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Are zooplankton food resources poor in the vegetated littoral zone of shallow lakes?

JOSHUA BOOKER* AND KENDRA S. CHERUVELIL*,†

*Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, U.S.A.

†Lyman Briggs College, Michigan State University, East Lansing, MI, U.S.A.

SUMMARY

1. The distribution of zooplankton in shallow lakes is negatively related to macrophyte density. However, the abundance of their food along density gradients of macrophytes is unknown. A common but untested assumption is that food quantity and quality for pelagic zooplankton is poor in the littoral zone owing to the deleterious influence of macrophytes on phytoplankton.

2. We tested this assumption with a combination of a field survey and laboratory experiments. We collected seston samples from the littoral and pelagic zones of four shallow temperate lakes and related food quantity (phytoplankton biovolume) and quality to macrophyte abundance (per cent volume infested). Seston food quality was assessed in three ways: N/C and P/C ratios, polyunsaturated fatty acid content and phytoplankton community composition. In the laboratory, we measured the growth and reproduction of *Daphnia pulicaria* on diets consisting of seston from the littoral and pelagic zones in one lake.

3. In our four study lakes, food quantity was not significantly influenced by macrophyte abundance, and food quality was generally high. Laboratory experiments showed increased juvenile growth, but no significant change in *D. pulicaria* reproduction, when feeding on littoral resources compared to pelagic resources.

4. Our results suggest that there is no nutritional cost to pelagic zooplankton inhabiting the littoral zone. Therefore, it is likely that other factors (e.g. predation, abiotic factors) are involved in determining zooplankton habitat use.

Keywords: *Daphnia*, food quality, macrophytes, phytoplankton, shallow lake

Introduction

Studies of the interaction between macrophytes and phytoplankton have led to the general conclusion that, when abundant, macrophytes tend to have negative effects on phytoplankton abundance (Gopal & Goel, 1993). Three mechanisms have been documented. First, macrophytes may outcompete phytoplankton by reducing the availability of nutrients (Carpenter & Lodge, 1986) and light (Mulderij, Van Nes & Van Donk, 2007). Second, allelopathic chemicals exuded by *Chara* and other macrophytes may inhibit phytoplankton growth (Gross *et al.*, 2007). Third, the physical structure of submersed macrophytes, such as *Ceratophyllum demersum* L., may reduce water movement and increase the net sinking loss of non-buoyant phytoplankton (Horppila & Nurminen, 2005). Competition between phytoplankton and macrophytes is especially evident in shallow lakes, as these systems tend to be dominated by

Correspondence: Joshua Booker, Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824, U.S.A.
E-mail: bookerj85@gmail.com
Present address: Joshua Booker, U.S. Fish and Wildlife Service, Texas Fish and Wildlife Conservation Office, 500 E. McCarthy Lane, San Marcos, TX 78666, U.S.A.

either one or the other (Scheffer *et al*., 1993). The consequences of this competition between primary producers is unknown for planktonic grazers of phytoplankton.

Macrophyte density changes with distance from the shore in many shallow lakes, forming different habitats. Zooplankton species of the order Cladocera typically partition their habitat according to this horizontal gradient of vegetation (Smiley & Tessier, 1998). Based on the known negative relationship between macrophytes and phytoplankton, the conventional explanation is that there is less seston in the vegetated littoral zone than in the pelagic. For example, Scheffer (2004) states that ''submerged weed beds with their low phytoplankton concentrations should be an unfavorable foraging habitat for pelagic zooplankton''. Although this would appear to explain the typical distribution of *Daphnia* within shallow lakes (higher densities in the pelagic zone), the hypothesis of reduced food quantity for zooplankton in the littoral zone remains untested.

Food quality may be even more important for *Daphnia* fecundity and growth than food quantity (Sterner, 1993; Kilham *et al*., 1997), and results from some studies suggest that food quality for *Daphnia* may be poor in the presence of macrophytes. Laboratory experiments show that macrophyte exudates can directly increase phytoplankton cell volume and colony formation in *Scenedesmus obliquus* (Turpin) Kützing (Mulderij, Mooij & Van Donk, 2005), rendering the cells less edible for *Daphnia*. In field observations of single European lakes, Vuille (1991) and Søndergaard & Moss (1998) observed a higher proportion of large and inedible algae in the presence of macrophytes than in their absence. Smiley & Tessier (1998) used a reciprocal transplant experiment with pelagic and littoral zooplankton species in one North American shallow lake to show that littoral resources were poorer than pelagic resources, but it was unclear whether this was attributable to low food quality or quantity. Although these studies provide some evidence supporting the hypothesis that macrophytes may lower daphniid food quality, research to date has been restricted to the laboratory or, if in the field, to single lakes and has involved only one measurement of zooplankton food quality. Therefore, the generality of the conclusion that macrophyte beds harbour poor resources for filter-feeding zooplankton remains uncertain.

A measure of food quality is required to determine how macrophytes affect *Daphnia* nutrition. The elemental content of phytoplankton, specifically the stoichiometric ratio of carbon (C) to nitrogen (N) and phosphorus (P), is currently the most widespread measurement of food quality. In many limnological studies, high food quality is often equated with high P/C or high N/C of lake seston (e.g. Sterner, 1993; Kilham *et al*., 1997; Elser, Hayakawa & Urabe, 2001). More recently, researchers have put biochemical focus on zooplankton resources. For example, the amount of polyunsaturated fatty acids (PUFAs) in algae has significant impacts on zooplankton growth (Gutseit, Berglund & Graneli, 2007) and egg production (Müller-Navarra *et al*., 2000). In particular, α-linolenic acid (C18:3n-3) is positively correlated with *Daphnia* growth (Wacker & Von Elert, 2001), and eicosapentaenoic (C20:5n-3) and arachidonic (C20:4n-6) acids are positively correlated with egg production (Müller-Navarra *et al*., 2000; Martin-Creuzburg & Von Elert, 2009). However, no studies have quantified differences in phytoplankton stoichiometry or fatty acids between the pelagic and littoral zones or along gradients of macrophyte abundance (Burks, Lodge, Jeppesen *et al*., 2002).

A third common way of measuring *Daphnia* food quality is by characterising the species composition of the phytoplankton (Demott & Gulati, 1999; Ravet & Brett, 2006). The logic of this approach is that different phytoplankton taxa vary in biochemical properties and digestibility (i.e. based on size, cell wall thickness and the presence of spines or mucilage). Cyanobacteria are considered poor-quality food for *Daphnia*, whereas diatoms and cryptophytes are considered high-quality food (Brett, Müller-Navarra & Park, 2000). However, although chlorophytes are described as an adequate food source (Brett *et al*., 2000), certain taxa have been shown to be poorly digested by *Daphnia* owing to their cell structure (*Sphaerocystis*, Porter, 1976; *Cosmarium*, Coesel, 1997).

We tested the hypothesis that *Daphnia* food quantity and quality is negatively influenced by macrophyte density using seston sampled from the littoral and pelagic zones of four shallow lakes. *Daphnia* food quality was quantified in several ways: elemental content (C, N, P), fatty acid content and species composition. We also measured food quality directly by conducting laboratory experiments on the growth and reproduction of *Daphnia* that were fed on lake

seston. We predicted that (i) there would be a negative relationship between macrophyte abundance and edible phytoplankton biomass, (ii) there would be a negative relationship between macrophyte abundance and food quality (as expressed by N/C, P/C, the content of total polyunsaturated fatty acids and the per cent high-quality taxa) and (iii) *Daphnia* growth and reproduction would be higher when feeding on pelagic resources than on food from littoral areas with dense macrophyte beds.

Methods

Lake field survey

Study lakes. Four small shallow (max depth < 5 m) lakes in south central Michigan, U.S.A., were sampled for lake seston on four different days during June 2009 (Table 1). These lakes were chosen because they do not stratify and have had no herbicide or algicide applications within the past 5 years. The shorelines of all lakes were undeveloped and forested. The lakes all have both inflow and outflow streams and harboured fish and invertebrate predators. All lakes were located in Michigan State Game Areas, which are natural parks that afford protection against vegetation disturbance and commercial use.

Quantifying lake conditions. For each lake, we measured Secchi depth at the deepest point. Within each lake, 14–18 sample sites were determined using a stratified random sampling protocol. The strata consisted of a littoral zone (i.e. water depth < 1.5 m or macrophytes visible from the surface) and a pelagic zone, with half of the sample sites allocated to each zone. At each sample point, lake seston, temperature, dissolved oxygen (DO) and macrophytes were sampled. Surface temperature and DO were measured with a probe (YSI 550A) at a depth of 0.5 m.

To sample lake seston, 2 L of water was collected from the entire water column at each sample point, stored cool and in the dark and then filtered in the laboratory. At sample points with dense macrophytes, water was collected using a manual hand pump and narrow tubing to minimise disturbance of the plants, thus avoiding accidental collection of periphyton. The tubing was continuously moved vertically to ensure that the entire water column was sampled during collection. In areas with low macrophyte density, a tube sampler was used to take a sample of seston, integrating the whole water column.

Macrophyte abundance estimates entailed combining water depth, macrophyte height and percentage macrophyte cover measurements to obtain a measure of the per cent volume infested (PVI) at each sampling point (calculated as the product of the percentage cover and plant height divided by the water depth; Canfield *et al.*, 1984). The PVI of free-floating macrophytes was measured using root length rather than macrophyte height (Meerhoff *et al.*, 2003). Percentage cover was determined using a buoyant 0.1- m^2 PVC quadrat and visually estimating the area occupied.

All macrophyte species were recorded at each sample point. To compare quantitatively macrophyte species among lakes, we calculated the frequency of macrophyte species within each lake using the following formula: (sampling points with a species/total sampling points) × 100 (Nichols, Weber & Shaw, 2000). These frequencies were then converted to relative frequencies by dividing by the sum of frequencies for all species. We also calculated species richness and Simpson's (1949) diversity index for each lake.

Quantifying zooplankton food quality. We evaluated seston food quality in three ways: elemental content, fatty acid content and taxonomic composition of the

Table 1 Characteristics of study lakes with latitude and longitude using the NAD83 coordinate system. All comparisons of surface temperature between lakes were significant (Tukey's honest significant difference; $\alpha = 0.05$). Dagget Lake had significantly lower dissolved oxygen (DO) than the other three lakes ($P < 0.05$). Total phosphorus (TP) values were obtained from single pelagic samples

Study lakes	Latitude, longitude (degrees minutes)	Sample date	No. of littoral samples	No. of pelagic samples	Area (ha)	Max depth (m)	Secchi depth (m)	Mean (SD) surface temperature (°C)	Mean (SD) DO mg L^{-1}	TP $\mu g\ L^{-1}$
Dagget	42°35′N-85°26′W	30 June 2009	8	7	7.2	4.5	1.6	24.3 (0.06)	5.3 (1.41)	34
Potter	42°47′N-84°24′W	12 June 2009	9	5	8.4	1.5	0.8	19.1 (1.73)	7.7 (2.04)	30
Muskrat	42°54′N-84°35′W	19 June 2009	9	9	17.4	4.3	0.1	22.9 (0.54)	9 (1.52)	313
Hall	42°36′N-85°28′W	25 June 2009	8	8	23.1	3.9	2.8	29.2 (0.59)	9.2 (0.76)	26

phytoplankton. All lake water was filtered three times through a 35 μm Nitex sieve to remove zooplankton and large, inedible algae. Therefore, all particles analysed were considered edible for most crustacean zooplankton (Demott, 1989). Vacuum pump filtration was used to collect seston on pre-combusted (500 °C for 2 h) Whatman GF/F filters. Three filters were obtained for each sample point within 7 h of collection: one for each of three different chemical analyses (particulate carbon and nitrogen, particulate phosphorus and fatty acid).

Filters for particulate carbon and nitrogen were dried and stored in a desiccator until analysis. The filters were packed into tin capsules and processed on a combustion analyser. Filters for particulate phosphorus were stored at 0 °C until analysis during October 2009. Particulate phosphorus was determined using a persulphate digestion (Menzel & Corwin, 1965), followed by a measurement of soluble reactive phosphorus (Murphy & Riley, 1962) with a Perkin Elmer Lambda 20 spectrometer.

Filters for fatty acid analysis were immediately placed in glass vials that had been washed with high-performance liquid chromatography–grade chloroform. The filters were immersed in chloroform, and the air space above the chloroform was flushed with nitrogen before replacing the Teflon-coated cap. The vials were stored at −20 °C until analysis during October 2009. Fatty acids filters were transesterified in 1 mL 1 N methanolic HCL (80 °C, 30 min) with 5 μg C15:0 fatty acid added for quantification. Subsequently, fatty acid methyl esters (FAMEs) were extracted by the addition of 1 mL 9% NaCl (w/v) and 1 mL hexanes, followed by 30 s of vortexing and centrifugation at 2000 *g* for 5 min. The hexane layer was transferred to a new glass tube and dried under a nitrogen gas stream. Hexane (100 μL) was added, and the redissolved FAMEs were transferred to GC vials. FAMES were analysed by gas chromatography on a HP 6890 GC equipped with a flame ionisation detector and a DB-23 (J&W Scientific, Santa Clara, CA, USA) capillary column, as described previously (Xu *et al*., 2005). The mass of each fatty acid detected was converted to relative mol % values for statistical analysis.

To characterise phytoplankton community composition, 250 mL of screened (35 μm) water from each sample point was reserved in a glass bottle and preserved with 1% Lugol's solution. Phytoplankton cells were settled for 36 h in 10- mL sedimentation chambers and then identified and counted at 200× and 400× on an inverted microscope (Wetzel & Likens, 2000). At least 400 algal units (single cells, colonies or filaments) per sample were identified; cells with a linear dimension greater than 35 μm were not counted. Cells were identified to genus when possible. Biovolume estimates were calculated from published equations (Hillebrand *et al*., 1999). Taxa were placed into either high-quality or low-quality food categories: high-quality food consisted of diatoms, cryptomonads and chlorophytes, and low-quality food included cyanobacteria and the genera *Cosmarium* (Coesel, 1997) and *Sphaerocystis* (Porter, 1976).

Characterising zooplankton communities. To quantify the population of cladocerans in each lake, we collected a zooplankton sample at each sample point with either a tube sampler (if the depth was < 1.5 m) or a zooplankton net (where depth was > 1.5 m). Zooplankton were filtered through 80 μm sieves and preserved in ethanol. Two composite samples per lake were formed: one by pooling all littoral samples and one by pooling all pelagic samples. At least five 1-mL subsamples were counted in Sedgwick-Rafter cells under a dissecting scope at 10× magnification (Wetzel & Likens, 2000). We identified cladocerans to genus and measured body lengths with a digitiser.

Laboratory experiments

Growth experiment. A *Daphnia* growth experiment was conducted from 19 to 23 July 2009. Female *Daphnia pulicaria* Forbes were taken from a clonal culture that was originally derived from a small, highly productive lake on the campus of Michigan State University. The clones were maintained in a *Daphnia* medium and fed a high-quality *Ankistrodesmus* culture (for culture information, see the study of Sarnelle & Wilson, 2005, 2008) until enough neonates were produced within 24 h of each other to use in the experiments ($n \approx 100$). Thirteen or 14 neonates were assigned randomly to one of four treatments: littoral water, pelagic water, littoral water supplemented with *Ankistrodesmus* and pelagic water supplemented with *Ankistrodesmus*. Each neonate was placed in a 30 mL vial with treatment water and attached to a plankton wheel that rotated at 1 revolution per minute in an 18:6 light–dark cycle at 23 °C. Animals were pipetted into clean vials with fresh treatment water daily.

Treatment water was obtained daily from Muskrat Lake (Table 1) using the methods described in the field survey. Littoral water was taken from a near-shore point with 100% macrophyte PVI that was dominated by *Nuphar* spp. but also had *Polygonum amphibium* L. and *Chara* spp. present. Pelagic water was taken from the deepest area of the lake with 0% macrophyte PVI using an integrated tube sampler. The water collected was screened through a 35- μm Nitex sieve and reached the experimental temperature (23 °C) before use.

Mean neonate length was determined by measuring 40 randomly selected neonates. The length of each neonate (top of head to base of tail spine) was measured after 4 days with a compound microscope (40×) and digitiser. Growth was measured as the daily increase in body length (final length − initial length/4 days).

Reproduction experiment. An experiment on *Daphnia* reproduction was conducted from 23 to 31 July 2009 (9 days). Adult females were used from the laboratory culture of *Daphnia pulicaria* described earlier. Fourteen adults were each placed in separate 60- mL glass vials, assigned randomly to the four treatments and kept under the same conditions as in the aforementioned growth experiment. All animals used in the experiment were 7 days old, had not previously reproduced and were fed a diet of *Ankistrodesmus* until the start of the experiment. The first clutches were observed on day five of the experiment. Offspring were counted and removed every day. At the end of the experiment, the total number of offspring produced and mean clutch size were calculated for each individual. All adults survived to the end of the experiment in all treatments.

To quantify the amount of food present in each treatment, we filtered unused treatment water onto Whatman GF/F filters on 4 days of the reproduction experiment (26 and 28–30 July 2009). Algal biomass was determined by fluorometric analysis of chlorophyll *a* on a Turner 10-AU-005 Fluorometer using the methods of Welschmeyer (1994).

Statistical analyses

We analysed the field data using general linear models that included lake as a factor, macrophyte PVI as a covariate and a lake x PVI interaction term. Our response variables were N/C, P/C, mol % total PUFA, mol % C20:5n-3, mol % C18:3n-3, mol % C20:4n-6, total algal biovolume and % high-quality phytoplankton. If the full model was not significant, we ran an analysis of covariance without the lake x PVI interaction term. Data within each lake were also analysed separately using simple linear regression models with macrophyte PVI as the predictor variable. We $\log_{10}$-transformed any non-normal data to fit the linear regression assumptions.

Juvenile growth rate, total offspring, mean clutch size and chlorophyll *a* concentration from the laboratory experiments were analysed by two-way analysis of variance (ANOVA) to measure the effects of habitat (littoral versus pelagic) and enrichment (added versus no added *Ankistrodesmus*). *Post hoc* comparisons were made using Tukey's honest significant difference test. These data met the assumption of homogeneity of variance so were not transformed. Field and laboratory data were analysed using R 2.8.0 software (R Foundation for Statistical Computing, Vienna, Austria) and α was set at 0.05.

Results

Lake field survey

Although the four study lakes had much in common, they differed in size, depth, clarity, temperature and DO (Table 1). Muskrat Lake had lower clarity than the other lakes, and the maximum depth of Potter Lake was less than half that of the next shallowest lake. Mean surface water temperature varied between lakes, ranging from 18 to 29 °C, which we believe was largely a consequence of sampling such shallow lakes on different dates. All lakes were oxygenated, with mean DO concentrations of *c.* 5 mg L^{-1} in Dagget Lake and >7 mg L^{-1} in all other lakes.

Macrophytes and abiotic factors. A total of 20 macrophyte species were found across the study lakes (species richness 11, 9, 9 and 5 for Dagget, Hall, Muskrat and Potter Lakes, respectively). Simpson's diversity indices were 7.38, 6.98, 6.82 and 4.48 for Dagget, Hall, Muskrat and Potter Lakes, respectively. Native floating-leaf (e.g. *Nuphar* and *Nymphaea* spp.) and submerged species (e.g. *Chara* spp. and *Utricularia macrorhiza* Leconte) were the most abundant macrophytes across all lakes. Muskrat Lake was dominated

Table 2 Summary of linear regressions between food quality variables and macrophyte per cent volume infested (PVI), with lake as a covariate. Parameters with significant *P*-values are bolded ($P < 0.05$)

Response variable	Parameter	DF	Sum of squares	Mean square	*F*-value	*P*-value
N:C	PVI	1	0.77	0.77	0.38	0.54
	Lake	**3**	**24.97**	**8.32**	**4.19**	**0.01**
	Residual error	58	115.33	1.99		
P:C	PVI	1	0.22	0.22	2.62	0.11
	Lake	**3**	**0.69**	**0.23**	**2.74**	**0.05**
	Residual error	57	4.77	0.08		
Mol % PUFA	PVI	1	0.01	0.01	0.31	0.58
	Lake	**3**	**7.76**	**2.59**	**64.90**	**<0.001**
	Residual error	58	2.31	0.04		
Mol % C20:5n-3	PVI	1	0.40	0.40	0.19	0.66
	Lake	3	1.37	0.46	0.22	0.88
	Residual error	58	119.46	2.06		
Mol % C18:3n-3	PVI	1	0.00	0.00	0.01	0.91
	Lake	**3**	**10.85**	**3.62**	**60.14**	**<0.001**
	Residual error	58	3.49	0.06		
Mol % C20:4n-6	**PVI**	**1**	**0.59**	**0.59**	**6.26**	**0.02**
	Lake	**3**	**5.13**	**1.71**	**18.16**	**<0.001**
	Residual error	58	5.4569	0.09		
Total edible algal biovolume	PVI	1	0.10	0.10	0.45	0.51
	Lake	**2**	**45.48**	**22.64**	**101.52**	**<0.001**
	Residual error	41	9.14	0.22		
% high-quality phytoplankton	PVI	1	.03	0.03	1.45	0.24
	Lake	**2**	**1.79**	**0.90**	**49.24**	**<0.001**
	PVI × Lake	**2**	**0.19**	**0.10**	**5.26**	**0.01**
	Residual error	39	0.71	0.02		

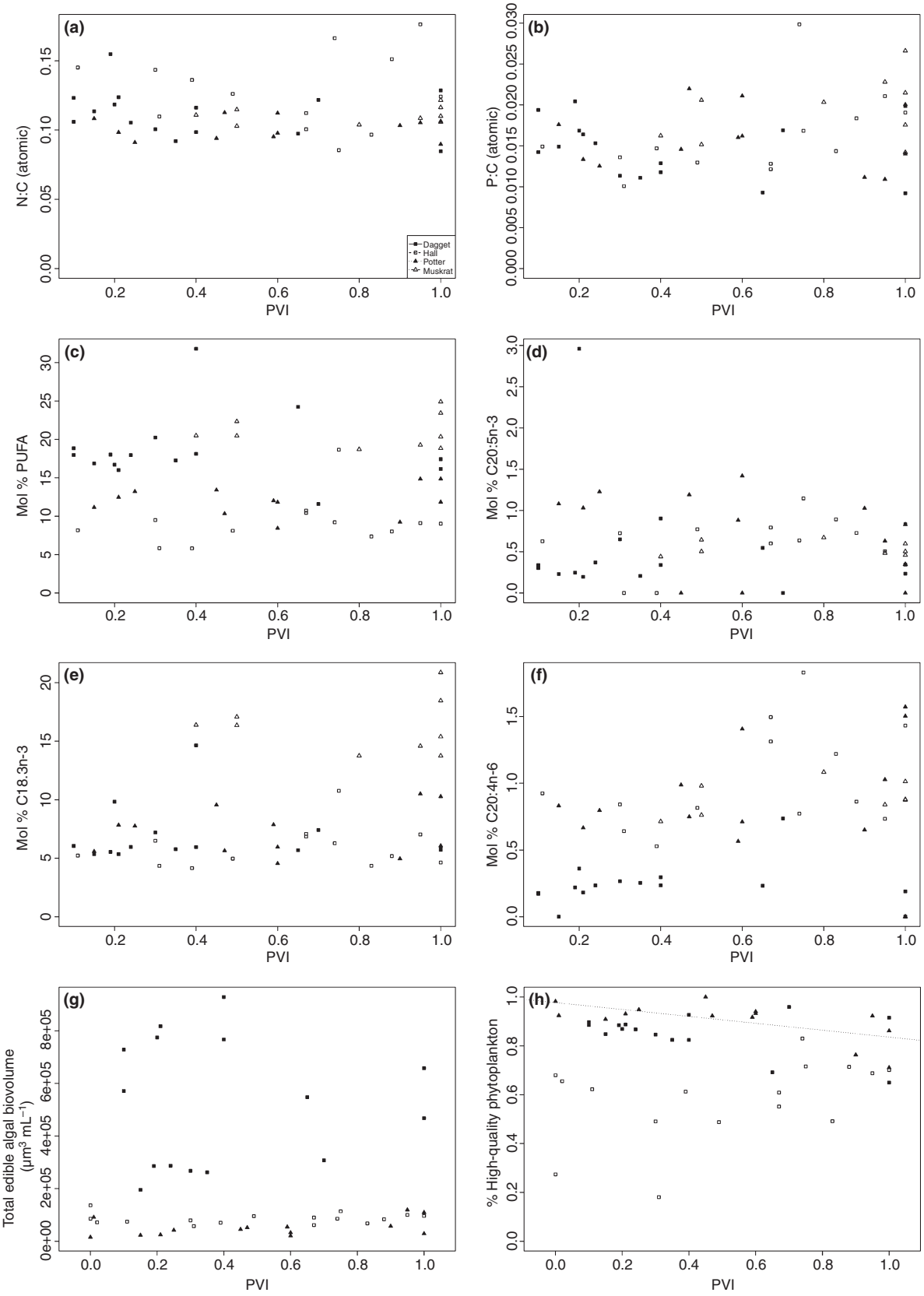
by floating-leaf macrophytes, probably due to low water clarity (Table 1). As expected, PVI was negatively correlated with water depth ($r < -0.60$ within each lake).

Zooplankton food quality and quantity. We analysed lake seston samples for food quantity and three different measurements of food quality (elemental ratios, fatty acid and phytoplankton taxa) to test for any negative relationship between food quantity or quality and macrophyte PVI. Algal food quantity, measured as total edible algal biovolume, was significantly different between lakes ($P < 0.001$; Table 2). Dagget Lake had five times the edible algal biovolume of Hall Lake, and Hall Lake had almost three times the biovolume of Potter Lake. Algal food quantity was not significantly related to PVI across lakes or within any individual lakes (Table 2).

Algal food quality, measured as the mean N/C and P/C (molar) of lake seston, ranged between 0.11–0.13 and 0.01–0.02, respectively. Both N/C and P/C varied significantly between lakes ($P < 0.05$; Table 2). We found no significant relationship between the elemental ratios and PVI across lakes ($P > 0.10$; Table 2) or within lakes (Fig. 1a,b).

Polyunsaturated fatty acids (PUFAs) comprised between 7.3 and 19.5% of all fatty acid among lakes. One of the fatty acids particularly important for *Daphnia* growth, α-linolenic acid (C18:3n-3), made up the highest proportion of total PUFAs, ranging from 4.8 to 14.7% of all fatty acid. The mol % of measured PUFAs differed significantly between lakes ($P < 0.001$; Table 2), except for eicosapentaenoic acid ($P = 0.88$; C20:5n-3). Arachidonic acid (C20:4n-6) was the only fatty acid measured that had a significant relationship with PVI across lakes ($P < 0.01$), although this rela-

Fig. 1 (a–h) Food quality variables plotted against macrophyte per cent volume infested. A linear regression line is shown for the case of % high-quality phytoplankton in Potter Lake (h; $P = 0.01$), the only variable for which such a regression was significant. Muskrat Lake is not represented in some panels because all phytoplankton taxa samples from Muskrat Lake were damaged.


(a)
N:C (atomic)
0.00
0.05
0.10
0.15
Dagget
Hall
Potter
Muskrat
0.2
0.4
0.6
0.8
1.0
PVI
(b)
P:C (atomic)
0.000
0.005
0.010
0.015
0.020
0.025
0.030
PVI
(c)
Mol % PUFA
0
5
10
15
20
25
30
PVI
(d)
Mol % C20:5n-3
0.0
0.5
1.0
1.5
2.0
2.5
3.0
PVI
(e)
Mol % C18.3n-3
0
5
10
15
20
PVI
(f)
Mol % C20:4n-6
0.0
0.5
1.0
1.5
PVI
(g)
Total edible algal biovolume
(µm3 mL−1)
0e+00
2e+05
4e+05
6e+05
8e+05
0.0
0.2
0.4
0.6
0.8
1.0
PVI
(h)
% High-quality phytoplankton
0.0
0.2
0.4
0.6
0.8
1.0
PVI

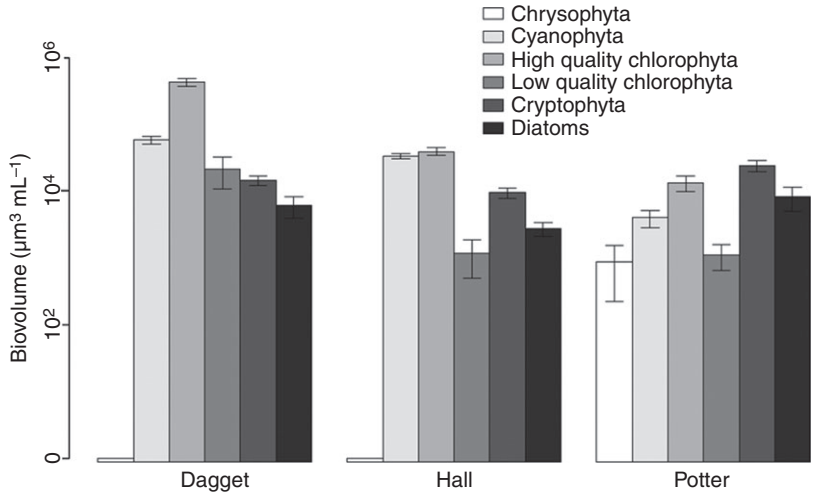


Fig. 2 Biovolume (means ± SE) of phytoplankton divisions, grouped by lake. Phytoplankton taxonomic groups are presented in the same order from left to right as the legend is ordered from top to bottom. Muskrat Lake is not represented because all phytoplankton taxa samples from Muskrat Lake were damaged beyond recovery.

tionship was not the negative one we had predicted (Fig. 1f). Within lakes, total PUFAs showed no trends with PVI, although individual PUFAs changed with PVI in different ways (Fig. 1c–f). There was no significant interaction between any of the PUFA variables and PVI (Table 2).

A total of 27 distinct phytoplankton taxa were found in Hall Lake, 28 taxa in Potter Lake and 29 in Dagget Lake. Muskrat Lake is not represented because all phytoplankton taxa samples from Muskrat Lake were damaged beyond recovery. Dagget Lake was dominated by high-quality chlorophytes, Potter Lake by cryptophytes and Hall Lake by cyanobacteria and high-quality chlorophytes (Fig. 2). The percentage of high-quality phytoplankton biovolume differed between lakes ($P < 0.001$; Table 2), with averages of 58%, 85% and 90% in Hall, Dagget and Potter Lakes, respectively. Within lakes, the only significant relationship between PVI and % high-quality phytoplankton was a negative relationship in Potter Lake ($P = 0.01$), but all samples in this lake were comprised of more than 70% high-quality taxa. In general, these

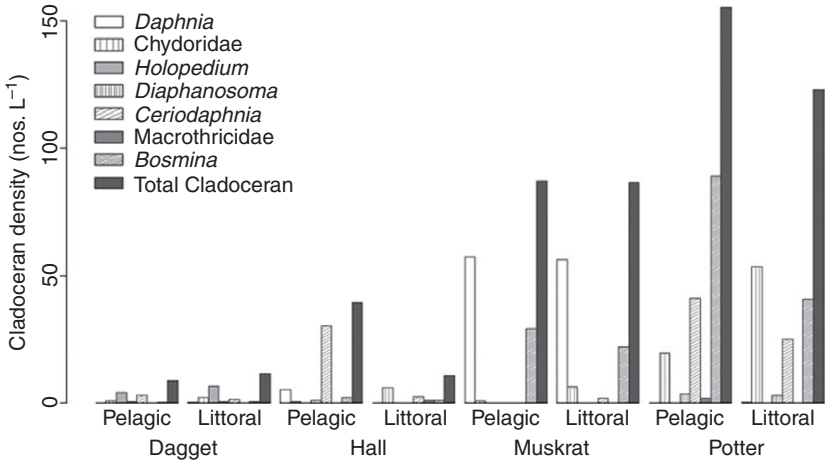


Fig. 3 Cladoceran density by lake and littoral or pelagic zone. Cladoceran taxonomic groups are presented in the same order from left to right as names in the key are ordered from top to bottom. Densities derived from composite samples of all littoral and all pelagic samples from each lake (n = 14–18 per lake).

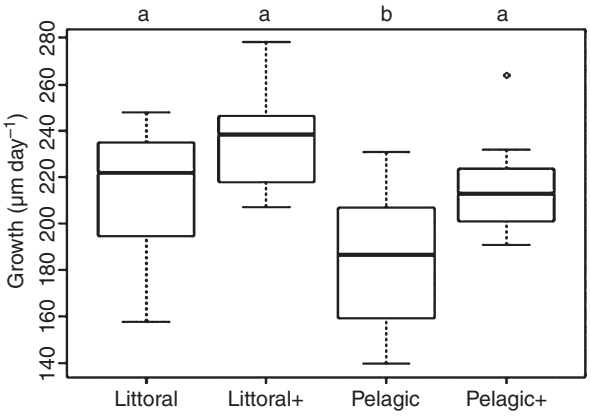


Fig. 4 Boxplot of growth experimental results. Treatments with (+) were supplemented with *Ankistrodesmus*. Treatments with the same letter are not significantly different from each other using Tukey's honest significant difference ($P > 0.05$). The ends of the whiskers represent data within 1.5 interquartile range of the upper and lower quartiles.

food quality results contradicted our expectations and indicated no trends in food quantity or quality across macrophyte abundance gradients.

Zooplankton communities. Total cladoceran densities ranged from 150 L^{-1} (Potter Lake) to less than 15 L^{-1} (Dagget Lake; Fig. 3). We found a similar density of *Daphnia* in the littoral zone of Muskrat Lake as in the pelagic zone, but *Daphnia* was restricted to the pelagic zone in the other lakes. *Daphnia* were rare in Potter and Dagget Lakes, with densities <1 L^{-1}. We found cladoceran mean lengths of 626, 508, 506 and 361 μm in Dagget, Muskrat, Hall and Potter Lakes, respectively. Cladoceran mean length differed across lakes ($P < 0.001$), except for in Muskrat and Hall Lakes ($P = 1.0$).

Laboratory experiments

We expected *Daphnia* juvenile somatic growth, total number of offspring and clutch size to be significantly lower in treatments using vegetated littoral water than those using unvegetated pelagic water. However, we found a significantly higher juvenile *Daphnia* growth rate in water from the littoral zone than in that from the pelagic zone ($F = 11.57$, $P = 0.001$; Fig. 4). Juvenile *Daphnia* grew faster when *Ankistrodesmus* was added to the pelagic water treatments ($P = 0.03$), indicating food limitation of somatic growth. Conversely, food did not limit growth in the littoral treatment ($P = 0.08$).

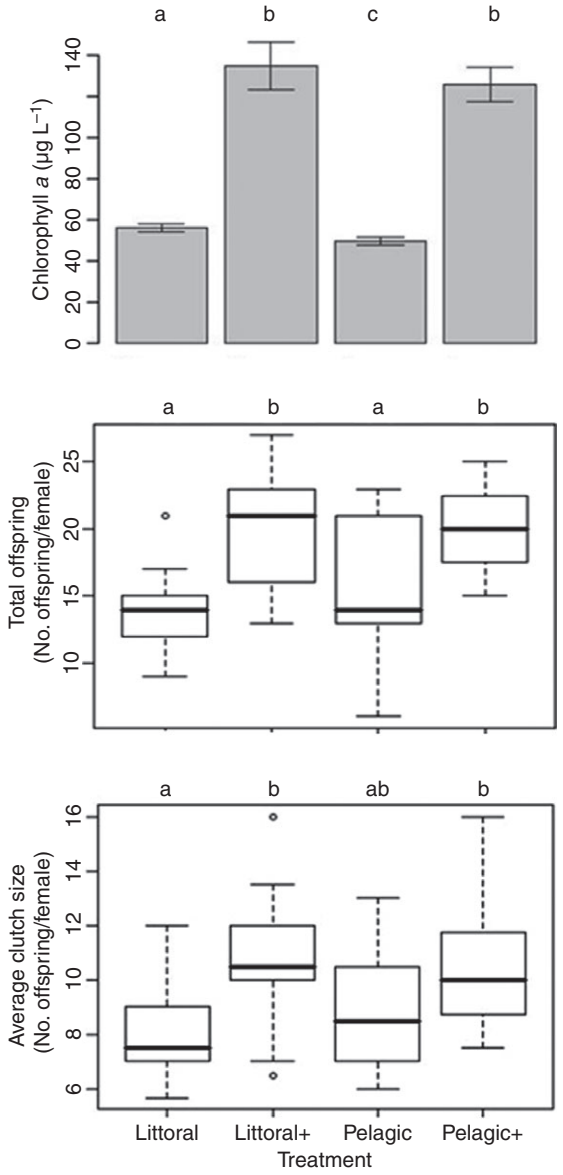


Fig. 5 Bar graph and boxplots of data (means ± SD) from the reproduction experiment. Treatments with (+) were supplemented with *Ankistrodesmus*. Treatments with the same letter are not significantly different from each other using Tukey's honest significant difference ($P > 0.05$). The ends of the whiskers represent data within 1.5 interquartile range of the upper and lower quartile.

In the reproduction experiment, enrichment increased the total number of offspring in both the littoral and pelagic water treatments ($F = 21.65$, $P < 0.001$; Fig. 5). Adding *Ankistrodesmus* increased the clutch size only in the littoral treatment ($P = 0.01$),

suggesting that the food quality in the pelagic water was already high. Habitat had no effect on either the total number of offspring ($F = 0.54$, $P = 0.47$) or the mean clutch size ($F = 0.12$, $P = 0.73$).

The chlorophyll *a* analysis associated with our reproduction experiment revealed a significantly higher algal biomass in the littoral zone as compared to the pelagic zone of Muskrat Lake ($P = 0.002$), but the magnitude of the difference was small (Fig. 5). Treatments with added *Ankistrodesmus* more than doubled the amount of food available to *Daphnia*, raising the chlorophyll *a* concentration to above 120 μg L^{-1}. Chlorophyll *a* concentrations in the natural Muskrat Lake seston (both pelagic and littoral) were three to four times greater than the concentration that maximises the feeding, assimilation and growth of *Daphnia* (6–15 μg L^{-1}; Sterner & Schulz, 1998). Therefore, because *Daphnia* in the littoral and pelagic treatments were saturated in terms of food quantity, the food limitation effects seen in our experiment probably corresponded to differences in food quality. The results of the reproduction and growth experiments generally suggest little difference between the littoral and pelagic habitats in terms of food quality, corroborating our lake survey results.

Discussion

It is generally assumed that resources for pelagic zooplankton are poor in quantity and quality in vegetated littoral zones. This assumption is based on the well-established negative relationship between phytoplankton and macrophytes that is attributed to allelopathy, competition for nutrients and light, and sedimentation. We tested this common assumption in four shallow lakes that differed in surface temperature, DO, macrophyte diversity and zooplankton community and in the relative strength of predation on zooplankton (as indicated by differences in cladoceran mean length across lakes (Carpenter *et al.*, 2001)). We found that food quantity and a variety of *Daphnia* food quality measurements showed no significant relationship with macrophyte abundance (measured as PVI). In addition, our experimental results suggested that overall food quantity and quality in turbid shallow lakes may be very similar in the littoral and pelagic zones. These results do not support the conventional wisdom that macrophytes negatively affect food resources for *Daphnia*.

Algal cell size is often considered an important determinant of resource quantity for *Daphnia*, because cells and colonies are inedible above a certain size threshold (Demott, 1995). Thus, it is often argued that food quantity is lower in the littoral zone owing to the higher proportion of large, inedible phytoplankton cells compared to the pelagic zone. Field observations in Denmark showed that algae were larger in the presence of macrophytes than in their absence (Søndergaard & Moss, 1998), and relatively larger cells were found in the littoral zone of a Swiss lake (Vuille, 1991). Laboratory experiments confirmed these observations, with a green alga having larger cell volume and more colony formation in the presence of macrophyte exudates (Mulderij *et al.*, 2005). However, all of our study lakes had a similar amount of edible phytoplankton cells, regardless of macrophyte density. Our results provide no evidence that the documented effect of macrophytes on cell volume has a negative effect on food quantity for zooplankton.

Phillips, Eminson & Moss (1978) hypothesised that phytoplankton can overcome the allelopathy of macrophytes when excess nutrients are available. In fact, some studies show that macrophytes may only have a growth-inhibiting effect on algae when nutrients are limiting (Fitzgerald, 1969; Lurling, Van Geest & Scheffer, 2006). Shallow lakes are typically nutrient-rich (Scheffer, 2004), and our study lakes were no exception (total phosphorus > 26 μg L^{-1}). Therefore, allelopathy may not have a strong effect on shallow lake phytoplankton biomass. In addition, above limiting nutrient thresholds, competition between macrophytes and phytoplankton for nutrients might not be sufficient to affect food quality for zooplankton. Therefore, other mechanisms, such as shading and reduced sediment resuspension, may be more important for driving macrophyte–phytoplankton interactions within shallow lakes.

However, shading and reduced sediment resuspension may be of little consequence to phytoplankton in shallow turbid lakes. In such lakes, including Muskrat Lake with its low water clarity, low light levels in macrophyte beds may not be a drastically different environment for phytoplankton from what they experience in the turbid pelagic zone. The dominance of floating-leaved plants in many similarly turbid lakes may also not increase net sinking rates of phytoplankton like more complex macrophyte growth forms do (Horppila & Nurminen, 2005). Therefore, it

makes sense that both our lake seston survey and laboratory experiments resulted in similar food quantity and quality for *Daphnia* in Muskrat Lake, regardless of habitat.

In less-turbid lakes, application of the light/nutrient hypothesis might be most appropriate for understanding the conventional negative relationship between macrophytes and zooplankton resources (Sterner *et al*., 1997). This hypothesis states that increased light availability in clear-water lakes leads to increased carbon fixation in phytoplankton, which decreases the amount of relative phosphorus in the cells and the overall food quality for *Daphnia*. In contrast, within the shaded environment of dense macrophyte beds, carbon fixation is lower; thus, P/C and food quality should be relatively high. However, we did not see a significant negative relationship with macrophytes and P/C in Dagget, Hall or Potter Lake, despite their relatively clear water. We also did not see this pattern with N/C in any of the lakes, as would be predicted by the light/nutrient hypothesis. In agreement with previous research that found high food quality in pelagic samples of Michigan shallow lakes (Tessier & Woodruff, 2002), food quality was generally high in all of our samples. The elemental ratios we measured were near the atomic ratios found in *Daphnia* populations (Andersen & Hessen, 1991) and were well above the P-limitation threshold of 0.003 P/C for *Daphnia* resources (Sterner, 1993). The results from our sampling across pelagic and littoral zones demonstrate that good food conditions for zooplankton hold across habitats within these shallow lakes.

Although vertical mixing in shallow lakes and its impact on phytoplankton is well studied (e.g. Macintyre, 1993; Huisman *et al*., 2002), horizontal mixing has received far less limnological attention. Horizontal mixing is believed to create patchiness in marine phytoplankton distributions (Martin, 2003), but we do not know how horizontal mixing impacts phytoplankton in shallow freshwater systems. In fact, horizontal mixing may help homogenise pelagic and littoral resources in shallow lakes despite clear differences in physical structure between the two habitats. For example, Barker, Irfanullah & Moss (2010) found similar algal biovolumes in littoral and pelagic sites within a shallow U.K. lake despite the presence of relatively still water within macrophyte beds. This homogeneous phytoplankton distribution was attributed to an active mixing force that allowed algal cells to cross the water boundary layers present around macrophytes, which might help explain why we found no relationships between food quantity or quality and macrophyte abundance in our study lakes.

Daphnia are able to consume periphyton, fungi, bacteria and detritus (Ojala *et al*., 1995; Pilati, Wurtsbaugh & Brindza, 2004; Siehoff *et al*., 2009), and it is often assumed that a high quantity of these resources are present among macrophytes (e.g. Burks *et al*., 2002). Past studies show increased reproductive success of *Daphnia* adults on non-algal resources (Sanders *et al*., 1996), so one might expect higher reproductive success for *Daphnia* when fed resources from macrophyte-dense areas. However, our reproduction experiments suggested no difference between littoral and pelagic resources. Our findings question the common, but rarely tested, assumption of macrophyte-dependent distribution of detritus, bacteria and fungi within lakes.

If significant and consistent gradients of resource quantity and quality associated with macrophyte density do *not* exist within shallow lakes, then future research can be directed towards understanding the other potential mechanisms behind the well-documented pattern of higher *Daphnia* densities in the open-water pelagic zone than the vegetated littoral zone (Scheffer, 2004). Habitat use by *Daphnia* in shallow lakes may represent adaptation to patchy environments that vary in mortality risk or abiotic factors instead of food resources. For example, chemical cues from invertebrate predators cause *Daphnia* to avoid densely vegetated areas (Lauridsen & Lodge, 1996), and macrophyte chemicals negatively affected *Daphnia* growth and reproduction, even on a controlled diet (Burks, Jeppesen & Lodge, 2000). Alternatively, abiotic factors often differ with macrophyte density (e.g. DO concentration is often lower among macrophytes, as in Dagget Lake), and this physical difference may play a role in the horizontal distribution of pelagic zooplankton. In deep, stratified lakes, much research has shown that *Daphnia* allocate time to different vertical habitats, thus optimising fitness based on abiotic and biotic factors (Lampert, Mccauley & Manly, 2003). Similar research on the horizontal habitat choice of *Daphnia* in shallow lakes is needed.

Although there has been much research on zooplankton food quality, little has examined quality *in situ* (Scheuerell *et al*., 2002). Of these field studies, most have focussed on variation in food quality in the

pelagic zone of lakes (Dobberfuhl & Elser, 2000; Tessier & Woodruff, 2002). Our observations shed light on the horizontal distribution of phytoplankton quality within several lakes, a topic that has received little attention in the literature. The distribution of phytoplankton quality within lakes has important implications for zooplankton productivity and foraging behaviour because they directly affect phytoplankton populations, water clarity and fish population dynamics. Despite our finding of no relationship between food quantity or quality and macrophyte PVI, the inherent patchiness of shallow systems was evident from the variability found for many of the food quality parameters we measured. Further studies of the distribution of zooplankton resources can identify possible mechanisms behind this variability. Future studies of zooplankton nutrition, as well as theoretical models of aquatic food webs, should also take into account this resource spatial heterogeneity and how it affects zooplankton habitat selection.

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