The trophic importance of epiphytic algae in a freshwater macrophyte system (*Potamogeton perfoliatus* L.): stable isotope and fatty acid analyses

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Abstract

Stable isotope and fatty acid analyses were used to study carbon sources for animals in a submerged plant bed. Epiphytes growing on *Potamogeton perfoliatus*, sand microflora, and alder leaves were the most important carbon sources. The most abundant macrophyte, *P. perfoliatus* was unimportant as a food source. Modelling (IsoSource) showed that epiphytes were the most important food source for the most abundant benthic invertebrates, the isopod *Asellus aquaticus* (annual mean contribution 64%), the amphipod *Gammarus pulex* (66%), and the gastropod *Potamopyrgus antipodarum* (83%). The mean annual contributions of sand microflora were respectively 21, 19, and 9% and of alder leaves, 15, 15, and 8% for these three species. The relative importance of carbon sources varied seasonally. The relative contribution of epiphytes was lowest for all three grazer species in July: *A. aquaticus* 38%, *G. pulex* 43%, and *P. antipodarum* 42%. . A decline in epiphyte biomass in summer may have caused this switch to less attractive food sources. *P. perfoliatus* provided habitat and shelter for consumers, but food was mainly supplied indirectly by providing space for attached epiphytes, which are fast-growing and provide a highly nutritious food source.

Key words: Epiphytes, Periphyton, Sand microflora, Grazing, IsoSource

Introduction

Shallow nutrient poor lakes dominated by aquatic plants are species-rich with complex structure and food webs (Jeppesen et al. 1997). In contrast to nutrient rich plankton-dominated lakes, nutrient poor lakes have a higher diversity of carbon sources for their fauna: the submerged plants, attached epiphytes, sand microflora, allochthonous material such as leaves, and phytoplankton. Under extreme nutrient conditions only one primary producer community – macrophytes or phytoplankton – is expected to dominate a lake ecosystem, but within a range of intermediate nutrient levels alternating stable states are possible (Peckham et al. 2006). Fauna associated with submerged plants includes isopods, amphipods, crayfish, gastropods, various insect larvae, and small or juvenile fishes (Jeppesen et al. 1997). The food web is often characterised by omnivory and a lack of dietary specialisation (Jones and Waldron 2003). The links among epiphytes, grazing invertebrates, and fish seem to be important in structuring these systems (Jones et al. 2002). Epiphytes on submerged plants provide food for grazing invertebrates, which, by removing dense accumulations of epiphytes, release macrophytes from competition for light and nutrients and facilitate their growth and survival (Brönmark 1994; Hughes et al. 2004; Jaschinski and Sommer 2008).

Our detailed knowledge of the effects of plant-associated invertebrates in lakes is, however, limited. Most studies of periphyton and grazing in freshwater systems have concentrated on periphyton growing on stones or sediment (see Feminella and Hawkins 1995 for review). The existing studies on epiphyte grazing suggest that grazing plant-associated invertebrate species can control epiphyte accumulation (Cattaneo and Kalff 1980; Jones et al. 2002).

Past research suggests that epiphytic algae (mostly small filamentous green algae and diatoms) may be important carbon sources in these communities (Underwood and Thomas 1990; James et al. 2000a; Jones and Waldron 2003; Hadwen and Bunn 2005). Macrophytes make the greatest contribution to the organic carbon pool in the littoral zones of these lakes, but may not be direct food sources for animals (Keough et al. 1996; Hecky and Hesslein

1995). The importance of periphyton/benthic productivity compared to pelagic productivity depends on the size and shape of the lake and on nutrient and light availability (Vadeboncoeur et al. 2008). A review on 193 studies showed that benthic productivity constituted on average 46% of whole-lake productivity, and that benthic and pelagic food webs can be strongly linked (Vadeboncoeur et al. 2002).

Analytical tools, such as stable carbon and nitrogen isotope, and fatty acid analyses, may add detail to these generalisations. (Desvilettes et al. 1997; James et al. 2000b; Jones and Waldron 2003; Hadwen and Bunn; 2005; Jaschinski et al. 2008a). The fractionation of $\delta^{13}C$ is though to be low – maximally 1‰ per trophic level – and $\delta^{13}C$ is therefore useful to identify different carbon sources. The $\delta^{15}N$ content is enriched by 3 to 4‰ per trophic level on average and can be used to construct food webs (Eggers and Jones 2000). Fatty acid analysis can trace food sources in aquatic food webs because specific fatty acids are conserved in structure (Lee et al. 1971; Brett et al. 2006). "Indicator" fatty acids, specific to diatoms, dinoflagellates or aerobic and anaerobic bacteria can be used as markers (Viso and Marty 1993; Desvilettes et al. 1997). Fungi, which provide an important link between detritus and invertebrates, might be traced indirectly by 18:1ω9 and 18:2ω6. These fatty acids originate from plant detritus. The strong correlation with ergosterol (a proxy for fungal biomass) supports the use as indicator fatty acid for fungi (Arts and Wainman 1999; Frostegård and Bååth 1996, Wurzbacher et al. 2010).

We tested the hypothesis that epiphytes growing on *Potamogeton perfoliatus* L.were the most important primary source of organic matter for animals in a lake and we used stable isotope and fatty acid analyses to do this.

Methods

Study site

The study was carried out from May to September 2003. Field work began when *P. perfoliatus* appeared in spring, and ended when the plants started to senescence. All samples were obtained from Schluensee in north Germany (54° 11′ 24″ N, 10° 28′ 12″ E). The lake is small (1.27 km^2), mostly shallow with one deep depression (45 m) and is surrounded by alder and willow trees. *P. perfoliatus* is the dominant submerged macrophyte down to 5 m water depth. Water temperature was about 12 ^oC in May, 20 ^oC in June, 22 ^oC in July, 23 ^oC in August, and 18 ^oC in September. The mean conductivity was 420 µS* cm^{-2} and the mean pH was 8.6±0.1SD (n = 5) from May to September. All measurements were made around midday. The sediment was sandy with a low content of organic material.

The nutrient supply was lowest in June and July with nitrate/nitrite concentrations of about 2.5 µmol l^{-1}, ammonium ~ 1µmol l^{-1}, silicate ~ 1.8 µmol l^{-1} and phosphate ~ 0.2 µmol l^{-1} (Table 1).

Sample collection

Samples of alder leaves (*Alnus* sp.), *P. perfoliatus*, attached epiphytes, sand microflora, and the most common macrozoobenthic organisms and fish species were analysed in this study. Samples were collected at 1 m water depth in a small wind exposed bay every month from May to September. We collected 20 *P. perfoliatus* shoots and 10 alder leaves from inside the plant stand on each sampling date. We swept the submerged plants with a net (mesh size 1 mm) to obtain invertebrate samples. At each sampling date we swept about six to ten times in a straight line from the shore to 1 m water depth (about 5 m distance) until we had at least 20 individuals of the main potentially epiphyte grazing species *Asellus aquaticus* and *Gammarus pulex*. We tried to sample - if possible – at least 3 individuals of other species present. The lines were approximately 1 m apart. Fish were caught with a dip net. Additionally we sampled 60 *Potamopyrgus antipodarum* from plants and sediment and 10 *Dreissena polymorpha* from stones. All samples were placed in plastic containers with water from the collection site and transported to the laboratory for sorting and further processing. We took 15 sediment cores (1 cm inner diameter) in plant-free patches within the macrophyte stand at each sampling date. The sediment cores were taken at 1 m water depth 1 m apart in a line parallel to the shore (distance from the shore about 3m). Additionally, we collected *Asellus aquaticus* (L.) from the alder leaves of a nearby unvegetated area.

Sample processing

In the laboratory, the plant material (*Alnus* sp. leaves and *P. perfoliatus* with epiphytes) was rinsed in distilled water to remove fine detritus and attached animals. Epiphytes were carefully scraped from the *P. perfoliatus* leaves and transferred to small amounts of distilled water using a plastic scraper and filtered on precombusted (450 ^{o}C, 24 h) Whatmann GF/F filters. The sediment cores were deep-frozen, the top 0.5 cm was cut off, and 3 at a time were pooled to yield a single sample (n = 5). Sand microflora, which consisted mostly of small epipsammic diatoms and bacteria in our study, was measured as detritus-free sediment. Visible detritus was manually removed and the sediment samples were carefully rinsed with water, which was discarded. We used this method, because the removal of the fine detritus particles was more important in this study than to loose a small part of the epiphyte biomass in form of epipelic diatoms. Observations with a dissecting microscope before and after the cleaning procedure of epiphytes and sediment showed the successful removal of unwanted material. All samples for stable isotope analysis were dried to constant weight (60 ^{o}C, 24 h) and stored in a dessicator. All samples for fatty acid analysis were deep-frozen at -80 ^{o}C. All invertebrate species were kept alive overnight in lake water to clear their guts. Muscle tissue was analysed for all fish species, the invertebrate species were processed as whole organisms. Consumer and plant samples for stable isotope analysis were dried to constant weight (60 ^{o}C, 48 h). The samples were ground in an agate mortar with a pestle as fine as possible and then stored in airtight plastic vials. The shells of the molluscs, apart from unavoidable small fragments were discarded before this procedure.

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Stable isotope analysis

P. perfoliatus and *Alnus sp.* leaf subsamples were transferred into tin cups. Consumer and sediment subsamples were transferred into silver cups, treated with 0.2 µl 10% HCl to remove carbonates and then dried again. The use of HCl to remove nondietary carbon in tissue used for stable isotope analysis has been questioned, because the $\delta^{15}N$ values can be influenced too, but the elimination of carbonates is absolutely necessary for some organisms, especially small gastropods and crustaceans that could only be sampled by crushing their shell or carapace. Preliminary analyses showed no statistically significant differences of $\delta^{15}N$ in acid or no-acid treatments (Jaschinski *et al.*, 2008a).

All consumer species were measured as individuals, except the small gastropod *Potamopyrgus antipodarum*, where 10 individuals were pooled in order to obtain sufficient material for analysis (n = 3). All samples were combusted in a CN-analyser (Fisons, 1500N) connected to a Finnigan Delta plus mass spectrometer. $\delta^{15}N$ and $\delta^{13}C$ values were calculated as

$$\delta X\ (‰) = [(R_{sample}/R_{standard})-1] \times 1000$$

where X = ^{15}N or ^{13}C and R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Pure N_2 and CO_2 gases were used as primary standards and calibrated against IAEA reference standards (N1, N2, N3, NBS22 and USGS24). Acetanilide was used as internal standard after every sixth sample. The overall analytical precision was ± 0.1‰ for $\delta^{15}N$ and $\delta^{13}C$.

The model of Phillips and Gregg (2003), which provides a range of feasible source mixtures, was used to determine the carbon sources:

$$\delta_M = f_A\delta_A + f_B\delta_B + f_C\delta_C$$

$$1 = f_A + f_B + f_C$$

f_A, f_B and f_C are the proportions of source isotopic signatures (δ_A, δ_B and δ_C) which coincide with the observed signature for the mixture (δ_M). All possible combinations of primary producer contributions were analysed with an increment of 1%. These predicted mixture signatures were compared with the measured values in the animals. If they were within a tolerance of 0.01%, they were considered feasible solutions. We only used $\delta^{13}C$ values in the modelling because of the sensitivity of the model to fractionation corrections (Connolly *et al.*, 2005). The fractionation is much larger for ^{15}N than for ^{13}C and can vary considerably between different species. We chose 0.2‰ as average fractionation increase of ^{13}C for freshwater ecosystems (France and Peters, 1997). Calculations were carried out with IsoSource, a Visual Basic program, which is available for public use (http://www.epa.gov/wed/pages/ models.htm). Epiphytes, *Alnus sp.* leaves and sand microflora were used as possible carbon sources for the most abundant consumers (*A. aquaticus*, *Gammarus pulex*, and *P. antipodarum*)

Fatty acid analysis

The plant and consumer samples were freeze-dried for 48 h, ground in an agate mortar with a pestle and weighed. Plants were processed as individuals, while consumers were pooled into three replicate samples, each containing three individuals, with the exception of *P. antipodarum* where 10 individuals were pooled to obtain sufficient material for analysis. Fatty acids were extracted with a mixture of CH_2Cl_2 and MeOH (2:1) with the addition of butylated hydroxytoluene (BHT; Sigma) to avoid auto-oxidation of the unsaturated fatty acids and trans-esterified with 2 ml of 3% H_2SO_4 in methanol for four hours at 70 ^oC following the method of Wiltshire *et al*. (2000). The Fatty Acid Methyl Esters (FAMEs) were analysed on a Hewlett Packard 5890 Series II gas chromatograph using the GC temperature settings of von Elert (2002). We used a JW Scientific column (30 m length, 0.25 mm I.D., and 0.25 µm film thickness). To quantify the fatty acid content an internal standard of heptadecanoic (17:0) and tricosanoic fatty (23:0) acid methyl esters was used.

Epiphyte and *P. perfoliatus* biomass

Epiphyte biomass was measured as chlorophyll *a*. Ten *P. perfoliatus* shoots were randomly selected on each sampling date. Epiphytes were carefully scraped from the shoots into small amounts of filtered lake water. This suspension was filtered on precombusted (450 ^{o}C, 24 h) Whatmann GF/F filters. Chlorophyll *a* and phaeophytin concentrations were measured according to Lorenzen (1967). The cleaned *P. perfoliatus* shoots were dried to a constant weight for 48 h at 60 ^{o}C and subsequently combusted for 8 h at 540 ^{o}C to determine the ash-free dry mass (AFDM). The surface area was calculated using the formula surface (mm^2) = AFDM (g) x 1362.4 (R^2=0.96), determined by measuring and weighing 100 shoots. All epiphytic chlorophyll concentrations were normalized to unit *P. perfoliatus* surface area.

Statistics

Temporal variability of carbon sources and trophic position of consumers was analysed using third-order polynomial regressions to find the best correlation between time and changes in food sources and trophic position (Statistica).

Results

Isotopic composition of food sources and consumers

Possible carbon sources fell into three categories based on $\delta^{13}C$ values during the main growing season: (1) an enriched source *P. perfoliatus*; (2) a source of intermediate value for epiphytes growing on *P. perfoliatus*; (3) depleted sources consisting of sand microflora and alder leaves (Fig. 1). Alder leaves always had the most depleted $\delta^{15}N$ signature (annual mean -0.9‰), whilst $\delta^{15}N$ values of epiphytes (annual mean 4.8‰) and *P. perfoliatus* (annual mean 4.5‰) were the most enriched. Sand microflora had intermediate $\delta^{15}N$ values (annual mean 2.2‰).

Observations with the microscope showed that epiphytes mostly consisted of filamentous green algae with an increasing amount of diatoms as the season progressed. Cyanobacteria were found in very small amounts with an increase in autumn. Sand microflora consisted mostly of prostrate diatoms, but green algae and cyanobacteria were also present. The proportions of these two algae groups increased from May to September.

Table 2 shows the consumers found and analysed in submerged macrophytes at the Schluensee in the growing season of 2003. The stable isotope signatures found are shown in Fig. 1. The crustaceans *A. aquaticus* and *G. pulex*, the ephemerid larvae *Cleon dipterum*, *Ephemera danica*, *Paraleptophlebia. submarginata* and *Torleya major* and the gastropod *P. antipodarum* had relatively similar $\delta^{13}C$ and $\delta^{15}N$ values suggesting herbivory with a mixed contribution of epiphytes, sand microflora and alder leaves. All other gastropods *Galba trunculata*, *Lymnea stagnalis*, *Radix ovata*, and *Theodoxus fluviatilis* had more positive $\delta^{13}C$ signals closer to that of *P. perfoliatus*. The $\delta^{15}N$ values ranged from 4.7‰ in *G. trunculata* to 7.3‰ for *T. fluviatilis*.

Predators were dragon fly larvae *Calopteryx virgo* and *Orthetrum cancellatus*, crayfish *Orconectes limosus*, fish including stone loach *Barbatula barbatula*, and three-spined stickleback *Gasterosteus aculeatus*, and juveniles of the European perch *Perca fluviatilis*. Their $\delta^{13}C$ signals indicated a mixed nutrition based ultimately on epiphyte, sand microflora, alder leaf, and *P. perfoliatus* carbon. The perch were probably still in their planktivorous phase. Small crayfish and perch had lower $\delta^{15}N$ values than the larger ones.

Seasonal change in nutrition of main consumer species

The most abundant macrozoobenthic organisms were *A. aquaticus*, *G. pulex* and *P. antipodarum* (Gohse-Reimann 2007). Modelling of feasible mixtures showed that epiphytes were the most likely carbon source for all three species, but the relative contributions varied with time and species. The relative contribution of epiphytes to the nutrition of *A. aquaticus* (Fig. 2a) was high in June (85%), decreased strongly in July (38%) and increased again in autumn (63-72%). Sand microflora and alder leaves inevitably had complementary contributions to *A. aquaticus* nutrition with the highest values in July (33% and 29%, respectively). In contrast, *A. aquaticus* collected in a nearby unvegetated area had significantly lower $\delta^{13}C$ values (around -29‰), and alder leaves (93-100%) were the most likely contributors to their nutrition (Table 3).

The relative contributions of epiphytes, sand microflora, and alder leaves to the nutrition of *G. pulex* (Fig. 2b) showed essentially the same pattern as for *A. aquaticus* in summer and autumn, with high values for epiphyte contribution in June (91%), a decrease in July (43%), and an increase in autumn (60-75%). The relative contribution of these autotrophs was intermediate in May (63%).

The most likely contributor to *P. antipodarum* nutrition was again epiphytes (90-100%), with the exception of July (Fig. 2c). The other possible carbon sources sand microflora and alder leaves had a low likelihood of making a substantial contribution to the gastropod's nutrition, but in July the importance of epiphytes as carbon source for *P. antipodarum* was strongly reduced (42%).

Biomarker fatty acids in dominant consumers

The biomarker fatty acid for *P. perfoliatus* 18:3ω3 (Rozentsvet et al. 2002) was present in all consumer species (Fig. 3a), but only in insignificant amounts (≤ 1.2%). Concentrations of 20:5ω3, characteristic for diatoms (Viso and Marty 1993; Desvilettes et al. 1997) were low in all consumers (Fig. 3b). A specific unsaturated fatty acid for green algae was not found in the epiphyte samples. We found 18:1ω9, a fatty acid occurring in high amounts in *Alnus* leaf litter, and thought to be characteristic for angiosperm detritus (Arts and Wainman 1999), in all animal species. *A. aquaticus* living on *Alnus* leaves (18%) and *G. pulex* (33%) had the highest levels of this fatty acid (Fig. 3c). The *G. pulex* value was derived from only one sample taken in September. Another biomarker fatty acid for angiosperm detritus 18:2ω6was found in lower amounts.

Epiphyte biomass and phaeophytin content

The biomass of epiphytes on *P. perfoliatus,* measured as chlorophyll *a* per surface area was highest in May, and then declined to approximately one third of the initial values in summer. Epiphyte biomass increased again in late summer/autumn (Fig. 4). The phaeophytin values were generally low and ranged from 1 to 5% of the chlorophyll *a* values.

Discussion

Plant beds in littoral zones provide a variety of allochthonous and autochthonous organic matter (Jeppesen et al. 1997). Our stable isotope and fatty acid analyses strongly argue in favour of a food web based mainly on epiphytic algae and to a lesser degree on sand microflora and alder leaves in Lake Schluensee from May to September. The negligible amount of the characteristic biomarker fatty acid for *P. perfoliatus* 18:3ω3 found in consumers strongly suggests that the macrophyte is of minor importance for the carbon flow in this food web. Feeding experiments with some of the dominant grazers in Lake Schluensee support this assumption (Gohse-Reimann 2007). Unfortunately, we found no characteristic fatty acid for green algae in the epiphyte samples to further support the results of the stable isotope analyses. The use of fatty acid analyses seemed to be somewhat limited in freshwater macrophyte systems, although the fatty acid composition of green algae is species-specific and under other circumstances a biomarker fatty acid for epiphytic green algae might be found.

The question of the importance of fresh macrophytes as food source is still in debate. Originally, submerged vascular plants were supposed to be only relevant as substratum for epiphytes and refuge from predation, and many studies found a low degree of herbivory (e.g. Porter 1977; Otto and Svensson 1981). But reviews of the literature and additional studies revealed that herbivory might be more common than generally thought (Newman 1991; Lodge 1991; Lodge et al. 1994; Jacobsen and Sand-Jensen 2006).

Epiphytes were the most important contributors to the nutrition of the most abundant invertebrates in our study. *P. antipodarum* had $\delta^{13}C$ values closest to those of epiphytes. *P. antipodarum* is indigenous to New Zealand and colonised freshwater and brackish habitats in Australia, Europe, and North America during the 19th and 20th centuries and can reach densities as high as 800.000 ind. m^{-2} (Kerans et al. 2005). It is a generalist feeder (i.e., both grazing herbivore and detritivore) and can feed on sand microflora, periphyton, fungi, bacteria, and detritus (James et al. 2000b; Aberle et al. 2005). Our findings concerning *P. antipodarum* nutrition are consistent with another study in New Zealand, where stable isotope signatures and gut analyses indicated that epiphytes are the predominant carbon source for this species (James et al. 2000a).

The most important source of nutrition for the two major crustacean species *A. aquaticus* and *G. pulex* were also epiphytes. Traditionally, both species are considered detrivorous shredders mainly feeding on decaying allochthonous leave material and on attached fungi and bacteria (Graça et al. 1993), but some studies suggest microscopic algae and macrophytes as additional food sources (Moore 1975; Marcus et al. 1978). In laboratory studies even predation and cannibalism occurred (Kelly et al. 2002; Gohse-Reimann, 2007). Our results imply that both grazers show a great plasticity in feeding habits in accordance with the food sources available to them (Moore 1975). *A. aquaticus* from a nearby unvegetated area had $\delta^{13}C$ values indicating alder leaves as ultimate carbon source. High content of 18:1ω9, a fatty acid found in high amounts in leaf litter supports this assumption.

The $\delta^{15}N$ values of both crustaceans (about 6.7‰) indicated a mostly herbivorous lifestyle. In particular, it was not possible that chironomid larvae were a preferred food item, as found in feeding experiments (Gohse-Reimann 2007). The chironomid larvae had higher $\delta^{15}N$ values (annual mean 7.9‰) than both crustacean species in the plant bed. In contrast, the *A. aquaticus* specimen from the unvegetated area showed unusually high $\delta^{15}N$ values (annual mean 13.4‰). The trophic fractionation from detritus to fungi and bacteria may explain the high $\delta^{15}N$ values of *A. aquaticus* as this species preferentially feeds on fungi and bacteria colonizing the leaves (Graça et al. 1993).Otherwise predation on chironomid larvae may be an option in this environment, where alder leaves with associated fungi and bacteria provided the main food source.

In July, the proportional contribution of epiphyte carbon to the nutrition of *P. antipodarum*, *A. aquaticus*, and *G. pulex* was reduced by half compared with all other months. The contributions of sand microflora and alder leaves increased accordingly. This drastic change in food sources occurred along with a strong decrease in epiphyte biomass. Reduction in the quantity of the preferred food seems to induce a switch in nutrition to less attractive food items. Furthermore, these results support the hypothesis that grazing invertebrates can control the density of epiphytes and, thus, the competition between epiphytes and macrophytes for light and nutrients (Jones et al. 2002). A strong increase in the abundances of main consumers in summer (Gohse-Reimann 2007) in combination with low nutrient levels probably reduced epiphyte biomass and production until autumn, when early storms and a decrease in consumer abundance changed the situation.

Our epiphyte chlorophyll *a* values are in the range of values found for epiphytes growing on *P. perfoliatus* in brackish water in the Chesapeake Bay (Neundorfer and Kemp 1993).The same seasonal development and similar absolute chlorophyll *a* values were found in a phosphate limitated temperate lake for epiphytes growing on *Scirpus subterminalis* (Burkholder and Wetzel 1989). The phaeophytin content, which might be used as an indirect indicator for bacteria in the epiphyte community, was about ten times higher than in our study indicating that bacteria play a minor role as food source at our study site.

Other gastropod species than *P. antipodarum* occurred only occasionally and in low densities. The stable carbon signatures of *R. ovata* and *T. fluviatilis* indicated a possible contribution of the macrophyte *P. perfoliatus* to their nutrition. May flies (*C. dipterum*, *E. danica*, *P. submarginata*, *T. major*) were only found in May and June and their $\delta^{13}C$ values suggested a mixed diet of epiphytes, sand microflora, and alder leaves.

Stable isotope analyses indicated that the main predators, American crayfish (*O. limosus*), loach (*B. barbatula*) and three-spined stickleback (*G. aculeatus*) ultimately depend on epiphyte, sediment microflora, *Alnus* leaves and *P. perfoliatus* carbon in about equal proportions. Crayfish are considered omnivorous, though preferring aquatic invertebrates, but may take macrophytes and leaf litter, when more nutritious food is scarce (Nyström et al. 1999; Dorn and Mittelbach 2004). Feeding experiments with *O. limosus* showed that this crayfish preferentially fed on animals, but small amounts of leaf litter and macrophyte tissue were also ingested (Gohse-Reimann 2007). The relatively low $\delta^{15}N$ values (annual mean 8.7‰) give further evidence for an omnivorous life style of this species.

The basic food of loach is dipteran larvae, mostly chironomids, while other insect larvae are fed upon only in spring, and benthic crustaceans, amphipods and isopods become more important in autumn (Michel and Oberdorff 1995). This is in good accordance with the $\delta^{15}N$

content of the loach, which decreased from 12.1‰ in spring to 9.1‰ in autumn while the $\delta^{15}N$ of chironomid larvae decreased simultaneously from 9.7‰ to 6.5. The $\delta^{13}C$ of the loach, however, suggested that chironomids are not the only food source.

Sticklebacks are the top predator in this macrophyte system showing the highest $\delta^{15}N$ values (14‰). This fish species is a generalist, feeding on insect larvae, benthic crustaceans, copepods and fish eggs, which may explain its high trophic position. The perch in our study were probably still in their zooplanktivorous phase (Hargeby et al. 2005).

Our assumption that there is no strong direct link between living macrophytes and higher trophic levels is consistent with other stable isotope analyses in plant-dominated lake systems (Hecky and Hesslein 1995; James, et al. 2000b; Jones and Waldron 2003). In contrast to most other studies on freshwater macrophyte systems, Keough et al. (1996) found that phytoplankton was the dominant carbon source in the littoral zone of Lake Superior and epiphytes played only a minor role in invertebrate nutrition. In addition, Solomon et al. (2008) showed that the importance of periphyton is species-dependent for aquatic insect larvae in a whole-lake ^{13}C addition experiment. In marine seagrass beds, similar analyses also supported the importance of epiphytic algae (Moncreiff and Sullivan 2001; Jaschinski et al. 2008b) and benthic microalgae can also be relevant in salt marsh food webs (Teal 1962; Sullivan and Moncreiff 1990).

Epiphytes on submerged plants in temperate regions are generally believed to have the potential to fix more carbon than the macrophytes they grow on (Cattaneo and Kalff 1980; Jaschinski et al. 2008b) despite their relatively low biomass and provide a more nutritious and less toxic or repellent food than most macrophytes. Epiphytes usually contain more nitrogen and phosphorus in relation to carbon compared to macrophytes and leaf litter, and the content of essential fatty acids (ω3 and ω6 groups) is higher than in *Alnus* leaves (Brepohl, unpubl. data). Both amount and nature of the food sources thus influence what animals eat.

Acknowledgements

We thank T. Hansen for the analysis of the stable isotope samples. We also thank N. Aberle-Malzahn for helpful comments on the manuscript. S. Flöder provided valuable assistance in the field. The Brazilian National Counsel of Technological and Scientific Development (CNPq) and the German Research Foundation supported this work (So 145/20).

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Table 1 Dissolved nutrients in the Schluensee from March to November 2003

Nutrient (µmol/l)	March	April	May	June	July	August	September	October	No
Nitrate/nitrite	21.448	2.260	3.334	2.847	2.068	1.643	1.394	2.769	[illegible]
Ammonium	0.774	3.546	1.417	1.149	0.928	0.816	1.798	1.124	
Silicate	2.977	0.731	6.435	1.574	2.057	22.697	21.980	47.672	[illegible]
Phosphate	1.741	0.204	0.212	0.199	0.165	0.133	0.082	0.179	

Table 2 Species analysed (SI/FA) in a *Potamogeton perfoliatus* stand in the Schluensee, Germany, 2003

Species	May	June	July	Aug.	Sept.
Crustacea					
Asellus aquaticus (Linnaeus)		10/9	10/9	10/9	10/9
Gammarus pulex (Linnaeus)	10/9	10/9	10/9	10/9	10/9
Orconectes limosus (Rafinesque)		10/5	10/5		
Diptera					
Chironomus sp.	10/0	10/0	10/10	10/0	
Ephemeroptera					
Cloeon dipterum (Linnaeus)	5/6				
Ephemera danica Müller	8/1	3/0			
Paraleptophlebia submarginata (Stephens)		4/0			
Torleya major (Klapalek)		3/0			
Odontata					
Calopteryx virgo (Linnaeus)		3/1	3/0	3/0	3/0
Oethetrum cancellatum (Linnaeus)				1/0	
Mollusca					
Bithynia tentaculata (Linnaeus)			1/0		
Dreissena polymorpha Pallas	10/3	10/3	10/3	10/3	10/3
Galba trunculata			1/0		
Lymnaea stagnalis (Linnaeus)			1/1		
Potamopyrgus antipodarum (J. E. Gray)	30/30	30/30	30/30	30/30	30/30
Radix ovata (Draparnaud)		1/1			
Theodoxus fluviatilis (Linnaeus)		3/0	3/0	3/0	3/0
Pisces					
Barbatula barbatula (Linnaeus)		1/1	1/1	1/1	1/1
Gasterosteus aculeatus Linnaeus	1/0		1/0		
Perca fluviatilis Linnaeus	4/4	4/4	4/4		

Table 3 Results of the IsoSource model for the most abundant consumers. Mean contributions of primary producers to consumer nutrition and the 1 to 99 percentile ranges are given (in parentheses).

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Month	Epiphytes	Alder leaves	Sand microflora
A. aquaticus			
June	85 (84-87)	6 (0-13)	9 (0-16)
July	38 (35-41)	29 (5-55)	33 (4-60)
August	63 (58-67)	16 (0-30)	22 (3-42)
September	72 (64-79)	11 (0-20)	18 (1-36)
A. aquaticus (unvegetated area)			
June	0	99 (99-100)	1 (0-1)
July	0	93 (88-98)	7 (1-12)
August	0	100	0
G. pulex			
May	63 (50-75)	13 (0-25)	25 (0-50)
June	91 (90-92)	4 (0-8)	5 (0-10)
July	43 (40-46)	28 (2-52)	28 (2-58)
August	60 (54-64)	18 (2-35)	23 (1-44)
September	75 (68-81)	11 (2-19)	15 (0-30)
P. antipodarum			
May	100	0	0
June	88 (87-89)	5 (0-9)	7 (2-13)
July	42 (39-45)	31 (5-55)	27 (0-56)
August	90 (89-91)	3 (0-7)	7 (2-11)
September	96 (95-96)	2 (1-2)	3 (2-4)

Figure legends

Fig. 1 Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ values (‰) for food sources and consumers collected from a *Potamogeton perfoliatus* stand in the Schluensee in 2003.

Fig. 2 Seasonal variation in carbon sources for the most abundant consumers (a) *Asellus aquaticus,* (b) *Gammarus pulex*, and (c) *Potamopyrgus antipodarum*.

Fig. 3 Biomarker fatty acids in dominant animals and primary food sources: (a) for *Potamogeton perfoliatus*, (b) for diatoms, and (c) for *Alnus* leaves. The dotted lines separate primary producers and consumer species. (PA = *Potamopyrgus antipodarum*, LA = *Asellus aquaticus* on leaves, EA = *A. aquaticus* on epiphytes, GP = *Gammarus pulex*, OP = *Orconectes limosus*, PF = *Perca fluviatilis*, BB = *Barbatula barbatula*, PP = *Potamogeton perfoliatus*, AL *Alnus* leaves, EP = epiphytes, SM = sand microflora).

Fig. 4 Epiphyte biomass and phaeophytin content in a *P. perfoliatus* stand in the Schluensee, Germany, from May to September. Shown are means and standard deviation.

Figure 1

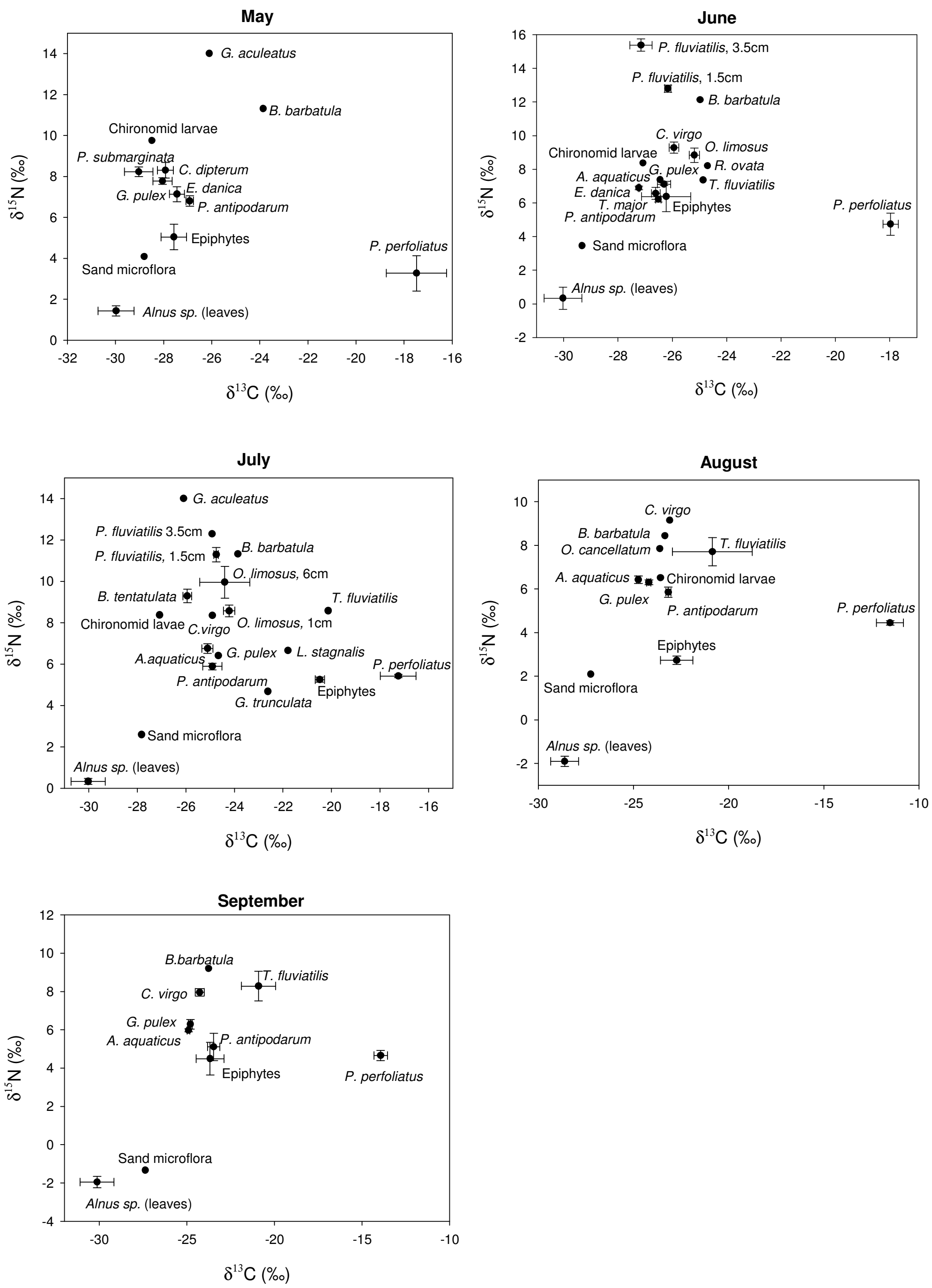
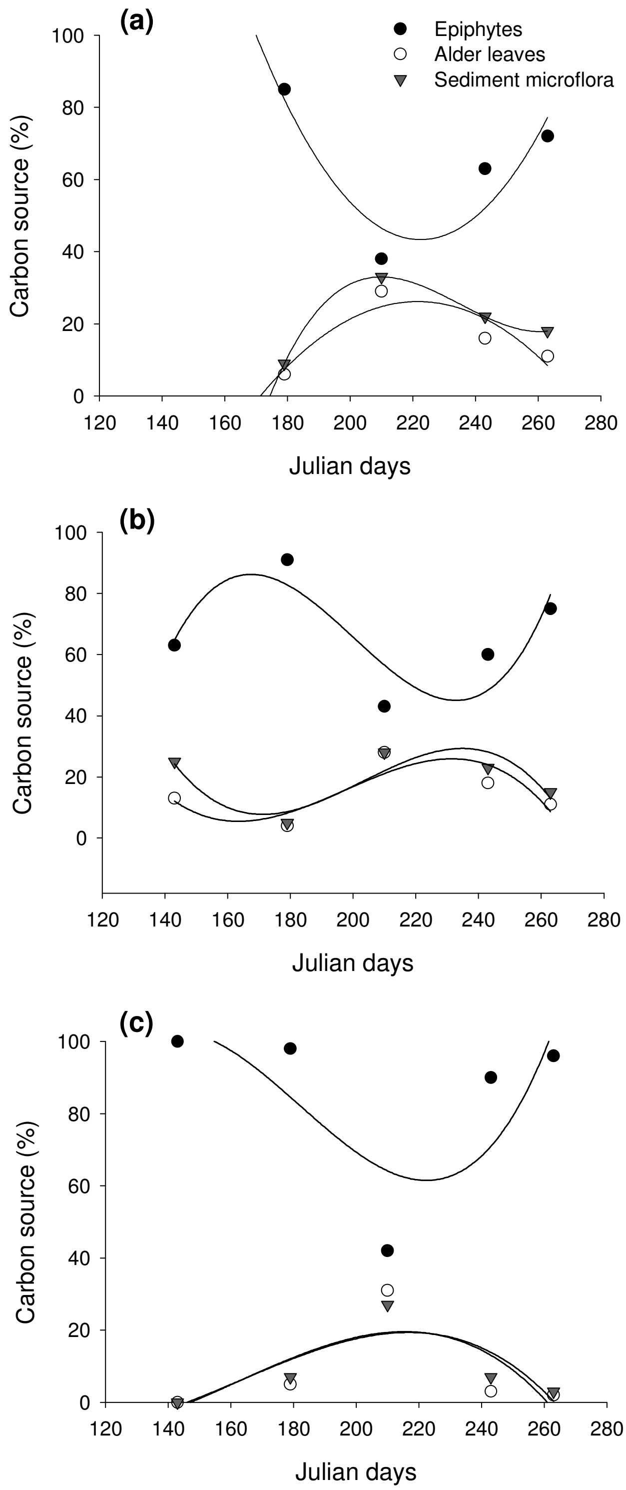

May
G. aculeatus
B. barbatula
Chironomid larvae
P. submarginata
C. dipterum
E. danica
G. pulex
P. antipodarum
Epiphytes
Sand microflora
P. perfoliatus
Alnus sp. (leaves)
δ15N (‰)
δ13C (‰)
June
P. fluviatilis, 3.5cm
P. fluviatilis, 1.5cm
B. barbatula
C. virgo
O. limosus
Chironomid larvae
R. ovata
A. aquaticus
G. pulex
T. fluviatilis
E. danica
T. major
Epiphytes
P. antipodarum
P. perfoliatus
Sand microflora
Alnus sp. (leaves)
δ15N (‰)
δ13C (‰)
July
G. aculeatus
P. fluviatilis 3.5cm
B. barbatula
P. fluviatilis, 1.5cm
O. limosus, 6cm
B. tentatulata
T. fluviatilis
Chironomid lavae
C.virgo
O. limosus, 1cm
A.aquaticus
G. pulex
L. stagnalis
P. perfoliatus
P. antipodarum
Epiphytes
G. trunculata
Sand microflora
Alnus sp. (leaves)
δ15N (‰)
δ13C (‰)
August
C. virgo
B. barbatula
O. cancellatum
T. fluviatilis
A. aquaticus
Chironomid larvae
G. pulex
P. antipodarum
P. perfoliatus
Epiphytes
Sand microflora
Alnus sp. (leaves)
δ15N (‰)
δ13C (‰)
September
B.barbatula
T. fluviatilis
C. virgo
G. pulex
A. aquaticus
P. antipodarum
Epiphytes
P. perfoliatus
Sand microflora
Alnus sp. (leaves)
δ15N (‰)
δ13C (‰)

Figure 2



(a)
Epiphytes
Alder leaves
Sediment microflora
Carbon source (%)
Julian days
(b)
Carbon source (%)
Julian days
(c)
Carbon source (%)
Julian days

Figure 3

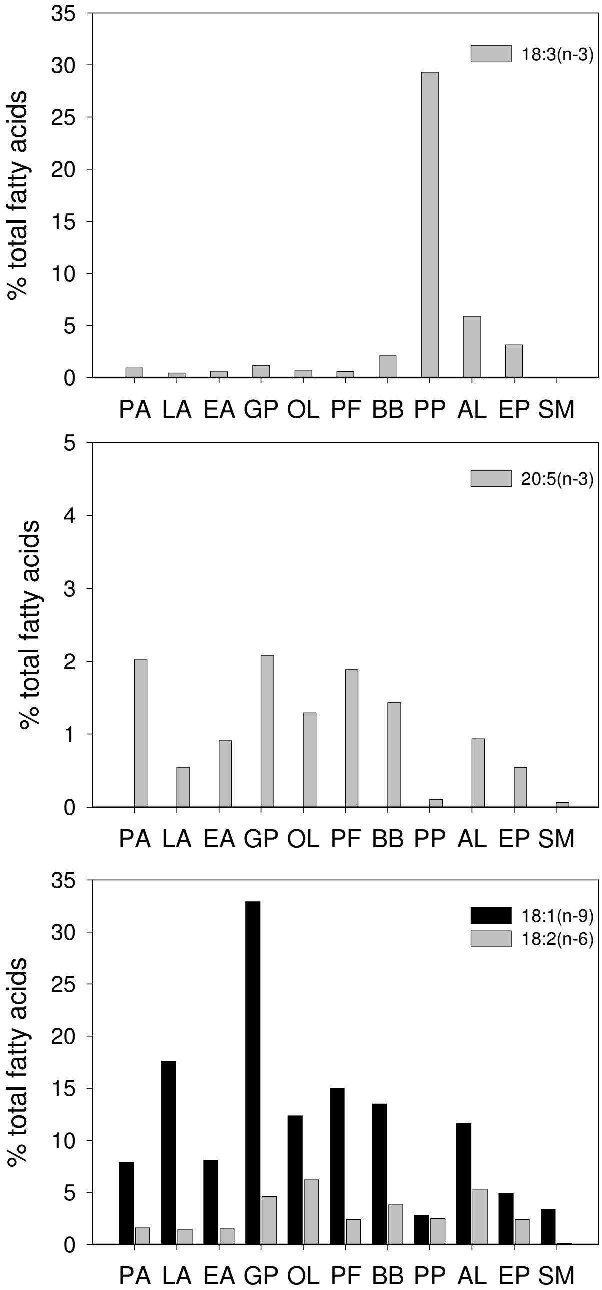
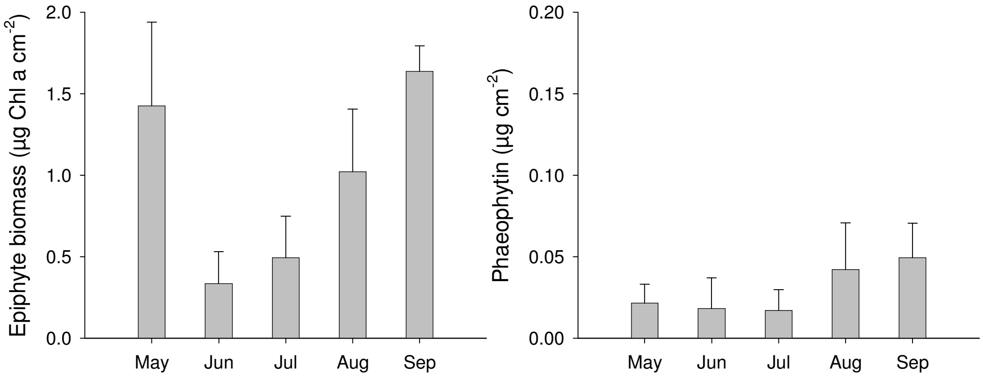


Figure 4



Epiphyte biomass (µg Chl a cm-2)
2.0
1.5
1.0
0.5
0.0
May
Jun
Jul
Aug
Sep
Phaeophytin (µg cm-2)
0.20
0.15
0.10
0.05
0.00
May
Jun
Jul
Aug
Sep

675