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Hélène Masclaux

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**Hélène Masclaux**

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**Diversité des interactions trophiques à l'interface  
microorganismes-microcrustacés dans une zone littorale à macrophytes :  
conséquences sur le transfert des acides gras essentiels**

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## **Résumé**

Les zones littorales à macrophytes ont un rôle fonctionnel important dans les hydrosystèmes fluviaux. Outre leur forte productivité, ces milieux se caractérisent également par une forte biodiversité. Cependant, les liens pouvant exister entre hétérogénéité spatiale, biodiversité et flux de matière restent encore peu connus. En milieu aquatique, les microcrustacés représentent un maillon clef entre les microorganismes eucaryotes, qui sont les principaux producteurs d'acide gras polyinsaturés (AGPI), et le compartiment piscicole. Dans ce travail de thèse, nous avons donc cherché à savoir comment la diversité des cladocères rencontrée dans une zone littorale à macrophytes pouvait affecter le transfert des AGPI dans les réseaux trophiques.

L'approche vers laquelle s'est orienté ce travail combine à la fois des études menées en milieu contrôlé, permettant des expériences de nutrition, ainsi que des études menées en milieu naturel. Cette double approche permet de faciliter l'interprétation des résultats issus des analyses lipidiques, isotopiques et isotopiques de composés spécifiques.

Nos résultats montrent ainsi qu'il n'existe pas de variabilité dans les capacités d'accumulation et de bioconversion des AGPI chez les cladocères coexistant dans une zone littorale à macrophytes. D'après ces résultats, il semble donc que le principal facteur influençant la variabilité des transferts d'AGPI vers le compartiment piscicole soit la stratégie alimentaire et le compartiment trophique exploité par les différentes espèces de microcrustacés. En effet, outre le seston, notre étude montre que certaines espèces de cladocères rencontrées en zone littorale sont capables d'exploiter l'épiphyton et le neuston. Les analyses lipidiques mettent de plus en évidence, que la diversité des compartiments trophiques exploités s'accompagne d'une variabilité d'apports en AGPI. Dans notre étude, l'épiphyton est ainsi significativement plus concentré en AGPI que le seston. A l'interface air-eau, le neuston se caractérise de plus, par une forte accumulation de matière organique d'origine allochtone qui, dans le cas d'une pluie de pollen, représente une source de carbone et d'AGPI non négligeable pour certaines espèces de microcrustacés. Dans les zones littorales à macrophytes, la complémentarité des espèces de cladocères entraîne donc une utilisation plus complète des ressources. La diversité des compartiments trophiques, associée à la diversité des microcrustacés, permet donc probablement une optimisation du transfert des AGPI vers les niveaux trophiques supérieurs.

## **Abstract**

Areas with littoral macrophytes play an important functional role in freshwater systems. In addition to the high productivity recorded in such areas, they also shelter a high diversity of organisms. However, possible links between spatial heterogeneity, biodiversity and energy pathways are still poorly known. As they constitute the major link between microorganisms and species higher in the food web, microcrustaceans play a key role in the transfer of polyunsaturated fatty acids (PUFA) to organisms at higher trophic levels. In this study, we wanted to assess if microcrustaceans diversity encountered in macrophytes littoral zones would lead to a variability of PUFA transfer in the food web.

This work combined controlled conditions experiment and studies run in natural environments in order to help the interpretation of results from lipid analysis, isotopic analysis and fatty acid isotope analysis.

Our results indicate that there are no differences of PUFA concentrations between cladocerans from a macrophyte littoral zone when they were exposed to the same pool of dietary PUFA. Hence, in heterogeneous feeding habitats such as macrophytes zones where these cladocerans often co-exist, foraging behavior of cladoceran species more than differences of metabolism may be crucial for determining PUFA transfer to upper trophic levels. In addition to seston, our study shows indeed that some cladoceran species are able to forage on the epiphytic and neustonic compartments. Lipid analyses highlight moreover that the diversity of trophic compartments lead to a variability of PUFA inputs to primary consumers. In our study, the epiphytic compartment is indeed significantly more concentrated in PUFA than seston. At the air-water interface, neuston is moreover characterized by important allochthonous organic matter accumulation. During a pollen rain, this organic matter represents an important source of carbon and PUFA for some microcrustacean species. In macrophytes littoral zones, cladocerans complementarity leads to a more complete use of PUFA sources. The association of trophic compartment diversity and microcrustacean diversity probably allows an optimization of PUFA transfer to higher trophic levels.

## Abréviations en français

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<b>AG</b>	Acide Gras
<b>AGPI</b>	Acide Gras Polyinsaturé
<b>CPG</b>	Chromatographie en Phase Gazeuse
<b>EMAG</b>	Ester Méthyliques d'Acide Gras
<b>IA-EA</b>	Agence Internationale de l'Énergie Atomique
<b>IR-MS</b>	Spectromètre de Masse - analyseur de Ratio Isotopique

## Abréviations en anglais

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<b>ALA</b>	$\alpha$ -Linolenic Acid
<b>ANOVA</b>	Analysis Of Variance
<b>ARA</b>	Arachidonic Acid
<b>BAFA</b>	Bacterial Fatty Acid
<b>B-EF</b>	Biodiversity and Ecosystem Functioning
<b>DHA</b>	Docosahexaenoic Acid
<b>EPA</b>	Eicosapentaenoic Acid
<b>FA</b>	Fatty Acid
<b>FAME</b>	Fatty Acid Methyl-Ester
<b>FA-SIA</b>	Fatty acid-Stable Isotope Analysis
<b>GC</b>	Gas Chromatography
<b>HF</b>	Heterotrophic Flagellates
<b>HUFA</b>	Highly Unsaturated Fatty Acid
<b>IA-EA</b>	International Atomic Energy Agency
<b>IR-MS</b>	Isotope Ratio-Mass Spectrometer
<b>LIN</b>	Linoleic Acid
<b>MUFA</b>	Monounsaturated Fatty Acid
<b>POM</b>	Particulate Organic Matter
<b>PON</b>	Particulate Organic Nitrogen
<b>PUFA</b>	Polyunsaturated Fatty Acid
<b>SAFA</b>	Saturated Fatty Acid
<b>SDA</b>	Stearidonic Acid
<b>SIA</b>	Stable Isotope Analysis
<b>t-POC</b>	terrestrial Particulate Organic Carbon





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# **CHAPITRE I : Introduction générale**





## I. Introduction

Depuis les travaux de Lindeman (1942), de nombreuses études ont cherché à comprendre les processus qui régulent l'efficacité des transferts de matière dans les réseaux trophiques (Gulati & DeMott 1997; Müller-Navarra *et al.* 1997). Si la production d'un consommateur peut être liée à la disponibilité de sa ressource, les rendements avec lesquels l'énergie est transférée d'un niveau trophique à un autre sont extrêmement variables, notamment à l'interface producteurs primaires-herbivores (Müller-Navarra *et al.* 1997). Il est maintenant largement accepté qu'à ce niveau, cette variabilité d'efficacité des transferts peut être attribuée aux différences de qualité nutritionnelle des ressources. En milieu aquatique, parmi les facteurs qui déterminent la qualité nutritionnelle d'une source de nourriture, la toxicité (Jungmann & Benndorf 1994), la digestibilité (Lurling & Van Donk 1997), la teneur en phosphore (et en particulier le rapport carbone/phosphore ; Hessen 1990), et la composition en acides gras polyinsaturés (AGPI ; Ahlgren *et al.* 1990; Müller-Navarra *et al.* 2000) sont le plus souvent mis en avant.

Les AGPI, et plus particulièrement ceux des séries  $\omega 3$  et  $\omega 6$ , sont des composés biologiques ayant un rôle-clé dans la physiologie membranaire et en tant que précurseurs d'hormones pour de nombreux animaux (Olsen 1998; Arts *et al.* 2001). Ils représentent donc des facteurs nutritionnels primordiaux pour le développement de la plupart des métazoaires (Arts *et al.* 2001). Cependant, les métazoaires sont incapables de synthétiser ces AGPI *de novo*, ou à des taux insuffisants pour répondre à leur besoins physiologiques. Ils vont donc largement dépendre des AGPI fournis par leur alimentation, ceux-ci étant alors considérés comme des molécules essentielles. En milieu aquatique, les microorganismes eucaryotes (algues et protistes hétérotrophes) sont les principaux producteurs d'AGPI (Brett & Müller-Navarra 1997; Desvilettes & Bec 2009), qui vont ensuite être retenus chez les consommateurs et, en général, transférés de manière inchangée le long de la chaîne alimentaire (Koussoroplis *et al.* 2011). La disponibilité des AGPI à la base des réseaux trophiques et leur transfert vers les niveaux trophiques supérieurs seront donc un facteur déterminant pour la production secondaire. En tant que principal maillon entre les microorganismes et les niveaux trophiques supérieurs, les microcrustacés vont alors jouer un rôle-clé dans le transfert de ces composés essentiels. Si le transfert des AGPI et l'efficacité des transferts de matière dans les réseaux trophiques aquatiques a fait l'objet d'un certain nombre d'études, celles-ci se sont le plus souvent placées dans le cadre de chaînes trophiques simplifiées et linéaires de type

« microorganismes-zooplancton-poissons », sans prendre en compte la diversité rencontrée au sein d'un même niveau trophique. Or, de récentes études cherchant à lier biodiversité et fonctionnement des écosystèmes ont montré que la diversité présente au sein d'un même niveau trophique pouvait avoir un impact majeur sur le fonctionnement de l'écosystème et notamment sur les transferts de matière et d'énergie (Duffy *et al.* 2007; Woodward 2009). Les auteurs soulignent alors la nécessité de rapprocher deux domaines de recherche encore largement séparés : l'étude des interactions entre les différents niveaux trophiques et la prise en compte de la diversité rencontrée au sein d'un même niveau trophique.

Dans les écosystèmes aquatiques continentaux, les zones littorales à macrophytes ont été décrites comme des milieux très productifs (Wetzel 1990), jouant un rôle important d'un point de vue fonctionnel (Burks *et al.* 2002; Van Donk & Van de Bund 2002; Walseng *et al.* 2006). De plus, la forte hétérogénéité spatiale de ces milieux permet le maintien d'une forte biodiversité, et notamment d'une forte diversité de microcrustacés (Walseng *et al.* 2006). Ces deux particularités font des zones littorales à macrophytes des zones d'intérêt particulier pour étudier les relations entre biodiversité et fonctionnement d'un écosystème. A l'interface microorganismes-microcrustacés, la diversité des microcrustacés pourrait notamment entraîner une variabilité de transfert des AGPI et donc conséquemment une variabilité de l'efficacité des transferts de matière et d'énergie dans le réseau trophique.

Afin de mieux prendre en compte la complexité des réseaux trophiques, et d'étudier les relations entre richesse spécifique et transfert d'AGPI, ce travail de thèse s'est attaché à tester dans un premier temps, à travers une étude expérimentale en conditions contrôlées, si la diversité spécifique des cladocères est associée à une diversité de métabolismes lipidiques. Puis, à travers l'utilisation combinée d'approches expérimentales en conditions contrôlées et d'approches en milieu naturel, nous avons cherché à déterminer les compartiments trophiques utilisés par les espèces de cladocères rencontrées dans une zone littorale à macrophytes. Nous avons ainsi pu apprécier si la diversité des cladocères était associée à une séparation des niches trophiques, et si celle-ci pouvait entraîner une variabilité dans les apports en AGPI à la base du réseau trophique.

## **II. Rôle des zones à macrophytes dans le maintien de la transparence de l'eau**

### **1. Influence des zones à macrophytes sur la resuspension des sédiments**

Dans les écosystèmes aquatiques, les mouvements de l'eau produisent une remise en suspension des sédiments qui va dépendre de la nature des sédiments, de la bathymétrie de l'écosystème et, bien sûr, de la force des mouvements d'eau. Dans les lacs profonds, les sédiments des zones littorales peuvent être mis en suspension par l'action de l'eau et concentrés dans les zones plus profondes. En revanche, dans les environnements peu profonds, l'absence de zones jouant le rôle de piège peut entraîner une remise en suspension continue des sédiments (Evans 1994). Horppila & Nurminen (2003) suggèrent ainsi que la resuspension de sédiments est, dans certains écosystèmes, le processus majeur du maintien de la turbidité de l'eau. Dans ces environnements aquatiques peu profonds, les macrophytes émergés vont alors jouer un rôle important dans les processus de sédimentation et de remise en suspension des sédiments (Dieter 1990; Braskerud 2001; Madsen *et al.* 2001; Van Donk & Van de Bund 2002; Horppila & Nurminen 2003). En effet, l'énergie des vagues et la vitesse du courant diminuent en présence de végétation (Madsen *et al.* 2001; Horppila & Nurminen 2003). Cette diminution des mouvements de l'eau conduit alors à une augmentation du taux de sédimentation des particules en suspension, et à une diminution de la remise en suspension des sédiments. Madsen *et al.* (2001) suggèrent que cet effet serait positivement corrélé à la surface occupée par les macrophytes ainsi qu'à leur biomasse. Les poissons fouisseurs ont également un rôle important dans la remise en suspension des sédiments (Roberts *et al.* 1995). En ayant un effet inhibiteur sur le comportement d'alimentation de ces poissons, les macrophytes aquatiques pourraient entraîner de manière indirecte une diminution de la remise en suspension des sédiments. Dans les deux cas, une diminution de la turbidité de l'eau est observée.

### **2. Influence des zones à macrophytes sur les communautés microalgales**

De nombreux auteurs observent une faible biomasse phytoplanctonique dans les zones à macrophytes (Jeppesen *et al.* 1997; Van Donk & Van de Bund 2002; Bicudo *et al.* 2007; O'Farrell *et al.* 2009). Ces études identifient alors plusieurs causes.

*a) Compétition pour la lumière et les nutriments*

En tant qu'organismes autotrophes, les macrophytes sont en compétition pour la lumière et les nutriments avec les microalgues du milieu (phytoplancton et microalgues attachées à un support). La diminution de la biomasse phytoplanctonique dans les zones littorales à macrophytes par rapport aux zones pélagiques peut ainsi être expliquée, au moins partiellement, par l'effet d'ombrage (Van Donk & Van de Bund 2002; O'Farrell *et al.* 2009). En effet, la persistance d'un couvert végétal représente un environnement contraignant pour les microorganismes autotrophes, limitant le développement de la biomasse phytoplanctonique. De manière générale, l'atténuation de la lumière par la végétation aquatique va dépendre de la biomasse des plantes (Ikusima 1970) ; une diminution allant jusqu'à plus de 95% de l'irradiance dans les premiers 20 cm de la colonne d'eau a été enregistrée dans un herbier d'*Elodea* (Pokorný *et al.* 1984). Cette atténuation de la lumière par les macrophytes est particulièrement remarquable dans le cas de macrophytes à feuilles flottantes. O'Farrell *et al.* (2009) suggèrent ainsi que la limitation lumineuse imposée par les macrophytes à feuilles flottantes est le principal facteur influençant la composition et l'abondance phytoplanctonique tandis que, dans les zones d'eau libre, les nutriments seraient le principal facteur limitant pour le phytoplancton.

Si l'atténuation de l'intensité lumineuse a un effet sur la biomasse des microalgues, elle joue également un rôle sur la composition des communautés en sélectionnant des espèces tolérantes à une faible luminosité (O'Farrell *et al.* 2003). Les environnements présentant une couverture en macrophytes partielle ou fluctuante pourraient alors être caractérisés par des assemblages phytoplanctoniques plus diversifiés (O'Farrell *et al.* 2003; O'Farrell *et al.* 2009).

Plusieurs autres études relient la faible biomasse phytoplanctonique fréquemment observée dans les zones à macrophytes à la compétition pour les nutriments, notamment azote, phosphore, et carbone inorganique dissous dans l'eau (Van Donk *et al.* 1993; Sondergaard & Moss 1998; Van Donk & Van de Bund 2002). Barko & James (1998) soulignent également que la plupart des macrophytes aquatiques sont capables de puiser les nutriments dans les sédiments. Cette source alternative de nutriments permet aux macrophytes de se développer même si la concentration en nutriments dans l'eau est faible, ce qui leur conférerait un avantage compétitif par rapport aux espèces microalgales. Bicudo *et al.* (2007) ont ainsi montré que la suppression de macrophytes entraîne une augmentation significative du phosphore et des biomasses phytoplanctonique et cyanobactérienne dans un réservoir tropical peu profond.

La végétation aquatique a également des effets indirects sur la disponibilité des nutriments. Deux mécanismes contradictoires sont alors mis en avant. D'une part, la présence de végétation aquatique réduit les mouvements de l'eau et donc la remise en suspension des sédiments (Madsen *et al.* 2001). Or, la remise en suspension des sédiments est un processus important de recyclage des nutriments, notamment du phosphore (Jeppesen *et al.* 1997; Horppila & Nurminen 2003). Les macrophytes aquatiques diminuent donc la quantité de nutriments qui pourraient être libérés dans l'eau et bénéficier aux microorganismes autotrophes. D'autre part, la grande quantité de matière organique en décomposition parfois accumulée au niveau des sédiments dans les zones à macrophytes entraîne une consommation d'oxygène, qui peut conduire à une situation d'anoxie. Celle-ci favorise alors la libération du phosphore et de l'azote des sédiments (Van Donk & Van de Bund 2002; O'Farrell *et al.* 2009).

La connaissance des mécanismes de compétition entre les macrophytes aquatiques et le phytoplancton, ainsi que les observations sur le terrain ont alors amené les écologues à penser que les écosystèmes aquatiques peu profonds possédaient deux états d'équilibre alternatifs (Fig. 1.1) : un état d'équilibre pour lequel l'eau est claire et où les macrophytes sont présents en grande quantité, et un état d'équilibre pour lequel le milieu est turbide, avec une forte biomasse phytoplanctonique et une absence de macrophytes. Cette théorie des équilibres alternatifs a été exposée par Scheffer *et al.* (1993) : elle prédit que les macrophytes aquatiques peuvent maintenir la transparence de l'eau dans un écosystème peu profond jusqu'à un seuil de concentration en nutriments relativement élevé. Cependant, une fois que le système a basculé vers un état de turbidité, la réduction de la concentration en nutriments doit être importante pour permettre la recolonisation des macrophytes. Les lacs Takern et Krankesjön, dans le sud de la Suisse représentent deux bons exemples permettant d'illustrer ces états. En effet, pendant plusieurs dizaines d'années, des périodes d'eau claire avec une végétation aquatique abondante ont alterné avec des périodes où le milieu était turbide et où l'on trouvait peu de macrophytes, sans qu'il y ait un changement significatif d'apport en nutriments (Blindow 1992). Bien que les mécanismes entraînant le passage d'un état d'équilibre à un autre n'aient pas été clairement mis en évidence, il semblerait que les changements de niveau d'eau dans le système puissent jouer un rôle important en ayant un impact négatif sur le développement des macrophytes (Blindow 1992; Scheffer & van Nes 2007).

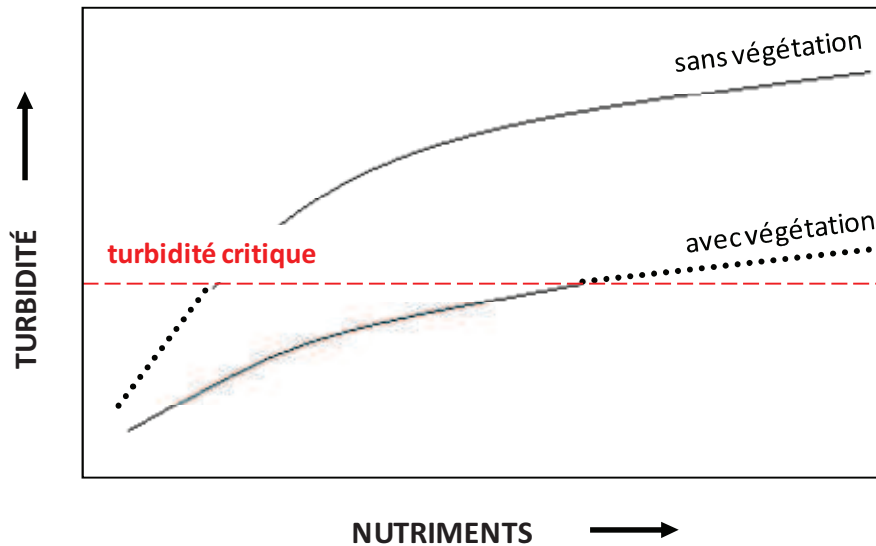


Figure 1.1 : Théorie des états d'équilibre alternatifs présentée par Scheffer *et al.* (1993). Sur cette figure, on voit que pour une même concentration en nutriments, un écosystème peut avoir deux états d'équilibre : avec ou sans macrophytes aquatiques. Il existe de plus un seuil de turbidité critique entraînant le passage d'un état à un autre.

#### b) Allélopathie

Van Donk & Van de Bund (2002) ont montré, dans des expériences en mésocosmes, que l'ajout de nutriments (phosphore et azote) n'avait aucun effet dans les mésocosmes contenant des macrophytes mais entraînait un bloom phytoplanctonique dans ceux n'en contenant pas. Dans cette étude, où la compétition pour la lumière et les nutriments ne pouvait être mise en cause dans l'inhibition du développement phytoplanctonique, d'autres phénomènes, tels que l'allélopathie ont alors été considérés (Van Donk & Van de Bund 2002). D'après Molisch (1937), l'allélopathie couvre l'ensemble des interactions biochimiques, positives ou négatives, entre les différents producteurs primaires ou entre les producteurs primaires et les microorganismes. La production de composés chimiques par les macrophytes aquatiques a ainsi été mise en avant comme étant une stratégie de défense efficace contre les autres organismes photosynthétiques en compétition pour la lumière et les nutriments, notamment épiphytiques et phytoplanctoniques. Ces composés appartiennent à différentes familles chimiques telles que des composés sulfurés, les polyacétylènes et les polyphénols (Gross 1999) et peuvent être produits par les macrophytes appartenant à différents genres : *Ceratophyllum*, *Stratiotes*, *Chara*, *Myriophyllum*, *Eichhornia* ou *Potamogeton* (Van Donk &

Van de Bund 2002; Gross 2003). Il a ainsi été montré que les faibles concentrations phytoplanctoniques observées dans les zones à macrophytes pouvaient être en partie liées à une inhibition chimique. La sensibilité des espèces phytoplanctoniques aux composés chimiques pourrait cependant dépendre des conditions du milieu. Ainsi, un environnement pauvre en nutriments rendrait les espèces phytoplanctoniques plus sensibles aux signaux chimiques libérés par les macrophytes. Il semblerait également que la sensibilité du phytoplancton soit dépendante de l'espèce concernée. Mulderij *et al.* (2003) ont par exemple montré que les espèces phytoplanctoniques *Selenastrum capricornutum* et *Chlorella minutissima* étaient plus affectées par les composés chimiques libérés par les macrophytes appartenant au genre *Chara* que *Scenedesmus obliquus*. La production de composés chimiques par les macrophytes aquatiques aurait donc un effet non seulement sur la biomasse mais également sur la structure des communautés phytoplanctoniques (Mulderij *et al.* 2003; Van Donk 2006).

#### *c) Contrôle par le zooplancton*

Les zones littorales à macrophytes sont des zones abritant de fortes densités zooplanctoniques (voir paragraphes suivants). En favorisant la survie des microcrustacés, parmi lesquels certaines espèces ont de fortes capacités de filtration, les macrophytes renforcent le contrôle du développement phytoplanctonique et donc permettent une stabilisation de la transparence de l'eau.

### **III. Influence des zones à macrophytes sur les communautés de microcrustacés**

#### **1. Les zones à macrophytes : des zones refuge pour les microcrustacés**

Dans les zones littorales, une forte densité de microcrustacés est souvent associée à la présence de macrophytes, qu'il s'agisse de macrophytes émergés ou à feuilles flottantes (Lauridsen *et al.* 1996; Stansfield *et al.* 1997; Balayla & Moss 2003; Cazzanelli *et al.* 2008; Van Onsem *et al.* 2010). Cazzanelli *et al.* (2008) montrent par exemple, qu'en présence de



plantes, la densité de cladocères est en moyenne soixante fois plus importante que dans la zone d'eau libre, avec des valeurs allant jusqu'à 15 000 individus par litre pour une communauté composée principalement de *Ceriodaphnia* et de *Bosmina*. Les contraintes liées à la présence de macrophytes et à l'hétérogénéité spatiale entraînent, cependant, des difficultés d'échantillonnage et amènent très probablement à une sous-estimation de la densité des microcrustacés existant réellement en zone littorale (Balayla & Moss 2003; Walseng *et al.* 2006).

Les fortes densités de microcrustacés enregistrées dans les zones à macrophytes semblent s'expliquer en partie par la diminution de la pression de prédation des poissons zooplanctonophages (Lauridsen *et al.* 1996; Jacobsen *et al.* 1997; Jeppesen *et al.* 1997; Stansfield *et al.* 1997). Bien que l'on ne connaisse pas la nature précise des mécanismes qui permettent aux macrophytes aquatiques d'offrir un refuge aux microcrustacés, plusieurs explications ont été avancées par les limnologues. L'augmentation du pH parfois observée au niveau des zones à macrophytes, par rapport aux zones de pleine eau, a été présentée comme un facteur pouvant diminuer l'activité des poissons et donc leur efficacité d'alimentation (Moss *et al.* 1998). Il a également été souligné que, dans le cas de poissons omnivores, la diversification du régime alimentaire dans les zones végétalisées entraîne une diminution de la pression de prédation sur les microcrustacés (Moss *et al.* 1998). Cependant, les deux facteurs explicatifs les plus souvent retenus sont une diminution de la luminosité sous le couvert végétal correspondant à une contrainte significative pour les chasseurs à vue (Scheffer 1998; Burks *et al.* 2002), ainsi que les interférences physiques entre poissons et végétaux diminuant l'efficacité de capture (Moss *et al.* 1998; Balayla & Moss 2003). L'importance relative de ces différents facteurs semble toutefois dépendre de la nature, de la densité, et de la structure de la végétation aquatique (Persson & Eklöv 1995).

## 2. Diversité des microcrustacés associée aux zones à macrophytes

Si la forte densité des microcrustacés en présence de macrophytes aquatiques est maintenant largement connue et documentée, certaines études montrent que la végétation soutient également une forte diversité (Walseng *et al.* 2006; Van Onsem *et al.* 2010). Les assemblages de microcrustacés changent en effet le long d'un gradient horizontal, de la zone littorale végétalisée à la zone de pleine eau (Lauridsen *et al.* 1996; Smiley & Tessier 1998; Cazzanelli *et al.* 2008). Chez les cladocères, les genres *Daphnia*, *Bosmina* et *Ceriodaphnia*

sont le plus souvent associés à des zones d'eau libre tandis que dans les zones littorales à macrophytes, en plus de ces espèces, apparaissent des genres inféodés à la végétation aquatique, tel que *Simocephalus*, *Eurycercus* ou *Sida* (Lauridsen *et al.* 1996; Balayla & Moss 2003; Lemke & Benke 2004; Walseng *et al.* 2006). En étudiant la diversité du zooplancton à l'échelle d'un lac, Walseng *et al.* (2006) montrent ainsi que les deux tiers des espèces de microcrustacés sont des espèces littorales. D'après ces auteurs, la forte hétérogénéité spatiale liée à la présence de macrophytes en zone littorale, offrirait un plus grand nombre de niches écologiques, et plus particulièrement de niches trophiques, et permettrait donc le maintien d'un grand nombre d'espèces. En plus de la matière organique particulière en suspension, certaines espèces zooplanctoniques pourraient en effet se nourrir des biofilms épiphytiques qui se développent sur les macrophytes. Outre les ressources trophiques, les changements de qualité de l'eau et de la pression de prédation le long du gradient horizontal de colonisation de la végétation aquatique a également un rôle structurant sur les communautés zooplanctoniques (Smiley & Tessier 1998).

### 3. Les contraintes associées aux zones à macrophytes

Bien que les zones à macrophytes constituent des zones refuge pour le zooplancton, un certain nombre de contraintes leur sont également associées. L'une des principales correspond à une pression de prédation élevée de la part des larves de poissons et des macroinvertébrés. Le bénéfice lié à l'évitement des prédateurs pélagiques peut donc être contrebalancé par le coût d'être confronté à des prédateurs de la zone littorale (Burks *et al.* 2002). En effet, si les zones à macrophytes constituent des zones refuge pour les microcrustacés contre les poissons zooplanctonophages adultes, elles le sont également pour les larves de poissons qui fuient la prédation des poissons piscivores (ex : perche, brochet). Ces larves exercent alors une forte pression de prédation sur les microcrustacés (Balayla & Moss 2003). Certaines études ont également mis en évidence qu'il existe une pression de prédation élevée par les macroinvertébrés (Gonzalez Sagrario *et al.* 2009; Gonzalez Sagrario & Balseiro 2010). Les macrophytes constituent en effet, l'habitat préférentiel de nombreux prédateurs invertébrés du zooplancton tels que les larves d'odonates, les dytiques, les notonectes, les corixidés, les hydres, les hydracariens et les larves de *Chaoborus* (Burks *et al.* 2002; Gonzalez Sagrario *et al.* 2009; Gonzalez Sagrario & Balseiro 2010).

Des effets allélopathiques ont également pu être mis en évidence entre certains macrophytes et le zooplancton. Ainsi, *Elodea canadensis* produit des substances chimiques qui réduisent le taux de croissance et de reproduction de *Daphnia* (Burks *et al.* 2000). Des temps d'exposition prolongés (5 jours) à des exsudats de nénuphars peuvent, de plus, entraîner une mortalité significative des *Daphnidae* (Sutfeld *et al.* 1996). Cependant, l'importance de telles interactions en milieu naturel, ainsi que la sensibilité d'espèces de microcrustacés inféodées aux zones à macrophytes restent encore peu connues (Van Donk & Van de Bund 2002).

Enfin, les faibles biomasses phytoplanctoniques enregistrées dans les zones à macrophytes pourraient constituer une contrainte pour le zooplancton qui pourrait ne pas y trouver une quantité de nourriture suffisante à son développement (Burks *et al.* 2002).

#### 4. La migration horizontale : un compromis entre évitement de la prédation et recherche de nourriture

Les contraintes liées à la vie dans les zones à macrophytes évoquées dans le paragraphe précédent laissent à penser qu'en l'absence de pression de prédation, certaines espèces de microcrustacés évitent la zone à macrophytes (Lauridsen & Lodge 1996; Gonzalez Sagrario & Balseiro 2010). Un compromis visant à minimiser les risques liés à la prédation et à maximiser leur taux de croissance et de reproduction conduit de nombreux cladocères, notamment des genres *Daphnia*, *Ceriodaphnia* et *Bosmina*, à effectuer une migration horizontale journalière entre la zone pélagique et la zone littorale à macrophytes dans les écosystèmes aquatiques peu profonds (Jacobsen *et al.* 1997; Stansfield *et al.* 1997; Burks *et al.* 2002; Wojtal *et al.* 2003; Pinel-Alloul & Ghadouani 2007; Cazzanelli *et al.* 2008). Cette migration semble être principalement gouvernée par la pression de prédation en zone pélagique (Balayla & Moss 2003; Wojtal *et al.* 2003; Pinel-Alloul & Ghadouani 2007). Durant la journée, les cladocères trouvent ainsi refuge dans les macrophytes afin d'éviter les prédateurs pélagiques, tandis que la nuit, ils éviteraient les contraintes associées aux zones à macrophytes tels que les phénomènes d'allélopathie et les faibles biomasses phytoplanctoniques. Comprendre les scénarii qui amènent les cladocères à effectuer une migration horizontale nécessite cependant la prise en compte des multiples paramètres interagissant dans les écosystèmes aquatiques peu profonds, notamment la densité des

macrophytes, les prédateurs en présence, la structure des communautés piscicoles (piscivores *versus* planctonophages), ainsi que les espèces de microcrustacés rencontrées.

#### **IV. Influence des zones à macrophytes sur les communautés piscicoles**

##### **1. Les zones à macrophytes : des zones refuge pour les poissons**

Les zones à macrophytes procurent une zone de refuge pour de nombreux organismes aquatiques. Elles ont ainsi été décrites comme des zones refuge pour les microcrustacés, mais elles le sont également pour plusieurs espèces de poissons (Eklov & Diehl 1994; Persson & Eklöv 1995; Chick & McIvor 1997; Eklov 1997; Sanchez-Botero *et al.* 2007). En présence de prédateurs, certaines études montrent ainsi que ces espèces de poissons se rencontrent préférentiellement dans les macrophytes (Persson & Eklöv 1995; Chick & McIvor 1997; Jacobsen *et al.* 1997). Ce rôle de refuge est lié principalement à la complexité structurale offerte par la végétation. En effet, la faible probabilité de rencontre entre le prédateur et sa proie, ainsi que les interférences physiques avec la végétation diminuent l'efficacité de capture de la plupart des poissons (Persson & Eklöv 1995; Burks *et al.* 2002). La diminution de la luminosité sous le couvert végétal pourrait également être un facteur affectant les chasseurs à vue (Burks *et al.* 2002). Toutefois, l'efficacité du refuge fourni par la végétation dépend de la densité et de la structure de celle-ci, et est variable selon l'espèce, la taille et le stade de développement des poissons (Persson & Eklöv 1995; Chick & McIvor 1997).

##### **2. Diversité piscicole associée aux zones à macrophytes**

Grâce à une étude réalisée sur plusieurs lacs américains, Tonn & Magnuson (1982) montrent que, quel que soit l'assemblage d'espèces piscicoles considéré, la richesse spécifique est fortement corrélée à la diversité de la végétation. Whitfield (1993) montre également que la richesse spécifique des poissons est plus importante dans la zone littorale des lacs côtiers où la diversité des habitats est la plus grande. Plusieurs études s'intéressant aux communautés piscicoles dans les écosystèmes aquatiques continentaux soulignent ainsi que la complexité structurale induite par la présence de végétation fournit une plus grande

diversité de microhabitats, et également une diversité accrue des sources de nourriture, propice au maintien d'un plus grand nombre d'espèces piscicoles (Benson & Magnusson 1992; Sanchez-Botero *et al.* 2007).

### 3. Les zones à macrophytes : des zones d'alimentation pour les poissons

Outre le refuge offert par la végétation, les zones littorales à macrophytes représentent des zones d'alimentation pour beaucoup d'espèces de poissons. Une forte densité et une importante diversité de sources de nourriture sont en effet associées aux macrophytes : petits poissons, macroinvertébrés, microcrustacés, biofilms périphytiques, macrophytes. Les juvéniles et les poissons de petite taille présents en forte densité, constituent ainsi une source de nourriture pour les poissons piscivores. Bien que pour ces derniers la complexité structurale de la végétation entraîne une diminution de l'efficacité de prédation, la végétation n'affecte pas de la même manière tous les prédateurs. Tandis que la perche (*Perca fluviatilis*) ou le sandre (*Stizostedion lucioperca*) voient leur efficacité de prédation diminuer avec l'augmentation de la complexité structurale de l'habitat (Petr 2000), il semblerait que des densités de végétation intermédiaires favorisent le brochet (*Esox lucius*) qui est un chasseur à l'affût (Persson & Eklöv 1995; Petr 2000). La végétation aquatique abrite également de fortes densités de proies invertébrées, qu'il s'agisse de microcrustacés (Balayla & Moss 2003; Van Onsem *et al.* 2010) ou de macroinvertébrés (Lalonde & Downing 1992; Thomaz *et al.* 2008), ainsi qu'une forte production primaire (macrophytes et périphyton associé) qui peut être une source de nourriture non négligeable pour certaines espèces de poissons herbivores ou omnivores. Petr (2000) rapporte qu'un certain nombre de poissons appartenant principalement à la famille des *Cyprinidae* dans les communautés piscicoles d'Europe, se nourrissent directement sur les macrophytes et contribuent à réguler l'extension des herbiers. Sanchez-Botero *et al.* (2007) précisent également qu'une large majorité d'espèces de poissons rencontrées dans les zones à macrophytes sont omnivores, ce qui pourrait suggérer que ces environnements sont favorables aux poissons ayant une forte plasticité dans leur comportement d'alimentation.

#### 4. Importance des zones à macrophytes pour la ponte et l'élevage des alevins

Beaucoup de poissons d'eau douce, même parmi ceux considérés comme étant typiquement pélagiques, utilisent les zones littorales et les macrophytes durant une partie de l'année pour pondre (Petr 2000) ou pendant le développement des larves et des juvéniles (Copp 1992; Baras & Nindaba 1999; Sanchez-Botero *et al.* 2007). Les poissons qui utilisent préférentiellement un substrat végétal comme support de ponte, dénommés poissons phytophiles, collent leurs œufs aux plantes émergées, vivantes ou mortes, pendant la période de ponte (Petr 2000). Dans les écosystèmes aquatiques continentaux d'Europe, la carpe commune (*Cyprinus carpio*), le gardon (*Rutilus rutilus*), la brème (*Abramis brama*), ou le brochet (*Esox lucius*) sont ainsi des poissons phytophiles. L'absence de végétation pendant la période de ponte de ces espèces peut donc avoir un effet négatif important sur leur succès de reproduction (Petr 2000). Une fois éclos, les larves et les juvéniles de poissons trouvent au sein des macrophytes, une zone de refuge contre les prédateurs, ainsi qu'un environnement plus chaud (en raison des faibles profondeurs d'eau) et riche en nourriture, qui leur permettra une croissance rapide (Copp 1992; Baras & Nindaba 1999; Petr 2000; Sanchez-Botero *et al.* 2007).

#### V. Importance fonctionnelle des bras morts et des zones à macrophytes associées en milieu fluvial

Un hydrosystème fluvial naturel est caractérisé par une diversité géomorphologique dont le moteur est l'érosion des berges et la sédimentation, qui entraînent la migration latérale du chenal principal. Ces processus d'érosion, de transport et de dépôts de sédiments créent une mosaïque d'habitats qui vont se différencier du chenal principal par leurs caractéristiques biotiques et abiotiques. Lorsque la pente est faible, la migration latérale du chenal conduit à la formation de méandres qui peuvent être recoupés naturellement à l'occasion d'une crue lorsque les rayons de courbure sont trop importants. Dans le cas d'une zone de tressage, lorsque la pente est plus forte, la migration du chenal peut aussi aboutir à l'abandon d'un bras de tressage. La partie relictuelle du méandre recoupé ou du bras de tressage devient alors un bras mort. Ce dernier va, dans un premier temps, être alimenté par le chenal, puis va se déconnecter et se combler petit à petit au fur et à mesure de son vieillissement, bien qu'un rajeunissement et une reconnexion au chenal principal puissent avoir lieu naturellement lors de crues.

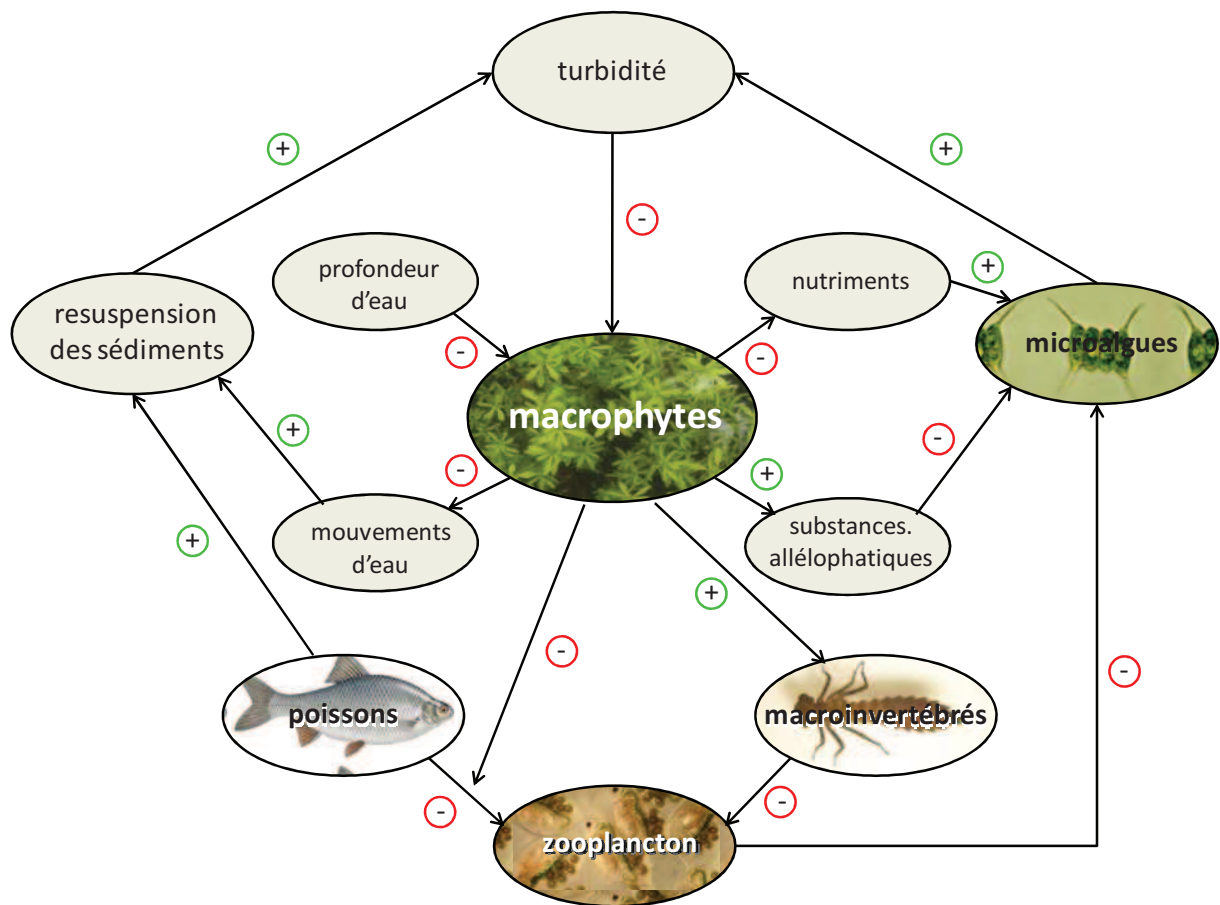


Figure 1.2 : Représentation schématisée des interactions biotiques et abiotiques relatives à la présence de macrophytes aquatiques (modifié d'après Scheffer *et al.* 1993).

Les bras morts sont caractérisés par une faible vitesse de courant, voire une absence de courant lorsqu'ils sont obstrués en amont. Or, le débit de l'eau est un déterminant majeur de l'habitat physique, qui va à son tour jouer un rôle important dans la colonisation par les organismes (Bunn & Arthington 2002). La diminution de la vitesse du courant dans les bras morts entraîne une sédimentation plus importante des particules en suspension dans l'eau. Le substrat y est donc plus fin et plus riche en matière organique et en nutriments que dans le chenal principal (Madsen *et al.* 2001). L'augmentation du temps de rétention de l'eau permet également, dans la plupart des cas, une augmentation de la température de l'eau, au printemps et en été (Schiemer *et al.* 2001; Amoros & Bornette 2002). Tandis que les conditions abiotiques du chenal principal des rivières, et notamment la vitesse du courant, ne permettent

pas à de nombreux organismes de boucler leur cycle de vie, les conditions rencontrées dans les bras morts sont propices au développement de nombreuses espèces. En effet, les bras morts sont caractérisés par une forte production primaire, aussi bien sous forme de microalgues que de macrophytes, qui ne pourraient se développer sur le substrat et avec les débits importants caractérisant le chenal principal (Madsen *et al.* 2001; Thorp & Delong 2002; Casper & Thorp 2007). Or, la production primaire et la structure de l'habitat offertes par les macrophytes aquatiques ont une influence déterminante sur la production secondaire et la diversité des organismes, comme cela a été souligné dans les paragraphes précédents. Plusieurs études mettent ainsi en avant les fortes densités et la forte diversité spécifique de microcrustacés mesurées dans les bras morts, et suggèrent que ces annexes hydrauliques sont importantes pour le maintien des communautés de microcrustacés dans les hydrosystèmes fluviaux (Baranyi *et al.* 2002; Casper & Thorp 2007; Rossetti *et al.* 2009; Vadadi-Fülöp *et al.* 2009). Au niveau des plaines d'inondation, la plupart des vertébrés rencontrés dans le chenal principal dépendent également largement, directement ou indirectement, de la production des annexes fluviales (Junk *et al.* 1989). De nombreux poissons utilisent des habitats différents au cours de leur développement. Ces poissons vont donc passer successivement du chenal principal aux bras morts, afin d'y trouver des conditions de température, de vitesse de courant, de substrat, ou de nourriture qui correspondent à leurs besoins (Amoros & Bornette 2002). Les poissons utilisent notamment les annexes hydrauliques lors de la reproduction car ils trouvent dans les zones à macrophytes des lieux de pontes favorables (Bunn & Arthington 2002). Les larves trouvent ensuite dans ces milieux une nourriture abondante et des conditions propices à leur développement (Copp & Penaz 1988; Schiemer *et al.* 2001). Enfin, les bras morts peuvent avoir un rôle refuge en cas de crue ou de pollution du chenal principal (Amoros & Bornette 2002). La disponibilité de bras morts fonctionnels va donc avoir un rôle crucial pour le recrutement et le maintien des populations de poissons dans les hydrosystèmes fluviaux (Amoros & Bornette 2002) à condition qu'une certaine connectivité soit maintenue entre les différents habitats (Cloern 2007).

Dans de nombreux hydrosystèmes fluviaux, la mosaïque d'habitats qui soutient une forte biodiversité et une forte productivité est menacée car la dynamique fluviale qui permet leur formation a été sévèrement altérée par les aménagements faits par l'homme. Cependant, depuis une vingtaine d'années, des efforts croissants ont été apportés à la compréhension de la dynamique fluviale ainsi qu'à la préservation et la restauration des milieux associés tels que les bras morts et les zones à macrophytes. On sait maintenant que les zones littorales à



macrophytes des lacs et des rivières sont des zones abritant de fortes densités d'organismes ainsi qu'une forte productivité. La structuration de l'habitat par la végétation semble, de plus, permettre le maintien d'une forte biodiversité. Cependant, il semble que les liens pouvant exister entre hétérogénéité spatiale, biodiversité, et flux de matière dans ces milieux sont encore peu connus.

## **VI. Organisation du mémoire de thèse**

Ce travail de thèse cherche à apprécier la variabilité des transferts de composés essentiels à la base des réseaux trophiques aquatiques dans les zones à forte hétérogénéité spatiale telles que les zones littorales à macrophytes. Plus particulièrement, nous avons voulu savoir si la diversité des microcrustacés dans ces milieux pouvait entraîner une variabilité des transferts d'AGPI à la base des réseaux trophiques. Compte tenu de la complexité du milieu et des difficultés à identifier de manière précise les sources de nourriture réellement utilisées par les microcrustacés en milieu naturel, ainsi que des faibles connaissances du métabolisme lipidique de ces organismes, l'utilisation conjointe d'approches en conditions contrôlées et en milieu naturel nous ont paru indispensables pour tester nos hypothèses.

Le chapitre II présentera, tout d'abord, les sites d'études et la méthodologie employée dans cette étude.

Le chapitre III de ce manuscrit correspond à une étude en milieu expérimental, visant à évaluer la variabilité interspécifique de la capacité d'accumulation et de bioconversion des AGPI chez les cladocères, microcrustacés abondants dans les zones à macrophytes. Cette évaluation nous a semblé indispensable comme première approche concernant la variabilité des transferts d'AGPI vers les niveaux trophiques supérieurs dans ces milieux particuliers et encore peu connus.

Le chapitre IV a pour objectif d'évaluer l'importance relative du seston et du périphyton en tant que source de nourriture de différentes espèces de cladocères, à la fois d'un point de vue quantitatif et qualitatif. Pour répondre à cet objectif, une étude expérimentale en conditions simplifiées ainsi qu'une approche en milieu naturel ont été menées. Pour cette dernière, l'utilisation des isotopes stables du carbone et de l'azote nous ont permis d'explorer les liens trophiques existant entre les différentes espèces étudiées et leurs sources de

nourriture, tandis que l'analyse lipidique de ces sources a permis d'évaluer leur qualité nutritionnelle.

Les zones littorales des écosystèmes aquatiques continentaux étant particulièrement exposées aux apports de matière organique du milieu terrestre, de fortes accumulations de grains de pollen sont parfois observées à la surface de l'eau, lors de la pollinisation de certaines espèces anémophiles. Dans le chapitre V, l'importance du neuston et d'apports allochtones de pollen pour la communauté de microcrustacés a donc été estimée. Une étude expérimentale en conditions contrôlées nous a, dans un premier temps, permis d'apprécier la qualité nutritionnelle du pollen pour les cladocères. Puis, une étude en milieu naturel a été conduite dans le but de mesurer l'effet des dépôts de pollen de pin sur les communautés microbiennes du neuston et sur la communauté de microcrustacés. Des analyses lipidiques et isotopiques ont également été réalisées afin de suivre le devenir de cet apport de carbone et d'AGPI dans l'écosystème.

Enfin, dans le dernier chapitre de ce manuscrit (chapitre VI), une discussion générale et des perspectives de ce travail de thèse sont présentées.



## **CHAPITRE II : Sites d'études et méthodes**



## I. Sites d'études

### 1. Présentation de la zone d'étude

Principal affluent de la Loire, l'Allier prend sa source à 1485 m d'altitude au pied du Moure de la Gardille en Lozère. Après avoir parcouru 425 km dans des paysages variés, des reliefs volcaniques aux bassins sédimentaires en aval, il se jette dans la Loire au Bec d'Allier (« Bec » étant le nom donné aux confluences dans le bassin ligérien) près de Nevers. Son bassin versant couvre 14 310 km<sup>2</sup>. Notre zone d'étude se situe entre Puy-Guillaume (Puy-de-Dôme, 63) et Saint-Yorre (Allier, 03), dans la plaine de la Limagne, sur une zone très peu pentue où la rivière forme de larges méandres (Fig. 2.1).

L'Allier et la Loire ont subi peu d'aménagements liés aux activités humaines comparativement aux autres cours d'eau européens et sont considérés comme les derniers grand cours d'eau sauvages en Europe. L'espace de liberté laissé à la rivière lui permet, en effet, d'avoir une dynamique fluviale très active qui entraîne la formation de nombreux bras morts en plaine. On y retrouve une importante diversité floristique et faunistique et plusieurs programmes ont été mis en place afin de préserver ces cours d'eau et la diversité des milieux associés (ex : réserve naturelle nationale du Val d'Allier, plan Loire grandeur nature).

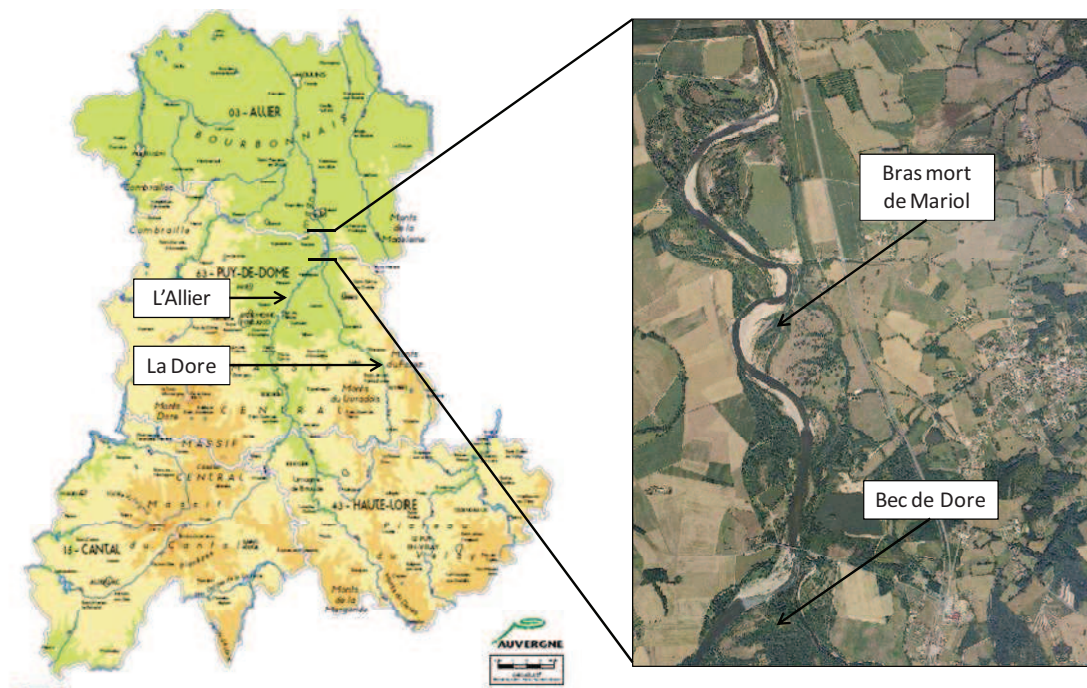


Figure 2.1 : Situation géographique de la zone d'étude

## 2. Présentation des sites d'études et stratégie d'échantillonnage

### a) *Bras mort de Mariol*

Le bras mort de Mariol se situe dans la plaine d'inondation de la rivière Allier, près de la commune du même nom, à 6 km en aval de Saint-Yorre dans le département de l'Allier (3°28'E, 46°01'N) (Fig. 2.1).

Le bras mort est composé d'une dépression profonde, au centre (profondeur maximale : 3 m), entourée d'une ceinture de macrophytes aquatiques moins profonde (profondeur moyenne : 0,8 m). Le bras mort est connecté uniquement en aval, de manière permanente, avec le lit principal de l'Allier via un chenal étroit et peu profond (largeur moyenne : 10 m ; profondeur moyenne : 0,8 m). Le chenal de connexion et la ceinture de macrophytes du bassin principal sont recouverts de diverses espèces de macrophytes aquatiques, parmi lesquelles les trois espèces dominantes sont la Callitriche (*Callitriche* sp.), l'Elodée du Canada (*Elodea Canadensis*) et la Jussie (*Ludwigia grandiflora*).

#### - Stratégie d'échantillonnage

Nous présenterons ici la stratégie d'échantillonnage mise en place sur le bras mort de Mariol. Les procédés méthodologiques propres à chaque étude seront présentés dans les chapitres concernés.

La campagne d'échantillonnage sur le bras mort de Mariol a été conduite entre mars et novembre 2008. Afin de prendre en compte l'hétérogénéité spatiale du milieu, le bras mort a été divisé en cinq zones (Fig. 2.2). Au sein de chaque zone, la structure de l'habitat était considérée comme homogène et les zones différaient les unes des autres par leur orientation, leur pente et la végétation aquatique présente. Ainsi, les zones 1, 2, 3 et 4 correspondent à la ceinture de macrophytes et la zone 5 à la zone centrale et plus profonde, sans macrophytes.

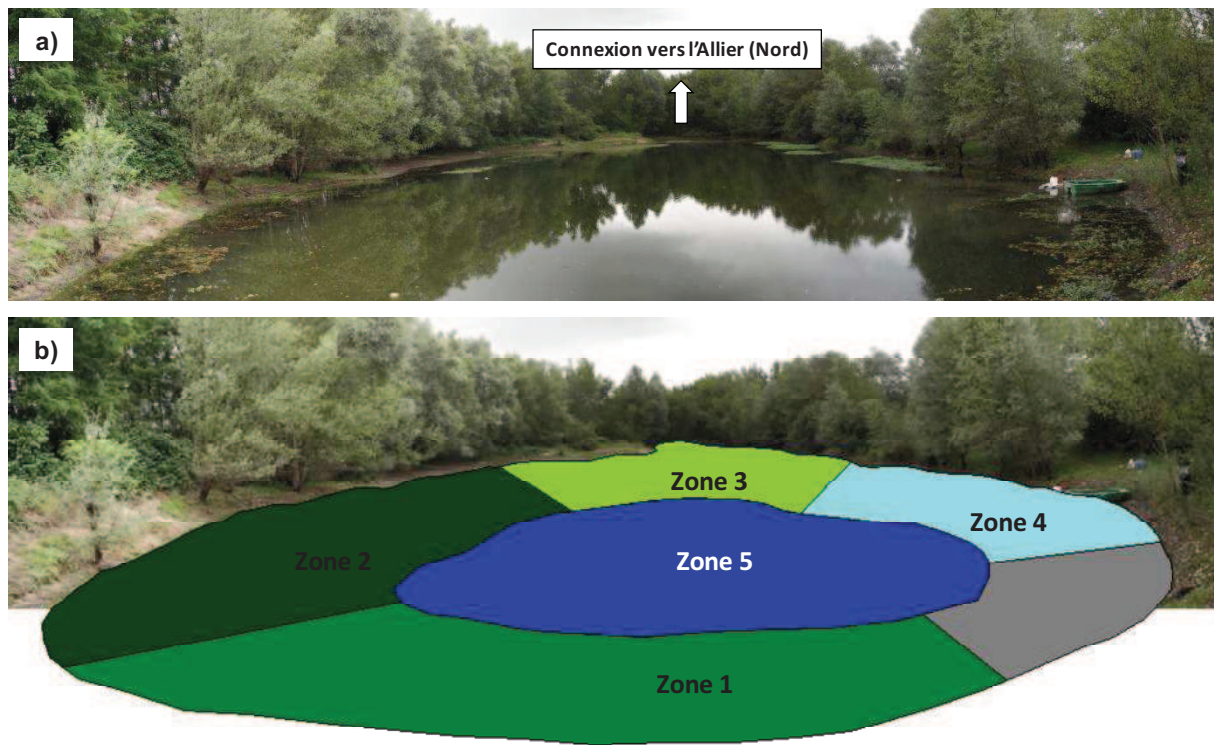


Figure 2.2 : a) Bras mort de Mariol, b) représentation des cinq zones d'échantillonnage. La zone grisée correspond à une zone d'entrée dans le bras mort qui n'a pas été échantillonnée. Les zones 1, 2, 3 et 4 correspondent à la ceinture de macrophytes tandis que la zone 5, plus profonde, représente une zone d'eau libre.



- Prélèvements des microcrustacés

A chaque date d'échantillonnage, trois prélèvements de microcrustacés ont été réalisés de manière aléatoire dans chacune des zones à l'aide de colonne en plexiglas, d'après une méthode d'échantillonnage proposée par Amoros (1980). Les colonnes utilisées étaient de différentes tailles afin de pouvoir prélever l'ensemble de la colonne d'eau, quelle que soit la profondeur échantillonnée (Fig. 2.3). Les espèces de microcrustacés vivant au niveau des sédiments, dans la colonne d'eau, accrochées aux macrophytes ou au niveau de l'interface air-eau ont donc à chaque fois été prélevés. Les organismes de chaque prélèvement ont été fixés au formaldéhyde (5%) sucrés, et conservés à température ambiante avant analyses. De plus, à partir du 15 avril, deux traits de filet à zooplancton ont été réalisés horizontalement, en parallèle, dans les cinq premiers cm de surface et à 50 cm, à l'aide d'un radeau télécommandé (Fig. 2.4). Chaque prélèvement a également été fixé au formaldéhyde (5%) sucrés, et conservé. Au laboratoire, les échantillons de microcrustacés fixés ont été comptés dans des cuves de Dolfus, sous loupe binoculaire (grossissement 50), et les différentes espèces de cladocères ont été identifiées grâce à une clef de détermination proposée par Amoros (1984). Ces comptages nous ont permis d'apprécier la structure et la dynamique temporelle de la communauté de microcrustacés dans chacune des zones et d'évaluer s'il existait une différence dans la structure des communautés vivant en surface et en profondeur. Les résultats sont brièvement présentés dans les figures 2.5, 2.6 et 2.7.

Les prélèvements quantitatifs de microcrustacés destinés aux analyses ont été faits à l'aide d'un filet, en effectuant des traits de filet verticaux dans chacune des zones. Ils ont été congelés sur le terrain dans de l'azote liquide, puis conservés à -40°C au laboratoire. Pour chacune des dates, les échantillons ont par la suite été décongelés et placés sous une loupe binoculaire équipée de lumière froide, tout en étant maintenu dans de la glace. Les principales espèces de cladocères ont ainsi été triées afin de préparer des échantillons destinés aux analyses lipidiques et isotopiques.



Figure 2.3 : Colonnes en plexiglas utilisées pour les prélèvements de zooplancton destinés aux comptages



Figure 2.4 : Radeau d'échantillonnage télécommandé

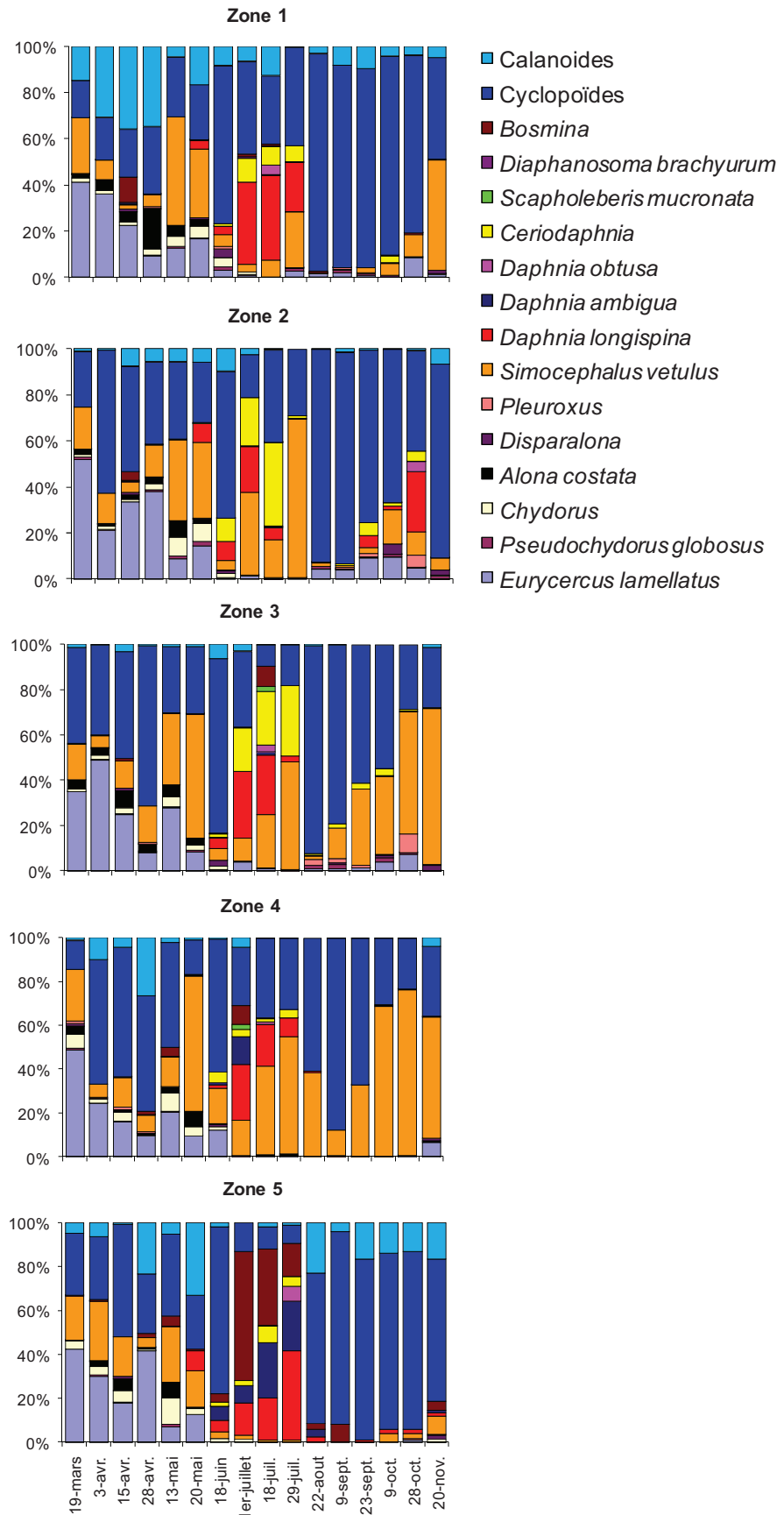


Figure 2.5 : Evolutions temporelles de l'abondance relative des microcrustacés prélevés dans chacune des cinq zones

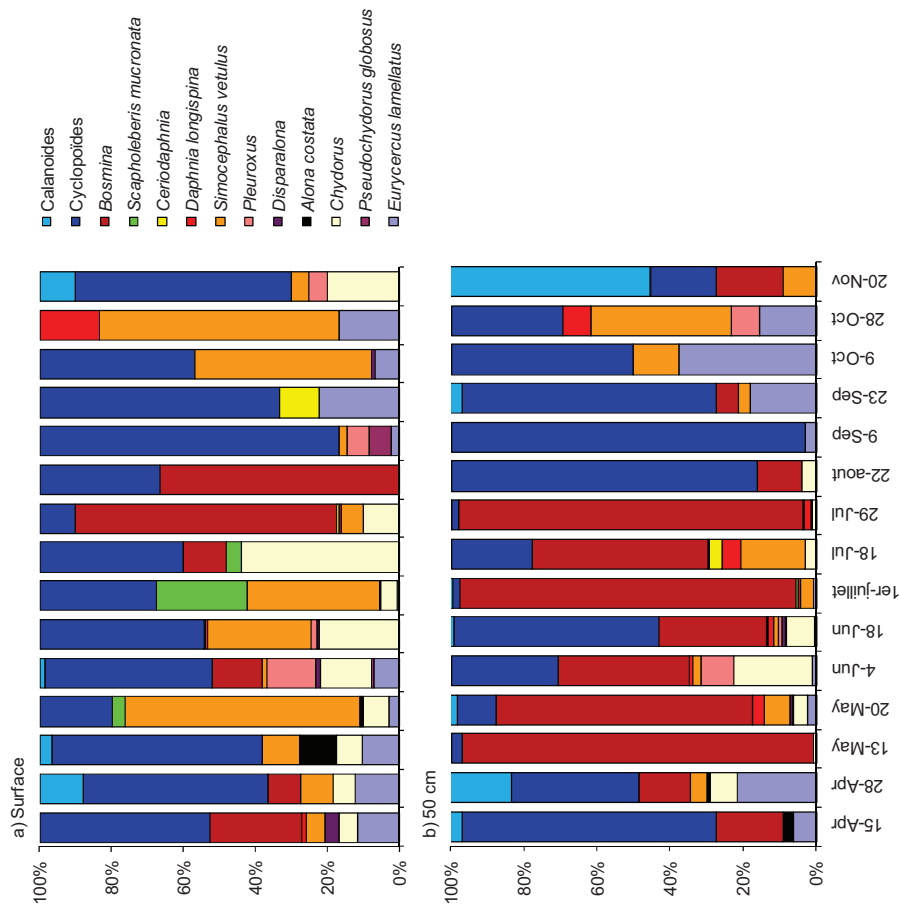


Figure 2.6 : Evolutions temporelles des abondances respectives des différents groupes de microcrustacés (mesurées en nombre d'individus par litre) a) dans la zone à macrophytes (zones 1, 2, 3 et 4 regroupées) et b) dans la zone d'eau libre (zone 5).

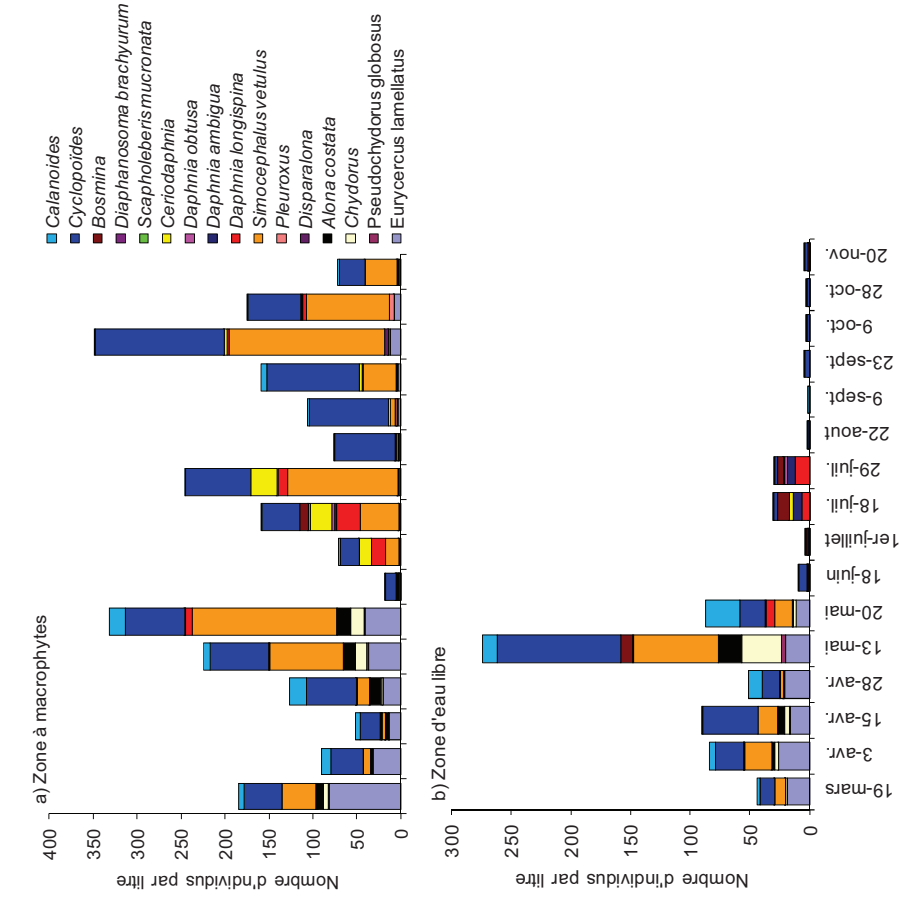


Figure 2.7 : Evolutions temporelles de l'abondance relative des microcrustacés prélevés a) en surface (5 premiers cm) et b) en profondeur (à 50cm).

- Prélèvements de seston et d'épiphyton

Les prélèvements de seston ont été réalisés à 50 cm de profondeur, à l'aide du radeau télécommandé (Fig. 2.4). Ce dispositif nous a permis à chaque date d'avoir un prélèvement horizontal intégré de l'ensemble du bras mort. Pour l'épiphyton, les trois espèces principales de macrophytes ont été prélevées dans chacune des zones correspondantes à la ceinture de macrophytes (Zones 1, 2, 3 et 4) et placées dans des sacs contenant de l'eau préfiltrée à 0.2  $\mu\text{m}$ .

Au laboratoire, les sacs contenant les macrophytes ont été placés dans un Stomacher (Bagmixer 400, Interscience, France) afin de détacher les communautés microbiennes des tiges et des feuilles (Bowker *et al.* 1986). La solution contenant l'épiphyton, ainsi que le seston ont été préfiltrés à 50  $\mu\text{m}$  de manière à ne conserver que la fraction inférieure à 50  $\mu\text{m}$  (c'est-à-dire la gamme de taille préférentiellement ingérée par les cladocères, Burns 1968). Celle-ci a ensuite été filtrée sur filtre GF/F (Whatman <sup>®</sup>) immédiatement après les échantillonnages, et conservée à -80°C pour les analyses lipidiques et isotopiques.

b) *Bras mort du Bec de Dore*

Le bec de Dore est un espace situé à la confluence de deux rivières : l'Allier et la Dore (3.48°W, 46.00°N) (Fig. 2.8). Ce site, classé « espace naturel sensible » par le conseil général du Puy de Dôme depuis 2008, comprends plusieurs bras morts. Le bras mort sur lequel nous avons travaillé est déconnecté. Sa profondeur varie entre 0,5 et 1 m. Sur ce site, en plus de la végétation rivulaire, on trouve de nombreux pin (*Pinus* sp.).

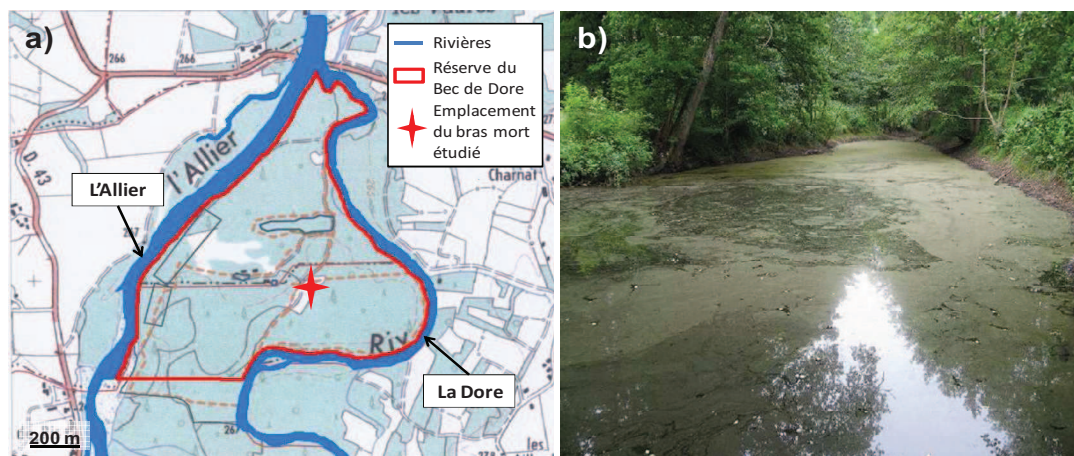


Figure 2.8 : a) Carte du site classé « espace naturel sensible » du Bec de Dore et b) photo du bras mort étudié.

- Stratégie d'échantillonnage

L'échantillonnage effectué sur ce bras mort est présenté de manière précise dans la deuxième partie du chapitre V.

## **II. Culture et maintien des organismes phytoplanctoniques et des cladocères au laboratoire**

### **1. Culture des espèces phytoplanctoniques**

Deux espèces phytoplanctoniques sont utilisées dans cette étude : une Cryptophycée, *Cryptomonas sp.* SAG 26.80, et une Chlorophycée, *Scenedesmus obliquus* SAG 276-3a. De nombreuses études ont montré que ces espèces se différenciaient par leur qualité nutritionnelle vis-à-vis des Cladocères, *Cryptomonas* contenant des AGPI à longue chaîne de carbone ( $\geq 20$  atomes de carbone) absent chez *Scenedesmus* (Ahlgren *et al.* 1990; Brett & Müller-Navarra 1997). Les espèces phytoplanctoniques sont cultivées dans des erlenmeyers de 3L sur milieu MWC modifié d'après Von Elert & Wolffrom (2001) dans lequel des vitamines ont été ajoutées dans le protocole du chapitre 3, et sur milieu Cyano (Jüttner *et al.* 1983) dans les protocoles des chapitres 4 et 5. Les cultures sont maintenues à 20°C avec une lumière permanente de  $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Le mode de culture est semi-continu : 25% du volume de la culture est prélevé et remplacé par du milieu neuf contenant des vitamines, tous les jours pour *Scenedesmus* et tous les deux jours pour *Cryptomonas*, afin de maintenir les cultures en phase exponentielle de croissance.

### **2. Préparation des solutions utilisées pour la nutrition du zooplancton**

Un sous-échantillon des cultures phytoplanctoniques est prélevé et centrifugé à 3000 tours par minute pendant 5 minutes. Le milieu de culture constituant la phase surnageante, est retiré et le culot contenant les cellules phytoplanctoniques est remis en suspension dans du milieu neuf. La concentration en carbone de la suspension ainsi obtenue est estimée grâce à un spectrophotomètre (longueur d'onde 800 nm), en utilisant les équations définies par Martin-Creuzburg (université de Bayreuth, Allemagne) qui a réalisé cet étalonnage:

$$\textit{Scenedesmus obliquus SAG 276-a } y = 313x + 20,7$$

$$\textit{Cryptomonas sp. SAG 26.80 } y = 271,38x - 13,157$$

où x correspond à la densité optique et y à la concentration de carbone en  $\text{mgC L}^{-1}$ .

### 3. Origine et maintien des différentes espèces de cladocères au laboratoire

*Daphnia magna* a été récoltée dans une mare située dans la plaine d'inondation de la rivière Allier. Cette espèce a été largement utilisée dans les études réalisées en laboratoire (Dawidowicz & Loose 1992; Müller-Navarra *et al.* 2000), Les autres espèces : *Ceriodaphnia* sp., *Daphnia longispina*, *Daphnia pulex*, *Eurycercus lamellatus*, *Scapholeberis mucronata* et *Simocephalus vetulus* proviennent toutes du bras mort de Mariol où elles dominent la communauté. Pour chacune des espèces, des femelles sont maintenues dans des flacons de 1L contenant 50% d'eau de la rivière ayant subi une filtration tangentielle sur  $0,2 \mu\text{m}$  (pompe Amicon®) pour éliminer les bactéries, les algues et les détritins fins, et de 50% d'un milieu de culture artificiel, le milieu ADaM (Klüttgen *et al.* 1994). Afin d'éviter des effets liés à la surpopulation, des densités inférieures à 20 individus par litre sont maintenues. Les espèces sont placées à  $20^{\circ}\text{C}$  avec une photopériode de 12 heures de nuit et 12 heures de jour. Elles sont nourries une fois par jour, à une concentration de  $2 \text{ mg C L}^{-1}$  avec *Scenedesmus obliquus*. Pour *E. lamellatus*, des tiges en verre sur lesquelles s'était développé du périphyton sont rajoutées dans chaque flacon.

Dans le chapitre 3, un mois avant le début de l'expérience, les espèces utilisées sont cultivées uniquement sur du milieu ADaM et nourries tous les jours avec une mixture de *Cryptomonas* sp. et de *Scenedesmus obliquus* (20%: 80% des biomasses respectives).

### III. Analyses lipidiques et isotopiques

#### 1. Analyse lipidique

##### a) *Extraction des lipides totaux*

L'extraction est réalisée selon la méthode de Folch *et al.* (1957) (Fig. 2.9). L'échantillon est dans un premier temps broyé dans un mélange chloroforme / méthanol (2 :1, v/v), afin de réaliser la rupture des liaisons protéines/lipides, puis il subit une sonication et un passage au bain-marie afin d'optimiser cette rupture. Après l'ajout d'une solution de NaCl (0.9%) suivi d'une centrifugation, l'extrait lipidique total est récupéré par évaporation sous flux d'azote et conservé dans de l'hexane (1 ml) à - 40°C.

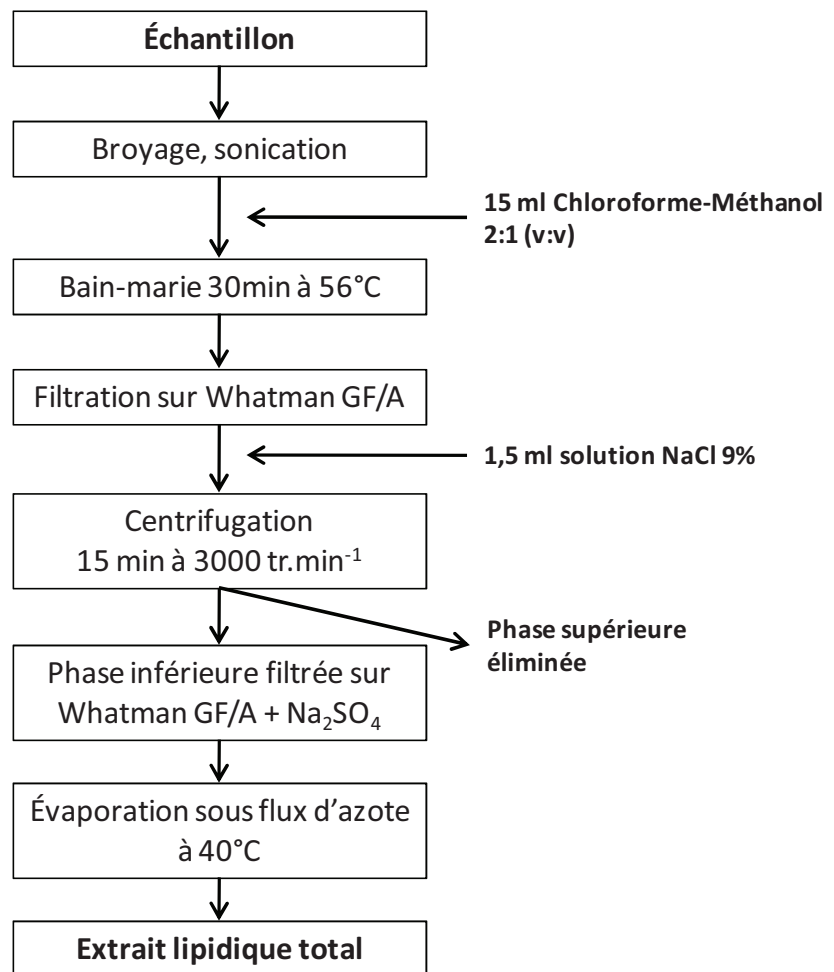


Figure 2.9 : Protocole d'extraction des lipides



*b) Préparation des esters méthyliques d'acides gras (EMAG)*

Afin d'identifier les acides gras (AG) par chromatographie en phase gazeuse (CPG), il est nécessaire de les convertir en molécules plus légères non polaires, les esters méthyliques d'acides gras (EMAG ; Fig. 2.10). Les lipides totaux sont estérifiés en une seule étape en utilisant du H<sub>2</sub>SO<sub>4</sub> méthanolique (2% ; Christie 1982). Les échantillons sont portés à 75°C pendant 2 heures dans un bain-marie à sec. L'adjonction d'un mélange eau-hexane (1:2, v/v) suivi d'une centrifugation permet de séparer les EMAG du matériel insaponifiable. Les EMAG sont récupérés à partir de la phase supérieure, après évaporation à 40°C sous flux d'azote, les échantillons sont repris dans 50-100 µl d'hexane puis stockés à -40°C.

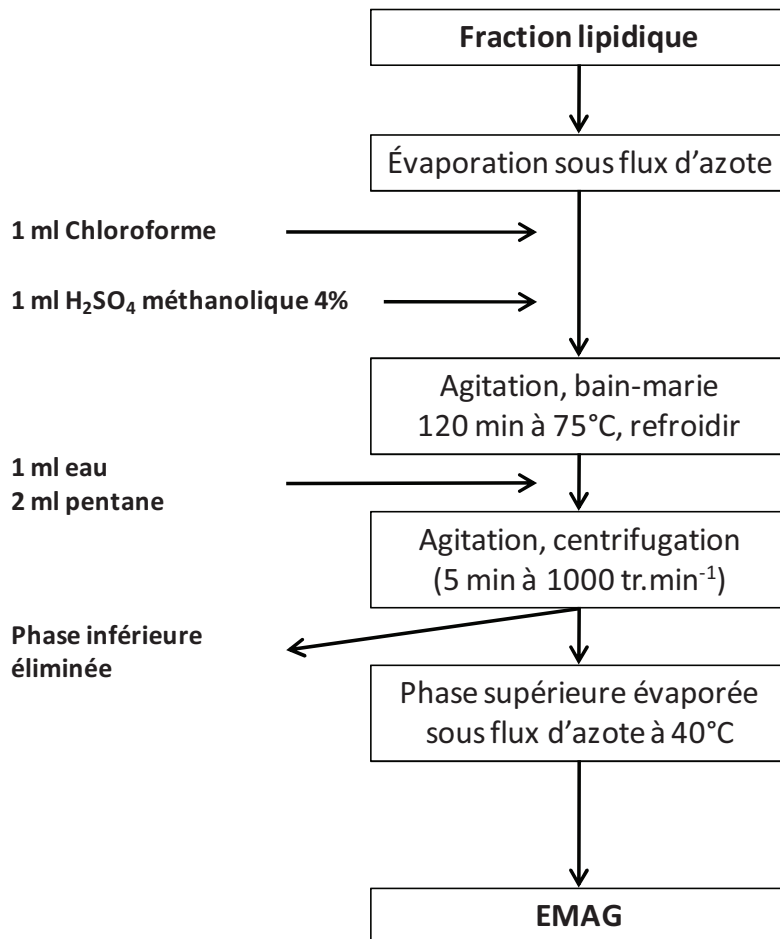


Figure 2.10 : Protocole de conversion des acides gras en esters méthyliques d'acides gras (EMAG)

c) Analyse des EMAG par chromatographie en phase gazeuse

Les EMAG sont séparés avec un chromatographe Agilent 6850 (Santa Clara, Etats-Unis) dans les conditions opératoires suivantes :

- Colonne capillaire : DB-Wax column (J&W Scientific), L = 30m,  $\varnothing=0,32\text{mm}$ , phase stationnaire polaire FFAB CB, épaisseur du film =  $0,25\mu\text{m}$ .
- Injecteur : mode SPLIT (1 :10)
- Détecteur à ionisation de flamme (DIF)
- Gaz vecteur : Hélium
- Conditions de température : injecteur =  $300^{\circ}\text{C}$ , détecteur =  $260^{\circ}\text{C}$
- Programmation de température :  $140^{\circ}\text{C}$  à  $3^{\circ}\text{C}/\text{min}$ .

L'intégration des pics est faite par le programme Mosaic Chrompack, alors que l'identification des AG est effectuée par comparaison avec un mélange d'AG connus (Fig. 2.11). Les AG sont quantifiés à l'aide d'un étalon interne incorporé en quantité fixe dans l'échantillon avant la conversion en EMAG (afin d'estimer les pertes éventuelles pouvant survenir pendant cette conversion). Celui-ci doit être absent des échantillons et se comporter de la même façon que les AG analysés. L'acide tridécanoïque (13:0) et l'acide triicosanoïque (23:0) ont été choisis comme double étalon interne car ils répondent bien à ces différents critères.

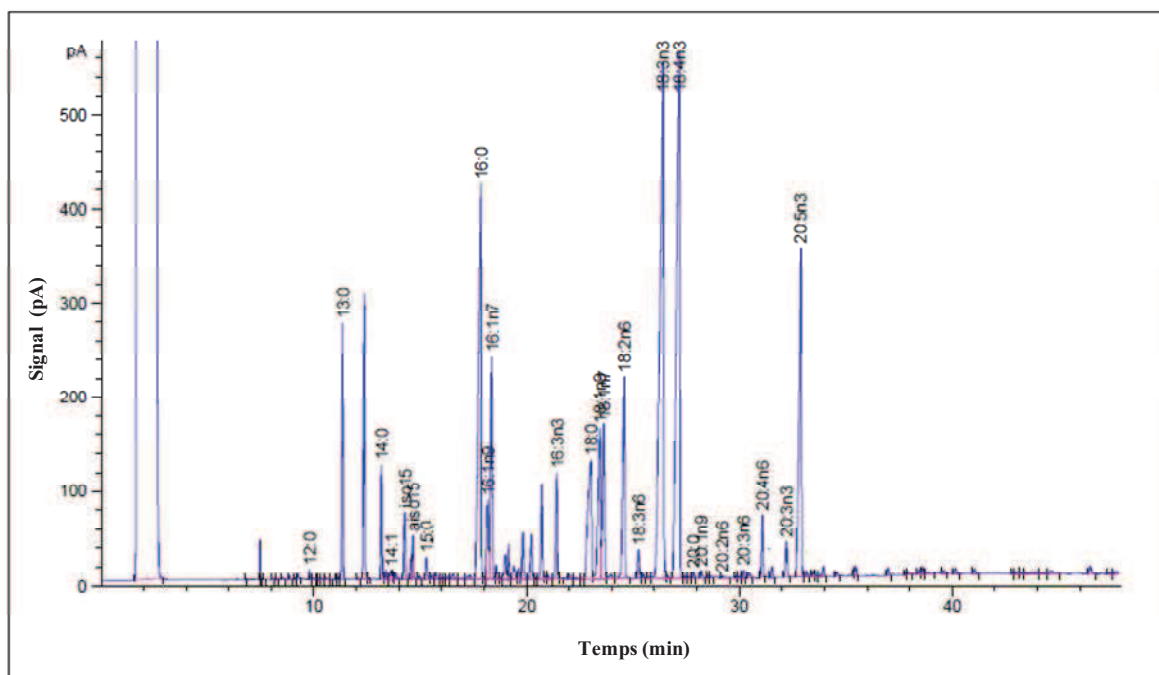


Figure 2.11 : Exemple de chromatogramme (échantillon: *Daphnia longispina*)

## 2. Analyse isotopique

Les échantillons lyophilisés sont broyés en poudre très fine et analysés sur un spectromètre de masse - analyseur de ratio isotopique (IR-MS) couplé à un analyseur élémentaire. Les isotopes stables du carbone et de l'azote des gaz délivrés par l'IR-MS (CO<sub>2</sub> et N<sub>2</sub>) sont analysés, et les résultats sont alors présentés comme la différence relative de rapport isotopique entre l'échantillon et la référence internationale, suivant les équations :

$$\delta^{13}\text{C}_{\text{échantillon}} = \left[ \left( \frac{^{13}\text{C}/^{12}\text{C}_{\text{échantillon}}}{^{13}\text{C}/^{12}\text{C}_{\text{PDB}}} \right) - 1 \right] \times 1,000$$

$$\delta^{15}\text{N}_{\text{échantillon}} = \left[ \left( \frac{^{15}\text{N}/^{14}\text{N}_{\text{échantillon}}}{^{15}\text{N}/^{14}\text{N}_{\text{AIR}}} \right) - 1 \right] \times 1,000$$

Les données obtenues sont corrigées à l'aide d'étalons analytiques définis par l'Agence Internationale de l'Energie Atomique (IAEA), analysés à intervalles réguliers pendant toute la durée des analyses. L'écart type entre deux analyses successives d'étalons est inférieur à 0,2 ‰.

Les échantillons du chapitre 4 ont été analysés sur un IR-MS Finnigan MAT Delta plus (Brême, Allemagne) couplé à un analyseur élémentaire EA 1110 (CE Instruments, Milan, Italy) à l'université de Vienne en Autriche.

Les échantillons du chapitre 5 ont été analysés sur un IR-MS Thermo-Finnigan Delta plus (Brême, Allemagne) couplé à un analyseur élémentaire Carlo Elba (Milan, Italie) au laboratoire « Stable Isotopes In Nature » de l'Université de New Brunswick au Canada.

Dans les deux cas, les conditions opératoires étaient :

- Température de la colonne de combustion : 1050°C
- Température colonne de réduction : 780°C
- Longueur de la colonne CPG (séparation) : 2 m
- Gaz vecteur : Hélium

## 3. Analyse isotopique de composés spécifiques

L'analyse de la composition isotopique des EMAG est réalisée à Davis (Californie) au laboratoire « Stable Isotope Facility » de l'Université de Californie, Etats-Unis. Les EMAG sont séparés sur un chromatographe Trace GC Ultra (Thermo Electron, Milan, Italie) puis injectés dans un spectromètre de masse - analyseur de ratio isotopique Finnigan Delta Plus IRMS (Thermo Electron, Brême, Allemagne) sous les conditions opératoires suivantes :

- Colonne capillaire : SGE Analytical science® BPX70™, L = 30m, Ø=0,25mm, phase stationnaire polaire CB, épaisseur du film = 0,25µm.
- Injecteur : mode SPLITLESS
- Gaz vecteur : Hélium (flux constant : 0,8 ml min<sup>-1</sup>)
- Conditions de température : injecteur = 260°C,
- Programmation de température : de 100°C à 190°C à 4°C min<sup>-1</sup>, 190°C pendant 10 min puis de 190°C à 250°C à 8°C min<sup>-1</sup>, 250°C pendant 5 min.

Les valeurs obtenues sont corrigées à l'aide d'étalons analytiques. Les valeurs sont également corrigées pour le groupement méthyle ajouté lors de la conversion des AG en EMAG suivant la formule :

$$\delta^{13}C_{AG} = [(n+1) (\delta^{13}C_{EMAG}) - \delta^{13}C_{MeOH}]/n$$

où  $\delta^{13}C_{AG}$  est la valeur de  $\delta^{13}C$  de l'AG avant la méthylation,  $\delta^{13}C_{EMAG}$  celle de son ester méthylique,  $\delta^{13}C_{MeOH}$  celle du méthanol utilisé pour la méthylation, et  $n$  le nombre d'atomes de carbone de l'AG. Le  $\delta^{13}C$  du méthanol utilisé est de -46.4 ‰.



## **CHAPITRE III : Variabilité interspécifique des métabolismes lipidiques chez les cladocères**



**Accumulation and bioconversion of polyunsaturated fatty acids by cladocerans: effects of taxonomy, temperature and food sources**

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**Soumis à « Freshwater Biology », en révision**

**I. Abstract**

Herbivorous zooplankton are known to play a key role for transferring dietary nutrients, including polyunsaturated fatty acids (PUFA), from the base to upper trophic levels of aquatic food webs. However, little is known about inter-species differences of herbivorous zooplankton found in the same habitat in conveying PUFA to upper trophic levels. We tested the hypothesis that the taxonomic composition of herbivorous zooplankton species richness affects PUFA retention and subsequent transfer from lower to higher trophic levels. Using laboratory experiments we investigated patterns of PUFA distribution among six freshwater cladocerans (*Ceriodaphnia* sp., *Daphnia longispina*, *D. magna*, *D. pulex*, *Scapholeberis mucronata*, and *Simocephalus vetulus*) exposed to two diets (*Scenedesmus obliquus* and *Cryptomonas* sp.). Moreover, we performed experiments at two different temperatures (14°C and 20°C) to assess the role of temperature in the trophic transfer of PUFA of these consumers. Results show that the variability of PUFA concentrations among these cladocerans was weak and widely controlled by dietary PUFA supply. However, preferential accumulation and/or bioconversion of some PUFA were recorded for all the cladocerans tested. As expected, PUFA concentrations of all cladoceran species were higher at cold temperature, irrespective of diet supply. Nevertheless, we found that the inter-species variability in the ability of PUFA accumulation and bioconversion of cladocerans was more pronounced at the cold temperature (14°C) for both food sources.

**Keywords:** food webs, trophic transfer, trophic up-grading, zooplankton richness



## II. Introduction

Areas with littoral macrophytes are used as nursery and feeding habitats by many fish in freshwater systems (Skov & Koed 2004; Sanchez-Botero *et al.* 2007). The development of larval fish depends then on the availability of nutritionally important compounds, such as polyunsaturated fatty acids (PUFA). Among them, the highly unsaturated FA (HUFA;  $\geq 20$  carbon atoms and  $\geq 3$  double bonds) are crucial for fish development due to their key role in physiological and biochemical processes (Sargent *et al.* 1999). However, because fish, and metazoan in general, cannot biosynthesize PUFA *de novo*, or not at rates sufficient to meet their physiological requirements, they largely depend on PUFA being supplied by their diets.

In aquatic systems, microorganisms such as algae and heterotrophic protists are major producers of PUFA (Brett & Müller-Navarra 1997; Desvillettes & Bec 2009) that can subsequently be retained in consumers and, in general, transferred conservatively in the food web (Koussoroplis *et al.* 2011). As they constitute the major link between microorganisms and species higher in the food web, zooplankton play therefore a key role in the transfer of PUFA to organisms at higher trophic levels, including fish. However, some recent studies have challenged the fact that PUFA are transferred unmodified from one trophic level to another (Müller-Navarra 2006; Persson & Vrede 2006; Smyntek *et al.* 2008). Indeed, these authors have found taxon-specific differences in the PUFA composition of zooplankton, especially between cladocerans and copepods, which were not related to edible sestonic PUFA composition. Ingested PUFA can be retained, metabolized, or, to some extent, converted to another PUFA (a process called “bioconversion”). One can expect that zooplankton, even within the same taxa, e.g. cladocerans, may differ in their PUFA accumulation, bioconversion and subsequent trophic transfer abilities. Therefore, in aquatic ecosystems where zooplankton communities are diversified, such as macrophyte littoral zones (Walseng *et al.* 2006), zooplankton diversity may affect PUFA transfer from microorganisms to higher trophic levels.

Our aim was thus to experimentally investigate changes of PUFA patterns among six cladoceran species exposed to the same pool of dietary FA. These species were fed on two algae differing by their FA composition: *Cryptomonas* sp., a HUFA-rich diet, which allowed us to test the capacity of different cladoceran species to accumulate HUFA, whereas we assessed the bioconversion capacity of these cladoceran species when fed on HUFA-deficient *Scenedesmus*. Moreover, in response to changes in temperature, it was shown that zooplankton retain more unsaturated PUFA at lower temperatures (Schlechtriem *et al.* 2006)

in an effort to maintain high membrane fluidity (Nishida & Murata 1996). We thus chose to perform food experiments at two temperatures as we assumed that these different cladoceran species have different biosynthetic abilities to adjust and maintain their PUFA necessary to survive at low temperatures.

### III. Methods

#### 1. Origin and maintenance of daphnids

The following six *Daphnidae* species were used for the experiments: *Ceriodaphnia* sp., *Daphnia longispina*, *D. magna*, *D. pulex*, *Scapholeberis mucronata* and *Simocephalus vetulus*. For detailed information, please refer to chapter 2.

#### 2. Cultures of autotrophic organisms

*Scenedesmus obliquus* SAG 276-3a and *Cryptomonas* sp. SAG 26.80 were used as food for the six species of *Daphnidae*. Details concerning the phytoplanktonic cultures were given in chapter 2.

#### 3. Experimental setup

Thirty individuals of each zooplankton species were randomly isolated and transferred to glass containers (1 L) in ADaM medium with a 13:11 light: dark cycle for each treatment. Experiments were performed at 14°C and 20°C on two food sources, resulting in a 2 X 2 factorial design for each species, with three replicates per treatments. The two food sources were *Cryptomonas* sp. SAG 26.80, a HUFA-rich diet, and *Scenedesmus obliquus* 276-3, a HUFA-deficient diet (Ahlgren *et al.* 1990; Brett & Müller-Navarra 1997). During the experiments, individuals were transferred every second day to fresh medium and fed every day under non-limiting food conditions (2 mg C L<sup>-1</sup>, i.e., well above the incipient limiting level that is reported to be approximately 0.5 mg C L<sup>-1</sup>; Lampert 1978). The experiments were stopped after 10 days, allowing the renewal of cladoceran fatty acids. All the individuals of

the population were collected, freeze-dried, weighed on a microbalance (Mettler Toledo UMX2 balance  $\pm 1 \mu\text{g}$ ), and subsequently frozen at  $-80^{\circ}\text{C}$  until further analyses.

#### 4. Fatty acid analysis

FA analyses of phytoplankton were performed on 2 mg triplicate samples of particulate organic carbon of *Scenedesmus* and *Cryptomonas* sp. filtered on pre-combusted GF/F filters (Whatman<sup>TM</sup>). At the end of the feeding experiments, FA analyses were performed on zooplankton of each container, i.e. in triplicates for each treatment.

The method used for lipid analysis was fully described in chapter 2. FAME were identified by comparing retention times with those obtained from Supelco<sup>®</sup> standards (37-Component FAME mix, Bacterial FAME Mix) and laboratory standards (Cod liver oil FAMEs) and quantified using internal standards (13:0 and 23:0).

#### 5. Data analysis

Differences in FA concentrations among species as well as FA concentration ratios of cladocerans reared at  $14^{\circ}\text{C}$  and  $20^{\circ}\text{C}$  were analyzed by one-way analysis of variance (ANOVA) and post-hoc comparisons (Tukey's Honestly Significant Difference [HSD],  $\alpha = 0.05$ ). All data were log-transformed prior to analysis to meet the assumptions of normal data distribution.

**Table 3.1 :** Results of ANOVA testing the difference of FA concentrations for six cladoceran species: *Scapholeberis*, *Ceriodaphnia*, *S. vetulus*, *D. longispina*, *D. pulex* and *D. magna* at 14°C and 20°C and testing the difference of FA concentrations between temperatures (14°C/20°C) for these cladoceran species. The results are shown for two different food sources: *Cryptomonas* or *Scenedesmus*.

		<i>Cryptomonas</i>		<i>Scenedesmus</i>	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
SAFA	14°C	$F_{5,12} = 8.4$	$p = 0.001$	$F_{5,12} = 5.1$	$p = 0.012$
	20°C	$F_{5,12} = 4.6$	$p = 0.013$	$F_{5,12} = 1.1$	$p = 0.404$
	14°C/20°C	$F_{5,42} = 3.9$	$p = 0.004$	$F_{5,42} = 8.5$	$p < 0.001$
MUFA	14°C	$F_{5,12} = 3.2$	$p = 0.043$	$F_{5,12} = 6.7$	$p = 0.004$
	20°C	$F_{5,12} = 7.3$	$p = 0.002$	$F_{5,12} = 2.9$	$p = 0.061$
	14°C/20°C	$F_{5,42} = 3.2$	$p = 0.013$	$F_{5,42} = 8.9$	$p < 0.001$
PUFA	14°C	$F_{5,12} = 2.1$	$p = 0.128$	$F_{5,12} = 2.2$	$p = 0.121$
	20°C	$F_{5,12} = 2.6$	$p = 0.079$	$F_{5,12} = 0.5$	$p = 0.768$
	14°C/20°C	$F_{5,42} = 2.1$	$p = 0.073$	$F_{5,42} = 3.9$	$p = 0.005$
LIN	14°C	$F_{5,12} = 1.7$	$p = 0.198$	$F_{5,12} = 1.7$	$p = 0.200$
	20°C	$F_{5,12} = 1.8$	$p = 0.183$	$F_{5,12} = 1.7$	$p = 0.195$
	14°C/20°C	$F_{5,42} = 2.4$	$p = 0.050$	$F_{5,42} = 6.0$	$p < 0.001$
ALA	14°C	$F_{5,12} = 1.8$	$p = 0.178$	$F_{5,12} = 2.9$	$p = 0.064$
	20°C	$F_{5,12} = 2.0$	$p = 0.144$	$F_{5,12} = 0.4$	$p = 0.806$
	14°C/20°C	$F_{5,42} = 2.2$	$p = 0.061$	$F_{5,42} = 3.6$	$p = 0.007$
SDA	14°C	$F_{5,12} = 2.2$	$p = 0.120$	$F_{5,12} = 2.4$	$p = 0.101$
	20°C	$F_{5,12} = 3.1$	$p = 0.047$	$F_{5,12} = 0.2$	$p = 0.948$
	14°C/20°C	$F_{5,42} = 3.7$	$p = 0.006$	$F_{5,42} = 2.5$	$p = 0.040$
ARA	14°C	$F_{5,12} = 5.5$	$p = 0.007$	$F_{5,12} = 11.2$	$p < 0.001$
	20°C	$F_{5,12} = 2.6$	$p = 0.077$	$F_{5,12} = 7.3$	$p = 0.002$
	14°C/20°C	$F_{5,42} = 3.2$	$p = 0.014$	$F_{5,42} = 15.9$	$p < 0.001$
EPA	14°C	$F_{5,12} = 3.2$	$p = 0.041$	$F_{5,12} = 7.1$	$p = 0.003$
	20°C	$F_{5,12} = 2.5$	$p = 0.082$	$F_{5,12} = 3.9$	$p = 0.026$
	14°C/20°C	$F_{5,42} = 1.6$	$p = 0.164$	$F_{5,42} = 11.7$	$p < 0.001$

## IV. Results

### 1. PUFA of phytoplankton

*Scenedesmus* was rich in C18-PUFA, with  $\alpha$ -linolenic acid (ALA, 18:3 $\omega$ 3) and linoleic acid (LIN, 18:2 $\omega$ 6) concentrations being the most abundant PUFA. However, HUFA were not detected in *Scenedesmus*. As was the case for *Scenedesmus*, *Cryptomonas* sp. was also rich in C18-PUFA concentrations, especially in ALA and stearidonic acid (SDA, 18:4 $\omega$ 3). However, PUFA concentrations differed from those of *Scenedesmus* by the presence of SDA, EPA, and DHA (Fig. 3.1).

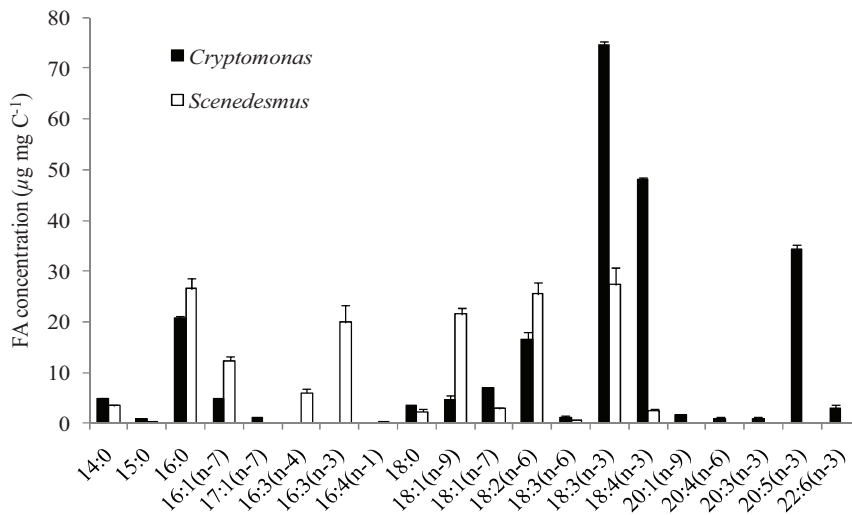


Figure 3.1 : Fatty acid concentrations (+SD) of *Scenedesmus* and *Cryptomonas*.

### 2. Fatty acid concentrations of cladocerans fed on *Cryptomonas*

No significant difference in the concentrations of LIN, ALA, SDA, and total PUFA were determined among the six cladocerans species fed on *Cryptomonas* at both temperatures (Table 3.1; Fig. 3.2). However, significant differences in SAFA and MUFA concentrations were found among these species. For ARA and EPA, concentrations were not significantly different when species were reared at 20°C, but significantly different when reared at 14°C

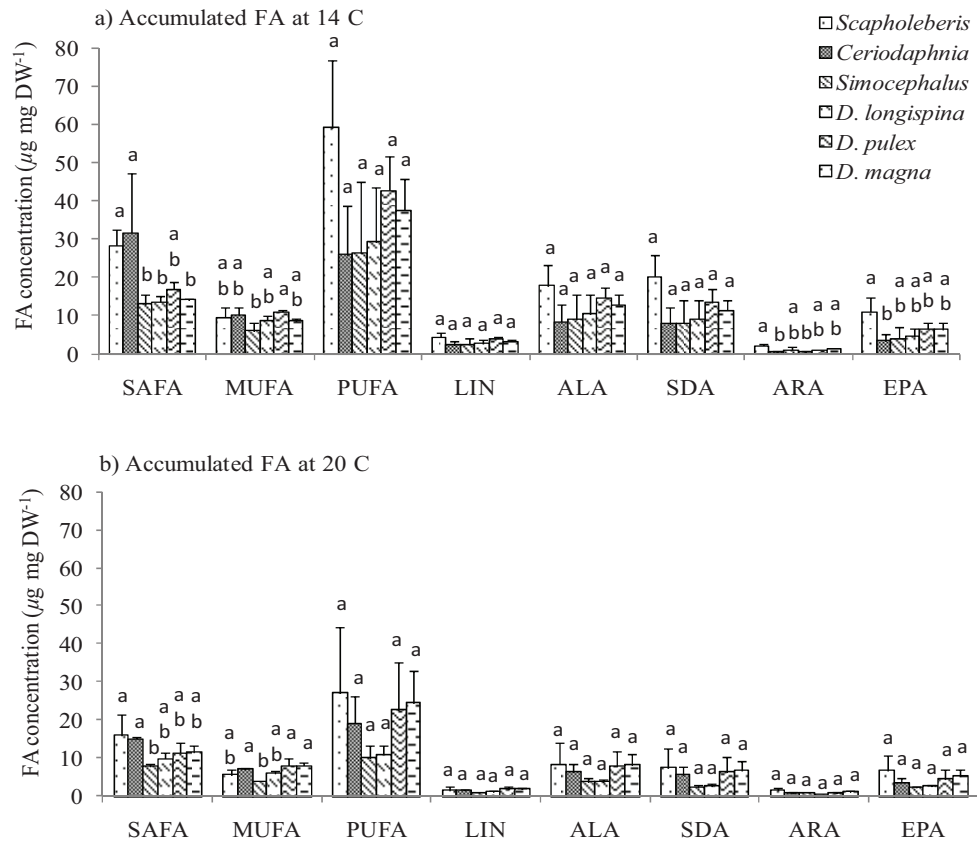
(Table 3.1). At this lower temperature, *Scapholeberis* had indeed the highest ARA and EPA concentrations (Fig. 3.2). In *Cryptomonas*, SDA concentrations were up to 19 x higher than in *Scenedesmus*. However, SDA concentrations of cladocerans feeding on *Cryptomonas* were only 4-12 x higher at 14°C and 5-11 x higher at 20°C than those detected in cladocerans feeding on *Scenedesmus*.

### 3. Fatty acid concentrations of cladocerans fed on *Scenedesmus*

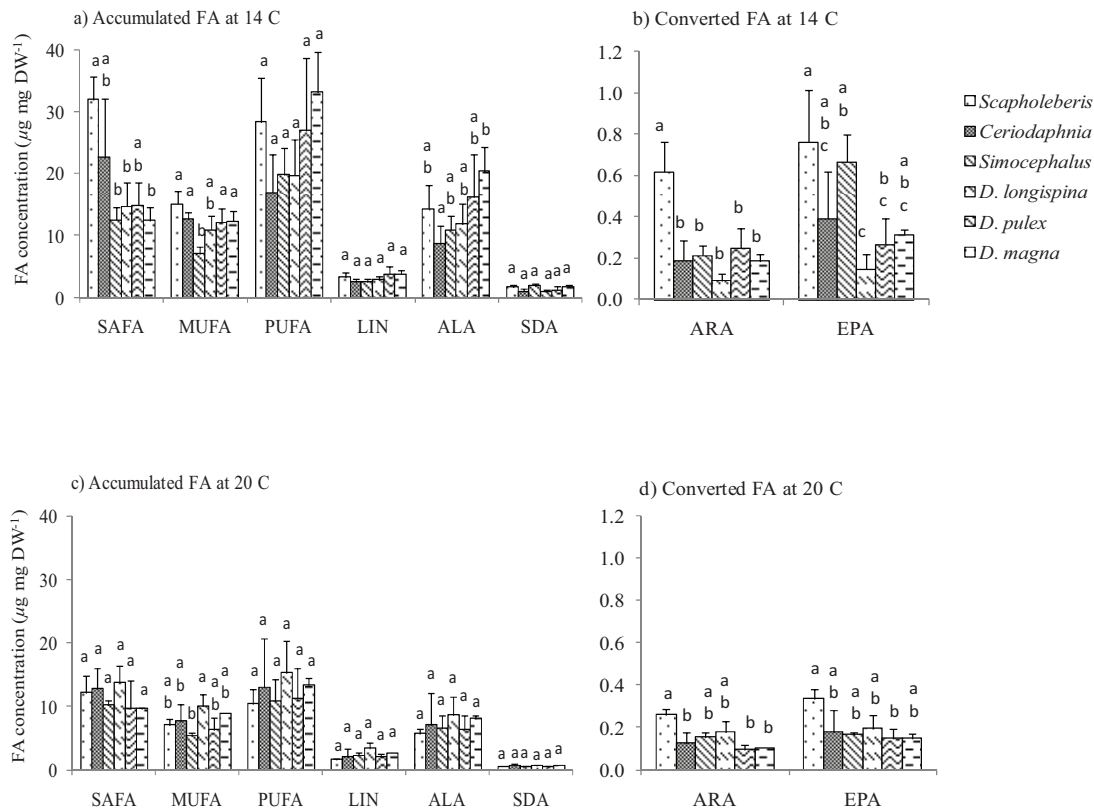
At both temperatures, concentrations of LIN, SDA, and total PUFA were not significantly different among species, but slight differences were recorded between MUFA concentrations (Table 3.1; Fig. 3.3). Total SAFA and ALA concentrations were not different at 20°C, but some differences were recorded among species reared at 14°C (Table 3.1; Fig. 3.3). Although EPA and ARA were not detected in *Scenedesmus*, these two FA were present in the six cladocerans species fed on *Scenedesmus*. However, ARA and EPA concentration were significantly different among species at both temperatures (Table 3.1). *Scapholeberis* exhibited the highest concentrations of ARA and EPA at both temperatures (Fig. 3.3).

### 4. Effect of temperature on fatty acid concentrations of cladocerans

Except for LIN, ARA, and EPA concentrations of *D. longispina* fed on *Scenedesmus* and ARA concentrations of *Ceriodaphnia* fed on *Cryptomonas*, all FA concentration ratios based on the two temperatures ( $FA_{14^{\circ}C}/FA_{20^{\circ}C}$ ) were  $>1$  indicating that cladocerans retained more FA at the colder temperature (Fig. 3.4). However, differences of FA concentrations between 14°C and 20°C varied according to FA, cladocerans species, and food sources. When cladocerans were fed on *Cryptomonas*, differences of LIN, ALA, EPA, and PUFA accumulation between 14°C and 20°C were not significantly different among the cladoceran species. Some slight differences were detected among SAFA, MUFA as well as SDA and ARA concentrations between the two temperatures. When cladocerans were fed on *Scenedesmus*, significant differences of FA concentrations between 14°C and 20°C were recorded for all the FA and FA groups (Fig. 3.4).



**Figure 3.2 :** Fatty acids (FA) concentrations at a) 14°C and b) 20°C by cladoceran species fed on *Cryptomonas*. Data are mean concentrations + SD for three replicates per treatment. Distinct letters for the comparisons of each FA indicate a significant difference among cladoceran species based on a one-way ANOVA with a Tukey’s HSD tests at  $\alpha = 0.05$ .



**Figure 3.3 :** Fatty acids (FA) concentrations at a) 14°C and c) 20°C and FA conversion at b) 14°C and d) 20°C by cladoceran species fed on *Scenedesmus*. Data are mean concentrations + SD for three replicates per treatment. Distinct letters for the comparisons of each FA indicate a significant difference among cladoceran species based on a one-way ANOVA with a Tukey's HSD tests at  $\alpha = 0.05$ .



## V. Discussion

Knowing how zooplankton species vary in their ability to modify their PUFA profiles relative to their dietary PUFA is an ecologically important, yet still unresolved research question. Whatever the cladoceran species and the temperature considered, our results showed that cladoceran PUFA composition exhibited a marked dietary influence. The high concentrations of ALA, SDA, and EPA of cladocerans fed on *Cryptomonas* (Fig. 3.1) clearly reflected the PUFA profile of their food source (Fig. 3.2). The same observation can be drawn for cladocerans fed on *Scenedesmus* that contained high ALA concentrations (Fig. 3.1 and 3.3). There is laboratory evidence that PUFA patterns of *Daphnia* generally match those of their dietary supply (Brett *et al.* 2006), however, with some as yet poorly understood intrinsic ability of *Daphnia* to modify dietary PUFA (Weers *et al.* 1997; von Elert 2002). Our study, performed on six cladoceran species fed during ten days with the same monospecific food source, corroborates these studies by showing that cladocerans accumulated dietary PUFA and, in addition, exhibit non-dietary HUFA such as ARA and EPA.

The retention of non-dietary HUFA in all of these studied cladocerans clearly suggested that these species are able to regulate, in part, their PUFA through bioconversion. Our results show that even if small DHA concentrations were detected in dietary *Cryptomonas* (Fig. 3.1), this HUFA was never detected in these cladocerans (Fig. 3.2). Similar results on the inability of daphnids to accumulate DHA were found in field studies (Persson & Vrede 2006; Smyntek *et al.* 2008; Kainz *et al.* 2009) and through laboratory supplementation experiments (Weers *et al.* 1997; von Elert 2002). Moreover, even if the green alga *Scenedesmus* lacks HUFA (Fig. 3.1), these six cladocerans fed on *Scenedesmus* contained ARA and EPA (Fig. 3.3). This indicates that these daphnids are able to bioconvert dietary LIN and ALA into ARA and EPA, probably through the successive use of  $\Delta 6$  and  $\Delta 5$  desaturases and elongases (Weers *et al.* 1997; von Elert 2002; Bec *et al.* 2003). In contrast to previous single-species lab experiments, our results provide experimental evidence that *Ceriodaphnia* sp., *Daphnia longispina*, *D. magna*, *D. pulex*, *Scapholeberis mucronata*, and *Simocephalus vetulus*, and perhaps cladocerans in general, may be all able to convert some dietary FA into EPA. This strongly suggests that cladocerans may be able to trophically upgrade a poor quality food source by producing EPA, an important PUFA that supports life history traits of cladocerans (Müller-Navarra *et al.* 2000; von Elert 2002). Finally, the variability of PUFA concentrations was lower in daphnids than in dietary phytoplankton. Lipids of cladocerans fed *Cryptomonas*, in

which SDA was up to 19X higher than in *Scenedesmus* (Fig. 3.1), were only 4-12X enriched in SDA compared to those of cladocerans fed on *Scenedesmus* (Fig. 3.2 and 3.3), ALA concentrations were 3X higher in *Cryptomonas* than in *Scenedesmus* (Fig. 3.1), but quite similar in cladocerans feeding on *Cryptomonas* and on *Scenedesmus* (Fig. 3.2 and 3.3). For cladocerans fed on *Scenedesmus*, one can expect that the lack of HUFA could thus be compensated by active accumulation of C18-PUFA.

In field studies, some authors have failed to find correlations between the PUFA compositions of zooplankton and their food sources, and suggested that the PUFA composition of zooplankton could be taxonomically regulated (Persson & Vrede 2006; Smyntek *et al.* 2008). However, field studies make it difficult, if at all possible, to clearly separate assimilated diet in zooplankton. Here, we assessed interspecific variability of cladocerans in the accumulation and bioconversion of PUFA under controlled experimental conditions. In general, our results showed low variability of PUFA concentrations among these six non selective zooplankton species when exposed to the same dietary PUFA pool. However, differences of PUFA accumulation were observed when cladocerans were fed with *Scenedesmus* at 14°C, with highest variability of dietary ALA concentrations among species (Fig. 3.3). Moreover, dietary *Scenedesmus* caused some differences of PUFA bioconversion abilities among species. Among all species tested, *Scapholeberis* accumulated ARA and EPA most efficiently, which also indicates that *Scapholeberis* can most efficiently bioconvert precursors to ARA and EPA at both temperatures (Fig. 3.3). These results suggest that *Scapholeberis* is able to regulate its PUFA composition better than the other cladoceran species.

In a general way, it is important to note that the interspecific variability of PUFA accumulation and bioconversion was more pronounced at the cold temperature for both food sources. One of the important roles of PUFA is to help maintaining membrane fluidity at low temperatures (Nishida & Murata 1996). Temperature is thus an important factor affecting PUFA concentrations (Farkas & Herodek 1964; Schleichriem *et al.* 2006) which tend to increase with decreasing temperatures in all of these tested cladocerans (see also: (Farkas & Herodek 1964; Jiang & Gao 2004; Schleichriem *et al.* 2006). Furthermore, the accumulation and bioconversion of some PUFA such as LIN, ALA, SDA, ARA, and EPA was on average higher at the colder temperature on both food sources (Fig. 3.4). However, interspecific differences in the abilities to metabolically regulate PUFA concentrations at the colder temperature were recorded, which may reflect inter-species differences of temperature

adjustment that may consequently improve survival success at lower temperatures. When individuals were fed with *Scenedesmus*, the difference of PUFA concentrations between the two temperatures was larger for *Simocephalus* and *Scapholeberis*, especially with respect to their SDA and EPA concentrations (Fig. 3.4). *Simocephalus* and *Scapholeberis* are two littoral cladocerans and as such can be exposed to substantial temperature variations. These results may suggest that littoral cladocerans have developed metabolic strategies to regulate their PUFA content to overcome constraints of their environment, as was proposed by Lemke & Benke (2003), suggesting that these two species could be more adapted to cold temperatures.

In conclusion, our study showed that all the cladocerans tested showed some abilities to regulate their PUFA concentrations through bioconversion, and are therefore able to trophically upgrade a poor food quality. Using controlled experimental conditions, this study test interspecific ability and variability of PUFA accumulation and bioconversion when different cladoceran species were exposed to the same dietary FA pool. We demonstrate that the variability among species increases with decreasing temperatures. Such differences of PUFA regulation are likely to depend on nutritional requirements and biochemical limitation of each species in response of constraints of their feeding habitats. However, for warmer temperature, cladocerans species tested show only minor differences in their PUFA accumulation and bioconversion. In littoral macrophytes zones, the high zooplankton species diversity leads to a trophic niche partitioning. In addition to suspended particulate organic matter, zooplankton can potentially feed on different diet sources, including benthic, epiphytic and neustonic diets that may differ in their biochemical/lipid quality. Hence, in heterogeneous feeding habitats such as macrophytes zones where these cladocerans often co-exist, foraging behavior of cladoceran species more than differences of metabolism may be crucial for determining PUFA transfer to upper trophic levels.

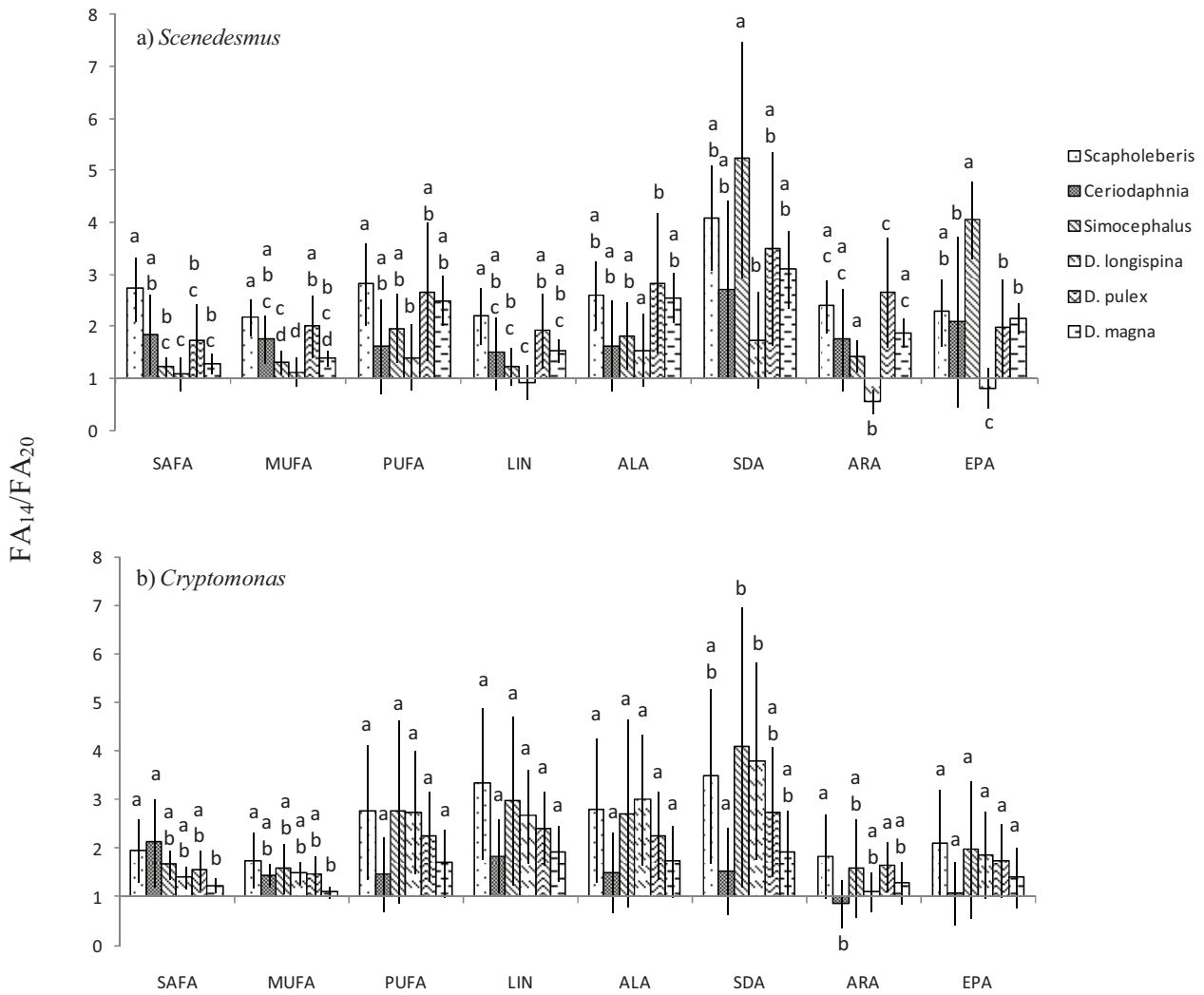


Figure 3.4 : Fatty acids concentrations ( $\mu\text{g mg DW}^{-1}$ ) ratios of cladocerans reared at 14°C (FA<sub>14</sub>) and 20°C (FA<sub>20</sub>) on, a) *Scenedesmus*, and, b) *Cryptomonas*. The error bars represent  $\pm$  SD. Bars labeled with the same letters are not significantly different (Tukey's HSD,  $\alpha = 0.05$ ). Values >1 represent increased accumulation of the respective fatty acid in cladocerans fed at 14°C compared to cladocerans fed at 20°C.



**CHAPITRE IV : Importance quantitative et qualitative du  
périphyton pour les cladocères d'une zone littorale à  
macrophytes**



## Préambule

Les microcrustacés représentent un maillon clef entre les microorganismes eucaryotes, principaux producteurs d'AGPI, et les niveaux trophiques supérieurs. Dans les milieux abritant une forte diversité de microcrustacés, tels que les zones littorales à macrophytes, nous avons émis l'hypothèse que la diversité des cladocères pouvait induire une variabilité du transfert des AGPI dans le réseau trophique. Afin de répondre à cette question, nous avons évalué, dans un premier temps, l'existence possible d'une variabilité interspécifique de la capacité d'accumulation et de bioconversion des AGPI. Cette étude menée en conditions contrôlées (chapitre 3), nous a montré qu'il n'existe pas de différences inter-espèces de composition en AGPI chez les six espèces de cladocères testées et donc, très probablement, chez les cladocères de manière générale, lorsqu'ils reçoivent les mêmes apports alimentaires. Puisque le métabolisme lipidique des cladocères ne semble pas varier d'une espèce à une autre, c'est le comportement alimentaire et la niche trophique de ces espèces qui pourraient être le principal déterminant du transfert des AGPI vers les niveaux trophiques supérieurs.

En effet, la séparation des niches trophiques est un des éléments qui permet la coexistence et le maintien d'une forte richesse spécifique dans un milieu (Chesson 2000; Tews *et al.* 2004). Dans les zones littorales, outre le seston, les biofilms périphytiques et neustoniques représentent des sources de nourriture potentielles pour les microcrustacés. Cette diversité des compartiments trophiques pourrait alors s'accompagner d'une diversité des apports en AGPI.

Dans les environnements peu profonds, le périphyton peut représenter 80 à 98% de la production primaire autochtone (Vadeboncoeur *et al.* 2003) et, à ce titre, fait l'objet d'un intérêt particulier depuis quelques années (Herwig *et al.* 2004; Rautio & Warwick 2006; Siehoff *et al.* 2009). Cependant, s'il est largement accepté que les cladocères de la famille des *Chydoridae* sont capables d'utiliser cette source de nourriture, et si Siehoff *et al.* (2009) ont récemment montré que *Daphnia magna* pouvait également se nourrir de périphyton, nous avons finalement assez peu de données sur le transfert de cette source de nourriture vers les niveaux trophiques supérieurs.

Dans la première partie de ce chapitre, nous avons donc étudié à travers une approche expérimentale en conditions simplifiées, la capacité de trois espèces de cladocères fréquemment rencontrées en zone littorale à exploiter le périphyton. Dans la deuxième partie,



l'analyse isotopique de quatre espèces de cladocères, ainsi que du seston et de l'épiphyton prélevés, nous a permis d'apprécier les interactions trophiques pouvant exister en milieu naturel entre ces sources de nourriture potentielles et les microcrustacés. De plus, nous avons déterminé la composition lipidique du seston et de l'épiphyton afin d'évaluer les apports en AGPI correspondant à chacun de ces compartiments trophiques.

## **Trophic partitioning among three littoral microcrustaceans: relative importance of periphyton as food resource**

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### **I. Abstract**

The high species richness of zooplankton communities in macrophytes littoral zones could result from the diversity of potential trophic niches found in such environment. In macrophytes littoral zones, in addition to phytoplankton, neustonic, benthic and epiphytic biofilms can also be potential components of the microcrustacean diet. Here, we investigated the ability of three large cladocerans: *Daphnia longispina*, *Simocephalus vetulus* and *Eurycerus lamellatus*, to develop on periphyton as their only food source or as a complement to a phytoplankton resource in scarce supply. *D. longispina* exhibited a very low growth and reproduction rates on the periphytic resource and as *S. vetulus* seems unable to scrape on periphyton. In contrast, *E. lamellatus* could not grow on phytoplankton, and appears to be an obligatory periphyton scraper. This latter finding contrasts with previous studies suggesting that periphyton could only be a complementary resource for some cladocerans. These differences in feeding strategy probably reflect the different trophic niches occupied by these three species in macrophytes littoral zones, and may explain at least in part their ability to coexist in the same environment.

Keywords: feeding behavior, food resources, trophic niches, periphyton

## II. Introduction

Trophic partitioning is a key element underlying the stable coexistence of competitors and the maintenance of species diversity in a given environment (Chesson 2000; Levine & HilleRisLambers 2009). Macrophytes littoral zones sustain a wide range of different life forms, ranging from those attached to surfaces to those that swim or float freely in the water column. Littoral or semilittoral species are major contributors to microcrustacean diversity, with about 75% of the zooplankton species inventoried in lakes being classified as littoral species (Walseng *et al.* 2006). In littoral zones, aquatic macrophytes play an important role in increasing the habitat complexity of the waterscape, thus providing more potential ecological niches. Indeed, zooplankton trophic niches are much more diversified in macrophytes littoral zones than in the pelagic environment, and, in addition to planktonic particles, they also contain neustonic, benthic, and epiphytic biofilms, which may be potential components of the microcrustacean diet.

Periphyton can make up a considerable proportion of the total primary production of lakes, especially in shallow lakes, where it can be responsible for 80-98% of primary production (Vadeboncoeur *et al.* 2003). However, little is known about the role of periphytic production in the food web dynamics of such an environment (Karlsson *et al.* 2009; Siehoff *et al.* 2009). Hitherto, the only cladocerans known to exploit periphytic biofilms were *Chydorideae* species (van de Bund *et al.* 1994). However in a recent study, Siehoff *et al.* (2009) show that *Daphnia magna* is also able to feed on periphytic resources when phytoplankton is scarce. A broader understanding of how the different food sources are used by littoral microcrustaceans helps to elucidate how such large numbers of zooplankton species can co-exist in the same environment. In this study, we conducted laboratory experiments involving three species of cladocerans found in littoral habitats: *Daphnia longispina*, *Simocephalus vetulus* (*Daphniidae*) and *Eurycercus lamellatus* (*Chydorideae*), which were fed with three food sources: periphyton, phytoplankton, and a mixture of periphyton and phytoplankton. The aim of this study was to investigate how common periphyton scraping is as mode of feeding among cladocerans in littoral zones.

### III. Material and methods

#### 1. Origin of the planktonic food source

*Scenedesmus obliquus* SAG 276-3a was used in this study. Details concerning the phytoplanktonic cultures were given in chapter 2.

#### 2. Origin of the periphytic food source

Four months before the experiments, glass rods (about 16 cm<sup>2</sup>) were immersed in a backwater of the Allier river (from February to May) to allow attached algae and other microorganisms to colonized them. Before they were placed in the experimental beakers, they were lightly rinsed in filtered river water in order to remove any particles likely to come off the rod and become planktonic. Moreover, as periphyton can be patchy in its distribution, glass rods having a homogeneous distribution of periphyton and a similar appearance were choosed. We thus expected that quantity and quality of periphyton were the same in all beakers.

#### 3. Origin and maintenance of zooplankton species

*Daphnia longispina*, *Simocephalus vetulus* and *Eurycercus lamellatus*, three large microcrustaceans found in littoral habitats, were used for this experiment. All three species were collected from a backwater sampling site of the Allier River, where they dominate cladoceran community.

For detailed information, please refer to chapter 2.

#### 4. Experimental set-up

##### a) *Somatic growth rate experiment*

*D. longispina*, *S. vetulus* and *E. lamellatus* were used for this experiment. One clone of each cladoceran species was isolated. The clones were then transferred to 1L containers containing 50% filtered river water (<0.2  $\mu\text{m}$ ) and 50% ADaM medium, and fed with *Scenedesmus ad libitum* every day. Rods on which periphyton had grown were added to the *E. lamellatus* containers. When the females produced offspring, the neonates (first generation) were separated, and the mothers removed. This step was repeated, and the second generation kept. After they had released their first clutch, females from the second generation were kept and the neonates removed. The individuals used in the experiments were third-brood offspring obtained from the second generation in order to limit variability due to maternal size and weight (Lampert 1993). Neonates were collected about 12 h after they had been released, and randomly placed in 200-mL beakers (10 individuals per beaker). Experiments were performed on four food sources with three replicates of each treatment. The four food sources used were: *Scenedesmus* at a nonlimiting concentration (2 mg C L<sup>-1</sup>, i.e. well above the incipient limiting level that is reported to be approximately 0.5 mg C L<sup>-1</sup>; Lampert 1978) (S<sup>+</sup>), *Scenedesmus* at a limiting concentration (0.3 mg C L<sup>-1</sup>) (S<sup>-</sup>), *Scenedesmus* at a limiting concentration (0.3 mg C L<sup>-1</sup>) plus periphyton (one rod) (S+P), and periphyton alone (two rods) (P). During the experiments, individuals were transferred into clean water and fed every day. The periphyton rods used in the different treatments were changed every day.

To determine the average initial dry weight ( $W_0$ ), randomly selected neonates were transferred into tared aluminum containers (two samples of 30 neonates for both *D. longispina* and *S. vetulus*, and two samples of 20 neonates for *E. lamellatus*), dried overnight at 60°C, cooled in a desiccator, and weighed on an electronic balance (Mettler Toledo UMX2 balance  $\pm 1 \mu\text{g}$ ). The experiments were stopped when the females reached maturity, which took between 4 and 7 days for *D. longispina*, between 6 and 9 days for *S. vetulus*, and between 6 and 7 days for *E. lamellatus*, depending on feeding conditions. Individuals were collected, the clutch size was measured (eggs per individual), and females were dried overnight at 60°C and then weighed to obtain the average individual weight per replicate ( $W_t$ ). Somatic growth rates (g) were calculated as

$$g = (\ln W_t - \ln W_0) / t$$

where  $t$  is the duration of each experiment in days, as somatic growth rate have been shown to be a good measure of cladoceran fitness (Lampert & Trubetskova 1996).

*b) Population experiment*

*D. longispina* and *E. lamellatus* were used in this experiment. For each of these two cladoceran species, 10 matures females who have just released their clutch were selected, and placed in 300 mL beakers. Experiments were performed at 18°C on three food sources, with two replicates for each treatment. The three food sources were: *Scenedesmus* at a non-limiting concentration (S) (2 mg CL<sup>-1</sup>), *Scenedesmus* at a limiting concentration (0.3 mg C L<sup>-1</sup>) plus periphyton (one rod) (S+P), and periphyton alone (two rods) (P).

During the experiments, individuals were fed every day; they were transferred to clean water every two days. The periphyton rods used for the different treatments were changed every two days. The experiment lasted 8 days. The individuals were counted every two days to allow us to determine changes in population size for each treatment.

## 5. Data analysis

In the somatic growth rate experiment, the effects of food source on somatic growth rate and clutch size of *D. longispina*, *S. vetulus*, and *E. lamellatus* were analyzed by a one-way ANOVA ( $\alpha < 0.05$ ). Pairwise comparisons were made with a *post hoc* test (Tukey's Honestly Significant Difference [HSD]) with Bonferroni adjustment ( $\alpha < 0.008$ ).

In the population growth rate experiment, the effects of food source on the population abundance of *D. longispina* and *E. lamellatus* at the end of the experiment were analyzed by one-way analysis of variance (ANOVA,  $\alpha < 0.05$ ). Pairwise comparisons were made with a *post hoc* test (Tukey's HSD) with Bonferroni adjustment ( $\alpha < 0.017$ ).

## IV. Results

### 1. Somatic growth rate experiment

#### a) *Effects of food sources on D. longispina, S. vetulus, and E. lamellatus clutch size*

For *D. longispina* and *S. vetulus*, the clutches of females fed on *Scenedesmus* at a non-limiting concentration ( $S^+$ ) were significantly larger than those of females fed on periphyton alone (P) (Fig. 4.1, Table 4.1). In spite of their intermediate values, the clutch sizes obtained for the treatments with *Scenedesmus* at a limiting concentration with or without periphyton ( $S+P$  and  $S^-$  respectively) were not significantly different from those for the other two treatments. However, even if the differences were not significant, clutch sizes were slightly larger for *S. vetulus* fed on the  $S+P$  mixture than for those of *S. vetulus* fed on  $S^-$ .

For *E. lamellatus*, maturity was reached only with the two treatments containing periphyton (Fig. 4.1). The clutch sizes for these two treatments were not significantly different, although the clutches of individuals fed on P appeared to be slightly larger (Table 4.1).

#### b) *Effects of food sources on the growth rates of D. longispina, S. vetulus, and E. lamellatus*

For *D. longispina*, the highest growth rates were recorded when the females were fed on  $S^+$ , and the lowest ones when the females were fed on P (Fig. 4.1, Table 4.1). Intermediate growth rates were recorded for the females fed on  $S^-$  and on  $S+P$ .

As for *D. longispina*, the lowest growth rates of *S. vetulus* were recorded when individuals were fed on P (Fig. 4.1, Table 4.1), but for this species, the  $S+P$  mixture produced the highest growth rates. The intermediate values obtained with the two concentrations of *Scenedesmus* did not differ significantly.

All the *E. lamellatus* individuals fed on *Scenedesmus* as their only food source died after 8 days on  $S^+$ , and after 14 days on  $S^-$  (Fig. 4.1). The individuals reached maturity on both the treatments containing periphyton, and growth rates on these treatments were the same (Table 4.1).

**Table 4.1 :** Results of one-way ANOVA testing the effect of food sources on *D. longispina*, *S. vetulus* and *E. lamellatus* clutch size and growth rate in the first experiment

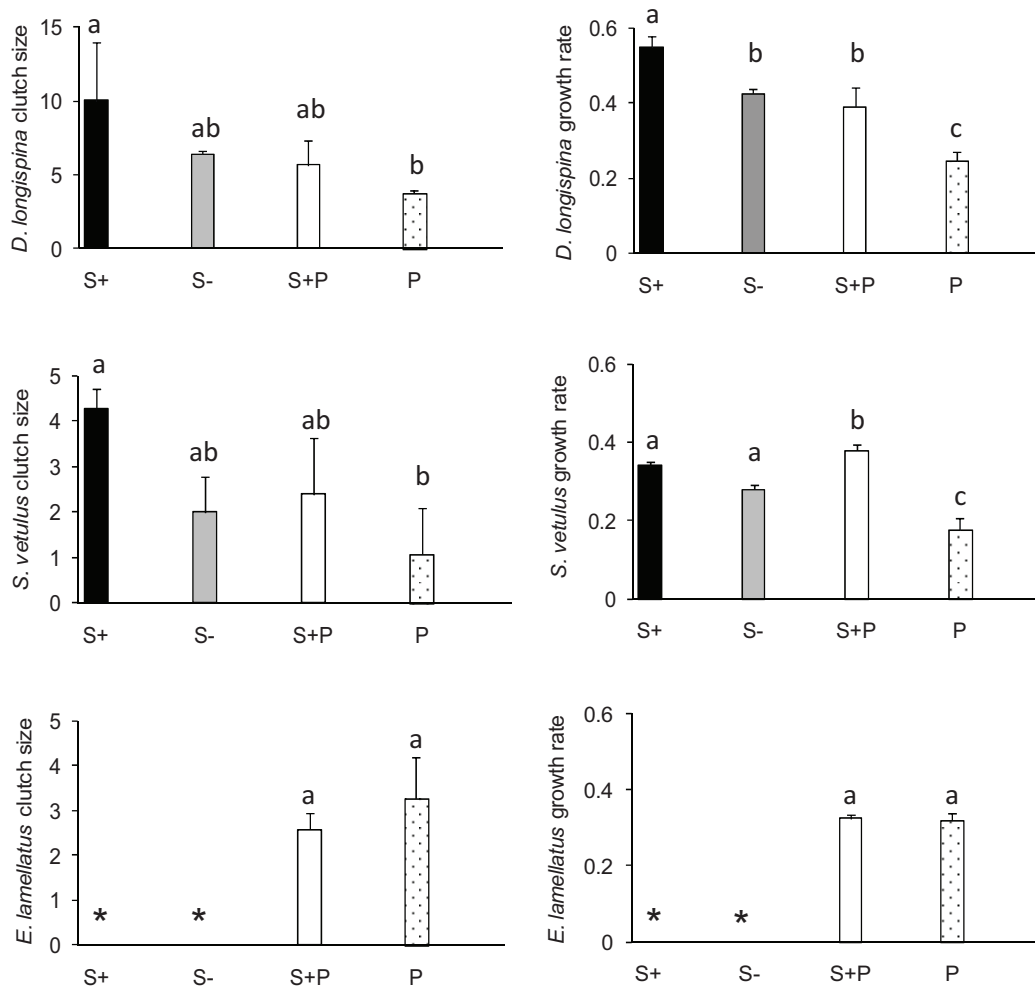
	<i>D. longispina</i>		<i>S. vetulus</i>		<i>E. lamellatus</i>	
	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value
Clutch size	$F_{3,8} = 4.398$	$p = 0.042$	$F_{3,8} = 6.296$	$p = 0.017$	$F_{3,8} = 36.46$	$p < 0.0001$
Growth rate	$F_{3,8} = 45.399$	$p < 0.0001$	$F_{3,8} = 81.986$	$p < 0.0001$	$F_{3,8} = 741.86$	$p < 0.0001$

## 2. Population growth rate experiment

For *D. longispina* and for *E. lamellatus*, the number of individuals increased until the end of the experiment whatever the food sources (Fig. 4.2). However, in the case of *D. longispina*, at the end of the experiment (d8), there were more individuals in the populations fed on *Scenedesmus* (326.5 individuals) than in the populations fed on periphyton (67 individuals) (Tukey's HSD,  $p=0.009$  following ANOVA,  $F_{2,6}=19.43$ ;  $p=0.019$ ). There are no significant differences between the number of individuals fed on *Scenedesmus* and on the mixture of *Scenedesmus* plus periphyton (263 individuals) (Tukey's HSD,  $p=0.240$  following ANOVA,  $F_{2,6}=19.43$ ;  $p=0.019$ ), and between the number of individuals fed on periphyton and on the mixture of *Scenedesmus* plus periphyton (Tukey's HSD,  $p=0.02$  following ANOVA,  $F_{2,6}=19.43$ ;  $p=0.019$ ). However, for these two last treatments, the population of *D. longispina* apparently grew better on the mixture of *Scenedesmus* plus periphyton than on periphyton alone.

For *E. lamellatus*, the lowest population abundance at the end of the experiment was obtained when individuals were fed on *Scenedesmus* alone (21.5 individuals) (Tukey's HSD, between P and S  $p=0.008$ , and between S and S+P  $p=0.015$  following ANOVA,  $F_{2,6}=22.678$ ;  $p=0.015$ ; Fig. 4.2). The number of individuals was higher in the presence of periphyton, whether alone (64.5 individuals) or as a complement with *Scenedesmus* (55 individuals). Although there were slightly more individuals on the treatment with periphyton alone, there was no significant difference between the number of individuals for the last two treatments (Tukey's HSD,  $p=0.252$  following ANOVA,  $F_{2,6}=22.678$ ;  $p=0.015$ ).





**Figure 4 1 :** Effects of food resource on clutch size (on the left) and growth rate (on the right) of *Daphnia longispina*, *Simocephalus vetulus* and *Eurycercus lamellatus*. Data are means  $\pm$  SD on three replicates per treatments. On treatments labeled with \* all the individuals died after a few days. Bars labeled with the same letters are not significantly different (Tukey's HSD,  $p < 0.008$ )

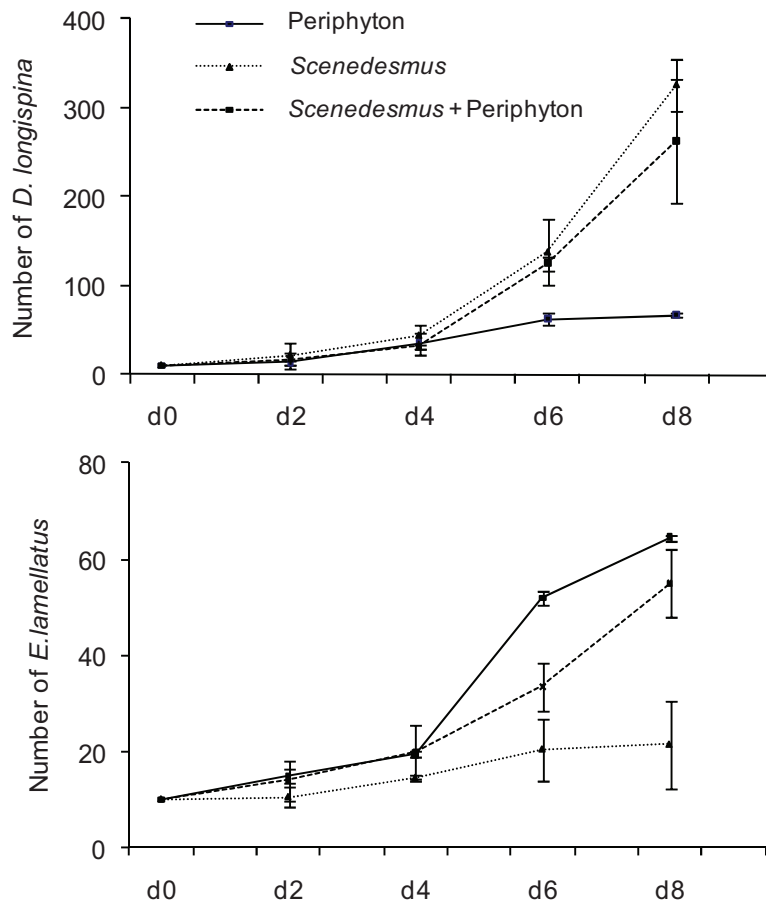


Figure 4.2 : Evolution of population size (mean values  $\pm$ SD) of *Daphnia longispina* and *Eurycerus lamellatus* fed on three food sources: *Scenedesmus*, periphyton and a mixture of *Scenedesmus*+periphyton

## V. Discussion

In this study, we aimed to assess how common periphyton scraping is as a mode of feeding among three large cladocerans of littoral zones. Indeed, we expected that differences in food resources use could be a factor of maintenance of species diversity in natural environment. Moreover, as periphytic production could be responsible for the main proportion of primary production in shallow environment (Vadeboncoeur *et al.* 2003), we feel that a better understanding of how periphytic resources is used by the main large microcrustaceans could help to assess energy transfer pathways in freshwater aquatic food webs. The first experiment, measuring clutch size and somatic growth rate, was performed as this last measure has been shown to be a good indicator of cladoceran fitness (Lampert & Trubetskova 1996). Nevertheless, as we supposed that the small size of neonates used in this experiment could have had an effect on their feeding behavior and their ability to forage on periphyton, and in order to take into account a potential interclonal variability (von Elert 2002), a population feeding experiment with individuals who have reached their maturity was performed for the two species with the most contrasting behavior, i.e. *D. longispina* and *E. lamellatus*.

In a recent study, Siehoff *et al.* (2009) showed that *Daphnia magna* is able to graze on periphyton. According to these authors, periphyton could be an alternative food source for the population of *D. magna*, especially when phytoplanktonic resources become scarce. In this study, we found that the highest growth rates of the daphniid *S. vetulus* were obtained on the *Scenedesmus*+periphyton mixture. However, this pattern was not observed for the fecundity data. Even if a previous field study suggested that a large proportion of the diet of this species could be from periphytic material (Balayla & Moss 2004), our results do not allow us to corroborate this hypothesis but rather suggest that *S. vetulus* fed mainly on planktonic particles.

*D. longispina* exhibit low somatic growth rates and clutch sizes when fed on periphyton. In the population experiment, a low population growth rate was also recorded when individuals were fed with periphyton as the only food source. Moreover, the addition of periphyton did not increase growth rates of *D. longispina* fed on limiting concentrations of *Scenedesmus*. These results prove the inability of *D. longispina* to scrape on periphyton. In this study, we cannot totally exclude the fact that some particles were detached from the support to become planktonic and therefore available for individuals. However the rods were rinsed before the experiment to minimize this bias. The slight increase of individual abundance during *D. longispina* population growth experiment may possibly be linked to

other factors. Siehoff *et al.* (2009) found an increase in *D. magna* population abundance during the first week of population experiments even when they were starved. Controlled experiments have moreover shown that mothers provision their offspring with energy and biochemical compounds (Goulden *et al.* 1987). Hence, in our study, the slight increase of *D. longispina* abundance on periphyton treatments could be due to maternal effects. It seems thus that *D. longispina* is mainly able to forage on planktonic particles. This species is furthermore generally considered to be an open-water species (Boronat & Miracle 1997; Waervagen *et al.* 2002), which could explain why scraping abilities had not been selected.

Finally, *E. lamellatus* exhibited high population and somatic growth rates and was able to produce eggs when individuals were fed on periphyton. It is interesting to note that even if there was twice the amount of periphyton in the P treatment compared to the S+P treatment, the population and the somatic growth rate were the same in both of them, suggesting that the amount of periphyton was not limiting in this study. Moreover, we found surprisingly that *E. lamellatus* cannot develop on the phytoplanktonic species *Scenedesmus*. Smirnov (1962) also found that this species died within a few days when fed on *Chlorella terricola*, another phytoplanktonic species. However, Chydorid cladocerans are usually considered to be able to exploit both periphyton and phytoplankton food sources (Meyers 1984). And Fryer (1963) and Beklioglu & Jeppesen (1999) suggest that *E. lamellatus* could be able to scrap periphyton as well as filter-feed on suspended matter. The experiments performed by these last authors aimed to assess habitat preference and behavioral responses of *E. lamellatus* at different food sources and exposed to fish cues, through short observations of 10 min. This protocol allowed them to highlight that this species prefer plant dwelling to avoid predation and because of the presence of epiphyton. However, their protocol was not performed to test the ability of *E. lamellatus* to feed on planktonic particle. To our knowledge, our study is the first to test through controlled laboratory experiments, the current consensus claiming that *Eurycerus* can use both resources, periphyton and suspended material. Our results strongly suggest that contrary to what we thought until now *E. lamellatus* is an obligatory scraper unable to feed on sestonic particles.

In shallow lakes, periphytic production can be responsible for the main proportion of primary production (Vadeboncoeur *et al.* 2003). *E. lamellatus*, with its feeding strategy that allows it to feed on a resource not available to other microcrustaceans, is probably very competitive in such an environment. Lemke & Benke (2004) reported a higher density of *Eurycerus vernalis* in the *Nymphaea* zone in mid to late spring. As phytoplanktonic species

are often not well developed at this time of year (Sommer *et al.* 1986), it is likely that the ability of *Eurycercus* to forage on a periphytic resource promotes its development and its biomass production. Moreover, at that time of year when the water is still cold, *Eurycercus* probably benefits from feeding on polyunsaturated fatty acid (PUFA)-rich algae. Indeed, periphyton composition is season dependent and is dominated by PUFA-rich diatoms (Ahlgren *et al.* 1990) in winter and spring (Leland *et al.* 1986; Vymazal & Richardson 1995). This means that the access to periphytic resource could not only confers a benefit in terms of food availability, but also provides periphyton scrapers with high quality food. It has been shown that food quality constraints on zooplankton development increase as temperature decreases, in part because of the high dietary requirements of zooplankton for PUFA at low temperatures (Masclaux *et al.* 2009). Thus, scraping a high quality periphyton food source may be of particular importance for microcrustacean development under such conditions.

Our study shows that the cladocerans found in littoral zones have differing abilities to forage on planktonic and periphytic components. *S. vetulus* and *D. longispina* appear to be able to feed only on plankton whereas *E. lamellatus*, contrary to what we thought until now, seems only able to graze on periphyton. Even if these results have to be verified in natural environment, we assume that feeding behavior could be a major factor determining the seasonal dynamics of cladoceran species in macrophyte-rich habitats. It also appears that further studies are required to evaluate variations in periphyton food quality, especially with regard to its lipid content, as polyunsaturated fatty acids are considered as essential compounds for higher trophic levels.

**Effects of food partitioning in the transfer of essential compounds in a macrophyte littoral zone**

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**En préparation**

**I. Abstract**

The main factor put forward for the maintenance of species diversity in a given environment have been strongly linked to trophic partitioning. In heterogeneous environment like macrophyte littoral zones, where high microcrustacean diversity has been recorded, it seems therefore senseless to consider the seston as the unique food resource for cladocerans. In a field study performed in a macrophyte-rich backwater of the river Allier, we assessed the trophic compartment (i.e. seston and/or epiphyton) used by the four dominant cladoceran species. Moreover, as we hypothesized that the diversity of food resources may lead to a variability of essential compounds inputs, the fatty acids analyses of the two trophic compartments were performed. Our results showed trophic niches partitioning among the four cladocerans studied. Indeed, while *Eurycerus* fed on epiphyton, *Daphnia*, *Ceriodaphnia* and *Simocephalus* foraged on the sestonic compartment. Moreover, in this last compartment, it seems that *Daphnia* and *Ceriodaphnia* did not exploit the same size fraction of particles. Furthermore, we highlighted that epiphyton was a higher quality food resource than seston, regarding its PUFA content. Variability of PUFA compositions of seston and epiphyton, and diversity of foraging behavior of cladoceran species, which represent the major link between microorganisms and species higher in the food web, may therefore have implications in energy pathways and could lead to a variable transfer of PUFA in the food web.

Key words: cladocerans, PUFA, habitat heterogeneity, trophic niches, transfer variability, stable isotopes

## II. Introduction

Since Hutchinson's (1959) famous paper on the question of animal diversity, a major focus of ecological research is to understand the mechanisms allowing biodiversity to be maintained. One of the main factors put forward for the maintenance of species diversity in a given environment has been yet strongly linked to trophic partitioning (Chesson 2000; Levine & HilleRisLambers 2009). Backwaters formed on the floodplain of large river systems are often associated with aquatic macrophytes vegetation which sustains a wide range of different life forms. Walseng *et al.* (2006) indeed recorded high microcrustacean diversity in such zones, with 75% of zooplankton species inventoried in freshwater systems being associated to littoral aquatic macrophyte zones. In this habitat, complexity of the waterscape, and support offer by the vegetation allow the development of neustonic, benthic and epiphytic biofilms which may be potential components of microcrustacean diets in addition to seston (i.e. suspended particulate material). Among these potential food resources, periphytic biofilms have been the subject of a growing interest (Herwig *et al.* 2004; Rautio & Warwick 2006; Siehoff *et al.* 2009) as it can be responsible for 80-98% of the autochthonous primary production in shallow lakes (Vadeboncoeur *et al.* 2003). However, if a few studies have shown that some cladoceran species are able to forage on periphytic resources (Siehoff *et al.* 2009), little is known about the transfer of this abundant food resource to primary consumers and higher trophic levels.

An increasing number of studies have furthermore focused on the relation linking biodiversity and ecosystem functioning (B-EF). However, most of the B-EF studies have been theoretical (Hooper *et al.* 2005; Duffy *et al.* 2007; Loreau 2010), and only a few of them have considered aquatic environments (Ward & Tockner 2001; Woodward 2009). The sestonic and epiphytic composition, regarding the relative proportions of organic and inorganic matter, the nature of their organic matter, or their taxonomic composition could lead to differences in food quality which can, in turn, lead to differences of energy transfer efficiency to primary consumer (Brett & Müller-Navarra 1997). Differences in polyunsaturated fatty acids (PUFA) composition, which has been reported to be one of the main factor determining the food quality for zooplankton (Müller-Navarra *et al.* 1997), could especially be encountered. PUFA are closely linked to key physiological and biochemical processes in many consumers, including zooplankton and fish (Müller-Navarra *et al.* 1997; Sargent *et al.* 1999; Arts *et al.* 2001). However, because they cannot biosynthesize PUFA *de novo*, or not at rate sufficient to meet their physiological requirements, consumers depend largely on PUFA being supply by

their diets. In aquatic systems, microorganisms such as algae and heterotrophic protists are major producers of PUFA (Brett & Müller-Navarra 1997; Desvillettes & Bec 2009), which are then incorporated into consumers and transferred conservatively in the food web (Koussoroplis *et al.* 2011). Diversity of PUFA composition of the basal food resources (e.g. seston and periphyton), and diversity of foraging behavior of zooplankton species, which represent the major link between microorganisms and species higher in the food web, may therefore lead to a variable efficiency of matter and energy transfer in the food web.

In order to understand in a better way the trophic interaction at the base of the food web in macrophyte littoral zone, we first aimed to assess the use of the two main trophic compartments, i.e. seston and epiphyton, by the different cladoceran species, in a macrophyte-rich backwater of the river Allier. As stable isotopes analyses have proven to be valuable ecological tools that can make energy pathways assessment easier (DeNiro & Epstein 1978), we used to measure the natural abundance of carbon and nitrogen stable isotopes of seston, epiphyton, and cladocerans to answer this question. Moreover, as we hypothesized that the diversity of food resources may lead to variability in essential compounds inputs, the fatty acids analyses of seston and epiphyton have also been performed.

### **III. Material and methods**

#### **1. Study site**

The study was carried out from March to November 2008. Field work began when aquatic macrophytes appeared in late winter, and ended when the plants started to senescence. All samples were obtained from a backwater (3°28'E, 46°01'N) of the river Allier, France.

The shallow zone of backwater was covered by aquatic macrophytes among which the three dominant were: *Callitriche* sp., *Elodea canadensis* and *Ludwigia granddiflora*.

For study site description please refer to chapter 2.



## 2. Sample collection

Samples of seston (i.e. suspended particulate material), epiphyton, and of the dominant cladoceran species were analyzed in this study. Samples were collected the 19<sup>th</sup> March, 13<sup>th</sup> May, 1<sup>st</sup> and 29<sup>th</sup> July, 9<sup>th</sup> October, and 20<sup>th</sup> November. These sampling dates were chosen based on the seasonal dynamics of the microcrustaceans in the backwater (chapter 2). For each date, horizontal integrated samples of seston were done by collecting 20 L of water at 50 cm of depth with a water sampler remote controlled. To collect epiphyton, the three dominant aquatic macrophytes of the backwater were picked by hand at different locations and put in a stomacher bag containing filtered water (<0.2  $\mu\text{m}$ , see below). Zooplankton was collected by towing vertically a 64  $\mu\text{m}$  plankton net several times. All the samples were stored in containers placed in ice, transported to the laboratory and processed immediately.

## 3. Sample processing

In the laboratory, stomacher bag containing the macrophytes were placed in a Stomacher (Bagmixer 400, Interscience, France) to removed microbial population attached on plant stems (Bowker *et al.* 1986). The stomacher was operated at normal speed (230 rpm) for 2 min. The solution containing the epiphyton removed with the stomacher, as well as the seston were then filtrated through a Nitex mesh of 50  $\mu\text{m}$  to keep the fraction <50  $\mu\text{m}$  of particulate organic matter (POM), i.e. the optimal size range of particles ingested by cladocerans (Burns 1968). For each compartment, this fraction of POM was then filtered onto precombusted GF/F-filters (Whatman<sup>TM</sup>) immediately after sampling, for lipid and isotopes analyses. All the samples (seston and epiphyton filters and zooplankton samples) were stored at -80°C until analysis.

## 4. Fatty acids analyses

Fatty acids analyses were performed in triplicate on seston and epiphyton samples.

The method used for lipid analysis was fully described in chapter 2.

FAME were identified by comparing retention times with those obtained from Supelco® standards (37-Component FAME mix, Bacterial FAME mix) and laboratory standards (Cod liver oil FAME) and quantified against internal standards (13:0 and 23:0).

## 5. Stable isotopes analyses

Stable isotopes analyses were performed in triplicate on seston and epiphyton samples as well as on dominant cladoceran species for each sampling date: *Eurycercus lamellatus* and *Simocephalus vetulus* in March, May, and October, *S. vetulus*, *Daphnia longispina* and *Ceriodaphnia* sp. in July and *S. vetulus* in November. Zooplankton samples were thawed, kept in ice, and separated manually (200 individuals per sample) under a binocular microscope equipped with a cold light.

The freeze dried samples of seston, epiphyton, and zooplankton were homogenized and put into tin capsules (~0.7 mg each).

For detailed information regarding isotope analysis, please refer to chapter 2.

We used 0.15% for  $\delta^{15}\text{N}$  and 0.10% for  $\delta^{13}\text{C}$  standard deviation of measurement.

Lipid content can affect results and then conclusions of  $\delta^{13}\text{C}$  analyses as lipids have more negative  $\delta^{13}\text{C}$  values relative to other major biochemical compounds in animal tissues (DeNiro & Epstein 1977). Hence, we normalized the  $\delta^{13}\text{C}$  values of zooplankton to a zero lipid content by calculating lipid extracted  $\delta^{13}\text{C}$  values using a mass balance correction model elaborated by Smyntek et al. (2007). All the  $\delta^{13}\text{C}$  values for zooplankton were reported as lipid normalized values.

## 6. Data analysis

Differences in  $\delta^{13}\text{C}$  values between seston and epiphyton and between the different cladoceran species, as well as differences in EPA and PUFA concentrations between seston and epiphyton were investigated using Mann-Whitney U-test. All calculations were performed using the XLStat-Pro 7.5 (Addinsoft). Significance level was set at  $\alpha = 0.05$ .

## IV. Results

### 1. Stable isotopes analyses

In the backwater,  $\delta^{13}\text{C}$  values of the two trophic compartments ranged from  $-34.7\pm 0.8\text{‰}$  to  $-31.8\pm 0.1\text{‰}$  for seston and from  $-33.0\pm 1.4\text{‰}$  to  $-26.5\pm 0.9\text{‰}$  for epiphyton and differed significantly (Mann-Whitney;  $U = 244$ ,  $p < 0.001$ ). Seston and epiphyton  $\delta^{15}\text{N}$  values ranged respectively from  $5.9\pm 0.1\text{‰}$  to  $16.4\pm 0.1\text{‰}$  and from  $6.8\pm 1.7\text{‰}$  to  $14.9\pm 5.4\text{‰}$  (Fig. 4.3). *Simocephalus*  $\delta^{13}\text{C}$  signatures were not significantly different from those of *Daphnia* and *Ceriodaphnia* (Table 4.2, Fig. 4.3). However,  $\delta^{13}\text{C}$  signatures of these last two species are significantly different from each other (Table 4.2, Fig. 4.3) and *Eurycercus*  $\delta^{13}\text{C}$  signatures were well separated from the three other cladocerans (Table 4.2, Fig. 4.3). *Eurycercus* was the more  $^{13}\text{C}$  enriched species with  $\delta^{13}\text{C}$  values ranging from  $-31.9\pm 0.5\text{‰}$  to  $-27.0\pm 0.3\text{‰}$  and *Daphnia* was on the contrary the more  $^{13}\text{C}$  depleted species with  $\delta^{13}\text{C}$  values ranging from  $-38.1\pm 2.4\text{‰}$  to  $-35.6\pm 0.2\text{‰}$ . *Ceriodaphnia* and *Simocephalus* presented intermediate  $\delta^{13}\text{C}$  values ranging respectively from  $-34.0\pm 0.3\text{‰}$  to  $-32.8\pm 0.3\text{‰}$  and from  $-37.0\pm 1.5\text{‰}$  to  $-30.0\pm 0.1\text{‰}$ , these latest species offering the largest range of  $\delta^{13}\text{C}$  values, with a variability of 7‰. Like for the trophic compartments, the  $\delta^{15}\text{N}$  signatures of the four cladocerans highly overlap (Fig. 4.3).

Table 4.2 : Results of the Mann-Whitney test comparing  $\delta^{13}\text{C}$  values of the four cladocerans species. The probability in bold indicate significantly different  $\delta^{13}\text{C}$  values between the two species.

	$\delta^{13}\text{C}$ values	
	<i>U</i> Value	<i>p</i> Value
<i>Simocephalus</i> vs. <i>Daphnia</i>	<i>U</i> = 14	<i>p</i> = 0.225
<i>Simocephalus</i> vs. <i>Ceriodaphnia</i>	<i>U</i> = 37	<i>p</i> = 0.115
<i>Simocephalus</i> vs. <i>Eurycercus</i>	<i>U</i> = 64	<b><i>p</i> = 0.009</b>
<i>Daphnia</i> vs. <i>Ceriodaphnia</i>	<i>U</i> = 0	<b><i>p</i> = 0.021</b>
<i>Daphnia</i> vs. <i>Eurycercus</i>	<i>U</i> = 0	<b><i>p</i> = 0.011</b>
<i>Ceriodaphnia</i> vs. <i>Eurycercus</i>	<i>U</i> = 0	<b><i>p</i> = 0.011</b>

## 2. Fatty acids analyses

Whatever the season, total FA concentrations of epiphyton were always higher than those of seston. Indeed, FA concentrations ranged from  $115.8 \pm 14.7$  to  $227.0 \pm 27.7$   $\mu\text{g FA mg C}^{-1}$  in epiphyton and from  $16.2 \pm 1.4$  to  $77.0 \pm 4.1$   $\mu\text{g FA mg C}^{-1}$  in seston (Table 4.3). Moreover, the concentrations of nutritionally important compounds such as PUFA were 4 to 14X higher and the concentrations of EPA were 3 to 18X higher in epiphyton compared to seston (Fig. 4.4). For both EPA and PUFA, the biggest differences between the two compartments were recorded in May and October (Fig. 4.4).

The FA concentrations of both trophic compartments were presented in Table 4.3. SAFAs concentrations ranged from  $7.1 \pm 0.6$  to  $36.7 \pm 0.9$   $\mu\text{g mg C}^{-1}$  and from  $47.7 \pm 4.1$  to  $106.9 \pm 7.6$   $\mu\text{g mg C}^{-1}$  for seston and epiphyton, respectively, while MUFAs ranged from  $3 \pm 0.3$  to  $24.9 \pm 1.7$   $\mu\text{g mg C}^{-1}$  and from  $37.8 \pm 4.6$  to  $102.6 \pm 10.1$   $\mu\text{g mg C}^{-1}$  for seston and epiphyton, and PUFAs ranged from  $3 \pm 1.3$  to  $7.4 \pm 1.5$   $\mu\text{g mg C}^{-1}$  and  $20.8 \pm 10.1$  to  $58.4 \pm 10.2$   $\mu\text{g mg C}^{-1}$  for seston and epiphyton, respectively (Table 4.3). Bacterial FA [BAFA, odd-saturated and branched chain FA, i.e. the sum of 15:0 and 17:0 and their iso-series and anteiso-series (Dalsgaard *et al.* 2003)] were about 1.2 to 9.8X more concentrated in epiphyton than in seston. In both seston and epiphyton, PUFA composition was dominated by C18-PUFAs while highly unsaturated fatty acids (HUFAs,  $\geq 20$  carbon atoms and  $\geq 3$  double bonds) were found in low concentrations, especially in seston.

Table 4.3 :  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰) and fatty acid concentrations ( $\mu\text{g mg C}^{-1}$ ) of seston and epiphyton (mean  $\pm$  SD). SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. nd = not detected

	19 <sup>th</sup> March		13 <sup>th</sup> May		1 <sup>st</sup> July		29 <sup>th</sup> July		9 <sup>th</sup> October		20 <sup>th</sup> November	
	seston	epiphyton	seston	epiphyton	seston	epiphyton	seston	epiphyton	seston	epiphyton	seston	epiphyton
$\delta^{13}\text{C}$	-34.1 $\pm$ 0.1	-27.1 $\pm$ 0.3	-33.3 $\pm$ 0.1	-26.5 $\pm$ 0.9	-31.8 $\pm$ 0.1	-30.5 $\pm$ 1.4	-34.7 $\pm$ 0.8	-30.8 $\pm$ 0.7	-33.4 $\pm$ 0.2	-30.0 $\pm$ 1.7	-33.0 $\pm$ 0.3	-33.0 $\pm$ 1.4
$\delta^{15}\text{N}$	8.0 $\pm$ 0.8	8.9 $\pm$ 0.9	7.0 $\pm$ 0.6	8.7 $\pm$ 0.4	8.5 $\pm$ 0.2	10.9 $\pm$ 0.8	15.2 $\pm$ 0.1	14.1 $\pm$ 2.0	16.4 $\pm$ 0.1	14.9 $\pm$ 5.4	5.9 $\pm$ 0.1	6.8 $\pm$ 1.7
FA concentrations ( $\mu\text{g mg C}^{-1}$ )												
14:0	1 $\pm$ 0.1	7.7 $\pm$ 0.3	2.3 $\pm$ 0.1	9.3 $\pm$ 1.9	1 $\pm$ 0.6	3.6 $\pm$ 1.4	1.3 $\pm$ 0.8	4.7 $\pm$ 1.3	1.2 $\pm$ 0.4	9.4 $\pm$ 1.7	2.7 $\pm$ 0.2	5.7 $\pm$ 1.7
16:0	5.4 $\pm$ 0.2	37.4 $\pm$ 12.3	2.9 $\pm$ 0.3	76.9 $\pm$ 6.7	4.6 $\pm$ 3.7	38 $\pm$ 9.9	5.4 $\pm$ 3	29.5 $\pm$ 1.8	5.3 $\pm$ 1.6	45 $\pm$ 9.6	17.6 $\pm$ 0	31.7 $\pm$ 8.4
18:0	1.7 $\pm$ 0.1	16.1 $\pm$ 16.6	1.5 $\pm$ 0.5	16.9 $\pm$ 2.5	2.1 $\pm$ 2.1	20.3 $\pm$ 2.7	1.9 $\pm$ 0.5	10.4 $\pm$ 2.6	2.4 $\pm$ 0.6	20.4 $\pm$ 4.9	14.7 $\pm$ 1.3	15.3 $\pm$ 6.4
22:0	0.1 $\pm$ 0	0.5 $\pm$ 0.4	0.1 $\pm$ 0	1.3 $\pm$ 0.2	0.2 $\pm$ 0.1	1.9 $\pm$ 0.5	0.2 $\pm$ 0.1	1.1 $\pm$ 0.2	0.1 $\pm$ 0	1.5 $\pm$ 0.5	0.9 $\pm$ 0.2	1.2 $\pm$ 0.3
24:0	0.1 $\pm$ 0	0.4 $\pm$ 0.3	0.1 $\pm$ 0	1.7 $\pm$ 0.1	0.1 $\pm$ 0.1	0.9 $\pm$ 0.6	0.2 $\pm$ 0.1	0.9 $\pm$ 0.4	0.2 $\pm$ 0.1	1.7 $\pm$ 0.8	0.3 $\pm$ 0	1.3 $\pm$ 0.9
SAFA	8.4 $\pm$ 0.3	62.6 $\pm$ 29.6	7.1 $\pm$ 0.6	106.9 $\pm$ 7.6	8.1 $\pm$ 6.6	66.4 $\pm$ 15.7	9.1 $\pm$ 4.4	47.7 $\pm$ 4.1	9.4 $\pm$ 2.7	80.2 $\pm$ 15.7	36.7 $\pm$ 0.9	56 $\pm$ 15.5
Bacterial FA	2.4 $\pm$ 0.5	5.9 $\pm$ 1	2 $\pm$ 0.1	9.1 $\pm$ 0.7	1.2 $\pm$ 0.6	11.8 $\pm$ 2.9	3.2 $\pm$ 1.9	6.9 $\pm$ 2.1	2.5 $\pm$ 0.9	16.2 $\pm$ 2.1	7.9 $\pm$ 1.8	9.4 $\pm$ 2
16:1n7	1.7 $\pm$ 0.2	34.5 $\pm$ 2.3	1 $\pm$ 0	33.3 $\pm$ 10.1	2.9 $\pm$ 2	11 $\pm$ 1.6	3 $\pm$ 2	9.1 $\pm$ 7.5	3.4 $\pm$ 1.5	30.3 $\pm$ 4.3	11.3 $\pm$ 1.3	24.8 $\pm$ 9.4
18:1n9	1.2 $\pm$ 0	5.3 $\pm$ 2.1	0.6 $\pm$ 0	58.5 $\pm$ 21.5	0.8 $\pm$ 0.5	35.3 $\pm$ 11.1	1.3 $\pm$ 0.6	10.5 $\pm$ 3.1	0.9 $\pm$ 0.2	12.7 $\pm$ 9.7	3.2 $\pm$ 0.1	7.2 $\pm$ 5.6
18:1n7	0.7 $\pm$ 0.1	2.7 $\pm$ 1.9	0.5 $\pm$ 0	9.6 $\pm$ 1	0.8 $\pm$ 0.5	17.1 $\pm$ 3.3	1.4 $\pm$ 0.8	8.5 $\pm$ 2.7	1.4 $\pm$ 0.5	11.9 $\pm$ 5.6	3.2 $\pm$ 0.4	5.4 $\pm$ 3.7
MUFA	4.6 $\pm$ 0.1	46.5 $\pm$ 11.7	3 $\pm$ 0.3	102.6 $\pm$ 10.1	4.8 $\pm$ 2.8	65.3 $\pm$ 15.7	7 $\pm$ 4.2	37.8 $\pm$ 4.6	7.3 $\pm$ 2.7	58.1 $\pm$ 15.4	24.9 $\pm$ 1.7	44.8 $\pm$ 15.7
C16 PUFA	1 $\pm$ 0	3.4 $\pm$ 0.5	0.7 $\pm$ 0.1	3.9 $\pm$ 0.8	1 $\pm$ 0.1	0.7 $\pm$ 0.5	0.8 $\pm$ 0.5	1.6 $\pm$ 0.6	1.3 $\pm$ 0.6	6.6 $\pm$ 1.3	1.1 $\pm$ 0.4	3.7 $\pm$ 1.1
18:2n6	1 $\pm$ 0	2.3 $\pm$ 0.4	0.5 $\pm$ 0.1	15.9 $\pm$ 3.5	nd	8.9 $\pm$ 7.4	0.5 $\pm$ 0.2	5.4 $\pm$ 4	0.5 $\pm$ 0.2	6.1 $\pm$ 1.4	0.7 $\pm$ 0	4.4 $\pm$ 3.1
18:3n3	1.2 $\pm$ 0.1	2.9 $\pm$ 1.3	0.5 $\pm$ 0	25.5 $\pm$ 8.2	0.4 $\pm$ 0.2	7.1 $\pm$ 6.4	1.1 $\pm$ 0.8	5.9 $\pm$ 3.6	1.2 $\pm$ 0.4	8.1 $\pm$ 1.3	0.7 $\pm$ 0.1	6.3 $\pm$ 3.7
18:4n3	0.9 $\pm$ 0.1	4.5 $\pm$ 5.4	1.1 $\pm$ 0	2.3 $\pm$ 2.2	0.7 $\pm$ 0.6	2.8 $\pm$ 2.2	1.4 $\pm$ 1.6	0.7 $\pm$ 0.6	1.2 $\pm$ 0.6	5 $\pm$ 5.6	1.1 $\pm$ 0.1	2.9 $\pm$ 3.4
20:4n6	0.1 $\pm$ 0	0.6 $\pm$ 0.2	0.1 $\pm$ 0	1 $\pm$ 0.3	nd	1.7 $\pm$ 1.8	0.2 $\pm$ 0.1	1.7 $\pm$ 1.4	0.1 $\pm$ 0	3.1 $\pm$ 1.6	0.1 $\pm$ 0	1.1 $\pm$ 1.1
20:5n3	0.3 $\pm$ 0	4 $\pm$ 0.8	0.3 $\pm$ 0	5.4 $\pm$ 1.3	0.3 $\pm$ 0.1	3.1 $\pm$ 3.3	1.3 $\pm$ 0.8	3.8 $\pm$ 3.2	1.8 $\pm$ 0.8	13.2 $\pm$ 5.4	0.7 $\pm$ 0	5.8 $\pm$ 4.4
22:6n3	0.1 $\pm$ 0	0.1 $\pm$ 0.1	0.3 $\pm$ 0	0.1 $\pm$ 0.2	nd	0.4 $\pm$ 0.5	0.3 $\pm$ 0.3	0.2 $\pm$ 0.2	0.4 $\pm$ 0.1	1 $\pm$ 0.9	nd	0.1 $\pm$ 0.2
PUFA	5.2 $\pm$ 0.2	20.8 $\pm$ 10.1	4.1 $\pm$ 0.4	58.4 $\pm$ 10.2	3 $\pm$ 1.3	31.2 $\pm$ 24.4	6 $\pm$ 4.4	23.3 $\pm$ 14.8	7 $\pm$ 2.8	50.8 $\pm$ 16.8	7.4 $\pm$ 1.5	28.7 $\pm$ 20.6
Total	20.7 $\pm$ 0.8	135.8 $\pm$ 49.9	16.2 $\pm$ 1.4	277 $\pm$ 27.7	17.1 $\pm$ 11.3	174.6 $\pm$ 40.3	25.2 $\pm$ 14.8	115.8 $\pm$ 14.7	26.2 $\pm$ 9	205.3 $\pm$ 42	77 $\pm$ 4.1	138.8 $\pm$ 48.5

## V. Discussion

This study aimed to understand in which way the two main trophic compartments of a macrophyte-rich habitat are used by cladocerans. We also wanted to determine if trophic niche partitioning could lead to a variability of essential compounds inputs for primary consumers.

Seston is typically the most considered food resource supporting zooplankton production in lakes. Recently however, there has been increased evidence that other food resources should be taken into consideration, especially in shallow heterogeneous environment. In particular, there has been a growing appreciation that periphytic production (including benthic and epiphytic biofilm) may also be an important resource for invertebrate consumers (Herwig *et al.* 2004; Rautio & Warwick 2006; Karlsson & Sävström 2009; Siehoff *et al.* 2009), as it can represent an important proportion of the autochthonous primary production, especially in clear water lakes (Vadeboncoeur *et al.* 2003). Here, we used stable isotopes analyses of C and N to assess the food sources used by different cladoceran grazers. In the backwater studied, seston and epiphyton were well separated from each others, allowing us to distinguish among food resources that support consumers. Seston had indeed a  $\delta^{13}\text{C}$  of  $-33.2 \pm 1.1\text{‰}$  which fell in range of  $\delta^{13}\text{C}$  observed for sestonic particles in other freshwater ecosystems (delGiorgio & France 1996; Matthews & Mazumder 2006), whereas epiphyton was comparatively enriched in  $^{13}\text{C}$  with  $\delta^{13}\text{C}$  of  $-30.0 \pm 2.4\text{‰}$ . In this case, it is likely that the high photosynthetic activity of macrophyte and epiphytes lead to a local competition for the dissolved inorganic carbon. This competition may lead to a decreased  $^{13}\text{C}$  discrimination and therefore to an enrichment of the  $\delta^{13}\text{C}$  value of epiphyton (Lajtha & Marshall 1994).

Except *Daphnia* and to some extent *Simocephalus* which had much depleted  $\delta^{13}\text{C}$  values, consumers fell in the range of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values observed for seston and epiphyton. Pelagic zooplankton depleted in  $^{13}\text{C}$  compared to seston as their putative food source is quite common in freshwater ecosystems (delGiorgio & France 1996; Grey *et al.* 2000, 2001). The depleted  $\delta^{13}\text{C}$  values of *Daphnia* probably reflected a differential assimilation of seston constituents. Sestonic carbon is indeed of multiple origins and the phytoplankton isotopic signature is often masked by allochthonous and littoral detritus which are usually enriched in  $^{13}\text{C}$  relative to phytoplankton (Hamilton & Lewis 1992; Delong & Thorp 2006). In the sestonic compartment, *Daphnia* is thus expected to preferentially assimilate phytoplankton and heterotrophic microorganisms which represent a more labile organic matter than detritus

and allochthonous inputs. The isotopic signatures of *Ceriodaphnia* and *Simocephalus* also suggested that these species mainly forage on the sestonic compartment. Differences of  $\delta^{13}\text{C}$  values between *Daphnia* and *Ceriodaphnia* could then be due to differences of assimilation or differences in the optimal size range of particles ingested. Indeed, allometric relations linking feeding behavior to body size is undoubted, and body size, more than taxonomic affiliation, can sometime explain trophic niche used by organisms (Rojo & Salazar 2010). The different sizes of these two species ( $\approx 2.5$  mm for *Daphnia* and 1 mm for *Ceriodaphnia*) may thus suggest that they exploit different size fraction of seston (Burns 1968). In the case of *Simocephalus*, the wide distributions of  $\delta^{13}\text{C}$  values could indicate that its food sources were varied. Indeed, isotopic variance had been highlighted to be a valuable tool for the measure of niche width (Bearhop *et al.* 2004). However, in our study, *Simocephalus* was also the only species which was found all along the sampling season, from March to November. It is thus likely that the higher  $\delta^{13}\text{C}$  variability encountered was due to the seasonal  $\delta^{13}\text{C}$  variability of its food source. In our case it is unclear which of these factors is the most likely and one can expect that both of them could be true. Finally, *Eurycercus* enriched  $\delta^{13}\text{C}$  values imply that this species was a primary consumer drawing its energy resources from epiphyton. This last result is not surprising as this genus, often associated with macrophytes-rich habitat (Balayla & Moss 2003; Lemke & Benke 2004), is known to be a scraper and as a recent experimental study has moreover suggested that periphyton may be the unique food source of *E. lamellatus* (Masclaux *et al.*, unpublished data). The differences in  $\delta^{13}\text{C}$  values of the four cladocerans studied here lead us to conclude that in heterogeneous environment like macrophytes littoral zones, trophic niches partitioning is certainly a mechanism allowing cladoceran biodiversity to be maintained.

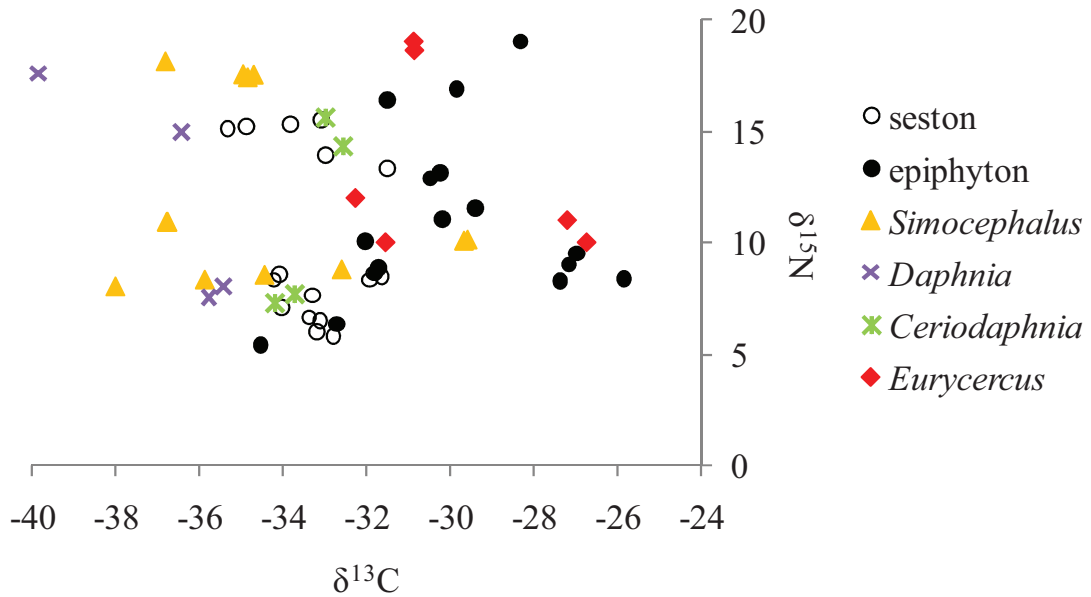


Figure 4.3 :  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of seston, epiphyton, *Simocephalus*, *Daphnia*, *Ceriodaphnia*, and *Eurycerus*.

The partitioning of resources by microcrustaceans has already been suggested based on the spatial distribution of species in a macrophytes littoral environment (Balayla & Moss 2003). However, the assessment of cladoceran trophic niches by stable isotopes analyses is only poorly documented (Matthews & Mazumder 2006). The trophic niches partitioning observed between cladocerans in our study could have implications in energy pathways and therefore implications for secondary consumer production. Indeed, Masclaux et al. (unpublished data) showed in a previous study that cladocerans do not differ in their abilities to accumulate and bioconvert PUFA. The foraging behavior and the trophic niches used by the different species will therefore be the main determinant of the variability of PUFA transfer to higher trophic level. Seston and epiphyton may indeed differ in their composition in freshwater environment. An argument supporting this is that, except phytoplankton and others microorganisms, allochthonous organic matter and littoral detritus composed the sestonic compartment (Hamilton & Lewis 1992; Delong & Thorp 2006). These differences could then lead to differences in food quality for microcrustaceans. One of the most important factor determining the food quality for microcrustaceans is the PUFA content (Brett & Müller-Navarra 1997). PUFA are linked to key physiological and biochemical process for zooplankton (Ahlgren *et al.* 1990; Brett & Müller-Navarra 1997) but also for secondary consumers, including fish (Arts *et al.* 2001). One of them, the eicosapentaenoic acid (EPA)



had especially been highlighted to be of crucial concern for cladoceran development. EPA is indeed a precursor of eicosanoids involved in reproduction processes (Harrison 1990) and some studies showed positive correlations between EPA content of the food source and cladoceran growth and reproduction rate (Müller-Navarra *et al.* 2000; Bec *et al.* 2003). Our FA analyses revealed that PUFA and EPA concentrations were by far higher in epiphyton than in seston all along the sampling season. These results therefore suggest that epiphyton was a higher quality food source and an important source of essential compounds for consumers. *Eurycerus*, and all the species able to scrape on periphyton, may therefore play a key role in the transfer of PUFA from periphytic production to fish.

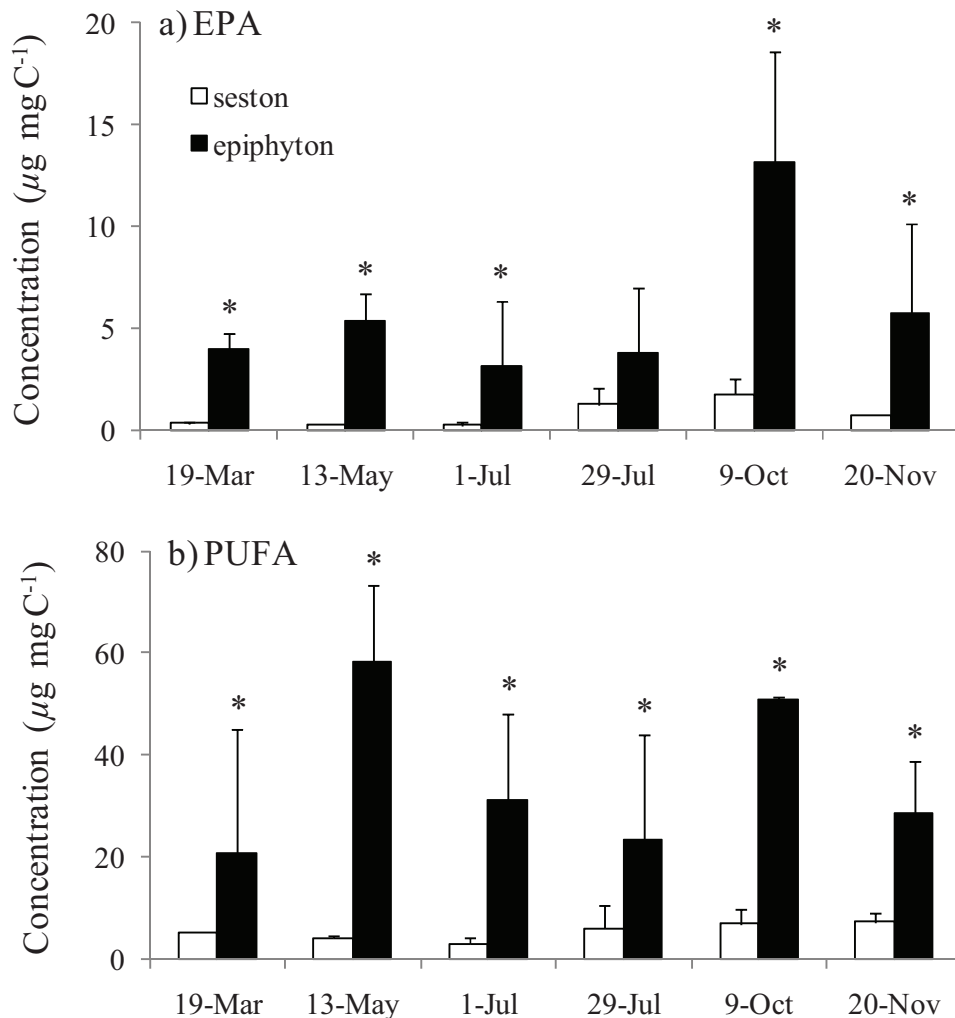


Figure 4.4 : Seasonal evolution of a) EPA and b) PUFA concentrations in seston and epiphyton. For the dates labeled with an asterisk, epiphytic concentrations are significantly higher than in sestonic concentrations (Mann-Whitney,  $p < 0.05$ )

In conclusion, we showed through the use of stable isotope analyses that seston and epiphyton represent two important food sources for cladocerans in shallow aquatic environment. The use of stable isotopes has provided an effective tool for studying trophic interactions in aquatic systems and, in this study, it allowed us to highlight a trophic niche partitioning of cladocerans in a macrophytes-rich habitat. Moreover, and for the first time, we suggest that differences of food quality between seston and epiphyton, regarding their PUFA content, and differences in foraging behavior of cladoceran species may lead to a variable transfer of PUFA to higher trophic level. The high cladoceran species richness in macrophytes littoral zones and the complementarity of species regarding their trophic niche may thus lead to a more efficient use of resource and to a higher transfer of essential compounds to secondary consumers. We think that macrophytes littoral zones and their associated biodiversity represent an interesting environment to perform field study linking biodiversity and ecosystem process and as such, should be more heavily studied.



**CHAPITRE V : Importance du neuston et des apports  
allochtones de matière organique pour la communauté de  
microcrustacés : étude du cas particulier d'une pluie de  
pollen**



## Préambule

Les résultats obtenus dans le chapitre précédent nous ont permis de mettre en évidence une séparation des niches trophiques entre les différentes espèces de cladocères coexistant dans une zone littorale à macrophytes, soulignant principalement une exploitation différentielle du seston et de l'épiphyton par les principales espèces présentes. Les analyses lipidiques de ces deux compartiments trophiques ont, de plus, révélé une variabilité des apports en AGPI d'un compartiment à un autre.

Les résultats préliminaires obtenus grâce aux comptages zooplanctoniques (présentés dans le chapitre 2), ont mis en évidence une hétérogénéité verticale de la répartition des espèces. En effet, certaines espèces, comme *Scapholeberis mucronata*, se maintiennent en surface, dans les premiers centimètres d'eau. Les observations faites sur le terrain nous ont de plus montré qu'il se formait souvent à l'interface air-eau un biofilm : le neuston. En raison de la difficulté à appréhender ce compartiment, peu d'études ont étudié la composition de ce biofilm neustonique (Södergren 1987; Burchardt & Marshall 2003; Hortnagl *et al.* 2010; Santos *et al.* 2010), et aucune à notre connaissance ne l'a considéré comme une source de nourriture potentielle pour les cladocères. Or, nous avons observé une localisation préférentielle de certaines espèces de cladocères à l'interface air-eau qui suggère que le neuston pourrait constituer une source de nourriture pour ces dernières. Nous avons donc choisi de considérer, dans le chapitre suivant, le neuston en tant que source de nourriture potentielle pour les microcrustacés.

De plus, les observations faites sur le terrain, tout au long de l'année nous ont permis de constater qu'il y avait une accumulation de matière organique d'origine allochtone au niveau du neuston. Notamment, une forte accumulation de grains de pollen est parfois observée à l'interface air-eau, lors de la période pollinisation des conifères, représentant une source de carbone allochtone non négligeable. Afin d'étudier l'importance d'une source de nourriture telle que le neuston pour les cladocères, il nous a donc semblé indispensable de considérer parallèlement, l'importance des apports allochtones de matière organique terrestre pour la production secondaire. Les grains de pollen appartiennent à la gamme de taille préférentiellement ingérée par les cladocères (Burns 1968; Manning 2006). Leur composition chimique (forte teneur en phosphore, Banks & Nighswander 2000) et biochimique (forte teneur en AGPI, Manning 2006) en font, de plus, une source de nourriture potentiellement de

bonne qualité (Brett & Müller-Navarra 1997; Elser *et al.* 2001). Le pollen nous a donc semblé être un apport de carbone terrestre intéressant à prendre en compte en zone littorale.

Dans la première partie de ce chapitre, une étude expérimentale nous a permis de tester la qualité nutritionnelle de différents pollens pour les cladocères, ainsi que l'importance des microorganismes dans le transfert de ce carbone allochtone vers les niveaux trophiques supérieurs. Nous avons ensuite, dans la deuxième partie du chapitre, évalué en milieu naturel, l'importance d'une pluie de pollen de pin sur la formation d'un biofilm neustonique, ainsi que les conséquences sur la production secondaire et la structuration des communautés de microcrustacés.

## Food quality of anemophilous plant pollen for zooplankton

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### I. Abstract

Pollen rain can lead to considerable allochthonous particulate organic carbon input into freshwater systems. However, the importance of allochthonous pollen deposition for zooplankton production has not so far been considered. Here, standardized growth experiments were performed to assess the quality of pollen from three species (*Alnus* sp., *Populus* sp., and *Cedrus* sp.) as a food source for two cladocerans (*Daphnia longispina* and *Simocephalus vetulus*). Although lipid analysis revealed high polyunsaturated fatty acid (PUFA) contents in pollen, both cladoceran species exhibited suboptimal development when directly fed on pollen (ranging from 0.2 to 0.3 d<sup>-1</sup>). The low food value of pollen was attributed to the presence of a refractory wall reducing its digestibility. In a second set of experiments, cladocerans were fed on a mixture of heterotrophic microorganisms (bacteria, chytrids, and protozoa) that had grown on pollen grains (*Cedrus* sp.). The introduction of microorganisms as an intermediate trophic level resulted in cladoceran growth rates that were about double those obtained on pollen alone. Hence, our findings suggest that pollen carbon could sustain zooplankton growth indirectly, and highlight the key role of microorganisms, and especially of chytrids, in transferring and upgrading pollen PUFA to higher trophic levels.



## II. Introduction

It has been suggested that inputs of allochthonous matter contribute significantly to the secondary production of aquatic food webs (Herwig et al. 2004; Pace et al. 2004; Cole et al. 2006). In some small and shallow humic lakes, Cole *et al.* (2006) estimated that between 33% and 73% of zooplankton carbon could be supplied by terrestrial particulate organic carbon. While there is an increasingly widespread view that zooplankton obtain a large proportion of their carbon from terrestrial sources, Brett *et al.* (2009) suggested that terrestrial particulate organic carbon (t-POC) inputs were overestimated in whole lake  $^{13}\text{C}$  addition experiments (Pace et al. 2004; Cole et al. 2006), and account for a disproportionately high proportion of zooplankton production. This scientific controversy is based on the quality of t-POC as a food source for aquatic consumers. In a controlled laboratory study, Brett *et al.* (2009) showed that leaf litter, used as an example of t-POC, is only weakly assimilated by *Daphnia* in contrast to most of the phytoplanktonic species tested. Indeed, the somatic growth and reproduction of *Daphnia* were lower when they were fed on t-POC than when they were fed cryptophytes, diatoms, and chlorophytes. According to Brett *et al.* (2009), these results can be attributed to the very low food quality of leaf litter, especially with regard to its polyunsaturated fatty acid content and poor digestibility.

Nevertheless, leaf litter is not the only form under which t-POC can enter aquatic ecosystems. Massive pollen deposition can constitute a large part of the allochthonous carbon input. Because of the haphazard nature of wind as a vector of pollination, anemophilous plants are characterized by their high pollen production, resulting in the deposition of huge quantities of pollen grains in the environment (in the form of 'pollen rains'). Estimates of ground pollen deposition, performed by placing pollen collectors fitted with a film of petroleum jelly (Lee *et al.* 1996a; Lee *et al.* 1996b; Lee & Booth 2003), have shown that the amount of pollen deposited by a mature pine forest in west central Manitoba ranged from 1600 and 2500 kg of pollen  $\text{km}^{-2}$  (Lee et al. 1996a), and could reach 2750 kg  $\text{km}^{-2}$  in pine stands in southeast Korea (Lee & Booth 2003). A large amount of pollen can end up in aquatic ecosystems during the floral bloom (Richerson *et al.* 1970; Banks & Nighswander 2000; Graham *et al.* 2006), and up to 4000 kg of conifer pollen can be deposited in Lake Tahoe (California) during a single floral season (Richerson et al. 1970). Most pollen grains from wind-pollinated tree species are in the optimal size range ( $<50 \mu\text{m}$ ) for particles preferentially ingested by cladocerans (Burns 1968; Manning 2006). Moreover, the

biochemical and mineral composition of pollens include high proportions of polyunsaturated fatty acids (Manning 2006) and a high phosphorus content (Banks & Nighswander 2000), two factors that determine the quality of a food source (Brett & Müller-Navarra 1997; Elser *et al.* 2001). We thus hypothesized that pollen could be ingested by freshwater zooplankton, and that unlike leaf litter detritus (Brett *et al.* 2009), anemophilous plant pollen carbon could represent a high quality food for these consumers.

However, in their mesocosm experiments, Graham *et al.* (2006) did not find any evidence of direct pollen consumption by zooplanktonic grazers, and suspected that jack pine pollen grains might be inedible due to their large size. Nevertheless, as early as the 1960s, some studies showed that pollen grains can be decomposed by zoosporic fungi, such as chytrids (Goldstein 1960; Skvarlaa & Anderegg 1972; Hutchison & Barron 1997). Once mature, the fungal sporangia release zoospores which are small enough ( $<5 \mu\text{m}$ ) for cladocerans to eat (Kagami *et al.* 2007). Thus, during pollen rain, we hypothesize that microorganisms, and especially zoosporic fungi, could play an important role in transferring carbon from pollen to a higher trophic level.

To evaluate the transfer of carbon from pollen to zooplankton, we conducted standardized growth experiments on two cladocerans (*Daphnia longispina* and *Simocephalus vetulus*) that were fed different pollen species (*Alnus* sp., *Populus* sp., *Cedrus* sp.). In a second set of experiments, we also investigated whether heterotrophic microorganisms could repackage pollen carbon and upgrade its quality for zooplankton.

### **III. Material and methods**

#### **1. Pollen collection**

*Alnus* sp., *Populus* sp., and *Cedrus* sp. pollens were used in the experiments (Fig. 5.1). *Alnus* (25  $\mu\text{m}$  diameter, C:N = 13.6) and *Populus* (30  $\mu\text{m}$  diameter, C:N = 6.57) pollen grains are both in the optimal size range for particles ingested by cladocerans (Burns 1968). Due to the presence of air bladders, *Cedrus* pollen grains are somewhat larger (75 x 50  $\mu\text{m}$ , C:N = 17.7), but they can sometimes be ingested, depending on the direction from which they

approach the bivalve shell. The three pollen species were collected directly from trees in a floodplain of the river Allier. *Alnus* and *Populus* pollen samples were collected in March 2009, and *Cedrus* pollen samples in October 2009. They were passed through a 100  $\mu\text{m}$  mesh to remove any insect and other plant detritus.

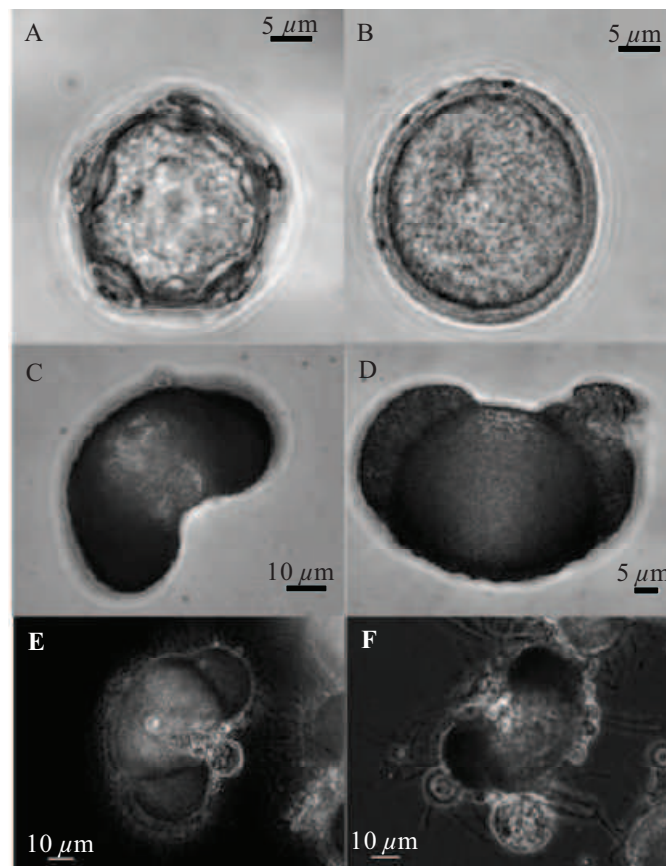


Figure 5.1 : Observations with an inverted microscope (magnification X63) of (A) *Alnus* pollen grain, (B) *Populus* pollen grain, (C) *Cedrus* pollen grain, (D) sonicated *Cedrus* (*Cedrus\**) pollen grain, (E, F) Chytrids sporangium on *Cedrus* pollen.

## 2. Origin and maintenance of daphnids

*Daphnia longispina* and *Simocephalus vetulus* were collected from a backwater sampling site of the river Allier, France.

For detailed information, please refer to chapter 2.

## 3. Culture of the autotrophic organism

*S. obliquus* (C:N = 30.7) was grown in Cyano medium according to the method described in chapter 2.

## 4. Culture of the heterotrophic microorganisms

During the pollen season of *Cedrus*, natural samples of water containing *Cedrus* pollen grains were taken from a backwater of the Allier River. Once in the laboratory, the samples were gently filtered through a 30  $\mu\text{m}$  membrane filter without a vacuum, and the particles retained on the filter were resuspended in filtered river water ( $<0.2 \mu\text{m}$ ). The suspension obtained was then observed under a microscope, and parasitized pollen grains on which sporangia of chytrids were visible were isolated with a Pasteur pipette and placed in Petri dishes containing filtered river water ( $<0.2 \mu\text{m}$ ) and pure *Cedrus* pollen grains previously collected from trees. The Petri dishes were maintained in darkness at 20°C. When microscopic observations showed that most of *Cedrus* pollen grains had been parasitized, the Petri dish cultures were transferred into an Erlenmeyer flask. The volume was adjusted to 50 mL with filtered river water, and pure *Cedrus* pollen grains were added. This step was then repeated using 100 mL, 250 mL, 500 mL, and 3-L Erlenmeyer flask. At each step, pure *Cedrus* pollen from trees was added in order to maintain a concentration of  $\sim 32$  mg of pollen per liter. Four days before beginning our experiments, we stopped adding the medium and pollen. Daily microscopic observations of culture aliquot samples revealed that the microorganism community was dominated by heterotrophic bacteria and chytrid zoospores ( $<5 \mu\text{m}$ ), although heterotrophic flagellates ( $>5 \mu\text{m}$ ) and ciliates were also present.

## 5. Experimental set-up

### a) *Food quality of pollen for cladocerans*

This experiment was performed in March 2009, just after the *Alnus* and *Populus* pollens had been collected. For each zooplankton species, one clone was isolated and transferred into a mixture of 50% filtered river water ( $<0.2 \mu\text{m}$ ) and 50% artificial medium. Cladocerans were fed on *S. obliquus* every day, and the culture medium was changed on alternate days. When the females produced offspring, the neonates (first generation) were separated, and the mothers discarded. This step was repeated, and the second generation kept. After they had released their first clutch, females from the second generation were kept, and the neonates discarded. The third-brood offspring from the second generation was finally used in the experiments in order to limit variability due to maternal size and weight (Lampert 1993). At  $20^\circ\text{C}$  and when fed on *S. obliquus*, generation times were slightly different for the two cladoceran species: it took *D. longispina* 5 days to reach maturity and 2 days more for the eggs to be delivered, whereas *S. vetulus* took 6 days to reach maturity, and another 3 days for the eggs to be delivered. Neonates were collected about 12 h after they had been released and randomly distributed in 200-mL beakers (12 individuals per beaker for each species). Average initial dry weight ( $W_0$ ) was measured by transferring randomly-selected neonates into pre-weighed aluminium cups (three samples of thirty neonates each), drying overnight at  $60^\circ\text{C}$ , cooling in a desiccator, and weighing on an electronic balance (Mettler Toledo balance  $\pm 1 \mu\text{g}$ ). Experiments were performed at  $20^\circ\text{C}$  with a 12:12 h light:dark cycle on four food sources with three replicates for each treatment. The four food sources were: *S. obliquus* in non-limiting concentrations ( $2 \text{ mg C L}^{-1}$ ; i.e., well above the incipient limiting level that is reported to be approximately  $0.5 \text{ mg C L}^{-1}$ ; Lampert 1978) (S+), *S. obliquus* in limiting concentrations ( $0.3 \text{ mg C L}^{-1}$ ) (S-), *Alnus* pollen ( $8 \text{ mg L}^{-1}$  i.e.,  $>3 \text{ mg C L}^{-1}$ ; *Alnus*) and *Populus* pollen ( $8 \text{ mg L}^{-1}$  i.e.,  $>3 \text{ mg C L}^{-1}$ ; *Populus*). Every day during the experiments, individuals were transferred to clean medium and fed. The experiments were conducted for 5 days for *D. longispina*, and 6 days later for *S. vetulus*. Females were collected and dried overnight at  $60^\circ\text{C}$  and weighed in order to obtain the average individual weight per replicate ( $W_t$ ). Somatic growth rates (g) were calculated as

$$g = (\ln W_t - \ln W_0)/t, \quad (1)$$

where  $t$  is the duration of each experiment in days.

*b) Trophic upgrading of pollen by microorganisms*

This experiment was performed in October 2009, just after the *Cedrus* pollen had been collected. The protocol and measurements of this experiment were the same as in the first experiment. The four food sources used here were: *S. obliquus* in non-limiting concentrations (2 mg C L<sup>-1</sup>) (S+), *Cedrus* pollen (8 mg L<sup>-1</sup> i.e., >3 mg C L<sup>-1</sup> *Cedrus*), *Cedrus* pollen that had been subjected to sonication for 4 min (at 7 watts) in order to disrupt the pollen air bladders and floatability (8 mg L<sup>-1</sup>; *Cedrus\**; Fig. 5.1), and the mixture of *Cedrus* pollen grains and microorganisms fed on it (C+M, C:N = 27.3). For this last treatment, 50 mL of the culture of heterotrophic microorganisms was added to the experimental beakers of daphnids containing 150 mL of medium (final volume: 200 mL).

## 6. Fatty acid composition of the food sources

Analyses were performed in triplicate of ~150 mg samples of pollen grains of each tree species, 2 mg triplicate samples of particulate organic carbon of *S. obliquus*, and duplicate pre-combusted GF/F filters (Whatman™) on which 50 mL of the mixture of the *Cedrus* pollen grains and microorganisms had been filtered. For the mixture of *Cedrus* pollen grains and microorganisms, analyses were repeated on the first, the fourth, and the sixth day of the experiment, in order to track the changes in the microorganism community, and thus any potential changes in lipid composition. Pollen samples were sonicated in a mixture of chloroform:methanol (v:v, 2:1) for 4 min one day before the lipids were extracted, and kept in this mixture over night. Lipids from the different food sources were then extracted using chloroform:methanol, following the method of Folch *et al.* (1957).

The method used for lipid analysis was fully described in chapter 2.

FAME were identified by comparing their retention times to those obtained for Supelco® standards (37-Component FAME mix, Bacterial FAME mix), laboratory standards (Cod liver oil FAME), and using an Agilent technologies 6850 gas chromatograph coupled to an Agilent technologies 5975B mass spectrometer and quantified against internal standards (13:0 and 23:0). The absolute amount of each FAME was normalized in terms of the independently-determined POC content of the samples. POC and particulate organic nitrogen (PON) were measured using a Flash 2000 analyzer (Thermo scientific).

## 7. Data analyses

The effects of the food source on the somatic growth rates of *D. longispina* and *S. vetulus* were analyzed by a one-way analyze of variance (ANOVA,  $\alpha < 0.05$ ). Pairwise comparisons were performed using a post hoc test (Tukey's Honestly Significant Difference [HSD]) with the Bonferroni adjustment ( $\alpha < 0.008$ ). All calculations were performed using the XLStat-Pro 7.5 (Addinsoft).

## IV. Results

### 1. Food quality of pollen for cladocerans

For both the crustacean species investigated here (*D. longispina* and *S. vetulus*), the best growth rates were obtained for individuals fed *S. obliquus* ad libitum (Fig. 5.2), while growth rates were 25% to 30% lower at the limiting *S. obliquus* concentration of 0.3 mg C L<sup>-1</sup>. The growth rates of *D. longispina* individuals fed on pollen were approximately half those of individuals fed on the optimum food source, with no significant difference attributable to the type of pollen, i.e., *Alnus* and *Populus* (Fig. 5.2). For *S. vetulus*, individuals fed on the *Populus* pollen had the lowest growth rate (Fig. 5.2). All the *S. vetulus* individuals fed on *Alnus* pollen died after the first 5 days of the experiment.

### 2. Trophic upgrading of pollen by microorganisms

For *D. longispina*, the fastest growth rates were obtained when they were fed on *S. obliquus*, and the lowest (about half the optimum growth rate) for individuals fed on *Cedrus* pollen alone, whether sonicated or not. However, individuals fed on the mixture of pollen grains and microorganisms exhibited an intermediate growth rate, only 25% below the optimum growth rates (Fig. 5.3). For *S. vetulus*, the lower growth rates were also obtained on pollen of *Cedrus* alone. However, *S. vetulus* fed on the mixture of pollen grains and

microorganisms exhibited individual growth rates as high as those on the optimum diet of *ad libitum* feeding on *S. obliquus* (Fig. 5.3).

### 3. Fatty acid composition of the different food sources

The FA contents of the different food sources are presented in Table 5.1. As for *S. obliquus*, the pollens tested were all rich in C<sub>18</sub> polyunsaturated fatty acids (C<sub>18</sub> PUFA). The pollens of *Alnus* and *Cedrus* contained very high amounts of linoleic acid (18:2 $\omega$ 6). Whereas *Populus* pollen exhibited a high proportion of eicosadineoic acid (20:2 $\omega$ 6), long-chain PUFA (>C<sub>18</sub>) were not detected in *S. obliquus*, and only small quantities of some of these C<sub>20</sub> or C<sub>22</sub> PUFA were detected in *Alnus* and *Cedrus* pollen. The mixture of *Cedrus* pollen and microorganisms contained higher amount of  $\alpha$ -linolenic acid (18:3 $\omega$ 3) and 18:2 $\omega$ 6 than the *Cedrus* pollen alone throughout the experiments. Furthermore, some long-chain PUFA, such as eicosapentaenoic acid (EPA, 20:5 $\omega$ 3), were not detected in the *Cedrus* pollen alone, but appeared in the mixture from the first day.



**Table 5.1 :** Fatty acid composition and content of *S. obliquus*, *Alnus* pollen, *Populus* pollen, *Cedrus* pollen, and of the mixture of *Cedrus* pollen and microorganisms (SAFA: saturated fatty acid, MUFA: monounsaturated fatty acid).

Fatty acids	<i>S. obliquus</i>				<i>Alnus</i> pollen				<i>Populus</i> pollen				<i>Cedrus</i> pollen				Mixture of <i>Cedrus</i> pollen and microorganisms									
	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)	Fatty acid content ( $\mu\text{g mg C}^{-1}$ )	Fatty acid composition (%)		
14:0	1.2±0.3	2.3±0.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.3±0	0.7±0	0.6±0	0.8±0	0.3±0	0.3±0	0.8±0	0.3±0	0.2±0	0.2±0	0.3±0	0.2±0	
16:0	8.8±2.1	17.4±1.1	2.8±0.6	11.2±0	1.1±0.1	16.6±0.3	2.4±0.8	10.9±0.1	8.8±0.9	21.3±0.1	10.9±0.4	14.4±0.6	15±3.4	8.8±0.9	21.3±0.1	10.9±0.4	14.4±0.6	15±3.4	8.8±0.9	21.3±0.1	10.9±0.4	14.4±0.6	15±3.4	8.8±0.9	21.3±0.1	
17:0	nd	nd	0.1±0	0.1±0	0.1±0	1.9±0	0.1±0	0.2±0	0.1±0	0.3±0	0.2±0	0.3±0	0.2±0.1	0.1±0	0.3±0	0.2±0	0.3±0	0.2±0.1	0.1±0	0.3±0	0.2±0	0.3±0	0.2±0.1	0.1±0	0.3±0	
18:0	0.8±0.5	1.5±0.5	0.3±0.1	1.3±0	0.2±0	2.6±0	0.3±0.1	1.4±0.1	1.9±0.1	4.5±0.2	3.3±0.9	4.4±1.3	4.4±1.3	1.9±0.1	4.5±0.2	3.3±0.9	4.4±1.3	4.4±1.3	1.9±0.1	4.5±0.2	3.3±0.9	4.4±1.3	4.4±1.3	1.9±0.1	4.5±0.2	
20:0	0.1±0.1	0.3±0.2	0.3±0	1.4±0.1	0.1±0	0.7±0	1.8±0.3	8.6±1.5	1.1±0	2.7±0.3	0.7±0.1	1.6±0.3	1.3±0.1	1.1±0	2.7±0.3	0.7±0.1	1.6±0.3	1.3±0.1	1.1±0	2.7±0.3	0.7±0.1	1.6±0.3	1.3±0.1	1.1±0	2.7±0.3	
22:0	nd	nd	0.6±0.1	2.2±0.1	0.1±0	1.1±0	0.7±0.1	3.3±0.8	0.1±0	0.2±0	0.1±0	0.2±0	0.1±0	0.1±0	0.2±0	0.1±0	0.2±0	0.1±0	0.1±0	0.2±0	0.1±0	0.2±0	0.1±0	0.1±0	0.2±0	
24:0	nd	nd	0.3±0.1	1±0.2	0.1±0	2.1±0.3	1.2±0.1	5.8±1.3	0.1±0	0.3±0.1	0.2±0	0.3±0.1	0.1±0	0.1±0	0.3±0.1	0.2±0	0.3±0.1	0.1±0	0.1±0	0.3±0.1	0.2±0	0.3±0.1	0.1±0	0.1±0	0.3±0.1	
Σ SAFA	10.9±2.7	21.4±1.1	4.4±0.9	17.2±0	1.7±0.2	25.5±0.6	6.4±1.3	30.2±3.7	12.3±1	30±0.5	16±1.3	21.2±1.9	21.8±10	12.3±1	30±0.5	16±1.3	21.2±1.9	21.8±10	12.3±1	30±0.5	16±1.3	21.2±1.9	21.8±10	12.3±1	30±0.5	
Σ Branched	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
16:1 ( <i>n</i> -7)	4.1±0.7	8.2±1	0.1±0	0.2±0	0.1±0	0.9±0.1	nd	nd	0.6±0.1	1.4±0.1	1.1±0.1	1.4±0.1	1.4±0.1	0.6±0.1	1.4±0.1	1.1±0.1	1.4±0.1	1.4±0.1	0.6±0.1	1.4±0.1	1.1±0.1	1.4±0.1	1.4±0.1	0.6±0.1	1.4±0.1	
18:1 ( <i>n</i> -9)	7.2±1.2	14.3±1.8	3.6±0.8	14±0.3	0.3±0	5.1±0.1	6.4±2.8	28.9±2.4	12.5±1.5	30.2±0.6	23.1±0.8	30.5±0.8	28.5±8.4	12.5±1.5	30.2±0.6	23.1±0.8	30.5±0.8	28.5±8.4	12.5±1.5	30.2±0.6	23.1±0.8	30.5±0.8	28.5±8.4	12.5±1.5	30.2±0.6	
18:1 ( <i>n</i> -7)	1±0.1	1.9±0.3	0.2±0	0.7±0.1	nd	nd	0.2±0.1	0.7±0	1±0.1	2.4±0.1	1.1±0.1	1.5±0.1	1.5±0.1	1±0.1	2.4±0.1	1.1±0.1	1.5±0.1	1.5±0.1	1±0.1	2.4±0.1	1.1±0.1	1.5±0.1	1.5±0.1	1±0.1	2.4±0.1	
Σ MUFA	12.3±2.1	24.4±3.1	3.8±0.8	14.9±0.2	0.4±0	6±0.3	6.6±2.8	29.7±2.4	14.1±1.8	34.1±0.8	25.3±0.8	33.5±0.7	45.3±3	14.1±1.8	34.1±0.8	25.3±0.8	33.5±0.7	45.3±3	14.1±1.8	34.1±0.8	25.3±0.8	33.5±0.7	45.3±3	14.1±1.8	34.1±0.8	
16:3 ( <i>n</i> -3)	6.7±3.2	12.4±2.4	nd	nd	0.1±0	0.7±0	nd	nd	0.1±0	0.2±0	nd	nd	nd	0.1±0	0.2±0	nd	nd	nd	0.1±0	0.2±0	nd	nd	nd	0.1±0	0.2±0	
18:2 ( <i>n</i> -6)	8.5±2.4	16.5±0.2	10.4±2.2	41.2±0.1	1±0.1	15.3±0.2	7.5±3.1	33.9±2.4	10.9±1.1	26.5±0	21.7±0.9	28.6±0.9	51±13.5	10.9±1.1	26.5±0	21.7±0.9	28.6±0.9	51±13.5	10.9±1.1	26.5±0	21.7±0.9	28.6±0.9	51±13.5	10.9±1.1	26.5±0	
18:3 ( <i>n</i> -6)	0.2±0.1	0.5±0.1	nd	nd	0.5±0.1	7.5±0.3	0.5±0	2.7±0.7	0.6±0.4	1.4±0.9	1±0.6	1.3±0.8	3±0.8	0.6±0.4	1.4±0.9	1±0.6	1.3±0.8	3±0.8	0.6±0.4	1.4±0.9	1±0.6	1.3±0.8	3±0.8	0.6±0.4	1.4±0.9	
18:3 ( <i>n</i> -3)	9.1±3.3	17.5±1.1	6±1.3	23.8±0.1	2.2±0.2	33.5±0.2	0.1±0.1	0.6±0.2	0.2±0	0.6±0.1	3.7±0.6	4.8±0.7	1.5±0.6	0.2±0	0.6±0.1	3.7±0.6	4.8±0.7	1.5±0.6	0.2±0	0.6±0.1	3.7±0.6	4.8±0.7	1.5±0.6	0.2±0	0.6±0.1	
18:4 ( <i>n</i> -3)	0.8±0.4	1.6±0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
20:2 ( <i>n</i> -6)	nd	nd	0.2±0	0.7±0.1	0.6±0.1	9.8±0.3	0.1±0	0.7±0.2	0.1±0.1	0.2±0.3	0.1±0.1	0.2±0.2	0.3±0	0.1±0.1	0.2±0.3	0.1±0.1	0.2±0.2	0.3±0	0.1±0.1	0.2±0.3	0.1±0.1	0.2±0.2	0.3±0	0.1±0.1	0.2±0.3	
20:3 ( <i>n</i> -6)	nd	nd	0.2±0	0.8±0.1	nd	0.5±0	0.2±0	0.8±0.1	0.2±0	0.6±0.1	0.5±0	0.7±0.2	0.6±0	0.2±0	0.6±0.1	0.5±0	0.7±0.2	0.6±0	0.2±0	0.6±0.1	0.5±0	0.7±0.2	0.6±0	0.2±0	0.6±0.1	
20:4 ( <i>n</i> -6)	nd	nd	0.1±0	0.2±0.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
20:4 ( <i>n</i> -3)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
20:5 ( <i>n</i> -3)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
20:5 ( <i>n</i> -6)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
22:2 ( <i>n</i> -6)	nd	nd	0.3±0.1	1.1±0.1	0.1±0	1.1±0.1	nd	nd	0.5±0	1.2±0.2	0.7±0	1±0.1	0.2±0	0.5±0	1.2±0.2	0.7±0	1±0.1	0.2±0	0.5±0	1.2±0.2	0.7±0	1±0.1	0.2±0	0.5±0	1.2±0.2	
22:6 ( <i>n</i> -3)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Σ ( <i>n</i> -3)	16.6±6.9	31.5±3.8	6.1±1.3	23.8±0.1	2.2±0.2	34.2±0.2	0.1±0.1	0.6±0.2	1.4±0.1	3.5±0.6	7.1±0.5	9.4±0.6	3.2±0.8	1.4±0.1	3.5±0.6	7.1±0.5	9.4±0.6	3.2±0.8	1.4±0.1	3.5±0.6	7.1±0.5	9.4±0.6	3.2±0.8	1.4±0.1	3.5±0.6	
Σ ( <i>n</i> -6)	8.7±2.5	17±0.2	11.2±2.3	44.1±0.2	2.2±0.3	34.3±0.4	8.4±3.2	38.3±1.4	12±1.4	29±0.5	23.6±1.1	31.1±1.2	55.2±14.8	12±1.4	29±0.5	23.6±1.1	31.1±1.2	55.2±14.8	12±1.4	29±0.5	23.6±1.1	31.1±1.2	55.2±14.8	12±1.4	29±0.5	
Σ PUFA	25.4±9.4	48.5±3.6	17.2±3.6	68±0.1	4.4±0.5	68.5±0.4	8.5±3.3	38.9±1.5	13.4±1.3	32.5±0.2	30.7±1.6	40.6±1.8	58.4±15.7	13.4±1.3	32.5±0.2	30.7±1.6	40.6±1.8	58.4±15.7	13.4±1.3	32.5±0.2	30.7±1.6	40.6±1.8	58.4±15.7	13.4±1.3	32.5±0.2	
Σ unidentified	3±1.2	5.8±0.6	nd	nd	nd	nd	0.2±0	1.2±0.2	0.1±0	0.3±0.1	1.3±0.4	1.7±0.5	0.3±0.2	0.1±0	0.3±0.1	1.3±0.4	1.7±0.5	0.3±0.2	0.1±0	0.3±0.1	1.3±0.4	1.7±0.5	0.3±0.2	0.1±0	0.3±0.1	

Values are given in percentages as means of  $n = 3 \pm \text{SD}$ . nd = not detected.

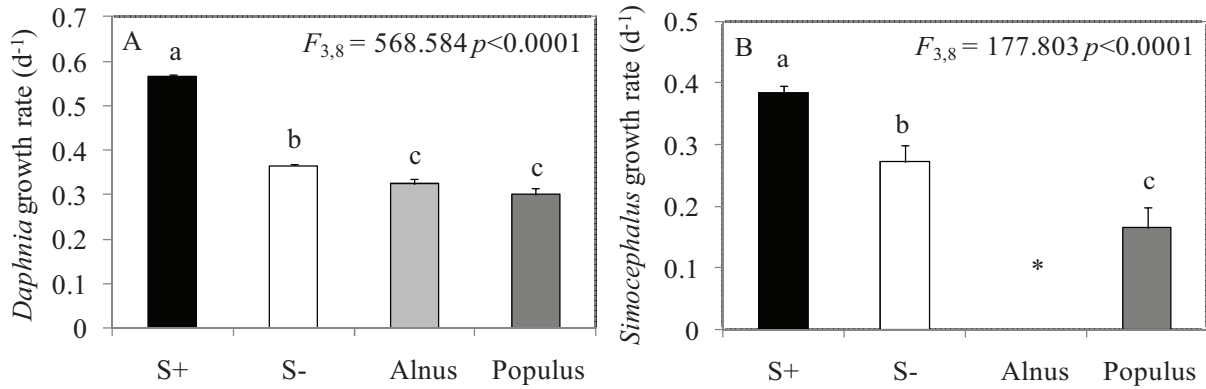


Figure 5.2 : (A) Growth rate ( $d^{-1}$ ) of *D. longispina* and (B) *S. vetulus* for four different food sources: *S. obliquus* in non-limiting concentrations ( $2 \text{ mg C L}^{-1}$ ) (S+), *S. obliquus* in limiting concentrations ( $0.3 \text{ mg C L}^{-1}$ ) (S-), *Alnus* pollen (Alnus), and *Populus* pollen (Populus). Data are means + SD for three replicates per treatment. On the treatment labeled with an asterisk all the individuals died after a few day. Results of the one-way analyses of variance (ANOVA) are presented at the top right corner of each graph ( $\alpha = 0.05$ ). Bars labeled with the same letters are not significantly different (Tukey's HSD,  $p < 0.008$ ).

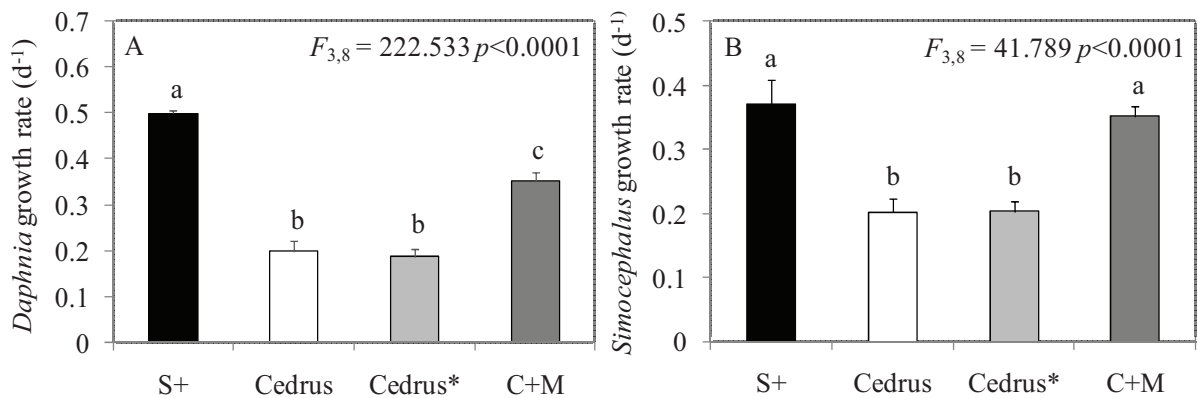


Figure 5.3 : (A) Growth rate ( $d^{-1}$ ) of *D. longispina* and (B) *S. vetulus* for four different food sources: *S. obliquus* in non-limiting concentrations ( $2 \text{ mg C L}^{-1}$ ) (S+), *Cedrus* pollen (Cedrus), sonicated *Cedrus* pollen (Cedrus\*), and a mixture of *Cedrus* pollen and microorganisms (C+M). Data are means + SD for three replicates per treatment. Results of the one-way analyses of variance (ANOVA) are presented at the top right corner of each graph ( $\alpha = 0.05$ ). Bars labeled with the same letters are not significantly different (Tukey's HSD,  $p < 0.008$ ).

## V. Discussion

Pollen rains can correspond to a quantitatively important particulate organic carbon input for freshwater systems (Richerson *et al.* 1970; Banks & Nighswander 2000). In the present study, we investigated whether pollen could support significant zooplankton growth, and whether the introduction of microorganisms as an intermediate trophic level enhances zooplankton development.

Compared to cladocerans fed on *S. obliquus*, which is usually considered to be a high quality food source (Lampert 1978; Ahlgren *et al.* 1990; Giebelhausen & Lampert 2001), individuals fed directly on the pollens tested exhibited low growth rates (Fig. 5.2, 5.3). All the individuals of *Simocephalus* even died after a few days when fed with *Alnus* pollen. These results suggest that pollen grains are a low quality food for zooplankton.

Pollen grains of the tree species tested herein belong to the optimal size range of particles ingested by cladocerans, as confirmed by the microscopic observations performed over the course of these experiments. However, in the case of *Cedrus* pollen, the presence of air bladders makes the pollen grains buoyant as neustonic particles (Hopkins 1950), which could reduce their accessibility to some planktonic grazers. The sonication treatment we performed damaged their air bladders (Fig. 5.1), resulting in decreased floatability and made the pollen more accessible and easier for cladocerans to ingest. However, cladocerans fed sonicated *Cedrus* pollen did not display a higher growth rate ( $0.19 \pm 0.01 \text{ d}^{-1}$  for *D. longispina*,  $0.20 \pm 0.01 \text{ d}^{-1}$  for *S. vetulus*) than those fed on untreated pollen ( $0.20 \pm 0.02 \text{ d}^{-1}$  for *D. longispina*,  $0.20 \pm 0.02 \text{ d}^{-1}$  for *S. vetulus*), which refutes the hypothesis that the low food quality of *Cedrus* pollen is related solely to its accessibility.

In addition to its accessibility, various other factors could also explain its low quality as a food source. These include its digestibility, which has been reported to be an important factor in the food quality for zooplankton (Van Donk *et al.* 1997; DeMott *et al.* 2010). Pollen grains are protected by an extracellular wall, known as the exine, which is composed of cellulose and sporopollenin, and which is highly resistant to degradation (Stanley & Linskens 1974). This is what makes pollen a good paleontological marker (Havinga 1967). Any animal consuming pollen grains has to degrade this refractory wall in order to access the cytoplasmic nutrients. Some studies of bird nutrition have shown that pollen grains were not degraded during passage through the avian digestive tract (Ford 1985; Brice *et al.* 1989). Amongst the invertebrates, some arthropods have developed adaptations to allow them to extract the

content from pollen, usually by inducing germination or the formation of instant pollen tubes as a result of osmotic shock (a process similar in appearance to naturally germinating pollen and referred to as ‘pseudo-germination’), or with digestive enzymes (Roulston & Cane 2000). However, in our study, it seems unlikely that cladocerans have the enzymatic equipment required to digest pollen as, as far as we are aware, no such ability has ever been documented. The low somatic growth of cladocerans when fed with the different kind of pollen as their only food source might therefore be explained in a great part by the low digestibility of pollen.

The introduction of microorganisms as an intermediate trophic level in the context of feeding with *Cedrus* pollen significantly increased the somatic growth of cladocerans ( $0.35 \pm 0.01 \text{ d}^{-1}$  for both cladoceran species). The microbial community present in the mixture was composed of heterotrophic bacteria, heterotrophic flagellates, ciliates, and chytrid fungi. Pollen grains are remarkably resistant to microbial degradation (Havinga 1967). Zoosporic fungi, especially chytrids, are among the few microorganisms able to overcome the barrier created by the exine and to grow on pollen (Goldstein 1960; Skvarlaa & Anderegg 1972; Czczuga & Muszynska 2001). Chytrids might thus play an important role in damaging the pollen wall in order to permit other organisms, such as bacteria, to gain access to the cytoplasmic content of pollen. In our study, we therefore expected that bacteria and bacterivorous protists would transfer pollen carbon to cladoceran grazers. On the other hand, zoospores released by chytrids could certainly be grazed by cladocerans (Kagami et al. 2004). Chytrid zoospores have been shown to accumulate PUFA and sterols extracted from their host (Kagami et al. 2007). Thus, in this case, chytrid zoospores could play a key role in transferring not only pollen carbon, but also pollen lipids compounds to higher trophic levels.

FA analysis of the different food sources showed that the compositions of the three pollens and the composition of *S. obliquus* have some similarities. As in the chlorophyte, large proportions of C<sub>18</sub> PUFA such as linolenic acid, 18:2 $\omega$ 6, and  $\alpha$ -linoleic acid, 18:3 $\omega$ 3, were recorded in all three pollen species. It therefore seems unlikely that the biochemical content of pollen grains was a limiting factor for cladoceran development. Like the previous food sources, the mixture of pollen and microorganisms contained large amounts of C<sub>18</sub> PUFA. However, lipid analysis revealed the emergence of some long chain PUFA (C<sub>20</sub> and C<sub>22</sub> PUFA), such as EPA, that were not detected in *Cedrus* pollen alone and which may be associated with the introduction of heterotrophic protists (Bec *et al.* 2006; Desvillettes & Bec

2009). Such long chain PUFA were highlighted to be of particular importance for the growth and reproduction of cladocerans (Müller-Navarra et al. 2000; Bec et al. 2003; Masclaux et al. 2009). In our study a microbial food web therefore appears to have transferred and upgraded pollen carbon to cladocerans.

This study therefore shows that the pollen of wind pollinated species cannot support zooplankton growth directly. The low quality of pollen grains as food could be mainly attributable to their refractory wall, which leads to a poor digestibility. Nevertheless, damage of the pollen grain wall by saprophytic microorganisms, especially chytrid fungi, can create a microbial food web transferring and upgrading pollen carbon to metazoan grazers. Further investigations of the role of chytrid fungi in the transfer of carbon and PUFA from pollen grains to zooplanktonic communities are therefore required. Finally, the massive deposition of pollen during the pollination of anemophilous species could have a significant effect on secondary production.

## **Trophic niche induction by pollen rain in aquatic systems: effects on secondary production and biodiversity**

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### **En préparation**

#### **I. Abstract**

Huge deposition of anemophilous plants pollen in aquatic ecosystems results in a massive accumulation of allochthonous organic matter at the water-air interface. As most pollen grains are edible-sized particles for freshwater filter-feeders, we thus hypothesized that pollen rain could increase habitat diversity and induce a temporary trophic niche, and might hence benefit to zooplankton diversity and secondary production. Our results show that a pine pollen rain event on an oxbow lake created a strong vertical heterogeneity of food resources and consumers in the water column. Bulk and fatty acids specific isotopes analysis showed that some zooplankton species (such as *Scapholeberis*) forage specifically on neuston induced from pollen rain deposition and subsequent repackaging and upgrading of pollen C by microorganisms/fungi. Pollen rains may thus not only act as an external input of nutrients but also as a structuring factor promoting habitat heterogeneity and supporting consumers diversity and production in aquatic ecosystems.

Key words: pine pollen, spatial heterogeneity, allochthony, foraging behavior, fatty-acid specific stable isotope analyses

## II. Introduction

Anemophilous plants produce large quantities of pollen to offset the unpredictability of wind pollination. Northern forest ecosystems which are dominated by wind pollinated trees release annually huge quantities of pollen ranging from 2-4 kg ha<sup>-1</sup> (Richerson *et al.* 1970; Lee & Booth 2003). Most pollen grains may be deposited within a relatively short distance from their site of production (Koski 1970), but may also be transported across long distances (i.e. up to 3000 km, Campbell *et al.* 1999). Thus, large amounts of pollen can end up in aquatic ecosystems during pollen rains (Richerson *et al.* 1970; Banks & Nighswander 2000; Graham *et al.* 2006).

Once in the water, pollen grains of most deciduous trees species sink. On the contrary, the presence of air bladders makes pollen grains of coniferous trees buoyant (Hopkins 1950). Air bladders have been reported to be an adaptation for increasing dispersal distance (Schwendemann *et al.* 2007) while buoyancy may be an effective mechanism of pollen capture and transport upwards into the ovule of coniferous trees (Leslie 2010). Anyway, during coniferous pollen rains, pollen grains accumulate at the water-air interface and become neustonic particles. In such yellow lakes, the high concentration of allochthonous organic matter at the water-air interface (neuston) creates thus an heterogeneity in the water column.

Mineral and biochemical compositions of pollen are characterized by high phosphorus (Banks & Nighswander 2000) and high polyunsaturated fatty acids (Manning 2006) contents. Adding the fact that most pollen grains are edible-sized particles for freshwater filter-feeders, those characteristics make pollen a potentially high-quality food for zooplankton (Brett & Müller-Navarra 1997; Frost *et al.* 2006). However, this hypothesis has been invalidated by a recent experimental study which showed that anemophilous plant pollen alone could not promote zooplankton growth (Masclaux *et al.* 2011) and its poor food quality has been attributed to its low digestibility (Masclaux *et al.* 2011). Pollen grains are protected by the exine, an extracellular wall, which make them highly resistant even to microbial degradation (Stanley & Linskens 1974). Chytrids (zoosporic fungi), are amongst the few microorganisms able to bypass the exine and grow on pollen (Goldstein 1960). The strong tendency of these fungi to form multiple infections of individual pollen grains weakens the wall and, undoubtedly, facilitates their further decomposition by other biological agents (Goldstein 1960). Therefore, in aquatic ecosystems, chytrid infection of pollen grains could initiate the

formation of a microbial food web composed of chytrids zoospores, heterotrophic bacteria and protozoan grazers which have been shown to transfer pollen carbon and upgrade its quality to promote zooplankton growth (Masclaux *et al.* 2011).

However, because buoyant pollen grains and associated microorganisms sit at the water–air interface, they might not be accessible to most zooplankton species which usually forage in the sestonic compartment below. In contrast, other zooplankton species that are able to feed on neuston compartment would benefit of a high quantity/quality food during pollen rains. For such species, solar radiations may be a constraint at the water-air interface but warm temperatures may also shorten their generation times.

As environmental variability in space and time is a primary mechanism allowing species that share resources to coexist (Shurin *et al.* 2010), we hypothesized that pollen rain could induce a trophic niche which may sustain secondary production and zooplankton biodiversity in aquatic ecosystems. Neuston and seston compartments of an oxbow lake were sampled during a pine pollen rain in order to evaluate whether (i) pollen deposition initiated a vertical heterogeneity gradient of food sources and consumers, (ii) pollen carbon ultimately contributed to the secondary production of zooplankton consumers.

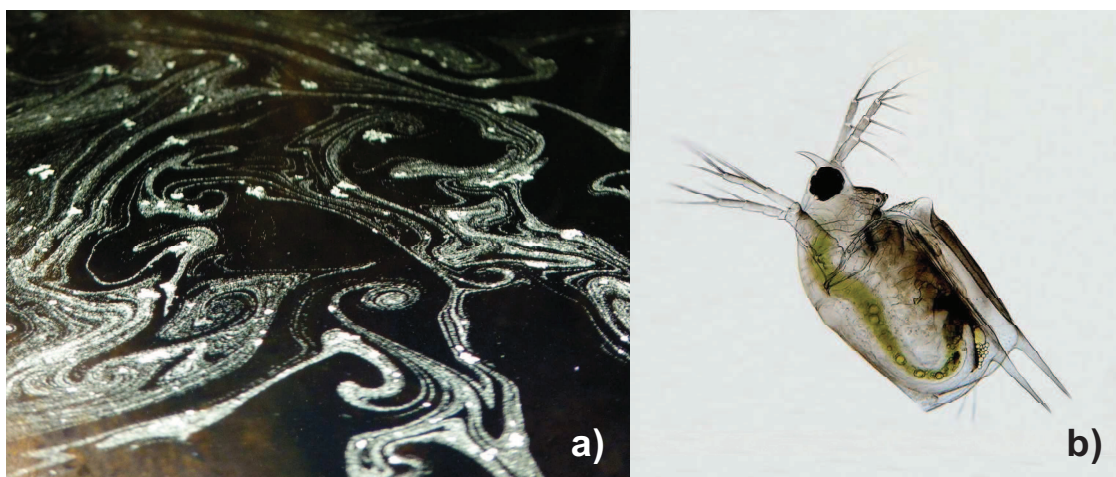


Figure 5.4 : a) Pollen deposition on the water surface of an oxbow lake, b) *Scapholeberis mucronata*, photo by Paul-Rainer Heck



### **III. Material and methods**

#### **1. Study site and sampling**

All the samples were obtained from an oxbow lake, located in the Natural Reserve of Bec de Dore (center of France 3.48°W, 46.00°N). For study site description, please refer to chapter 2.

The study was conducted in May 2009 during the episodic pollination of *Pinus* sp. The oxbow lake was sampled from the estimated beginning of the pollination (May 11<sup>th</sup>), and every two days for the following ten days. For each sampling date, neuston was sampled by collecting water at the air-water interface (surface layer  $\approx$  2mm) with a modified oil sampler acc. to Schomaker (Agogué *et al.* 2004) and seston was sampled from water collected at a 50 cm depth. For each compartments, water was screened on a 50  $\mu$ m-mesh filtercup to remove zooplankton and plant debris, and then filtered onto precombusted GF/F-filters (Whatman<sup>TM</sup>) immediately after sampling, for lipid and isotope analyses. In addition, water samples (<100  $\mu$ m) were fixed for heterotrophic microorganisms abundance (with formaldehyde for bacteria, glutaraldehyde 1% for flagellates and with lugol for ciliates). Subsamples from the neuston and seston were preserved in 4% formaldehyde solution saturated with sucrose for zooplankton enumeration and the other part was immediately frozen for lipid and isotopic analyses. All the samples for lipid and isotopic analyses were stored at -80°C until analyses.

#### **2. Abundance of bacteria, heterotrophic flagellates, ciliates, zooplankton, and pollen grains**

Bacterial abundances were determined on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, Calif.) equipped with a 15-mW 488-nm air-cooled argon-ion laser and a standard filter setup. Samples were stained with SYBR Green I, and delivered at a calibrated rate of 77  $\mu$ L/min (Marie *et al.* 1999).

Flagellates were enumerated by standard epifluorescence microscopy after primulin staining. Samples were filtered onto black 0.8  $\mu$ m pore-size polycarbonate filters, before

mounting on microscope slides. Abundances were determined with a Zeiss Axiovert 200M epifluorescence microscope. In addition, the abundance of pollen grains could be determined from the same slides.

Ciliate counts were performed under an inverted microscope using Utermöhl method.

Zooplanktonic species were identified and counted under a binocular microscope in Dolffus chambers.

### 3. Bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses

Stable isotopes analyses were performed in triplicate on neuston, seston and on four zooplankton taxa: the three cladocerans *Chydorus* sp, *Bosmina* sp. and *Scapholeberis mucronata* and calanoid copepod. Zooplankton was kept in ice and manually sorted under a binocular microscope.

For detailed information, please refer to chapter 2.

Lipid content can affect results and then conclusions of  $\delta^{13}\text{C}$  analyses as lipids have more negative  $\delta^{13}\text{C}$  values relative to other major biochemical compounds in animal tissues (DeNiro & Epstein 1977). Hence, zooplankton  $\delta^{13}\text{C}$  values were normalized to a zero lipid content by calculating lipid extracted  $\delta^{13}\text{C}$  values using a mass balance correction model elaborated by (Smyntek *et al.* 2007). mean shift =  $1.3 \pm 0.7\text{‰}$ ).

### 4. Fatty acids analyses

Fatty acids (FA) analyses were performed in triplicate on neuston and seston samples as well as on two dominant zooplanktonic taxa: calanoid copepod and *Scapholeberis*. Zooplankton samples were thawed and manually sorted (200 individuals per sample) under a binocular microscope.

The method used for lipid analysis was fully described in chapter 2.

FAME were identified by comparing retention times with those obtained from Supelco® and laboratory standards and quantified against internal standards (13:0 and 23:0).

## 5. Stable carbon isotopes of FA

Extracted FAME of the samples corresponding to peak of pollination (May 13<sup>th</sup>) were analyzed for their C stable isotope compositions at the UC Davis Stable Isotope Facility for CSIA with a gas chromatography (GC)-combustion-isotope ratio mass spectrometer (IRMS) system: a Trace GC Ultra (Thermo Electron) was interfaced with a Finnigan Delta Plus IRMS (Thermo Electron) and installed with a VF-5 GC column (30.0 m length, 0.25 mm ID, 0.25  $\mu\text{m}$  film thickness). Samples were injected in splitless mode (inlet temperature 250°C; carrier gas: helium; constant flow rate of 2.2 ml min<sup>-1</sup>; oven temperature rise from 110 to 220°C at 4°C min<sup>-1</sup> with 10 min hold, then 220 to 290°C at 10°C min<sup>-1</sup> with 4 min hold). An isotopically calibrated CO<sub>2</sub> reference was introduced at the beginning and end of each GC run to convert the raw data for combusted FAME peaks into  $\delta^{13}\text{C}$  values according to the formula:

$$\delta^{13}\text{C}_{\text{sample}} = \left[ \left( \frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{CPDB}}} - 1 \right) \times 1000 \right]$$

$\delta^{13}\text{C}$  values were corrected using working standards composed of several FAMES calibrated against National Institute of Standards and Technology standard reference materials. FAME  $\delta^{13}\text{C}$  values were corrected for the methyl-group addition during methylation according to the formula:

$$\delta^{13}\text{C}_{\text{FA}} = [(n + 1) (\delta^{13}\text{C}_{\text{FAME}}) - \delta^{13}\text{C}_{\text{MeOH}}] / n$$

where  $\delta^{13}\text{C}_{\text{FA}}$  represents the FA  $\delta^{13}\text{C}$  prior to methylation,  $\delta^{13}\text{C}_{\text{FAME}}$  and  $\delta^{13}\text{C}_{\text{MeOH}}$  are the  $\delta^{13}\text{C}$  values of the measured FAME and methanol used during methylation, respectively, and  $n$  is the number of carbon atoms in the (non-methylated) FA. For the present study, the  $\delta^{13}\text{C}$  of the methanol used for FAME preparation was -46.40 ‰. In addition to essential FA (i.e. 18:2 $\omega$ 6, 18:3 $\omega$ 3, 20:4 $\omega$ 6, 20:5 $\omega$ 3, and 22:6 $\omega$ 3), the FA that presented the greatest abundance, were present in all samples and for which no coelutions occurred were analyzed for their  $\delta^{13}\text{C}$  values (i.e. 14:0, 16:0, 18:0, 22:0, 16:1 $\omega$ 7, and 18:1 $\omega$ 9).

## 6. Data analysis

Differences in bacterial, HF and ciliate abundances as well as differences in  $\delta^{13}\text{C}$  values between seston and neuston were investigated using Wilcoxon tests. All calculations were performed using the XLStat-Pro 7.5 (Addinsoft). Significance level was set at  $\alpha = 0.05$ .

## IV. Results

The sampling was performed during a calm and sunny period with the exception of the 15<sup>th</sup> May on which it rained. There was no strong wind during the sampling period, suggesting modest influence of mixing.

### 1. Characterization of vertical heterogeneity: description of neuston and seston

As hypothesized, the presence of air bladders on pine pollen grains makes them buoyant as neustonic particles (Fig. 5.4a). The number of pollen grains was by far higher in the neuston (between 544 and 3527 pollen grains mL<sup>-1</sup>) than in the seston (between 17 and 61 pollen grains mL<sup>-1</sup>) during the pollination peak, between the 11<sup>st</sup> and the 15<sup>th</sup> May (Fig. 5.5). POC concentrations were on average six times higher ( $W=36, p=0.002$ ) and PON concentrations twelve times higher ( $W=36, p=0.002$ ) in neuston than in seston. As a result, C/N ratios in neuston were lower than those in seston ( $W=0, p=0.004$ ).

Neuston and seston presented moreover pronounced differences in the concentration and structure of the microbial communities (Fig. 5.5). The concentrations of bacteria ( $W=61, p=10^{-3}$ ), Ciliates ( $W=52.5, p=0.03$ ), HF  $< 5 \mu\text{m}$  ( $W=64, p=10^{-4}$ ) were significantly higher in the neuston than in the seston all along the sampling period (up to 10 times for bacteria and 100 times for HF, Fig. 5.5). In neuston, the number of HF  $< 5 \mu\text{m}$  reached indeed a value as high as  $8.1 \cdot 10^5$  cells mL<sup>-1</sup> the 21<sup>th</sup> May. In contrast, the concentrations of HF  $> 5 \mu\text{m}$  were not significantly different between the two compartments ( $W=50, p=0.06$ ).

The structure of zooplanktonic communities differed between neuston and seston (Fig. 5.6). In the sestonic compartment, the genus *Bosmina* was indeed the most abundant whereas *Scapholeberis* and *Chydorus* were dominant in neuston. Moreover, the structure of zooplanktonic community remained stable in seston all along the sampling period but changed in neuston with an increase of the relative abundance of *Scapholeberis* and chydorids cladocerans after the pollen rain, which represented up to 80% of the relative abundance of neustonic zooplankton between the May 13<sup>th</sup> and 19<sup>th</sup> (Fig. 5.6).

## 2. Assessment of allochthonous pollen carbon contribution to zooplankton production

### a) *Fatty acid analyses*

The fatty acids (FA) composition of pine pollen was characterized by the dominance of two FA, the oleic acid (18:1 $\omega$ 9) and the linoleic acid (18:2 $\omega$ 6), which represented 62.5% of total FA weight. Long-chain polyunsaturated fatty acids (long-chain PUFA,  $\geq 20$  carbon atoms) were not detected in pollen (Table 1).

Total FA concentrations in neuston were significantly higher than in seston (1.8 times on average,  $W=31$ ,  $p=0.04$ ). Moreover, the content in nutritionally important compounds such as  $\omega$ 3-PUFA was 1.4X to 4.2X higher in neuston compared to seston (Table 1).

The relative proportions of the different FA were presented in table 1. A principal component analysis conducted on FA relative proportions in neuston and seston did not detect any striking differences in their FA composition (results not shown). In both seston and neuston, PUFA composition was dominated by C18-PUFA. However, long-chain PUFA were found in lower levels (<5% of total FA weight) in seston than in neuston ( $W=36$ ,  $p=0.005$ ) where they always contributed >5% of total FA weight, with the highest value recorded the 21<sup>st</sup> May (9.5%).

**Table 5.2 :** POC and PON concentrations,  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰) values (mean  $\pm$  SD), and fatty acid (FA) composition (mean  $\pm$  SD) of pine pollen, neuston, and seston. PUFA: polyunsaturated fatty acids. nd = not detected. Bacterial FA: the sum of 15:0 and 17:0 and their iso- and anteiso-series.

	11-May		13-May		15-May		17-May		19-May		21-May	
	neuston	seston	neuston	seston	neuston	seston	neuston	seston	neuston	seston	neuston	seston
POC ( $\mu\text{g L}^{-1}$ )	13126	3107	29312	3087	5042	3213	13455	2450	11420	3592	38630	3547
PON ( $\mu\text{g L}^{-1}$ )	2342	69	5561	404	833	487	2644	356	2030	470	6876	509
C/N	17.7	5.6	10.7	7.7	6.1	6.6	5.1	6.8	5.6	7.6	5.6	7.0
$\delta^{13}\text{C}$	-26.5 $\pm$ 0.0	-33.0 $\pm$ 0.5	-28.5 $\pm$ 1.0	-33.3 $\pm$ 0.0	-29.7 $\pm$ 0.0	-31.9 $\pm$ 0.5	-28.2 $\pm$ 0.0	-32.9 $\pm$ 0.2	-28.7 $\pm$ 0.2	-32.2 $\pm$ 0.1	-27.5 $\pm$ 0.1	-30.8 $\pm$ 0.4
$\delta^{15}\text{N}$	3.4 $\pm$ 0.3	1.8 $\pm$ 0.3	6.3 $\pm$ 0.4	1.8 $\pm$ 0.3	1.7 $\pm$ 0.4	0.5 $\pm$ 1.1	0.8 $\pm$ 0.5	0.2 $\pm$ 1.0	1.3 $\pm$ 0.5	1.3 $\pm$ 0.1	6.6 $\pm$ 0.2	3.1 $\pm$ 0.7
FA percentage (%)												
14:0	0.3 $\pm$ 0.2	11.4 $\pm$ 1.1	5.4 $\pm$ 0.7	19 $\pm$ 0.2	5.7 $\pm$ 0.5	7.8 $\pm$ 0.3	4.4 $\pm$ 0.6	12 $\pm$ 0.9	6.2 $\pm$ 0.6	12.7 $\pm$ 0.7	5.8 $\pm$ 0.6	23.3 $\pm$ 0.4
16:0	23.5 $\pm$ 0.3	22.1 $\pm$ 0.1	19.1 $\pm$ 0.5	16.3 $\pm$ 0.4	20.5 $\pm$ 0.5	19.6 $\pm$ 1	19.2 $\pm$ 0.4	21.1 $\pm$ 1.4	23.8 $\pm$ 0.9	19 $\pm$ 1.2	20.8 $\pm$ 0.9	10.5 $\pm$ 0.9
18:0	6.3 $\pm$ 0.5	5.1 $\pm$ 0.3	13.5 $\pm$ 1.1	3.4 $\pm$ 0.3	11.9 $\pm$ 2.7	8.3 $\pm$ 0.2	12.2 $\pm$ 1.7	5 $\pm$ 0.8	18.5 $\pm$ 6.2	5.3 $\pm$ 0.3	12 $\pm$ 1	1.5 $\pm$ 0.2
22:0	2.0 $\pm$ 0.2	0.9 $\pm$ 0.2	2.3 $\pm$ 0.2	0.5 $\pm$ 0.1	1.8 $\pm$ 0	4.5 $\pm$ 0.4	1.8 $\pm$ 0.2	0.8 $\pm$ 0.1	1.7 $\pm$ 0.2	1 $\pm$ 0.2	3.9 $\pm$ 2.7	0.2 $\pm$ 0
24:0	0.3 $\pm$ 0.3	1 $\pm$ 0.3	2.4 $\pm$ 0.4	0.7 $\pm$ 0.1	2 $\pm$ 0.1	2.5 $\pm$ 0.3	3.4 $\pm$ 1.8	0.9 $\pm$ 0.2	nd	1.5 $\pm$ 0.4	3.4 $\pm$ 0.3	0.5 $\pm$ 0.2
Bacterial FA	nd	3.5 $\pm$ 0.1	6.7 $\pm$ 0.2	3.4 $\pm$ 0.4	4.3 $\pm$ 0.4	2.9 $\pm$ 0.5	6.9 $\pm$ 0.8	3.5 $\pm$ 0.5	5.5 $\pm$ 0.7	5.4 $\pm$ 0.4	6.8 $\pm$ 0.7	3.9 $\pm$ 0
16:1 $\omega$ 7	0.3 $\pm$ 0.0	16.5 $\pm$ 2	12.7 $\pm$ 1.4	7.5 $\pm$ 0.3	20.8 $\pm$ 1.7	11.7 $\pm$ 0.8	16.5 $\pm$ 0.6	11.9 $\pm$ 1.4	15 $\pm$ 2.4	13.6 $\pm$ 1	15.1 $\pm$ 1.1	3.2 $\pm$ 0
18:1 $\omega$ 9	26.0 $\pm$ 0.1	7.5 $\pm$ 0.3	6.7 $\pm$ 0.5	3.9 $\pm$ 0.1	5.7 $\pm$ 0.1	8.7 $\pm$ 1	6 $\pm$ 0.7	13 $\pm$ 1	6.2 $\pm$ 0.2	6.1 $\pm$ 0.4	6.2 $\pm$ 0.4	2.5 $\pm$ 0.2
18:1 $\omega$ 7	0.9 $\pm$ 0.0	4.9 $\pm$ 0.2	5.2 $\pm$ 0.2	2.6 $\pm$ 0.1	8.1 $\pm$ 0.4	5.5 $\pm$ 0.5	8.6 $\pm$ 0.2	3.4 $\pm$ 0.2	6.3 $\pm$ 1	3.9 $\pm$ 0.2	5.6 $\pm$ 0.3	1.4 $\pm$ 0
C16-PUFA	nd	4.7 $\pm$ 0.3	11.2 $\pm$ 0.3	1.7 $\pm$ 0.2	5.2 $\pm$ 0.3	6.8 $\pm$ 1	9.8 $\pm$ 0.6	2.6 $\pm$ 0.3	3.8 $\pm$ 0.8	4.7 $\pm$ 0.9	10.4 $\pm$ 1.2	1 $\pm$ 0
18:2 $\omega$ 6	36.5 $\pm$ 0.3	5.2 $\pm$ 0.3	4.1 $\pm$ 0.8	3.7 $\pm$ 0.1	2.3 $\pm$ 0.2	5 $\pm$ 0.5	2.5 $\pm$ 0.2	7.2 $\pm$ 0.4	2.4 $\pm$ 0.2	3.5 $\pm$ 0.5	2 $\pm$ 0.3	2.8 $\pm$ 0.2
18:3 $\omega$ 3	1.5 $\pm$ 0.6	5.1 $\pm$ 0.1	4.3 $\pm$ 0.1	7.3 $\pm$ 0.1	5.3 $\pm$ 0.3	3.5 $\pm$ 0.1	3.6 $\pm$ 0.1	4.4 $\pm$ 0.4	4.6 $\pm$ 0.7	5.3 $\pm$ 0.4	2.8 $\pm$ 0.4	8.2 $\pm$ 0.6
18:4 $\omega$ 3	2.4 $\pm$ 0.1	6.5 $\pm$ 0.7	3.3 $\pm$ 0.1	21.8 $\pm$ 0.7	2.7 $\pm$ 0.2	5 $\pm$ 0.1	2.1 $\pm$ 0.1	9.7 $\pm$ 2.8	3.4 $\pm$ 0.1	13 $\pm$ 1.6	2.9 $\pm$ 0.1	31.5 $\pm$ 0.8
20:4 $\omega$ 6	nd	0.7 $\pm$ 0.1	0.5 $\pm$ 0	0.5 $\pm$ 0	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0	0.5 $\pm$ 0	0.9 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0.1	0.5 $\pm$ 0
20:5 $\omega$ 3	nd	2.7 $\pm$ 0.3	1.5 $\pm$ 0.1	1.7 $\pm$ 0.1	1.7 $\pm$ 0.2	1.8 $\pm$ 0.1	1.5 $\pm$ 0.1	1.6 $\pm$ 0.2	1.8 $\pm$ 0.2	1.7 $\pm$ 0.1	1.8 $\pm$ 0.3	1.1 $\pm$ 0.1
22:4 $\omega$ 6	nd	1.1 $\pm$ 0.3	nd	3.9 $\pm$ 0.1	nd	3.3 $\pm$ 0.1	nd	2.1 $\pm$ 0.5	nd	1.9 $\pm$ 0.5	nd	5.8 $\pm$ 0.6
22:6 $\omega$ 3	nd	1 $\pm$ 0.3	1.4 $\pm$ 0.3	2.1 $\pm$ 0	1 $\pm$ 0.1	1.8 $\pm$ 0.4	0.8 $\pm$ 0.2	1 $\pm$ 0.1	nd	0.9 $\pm$ 0.2	nd	2.1 $\pm$ 0.4
$\Sigma$ PUFA (%)	40.4 $\pm$ 0.9	27 $\pm$ 0.1	26.2 $\pm$ 0.9	42.8 $\pm$ 1.1	19.1 $\pm$ 0.9	27.7 $\pm$ 2	21 $\pm$ 0.3	30.5 $\pm$ 1.9	16.9 $\pm$ 2	31.6 $\pm$ 2.2	20.6 $\pm$ 0.3	53 $\pm$ 1.2
$\Sigma$ PUFA ( $\mu\text{g mg C}^{-1}$ )		3.6 $\pm$ 0.6	7.1 $\pm$ 0.5	6.8 $\pm$ 0.9	1.1 $\pm$ 0.2	4.5 $\pm$ 0.8	2.0 $\pm$ 0.4	5.0 $\pm$ 0.1	0.8 $\pm$ 0.2	3.7 $\pm$ 0.3	1.5 $\pm$ 0.4	12.4 $\pm$ 2.4
Total FA concentrations ( $\mu\text{g mg C}^{-1}$ )		13.1 $\pm$ 2.3	27.2 $\pm$ 1.9	16.9 $\pm$ 1.7	5.7 $\pm$ 0.6	14.2 $\pm$ 4.8	9.5 $\pm$ 1.9	14.1 $\pm$ 4.2	4.9 $\pm$ 0.9	11.6 $\pm$ 0.5	7.3 $\pm$ 2.0	23.4 $\pm$ 5.0

b) *Bulk isotope analysis (SIA)*

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of pine pollen were respectively  $-26.5 \pm 0.0\text{‰}$  and  $3.4 \pm 0.3\text{‰}$ . Seston was more  $^{13}\text{C}$  depleted than neuston with  $\delta^{13}\text{C}$  values ranging from  $-33.5\text{‰}$  to  $-30.3\text{‰}$  for seston and from  $-29.8\text{‰}$  to  $-27.4\text{‰}$  for neuston ( $W=36$ ,  $p=0.002$ ), hence highlighting marked isotopic differences between the two endmembers for C but not for N isotopes ( $W=24.5$ ,  $p=0.33$ , Fig. 5.7). Calanoid copepods and *Bosmina* were the most  $^{13}\text{C}$  depleted consumer taxa ( $\delta^{13}\text{C} = -39.0 \pm 0.8\text{‰}$  for the calanoides and  $\delta^{13}\text{C} = -34.5 \pm 1.9\text{‰}$  for the *Bosmina*), with  $\delta^{13}\text{C}$  values that were even below those of seston. *Chydorus* had intermediate values ( $\delta^{13}\text{C} = -30.0 \pm 0.8\text{‰}$ ) and *Scapholeberis* was the most  $^{13}\text{C}$  enriched species ( $\delta^{13}\text{C} = -27.2 \pm 0.7\text{‰}$ ) with  $\delta^{13}\text{C}$  values that matched those of neuston.  $\delta^{15}\text{N}$  values of neuston and *Scapholeberis* exhibit large variation during sampling period with values ranging from  $0.2\text{‰}$  to  $6.7\text{‰}$  for neuston and  $-0.8\text{‰}$  to  $2.8\text{‰}$  for *Scapholeberis* (Fig. 5.7).

c) *FA isotope analysis (FA-SIA)*

The FA selected for their  $\delta^{13}\text{C}$  analyses were 14:0, 16:0, 18:0, 22:0, 16:1 $\omega$ 7, 18:1 $\omega$ 9, 18:2 $\omega$ 6, 18:3 $\omega$ 3, 20:4 $\omega$ 6, 20:5 $\omega$ 3 and 22:6 $\omega$ 3.  $\delta^{13}\text{C}$  values of pollen FA fell within a relatively narrow range, between  $-27.9\text{‰}$  to  $-25.3\text{‰}$ , consistently with pollen bulk  $\delta^{13}\text{C}$  value (Fig. 5.8).  $\delta^{13}\text{C}$  values of the neuston FA ranged from  $-32.3\text{‰}$  to  $-24.7\text{‰}$ , and long-chain PUFA (20:5 $\omega$ 3 and 22:6 $\omega$ 3) that could be detected in neuston but not pollen showing  $\delta^{13}\text{C}$  values around  $-30\text{‰}$ . A greater intermolecular variability was recorded for  $\delta^{13}\text{C}$  values of the seston (Fig. 5.8). Indeed, seston  $\delta^{13}\text{C}$  values ranged from  $-25.3\text{‰}$  for the 18:0 and  $-26.6\text{‰}$  for the 22:0, down to  $-37.3\text{‰}$ ,  $-38.2\text{‰}$ , and  $-38.5\text{‰}$  for the 18:2 $\omega$ 6, 18:3 $\omega$ 3, and 16:1 $\omega$ 7 respectively, leading to an intermolecular variability of 13 $\text{‰}$ . The  $\delta^{13}\text{C}$  values of saturated FA were  $<3\text{‰}$  different between neuston and seston, while mono- and polyunsaturated FA  $\delta^{13}\text{C}$  values were  $\sim 8\text{--}10\text{‰}$  lower in seston than in neuston. For all the FA considered herein,  $\delta^{13}\text{C}$  values of *Scapholeberis* were very close to those of the neuston (from  $-33.2\text{‰}$  to  $-24.1\text{‰}$ ) while FA of the calanoids exhibited  $\delta^{13}\text{C}$  values that were  $>10\text{‰}$  lower.

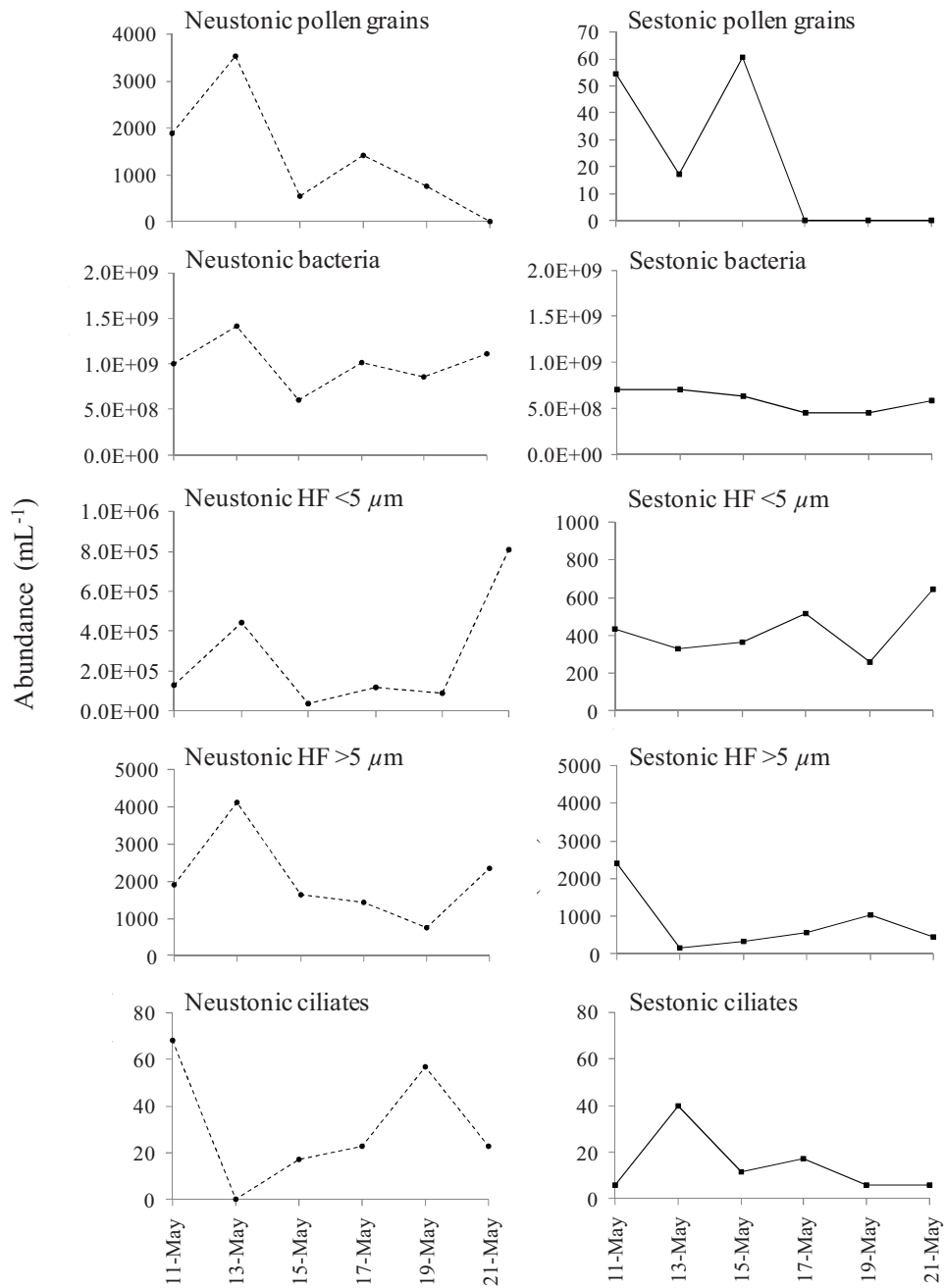


Figure 5.5 : Neustonic and sestonic abundances of pollen grains and heterotrophic microorganisms during the sampling period.



## V. Discussion

### 1. Vertical heterogeneity: pollen, carbon, fatty acids, microorganisms and zooplankton species distribution

Buoyancy, electrostatic attraction, physical and chemical adsorption, and surface tension are interacting processes capable of holding various materials at the water-air interface and contribute to considerable heterogeneity in the water column (Södergren 1987). Such heterogeneity is particularly marked during striking seasonal events such pollen rains (Fig. 5.4a). Indeed, our study showed that pine pollen grains buoyancy lead to a massive accumulation of allochthonous organic matter at the water-air interface. POC ( $<50 \mu\text{m}$ ) was indeed 2 to 18 times more concentrated in neuston than in seston.

Consequently, pollen deposition at the water surface induced the development of very abundant heterotrophic microorganisms (bacteria, flagellates, ciliates), at concentrations significantly higher in neuston than in seston. This was particularly the case for small ( $<5 \mu\text{m}$ ) heterotrophic flagellates (HF) which concentration reached  $8.1 \cdot 10^5 \text{ cells mL}^{-1}$  the 21<sup>th</sup> May (Fig. 5.5). This last value should be underlined as such high concentration is rarely found even in eutrophic lakes (Auer & Arndt 2001 but see Berninger *et al.* 1991). Microscopic observations of fresh samples, as well as the presence of infected pollen grains, lead us to consider that most of these small HF were released zoospores of chytrid fungi. Thus, we can reasonably assume that accumulation of pollen grains at the water-air interface and development of key microorganisms such as zoosporic chytrid fungi may have initiated a pollen-based microbial food web in the neuston compartment. In a previous experimental study, such a pollen-based microbial food web has been shown to transfer pollen carbon and upgrade its quality to promote zooplankton growth (Masclaux *et al.* 2011). Trophic upgrading of food quality by microorganisms has been associated to increasing digestibility and essential lipid compounds production such as C20 and C22-PUFA (Bec *et al.* 2006). Indeed, long-chain PUFA and especially 20:5 $\omega$ 3 are key essential compounds for metazoan (Arts *et al.* 2001; Arts & Kohler 2009) and their dietary availability may strongly determine secondary production in aquatic ecosystems (Müller-Navarra *et al.* 2000; Muller-Navarra *et al.* 2004; Ravet *et al.* 2010). In our study, high concentrations of microorganisms at the water-air interface may certainly explain why neuston samples exhibit large amounts of long-chain PUFA contrary to pollen grains. In the same way, compared to pollen grains, neuston samples

are characterized by lower C:N ratio indicating a better food quality for freshwater consumers (Elser *et al.* 2001; Cebrian *et al.* 2009). Thus, from both stoichiometric and biochemical points of view, neustonic habitat could potentially provide zooplankton species with a high quantity of food with a better nutritional value.

The uneven distribution of zooplankton species in neuston and seston compartments may suggest different foraging behaviors as well as trophic partitioning (Fig. 5.6). The cladoceran *Bosmina* and the calanoid copepods were the most encountered taxa in seston whereas the cladocerans *Chydorus* and more particularly *Scapholeberis* were the dominant species in neuston. Morphological characteristics of *Scapholeberis*, with modified ventral rim of valves (Fig. 5.4b), allow the animals to hang updown at the underside of the surface film of the water (Dumont & Pensaert 1983). These morphological particularities, as well as a high melanin pigment content which serves as protection against high UV radiations at the water–air interface (Kerfoot 1982), would allow *Scapholeberis* to exploit neustonic habitat (De Meester *et al.* 1993).

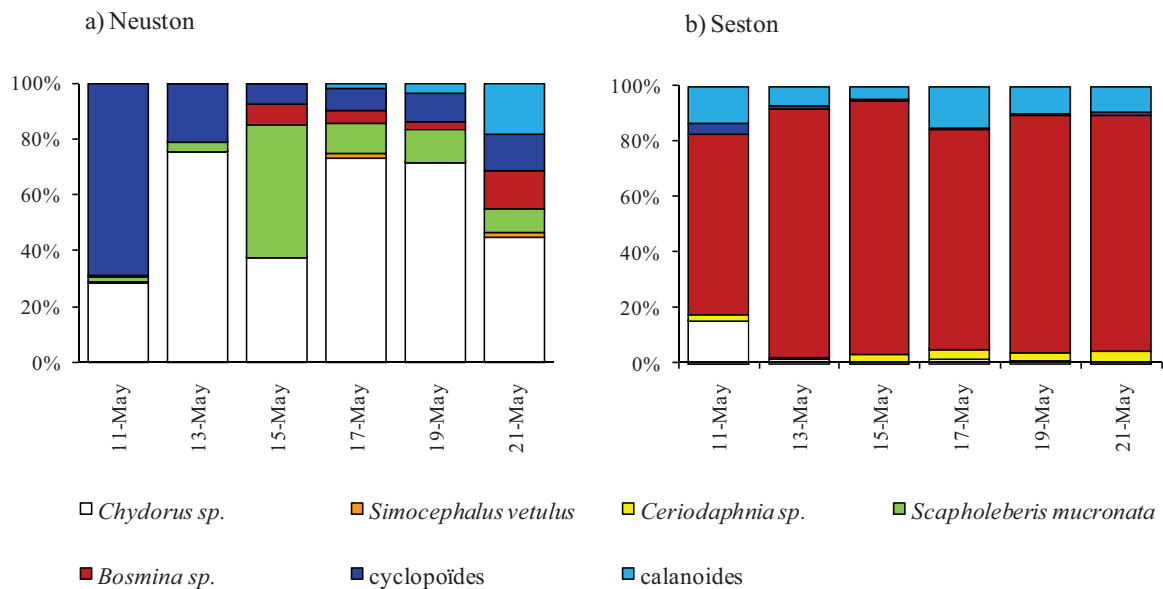


Figure 5.6 : Variations of the distribution of the different zooplankton groups in a) neuston and b) seston during the sampling period.

## 2. Bulk isotope analysis

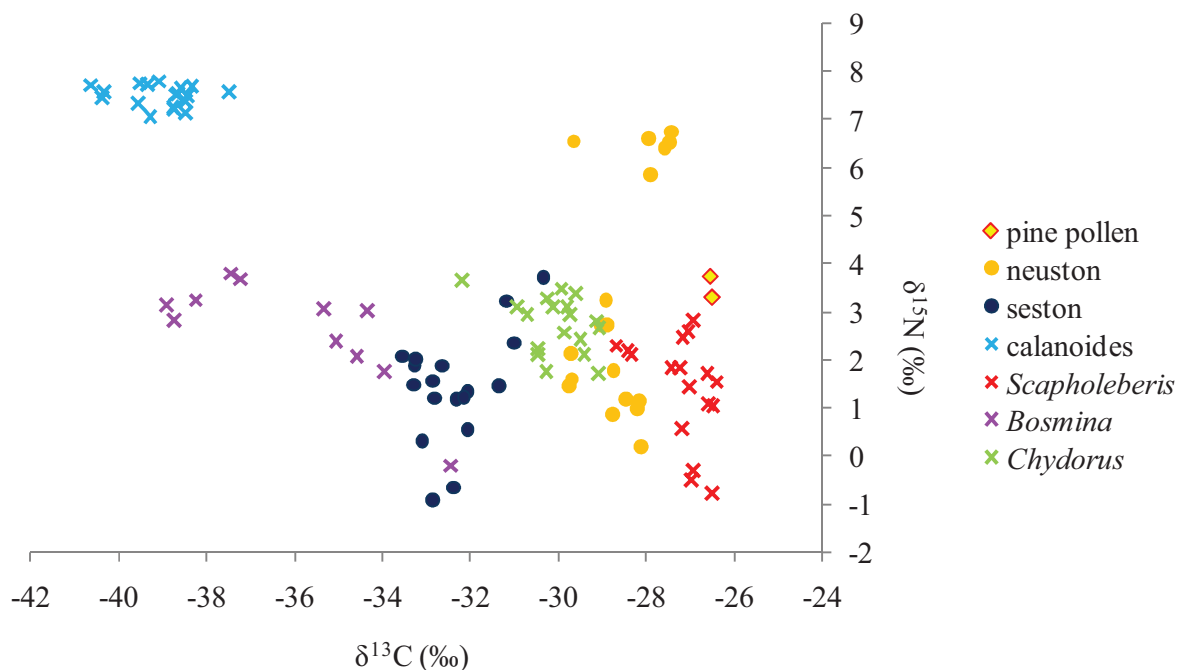
Compared to neuston and seston, pine pollen exhibits a more enriched isotope signature (-26.5‰). Bulk isotope analysis highlight marked  $\delta^{13}\text{C}$  differences between neuston and seston (Fig. 5.7). Indeed neuston  $\delta^{13}\text{C}$  values, ranging from -29.8‰ to -27.4‰, were significantly enriched compared to their seston counterparts and tend to confirm a strong pollen influence. Concerning zooplankton species, the enriched bulk  $\delta^{13}\text{C}$  values of *Scapholeberis* may thus confirm that it mainly relies on neustonic food sources. The intermediate bulk carbon isotope signature of *Chydorus* may suggest that this species exhibits a more opportunistic behavior and forage in both neuston and seston habitats. Finally, bulk carbon isotope signatures of *Bosmina* and calanoides copepods were much more depleted in  $^{13}\text{C}$ , even compared to seston values. However, pelagic zooplankton depleted in  $^{13}\text{C}$  compared to seston as their putative food source is quite common in freshwater ecosystems (delGiorgio & France 1996; Grey *et al.* 2000, 2001). *Bosmina* and copepods, which are known as selective grazers (DeMott 1982) may have ingested and/or assimilated particulate carbon (e.g. algal carbon) that had a lower  $\delta^{13}\text{C}$  (delGiorgio & France 1996) and whose signature is masked by other carbon sources that had higher  $\delta^{13}\text{C}$ . Sestonic carbon is indeed of multiple origins and the phytoplankton isotopic signatures is often masked by allochthonous and littoral detritus which are usually enriched in  $^{13}\text{C}$  relative to phytoplankton (Hamilton & Lewis 1992; Delong & Thorp 2006).

## 3. FA isotope analysis

Seston FA  $\delta^{13}\text{C}$  values range from -25.3‰ down to -38.2‰. This high intermolecular variability (i.e. 13‰) would corroborate the multiple origins of carbon in seston (Koussoroplis *et al.* 2010; Bec *et al.* 2011). 22:0 which is usually considered as a terrestrial marker had an enriched  $\delta^{13}\text{C}$  value in seston, whereas the ubiquitous 16:0 had an intermediate value. Unsaturated FA like 16:1 $\omega$ 7, 18:2 $\omega$ 6 and 18:3 $\omega$ 3, typical for aquatic algae (Brett & Müller-Navarra 1997; Bec *et al.* 2010) exhibited depleted  $\delta^{13}\text{C}$  values, consistent with phytoplankton carbon being the most  $^{13}\text{C}$ -depleted component of seston. Remarkably depleted isotopic values of phytoplankton may reveal methane-derived  $\text{CO}_2$  uptake (Bastviken *et al.* 2003; Jones & Grey 2011). In our oxbow lake, the important organic matter loading in the shallow sediments would probably provide methane to methane-oxidizing bacteria to produce depleted  $\text{CO}_2$  which could result in remarkably depleted phytoplankton signatures. Copepod

FA and especially PUFA  $\delta^{13}\text{C}$  values were remarkably depleted and range from -49.8‰ to -35.3‰. Stable isotope compositions of PUFA are globally transmitted from the diet to zooplankton lipids (Bec *et al.* 2011) and would indicate that copepod fed obtained their FA, and especially PUFA from a phytoplanktonic source. This result highlight that the common isotopic difference observed between zooplankton grazers and seston arises from selective grazing of phytoplankton-derived particles within seston. In contrast, the  $\delta^{13}\text{C}$  values of *Scapholeberis* FA matched those of neuston, confirming that they obtained all their FA, and especially PUFA, solely from neuston.

Provided the endmembers are substantially isotopically different, fatty-acid specific stable isotope analyses can provide valuable insights on the pathways conveying essential FA to consumers, while bulk isotope analysis would allow assessing the contribution of a dietary source to the consumer's C biomass. Hence, the combined use of SIA and FA-SIA is of great interest as it has previously permitted to document an uncoupling between essential compounds and major organic matter transfers in heterogeneous environments (Koussoroplis *et al.* 2010). Here, the closed  $\delta^{13}\text{C}$  values between FA of *Scapholeberis* and neuston strongly indicate that the water-air interface is the only foraging habitat for this cladoceran species.



**Figure 5.7 :**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of pine pollen, neuston, seston, calanoides copepods, *Scapholeberis*, *Bosmina* and *Chydorus*.

#### 4. Conclusion

Whole lake  $^{13}\text{C}$  addition experiments and bulk isotope analysis suggested that allochthonous matter inputs contribute largely to the secondary production of aquatic food webs (Pace *et al.* 2004; Cole *et al.* 2006). Indeed, Cole *et al.* (2006) estimated that between 33% and 73% of zooplankton carbon could be supplied by terrestrial particulate organic carbon. While there is an increasingly widespread view that zooplankton obtain a large proportion of their carbon from terrestrial sources, the degree of terrestrial support of aquatic consumers still remains debated as it appears that allochthonous support of zooplankton production has been vastly overstated (Brett *et al.* 2009; Francis *et al.* 2011). This scientific controversy lies on the fact that terrestrial organic matter food quality is too low to support zooplankton growth (see Brett *et al.* 2009). Moreover, heterogeneity in the vertical distribution of zooplankton and their food resources has been ignored in most efforts to quantify the energetic support of zooplankton (see Francis *et al.* 2011). Even if we totally agree with Brett *et al.* (2009) and Francis *et al.* (2011), our study using bulk isotope as well as compound specific isotope analysis demonstrates that terrestrial-derived carbon contributes sometimes largely to biomass and polyunsaturated fatty acids contents of some zooplankton species. Nevertheless, we add that a microbial food web (initiated here by zoospore fungi) is required to upgrade and transfer allochthonous carbon. Finally, we show here that pine pollen rains creates an heterogeneity in the water column allowing trophic partitioning which may sustain zooplankton production and diversity.

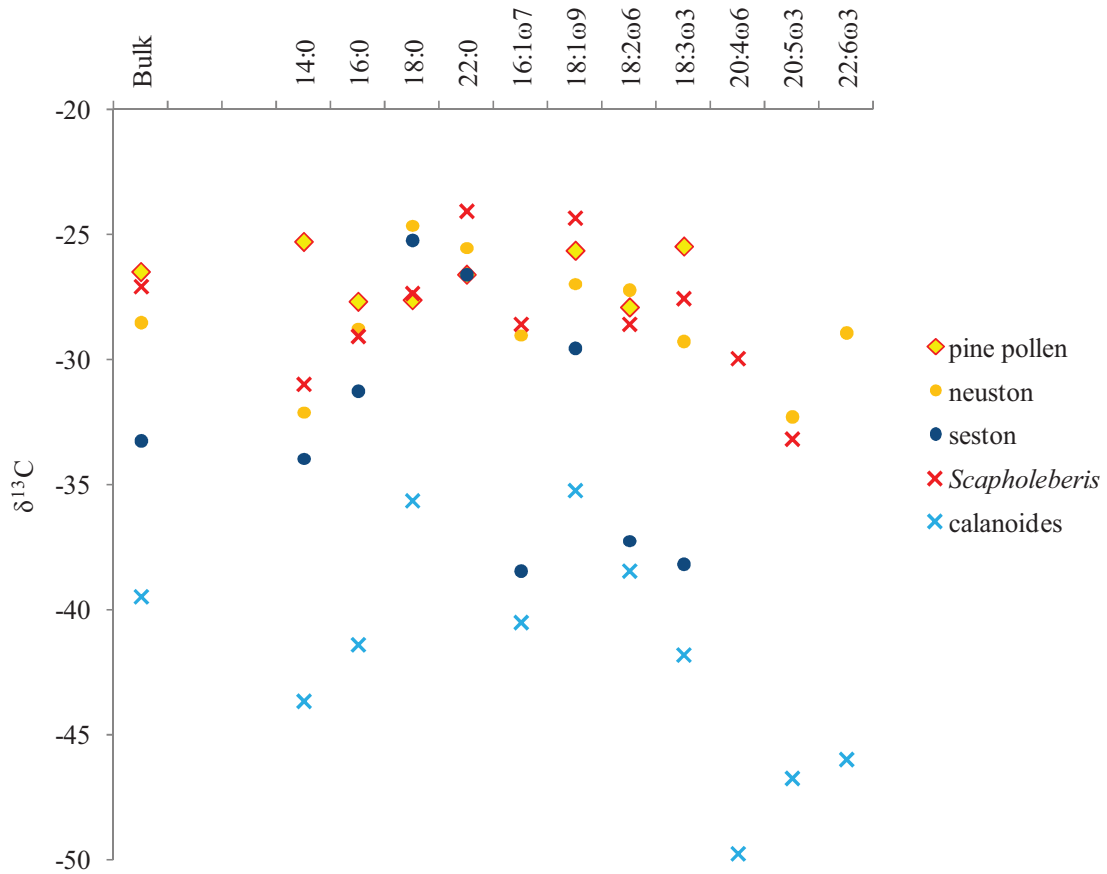


Figure 5.8 :  $\delta^{13}\text{C}$  values of bulk and individual fatty acids within total lipids of pine pollen, neuston, seston, *Scapholeberis* and calanoides.  $\delta^{13}\text{C}$  values of bulk and fatty acids are mean of triplicates for the 13<sup>th</sup> May.



## **CHAPITRE VI : Conclusions générales et perspectives**





Dans les milieux d'eau courante, aux phénomènes d'érosion des berges et de sédimentation sont associées à la fois une très grande hétérogénéité dans l'espace et une forte variabilité dans le temps. La dynamique fluviale conduit notamment à la formation de bras morts par recoupement de méandres ou abandon de bras de tressage. Ces bras morts, et les herbiers aquatiques qui y sont souvent associés, ont un rôle fonctionnel très important pour l'ensemble du système fluvial. En effet, grâce à une forte diminution voire une absence de courant, on retrouve dans ces milieux, une forte production primaire (Wetzel 1990) et des communautés de microcrustacés et de macroinvertébrés abondantes (Lalonde & Downing 1992; Balayla & Moss 2003; Thomaz et al. 2008; Van Onsem et al. 2010). De plus, de nombreuses espèces de poissons y trouvent des zones de refuge, d'alimentation ainsi que des zones de ponte et d'élevage des alevins (Petr 2000; Sanchez-Botero et al. 2007). Mais, outre l'abondance d'organismes et la forte productivité enregistrée dans ces milieux (Wetzel 1990), l'hétérogénéité spatiale permet également le maintien d'une forte biodiversité.

La dynamique fluviale permettant la formation de ces milieux a cependant été sévèrement altérée par les aménagements du paysage, dans de nombreux hydrosystèmes fluviaux. Ces aménagements, visant principalement à contrôler les crues, créer des voies navigables, ou récupérer des terres agricoles, ont eu pour conséquence de diminuer, voire d'empêcher la migration latérale du chenal principal et donc de diminuer la diversité géomorphologique, supprimant ainsi les bras morts et les herbiers aquatiques associés. La dégradation des milieux a alors eu un impact négatif sur la biodiversité et le fonctionnement des écosystèmes, et, en définitive, sur les services écosystémiques rendus à l'homme. Depuis une vingtaine d'années, des efforts importants ont donc été fournis pour une meilleure compréhension du fonctionnement des bras morts et des zones littorales à macrophytes en général, dans le but de les préserver et de les restaurer. Or, si on sait maintenant que ces milieux abritent une forte densité et une forte diversité d'organismes, les liens pouvant exister entre hétérogénéité spatiale, biodiversité et flux de matière restent encore peu connus.

## **I. Richesse spécifique des cladocères et transfert des AGPI**

Bien que la biodiversité se définisse à différentes échelles, la richesse spécifique est la mesure de la biodiversité horizontale la plus communément rencontrée, notamment dans les études portant sur les relations entre biodiversité et fonctionnement des écosystèmes (Downing & Leibold 2002; Duffy *et al.* 2007; Woodward 2009; Costantini & Rossi 2010). De

même que la biodiversité, le fonctionnement des écosystèmes est un terme qui a de multiples facettes parmi lesquelles une nous intéresse particulièrement : les flux de matière et de composés essentiels dans les réseaux trophiques. Dans les écosystèmes aquatiques continentaux, les zones littorales à macrophytes représentent des zones d'intérêt pour étudier les relations entre hétérogénéité spatiale, biodiversité et flux de matière. Ces milieux abritent en effet une importante diversité de microcrustacés (Walseng *et al.* 2006) qui, grâce à l'hétérogénéité de l'habitat, ont potentiellement une plus grande diversité de sources de nourriture qu'en milieu pélagique. En tant que principal maillon entre les microorganismes, principaux producteurs d'AGPI en milieu aquatique, et les niveaux trophiques supérieurs, les microcrustacés jouent un rôle clef dans le transfert de ces composés essentiels dans les réseaux trophiques (Brett & Müller-Navarra 1997; Desvillettes & Bec 2009). En effet, les AGPI, et plus particulièrement ceux des séries  $\omega 3$  et  $\omega 6$ , sont des composés biologiques ayant un rôle important dans la physiologie membranaire et en tant que précurseurs d'hormones pour de nombreux animaux (Olsen 1998; Arts *et al.* 2001). Ils représentent donc des facteurs nutritionnels primordiaux pour le développement de la plupart des métazoaires (Arts *et al.* 2001). Cependant, contrairement aux végétaux, la majorité des animaux ne disposent pas des enzymes nécessaires à la formation de AGPI, notamment à la formation du 18:3 $\omega 3$  et 18:2 $\omega 6$ , précurseurs des AGPI des séries  $\omega 3$  et  $\omega 6$ . De plus, si certaines métazoaires sont capables de convertir ces précurseurs à 18 atomes de carbone en AGPI à longue chaîne ( $\geq 20$  atomes de carbone), ces conversions se font à des taux insuffisants pour répondre à leur besoins physiologiques. Les animaux, et en milieu aquatique les poissons, vont donc largement dépendre des AGPI fournis par leur alimentation. On peut alors penser que dans les zones littorales à macrophytes, la diversité des cladocères induit une variabilité dans les transferts d'AGPI vers les niveaux trophiques supérieurs.

Un des premiers facteurs à prendre en compte dans la variabilité des transferts d'AGPI vers les niveaux trophiques supérieurs nous a semblé être la variabilité de la capacité d'accumulation et de bioconversion pouvant exister d'une espèce de cladocère à une autre. La première étape de ce travail a donc été d'évaluer cette variabilité, en conditions contrôlées, chez les principaux cladocères vivant dans une zone à macrophytes. Notre étude montre ainsi qu'à 20°C, il n'existe pas de différences de composition en AGPI entre les six espèces de cladocères testées, lorsqu'elles reçoivent un même *pool* alimentaire d'acides gras. A une température plus faible (14°C), la variabilité interspécifique semble cependant être plus importante. Ce dernier résultat indique qu'il serait intéressant d'étudier de manière plus

approfondie l'effet de la température sur les capacités d'accumulation, de bioconversion et donc de transfert des AGPI des différentes espèces. Néanmoins, plus que la variabilité interspécifique des métabolismes lipidiques, il semble, d'après nos résultats, que le principal facteur pouvant influencer la variabilité des transferts d'AGPI vers le compartiment piscicole soit le comportement alimentaire et les ressources trophiques exploitées par les différentes espèces de microcrustacés.

Dans les zones littorales à macrophytes, l'hétérogénéité spatiale offre aux microcrustacés plus de possibilités dans l'exploitation des ressources trophiques qu'en milieu pélagique. Nous avons ainsi montré qu'outre le seston, l'épiphyton et le neuston étaient des compartiments trophiques à considérer en tant que sources de nourriture potentielles des microcrustacés. Parmi les espèces étudiées, certaines se sont révélées être spécialisées dans l'exploitation d'un compartiment trophique. En effet, les résultats des analyses isotopiques en milieu naturel ont montré que *Ceriodaphnia* et *Daphnia longispina* semblaient se nourrir uniquement sur le compartiment sestonique. *Eurycercus lamellatus* s'est révélé être capable d'exploiter uniquement le matériel périphytique. Quant à *Scapholeberis mucronata*, les analyses isotopiques de *bulk* et de composés spécifiques ont mis en évidence que la position de cette espèce dans la colonne d'eau s'expliquait certainement en grande partie par sa spécialisation dans l'exploitation du biofilm neustonique. En revanche, d'autres espèces pourraient être plus opportunistes. *Daphnia magna* peut, en effet, se nourrir de matière organique en suspension, mais également de matière organique périphytique (Siehoff *et al.* 2009). Dans notre étude, nous avons de plus pu nous rendre compte que le genre *Chydorus*, par ailleurs connu pour avoir un comportement de type « racleur », pouvait également se nourrir de particules sestoniques et neustoniques.

Les résultats de ce travail de thèse ont montré que la diversité des compartiments trophiques s'accompagnait, en outre, d'une diversité d'apports en AGPI. Dans notre étude, le seston était, en effet, moins concentré en acides gras essentiels que le neuston et l'épiphyton. Ces différences s'expliquent probablement par la composition de chaque compartiment trophique : proportions relatives de matière organique et inorganique, nature de la matière organique, compositions taxonomiques des communautés de microorganismes. Ainsi, dans les bras morts, le seston est le plus souvent composé de phytoplancton mais également d'une proportion non négligeable de matière organique allochtone et de détritiques, plus pauvres en AGPI (Hamilton & Lewis 1992; Delong & Thorp 2006), tandis que les biofilms périphytiques sont, dans ces milieux, composés en grande partie de microalgues (Leland *et al.* 1986;

Vymazal & Richardson 1995). Situé à l'interface air-eau, le neuston est, quant à lui, fortement influencé par les apports organiques d'origine allochtone. Plusieurs études ont mis en avant que la matière organique d'origine terrestre pouvait largement contribuer à la production secondaire et notamment à la production zooplanctonique (Pace *et al.* 2004; Cole *et al.* 2006; Pace *et al.* 2007; Cole *et al.* 2011). Cependant, Brett *et al.* (2009) ont récemment argumenté que le carbone organique particulaire d'origine terrestre (un mélange de feuilles mortes broyées dans leur étude) était d'une faible qualité nutritionnelle pour le zooplancton comparativement aux espèces phytoplanctoniques, et qu'il ne contribuait donc qu'en faible proportion à la production zooplanctonique. Or, pour la première fois, nous avons considéré ici les apports allochtones de pollen et leur importance pour la production secondaire en milieu aquatique. Nous avons ainsi mis en évidence que les AGPI, fortement concentrés dans le pollen, pouvaient être transférés, grâce aux microorganismes hétérotrophes neustoniques, jusqu'à certaines espèces de cladocères.

La diversité des compartiments trophiques procure une large gamme de ressources nutritionnelles (Duffy *et al.* 2007), et dans notre cas des apports variés en AGPI. Cependant, pour pouvoir entraîner un meilleur transfert de matière et d'énergie, l'hétérogénéité spatiale et la diversité des ressources doivent être combinées à des différences de capacités d'exploitation de ces ressources par les consommateurs (Griffin *et al.* 2009). Le degré de spécialisation des espèces joue, en effet, un rôle important sur la manière dont la richesse spécifique influence le fonctionnement de l'écosystème (Duffy *et al.* 2007). Dans les environnements tels que les zones littorales à macrophytes, la complémentarité des espèces de cladocères entraîne donc une utilisation plus complète des sources de carbone et d'AGPI. La diversité des compartiments trophiques, associée à la diversité des microcrustacés, permet donc très probablement une optimisation du transfert des AGPI vers les niveaux trophiques supérieurs, et notamment vers le compartiment piscicole.

## **II. Perspectives**

Au terme de ce travail, plusieurs perspectives intéressantes s'offrent à nous afin d'améliorer la compréhension des liens existant entre la diversité des cladocères et le transfert des AGPI dans les réseaux trophiques.

Tout d'abord, notre étude portant sur la capacité d'accumulation et de bioconversion des AGPI de différentes espèces de cladocères, réalisées en conditions contrôlées, révèlent qu'il

n'y a pas de différences de composition en AGPI lorsque les espèces reçoivent un même *pool* alimentaire d'acides gras. Il serait intéressant d'étudier, parallèlement à la composition lipidique des compartiments trophiques, la composition lipidique des espèces de microcrustacés qui les exploitent, en milieu naturel. Nous pourrions ainsi évaluer l'influence de la variabilité de la composition en AGPI des compartiments trophiques, associée à la diversité des comportements alimentaires des cladocères, sur le transfert des AGPI. En effet, on peut imaginer que contrairement à ce qui est observé expérimentalement, la fraction de matière organique particulaire analysée ne correspond pas exactement à la matière organique réellement assimilée par les consommateurs. Dans ce cas, les fortes concentrations en AGPI enregistrées dans certains compartiments, à la base du réseau trophique, ne se retrouveraient peut être pas au niveau des cladocères, et entraîneraient une variabilité moindre dans le transfert des AGPI.

De plus, nous avons pu mettre en évidence, que certaines espèces de cladocère étaient capable de se nourrir sur plusieurs compartiments trophiques. Il serait intéressant de voir si des changements de qualité des sources de nourriture peuvent avoir un effet sur le comportement alimentaire de ces espèces. Un suivi temporel de la composition lipidique de différents compartiments trophiques en milieu naturel pourrait ainsi être envisagé. Des analyses isotopiques permettraient alors de voir s'il existe une corrélation positive entre le degré d'utilisation d'une ressource et sa qualité nutritionnelle.

Il n'est pas impossible, de plus, que dans le cas d'espèces de microcrustacés ayant la possibilité de se nourrir sur plusieurs compartiments trophiques, on observe un découplage entre les principaux flux de matière et les flux de composés essentiels. En effet, comme il a été montré par Koussoroplis *et al.* (2010) dans un milieu lagunaire, des sources de matière organique représentant un apport énergétique mineur pour un organisme pourrait avoir une grande importance en tant que source principale de certains composés essentiels. L'utilisation d'analyses isotopiques de composés spécifiques combinées à des analyses isotopiques de l'ensemble de la matière organique serait, dans ce cas, d'un grand intérêt pour apprécier la provenance des AGPI.

Enfin, nous avons pu mettre en évidence que toutes les espèces de cladocères présentes dans les zones littorales à macrophytes n'utilisent pas de la même manière les différentes sources de carbone du milieu. Les analyses lipidiques nous ont également révélé que cette diversité de ressources trophiques s'accompagnait d'une variabilité des apports en composés essentiels tels que les AGPI à la base du réseau trophique. Cependant, au terme de notre

travail, la proposition selon laquelle la diversité des compartiments trophiques, associée à la diversité des microcrustacés, pourrait entrainé une optimisation du transfert des AGPI vers le compartiment piscicole ne reste qu'une hypothèse. Afin d'aller plus loin dans la compréhension de ces transferts, des expériences en conditions contrôlées pourraient être envisagées. Des expériences en mésocosmes, recréant des réseaux trophiques à trois niveaux (microorganismes - microcrustacés - poissons zooplanctonophages) pourraient ainsi être mises en place. En manipulant les deux premiers niveaux de ce réseau, l'effet de la diversité des sources de nourriture à la base du réseau trophique (seston, neuston et périphyton) et de la diversité des cladocères sur la biomasse et la composition en AGPI des poissons pourrait alors être apprécié.

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## **Annexe**

## Combined effects of food quality and temperature on somatic growth and reproduction of two freshwater cladocerans

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### Abstract

We investigated the effect of food quality on somatic growth and reproduction of zooplankton at different temperatures (12°C, 15°C, 20°C, and 25°C). Standardized growth experiments of two cladocerans, *Daphnia magna* and *Simocephalus vetulus*, were performed on (1) high-quality food (*Cryptomonas* sp.), (2) relatively low-quality food (*Scenedesmus obliquus*), and (3) intermediate-quality food (*Cryptomonas*:*Scenedesmus* mixture). Food quality constraints on somatic growth and reproduction of the two cladocerans decreased with increasing temperature. For *D. magna* and for *S. vetulus*, differences between clutch size and growth rate of individuals fed on the three food sources were highly pronounced when they were reared at 12°C and 15°C; however, such differences decreased at 20°C and were negligible at 25°C. Variations in food quality constraints with temperature can be explained by the variability of dietary polyunsaturated fatty acids, such as eicosapentaenoic acid and stearidonic acid requirements of these cladocerans. We conclude that dietary constraints exerted by food quality for zooplankton development vary as a function of different temperature conditions.

Since the conceptual work of trophodynamics by Lindeman (1942), determining factors that affect matter transfer efficiency is a key issue in ecology. In aquatic food webs, the efficiency of energy transfer at the plant–animal interface is highly variable (Brett and Müller-Navarra 1997), consequently resulting in variable secondary production. Thus far, several studies have been motivated by this variability of dietary energy transfer at the phytoplankton–zooplankton interface, and it has been clear for many years that these variations can be attributed to the variation in food quality of algae for herbivorous zooplankton (Ahlgren et al. 1990; Müller-Navarra and Lampert 1996; Brett and Müller-Navarra 1997). Among the variety of factors determining their food quality, the following are usually highlighted: P content and stoichiometric composition (C:P ratios in particular), fatty acid composition (in particular polyunsaturated fatty acids: n-3 PUFA; Ahlgren et al. 1990; Hessen 1990; Müller-Navarra et al. 2000), digestibility (Lüring and Van Donk 1997), and toxicity (Jungmann and Benndorf 1994). However, as proposed by Sterner (1997), “attention now turns to the more sophisticated question about when and where food quality constraints are important.”

Temperature is one of the most important abiotic factors in aquatic environments, as it presents great variations on a spatial as well as on a temporal scale. Moreover, temperature strongly influences organisms, especially ectothermic organisms, as it affects both their physiology and their life history traits (Angilletta et al. 2004). Temperature is an external factor determining, at least in part, the habitat selection of ectothermic organisms. In nature, cladocerans have thus to optimize their fitness with a

trade-off between temperature and food availability. Earlier work from Farkas et al. (1984) investigated the effect of temperature on zooplankton’s ability to adapt their cell membrane lipid composition. Unlike the complete fatty acid adaptation in membranes of *Cyclops vicinus* to changing temperature, Farkas et al. (1984) argued that *Daphnia magna* fails to incorporate PUFA into phospholipids at lower temperatures and suggested that failure to overwinter in an active state is related to their ability to adapt the lipid composition of their cell membranes to lower (winter) temperatures. However, although the trade-off between food quantity and water temperature for maximizing fitness of the key herbivore *Daphnia* has been the subject of several studies (Dawidowicz and Loose 1992; Mitchell 1997; Lampert et al. 2003), the interaction between food quality and temperature remains still poorly understood. In an in situ study, Cole et al. (2002) hypothesized that such an interaction between food quality and temperature exists and that it might be important in understanding zooplankton distribution and production in lakes. However, this hypothesis has not been tested yet. In an effort to better understand this important ecological interaction, we conducted laboratory experiments to test how the importance of food quality for zooplankton growth and reproduction varies with temperature.

### Methods

*Origin and maintenance of daphnids*—*Daphnia magna* and *Simocephalus vetulus* were used for the experiments, as their natural habitat exhibits strong temperature variations. *D. magna*, an organism widely used in laboratory experiments (Dawidowicz and Loose 1992; Müller-Navarra et al. 2000), was collected from a pond of the Allier River

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floodplains (France). *S. vetulus*, a common species in backwaters of the Allier River but also found in many aquatic ecosystems worldwide, was collected from a backwater sampling site of the Allier River. Females were maintained in 1-L containers of filtered river water ( $<0.2 \mu\text{m}$ ) with a maximum of 20 individuals per liter on a 14:10 h light:dark cycle at  $15^\circ\text{C}$ . They were fed once a day ad libitum using a mixture of *Cryptomonas* sp. strain SAG 26.80 and *Scenedesmus obliquus* strain SAG 276-3a (20%:80% of respective biomass).

**Cultures of autotrophic organisms**—*S. obliquus* SAG 276-3a and *Cryptomonas* sp. SAG 26.80 were used as food for the two species of Daphnidae. The two algae were grown in modified WC (Woods Hole modified CHU-10) medium with vitamins (Von Elert and Wolffrom 2001) at  $20^\circ\text{C}$  and cultured semicontinuously at a dilution rate of  $0.25 \text{ d}^{-1}$  using aerated 3-L vessels. This medium provided sufficient amounts of P to algae. Stock solutions of the autotrophic organisms for the growth experiments were prepared by centrifugation and resuspension of the cultured cells in WC medium lacking vitamins. The carbon concentrations of the autotrophic food suspensions were estimated from photometric light extinction (800 nm) and from carbon-extinction regressions determined previously (D. Martin-Creuzburg pers. comm.).

**Experimental setup**—For each zooplankton species, one clone was isolated. In preparation for our experiments, four neonates of each clone were transferred to 1-L containers and randomly assigned to one of the four experimental temperatures:  $12^\circ\text{C}$ ,  $15^\circ\text{C}$ ,  $20^\circ\text{C}$ , and  $25^\circ\text{C}$ . They were transferred to filtered water ( $0.2 \mu\text{m}$ ) and fed on the mixture of *Cryptomonas* sp. and *S. obliquus* (20%:80% of respective biomass) every day. When the females produced offspring, the neonates (first generation) were separated and the mothers removed. This step was repeated to keep the second generation. After they had released their first clutch, females from the second generation were kept and the neonates removed. The third-brood offspring from the second generation were finally used for the experiments in order to limit variability due to maternal size and weight (Lampert 1993). For *D. magna*, depending on temperature, it took between 4 and 11 d to reach maturity and between 3 and 8 d more so that eggs are delivered. For *S. vetulus*, it took between 3 and 14 d to reach maturity and between 2 and 7 d more so that eggs are delivered. Each species were thus acclimated between 3 and 6 weeks at each temperature. Neonates were collected about 8 h after they had been released and randomly distributed in 200-mL beakers (eight individuals per beaker for *D. magna* and 12 individuals per beaker for *S. vetulus*). Experiments were performed at  $12^\circ\text{C}$ ,  $15^\circ\text{C}$ ,  $20^\circ\text{C}$ , and  $25^\circ\text{C}$  on three food sources, leading to  $4 \times 3$  factorial design for both clones and with three replicates for each treatment. The three food sources were *Cryptomonas* sp. SAG 26.80 (considered as relatively “high-quality food” [HQ]), a *Cryptomonas*:*S. obliquus* mixture (20%:80% of respective biomass, used as “medium-quality food” and considered as reference food [RefQ]), and *S. obliquus* SAG 276-3a (used as relatively “low-quality

food” [LQ]) compared to *Cryptomonas*). Previous studies indicated that these two algal species differ in their nutritional quality; that is, PUFA concentrations, potentially conducive to increased somatic growth of daphnids, were higher in Cryptophyceae than Chlorophyceae (Ahlgren et al. 1990; Brett and Müller-Navarra 1997; Brett et al. 2000). In addition to the nutritionally less favorable fatty acid profile of *S. obliquus*, we note that chlorophytes may be digested differently than cryptophytes because of different cell wall characteristics.

During the experiments, individuals were transferred every day to clean water and fed under nonlimiting food conditions ( $2 \text{ mg CL}^{-1}$ , i.e., well above the incipient limiting level that is reported to be approximately  $0.5 \text{ mg C L}^{-1}$ ; Lampert 1978). To measure the average initial dry weight ( $W_0$ ), randomly selected neonates were previously transferred into preweighed aluminum containers (three samples of 15 neonates for *D. magna* and three samples of 30 neonates for *S. vetulus*), dried overnight at  $60^\circ\text{C}$ , cooled in a desiccator, and weighed on an electronic balance (Mettler Tobelo UMX2 balance  $\pm 1 \mu\text{g}$ ). The experiments were stopped when the females reached maturity, which took, depending on temperature and food conditions, between 4 and 18 d for *D. magna* and 4 and 15 d for *S. vetulus*. Individuals were collected, clutch size was measured (eggs per individual), and females were dried overnight at  $60^\circ\text{C}$  and weighed to obtain the average individual weight per replicate ( $W_t$ ). Somatic growth rates ( $g$ ) were calculated as

$$g = (\ln W_t - \ln W_0) / t \quad (1)$$

where  $t$  is the duration of each experiment in days.

**Analysis of *S. obliquus* and *Cryptomonas* sp. fatty acids**—To analyze fatty acids (FA) of the phytoplanktonic species used in this study, 2 mg of particulate organic carbon of *S. obliquus* and *Cryptomonas* sp. were filtered (with three replicates for each species) on precombusted GF/F filters (Whatman™). Lipids were extracted using the method of Folch et al. (1957). Fatty acid analyses were performed on total lipids (TL). The methyl esters were prepared by esterifying the lipid extract in  $\text{BF}_3\text{-CH}_3\text{OH}$  (10% w/w) at  $90^\circ\text{C}$  for 45 min. Subsequently, fatty acid methyl esters (FAME) were extracted  $3\times$  with a mixture of water-pentane (1:2, v/v). The supernatant was transferred to another tube, dried under nitrogen, and stored at  $-40^\circ\text{C}$  in hexane. FAME were analyzed on a Chrompack CP9001 gas chromatograph equipped with a Supelco® Omega-coax™ column and a FID detector ( $260^\circ\text{C}$ ; split injection; carrier gas: helium; oven temperature ramp  $140\text{--}300^\circ\text{C}$  at  $3^\circ\text{C min}^{-1}$ ). FAME were identified by comparing retention times with those obtained from Supelco® standards and laboratory standards and quantified using 13:0 and 23:0 as internal standards added before the derivatization of FA.

**Data analysis**—Effects of temperature and food quality of the different food source on growth rate and clutch size were analyzed by one-way analysis of variance (ANOVA)

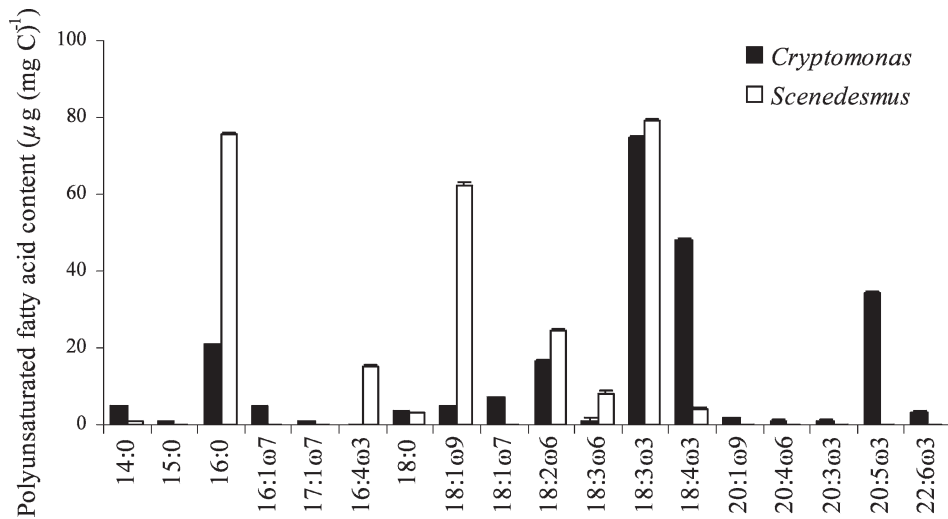


Fig. 1. Fatty acid concentrations of *S. obliquus* and *Cryptomonas* sp. Data are means  $\pm$  SD for three replicates.

and post hoc comparisons (Tukey's Honestly Significant Difference [HSD],  $\alpha = 0.05$ ). Two-way ANOVAs were used to test a possible interaction between temperature and food quality. Moreover, in order to emphasize this possible interaction and to assess how food quality constraints exercised on zooplankton development vary according to temperature, the mixture *Cryptomonas*:*S. obliquus* (RefQ) was taken as reference for standard in this experiment. Then, for each cladoceran, at all temperatures, clutch size obtained for individuals fed on *Cryptomonas* sp. (HQ) and *S. obliquus* (LQ) were expressed as a deviation (%) to clutch size of individuals fed on the reference diet (RefQ):

$$(C_{\text{HQ}}/C_{\text{RefQ}}) \times 100 \quad (2)$$

and

$$(C_{\text{LQ}}/C_{\text{RefQ}}) \times 100 \quad (3)$$

where  $C_{\text{HQ}}$  is the clutch size obtained for individuals fed on HQ food,  $C_{\text{RefQ}}$  the clutch size obtained for individuals fed on the RefQ food, and  $C_{\text{LQ}}$  the clutch size obtained for individuals fed on the LQ food.

The same procedure was followed for somatic growth rates:

$$(G_{\text{HQ}}/G_{\text{RefQ}}) \times 100 \quad (4)$$

and

$$(G_{\text{LQ}}/G_{\text{RefQ}}) \times 100 \quad (5)$$

where  $G_{\text{HQ}}$  is the somatic growth rate obtained for individuals fed on HQ food,  $G_{\text{RefQ}}$  the growth rate obtained for individuals fed on the RefQ food, and  $G_{\text{LQ}}$  the growth rate obtained for individuals fed on the LQ food.

## Results

**PUFA of phytoplankton**—*S. obliquus* was rich in  $C_{18}$ -PUFA, with  $\alpha$ -linolenic acid (ALA, 18:3n-3) concentrations

being the most abundant. However, long-chain PUFA ( $>C_{18}$ ) were not detected in *S. obliquus*. As was the case for *S. obliquus*, *Cryptomonas* sp. was also rich in  $C_{18}$ -PUFA concentrations, especially in ALA and stearidonic acid (SDA, 18:4n-3). However, PUFA concentrations differed from those of *S. obliquus* by the presence of SDA concentrations and the long-chain PUFA eicosapentaenoic acid (EPA, 20:5n-3; Fig. 1).

**Effects of temperature on *D. magna* and *S. vetulus* clutch size**—Clutch size of *D. magna* and *S. vetulus* fed on *Cryptomonas* sp. decreased significantly with increasing temperature (Table 1). For *D. magna*, the clutch size decreases from 24.3 eggs per female (e.f.<sup>-1</sup>) at 12°C to 15.7 e.f.<sup>-1</sup> at 25°C. For *S. vetulus*, it decreases from 16.3 e.f.<sup>-1</sup> at 12°C to 7.3 e.f.<sup>-1</sup> at 25°C. This trend was less noticeable when individuals were fed on the mixture of *Cryptomonas*:*S. obliquus*, but the largest clutch sizes were always obtained for the treatments run at 12°C (Table 1). The clutch size decreased from 21.3 e.f.<sup>-1</sup> at 12°C to 14.9 e.f.<sup>-1</sup> at 25°C for *D. magna* and from 11.9 e.f.<sup>-1</sup> at 12°C to 7.2 e.f.<sup>-1</sup> at 25°C for *S. vetulus*. Fed on *S. obliquus*, this trend disappeared for *D. magna* and was reversed for *S. vetulus* (Table 1). For *S. vetulus*, the lowest clutch sizes were recorded at 12°C and 15°C (3.3 e.f.<sup>-1</sup> and 3.7 e.f.<sup>-1</sup>, respectively).

**Effects of temperature on *D. magna* and *S. vetulus* somatic growth rate**—For all the food sources considered, somatic growth rates of both species increased with increasing temperature (Table 1). For *D. magna*, it increased from 0.29 d<sup>-1</sup> at 12°C to 0.71 d<sup>-1</sup> at 25°C when individuals were fed on *Cryptomonas*, from 0.27 d<sup>-1</sup> at 12°C to 0.78 d<sup>-1</sup> at 25°C when fed on the mixed diet (*Cryptomonas*:*S. obliquus*), and from 0.19 d<sup>-1</sup> at 12°C to 0.76 d<sup>-1</sup> at 25°C when they were fed on *S. obliquus*. For *S. vetulus*, somatic growth increased from 0.25 d<sup>-1</sup> at 12°C to 0.76 d<sup>-1</sup> at 25°C when individuals were fed on *Cryptomonas*, from 0.22 d<sup>-1</sup> at 12°C to 0.75 d<sup>-1</sup> at 25°C when fed on

Table 1. Results of ANOVA testing the effect of temperature on *D. magna* and *S. vetulus* clutch size and growth rate for three different food sources: *Cryptomonas* spp. (HQ food), *Cryptomonas*:*Scenedesmus* mixture (RefQ food), or *S. obliquus* (LQ food).

		HQ food		RefQ food		LQ food	
		F Value	p Value	F Value	p Value	F Value	p Value
<i>D. magna</i>	Clutch size	$F_{3,8}=82.6$	$p<0.001$	$F_{3,8}=7.5$	$p=0.014$	$F_{3,8}=61.6$	$p<0.001$
	Growth rate	$F_{3,8}=3844.4$	$p<0.001$	$F_{3,8}=334.7$	$p<0.001$	$F_{3,8}=865.8$	$p<0.001$
<i>S. vetulus</i>	Clutch size	$F_{3,8}=168.8$	$p<0.001$	$F_{3,8}=144.1$	$p<0.001$	$F_{3,8}=84.5$	$p<0.001$
	Growth rate	$F_{3,8}=9734.4$	$p<0.001$	$F_{3,8}=5105.7$	$p<0.001$	$F_{3,8}=1697.2$	$p<0.001$

the *Cryptomonas*:*S. obliquus* mixture, and from  $0.16 \text{ d}^{-1}$  at  $12^\circ\text{C}$  to  $0.67 \text{ d}^{-1}$  at  $25^\circ\text{C}$  when fed on *S. obliquus*. The growth rate was significantly smaller at  $25^\circ\text{C}$  than at  $20^\circ\text{C}$  only for *D. magna* reared on *Cryptomonas* sp. ( $0.71 \text{ d}^{-1}$  and  $0.74 \text{ d}^{-1}$ , respectively; Table 1).

**Effects of food quality on *D. magna* and *S. vetulus* clutch size**—The lowest clutch sizes were recorded when zooplankters were fed on *S. obliquus*; with the exception of *D. magna* fed on *S. obliquus* at  $25^\circ\text{C}$ , no significant difference was observed among the three nutritional qualities tested (Table 2). At  $12^\circ\text{C}$  and  $15^\circ\text{C}$ , the largest clutch sizes were obtained when the animals were fed on *Cryptomonas* sp. At  $20^\circ\text{C}$  and  $25^\circ\text{C}$ , largest clutch sizes were obtained on *Cryptomonas* sp. and on the *Cryptomonas*:*S. obliquus* diet mixture (Fig. 2).

**Effects of food quality on *D. magna* and *S. vetulus* growth rate**—Zooplankters fed on *S. obliquus* had the significantly lowest somatic growth rate, except when the treatment was run at  $25^\circ\text{C}$  with *D. magna*. The highest growth rates were obtained on *Cryptomonas* sp., except for the treatments run at  $20^\circ\text{C}$  and  $25^\circ\text{C}$  with *S. vetulus*, where the mixture of *Cryptomonas*:*S. obliquus* also caused a high somatic growth rate (Table 2; Fig. 3).

**Combined effects of temperature and food source**—Clutch size and somatic growth rate of both zooplankters were affected by temperature and food quality. Results of two-way ANOVA showed that there was a significant interaction between the effects of temperature and food quality on the clutch size of *D. magna* ( $F_{6,24} = 29.3$ ,  $p < 0.001$ ) and *S. vetulus* ( $F_{6,24} = 135.7$ ,  $p < 0.001$ ) as well as on their growth rate: *D. magna* ( $F_{6,24} = 32.9$ ,  $p < 0.001$ ) and *S. vetulus* ( $F_{6,24} = 27.6$ ,  $p < 0.001$ ).

Differences between clutch sizes of individuals fed on different food quality decreased with increasing temperature (Fig. 4). These differences disappeared when females were fed on *Cryptomonas* sp. and on the mixture *Cryptomonas*:*S. obliquus* at  $20^\circ\text{C}$  for both zooplankters and on the three food source at  $25^\circ\text{C}$  for *D. magna*. Differences between growth rates of *D. magna* fed on different food sources decreased with increasing temperature (Fig. 5). Concerning *S. vetulus* growth rates, the distances were similar at  $12^\circ\text{C}$  and  $15^\circ\text{C}$ , decreased at  $20^\circ\text{C}$  and  $25^\circ\text{C}$ , and disappeared between individuals fed on *Cryptomonas* sp. and on the diet mixture *Cryptomonas*:*S. obliquus* at  $25^\circ\text{C}$ .

## Discussion

We experimentally investigated the effects of temperature and food quality on somatic growth and reproduction of zooplankton and showed that constraints exerted by food quality on somatic growth and reproduction of the two cladocerans used in this study decrease with increasing temperature. The two cladocerans used in this study are representatives of metazoan grazers of littoral zones and backwaters. In such zones, because of the low depth and strong contributions of autochthonous as well as allochthonous organic matter, high microbial production is observed (Testard 1995). As a consequence, these ecotones exhibit long periods during which zooplankton are not limited by food quantity, and they are suitable environments for studying the importance of food quality. Moreover, low water depth can substantially influence diurnal and seasonal temperature variations, which in turn can strongly affect physiological activity and life history traits of zooplankton (Giebelhausen and Lampert 2001).

Table 2. Results of ANOVA testing the effect of food quality (*Scenedesmus obliquus* [LQ food], *Cryptomonas*:*Scenedesmus* mixture [RefQ food], and *Cryptomonas* spp. [HQ food]) on *D. magna* and *S. vetulus* clutch size and growth rate grown at four temperatures:  $12^\circ\text{C}$ ,  $15^\circ\text{C}$ ,  $20^\circ\text{C}$ , and  $25^\circ\text{C}$ .

		$12^\circ\text{C}$		$15^\circ\text{C}$		$20^\circ\text{C}$		$25^\circ\text{C}$	
		F Value	p Value	F Value	p Value	F Value	p Value	F Value	p Value
<i>D. magna</i>	Clutch size	$F_{2,6}=94.0$	$p<0.001$	$F_{2,6}=60.4$	$p<0.001$	$F_{2,6}=7.0$	$p=0.026$	$F_{2,6}=0.9$	$p=0.433$
	Growth rate	$F_{2,6}=57.3$	$p<0.001$	$F_{2,6}=50.3$	$p<0.001$	$F_{2,6}=54.8$	$p=0.026$	$F_{2,6}=83.2$	$p<0.001$
<i>S. vetulus</i>	Clutch size	$F_{2,6}=354.9$	$p<0.001$	$F_{2,6}=322.2$	$p<0.001$	$F_{2,6}=68.4$	$p<0.001$	$F_{2,6}=61.1$	$p<0.001$
	Growth rate	$F_{2,6}=513.7$	$p<0.001$	$F_{2,6}=164.4$	$p<0.001$	$F_{2,6}=79.5$	$p<0.001$	$F_{2,6}=149.8$	$p<0.001$



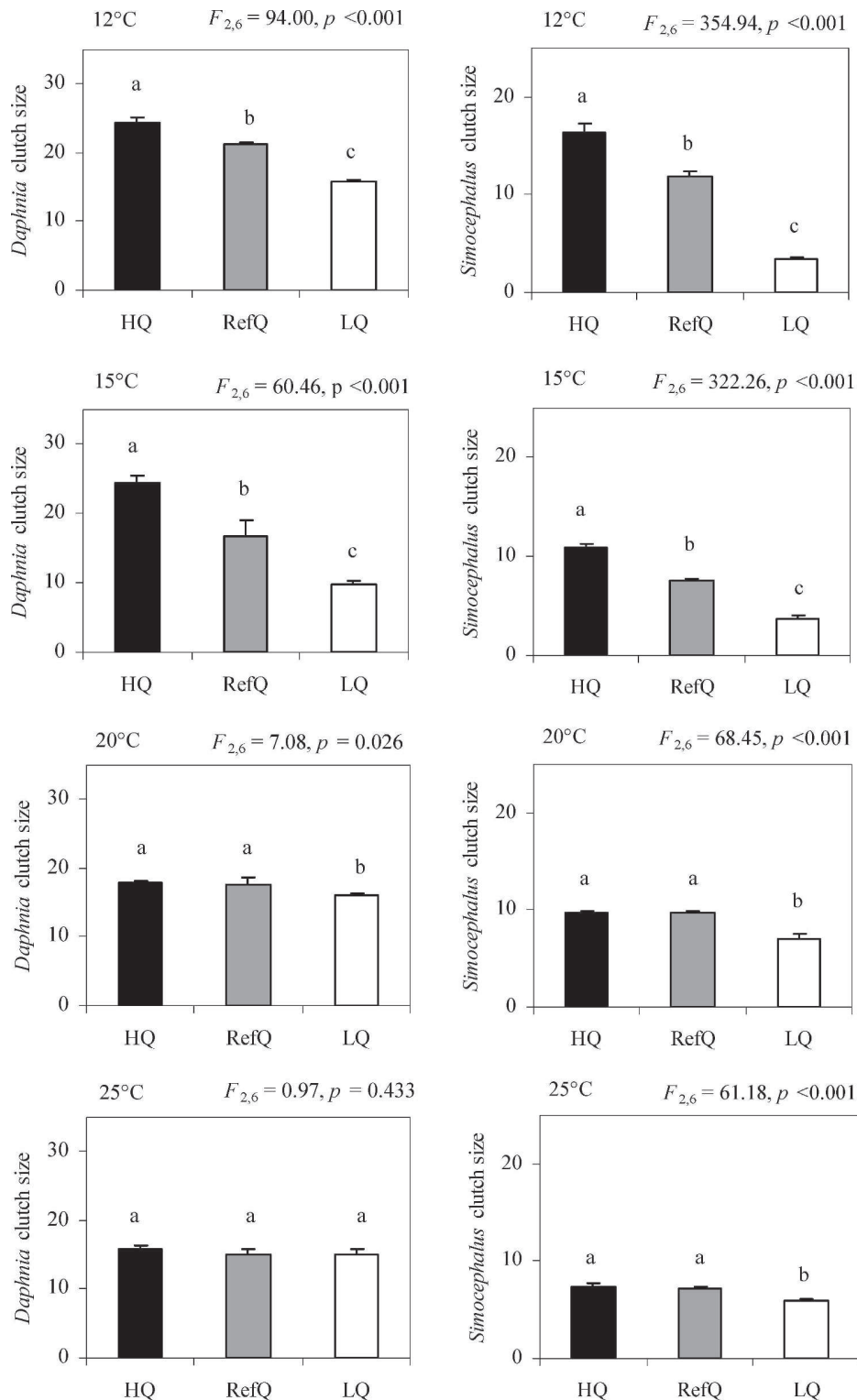


Fig. 2. Effects of food quality on *D. magna* (on the left) and *S. vetulus* (on the right) clutch size grown at four temperatures: 12°C, 15°C, 20°C, and 25°C. Data are means ± SD for three replicates per treatment. Results of the one-way ANOVA are presented at the top right corner of each graph. Bars labeled with the same letters are not significantly different (Tukey's HSD,  $p < 0.05$ ).

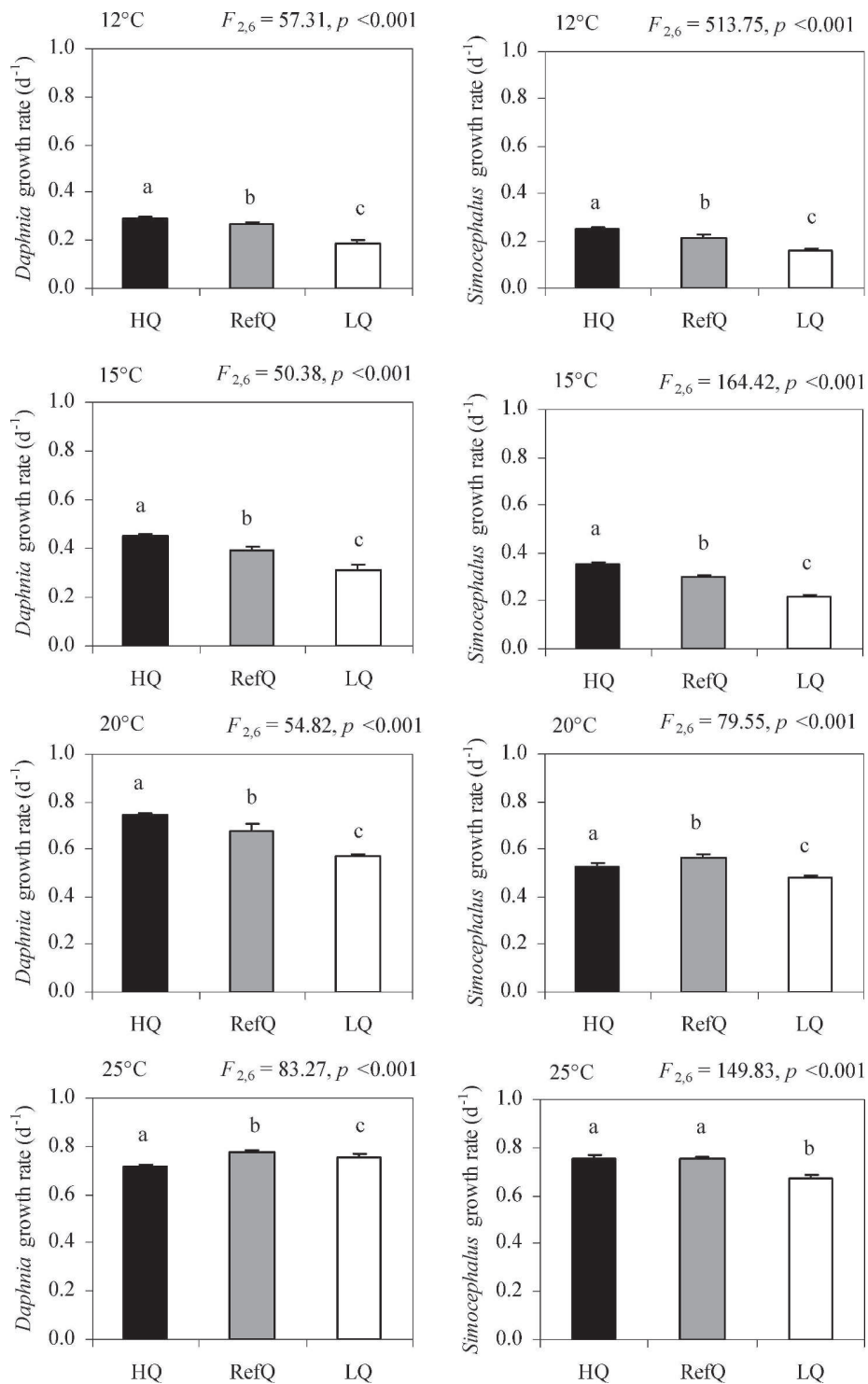


Fig. 3. Effects of food quality on *D. magna* (on the left) and *S. vetulus* (on the right) growth rate grown at four temperatures: 12°C, 15°C, 20°C, and 25°C. Data are means  $\pm$  SD for three replicates per treatment. Results of the one-way ANOVA are presented at the top right corner of each graph. Bars labeled with the same letters are not significantly different (Tukey's HSD,  $p < 0.05$ ).

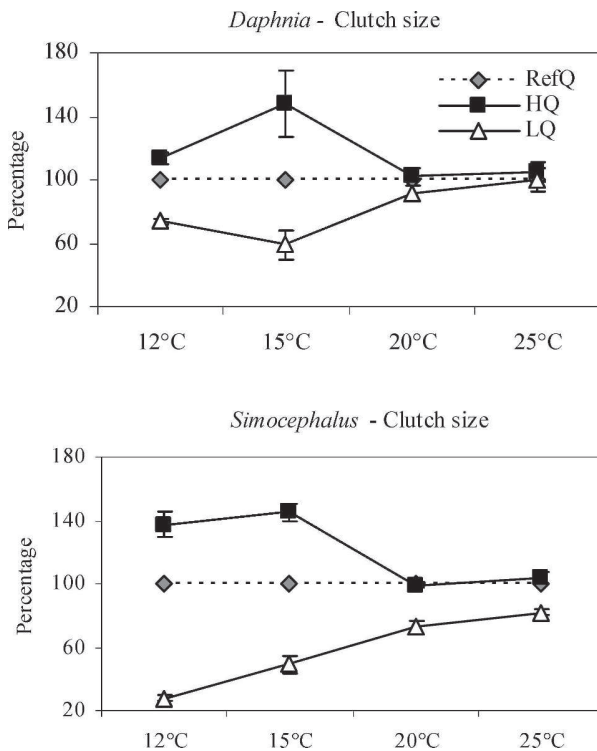


Fig. 4. Combined effects of temperature and food quality on *D. magna* and *S. vetulus* clutch size. Data are means  $\pm$  SD for three replicates per treatment. The mixture *Cryptomonas*:*Scenedesmus* (RefQ) was taken as reference for nutritional quality. Results obtained with *D. magna* and *S. vetulus* fed on HQ food and LQ food are expressed as a deviation (%) of results obtained with individuals fed on this reference quality food (RefQ).

We applied three food sources to supply different food quality to cladocerans. As individuals were fed ad libitum, we infer that variations observed for a same temperature were due to variation in food quality of the food source. Best growth and reproduction rates were obtained when individuals were fed on *Cryptomonas* sp. at 12°C and 15°C. In contrast, the lowest growth and reproduction rates were obtained on *S. obliquus*, and intermediate rates were obtained on the mixture *Cryptomonas*:*S. obliquus*. These results are in agreement with previous studies that examined the nutritional quality of phytoplankton species as food source for *Daphnia* (Ahlgren et al. 1990; Brett et al. 2000). The decrease of growth and reproduction rates with decreasing food quality is less pronounced at 20°C and almost inexistent at 25°C, suggesting that there is an interaction between the temperature and the food quality effects.

Somatic growth rates of both cladocerans were positively affected by increasing temperature. This result is in line with other studies on zooplankton and particularly on cladocerans (Dawidowicz and Loose 1992; Lemke and Benke 2004), although growth rates can vary among species. As for all poikilotherms, reduced metabolic rates are generally induced by decreasing temperature and account for slower somatic growth (Angilletta et al. 2004). However, *D. magna* fed on HQ food exhibited

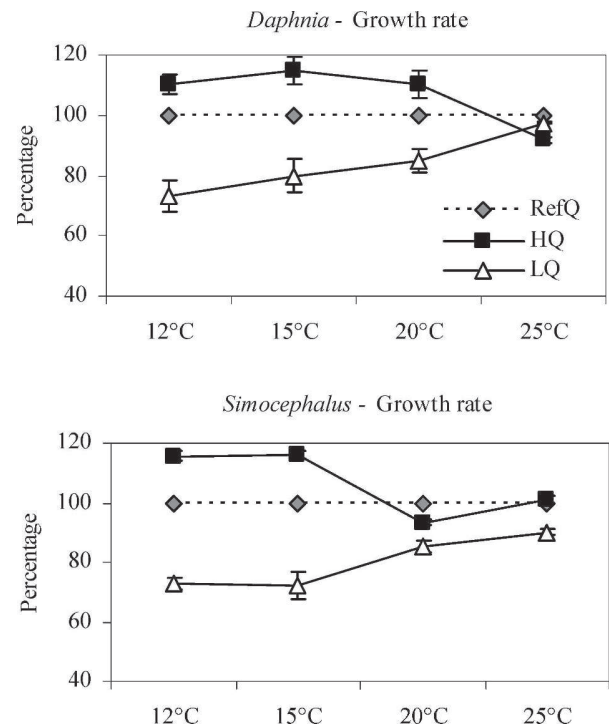


Fig. 5. Combined effects of temperature and food quality on *D. magna* and *S. vetulus* growth rate. Data are means  $\pm$  SD for three replicates per treatment. The mixture *Cryptomonas*:*Scenedesmus* (RefQ) was taken as reference for nutritional quality. Results obtained with *D. magna* and *S. vetulus* fed on HQ food and LQ food are expressed as a deviation (%) of results obtained with individuals fed on this reference quality food (RefQ).

slightly lower growth rates at 25°C than at 20°C. This suggests that the range of optimum temperatures for the clones of *D. magna* used in this study do not exceed 20°C. Although some studies found that *D. magna* grew best at and above 25°C (Mitchell and Lampert 2000; Mitchell et al. 2004), Giebelhausen and Lampert (2001) also found highest somatic growth at 20°C for *D. magna* but decreasing growth rates at higher temperatures.

Contrary to growth rates, the effects of temperature on clutch size varied with different food quality. When zooplankton was fed on the HQ food, clutch sizes of *D. magna* and *S. vetulus* decreased with increasing temperature. This trend had previously been observed in other studies on *Daphnia* and *Simocephalus* (Perrin 1988; Giebelhausen and Lampert 2001). These authors showed that temperature increases caused smaller body size and smaller clutch size of daphnids. It seems that there is a general "temperature-size" rule in ectotherms predicting that delayed maturation provides a benefit for greater fecundity because fecundity increases with increasing body size (Angilletta et al. 2004). However, when individuals were fed on the RefQ food, the decrease of clutch size with increasing temperature is less visible and disappears on the LQ food. Our data suggest that there are combined effects of temperature and food quality and contribute, from a food quality perspective, to previous studies on temperature and food quantity (Mitchell 1997; Giebelhausen and

Lampert 2001) that found differences in the temperature response measured under different food concentration, with greater increase of *Daphnia* growth rate with increasing temperature at a high food concentration. Hence, it seems that there are reduced temperature effects under reduced food conditions (with respect to quantity as well as quality) challenging such a temperature-size rule.

In an in situ study, Cole et al. (2002) hypothesized that such interaction between food quality and temperature exists, but it has not been tested yet. In the present study, the mixture *Cryptomonas*:*S. obliquus* was taken as a reference (RefQ). In order to better understand variations in food quality constraints with temperature, results obtained on *Cryptomonas* sp. (HQ) and *S. obliquus* (LQ) were normalized to this reference diet. Our results show that constraints caused by food quality on growth and reproduction of the two cladocerans decrease with increasing temperature. When the temperature increased, the differences between clutch sizes of females fed on the different quality foods decreased. There was no difference between clutch size of females fed on HQ food and on the RefQ food at 20°C and for the three food sources at 25°C for *D. magna*. At 12°C, higher *D. magna* mortality was observed, and males appeared, especially on the RefQ food (12.5% of mortality and 29.2% of male) and the LQ food (75% of mortality and 8.3% of male), indicating that this temperature was below that of the fitness optimum for the organisms used in this study. As a consequence, the number of females taken into account for the measurements decreased and may have accounted for the unexpected results at this temperature. Concerning somatic growth rates, the differences between individuals fed on the three food types decreased with increasing temperature for *D. magna*. For *S. vetulus*, the difference remained stable at 12°C and 15°C but decreased at 20°C and 25°C. There was no difference observed between somatic growth rate of *S. vetulus* fed on the HQ food and the RefQ food at 25°C. These data suggest that food quality constraints for zooplankton development vary with temperature.

Different factors could be taken into account to characterize the food quality and to explain the combined effects of temperature and food. In our study, the two phytoplanktonic species used may differ in their digestibility. Such difference in digestibility has been found in particular when phytoplankton is limited by nutrients (Lüring and Van Donk 1997). For example, the cell wall of *S. obliquus* can thicken, and metabolically costly amylases and cellulases are required to digest them. In our study, the algae were not limited by nutrients. Moreover, although we consider *S. obliquus* a low quality food compared to *Cryptomonas* sp., *S. obliquus* is widely regarded as good-quality food in studies dealing with zooplankton development (Lampert 1978; Ahlgren et al. 1990; Giebelhausen and Lampert 2001). But we cannot exclude that some slight difference in digestibility between these two phytoplanktonic species exists and that such difference may have been enhanced by lower temperatures. The metabolic constraints of using amylases and cellulases may increase with decreasing temperature and may partly explain the

variation of food quality constraints for zooplankton development with temperature. Clearly, additional research is needed to investigate how digestibility of algae at different temperatures affects life history traits of zooplankton.

As for the biochemical composition of primary producers, it is now widely accepted that nutritional quality of phytoplankton can be defined, at least in part, by its PUFA composition (Müller-Navarra et al. 2000). Some studies reported accumulation of EPA in total lipids of daphnids compared to the EPA content of the seston they fed on (Kainz et al. 2004; Hessen and Leu 2006). Moreover, several studies established a positive correlation between EPA concentrations of phytoplanktonic species and growth of *Daphnia* in the laboratory (Ahlgren et al. 1990; Weers and Gulati 1997) and field studies (Müller-Navarra et al. 2000; Wacker and Von Elert 2001). It was also shown that EPA enhances egg production of laboratory-raised cladocerans (Bec et al. 2003, 2006). Lipid analyses of our phytoplanktonic species show that *Cryptomonas* sp. was rich in long-chain PUFA and particularly in EPA and SDA, which were absent or occurred only at low concentrations in *S. obliquus*. Moreover, we argue that phytoplankton species were not limited by P, which was abundant in the culture medium. Therefore, we suggest that differences in EPA and SDA concentrations among the three food sources account for biochemical differences in their nutritional quality for these cladocerans. We recognize the possibility that cladocerans may be able, at least in part, to synthesize EPA from ALA via enzymatic elongation and desaturation (Bec et al. 2003). However, such EPA biosynthesis does not seem to be sufficient for optimizing somatic growth, as dietary EPA supplementation further improved *Daphnia* growth rates (Von Elert 2002). In addition, Schlechtriem et al. (2006) found that retention of EPA by daphnids was clearly influenced by temperature because significantly higher levels of EPA were found in individuals reared at 11°C compared with those kept at 22°C, suggesting that EPA requirements of cladocerans increase with decreasing temperature. Farkas (1984) argued that the ability of zooplanktonic species to adapt their membranes at low temperature depends on their ability to incorporate PUFA. More generally, the level of unsaturation of fatty acids of poikilotherms increases at low temperature in membrane lipids, lending support to Schlechtriem's and Farkas's results (Nishida and Murata 1996). It seems thus that dietary PUFA, such as EPA and SDA, are important for maintaining membrane fluidity of cladocerans at low temperatures. In addition to EPA, SDA concentrations were significantly higher in *Cryptomonas* sp. than in *S. obliquus*. Although there is still little known about the physiological role of this n-3 PUFA, SDA is retained in daphnids when supplied by diet (Brett et al. 2006); consequently, the higher diet supply of SDA by *Cryptomonas* sp. may, in concert with EPA, have favored higher clutch sizes in both cladocerans at lower temperatures as observed in this study. Hence, we suggest that variations in food quality constraints according to temperature can be explained by the variability of dietary EPA and possibly SDA requirements of these cladocerans.

In freshwater systems of the temperate zone, the taxonomic composition of the phytoplankton community shifts during the year. During the wintertime and the early spring, diatoms and cryptophytes, which are known to be rich in EPA (Ahlgren et al. 1990), can dominate phytoplankton composition, while in summer they are replaced by chlorophytes and cyanobacteria that lack EPA and that are generally poorer in PUFA (Ahlgren et al. 1990; Amblard and Pinel-Alloul 1995). In a field study, Maazouzi et al. (2008) also found that exposure of planktonic organisms to overheated water resulted in increased saturated and monounsaturated FA content at the expense of PUFA. Thus, with regard to seasonal changes of phytoplankton in ponds and lakes, the importance of variability of food quality constraints with temperature may be offset by the presence of PUFA-rich phytoplankton communities during the cold season. Nevertheless, in shallow lakes and littoral zones, zooplankton can undergo sudden variations of temperature. These variations of temperature could be too fast to allow phytoplanktonic communities to shift, and in such cases, combined effects of temperature and food quality may constrain somatic growth and reproduction of zooplankton in natural systems. In the same way, daphnids can undergo great variations of temperature during diel vertical migration in the pelagic environment. Clearly, further research is required to understand the importance of such "diet-temperature" interactions in natural ecosystems.

We conclude that constraints exerted by food quality on somatic growth and reproduction of cladocerans decrease with increasing temperature. In the light of predicted climate change, further studies, including studies of various aquatic ecosystems, are needed to better understand and eventually predict how changing diet composition affects life history traits of natural populations with changing temperatures.

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