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Grazing impact on periphyton

De Montpellier d'Annevoie, Géraldine

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GRAZING IMPACT ON PERIPHYTON

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Grazing impact on periphyton

DE MONTPELLIER D'ANNEVOIE Géraldine

Abstract

Much research has been devoted to the ecological interactions in food webs in the littoral. Different kinds of grazers such as snails play a very important role in this ecological system. I investigated the snail-periphyton interactions in 3 laboratory experiments. In the first experiment, I tested whether different snails select their food based its quality. The results showed that *Valvata viviparum* chose the high P-content algae, whereas *Theodoxus fluviatilis* had no preference and *Bythinia tentaculata,* a more sedentary species, did not discriminate between food items.

The second experiment (6 d) dealt with the influence of grazing by these same three snails on periphytic biomass and algal growth forms. A shift in algae composition, removing unicellular with raphe and chains diatoms to allow green colonies to grow up, was caused by *T. fluviatilis* and less intensely by *V. viviparum,* but not by *B. tentaculata*. The biomass of the periphyton was not significantly affected, probably because of the short duration of this experiment.

Experiment three studied the impact of grazing in a long-term laboratory experiment (23 d) on epilithic periphyton with two different grazers (*T. fluviatilis* and *B. tentaculata*). The biomass of the periphython decreased in the "grazed" treatments. Moreover, grazing enhanced the spatial heterogeneity of periphton. This decrease of difference of Chl.*a* was not explained by the distances between each tile. The snails grazed periphyton in a homogeneous way: they were not concentrated to feed on the algae, which were close to each other. The grazing pressure of these two snails and its effetcs on spatial heterogeneity were similar.

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PRESENTATION OF THE STUDY

Periphyton, which comes from Greek language "peri" ("around"), and "phuton" (all what it is growing or plant) is a typical community of the freshwater ecosystems. This community is an assemblage of benthic algae living in close connection with meiofauna, fungi, bacteria, and organic and inorganic non-living material (detritus) embedded in a mucopolysaccharide matrix. The particularity of the periphyton is its connection to a substrate, like rocks, macrophytes, wood, sand, ... in opposite to the phytoplankton, which is suspended in the water column.

The periphyton plays a strategic role in an aquatic ecosystem. In certain rivers or lakes, the equilibrium of all trophic web rests on periphyton.

On one hand, this benthic algae community are regulated by abiotic factors, mostly the light and the nutrients. On the other hand, the consumption by predators like snails (grazers), called grazing has a negative impact on the periphyton because of the decrease of the biomass, but it permits the release of nutrients and thus a positive impact on periphyton.

Much research has been devoted to this ecological interaction, studying diverse aspects. My project will be focused on three different aspects of this interaction.

1) A lot of studies proved that snails can select algae according to their mechanical feeding apparatus. However, nobody tried to investigate if this selection could be based on the food quality instead of the taxonomy of the algae, by a "feeling" behaviour. To test this idea, pre-colonized tiles are transferred to the laboratory and placed into 4 aquaria. Then, each aquarium is assigned to one out of 4 nutrient-enriched treatments: addition of N, addition of P, addition of both, addition of none. 24h after the nutrient pulse, the tiles are placed in other aquaria (one tile from each nutrient treatment), together with one individual of each grazer species. The choice of the herbivore is monitored and repeated for all the individuals for each herbivore. The results are compared to random choices to test for selection. Sample for Chlorophyll a and C:N:P are used to verify that the treatments differed in nutrient content, but not in algal biomass.

2) Another aspect of this interaction is the impact of grazer on the composition of the periphyton growth form. To investigate the impact of three different snails on the periphyton, the following design was mounted: pre-colonized tiles are transferred to the laboratory and

placed into aquaria, together with randomly assigned grazer treatments. At day 6, all tiles will be sampled quantitatively and analysed for chlorophyll a and algal groups.

3) Finally, the grazing will be investigated with regards to its effect on the biomass, and on the spatial heterogeneity. 12 aquaria (2 treatments x 4 replicates) in the laboratory were filled with- precolonised ceramic tiles, with 36 tiles per aquarium. The treatments consisted of no addition of grazers or equal biomass of the two species in question. At each sampling day, three tiles will be removed (determined at random) by transferring them under water onto petri dishes and then replaced by empty tiles. From these three tiles, the biomass will be studied. The last day of the experiment (d 23), a sampling was done for 16 tiles chosen at random, and the biomass was analysed with chlorophyll *a* analysis. The position of each tile was recorded in order to measure spatial autocorrelation.

INTRODUCTION

1 : The periphyton, a community raising from oblivion?

Especially since the eighties, the interest increases for this community, called the periphyton. "Peri", which comes from Greek language, means "around" and " phyton", plant or all what is growing. Indeed, benthic algae live in close connection with meiofauna, fungi, bacteria, and organic and inorganic non-living material (detritus) embedded in a mucopolysaccharide matrix (Burkholder, 1996). This assemblage is defined here as a benthic algal community or periphyton. This typical community is present in the littoral zone of lake (lentic) or in the bottom of streams (lotic).
Almost all surfaces receiving light sustain this community, which is dominated by benthic phototrophs and thus can be important primary producer of the littoral zone.

The periphyton can be categorised according to its occurrence on stones (epilithon), soft sediments (epipelon) and plants (epiphyton), which are mostly macrophytes, particularly angiosperms. Unlike epipelic species, which are motile and easily swept away, the epiphytic and epilithic taxa are usually attached by secretions (polysaccharides) or a stalk to the ground avoiding to be carried away by water movements (Allan, 1995).

Diatoms represent the dominant taxa within the microalgae community although green algae and cyanobacteria are well represented and can predominate the benthic algal assemblage under certain circumstances. There exists different growth forms such as prostrate and filamentous and a mature periphyton mat can have a three-dimensional structure similar to that of terrestrial plant communities. Some periphyton species are in contact with the substrate (or host epidermis in the case of epiphytes) along the entire cell wall, colony or filamentous system. This growth form is termed and pressed, and contrasts with erect (pedunculate) forms in which only a basal cell or basal mucilage contacts the substrate. As a consequence of this variety in growth form and life style, a close look at a periphyton community reveals much structural diversity (Fig.1). In general, there are prostrate forms in the lowest level, stalked species in the middle level and filamentous algae reaching from the substrate to the upper level.

2 : Importance of the periphyton in the trophic web

The role of benthic algae in aquatic food webs has received relatively little attention when compared to studies of the function of phytoplankton in pelagic food web (McQueen et al., 1989). In freshwater ecosystems, considerable attention in the past two decades has been directed to lotic periphyton, for the reason that attached algae usually dominate the algal communities of flowing waters (Minshall, 1988).

Organic carbon in aquatic ecosystems derives from two principal sources: material contributeded externally by the sourrounding terrestrial system (allochthonous carbon) or material synthesized internally by autotrophic organisms within the system (autochthonous carbon).

2 a : Aquatic energy budgets in lakes and in rivers

Lake littoral zones are characterized by diverse energy sources. Autochthonous resources include periphyton, macrophytes, and metaphyton (floating algae). Allochthonous resources include pelagic plankton transported to littoral zones by currents, organic matter carried by inflowing streams, and detritus contributed by shoreline vegetation. In lakes, littoral periphyton production generally is not limited by riparian shading, although suspended matter and macrophyte beds can reduce light penetration and thus benthic primary production.

Benthic algae are the dominant primary producers in most streams (Bott, 1983). Streams contain many of the same carbon resources as lakes, but in differing proportions. For example, phytoplankton is often less abundant in flowing waters than in lakes, although even small streams can contain "potamoplankton" (Burkholder and Sheath, 1984). In small streams, benthic algal production is inversely correlated with the amount of cover by riparian canopy (Hawkins *et al.*, 1982; Feminella *et al.*, 1989; Tait *et al*, 1994). The river continuum concept predicts that benthic primary production should be maximized in mid order streams (Vannote *et al.*, 1980). At the other extreme, large rivers usually are turbid, which limits periphyton production.

In streams ecosystems (even of approximely the same size or found in the smae biome), autotrophic production accounts for a wide range of total energy inputs. Most energy budgets have been develop for small (order 1-2 streams, for which autotrophic production accounts for <1% to 60% of the total energy). Primary producers in streams up to fourth-order are dominated by periphyton and bryophytes. With increasing stream size, autotrophic production

also increase (as a proportion of total energy), but the relative contribution of periphyton declines as plant communities shift to macrophytes and phytoplancton in large rivers (e.g. Naiman *et al.,* 1987). In littoral zones of lakes, autotrophic production (from macrophytes, benthic algae, and settled plankton) usually dominates energy inputs to benthic food web. In some cases, however, inputs from terrestrial vegetation, inflowing rivers, or atmospheric deposition can be significant. This allochtonous matter can be transported by currents and wind or moved by gravity to deeper parts of the lake. Benthic algal production can account for a substantial proportion of total lake primary productivity. Wetzel (1983) summarised data from eight lakes and found that littoral, attached algae accounted for anywhere form 1 to 62% (mean = 21%) of whole-lake primary productivity. Many factors influence this percentage, including basin morphology, shoreline development, depth of the euphotic zone, sediment type, and nutrient loading. In several small lakes in northern Michigan, periphyton on littoral zone sediments and wood (a frequent ignored substrata) accounted for approximately 50 % of total epilimnetic chlorophyll *a* and primary production (Y. Vadeboncoeur and D. Lodge, unpublished data).

2 b : Fate and utilisation

Periphyton biomass produced in aquatic ecosystems can be allocated to several possible energetic "compartments": (1) accumulation as standing crop of algae, (2) respiration to CO_2 (i.e., decomposition), (3) consumption by herbivores (i.e., grazing), or (4) export to suspended matter. Each of these fates involves different processes and has different implications for energy flow through aquatic food webs (Fig.2).

Then benthic algae clearly are a major component of aquatic food webs. Many, if not most, benthic consumers in aquatic ecosystems ingest periphyton because of a high degree of omnivory. The interplay between the physical template (productive capacity) and utilisation by grazers (consumptive efficiency) in time and space may determine the ultimate role of periphyton in aquatic food webs.

2 c : Are benthic grazers food-limited by periphyton?

Because periphyton is a valuable food resource in aquatic ecosystems, its abundance may influence the physiological fitness of herbivores. Hill *et al.* (1992) demonstrated that *Elimia* snails and *Neophylax* caddis flies were strongly food-limited in a small woodland stream, and

nutrients, and grazing pressure covary on both temporal and spatial scales (Hart and Robinson, 1990).

3 a : Bottom-up and top-down controls

Among trophic interactions, *bottom-up* control implies that all trophic levels are food-limited, which begins with limitation of the lowest trophic level, normally primary producers (White, 1978). Increases in resources for primary producers (e.g., light or nutrients) are postulated to boost productivity through the food web. The magnitude of the response, however, should decline with trophic distance from producers owing to inefficiencies in energy transfer (Hairston, 1993). *Top-down* control means that control is exerted from the top level of the food web and alternating trophic levels are either predator- or resource-limited. For example, top predators in the food web exert control on the next lower trophic level (e.g., herbivores). The next lower trophic level (e.g., producers) thus becomes resources-limited because their consumers are controlled by predators.

3 b : Bottom-up experiments

Bottom-up experiments proved that nutrients (Bothwell, 1985) and light (Steinmann, 1992) are the most important abiotic factors regulating periphyton community. For example, a long-term enrichment has been conducted in the Kupauk River in the Alaskan tundra. Fertilisation of the river with phosphorus has resumed in stimulation of the entire food web (Peterson *et al.*, 1985, 1993) (Fig.3: example 6). Positive effects have been measured for bacteria, algae, bryophytes, invertebrates, and fish in this relatively simple community. However, feedback effects of consumers on food resources, starting in the third year of enrichment, have limited the accumulation of the biomass in the stream. Another experiment was done in desert streams (Tait *et al.*, 1994), and a positive association was found between light and herbivores abundance, but predaceous fishes were negatively affected, owing to the concurrent increase in water temperature (Tait *et al.*, 1994) (Fig. 3: example 4). Enrichment with sucrose increased the biomass of bacteria (*Sphaerotilus natans*) substantially, thus stimulating the heterotrophycally based food web (Warren *et al.*, 1994) (Fig.: example 5). Benthic invertebrates, especially chironomid midges, increased 2- to 10- fold and cutthroat trout production increased by seven times. Autotrophic production was not stimulated by the

sucrose addition, but was increased by an associated light-enhancement experiment (by canopy removal).

3 c : Top-down experiments

The effects of an aquatic predator on two or more trophic levels, including periphyton, have been examined experimentally. In prairie streams, predaceous bass (*Micropterus* spp.) determined the local distribution of algivorous minnows (*Campostoma anomolum*), thereby indirectly affecting the standing crop of benthic algae (Power and Matthews, 1983) (Fig.3: example 1). Bass pools tended to have high algal standing crop, whereas minnow pools had low algal standing crop. Piscivorous wading birds initiated a similar three-level "cascade" in stream pools of the Rio Frijoles in Panama (Power, 1984) (Fig.3: example 2). Birds such as heron excluded grazing catfish from shallow margins of streams pools, resulting in a bloom of macroalgae in shallow water. In a California stream, juvenile steelhead trout and invertivorous roach triggered a trophic cascade that involved predaceous damselflies, chironomid larvae, and filamentous algae (Power, 1990) (Fig.3: example 3).

3 d : Bottom-up and top-down experiments

McQueen *et al.* (1986, 1989) proposed that freshwater pelagic systems were simultaneously regulated by both bottom-up and top-down processes (the BU:TD model). As demonstrated early, both resources and predators can affect food web structure of benthic systems. Perhaps the most promising approach to determine the relative importance of BU:TD effects is to conduct experiments involving two benthic trophic level, various investigators have manipulated light and grazers, and nutrients and grazers. These studies suggest that periphyton productivity is ultimately controlled by abiotic factors, but that grazing exerts proximate control over standing crop. Positive effects on productivity often are manifested in higher growth rates of grazers (Lamberti *et al.*, 1989; Rosemond *et al.*, 1993).
Hill and Harvey (1990) incorporated a third trophic level by simultaneously manipulating predators (creek chubs) and grazers (snails) under different natural light regimes. They found that fish had no effect on lower trophic levels, but that both light regime and grazing simultaneously affected periphyton (Rosemond *et al.*, 1993) (Fig.3: example 11). This dual control of plants by herbivores and resources appears to be a recurring deduction from benthic food web experiments.

3 e : Intermediate regulation

A central element of the pelagic BU:TD model is the trophic "uncoupling" that can occur at the zooplankton-phytoplankton link (McQueen *et al.*, 1989). That is, predator or resource influence often breaks down at that interface because effects diminish with distance up or down a food web. In benthic systems, by contrast, the herbivores-periphyton linkage appears to be strong. Similar to pelagic systems, however, effects of top predators or abiotic resources tend to weaken in either direction. This suggests a central role for periphyton and grazers in benthic systems.

In benthic communities, the combined effects of resources and predators frequently are mediated by primary consumers (herbivores), which may serve as keystone species (Paine 1969). Paine considered keystone species normally to be predators, such as starfish in the marine intertidal zone or otters in kelp beds, that exert direct control over prey abundance and species composition and thereby indirect control of primary producers. This idea has since been extended to encompass any consumer that exerts an overriding influence on the structure of a food web. Hairston (1993) reemphazed the importance of studying species that constitute the "dominant bulk" of the biomass or that exercise "exceptional influence" at each trophic level. There are few examples from lotic and lentic ecosystems in which top predators regulate the structure of food web, but many examples that suggest other controls. Evidence is mounting to suggest that dominant consumers intermediate in the food web can exert control over the food web. In benthic systems, the influence of a dominant midlevel consumer can extend laterally (at the same trophic level) or, indirectly, even upward (to higher trophic levels) in a food web, thus resulting in "intermediate regulation".
For example, in a northern Wisconsin lake, *Orconectes rusticus* crayfish controlled the abundance of littoral macrophytes, snails, and periphyton (Lodge *et al.*, 1994) (Fig.4: example 12). *Orconectes propinquus* was a key species in regulating the benthic community structure of a productive, southern Michigan stream (Creed, 1994) (Fig.5: example 10). Crayfish reduced the abundance of the filamentous green alga *Cladophora glomerata,* thereby clearing space for microalgae and small sessile grazers. In a less productive, northern Michigan stream that did not contain *Cladophora, Orconectes rusticus* reduced abundances of benthic invertebrates (including grazers), which indirectly increased the productivity of microalgae (Charlebois, 1994) (Fig.4: example 13). There was no evidence that crayfish were controlled

by fish predators in either Michigan stream. These studies suggest that *Orconectes* can regulate that structure of benthic communities.

In northern California streams, the caddis fly *Helicopsyche borealis* dramatically alters the benthic landscape and reaches densities of 8000 m^{-2}, but has few invertebrates predators (Lamberti *et al.,* 1987). Another example of an "intermediate regulator" may be the well-studied minnow *Campostoma anomalum*, which strongly influences autotrophic production and detritus processing in North American prairie streams (Steward, 1987) (Fig.4: example 9). Pleurocerid snails appear to act as intermediate regulators in many North American streams. For example, *Elimia clavaeformis* greatly depletes periphyton in eastern Tennessee streams, thereby creating food-limited conditions for its own population and those of other benthic grazers (Rosemond *et al.*, 1993) (Fig.4: example 11). The snail apparently is unaffected by common fish. In many streams of the Pacific Northwest, *Juga silicula* dominates consumer populations and has negative effects on periphyton standing crop and the densities of other invertebrates including competing grazers (Hawkins and Furnish, 1987; Lamberti *et al.* (1989) (Fig.4: example 8). Furthermore, *Juga* may have negative indirect effects on higher trophic level (e.g., fish) because it consumes fish prey items whereas it is rarely consumed by fishes.

4 : Impact of the grazers on the periphyton

Grazing is considered as one of the most important regulator of the periphyton biomass (Hillebrand and Kalhert, 2001; Rosemond 1993). Grazing, i.e. consumption of living plants, is the subject of a broad range of studies. Although less attention has been paid to grazing in freshwater benthic ecosystems than to grazing (= herbivory) in terrestrial or pelagic ecosystems, there is still quite a number of investigations on plant animal-interactions in freshwater benthic regions with different approaches. The majority of these investigations were conducted in streams as field experiments or in laboratory experiments under running water conditions (Feminella and Hawkins, 1995). Few studies were realised in lentic communities. As will be evident, some parameters have been examined much more frequently (biomass, taxonomic structure) than others (nutrient cycling).

4 a : Consumption

A large number of investigations concerning grazer-periphyton interactions reveal that in general, the periphyton biomass decreases at grazer presence (Hillebrand and Kahlert, 2001).

Kehde & Wilhm (1992) and Hart (1985) reported that grazed periphyton had a higher biomass than ungrazed treatments. This paradoxe shows the importance to qualify the interactions between herbivores and benthic algae. This enhancement of the productivity may be caused by several mechanisms : (i) grazing reduces the number of cells and thickness of the algal layer and thus prevents light and nutrient limitation, the latter probably because of a shorter boundary layer, (ii) grazing remobilizes nutrients and stimulates therefore nutrient turnover rates and in turn algal cell division and growth. There are at least two reasons why algal biomass may not decline when herbivores are present: (1) biomass reduction is a density-dependant response, and grazer density and consumption rate are insufficient to result in a measurable decline; (2) the grazer's feeding morphology is not well matched with the dominant algal growth form(s).

Studying the periphyton-grazer interactions, different parameters were used to measure the response of benthic algae to herbivory. Those being most frequently studied were variables such as Chl *a* and biovolume to quantify periphyton biomass. The main grazer types investigated encompass insect larvae like mayflies and caddisflies (e.g. Fuller *et al.*, 1998), crustaceans (Sommer, 1997), vertebrates like tadpoles (Dickman, 1968) as well as snails (e. g. Cattaneo and Kalf, 1986; Cuker, 1983; Rosemond *et al.*, 2000).

Karouna and Fuller (1992) investigated the mouthpart of mayflies and caddies flies concluding that the mouthpart morphology caused distinct responses of the periphyton. Cattaneo and Kalf (1986) showed that snail grazing depressed filamentous algae and diatoms. Snail grazing was often related with the removal of filamentous algae (e.g. Tuchman and Stevenson, 1991) especially the filamentous parts of *Stigeoclonium tenue* (Rosemond *et al.*, 2000).

4 b : Choice

The type of mouthpart morphology and others structures of the grazer will influence the zone in which it is best adapted to feed. For example, the larvae of many mayflies species have gathering-collector feeding structures (Merritt and Cummins, 1984) and tend to feed at the outer layers, or loosely attached, portions of the periphyton mat (Hill and Knight, 1987). Caddis fly larvae and snails, with scraping and rasping mouthparts, respectively, are better suited to feed in zones where low-profile, tightly attached algae grow.

Selectivity, in the pure sense, refers to a directed behaviour on the path of the herbivore, there appears to be little evidence that freshwater benthic grazers possess the sensory equipment necessary for discriminating algal taxa. Cruz-Rivera and Hay (2000) investigated food selection, compensatory feeding and fitness of marine mesograzers. In these experiments it appears that numerous mesoconsumers utilise a wide variety of foods, but also that most will benefit significantly from selecting nutrient-rich foods, like animal tissue, or from mixed diets. Although the potential importance of prey nutritional value has been recognised (White, 1993), fewer studies have explicitly addressed this aspect of prey-consumer interactions, especially in benthic systems (Hay and Fenical, 1996). Understanding food utilization by animals requires to establish the link between feeding behaviour and fitness (Cruz-Rivera and Hay, 2000). Differential efficiency, on the other hand, involves no specialized sensory recognition; grazers exhibit a standard type of feeding behaviour that involves harvesting items with different efficiencies. For instance, the removal of overstory growth forms most likely can reflect differential efficiency rather than true selection. Indeed, the removal of the algae depends more upon morphological constraints of the grazer feeding apparatus than to sensory recognition *per se*.

No experiments has been realized so far to investigate whether the grazers are able to select the best nutritional quality food by a "feeling" behaviour, irrespective of the best adapted algae morphology to its mechanical food apparatus. In order to test this hypothesis 1, in the first experiment: "*The macrograzers are able to select high quality food and this is different for different grazers*", I chose three different snails as grazers and placed them in an aquarium with periphyton differing in nutrient quality. After, when I saw how many grazers chose each kind of quality food, the results were analysed by a statistical test to check if the choices of the grazer in question were a consequence of random feeding or if they made a real choice by" feeling" behaviour. I called this experiment: "Impact of periphyton nutrient status on grazer feeling selection".

4 c : Morphology composition

Morphology refers to the study of form and structure in natural communities (Whittakers, 1975). Among benthic algal communities, physiognomy exhibits strikingly consistent patterns in response to herbivory. However sometimes exceptions may occur. Results from the literature review indicate that most of the studies reported a decline in percentage overstory in response to grazing. Similarly, an increase in percentage understory occurs in response to

grazing. This response has been observed with mayfly larvae (Hill and Knight, 1987), minnows (Gelwick and Matthews, 1992), and crayfish (Vaughn *et al.*, 1993). The most dramatic effects were observed when the grazers used were either caddisfly larvae (Feminella *et al.*, 1989) or snails (Rosemond *et al.*, 1993). The declines in overstory forms are a direct consequence of their vulnerable position in the assemblage. Even if they are not directly grazed, their peripheral location and often loose attachment make them susceptible to dislodgement as grazers manoeuver through an assemblage (Lamberti *et al.,* 1989). Although declines in overstory forms have been quantified in terms of relative abundance, this pattern holds for absolute numbers cells as well (Rosemond *et al.*, 1993). Conversely, increase in the percentage of understory forms may result either from an indirect or from a direct consequence of grazing activity. The indirect response results from the removal of the more vulnerable overstory form, which may result in either increased percentage abundance of the less vulnerable understory forms or in more resource reaching the understory forms. Whether this increase in resource can stimulate growth rates to the point that they exceed grazer-mediated losses, is uncertain, and is in need of rigorous experimentation. The direct response results from nutrient regeneration from the grazers excretion, which may provide a direct means of stimulating an increase of an absolute abundance of understory forms. McCormick and Stevenson (1991) proposed this hypothesis to explain amounts of *Stigeoclonium* basal cells in response to grazing by the snail *Elimia* (by contrast, Hill, (1992) reported declines in absolute amounts of *Stigeoclonium* basal cells in the presence of snails of the same genus).

Grazers do not always cause a reduction in overstory forms, however. Several researchers working with the filamentous chlorophyte *Cladophora* have reported that grazing may increase overstory biomass (Sarnelle *et al.,* 1993). Apparently, the combination of firm basal attachment, coarse texture, and the grazers' ability to remove epiphytes results in a net positive effect of grazing for this alga. Alhtough grazing may lead to an increased relative abundance of understory forms, the absolute numbers of understory cells usually decline (Hill, 1992). Sarnelle *et al.* (1993) reported that mean understory biomass was reduced at moderate snail densities, as expected, but understory biomass was greatest at the highest snail densities. They suggested that the tufted nature of the overstory filaments of *Cladophora* may have physically prevented the snails from reaching the understory.

Although physiognomic responses permit reseachers to make generalization about benthic algal responses to grazing, it is obvious that these generalizations are not without exceptions. Based on the foregoing studies the ultimate algal response will be dependent, algal species and/or growth form, grazer density and on grazer species. In order to investigate the impact

of macrozoobenthos on morphology composition of periphyton, three different snails were used. The second experiment deals with the hypothesis 2: *"Grazing by different grazer species affects the proportion of different food algae composition differently"*. Thus three grazers were placed in different aquaria with the same periphyton composition, and then the proportion of biovolume of each algal form was analyzed.

4 d : Nutrient cycling and regeneration

Organisms differ in the proportions of major elements that they contain, including N and P, which are known to be highly dynamic and potentially limiting the production of aquatic ecosystems. Such contrasting elemental composition between, for example, algae and herbivores, or between different herbivores, generates a suite of ecological predictions and opens up new possibilities. Production of consumer biomass out of resources is similar to a complex chemical reaction in which resources are reactants and consumer biomass and wastes are products. Evolution favors organisms that perform this manufacturing process efficiently, optimizing the yield of the product, biomass, relative to the amount of reactant, resources, consumed in the reaction. However, as in all chemical reactions, ecological yields are dependent on the proportions of all reactants. Thus stoichiometry can be an important rate-determining factor of ecological relationships. All organisms have a set of nutritional requirements for metabolism and growth. When these required substances are balanced in optimal proportion, production efficiencies (production / ingestion) for bulk food and all essential substances, including carbon or energy, will be equal. Imbalance, on the other hand, results in less efficient use of substances that are overabundant, and highly efficient use of substances in short supply (Sterner and Hessen, 1994).

Ecologists are increasingly recognizing the importance of consumers in regulating ecosystem processes such as nutrient cycling (Elser and Urabe, 1999). Ecologists have recently made considerable progress in understanding nutrient cycling and trophic interactions in pelagic systems by applications of ecological stoichiometry to consumer-driven processes. Stoichiometric theory shows that grazer and algal elemental composition are critical parameters influencing rates and ratios of nutrient release. Thus, the stoichiometry of nutrient recycling is a feed-back mechanism linking grazer dynamics and algal nutritional status. Incorporations of such effects into a fully dynamic stoichiometric model generates profound changes in the predicted dynamics of algae and grazers, suggesting that adoption of a stoichiometric view may substantial alter our view of the interaction between trophic

dynamics and nutrient cycling. The basic predictions of stoichiometric models of nutrient release are generally supported by experimental data showing that N:P release ratios are primarily a function of algal N:P ratio and secondarily a function of grazer N:P ratio, and that rates of P release by grazers are also related to food P:C. Furthermore, evidence for effects of nutrient release stoichiometry on phytoplankton communities and pelagic ecosystem function is accumulating, including data showing consistent alterations in algal physiological status and ecosystem-scale changes in N fixation in response to altered grazer community structure and elemental composition (Elser and Urabe, 1999).

A positive effect of grazers on algae, which can compensate for biomass removal, is the nutrient supply from animal excretions. In the pelagic food web in lakes, recycled nutrients originating from zooplankton are an important nutrient source for phytoplankton (Elser *et al.*, 1995). Moreover, nutrient ratios in excretion may vary according to the body stoichiometry of the grazers and induce different nutrient limitation in phytoplankton (Sterner *et al.*, 1992). Similar effects can be expected in the benthic food chain of rivers and lakes, provided that external nutrient loading is small. Lack of continuous water transport in lakes might cause a closer coupling of benthic algae and grazers than in streams, and might result in a more distinct improvement of algal nutrient status and biomass in lakes than in streams. Thus, nutrient excretion by benthic animals in lakes may be an important nutrient source both for algae attached to animal substrates and for other benthic organisms receiving nutrients from mobile grazers. Results of Hillebrand and Kalhert (2001) and Rosemond *et al.* (1993) show that benthic algae in lakes benefit from nutrients released by animals. Benthic algae had a higher N and P content when associated with mobile macrograzers than with inert substrates, such as stones and rocks. Nutrients were probably taken up as food by the grazers, excreted as dissolved and particulate forms in the feces, and then remineralized into forms available for algae, causing close coupling between algal and animal assemblages, and low benthic algal C:N and C:P ratios (Hillebrand and Kahlert, 2001).

4 e : Spatial heterogeneity

The micro-distribution of periphyton can affect not only the behaviour, distribution, and diversity of grazers but also the growth and diversity of periphyton. Thus, the micro-distribution of periphyton can be an important ecological factor that can possibly influence ecological phenomena at larger scales.

Several factors can be considered in order to elucidate the spatial distribution of periphyton. Previous studies have focused on the effects of the heterogeneity of substrates and currents on the spatial distribution of algae (e.g., Biggs, 1996). The heterogeneous spatial distribution of currents over a topography complex streambed may generate physically separated patches (Poff and Ward, 1992). Grazing is also an important factor affected the spatial distribution of periphyton (Sommer, 2000). Sarnelle *et al.* (1993) showed that snail grazing causes the fragmentation or patchy distribution of periphyton. The interactions among neighbouring algae and snail grazing might be an important factor creating the spatial heterogeneity of periphyton even on homogeneous substrates (Kawata et *al.*, 2001). The spatial distribution of resources is dynamic and under the influence of competition and predator-prey interactions. Thus, the spatial distribution of resources is generated and modified through complex interactions between the resources themselves, between resources and consumers, and between resources and abiotic factors.

In terrestrial plant communities, the mode of competition between neighbouring individuals can affect the spatial patterns of individual plant sizes (Yokozama *et al.*, 1998). Although detailed studies documenting the importance of competitive interactions in benthic algal assemblages in aquatic habitats are lacking, competition may be an important factor determining spatial distribution (Kawata *et al.*, 2001).

Kawata and Agawa (1999) showed that snails recognize algal patches as heterogeneous when the size of patches is 2.5 cm to 5 cm in length: the snails moved more slowly when they were on a patch than when they were not if the size of patches was larger than about 2.5 cm. Moreover, the snails might have grazed for longer times on the periphyton patches. Thus, spatial scales smaller than 2.5 cm are important in determining grazers' behaviour and spatial heterogeneity of algae. In the experiments of Kawata (2001), the simulations suggested that when grazing effects creates patches, grazers should have a tendency to graze the same area.

But this tendency to graze the same area may be explained by the short distance to find new resources. For the last experiment, two different snails species were used. They are both grazers, that means they are the best at finding new patches more quickly but leave more resource behind (compared to "diggers" for example which are the best at exploiting local resources (Chase *et al.*, 2001)). This experiment dealt with the third hypothesis: *"Grazing has an impact on the spatial heterogeneity of the periphyton and this spatial heterogeneity is correlated to the distance".*

Almost all studies investigating grazing effects on periphyton were conducted either in stream or marine habitats. In contrast to those, lakes have a different combination of abiotic constraints (currents, salinity) and composition of biota (periphyton, grazers, guilds). These different conditions might have a different impact on behaviour and interactions of the organisms involved. To have a periphyton community close to the natural community in the lake, I chose periphyton grown in a lake. The utilization of natural grown periphyton was done, because of a better comparability of the results in laboratory to field results. I used algal-dominated periphyton grown on hard substrate and the snails *Theodoxus fluviatilis*, *B. tentaculata* and *V. viviparum* as its consumer. These snails were chosen for a practical reason, they were the most common organisms living in the lake of Erken, and the easiest to sample.

3 : STUDY SITE

STUDY SITE

Lake Erken (59 ° 51' N, 18 ° 36 ' E) is situated in the middle-eastern part of Sweden, 80 km north of Stockholm. The lake comprises a surface area of 24 km^2 within a catchment area of 141 km^2, has a maximum depth of 21 m and a mean depth of 9 m. Erken is a mesotrophic dimictic lake covered with ice for approximately four months of the year.
The pH is around 8, and mean conductivity is 289 $\mu S\ cm^{-1}$.

Once a part of the Baltic Sea, the lake was formed about 2000 years ago due to the postglacial uplift. Therefore, Erken is located only 11 meter above sea level. Organisms having their origin in the marine environment, such as red and brown algae, are still living in the lake.

The ground in the area consists of a thick layer of carbonate-rich clay deposited on top of Precambrian bedrock (granite). This clay gives the lake features such as high pH, good buffering capacity and a moderately high nutrient status. There is a shift during the year in nutrient limitation. In general, phytoplankton and the benthic algae are P limited in spring. This limitation lasts longer for periphyton. In summer, the lake often becomes N limited for the phytoplankton and the periphyton (Hillebrand and Kahlert, 2001).
The littoral substrate consists of bedrocks, cobblestones and sediments.

Because of its geographical situation, the lake is strongly wind exposed, mostly from westerly and northern directions. The lake is stratified in summer for about three months. Lake Erken has several small inlets and one outlet. Three small islands can be found in Erken, one of them contains a weather station delivering data on air, water, temperature and wind velocity to the field station. Also, the lake serves as drinking water supply for the town of Norrtälje, about 10 km South of Erken. Limnological research has been performed at Lake Erken since 1928.

4 : EXPERIMENT ONE :

”Impact of periphyton nutrient status on grazer feeling selection”

EXPERIMENT ONE : "Impact of periphyton nutrient status on grazer feeling selection"

1 : Material and methods

1.1 : Experimental set-up: general conditions

This experiment was conducted in order to investigate the hypothesis one: *"The macrograzers are able to select high quality food and this is different for different grazers".*

1.1 a : Periphyton

Unglazed ceramic tiles were used as standard substrata. On the 18th of February tiles (ceramic tiles), glued to one concrete plate (concrete; 17 cm to side, normally used for road surfaces kindly provided by the Erken laboratory), were fetched from the water. These tiles (2.5 cm x 2.5 cm = 6.25 cm^2) had been lying there since November 2001. They were incubated in the littoral zone of Lake Erken at approximately 2 m water depth, allowing the colonization of a natural epilithic periphyton community. Periphyton tiles were stripped from all visible macrozoobenthos.

1.1 b : Grazers

For the grazers, three snails were used: *Theodoxus fluviatilis* (L.), *Bithynia tentaculata* (L.) and *Valvata viviparum* (L.) (Gastropoda). *T. fluviatilis*, which means "living in rivers", is called "squares of a chess board-snail" because it has regular pattern of dots (Tachet, 2001). It likes hard substratum and is the most common fresh water snail in Lake Erken. *B. tentaculata*'s features are the high spire on the shell and the tall light tentacles. It likes to hide. *V. viviparum* is known to live between 22 cm and 1 m of depth. It is also one of the most common snail in the Lake Erken (Tachet, 2001). These three snails were chosen for practical reasons in the sampling.

T. fluviatilis and *V. viviparum* have been cultivated in the laboratory three months prior the experiment. They were cultured with algal covered stones in small containers with water from the lake. The snails *B. tentaculata* were collected from the outflow of Lake Erken the 18th of

January. They were placed in the same room as *B. tentaculata* and *V. viviparum*, in water from Lake Erken. The culture room had a constant temperature of 16 ° C. Additionally, an aeration system was installed to guarantee a sufficient supply of oxygen from an aquarium pump. The water was changed once a week and the substrata were exchanged approximately once a month. Duration of light exposure was 24 hours per day. The increasing population indicated the suitability of the conditions. The snails did not receive any food four days prior to the experiment.

1.1 c : Experimental set-up

The set-up was as follows: 16 pre-colonised tiles sampled from lake Erken were transferred to the laboratory and placed into 4 aquaria, filled with unfiltered water from Lake Erken. After 24 h adjustment to laboratory conditions, each aquarium was assigned to one out of 4 nutrients treatments: addition of N (16.0 mM as KNO_3), addition of P (1 mM as NaH_2PO_4), addition of both N and P, addition of none (control). 24 h after the nutrient pulse, one of each nutrient treatment tiles were placed in another aquarium, in common with one tile from each other treatment (in total 4 tiles per aquaria).
They were all placed next to each other at random to form a big square.

One snail was placed in the box in the middle of the four different treatment tiles. It was carefully noted which tile the snail chose. It was considered there was no choice when nothing happened after 5-10 minutes. At each individual, the position of the tiles was changed. The choice of the snail was monitored and repeated for all the replicates. For each species a different number of individuals was used: 25 for *T. fluviatilis*, 17 for *B. tentaculata*, and 10 for *V. viviparum.*

1.2. : Sampling and analysis

1.2 a : Sampling

Samples for Chl.*a* and algal C, N content were taken to verify that the treatment effectively affect the nutrient content and biomass of the periphyton. The algae were scraped from the substratum with a scalpel and suspended in 50 ml filtered water. From this suspension subsamples were filtered on precombusted GF/C (Whatman, 47 mm) filters. Two filters were

used, one for C:N:P (25 ml), the other for periphyton biomass as Chl.*a* (25 ml). Filters were stored at – 20 ° C before analysis.

Chl.a

There are many methods for measuring Chl.*a*. The one used in the experiments described above is rather simple but takes longer time to perform. Here Chl.*a* was extracted using the acetone method according to Cattaneo and Kalf (1986) and Gresens (1995). The sample was vacuum-filtered on small filters. The filters were collected on plastic plates and stored in the freezer. After a few days they were picked up from the freezer and placed in glass scintillation vials. The vials were filled with 9 ml Acetone (90%) and are placed at 4 ° C overnight. After about 24 hours the contents of chlorophyll a were measured in a spectrophotometer. Three ml from each vial was used and the spectrophotometer measured absorption at four different wavelengths: 750 nm, 665 nm, 645 nm, 630 nm. The value at 750 nm is substracted from the other absorption values since it represents turbidity. Chl.*a* was calculated from the following equation. The value is calculated in $\mu g\ L^{-1}$ and transformed to $\mu g\ cm^{-2}$. The absorbance was studied in Units of Absorbance, U. A.

$$\text{Chl.}a\ [\mu g\ cm^{-2}] = \frac{[11.6 \times (E665\ U.A. - E750\ U.A.) - 0.14 \times (E630\ U.A. - E750\ U.A.) - 1.31 \times (E645\ U.A. - E750\ U.A.)] \times 9]}{\text{Tile area}\ [cm^2]}$$

C:N:P ratios

The filter for C N P analysis was cut in two halves: one for P determination and the other for C and N.

Particulate P was measured as phosphate after hydrolysis with potassium persulphate (Grasshoff et al. 1999). The filters were autoclaved together with 10 ml H_2O and 2 ml of a solution of potassium persulphate ($K_2S_2O_8$; 5%; Merck) at 120 ° C for 60 minutes (Certoclav Type CVII/1600).

After cooling, 2 ml of a reacting solution was added. The reacting solution contained 70 % mixing solution (2.5 M H_2SO_4: $(NH_4)_6\ Mo_7O_{24}$ x 4 H_2O (4%) : $K(SbO)C_4H_4O_6$ x 0,5 H_2O (1

mg Sb ml^{-1})) with the ratio 10:3.33:1 and 30% 0.1 M ascorbic acid. After 10 minutes, orthophosphate could be measured with a spectrophotometer at 882 nm, and P concentration was calculated using standard calibration curve.

C and N contents were measured simultaneously with a CHN analyzer (LECO CHN-932). Filters were dried at 60 ° C for 48 h and then transferred into tin capsules (4 x 9 mm) for CN analyses. The amounts of both elements were obtained with standard calibration curves. For the C:N:P ratios, the values were recalculated on molar basis. The cellular nutrient ratios are a useful approach for the detection of nitrogen or phosphorus limitation in microalgae. An optimal stoichiometric ratio of C:N:P = 119:17:1 could be deduced for benthic microalgae, which is slightly higher than the Redfield ratio (106:16:1) considered typical for optimally growing phytoplankton (Redfield, 1958). Cellular nutrient ratios are proposed as an indicator for nutrient status in periphyton.

The following constraints should be observed: (1) the cellular C:P ratio is an index of P limitation, (2) the cellular C:N ratio indicates N limitation in general and (3) the cellular N:P ratio distinguishes between N or P limitation. With N:P ratio < 13 and C:N ratio > 10, the periphyton can be N-limited. With N:P ratio > 22 and C:P ratio > 180, the microbenthic assemblage is P-limited (Hillebrand and Sommer, 1999).

1.3 : Statistical analysis

The χ^2 test was used to check whether there was an effect of the treatments on the choice of the snails.

It was necessary to see if there was any difference in biomass between the nutrient-enriched plates and the control ones: the snails could have chosen the enriched plates because of the biomass instead of the higher nutrients content. Biomasses were compared using one-way Anova.

2 : Results

2.1 : CHL *a* CONTENT

There was no statistically significant difference between the treatments observed (Fig.1). Then, the results of the choice were considered as based on food quality and not on food quantity.

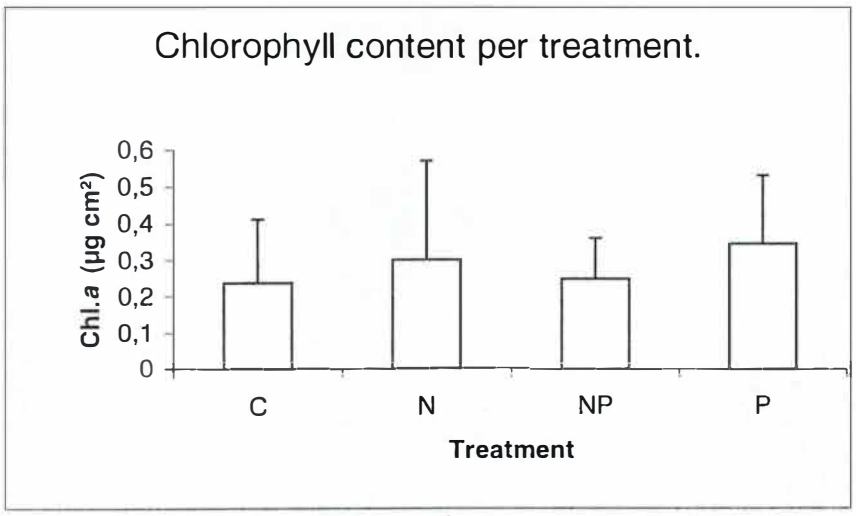


Fig.1: Chlorophyll content (µg Chl.*a* / cm^2) of the periphyton in different treatments as mean +- SD. C = control, N = nitrogen-enriched treatment, NP = nitrogen and phosphorus-enriched treatment; P = phosphorus-enriched treatment.

2.2 : C:N:P ratios

The epilithic community in the control was strongly N and P colimited (Fig.2).

Addition of nitrogen mostly affected C:N ratios (Fig.2). This treatment was still N-limited. It indicated that the periphyton was able to take up the nitrogen, but not in sufficient amount as its C:N ratio indicate N-limitation. However, this N-limitation was less strong than in the control. The low C:P ratios showed that algae could be able to take up more phosphorus with N addition, because no more P-limitation was found after N addition.

Addition of phosphorus had the same effects on C:N and C:P ratios than with the N-enriched treatment (Fig.2). Then the periphyton of this treatment was N-limited.

Addition of both phosphorus and nitrogen led to a decrease of C:N ratios and C:P ratios. These ratios did not indicate a P-limitation, but still N-limitation (Fig.2). Nevertheless, this N limitation was the weakest than in the two others enriched treatments.

The highest difference in C:P ratios was found between the control and the NP enriched treatment, which had the highest content of phosphorus. Concerning N:P ratios, the control showed the lowest content in nitrogen and the enriched treatment indicated a higher nitrogen content, but still a N limitation.

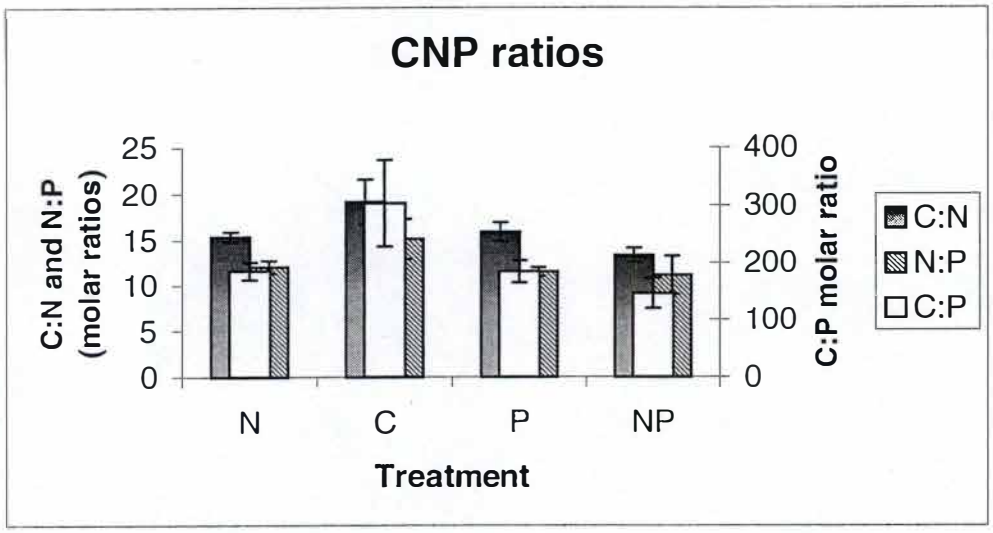


Fig.2: C:N, N:P, and C:P molar ratios of the periphytic community exposed under different treatments (C = control, N = nitrogen enriched-treatment, NP = nitrogen and phosphorus enriched-treatment; P = phosphorus-enriched treatment) for the first experiment, depicted as mean +- SE.

2.3 : CHOICE

V. viviparum

When these tests were conducted, 7 of 10 individuals made a choice. 5 individuals chose the P-enriched treatment, one the N&P-enriched tratment, one with N-enriched treatment, 0 the control.

The χ^2 test found an impact of the treatments on the choice of *V. viviparum* ($p < 0.05$). *V. viviparum* had a preference for P-enriched food compared to the three others treatments together (Table 1).

Table 1: Values resulted of a χ^2 test with 7 individuals choosing one of the four treatments, concerning *V.viviparum.*

treatment	n choosing	E	(O-E)^2/E
control	0	1.75	1.75
NP-treatment	1	1.75	0.32
P-treatment	5	1.75	0.32
N-treatment	1	1.75	6.03
	7	χ^2 obs	**8.43**
		χ^2 table a=0.05	**7.81**

T. fluviatilis

These tests were conducted with 22 of 25 grazers, which made a choice. Five individuals chose with P-enriched treatment, 7 the N&P-enriched treatment, three the N-enriched treatment, and 7 chose the control. Three did not choose at all.

The χ^2 test did not find an impact of the treatments on the choice of *V. viviparum* ($p > 0.05$) (Table 2).

Table 2: Values resulted of a χ^2 test with 22 individuals choosing one of the four treatments, concerning *T fluviatilis.*

treatment	n choosing	E	(O-E)^2/E
control	7	5.5	0.41
NP-treatment	7	5.5	0.41
P-treatment	5	5.5	0.04
N-treatment	3	5.5	1.14
	22	χ^2obs	**2**
		χ^2table a=**0.0**5	**7.81**

B. tentaculata

On 17 individuals, only two of them chose. Subsequently for this one, I was not able to discuss the possible selection of none treatment.

3 : Discussion

Reality of C:N:P ratios :

In this experiment, there was no difference in quantity, so that all choices, which occur, may be originited from the differences in food quality. The periphyton of the control was N and P colimited. Compared to that, the N addition resulted in a periphyton less but still N limited, but strangely there was no more P limitation. We can wonder where did the phosphorus taken up by the periphyton come from in this treatment, because I normally just made only a N addition. Maybe a contamination of phosphorus occurred when the four tiles (from each different treatment) were placed together next to each other to form a big square. Indeed, some water rich in PO_4^{3-} could be transferred from the P-enriched aquarium to the new boxes with the 4 different tiles. Moreover, the choice experiment lasted about two hours, enough time for nutrient-deficient algae to take up the nutrients released by the periphyton from another enriched treatment tile. In addition, the snails could excrete nutrients during the time they were choosing. This could lead to a variation in nutrient content during the duration of the experiment. Remember that the C:N:P ratios presented here were obtained from algae taken up at the end of incubations.

The same problem was observed for the periphyton which came from the P addition treatment. As expected, it showed no more P limitation, but the N limitation was less strong than in the control. The explanation for that could be also the contamination and the excretion of the snails. The results of the NP-enriched periphyton should be logic : it contained the highest content in nitrogen and phosphorus. The periphyton of control was probably also affected by the possible contaminations, but the algae remained more nutrient-limited than the others. That may be probably explained by the fact that they were more nutrient-limited at the start of the experiment.

In conclusion, it seems that there was no more differences in the C:N:P ratios between the 3 nutrient-added treatments at the end of the grazer choice incubations. Differences between the control and the 3 nutrient-added treatments still remained. These observations lead us to the impossibility to really identify which the nutrient treatment may have been chosen by the snails.

Selectivity :

Our experimental design was constructed to test the preference of the snails in presence of 4 nutrient-treatments. This design can not show the preference for one treatment compared to another one, because the 4 tiles were always placed together. However, it can allow to test whether the treatment of the tiles had a statistically significant influence on the choice of the grazer. Unfortunately, as discuss before, we are not able to really identify any chosen nutrient.

Generally the selectivity was low among tested snail species. We observed a selection of some tiles for *V. viviparum*. No statistically significant selection was found in *T. fluviatilis*, which refused to move.

These results do not completely agree with the tested hypothesis. The ability of *"the macrograzers to select high quality food"* was confirmed in this experiment for *T. fluviatilis* (it did not choose), but not for *V. viviparum* and *B. tentaculata*. The question as if there was a species-specific choice is difficult to answer because only one of of the macrograzers chose. The problem was to know what did it mean "high quality" for a snail ? The needs in P and N for each species were probably not the same, but they have not been quantified.

V. viviparum chose the P-enriched treatment, which had low C:N and C:P ratios, meaning good quality. This treatment was characterized by high P content and low N limitation (C: N ratio near to the limit value). We can hypothesize that *V. viviparum* was able to sense great P content, but it did not choose the N-enriched treatment although it had a P content similar to the P treatment. It becomes difficult to find a reason. We do not know which molecule really attracts the snail, and how is the mechanism of "sensing" food. For *V. viviparum* the needs in nitrogen were maybe lower than phosphorus.

We can wonder why *B. tentaculata* did not move during our experiment. A possible explanation was that it could choose but,as this species is also a filter feeder, it chose to filter rather than to graze. (Tachet, 2001). *B. tentaculata* is also characterized by a sedentary behaviour. This kind of observation was also noticed in the experiment of Cruz–Rivera (2000). Due to this behaviour, longer experiment may be necessary to show a selectivity in this species.

That forces to think about the real meaning of selectivity: it is possible to test an attraction for a special kind of nutrient quantity present in certain algae in maximum 10 minutes? Moreover, the selectivity should take more time in natural condition, when the snails were not starving (like in this experiment). That means without stress situation, which could perhaps modify the "sensing food behaviour" tested here. In addition, the "sensing food" behaviour could be associated to or interact with morphology selectivity, and not based only on the "sensing". Thus the senses of touch, taste, and smell could interact in the process of selecting the best adapted food for their growth and reproduction.

5 : EXPERIMENT TWO : "Impact of grazer species on algal community composition"

EXPERIMENT TWO : "Impact of grazer species on algal community composition"

1 : Material and Methods

1.1 : Experimental set-up: general conditions

This second experiment was conducted to deal with the hypothesis two, which states that *"Grazing by different grazer species affects the proportion of different food algae composition differently"*.

1.1 a : Periphyton

The incubation of the tiles was the same as in experiment one. They were fetched from the lake one week (the 11th of March 2002) before starting the experiment (the 18th of March 2002), taken off and moved to the culture room.

1.2 b : Grazers

Three grazers types (BIT treatment = *B. tentaculata*, THE treatment = *T. fluviatilis*, VAL treatment = *V. viviparum*) were used and cultured as described in the experiment one. Some these snails were already used for the precedent experiment, but a new sampling was done. These new snails were placed in the culture room of Erken laboratory with the same conditions as for experiment one.

1.2 c : Experimental set-up

This experiment was conducted in two plastic boxes (40 cm X 3 cm X 36 cm) subdivided in twelve 10 cm x 10 cm small aquaria, each with a maximal volume of 420 ml. All the aquaria were filled in with 150 ml-filtered water from Lake Erken .The water was filtered (GF/C filter) so that algae and small animals that could disturb or have an effect on the results of the experiment were removed. The incubated tiles were randomly placed in small aquaria. The experimental set-up used three different grazer treatments and one control, each replicated four times. This design resulted in 16 treatments that were randomly distributed in the two boxes. Ungrazed treatments served as control for all others treatments.

There was one individual for the VAL and THE treatment, and two for the BIT treatment (because they are slower).

Four tiles were put together to form a square in each aquarium. The total surface was 4 x 6.25 cm^2 = 26 cm^2. In order to provide these aquaria with oxygen, each box was connected by the main aeration system with a small tube. Abiotic conditions were kept constant as in experiment one. The experiment lasted 6 days.

1.2 : Sampling and analysis

1.2 a : Sampling

At day 6, the algae were scraped from the substrata with a scalpel and suspended in filtered water to estimate the Chl a content as well as the "form" groups of algae.
From this slurry (50 ml), an aliquot (10 ml) was filtered on GF/C filters for the analysis of Chl a (all filtered samples were stored frozen until analysis). Another aliquot was preserved with three drops of Lugol's iodine for the determination of algae abundance (cells cm^{-2}) and biovolume.

Chl.a

The analysis of the chl a content was done exactly like in the experiment one.

Algae abundance and biovolume

The identification of algae was done to "form" group (Fig. 2.5). Counting was done with three ml Utermöhl chambers under an inverted microscope at 40x magnification. At least 400 cells were counted per sample.
The total abundance (cells number cm^{-2}) and the biovolume for each group was calculated with best fitting geometric models as described in Hillebrand *et al*, 1999.
This method presents a set of geometric shapes and mathematical equations for calculating biovolumes of more than 850 pelagic and benthic marine freshwater microalgal genera. The equations were designed to minimize the effort of microscopic measurement. In this case, a

shape can be applied to the entire colony or filament. The algae had to be recognised by groups and distinguished by shape (Fig.1).

In order to have an idea about the proportion present of each group in each treatment, the percentage of biovolume per algal group and per treatment was calculated.

DIATOMS	VOLUME CALCULATION	
Unicellular attached without raphe	prism on elliptic base	V= p/4.a.b.c
Unicellular with raphe	prism on elliptic base	V= p/4.a..b.c
Chains	cylinder	$V= p.r^2.h = p/4.d^2.h$
Tubes	cymbelloid	V= 1/6p. (2b)2.a.b/360
Gomphonemoid	gomphonemoid	V= b.c.((p.e/4)+((f-e)/3))
GREEN ALGAE		
Filamentous	cylinder	$V= p.r^2.h = p/4.d^2.h$
Colonist	prolate spheroid	$V= p/6.d^2.h$
CYANOBACTERIA		
Filamentous	cylinder	$V= p.r^2.h = p/4.d^2.h$
Colonist	prolate spheroid	$V= p/6.d^2.h$
ZYGNEMATOPHYTA		
Filamentous	cylinder	$V= p.r^2.h = p/4.d^2.h$

Fig.1: Geometric shapes equations for the calculation of biovolume. Shapes are drawn in a three-dimension version in cross-section. Equations are given, using standard abbreviations for the linear dimensions to be measured. Abbreviations: V = volume; r = radius; d = diameter; h = height; a = apical axis (length); b = transapical axis (width); b = angle between the two transapical sides, to be calculated as sin b/2= c/(2.b); c = pervalvar axis (height); Z = height of cone; l = length of one side; m = height of a triangle; p = 3,1416.

Fresh weight of snails

The snails were picked up on paper and cotton pads to dry (5 seconds) and measure their fresh weights after the experiment (by a microbalance, Co. AND HA-180M).

2.3 : Statistical analysis

For this experiment, statistics were performed using one-way Anova. This statistical analysis aimed to see if there was a significant difference in Chl.*a* between different treatments (grazers & control). Another one-way was used to investigate if there was a significant difference in biovolume between the different treatments (grazers and control). As a significant difference was found, a Tukey test was used to distinguish between effects of different treatment levels.

2 : Results

2.1 : FRESH WEIGHT OF SNAILS

B. tentaculata had the biggest body size, following by *V. viviparum* and after by *T. fluviatilis* (Table 2). The individual mean weight of *B. tentaculata* (161.5 mg) was almost the same of *V. viviparum* (138.7 mg). The individual mean weights of these two precedent snails were three times greater than that of *T. fluviatilis* (56.7 mg).

Table 2: Fresh weight (mg) of the snails (*B. tentaculata, T. fluviatilis* and *V. viviparum*) in the second experiment. The mean (mw) was calculated for *B. tentaculata.*

	Grazers	mg	mg	mw
1	Bithynia	133.4 (a)	119.17 (b)	126,3
2	Bithynia	84.2 (a)	192.1(b)	138.1
3	Bithynia	177.6 (a)	230.2 (b)	203.9
4	Bithynia	173.7 (a)	182 (b)	177.8
13	Theodoxus	54.7		
14	Theodoxus	47.7		
15	Theodoxus	65.5		
16	Theodoxus	58.9		
21	Valvata	149.2		
22	Valvata	92.2		
23	Valvata	153.5		
24	Valvata	159.9		

2.2 : PERIPHYTON BIOMASS

2.2.a : CHL.*a* CONTENT IN FOUR TREATMENTS AFTER GRAZING INCUBATION

The mean was quite different among treatments (Fig.3). Therefore, there seemed to be a grazing impact, which unfortunately could not be proved as statistically significant (Table 1).

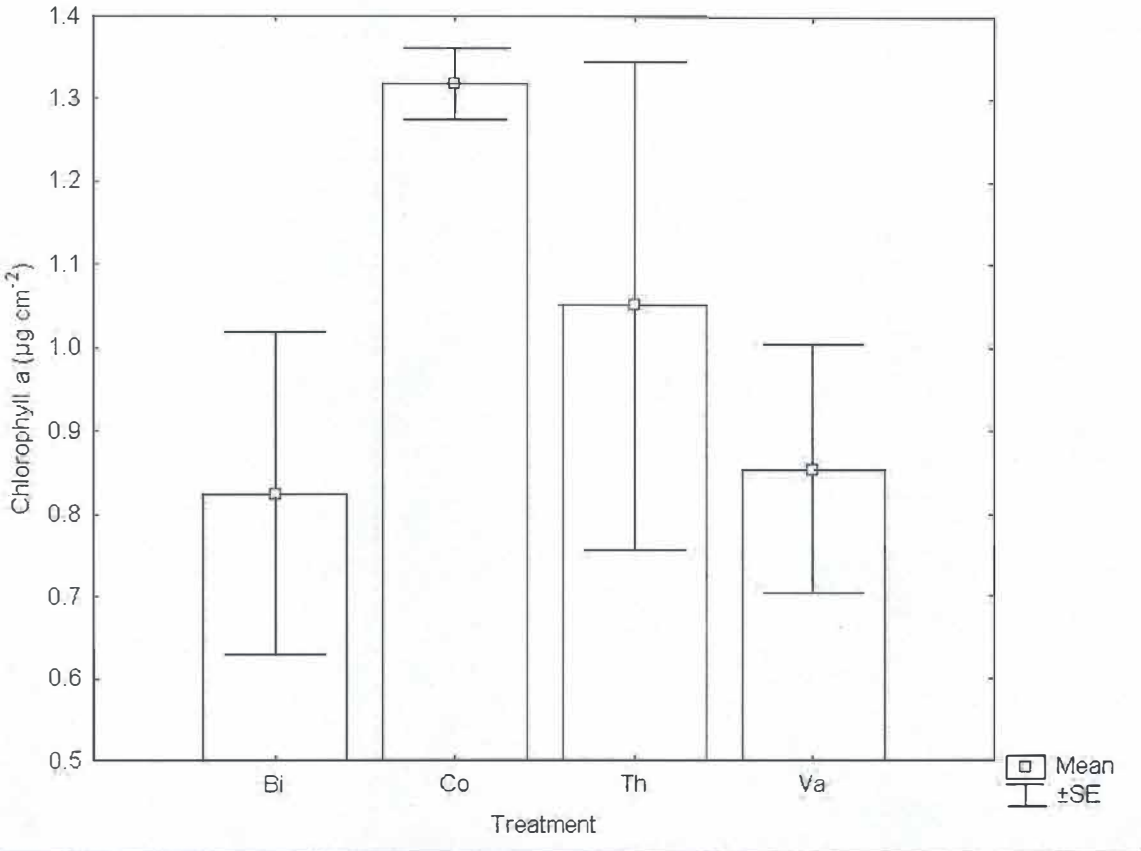


Fig.3: Chl.*a* content (mg cm^{-2}) with no significant difference, in the experiment two, in four grazer treatments (Bi = *B. tentaculata*; Co = control; Th = *T. fluviatilis*; Va = *V. viviparum*), depicted as mean +- SE.

Table 1: Results of a one-factor ANOVA between different treatments on Chl.*a* content, in four different grazer treatments. The variance was homogenous.

Between subjects	df	MS	F	p
Treatment	3	0.2	1.39	0.29
Error	12	0.15		

2.2.b : CELLS DENSITY

The results show a difference (Table 2) in cell density between the control and the grazer treatments. Cell density was similar for the three different grazers. The control showed a density three times higher than the grazer treatments. The SE were very low (Fig 4).

Then a great impact of the grazers was evident on the density of cells. The reduction of density was similar for all grazer treatments compared to the control (Table 3).

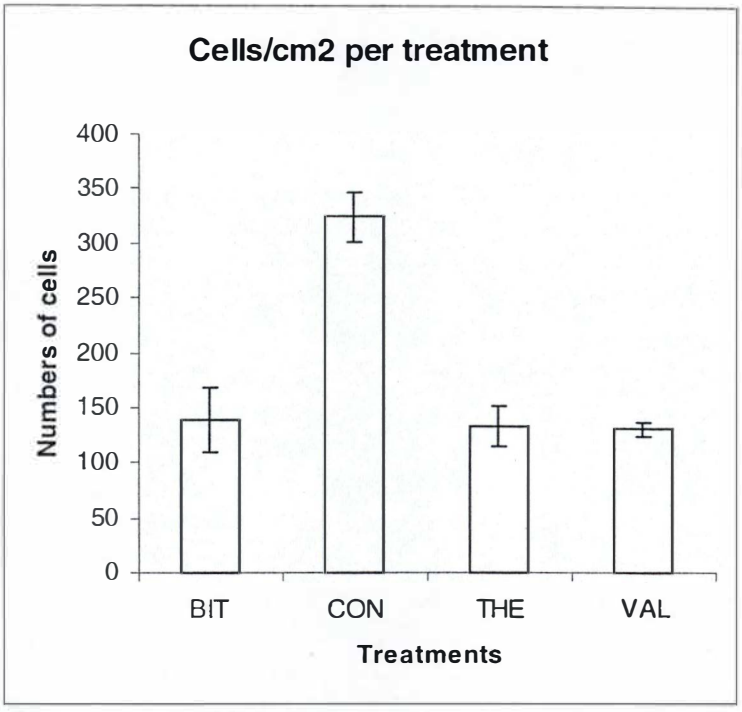


Fig.4: Mean cell number / cm^2 in four different grazer treatments (BIT= *B. tentaculata*; CON = control; THE = *T. fluviatilis* and VAL = *V. viviparum*), depicted as mean +- SE.

Table 2: Results of a one-factor ANOVA between different treatments on cells density, in four different grazer treatments. The variances were homogeneous.

Between subjects	df	MS	F	p
Treament	3	36159.06	20.3	5.39E-05*
Error	12	1780.50		

Table 3: Tukey test on grazer treatments.

	{1}BIT	{2}CON	{3}THE	{4}VAL
{1}BIT		0,0003*	0,9986	0,9956
{2}CON	0,0003*		0,0003*	0,0003*
{3}THE	0,9986	0,0003*		0,9998
{4}VAL	0,9956	0,0003*	0,9998	

2.2.c : BIOVOLUME

The total biovolume was highest in the control. It was followed by THE treatment, which removed 1/3 of the algae (Fig.5). Subsequently, the BIT and VAL treatments showed the same total biovolume, where 2/3 of the algae were removed by each of them (Fig.5).

The ANOVA test showed a significant difference on biovolume between the four treatments (Table 4). Furthermore, a Tukey test (Table 5) found a significant difference between the control and BIT treatment, and also between the control and VAL treatment.

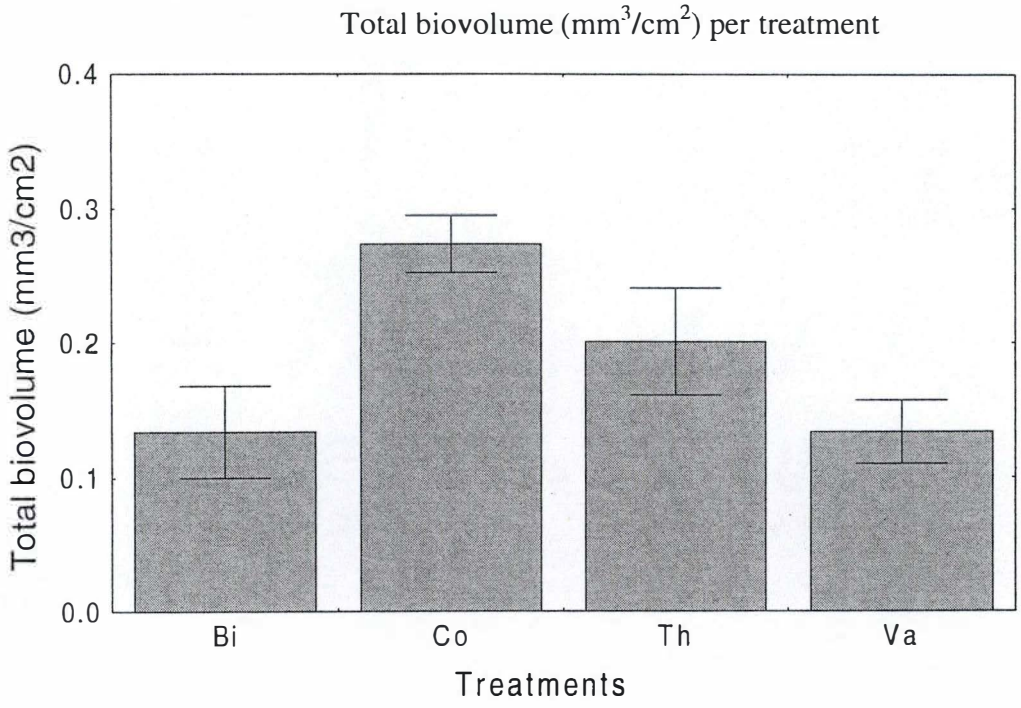


Fig.5: Total biovolume (mm^3 cm^{-2}) per grazer pressure treatment, in four grazer pressure treatments: Bi = *B. tentaculata*, Co = control, Th = *T. fluviatilis*, Va = *V. viviparum* for the second experiment, depicted as mean +- SE.

Table 4: Results of a one-factor ANOVA between different treatments on biovolume, in four different grazer treatments. The variances were homogeneous.

Between subjects	df	MS	F	p
Treatment	3	0.18	4.75	0.02*
Error	12	0.0038		

Table 5: Tukey HSD test on grazer treatments.

	BIT	CON	THE	VAL
BIT		0.032*	0.45	1.0
CON	0.032*		0.37	
THE	0.45	0.37		0.032*
VAL	1.0	0.032*	0.44	0.44

The most important groups were diatoms (unicellular, unicellular with raphe, chains and gomphonemoid), green algae (colony and filament), and cyanobacteria. The Zygnematophyta were not found here. The seasonal pattern of spring is characterized by diatom dominance

(Hillebrand and Kalhert, 2001). The effects of grazers on the forms groups were mainly the reduction in unicellular diatoms with raphe and band-forming species (chain diatoms) (Fig. 6). They were reduced from almost 50 to less than 5 % of the total biovolume. Instead, colonies of green algae and cyanobacteria began to increase in the grazed assemblages. But these effects differed between grazed treatments.

On the one hand, unicellular diatoms and especially those with raphe decreased with *T. fluviatilis*. The chain diatoms were removed by *T. fluviatilis* and *V. viviparum*. There was no high impact on gomphonemoid diatoms by grazers, only a weak decrease was observed in the THE treatment.

On the other hand, green algae filaments showed a high increase in THE treatment, and the green colonies appeared in greater numbers in the THE and VAL treatment. Cyanobacteria rarely appeared in these two treatments.

The removal of most diatoms allowed green colonies and filaments to grow, seemingly because they were not removed by grazers. This "grazing resistance" could have resulted from greater cell wall thickness or physiognomy, which rendered them less edible. Another possibility is that *T. fluviatilis* had a preference for understory algae still present after 6 days. The BIT treatment showed no difference with the control in the algae proportion. (Fig. 6).

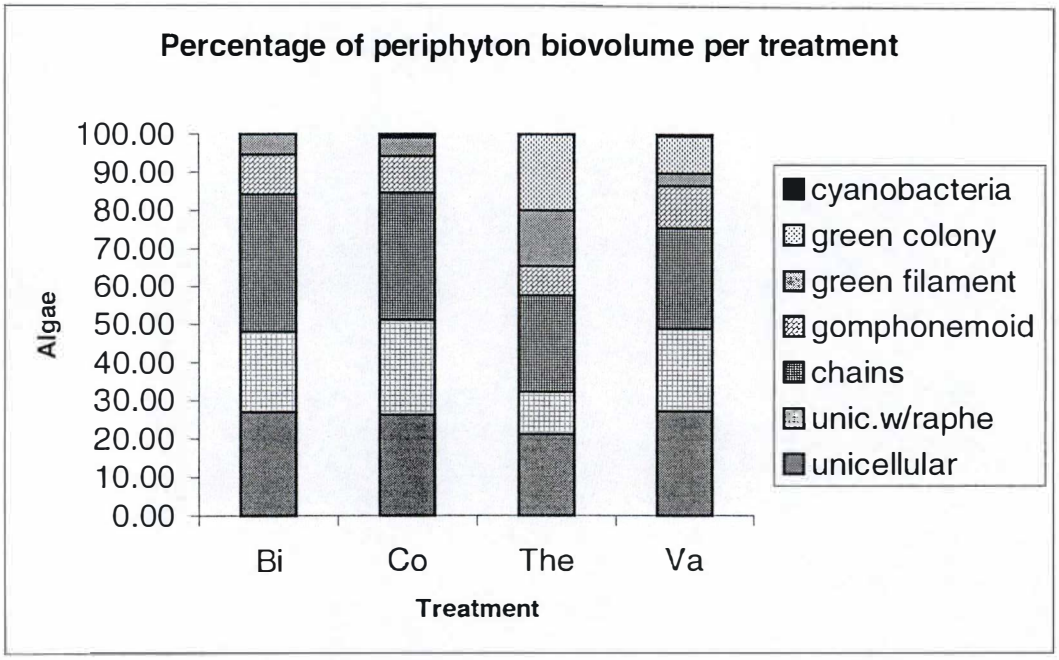


Fig.6: Percentage of periphyton biovolume in four grazer pressure treatments: Bi = *B. tentaculata*, Co = control, The = *T. fluviatilis*, Va = *V. viviparum* for the second experiment.

3 : Discussion

Grazing on periphyton biomass:

This experiment used snails whose the individual fresh weight was the highest for *B. tentaculata* and *V. viviparum,* followed by *T. fluviatilis*. The individual fresh weight of the last snail represented the third of the individual fresh weight of the two precedent snails. The grazing of each species of snail had a negative impact on periphyton biomass in terms of Chl.*a*, which however was not significant, due to high variability in a serie of measurements. Moreover, a great impact of the grazers was evident on the density of cells and the biovolume: the reduction of cells density was the same (almost 1/3) for all the grazer treatments compared to the control.

The three different species consume different amount of algal biomass (Fig.5). A relation could be expected between snail body size and amount of periphyton biomass removed: *B. tenticulata* and *V. viviparum* grazed 2/3 of periphyton compared to *T. fluviatilis* which removed 1/3 of the periphyton. Thus grazing by different snail species affected differently algal biovolume. In fact, the impact of the *V. viviparum* and *B. tentaculata* on the periphyton biomass was similar and differed significantly from the control. *T. fluviatilis* affected periphyton biovolume to a lesser extent.

Grazing and algae composition:

Regarding the growth form composition, the most important results was that grazing by *T. fluviatilis* removed unicellular with raphe and chains diatoms. Moreover, it allowed green colonies to grow. For the two others snails, the effects on algal composition were small, except that *V. viviparum* increased also green colonies and reduced diatom chains. The effect on growth forms was thus reduction in chains, but not green filaments (which however were of generally low importance) and then the decrease in raphe-bearing diatoms (with THE) and the increase in colonies (with THE and VAL treatments). The hypothesis two, testing whether the *"grazing by different grazer species affects the proportion of different food algae composition differently"*, was confirmed here. *T. fluviatilis* removed chains, unicellular with raphe diatoms to allow colonies to grow. But there was no decrease in green colonies and filaments as reported in other studies. In fact, in the experiment of Hillebrand and Kalhert (2001) "filamentous algae (chlorophytes) were reduced most by grazing whereas the relative importance of filamentous cyanobacteria was often enhanced by grazer presence".

Here I did not find an enhancement of cyanobacteria excepted weakly in VAL treatments, where the snail may have avoided this kind of algae because it was not edible.

There was no negative impact on overstory (filamentous) algae and green colonies as shown in most studies. It was maybe a consequence of cell wall thickness or of algae morphology. Other possibilities could be that they were not edible or that *T. fluviatilis* had a preference for understory algae still present after 6 days. The impact of *V. viviparum* and *B. tenticulata* was weaker as described before. *B. tenticulata* has scarcely, if anything, modified the growth form composition.

A shift in algae composition, resulting from selectivity in the grazing of the snail, was also found in the experiment of Sommer (1997), Hillebrand and Kahlert (2002) where "(...) grazing negatively affects most filamentous species. The chain forming diatoms decreased with grazer presence. Grazing had less impact on single celled species ".

6 : EXPERIMENT THREE : "Grazing impact on periphyton biomass and spatial heterogeneity on the algae distribution"

EXPERIMENT THREE : "Grazing impact on the periphyton biomass and spatial heterogeneity on the algae distribution"

1 Material and methods

1.1 Experimental set-up: general conditions

This experiment was designed to test effects of two grazer types on epilithic periphyton in a longer experiment than the precedent. The question was "how do different grazer types (a "clean-sweaper" = *T. fluviatilis* and a "trail wake" *B. tentaculata*) affect the biomass of periphyton, and do they generate spatial heterogeneity? Therefore, this third and last experiment was conducted to test the hypothesis three, wich states: *"Grazing has an impact on the spatial heterogeneity of the periphyton and this spatial heterogeneity is correlated to the distance".*

1.1 a : Periphyton

The periphyton communities were pre-grown in Lake Erken on ceramic tiles for at least 3 months like in the experiment two. They were covered by dense periphyton at the start of this experiment.

1.1 b : Grazers

T. fluviatilis and B. *tentaculata* were chosen as grazer organisms. Both were "bulldozer" (they graze on the bottom). They differed in mobility: *T. fluviatilis* could be characterised by high mobility in contrary to *B. tentaculata.* Their grazing behavior was also different. For this reason, I called *T. fluviatilis* "clean-sweaper" because they are characterized by a high grazing effect, and *B. tentaculata* "trail wake" because they let tracks behind them after they grazed (Tachet, 2000). Both belong to the most common benthic invertebrates in Lake Erken (Hillebrand, 2001). Before the experiment started (the 22nd of April), both periphyton and grazers were transferred to the laboratory and kept for several days at experimental conditions to adjust to laboratory conditions. The two snails used, *T. fluviatilis* and *B. tentaculata*, were cultured in the same conditions as in experiments one and two.

1.1 c : Experimental set-up

This experiment was conducted with three different grazer treatments (BIT treatment = *B. tentaculata* and THE treatment = *T. fluviatilis* and the control), being replicated four times and resulting in 12 treatments. All treatments were randomly distributed as shown in Fig.1. This design resulted in 12 aquaria. Ungrazed treatments served as control for all other treatments. Subsequently, twelve plastic aquaria were filled in with 3.1 liters lake water filtered on GF/C. In each of these aquaria, 36 pre-grown tiles were placed (tile area 6.25 cm^2 and total grazable area 225 cm^2). The light was provided from above by Osram biolux and Fluora lamps and the photoperiod was 12 : 12 light : dark . The pH was around 7.0-7.5 in all the boxes. Photon flux was 58 $\mu mol\ m^{-2}\ s^{-1}$. Additionally, an aeration system was installed as in the two precedent experiments. BIT treatments were stocked with 10-13 individuals of *B. tentaculata* (mean blotted fresh weight per aquarium +- S.E. = 1.84 g +- 0.01), the THE treatments were stocked with 9-13 individuals of *T. fluviatilis* (mean blotted fresh weight per aquarium +- S.E. = 1.26 g +- 0.05).

Box 1

CON2	BIT2	BIT1
CON3	THE2	BIT3

Box 2

THE1	CON4	CON1
THE4	BIT4	THE3

Fig.1: Random distribution of two different grazer levels (BIT = *B. tentaculata*, THE = *T. fluviatilis*) and one control (CON = control), resulting in 12 treatments (Box 1-2).

1.2 : Sampling and analysis

1.2 a : Sampling

At the beginning of the experiment each tile was assigned a random number between 1 and 36. Each sampling day (0, 2, 4, 8, 15 and 23 days after the start of incubation), three tiles were removed at random following the scheme (Fig.2), and replaced by reserve tiles pre-grown in Erken Lake. From the three sampled tiles, the periphyton was removed with razor blades,

algae conglomerates were carefully separated with scissors and forceps. The suspension was adjusted to a defined volume of 50 ml and was divided into six different subsamples.

27	9	19	23	14	33
5	16	12	31	4	26
2	28	15	13	1	7
6	21	34	30	29	25
17	35	22	11	24	18
32	8	3	20	36	10

Fig.2: Random distribution of the pre-grown colonised tiles in each box of the third experiment.

From this slurry, the following subsamples will be taken: 5 ml with Lugol to count algae, 5 ml with glutaraldehyde for counting bacteria and flagellates, 10 ml for ciliates, 5 ml for live counts, 20 ml fixed for meiofauna, 2.5 ml filtered for Chl.*a* (half filters), 2.5 ml filtered for C : N : P (half filters).
Only data on Chl.*a* are presented here. The experiment lasted 23 days.

Every day the temperature and the snails were checked. If one snails was dead, another of similar weight and size immediately replaced it. Note that only 3 individuals (two *B. tentaculata* and one *T. fluviatilis*) were replaced during all the experiment.

Concerning the spatial heterogeneity, a sampling was done at the last day (d 23) for tiles 19-34; the biomass of each tile was scraped off and filtered on one filter, to be used for Chl.*a* analysis.

*Chl.*a

This analysis used the same method than in the experiment one and two.

Spatial autocorrelation

This equation was used to calculate the diffence in Chl.*a* content between two tiles:

$$\left| \frac{\text{Chl.a}_i - \text{Chl.a}_j}{d_{i-j}} \right|$$

i = tile

j = another tile

d = distance between the two studied tiles

This difference in Chl.*a* content was calculated between all the tiles (19-34), and recorded to the distance separating the two studied tiles.

1.3 : Statistical analysis

Repeated measurement ANOVA was used to detect significant effects of grazer treatments (*T. fluviatilis, B. tenticulata*, control) on algal biomass (Chl.*a*) and significant changes over time within treatments. The values of Chl.*a* were log-transformed to have homogeneous variance.

A Tukey test was used to distinguish between effects of different treatment levels.

Another Tukey test was used to contrast effects of each different time against each other during the experiment.

The impact of grazer presence on the spatial variation in algal biomass was investigated by calculating the coefficient of variation (CV), which is the standard deviation as percent of the mean. CV is a measure of variation around the mean using all replicates of one treatment combination.

A linear regression was realized with the percentage of relation (R^2) between difference in Chl.*a* and the distance between the tiles in order to investigate whether the spatial heterogeneity was correlated to the distance.

2 : Results

2.1 : CHL a CONTENT

Grazing had a strong impact on algal biomass (Fig.3 and Table 2). In fact, compared to the initial amount of Chl.*a*, the biomass of the periphyton began to increase in the control and afterwards decreased. For the grazer treatments, Chl.*a* on the substrata decreased step by step to reach the level 0 (Fig.3).

The ANOVA showed a significant difference between grazer treatments (Table 2). Furthermore a Tukey test indicated that the control differed from the two grazer treatments. However, the two grazer treatments did not differ from each other (Table 3). They affected the variation of the periphyton similarly.

The ANOVA test found also a significant difference among the dates (Table 2). A Tukey test (Table 4) showed an effect of the time almost everywhere. That indicated that the total algal biomass varied during the experiment. Then it was evident that the variation of Chl.*a* consumption during the 23 days of experiment was influenced by the grazers.

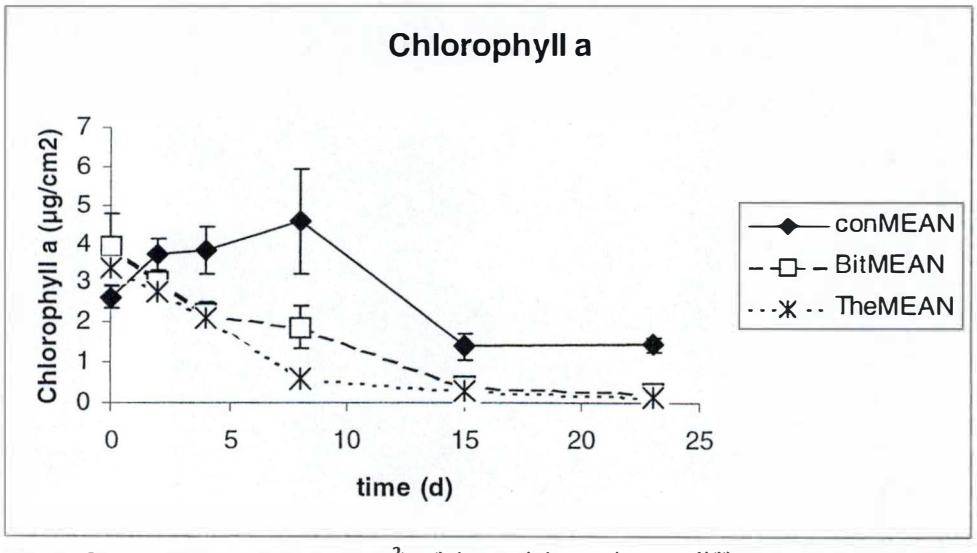


Fig.3: Chlorophyll content (µg cm^{-2}) of the periphyton in two different grazer pressure treatments (BitMEAN = *B. tenticulata*, TheMEAN = *T. fluviatilis*) and one control (conMEAN = control), at each sampling dates for the third experiment. Mean +- SE.

Table 2: Repeated measurement ANOVA on algal biomass (Chl.*a*) in the experiment on temporal variation, with grazing (*T. fluviatilis, B. tenticulata*, control) and time as independent factors. The table gives the degrees of freedom (df), mean squares (MS), the F-ratio (F) and the significance level (p). This statistical analysis was performed on log-transformed data in order to obtain homogeneous variances.

	df	MS	F	p
Between subjects				
Grazer	2	0.29	17.57	0.0078
Error	9	0.016		
Within Subjects				
Time	5	0.46	59.21	0
Time X Grazer	10	0.048	6.1	0.000009
Error	45	0.0079		

Table 3: Tukey test HSD on grazer treatments. The values had significant p levels.

	CON	BIT	THE
CON		0.00804*	0.00084*
BIT	0.00804*		0.22501
THE	0.00083*	0.25017	

Table 5: Tukey test HSD on days. The values had significant p.levels

days	0	2	4	8	15	23
	0.62	0.61	0.55	0.44	0.21	0.18
0		0.99	0.41	0.00042*	0.00014*	0.00014*
2	0.99		0.52	0.00064*	0.00014*	0.00014*
4	0.41	0.52		0.067	0.00014*	0.00014*
8	0.00041*	0.00064*	0.67		0.00014*	0.00014*
15	0.00014*	0.00014*	0.00014*	0.00014*		0.97
23	0.00014*	0.00014*	0.00014*	0.00014*	0.97	

2.3 : SPATIAL HETEROGENEITY

The CV was used as an indicator of the spatial heterogeneity for the three different treatments: it was calculated to compare each difference of Chl.*a* to the mean of the respective treatment. The THE treatment had the highest variation between each Chl.*a* content of each tile. The BIT treatment showed the same tendency but less strongly. The control had lower values (Fig.4). Furthermore, the t-test showed a significant difference between the CVs of the control and BIT (CON us BIT, $t_{(4)} = -4.93$, $p = 0.008$). The same value was obtained between the control and THE (CON us THE, $t_{(4)} = -4.98$, $p = 0.0078$). Finally, the t-test made between the two grazer treatments showed a value near to the significance limit (BIT us THE, $t_{(4)} = 2.78$, $p =$

0.05). The impact of these two grazers was almost significantly different from each other. That allowed confirming that grazing increased heterogeneity of the biomass.

The differences in Chl.*a* content between each tile of a same aquarium were lower in grazer treatments (Fig.6 and 7), compared to the control (Fig.5). These low biomass levels were due to the grazing. The distance between tiles did not explain the difference in Chl.*a* content. In fact, for all the different treatments, the R^2 of the coefficients of determination were very low (Fig.5, 6 and 7).

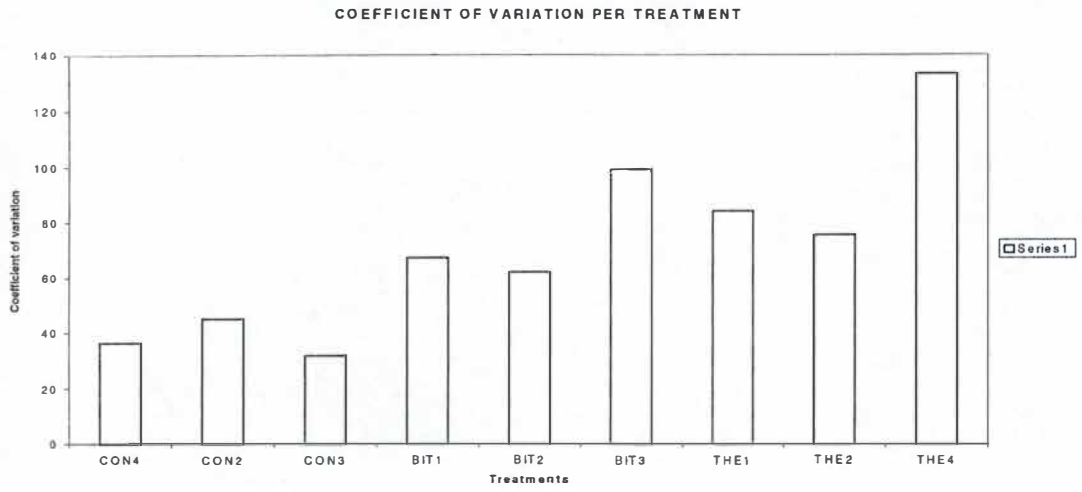
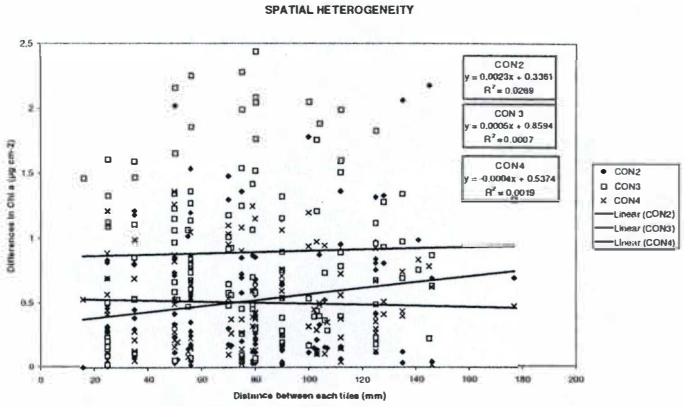
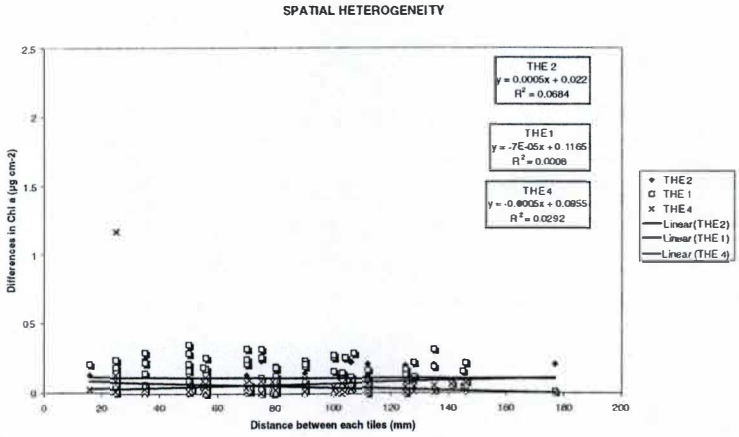


Fig.4: Results of the coefficient of variation (CV) between each treatment during the third experiment.





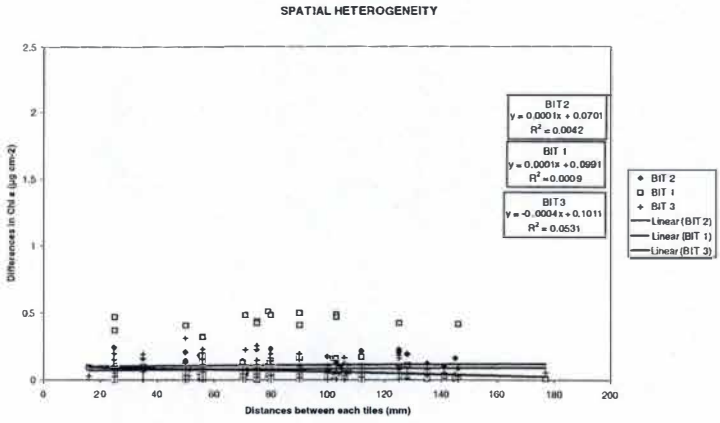


Fig.5, 6 and 7: Results of a linear regression with the percentage of relation between difference in Chl.*a* and the distance between tiles, in the control, in THE treatments (*T. fluviatilis*) and in the BIT treatment (*B. tentaculata*) during the third experiment.

3 : Discussion

Grazing on periphyton biomass:

In this experiment, snail grazing significantly reduced algal biomass of the grazer treatments, which is a consistent result for freshwater (Feminella and Hawkins, 1995). The control differed from the two grazer treatments, but the two grazer treatments affected the periphyton similarly.

B. tentaculata and *T. fluviatilis* consumed the similar algal biomass during the experiment, however the consumption of algae varied with time according to the two grazers species: *B. tentaculata* decreased the biomass slower than *T. fluviatilis*. Already after day 8, *T. fluviatilis* had grazed almost all the algae.

For *B. tentaculata*, this level was reached 8 days later. That could be due to the feeding rate of *B. tentaculata*, which seems to be slower than that of *T. fluviatilis*. Moreover, the fresh weight of the two grazers was significantly different: the fresh weight of *B. tentaculata* was greater than *T. fluviatilis*.

B. tentaculata had perhaps filtered nutrients in the water column, and after the decreasing of the nutrients in the water column, it became to graze more instead of filtering.

Grazing on spatial heterogeneity:

On one hand, by analysing the coefficient of variation for the different treatments, the CVs of Chl.*a* were much higher in grazer presence (60%) than without (30%). The t-test confirmed this observation by a significant difference in the CV of Chl.*a* between the control and the two grazer treatments. Thus grazing increased spatial heterogeneity by forming tracks. In fact after some days, there was a diverse mosaic of fresh grazing tracks. The first part of the hypothesis three ("*Grazing has an impact on the spatial heterogeneity of the periphyton*") was confirmed. Grazing had an impact on the heterogeneity of the biomass, and moreover the results showed a increase of spatial heterogeneity. This significant impact of grazing organisms on the spatial distribution of the periphyton was already found in other studies with others organisms (Sommer, 1999; Kawata *et al.*, 2001).

This effect found here could be linked with the interaction between the way they grazed and the existing spatial pattern of periphyton existing on the tiles. Moreover the impact of grazing could depend also on the distribution of the grazers and on their behaviour. Here, the design

did not allow them to escape from the area in the aquaria, and kept them in a homogeneous distribution with snails only of the same species. The presence of a competitor of predator could make this impact of the snail less strong. Did this result reflect the reality of the grazing on natural benthic algae heterogeneity? Indeed, the impact of grazing on the heterogeneity of the algal resource had different implication for small spatial scales (equivalent to the action range of individuals) and larger spatial scales (equivalent to the effects of the grazer population).

The second part of the last hypothesis states that *"this spatial heterogeneity is correlated to the distance"*. The variation in differences in Chl.*a* (spatial heterogeneity) was not correlated to the distance (R^2 very low). Grazing did not change the pattern between distance (between each tiles) and Chl.*a* in the control as well as grazed treatments.

An explanation could be that the distance between tiles was too small (25 mm to 150 mm). The grazed tiles were quite homogeneously distributed on the total surface of each aquarium. We can thus suspect that the snails of a same aquarium did not grazed in patches. As all used tiles were pre-grown in the same conditions, we can suspect that the community present in each tile were quite the same than the others. It may justify why we do not observed a selection of some tiles. They fed what they met on their way.

In addition, the increase of spatial heterogeneity by grazing could depend on the distribution of the grazers and on their behaviour. At small scale, spatial heterogeneity increased with grazing pressure for other grazer species than these studied here (Kawata 2001). The distribution of grazers here did not reflect the real distribution in the natural environment: the density was probably too high and did not favour the assembly of the organisms: they were spread on the all area.

Therefore, the spatial distribution of algae could be dynamic and under the influence of competition among grazers and spatial heterogeneity.

The spatial heterogeneity caused by grazing should have also an effect on the species richness and composition with creating patches. The diversity of algae creating by grazers could have a feedback effect on the grazing, and then modify the impact of grazing on spatial heterogeneity, in the long run. This experiment was a short-term one, and could not investigate the temporal effect, for period longer than 4 weeks (Feminella and Hawkins, 1995).

7 : CONCLUSIONS AND PERSPECTIVES

CONCLUSIONS AND PERSPECTIVES

Experiment one shows that one of the studied macrograzers may be able to select high quality food. In fact, the different treatments of the four tiles had an influence on *V. viviparum* and it preferred the P-enriched treatment in presence of the other treatments, although this treatment was N-limited. *T. fluviatilis* did not show any preference. Finally, *B. tentaculata* refused apparently made no choice. However, these results must be treated carefully because of the problem of nutrient contamination, probably varyiable during the experiment. Another design could be realized to avoid this contamination. I suggest to place a fifth tile in each nutrient-pulse treatment in order to measure, prior to start to the choice incubations, the algal C:N:P ratios for future comparisons with the ones after incubations with the snails. That could confirm if there was a contamination during the incubations with the snails. Another solution, more exhaustive, should be to prepare much more tiles with periphyton for each treatment and to use them only once (for each snail), and also to change the water between each snail. A "dried algae" method (Van Donk *et al.*, 1993; Elser, 2000) could resolve the contamination, destroying the enzyme mechanisms of taking up the nutrients.

With this method, it could be interesting to investigate the selection for only one treatment, in presence of another treatment. A same design could be imagined with only two tiles with the same biomass but differing in nutrient status. This kind of experiment could also be conducted for a longer period of time (for example 2 days) in order to identify the most intensely grazed tiles. In this case, the selection of one tile made by the snail must be considered as a more complex behaviour, not only based upon the 5-first minutes of valuation of its environment.

The experiment two shows that the grazing of the three species decreased significantly the algae biovolumes and densities but not biomasses. The proportion of the different food items (algae) were altered in two of three grazer treatments. The most drastic changes were observed in the treatment with *T. fluviatilis*. This species removed unicellular and chains diatoms. These diatoms were mostly replaced by filamentous and colonial green algae. Due to their larger sizes, these algae were probably less easily edible. The same effect, altough less strong, was observed for *V. viviparum*. In contrast, *B. tentaculata* did not change the proportion of the algae, compared to the control.

The experiment three shows that the biomass of the periphyton was strongly affected by the two different grazers, *T. fluviatilis* and *B. tentaculata*. Moreover the Chl.*a* content decreased more rapidly with *T. fluviatilis* than with *B. tentaculata*. The grazing of both species increased with the same magnitude the spatial heterogeneity of algae. The grazing pressure was thus not everywhere at the same level. This disturbance due to the grazers may favor some specific algae but we did not test it. Worth noting is that the difference in Chl.*a* between two tiles of the same aquarium was not correlated by the distance between these two tiles, meaning that the difference in the grazing pressure was not explained by the spatial position of the tiles. Then the snails grazed periphyton in a homogeneous way: they were not concentrated to feed the algae, which were close to each other. To investigate the role of the distance in the grazing, another laboratory experiment in a larger area, al least 1 m^2, could be proposed.

An experiment under natural conditions could also increase our knowledge about the impact of the grazing on algal spatial heterogeneity and biodiversity. We may carry out a survey of the mean benthic grazer densities (gastropods, crustaceans, insects...) of the littoral zone in, for example, 50 swedish lakes where, during all the growing season, an artificial substrate (for example a tile of 1 m^2). At the end of the season, the Chl.*a* could be quantified in 20 small areas of the artificial substrate. The observed spatial heterogeneity of algae grown on the substrate of each lake could be compared with the actual mean densities or biomasses of *in situ* benthic grazers. The influence of grazer species or densities on algal biodiversity may also be tested. The major difficulty of this survey is to choose 50 lakes with expected contrasting biomass in herbivores, but with quite the same characteristics of abiotic factors as water quality, nutrient enrichment or solar irradiation exposure. It may be done by choosing lakes from the same geological area with non anthropogenic nutrient sources. Differences in herbivore densities may be obtained in choosing lakes with different fish community composition.

8 : LITERATURE

LITERATURE

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9 : APPENDIX

APPENDIX

Experiment 1

Table 1 : Content of Chl.*a* (µg/cm2) in each treatment.

treat	Chl.*a* (µg/cm2)
C1	0,24
C2	0,48
C3	0,15
C4	0,08
N1	0,28
N2	0,68
N3	0,21
N4	0,04
NP1	0,08
NP2	0,29
NP3	0,29
NP4	0,33
P1	0,43
P2	0,26
P3	0,56
P4	0,14

Table 2 : C:N:P ratios in each enriched-treatments and the control.

molar ratios	1) C : N	2) C : P	3) N : P
N1	16,65407343	218,1872371	13,10113337
N2	15,91078397	199,6707881	12,54939974
N3	14,41074951	181,995489	12,62914804
N4	14,46187488	145,4597671	10,05815417
C1	24,2384106	491,0667913	20,25985942
C2	19,32931424	322,4452555	16,68167072
C3	20,40286892	274,8839369	13,47280806
C4	12,68330513	128,2782028	10,11394124
P1	15,12065387	164,7988506	10,89892355
P2	17,34742932	204,6148003	11,79510789
P3	17,82723577	228,4022486	12,81198339
P4	13,304496	141,4696478	10,63322111
NP1	11,49895833	141,5734177	12,31184717
NP2	13,41935644	222,559232	16,58494079
NP3	15,45784006	109,6177654	7,091402485
NP4	12,97202797	111,508629	8,596082994

Experiment 2

Table 1 : Content of Chl.*a* (µg/cm2), biovolume and cells numbers per cm2 in each treatment.

treatments	Chl.*a* (µg/cm2)	biovol.(mm3/cm2)	cells/cm2
Bi	1,10	0,12	149,53
Bi	0,29	0,05	54,1
Bi	0,77	0,17	155,57
Bi	1,13	0,20	191,91
Co	1,26	0,22	270,48
Co	1,28	0,28	331,43
Co	1,45	0,28	313,34
Co	1,28	0,32	381,91
Th	0,69	0,11	78,05
Th	0,78	0,30	147,95
Th	1,93	0,18	163,19
Th	0,81	0,22	144,46
Va	0,68	0,20	131,12
Va	1,27	0,10	116,92
Va	0,87	0,11	129,54
Va	0,60	0,12	147,31

Experiment 3

Table 1 : Content of Chl.*a* (µg/cm2) in each treatment, at each sampling date.

day	con: Chl.a (µg/cm2)	bit: Chl.a (µg/cm2)	the: Chl.a (µg/cm2)
0	3,22	5,40	3,48
0	2,78	4,95	2,39
0	2,59	3,70	4,34
0	1,92	1,75	3,26
2	4,51	2,78	2,70
2	2,62	3,01	2,73
2	4,09	2,78	2,14
2	3,64	3,61	3,42
4	2,98	1,47	2,14
4	3,20	2,34	1,70
4	5,59	2,06	1,48
4	3,45	3,00	3,09
8	8,69	1,87	0,45
8	3,20	3,20	0,74
8	3,45	1,84	0,85
8	3,03	0,61	0,29
15	1,84	0,48	0,27
15	0,71	0,70	0,21
15	2,06	0,28	0,41
15	1,00	0,21	0,33
23	1,82	0,21	0,14
23	0,97	0,28	0,14
23	1,56	0,20	0,20
23	1,41	0,39	0,13

Table 2 : T-tests between the different treatments.

	T-tests Group 1:THE treatment Group 2: BIT treatment					
Variable	Mean THE	Mean BIT	t-value	df	p	Valid N THE
Var2	87,45	64,48	2,78	4	0,05	3

	T-tests Group 1: CON (control) Group 2: BIT treatment					
Variable	Mean CON	Mean BIT	t-value	df	p	Valid N CON
Var2	41	64,48	-4,93	4	0,008	3

	T-tests Group 1:THE treatment Group 2: BIT treatment					
Variable	Mean CON	Mean THE	t-value	df	p	Valid N CON
Var2	41	87,45	-4,98	4	0,0078	3