




# Stable isotope measurements confirm consumption of submerged macrophytes by macroinvertebrate and fish taxa

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**Abstract** Many macrophyte species in lowland streams exhibit signs of grazing and herbivore damage, even though herbivory by aquatic macroinvertebrates and fish is generally considered to be of little importance. In this study, we collected evidence for the hypothesis that herbivory on macrophytes by macroinvertebrates and fish is more widespread than assumed. We measured the dual stable isotope signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of organic matter, epiphyton, submerged macrophytes, macroinvertebrates and fish in a Belgian lowland stream. There was a clear distinction in isotopic signatures of the different basal

resources, allowing the use of the SIAR mixing model. These calculations revealed the consumption of macrophyte tissue not only by the phytophagous larvae of *Nymphula nitidulata* Hufnagel (Lepidoptera: Crambidae), but also by Baetidae nymphs (Ephemeroptera), Orthocladiinae larvae (Diptera: Chironomidae), the crayfish *Orconectes limosus* Rafinesque (Decapoda: Cambaridae) and the fish *Gobio gobio* L. (Cypriniformes: Cyprinidae) which are classified as feeding on other resources. Although the potential share of macrophyte biomass in the diet of macroinvertebrates and fish was demonstrated to be up to 49%, this amount is only a small percentage of the total standing macrophyte biomass in a lowland stream. However, the impact of this herbivory may still be substantial because consumption may comprise a significant fraction of the daily primary production. Additionally, small-scale herbivory may still have a negative impact on macrophyte growth and survival, for example through consumption of apical meristems and the increased susceptibility to diseases and toxins if the macrophyte's epidermis is damaged.

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## Introduction

Even though aquatic macrophytes can develop substantial biomass within lowland streams and rivers (Champion and Tanner 2000), it is commonly thought that living plant parts are rarely consumed by macroinvertebrates and fish (Lampert and Sommer 2007; Mann 1988; Moore and De Ruiter 2012). Instead, the majority of macrophyte primary production is assumed to enter the aquatic food web as detritus (Polunin 1984). The scarcity of direct macrophyte consumption by invertebrates and fish is probably not driven by its nutritive quality, as freshwater macrophytes generally have lower C:N ratios and contain lower amounts of hard to digest carbon-rich structural compounds, such as lignin and cellulose, in comparison with many terrestrial plant species (e.g. Bakker et al. 2016; Lodge 1991). However, many macrophyte species possess inhibitory secondary metabolites, such as alkaloids, glucosinolates and polyphenolics, which can act as a chemical defence against herbivory (Gross and Bakker 2012; Sotka et al. 2009). Feeding trials with omnivorous macroinvertebrates and fish confirm a preference for macrophytes with low concentrations of deterring chemicals (e.g. Dorenbosch and Bakker 2011; Li et al. 2004).

Despite this general consensus, there are numerous cases where significant amounts of invertebrate and fish-induced herbivore damage on aquatic vascular plants have been observed under natural conditions, both in older and recent literature (Bakker et al. 2016; Cronin et al. 1998; Jacobsen and Sand-Jensen 1992; Körner and Dugdale 2003; Wood et al. 2017). However, only a few macroinvertebrate and fish taxa are actually known to directly consume living macrophyte parts, including specialist Lepidoptera, Coleoptera and Diptera taxa, generalist omnivorous crabs and crayfish and generalist herbivorous fish (Cronin et al. 1998; Dorenbosch and Bakker 2011; Newman 1991; Olsen et al. 1991).

Although aquatic food webs have been reconstructed before, using both consumer stomach content and stable isotope analyses, aquatic macrophytes have often been excluded as possible food sources for macroinvertebrates and fish (e.g. Finlay 2001; Hamilton et al. 1992). However, studies that did include macrophytes as a food source reported varying results regarding macrophyte consumption; they either included only emergent and generally unpalatable species (Reid et al. 2008), observed

little evidence for direct macrophyte consumption (e.g. Jaschinski et al. 2011), or did indeed observe incorporation of macrophyte-derived carbon in the aquatic food web, including consumption of living macrophytes by macroinvertebrate shredders (Syväranta et al. 2016; Watson and Barmuta 2011).

This study hypothesises that herbivory on aquatic macrophytes by macroinvertebrates and fish occurs in more generalist consumer taxa than generally assumed and will consequently also have a larger impact on the standing macrophyte biomass. This hypothesis is based both on personal observations of grazing damage on aquatic macrophytes in field conditions and on the expectation from an evolutionary point of view, large feeding niches such as macrophytes can hardly be expected to remain unoccupied (e.g. Lodge 1991). In order to confirm or reject our hypothesis, we reconstruct the instream food web of a slow-flowing Belgian lowland stream using stable isotope measurements of typical producers and generalist consumers and by analysing the consumers' diet composition using a stable isotope mixing model.

## Materials and methods

### Study site

Fieldwork was performed in the 18th and 19th of May 2015 in the Desselse Nete, a slow-flowing sand bottom lowland stream in the north of Belgium (51°14'53"N, 5°4'53"E) with a stream width varying between 3.5 and 5.5 m and an average depth of 60 cm. Summer nutrient concentrations were  $95.0 \pm 35.7 \mu\text{g l}^{-1}$  N-NH<sub>4</sub><sup>+</sup>,  $672.5 \pm 60.5 \mu\text{g l}^{-1}$  N-NO<sub>3</sub><sup>-</sup> and  $17.3 \pm 4.0 \mu\text{g l}^{-1}$  P-PO<sub>4</sub><sup>3-</sup>, with an average pH of 7.45 (VMM—Flemish Environment Agency 2017). Catchment land use is mainly agricultural and anthropogenic impact on the stream is substantial, with stressors including hydromorphological degradation and water quality issues (i.e. pollution and agricultural nutrient inputs).

### Sample collection, processing and isotopic analysis

In order to accurately reconstruct the aquatic food web, samples of all trophic levels were collected, including coarse particulate organic matter (CPOM),

fine particulate organic matter (FPOM), aquatic macrophytes, epiphyton, macroinvertebrates and fish. Sestonic FPOM was filtered from collected river water over 55  $\mu\text{m}$  Whatmann glass-fibre filters (GF/C), while CPOM was collected in both sestonic and benthic form, by sieving it from the river water and the upper 5 cm of the sediment, respectively. Aquatic macrophytes with their associated epiphyton and macroinvertebrates were collected using a cylindrical box sampler [inner dimensions: 23.5 cm  $\times$  19 cm (length  $\times$  diameter); mesh size: 500  $\mu\text{m}$ ; total volume: 6663 cm<sup>3</sup> (method after Wolters et al. 2018)], which was gently lowered over the vegetation stand, after which its two halves were gently closed and the aquatic vegetation within the sampler was cut off by hand with a sharp knife. Plant biomass was collected from three separate locations in the stream in order to obtain sufficient biomass and to account for small-scale spatial variability. Macroinvertebrates associated with sediment were additionally collected by taking five core samples spread over bare and vegetated sections of the stream, using a plastic core sampler (diameter 5.4 cm). Immediately after collection, samples were stored in 5-L plastic buckets and transported back to the laboratory where macroinvertebrates were separated from vegetation and stored at  $-20\text{ }^{\circ}\text{C}$  until further identification and processing. Macroinvertebrate guts were not removed, nor was gut clearance time provided, because these procedures were shown to not significantly affect stable isotope signatures of herbivorous and detritivorous macroinvertebrates (Jardine et al. 2005). To be able to reconstruct the food web, macroinvertebrates were identified to the lowest taxonomic level practical. Care was taken to remove carbonate shells of molluscs by dissection, to prevent it biasing the  $^{13}\text{C}$  measurements (Jacob et al. 2005). Epiphyton was manually scraped from the macrophytes, using tweezers for large fragments and by carefully brushing the macrophytes with gloved fingers and rinsing them with distilled water for more tightly attached fragments. This was then stored at  $-20\text{ }^{\circ}\text{C}$  until further processing. Macrophytes that were cleaned of epiphyton in this way were sorted by species and oven-dried at  $70\text{ }^{\circ}\text{C}$  to a constant weight (at least 48 h). Fish were captured by electrofishing. After identification, three individuals were collected per species and dissected to obtain muscle tissue, which was subsequently stored at  $-20\text{ }^{\circ}\text{C}$  until further processing. As is common in

isotope food web studies, only muscle tissue was extracted for isotope analysis in fish and the crayfish *Orconectus limosus* Rafinesque (Decapoda: Cambaridae), due to its intermediate turnover rate and low variability compared to other tissues (Pinnegar and Polunin 1999; Tieszen et al. 1983). All other macroinvertebrates were stored and analysed as complete animals. Organic matter, epiphyton, macroinvertebrates and (cray-)fish tissue samples were subsequently freeze-dried, using a Heto PowerDry LL3000 (Thermo Scientific) and ground using a Retsch mixer mill (MM301). Dried macrophytes were all ground with a Retsch ZM200 ultra-centrifugal mill.

Subsamples of the powdered material were weighed in silver cups and acidified with one drop of 5% hydrochloric acid, to remove any carbonates (Jacob et al. 2005), and oven-dried at  $60\text{ }^{\circ}\text{C}$  for 4 h after which the cups were folded and analysed. 5 mg of sample was used for organic matter, macrophytes and epiphyton, and 1 mg was used for macroinvertebrates and fish. Whenever possible, macroinvertebrates were measured per separate vegetation or sediment sample in which they occurred, although it was sometimes necessary to pool animals from different samples in order to obtain enough biomass for stable isotope analyses. Fish muscle tissue was analysed for three separate individuals, although some species were caught in fewer numbers. Sample carbon and nitrogen content was measured using a Flash EA 1112 Elemental Analyser (Thermo Finnigan) or a EuroEA3000 (Eurovector) Elemental Analyser. The  $^{13}\text{C}$  and  $^{15}\text{N}$  stable isotope signatures were measured using a Delta V Advantage isotope ratio mass spectrometer (Thermo Finnigan) that was coupled, via a ConFlo III interface (Thermo Finnigan), to the Elemental Analysers.

#### Data analysis

To assess the relative importance of the food sources in the consumer's diet, the stable isotope mixing model 'Stable Isotope Analysis in R' (SIAR, Parnell and Jackson 2013) package (version 4.2) was used under R 3.3.2 (R Development Core Team 2016). This Bayesian mixing model incorporates variation in the stable isotope (i.e.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) signatures of the different food sources and consumers and, based on this information, calculates density plots of credible intervals for the estimated dietary proportion of each

food source (Parnell and Jackson 2013; Parnell et al. 2010). Additionally, this mixing model has the ability to incorporate carbon and nitrogen content of food sources, allowing for a better resolution when analysing food sources with vastly different C and N concentrations (Phillips and Koch 2002), for example for omnivores that may consume both nitrogen-poor detritus and nitrogen-rich animal material.

The model was applied to the following macroinvertebrate and fish taxa that, based on literature surveys and our own experience, were expected to potentially include macrophytes in their diets, either as living macrophyte parts or macrophyte-derived detritus (i.e. groups for which the mixing model analysis was useful cf. Phillips et al. 2014): *Pisidium* sp. (Bivalvia: Sphaeriidae), *Baetis* sp. (Ephemeroptera: Baetidae), Chironomini (Diptera: Chironomidae), Orthocladiinae (Diptera: Chironomidae), *Simulium* sp. (Diptera: Simuliidae), *Hydroptila* sp. (Trichoptera: Hydroptilidae), *Asellus aquaticus* L. (Isopoda: Asellidae), *Orconectes limosus* Rafinesque (Decapoda: Cambaridae), *Gobio gobio* L. (Cypriniformes: Cyprinidae) and *Perca fluviatilis* L. (Perciformes: Percidae). The phytophagous larvae of *N. nitidulata* Hufnagel (Lepidoptera: Crambidae) were also included in the model as a reference group that is known to purposefully consume macrophytes (Gaevskaya 1969; Palm 1986).

Organic matter, epiphyton and macrophytes, being the three basal resources measured in this study, were used as the three possible food sources for the herbivorous/detritivorous invertebrate taxa in the SIAR model calculations. For the two omnivorous fish species and the crayfish *O. limosus*, macroinvertebrates were added as an additional possible food source, of which the mean stable isotope signature was calculated as the weighted average of all different measured invertebrate taxa (Phillips et al. 2005). Additionally, fish were included as a possible food source in the diet of *P. fluviatilis*, as our collected individuals measured over 10 cm and were thus large enough to have possibly undergone the ontogenetic shift to a piscivorous lifestyle (e.g. Mittelbach and Persson 1998). Again, the mean stable isotope signature of this food source was calculated as the weighted average of the relevant fish taxa (Phillips et al. 2005).

Before incorporation in the model, the food source carbon and nitrogen stable isotope signatures were corrected for trophic fractionation by adding 0.8‰ and

2.6‰, respectively, for animals of which only muscle tissue was analysed (i.e. *O. limosus* and fish) and by adding 0.4‰ and 2.3‰, respectively, for the remaining macroinvertebrates that were analysed in one piece, according to McCutchan et al. (2003). No  $\delta^{15}\text{N}$  differentiation was used for *Hydroptila* sp. because these animals are considered fluid feeders and should therefore not be corrected for nitrogen (Keiper 1998; McCutchan et al. 2003).

After the calculation of the macrophyte proportion in the diet of invertebrate and fish consumers, the quantitative impact of macrophyte consumption by Orthocladiinae, *Baetis* sp. and *N. nitidulata* on the macrophyte standing biomass within the stream was calculated, based on these dietary composition data and literature data on macroinvertebrate feeding rates and distribution in the Desselse Nete. This was done by multiplying the calculated potential macrophyte proportion in the consumer's diet with its individual daily feeding rate, which was obtained from various literature sources (Cattaneo and Mousseau 1995; Monakov 2003; Nolte 1990; Winterbourn et al. 1985; Zelinka 1984). Furthermore, the animal's distribution data from the Desselse Nete were used from the literature. These specimens were collected on macrophytes (i.e. *Sparganium emersum* Rehmman (Sparganiaceae), *Potamogeton natans* L. (Potamogetonaceae) and *Callitriche obtusangula* Le Gall (Plantaginaceae), the same plant species as in this study) with a known plant biomass in the Desselse Nete on August 2014 and June 2015 (Wolters et al. 2018). Data from both years were hereby used in the calculation, in order to incorporate a degree of the seasonal differences in abundance that naturally occur in macroinvertebrate populations (Gross et al. 2002; Jacobsen and Sand-Jensen 1992). This calculation should be seen as an indicator of the scale of impact of macroinvertebrate herbivory, rather than a definite account, as the time scale of the study cannot fully capture the natural fluctuations in herbivore population size.

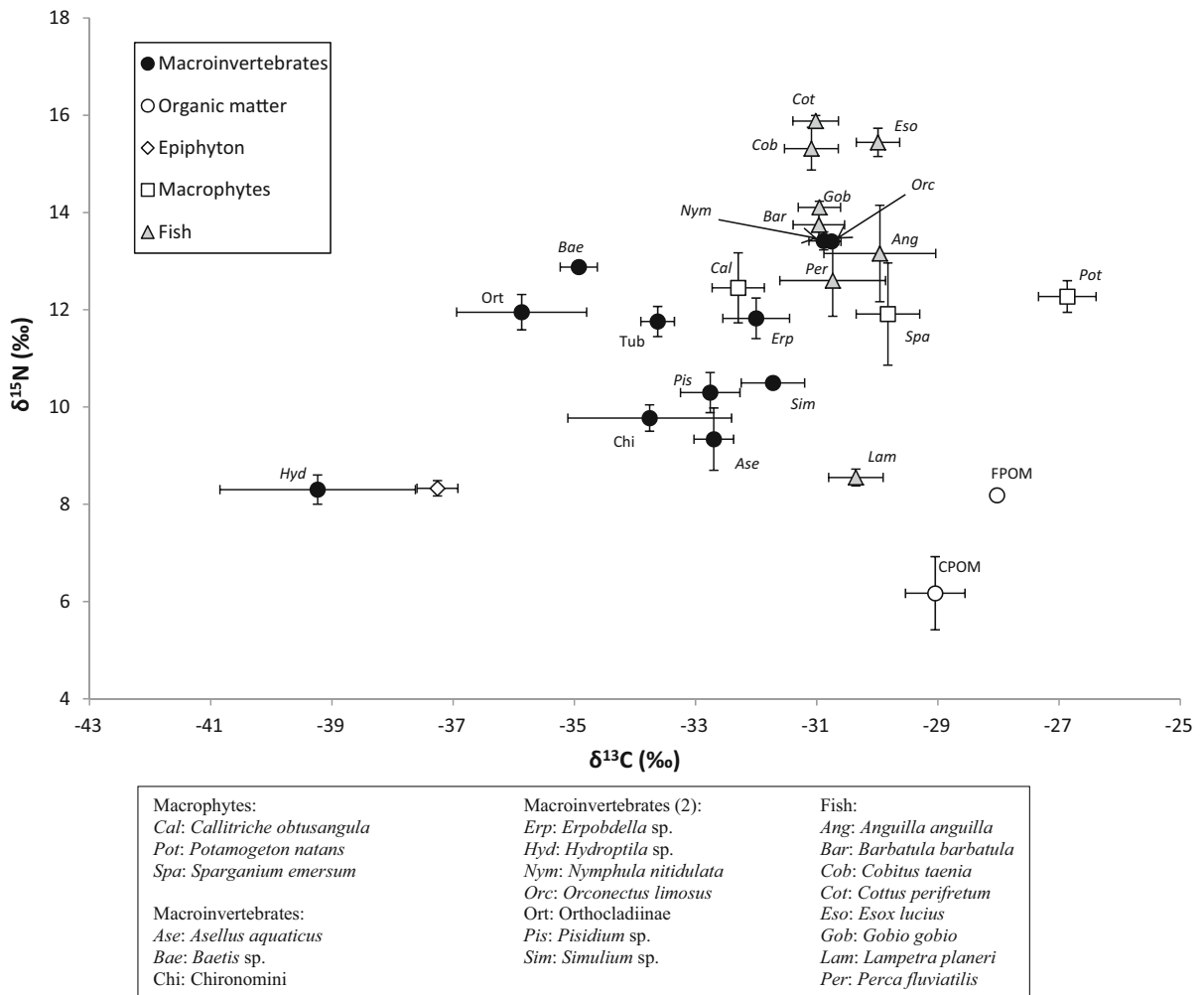
## Results

### Natural $^{13}\text{C}$ and $^{15}\text{N}$ abundance

Clear differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of different food web components were observed within

the Desselse Nete (Fig. 1). A clear distinction is hereby visible in the isotopic signatures of the basal resources in the food web: organic matter, epiphyton and macrophytes. With a relatively high  $\delta^{13}\text{C}$  and a low  $\delta^{15}\text{N}$  value, compared to other food web components, FPOM and CPOM were positioned at the lower right corner of the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  isotope biplot. Epiphyton on the other hand, with the majority of its biomass consisting of the green alga *Cladophora* sp. (Cladophoraceae), displayed the greatest depletion in  $^{13}\text{C}$  values. This, combined with relatively low  $\delta^{15}\text{N}$  values, resulted in its bottom left position within the isotope biplot. The macrophytes *S. emersum*, *P. natans* and *C. obtusangula* showed mean  $\delta^{13}\text{C}$  values that

ranged from  $-32.2\text{‰}$  (*C. obtusangula*) to  $-26.9\text{‰}$  (*P. natans*), in addition to mean  $\delta^{15}\text{N}$  values that were higher than those of most invertebrate consumers, with the exception of *N. nitidulata* and *O. limosus*, and ranged from  $11.9\text{‰}$  (*S. emersum*) to  $12.5\text{‰}$  (*C. obtusangula*). After correcting for trophic fractionation, macroinvertebrate isotopic signatures were generally positioned within the mixing triangle of the basal resources, except for the  $^{15}\text{N}$  enriched *N. nitidulata* and *O. limosus* and the  $^{13}\text{C}$  depleted *Hydroptila* sp., Orthocladiinae and *Baetis* sp. taxa. The group with the highest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures were the fishes, with the exception of the FPOM filtering *Lampetra planeri* Bloch



**Fig. 1** Stable isotope signatures (mean  $\pm$  SE) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of organic matter, epiphyton, macrophytes and different macroinvertebrate and fish taxa collected in the Desselse Nete.

Abbreviations within the graph are explained in the textbox below the graph



(Petromyzontiformes: Petromyzontidae), and the aforementioned macroinvertebrates *N. nitidulata* and *O. limosus* which also possessed high  $\delta^{15}\text{N}$  signatures.

### Mixing model analysis

Besides differences in stable isotope signatures of organic matter, epiphyton, macrophytes and macroinvertebrates, there were also marked differences in the C:N ratios of these possible food sources. Organic matter, with a C:N ratio of  $21.3 \pm 4.8$  (mean  $\pm$  SE), was hereby relatively the poorest in nitrogen, and macroinvertebrates were relatively rich in nitrogen with a C:N ratio of  $5.7 \pm 0.3$ . Epiphyton and macrophytes were situated in between with C:N ratios of  $10.8 \pm 0.3$  and  $13.2 \pm 0.7$ , respectively. The mixing model analysis revealed varying potential contributions of macrophytes in the diet of the analysed macroinvertebrate and fish taxa, with median values ranging from 7% for *A. aquaticus* and Chironomini to 49% for *Baetis* sp. and *N. nitidulata* (Fig. 2). Macrophyte consumption was significantly ( $P < 0.05$ ) above 0 (i.e. the 5th percentile was higher than 0) for Orthocladiinae, *O. limosus*, *G. gobio*, *Baetis* sp. and *N. nitidulata*, indicating macrophyte consumption by these taxa (Fig. 2). On the other hand, *A. aquaticus*, Chironomini, *Pisidium* sp., *Hydroptila* sp., *Simulium* sp. and *P. fluviatilis* did not consume significant portions of macrophyte tissue (Fig. 2).

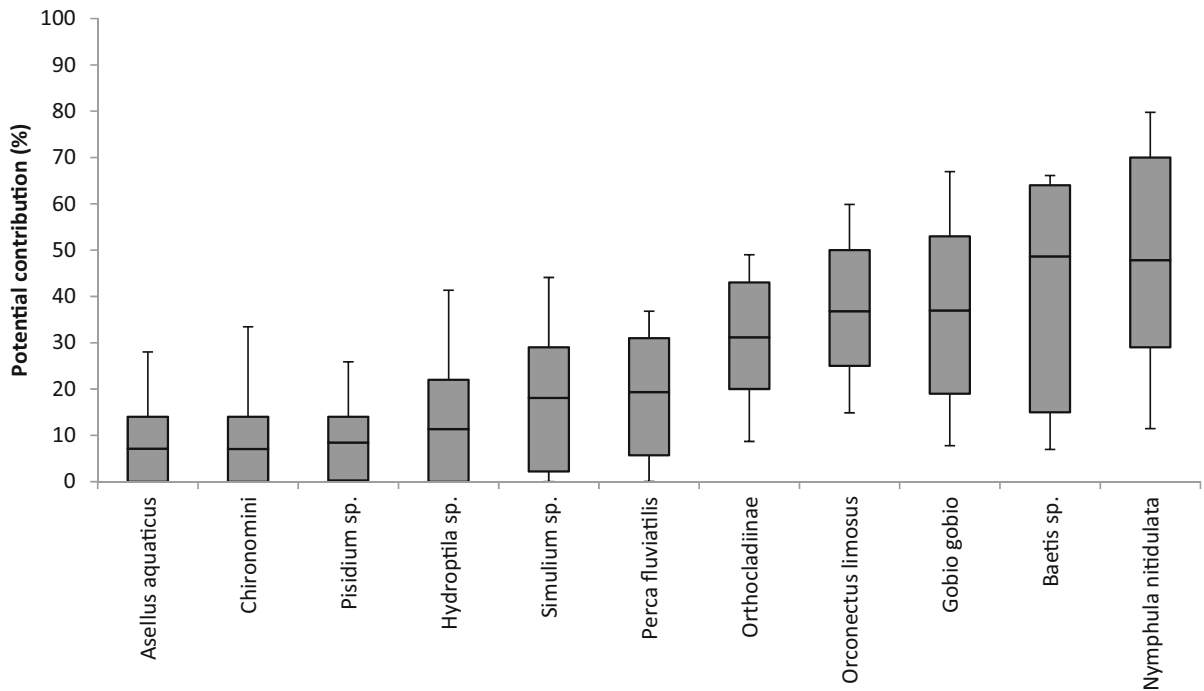
From the calculated diet composition of Orthocladiinae, *Baetis* sp. and *N. nitidulata*, which were coupled to quantitative literature data of macrophyte biomass, macroinvertebrate distribution and the feeding rates of these animals, the relative daily consumption of macrophyte standing biomass was calculated (Table 2). Although considerable variation was observed between the different years, invertebrate taxa and plant species, the general calculated consumption of macrophyte standing biomass was low with median values ranging from 0.72 (*P. natans* in 2014) to 3.35% macrophyte biomass consumed day<sup>-1</sup> (*S. emersum* in 2014) (Table 2).

## Discussion

### Reconstruction of consumers' diets

With a potential median contribution of 49%, macrophytes formed the most important food source for the aquatic larvae of the lepidopteran *N. nitidulata* and the ephemeropteran nymph *Baetis* sp. The fact that the rest of the diet of *N. nitidulata* consisted of epiphytic algae was unexpected since these larvae are typically described as oligophagous macrophyte specialists that only feed on a number of host plants (Gaevskaya 1969; Palm 1986). A most likely explanation for this observation could be the ingestion of attached epiphyton during macrophyte consumption. Similarly, yet the other way around, the median potential inclusion of 49% and 31% macrophyte biomass in the diets of *Baetis* sp. and Orthocladiinae, respectively, could very well be caused by accidental ingestion of macrophyte tissue during the grazing of epiphyton. These two taxa are both known to feed on epiphytic algae, whereby *Baetis* nymphs are generally classified in the scraper functional feeding group (cf. Cummins 1973) and Orthocladiinae larvae as gatherers. *Baetis* nymphs hereby mostly feed on algal species that are tightly attached to the macrophytes whereas larvae of many Orthocladiinae species prefer to feed on the more loosely attached algal species in the outer epiphyton layer (Maasri et al. 2010; Tall et al. 2006). The fact that *Baetis* nymphs consume epiphyton closer to the macrophyte surface than Orthocladiinae larvae might result in a higher accidental scraping of macrophyte tissue during grazing, which is also reflected in the animals' diet calculated in this study (Fig. 2). The accidental consumption and incorporation of aquatic macrophyte tissue by gathering and scraping macroinvertebrates is rarely mentioned in studies (but see Yule 1986), yet might be more common than based on the records in the literature alone. Another possible route for the consumption of macrophyte-derived material might be the direct or accidental consumption of senescing macrophyte parts, which are generally colonised by many epiphytic algae and bacteria, are softer and are poorer in inhibitory secondary metabolites (Newman 1991; Suren 1989; Suren and Lake 1989).

Although macrophyte leaf damage was not assessed in this study, other researchers also observed leaf erosion through accidental scraping by



**Fig. 2** Potential contribution ranges of macrophytes to the consumers’ diets. Boxplots show median (line), 25–75 percentile range (box) and 5–95 percentile range (whiskers)

**Table 1** Potential contributions of the different basal resources to the consumers’ diets

	Macrophytes	Epiphyton	Organic matter	Macroinvertebrates	Fish
<i>Asellus aquaticus</i>	0.00–0.28 (0.07)	<b>0.39–0.61 (0.50)</b>	<b>0.18–0.57 (0.42)</b>		
Chironomini	0.00–0.33 (0.07)	<b>0.18–0.79 (0.50)</b>	<b>0.07–0.69 (0.41)</b>		
<i>Pisidium sp.</i>	0.00–0.26 (0.08)	<b>0.35–0.64 (0.50)</b>	<b>0.20–0.57 (0.41)</b>		
<i>Hydrophtila sp.</i>	0.00–0.41 (0.11)	<b>0.17–0.97 (0.59)</b>	<b>0.00–0.57 (0.24)</b>		
<i>Simulium sp.</i>	0.00–0.44 (0.18)	<b>0.13–0.65 (0.40)</b>	<b>0.09–0.65 (0.41)</b>		
<i>Perca fluviatilis</i>	0.00–0.37 (0.19)	0.02–0.37 (0.21)	0.01–0.41 (0.24)	0.00–0.38 (0.19)	0.00–0.34 (0.16)
Orthoclaadiinae	<b>0.09–0.50 (0.31)</b>	<b>0.23–0.86 (0.57)</b>	0.00–0.38 (0.11)		
<i>Orconectus limosus</i>	<b>0.15–0.60 (0.37)</b>	0.05–0.35 (0.20)	0.00–0.27 (0.10)	<b>0.00–0.57 (0.31)</b>	
<i>Gobio gobio</i>	<b>0.08–0.67 (0.37)</b>	0.01–0.36 (0.18)	0.00–0.35 (0.12)	<b>0.00–0.58 (0.30)</b>	
<i>Baetis sp.</i>	<b>0.07–0.66 (0.49)</b>	<b>0.18–0.83 (0.45)</b>	0.00–0.27 (0.07)		
<i>Nymphula nitidulata</i>	<b>0.11–0.80 (0.49)</b>	0.01–0.47 (0.27)	<b>0.00–0.55 (0.26)</b>		

Values are presented as proportions of the total diet (between 0 and 1). Empty cells indicate that a food source was not incorporated in the mixing model. Ranges represent 90% credible intervals (5–95 percentile ranges) with median contribution in parentheses, calculated using the SIAR mixing model. Food web resources with high potential contribution are indicated in bold (95 percentile ≥ 50%)

Ephemeroptera nymphs, whereby Ephemeroptera taxa that consumed more adnate algal taxa caused more erosion than taxa that consumed algae that were farther removed from the leaf surface (Karouna and

Fuller 1992). Furthermore, the larvae of the *Hydrophtila* genus of microcaddisflies, another algivorous taxon, are known to feed on green filamentous algae (e.g. *Cladophora sp.*) which are far removed from

**Table 2** Daily consumption of macrophyte standing biomass by Orthocladinae larvae, *Baetis* sp. nymphs and *N. nitidulata* larvae in the Desselse Nete as calculated from dietary composition data from this study and quantitative distribution data and consumption rates from the literature

	2014		2015			2014		2015	
	<i>S. emersum</i>	<i>P. natans</i>	<i>C. obtusangula</i>	<i>S. emersum</i>	<i>P. natans</i>	<i>C. obtusangula</i>	Total	Total	Total
Macrophyte dry weight (g sample <sup>-1</sup> ) <sup>a</sup>	0.79 ± 0.17	4.27 ± 0.9	3.89 ± 1.14	0.63 ± 0.07	2.96 ± 0.52	3.78 ± 0.55	3.00 ± 0.56	2.60 ± 0.36	2.60 ± 0.36
Orthocladinae larvae sample <sup>-1</sup> <sup>a</sup>	5.59 ± 1.56	3.23 ± 0.56	9.24 ± 2.53	85.48 ± 12.37	193.72 ± 40.51	496.56 ± 94.34	6.02 ± 1.08	258.59 ± 47.56	258.59 ± 47.56
<i>Baetis</i> sp. nymphs sample <sup>-1</sup> <sup>a</sup>	9.00 ± 2.52	31.00 ± 6.96	105.00 ± 30.89	0.33 ± 0.24	11.89 ± 1.98	23.78 ± 7.69	48.33 ± 12.97	12.00 ± 3.16	12.00 ± 3.16
<i>N. nitidulata</i> larvae sample <sup>-1</sup> <sup>a</sup>	2.78 ± 0.81	0	0.89 ± 0.26	0	0	0	1.22 ± 0.36	0	0
Orthocladinae feeding rate (mg dry matter ind <sup>-1</sup> day <sup>-1</sup> ) <sup>b,c</sup>	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
<i>Baetis</i> sp. feeding rate (mg dry matter ind <sup>-1</sup> day <sup>-1</sup> ) <sup>d,e</sup>	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
<i>N. nitidulata</i> feeding rate (mg dry matter ind <sup>-1</sup> day <sup>-1</sup> ) <sup>f</sup>	11.54	11.54	11.54	11.54	11.54	11.54	11.54	11.54	11.54
Biomass consumption Orthocladinae (% macrophyte standing stock consumed day <sup>-1</sup> )	0.01–0.06 (0.04)	0.002–0.01 (0.008)	0.003–0.02 (0.01)	0.24–1.29 (0.84)	0.14–0.75 (0.48)	0.3–1.62 (1.04)	0.006–0.03 (0.02)	0.23–1.22 (0.79)	0.23–1.22 (0.79)
Biomass consumption <i>Baetis</i> sp. (% macrophyte standing stock consumed day <sup>-1</sup> )	0.13–1.19 (0.89)	0.10–0.96 (0.71)	0.27–2.54 (1.89)	0.006–0.06 (0.05)	0.06–0.56 (0.41)	0.08–0.77 (0.57)	0.17–1.57 (1.16)	0.05–0.46 (0.34)	0.05–0.46 (0.34)
Biomass consumption <i>N. nitidulata</i> (% macrophyte standing stock consumed day <sup>-1</sup> )	0.47–3.42 (2.05)	0	0.05–0.38 (0.23)	0	0	0	0.17–1.27 (0.76)	0	0
% Ingestion total	0.69–5.26 (3.35)	0.10–0.97 (0.72)	0.42–3.78 (2.74)	0.32–1.74 (1.13)	0.20–1.31 (0.9)	0.38–2.38 (1.61)	0.39–3.22 (2.19)	0.30–1.81 (1.22)	0.30–1.81 (1.22)
% Ingestion of daily vegetation growth rate <sup>g,h</sup>	13.70–105.29 (67.00)	2.60–24.31 (18.00)	6.43–58.17 (42.15)	6.40–34.82 (22.60)	4.99–32.66 (22.50)	5.91–36.62 (24.77)			

Macrophyte biomass and consumer distribution data are mean ± SE, while calculated potential macrophyte consumption rates are presented as 90% credible intervals (5–95 percentile ranges) with median contribution in parentheses. Data from <sup>a</sup>Wolters et al. (2018), <sup>b</sup>Nolte (1990), <sup>c</sup>Cattaneo and Mousseau (1995), <sup>d</sup>Zelinka (1984), <sup>e</sup>Winterbourn et al. (1985), <sup>f</sup>Monakov (2003), <sup>g</sup>Nielsen et al. (1985) and <sup>h</sup>Madsen et al. (2001)



their macrophyte substrates (e.g. Keiper 1998). It is therefore no surprise that macrophytes did not seem to be part of the diet of *Hydroptila* sp. larvae at all (Fig. 2). This theory of accidental leaf erosion could be tested in future studies by assessing the damage done to macrophyte leaves by different groups of grazing macroinvertebrates, using electron microscopy (cf. Karouna and Fuller 1992).

Other macroinvertebrates that did not include significant potential fractions of macrophytes in their diet were Chironominae larvae and *A. aquaticus* (Fig. 2). These animals are generally considered to be predominantly detritivorous, though macrophyte miners like some representatives of the genus *Endochironomus* exist, and are classified in the gatherer and shredder functional feeding group, respectively (e.g. Moller Pillot 2009; Usseglio-Polatera et al. 2000). It is hereby interesting to note that, while the calculated diet of the Chironominae larvae is in accordance with the literature (e.g. Moller Pillot 2009), the calculated diet of *A. aquaticus* deviates from the assumption that this animal lives from allochthonous and autochthonous detritus colonised by a bacterial/fungal biofilm (e.g. Graca et al. 1994). A possible explanation for this deviation could be that *A. aquaticus* supplements its relatively nitrogen-poor diet with small amounts of alternative food sources which are richer in nitrogen such as epiphytic algae and dead animals, which has previously been shown for a variety of detritivorous invertebrates that mostly feed on nutritionally poor resources such as leaf litter (Anderson 1976; Crenier et al. 2017).

Furthermore, no significant potential contributions of macrophyte tissue were observed in the diet of the filter-feeding invertebrates *Pisidium* sp. and *Simulium* sp. (Fig. 2). This is in accordance with the literature that names bacteria, algae and FPOM as major food sources for these taxa (Monakov 2003; Wallace and Merritt 1980). However, it can be expected that macrophyte-derived detritus might be consumed by these animals, particularly at the end of the growing season when most macrophytes decay and are broken down, as this is also a more nutritious resource than living macrophyte tissue (Newman 1991).

Finally, a significant dietary fraction of macrophyte biomass was calculated in the diet of the omnivorous crayfish *O. limosus* and the fish *G. gobio* (Fig. 2), confirming earlier observations of macrophyte consumption by these taxa (Lodge et al. 1994; Michel and

Oberdorff 1995). Although it can be expected that omnivorous taxa might only consume macrophytes when more nutritious food sources such as animal prey are in short supply (e.g. Dorenbosch and Bakker 2011), some studies have instead demonstrated distinct preferences for either macrophytes or detritus in certain aquatic omnivores (Gherardi et al. 2004; Gherardi and Barbaresi 2007). It is hereby expected that other factors, such as a higher carbon assimilation efficiency of macrophytes, together with their higher availability and easier handling, contribute to this selectivity (e.g. Gherardi et al. 2004). More research, including behavioural studies, is needed in order to clarify the driving forces that determine feeding preferences in freshwater omnivores.

### Conclusions and ecological relevance

Based on stable isotope measurements and mixing model calculations, this study observed the consumption of macrophyte tissue in a number of herbivorous and omnivorous macroinvertebrate and fish taxa in a Belgian lowland stream. Besides the taxa that purposefully consume living macrophytes (e.g. *N. nitidulata* and *O. limosus*), evidence was found for the incorporation of macrophyte-derived carbon and nitrogen in the macroinvertebrate taxa Orthocladinae and *Baetis* sp., which are normally described as algivores feeding on periphyton (Elliott and Humpesch 2010; Moller Pillot 2013). The magnitude and timing of this behaviour is unknown, as this study only reconstructed the food web of a single stream at a single moment. Yet we argue that this study may be the first explicitly demonstrating the assimilation of macrophyte tissues in algivorous macroinvertebrates.

While the destructive effects of (invasive) crayfish and crabs, that actually consume very little of what they destroy, are well known (e.g. Jin et al. 2003; Lodge et al. 1994), the ecological effects of smaller-scale consumption are less well known and can potentially be underestimated. In this study, we demonstrate the potential relevance of macrophytes in the diet of macroinvertebrates, although the scale of this herbivory on the standing macrophyte biomass remains relatively small, with only a few percentage consumed by the dominant macroinvertebrate taxa each day (Table 2). In comparison, the daily consumption of epiphyton standing biomass has been estimated at 50–70% by some authors (Armitage et al.

1995; Kesler 1981). However, when compared to the macrophytes' growth rates, the percentage of macrophytes consumed each day by the macroinvertebrates accounts for 18–105% of the daily primary production (Table 2), meaning that these animals can potentially hamper or even restrict the growth of macrophytes. However, it should be noted that herbivore density in these calculations is based on only two sampling events. This may only provide a limited image of the actual seasonal fluctuations in herbivore population size and makes it easy to miss annual peaks in herbivore abundance, leading to a potential underestimation of macrophyte consumption (Gross et al. 2002; Jacobsen and Sand-Jensen 1992).

Although complete consumption of macrophyte biomass was not observed in this study, the consequences of herbivory by macroinvertebrates and fish on the instream aquatic vegetation can vary in magnitude depending on the prevailing environmental conditions and the nature of the macrophyte consumption. Small-scale consumption of plant tissue can possibly induce vigorous regrowth combined with additional branching and investment in new undamaged shoots (e.g. Belsky et al. 1993; Pieczyńska 2003). On the other hand, damaging of the macrophyte cuticle and epidermis might expose the plant to bacterial and fungal infections or toxic compounds, which can lower the overall fitness of the vegetation (Suren 1989). The negative effects of macrophyte consumption on standing plant biomass are likely to be more pronounced under prevailing ambient conditions that already have a negative influence on macrophyte growth, such as a turbid water layer and high epiphyton cover. Under these conditions, even small amounts of herbivory can lead to a significant decline in underwater vegetation (Hidding et al. 2016). More research may be needed to quantitatively assess the role of small-scale herbivory on the development and functioning of the aquatic macrophyte community.

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