  $0.94$  and  $0.11 \text{ mg L}^{-1}$ , respectively. A two-way interaction between fish species and biomass/size was also apparent ( $P = 0.003$ , Table 2c). Analysis revealed that mesocosms with one large carp had significantly greater quantities of TSS than mesocosms containing one roach, no carp or four small carp.

*Cyclops* spp. were the most abundant zooplankton with an overall average, for all weeks, of 130 individuals  $\text{L}^{-1}$ . The Cladocera were dominated by *D. hyalina* females and together with *Diaptomus gracilis* (Sars) and *Cyclops* spp. contributed 86% of all the individuals counted over the experiment. Most of the biomass consisted of *Daphnia* spp. and copepods with an overall average, for all sampling weeks, of 99%. Of that 99%, copepods made up about 15% and *Daphnia* spp. 84%. The dominant *Daphnia* sp. was *D. hyalina* with an overall average of 66% of the total biomass for all weeks.

Zooplankton size was significantly affected by several of the main factors and their interactions. Time was nearly always significant for each species but certainly for the overall small and large groupings ( $P = 0.001$  for both). Further analysis revealed that there were more small and large zooplankton in weeks 7 (360 and 63 individuals  $\text{mL}^{-1}$ , respectively) and 9 (310 and 120 individuals  $\text{mL}^{-1}$ , respectively) than in weeks 3 (120 and 28 individuals  $\text{mL}^{-1}$ , respectively) and 5 (86 and 12 individuals  $\text{mL}^{-1}$ , respectively). Within the small group, the uncommon *B. longirostris* were influenced by time, as described above, fish biomass/size and a two-way interaction between fish species and time ( $P = 0.019$  and  $0.048$ , respectively). In the case of biomass/size there were more *B. longirostris* in mesocosms stocked with one large fish (overall mean = 1.2 individuals  $\text{L}^{-1}$ ) than mesocosms containing either none or four small fish (overall mean = 0.5 individuals  $\text{L}^{-1}$  for both) (Table 2b). For the two-way interaction carp mesocosms, in week 9, had a higher density of *B. longirostris* than roach mesocosms of the same week. *Cyclops* spp. density was significantly affected by a two-way

interaction between fish biomass/size and time ( $P = 0.001$ ). Further analysis found that, in week 7, mesocosms stocked with four fish had a higher number of *Cyclops* spp. individuals than mesocosms stocked with none or one fish in the same week. In addition, in week 9, mesocosms with one fish had higher densities of *Cyclops* spp. than mesocosms of the same week stocked with either no fish or four fish. As a group, the small zooplankton were affected by a two-way interaction between fish biomass/size and time as described above for *Cyclops* spp. ( $P = 0.038$ ). The large zooplankton group were affected by a two-way interaction between fish species and time ( $P = 0.042$ ). Mesocosms in week 9 containing carp had more numerous large zooplankters than mesocosms containing roach in week 9.

Chlorophyll *a* was affected significantly by time ( $P = 0.001$ ) and a two-way interaction between fish species and biomass/size ( $P = 0.015$ ; Table 2c). LSD analysis found that low concentrations in weeks 3 and 5, of around  $3 \mu\text{g L}^{-1}$ , rose significantly by week 7 to an overall average of  $32 \mu\text{g L}^{-1}$  and then fell significantly by week 9. For the two-way interaction between fish species and biomass/size, mesocosms with one large carp had higher concentrations of chlorophyll *a* than those stocked with either one large roach, no carp or four small carp (Table 2c).

As a total overall average for all experimental weeks there were 3600 phytoplankton cells  $\text{mL}^{-1}$ . Of these the most numerous were the Chrysophyta and Cryptophyta with 1700 and 1600 cells  $\text{mL}^{-1}$ , respectively. The two most prolific species were both from the Cryptophyta: *Cryptomonas erosa* (Ehrenberg) with on average 900 cells  $\text{mL}^{-1}$  and *R. minuta* with 730 cells  $\text{mL}^{-1}$ . None of the fish treatments or their interactions were significant in determining the total numbers of phytoplankton. Furthermore when phytoplankters were split into either edible ( $< 30 \mu\text{m}$ ) or inedible ( $> 30 \mu\text{m}$  or filamentous or largely mucilagenous) forms only time was significant in determining the phytoplankton structure ( $P = 0.001$ ). Analysis found that in week 7 there were more edible phytoplankton (overall mean = 6500 cells  $\text{mL}^{-1}$ ) than in any of the other weeks (overall mean = 2200 cells  $\text{mL}^{-1}$ ). No main fish factor or interaction was significant.

Fish grew during the experiment, however, none of the mesocosms stocked with  $800 \text{ kg ha}^{-1}$  of fish increased beyond  $1000 \text{ kg ha}^{-1}$ . Dead fish were rarely

seen but when they were observed they were replaced immediately with fish from a mesocosm, located in Little Mere, containing spare stock.

## Discussion

This discussion covers three issues. The first is the effects of fish species, biomass and size on macrophyte abundance and possible causes for the effects. The second and third discuss the mechanisms that may lie behind the causes:

- top-down effects of fish in removing zooplankton and macro-invertebrate grazers that may lead to macrophyte shading through reduced phytoplankton and epiphyte removal as well as direct macrophyte ingestion by fish.
- bottom-up effects of fish that release nutrients from disturbed sediment or via fish excretion which aids the growth of phytoplankton and epiphytes and along with disturbed sediment can shade out macrophytes.

An overall summary of the statistically significant main and interactional effects of fish species, biomass and size on measured parameters for both years are presented in Tables 1 and 2.

### *The effects of fish species, biomass and size on macrophyte abundance and their possible causes*

In the first experiment there was a strong negative relationship between fish biomass and macrophyte abundance. This supports the work carried out by Hill *et al.* (1987). There was no significant effect of fish species alone in relation to macrophyte biomass, in contrast to work carried out by others. This may have been because of the small size of fish used (15 cm fork length) such that adult characteristics did not show themselves. All were largely zooplanktivorous and did not differ in their functional characteristics from one another (Hartley, 1940). This was consistent with a lack of significant differences within the benthic macro-invertebrate data for any of the mesocosms in 1996.

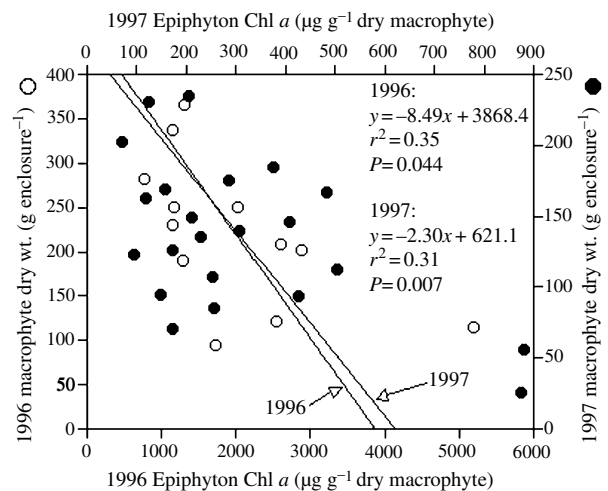
During the 1997 experiment, fish size was increased and results indicate a strong effect of fish species on macrophyte biomass. Mesocosms stocked with carp had significantly less macrophytes than roach mesocosms. This agrees with Crivelli (1983). In addition it confirms the general consensus that carp reduce macrophyte abundance hence the removal of carp

from lakes is often needed for recovery of macrophytes within them (Ozimek *et al.*, 1990; Wright & Phillips, 1992).

Fish biomass, irrespective of fish species, was not significant in determining the macrophyte biomass in 1997. The reason for this disparity between years is unclear. However in 1996, by the end of the experiment, the dominant macrophyte species was *P. Berchtoldii*, whilst in 1997 it was *C. demersum*; this may be key to understanding the disparity. It may be that the more delicate *P. berchtoldii* were affected by fish movement and disturbance irrespective of fish species as well as being affected by species-specific indirect effects of fish. The more robust *C. demersum* may have only been affected by indirect effects of fish species and not general fish presence.

The combined macrophyte biomass reduction with fish biomass and species, over the 2 years, could be for several potential reasons as described earlier in the introduction. However in both years there was a very clear and strong negative relationship between the epiphyton and macrophyte abundance (1996:  $P = 0.044$ , 1997:  $P = 0.007$ ; Fig. 5).

It is likely that the decline in macrophytes was because of epiphyton that shaded macrophytes or competed with them for  $\text{CO}_2$  or both. This agrees with work carried out by Phillips *et al.* (1978), Sand-Jensen (1983) and Simpson & Eaton (1986). It could be argued



**Fig. 5** Linear regression between macrophyte dry weight biomass ( $\text{g enclosure}^{-1}$ ) and epiphyton chlorophyll *a* concentration ( $\mu\text{g g}^{-1}$  dry macrophyte) in experimental mesocosms in Little Mere, 1996 and 1997 including linear equation,  $r^2$  value and  $P$ -value.

that the inverse relationship is simply a response of epiphyton to an improved light climate resulting from a direct impact of fish on macrophytes. However no evidence for uprooting or plant ingestion was found. Moreover, the lake itself has an abundant macrophyte community and an excellent light climate, clear to the bottom throughout the year, yet epiphyton growth is not extensive. Thus, indirect effects of fish that favour epiphyton, which itself is detrimental to macrophytes, is the most likely explanation of macrophyte decline in the mesocosms. However, questions remain regarding the mechanisms that lie behind this epiphyton-induced macrophyte decline. Does fish excreta or disturbed/undisturbed sediment release nutrients that aid epiphyton growth and shading or are there other factors involved such as macro-invertebrate interactions? In addition, does disturbed sediment or an enhanced phytoplankton abundance increase the degree of shading and further affect macrophytes?

#### *Top-down effects of different species, biomass and sizes of fish*

The number of macrophyte-associated macro-invertebrates was, for the most dominant species, significantly higher in mesocosms stocked with carp than roach. This could be because, roach removed macro-invertebrates from macrophytes with a greater efficiency than carp. However, if macro-invertebrates were having a significant grazing effect on the epiphyton, as suggested by Bronmark & Weisner (1992), then it might be predicted that the carp-mesocosms would contain macrophytes with less epiphyton. This, however, was not the case and significant regressions between epiphyton (chlorophyll *a*  $\mu\text{g g}^{-1}$  dry macrophyte) and macro-invertebrates (no.  $\text{g}^{-1}$  dry macrophyte) were all positive. The total number of oligochaetes was most closely correlated with epiphyton abundance ( $P = 0.001$ ,  $r^2 = 0.71$ , Fig. 6). The effect of macro-invertebrate grazing on keeping macrophytes epiphyton-free was therefore negligible; as the quantity of epiphyton increased, the quantity of macro-invertebrates feeding on the epiphyton increased. It should be noted that there was a distinct scarcity of snails in the macro-invertebrate community. The lack of snails is unlikely to be related to the density of fish within the lake as this is low, typical non-piscivorous biomass being  $<20 \text{ kg ha}^{-1}$ . The scarcity is thought to be a natural

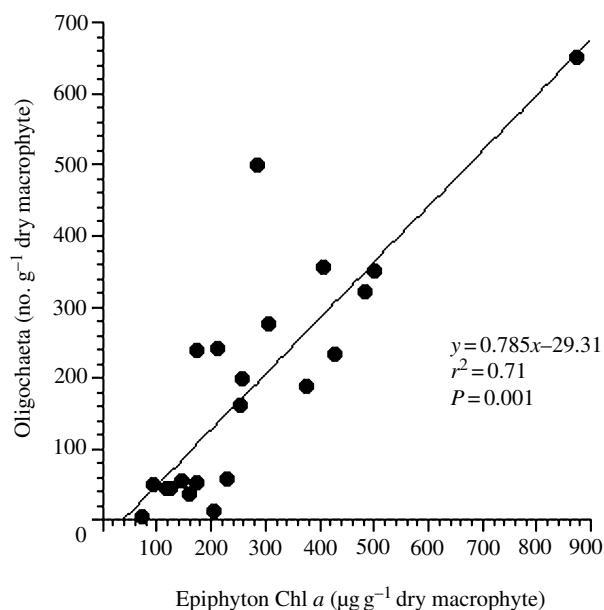


Fig. 6 Linear regression between macrophyte-associated Oligochaeta density (no.  $\text{g}^{-1}$  dry macrophyte) and epiphyton chlorophyll *a* concentration ( $\mu\text{g g}^{-1}$  dry macrophyte) in experimental mesocosms in Little Mere, 1997 including linear equation,  $r^2$  value and  $P$ -value.

phenomenon as identified in other aquatic systems where macro-invertebrate communities are dominated by more numerous, smaller macro-invertebrates (Gresens, 1995).

As found elsewhere (Hrbacek *et al.*, 1961; Lynch, 1979), fish biomass was significant in determining zooplankton abundance. The total number of small zooplankton was significantly greater when  $700 \text{ kg ha}^{-1}$  of fish were present, than when either  $200$  or  $0 \text{ kg ha}^{-1}$  were present. Conversely, the number of large zooplankton decreased significantly as fish biomass rose from  $0$  to  $700 \text{ kg ha}^{-1}$ . In 1997, although the findings were not as strong, there was significantly more small zooplankton in mesocosms stocked with four fish, in week 7, than those stocked with no or one fish. In addition there were more small zooplankton in mesocosms containing one fish, in week 9, than mesocosms stocked with either no or four fish. Overall this supports the Size-Efficiency Hypothesis put forward by Brooks & Dodson (1965). However, whether this had any effect on the shading potential of phytoplankton was important to assess.

In the 1996 experiment, there were lower abundances of chlorophyll *a*, phytoplankton and zooplankton than during the second experiment. To determine

whether zooplankton were grazing the phytoplankton crop, regressions between zooplankton density (dry weight  $\mu\text{g L}^{-1}$  and number  $\text{L}^{-1}$ ) and phytoplankton abundance (chlorophyll *a*  $\mu\text{g L}^{-1}$ ) were performed. In the 1996 experiment, the significant relationships were, on the whole, positive. It is therefore unlikely that zooplankton were having a significant grazing effect on phytoplankton; they appeared to generally increase in number with increasing food (phytoplankton) supply. The opposite was however true for the second experiment, where all regressions were negative and as such grazing was likely to have been significant. This relationship was especially true for *Daphnia* spp., and supports a relationship observed by Moss, McGowan & Carvalho (1994) who found a strong inverse correlation between chlorophyll *a* and Cladocera in shallow West Midland meres.

Estimated grazing and phytoplankton reproduction rates provided further insight into the results of the 1997 experiment. Cladocerans are understood to potentially ingest about 100% of their biomass each day and copepods about 50%, in terms of phytoplankton standing biomass (Jeppesen *et al.*, 1994). Assuming that phytoplankton have about a 30%  $\text{day}^{-1}$  reproduction rate during summer (Fogg, 1975), then it is possible to determine the impact of grazing. Zooplankton grazing and phytoplankton reproduction rates ( $\text{mg L}^{-1} \text{day}^{-1}$ ) were calculated, on a fortnightly basis, using the percentages given above and biomass calculations as explained in the methodology. In nearly all mesocosms, zooplankton grazing exceeded phytoplankton reproduction. As such, in 1997, zooplankton were grazing phytoplankton to a large degree, but they were doing this irrespective of fish presence, whilst in 1996 zooplankton seemingly had little effect on phytoplankton densities. Furthermore, in both years, the main effects of fish species, biomass or size had no effect on the total number of phytoplankton cells. The only doubt to this was raised in 1996, where mesocosms containing bream, particularly  $700 \text{ kg ha}^{-1}$  of bream, had more numerous small edible phytoplankton. These mesocosms also had more small zooplankters than other mesocosms. This was presumably as a result of bream removing the larger zooplankton and allowing the small zooplankton to be competitively advantaged and dominate. Small zooplankton are less-efficient grazers (Burns, 1968), and as a result the development of inedible colonies is less likely (Lynch & Shapiro,

1981). However, there were no fewer large zooplankters in mesocosms containing  $700 \text{ kg ha}^{-1}$  of bream than the other mesocosms, just more small zooplankton and more numerous edible phytoplankters. As such, some factor other than grazing may have been important in determining the phytoplankton population, for instance, nutrient availability. Furthermore, chlorophyll *a* concentration was the same in all 1996 mesocosms, so any differences in shading impact because of different phytoplankton communities would likely be minimal. Therefore, although fish presence can influence zooplankton abundance, this probably did not affect the degree of phytoplankton shading through zooplankton removal and subsequent grazing reduction.

In 1997, zooplankton were significantly grazing phytoplankton, but in mesocosms containing one fish or carp there were generally not only greater numbers of zooplankters, but also higher chlorophyll *a* concentrations. It is thus possible that the phytoplankton crop was growing faster, in these carp-mesocosms, than in other mesocosms. It is feasible that this relative increase in phytoplankton biomass could have been because of the release of nutrients from disturbed sediment or fish excreta (Hilsenhoff, 1965; Lamarra, 1975; Andersson *et al.*, 1988).

Overall it seems that, although fish can affect the abundance of macro-invertebrates and zooplankton, the effect this has on macrophyte shading, either from epiphyton or phytoplankton reduction or increase, is probably minimal and as such the top-down effects of fish on these processes did not influence the abundance of macrophytes. Furthermore, direct fish-induced macrophyte removal by ingestion or uprooting was not apparent from fish gut contents or visual data.

#### *Bottom-up effects of fish in release of nutrients*

Epiphyton and phytoplankton are likely to be affected by nutrient levels in similar ways, although epiphyton may gain additional nutrients directly from macrophytes (Allen, 1971). However, Carignan & Kalff (1982) suggest that epiphytic algae obtain <10% of their phosphorus from the host macrophyte, whilst Cattaneo & Kalff (1979) suggest that macrophytes act as a neutral substrate for algal growth. Hence, as epiphyton samples were collected only at the end of both experiments, factors that might have affected the epiphyton will be inferred from the

phytoplankton/nutrient results which were collected weekly or fortnightly.

Phosphorus is usually relatively scarce in temperate regions and therefore becomes limiting in many waterbodies. However, the deeper, stratified West Midland meres have a low N : P ratio and Reynolds (1979) and Moss *et al.* (1994) suggested that, on the whole, the phytoplankton crops in these are nitrogen limited. Over the last few years in Little Mere, which is fed from such a deep mere, Mere Mere, summer SRP concentrations have been in the region of 30–150  $\mu\text{g L}^{-1}$ , whilst DIN concentrations have been low or undetectable. It is therefore believed that, in Little Mere, the summer phytoplankton crop, and by association, the epiphyton is limited by nitrogen availability (Stephen, 1997).

Several factors can be used to examine the validity of this hypothesis. The N : P ratio during the entire 1996 experiment was, by weight, on average  $0.5 \pm 0.9$  and for 1997  $1.0 \pm 0.7$ , a great deal lower than that which might be expected, were the system not nitrogen limited ( $> 10.0$  by weight). The A480 : A663 phytoplankton photosynthetic pigment ratios were nearly always  $> 1.3$ , suggesting that the phytoplankton crop was either nitrogen limited or decaying (Moss, 1967). The dominance of nitrogen-fixing Cyanophyta within the epiphyton and its abundance within the phytoplankton, during both years, may also be an indication of nitrogen limitation (Schindler, 1977).

Phytoplankton crop (chlorophyll *a*  $\mu\text{g L}^{-1}$ ) and epiphyton abundance (chlorophyll *a*  $\mu\text{g g}^{-1}$  dry macrophyte) when regressed with available nutrients such as TP, SRP and DIN were occasionally related significantly. Both years showed varied and different positive and negative relationships, however, overall, it seems that at the start of each experiment, phosphorus was not limiting. Phosphorus concentrations were, in relation to nitrogen, relatively high at the start of each experiment. SRP was, when significantly related to phytoplankton and epiphyton crop, nearly always negatively related, i.e. as algal crop increased the amount of SRP decreased, but probably never became limiting because of the large initial amount. If DIN was limiting throughout the summer, significant regressions might be expected between algae abundance and DIN for all weeks. This, however, was not the case, although regressions indicated that it became limiting towards the end of both experiments. In addition, several phytoplankton species decreased in

abundance as the experiments progressed, whilst notably *A. flos-aquae* began to increase in abundance towards the end of the 1996 experiment. Whilst this may have been as a result of seasonal weather factors or grazing, it may also indicate a competitive advantage gained from its ability to fix nitrogen in a nitrogen-limited environment.

Dissolved Inorganic Nitrogen concentration was always low ( $< 140 \mu\text{g L}^{-1}$ ) throughout both summers and it is possible that pulsed release, followed by immediate uptake, may have been occurring. As a result strong and obvious regressions between DIN and algal growth for all weeks are not likely to be apparent.

Overall, the findings suggest that the phytoplankton crop and epiphyton were removing nutrients from the water column and that nutrient availability, in particular nitrogen, may have been limiting algal growth. However, what effect did the fish have in making nutrients available to the algal communities?

Total Suspended Solids and therefore disturbed sediment increased as time passed in both years and it could be argued that the rise in TSS, or indeed another factor such as fish excreta, was increasing the available nutrients in the water column (Hilsenhoff, 1965; Lamarra, 1975; Andersson *et al.*, 1988). TSS will be influenced by phytoplankton biomass and its rise with time could just mirror a rise in phytoplankton abundance and not just disturbed sediment. However, mesocosms containing a higher biomass of fish (1996) or larger fish (1997) had significantly higher quantities of TSS but no corresponding significant differences in total phytoplankton abundance (no.  $\text{mL}^{-1}$  or chlorophyll *a*  $\mu\text{g L}^{-1}$ ). Therefore, TSS did include a quantity of disturbed sediment. This increased in mesocosms containing a greater biomass of fish and in particular those containing large carp, probably as a result of general fish movement and increased benthic feeding, as might be expected. This was in part indicated by the second experiment's benthic macro-invertebrate data, where there were more benthic macro-invertebrates in control enclosures than mesocosms stocked with fish.

The physico-chemical data suggest that this bioturbated, de-oxygenated, sediment was releasing phosphorus (Graneli, 1979; Phillips *et al.*, 1994) and that disturbed sediment was also releasing phosphorus in particulate form whilst DIN, which is not released in the same way, fell as the phytoplankton crop increased over time. Levels of fish-induced nutrient

release, either from disturbed sediment (Hilsenhoff, 1965) or excreta (Lamarra, 1975; Andersson *et al.*, 1988) are of direct relevance. Lamarra (1975) found that 200 kg ha<sup>-1</sup> of carp can increase the concentration of SRP by 0.52 mg m<sup>-2</sup> day<sup>-1</sup>, and TP by between 1 and 2 mg m<sup>-2</sup> day<sup>-1</sup>, whilst 100 kg ha<sup>-1</sup> of bream can excrete 0.67 µg TP L<sup>-1</sup> day<sup>-1</sup> (Andersson *et al.*, 1988). However, in the current experiments there were no differences in SRP concentration between fish treatments, and phosphorus levels in 1996 rose in all mesocosms including those with no fish. Phosphorus release from sediment is well documented (Marsden, 1989). Indeed, phosphorus release experiments by Beklioglu (1995) and Stephen (1997), both indicate that Little Mere sediment can release about 30 mg m<sup>-2</sup> day<sup>-1</sup>. Therefore, although fish excreta or sediment disturbance may have added to phosphorus release, this release was occurring independently of fish treatment. As such, nitrogen was probably limiting and disturbed sediment had little effect on levels. The release of nitrogen from fish excreta was therefore the most likely catalyst of increased epiphyton.

In both mesocosm experiments, direct shading from disturbed sediment or phytoplankton seemed unlikely as there were no significant regressions with TSS or phytoplankton chlorophyll *a* and the abundance of macrophytes. Indeed Phillips *et al.* (1978) suggested an increase in the phytoplankton crop which is sometimes an after effect of macrophyte decline rather than its cause.

## Conclusion

In conclusion, the decrease in macrophyte abundance was most likely to be the result of an increase in the epiphytic load and a subsequent reduction in available light and CO<sub>2</sub> to the plants (Phillips *et al.*, 1978; Sand-Jensen, 1983; Simpson & Eaton, 1986). The direct impact of fish in uprooting or ingesting macrophytes appears minimal. Therefore, the likelihood of epiphyton proliferating in response to a decreasing macrophyte population and increased light availability, although possible, is unlikely to be the cause of macrophyte decline. Shading by phytoplankton or disturbed sediment may become important, or complement the effect of epiphyton, once macrophytes have begun to diminish, however, the direct effect of these two processes seems to be minimal in Little Mere. Indeed, Moss (1981) used palaeolimnological

records, from lakes where macrophytes had disappeared, to suggest that epiphytic algae increased with nutrient addition and that this happened before any changes in the abundance of phytoplankton were detectable.

The algal crop in Little Mere is most likely controlled by nutrient limitation, especially nitrogen. Top-down grazing may partly reduce the algal crop but does not control it. Fish probably fed on macro-invertebrates present on the macrophytes, although it is unlikely that this had any significant impact on the amount of epiphyton present in these experiments. Zooplankton occasionally grazed the phytoplankton by a significant degree, although it remains possible that in certain mesocosms, where increased nutrient availability was apparent, the phytoplankton crop was growing faster than it could be cropped by zooplankton.

It was evident from benthic macro-invertebrate data that larger fish were feeding on the benthos and that in mesocosms stocked with large fish, especially carp, there was an increase in the amount of disturbed sediment which may have increased phosphorus levels. However, phosphorus release from sediment was occurring, irrespective of fish treatment and was comparatively high in comparison with available nitrogen. Nitrogen was therefore the likely limiting nutrient and was seemingly more available in mesocosms stocked with carp, a higher biomass of fish and larger fish, probably as a result of an increase in fish excreta. However, an almost immediate response, by algae, to take up nitrogen that becomes available is important to understanding the findings.

Stephen *et al.* (1998) presented work carried out in Little Mere during 1995 and 1996 and concluded that phytoplankton in Little Mere was not nitrogen limited during the growing season. Although nitrogen availability sets a maximum potential phytoplankton biomass; this level was not realised owing to control by zooplankton grazing. It is therefore possible, and likely, that the controlling processes in Little Mere are undergoing change as they have in Lake Zwemlust (Netherlands) (Ozimek *et al.*, 1990). It is also quite probable that both top-down and bottom-up processes play important roles within Little Mere and indeed other lakes but that the relative importance of each process may change from year to year possibly in conjunction with changing macrophyte communities.

## Acknowledgments

We would like to thank: the Economic and Sociological Research Council and English Nature for funding, Mike Sheehan of the Mere Golf and Country Club for allowing me to use Little Mere and David Holliday, Paula Knight, Jim McGill, Suzanne McGowan, Debbie Stephen, Derek Ward, David Wilson and Eddie Wright for field and laboratory assistance.

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(Manuscript accepted 26 June 2002)