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Couvertures : «**Un jour, un hiver, une rive**»

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Une thèse de doctorat a la particularité d'être à la fois un travail personnel et le résultat d'une équipe.

Au moment de remercier toutes ces personnes qui m'ont entouré au cours de ces années de recherche, je jette un œil, nostalgique, dans le passé, et revois avec tendresse tous ces visages qui ont passé de nombreuses heures à m'enseigner les rudiments d'une langue ancienne, de la théorie des ensembles ou la rigueur de la systématique. Merci à tous ces enseignants, qu'ils trouvent dans ce travail le résultat de leur passion et de leur enthousiasme.

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D'ici quelques années, quand je me retournerai vers ces belles années de recherche, les journées de terrain passées sur la passerelle à Esch-sur-Sûre resteront à coup sûr parmi les souvenirs les plus vivaces. La très belle ambiance qui nous a toujours unis sur ces planches en est certainement la cause. La responsabilité en revient d'abord à Isabelle Thys, qui a toujours su organiser de maître façon ces journées où il fallait souvent concilier les envies de multiples chercheurs. Isabelle, merci de m'avoir si chaleureusement accueilli dans votre équipe ! Merci aussi à tous ces chercheurs qui se sont succédés sur le ponton et ont permis à chacun d'obtenir de belles séries de données sur notre lac modèle. Merci à Véronique, Bruno, Raphaël, Henry-Michel, Jeff, et tous ceux que j'oublie, pour ces fabuleux souvenirs !

Isabelle ! Ta contribution à ce travail ne se limite évidemment pas à ta présence sur la passerelle. Ta joie de vivre, ton sourire, ton courage, ton accueil auront été chacun un stimulant incroyable que je souhaite à chaque doctorant. Notre tandem, complémentaire, n'aura

pas si mal fonctionné. Tu sais mieux que moi tout ce que je te dois ! A très bientôt, chère Docteur.

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Enfin, il est des moments où les mots n'ont plus leur place. A mes proches, ma famille, mes amis ...

A Kaki

A mes étudiants

Responses of zooplankton to variation in elemental composition of algae : regulations at individual and ecosystem levels

by François DARCHAMBEAU

Abstract

The aim of this work was to improve our knowledge about the responses and adaptations of zooplankton, and especially *Daphnia*, when faced with food sources that are deficient in essential elements.

First, we have modelled the constraints of feeding, digestion and growth in *Daphnia*. We have hypothesized that *Daphnia* possesses the intrinsic ability to initiate behavioural (i.e. filtration rate) and/or physiological (i.e. excretion rate of digestive enzymes) responses designed to cope with dietary phosphorus (P) deficiency. This first stoichiometrically explicit model of *Daphnia* filtration and digestion shows that an increase in feeding rate is an appropriate response to cope with P-deficiency. This mathematical prediction is in agreement with field observations on a *Daphnia galeata* population. Indeed, the filtration rate of lake *Daphnia* was positively correlated with lake seston carbon:phosphorus (C:P) ratios. So, if algae are deficient in P (high C:P ratios), *Daphnia* filter more water, and so feed more intensively. This behaviour may be seen as a use of C assimilated in excess from the algae, this to generate more energy that can then be used to obtain more P, through increased feeding and digestion rates.

Behavioural and physiological adaptations taken into account in the model are however not immediate. After an acclimation period when the growth rate must remains low, I predict that we can observe an increase of growth rate as soon as these functional responses are triggered. This is exactly what it is observed in a meta-analysis of published *Daphnia* growth rates. This analysis shows that the growth rate of juvenile *Daphnia* submitted to P-limited food first drops, then progressively increases.

Moreover, we have conducted laboratory experiments to demonstrate enhanced respiration in daphniids fed with P-depressed algae. Once again, this observation is in total agreement with the predictions of the model. This increase in respiration rate is, of course, the expression of enhanced metabolism. From a stoichiometrical perspective, the increase in respiration allows the disposal of excess C with respect to P. In the same study, we have also shown the higher excretion of organic C by P-limited *Daphnia*.

Beyond individual responses, this work was also concerned with the global impact of these adaptations to fluxes of matter in the ecosystem. As

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specific needs for nitrogen (N) and P of each zooplankton species probably lead to species-specific regulations of both elements, the relative composition in C, N and P of faeces produced by zooplankton at a certain time must reflect the average elementary requirements of the entire zooplankton community. We have indeed observed a good correlation between the stoichiometrical characteristics of the zooplankton community (C:N:P ratios) in the Esch-sur-Sûre reservoir (Grand-Duchy of Luxemburg) and C, N and P sedimentation rates. So, when e.g. the zooplankton community was composed of crustacean species with high bodily demand in P, the N:P ratio of small sedimenting particles increased, indicating high assimilation of P by zooplankton, and the corresponding N enrichment of faeces.

Réponses du zooplancton aux variations de la composition élémentaire des algues : régulations au niveau de l'individu et à l'échelle de l'écosystème

par François DARCHAMBEAU

Résumé

Ce travail a pour objectif de mieux comprendre les réponses adaptatives du zooplancton, et en particulier des Daphnies, lorsqu'elles qui doivent faire face à des carences alimentaires en éléments essentiels.

Nous nous sommes tout d'abord attaché à modéliser les contraintes d'alimentation, de digestion et de croissance d'une Daphnie. Nous avons posé comme hypothèse que les Daphnies ont la capacité intrinsèque de mettre en œuvre des réponses comportementale (le taux de filtration) et/ou physiologique (le taux d'excrétion des enzymes digestives) leur permettant de s'adapter à une déficience alimentaire en phosphore (P). Ce premier modèle explicitement stœchiométrique de la filtration et de la digestion d'une Daphnie nous apprend qu'une augmentation de l'activité d'ingestion est une réponse appropriée afin de faire face à une carence nutritionnelle en P. Cette prédiction mathématique corrobore nos observations de terrain menées sur une population de *Daphnia galeata*. Les individus de cette population montrent en effet une corrélation positive de leur taux de filtration de l'eau avec le rapport carbone:phosphore (C:P) des algues du milieu. Ainsi, si les algues se trouvent carencées en P (augmentation du rapport C:P), les Daphnies se nourrissent plus intensément. Ce comportement peut être compris comme une mise à profit du C excédentaire rencontré dans les algues pour acquérir plus d'énergie. Cette énergie est ensuite consommée par des activités de filtration et de digestion plus intenses permettant ainsi d'obtenir plus de P.

Les réponses comportementale et physiologique prises en compte dans le modèle ne sont certainement pas immédiates, et après une période d'adaptation où le taux de croissance des animaux doit être faible, on peut prédire qu'il est possible d'observer une augmentation du taux de croissance au fur et à mesure que ces réponses fonctionnelles se mettent en place. C'est en effet ce que nous avons pu observer en étudiant un grand jeu de données de la littérature reprenant le taux de croissance mesuré en laboratoire de diverses espèces de Daphnies. Cette analyse montre que lorsque l'on augmente la carence en P des algues qui les nourrissent, le taux de croissance des Daphnies diminue d'abord, puis remonte progressivement.

Des expériences en laboratoire ont également mis en évidence la plus forte respiration des Daphnies nourries par des algues carencées en P, tel que prédit par notre modèle. Cette augmentation de la respiration traduit

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une activité métabolique plus intense, et, d'un point de vue stœchiométrique, est une voie de sortie pour le C ingéré en excès par rapport au P. On a également pu mettre en évidence au cours de la même étude une plus forte excrétion de C organique par les Daphnies carencées.

Au-delà des réponses individuelles, ce travail s'est aussi intéressé à l'impact global de ces adaptations sur les flux de matière dans l'écosystème. Puisque les besoins spécifiques en azote (N) et en P de chaque espèce du zooplancton induisent très probablement une régulation elle aussi spécifique de l'assimilation de ces 2 éléments, la composition relative en C, N et P des fèces produites par le zooplancton à un moment donné doit traduire les besoins élémentaires moyens de l'ensemble de la communauté. Nous avons en effet pu montrer une bonne corrélation entre les caractéristiques stœchiométriques de la communauté zooplanctonique (rapports C:N:P) de la retenue d'Esch-sur-Sûre (Grand-Duché du Luxembourg) et les taux de sédimentation en C, N et P. Ainsi, lorsque la communauté zooplanctonique s'enrichissait, par exemple, en espèces de crustacés à forte demande en P, le rapport N:P des petites particules en voie de sédimentation augmentait, traduisant ainsi la forte assimilation du P par le zooplancton et l'enrichissement des fèces en N.

Chapter 1

Introduction

HISTORY

At the moment where many ecologists call to the reintegration of science into the human society (Bradshaw and Bekoff 2001), Reynolds (1998) advocates that the generally short time scales at which aquatic ecosystems operate provide excellent opportunities for verifying the hypothesized mechanisms underpinning the patterns of large-scale ecosystem behavior. So aquatic ecologists are favorably situated to tackle the main criticism addressed to ecology: its imprecision and lack of fundamental rules (Peters 1991). Lawton (1999) points out that in order to realize this ambition, attention needs to be concentrated, among others, on the compliance of biological systems to the physical laws of thermodynamics and entropic dissipation, of the chemistry of biomaterials and the stoichiometry of their composition. Reynolds (2000) notes that aquatic ecologists are already embarked upon these concepts and thus "are in a privileged position to tackle studies towards the casting of robust laws in ecology".

Simultaneously to these reflections, we observe that limnology and oceanography are at the crossroads between a descriptive, pattern-based discipline and a more functional, predictive, process-focused science (Harris 1994, Bourget and Fortin 1995). This trend is wanted, first of all, and pragmatically, by the decision-makers who call for both commonality and causality (Wetzel 2000). However, limnology has quite recently emerged from the descriptive stages and is just beginning to evaluate controlling mechanisms that regulate and produce the observed biological and chemical results. Since Forel's founding studies on Lake Geneva (Forel 1892, 1895, 1904), aquatic scientists must recognize that patterns identified and concepts developed within aquatic systems rarely translate into ecological theory and tends to stay exclusively 'wet' (Reynolds 1998). Yet, few aquatic core synthesizing thoughts should merit this transfer. I think firstly about Lindeman's development of trophic dynamics (Lindeman 1942), resource competition theory (Tilman 1982), and trophic cascade (Hrbáček et al. 1961, Carpenter et al. 1985). During this last decade, a new, refreshing conceptual framework stimulates imaginations and works of some limnologists around the world. Built step by step, ecological stoichiometry has the potential to become a significant milestone in the development of modern ecology (Vitousek 2002) and in the congruent reconnection of biological disciplines at the moment highly fragmented (Sterner and Elser 2002). The thesis that you have in your hands intends to add some modest personal pieces to the highly motivating building of this refreshing framework. Without the instrumental and stimulating studies of a few aquatic researchers, Dag O. Hessen, Tom Andersen, Robert W. Sterner, James J. Elser, and Jotaro Urabe, my work during these last 6 years would have been totally different, as

would have been the resulting thesis. Unassumingly, today by the present work, I would like acknowledge these stimulators for the real delight that their ideas have procured me.

Stoichiometry is a branch of chemistry that deals with the application of the laws of definite proportions and the conservation of mass and energy. Its most popular application is found in the balancing of chemical reactions where proportions of elements must stay equivalent between reactants and products. Similarly, ecological stoichiometry represents organisms as single abstract molecules where constituent elements exhibit mass conservation (Sterner and Elser 2002). Chemical reactions are transfigured into trophic relationships and biogeochemical fluxes. By definition, the application of the law of mass conservation into ecology implies the stoichiometry of trophic relationships and biogeochemical fluxes. We might retort that this approach is too simple to be really promoting and innovatory, but we will see that a stoichiometric approach of ecosystem functioning leads to multiple negative and positive feedbacks with great ecological implications. Stoichiometry, as a fundamental rule, may bring to ecology some of the requested commonality helping to understand observed biological and chemical patterns and therefore the functioning of ecosystems. Note here that since the initial papers showing the first thoughts about implications of stoichiometry into ecology (we were in the start of 90'ies), more than 200 explicitly stoichiometric papers have been published in international peer-reviewed ecological journals. Recently, two limnologists, among the first who have developed stoichiometric ecology principles, have synthesized 10 years of studies and their hopes for future research into a book: "Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere" (Sterner and Elser 2002).

The ecological stoichiometry starts at the corner between 80'ies and 90'ies from the evidence of elemental homeostasis of many zooplankton species (Hessen 1988, Hessen 1990, Hessen and Lyche 1991, Andersen and Hessen 1991). Without homeostasis, ecological stoichiometry would be a dull subject (Sterner and Elser 2002). The homeostasis, a concept familiar to physiologists, indicates the physiological regulation of an organism's internal environment reducing changes within the organism. Kooijman (1995) defined homeostasis in a stoichiometic context as follows: "The term homeostasis is used to indicate the ability of most organisms to keep the chemical composition of their body constant, despite changes in the chemical composition of the environment, including their food." Thus, proportions of major elements (carbon [C], nitrogen [N] and phosphorus [P]) were found very stable into individual taxa of zooplankton, whatever environmental conditions and especially elemental composition of their food (Fig. 1.1). Surprisingly also, if intra-specific variations are low, inter-specific

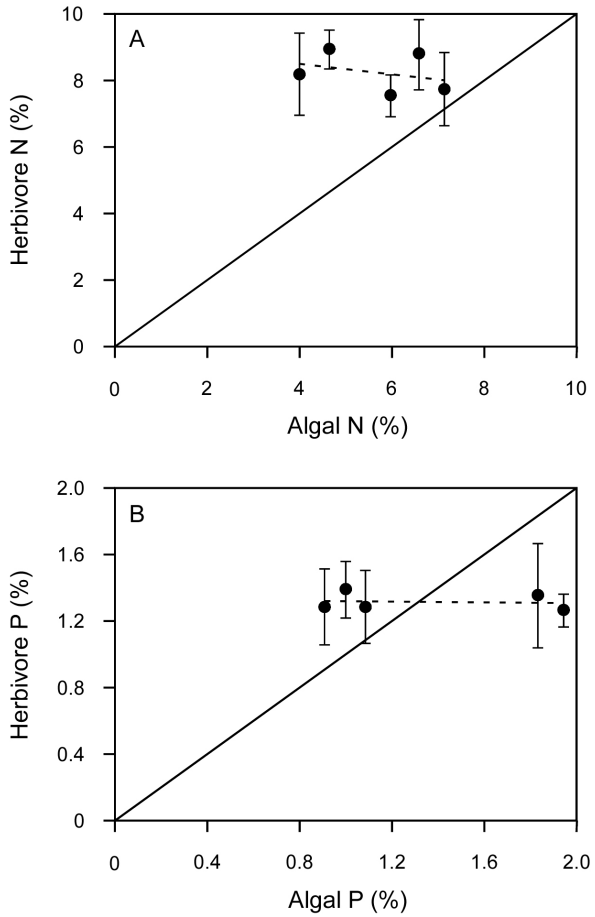

Fig. 1.1: Homeostatic regulation of (A) nitrogen, N, and (B) phosphorus, P, content of *Daphnia magna*. The animals were fed during 11-15 days with varying algal elemental composition. The final % N and P in the herbivore (mean ± standard deviation) were independent of diet nutrient content. Drawn with data from Hessen (1990).

variations are high, and we can easily distinguish zooplankton species as a function of their composition in C, N and P (Fig. 1.2). As already noted, these fundamental observations have directly opened the limnologists to the stoichiometry of trophic relationships. Two thoughts have immediately emerged: (1) if proportionate demands in elements of the herbivore are different from its food, the elemental composition of food can limit the growth of their consumers (Hessen 1992, Urabe and Watanabe 1992), and (2) the ratio of elements ingested in excess and thus resupplied by herbivores to the environment is dependent on both food composition and consumer demands (Sterner 1990, Hessen and Andersen 1992).

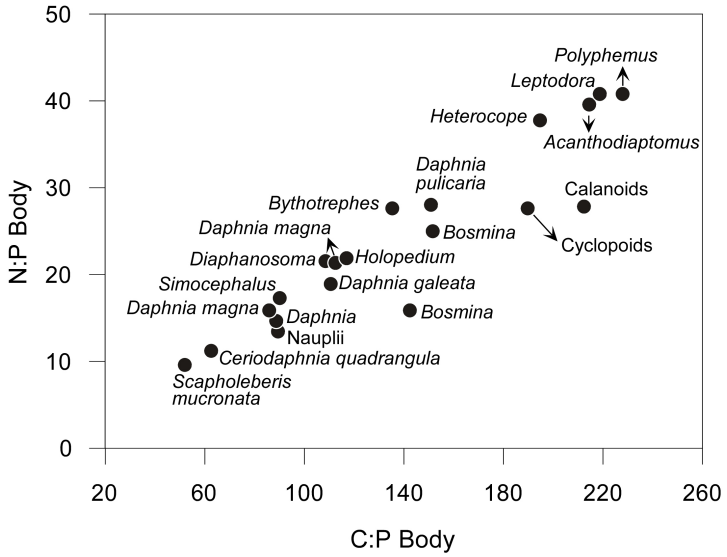

Fig 1.2: Interspecific variation in the elemental composition (in molar units) of various taxa of freshwater zooplankton (cladocerans: *Bosmina*, *Bythotrephes*, *Ceriodaphnia*, *Daphnia*, *Diaphanosoma*, *Holopedium*, *Polyphemus*, *Scapholeberis*, *Simocephalus*; copepods: others). From Sterner and Elser (2002).

FOOD QUALITY LIMITATION

The first prediction will be largely discussed in the literature (see e.g. the discussion between Brett 1993, Urabe and Watanabe 1993, and Hessen 1993). A special and increasing interest has focused on *Daphnia* species and on their putative limitation by P-depressed algae. Indeed phytoplankton in lakes often exhibits very high C:P ratios in comparison with consumer C:P ratios (Hecky et al. 1993, Elser and Hassett 1994, Hassett et al. 1997). By their high demands in P relative to C (see Fig. 1.2), species of the genus *Daphnia* are the most susceptible to experience food with non-equilibrated elemental composition. Then attention was immediately drawn to experimental observation of growth penalty due to low food quality (e.g. Sterner 1993). The causality of the relationship between diet P deficiency and low growth rates was naturally addressed, e.g. by Brett (1993), and it was suggested that P deficiency actually masks the depression of algae in fatty acids, especially some highly unsaturated fatty acids (HUFA) as eicosapentaenoic acid (Müller-Navarra 1995). After further experimental evidence (Urabe et al. 1997, Boersma 2000, Boersma et al. 2001, Elser et al. 2001), it is now admitted that both P element and HUFA can directly and independently limit the growth of *Daphnia* in the laboratory and, more

fundamentally, in the field. Nevertheless it appears that when both resources are depressed in the food (i.e. C:P ratio > 300 in atomic values), the P demand must be met first (Boersma 2000, Becker and Boersma 2003).

Comparatively with this abundant literature on growth penalty induced by low food quality, the attention was rarely drawn on the ability of physiological regulation in the consumer. Yet, paradoxically, this regulation is the core of homeostasis definition, and homeostasis is a central axiom of stoichiometry theory. After initial works by Hessen and his colleagues (*op. cit.*), the model of strict elemental homeostasis in zooplankton was rapidly adopted and, since, rarely tested against experimental evidence. Only recently, strict homeostasis was questioned by results of DeMott et al. (1998) and Plath and Boersma (2001). However, as pointed out by Sterner and Elser (2002), even if the initial axiom of the strict homeostasis does not stand up to analysis, the results of DeMott et al. (1998) and of Plath and Boersma (2001) also showed that internal variations in P contribution to total *Daphnia* biomass stay largely below the ones of the environment, and especially of the food. This physiological regulation of internal environment reducing elemental changes within the organism thus demonstrates the strongly homeostatic nature of P in *Daphnia* (Sterner and Elser 2002).

As zooplankton clearly lives and grows also when elemental composition of their food is not strictly balanced with its demand, we must agree that the consumer is able at least partly to elaborate some compensatory mechanisms. Homeostatic regulation of stoichiometry can occur in multiple ways. It can result from food choice, habitat selection, assimilation, or excretion (Sterner and Elser 2002). The first models of the stoichiometric relationship between planktonic autotrophs and consumers have explicitly taken into account the ability of an assimilation regulation (Urabe and Watanabe 1992, Hessen 1992, Sterner 1997). For instance, the first stoichiometric model of the physiology of ingestion and growth of herbivorous freshwater zooplankton, the Urabe and Watanabe's model (Urabe and Watanabe 1992), stipulated that the gross growth efficiencies for N and P are both set to 1 (it means that all ingested N and P are assimilated through the gut wall and converted into new biomass without release) while the gross growth efficiency for C ranged from 0.2 to 0.4 for *Daphnia*. All these models led to a Threshold Elemental C:P Ratio (TER) between 120–300 (atomic values). Above the TER, growth of *Daphnia* is expected P-limited while below the TER growth is expected C-limited. The assumptions upon which their models are based have been tested very rarely. Particularly, ability of zooplankters to assimilate C differently from P and to modify their assimilation efficiency depending on diet composition have to date been tested only once in *Daphnia* (DeMott et al. 1998). Yet, predictions of these models were largely used to investigate the probability to observe P-

limitation of *Daphnia*'s growth in lakes (e.g. by Hessen and Faafeng 2000). The present thesis extends these assumptions and model predictions into a more biochemical framework (Chap. 2). Particularly, I will focus on the biochemical link between C and P assimilation.

Sterner and Hessen (1994) have suggested that ingestion is also largely under control of the consumer and might help to balance incorporated elements with requirements. The stoichiometric advantages of optimal foraging stayed largely unclear until recently and the observations of Plath and Boersma (2001). They showed that *Daphnia* slightly increases beat rate of its feeding appendages if food become P-deficient. They suggested that this might increase the expenditure of energy (and thus of C) and lead to lower C:P ratio of incorporated elements. This hypothesis must necessarily be integrated in a global perspective where ingestion and assimilation are linked. Indeed, higher ingestion means also lower residence time of particles in the gut, and thus probably lower assimilation of elements. This combined perspective was modeled in the present thesis (Chap. 2) and obtained predictions tested against field evidence (Chap. 3) and literature data on *Daphnia* growth (Chap. 4).

CONSUMER-DRIVEN NUTRIENT RECYCLING

The second thought directly emerging from observation of zooplankton composition homeostasis translates into stoichiometric perspectives the influence of zooplankton on nutrient cycling. The net positive impact on phytoplankton growth of single nutrient excretion by planktonic heterotrophs was already revealed by Lehman (1980) and Sterner (1986). And very soon, some oceanographs and limnologists had forewarned that the ratio of N and P resupplied to the environment by zooplankton is probably dependent upon consumer's body N:P proportions (Ketchum 1962, Corner and Davies 1971). In 1990, the studies of Hessen and his colleagues (*op. cit.*) on zooplankton elemental homeostasis have re-highlighted the gap between seston and zooplankton elemental composition. The different nutrient composition among zooplankton taxa (see Fig. 1.2) implies a relatively higher demand for P in *Daphnia* and a higher demand for N in the copepods, hence a higher N:P ratio in the release products would be expected from a *Daphnia* relative to a copepod. So, the homeostasis of the consumer should lead to limitation of autotrophs growth by the nutrient the most highly demanded by the consumer (Sterner 1990, Hessen and Andersen 1992). These predictions were later corroborated by *in situ* observations of the effects of a whole-lake food-web manipulation on the identity of the nutrient limiting phytoplankton growth (Sterner et al. 1992). The manipulation shifted the zooplankton

community from *Daphnia* dominance to copepod dominance, resulting in a corresponding change in the identity of the limiting nutrient: initially P-limited, phytoplankton became N-limited when copepods dominated the zooplankton community (Elser et al. 1988). Zooplankton assemblages dominated by *Daphnia* (low body N:P, see Fig. 1.2) likely retained P in biomass at high efficiency while recycling N at a relatively high rate, and thus shifted phytoplankton growth toward P limitation. Inversely, when copepods dominated, the zooplankton community retained N while preferentially recycling P, leading to N limitation of algae (Sterner et al. 1992). Also empirically, Elser and Urabe (1999) observed, by the compilation of literature data, a strong positive relationship between N:P released by zooplankton and diet N:P, and a negative relationship between the residuals of the first relationship and zooplankton N:P. These observations provided strong support for stoichiometric predictions on consumer-driven nutrient recycling (Sterner 1990, Hessen and Andersen 1992).

Moreover, the indirect effect of zooplankton on autotrophs via nutrient recycling should also have a negative feedback effect on consumer growth. Indeed, as the grazer favors the growth limitation of algae by the highest requested nutrient, the algae nutrient:C ratio will rapidly drop and induce a further mineral growth limitation of the grazer. This negative feedback loop involving nutrients recycled by herbivores, autotrophs nutrient content, and consequent putative food quality limitations for herbivores naturally questions the dynamics of the algae-grazer system (Hessen and Andersen 1992). This question was fundamentally addressed by modeling (see e.g. Andersen 1997, Hessen and Bjerkeng 1997, Daufresne and Loreau 2001). These works showed that the final state of the system may vary (non-zero stable state, limit cycle, grazer extinction or even deterministic chaos) and is largely dependent on many parameters, including initial biomass and stoichiometric parameters, such as consumer C:P ratio and minimal C:P of algae.

All these reflections always implied that nutrients released by heterotrophs are directly bioavailable for autotrophs. Actually, the disposal of nutrient ingested in excess may be encountered either by its non-assimilation and its final egestion, or by its metabolization and its final excretion. Both forms are potentially opposite in their fate. Metabolized nutrients are excreted in a form (NH_4^+ for N and PO_4^{3-} for P) directly bioavailable for autotrophs, while egested nutrients are still particulate and largely linked to biochemical compounds. These last have higher probabilities to sediment outside the mixed, upper, productive layer. As both processes very likely occur in the stoichiometric regulation of incorporated elements, the final fate of released nutrients must deserve our attention (Elser and Urabe 1999). For instance,

Elser and Foster (1998) have observed in a survey of 12 temperate lakes that the N:P ratio of sedimented particles was inversely related to the N:P ratio of the zooplankton community. This illustrated the importance of nutrients egested by zooplankton on sedimentation fluxes. It also demonstrated the stoichiometric influence of zooplankton on nutrient biogeochemical cycles. This question is also addressed in the present thesis (Chap. 6) with special focus on the global effect of *Daphnia* on total P flux.

The precedent perspectives only deal with N and P, but from an ecological and biogeochemical point of view, the fate of C ingested in excess is also totally crucial (Sterner and Hessen 1994). At the moment where special focus is addressed in the study of C cycle and its impact on global change, the stoichiometric approach of trophic relationships also leads to meaningful effects on the C cycle (Falkowski et al. 2000, Sterner and Elser 2002). Nutrient deficiency of the food results in the ingestion by the consumer of C in excess relative to its demand. The disposal of this excess C induces by definition a reduction of carbon growth efficiency and a lower energy transfer in food webs (Hessen and Faafeng 2000). The mechanisms used by herbivores for disposal of excess C may have important ecological and biogeochemical implications. Here we report an experimental study on the fate of C ingested in excess by a primary consumer feeding on low quality (high C:P ratio) food (Chap. 5).

OVERVIEW

The thesis is divided in 2 parts. Part A (containing Chap. 2–5) groups together my personal contributions to the understanding of food quality effects on *Daphnia* growth. Testing the ability of *Daphnia* to adapt to low food quality specially attracted my attention. Four papers were produced from my work. First, in Chap. 2, the reader will discover a new deterministic model predicting both optimal foraging and digestive investment of *Daphnia* in function of food quality and food quantity (Darchambeau submitted). The behavioral prediction of the model will then be tested in Chap. 3 on a natural population of *Daphnia galeata*. There I have attempted to observe whether seston food quality can influence the filtration rate of the population in a way predicted by the model (Darchambeau and Thys submitted). Then, in Chap. 4, I have used literature data on *Daphnia* growth rate to explore to what extent *Daphnia* is able to exploit some regulatory processes reducing the growth penalty due to low food quality (Darchambeau to be submitted). Finally, through an experimental study, Chap. 5 explores the fate of C ingested proportionally in excess relative to P in *Daphnia magna* (Darchambeau et al. 2003).

In the second part of the manuscript, the reader will find new data and perspectives on the stoichiometry of consumer-driven nutrient recycling. In Chap. 6, I report an investigation on field seasonal surveys involving zooplankton composition and nutrient sedimentation rates to unravel the effects of herbivores on nutrient ratios in the sinking flux (Darchambeau et al. submitted). Special attention has been given to P flux and *Daphnia* nutrient drain.

The last Chapter of the manuscript summarizes what this thesis has permitted to learn in the increasingly fast-growing field of stoichiometry theory. Some perspectives are proposed.

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Chapter 2

Filtration and digestion responses of *Daphnia* to changes in food quality: a predictive model

In my research work, I have first developed a deterministic model of filtration, assimilation and digestion in Daphnia facing a dietary deficiency in phosphorus. This model is presented in the present Chapter. This explicitly stoichiometric model will allow us to test the behavioural and physiological responses of Daphnia that make it possible to cope with different food quality and quantity. These predictions will subsequently be confronted to experimental and field evidence.

Filtration and digestion responses of *Daphnia* to changes in food quality: a predictive model

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ABSTRACT

In the study of the stoichiometric relationship between autotrophs and herbivores, attention was largely focused on effects of the encountered mismatch between needs and supplies on herbivore growth and ecosystem process. Rarely, herbivore adaptation to bad food quality was investigated. As a first step, this study presents a predictive model of feeding, assimilation and digestion of *Daphnia* facing dietary deficiency in phosphorus. Biochemical compounds of the food were divided into phosphorous and non-phosphorus compounds. It was assumed that *Daphnia* is able to differently assimilate both compounds by regulation of target-specific digestive enzymes. Feeding rate was regulated by optimal gut residence time of food particles, and assimilation efficiency by gut residence time and optimal secretion of both digestive enzymes. In case of food P-deficiency, the model predicted increased *Daphnia* filtration rate compensated by increased secretion of both digestive enzymes. Effects of food quality on filtration and growth rates were independent of food biomass. Carbon assimilation efficiency was reduced with increased P-deficiency while effects on P assimilation efficiency was dependent upon food biomass. The sensitivity analysis showed that proportion of biochemical compounds into the food, with algal carbon content and costs of filtration, were the most influencing parameters of the model, and therefore need accurate estimations. The present study also emphasizes the role of all the metabolic feeding costs (filtration, production of digestive enzymes, and digestion costs) in the feeding process. In conclusion, the model confirms that carbon ingested in excess may generate more energy that can be used to obtain more phosphorus by increased feeding rate.

INTRODUCTION

"The study of zooplankton nutrition has been extremely active of late" (Sterner and Schulz 1998, and review therein). Research has been recently focusing on stoichiometry of the trophic relationship between autotrophs and herbivorous consumers, leading to a new discipline, the ecological stoichiometry (Sterner and Elser 2002). This conceptual framework emerged from the evidence of homeostatic regulation of elemental contents of many herbivorous zooplankters (Hessen 1990, Hessen and Lyche 1991, Andersen and Hessen 1991, Sterner and Elser 2002). This elemental homeostasis of herbivorous contrasts with observed variability in algae nutrient content (Hessen and Andersen 1992, Sterner and Hessen 1994). The mismatch encountered by zooplankters between their elemental needs and supplies provided by planktonic algae could lead to reduced somatic and population growths of herbivores (Hessen 1997, Sterner et al. 1998, Hessen and Faafeng 2000). This is particularly true for zooplankters of the genus *Daphnia* which maintain high body proportion of phosphorus (P) whilst this element often limits phytoplankton growth in temperate lakes.

Some compensatory mechanisms to cope with this mismatch have been proposed and studied for *Daphnia* feeding on diets with varying C:P-ratio. Very low P release rates when *Daphnia* were fed on P-limited algae were reported (Lehman 1980, Olsen and Østgaard 1985, Van Donk et al. 1993). Also respiration and extracellular excretion of C compounds have been demonstrated as a means to dispose of C assimilated in excess from the food (Darchambeau et al. 2003). Moreover, assimilation from food of the deficient element may be improved. But DeMott et al. (1998) observed that both C and P assimilation efficiencies of *Daphnia* fed with P-deficient algae were reduced. Note that the reduction of P assimilation efficiency was yet lower than the one for C, leading to a more balanced C:P ratio of incorporated elements relative to the grazer demands.

Assimilation of elements from food is controlled by digestive enzymes secreted through the gut wall and by residence time into the gut of food particles. C and P are available from ingested biochemical compounds, such as proteins, lipids or carbohydrates, and are assimilated in these forms or even already slightly hydrolysed, but not in the elemental form (if we except the known ability of *Daphnia* to incorporate inorganic P directly into its body from the surrounding water when that P is in very high concentration in the water, see e.g. Parker and Olsen 1966). Some biochemical compounds are richer in P than others. In plants, P is found under the form of polyphosphate (Pi) chains (included in polyphosphate bodies), in nucleic acids (RNA and DNA), in P-lipids (essentially included in membranes, with e.g. lecithin) and in P-esters (glucose 6-phosphate, ATP, etc). They are

present in various proportions, with polyphosphates, RNA and, for a short period, also ATP playing an important role in the storing of P (Melzer and Steinberg 1983). In young leaf, P is found in the proportion of Pi 10, RNA 2, DNA 0.15, P-lipid 1.5, P-ester 10 on a weight basis (Bieleski 1973 in Bieleski and Ferguson 1983). Some macromolecules, as proteins or carbohydrates of the cell wall (essentially cellulose, hemicellulose and pectin), do not contain any P. The hydrolysis of these compounds into the gut lumen is controlled by the secretion of specific digestive enzymes (carbohydrases, proteases, lipases, etc). So, by regulation of the secretion of some specific enzymes, animals can probably, at least partly, control the assimilation of some specific elements.

Beside enzymatic regulation, another mechanistic way to improve the assimilation efficiency of the limiting element is increasing the retention time of food particles in the gut. In filter feeders, this increase may be the result of decreased filtration rates. Some experiments have shown this response in the clearance rate of daphniids feeding on nutrient-deficient food (Butler et al. 1989, Gerritsen 1991, Sterner et al. 1993, Lürling and Van Donk 1997). But, except Gerritsen (1991), all ingestion or clearance rates were based upon decrease of algal density over time in presence of grazers. Now, some have observed an increased passage through the gut of intact algal cells due to increased cell-wall thickness of some nutrient-limited algae (Van Donk and Hessen 1993, 1995). Therefore, these experiments might not separate the grazing effect from algal disappearance rates. In some other experiments where feeding rates were measured by direct observations of ingestion of tracer food particles, nutrient-deficiency of algal cells had no effect on ingestion rates (Rothhaupt 1995, Van Donk et al. 1997, DeMott et al. 1998, Hessen et al. 2002, Darchambeau et al. 2003).

By contrast, it has also been argued that increased filtration activity must lead to higher energy (thus C) expenditures, reducing the C:P ratio of net incorporated materials (Plath and Boersma 2001). A behavioural response of animals to dietary nutrient-deficiency thus might be to increase ingestion rate. Plath and Boersma (2001) have indeed shown that the beat rate of the feeding appendages of *D. magna* increased when food became P-deficient. But, as soon as the gut is filled, a further increase in ingestion rate should result in lower gut residence time, and decreased assimilation efficiencies of all elements (Willows 1992). As less elements are assimilated per unit of time, it means also that less C are in excess and can be used to generate more energy for foraging.

Thus, following the reasoning above, there could be contrasting foraging responses to cope with different food quality and quantity. Accurate evaluations of these processes and of their links seem useful to predict the

direction of the filtration and assimilation responses for animals facing nutrient-deficient food. To my knowledge, there has been no attempt to develop a model of the effects of food quality [defined as the ratio between 2 essential elements] simultaneously on ingestion, assimilation and growth of the consumer. Therefore, I apply a modified version of the Willow's model (Willows 1992) of feeding behaviour and physiology of filter-feeders to the case of stoichiometrically constrained homeostatic growth.

DESCRIPTION OF THE MODEL

The initial model of Willows (1992) was designed for observing optimal behavioural (i.e. the filtration rate or gut residence time) and physiological (i.e. the production rate of digestive enzymes) responses of *Mytilus edulis* facing off variable dietary biomass and composition. I have adapted, applied and parameterised the model for *Daphnia magna* facing off variable food biomass and qualities (in terms of C:P ratios). Homeostatic growth constrained the optimal responses. Note that all symbols and units are described in Table 2.1.

Willows (1992) assumed that the volume of food particles may be reduced as they are digested and absorbed in the gut. The evolution over time of particle volume in the gut can thus be defined as a function of gut residence time, T, and production rate of digestive enzymes, S. This function is of the same functional form as the absorption of element (Taghon et al. 1978), but, as Willows (1992), I assume that the reduction of particle volume can occur at a rate, μ, lower than the one for elemental absorption, β (i.e. $\mu \leq \alpha = \beta$). The time evolution of particle volume in the gut is thus given by (Willows 1992)

$$V(S,t) = V_{if} + (V_{i0} - V_{if}) \exp(-\mu S t^2), \quad (1)$$

where V_{i0} and V_{if} are, respectively, the initial (undigested) volume of algal cell and the minimal potential (egested) volume of algal cell, and t is time. Hence,

$$\int_{t=0}^{t=T} V(S,t)dt \quad (2)$$

represents the amount of digestive capacity utilized by a food particle (in ml h). If V_g is the volume capacity of the gut, then the filtration rate F is derived as

$$F = \frac{V_g}{D\int V(S,t)dt}, \quad (3)$$

where D is the density of food particles in the water.

Table 2.1: Explanations of symboils used in the feeding homeostatic model, with values of parameters.

Optimized parameters		
S_N	Production rate of digestive enzymes hydrolyzing non-P compounds, µg atomic C h^{-1}	
S_P	Production rate of digestive enzymes hydrolyzing P compounds, µg atomic C h^{-1}	
T	Gut residence time, h	
Fixed parameters		
α	Rate parameter for elemental absorption from non-P compounds, [µg atomic C]$^{-1}$ h^{-1}	30.8
β	Rate parameter for elemental absorption from P compounds, [µg atomic C]$^{-1}$ h^{-1}	30.8
γ	Resorption rate parameter for digestive enzymes, h^{-1}	2.30
μ	Rate parameter for volume reduction of algal cell, [µg atomic C]$^{-1}$ h^{-1}	23.1
Q_T	Total carbon content of algal cell, µg atomic C $cell^{-1}$	8.03E-8
ρ	Fraction out total food C of refractory C (no units)	0.20
ν	Fraction out total food C of C into non-P compounds (no units)	0.20
π	Fraction out total food C of C into potentially P compounds (no units)	0.60
θ_Z	Consumer C:P ratio, µg atomic C [µg atomic P]$^{-1}$	100
V_g	Volume capacity of the gut, ml	8.26E-6
V_{i0}	Initial (undigested) volume of algal cell, ml	4.19E-12
V_{if}	Min potential (egested) volume of algal cell, ml	1.05E-12
g_1	Filtration cost coefficient, µg atomic C h ml^{-2}	4.60E-3
g_2	Cost coefficient linked to the production of digestive enzymes	0.2
g_3	Digestion/absorption cost coefficient	0.2

Variables		
D	Concentration of algal cells in the water, cells ml^{-1}	2.07E5 – 51.8E5
θ_T	Total C:P ratio of algal cell, µg atomic C [µg atomic P]$^{-1}$	20 – 800
P_T	Total P content of algal cell, µg atomic P cell^{-1}	$= Q_T / \theta_T$
Q_R	C content of refractory fraction, µg atomic C cell^{-1}	$= \rho Q_T$
Q_N	C content of non-P compounds, µg atomic C cell^{-1}	$= \nu Q_T$
Q_P	C content of P compounds, µg atomic C cell^{-1}	$= \pi Q_T$
Rates		
F	Filtration rate, ml h^{-1}	
L_C	Rate of C loss by egestion of digestive enzymes, µg atomic C h^{-1}	
B_C	Gross rate of absorption of C from feeding activity, µg atomic C h^{-1}	
R_C	Net rate of absorption of C from feeding activity, µg atomic C h^{-1}	
R_P	Net rate of absorption of P from feeding activity, µg atomic P h^{-1}	
D_C	Net rate of metabolic feeding cost, µg atomic C h^{-1}	
G_C	Net rate of C growth, µg atomic C h^{-1}	
G_P	Net rate of P growth, µg atomic P h^{-1}	

Carbon (C) and P are combined in various compounds within the bodies of organisms. I thus distinguish food biochemical compounds into non-phosphorous and P-containing compounds. Assimilation over the gut wall of P compounds provides C and P to the animal, while assimilation of non-P compounds brings C and no P. But considering that animals do not produce digestive enzymes specific to P compounds but more generally specific to large groups of biochemical compounds (e.g. production of lipase, protease, etc), I regroup in P compounds all protoplasmic compounds which may contain P (carbohydrates, lipids, nucleic acids) and in the non-P compounds we find just proteins. I also distinguish the molecules who are never or uneasily assimilated and defined as refractory: essentially the carbohydrates of the cell wall which does not contain any P. Thus, total C of the food is divided into 3 fractions:

$$Q_T = Q_N + Q_P + Q_R \,. \tag{4}$$

Let us define ρ as the fraction of the refractory C, ν as the fraction out total C of C into non-P compounds, π as the fraction out total C of C into potentially P compounds, and θ_T as the total C:P ratio of food ($\theta_T = Q_T / P_T$). I assume that ρ, ν and π are independent of food quality, and that the variation of θ_T reflects the variation in the richness of P in P compounds.

Thus, I use a model that allows absorption of C through the assimilation of Q_N and/or of Q_P. As I divide biochemical compounds into 2 digestible fractions, I also divide the digestive enzymes into enzymes appropriate to the digestion of P compounds (lipase, esterase, etc), with their own production rate S_N, and enzymes specific to the digestion of non-P compounds (essentially protease), with their own production rate S_P. The gross absorption rate of C from ingested food, B_C, increases with both the production rates of digestive enzymes (S_N and/or S_P) and gut residence time T:

$$B_C = FCQ_N\left[1 - \exp\left(-\alpha S_N T^2\right)\right] + FCQ_P\left[1 - \exp\left(-\beta S_P T^2\right)\right], \tag{5}$$

where α and β are the rate parameter for C absorption from, respectively, non-P and P compounds.

Part of the digestive enzymes may be egested with non-assimilated food. The resorption by the gut wall of the secreted digestive enzymes themselves depends on gut residence time and may occur at its own rate γ. The rate of C loss through egestion of both digestive enzymes which are not reabsorbed during passage through the gut is thus given by

$$L_C = \left(S_N + S_P\right)\exp\left(-\gamma T\right), \tag{6}$$

where γ is the resorption rate parameter for both digestive enzymes. This loss of C is a first cost of the feeding process. The net rate R_C at which C is truly absorbed is thus equal to the difference between B_C and L_C:

$$R_C = B_C - L_C \,. \tag{7}$$

Assimilation of P is achieved only by absorption of P compounds. As in these compounds, C and P are linked, the rate of P assimilation is equivalent to the one for C from P compounds. But, as no amino acids contain P, I can postulate that the digestive enzymes are not constituted by P and that consequently there is no loss of P through the egestion of enzymes. Thus, the net rate at which P is absorbed (R_P) is easily described by:

$$R_P = FCP_T \left[1 - \exp\left(-\beta S_P T^2\right)\right]. \quad (8)$$

Note that $[1 - \exp(-\beta S_P T^2)]$ represents the assimilation efficiency of P compounds (A_P). This is also by definition equivalent to the P assimilation efficiency. $[1 - \exp(-\beta S_N T^2)]$ represents the assimilation efficiency of non-P compounds (A_N). The C assimilation efficiency (A_C) can be calculated by the weighted average between A_P and A_N:

$$A_C = \nu A_N + \pi A_P . \quad (9)$$

However, the net C absorption efficiency, i.e. the net balance of C absorbed by the feeding process, is given by R_C / FCQ_T.

As I have divided the production rate of digestive enzymes S into S_N and S_P, I need to redefine S in Eq. 1 as the weighted average between S_N and S_P:

$$S = \nu S_N + \pi S_P . \quad (10)$$

In the model, metabolic costs of feeding are divided into three parts. First, there are the metabolic costs associated with beating of filtering appendages. In agreement with Lehman (1976), I assume that they are proportional to the square of the filtration rate. Second, there is a cost directly proportional to the production rate of digestive enzymes. Third, I assume a cost proportional to the amount of digestion and absorption that occurs, including components due to both food and reabsorption of the digestive enzymes. The total metabolic feeding costs (D_C), excluding that part of the digestive enzymes that is not resorbed, are then given by

$$D_C = g_1 F^2 + g_2\left(S_N + S_P\right) + g_3 R_C , \quad (11)$$

where $g_{1,2,3}$ are the appropriate cost coefficients (see Table 2.1). The net rate at which the animal gains C as a result of its feeding activity (G_C) is then simply given by

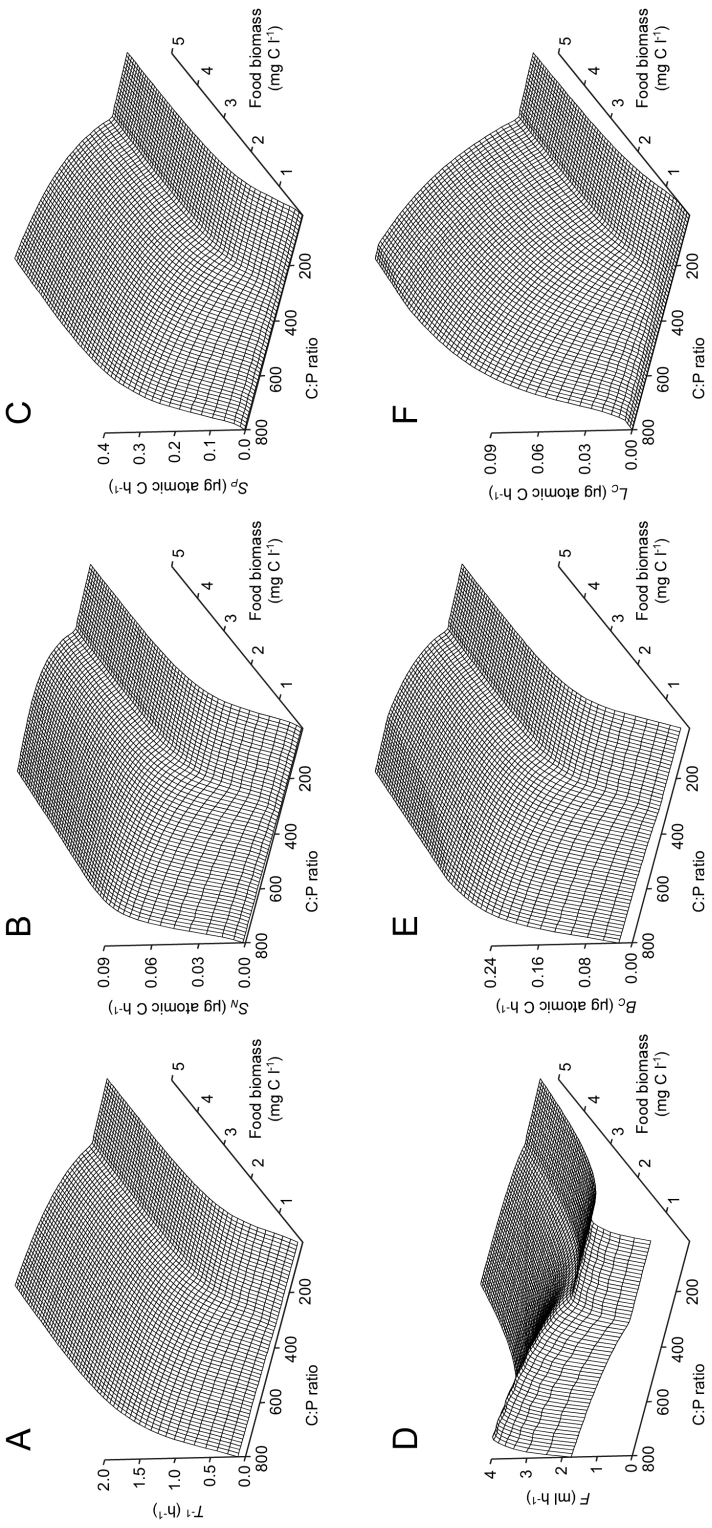
$$G_C = R_C - D_C , \quad (12)$$

whilst for P I assume no metabolic costs, leading to the definition of P gain rate (G_P):

$$G_P = R_P . \quad (13)$$

In my model, homeostatic growth of animals constrains the net rate of C growth :

$$G_C \le G_P \theta_Z , \quad (14)$$


A
B
C
D
E
F
C:P ratio
Food biomass (mg C l-1)
T-1 (h-1)
SN (µg atomic C h-1)
SP (µg atomic C h-1)
F (ml h-1)
BC (µg atomic C h-1)
LC (µg atomic C h-1)

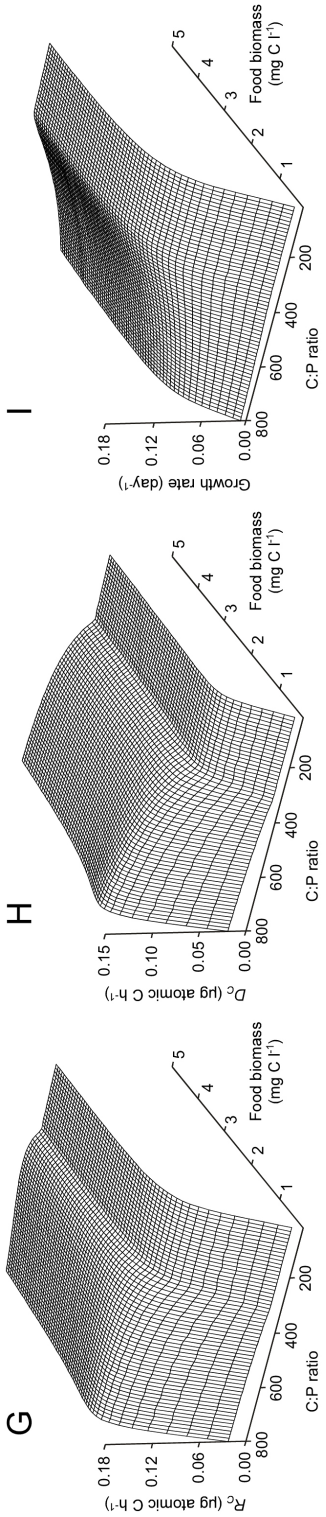


Fig. 2.1: Modelled effect of food biomass and food quality (C:P ratios) on (A) optimal gut residence time (note that T^{-1} is plotted instead of T), (B) optimal production rate of digestive enzymes for non-P compounds S_N, (C) optimal production rate of digestive enzymes for P compounds S_P, (D) resulting filtration rate F, (E) gross rate of C absorption B_C, (F) egestion rate of digestive enzymes L_C, (G) net rate of C absorption R_C, (H) total metabolic feeding cost D_C, and (I) growth rate (calculated by dividing the net rate of C growth G_C by body C mass) of an adult *Daphnia magna*. All values of parameters are presented in Table 2.1 and discussed in Appendix.

where θ_Z is the body atomic C:P ratio of the herbivore. The gain in P may be proportionally higher than the one for C, because excess P will be simply excreted with a C cost already taken into account into the metabolisation of R_C.

The optimization problem is to find values for the gut residence time, T, and both production rates of digestive enzymes, S_N and S_P, which maximize G_C in respect of the homeostatic constraint defined in Equ. 14. Optimization was realized for each set of parameters with DONLP2 (Spellucci 1998), an algorithm implemented for nonlinearly, constrained optimization problems and accessible via the NEOS Server (Gropp and Moré 1997, Czyzyk et al. 1998). Parameterisation was made for an adult *Daphnia magna* of 150 µg C (total body C) feeding on small *Chlorella* cells. Details of parameter estimation are given in Appendix. Optimizations were made for ranges of food biomass from 0.2 to 5 mg C l^{-1} and food C:P ratios from 20 to 800.

To see how the outcome of the model may vary over the range of plausible parameter values, I performed a sensitivity analysis. I perturbed each parameter to its lowest and highest plausible expected value in turn (see Appendix), recording the response of the model, whilst holding all other parameters constant at their most likely point estimates figured in Table 2.1. The analysis was performed for each perturbation at 0.2, 0.6, 1 and 5 mg C l^{-1}, each with a food C:P ratio of 100 and 800. I computed mean squared relative error of C gain rate for each perturbation [Σ(residual/expected value)² divided by 8]. I also recorded the effect of food quality on filtration rate and C and P assimilation efficiencies.

RESULTS

The optimal gut residence time (Fig. 2.1A) is inversely proportional to both optimal production rates of digestive enzymes (Figs. 2.1B and C). When gut residence time is low, the production of both digestive enzymes is predicted high. The resulting optimal filtration rate depends both on food quantity and quality (Fig. 2.1D). Along the food biomass gradient, the filtration rate first increases to a maximum reached near 0.5 mg C l^{-1} (by definition, it is the incipient limiting level, ILL), and then slowly decreases to an asymptotic value around 0.54–0.86 ml h^{-1}, depending on food quality. The filtration rate is stable for food quality from 20 to 260-300 (Figs. 2.1D and 2.2), and largely increases when food becomes more P-deficient (C:P > 260-300). Note that the C:P value upper which filtration rate increases is depending on food biomass (Fig. 2.2). However, the overall relative effect of food quality on filtration rate is predicted uninfluenced by food quantity.

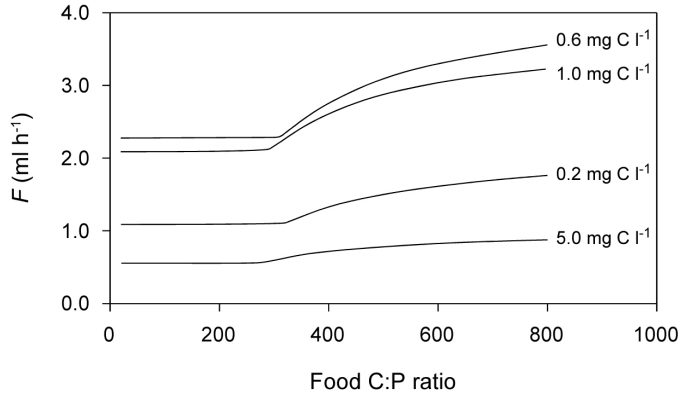


Fig. 2.2: Predicted filtration rate, *F*, as a function of food biomass and food C:P ratio.

The outcome of the predicted greater ingestion of algae when food becomes P-deficient is an increasing gross absorption of C along the C:P gradient (Fig. 2.1E). The predicted higher production of digestive enzymes associated with the reduction of gut residence time induces a higher egestion of digestive enzymes (Fig. 2.1F) resulting in a reduced benefit in the net C absorption (Fig. 2.1G).

Total metabolic feeding cost, i.e. the filtration cost, plus the cost associated to production of digestive enzymes, plus the digestion cost, is predicted to largely increase with food C:P ratio (Fig. 2.1H). The result of the change of net C absorption and total feeding cost is that the growth rate is maximal when food is abundant and with a C:P ratio below 260-340, depending on food biomass (Fig. 2.1I). Interestingly, the effect of food quality on growth rate is present for the whole gradient of food biomass (Fig. 2.3).

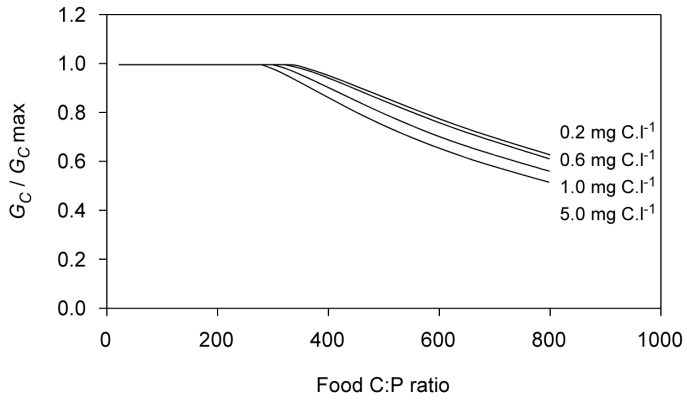


Fig. 2.3: Predicted relative growth rate, G_C / G_C max, as a function of food biomass and food C:P ratio.

The optimization of S_N, S_P and T in function of food quantity and quality is constrained by the homeostatic growth. The higher total feeding C cost (Fig. 2.1H) plus the higher loss of C through egestion of digestive enzymes (Fig. 2.1F), both predicted with the increase of food C:P ratio, maintain an homeostatic but reduced growth (Fig. 2.1I). These predicted rises in C costs are essentially linked, at high food biomass, to the increase of costs due to the digestive enzymes: the production cost of their production and the egestion of a part of them (Fig. 2.4A). On the other hand, at biomass near the ILL, it is the filtration cost which is mainly responsible of total costs and of its rise with the increase of food C:P ratio (Fig. 2.4B).

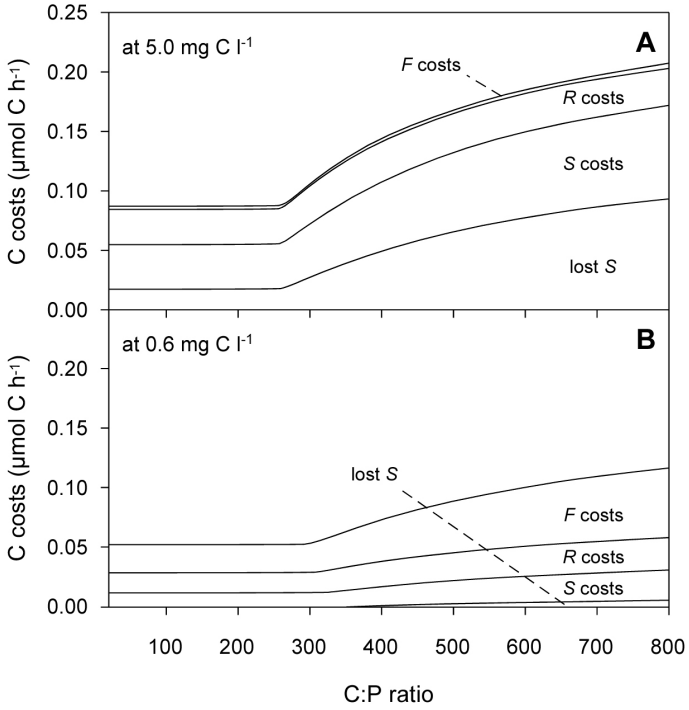


Fig. 2.4: Metabolic feeding costs partitioned between those due to the filtration rate (F costs, g_1F^2), the production of digestive enzymes (S costs, g_2S), and the absorption and digestion (R costs, g_3R_C), plus the egestion of digestive enzymes (lost S, $S\exp(-\gamma T)$). (A) for food biomass of 5 mg C l^{-1}. (B) for food biomass of 0.6 mg C l^{-1}.

The evolution of P assimilation efficiency in function of C:P ratio is predicted depending on food biomass (Fig. 2.5A). In all cases, P assimilation efficiencies are high. They are predicted increasing with C:P ratio for low food biomass and decreasing when food is abundant. Note nevertheless that

the influence of C:P ratio on P assimilation efficiency seems very low. However, C assimilation efficiency is always predicted decreasing with the rise of C:P ratio (Fig. 2.5B), and more influenced by C:P ratio than is P assimilation efficiency. These tendencies are reinforced in the case of the net C absorption efficiency because this efficiency takes into account the increased egestion of digestive enzymes with the rise in C:P ratio (Fig. 2.5C).

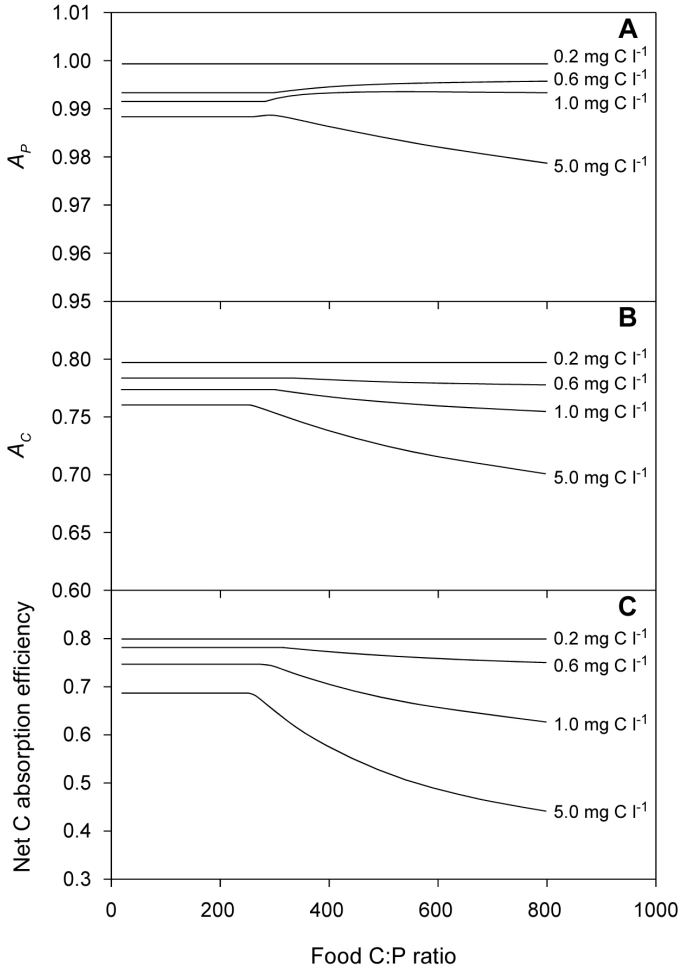


Fig. 2.5: Assimilation efficiency of P, A_P (A), and C, A_C (B), as a function of food biomass and food C:P ratio. Net C absorption efficiency (C). Note the differences in the scale of the axis.

My model assumes that *Daphnia* can take advantage simultaneously of both responses, the behavioural one (optimization of the gut residence time) and the physiological one (regulation of the production of digestive enzymes). We may suppose that there is a time-lag between both responses: the behavioural one is probably quicker than the physiological one. I may simulate the effect of this unique, first response of a *Daphnia* submitted to a change in food quality by maintaining constant both production rates of digestive enzymes (S_N and S_P). It may correspond to a short-term change to compare to a long-term acclimation illustrated previously. In Fig. 2.6, we observe the predicted filtration rate of a *Daphnia* acclimated to a food C:P ratio of 100 and then feeding on algae with higher C:P ratios. Only gut residence time is optimized and values of S_N and S_P maintain at the same levels as for a C:P ratio of 100. The short-term response of filtration rate to dietary P-deficiency is going to the same direction than the long-term response but reduced: the filtration rate increases with C:P ratio but more slowly, except at high food biomass (Fig. 2.6A). However, P and C assimilation efficiencies are much more reduced than for the long-term adaptation, i.e. when S_N and S_P are also optimized (Figs. 2.6B and C). These largely decreased assimilation efficiencies induce growth rate much more reduced than when S_N and S_P are also optimized (Fig. 2.6D).

The sensitivity analysis indicates parameters which largely influence the output of the model (Table 2.2). Net C gain rate seems largely robust to perturbations of most parameters. The model is highly insensitive to perturbations of α, β, μ and γ, 4 parameters for which I do not have very accurate estimates (see Appendix). Logically, the C gain rate is directly influenced by the C content of algae, Q_T, and by its digestibility (ρ:ν:π). C cost of filtration rate, g_1, also needs accurate estimation. More surprisingly, the C:P ratio of consumer, θ_Z, determines significantly its growth rate. The higher is the demand in P by the consumer, the lower is its growth rate in case of P-deficiency. It is interesting to note that, in the 104 simulations (13 parameters x 2 extreme values by parameter x 4 food biomass) of effect of food quality on grazer rates that this sensitivity analysis brings us, only once the filtration rate was decreased by food P deficiency. This observation was made when g_1 was put to its lowest value and when food biomass was low (0.2 mg C l^{-1}). In all the 103 other cases, filtration was increased by food P deficiency. The sensitivity analysis also showed that P assimilation efficiency was increased (although always very little) by lowered food quality in 74 cases on 104 simulations. By opposite, only once C assimilation efficiency increased if food quality was lowered.

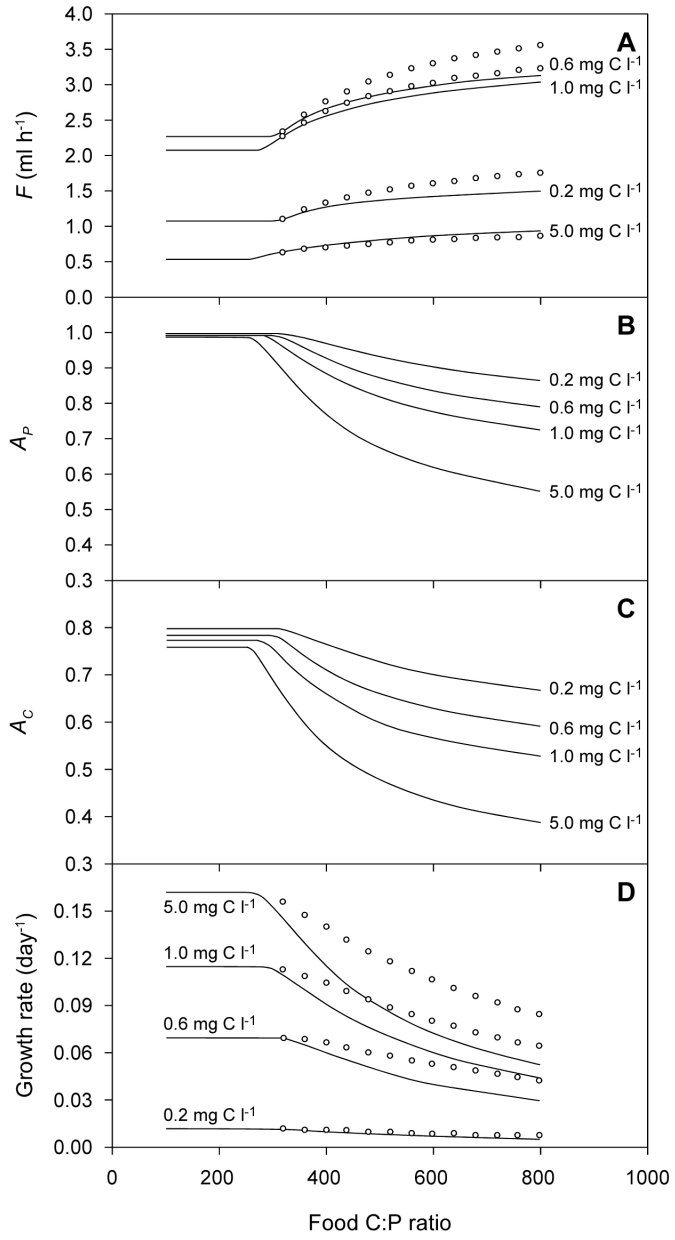


Fig. 2.6: Predicted responses to short-term change to C:P ratio. Values of S_N and S_P are maintained to the ones predicted for a food C:P ratio of 100. Only gut residence time is optimized in function of C:P ratio. (A) Filtration rate, F. (B) P assimilation efficiency, A_P. (C) C assimilation efficiency, A_C. (D) Growth rate. In A and D, respectively filtration and growth rates obtained by the optimization of T, S_N and S_P are redrawn (open circles) for comparison.

Table 2.2: Sensitivity analysis of the model. Each parameter was perturbed to its lowest and highest plausible expected value in turn whilst holding all other parameters at their estimates of Table 2.1. 4 food biomass were tested (0.2, 0.6, 1 and 5 mg C l^{-1}) for 2 food C:P ratio (100 and 800). The mean squared relative error of C gain rate (*MSRE*) was computed for each perturbation. High value indicates that the model is sensitive to the parameter.

	Perturbation to the lowest value of parameter		Perturbation to the lowest value of parameter	
Parameters	Value of parameter	*MSRE*	Value of parameter	*MSRE*
Algal length (µm) [a]	1	0.019	10	0.068
Q_T (µg atomic C $cell^{-1}$)	3.75E-8	**0.304**	12.7E-8	**0.375**
$\rho:\nu:\pi$ (in % of total C)	5:20:75	**0.129**	30:30:40	0.053
V_{if} as a % of V_{i0} [b]	10 %	0.008	40 %	0.000
μ as a % of β [c]	25 %	0.001	100 %	0.000
V_g (ml)	6.19E-6	0.067	10.3E-6	0.068
α ([µg atomic C]$^{-1}$ h^{-1})	23.1	0.001	38.6	0.000
β ([µg atomic C]$^{-1}$ h^{-1})	23.1	0.000	38.6	0.000
γ(h^{-1})	1.73	0.005	2.88	0.003
g_1 (µg atomic C h ml^{-2})	2.66E-3	**0.149**	10.2E-3	**0.138**
g_2	0.16	0.004	0.24	0.003
g_3	0.16	0.005	0.24	0.005
θ_z (µg atomic C [µg atomic P]$^{-1}$)	90	0.003	170	**0.104**

Notes: (a) algal length is not a direct parameter of the model, but it affects V_{i0}, V_{if} and Q_T (see Appendix), (b) perturbation of V_{if} also affects V_g, (c) perturbation of μ also affects V_g.

DISCUSSION

My aim was to build up a realistic model of *Daphnia* feeding, able e.g. to correctly represent the well-known kinetics of filtration rate in function of food biomass. The Willows's model (Willows 1992) showed this property because based upon volume of the gut, volume of food particles and gut residence time of particles. My improvements of this model were designed to predict influence of both food quantity and quality on a filter feeder with an homeostatic elemental content. Two potential responses are attributed to a *Daphnia* dealing with P-deficient algae: regulation of its filtration rate, and improvements of the assimilation of elements from food, particularly of the deficient nutrient. As food elements are combined into different biochemical fractions, elements can not be assimilated alone. Following the original idea of Anderson and Hessen (1995) for copepods with C and nitrogen, I have separated food biochemical components into P and non-P compounds. However contrary to Anderson and Hessen (1995) for the C:N ratio, I have considered that the C:P ratio of P compounds is not fixed, and that the total C:P ratio reflects the richness in P of P compounds. I postulate that these 2 fractions can be assimilated separately from each other. Indeed, in plants, P is essentially found in nucleic acids (RNA and DNA), in P-lipids (e.g. membrane phosphoglycerides), in some esters (e.g. glucose 6-phosphate, ATP) and sometimes abundantly in polyphosphate bodies (Bieleski and Ferguson 1983). We may assume that the secretion into the gut of some digestive enzymes specific to P compounds (some carbohydrases, esterases, lipases) can be regulated by animals. Other digestive enzymes (e.g. proteases) specifically deal with non-P compounds. The model thus allows to distinguish regulation of these 2 categories of digestive enzymes, inducing separate assimilation of P compounds and non-P compounds. The C assimilation results from the assimilation of both compounds while P only comes from assimilation of P compounds. Also, always with the aim to be realistic, I separate a non-digestible fraction, which contains C refractory to the digestion. It may e.g. realistically correspond to the cell wall rich in carbohydrates.

The final output of the model is the gain of P and C by the animal. Feeding and digestive costs are added for C in order to obtain the net gain of C. No analytical solution was performed for this complex, non-linear model. Homeostatic growth constrains the 3 non-fixed parameters: gut residence time, production rate of digestive enzymes for P compounds, and production rate of digestive enzymes for non-P compounds. The 3 parameters were optimized for each set of food characteristics (quantity and quality) by an SQP algorithm implemented by DONLP2 (Spellucci 1998). As I did not only want to predict the direction of regulations used by the consumer, but also to observe their respective magnitude in a realistic range of food

conditions, I have tried to use estimates from the literature for fixed parameters. Thus, realistic assumptions about the feeding process and parameterization should produce a feeding model giving correct prediction.

Two situations were tested: one corresponding to a long-term acclimation of changes in food characteristics (gut residence time and both production rates of digestive enzymes can be optimized) and the other to a short-term where just gut residence time is able to be adjusted (both production rates of digestive enzymes are maintained constant).

In long-term acclimation, i.e. when gut residence time and production of digestive enzymes for non-P and P compounds absorption are optimized as a function of food characteristics, my model predicts a double response of daphniids to food nutrient deficiency: a reduction of gut residence time (leading to an increase of filtration and ingestion rates) and a simultaneous increase of the production of digestive enzymes (see Figs. 2.1A-D). The rise of feeding activity seems a common response of animals to dietary nutrient-paucity (Plath and Boersma 2001). This phenomenon allows a greater ingestion of food in the same unit of time. The reduced gut residence time of particles is efficiently counteracted by a higher excretion of digestive enzymes. From a stoichiometrical point of view, the phenomenon may also be seen as a way to dispose of C assimilated in excess in accentuating the feeding process bringing to the consumer more of the deficient nutrient. This leads to a reduced but more equilibrated C:P ratio of incorporated elements. Nevertheless, both responses, even simultaneously present, do not avoid a significant reduction in growth rate of the consumer feeding on nutrient-deficient diet, whatever the food biomass (see Fig. 2.1I).

A high reduction in assimilation efficiencies is only predicted if production rates of digestive enzymes are not optimized (see Figs. 2.6B and C). This may correspond to a transient, first response of *Daphnia* to dietary P-deficiency. This prediction may adequately explain some observations made by DeMott et al. (1998). In one of their experiments, they submitted during 48 h juveniles of *D. magna* to a range of food C:P ratio from 80 to 900 at a biomass level of 0.5 mg C l^{-1}. Both C and P assimilation efficiencies (calculated over the 2-day period) were shown decreasing along the C:P ratio, with a higher decrease for C than for P. Their observations really mimic my results figured in Figs. 2.6B and C. Unfortunately, they did not observe, in the same experiment, any influence of food C:P ratio on weight-specific ingestion rate, maybe due to the relative high variance of some of their measurements. However, Plath and Boersma (2001) observed the feeding-appendage beat rates of *D. magna*. They found that the rate was not influenced by food quality along a C:P range of ~30 to ~250, but was largely

increasing when food became more and more P-deficient (C:P of 360 and 690). My model predicts the same change in filtration activity.

One important assumption of my model is that the assimilation of both elements (C and P) is controlled by specific digestive enzymes leading to differing assimilation efficiencies from ingested food for C and P. While I do not know if this may correspond to the reality, some experimental evidences point in this way. DeMott et al. (1998) observed simultaneously assimilation rates of C and P in *D. magna*. They found differences between the coefficients of assimilation efficiency going to 0.38 (e.g. 0.95 for P and at the same time only 0.57 for C). But, as already pointed out by Darchambeau et al. (2003), an important restraint must be considered for C assimilation efficiency in the study by DeMott and its colleagues, as they most probably measured a rate between the real total assimilation rate and net incorporation rate of C into biomass. Gulati et al. (2001) also observed a significantly higher assimilation efficiency for P than for C in *D. galeata* feeding on a cyanobacterium. Anderson (1994) with the data of Head (1992) calculated very large differences in the assimilation efficiencies of C, proteins, carbohydrates and lipids in herbivorous marine copepods. And finally Tang and Dam (1999), reinterpreting the data of 7 studies on assimilation in copepods, put in evidence that the assimilation efficiency of non-N compounds is indeed lower than the one of N compounds. So, differential assimilation of biochemical compounds seems to exist in herbivorous crustacean zooplankters. However, interestingly, at an upper trophic level, Lehman (1993) found no difference between C and P assimilation efficiencies in *Bythotrephes cederstroemi*, a cladoceran predator of invertebrates.

The model assumes that C and P can be assimilated in differing rates, but also that the consumer can regulate its production of digestive enzymes in function of food biomass and nutrient-richness. So far, this second assumption is confirmed by few experimental results. We know that copepods can control their total digestive enzymatic activity as a function of food biomass (Mayzaud and Poulet 1978, but see discussion in Mayzaud et al. 1998), but nothing is known about the same ability in cladocerans. I must underline here that the single observation of variable assimilation efficiencies as a function of food quality is not a proof of digestive enzyme regulation because assimilation is primarily under the control of gut residence time and therefore of ingestion rate. If the production of digestive enzymes and/or their activities can not be regulated as a response to food quality, the control of filtration rate alone is sufficient to achieve homeostatic growth. However, the resulting growth will still be lower than with enzymatic regulation (see Fig. 2.6D). The absence of enzymatic regulation should also induce decreasing C and P assimilation efficiencies at

high C:P ratios. This is a result of reduced gut residence time. As already suggested by Plath and Boersma (2001), it may help to understand why DeMott et al. (1998) observed a decrease in P-assimilation efficiency when daphniids were fed algae with a very high C:P ratio. This could also explain why daphniids keep on excreting P, even when severely P-limited (Olsen et al. 1986, DeMott et al. 1998).

Another important assumption made here is that a significant part of the digestive enzymes is lost via egestion. Although this process has been largely documented in mussels (Hawkins and Bayne 1985, Hawkins et al. 1986, 1990, Bayne et al. 1988), there is no evidence that it may occurs in daphniids. Probably due to the high fluidity of their faeces, it seems difficult to show the egestion of digestive enzymes in these animals. Note that Darchambeau et al. (2003) observed in *D. magna* high release of endogenous C-rich compounds. This excretion of endogenous material was significantly increased when animals were fed with P-deficient algae. Does this excretion correspond to the predicted higher egestion of digestive enzymes?

Although Plath and Boersma (2001) only incorporated costs due to the filtration in their model, they also suggested that additional energy costs may arise from a greater egestion of digestive enzymes out of the gut. My model takes into account 4 energy (C) costs linked to the feeding process. At low food biomass, mainly the high filtration rates are responsible of the rise of total metabolic cost observed when C:P ratio increases (Fig. 2.4B). This relative high cost of appendage beating (45 – 50 % of total costs, depending of food C:P ratio) is at the basis of the important reduction of filtration rate predicted by the model and experimentally observed in daphniids at low food biomass (e.g. Philippova and Postnov 1988). Inversely, at high food biomass, the predicted reduced gut residence time and increased production rates of digestive enzymes raise the contribution of digestive enzymes in the total metabolic cost. Thus, even if at high biomass the contribution of filtration cost in total metabolic cost is rather low (~ 1.5 % of total costs), the whole feeding process creates higher costs, maintaining the role of feeding in the dissipation of excess C. The link between high dietary C:P ratios and increased dissipation of C by respiration has been recently shown by Darchambeau et al. (2003). The present study emphasizes the role of all the metabolic feeding costs in the disposal of excess C.

Some predictions of the model have already been confirmed by experimental results. The reduced growth rates of *Daphnia* feeding on P-deficient algae, although firstly largely debated (see the syntheses of Gulati and DeMott 1997, and Sterner and Schulz 1998), was finally brilliantly demonstrated by Urabe et al. (1997). Darchambeau et al. (2003) explored the influence of food quality on *Daphnia* respiration rate. They confirmed an increase in

respiration rate in case of dietary P-deficiency. The reduction of both C and P assimilation efficiencies was observed in one study (DeMott et al. 1998) and needs confirmation, particularly for natural populations well acclimated to P-deficiency. Some other predictions are not yet supported by experimental data. Effects of food quality on digestive enzyme production and activity can be easily demonstrated. Differential responses as a function of enzyme substrates needs to be further studied. The study of the effect of food quality on filtration and ingestion rates is perturbated by the modification of cell-wall thickness observed in some nutrient-deficent algae (Van Donk and Hessen 1993, 1995). This complicates the discussion of results about long-term clearance rates, as studied e.g. by Sterner et al. (1993), Sterner and Smith (1993), Van Donk et al. (1997), Lürling and Van Donk (1997). Plath and Boersma (2001) clearly showed a positive effect of dietary P-deficiency on *Daphnia* feeding-appendage beat rates. But they did not distinguish in their experiment the direct effect of food quality from an indirect effect via the observed reduced growth and thus lower size of animals at the moment of beat rate measurements. Moreover feeding-appendage beat rates is not *stricto sensu* a measure of ingestion. Much particles may be captured but not ingested. I thus clearly support further research on effect of food quality on ingestion, particularly in natural *Daphnia* populations acclimated to P-deficiency.

In conclusion, my results emphasize the importance of P availability in resources for *Daphnia* success. The predicted increase of filtration rates when algal C:P increases, is an appropriate adaptive behavioural response to nutrient deficiency. The reduction of gut residence time implies lower absorption efficiencies of both elements, and especially C. Regulation of digestive enzymes may reduce the negative effect of food quality on growth rate. Note here that the higher turnover rate of P may counteract the P-limitation of algae, and improve the primary production of planktonic systems. If regulatory processes are confirmed *in vivo*, it claims for a re-exploration of herbivorous effects on nutrient cycling.

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APPENDIX: Parameterization of the model

Estimation of α and β

If I postulate a C assimilation efficiency of 70 % after a gut residence time of 30 min, we have that $\beta = \frac{-\ln(0.3)}{(30/60)^2 S}$ (see Equ. 1d of Willows 1992). If I assume that in mean S is expected to be in the same magnitude than G_C (see Fig. 2 of Willows *op.cit.*), we have that for an adult *D. magna* of 150 µg C with an expected growth rate of 0.3 day^{-1}, S = 45 µg C day^{-1} = 0.156 µg atomic C h^{-1}. Finally, β = 30.8 [µg atomic C]$^{-1}$ h^{-1}. I assume the same value for α. For the sensitivity analysis, I perturb α and β to – 25 % and + 25 % of their mean values.

Estimation of μ

The model allows to separately define the rate parameter μ for volume reduction of algal cell through the gut and the rate parameters α and β for material absorption. I consider here a scenario where μ = 0.75 α = 23.1 [µg atomic C]$^{-1}$ h^{-1}. For the sensitivity analysis, I consider that μ may vary from 25 % to 100 % of α.

Estimation of γ

I postulate that 10 % of the secreted digestive enzymes are not reabsorbed after a gut residence time of 60 min. Thus, $\gamma = \frac{-\ln(0.10)}{(60/60)} = 2.30$ h^{-1}. For the sensitivity analysis, I set γ to – 25 % and + 25 % of its mean value.

Estimation of V_g

McMahon (1970) estimated the gut residence time of an adult *D. magna* feeding on *Chlorella* cells above the ILL: T = 40 min. McMahon and Rigler (1965) founded the maximal ingestion rate of the same *Daphnia* feeding on the same algae: 0.017 mm³.h^{-1}. If μ = 23.1, S = 0.156, and V_{if} = 25 % of V_{i0}, I can easily obtain the volume capacity of the gut (V_g) by Equ. 8 of Willows (*op. cit.*): V_g = 8.26E-6 ml. For the sensitivity analysis, I set V_g to – 25 % and + 25 % of its mean value.

Estimation of V_{i0}

I use the mean volume corresponding to a spherical *Chlorella* cell of 2-µm diameter: V_{i0} = 4.19 µm³ = 4.19E-12 ml. For the sensitivity analysis, I consider an algae of 1 µm and 10 µm.

Estimation of V_{if}

I postulate that the volume of algal cell may maximally be reduced to 25 % of initial volume. As V_{i0} = 4.19 µm³, V_{if} = 1.05 µm³ = 1.05E-12 ml. For the sensitivity analysis, I consider that V_{if} may vary from 10 % to 40 % of V_{i0}.

Estimation of Q_T

Eppley et al. (1970) estimated conversion factors between volume and C content of some algae. I use the factors for a non-diatomous spherical alga of 2-µm diameter: Q_T = 8.03E-8 µg atomic C cell^{-1}. For the sensitivity analysis, I found in Gosselain et al. (2000) a list of equations joining C content with cell biovolume. I used the 2 equations giving respectively the lower and the higher estimation of C content for a biovolume of 4.19 µm³.

Estimation of ρ, ν and π

There are very few indications of C content in cell wall of algae. In higher plants, cell wall constitutes 0.5–2 % of fresh weight (Buchanan et al. 2000). If I consider a dry weight:fresh weight ratio of 10 % and that dry residuals are constituted at 50 % of C, we found that cell wall may contain from 10 to 40 % of total C. I thus define ρ as equal to 0.2. Fraction of proteins in total biochemical compounds is also highly variable, and constitutes in average near 20 % of total dry weight. I thus define ν as equal to 0.2, and by difference π is equal to 0.6. For the sensitivity analysis, I considered two contrasting situations about refractory C: ρ:ν:π ratios = 5:20:75 and 30:30:40.

Estimation of g_1

Bohrer and Lampert (1988) estimated simultaneously respiration and assimilation rates for adult *D. magna*. The intercept on the *y*-axis of their regression of respiration on assimilation rates when feeding below the ILL gives us the respiration rate due to only filtration and not due to filtration

plus assimilation and metabolization: 1.68 µg C mg^{-1} DW h^{-1} (see their Fig. 5). McMahon and Rigler (1965) estimated the maximal filtration rates for adult *D. magna* feeding on 11 different types of algae. The mean of these rates was equal to 2.9 ml ind^{-1} h^{-1}. Thus, if respiration rate due to filtration = g_1F^2, g_1 = 5.52E-2 µg C h ml^{-2} = 4.60E-3 µg atomic C h ml^{-2}. For the sensitivity analysis, I used the standard error of Bohrer and Lampert's estimation to calculate 95 % confidence interval lower and higher limits. Combined with the lowest and highest estimations of filtration rates given by McMahon and Rigler (1965), it gives me the 2 realistic most extremes values of g_1.

Estimation of g_3

This coefficient is equivalent to the part of assimilated C respired for its metabolization. This cost associated to the biochemical transformation of ingested food is called the "specific dynamic action" in the literature on zooplankton and fish budget (see e.g. Philippova and Postnov 1988). This coefficient corresponds to the slope of the regression of respiration on assimilation rates. I use the value found by Bohrer and Lampert (1988) for adult *D. magna*: g_3 = 0.20. Note that Urabe and Watanabe (1990) found a very equivalent coefficient for *D. galeata*: 0.196. For the sensitivity analysis, I set g_3 to 0.16 and 0.24.

Estimation of g_2

I use the same coefficient than for g_3: g_2 = 0.20.

Estimation of θ_Z

Although P content in *Daphnia* seems not so homeostatic as previously demonstrated and linked to the C:P ratio of ingested food (see e.g. Plath and Boersma 2001), I use a constant body C:P ratio of *D. magna* equal to 100 µg atomic C [µg atomic P]$^{-1}$. For the sensitivity analysis, I followed the 2 most extreme values of θ_Z observed by Plath and Boersma: 90 and 170.

Chapter 3

In situ filtration responses of *Daphnia galeata* to changes in food quality

In the previous Chapter, I have theoretically demonstrated that the increase of Daphnia ingestion rate can be an appropriate adaptative behavioural response to dietary phosphorus deficiency. In this Chapter, we tested this prediction against evidence obtained on a natural D. galeata population.

In situ filtration responses of *Daphnia galeata* to changes in food quality

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ABSTRACT

Optimal ingestion rate is regulated by a balance between benefits and costs. A controversy exists about how *Daphnia* species regulate their feeding when submitted to low-quality food (i.e. high dietary carbon:phosphorus ratio). In this study, we gathered data over 3 years on *in situ* filtration rates of a *D. galeata* population, by conducting grazing experiments. Observed filtration rates were correlated with population and environmental variables. Mean body length of *Daphnia* individuals was the best predictor of filtration rate (r^2 = 0.639), followed by water temperature and phosphorus concentration in the seston. When combined with body length, seston C:P or N:P ratios provided the best predictive regression model of filtration rate (r^2 = 0.852–0.897). The filtration rate was always correlated negatively with P concentration in the food. It means that *Daphnia* reacts to a decrease of food quality (i.e. higher C:P ratio) by an increase of its feeding rate. The Bayesian analysis of the model containing all significant variables showed that the filtration response to food quality is inhibited by high population density. We suggest that, when food quality is low and population density high, this inhibition may favour fitness of future conspecific generations. In conclusion, our results emphasize the role of P availability on transfer rate of organic matter between lacustrian autotrophs and herbivores, and question about effects on P biogeochemical cycle.

INTRODUCTION

Herbivorous consumption is a key process transferring organic matter and energy at the basis of the food web. The feeding process is mainly controlled by the herbivore, and its ingestion rate results from an optimisation between costs and benefits (Taghon 1981, Darchambeau submitted). Beyond this simplistic assertion, the definition of benefits hides. This is particularly true in the pelagic of freshwater systems, which does not appear always green to zooplankton (Sterner and Schulz 1998). When the pelagic world is not green enough, the collection of food particles is increased as long as new benefits are higher to supplementary costs. Thus the dependence of zooplankton filtration rate vs. food concentration fits to a unimodal curve (Lampert 1987). Less straightforward is the feeding response of consumers to food quality. For example, if the food quality is defined in terms of the equilibrium among resources, it implies that one resource may be ingested in excess while another may be deficient. In this case, what is the best response of ingestion rate to disequilibrated resources? If the collection of food particles is increased, the deficient element is ingested with a higher rate, but there is much more excess of the abundant resource, accentuating the problem of its disposal. By contrast, if the collection is reduced, less resources are ingested in excess but the consumer has still got less of the deficient element.

This discussion was the hidden core of numerous papers on effects of food quality on zooplankton ingestion (from Libourel Houde and Roman 1987 to Darchambeau et al. 2003). Food quality is there defined as the ratio between 2 useful elements, often carbon (C) and phosphorus (P) because these 2 important elemental resources may be encountered in misappropriate proportions into food of some zooplanktonic species, like *Daphnia* (Hessen and Lyche 1991, Hessen 1992). Attempts were made to predict the feeding response to food quality. Physiological models, dealing with the balance between benefits and costs, predict the increase of food ingestion with the rise of dietary C:P ratio (Plath and Boersma 2001, Darchambeau submitted). These predictions are contradicted by numerous experiments on *Daphnia* species in which a decrease (e.g. Sterner et al. 1993, Van Donk and Hessen 1993) or a stability (e.g. Rothhaupt 1995, Van Donk et al. 1997, Lürling and Van Donk 1997, DeMott et al. 1998, Hessen et al. 2002, Darchambeau et al. 2003) of the clearance rate is observed if food quality is lowered. To our knowledge, only the study of Plath and Boersma (2001) demonstrated a positive response of feeding-appendage beat rate of *Daphnia* to dietary nutrient deficiency. These *a priori* contradictory results need to be reconciled.

Some arguments may be found in the fact that all experiments did not follow the same experimental design. Several authors observed disappearance rates of algal cells in presence of consumers and translated the observed decrease in the number of cells into clearance rates (e.g. Sterner et al. 1993, Sterner and Smith 1993, Van Donk and Hessen 1993, 1995, Van Donk et al. 1997, Lürling and Van Donk 1997, Hessen et al. 2002). This method may lead to inconclusive results about grazing rates, as it was demonstrated that the passage through the consumer gut of intact viable cells is increased when algae are nutrient-deficient (Van Donk and Hessen 1993). Other studies have used labelled algae or beads, stopping ingestion of animals before egestion of the markers occurs (e.g. Butler et al. 1989, Van Donk et al. 1997, Lürling and Van Donk 1997, DeMott et al. 1998, Darchambeau et al. 2003) . In these experiments, except in DeMott et al. (1998), animals were never acclimated to food quality before grazing measurements. As the increase of filtration rate may result from a behavioural response (increased feeding-appendage beat rate, Plath and Boersma 2001) but also from a morphological one (larger filter screens and/or finer meshes, Lampert 1994), and as both responses may be facilitated by a higher secretion of digestive enzymes (Darchambeau submitted), it seems more appropriate to consider that these responses may occur only at mid- (some hours for the behavioural one) to long-term scales (some days for the morphological one) (see Rothhaupt and Lampert 1992). Note also that all these responses might be phenotypic but also genotypic, and, in natural populations, the success of different clones in the seasonal succession may result from their ability to adapt to nutrient deficiency.

All these considerations call for large studies on natural populations of *Daphnia* feeding on algae of varying quality. In a 3-year study, we conducted *in situ* grazing experiments to observe seasonal variations of filtration rate of a *Daphnia galeata* population from a reservoir. We analysed these variations as a function of seston elemental composition (C:N:P ratios) but also composition and biomass of phytoplankton, and other variables. As we focus on the effect of dietary nutrient deficiency, the variance of filtration rate has been carefully divided among all potentially explanatory variables by means of multiple regressions. Significant results were highlighted by a Bayesian analysis of the best regression model.

MATERIAL AND METHODS

Field data acquisition

This field study was conducted in the Esch-sur-Sûre reservoir which lies in the Northern part of the G.-D. of Luxembourg. According to the OECD

classification (1982), the reservoir is considered as a meso-eutrophic waterbody (Dohet and Hoffmann 1995). *Daphnia galeata*, *Bosmina coregoni* and *Diaphanosoma brachyurum* are the dominant cladocerans in the reservoir (Dohet and Hoffmann 1995). The seasonal survey was conducted at a station (maximum depth 30 m) located in the middle of the lake, representative of whole lake conditions (Thys et al. 1998).

Zooplankton density was measured in parallel with seston analysis from January 1998 to December 2000. Samplings were conducted weekly from April to October and monthly during winter. Stratification layers were delimited according to the limnological profiles obtained using a Hydrolab DS-4 multiprobe. The zooplankton community was sampled with a 50-cm diameter, 50-µm mesh net towed in the epilimnion during the stratification period and in the 0-10 m layer during the mixing periods. Triplicate samples were collected and pooled to reduce heterogeneity in zooplankton horizontal distribution and sampling variability. For seston analysis, a pooled sample was constituted from discrete samples collected with a 3 l Ruttner bottle. Discrete samples were taken every meter in the same layers as those for zooplankton sampling.

Zooplankton was immediately narcotized in soda water, rinsed and preserved with a 4 % formalin solution (Haney and Hall 1973). In the laboratory, the cladocerans were counted at an inverted microscope. At least 200 individuals of each species were counted and *D. galeata* individuals measured from the upper edge of the eye to the base of the tail spine.

Hundred ml of the pooled seston sample was preserved with acid Lugol for microscopic analysis. Edible particles for cladocerans (Gliwicz 1977, Gliwicz and Siedlar 1980, Kasprzak and Lathrop 1997) were separated from total seston by sieving part of the pooled sample on a 28-µm Nytex screen. Water from both edible and total fractions was divided in 2 parts. One was filtered *in situ* on a 47-mm GF/C (porosity of 1.2 µm) Whatman filters and directly frozen in liquid nitrogen for pigment analysis using HPLC. The other part was filtered *in situ* on 6 pre-ignited 25-mm GF/C Whatman filters and directly frozen in dry ice for elemental analysis.

Pigments were extracted and analysed following Descy et al. (1999), using the HPLC protocol of Wright et al. (1991). Pigments were detected by a Waters 996 PDA detector and a Waters 470 fluorescence detector, and calibration was achieved using external standards (see details in Pandolfini et al. 2000). Chlorophyll *a* biomass of each algal class was estimated using the CHEMTAX program (Mackey et al. 1996), from the concentration of selected marker pigments.

From the 6 filters collected by date and by fraction for the elemental analysis, 3 were analyzed for particulate C and N contents and 3 for particulate P content. Particulate C and N were analyzed with a Carlo-Erba CN NA1500 elemental analyzer. Total P was analyzed by spectrophotometric determination of phosphate after potassium persulphate digestion (Greenberg et al. 1992). Seston elemental ratios were expressed as the ratio of the means in molar units corrected for the variance of denominator (Dagnelie 1992).

Feeding rates

We measured *in situ* grazing rates of *D. galeata* in parallel with variables expected to affect filtration rates: temperature, density of the zooplankton community, mean body size of the *D. galeata* population, sestonic biomass (in terms of C, N, and P) and C:N:P ratios in total and edible (< 28 µm) size fractions, algal biomass (Chl *a*) and composition in both fractions. All these factors were measured in the layer where the grazing experiment was conducted. Experiments were essentially run at mid-depth in the epilimnion but also sometimes in the metalimnion when *D. galeata* density was sufficient. In total, 89 grazing experiments were conducted from April 1998 to October 2000.

The technique used for *in situ* determination of individual filtration rates involved incubation with radioactively labelled algae in a 6.5 l transparent grazing chamber (Haney 1971) designed after Gawler and Chapuis (1987). The cultivated green alga *Kirchneriella subcapitata* (syn. *Selenastrum capricornutum*) (length 7-23 µm, wide 1.2-5 µm) was incubated at least 24 h before experiments with 250 µCi $NaH^{14}CO_3$ per 250 ml of culture medium. Unincorporated labelled bicarbonate was removed by repeated centrifugation and rinsing. The algal suspension was then stored in a dark cooler until use in the field. The volume of labelled suspension injected in the chamber food compartment was adjusted to correspond to less than 10 % of the algal biomass in the lake, as estimated by Chl *a* measured the preceding day. The duration of incubations was around 6 minutes from the closure of the chamber to the collection of zooplankton. This incubation time is normally shorter than the gut passage time of most zooplankters studied (Murtaugh 1985, Cauchie et al. 2000). At the end of incubation, the zooplankton was collected on a 100-µm sieve, rinsed with filtered lake water, anaesthetized for 2 minutes in soda water, resuspended in filtered lake water and deep-frozen in liquid nitrogen. Incubations were run at least in triplicate. In the laboratory, the samples were thawed one at a time. *D. galeata* individuals were sorted by size classes (< 0.5, 0.5 – 0.75, 0.75 – 1, 1 – 1.25, > 1.25 mm), measured, and placed on 5-µm nitro-cellulose filters. When density of *D. galeata* was high, sub-samples of individuals of the same size were sorted

for replicating the counting. When density of *D. galeata* was low, they were not sorted by size.

For each incubation, a fraction of the suspension was collected on 0.45-µm filters (Millipore) for determination of total suspended radioactivity. Control incubations without radioactively labelled food were also run and all measurements were corrected for background radioactivity. All samples were placed in 20 ml scintillation vials added with liquid scintillation cocktail (FilterCount®) and radioactivity (dpm) was measured with a Beckman LS 6000IC scintillation counter. Preliminary experiments revealed a constant radioactivity loss in sorted samples due to freezing and thawing (Thys 2003). A correcting factor of 1.56 was thus applied to radioactivity measurements before calculation of clearance rates. Individual filtration rates (ml individual^{-1} h^{-1}) were calculated according to Peters (1984).

Data processing

Our purpose was to determine the relative effect of P concentration in the food on *D. galeata* filtration rate. But, as P is not the only variable influencing filtration rate in natural zooplankton communities, we need to appropriately distinguish the respective effect of each potentially explanatory variable. Therefore, simple and multiple linear regressions obtained using the Statistica® statistical package (StatSoft, Inc., 1996) were firstly computed. Secondly, the interacting influences of predictors including C:P or N:P ratios on filtration rate were visualized by probability distributions of predicted filtration rate. These distributions were obtained by Bayesian analysis of a multiple regression model (Gelman et al. 1995).
The analysis resembles a conventional frequentist multiple regression but the interpretation is completely different (Ellison 1996). The Bayesian analyses yields probability distributions for predicted values of filtration rate. These distributions give the probability that a particular predicted value will be actually observed. In contrast, frequentist multiple regression analyses used most commonly in ecology predict confidence intervals that include the true value of the response variate in a given percentage of independent, identical studies (Ellison 1996). In this sense, and as Bayesian analyses tell us how probable our hypotheses actually are given the available data [i.e. $P(H_0|\, x)$], they allow stronger conclusions to be drawn from large-scale ecological experiments with few replicates (Ellison 1996).

Our analysis assumed that the predicted probability distributions depend on our data alone, and did not depend on any additional or external information. This assumption is termed a noninformative prior distribution (Gelman et al. 1995). Probability distributions of filtration rate were calculated using the model

$$F = X + E, \quad (1)$$

where F is the vector of response variate (the filtration rate). E is an independently, identically and normally distributed prediction error with mean 0 and variance s^2. X is the matrix of predictors with 1, all selected predictors and their interactions. The posterior predictive probability distribution of F for new data (or scenarios) conditional on X [i.e. $P\left(\widetilde{F}\middle|\widetilde{X}, X\right)$] is a t-distribution with $n - k$ degrees of freedom where n is the number of observations and k is the number of parameters (1 plus the predictors plus their interactions) (Gelman et al. 1995). The mean of the t-distribution is

$$\mathrm{E}\left(\widetilde{F}\middle|\widetilde{X}, X\right) = \widetilde{X}\hat{\beta}, \quad (2)$$

where

$$\hat{\beta} = \left(X^T X\right)^{-1} X^T F. \quad (3)$$

The variance of the t-distribution is

$$\mathrm{var}\left(\widetilde{F}\middle|s^2, F\right) = \left(I + \widetilde{X} V_\beta \widetilde{X}^T\right) s^2, \quad (4)$$

where

$$V_\beta = \left(X^T X\right)^{-1}, \quad (5)$$

$$s^2 = \frac{1}{n-k}\left(F - X\hat{\beta}\right)^T \left(F - X\hat{\beta}\right), \quad (6)$$

and I is the identity matrix.

RESULTS

Resource composition

The pattern of phytoplankton development in the reservoir was relatively similar from one year to another. Chl *a* biomass showed a marked spring peak followed in 1998 and 1999 by a clear-water phase (Fig. 3.1A). The summer assemblage comprised a greater proportion of algal units larger than 28 μm, while smaller algae became dominant in fall.

Phytoplankton composition varied with time (Fig. 3.1B). Throughout April and May, diatoms (among others, *Cyclotella* sp., *Stephanodiscus* sp., *Asterionella* sp.) and cryptophytes (*Rhodomonas* sp. and *Cryptomonas* sp.) dominated the plankton, sometimes with chrysophytes (mainly *Chrysidalis* sp.). In June 1998 and 1999, there was a marked clear-water phase during which cryptophytes were virtually the only 'edible' algae left. In July, large algae dominated the phytoplankton, mostly diatoms (*Fragilaria* sp.), green algae (mainly colonies of *Eutetramorus* sp.) or chrysophytes (*Chrysochoccus* sp. or *Mallomonas* sp.). Cyanobacteria were present throughout the growing season and they developed further through August and September to become

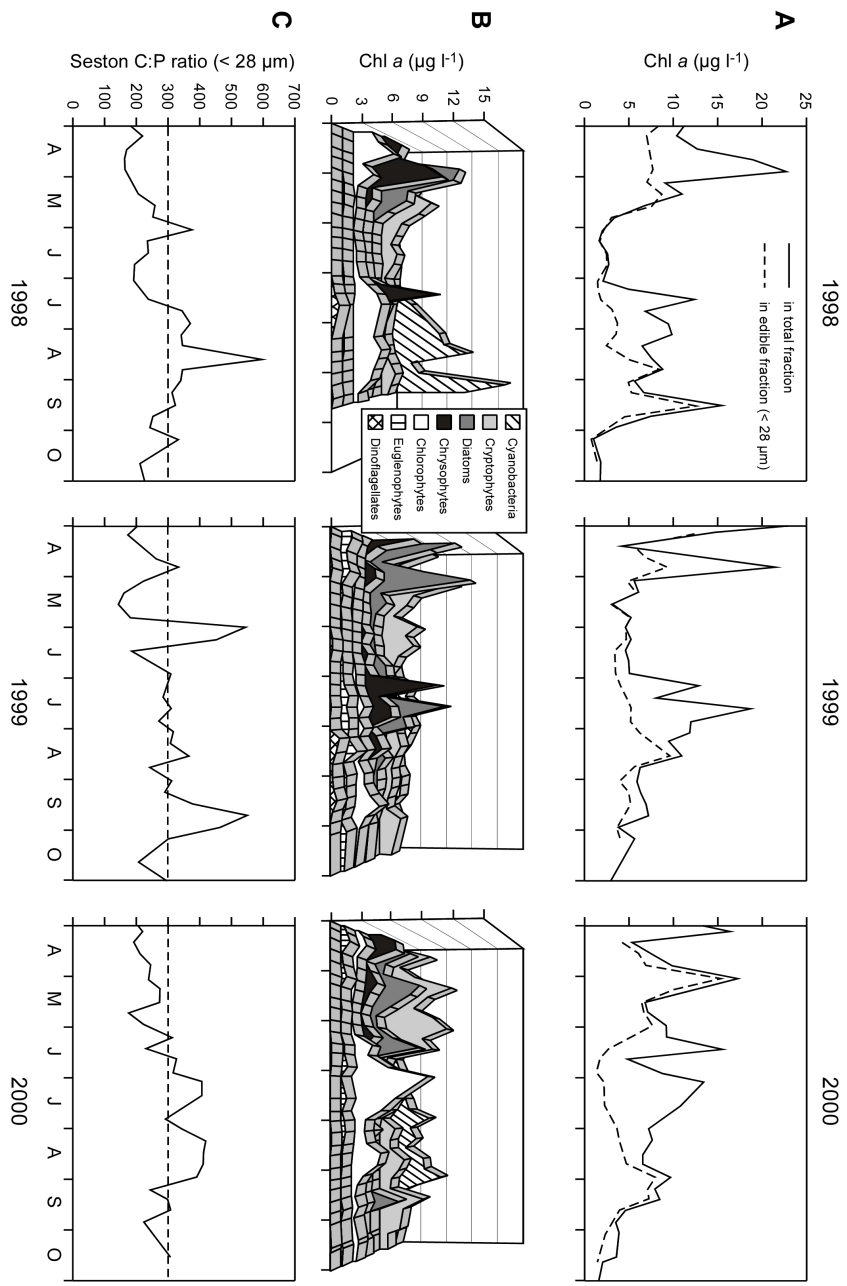

Fig. 3.1: Seasonal variations of algal community from 1998 to 2000 in the epilimnion of the Esch-sur-Sûre reservoir. (A) Total and edible algal biomass expressed by Chl *a*. (B) Class-specific phytoplankton biomass estimated through the HPLC analysis of specific marker-pigments. (C) C:P ratio in edible seston. The broken line represents the theoretical quality threshold ratio above which animal growth is predicted limited by food quality (Darchambeau submitted).

the dominant phytoplankton fraction, except in 1999, when large diatoms (*Fragilaria* sp.) prevailed. Cyanobacteria were single cells or colonies of Chroococcales or filamentous forms (mainly *Pseudanabaena*, *Planktothrix*, *Anabaena*, *Aphanizomenon* and *Leptolyngbya*).

The sestonic C:P ratio increased progressively over the year, reaching a first maximum during the clear-water phase in 1998 and 1999 and a second more marked maximum in August or September (Fig. 3.1C). The C:P ratio was frequently above the quality threshold for *Daphnia* (estimated ≈ 300 by, among others, Darchambeau submitted). Edible seston (i.e. < 28 µm) was significantly slightly poorer in N (proportionally to C) than the total fraction, whereas there was no difference in both C:P and N:P ratios between fractions (Table 3.1). The C:P ratios were also highly significantly correlated with C:N and N:P ratios, whereas there was no significant correlation between C:N and N:P ratios.

Table 3.1: Correlations between sestonic C:N:P ratios in the Esch-sur-Sûre reservoir from April to October in 1998, 1999 and 2000. *r* are Pearson coefficients.

x	y	n	*r*	*P* [a]	slope [b]	*P* [c]
Correlations between size fractions						
C:N total	C:N < 28 µm	80	0.808	< 0.001	1.070	0.004
C:P total	C:P < 28 µm	78	0.832	< 0.001	0.921	0.444
N:P total	N:P < 28 µm	78	0.768	< 0.001	0.954	0.885
Correlations between elements (all fractions)						
C:N	C:P	165	0.440	< 0.001		
C:N	N:P	165	0.198	0.801		
C:P	N:P	165	0.900	< 0.001		

Notes: (a) probability than r = 0, (b) slopes of the major axis regression, (c) two-tailed t-test for paired samples.

Daphnia galeata abundance and feeding response

Population dynamics of *D. galeata* were similar in all years investigated (Fig. 3.2). High abundances during the clear-water phase were followed by a decline in mid-June resulting in very low population densities (mid-summer decline). *Daphnia* remained scarce throughout the rest of the year except in 2000, when they developed a small autumnal peak. The lowest abundances were observed in 1999 (<5 ind l^{-1}) and the highest in May 2000 (± 20 ind l^{-1}).

In total, 89 grazing experiments were run, leading to 36 observations differing in dates and/or *Daphnia* body length. Observed *D. galeata* filtration rates varied between 2.5 – 13.5 ml ind^{-1} day^{-1} (mean, 7.0 ml ind^{-1} day^{-1}). Fourteen data out of 36 represented the filtration rate of the whole *D. galeata* population, while the resting 22 were from samples sorted by *Daphnia* size. Filtration data for the whole population are presented in Fig. 3.2.

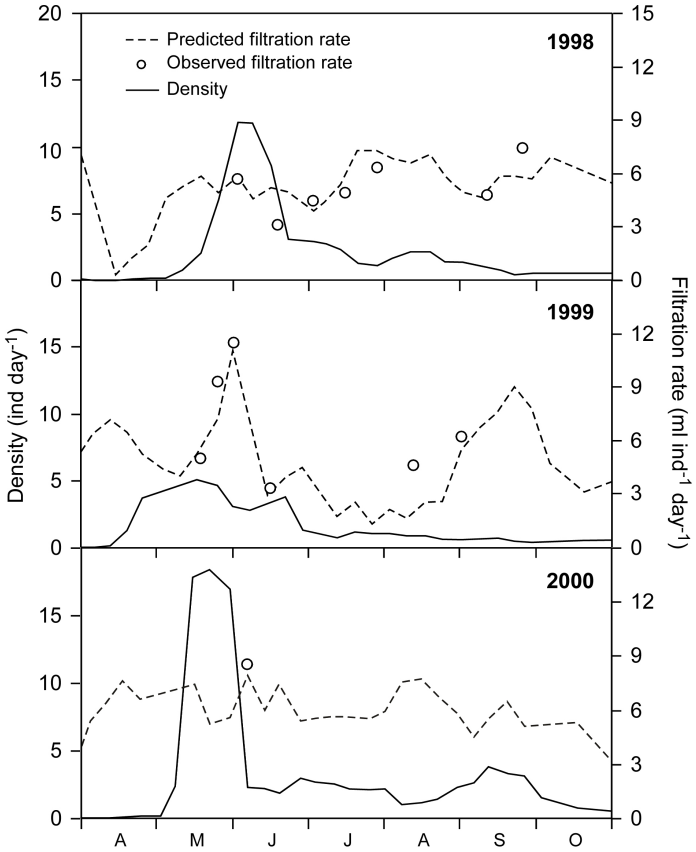

Fig. 3.2: Density in the upper layer and filtration rates of *Daphnia galeata* in the Esch-sur-Sûre reservoir from 1998 to 2000. Full lines represent moving averages on three data of density. Empty circles represent observed filtration rates of whole *D. galeata* population. Samples sorted by size are not figured. Dotted lines are population filtration rates predicted from the multiple linear regression model (see Table 3.3).

Table 3.2: Simple linear regressions between filtration rates of *D. galeata* and environmental explanatory variables ($y = b_0 + b_1 x$). Variables are classified in descending order of explained variance (r^2). *r* are Pearson coefficients. n = 36.

Explanatory variables x	Min	Mean	Max	*r*	r^2	*P* [a]
Body length (mm)	0.35	0.86	1.37	0.80	0.639	< 0.001
Temperature (°C)	9.9	17.2	22.1	-0.51	0.262	0.001
C:P ratio (< 28 µm)	187	304	454	0.49	0.241	0.002
N:P ratio (< 28 µm)	21	38	61	0.46	0.210	0.005
Particulate P (< 28 µm) (µg l^{-1})	1.0	2.6	5.2	-0.45	0.200	0.006
N:P (total)	15	39	71	0.35	0.122	0.037
Particulate P (total) (µg l^{-1})	1.5	4.1	7.4	-0.34	0.116	0.042
C:P (total)	142	303	549	0.34	0.115	0.043
% of inedible algae [b]	0	27	77	-0.34	0.114	0.044
Chlorophytes (µg Chl*a* l^{-1})	0.0	0.3	1.7	-0.33	0.111	0.047
Euglenophytes (µg Chl*a* l^{-1})	0.0	0.1	0.4	-0.27	0.074	0.110
Particulate C (< 28 µm) (µg l^{-1})	154	287	597	-0.27	0.073	0.110
Chl*a* (total) (µg l^{-1})	2.2	6.0	12.7	-0.27	0.073	0.092
Crustacean density (ind l^{-1})	1.8	16	41	-0.27	0.072	0.114
Chrysophytes (µg Chl*a* l^{-1})	0.0	0.2	1.3	-0.26	0.067	0.127
Particulate N (< 28 µm) (µg l^{-1})	22	43	83	-0.26	0.066	0.130
Dinoflagellates (µg Chl*a* l^{-1})	0.0	0.1	0.9	-0.24	0.056	0.165
Particulate C (total) (µg l^{-1})	204	450	888	-0.23	0.052	0.180
Particulate N (total) (µg l^{-1})	25	70	161	-0.17	0.030	0.310
C:N (total)	6.4	7.9	9.7	-0.15	0.023	0.377
Cyanobacteria (µg Chl*a* l^{-1})	0.0	0.6	3.8	-0.14	0.021	0.404
Chl*a* (< 28 µm) (µg l^{-1})	1.4	4.0	8.6	-0.14	0.020	0.405
Diatoms (µg Chl*a* l^{-1})	0.0	0.2	0.9	-0.07	0.005	0.678
C:N (< 28 µm)	7.0	8.0	8.9	0.05	0.003	0.750

Notes: (a) probability than $b_1 = 0$, (b) units : Chla:Chla 100.

Table 3.2 gives the results of simple linear regressions of filtration rates against environmental variables. Body length emerged as the most important factor explaining *D. galeata* filtration rates (63.9 % of total variance). As expected, filtration activity increased proportionally to body size. The water temperature is the second best explanatory variable (26.2 %), with reduction of filtration rates when temperature increases. Next, several variables expressing seston phosphorus content were singled out: C:P ratio, N:P ratio

and particulate P concentration of the edible fraction (respectively, 24.1, 21.0 and 20.0 %), followed by N:P ratio, particulate P concentration and C:P ratio of the total fraction (12.2, 11.6 and 11.5 %). When particulate P became deficient (lower particulate P concentrations or higher C:P or N:P ratios), filtration activities increased. Worth noticing is that Chl *a* biomass, both in total and < 28 μm fractions, was not significant.

Due to the likely high correlations of body length with some variables, it seemed more appropriate to test the significance of multiple models containing the mean body length and one of the other explanatory variables (Table 3.3). Once again, all C:P and N:P ratios are significant and, in linear combinations with mean body size, provide highly explanatory models of individual filtration rates ($r^2 > 0.85$). Water temperature was highly correlated with body length and explained a part of the variance of filtration rate already explained by mean body length.

Table 3.3: Multiple linear regressions between filtration rates in ml ind^{-1} day^{-1} of *D. galeata* and 2 environmental explanatory variables ($y = b_0 + b_1 x_1 + b_2 x_2$). Same units as in Table 3.2. Models are classified in descending order of explained variance (r^2). Only models with both slopes significantly different from 0 ($P < 0.05$) are showed. n = 36.

x_1	x_2	r^2	b_0	b_1	b_2	*P* for b_1	*P* for b_2
Body length	N:P (total)	0.897	-8.24 [a]	12.9 [a]	0.108 [a]	< 0.001	< 0.001
Body length	C:P (total)	0.890	-8.51	12.9	0.0148	< 0.001	< 0.001
Body length	N:P (< 28)	0.881	-8.45	11.8	0.140	< 0.001	< 0.001
Body length	C:P (< 28)	0.852	-8.02	11.2	0.0178	< 0.001	< 0.001
Body length	Cryptophytes	0.708	-4.20	11.3	0.595	< 0.001	0.009
Body length	Crust. density	0.696	-1.40	11.4	-0.0837	< 0.001	0.019
Body length	C:N (< 28)	0.693	7.30	12.7	-1.40	< 0.001	0.021
Body length	C:N (total)	0.690	2.98	11.8	-0.763	< 0.001	0.026

Note: (a) they are the model and parameter values used for predicting filtration rate during the whole study period figured in Fig. 3.2.

For exploring further the role of seston P deficiency on filtration rate, we have constructed a multiple model using a forward selection procedure (*F* to enter = 1). The best variable association selected 3 variables (mean *Daphnia*

body length, total sestonic N:P ratio and crustacean density in the upper layer) providing in linear combination a model explaining 92.0 % of filtration rate. Matrix X was filled for the 36 observations of filtration rate with an independent term, the predictors and their interactions:

$$X = [1\ S\ NP\ D\ S \times NP\ S \times D\ NP \times D\ S \times NP \times D], \quad (7)$$

where S is the mean body size of *D. galeata*, NP is the N:P ratio in total seston, and D is the crustacean density. The predicted probability distributions of filtration rate were obtained by Bayesian analysis of the multiple regression model. Probability distributions indicate the responses of filtration rate to selected combinations of mean size, N:P ratio, and crustacean density (Fig. 3.3). For given values of mean *Daphnia* body length and density, an increase of seston N:P ratio causes filtration rate to increase. For example, when mean size is 1.2 mm and crustacean density is 5 ind l^{-1}, a N:P ratio of 15 yields a slightly variable filtration rate with a mode of 9.2 ml ind^{-1} d^{-1}. If N:P ratio is increased to 60, filtration rate becomes higher with a modal value of 15.1 ml ind^{-1} d^{-1}. A higher crustacean density generally yields more variable filtration rates with lower modes, except for small animals feeding on P-rich seston (N:P ratio = 15) for which filtration rate is slightly increased. Interestingly, the influence of N:P ratio on filtration rate is decreased under high crustacean density.

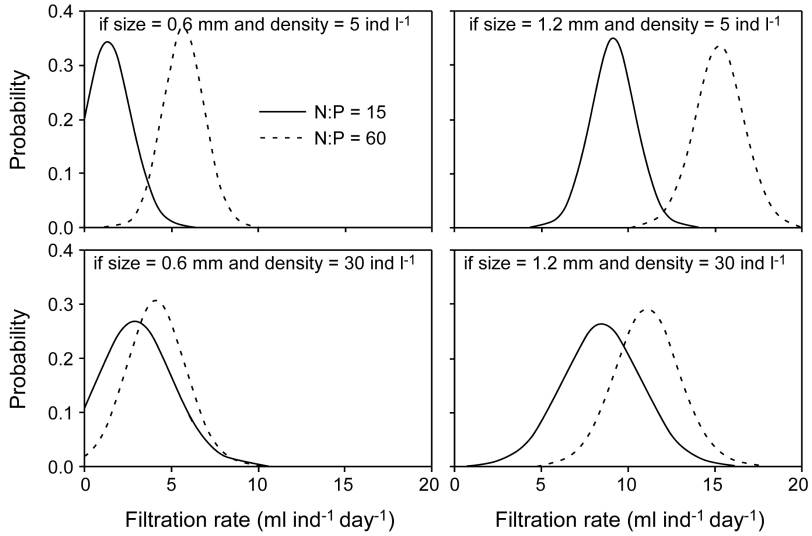

Fig. 3.3: Probability distributions of filtration rate of *D. galeata* for eight scenarios: all combinations of low and high values for seston N:P ratio (in total fraction), mean size, and crustacean density. The area under each curve is 1 (d.f. = 36 – 8 = 28). In the lake, N:P = 15 and 60 are equivalent respectively to C:P = ~150 and ~592.

DISCUSSION

The prediction of Darchambeau's model (Darchambeau submitted) and *in situ* observations both point to the same evidence: the *Daphnia* filtration rate increases when food becomes P-deficient. The model predicts a double response of daphniids to food nutrient-deficiency: a decrease in gut passage time (leading to an increase of filtration and ingestion rates) and a simultaneous increase in the secretion of digestive enzymes (Darchambeau submitted). Our experimental results on *in situ* filtration rates of *D. galeata* can only directly support the first response.

This study corroborates experimental works of Libourel Houde and Roman (1987) on the copepod *Acartia tonsa* and of Plath and Boersma (2001) on the cladoceran *Daphnia magna*. But it invalidates the results of Van Donk et al. (1997), Lürling and Van Donk (1997), DeMott et al. (1998), Hessen et al. (2002) and Darchambeau et al. (2003). All these authors failed to detect any (positive or negative) influence of food C:P ratio on *Daphnia*'s filtration rate. In most experimental designs, animals were not acclimated to food quality before grazing experiments. In DeMott et al. (1998), however, animals were acclimated for 40 h at 5 different food qualities (C:P range going from 120 to 900) before the measurement of feeding rate. Their analysis of variance (ANOVA) showed no effect of the treatment on weight-specific ingestion rate but their results were affected by high intra-group variances (see Fig. 6 in DeMott et al. 1998). To reconcile these contrasting experimental results, we can hypothesize that the filtration response of herbivores to food nutrient-deficiency is not immediate, but needs some hours to develop. This response can not be observed in studies without acclimation. In the present study, dealing with natural communities, we observe animals displaying long-term phenotypic and even genotypic adaptations to low food quality.

The results of Plath and Boersma (2001) suggest that the increased filtration rates observed in animals feeding on P-deficient seston may result from higher beat rates of feeding appendages. Morphological adaptations may also lead to more efficient collection of particles. As already observed in low-food conditions (Lampert 1994, Lampert and Brendelberger 1996), we may suspect that *Daphnia* adapted to P-deficient diet have larger filter screens and/or finer meshes. It should be interesting to observe whether these morphological differences do exist among natural (monoclonal or non monoclonal) populations of different sites with varied food conditions.

It does not seem so evident to conclude, as do Plath and Boersma (2001), that the rise of feeding activity is a common response of animals to dietary nutrient-paucity. Some evidence in insects are indeed pointing in this

direction (Slansky and Feeny 1977, Reynolds 1990). In these cases, clearly greater ingestion allows higher access in the same unit of time to the deficient element. However dietary amino acid deficiency is known to reduce feed intake in fish (de la Higuera 2001) at the inverse of poultry and pigs (respectively, Boorman 1979 and Henry 1985). A possible explanation of intake reduction in fish is that this behaviour would prevent or delay the onset of metabolic disorders (de la Higuera 2001). We may suspect that the ingestion response to food quality is species-dependent and, above all, depends on the nature of the deficient element. Energy-lacking food may probably not induce an increase of food intake, while the deficiency of structural elements may be compensated by increased ingestion.

An *a priori* curious response of filtering rates to the density of zooplankton was detected: the filtration rate is depressed by high zooplankton density only at high seston N:P ratio (Fig. 3.3). The known inhibitory effects of crowding on the ingestion rates of *Daphnia* species (Hayward and Gallup 1976, Helgen 1987, Matveev 1993) are mediated by released chemicals and, although still largely unclear, may provide between conspecifics an additional, density-dependent mechanism of population regulation (Burns 2000). Simultaneously with reduction of ingestion rates, crowding induces in some species a reduction in clutch size, but with bigger egg size and longer survival of juveniles without food (Burns 1995, Cleuvers et al. 1997). This mutual intraspecific influence on reproduction parameters, called life-strategy shift by intraspecific interaction (LiSSII), may be an adaptive response at unfavourable environmental conditions (Cleuvers et al. 1997). As the LiSSII, the reduction of ingestion observed under high density conditions may be seen as an adaptive behaviour favouring fitness of future conspecific generations, and especially when food quality is low. This hypothesis needs confirmation, e.g. by the research on a possible LiSSII in natural *Daphnia* populations facing off low-quality food.

Increased food ingestion implies reduced assimilation efficiency, higher nutrient turnover, and lower secondary production (Darchambeau submitted). Indirect effects of consumer behavioural response to changes in food quality need further investigations. For example, it should be very interesting to investigate the indirect effect of food quality and grazing on the stimulation of algal growth observed by Sterner (1986). Another important question is the fate of the non-assimilated elements, knowing the importance of egested materials in total sedimentation (e.g. Sarnelle 1999). What is the effect of increased grazing on the fraction of egested materials sinking out the mixed layer, and on stoichiometry of sinking particles (Elser et al. 1996)? How is influenced the biogeochemical cycle of P by increased ingestion of primary consumers in P-deficient lakes?

In conclusion, our results emphasize the importance of phosphorus availability in resources for *D. galeata* in natural conditions. The predicted and observed increase of filtration rates with the rise of seston P-deficiency is an appropriate adaptive behavioural response to nutrient-deficiency. Direct and indirect effects of this response should be further studied, with a particular focus on the biogeochemical cycle of the deficient element.

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Chapter 4

Can *Daphnia* phenotypically adapt to low food quality? A meta-analysis

In my predictive model of Chapter 2, I have hypothesized that Daphnia can adapt filtration and digestive enzymes production rates to cope with varying food quality. If existent, these responses must result in increased somatic growth rate. The reality of this phenomenon has not yet been demonstrated. In this Chapter, I show that we can actually observe a progressive increase of Daphnia growth rate, resulting from their acclimation to low-quality food.

Can *Daphnia* phenotypically adapt to low food quality? A meta-analysis

To be submitted

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ABSTRACT

A meta-analysis stresses the ability of *Daphnia* to adapt to stoichiometric food quality. A large literature survey gathered 96 published growth rates of *Daphnia* juveniles, a half observed for animals fed with P-rich algae and the other half observed in the same experimental conditions but for animals fed with P-low algae. Multiple regression analyses of growth rates and experimental conditions highlight the length of time between the transfer of animals to P-low algae and the half-time of the growth determination experiment in the explanation of the variance of P-limited growth rate. The relationship was significantly positive, meaning that the higher the length of time between transfer and half-time of growth determination, the higher the animal growth rate observed for P-low food. I support that this positive relationship results from the still largely unstudied ability for *Daphnia* to compensate diet P-deficiency by rapid physiological and/or behavioural responses, such increased filtration rate or digestive enzyme secretion.

INTRODUCTION

Many recent papers have clearly defined and demonstrated the effect of food quality on *Daphnia* growth (e.g. Weers and Gulati 1997, Urabe et al. 1997). Many factors influence food quality for consumers. The respective roles of one class of poly-unsaturated fatty acid, the highly unsaturated fatty acids (HUFA), and phosphorus (P) have been demonstrated (Sterner and Schulz 1998). The diet deficiency in P combined or not with a deficiency in HUFA was proven largely depressing for growth rates of several *Daphnia* species (e.g. Boersma 2000). Recently, some compensatory physiological mechanisms adopted by the consumer have been proposed and studied. For instance, in a study of influence of diet C:P ratio on *Daphnia magna* assimilation rate, DeMott et al. (1998) have shown that P assimilation efficiency rapidly decreased as diet C:P ratio rises, but more slowly than the decrease of C assimilation efficiency. This depressed assimilation efficiency for both elements led to more equilibrated resource absorption. A decrease in assimilation efficiency may be the consequence of higher ingestion rate (Darchambeau submitted). Accordingly, Plath and Boersma (2001) observed that *Daphnia* individuals increase their feeding-appendage beat rates in case of diet P-deficiency. The ingestion rate of a field *D. galeata* population was also increased if seston C:P increased (Darchambeau and Thys submitted). All these compensatory mechanisms may reduce the negative effect of low food quality on growth rate (Darchambeau submitted). The present study aims to demonstrate whether or not *Daphnia* submitted to a low P diet can improve with time its fitness, in particular its growth.

STRATEGY

A large literature survey about the influence of diet C:P ratio on growth rate of daphniid species has allowed to gather data on growth rate observed under very contrasting conditions. We conducted a meta-analysis (*sensu* Osenberg et al. 1999) of these data. All experiments measured the body weight of juveniles of various *Daphnia* species under at least two opposite food conditions: one with P-saturated food and the other with a P-depressed diet. Experiments were carried out with or without an acclimation time to food quality before actual growth experiment. Based on this experimental design, a simple model was designed to show the expected effect of adaptation to low food quality on growth rate (Fig. 4.1). The specific growth rate (*SGR*) is defined as the instantaneous exponential rate of change of biomass of the organism normalized to its biomass:

$$W_t = W_0 \exp(\mu t) \Leftrightarrow \mu = \frac{\ln(W_t/W_0)}{t} \quad (1)$$

where W_0 and W_t are, respectively, the individual body weight at time 0 and t, μ is the instantaneous growth rate (day^{-1}), and t is the time interval

between measurements of W_0 and W_t (day). Following Winberg and Duncan (1971), the instantaneous growth rate was assumed a negative function of body weight. For simplicity, I used an exponential negative relationship between growth rate and body weight:

$$\mu = a\left[1 - \exp\left(-bW_t\right)\right]. \quad (2)$$

Note that this assumption does not alter my predictions. Both equations (1) and (2) were used in an iterative process to model the time evolution of instantaneous growth rate. When food quality is high, the instantaneous growth rate shows a curvilinear decrease over time (Fig. 4.1). The change in food quality (P-depressed algae) was considered as depressing growth rate to 20 % of the P-saturated growth rate. If *Daphnia* is unable to adapt to food quality, the instantaneous growth rate stays a negative curvilinear function of time. A third scenario was computed, considering that the penalty due to low food quality starts at 20 % and reaches a maximum of 60 % of the weight-relative P-saturated growth rate about 7 days later (Fig. 4.1). The model shows that, if *Daphnia* has some ability to adapt to food P-deficiency, the specific growth rate increases during some days just after the change in food quality. Seeing these predicted time evolutions, I decided to gather, for each study of the literature, the growth rate in high quality and low quality treatments. In addition, the experimental design was recorded for each experiment by two variables: (1) the age of animals when they were transferred to low-quality food (*AT*), and (2) the length of time between transfer and half-time of growth rate determination (*LT*) (see Fig. 4.1). I predict that, if *Daphnia* is able to adapt to food P-deficiency, we may expect to observe a positive relationship between the growth rate of the low-quality treatment and the length of time between transfer and half-time of growth determination. Inversely, if *Daphnia* is unable to adapt, the same relationship should be negative. Other experimental variables were recorded, such as food biomass, algae and *Daphnia* species used, and finally food C:P ratios in both low quality and high quality treatments. Multiple linear regressions were used to separate the effect of each experimental variable and so highlight the actual slope of the relationship between growth rates and length of time between transfer and half-time of growth determination. All regressions were made with the help of the Statistica software package (StatSoft, Inc.).

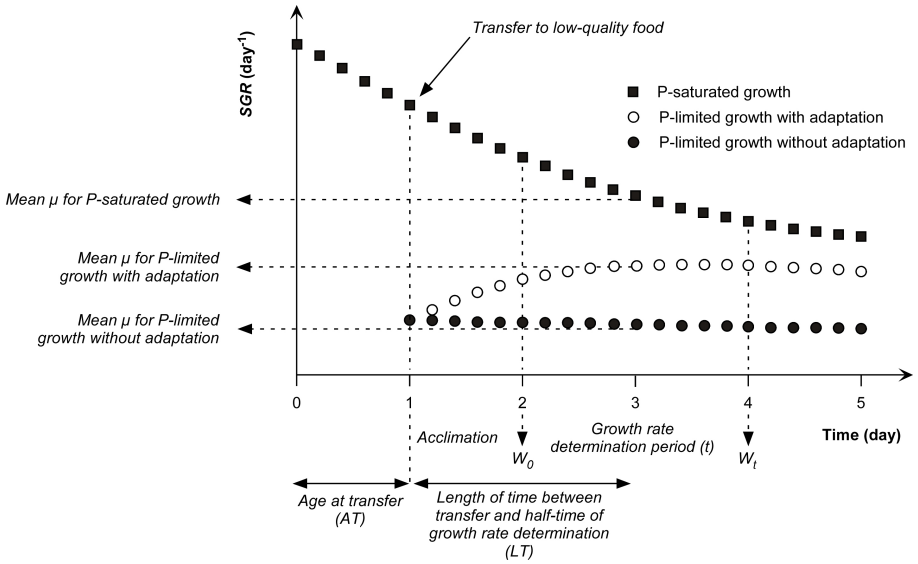

Fig. 4.1: Modelled specific growth rate (*SGR*) of *Daphnia* as a function of time and experimental conditions. The figure illustrates the general experimental design observed in the literature of effects of food quality on *Daphnia* growth rate. In all cases, the juveniles are initially fed during some hours with P-rich algae. In the high-quality control, the animals are fed during the entire experiment with P-saturated algae (filled squares). In the low-quality treatment, the animals, after some hours (here one day), are transferred to P-deficient algae. Two situations may occur: either the animals are able to partly adapt to the dietary P deficiency, and after an initial decrease of the growth rate, an increase is expected (empty circles), or the animals are unable to adapt and the growth rate continues to decrease (filled circles). The initial weight (W_0) may be determined directly or after an acclimation time. The final weight (W_t) is determined some days later. The observed growth rate is obtained by $\ln(W_t/W_o)/t$. In the meta-analysis, 2 variables describing the design of each literature experiment were recorded and are here figured: the age of animals when they were transferred to low-quality food (*AT*), and the length of time between transfer and half-time of growth rate determination (*LT*). This figure illustrates that, if *Daphnia* has some ability to adapt to dietary P-deficiency, a positive relationship can be predicted between the P-limited growth rate and *LT*. Inversely, if *Daphnia* are unable to adapt, the same relationship is expected negative.

DATASET

Seventeen published studies, corresponding to 29 different experiments with *Daphnia* spp., were examined (see list of studies in Appendix). In each experiment, the used *Daphnia* juveniles or their mothers were grown under P-sufficient food, and their growth was compared with contrasting food (C:P) qualities. This survey has led to the compilation of 320 growth rates. For each rate, 6 corresponding variables were also observed: the algal species, the food biomass, the food C:P ratio, the *Daphnia* species, *AT* and *LT*.

To have a better discrimination between variables potentially explaining the difference between growth rates observed under P-saturated and P-deficient food, I have focused on experiments where a comparison was possible between a treatment with P-low and another with P-rich food led under the same experimental conditions. So, from the 320 gathered growth rates, 95 comparisons between a P-rich and P-low treatments were made possible. As the range of food C:P ratio was quite large for both P-rich and P-low treatments (respectively 51-400 and 400-2266, in atomic values), I have limited the dataset to more comparable ranges: C:P ratio ≤ 200 for P-rich, and ≥ 600 for P-low. So, I have obtained for the meta-analysis 61 possible comparisons between a P-saturated and a P-limited growth rate satisfying these both criteria. *Daphnia magna* was the most used species (29 times), followed by *D. obtusa* (19), *D. galeata* (5), *D. pulicaria* (3), *D. pulex* (3) and finally *D. dentifera* (2). To be sure to not observe a species effect in the analysis, I have performed a separate meta-analysis for each *Daphnia* species occurring in sufficient number: *D. magna* and *D. obtusa*. The descriptive statistics of variables for the selected experiments are presented in Table 4.1.

The 3 first variables of Table 4.1, plus the corresponding C:P ratio, and algal species coded as dummy variable (0 for absence, 1 for presence), were used as independent variables in multiple linear regressions of both growth rates. Regressions were performed independently for each *Daphnia* species.

Table 4.1: Range and mean of variables for the selected experiments in the meta-analysis. *AT* is the age of animals when they were transferred to low-quality food. *LT* is the length of time between transfer and half-time of growth rate determination (see Fig. 4.1).

	Food biomass (mgC l^{-1})	*AT* (day)	*LT* (day)	C:P ratio in P-rich treatment (molar)	C:P ratio in P-low treatment (molar)	Growth rate in P-rich treatment (d^{-1})	Growth rate in P-low treatment (d^{-1})
In the D. magna experiments (n = 29):							
Min	0.06	0.25	0.21	80	900	0.03	-0.12
1st quartile	0.20	0.50	1.00	80	925	0.25	0.13
Median	1.00	0.50	2.50	120	1000	0.32	0.16
Mean	1.07	1.67	2.36	122	1275	0.36	0.16
3d quartile	1.00	3.00	3.50	152	1820	0.44	0.18
Max	5.00	4.00	4.75	168	1820	0.70	0.28
Three different algal assemblages were used: *Scenedesmus* (21 times), *Selenastrum* (5), and a mixture of *Scenedesmus* and *Synechococcus* (3).							
In the D. obtusa experiments (n = 19):							
Min	0.42	0.21	2.50	93	678	0.20	0.07
1st quartile	0.50	0.25	3.00	106	1283	0.27	0.11
Median	0.50	0.50	3.50	153	1585	0.33	0.18
Mean	1.54	0.42	5.16	148	1546	0.35	0.15
3d quartile	2.17	0.50	7.50	186	1585	0.43	0.18
Max	5.35	0.50	10.00	200	2266	0.47	0.19
Scenedesmus was used as food in the all 19 selected experiments.							

RESULTS AND DISCUSSION

First, two multiple regression analyses, one for the *D. magna* experiments, the other for the ones with *D. obtusa*, were carried out with the observed growth rates in the P-rich treatment as dependent variable. The best explanatory variables were selected by a forward stepwise procedure (*F*-to-enter = 1, Table 4.2). The first variable explaining the value of the observed growth rate in the P-rich treatment is *LT* for both *Daphnia* species. The significant negative slope of these relationships agrees with my simple predictive model of time variations of instantaneous growth rate (Fig. 4.1): in P-rich treatment, *Daphnia* growth rate slowly decreases over time. For *D. obtusa*, this negative effect of time on growth rate is also detected by the negative significant slope of *AT* in the model (Table 4.2).

Table 4.2: Results of the multiple regression analyses of *Daphnia* growth rate observed in the 29 P-rich treatments with *D. magna* and in the 19 with *D. obtusa*. The variables are classified from the top to the bottom in the order of their addition in the model. Values of the significant variables (*P*-value < 0.05) are highlighted.

	Cum. R^2 [a]	slope [b]	*P*-level [c]
For the *D. magna* growth rates:			
LT	**0.24**	**- 0.044**	**0.033**
Food biomass	**0.27**	**0.075**	**0.045**
Selenastrum as food	0.36	- 0.219	0.079
For the *D. obtusa* growth rates:			
LT	**0.63**	**- 0.023**	**< 0.001**
AT	**0.78**	**- 0.308**	**0.004**
C:P ratio	0.79	3.0E-4	0.332

Notes: (a) cumulated R^2 for the model with successively 1, 2 and 3 variables, (b) slope of each variable in the complete model with the 3 variables, the intercept was equal to 0.423 for the *D. magna* model and 0.551 for the *D. obtusa* model, (c) probability that the slope of each variable in the complete model with the 3 variables = 0.

Second, two supplementary multiple regression analyses were carried out with the observed growth rates in the P-low treatment as dependent variable (Table 4.3). Conversely to P-saturated growth, a significant positive relationship is found for both *Daphnia* species between P-limited growth rates and *LT*. This observation supports the idea of the ability of *Daphnia* to, at least partly, adapt to food P-deficiency (see Fig. 4.1). Note that the growth

rate on P-depressed algae stayed obviously lower than the one observed when animals fed P-saturated food (one-tailed *t*-test for dependent samples, n = 49, $P < 0.001$). But the multiple regression demonstrates that, after the initial reduction of growth rate due to their transfer to low-quality food, the growth rate of the animals is improved over time.

Table 4.3: Results of the multiple regression analyses of *Daphnia* growth rate observed in the 29 P-low treatments with *D. magna* and in the 19 with *D. obtusa*. The variables are classified from the top to the bottom in the order of their addition in the model. Values of the significant variables (*P*-value < 0.05) are highlighted.

	Cum. R^2 [a]	slope [b]	*P*-level [c]
For the *D. magna* growth rates:			
C:P ratio	0.20	- 1.8E-5	0.666
AT	**0.23**	**0.035**	**0.025**
LT	**0.36**	**0.031**	**0.034**
For the *D. obtusa* growth rates:			
LT	**0.38**	**0.009**	**0.011**
AT	**0.58**	**0.224**	**0.004**
C:P ratio	0.65	-3.4E-5	0.098

Notes: (a) cumulated R^2 for the model with successively 1, 2 and 3 variables, (b) slope of each variable in the complete model with the 3 variables, the intercept was equal to 0.049 for the *D. magna* model and 0.062 for the *D. obtusa* model, (c) probability that the slope of each variable in the complete model with the 3 variables = 0.

As the median value for *LT* is < 4 days, we may think that this regulation was carried out quickly and produced significant results on growth rate already at very short term. This can make acclimation effect on *Daphnia* growth rate difficult to directly observe for experimental researchers. It will need very accurate measurements of body weights recorded at short time-intervals, e.g. every day directly after the transfer.

The results of both multiple regressions may serve to isolate the effect of *LT* on growth rates. I made here the exercise for *D. magna*. If all other variables are equal to 0, the P-saturated growth rate of *D. magna* is equal to 0.423 – 0.044 *LT* (Table 4.2, Fig. 4.2) and the P-limited growth rate is equal to 0.049 + 0.031 *LT* (Table 4.3, Fig. 4.2). I calculated from these two equations the relative growth rate (*RGR*), i.e. the animal growth rate on low-P food divided

by animal growth rate on high-P food (Sterner and Elser 2002) within the studied range of *LT* (Fig. 4.2). Note, however, that *RGR* values for *LT* below 1 and above 4 are uncertain because few data points were available outside this range (see quartiles of *LT* in Table 4.1) and linear regressions were used. The evolution of *RGR* of *D. magna* stays really sharp, even in the *LT*-range of 1 to 4 days (Fig. 4.2). This highlights the rapid and important response evolved by *Daphnia* to compensate food P-deficiency.

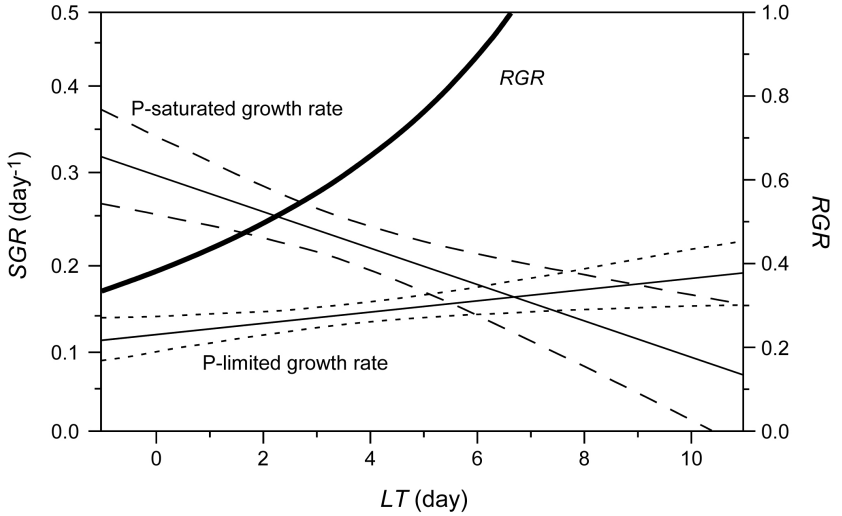

Fig. 4.2: Relationships between P-saturated, P-limited specific growth rates (*SGR*), and relative growth rate (*RGR*) of *Daphnia*, and the length of time between transfer of animals to low-quality food and half-time of growth rate determination (*LT*). Growth rates are linear regressions obtained by fitting with experimental data when once subtracted the effect of other variables of the respective multiple regression analysis (see Tables 4.2 and 4.3). Dotted curves are regression 95 % confidence intervals.

The observed phenotypic adaptation to low food quality may be attained by various physiological and behavioural regulations. Darchambeau (submitted) found by optimization of foraging effort and digestive investment that an increase of digestive enzymes secretion and/or a higher filtration rate can partly compensate the loss of growth due to bad food quality. Recently, Darchambeau and Thys (submitted) demonstrated that the filtration rate of a natural *D. galeata* population was positively correlated with lake seston C:P or N:P ratios. DeMott et al. (1998) also showed an important regulation of C and P assimilation efficiencies already after 36 h of acclimation to P regime. Admittedly, regulatory mechanisms behind these observations still need further study, but ways of research are already opened up.

The significant positive effects of age at transfer (*AT*) on P-limited growth rates (Table 4.3) are worth comments. It may illustrate the higher ability of older *Daphnia* to adapt to low-quality food. Indeed, the observed positive relationships mean that the later the transfer to low-quality food, the higher the consecutive growth rate. If confirmed, this observation may accredit the link between growth rate and P demand (Sterner and Elser 2002). Indeed, in *Daphnia*, the contribution of P to total biomass decreases slightly with the age (Main et al. 1997, Dobberfuhl 1999, Hessen and Faafeng 2000). This may result in lower P demand in older stages and lower sensitivity to dietary P deficiency. Elser al. (2000) showed that the *RGR* of temperate *D. pulex* populations was higher than the one of arctic *D. pulex* populations. The temperate populations were characterized by lower body % P than the arctic populations. We can draw a parallel between this latitudinal gradient in the *D. pulex* body % P resulting in difference in the *RGR* and my significant relationships between the age at transfer and ability to adapt to low-P food. In both cases, temperate populations or older *Daphnia* suffer less intensively the diet P deficiency than arctic populations or younger *Daphnia*. I suggest that the parallelism may come from their lower body % P.

To conclude, this meta-analysis confirms the phenotypic ability of *Daphnia* to adapt to food quality. This adaptation allows *Daphnia* to partly but significantly reduce the negative effect on their growth rate of P-deficient food. These results stress that *Daphnia* are usually regarded as phenotypically invariable animals, while regulations to environment are probably powerful and call for further investigations.

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APPENDIX

List of publications observed for the meta-analysis.

References	*Daphnia* species	Data sources	
Sterner et al. 1993	*D. obtusa*	Fig. 6	
Sterner 1993	*D. obtusa*	Fig. 2	*
	D. obtusa	Fig. 4	*
Sterner and Robinson 1994	*D. magna*	Table 2 and Table 3	
Müller-Navarra 1995	*D. galeata*	Fig. 1	
Urabe and Sterner 1996	*D. obtusa*	Fig. 2	*
	D. obtusa	Fig. 3	*
Weers and Gulati 1997	*D. galeata*	Table 4	
Sundbom and Vrede 1997	*D. galeata*	Fig. 4	*
DeMott et al. 1998	*D. magna*	Fig. 1	*
	D. magna	Table 2	*
	D. magna	Table 3 and Fig. 4	*
DeMott 1998	*D. magna*	Table 3 and Fig. 2	*
	D. pulicaria	Table 3 and Fig. 2	*
	D. pulex	Table 3 and Fig. 2	*
	D. galeata	Table 3 and Fig. 2	*
	D. magna	Table 4 and Fig. 4	
	D. pulicaria	Table 4 and Fig. 4	
	D. pulex	Table 4 and Fig. 4	
	D. galeata	Table 4 and Fig. 4	
Schulz and Sterner 1999	*D. magna*	Fig. 2	*
Boersma 2000	*D. magna*	Fig. 2	
Urabe and Sterner 2001	*D. obtusa*	Table 2	*
Elser et al. 2001	*D. dentifera*	Fig. 2	*
Urabe et al. 2002	*D. dentifera*	Fig. 5	
Park et al. 2002	*D. magna*	Fig. 3 and Fig. 4	
Hessen et al. 2002	*D. magna*	Fig. 6	
	D. magna	Fig. 8	*
Darchambeau et al. 2003	*D. magna*	Fig. 3	

Note : * = experiments used in the meta-analysis.

Chapter 5

How *Daphnia* copes with excess carbon in its food

After focusing on filtration and growth responses of Daphnia, let us switch our attention to the carbon budget of Daphnia feeding. The effects of food quality on assimilation and respiration will be the core of this Chapter.

How *Daphnia* copes with excess carbon in its food

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ABSTRACT

Animals that maintain near homeostatic elemental ratios may get rid of excess ingested elements from their food in different ways. Carbon (C) regulation was studied in juveniles of *Daphnia magna* feeding on two *Selenastrum capricornutum* cultures contrasting in phosphorus (P) content (400 and 80 C:P atomic ratios). Both cultures were labelled with ^{14}C in order to measure *Daphnia* ingestion and assimilation rates. No significant difference in ingestion rates was observed between P-low and P-rich food, whereas the net assimilation of ^{14}C was higher in the treatment with P-rich algae. Some *Daphnia* were also homogeneously labelled over 5 days on radioactive algae to estimate respiration rates and excretion rates of dissolved organic C (DOC). The respiration rate for *Daphnia* fed with high C:P algae (38.7 % of body C day^{-1}) was significantly higher than for those feeding on low C:P algae (25.3 % of body C day^{-1}). The DOC excretion rate was also higher when animals were fed on P-low algae (13.4 % of body C day^{-1}) than on P-rich algae (5.7 % of body C day^{-1}) . When corrected for respiratory losses, total assimilation of C did not differ significantly between treatments (around 60 % of body C day^{-1}). Judging from these experiments, *D. magna* can maintain its stoichiometric balance when feeding on unbalanced diets (high C:P) primarily by disposing of excess dietary carbon via respiration and excretion of DOC.

KEY-WORDS

Assimilation, Carbon:phosphorus ratios, Dissolved organic carbon excretion, Homeostasis, Respiration

INTRODUCTION

The highly variable nutrient stoichiometry in autotrophs may be a challenge for grazers that have a tighter elemental regulation. There is commonly a mismatch in elemental ratios between requirements and supplies (Hessen 1992; Urabe and Watanabe 1992; Elser et al. 2000). In daphniids, which commonly are the most important grazers in lakes, phosphorus (P) requirement for somatic growth is high whereas freshwater algae are frequently P deficient (Hessen 1992; Sterner 1993). Phytoplankton in lakes often shows very high carbon(C):P ratios in comparison with consumer C:P ratios (Elser and Hassett 1994) and this could lead to P limitation of daphniid growth and lower food chain production (Sterner and Hessen 1994; Hessen 1997; Sterner et al. 1998; Hessen and Faafeng 2000). Two parallel physiological adaptations which may be used by animals to cope with this excess dietary C have been suggested : (1) improvement of the assimilation of the limiting element, and (2) reduced intake, storage or disposal of the element in excess. There are three potential physiological solutions to the problem of excess C in a herbivore's diet (Sterner and Hessen 1994). First, P-rich animals could reduce the C assimilation efficiency across the gut, while maintaining a high P assimilation efficiency. Or, if assimilated, excess C may be stored internally in C-rich compounds like lipids. Finally, assimilated C may be disposed of through respiration or extracellular release of organic C compounds.

The first hypothesis, a reduction of C assimilation efficiency, was advocated in a study by DeMott et al. (1998) on *Daphnia magna*. They fed animals with a mixture of P-sufficient and P-deficient *Scenedesmus*, and observed a decrease of 30% in C assimilation efficiency while P assimilation efficiency remained constant when the atomic food C:P ratio increased from 80 to 164. The enzymatic responses at the basis of this regulation have not been explored. The second hypothesis, storage of excess C, does not seem to be very realistic in fast-growing homeostatic organisms (i.e. during juvenile development). Feeding zooplankters with P-enriched seston did not alter the P:dry weight (DW) ratio of *Daphnia longispina* and *Bosmina coregoni* (Andersen and Hessen 1991). Still the use of adipose tissue for energy storage is widespread among marine copepods (Båmstedt 1986), and also *Daphnia* may display visible stores of internal lipids in the last juvenile instar and in adults (Tessier and Goulden 1982; Goulden and Place 1990). Yet, it is not clear how this would affect body C:P ratios. Lipid accumulation in *Daphnia* could be a sign of a P-deficient diet (Groeger et al. 1991; Sterner et al. 1992; Sterner 1993). Still the rather tight homeostatic control in *Daphnia*, unless dietary C:P is extremely high, suggests that C storage is not very pronounced. DeMott et al. (1998) observed marked declines in P:DW when feeding *D. magna* with algae with very high C:P ratio (920). These

findings are probably more likely to originate from a P deficiency in body tissue than from an accumulation of C.

The third hypothesis has been less well explored. The ability of zooplankters to modify their respiratory rate or dissolved organic carbon (DOC) excretion rate when facing low food quality can induce relevant impacts on secondary production and elemental C pathways in ecosystems (Sterner 1997). More precisely, release of DOC vs. CO_2 might have marked consequences on the transfer of algal C to the microbial loop and the CO_2 balance of the system. Release of DOC through grazing has been well studied since Lampert's (1978) seminal works (e.g. Copping and Lorenzen 1980; Olsen et al. 1986; Richardot et al. 2001) and seems to originate from three different sources : (1) losses of organic substrates from prey during handling and feeding ("sloppy feeding"), (2) leakage of DOC from the faeces, and (3) actual secretion of DOC by the animal. Studies on the impact of grazing on DOC and bacterial uptake do not commonly distinguish between these three sources, and the contribution of each source to total flux is still unclear. Sloppy feeding cannot be element specific. We can assume that in this process all elements are lost in a proportion equivalent to that in the original food. So sloppy feeding cannot be used as a process for homeostatic regulation. Release from faeces is linked to the regulation of assimilation efficiency. If less C is assimilated from food during transfer in the gut, more C will potentially be lost via faeces. Direct excretion of dissolved organic carbon (Gardner and Paffenhöfer 1982) could be an interesting way for daphniids to get rid of previously assimilated C-rich, P-poor macromolecules like proteins (Elser et al. 1996), but this process has never been quantitatively studied. Likewise, although suggested by Plath and Boersma (2001), the ability of daphniids to increase their respiration rates when feeding on C-rich algae has never been studied directly. Thus Sterner's (1997) humorous remark : "the animal doing extra work in its environment (swimming ?) in order to maintain its homeostasis" is still largely undebated, although studies by Sterner himself (Sterner et al. 1993) indicate, in fact, that strongly P-deprived animals become notoriously sluggish. This response will depend not only on the access to P relative to C, but also on the absolute availability of P for the animals.

In this study we attempted, by use of tracer experiments, to reveal the impact of a food-quality deficiency on ingestion, C assimilation, respiration and DOC excretion rates in *Daphnia magna* Straus.

MATERIAL AND METHODS

Algae

The green alga *Selenastrum capricornutum* Printz was grown in the same continuous culture system as described by Hessen et al. (2002). Cultures were run in COMBO medium (Kilham et al. 1998) with a dilution rate of 0.4 day^{-1} under two P regimes: 50 µM (P saturation) and 2.5 µM (P limitation). Both received a nominal light intensity of 70 µmol quanta m^{-2} s^{-1}. These two regimes, referred to respectively as high quality (HQ) and low quality (LQ) treatments, supplied algae with C:P ratios nearing respectively 80 and 400 (atomic values). *Selenastrum* cultures were allowed to grow for two weeks before the start of labelling and experiments with *Daphnia*, in order to obtain a stable level of cell numbers, cell volumes and particulate C, nitrogen (N) and P. For *Daphnia* incubations and labelling, the algae were labelled with 7.3 MBq of $NaH^{14}CO_3$ l^{-1} for a 2-day period in batch cultures. Medium and radioactive substrate were replaced twice a day at the same dilution rate that algae underwent in the continuous system. This procedure provided saturating, uniform algal labelling with algal C:P ratios similar to those observed in the algae taken directly from the continuous cultures (data not shown). High specific radioactivities were obtained (ca. 25,000 and 15,000 dpm μg^{-1} C, respectively for P-rich and P-poor *Selenastrum* cultures). Algae from all cultures, i.e. from all P treatments and from continuous or batch cultures, were diluted to standardized particulate C concentrations of about 4 mg l^{-1} before being fed to the *Daphnia* cultures.

Daphnia *cultures and experimental conditions*

The zooplankters *D. magna* were taken from a non-monoclonal laboratory culture. They have been grown on *S. capricornutum* for > 5 years. For our experiments, all neonates produced by several mothers in < 24 h were isolated and fed with P-rich algae in saturating concentrations. All experiments were performed at 20°C and subdivided into 2 treatments : animals fed HQ- or LQ-*Selenastrum* cells.

Ingestion experiment

Fifteen beakers per treatment were filled with 25 ml of LQ- or HQ-unlabelled algae at 4 mgC l^{-1}. This concentration is far above the incipient limiting level for *D. magna* (Lampert 1987). Five 6-day-old *Daphnia* were gently transferred to each beaker and acclimatized to food concentration and quality. After 1 h, unlabelled algae were replaced by labelled algae with the corresponding qualities. After 0, 1, 3, 6 and 10 min, three beakers per treatment were filtered and all *Daphnia* were carefully rinsed in cold

medium before being transferred to scintillation vials for liquid scintillation counting. Five millilitres from each beaker was sampled for counting food radioactivity. The mass-specific amount of ingested C was calculated for each beaker by the following equation:

$$I = \frac{A_{zoo}}{SA\ N\ W} 100$$

where I = mass-specific amount of ingested C (*% of body C*), A_{zoo} = activity in daphniids (*dpm*), SA = specific activity in algae (*dpm* μg^{-1} *C*), N = number of valid *Daphnia* in the beaker (*individuals; ind*), W = mean body weight of a 6-day-old *Daphnia* ($\mu gC\ ind^{-1}$). The mass-specific ingestion rate of *Daphnia* was calculated for each treatment by a linear least-squares regression of mass-specific amounts of ingested C against feeding time on labelled algae.

Assimilation experiment

Four beakers per treatment were filled with 50 ml of LQ- or HQ-labelled algae. Ten 6-day-old *Daphnia* were gently transferred to each beaker. After 1 h and 3 h, two beakers per treatment were filtered and the animals were well rinsed, harvested in groups of two to six, and their radioactivity measured. Samples of water were taken at the start and at the end of each incubation for counting C and radioactivity in algae. The net C assimilation rate was calculated for each *Daphnia* activity count by:

$$net\ \rho_{01} = \left(\frac{A_x}{SA\ N\ W} 100 - DT \right) \frac{1}{t} \quad (1)$$

where $net\ \rho_{01}$ = net C assimilation rate (*% of body C* h^{-1}), A_x = activity in *Daphnia* after x hours (*dpm*), N = number of harvested *Daphnia* (*ind*), DT = estimated C mass in digestive tract (*% of body C*), t = time (*1 or 3 h*). The y-intercept of a linear least-squares regression of all values of

$$\frac{A_x}{SA\ N\ W} 100 \text{ (\% of body C)}$$

versus time of incubation was considered as a good approximation of DT. As a part of the freshly assimilated C is already respired during the incubations, we need to correct the net assimilation values for ^{14}C losses before estimation of total assimilation rates (Lampert 1977). We used respiration rates calculated from the respiration experiment (see below) to estimate the losses. So, the total C assimilation rate is expressed as:

$$\rho_{01} = \frac{TA_x}{t} \quad (2)$$

where ρ_{01} = total C assimilation rate (*% of body C* h^{-1}), TA_x = total assimilated C after x hours (*% of body C*), with:

$$TA_x = \left(\frac{A_x}{SA\ N\ W} 100 - DT_C \right) (1 + L_x) \quad (3)$$

where DT_C = corrected C mass in digestive tract (*% of body C*), L_x = estimated relative losses by respiration of freshly assimilated ^{14}C after x hours (no units), defined by Eq. 6 (see below). DT_C was estimated by calculating the y-intercept of a linear least-squares regression of all values of TA_1 and TA_3 versus time. As knowledge of DT_C is needed to calculate TA_x, and vice versa, both values were successively processed until stabilisation to the third decimal was reached.

Respiration/Excretion experiment

One-day-old *Daphnia* were fed with ^{14}C labelled P-rich *Selenastrum*. This procedure made it possible to obtain a perfectly stable specific activity in animals after only 4 days (data not shown). Six-day-old labelled *Daphnia* were gently rinsed and incubated in groups of five in beakers filled with 10 ml HQ- or LQ-unlabelled algal suspensions. Five beakers were used per treatment. All incubations were made in the dark to avoid re-incorporation of $^{14}CO_2$ by algae. One *Daphnia* was collected with 2 ml of suspension from each beaker after 10, 20, 30, 60 and 120 min. *Daphnia* radioactivity, total dissolved ^{14}C and $DO^{14}C$ were measured. We made use of the expected increases in both $^{14}CO_2$ and $DO^{14}C$ in the beakers so as to estimate, respectively, respiration and DOC excretion rates. $^{14}CO_2$ and $DO^{14}C$ data were first expressed in percentage of initial radioactivity in the *Daphnia*, and as animals were homogeneously labelled then in percentage of body C.

Analytical protocols

Radioactivity counting - Animals were dissolved in 1 mL of tissue solubilizer (Soluene®-350, Packard), and were dried overnight at 60°C. The water samples for counting specific activity in algae were filtered on GF/F Whatman filters. Filters were well rinsed with 0.1 N HCl before counting. Radioactivity in filtered water was measured with or without addition of 25 µl ml^{-1} of 1 N HCl. The acidified water was gently shaken overnight before counting. The radioactivity in filtered water without acid was due to total dissolved ^{14}C while the radioactivity in acidified water resulted from $DO^{14}C$. The difference between both is a measure of $^{14}CO_2$. All scintillation vials were filled with 10 mL of an environmentally friendly liquid scintillation cocktail (Ultima Gold, Packard) and were counted for radioactivity with a Packard scintillation counter. Quenching was corrected by a procedure using an internal standard.

Elemental analyses - Algal cells were collected on acid-washed and pre-ignited GF/F Whatman filters. Six-day-old *Daphnia*, reared in parallel to

those used in experiments, were placed in tin capsules and dried (60°C, 24 h). Particulate C in both algae and *Daphnia* was analyzed on a Carlo-Erba CHN 1106 elemental analyser. Total P was analyzed by a modified procedure with peroxi-disulfate digestion followed by spectrophotometric determination of phosphate (Hessen et al. 2002).

Tracer kinetic modelling

The results of the respiration experiment, i.e. the increase in $^{14}CO_2$ in water or the corresponding decrease in *Daphnia*'s body radioactivity due to respiration, were fitted with an adjustment of Lampert and Gabriel's (1984) model. The resulting respiration rates were used to estimate the relative losses of freshly assimilated labelled C in the assimilation experiment. The physiological model of Lampert and Gabriel (1984), presented in Fig. 5.1, is based on the assumption that the kinetics of the C tracer in small zooplankton is well described by a two-compartment model consisting of a small metabolic pool with a fast turnover rate and a large structural pool with a slow turnover rate (Conover and Francis 1973; Lampert 1975).

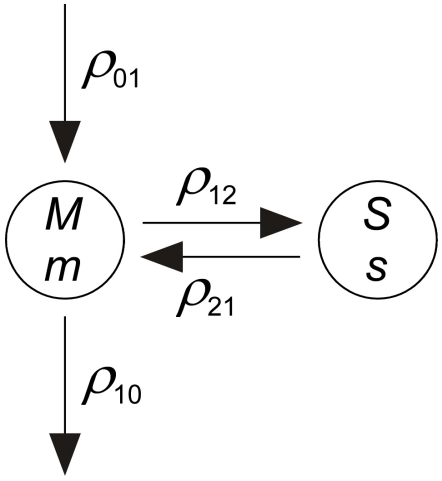

Fig. 5.1: Two-compartment model for tracer kinetics in Daphnia (modified from Lampert and Gabriel 1984). ρ_{01} C assimilation, ρ_{12} transfer rate of C from metabolic to structural pool, ρ_{21} transfer rate of C from structural to metabolic pool, ρ_{10} respiration rate, *S* relative size of structural pool, *s* quantity of radioactive tracer in structural pool, *M* relative size of metabolic pool, *m* quantity of radioactive tracer in metabolic pool.

Modelling of kinetics in the respiration experiment – We first used this model to develop equations for calculating the variations of radioactivity over time in homogeneously labelled *Daphnia* feeding on unlabelled algae (as was the case in the respiration experiment). The entire demonstration of the model is presented in Appendix A. All symbols are explained in Table 5.1.

The kinetic of total *Daphnia* radioactivity, $m + s$, as a function of time is described by:

$$m + s = a_1 \exp(\lambda_1 t)\left(1 + \frac{\rho_{12}/M}{\lambda_1 + \rho_{21}/S}\right) + a_2 \exp(\lambda_2 t)\left(1 + \frac{\rho_{12}/M}{\lambda_2 + \rho_{21}/S}\right) \quad (4)$$

with:

$a_1 = m_0 - a_2$,

$$a_2 = \left[\frac{\lambda_1 + \rho_{21}/S}{\rho_{12}/M} s_0 - m_0\right] \frac{\lambda_2 + \rho_{21}/S}{\lambda_1 - \lambda_2},$$

$$\lambda_1 = -\frac{1}{2}(\rho_{12}/M + \rho_{21}/S + \rho_{10}/M) + \frac{1}{2}\sqrt{(\rho_{12}/M + \rho_{21}/S + \rho_{10}/M)^2 - 4\,\rho_{10}\rho_{21}/MS},$$

$$\lambda_2 = -\frac{1}{2}(\rho_{12}/M + \rho_{21}/S + \rho_{10}/M) - \frac{1}{2}\sqrt{(\rho_{12}/M + \rho_{21}/S + \rho_{10}/M)^2 - 4\,\rho_{10}\rho_{21}/MS}.$$

Thus, Eq. 4 is based on seven parameters: M, S, m_0, s_0, ρ_{10}, ρ_{21} and ρ_{12}. M and S are assumed to be absolutely invariant, and well estimated by Lampert and Gabriel (1984), equalling 1.6 and 98.4, respectively. m_0 and s_0 are measured at the start of the experiment:
$m_0 = (m+s)_0\, M$ and $s_0 = (m+s)_0\, S$.

The 3 remaining parameters, ρ_{10}, ρ_{21} and ρ_{12}, are connected by (Lampert and Gabriel 1984):

$$\rho_{12} = \frac{S}{M+S}(\rho_{01} - \rho_{10}) + \rho_{21}. \quad (5)$$

After substitution of ρ_{12} in Eq. 4 by Eq. 5, and as the C assimilation rate, ρ_{01}, is estimated from the assimilation experiment, in Eq. 4 ($m + s$) we are left with 2 unknowns, ρ_{10} and ρ_{21}, that were estimated by fitting experimental data from the respiration experiment with Eq. 4. The experimentally observed values for ($m + s$) were obtained by subtracting respired $^{14}CO_2$ (*in dpm*) from $(m + s)_0$.

Table 5.1: Presentation of symbols used in the two-compartment model for tracer kinetics in *Daphnia*.

Symbols	Descriptions	Units
M	relative size of metabolic pool	% of total body C
S	relative size of structural pool	% of total body C
m	quantity of radioactive tracer in metabolic pool	dpm
s	quantity of radioactive tracer in structural pool	dpm
ρ_{01}	C assimilation rate	% of total body C h^{-1}
ρ_{12}	transfer rate of C from metabolic to structural pool	% of total body C h^{-1}
ρ_{21}	transfer rate of C from structural to metabolic pool	% of total body C h^{-1}
ρ_{10}	respiration rate	% of total body C h^{-1}
t	time	h
m_0	quantity of radioactive tracer in metabolic pool at start of respiration/excretion experiment	dpm
s_0	quantity of radioactive tracer in structural pool at start of respiration/excretion experiment	dpm
$(m + s)_0$	quantity of radioactive tracer in total body at start of respiration/excretion experiment	dpm
W	body weight of a *Daphnia*	µg C
SA	specific activity in algae	dpm μg^{-1} C

Modelling of kinetics in the assimilation experiment – The same model was again used to develop equations for calculating the variations of radioactivity over time in each pool of unlabelled *Daphnia* feeding on labelled algae (as was the case in the assimilation experiment). The entire demonstration of the model is presented in Appendix B. The model was used to estimate the relative losses by respiration of freshly assimilated ^{14}C after x hours, L_x:

$$L_x = \frac{\int_0^x \frac{\rho_{10}}{M} m_t \, dt}{m_x + s_x} \tag{6}$$

with:

$$m_t = a_1 \exp(\lambda_1 t) + a_2 \exp(\lambda_2 t) + \frac{\rho_{01}}{\rho_{10} 100} W M SA ,$$

$$s_t = \frac{\rho_{12} a_1 / M}{\lambda_1 + \rho_{21}/S} \exp(\lambda_1 t) + \frac{\rho_{12} a_2 / M}{\lambda_2 + \rho_{21}/S} \exp(\lambda_2 t) + \frac{\rho_{12}\rho_{01}}{\rho_{10}\rho_{21}100} W\ S\ SA,$$

$$a_1 = -\left(a_2 + \frac{\rho_{01}}{\rho_{10}100} W\ M\ SA \right),$$

$$a_2 = \frac{\rho_{01}}{\rho_{10}100} W\ M\ SA \left(1 - \frac{\lambda_1 + \rho_{21}/S}{\rho_{21}/S} \right) \frac{\lambda_2 + \rho_{21}/S}{\lambda_1 - \lambda_2},$$

λ_1 and λ_2 as defined above.

Repeated estimation of the rates – In Eq. 6 the respiration rate ρ_{10} is used for evaluating respiratory losses L_x (needed for a correct estimation of the total C assimilation rate ρ_{01}, see Eq. 2 and 3) while at the same time the determination of ρ_{01} is required for the fitting of Eq. 4 and then estimation of ρ_{10}. Therefore, an iterative process was computed. Firstly, the data set of the assimilation experiment was used for estimating ρ_{01} by Eq. 2 and 3 but without correction for respired C. Secondly, the data set of the respiration experiment and the value found for ρ_{01} were used for estimating ρ_{10} and ρ_{21} by Eq. 4, and ρ_{12} by Eq. 5. The values found were then inserted in Eq. 6 to obtain the relative quantity of freshly assimilated C, L_x, that had already been respired. This was then re-used for estimating ρ_{01}, and so forth. The iterative process was pursued until stabilisation at the 3rd decimal of all values.

DOC excretion modelling

In order to estimate DOC excretion rates from labelled *Daphnia* feeding on unlabelled algae, we have applied a model describing the kinetics of $DO^{14}C$ accumulation in water during incubations. As there was no time lag during transfer from labelling culture to unlabelled algae except for 30 seconds of washing in COMBO medium, the digestive tracts of animals were most probably initially filled with labelled algae. As a consequence, $DO^{14}C$ entering the water during incubations could be from two sources : release in a dissolved form of organic ^{14}C from digestive tracts or faeces, and actual secretion of metabolised organic C-rich compounds from labelled body tissues. As gut passage time of *D. magna* is about 10 minutes, and knowing the extremely rapid diffusion of dissolved compounds from zooplankton faeces (90 % in less than 1 s for this kind of faeces, Jumars et al. 1989), we used the accumulation of $DO^{14}C$ only during the 20-min to 2-h interval as the basis for our model, assuming that this was primarily accounted for by metabolised DOC. Accumulation of $DO^{14}C$ in water over time was matched by a saturation curve with a positive non-zero intercept on the *y*-axis. This intercept, *F*, corresponds to the quantity of unassimilated organic ^{14}C leaked from digestive tracts or feces. The curvature parameter, ρ_{DOC}, corresponds to the actual excretion rate of DOC from body tissues, and the saturation level,

D, must be equal to the size of the kinetically homogeneous metabolic pool from which organic C is excreted. As *Daphnia* labelling was identical for all animals irrespective of the treatment they underwent during the excretion experiment, we can postulate that *F* was similar between treatments. Data from both treatments were thus used simultaneously for estimating *F*, whilst excretion rates and *D* were assumed to be treatment specific. Data were fitted by:

$$DOCe = F + \alpha D_{HQ}\left(1 - e^{-\frac{\rho_{DOC-HQ}}{D_{HQ}}t}\right) + (1-\alpha)D_{LQ}\left(1 - e^{-\frac{\rho_{DOC-LQ}}{D_{LQ}}t}\right) \quad (7)$$

where *DOCe* = total excreted $DO^{14}C$ after time *t* for both treatments (*% of total body C*), *F* = quantity of unassimilated organic ^{14}C leaked from digestive tracts or faeces (*% of total body C*), α = dummy variable, coded 0 for LQ data and 1 for HQ data (*no units*), D_{HQ} = size of metabolic pool source of organic C excretion in HQ treatment (*% of total body C*), D_{LQ} = size of metabolic pool source of organic C excretion in LQ treatment (*% of total body C*), $\rho_{DOC\text{-}HQ}$ = DOC excretion rate in HQ treatment (*% of total body C* h^{-1}), $\rho_{DOC\text{-}LQ}$ = DOC excretion rate in LQ treatment (*% of total body C* h^{-1}), *t* in hours.

We did not apply a multiple-compartment model for DOC kinetics, as we did for CO_2 kinetics, because the published background literature is still insufficient. Thus, this one-compartment model does not take into account any transfer during incubations of organic labelled compounds from another pool to *D*.

RESULTS

Ingestion

The incorporation of labelled algae was linear for the three first samples (up to 6 minutes) (Fig. 5.2). Judging from the increased variability of the 10-min sample, and also from the reduced rate of increase, some animals had a gut passage time <10 min, and thus the 10-min set was excluded from the regression on ingestion values. Ingestion rates did not differ between treatments (*two-tailed test for parallelism*, $P>0.60$, Fig. 5.3A).

Assimilation

The net assimilation rates were calculated by Eq. 1 (Fig. 5.4A). These rates differed significantly between treatments (*two-tailed t-test*, $P<0.001$). We used respiration rates calculated from the respiration experiment (see below)

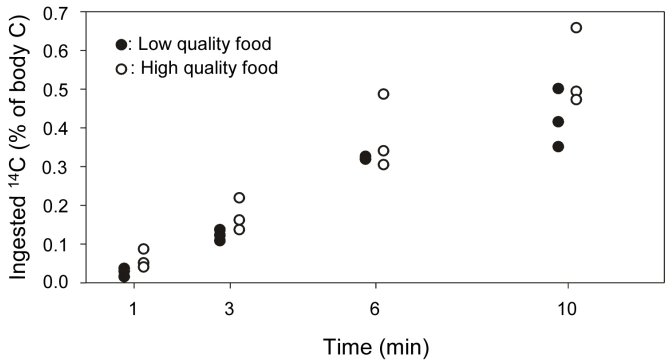

Fig. 5.2: Incorporation of ^{14}C by unlabelled *Daphnia* feeding on labelled algae. Each value represents one beaker with 5 *Daphnia*. Calculated ingestion rates are presented in Fig. 5.3A.

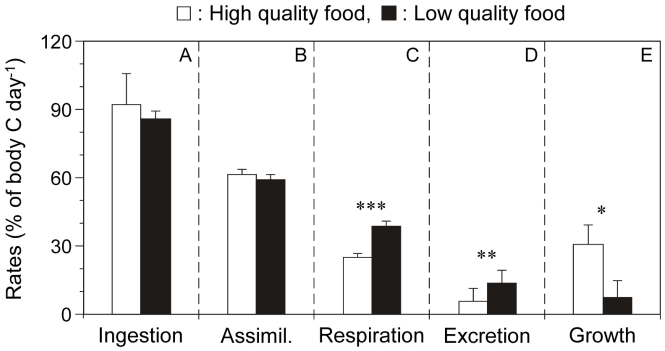

Fig. 5.3: Synthesis of estimated rates in *Daphnia*. **A** Ingestion rate, **B** total assimilation (*Assimil.*) rate, **C** respiration rate, **D** dissolved organic C (DOC) excretion rate, **E** growth rate. *Vertical bars* represent 1 SE. Respiration and DOC excretion rates were statistically higher in the low quality treatment, while growth rate was lower (one-tailed *t*-test, ∗ $P<0.05$, ∗∗ $P<0.025$, ∗∗∗ $P<0.001$).

to estimate losses by respiration of freshly assimilated ^{14}C during assimilation incubations. Eq. 6 yielded respiratory losses of ^{14}C equal to 19.6 and 29.1 % of net assimilated ^{14}C after respectively 1 and 3 h of incubation for the HQ treatment, and 41.7 and 78.9 % for the LQ treatment. After correcting net assimilated ^{14}C data for respiration losses (see Eq. 2 and 3), the difference in total C assimilation rate between HQ and LQ treatments

became insignificant (P>0.50, Fig. 5.4B). Thus, gross C assimilation efficiencies (69.3 % and 68.0 % respectively for LQ and HQ treatments) were similar between treatments.

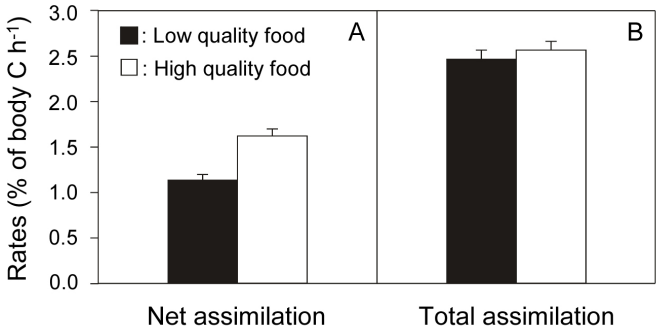

Fig. 5.4: Assimilation rates of ^{14}C by unlabelled *Daphnia* feeding on labelled algae. *Vertical bars* represent 1 SE. **A** Net assimilation rates are calculated without correction of incorporation values for ^{14}C losses by respiration. They are highly significantly different between treatments (two-tailed *t*-test, P<0.001). **B** Total assimilation rates are calculated after correction for respiratory losses. They are not significantly different between treatments (P>0.50).

Respiration

Higher accumulations of $^{14}CO_2$ were found in the beakers where *Daphnia* were fed with LQ food compared with the HQ treatment (Fig. 5.5). This suggests a higher respiration rate for *Daphnia* fed with low-P algae. Our data gave close fits with the two-compartment model (r^2 = 0.90 and 0.95 respectively for LQ and HQ treatments). Fitting data allowed us to estimate ρ_{10} and ρ_{21} for both treatments, and then ρ_{12} was calculated by Eq. 5 (Table 5.2). The estimated respiration rate (ρ_{10}) for the LQ treatment was higher than the one in the HQ treatment (*one-tailed t-test*, P< 0.001, Fig. 5.3C). However, the transfer rate between metabolic and structural pools (ρ_{12}) and the inverse flux (ρ_{21}) were both much lower in the LQ than in the HQ treatment (P<0.001 for ρ_{12}, P<0.025 for ρ_{21}). It is worth mentioning that, after 10 min of incubation, the amount of $^{14}CO_2$ released by *Daphnia* was already higher in the LQ treatment than in the HQ treatment (P<0.025).

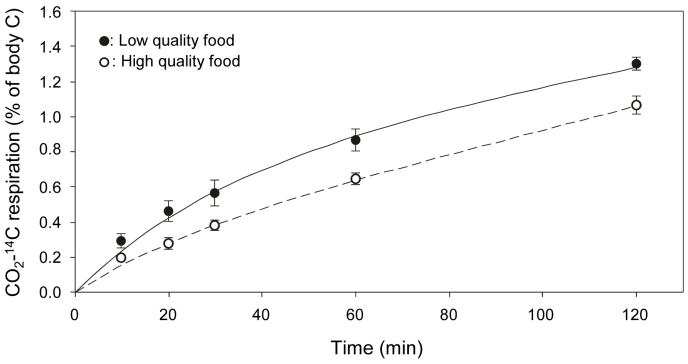

Fig. 5.5: Respiration of $^{14}CO_2$ by labelled *Daphnia* feeding on unlabelled algae. *Vertical bars* represent ± 1 SE. Lines are adjusted by modelling, not by regressions. Calculated respiration rates are presented in Fig. 5.3C.

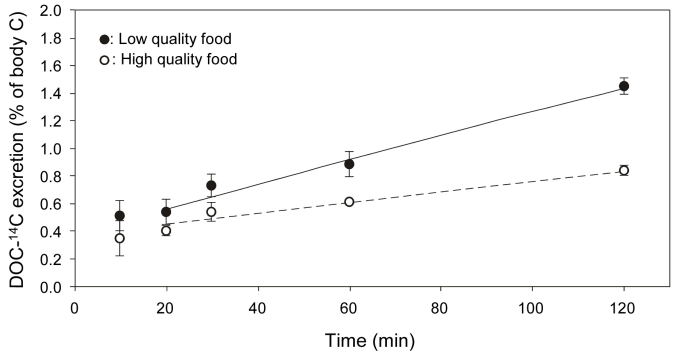

Fig. 5.6: Excretion of DO^{14}C by labelled *Daphnia* feeding on unlabelled algae. *Vertical bars* represent ± 1 SE. Lines are adjusted by modelling, not by regressions. Values at time=10 min are plotted, but were not used for modelling. Calculated DOC excretion rates are presented in Fig. 5.3D.

DOC excretion

As for release of $^{14}CO_2$, accumulations of DO^{14}C in beakers differed significantly (*two-tailed t-test*, e.g. $P<0.05$ for 1-h-data and $P<0.001$ for 3-h-data) indicating a higher DOC excretion rate for *Daphnia* feeding on P-deficient algae (Fig. 5.6). The *y*-variance of these data was well explained by the model of Eq. 7 (r^2 = 0.78 and 0.73, respectively, for LQ and HQ

treatments). Results of modelling are presented in Table 5.2. The estimated excretion rate of DOC was higher in the LQ treatment than in the HQ treatment (*one-tailed t-test*, $P<0.025$, Fig. 5.3D).

Table 5.2: Results of the assimilation and respiration/excretion experiments of *Daphnia magna* feeding on both *Selenastrum capricornutum* cultures of varying phosphorus quality. Algal C:P ratio was equal to 80 and 400, respectively in high quality (HQ) and low quality (LQ) treatments. C assimilation rate (ρ_{01}), respiration rates (ρ_{10}), transfer rates of C from structural to metabolic pool (ρ_{21}), transfer rates of C from metabolic to structural pool (ρ_{12}) and DOC excretion rates (ρ_{DOC}) are in % of body C ind^{-1} h^{-1}. The quantity of unassimilated organic ^{14}C leaked from digestive tracts or faces (F) and sizes of metabolic pool source of organic C excretion (D_{HQ} and D_{LQ}) are in % of body C $individual^{-1}$. Data are means ± 1 SE. Values are obtained by either direct measurement (M), or indirect calculation (C), or fitting with experimental observations.

	HQ food	LQ food	
Respiration			
ρ_{01}	2.56 ± 0.10	2.47 ± 0.10	M
ρ_{10}	1.05 ± 0.07	1.61 ± 0.10	F
ρ_{21}	1.58 ± 0.41	0.56 ± 0.23	F
ρ_{12}	3.06 ± 0.42	1.41 ± 0.26	C
Excretion			
F	0.38 ± 0.09		
ρ_{DOC}	0.24 ± 0.23	0.56 ± 0.23	F
D_{HQ} *and* D_{LQ}	6.94 ± 165	10.0 ± 65.1	F

DISCUSSION

The offered food "quality" in our experiments represented a C:P ratio (by atoms) of 80 and 400. Food concentrations were kept above the incipient limiting level. Using Sterner's (1997) model, we calculated the food C:P threshold for the onset of P limitation in the consumer. When using our ingestion, assimilation and respiration data, an atomic C:P ratio in *D. magna* of 80 (Vrede et al. 1999) and a P assimilation efficiency of 0.8 (DeMott et al. 1998), we obtained food C:P thresholds between 120 and 160. Thus, while a C:P of 400 is well below the applied maximum of ca. 1,500 that may be observed in *S. capricornutum* under severe P deficiency (Hessen et al. 2002), our LQ treatment should nevertheless represent food quality causing P limitation and thus C in excess. Inversely, the food C:P ratio in the HQ treatment (80) was well below these limits and thus growth of *Daphnia* in this case was not limited by P. All experiments were made with animals grown on P-saturated food in order to obtain equally fit animals, and thus to highlight the immediate effects of dietary P depletion on digestion and respiration traits. Resource depression is a common seasonal process in nature (see e.g. Sterner 1998).

Ingestion and assimilation

Filtration and C ingestion rates measured in our short-term incubations (< 10 min) were not affected by food quality. These observations agree with results from short-term experiments with the rotifer *Brachionus rubens* (Rothhaupt 1995) and *D. magna* (DeMott et al. 1998). Yet some studies have shown apparently reduced algal clearance rates in long-term (20 h - 2 day) incubations of daphniids feeding on P-limited chlorophytes (Van Donk and Hessen 1993; Sterner et al. 1993; Sterner and Smith 1993). So far these results do not really offer the possibility to draw conclusions about the regulation of ingestion rates because, as already pointed out by Sterner and Hessen (1994), these data were influenced by the gut passage of intact algal cells as observed for extremely P-limited algae (C:P > 1000) in batch culture (Van Donk and Hessen 1993). Experiments using semicontinuous cultures or chemostats have been unable to verify this effect (DeMott et al. 1998; Hessen et al. 2002).

In our tested range of algal C:P ratios (80-400), we failed to observe any difference in total C assimilation rate and C assimilation efficiency between treatments in *D. magna*, in contrast with DeMott et al. (1998). In experiments with *Scenedesmus* as food they observed a constant decrease in C assimilation efficiency (from 0.92 to 0.50) when food C:P increased from 80 to 475 (see their Fig. 7). This could in part be attributed to differing algal species. Moreover our estimated assimilation rates were based on ingestion

of labelled food during a 3-h period corrected for C losses due to respiration during incubations. By contrast, the rates measured by DeMott et al. (1998) were not corrected for respiratory losses of C from the metabolic pool during their 40-min incubations. As we observed an increased respiration rate in the LQ treatment, the reduced accumulation (net assimilation) of labelled C in the LQ treatment relative to the HQ one would also be due to an immediate loss of label from the metabolic pool. If the increased respiration rate is not corrected for, this will cause underestimation of assimilation rates when food C:P increases. This alone could explain the differences in assimilation rates that DeMott et al. (1998) observed between food of contrasting food C:P ratios. Also the rapid decline of the apparent assimilation rates observed in their experiment after only 30 min of feeding with P-deficient algae could be explained by rapid increases in respiration rate, as observed in our experiment after only 10 min.

Respiration

The two-compartment model (Lampert and Gabriel 1984) described satisfactorily the kinetics of the radioactive C tracer in *Daphnia* (see Fig. 5.5). The CO_2 excretion rate we measured for P-saturated algae (HQ treatment) is very similar to that observed by Lampert and Bohrer (1984) in the same species at high algal biomass, i.e. 25.3 % and 23.5 %, respectively, of total body C day^{-1}. A deficiency of P in the diet (LQ treatment) led to a higher respiration rate (38.7 % of total body C day^{-1}) and to an important decrease in transfer rates between metabolic and structural pools. Thus, when food becomes P-limited, more C is lost through respiration, and less C is allocated to the body structure. This important decrease in transfer rate between metabolic and structural pools could explain the reported lower growth rates when herbivores feed on mineral-deficient food (see e.g. Sterner et al. 1993; Hessen et al. 2002). We do not know whether this represents a transient situation, however. We transferred animals from labelled HQ food to unlabelled LQ food, and thus a full metabolic adjustment to LQ food may not have occurred. It is not clear how this increased respiration (and thus extra gain of energy and ATP) may affect the animals in the long run.

Plath and Boersma (2001) observed highly increased appendage beat rates of *D. magna* feeding on P-deficient algae in comparison with when feeding on algae richer in P. The authors suggested that the predicted resulting increase in filtration rate, an energy (thus C) costly process, offers a mechanistic explanation of the observed relative homeostasis of the animal. Our data confirm that the increase in the respiration rate is a process used by this species when facing a dietary nutrient deficiency, but they offer no identification of the energy expending process. We measured the filtration

rate only during the first 10 min after the transfer to the LQ food and observed no difference with the one for HQ algae, whereas the respiration rates were estimated over a period of 2 hours. Of course, we do not know how the filtration rate may have evolved after the first 10 minutes. But the fact that already after 10 min the respiration rate in the treatment with LQ algae was higher than in the one with HQ food suggests that filtration is certainly not the only energy expending process which increases in the case of dietary nutrient deficiency.

Our observation of increased respiration rate in a crustacean zooplankter when feeding on mineral-deficient algae supports data on heterotrophic bacteria. Both theoretical and experimental studies have demonstrated higher individual respiration rates and lower gross growth efficiencies in marine bacteria when substrate C:N ratios increase (Harder and Dijkhuizen 1983; Billen 1984; Lancelot and Billen 1985; Goldman et al. 1987; Hopkinson et al. 1989). Similar trends were more recently observed for freshwater bacteria with increasing substrate C:P and C:N ratios (Cimbleris and Kalff 1998; Biddanda et al. 2001). Higher seston C:N or C:P ratios indicate lower substrate availability for bacteria, which generates heavier costs of biosyntheses and increases catabolism of carbon compounds (Goldman et al. 1987; Cimbleris and Kalff 1998). These effects could thus be a general property for heterotrophs facing an unbalanced diet with high C:P or C:N. Note that the same principle may hold for photorespiration observed in green algae and C-3 higher plants when improving high light levels. Although resorting to a completely different biochemical pathway, this extracellular release of CO_2 can also be described as a process preserved through evolution allowing organisms to maintain cellular homeostasis in case of high energy input (see e.g. Salisbury and Ross 1992; Reynolds 1997).

DOC excretion

To our knowledge, this is the first time that excretion rates of DOC directly released from body tissues have been estimated in crustacean zooplankton. In the literature, impacts of grazing on DOC stocks and bacterial uptakes have often been mentioned (e.g. Hygum et al. 1997). Due to methodological constraints, however, the mechanisms of DOC release have rarely been investigated (Park et al. 1997, Strom et al. 1997). In experiments where animals are fed with labelled algae, the release of DOC from different sources (sloppy feeding, egestion of unassimilated compounds and release from faeces) has rarely been considered, and direct DOC excretion is rarely taken into account.

Nevertheless we can try to compare the DOC excretion rates we estimated with those of three previously published studies : Lampert (1978) and Olsen

et al. (1986) who both estimated DOC release by using radiotracers, and Strom et al. (1997) who simply measured the quantity of released DOC with or without grazers. We indirectly estimated *Daphnia pulex* DOC excretion rates in Lampert's experiments (Lampert 1978) by calculating the mean difference between his second (long-term, 3 h) and his first (short-term, 15 min) series of incubations (see his Table 1). This difference is an indirect measurement of the fraction of the ingested food released as DOC by any process but that of sloppy feeding. It was found to be equal to 4.7 % of ingested C. Olsen et al. (1986) found $DO^{14}C$ release rates in the same order of magnitude (5 % of ingested C) for *D. magna* feeding on unlabelled *Scenedesmus acutus* after 1 h of labelling. In our experiment, we estimated a very similar DOC excretion rate of 6.1 % of ingested C when animals were fed on P-saturated algae (HQ treatment). We observed that *Daphnia*, when shifted to a diet of P-deficient algae, increased its DOC excretion rate to 15.6 % of ingested C (LQ treatment). This rather high rate is still within the range of previous studies. Strom et al. (1997) estimated *Calanus pacificus* DOC production due to direct excretion to be between 16 and 21 % of ingested C. As for the respiration estimates above, it should also be remarked upon here that the high rate of DOC excretion observed in the LQ treatment could partly be a transient situation since animals were transferred from HQ food to LQ food. Hence while the general difference between HQ and LQ treatments stands, the absolute rate in the LQ treatment should be treated with some caution.

The DOC excretion rate in *D. magna* was more than twice as high when it feed on LQ algae (high C:P ratio) than on P-rich algae. Thus, to regulate excess C in their diet, daphniids could greatly enhance the metabolization of stored or structural compounds assimilated before changes in the quality of food took place. It means that daphniids, depending on food quality, could be able to regulate their disposal of C-rich compounds through DOC excretion. Biochemical description of these excreted organic compounds as a function of food C:P ratio would be of great interest in the understanding of zooplankton grazing impact on bacterial productivity (Rosenstock and Simon 2001; Richardot et al. 2001).

Impact on growth rate and carbon flux

We calculated growth rate for both treatments as the difference between assimilation rate and loss rates (respiration and DOC excretion) (Fig. 5.3E). The observed differences in loss rates between treatments resulted in a 4-fold reduction of *Daphnia* growth rate when feeding on LQ food (*one-tailed t-test*, $P<0.05$). Although this reduction supports the general findings that growth of *Daphnia* may be limited by low P in the food, the reduction of growth is more severe than reported in previous studies (e.g. Sterner 1993,

Urabe et al. 1997, DeMott et al. 1998, Hessen et al. 2002). Growth rates cited in the literature are often obtained after long-term (at least a few days) incubations of animals, whereas in our study we calculated instantaneous rates obtained without prior acclimatization to food quality (with the exception of 1 h acclimatization for assimilation rates). Although different algae could yield different growth rates both under high and low C:P, it may also emphasize the phenotypic capacity of *Daphnia* to gradually implement other physiological mechanisms, like increased assimilation efficiency for P relative to C, in order to tackle dietary deficiency.

In conclusion, our data suggest that increased respiration and increased release of C-rich compounds may be important mechanisms for metazoans to regulate excess C in food in order to maintain a rather rigid (though not totally homeostatic) control of elemental composition. In systems with temporal algal limitation by P, the expected increases in respiration and DOC excretion by herbivorous zooplankton could probably increase CO_2 output to the atmosphere, decrease zooplanktivorous fish production and favour the planktonic microbial loop. Since mineral limitation also seems to be a general phenomenon in marine and particularly terrestrial ecosystems (Elser and Hassett 1994; Elser et al. 2000), these regulation processes may potentially have great impacts on fluxes of C both on ecosystem and global scales.

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APPENDIX A: Demonstration of equations modelling evolution over time of total radioactivity in homogeneously labelled *Daphnia* feeding on unlabelled algae

The kinetics of the tracer in each pool is described by a system of two differential equations (see Table 5.1 for explanation of symbols):

$$\begin{cases} \frac{d(m)}{dt} = \frac{\rho_{21}}{S} s - \frac{\rho_{12}}{M} m - \frac{\rho_{10}}{M} m \\ \frac{d(s)}{dt} = \frac{\rho_{12}}{M} m - \frac{\rho_{21}}{S} s \end{cases}$$

We know that this kind of differential system has a solution of the type:

$$\begin{cases} m = a \exp(\lambda_j t) \\ s = b \exp(\lambda_j t) \end{cases} \quad (8)$$

In using this type of solution in the system, we found:

$$\begin{cases} \lambda_j a \exp(\lambda_j t) = \left[-\left(\frac{\rho_{12} + \rho_{10}}{M} \right) a + \frac{\rho_{21}}{S} b \right] \exp(\lambda_j t) \\ \lambda_j b \exp(\lambda_j t) = \left(\frac{\rho_{12}}{M} a - \frac{\rho_{21}}{S} b \right) \exp(\lambda_j t) \end{cases}$$

In simplifying this by $\exp(\lambda_j t)$, we found algebraic equations for a and b:

$$\begin{cases} -\left(\lambda_j + \frac{\rho_{12} + \rho_{10}}{M} \right) a + \frac{\rho_{21}}{S} b = 0 \\ \frac{\rho_{12}}{M} a - \left(\lambda_j + \frac{\rho_{21}}{S} \right) b = 0 \end{cases} \quad (9)$$

This system will give a non-trivial solution only if the next determinant

$$\begin{vmatrix} -\left(\lambda_j + \frac{\rho_{12} + \rho_{10}}{M} \right) & \frac{\rho_{21}}{S} \\ \frac{\rho_{12}}{M} & -\left(\lambda_j + \frac{\rho_{21}}{S} \right) \end{vmatrix} = \lambda_j^2 + (\rho_{12}/M + \rho_{21}/S + \rho_{10}/M)\lambda_j + \rho_{10}\rho_{21}/MS = 0$$

is equal to 0. This gives a quadratic equation in λ_j with 2 solutions:

$$\lambda_1 = -\tfrac{1}{2}(\rho_{12}/M + \rho_{21}/S + \rho_{10}/M) + \tfrac{1}{2}\sqrt{(\rho_{12}/M + \rho_{21}/S + \rho_{10}/M)^2 - 4\rho_{10}\rho_{21}/MS} \quad (10)$$

and

$$\lambda_2 = -\tfrac{1}{2}(\rho_{12}/M + \rho_{21}/S + \rho_{10}/M) - \tfrac{1}{2}\sqrt{(\rho_{12}/M + \rho_{21}/S + \rho_{10}/M)^2 - 4\rho_{10}\rho_{21}/MS} \quad (11)$$

We then rewrite the equations in system 8 (Eq. 8) under their decomposed form:

$$\begin{cases} m = a_1 \exp(\lambda_1 t) + a_2 \exp(\lambda_2 t) \\ s = b_1 \exp(\lambda_1 t) + b_2 \exp(\lambda_2 t) \end{cases} \quad (12)$$

We then rewrite the second equation in system 9 (Eq. 9) with decomposition of λ_j into λ_1 and λ_2:

$$\frac{\rho_{12}}{M} a_1 - \left(\lambda_1 + \frac{\rho_{21}}{S} \right) b_1 = 0 \Leftrightarrow b_1 = \frac{\rho_{12} a_1 / M}{\lambda_1 + \rho_{21}/S}$$

and

$$\frac{\rho_{12}}{M} a_2 - \left(\lambda_2 + \frac{\rho_{21}}{S} \right) b_2 = 0 \Leftrightarrow b_2 = \frac{\rho_{12} a_2 / M}{\lambda_2 + \rho_{21}/S}$$

If we insert these two equations into system 12 (Eq. 12), we obtain a new system of two linear equations with 2 variables a_1 and a_2:

$$\begin{cases} m = a_1 \exp(\lambda_1 t) + a_2 \exp(\lambda_2 t) \\ s = \dfrac{\rho_{12} a_1 / M}{\lambda_1 + \rho_{21}/S} \exp(\lambda_1 t) + \dfrac{\rho_{12} a_2 / M}{\lambda_2 + \rho_{21}/S} \exp(\lambda_2 t) \end{cases} \quad (13)$$

$$\Rightarrow m + s = a_1 \exp(\lambda_1 t) \left(1 + \frac{\rho_{12}/M}{\lambda_1 + \rho_{21}/S} \right) + a_2 \exp(\lambda_2 t) \left(1 + \frac{\rho_{12}/M}{\lambda_2 + \rho_{21}/S} \right)$$

Under initial conditions, system 13 (Eq. 13) becomes:

$$\begin{cases} m_0 = a_1 + a_2 \Leftrightarrow a_1 = m_0 - a_2 \\ s_0 = \dfrac{\rho_{12} a_1 / M}{\lambda_1 + \rho_{21}/S} + \dfrac{\rho_{12} a_2 / M}{\lambda_2 + \rho_{21}/S} \end{cases}$$

Thus,

$$s_0 = \frac{\rho_{12}}{M} \left(\frac{m_0 - a_2}{\lambda_1 + \rho_{21}/S} + \frac{a_2}{\lambda_2 + \rho_{21}/S} \right)$$

$$\Leftrightarrow a_2 = \left[\frac{\lambda_1 + \rho_{21}/S}{\rho_{12}/M} s_0 - m_0 \right] \frac{\lambda_2 + \rho_{21}/S}{\lambda_1 - \lambda_2}$$

APPENDIX B: Demonstration of equations modelling evolution over time of radioactivity in unlabelled *Daphnia* feeding on labelled algae

The kinetics of the tracer in each pool is described by (see Table 5.1 for explanation of symbols):

$$\begin{cases} \dfrac{d(m)}{dt} = \dfrac{\rho_{21}}{S} s - \dfrac{\rho_{12}}{M} m - \dfrac{\rho_{10}}{M} m + \dfrac{\rho_{01}}{100} W \ SA \\ \dfrac{d(s)}{dt} = \dfrac{\rho_{12}}{M} m - \dfrac{\rho_{21}}{S} s \end{cases} \quad (14)$$

Let us define

$$m_t = o_t + \alpha \tag{15}$$

and

$$s_t = u_t + \beta \tag{16}$$

that we insert in the system of differential equations:

$$\begin{cases} \frac{d(o)}{dt} = -\frac{(\rho_{12} + \rho_{10})}{M} o + \frac{\rho_{21}}{S} u - \frac{(\rho_{12} + \rho_{10})}{M} \alpha + \frac{\rho_{21}}{S} \beta + \frac{\rho_{01}}{100} W \ SA \\ \frac{d(u)}{dt} = \frac{\rho_{12}}{M} o - \frac{\rho_{21}}{S} u + \frac{\rho_{12}}{M} \alpha - \frac{\rho_{21}}{S} \beta \end{cases} \tag{17}$$

We can now determine α et β which nullify the non-homogeneous terms:

$$\begin{cases} \frac{(\rho_{12} + \rho_{10})}{M} \alpha - \frac{\rho_{21}}{S} \beta = \frac{\rho_{01}}{100} W \ SA \\ \frac{\rho_{12}}{M} \alpha - \frac{\rho_{21}}{S} \beta = 0 \end{cases}$$

$$\Leftrightarrow \begin{cases} \alpha = \frac{\rho_{01}}{\rho_{10} 100} W \ M \ SA \\ \beta = \frac{\rho_{12} \rho_{01}}{\rho_{10} \rho_{21} 100} W \ S \ SA \end{cases}$$

Both equations of system 17 (Eq. 17) become homogeneous:

$$\begin{cases} \frac{d(o)}{dt} = -\frac{(\rho_{12} + \rho_{10})}{M} o + \frac{\rho_{21}}{S} u \\ \frac{d(u)}{dt} = \frac{\rho_{12}}{M} o - \frac{\rho_{21}}{S} u \end{cases}$$

As for the problem defined in Appendix A, we know that the solution of this linear differential system is a linear combination:

$$\begin{cases} o_t = a_1 \exp(\lambda_1 t) + a_2 \exp(\lambda_2 t) \\ u_t = b_1 \exp(\lambda_1 t) + b_2 \exp(\lambda_2 t) \end{cases} \tag{18}$$

where, as in Appendix A, b_1 and b_2 are defined, respectively, by $b_1 = \frac{\rho_{12} a_1 / M}{\lambda_1 + \rho_{21}/S}$, and $b_2 = \frac{\rho_{12} a_2 / M}{\lambda_2 + \rho_{21}/S}$,

and λ_1 and λ_2, respectively, by Eqs. 10 and 11.

Under initial conditions, $m_0 = 0$ and $s_0 = 0$.

The insertion of Eqs. 15 and 16 into system 18 (Eq. 18) gives:

$$\begin{cases} -\alpha = a_1 + a_2 \Leftrightarrow a_1 = -(a_2 + \alpha) \\ -\beta = b_1 + b_2 \end{cases}$$

Thus,

$$-\beta = \frac{\rho_{12}}{M} \left[\frac{-(a_2 + \alpha)}{\lambda_1 + \rho_{21}/S} + \frac{a_2}{\lambda_2 + \rho_{21}/S} \right]$$

$$\Leftrightarrow a_2 = \left(\alpha - \frac{\lambda_1 + \rho_{21}/S}{\rho_{12}/M} \beta \right) \frac{\lambda_2 + \rho_{21}/S}{\lambda_1 - \lambda_2}$$

Chapter 6

Effects of zooplankton on phosphorus sedimentation

Beyond individual responses explored previously, we are now looking for the impacts of stoichiometric interactions at the algae-grazer interface on ecosystem processes. Particularly, this Chapter will explore the effects of zooplankton on nutrient sedimentation in a reservoir.

Effects of zooplankton on phosphorus sedimentation

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ABSTRACT

The global assessment of the effect of zooplankton on nutrient sedimentation is dependent on multiple factors, such as ecosystem morphometry, phytoplankton community and zooplankton species composition. Stoichiometric effects are expected on nutrient vertical fluxes. For instance, the role of P-rich cladocerans may be questioned. Their grazing activity should reduce the vertical P-flux, but the sedimentation of their remains, potentially P rich, may counterbalance this effect. A sedimentation study was conducted in the Esch-sur-Sûre reservoir, with the aim of clearly separating effects of grazing from effects of sedimenting remains or cast exuviae's on vertical nutrient fluxes. We used a sediment trap placed below the metalimnion for collecting sinking particles during a 2-year period, and sedimentation rates were correlated with phytoplankton production and zooplankton characteristics. Clearly, in this reservoir, zooplankton grazing reduced the export ratio of phytoplankton production. But, as expected, zooplankton contributed also significantly to loss of P associated to sedimenting large particles (> 250 μm). Nevertheless, more P was saved from sedimentation by zooplankton grazing than lost by sedimenting P-rich zooplankton remains. Significant relationships were also found between the time-variations of C:P and N:P ratios in zooplankton and the corresponding variations of C:P and N:P ratios in the sedimented small particles. These results support the influence of stoichiometry at the algae-grazer interface on nutrient ratios of sinking material. The sedimentation below the mixed layer of released nutrients questions the actual feedback effect of grazers on algae.

INTRODUCTION

In lakes and oceans, the vertical flux of small particulate matter is essentially constituted of two components (Sarnelle 1999): the directly sedimenting algae (e.g. Peinert et al. 1989, Larocque et al. 1996) and the faeces or faecal pellets of planktonic primary consumers (e.g. Honjo and Roman 1978, Pilskaln and Honjo 1987). Both particles will sediment below the thermocline if their sinking rate is higher than their mineralization rate. A simple heuristic model (Elser et al. 1995) explicitly represented the processes involved:

$$S_x = r_{z,x}\, e_x\, g\, P_x + r_x\, s_x\, (1-g) P_x$$

where S_x is the sedimentation rate of the element x, $r_{z,x}$ is the fraction refractory to mineralization of the egested (e_x), grazed (g) fraction of the production rate of element x (P_x), and r_x is the corresponding refractory fraction for the sedimenting (s_x), ungrazed ($1 - g$) fraction of the production rate of element x. For any given time interval we can express S_x as a fraction of element incorporated by autotrophic activity during that interval. In prior studies (Eppley and Peterson 1979, Baines and Pace 1994), this metric is referred to as the "export ratio" (ER) and can be defined for any element x (ER_x) (Elser et al. 1995):

$$ER_x = (S_x / P_x) = r_{z,x}\, e_x\, g + r_x\, s_x\, (1-g)$$
$$\Leftrightarrow ER_x = r_x\, s_x + g\,(r_{z,x}\, e_x - r_x\, s_x) \qquad (1)$$

This expression defines a relationship between export ratio and grazing intensity (g) in which ER_x is a linear function of g with a Y-intercept of $r_x\, s_x$ and a slope of ($r_{z,x}\, e_x - r_x\, s_x$). The slope of this formula explicitly formalizes the influence of zooplankton on sedimentation of autotrophic production. Zooplankton, by its grazing activity, enhances the export ratio if faeces are more refractory to mineralization than algae ($r_{z,x} > r_x$) and/or if the egested fraction of the ingested material is higher than the fraction of algae sedimenting ($e_x > s_x$). However, inversely, zooplankton will decrease the export ratio if $r_x\, s_x > r_{z,x}\, e_x$. The direction of the relationship between zooplankton (via its grazing) and sedimentation is thus depending on (1) lake morphometry, which determines the fraction of algae which directly sediments (s_x), (2) phytoplankton community characteristics (via r_x and s_x), and (3) zooplankton community characteristics (via $r_{z,x}$ and e_x) (Elser et al. 1995). This last point needs some explanations. The egested fraction from ingested food (e_x), i.e. the inverse of the assimilation efficiency, is function of the digestive physiology of animals. The stoichiometric theory indicates that it may also be a function of consumer elemental needs, and thus can vary in function of species and elements (Sterner and Elser 2002). E.g., daphniids, which are bodily rich in P (Andersen and Hessen 1991, Hessen and Lyche 1991), have high demands in P from food, and thus the fraction of P egested from *Daphnia* must be lower than for other genera with lower

body content in P. The fraction of faeces refractory to mineralization ($r_{z,x}$) also depends on the zooplankton community. For instance, copepods surround their faeces of a peritrophic membrane, largely increasing their cohesion and therefore their probability to sediment outside of the upper mixed layer.

Eq. 1 only deals with the fate of autotrophic production. The assimilated fraction ($1 - e_x$) of the ingested algae are considered by definition as excluded from the export ratio. Actually assimilated elements are excreted as a dissolved form or contribute to the production of new tissues. Secondary production must also necessarily sediment, and thereby zooplankton also contributes to the vertical elemental flux. Particularly, cladocerans periodically release their old exoskeleton in the water after molting. In *Daphnia*, ~ 14 % of the total P content is found in the carapace, and its reabsorption before molting is still uncertain (Vrede et al. 1999). Moreover, nonconsumptive mortality of zooplankters is also known to add large particles into sediment traps, with some high seasonal peaks of crustacean species (Gries and Güde 1999). We can therefore question about the importance of remains of P-rich species in the vertical P-flux.

The antagonistic role of these P-rich species in the vertical P-flux, for which we predict sometimes a decrease of P sedimentation rate due to P-poor faeces, sometimes an increase of P vertical flux by sinking carapaces, needs further investigations. Our present study is focusing on the role of zooplankton on distinct P sedimentation of small and large particles. This paper presents our data on primary planktonic production, sedimentation and zooplankton recorded during a 2-year study in a meso-eutrophic reservoir.

MATERIAL AND METHODS

Field data acquisition

This field study was conducted in the Esch-sur-Sûre reservoir which lies in the Northern part of the G.-D. of Luxembourg. A map and a summary of morphometric and ecological characteristics are provided in Fig. 6.1. According to the OECD classification (1982), the reservoir is considered as a meso-eutrophic waterbody (Dohet and Hoffmann 1995). *Daphnia galeata*, *Bosmina coregoni*, *Diaphanosoma brachyurum* (cladocerans), *Eudiaptomus gracilis*, *Cyclops vicinus* and *Halicyclops christianensis* (copepods) are the dominant zooplankton in the reservoir (Dohet and Hoffmann 1995). The seasonal survey was conducted at a station(maximum depth 30 m) located in the middle of the lake, representative of whole lake conditions (Thys et al. 1998).

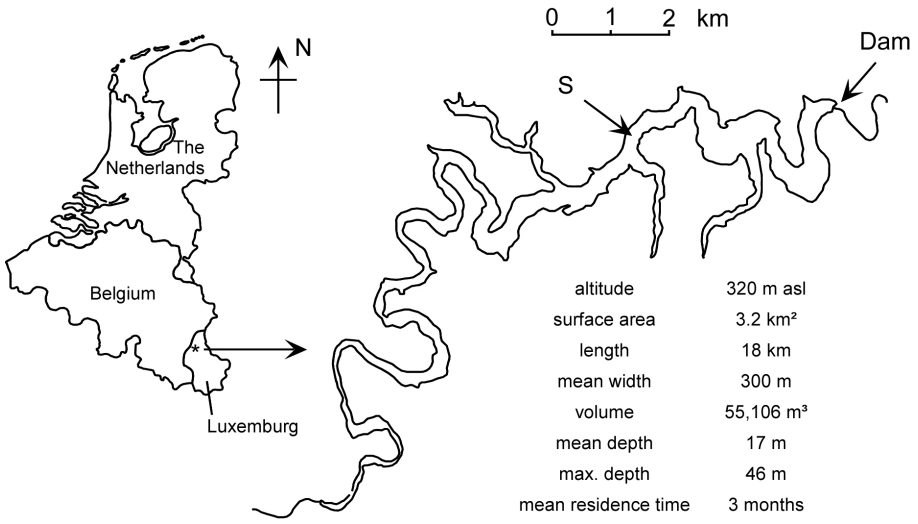

Fig. 6.1: Location of the Esch-sur-Sûre reservoir, with morphometric and ecological summary information. The location of the main sampling and sediment trap deployment station is indicated (S).

Seston and zooplankton community were sampled weekly during the stratification period (globally from April to October) in 1999 and in 2000. Stratification layers were delimited according to the limnological profiles obtained using a Hydrolab DS-4 multiprobe. The zooplankton community was sampled with a 17-cm diameter, 250-µm mesh net towed in the epilimnion. Six samples were collected and immediately filtered on 25-mm diameter, pre-ignited (12 h at 450°C), pre-weighted, Whatman GF/C filters and directly frozen in dry ice for elemental analysis. The zooplankton community was also sampled weekly with a 50-cm diameter, 50-µm mesh net towed in the same layer. Triplicate samples were collected and pooled to reduce heterogeneity in zooplankton horizontal distribution and sampling variability. The collected zooplankton was immediately narcotized in soda water, rinsed and preserved with a 4 % formalin solution (Haney and Hall 1973) for later counting. For seston analysis, pooled samples were constituted from discrete samples spaced every meter and collected with a 3 L Ruttner bottle. A pooled sample was constitued for the epiminion layer, and another for the metalimnion. The water from each sample was divided in 2 parts; one was filtered *in situ* on a 47-mm GF/C (porosity of 1.2 µm) Whatman filters and directly frozen in liquid nitrogen for pigment analysis using HPLC; the other part was filtered *in situ* on 6 25-mm diameter, pre-ignited, GF/C Whatman filters and directly frozen in dry ice for elemental analysis.

A sediment trap was deployed at the top of the hypolimnion. The trap consisted of one 15.4-cm diameter, 133-cm long PVC collection tube that was suspended from a floating pontoon. The trap was initially filled with GF/C filtered lake water taken at the immersion depth of the trap. One L of an inhibitory, high-density solution (180 µM $HgCl_2$, 10 % w/w NaCl) was added, using a small tube, at the bottom of the trap. This solution was proven to inhibit the breakdown of entrapped material without catching swimmers (Lee et al. 1992, Darchambeau comm. pers.). The trap was removed every 2 weeks. The trap was firstly gently taken back at the surface and the upper 4 litres were discarded. The remaining volume was carefully poured into a large basin and gently mixed. Six sub-samples were passed through a 250-µm Nitex screen and collected on 25-mm diameter, pre-ignited, Whatman GF/C filters for later elemental analysis. Particles > 250 µm collected on the screen were resuspended with filtered lake water and collected on 6 pre-ignited, pre-weighted, Whatman GF/C filters for later weight determination and elemental analysis. In addition, lake water was collected with a Van Dorn bottle at the same depth as the trap immersion depth. The same analyses were carried out on these water samples as on trap samples for deducing concentrations of suspended particles from concentrations of entrapped material.

Laboratory analyses

Phytoplankton pigments were extracted and analysed following Descy et al. (1999), using the HPLC protocol of Wright et al. (1991). Chl*a* was detected by a Waters 996 PDA detector and a Waters 470 fluorescence detector, and calibration was achieved using external standards.

The crustaceans were counted with an inverted microscope. At least 200 individuals of each species were counted, measured and their dry weight was estimated from body length, using literature values (Bottrell et al. 1976) except for *Daphnia galeata* and *Eudiaptomus gracilis* for which we have established our own length-weight relationships (for *D.g.* : $DW = -2.26 + 1.3 \exp(1.66\ L)$, and for *E.g.* : $DW = 8.08\ L^{2.33}$, with *DW* in µg and *L* in mm).

Filters with zooplankton and filters with sedimented particles > 250 µm were dried at 60°C for 2 days before dry weight determinations at the nearest 10 µg. From the 6 filters collected by date for zooplankton, seston and sedimenting particles, 3 were analyzed for particulate C and N contents and 3 for particulate P content. Particulate C and N were analyzed with a Carlo-Erba NA1500 elemental analyzer. Total P was analyzed by spectrophotometric determination of phosphate after potassium persulphate digestion (Greenberg *et al.* 1992). The elemental ratios were expressed as the

ratio of the means in molar units corrected for the variance of denominator (Dagnelie 1992).

Primary production

Primary production experiments were conducted on the day of trap removal, using the ^{14}C uptake method (Steeman-Nielsen 1952). Eighteen 100-ml glass bottles filled with water from a pooled epilimnion sample were incubated *in situ* just below the surface with ca. 10 µCi $NaH^{14}CO_3$ per bottle. The bottles were placed in duplicate into a 9-cases surface incubator providing a range of relative light energy, around noon, for 2-3 h. Continuous irradiance recordings from a Li-Cor sensor were available throughout the study. Three 0.5-ml aliquots were sampled randomly for total radioactivity determination. Labelled phytoplankton was collected on 25-mm Whatman GF/C filters, which were carefully rinsed with HCl 0.1 N before radioactivity measurements with a liquid scintillation counter (Beckman LS 6000 SC). Rates of C uptake (mg C m^{-3} h^{-1}) were determined as in Vollenweider (1974). Photosynthesis parameters were determined by fitting production vs. irradiance (corrected for surface reflection), using Smith's equation (Smith 1936), and depth-integrated daily primary production (mg C m^{-2} day^{-1}) was computed for the epilimnion and the metalimnion using 1 cm depth-intervals and 0.25 h time-intervals. Phosphorus uptake rates (mg P m^{-2} day^{-1}) were estimated by multiplying C uptake of each layer by the corresponding seston P:C ratio. For all days of the trap deployment period, daily production was estimated from measured irradiance and from values interpolated between weekly (light attenuation coefficient, Chl*a*, P:C ratios) or bi-weekly (photosynthesis-light parameters) measurements.

The export ratio was calculated for C and P. The export ratio is simply the fraction of production that is sedimented. The C or P sedimentation rate of particles < 250 µm was simply divided by the sum of daily C or P production rates occurring during the same period. This calculation assumes that the material sedimenting during a given interval has been produced during that interval. However, accumulation of phytoplankton biomass or faeces that sediments at a later date may lead to situations in which materials sedimenting during an interval were produced during previous intervals. The long time deployment (2 weeks) of the trap should reduce the effect of this time-lag.

Statistical analyses

Regression analyses were used to investigate the role of some planktonic descriptors on sedimentation variables. The trap was removed every 2 weeks, while plankton was examined weekly. We chose to exploit mid-trap

deployment data for planktonic descriptors, instead of weighted average between data from first day, mid-trap and last day of trap deployment, because how respective points contribute to sedimentation is unknown. First of all, we calculated the contribution into zooplankton biomass of cladoceran biomass calculated from zooplankton numbers. As an index between P-rich and P-poor animals, the role of this percentage on zooplankton C:P and N:P ratios was investigated. The C:P and N:P ratios of sedimented particles < 250 µm were then tested against seston and zooplankton C:P and N:P ratios. The P export ratio was regressed against zooplankton biomass, as an index of grazing, actually against total zooplankton dry weight and against cladoceran biomass. Finally, we examined whether P sedimentation rates of particles > 250 µm could be explained by zooplankton and cladoceran biomass. All regressions were made with the help of the Statistica software package (StatSoft, Inc.).

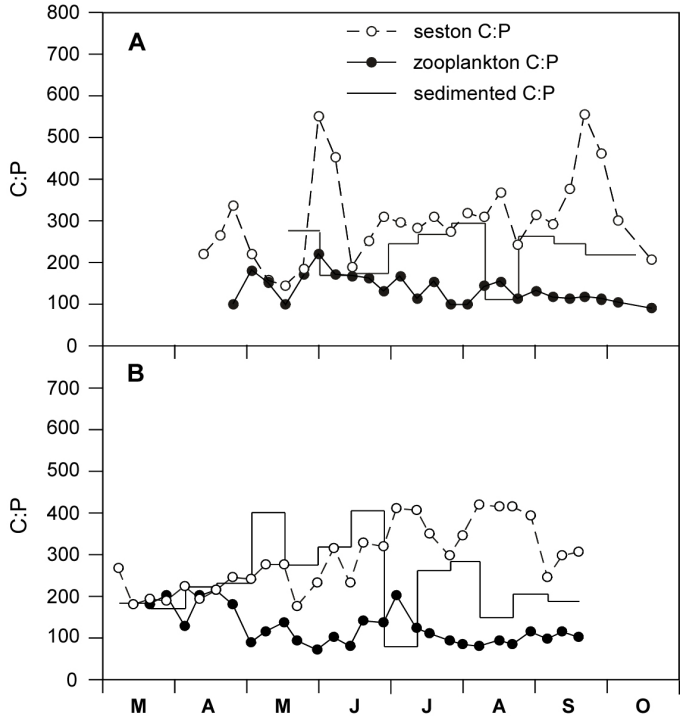

Fig. 6.2: C:P ratios of entrapped particles < 250 µm, epilimnetic seston and zooplankton (> 250 µm) in 1999 (A) and 2000 (B) in the Esch-sur-Sûre reservoir. All ratios are in atomic values.

RESULTS

In 1999 and 2000, the zooplankton biomass (> 250 µm) was dominated (> 98 %) by *Eudiaptomus gracilis* (calanoid copepod), Cyclopoids copepods, *Bosmina coregoni*, *Diaphanosoma brachyurum, Daphnia galeata* and *Daphnia cucullata* (cladocerans), which accounted for, respectively, 29 %, 24 %, 16 %, 11 %, 11% and 6 % of total zooplankton biomass (> 250 µm). The C:P ratio of the zooplankton community ranged from 70-220:1 (Fig. 6.2) and N:P ratio from 14-40:1. There were strong negative relationships between zooplankton C:P (R^2 = 0.39, n = 59, P < 0.001) and N:P ratio (R^2 = 0.22, n = 59, P < 0.001) and percentage of cladocerans in total biomass of zooplankton community.

The C:P ratio of entrapped particles < 250 µm was also highly variable, and no clear seasonal trend could be observed (Fig. 6.2). Seston analysis indicated P-limitation at the end of the growing season, with C:P ratio above 400 (Fig. 6.2). Variability of C:P ratio was nearly equivalent between the 3 fractions (CV = 31.1, 30.1 and 31.3 % for C:P ratios of, respectively, seston, zooplankton and entrapped material).

We tested the correlations between the C:P and N:P ratios in the trap (< 250 µm) and C:P and N:P ratios of zooplankton (> 250 µm) and seston observed at half-time of trap display (Table 6.1). While we failed to detect any significant relationships when data were analysed year per year, a significant negative relationship was found between sedimented C:P and zooplankton C:P when all data were plotted together. Intrigued by the important time-variations of the sedimented C:P and N:P ratios, we wondered whether these variations could be explained by time-variations of plankton predictors. To investigate this, we worked with C:P and N:P variations between consecutive trap samplings. Significant relationships were found between variations in zooplankton C:P and sedimented C:P ratios (Fig. 6.3A, Table 6.1). The relationships were negative, which means that, when zooplankton C:P increased (Δ zooplankton C:P > 0), sedimented C:P decreased (Δ sedimented C:P < 0), and inversely. Variations in N:P ratios of sedimented particles < 250 µm were also significantly correlated with variations in zooplankton N:P (Fig. 6.3B, Table 6.1).

The C primary production rates and C sedimentation rates of particles < 250 µm are presented in Fig. 6.4. The ratio between sedimentation and production gives the export ratio. All rates seemed highly variable and we observed no clear seasonal trends in the export ratio. Export ratios of P were well explained by dry weight of the zooplankton in the epilimnion (Fig. 6.5A, Table 6.2). In 1999, the relationship was near the significant level while in 2000 the relationship was significantly negative. When all data were

Table 6.1: Results of regressions of sedimented C:P and N:P ratios < 250 µm (and their time-variations indicated by Δ) with some planktonic predictors (and their time-variations). Only parameters for regression with a *P*-value < 0.10 are given. *P*-value < 0.05 are highlighted.

Dependent var.	Independent var.		R^2	P [a]	n	Intercept ± SE	Slope ± SE
Sedimented C:P < 250 µm	Seston C:P	1999	0.16	0.257	10		
		2000	0.02	0.675	14		
		all data	0.05	0.310	24		
	Zooplankton C:P	1999	0.14	0.281	10		
		2000	0.19	0.124	14		
		all data	0.18	**0.041**	24	350 ± 55	-0.820 ± 0.377
Sedimented N:P < 250 µm	Seston N:P	1999	0.30	0.102	10		
		2000	0.01	0.703	14		
		all data	0.02	0.562	24		
	Zooplankton N:P	1999	0.19	0.215	10		
		2000	0.10	0.267	14		
		all data	0.12	0.100	24	43.4 ± 10.0	-0.685 ± 0.399
Δ sedimented C:P < 250 µm	Δ seston C:P	1999	0.15	0.307	9		
		2000	0.03	0.562	13		
		all data	0.06	0.292	22		
	Δ zooplankton C:P	1999	0.45	**0.047**	9	-38.4 ± 20.9	-1.88 ± 0.78
		2000	0.39	**0.022**	13	-16.2 ± 31.2	-2.11 ± 0.79
		all data	0.40	**0.002**	22	-25.5 ± 19.7	-2.06 ± 0.57
Δ sedimented N:P < 250 µm	Δ seston N:P	1999	0.27	0.149	9		
		2000	0.01	0.691	13		
		all data	0.02	0.517	22		
	Δ zooplankton N:¨P	1999	0.26	0.159	9		
		2000	0.53	**0.005**	13	-5.98E-1 ± 29.88E-1	-1.80 ± 0.51
		all data	0.46	**0.001**	22	-8.77E-1 ± 19.97E-1	-1.57 ± 0.38

Note: (a) probability that the slope = 0.

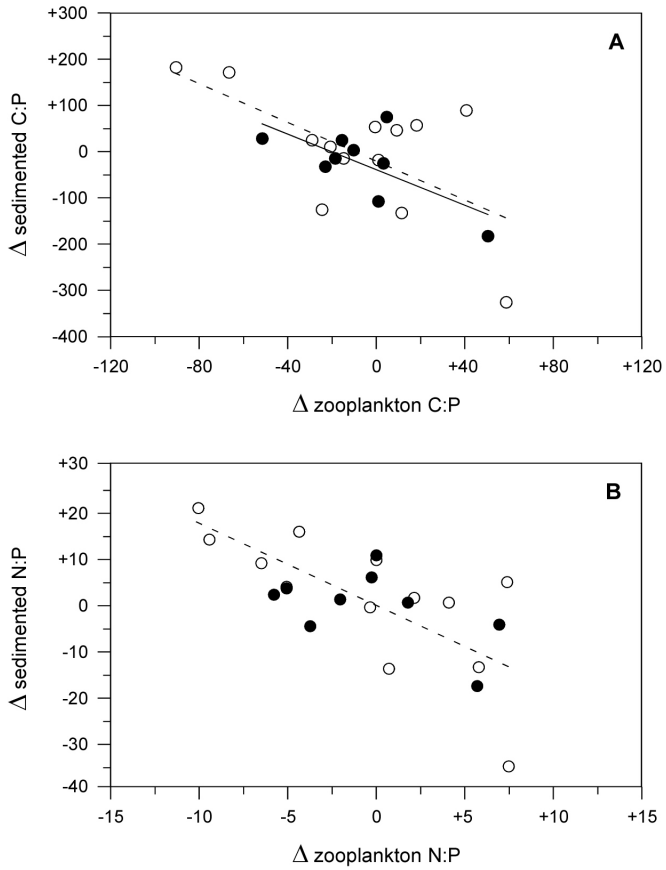

Fig. 6.3: Relationships between time-variations (Δ) in sedimented C:P or N:P < 250 µm and time-variations in zooplankton C:P (A) or N:P (B). Full circles and continuous line for 1999-data, empty circles and dashed line for 2000-data. The regression details are given in Table 6.1. Regressions were not figured when P > 0.10.

taken into account, the relationship stayed highly significant (Table 6.2). However, there were no significant relationships between cladoceran biomass and export ratios of P (Fig. 6.5B, Table 6.2).

Entrapped particles collected on 250-µm Nitex screen were also analysed. Calculated P sedimentation rates from these particles ranged from 0.09-1.45 mg P m^{-2} day^{-1} (mean: 0.51 mg P m^{-2} day^{-1}), which corresponds to 2-47 % of total P sedimentation rates (mean: 16 %). P sedimentation rates of particles > 250 µm were significantly positively correlated with both total zooplankton dry weight and cladoceran biomass (Fig. 6.6, Table 6.2).

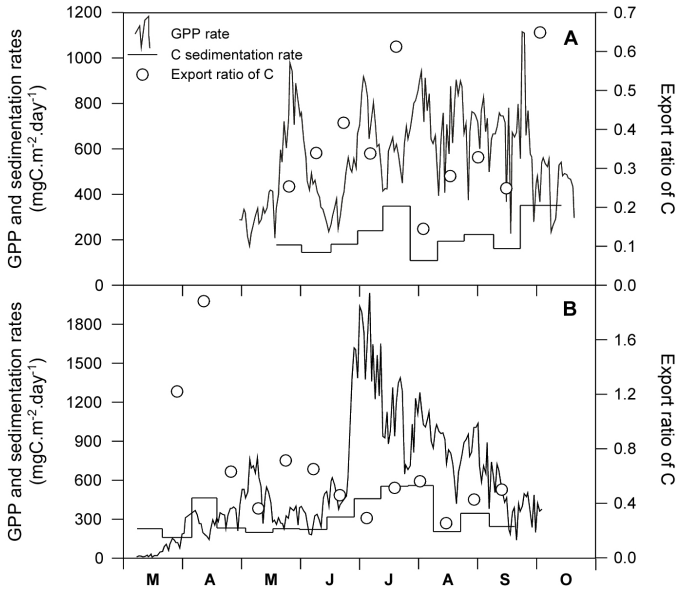

Fig. 6.4: Daily gross primary production (GPP) rates, C sedimentation rates of particles < 250 µm and export ratios of C in 1999 (A) and 2000 (B) in the Esch-sur-Sûre reservoir. Note the differences in scales between 1999 and 2000.

DISCUSSION

In the Esch-sur-Sûre reservoir, zooplankton had a significant negative effect on P export ratio (Fig. 6.5A). The Y-intercept of this relationship was equal to 1.0 (Table 6.2). From Eq. 1, we observe that this intercept corresponds to $r_x s_x$, i.e. the refractory fraction of the sedimenting fraction of algae. Thus, in this lake, both r_x and s_x are equal to 1; that means that the non-ingested algae sediment rapidly (at least during the 15 days after their synthesis) and do not undergo significant breakdown before and during their sinking out of the mixed layer and the metalimnion. In these conditions, the slope of the relationship between the export ratio and zooplankton biomass is negative (Table 6.2). In mathematical terms, $r_{z,x}\ e_x < r_x\ s_x = 1$ (see Eq. 1). Obviously, zooplankton assimilation is not null and all ingested elements are not egested ($e_x < 1$). Thus, in this lake, zooplankton, by its grazing activity, retains elements in the upper layers of the lake.

This situation is comparable to lake L110 of the Experimental Lakes Area in Ontario studied by Elser et al. (1995). A significant negative relationship was also found in this lake between elemental export ratios and zooplankton

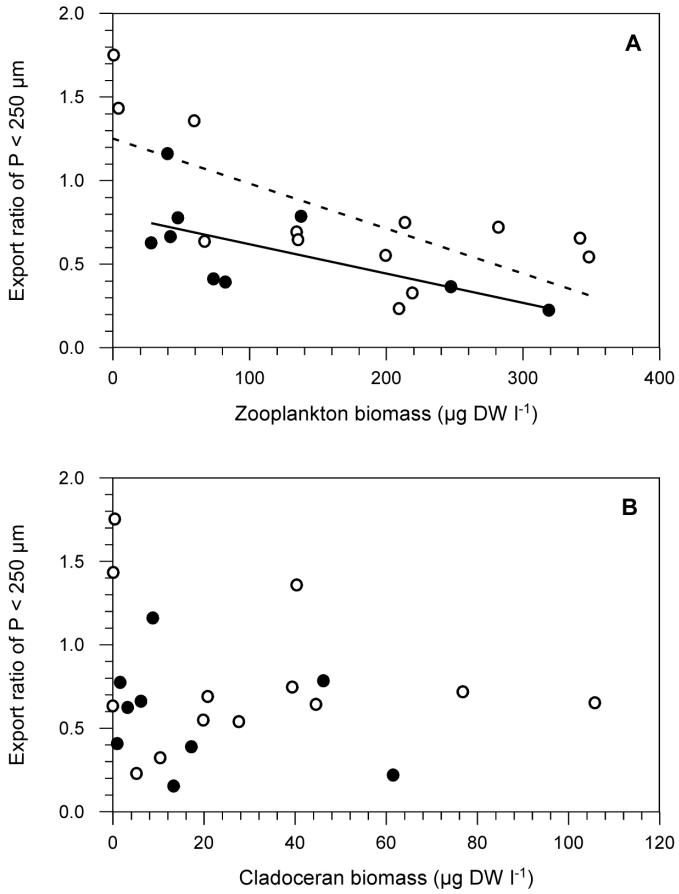

Fig. 6.5: Relationships between export ratios of P < 250 µm and zooplankton biomass (A) or cladoceran biomass (B) in the Esch-sur-Sûre reservoir. Full circles and continuous line for 1999-data, empty circles and dashed line for 2000-data. The regression details are given in Table 6.2. Regressions were not figured when P > 0.10.

biomass with an intercept around 1, at the inverse of L240 of the same study with a positive relationship and an intercept around 0.2. But contrary to L110, which is small and wind-protected, the Esch-sur-Sûre reservoir is large and wind-submitted, as L240. During the whole stratification period, the thermocline was well marked and the depth of the metalimnion varied between 6-11 m to 10-15 m. These limnological conditions seem *a priori* unfavourable to sedimentation, favouring mixing in the epilimnion, as in L240. Nevertheless, direct sedimentation of algae was maximal and, in these conditions, zooplankton grazing can obviously only reduce the sedimentation rate of assimilated elements.

Table 6.2: Results of regressions of export ratios of P and sedimented P > 250 µm with zooplankton and cladoceran dry weight (DW). Only parameters for regression with a *P*-value < 0.10 are given. *P*-value < 0.05 are highlighted.

Dependent var.	Independent var.		R^2	P [a]	n	Intercept ± SE	Slope ± SE
Export ratio of P < 250 µm	Zooplankton DW [b]	1999	0.40	0.068	9	0.794 ± 0.122	-1.75E-3 ± 0.81E-3
		2000	0.50	**0.007**	13	1.25 ± 0.17	-2.70E-3 ± 0.82E-3
		all data	0.33	**0.005**	22	1.00 ± 0.12	-2.00E-3 ± 0.64E-3
	Cladoceran DW [b]	1999	0.07	0.481	9		
		2000	0.02	0.606	13		
		all data	0.01	0.611	22		
Sedimented P [c] > 250 µm	Zooplankton DW [b]	1999	0.57	**0.019**	9	0.345 ± 0.133	3.13E-3 ± 1.04E-3
		2000	0.32	0.068	11	0.250 ± 0.115	1.54E-3 ± 0.74E-3
		all data	0.33	**0.008**	20	0.313 ± 0.095	2.00E-3 ± 0.67E-3
	Cladoceran DW [b]	1999	0.61	**0.013**	9	0.385 ± 0.116	1.41E-2 ± 0.43E-2
		2000	0.53	**0.011**	11	0.208 ± 0.094	1.22E-2 ± 0.39E-2
		all data	0.52	**< 0.001**	20	0.284 ± 0.077	1.33E-2 ± 0.30E-2

Notes: (a) probability that the slope = 0, (b) in µg l^{-1}, (c) in mg m^{-2} day^{-1}.

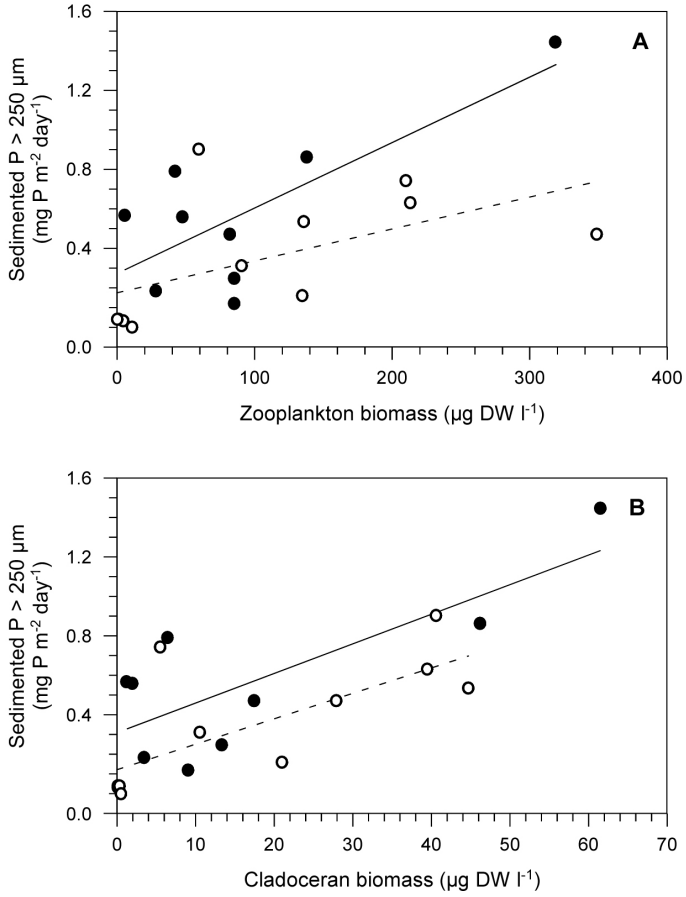

Fig. 6.6: Relationships between sedimented P of particles > 250 µm and zooplankton (A) or cladoceran (B) biomass in the Esch-sur-Sûre reservoir. Full circles and continuous line for 1999-data, empty circles and dashed line for 2000-data. The regression details are given in Table 6.2. Regressions were not figured when P > 0.10.

The significant negative correlations observed between zooplankton C:P and N:P ratios and % of cladocerans in total zooplankton biomass were not surprising. The 4 cladoceran species, *Daphnia galeata* and *D. cucullata*, *Bosmina coregoni* and *Diaphanosoma brachyurum*, are known to have body C:P ratios < 160, while other zooplankters, e.g. adult calanoid and cyclopoid copepods, have C:P ratios > 190 (Sterner and Elser 2002).

Zooplankton effects on sedimentation were also observable in the sedimented C:N:P ratios. When the zooplankton was composed of species

with higher P content (lower zooplankton C:P and N:P ratios), the C:P and N:P ratio of sedimented small particles increased (see Fig. 6.3 and Table 6.1). This observation is totally in agreement with stoichiometric theory and with the first results of Elser and Foster (1998). The stoichiometric theory predicts that, due to their higher body P content than many other zooplankton species (Andersen and Hessen 1991, Hessen and Lyche 1991), species like *Daphnia*, *Bosmina* and *Diaphanosoma*, must retain more efficiently ingested P than other zooplankton. As a consequence, material egested from P-rich cladocerans, and the resulting sinking flux, must be poorer in P than that from other species. Elser and Foster (1998) confirmed this prediction with the survey of 12 lakes located at the Experimental Lakes Area in Ontario. They found a significant negative relationship between sedimented N:P and zooplankton N:P, which was in that study a signature of *Daphnia* contribution to total zooplankton biomass. Both variables were measured once in each lake during thermal stratification, while we studied variation over time in a single lake. In other words, the variations we observed in sedimented C:P and N:P are not site-dependent, but time-dependent. Therefore, factors varying slowly over time can not explain the changes in sedimented C:P and N:P. We think essentially about hydrodynamic conditions, thickness of the metalimnion and microbial activity in the metalimnion, all factors known to be strongly site-dependent and to alter particle settling time and C:N:P composition (Elser et al. 1995), and therefore potentially involved in the differences in sedimented N:P observed by Elser and Foster (1998).

If zooplankton saved some P from direct sedimentation, part of this P was stored in body tissues, and among others in the exoskeleton of daphniids (Vrede et al. 1999). We suggest that the sedimentation of P-rich remains or cast exuviae's may contribute to the positive relationship that we observed between epilimnetic cladoceran biomass and P sedimentation of particles > 250 µm (see Fig. 6.6B). Part of the P saved from direct sedimentation by zooplankton grazing thus reintegrates the sedimentation flux by this way. In comparison to total vertical P flux, the sedimentation of cast exuviae's does not seem a negligible process (between 2-47 % of total vertical P flux) in pelagic phosphorus dynamics of the Esch-sur-Sûre reservoir. Note that this is opposite to the suggestion of Sommer et al. (2003).

To estimate the global effect of zooplankton on P sedimentation, we have calculated the average quantity of saved P by multiplying the diminution of the ER of P created by a mean zooplankton biomass (ER of P = 1 – 0.002 x 142 µg DW l^{-1}, see Table 6.2) by the average P production rate (5.35 mg P m^{-2} d^{-1}). We find that on average zooplankton saved 1.51 mg P m^{-2} d^{-1} from sedimentation by its grazing. Fig. 6.6A indicates that the maximal zooplankton effect on P sedimentation > 250 µm was ~ 1 mg P m^{-2} d^{-1}. We

therefore may suggest that on average more P is saved from sedimentation by zooplankton grazing than lost by sedimenting P-rich remains or cast exuviae's.

If the stoichiometric effect of zooplankton grazing on elemental sedimentation is widespread, it stresses the importance of separating excretion from egestion processes in the study of impacts of zooplankton-driven nutrient recycling on phyto-zooplankton interactions (Elser and Urabe 1999). Indeed, these two types of loss differ in physical form: excreted nutrients are solutes and egested nutrients are solid or semisolid. The processes also likely differ in the time scale over which nutrients become available for re-uptake, and egested material has higher probability to sediment out of the water column. The regulation of homeostasis, particularly for the element in excess in the food, may be driven by the non-assimilation of the element and thus by its final egestion, or, if assimilated, by its metabolization and its final excretion. The relative importance of both processes remains difficult to estimate but most probably each one plays a significant role in the final regulation. If the element in excess (e.g. P for copepods) is egested instead of excreted, it will rapidly be depleted in the upper layers. Thus, zooplankton species with low demand in N, as *Daphnia*, *Diaphanosoma* and *Bosmina*, will produce faeces with high N:P ratios and lead to low N resupply in the epilimnion, favoring N-limitation of algae instead of P-limitation unfavorable to P-rich zooplankton. Inversely, zooplankton species with low demand in P, as many herbivorous copepods, will produce faeces with low N:P ratios and lead to low P resupply in the epilimnion, favoring P-limitation of algae rather than N-limitation unfavorable to N-rich copepods. This view promoting a positive feedback loop between consumers and producers is the opposite of the usual explicitly stoichiometrical nutrient recycling theory which does not distinguish egestion from excretion and therefore considers that all resupplied nutrients are rapidly bioavailable (e.g. Sterner 1990). In these theories, elements are conservative in the studied system and a higher elemental demand of one trophic level, e.g. herbivorous consumers, induces a limitation of growth to other trophic levels, e.g. autotrophs, because of the deficiency of the element. Consequently the growth of the consumer is negatively affected by the consecutive deficiency of its food in the highly requested element. This negative feedback between consumers and producers leads to multiple stable and unstable equilibrium points defined by the initial conditions (Andersen 1997). When systems are uncoupled, e.g. an upper productive layer and a lower detrital layer collecting egested material from the upper layer, with non-conservative budgets of elements in each layer, we observe that the algae-grazer stoichiometric interactions may lead to a putative positive feedback loop in the upper layer. Whether there are multiple or single equilibrium points in these conditions, and if so, where they are situated, are

stimulating questions still unresolved. Ecological stoichiometry therefore will offer a theoretical basis which will possibly allow to better understand the complex dynamics observed at the largely unpredictable algae-grazer interface (Harris 1994, Sterner and Elser 2002).

In conclusion, our data clearly demonstrate the important role of zooplankton elemental composition in sedimentation. In spite of the role of P-rich remains and cast exuviae's in P sedimentation, zooplankton negatively affected the sinking of elements in the Esch-sur-Sûre reservoir. However, the egestion of elements in excess in the food favors their sedimentation out of the productive water column. Feedback effects of this sedimentation on resupplied elemental ratios and autotrophic production needs further modelling and observations.

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Chapter 7

Summary and future directions

WHAT HAVE WE LEARNED?

Science generally proceeds from observation to theory, from particular to general. Ecological stoichiometry is still at the start of accumulation of observations. Modeling, lab experiments, and field surveys have been used in this thesis to add some additional pieces in the challenging construction of ecological stoichiometry.

Where has this dissertation really taken us to? Hopefully, my thesis leads to a more comprehensive framework of multiple interactions in the nutrient-algae-*Daphnia* system. I have drawn Figs. 7.1 and 7.2 to help the integrative understanding of major findings of the present thesis.

To begin with, in Chapter 4, a meta-analysis of literature data on experimental growth rate of *Daphnia* juveniles has confirmed the negative effect of low elemental food quality (high C:P ratio) on consumer growth rate (Figs. 7.1 and 7.2, # 7). The meta-analysis has also revealed the potential for *Daphnia* to adapt to food quality, and to improve its growth rate after an adaptation time to low food quality.

Some mechanisms that may be used by *Daphnia* for this adaptation are revealed in Chapter 2. By modeling, I have, first of all, confirmed that ingestion and assimilation are coupled processes (# 3). A variation in residence time of particles in the gut implies the corresponding opposite variation in assimilation efficiency. The modeling has highlighted the importance of two putative responses of grazers in food quality regulation. The biochemical link between C and P assimilations has indeed allowed accurate modeling of filtration and digestion responses to dietary P-deficiency. Firstly, when food quality is low (high diet C:P ratio), the optimal filtration rate is higher than with high food quality (# 1). This implies higher ingestion and corresponding egestion rates (# 5). A second putative response of grazer to food quality deficiency is predicted: the higher secretion of digestive enzymes leading to increased nutrient assimilation efficiency (# 2). Combined, filtration and digestive responses lead to the decrease of nutrient assimilation. If realized, both responses have the potential to diminish the penalty on growth due to low food quality (# 4) and might be a mechanistic explanation to the observed adaptation revealed in Chapter 4.

In Chapter 3, I have tested the predicted filtration response to food quality against field data of individual filtration rates of *Daphnia galeata*. These results were obtained in the Esch-sur-Sûre reservoir (Grand-Duchy of Luxemburg) and revealed a significant positive relationship between seston

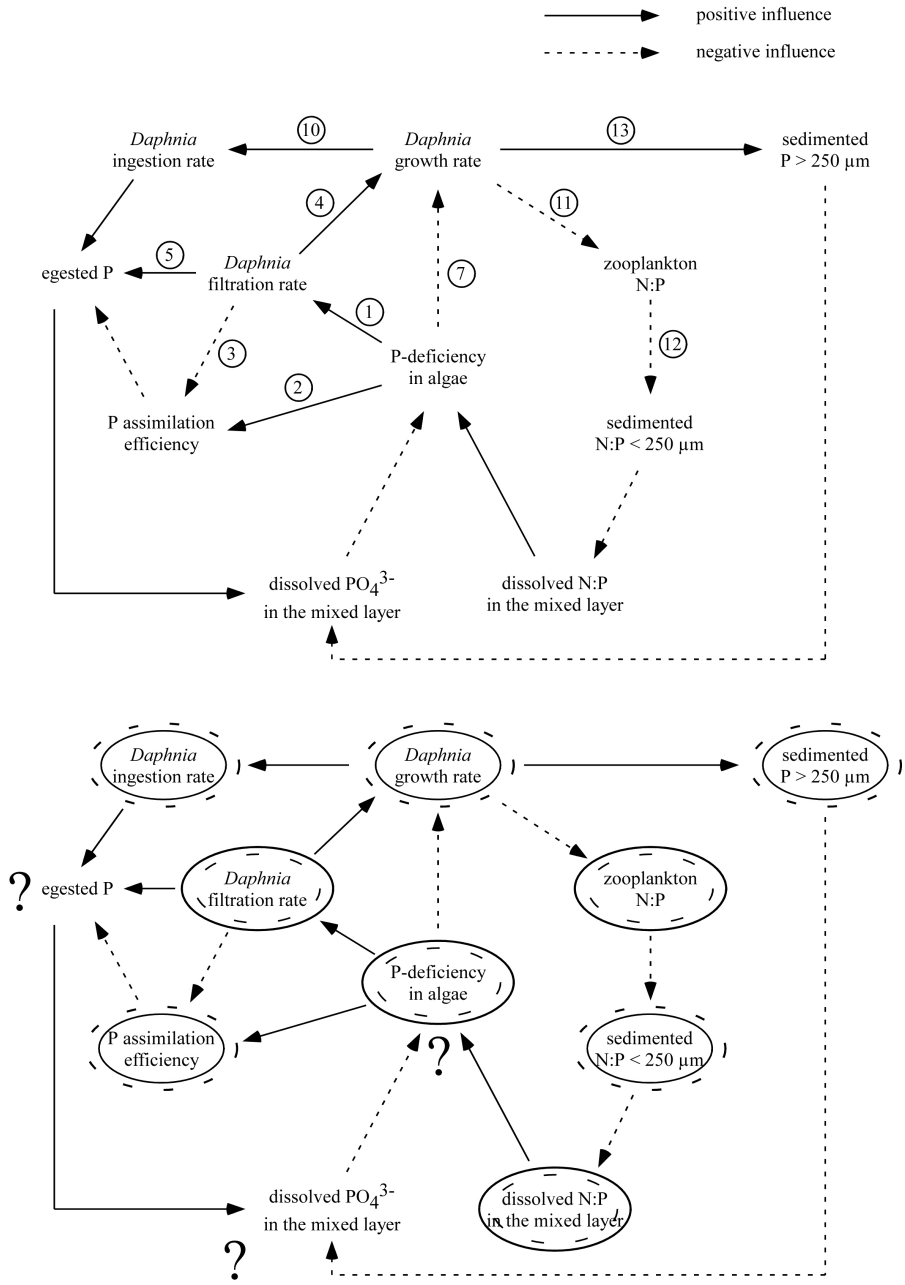

Fig. 7.1: Schematic framework synthesizing advancements of the present thesis in the multiple interactions of the nutrient-algae-*Daphnia* system dealing in the phosphorus cycle. In the upper panel, numbers refer to the interactions highlighted in the thesis, and are resumed in the text. In the lower panel, a scenario of increased P-deficiency of algae is tested. The dashed circles indicate the level of rates/biomass before the rise of P-deficiency. Full circles are the levels after the rise of P-deficiency.

N:P ratio and *Daphnia* filtration rate. It means that, when food quality was low, daphniids filtered water more intensively. This observation totally agrees with the prediction of Chap. 2 (# 1). The higher the filtration rate, the lower the assimilation efficiency (# 3), the higher the egestion rate (# 5). Recycling of nutrients by grazing was thus also enhanced, at the moment when algae suffered nutrient limitation. This putative positive feedback loop might potentially help stabilize the algae-*Daphnia* system.

The model of Chap. 2 also predicts the increase of *Daphnia* respiration rate when food quality is low. Predicted increases of filtration rate and metabolic activity might influence positively the respiration rate (# 6). Excretion of digestive enzymes is also predicted, particularly if food quality is low (# 9). In Chap. 5, experimental studies on the *Daphnia* C budget indicated higher respiration and organic carbon excretion rates when animals were fed with low quality algae in comparison with high quality algae. Interestingly, both rates increased very rapidly after the transfer of animals to low-quality food, while filtration rate still remained constant. These observations suggest a direct influence of food P-deficiency on both rates (# 8-9). Stoichiometrically, both responses might be observed as physiological processes used to dissipate C ingested in excess. These dissipations of C indeed lead to more balanced nutrient ratios incorporated into biomass.

Note here that all these physiological individual rates (filtration, respiration, excretion, egestion, and growth rates) are weight-specific. It implies that, if growth is reduced by low food quality, body weight will increase more slowly, reproductive maturity will be attained later, and all weight-specific rates will be reduced at both individual and population levels (# 10).

Chapter 6 focuses on effects of zooplankton individual grazing and nutrient egestion at the ecosystem level in the Esch-sur-Sûre reservoir. Both processes, directly and indirectly, influence nutrient cycling and the algae-grazer system. First of all, the relationship between zooplankton composition and zooplankton C:N:P ratios was confirmed (# 11). From the 6 dominant taxa in the reservoir, the 4 cladocerans (*Diaphanosoma brachyurum*, *Bosmina coregoni*, *Daphnia galeata* and *Daphnia cucullata*) were defined as P-rich, N-low species, while the 2 copepod taxa (a calanoid, *Eudiaptomus gracilis*, and some unidentified cyclopoids) were N-rich, P-low species. Elemental composition of egested particles was directly opposed to that of zooplankton community. Bodily P-rich community produced P-poor feces, and inversely (# 12). Excess nutrients found in the food have thus higher probabilities to sediment below the mixed layer than nutrients highly required by zooplankton and maintained into secondary biomass. This putative feedback loop between nutrient, algae and grazer might also stabilize the trophic system. However, secondary production also sediments

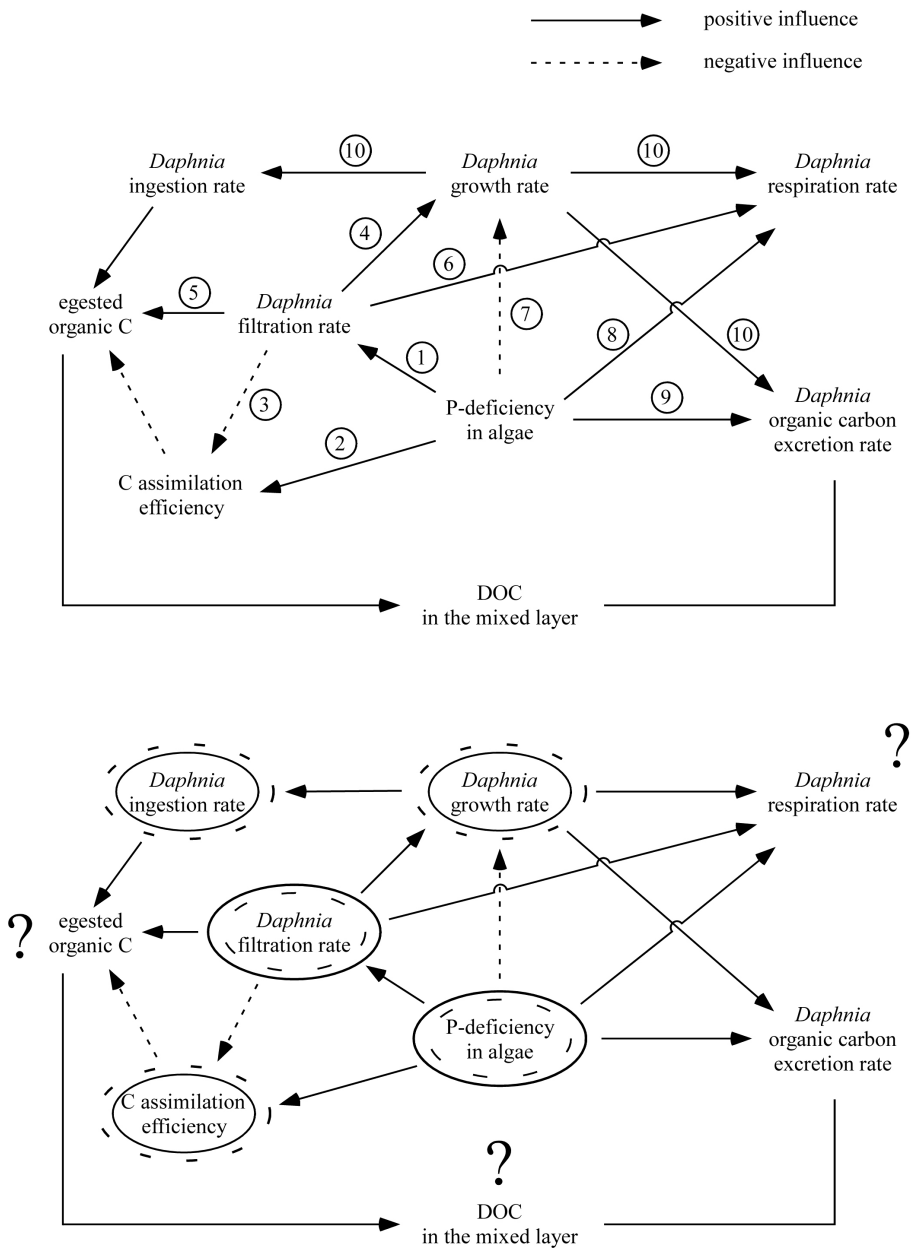

Fig. 7.2: Schematic framework synthesizing advancements of the present thesis in the multiple interactions of the nutrient-algae-*Daphnia* system dealing in the carbon cycle. In the upper panel, numbers refer to the interactions highlighted in the thesis, and are resumed in the text. In the lower panel, a scenario of increased P-deficiency of algae is tested. The dashed circles indicate the level of rates/biomass before the rise of P-deficiency. Full circles are the levels after the rise of P-deficiency.

outside the mixed layer. For instance, I have confirmed the significant vertical flux of remains and cast exuviae's associated to zooplankton biomass. P-rich species, as encountered in cladocerans, significantly contributed to P sedimentation of gross particles. This enhancement of loss of particulate P by cladocerans naturally questions their relative role in recycling of P. Note nevertheless that in the Esch-sur-Sûre reservoir, zooplankton contributed significantly to the maintenance of nutrients in the epilimnion because direct sedimentation of algae was very high. Thus all ingested nutrients are saved from direct sedimentation and may be recycled by egestion or excretion, or maintained into consumer biomass until probable later sedimentation or transfer to predator biomass.

When all the interactions between nutrient, algae, and zooplankton consumers observed and studied during the thesis are drawn all together, the pictures become rapidly complex and *a priori* impenetrable (Figs. 7.1 and 7.2). Interactions are sometimes opposite, and clear resulting tendencies are not straightforward. I have illustrated the case of the accentuation of P-deficiency of algae.

In the lower part of Fig. 7.1, we see that the accentuation of algae P-deficiency undoubtedly promotes individual *Daphnia* filtration rate and brings down P assimilation efficiency. But resulting lower growth rate and consequently lower population ingestion rate diminish egestion and recycling of P. Thus the global effect on P egestion becomes at this step unpredictable. The lower growth rate of *Daphnia* (and other P-rich species) increases the zooplankton N:P ratio, which in turn influences negatively the N:P ratio of sinking egested material. It strenghtens P-limitation of algae. However, lower P in zooplankton body tissues implies lower sedimentation of P attached to carapaces and cast exuviae's. Thus, the final effect on P recycling remains unknown. Particularly, the transition between individual responses to population changes is not modeled in the present work. Delay in age at maturity and consequent reproductive implications are still to be studied, and at this moment, stop our ability to predict global effect at ecosystem scale. We are just starting to observe and better understand implications of stoichiometry at the individual level.

The same applies to the C cycle (Fig. 7.2). Once again, individual weight-specific respiration and organic carbon excretion rates are predicted, but what is their evolution at population scale? Lower growth rate might considerably reduce their significance at ecosystem level.

For both nutrients, recycling of DOC and PO_4^{3-} may alter the fitness of some algae and induce shifts in algal community composition, depending on the time scale involved. Interactions between algae and bacteria may also be

altered. Indirect stimulation or inhibition of food growth will in turn alter grazer success. At the light of the present work, the real direction of the stimulation appears more complex than initially envisaged and still remains largely unresolved. The strong links between direct and indirect effects is a further argument against a strict top-down or bottom-up view of aquatic food webs. Grazing, predation, recycling and stoichiometry are all linked processes from a single whole. Each brings its part in the understanding of ecosystems structure and function.

FUTURE DIRECTIONS

As is often the case in research, the larger impact appears to be that I have raised more questions that I have resolved. Perspectives of further research are presented as a list of questions. My hope is that one or two researchers draw cheerfully from this list the source of their brainwave.

At the physiological level:

- What are respective magnitudes of non-assimilation and excretion in the homeostasis regulation? Are they species-, element-dependent?
- What is the composition of excreted organic products? Are they food quality-dependent? Are they a signature of food quality deficiency?
- How do herbivorous copepods regulate with diet elemental imbalances? Particularly, what are their ingestion, assimilation and respiration responses to food N-deficiency?

At the population level:

- What are population impacts of individual regulation? This question must certainly be attained firstly by modelling. Kooijman's works [1] (Vrije Universiteit van Amsterdam) on the Synthesizing Unit might certainly offer encouraging effort on this way. Secondly laboratory long-term experiments in chemostats might help model parameterization and direct observations of predicted population effects.

At the community and ecosystem levels:

- What is the influence of excreted organic carbon on algae-bacteria coupling?
- How do the regulation responses of the grazer and sedimentation of nutrients influence the stability of the nutrient-algae-grazer system?
- Herbivores: sink or link in the pelagic nutrient cycles?

At the environmental level:

- How can an ecologist at the end of his thesis give back to the society the investment that it gives him during his studies? My personal answer may be inspired from Albert Jacquard 's thought: «Le rôle du scientifique est celui d'un veilleur, qui donne l'alerte lorsqu'il voit se répandre des contrevérités ou quand il assiste à des actes inacceptables.» (« The role of a scientist is the one of a watchman, who alerts when he observes the spreading of falsehoods or when he's the witness of unacceptable acts. »).

[1] **Kooijman, S.A.L.M.**, 1998. The Synthesizing Unit as a model for the stoichiometric fusion and branching of metabolic fluxes. *Biophysical Chemistry*, 73: 179-188.

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