Epiphyte biomass and elemental composition on submersed macrophytes in shallow eutrophic lakes

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Abstract

Epiphytes cause a strong light attenuation for submersed macrophytes. Various abiotic and biotic factors determine epiphyte density, among them nutrients, water clarity and possibly allelopathy. We compared epiphyte biomass on the major occurring macrophytes, among them allelopathically active species in four shallow eutrophic lakes in Upper Swabia (Southern Germany). We determined epiphyte chlorophyll *a*, organic carbon, nitrogen and ash-free dry mass. Mean epiphyte chlorophyll *a* per macrophyte ranged between 100 and 400 μg g^{-1} dm in summer and increased up to 1700 μg g^{-1} dm in autumn. Few differences between species and lakes were observed. No apparent relation was found between epiphyte density and physical or chemical variables of the lakes. Epiphytes had a molar stoichiometry of organic carbon to nitrogen (C:N) slightly below 7:1, a ratio considered to be optimal also for benthic algae. Lakes with higher concentrations of humic compounds exhibited a significant higher ratio of organic carbon to epiphytic chlorophyll *a*, indicating a higher proportion of heterotrophs in the epiphyte layer. In one lake, one phenotype of *Elodea nuttallii* exhibited a significant higher C:chl a ratio than other macrophytes. Allelopathy as a factor responsible for this difference is discussed.

Introduction

Submersed macrophytes improve water quality in shallow eutrophic lakes through various mechanisms (Scheffer et al., 1993). They also greatly increase the colonization area in lakes for bacteria, cyanobacteria, algae and invertebrates. Epiphytes compete with macrophytes mainly for light and carbon, sometimes also for nutrients. Rooted submersed macrophytes retrieve nutrients mainly from the sediment (Best & Mantai, 1978; Carignan & Kalff, 1980), although significant uptake can also occur via shoots under eutrophic conditions (Ozimek et al., 1993). Generally, however, nutrient uptake of epiphytes is faster than that of macrophytes (Pelton et al., 1998). Light is generally considered to be the major limiting factor for both submersed macrophytes and epiphytes. Light attenuation increases with water depth, water turbidity and the thickness of the epiphyte layers (Sand-Jensen, 1990; Sand-Jensen & Borum, 1991). Especially epiphytes may cause the largest light attenuations for submerged macrophytes (Roberts et al., 2003). Optimal photosynthetic activity also depends on sufficient availability of carbon dioxide or bicarbonate. Often, light and carbon limitation of macrophytes co-occur, and interactive effects of light and carbon availability have been demonstrated experimentally (Madsen & Sand-Jensen, 1994).

This study was designed to gain quantitative and qualitative estimates of epiphyte biomass in four selected shallow lakes in Southern Germany. We asked whether epiphyte biomass would vary depending on macrophyte species, water column nutrients or clarity of the lake. We further estimated from epiphytes the C:N stoichiometry, indicative of carbon or nitrogen limitation, and the carbon to chlorophyll *a* ratio (C:chl *a*) representing the proportion of heterotrophic to autotrophic organisms.

Description of sites studied

We selected four shallow eutrophic lakes in Upper Swabia, Southern Germany. Ebenweiler See (ES), Lengenweiler See (LS), Rohrsee (RS) and Vorsee (VS) are glacially scoured shallow eutrophic lakes, located in rural areas. They all experienced eutrophication during the last decades. Further information can be found at: http://www.seenprogramm.de/ or Herz (2001). Macrophyte abundance and species composition varied between lakes. The abundance of macrophytes was estimated as PVI – plant volume infested, calculating the lake volume inhabited by macrophytes by percent coverage and height in the water column.

Ebenweiler See (7 ha, mean depth 1.8 m, max. depth 3.2 m, PVI <1%) was considered oligotrophic until 1985, exhibiting rich stands of submersed macrophytes. Since then, most submersed macrophytes have disappeared and have been replaced by *Nymphaea alba* L. and *Nuphar lutea* (L.) J. E. Smith in Sibthorp et J. E. Smith. In addition to eutrophication, resulting in part from liquid manure use on the pastures in the vicinity, stocking with grass carp (*Ctenopharyngodon idella*) may have caused submersed macrophyte decline. Few stands of *Myriophyllum verticillatum* L. and *Potamogeton crispus* L. remain close to a bathing area. The lake is located in a peat area and slightly dystrophic.

Lengenweiler See (5.5 ha, mean depth 2.8 m, max. depth 5.0 m, PVI 15%) has developed large stands of canopy-forming *Ceratophyllum demersum* L. during the last years, interfering with recreational use by swimmers and anglers. The pot-like morphometry of the lake – with steep slopes soon reaching 2.5 m depth and more – probably prevent the establishment of more rooted macrophytes. Only a few stands of *Potamogeton crispus* L. and *P. lucens* L. can be found. However, *C. demersum* apparently improved Secchidepth and increased the density of large zooplankton in this lake. We sampled these three macrophytes throughout the summer, only *P. lucens* was absent in September.

Rohrsee is the largest lake (56 ha, mean depth 1.0 m, max. depth 2.5 m, PVI 85%) in our study. It is a European ornithological nature reserve located at the watershed of the Rhine and Danube. Waterlevel fluctuates strongly depending on rainfall in this endorheic basin. In this weakly buffered lake, pH 10 is frequently observed. A diverse and almost impenetrable community of submersed macrophytes stabilises the sediment and prevents resuspension. Dominant, canopy-forming species are *C. demersum, Myriophyllum spicatum* L. and *Elodea nuttallii* (Planchon) St. John. *E. nuttalli* has replaced *E. canadensis* L. C. Richard in Michaux fil. in this lake during the last decade. *E. nuttallii* occurs in two growth forms, one with typical long and narrow leaves, one with shorter and broader leaves resembling *E. canadensis*. We refer to these phenotypes as ENl and ENs in this study. At first, we considered ENs as *E. canadensis*, but close chemical and molecular taxonomic analysis revealed that it is not (D. Erhard, V. Haid and E. M. Gross, unpubl. results). *E. nuttallii* is known to have differently shaped leaves that are sometimes difficult to distinguish from other *Elodea* species (Vanderpoorten et al., 2000). We sampled *C. demersum*, both phenotypes of *E. nuttallii, M. spicatum* and *P. lucens* in this lake.

Vorsee (8.7 ha, mean depth 1.4 m, max. depth 2.2 m, PVI 38%), surrounded by forest, is located in a peat area and slightly dystrophic. This lake is used solely by anglers and has developed a large stand of canopy-forming *M. spicatum*. During our survey no other submersed macrophytes were found. Fish biomass is high and dominated by cyprinids, also grass carp is present.

Materials and methods

Sampling

Lakes were sampled three times in 2000, in June/July, August, and at the end of September. At the lake, we measured Secchi depth, pH, oxygen content and temperature. Water samples were taken with a tube sampler integrating the whole water column at the centre of the lake. Water samples for chemistry were stored in amber 2.5 l glass bottles that had been acid-washed. All samples were transported back to the laboratory in a cooler on ice.

Epiphytes were sampled from 25 cm apical shoots of macrophytes in the respective lakes. Shoots reaching to the water surface were transferred directly in plastic beakers held next to the plants before detaching from the main stem. Deeper shoots were collected with plastic tubes closed at one end with mesh (20 μm mesh width). These procedures tried to minimize sampling loss of loosely attached algae as much as possible. Five replicates per plant species were collected and epiphytes removed by shaking twice in 150 and 200 ml of deionized water, respectively using a modified method by Galanti & Romo (1997). The

plant shoot was placed in a tightly closed plastic bag for further analysis in the laboratory.

Analysis of samples

In the laboratory, we measured alkalinity by titration of 100 ml of unfiltered water with 0.05 M HCl and methylorange to pH 4.5. Alkalinity was calculated as the amount of 0.1 M HCl needed and presented as mequiv l^{-1}. Colour of the water caused by humic compounds was measured with filtered (GF/F) water in 5 cm cuvettes at 400 nm. The resulting absorption was converted into equivalents of humic compounds (HC) using the formula HC (mg l^{-1})=$A_{400nm} \times 20/0.39$ (Mäekivi & Arst, 1995). For up to 6 mg l^{-1} of humic compounds, the lake is considered clear; between 6 and 12 mg l^{-1}, yellow; and above 12 mg l^{-1}, brown. The amount of total suspended solids (TSS) was determined by filtration of up to 250 ml of lake water on pre-weighed GF/F filters (Whatman). Total phosphorus and total nitrogen were determined using standard procedures with an autoanalyser. Phytoplankton chlorophyll *a* was determined according to the method of Nusch (1980) using hot 90% ethanol. Aliquots of up to 1 l of lake water were filtered on GF/C filters and stored in a freezer for up to 3 weeks before analysis.

Chlorophyll *a* content of the epiphyte suspension was determined as described above, using 10–50 ml each. Dry mass (dm) and ash free dry mass (afdm) were determined using preweighed GF/F filters. Carbon and nitrogen content of the suspension was determined with samples filtered on GF/F filters and incineration in a Carbon-Nitrogen-Sulphur Analyser (NCS 2500, CE Instruments). The amount of organic carbon was determined after treating filters with hydrochloric acid fumes to remove inorganic carbon.

Results

Results of the water chemistry analysis are presented in Table 1. Generally, lakes did not differ much in their nutrient content. All variables related to water clarity (TSS, Secchi depth, phytoplankton chl *a*, colour) were lowest in macrophyte-rich RS, followed by LS and ES. VS had the most suspended solids, the highest colour and most shallow Secchi depth of all lakes.

In July and August, epiphyte chlorophyll *a* per macrophyte ranged between 100 and 500 μg g^{-1} plant dm in summer, in three out of four lakes, mean values
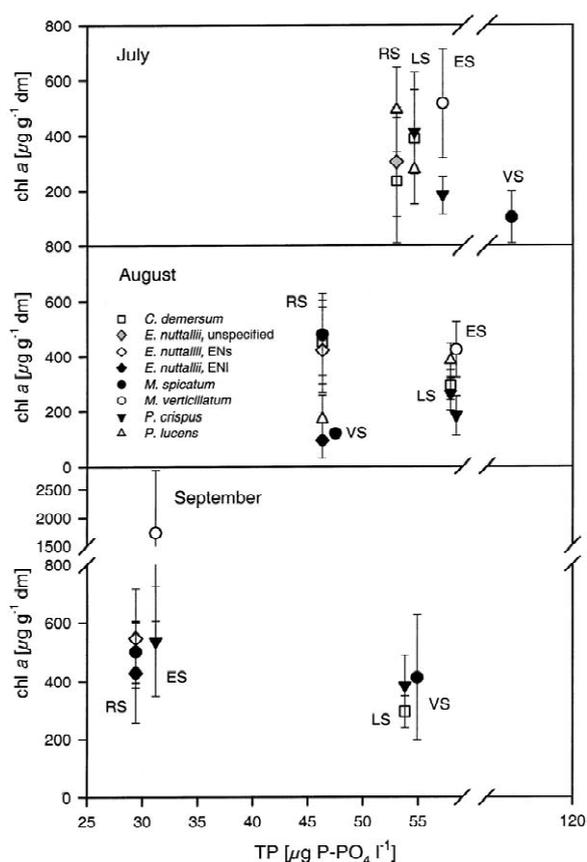

Figure 1. Epiphyte chlorophyll *a* values on different submersed macrophytes in four shallow eutrophic lakes from July through September 2000. ES – Ebenweiler See, LS – Lengenweiler See, RS – Rohrsee, VS – Vorsee. Data represent mean±SD of three to five replicates per macrophyte.

were 300–350 μg g^{-1} plant dm. Only *M. spicatum* in VS had lower mean epiphyte chlorophyll *a*, approximately 110 μg g^{-1} plant dm, however, this difference was not statistically significant. Variability in epiphyte chlorophyll *a* was high, with standard deviations of up to 100%. Within one lake a few differences were found between macrophytes. In ES, *P. crispus* consistently had fewer epiphytes than *M. verticillatum* (one-way ANOVA, df=1, *F*=19.1, *P*=0.01). In August, *Elodea nuttallii* ENl (long, narrow leaves) had significantly less epiphytes than ENs (short, rounded leaves) and other macrophytes (one-way ANOVA, df=4, *F*=9.7, *P* < 0.001). *M. spicatum* in VS exhibited lower epiphyte biomass than in RS in August. However, comparing epiphyte biomass on this macrophyte in both lakes during August and September revealed no significant differences (two-way ANOVA, *P* > 0.75 for lake, time and lake×time). In September, mean

Table 1. Water chemistry, phytoplankton and epiphyte chlorophyll *a* in shallow eutrophic lakes in Upper Swabia

Lake	Date	Conductivity (μS cm^{-1})	pH	Alk (mequiv. l^{-1})	TP (μg l^{-1})	TN (mg l^{-1})	Secchi depth (m)	Humic compounds (mg l^{-1})	TSS (mg l^{-1})	phytopl. chl *a* (μg l^{-1})	Epiphyte chl *a* (μg g dm) mean±SE	Epiphyte samples *n*
Ebenweiler See	6.7.00	561	8.5	5.2	55.3	1.18	1.0	5.1	14.5	104.9	350±92	10
	1.8.00	541	8.0	4.8	58.4	1.74	1.1	3.6	9.3	23.2	304±48	10
	19.9.00	654	7.8	6.2	31.2	1.24	1.6	5.1	5.5	31.1	1133±311	10
Lengenweiler See	3.7.00	415	8.6	3.5	52.9	1.10	1.7	3.6	8.4	37.5	360±56	15
	10.8.00	379	9.0	3.4	57.8	1.15	1.1	4.1	10.2	34.4	314±20	15
	25.9.00	395	7.9	3.3	53.8	1.38	1.0	4.6	6.0	58.0	468±41	10
Rohrsee	29.6.00	138	9.9	1.2	51.4	0.96	bottom	4.1	3.6	n.d.	334±65	11
	7.8.00	150	9.0	0.4	46.3	0.94	bottom	3.6	3.6	19.1	324±40	25
	19.9.00	146	9.2	1.5	29.4	1.07	bottom	4.1	0.8	11.2	492±39	15
Vorsee	29.6.00	165	9.7	1.3	115.0	2.78	0.3	7.7	42.9	24.9	103±42	5
	10.8.00	183	9.4	1.6	47.5	1.61	0.7	8.2	16.0	19.2	121±8	5
	25.9.00	218	8.8	2.0	54.9	1.90	0.7	7.2	40.4	71.6	412±96	5

Table 2. C:N ratio and ash-free dry mass (afdm) of epiphytes on different submersed macrophytes during August 2000. Data represent mean ±/− SE. Organic carbon was used for calculation of the molar ratio

Lake	Macrophyte	C:N ratio (molar)	afdm (mg g^{-1} dm)	*n*
Ebenweiler See	all	6.3±0.5	182.1±30.6	10
	M. verticillatum	5.7±0.1	280.3±25.7	5
	P. crispus	6.7±1.1	110.7±17.2	5
Lengenweiler See	all	6.8±0.3	70.0±13.1	15
	C. demersum	7.2±0.3	25.8±22.8	5
	P. crispus	6.1±0.1	80.3±6.4	5
	P. lucens	7.2±0.9	103.7±20.9	5
Rohrsee	all	6.0±0.2	194.2±29.0	22/25
	C. demersum	5.6±0.1	276.4±70.6	5
	E. nuttallii/ENl	5.7±0.2	166.4±71.7	2/5
	E. nuttallii/ENs	5.9±0.1	273.1± 77.0	5
	M. spicatum	5.5±0.2	167.7±42.9	5
	P. lucens	7.1±0.6	87.5±29.3	5
Vorsee	*M. spicatum*	6.7±0.2	111.5±10.2	5

epiphyte chlorophyll *a* increased significantly in all lakes compared to summer and reached up to 1700 μg g^{-1} plant dm (Fig. 1; one way ANOVA, df=2, *F*=29.5, *P* < 0.01).

The C:N molar ratios of epiphytes were generally below 7:1 (Table 2). Differences between lakes exist (Kruskal–Wallis–ANOVA on ranks, df=3, H=16.1, *P*=0.001), however, pairwise comparison did not reveal a statistically significant difference between specific lakes (Dunn's method). In RS, the C:N ratio of epiphytes on *P. lucens* was significantly higher than that of epiphytes from *M. spicatum* (Dunn's Method for pairwise multiple comparison, Q=3.2, *P* < 0.05).

The ratio of organic carbon to chlorophyll *a* (C:chl *a*) in August ranged from 113:1 to 403:1 (Fig. 2A). Significant differences existed between lakes (Kruskal–Wallis one way ANOVA on ranks, H=21.7, *P* < 0.001). We observed highest ratios in ES and VS, with median values of 273:1 and 403:1, respectively. The C:chl *a* ratio varied also among different macrophytes within one lake. In RS, *E. nuttallii* ENl had a significantly higher ratio than ENs and all other macrophytes (Fig. 2B, one way ANOVA, *F*=5.2, *P*=0.006). Differences between the C:chl *a* ratio of macrophytes found in ES and LS are presented in Fig. 2C.

Discussion

We did not observe significant differences of epiphyte biomass depending on water chemistry. Even in lakes with greater differences in TP, no correlation between TP and epiphyte biomass was found (Lalonde & Downing, 1991). The lower epiphyte biomass on *M. spicatum* in VS is probably due to the shallow Secchi depth and high concentration of humic compounds in this lake. Our results confirm only for one lake (ES) that filamentous-leaved macrophytes (*M. verticillatum*) tend to have higher epiphyte chlorophyll *a* than laminar leaved plants (*P. crispus*) (Lalonde & Downing, 1991). In LS, epiphyte chlorophyll *a* on *C. demersum* was intermediate between that on *P. crispus* and *P. lucens*.

Differences in epiphyte biomass depending on the allelopathic activity of the respective host plants was suggested, e.g. for *Chara* (Wium-Andersen et al., 1982). Other studies did not find such differences when comparing *Chara* and *Potamogeton* (Blindow, 1987). The lakes investigated in this study did not have *Chara*, but they had other macrophytes with known allelopathic activity, such as *C. demersum* (Wium-Andersen et al., 1983; Gross et al., 2003), *E. nuttallii* (D. Erhard & E. M. Gross, unpubl. results) and *M. spicatum* (Gross et al., 1996; Nakai et al., 1999; Leu et al., 2002). In general, these allelopathically active macrophytes did not have lower epiphyte biomass than other macrophytes with the exception of *E. nuttallii* ENl in RS in August. Whether this difference can be associated with the excretion of allelopathically active compounds, needs to be determined. Other factors, such as a higher ratio of invertebrate grazers, may also have accounted for this difference, especially considering the higher C:chl *a* ratio in this species (see below).

Although we found some differences in the organic C:N ratios of epiphytes among lakes, and between different macrophytes in one lake (RS), we question whether these differences are ecologically important. Marine benthic microalgae were carbon limited when the C:N ratio was below 7:1, above this ratio, they were considered nitrogen limited (Hillebrand & Sommer, 1999). The mean C:N ratio for all epiphyte samples in our study was (6.4$\pm$1.2):1 (mean$\pm$1 SD), thus not notably different from 7:1.

Interestingly, we found significant differences when looking at the ratio of heterotrophic to autotrophic biomass in the epiphytes, determined as the ratio of organic carbon to chlorophyll *a* (C:chl *a*). Only lakes exhibiting higher absorption of water at 400 nm, indicative of humic compounds, such as ES and VS, had high C:chl *a* ratios. Few studies have investigated this ratio for epiphytes before. Epiphytes from floating leaved macrophytes tended to have a higher C:chl *a* ratio than epiphytes from submersed species (Cattaneo et al., 1998). Seston in a dystrophic lake exhibited C:chl *a* ratios of more than 500:1 (Bianchi & Argyrou, 1997). It is conceivable that even in slightly dystrophic lakes both the availability of organic carbon and light attenuation by humic compounds should favour heterotrophy in biofilms, such as epiphyte layers.

That this effect may not be uniform among all macrophytes within one lake, is apparent with the significantly higher C:chl *a* ratio on *E. nuttallii* ENl compared to ENs and all other macrophytes in RS. Whether inhibition of algae and cyanobacteria due to allelopathic interference is responsible for this difference, remains to be seen.
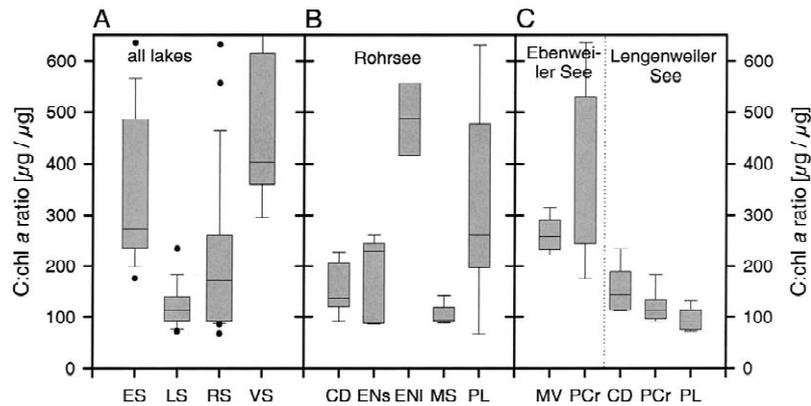
Figure 2. Ratio of heterotrophic to autotrophic biota in epiphyte suspension in August 2000 expressed as organic carbon to chlorophyll ratio (C:chl *a*). (A) Mean values for all macrophytes in a lake. $N = 10$ in Ebenweiler See/ES, $N = 15$ for Lengenweiler See/LS, $N = 22$ for Rohrsee/RS and $N = 5$ for Vorsee/VS. (B) C:chl *a* for different macrophytes in RS. (C) C:chl *a* for different macrophytes in dystrophic ES and clear LS. CD/*Ceratophyllum demersum*; EN l and EN s/*Elodea nuttallii* with long and short leaves, respectively, MS/*Myriophyllum spicatum*; MV/*M. verticillatum*; PCr/*Potamogeton crispus*; PL/*P. lucens*. Box plots show median, 25th and 75th percentile (lower and upper limit of box), 10th and 90th percentile (whiskers) and outlying points (dots).

Conclusions

Due to the inherent heterogeneity of the habitat, studying factors affecting epiphyte biomass is extremely challenging. Epiphyte biomass was determined not to be a function of light, nutrients, or water clarity, as we suspected, likely due to insufficient sample numbers. Many more replicates would have been optimal, but are difficult to analyse simultaneously. Allelopathic effects on epiphyte density cannot be excluded, but seem impossible to prove under field conditions. Still, the differences found in the ratio of heterotrophic to autotrophic production both among lakes and among different macrophytes within one lake were surprising. Future studies should incorporate this analysis to test whether our results reflect a general pattern.

Acknowledgements

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