PRIMARY RESEARCH PAPER

Temporal changes in essential fatty acid availability in different food sources in the littoral macrophyte zone

Hélène Masclaux · Gilles Bourdier ·
Lionel Jouve · Emilie Duffaud · Alexandre Bec

Abstract In littoral environments, different food resources are available for zooplankters. In addition to seston, species may feed on biofilms growing on sediments, plants (epiphyton), and at the air–water interface (neuston). However, despite a growing interest in these different biofilms, little is known about their food quality for microcrustaceans. In a field study, we measured changes in the food quality over time in terms of the essential fatty acid (EFA) content of different potential food sources for littoral consumers. The food quality of seston, neuston, and epiphyton growing on three different aquatic macrophytes were assessed. Our results showed that there is an important seasonal variability within each food source. However, in the system studied, epiphytic biofilms, especially those of *Ludwigia* and *Callitriche* offered the highest food quality, in terms of EFA content, throughout the year. As the highest EFA concentrations in each food source were found consecutively, high concentrations of these physiologically important compounds are maintained in the system throughout the year. Therefore, greater diversity of food resources could affect ecosystem productivity.

Keywords Food quality · Polyunsaturated fatty acids · Seston · Epiphyton · Neuston

Handling editor: Katya E. Kovalenko

H. Masclaux (✉) · G. Bourdier · E. Duffaud · A. Bec
LMGE, Clermont Université, Université Blaise Pascal,
BP 10448, 63000 Clermont-Ferrand, France
e-mail: helenemasclaux@yahoo.fr

H. Masclaux · G. Bourdier · E. Duffaud · A. Bec
CNRS, UMR 6023, LMGE, 63173 Aubiere, France

L. Jouve
Institut Universitaire et Technologique (IUT) Mesures
Physiques, 24 avenue des Landais, 63172 Aubière, France

Introduction

The factors that regulate energy transfer efficiency between primary producers and consumers have been largely documented and it is now accepted that differences in this efficiency can be attributed to the variability of food quality (Ahlgren et al., 1990; Brett & Müller-Navarra, 1997). Among the factors determining food quality, concentrations of some polyunsaturated fatty acids (PUFA) have been highlighted to be one of the most important in aquatic ecosystems (Müller-Navarra, 1995; Wacker & von Elert, 2001). In a field study, Müller-Navarra et al. (2000) showed a positive correlation between zooplankton growth and the concentration of some PUFA, especially the concentration of eicosapentaenoic acid (EPA,

20:5ω3) in seston (i.e., suspended particles). The importance of PUFA for zooplankton development have been confirmed in several field studies (Wacker & von Elert, 2001; Müller-Navarra et al., 2004; Gladyshev et al., 2008). However, almost all the studies thus far dealing with food quality and its seasonal variations for microcrustaceans in natural environments have concentrated on sestonic food sources only (Müller-Solger et al., 2002; Maazouzi et al., 2008).

Littoral macrophyte zones harbor 75% of known microcrustacean species (Walseng et al., 2006), and this species richness leads to trophic niche separation (Chesson, 2000; Levine & HilleRisLambers, 2009). In littoral macrophyte zones, different foraging strategies therefore coexist. Some zooplankton species have morphological adaptations allowing them to exploit different resources. The modified ventral rim of the valve in the genus *Scapholeberis* allows them to feed at the underside of the surface biofilm of the water, i.e., the neuston (Dumont & Pensaert, 1983; Masclaux et al., 2013). Some chydorid cladocerans, including *Eurycercus lamellatus*, are considered to be scrapers and feed mostly on periphyton (Masclaux et al., 2012a, 2014). Moreover, while the *Eurycercus* genus appears to have a specialized diet, others are able to feed intermittently on different biofilms. For example, *Chydorus* can feed on either neuston (Masclaux et al., 2013) or periphyton (van de Bund et al., 1994). Therefore, some microcrustaceans exploit the seston in littoral environments, while other species feed on biofilms growing on sediments and plants (i.e., periphyton), or at the air–water interface (i.e., neuston).

Few studies have assessed the taxonomic composition of the neuston (Burchardt & Marshall, 2003; Hortnagl et al., 2010), despite its presence in lentic systems for most of the year. On the contrary periphyton (including benthic and epiphytic biofilms) has received growing attention in the last decade. Some studies have attempted to describe periphyton taxonomic composition (Leland et al., 1986; Vymazal & Richardson, 1995) as well as its quantitative importance for the overall primary production (Vadeboncoeur et al., 2003; Liboriussen & Jeppesen, 2009). Recently several studies have highlighted that periphytic production can be an important food source for invertebrates (Herwig et al., 2004; Rautio & Warwick, 2006), especially for microcrustaceans (Cazzanelli

et al., 2012; Masclaux et al., 2012a). Despite a growing interest in these different biofilms there have only been a few studies describing their food quality (Hill et al., 2011; Mariash et al., 2011).

We hypothesize that the different food sources available for microcrustaceans in a littoral macrophyte zone differ in terms of food quality, particularly in terms of essential fatty acid (EFA) content. These differences are important for understanding ecosystem functioning as the food quality of resources can determine secondary production. In littoral macrophyte zones, the diversity of microcrustacean feeding strategies may imply a link between the temporal changes of EFA availability in the different food sources and the seasonal dynamic of microcrustacean species. In this field study, we assessed the temporal changes of the food quality, in terms of PUFA composition, of five potential food sources for microcrustaceans of a freshwater littoral zone. The five potential food sources included seston, neuston, and epiphyton growing on three different macrophyte species.

Materials and methods

Study site

The study was carried out from March to November 2008. Field work began when aquatic macrophytes started their development in late winter, and ended when most of the plants started to senesce. All samples were obtained from a backwater (3°28′E, 46°01′N) of the river Allier, in France. The backwater was composed of a deeper basin in the center (maximal depth 3 m), surrounded by a shallow littoral macrophytes zone (mean depth 0.8 m). The backwater is connected to the riverbed all along the year via a single, narrow (mean width 10 m), and shallow (mean depth 0.8 m) channel. The channel and the shallow zone of the main basin were covered by three dominant aquatic macrophytes: *Callitriche* sp., *Elodea canadensis*, and *Ludwigia granddiflora*, which occupied around 30% of the total area of the backwater.

Sample collection

Seston was collected on 12 sampling dates from March to November 2008. Neuston was collected at 11

sampling dates from May to November 2008. Horizontal integrated samples of seston were done by collecting 40 l of water at 50 cm water depth (euphotic zone) using a remote controlled catamaran equipped with a pump (3 × 30 m transects). Horizontal sampling of neuston was done by collecting 40 l of water at the air–water interface (surface layer ≈ 2 mm) with a modified oil sampler according to Schomaker (Agogué et al., 2004). Seston and neuston were collected in the area not covered by macrophytes. To collect epiphyton, the three dominant aquatic macrophytes in the backwater were picked by hand at different spots and put in different stomacher bags containing filtered water (<0.2 μm, see below). Epiphytic biofilms of *Callitriche* and *Ludwigia* were collected at six sampling dates from March to November 2008, whereas epiphytic biofilm of *Elodea* was collected at four sampling dates from July to November 2008. All samples were stored on ice, transported to the laboratory, and processed immediately.

Sample processing

In the laboratory, stomacher bags containing the different macrophyte species were placed in a Stomacher (Bagmixer 400, Interscience, France). The bags were vigorously pounded on their outer surface by metal paddles to remove microbial community attached on plant stems (see Bowker et al. (1986) for a complete description of the method). The stomacher was operated at normal speed (230 rpm) for 3 min. The solutions containing the epiphyton removed from the three macrophyte species with the Stomacher, as well as seston and neuston were then filtered through a Nitex mesh (50 μm) to keep the <50 μm fraction of particulate organic matter (POM), i.e., the optimal size range of particles ingested by cladocerans (Burns 1968). For each food source, this POM fraction was then filtered onto pre-combusted GF/F-filters (Whatman[TM]) immediately after sampling for subsequent lipid analyses. All samples were stored at −80°C until analysis.

Fatty acid analysis

Lipids were extracted in triplicate for seston and neuston, and in duplicate for epiphyton of each macrophyte species, using chloroform: methanol,

Table 1 Abbreviations used for essential fatty acids or groups of fatty acids

Full name	Structure	Abbreviation
Linoleic acid	18:2ω6	LIN
α-Linolenic acid	18:3ω3	ALA
Arachidonic acid	20:4ω6	ARA
Eicosapentaenoic acid	20:5ω3	EPA
Docosahexaenoic acid	22:6ω3	DHA
ω3-Polyunsaturated fatty acids	–	ω3-PUFA
ω6-Polyunsaturated fatty acids	–	ω6-PUFA

following the method of Folch et al. (1957). Fatty acid (FA) from total lipid extracts were converted into FA methyl-esters (FAME) after the addition of non-methylated 13:0 and 23:0 as internal standards. FAME were generated by acid catalyzed trans-esterification according to a modified protocol of Christie (1982) (4% H_2SO_4 in methanol at 75°C for 2 h). FAME were analyzed on an Agilent technologies 6850 gas chromatograph equipped with a DB-Wax column (J&W Scientific), and a flame-ionization detector (FID; 250°C; split injection; carrier gas: helium; oven temperature ramp 150–240°C at 3°C min^{-1}). FAME were identified by comparing retention times with those obtained from Supelco® standards (37-Component FAME mix, Bacterial FAME mix) and quantified using internal standards (13:0 and 23:0). The absolute amount of FAME was normalized in terms of the independently determined POC content of the samples. POC was measured using an elemental analyzer (EA 1110, CE Instruments, Milan, Italy). Abbreviations used for EFAs are presented in Table 1.

Data analysis

Differences in EFA concentrations between the different food sources were assessed by repeated measures ANOVA for six sampling dates for which data on seston, neuston, and epiphyton were available (i.e., 19 March, 13 May, 1 July, 29 July, 9 October, and 20 November). Repeated measures ANOVA allowed us to take into account the effect of repeated measurements on the same food source over time, and of the interaction between time and food sources on the concentrations of EFA. Pair-wise comparisons were performed using a post-hoc test (Tukey's HSD) with the Bonferroni adjustment ($\alpha = 0.005$). All
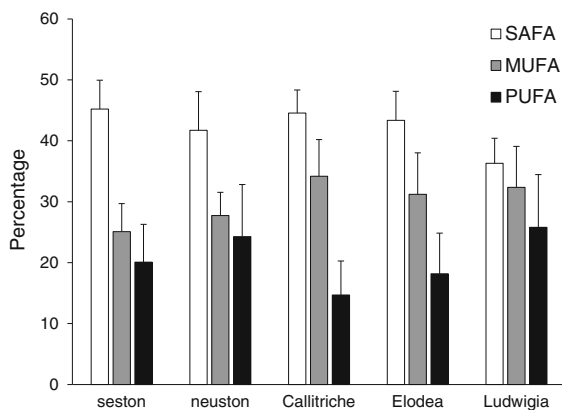
Fig. 1 Percentages (annual mean ± SD) of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in seston, neuston, and epiphyton growing on *Callitriche*, *Elodea*, and *Ludwigia* of a littoral macrophyte zone

calculations were performed using the XLStat-Pro 7.5 (Addinsoft).

Results

The relative proportions of saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA), and PUFA varied depending on the food sources considered (Fig. 1, see supplementary material for detailed compositions of FA in the food sources). However, on an annual basis, the average concentrations of PUFA were higher in the epiphyton (29.5 ± 22.5, 22.4 ± 9.7, and 49.9 ± 19.1 μg of PUFA/mg C for *Callitriche*, *Elodea* and *Ludwigia*, respectively) than for seston (4.3 ± 1.90 μg of PUFA/mg C) and neuston (6.6 ± 4.2 μg of PUFA/mg C).

The repeated measures ANOVA showed significant interaction effects between food sources and time, which can be explained by the non-independence of samples on a same food source over time (Table 2). The concentrations of physiologically important FA, or groups of FA, in the five diets studied showed that the concentrations of EFA were significantly different between the five diets (Table 2), with higher concentrations often found in epiphyton throughout the sampling season (Fig. 2). However, a high temporal variability was recorded (Fig. 3, Table 2). For instance, the concentration of EPA in neuston is 100 times higher in early July compared to early June. For

the epiphyton of *Callitriche* and *Ludwigia*, the highest concentrations of LIN and ALA were recorded in spring and early summer. At that time, values reached 18.8 μg of LIN/mg C and 32.5 μg of ALA/mg C in the epiphyton of *Callitriche*, and 18.5 μg of LIN/mg C and 18.5 μg of ALA/mg C in the epiphyton of *Ludwigia*. On the contrary, the highest concentrations of ARA and EPA were measured in the fall for the three kinds of epiphyton. EPA concentrations in epiphyton ranged from 1-3 μg/mg C up to 10.4, 9.5, and 19.7 μg/mg C for *Callitriche*, *Elodea*, and *Ludwigia*, respectively. The highest concentrations of DHA were also recorded in fall in seston and in epiphyton of *Ludwigia* and *Elodea* but this value never exceeded 2 μg of DHA/mg C (Fig. 3). Finally, if the concentration of EFA remained low in seston and neuston most of the year, increases of ALA, EPA, and ω3-PUFA concentrations were measured in neuston in summer (Fig. 3). In this food source, ALA concentration reached 4.5 μg/mg C in June, and EPA and ω3-PUFA reached 4.4 μg/mg C and 10.7 μg/mg C in July, respectively. Interestingly, the highest concentrations of LIN, ω3- and ω6-PUFA, and to a lesser degree of ALA were not always recorded in the same food source of the backwater, depending on the sampling date.

Discussion

LIN (18:2ω6) and ALA (18:3ω3) are the two FA considered to be strictly essential, as most animal including zooplankton species are not able to synthesis them de novo. Some aquatic consumers are then able to convert LIN to ARA (20:4ω6), and ALA to EPA (20:5ω3) and DHA (22:6ω3), probably through the successive use of Δ6 and Δ5 desaturases and elongases (Bec et al., 2003; Masclaux et al., 2012b). However, the rates of these bioconversion processes are too low to meet the physiological requirements of consumers, and these PUFA must also be supplied in the diet (Sargent et al., 1999; Arts et al., 2001). ARA, EPA, and DHA are therefore also grouped frequently as EFA. The sources of EFA in freshwater systems are an important issue to resolve as they play key roles in many physiological processes in microcrustacean and fish (Parrish, 2009), especially ARA, EPA, and DHA which are the most physiologically active ones. ARA and EPA are precursors of hormones, the

Table 2 Results of the repeated measures ANOVA testing the effects of diet, time, and of the interactions between time and diet on the concentrations of EFA

	LIN	ALA	ARA	EPA	DHA	ω3-PUFA	ω6-PUFA
Diet	$F_{(4,7)} = 598.5$	$F_{(4,7)} = 476.6$	$F_{(4,7)} = 442.8$	$F_{(4,7)} = 257.4$	$F_{(4,7)} = 154.6$	$F_{(4,7)} = 215.3$	$F_{(4,7)} = 199.7$
	MS = 204.8	MS = 278.1	MS = 16.5	MS = 163.9	MS = 1.0	MS = 1739.4	MS = 526.7
	P < 0.0001	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**
Time	$F_{(5,35)} = 232.0$	$F_{(5,35)} = 215.8$	$F_{(5,35)} = 136.2$	$F_{(5,35)} = 117.0$	$F_{(5,35)} = 53.9$	$F_{(5,35)} = 41.4$	$F_{(5,35)} = 55.9$
	MS = 42.7	MS = 101.1	MS = 4.2	MS = 69.3	MS = 0.6	MS = 340.7	MS = 63.4
	P < 0.0001	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**
Diet × time	$F_{(20,35)} = 180.9$	$F_{(20,35)} = 160.9$	$F_{(20,35)} = 41.9$	$F_{(20,35)} = 29.3$	$F_{(20,35)} = 20.7$	$F_{(20,35)} = 22.0$	$F_{(20,35)} = 44.4$
	MS = 33.3	MS = 75.4	MS = 1.3	MS = 17.4	MS = 0.2	MS = 181.2	MS = 50.5
	P < 0.0001	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**	**P < 0.0001**

P value in bold indicates a significant effect

eicosanoïdes, involved in inflammation, immunity, energy allocation, mineral balance, and reproduction (Schmitz & Ecker, 2008; Parrish, 2009). DHA have been shown to be associated with neural tissue and eye structure (Arts et al., 2001). More generally, ω3- and ω6-PUFA are thought to be physiologically important, especially for maintaining membrane fluidity at low temperatures (Farkas et al., 1984; Schlechtriem et al., 2006). Several studies have consequently shown that the availability of dietary EFA improve the somatic growth, reproduction, and survival of many aquatic consumers (Copeman et al., 2002; Ravet et al., 2003).

In this study, we showed that, whatever the food source, there was an important seasonal variability in the EFA concentrations, with different patterns from on food source to another. EFA concentrations varied sometimes by a factor of 100 in the same food source (e.g., neuston). The seston is the most studied food source when dealing with food quality for microcrustaceans (Müller-Navarra et al., 2004; Gladyshev et al., 2008). However, in the backwater studied here, the seston exhibited the lowest food quality for microcrustaceans according to its EFA concentrations. It is interesting to note that, almost all along the year, sestonic EPA concentrations were lower than the saturation threshold of 1.3 ± 0.3 μg EPA (mg C)$^{-1}$ established by Ravet et al. (2012). EPA is one of the most important PUFA for cladoceran's development (Bec et al., 2006; Masclaux et al., 2009; Sperfeld & Wacker, 2012). Ravet et al. (2012) suggested, based on supplementation experiments, that *Daphnia* growth and reproduction demands for EPA will be almost entirely met when their diets reach this value. Our results are not surprising as sestonic carbon comes from multiple origins. Eukaryotic microorganisms, which are the major potential sources of PUFA (Brett & Müller-Navarra, 1997; Desvilettes & Bec, 2009), can be mixed in with allochthonous or littoral detritus (Delong & Thorp, 2006), rich in recalcitrant components such as lignin and cellulose. This important detrital component, and the high bacterial biomass associated with it, can thus explain the low sestonic PUFA content.

The importance of neuston in terms of organic matter quantity varied throughout the year and its EFA concentrations were relatively low most of the times in the system studied. Nevertheless, in summer, ω3-PUFA concentrations increased greatly in neuston. These increasing concentrations may reflect a
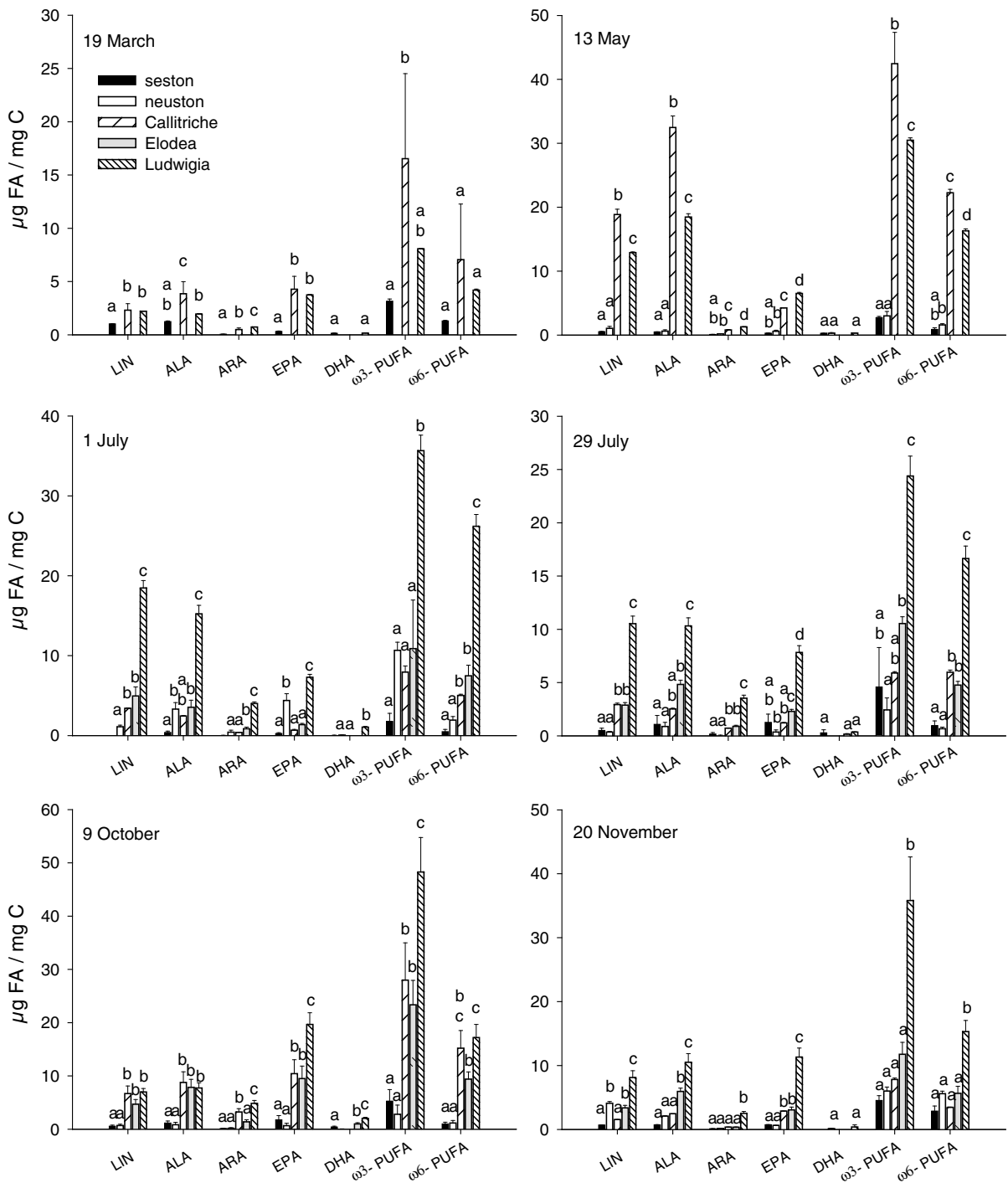
Fig. 2 Essential fatty acid concentrations (mean ± SD) in seston, neuston, and epiphyton growing on *Callitriche*, *Elodea*, and *Ludwigia* of a littoral macrophyte zone. *Distinct letters* for the comparisons of each fatty acid, or group of fatty acids, indicate a significant difference between diets (ANOVA with a Tukey's HSD tests, α = 0.005 after the Bonferroni adjustment). *LIN* linoleic acid (18:2ω6), *ALA* α-linolenic acid (18:3ω3), *ARA* arachidonic acid (20:4ω6), *EPA* eicosapentaenoic acid (20:5ω3), *DHA* docosahexaenoic acid (22:6ω3), *ω3- and ω6-PUFA* ω3- and ω6-polyunsaturated fatty acids

Fig. 3 Temporal changes in the concentrations (mean ± SD) of physiologically important fatty acids in **a** seston, **b** neuston, and **c** epiphyton growing on the three dominant macrophyte species (*Callitriche*, *Elodea*, and *Ludwigia*). Note the differences in scale; for direct comparisons across food sources see Fig. 2
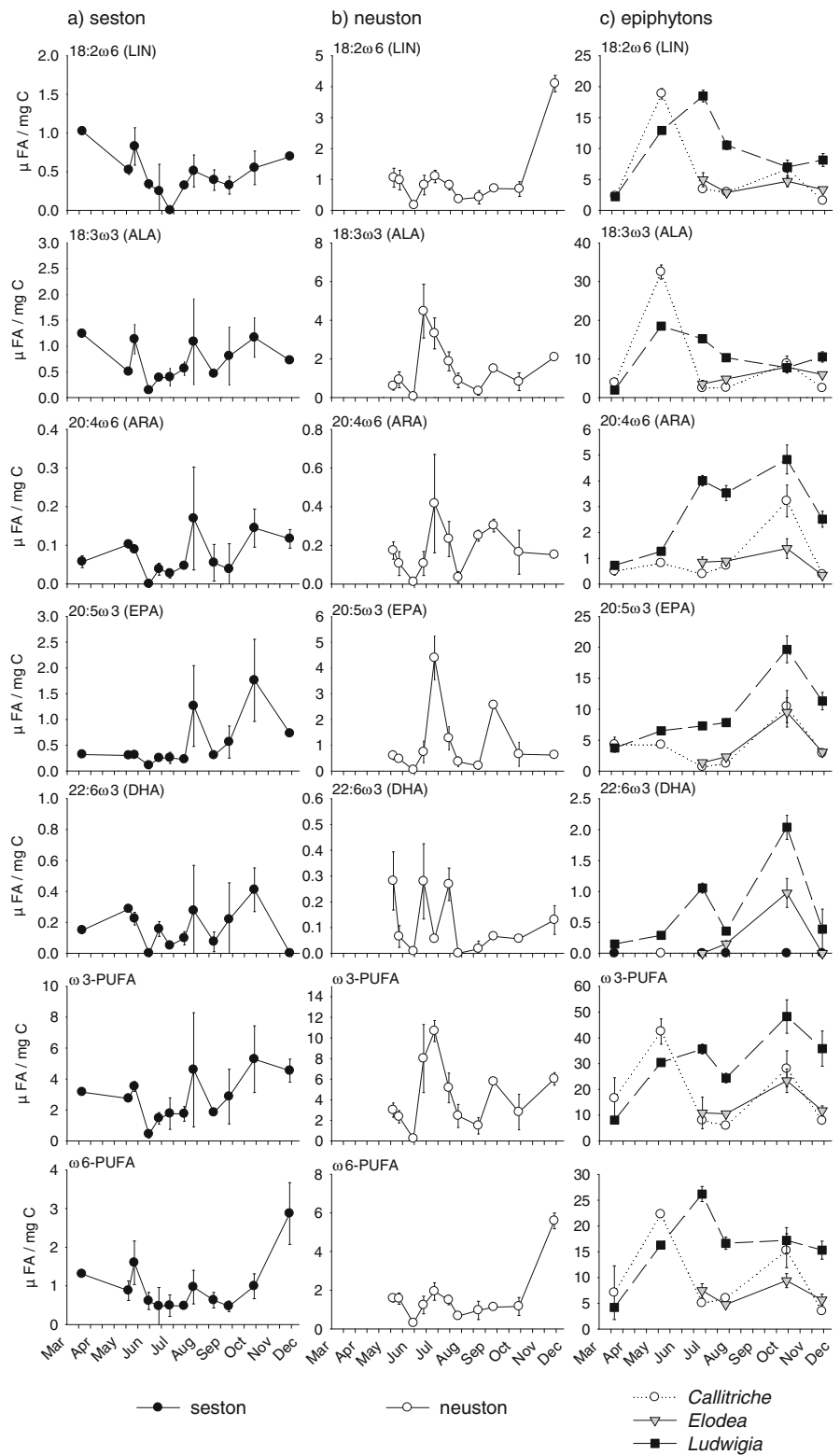
proliferation of eukaryotic microorganisms such as microalgae at the air–water interface (Desvilettes & Bec, 2009; Bec et al., 2010). The air–water interface has been poorly documented, especially in freshwater environments. However, a recent field study showed that it can be exploited by some species, including *Scapholeberis mucronata*. *Scapholeberis mucronata* benefits from food high in both quantity and quality in neuston during episodic deposition of pine pollen (Masclaux et al., 2013). The results of our study corroborate the proposition that neuston could sometimes provide such species with a PUFA-rich resource, which exceed the minimum threshold value of 1.3 ± 0.3 µg EPA (mg C)$^{-1}$ reported by Ravet et al. (2012). These results reinforce the idea that further studies are needed on the nutritional importance of neuston for aquatic organisms.

Finally, FA quantification showed that throughout the sampling season epiphytic biofilms, and those of *Ludwigia* and *Callitriche* in particular, offered the highest concentrations of physiologically important FA. Except for the epiphyton growing on *Callitriche* on 1 and 28 July, the concentrations of EPA in the different epiphyton were always higher than the limiting value of 1.3 ± 0.3 µg EPA (mg C)$^{-1}$ (Ravet et al., 2012). Biofilms are complex assemblages of microorganisms which can be composed of an important bacterial and detrital component when they develop at the sediment–water interface. Epiphytic biofilms are distinguished from benthic biofilms as they are richer in microalgae and are often dominated by PUFA-rich diatoms (Ahlgren et al., 1990; Karosienė & Kasperovičienė, 2008). Periphyton has received more attention in the last decade. Several studies used isotope tracers to show that periphytic production can be an important resource for invertebrates (Rautio & Warwick, 2006; Cazzanelli et al., 2012). Periphyton was mostly quantified as an important part of primary production in aquatic systems (Vadeboncoeur et al., 2003), but very few studies assessed its food quality (Hill et al., 2011; Mariash et al., 2011). Our results showed that periphyton, and more specifically epiphyton, is not only important for consumers with regards to its quantity, as suggested by the previous studies (Rautio & Warwick, 2006; Cazzanelli et al., 2012), but quality in terms of EFA content is also important. Differences in EFA composition of the three kinds of epiphyton had nevertheless been measured in our study. The epiphyton of

Ludwigia showed the highest concentrations of ARA, EPA, and DHA throughout the year. While the epiphyton of *Callitriche* reached the highest concentrations of ω3- and ω6-PUFA in spring, the highest concentrations of ω3- and ω6-PUFA in the epiphyton of *Ludwigia* were reached in fall and summer, respectively. These differences could have several explanations. First, epiphyton occurrence in aquatic systems is in part determined by availability of substrates. The three macrophytes species considered here had phenological differences. *Callitriche* is the first to develop in spring, followed by *Ludwigia* and *Elodea*. These phenological differences could be accompanied by differences in the development stages of epiphytic biofilm which may explain in part the differences in FA composition. The different compositions of epiphyton could reflect physical differences in the substrate. Compared to *Callitriche* and *Elodea*, *Ludwigia* had very thick and solid stems which could provide better support for epiphyte development, especially from July when the species reached their maximal development. Finally, we cannot exclude that differences in epiphyton composition could be due to allelopathic processes, as for rooted submersed macrophytes allelopathy might be an effective strategy to prevent light limitation due to shading by phytoplankton or epiphytes.

We think that the differences of EFA concentrations in the different food sources have important implications for ecosystem functioning. The overall amount of carbon was probably higher in seston and, therefore, there was more sestonic EFA in the system studied. However, most of the cladoceran species, which represent one of the major links between microorganisms and higher trophic levels, are non-selective feeders. The presence of detritus and poor quality particles in seston thus affect their somatic growth and reproduction rates. For these species, the lower food quality of seston is probably not compensated by its quantity. Furthermore, previous studies have showed that some cladoceran species do not exploit seston. Chydorid cladocerans, and among them *Eurycercus lamellatus*, mostly feed on periphyton (Masclaux et al., 2012a, 2014), while *S. mucronata* feed on neuston (Dumont & Pensaert, 1983; Masclaux et al., 2013). In ecosystems where the different food sources are available, these species may thus benefit from feeding on EFA-rich food sources compared to species feeding on seston. They may represent the main EFA pathway

to higher trophic levels. Some consumers may more-over be opportunistic and exploit different food sources. They would then rely mainly on seston for their carbon input, but their source of EFA could be partially or totally epiphytic or neustonic, if seston cannot provide them with sufficient concentrations of these essential compounds. Such a decoupling between the sources of carbon and EFA has indeed already been documented for fish in a Mediterranean lagoon (Koussoroplis et al., 2010). The authors showed that although the production of settled fish was mostly supported by benthic dietary sources, the EFA DHA had a planktonic origin. Finally, we showed that depending on the food source considered, the highest EFA concentrations were not recorded at the same time of the year. The maintenance of macrophyte and food resource diversity thus enabled the maintenance of high concentrations of EFA throughout the year in the system. This EFA dynamics in the different food sources could also explain, at least in part, the microcrustacean species seasonal changes. Interest-ingly, the highest concentration of *Chydorideae*, specialized in the exploitation of periphyton, and the appearance of *S. mucronata*, specialized in the exploi-tation of neuston, were recorded in spring and fall, for the former, and in July for the latter (data not shown). This corresponds with when their respective food sources exhibited high concentrations of ω3- and ω6-PUFA. This link between the temporal changes of EFA availability in the different food sources, and the seasonal dynamics of microcrustacean species, needs further investigation.

Conclusion

Our study is the first to simultaneously assess the temporal changes of the food quality of three zooplankton food sources available in a freshwater system: seston, neuston, and epiphyton. An important seasonal variability was highlighted within each food source. We showed that seston exhibited the poorest concentration of EFAs in the system studied, probably because of its important detrital component. On the contrary, epiphyton showed the highest concentrations of EFA throughout the year. One of the original aims of our study was to consider epiphyton growing on three different aquatic macrophytes. We were able to show that there were some differences, in terms of FA

concentrations and thus in terms of food quality, between the different epiphytic biofilms, depending on the macrophytes on which they develop. Finally, we showed that neuston, which has been largely ignored until now, may sometimes be a high quality food resource in terms of EFA content for species able to exploit it. The diversity of food resources available for microcrustaceans and others consumers enabled the maintenance of high concentrations of physiologically important FA at the base of the food web throughout the year. Further studies have to be carried out on the influence of resources and essential compound input diversity on secondary production. This diversity, associated with consumer diversity, could allow higher efficiency of EFA transfers to higher trophic levels and therefore, a greater secondary productivity in the system.

References

Agogué, H., E. O. Casamayor, F. Joux, I. Obernosterer, C. Dupuy, F. Lantoine, P. Catala, M. G. Weinbauer, T. Reinthaler, G. J. Herndl & P. Lebaron, 2004. Comparison of samplers for the biological characterization of the sea surface microlayer. Limnology and Oceanography: Meth-ods 2: 213–225.

Ahlgren, G., L. Lundstedt, M. T. Brett & C. Forsberg, 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. Journal of Plankton Research 12: 809–818.

Arts, M. T., R. G. Ackman & B. J. Holub, 2001. "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. Canadian Journal of Fisheries and Aquatic Sciences 58: 122–137.

Bec, A., C. Desvilettes, A. Vera, D. Fontvieille & G. Bourdier, 2003. Nutritional value of different food sources for the benthic *Daphnidae Simocephaius vetulus*: role of fatty acids. Archiv fur Hydrobiologie 156: 145–163.

Bec, A., D. Martin-Creuzburg & E. von Elert, 2006. Trophic upgrading of autotrophic picoplankton by the heterotrophic nanoflagellate *Paraphysomonas* sp. Limnology and Oceanography 51: 1699–1707.

Bec, A., M. E. Perga, C. Desvilettes & G. Bourdier, 2010. How well can the fatty acid content of lake seston be predicted from its taxonomic composition? Freshwater Biology 55: 1958–1972.

Bowker, D. W., W. van Teutem & J. C. Fry, 1986. A note on 'stomaching' for the quantitative sampling of epiphyton. Freshwater Biology 16: 123–125.

Brett, M. T. & D. C. Müller-Navarra, 1997. The role of highly unsaturated fatty acids in aquatic food web processes. Freshwater Biology 38: 483–499.

Burchardt, L. & H. G. Marshall, 2003. Algal composition and abundance in the neuston surface micro layer from a lake and pond in Virginia (U.S.A.). Journal of Limnology 62: 139–142.

Burns, C. W., 1968. Relationship between body size of filter-feeding Cladocera and maximum size of particle ingested. Limnology and Oceanography 13: 675–678.

Cazzanelli, M., L. Forsstrom, M. Rautio, A. Michelsen & K. S. Christoffersen, 2012. Benthic resources are the key to *Daphnia middendorffiana* survival in a high arctic pond. Freshwater Biology 57: 541–551.

Chesson, P., 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31: 343–358.

Christie, W. W., 1982. Lipid Analyses, 2nd ed. Pergamon, Oxford.

Copeman, L. A., C. C. Parrish, J. A. Brown & M. Harel, 2002. Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. Aquaculture 210: 285–304.

Delong, M. D. & J. H. Thorp, 2006. Significance of instream autotrophs in trophic dynamics of the Upper Mississippi River. Oecologia 147: 76–85.

Desvilettes, C. & A. Bec, 2009. Formation and transfer of fatty acids in aquatic microbial food webs: role of heterotrophic protists. In Arts, M. T., M. T. Brett & M. J. Kainz (eds.), Lipids in Freshwater Ecosystems. Springer, New York: 25–42.

Dumont, H. J. & J. Pensaert, 1983. A revision of the *Scapholeberinae* (Crustacea Cladocera). Hydrobiologia 100: 3–45.

Farkas, T., G. Y. Nemecz & I. Csengeri, 1984. Differential response of lipid metabolism and membrane physical state by an actively and passively overwintering planktonic crustacean. Lipids 19: 436–442.

Folch, J., M. Less & G. Stanley, 1957. A simple method for the isolation of and purification of total fatty acids from an animal tissues. The Journal of Biological Chemistry 226: 497–509.

Gladyshev, M. I., N. N. Sushchik, O. P. Dubovskaya, O. N. Makhutova & G. S. Kalachova, 2008. Growth rate of *Daphnia* feeding on seston in a Siberian reservoir: the role of essential fatty acid. Aquatic Ecology 42: 617–627.

Herwig, B. R., D. A. Soluk, J. M. Dettmers & D. H. Wahl, 2004. Trophic structure and energy flow in backwater lakes of two large floodplain rivers assessed using stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences 61: 12–22.

Hill, W. R., J. Rinchard & S. Czesny, 2011. Light, nutrients and the fatty acid composition of stream periphyton. Freshwater Biology 56: 1825–1836.

Hortnagl, P., M. T. Perez, M. Zeder & R. Sommaruga, 2010. The bacterial community composition of the surface microlayer in a high mountain lake. FEMS Microbiology Ecology 73: 458–467.

Karosienė, J. & J. Kasperovičienė, 2008. Seasonal succession of epiphyton algal communities on *Phragmites australis* (Cav.) Trin. ex Stend. in a mesoeutrophic lake. Ekologija 54: 32–39.

Koussoroplis, A. M., A. Bec, M. E. Perga, E. Koutrakis, C. Desvilettes & G. Bourdier, 2010. Nutritional importance of minor dietary sources for leaping grey mullet *Liza saliens* (Mugilidae) during settlement: insights from fatty acid delta C-13 analysis. Marine Ecology Progress Series 404: 207–217.

Leland, H. V., V. F. Steven, L. C. James & A. D. Mahood, 1986. Composition and abundance of periphyton and aquatic insects in a sierra navada stream. Great Basin Naturalist 46: 595–611.

Levine, J. M. & J. HilleRisLambers, 2009. The importance of niches for the maintenance of species diversity. Nature 461: 254–258.

Liboriussen, L. & E. Jeppesen, 2009. Periphyton biomass, potential production and respiration in a shallow lake during winter and spring. Hydrobiologia 632: 201–210.

Maazouzi, C., G. Masson, M. S. Izquierdo & J. C. Pihan, 2008. Midsummer heat wave effects on lacustrine plankton: variation of assemblage structure and fatty acid composition. Journal of Thermal Biology 33: 287–296.

Mariash, H., M. Cazzanelli, M. J. Kainz & M. Rautio, 2011. Food sources and lipid retention of zooplankton in subarctic ponds. Freshwater Biology 56: 1850–1862.

Masclaux, H., A. Bec, M. J. Kainz, C. Desvilettes, L. Jouve & G. Bourdier, 2009. Combined effects of food quality and temperature on somatic growth and reproduction of two freshwater cladocerans. Limnology and Oceanography 54: 1323–1332.

Masclaux, H., A. Bec & G. Bourdier, 2012a. Trophic partitioning among three littoral microcrustaceans: relative importance of periphyton as food resource. Journal of Limnology 71: 30–35.

Masclaux, H., A. Bec, M. J. Kainz, F. Perrière, C. Desvilettes & G. Bourdier, 2012b. Accumulation of polyunsaturated fatty acids by cladocerans: effects of taxonomy, temperature and food. Freshwater Biology 57: 696–703.

Masclaux, H., M. E. Perga, M. Kagami, C. Desvilettes, G. Bourdier & A. Bec, 2013. How pollen organic matter enters freshwater food webs. Limnology and Oceanography 58: 1185–1195.

Masclaux, H., G. Bourdier, P. Riera, M. J. Kainz, L. Jouve, E. Duffaud & A. Bec, 2014. Resource partitioning among cladocerans in a littoral macrophyte zone: implications for the transfer of essential compounds. Aquatic Sciences 76: 73–81.

Müller-Navarra, D. C., 1995. Biochemical versus mineral limitation in *Daphnia*. Limnology and Oceanography 40: 1209–1214.

Müller-Navarra, D. C., M. T. Brett, A. M. Liston & C. R. Goldman, 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403: 74–77.

Müller-Navarra, D. C., M. T. Brett, S. Park, S. Chandra, A. P. Ballantyne, E. Zorita & C. R. Goldman, 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427: 69–72.

Müller-Solger, A. B., A. D. Jassby & D. Müller-Navarra, 2002. Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento–San Joaquin River Delta). Limnology and Oceanography 47: 1468–1476.

Parrish, C., 2009. Essential fatty acids in aquatic food webs. In Arts, M. T., M. T. Brett & M. J. Kainz (eds.), Lipids in Aquatic Ecosystems. Springer, New York: 309–326.

Rautio, M. & F. V. Warwick, 2006. Benthic and pelagic food resources for zooplankton in shallow high-latitude lakes and ponds. Freshwater Biology 51: 1038–1052.

Ravet, J. L., M. T. Brett & D. C. Müller-Navarra, 2003. A test of the role of polyunsaturated fatty acids in phytoplankton food quality for *Daphnia* using liposome supplementation. Limnology and Oceanography 48: 1938–1947.

Ravet, J. L., J. Persson & M. T. Brett, 2012. Threshold dietary polyunsaturated fatty acid concentrations for *Daphnia pulex* growth and reproduction. Inland Waters 2: 199–209.

Sargent, J., G. Bell, L. McEvoy, D. Tocher & A. Estevez, 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177: 191–199.

Schlechtriem, C., M. T. Arts & I. D. Zellmer, 2006. Effect of temperature on the fatty acid composition and temporal trajectories of fatty acids in fasting *Daphnia pulex* (crustacea, cladocera). Lipids 41: 397–400.

Schmitz, G. & J. Ecker, 2008. The opposing effects of *n*-3 and *n*-6 fatty acids. Progress in Lipid Research 47: 147–155.

Sperfeld, E. & A. Wacker, 2012. Temperature affects the limitation of *Daphnia magna* by eicosapentaenoic acid, and the fatty acid composition of body tissue and eggs. Freshwater Biology 57(3): 497–508.

Vadeboncoeur, Y., E. Jeppesen, M. J. Vander Zanden, H. H. Schierup, K. Christoffersen & D. M. Lodge, 2003. From Greenland to green lakes: cultural eutrophication and the loss of benthic pathways in lakes. Limnology and Oceanography 48: 1408–1418.

van de Bund, W. J., O. E. Krips & C. Davids, 1994. Potential food sources for littoral meiobenthos: culture experiments with Chydorus piger. In van de Bund, W. J. (ed.), Food Web Relations of Littoral Macro- and Meiobenthos. Amsterdam: 69–78.

Vymazal, J. & C. J. Richardson, 1995. Species composition, biomass, and nutrient content of periphyton in the florida everglades. Journal of Phycology 31: 343–354.

Wacker, A. & E. von Elert, 2001. Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata*. Ecology Letters 82: 2507–2520.

Walseng, B., D. O. Hessen, G. Halvorsen & A. K. Schartau, 2006. Major contribution from littoral crustaceans to zooplankton species richness in lakes. Limnology and Oceanography 51: 2600–2606.