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The role of meiofauna in the nitrogen cycle of a cold marine mesocosm

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ABSTRACT

The nitrogen cycle is the key process in marine mesocosms. This cycle involves mineralizing and nitrifying bacteria which are preyed upon by meiofauna. Large populations of meiofauna (20-40 $g \cdot m^{-2}$ in the top 2.5 cm of sand) are found in the sand filters of the St. Lawrence marine mesocosm at the Montreal Biodome. A nitrogen budget was prepared for 1995 to quantify the importance of mineralization and nitrification in the mesocosm. Of the input nitrogen, 15% to 31% were transformed into ammonia through bacterial mineralization, and 71% passed through the nitrification process. Filtration and cleaning removed 20% of the input nitrogen, and only 5% were transformed into animal biomass. Experiments were then conducted in microcosms to see how filter meiofauna affect the apparent mineralization rate (AMR) and apparent nitrification rate (ANR). Microfauna and meiofauna abundances were manipulated to quantify their effect on AMR and ANR by linear regression analysis. Harpacticoid copepods dominated in number (87%) and biomass (98%). Neither AMR nor ANR were related to microfauna densities. AMR was inversely related to meiofaunal mass. Particle size affected the effect of meiofauna whereas the C:N ratio affected the AMR_0, i.e. the AMR in absence of meiofauna. Meiofauna biomass had no effect on nitrition but had a variable effect on nitration. This effect varied from –20% to +571%. The slope of the ANR vs Meiofauna regression was related to the particulate organic nitrogen content of the sediment. Meiofauna seems to exert a direct action by preying on nitrifiers and an indirect action by reducing the mineralizing bacteria through grazing. Results suggest that meiofauna plays an important role in water treatment by reducing the production of nitrate and more rapidly eliminating nitrite which are toxic to fish.

RÉSUMÉ

Le cycle de l'azote est le plus important des cycles dans un mésocosme marin et il implique des bactéries minéralisantes et nitrifiantes dont se nourrit la méiofaune. Les filtres à sable du Saint-Laurent marin du Biodôme de Montréal renferment de fortes concentrations de méiofaune (20-40 $g \cdot m^{-2}$ dans les 2.5 premiers cm de sable). Un budget de l'azote a été établi en 1995 pour mesurer l'importance de la minéralisation et de la nitrification dans le mésocosme. La minéralisation bactérienne a atteint 15-31% des intrants en azote et la nitrification 71%. La filtration et l'entretien ont enlevé 20% des intrants et seulement 5% ont été intégrés à la biomasse animale. Des expériences en microcosmes ont été ensuite menées pour mesurer l'effet de la méiofaune des filtres sur les taux apparents de minéralisation (TAM) et de nitrification (TAN). L'abondance de la microfaune et la méiofaune ont été manipulées pour quantifier leur effet sur le TAM et le TAN par régression linéaire. Les copépodes harpacticoïdes dominaient la méiofaune en nombre (87%) et en masse (98%). La densité de microfaune n'a pas fait varier le TAM ni le TAN. Le TAM a été inversement corrélé à la masse de la méiofaune. La taille des particules du substrat a affecté l'effet de la méiofaune tandis que le rapport C:N a affecté le TAM_0, i.e. le TAM en l'absence de méiofaune. La méiofaune n'a eu aucun effet sur la nitrition mais a eu un effet variable sur la nitration. Cet effet a varié entre -20% à +571% selon le contenu en azote organique particulaire dans les sédiments. La méiofaune exercerait une double action de prédation des bactéries nitrifiantes et de réduction de la compétition par broutage des bactéries minéralisantes. Les résultats suggèrent que la méiofaune joue un rôle utile dans le traitement de l'eau en réduisant la production des nitrates et en éliminant plus rapidement les nitrites, toxiques pour les poissons.

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As for any project of this size, this thesis would not have been possible without the moral, physical and financial assistance of many people. I think it is essential to name as many as possible here not only as an expression of my most sincere gratitude but also to show that each of us can contribute the advancement of science in her or his own way.

First of all, I would like to thank the management of the Montreal Biodome who accepted me as part of the Research and Development team in 1992. They convinced me to undertake this Ph.D. so as to better understand the biological processes of the St. Lawrence marine mesocosm which was built as part of the Montreal Biodome to make the population more aware of global thinking and local action. The Biodome management were confident that I could do it and provided me with all the available resources. Most of all, they allowed me to do this work as part of my job. By doing so they put me in the very privileged situation of not having to seek personal financial support. The Montreal Biodome has been the best incubator for research I have seen in my life and I hope it will stay that way for a long time. Special thanks to Mayor Pierre Bourque, the founder of the Montreal Biodome, Rachel Léger, Head of the Live Collections Division, Jean Bouvrette, Head of the Engineering Division and Daniel Gagnon, Head of the Research and Development Division from 1992 to 1996. Dr. Gagnon was instrumental in my being accepted at the University of Ottawa. He was always there for advice and support, he paid for part of my research out of his personal grants and, most of all, he has been an outstanding mentor during all these years.

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INTRODUCTION

The nitrogen cycle in a closed system

A newly established aquarium can be considered oligotrophic, because it is deficient in carbon, phosphorus and nitrogen. The ammonia level is many times higher at the outset than it is days later, when sufficient numbers of nitrifying bacteria have become established in the filter bed (Spotte 1979a). Nitrate is low because nitrification initially converts ammonia into nitrite. In the nitrification sequence, nitrite is the precursor to the formation of nitrate.

As the aquarium ages, levels of nitrate, inorganic phosphorus and dissolved organic carbon increase due to the regular food additions, and conditions become eutrophic. Only nitrogen appears to accumulate indefinitely: carbon and phosphorus eventually reach equilibrium. Eutrophic conditions make aquarium management troublesome. Some of the nutrients produced by animals, plants and bacteria – notably ammonia – are directly toxic to many organisms. In addition, dissolved organic matter increases the level of substrates available for oxidation by heterotrophic bacteria. The proliferation of these bacteria in the form of biofilm on the surfaces of the aquarium (walls, sediment, filter sand, trickling filter bioballs, fluidized bed, etc.) increases the biomass in the aquarium. Overall eutrophication in seawater aquaria reduces the carrying capacity of the water and interferes with the aquaria's ability to support life.

Inorganic nitrogen in aquarium water occurs mainly as ammonia, nitrite and nitrate. Urea, amino acids and peptides may also be present, but are usually found in small concentrations (Spotte 1979a). Particulate substances with nitrogen are free-floating in the water column, either as nonliving entities or as protein-based compounds in living organisms.

Besides food additions for the animals living in the aquarium that represent the biggest input of nitrogen, there are three other pathways by which nitrogen enters aquarium water: (1) diffusion from the atmosphere or subsurface air bubbles; (2) excretion by benthic macroalgae; and (3)

oxidation processes of heterotrophic bacteria. Nitrogen fixation is usually considered unimportant in aquaria because it requires much natural light and because inorganic nitrogen is abundant in the aquarium. Planktonic organisms play no significant role in the cycling of nitrogen through seawater aquaria since they are quickly filtered out. This is contrary to the situation in the ocean where 95% of the organic matter is recycled within the water column (Clarholm 1994).

The nitrogen cycling starts with the release of nitrogen into aquarium water by animals and plants. Marine benthic algae release amino acids, peptides and complex proteinaceous materials but most nitrogenous material in aquarium water originates from animals. The three main end products of nitrogen breakdown are ammonia, urea and uric acid. Most aquatic animals excrete ammonia, which is very soluble and easily eliminated into the surrounding water.

The nitrogenous components of animal and plant excretory products are acted upon quickly by bacteria. They are broken down by heterotrophic bacteria into ammonia and other inorganic constituents, a process called mineralization. The ammonia excreted by animals, or resulting from mineralization, is oxidized to nitrate by autotrophic nitrifying bacteria. The high level of nitrate produced as a result of nitrification is reduced somewhat by denitrification carried out by anaerobic bacteria, assimilation by benthic algae (periphyton) and, when the C:N ratio of their food is high, assimilation by heterotrophic bacteria (Verhagen and Laanbroek 1992). Denitrification reduces nitrate to molecular nitrogen or nitrous oxide (N_2O). Excess molecular nitrogen is then lost to the atmosphere by diffusion.

The sum of the processes carried out by fixed bacteria and benthic algae – mineralization, nitrification, denitrification and assimilation – is called biological filtration. It is the most important type of filtration used in aquaria to remove unwanted substances, primarily ammonia (Spotte 1973).

The organic nitrogen in aquaria has many sources, but dead organisms and bits of uneaten food are the most significant. Some of the organic nitrogen in solution is probably bound up in

aggregates and detritus. Techniques for lowering the total nitrogen level include periodic cropping of benthic algae, removal of dead organisms and uneaten food, and mechanical filtration.

The total inorganic nitrogen (TIN) concentration in seawater is the sum of ammonia, nitrite and nitrate. TIN levels in the Antarctic may reach 40 μg-at $N \cdot L^{-1}$ while the Northeast Pacific has a typical range of 8 to 15 μg-at $N \cdot L^{-1}$ (Spotte 1991). In comparison, TIN concentration in aquarium water can be staggering. Values for nitrate can vary between 3500 and 22000 mg NO_3-$N \cdot L^{-1}$ (Spotte 1991).

As stated above, different bacteria are responsible for different processes. Mineralization is carried out by heterotrophic bacteria (ammonifiers), nitrification by autotrophic aerobic bacteria (nitrifiers) and dissimilation by autotrophic anaerobic bacteria (denitrifiers). Most of the bacterial activity in seawater aquaria occurs on biofilms attached to the walls, the substrate, i.e. sand or gravel, and other solid surfaces (Spotte 1979a). Biofilms are produced by the bacteria inhabiting them, and consist mostly of polysaccharides and gums. Their thickness is limited by the nutrient load and dissolved oxygen concentration in the environment. Spotte (1979a) reported slimes 1 to 5 mm thick on membrane filters and heavy films on the surface of gravel grains in trickling filters. Normal population densities per gram of filter sand are 10^7 for total aerobes and 10^5-10^6 for nitrifiers. Most of the bacteria live in the upper 5 cm of the filter bed (Spotte 1991).

Meiofauna

Meiofauna was once thought to be an artificial assemblage because it was defined as a group of animals that pass through a 1 mm sieve but are retained by a 42 μm sieve (Higgins and Thiel 1988). However, recent reviews have shown that, at least in marine habitats, meiofauna represents a separate, biologically and ecologically defined group of animals, a concept long accepted in the case of the interstitial meiofauna of coarse sands (Remane 1933). Studies on the

size spectra of marine benthic fauna infer that marine meiobenthos can be distinguished from macrofauna (the group of animals retained by the coarse sieve, such as molluscs, echinoderms, large crustaceans and large polychaetes) by species size distribution (Warwick 1984; Warwick *et al.* 1986), and from both nanofauna (those passing through the 45 μm sieve, such as bacteria, fungi, flagellates and ciliates) and macrofauna by specimen size spectrum (Schwinghamer 1981, Gerlach *et al.* 1985). Moreover, Gerlach *et al.* (1985) found that the metabolism of meiofauna, expressed per unit of mass is, on average, four times higher than the metabolism of macrofauna.

In nature, meiofauna eat bacteria, microalgae, protozoans, detritus and other meiofauna (Montagna 1995). Phytobenthos is limited to the photic zone of the oceans and is mostly eaten by harpacticoid copepods whose grazing rate can reach 1% per hour (Montagna 1983; Nilsson *et al.* 1991). Organic detritus itself plays a minor role, whereas bacteria attached to detritus are the major food source for detritus feeders (Meyer-Reil and Faubel 1980). This is especially true for nematodes that typically comprise between 70 and 90% of the meiofaunal biomass (Escaravage *et al.* 1989). The meiofauna dominates the nanofauna in both biomass and trophic position (Tenore and Rice 1980). Together, they form the "small food web" which under certain circumstances consumes 70 to 80% of all the organic carbon being deposited on the bottom (Kuipers *et al.* 1981). Per biomass unit, meiofauna consume 60 times more organic matter than macrofauna, and releases 4800 times more nutrients (Kuipers *et al.* 1981).

The results obtained by Montagna (1984) and Nilsson *et al.* (1991) show that meiofauna plays an important role in regulating bacterial biomass and growth. This regulation is exerted in the following ways:

1. Meiofauna consumes up to 3% of the bacterial biomass per hour (Montagna 1984; Epstein and Shiaris 1992). A reduction in bacterial biomass rarely results from grazing since bacteria are highly productive. Rather, grazing stabilizes the bacterial population, eliminates senescent

cells and maintains colonies in exponential growth (Yingst and Rhoads 1980; Meyer-Reil and Faubel 1980; Montagna 1984).

2. The bulk of meiofauna, notably nematodes (Heip *et al.* 1985) and harpacticoid copepods (Hicks 1983), carry out "bactericulture" which increases bacterial biomass. Nematodes and harpacticoid copepods secrete mucous structures – tunnels, balls – which are quickly colonized by microorganisms. They then prey upon these, either by grazing the surfaces or eating the structures. Nematodes and harpacticoid copepods also secrete soluble metabolites rich in nitrogen and phosphorus which serve as nutrients to bacteria and favour their growth (Riemann and Schrage 1978; Hicks and Grahame 1979).

3. Meiofauna bioturbates sediments through mucous tunnels (nematodes), holes and burrows (ostracods and copepods). This bioturbation increases geochemical fluxes, notably the rate of oxygen diffusion (Aller and Aller 1992). It results in a deeper oxic habitat, larger faunal biomass and therefore an increased rate of mineralization of organic matter.

4. Meiofauna fragments detritus and has a catalytic effect on bacterial decomposition (Tenore *et al.* 1977). In terrestrial habitats, it produces a faster dissolution of cellular products and an increase in the total detrital surface available to microorganisms (Lebrun 1987).

5. Dead meiofauna becomes food for bacteria.

6. Lastly, some specialized meiofaunal species, including many nematodes, are symbiotic partners with bacteria (Heip *et al.* 1985).

With a mean biomass of 1 g $C \cdot m^{-2}$ in littoral sands, meiofauna represents only 3 to 6.6% of the macrobenthos biomass (Kuipers *et al.* 1981). However, its contribution to the energy flow in benthos may be as important as that of macrofauna. According to Platt (1981), with a biomass five times smaller, meiofauna consumes the same amount of organic matter as macrofauna (6% detritus and 94% bacteria, protozoa and phytobenthos) and produces as much organic matter.

Gerlach (1978) measured similar production:biomass ratios in subtidal silty sand marine sediments.

What would happen if the meiofauna were removed from an aquatic ecosystem? In two terrestrial habitats, Moore *et al.* (1993) found that the removal of bacterivorous nematodes reduced overall biomass production (B_{tot}) by 12%, a value 4-5 times higher than the nematodes' biomass production (P_{meio}). By plotting B_{tot}/P_{meio} ratios against the trophic position of different functional nanofaunal and meiofaunal groups, the authors found that the drop in productivity was proportionate to trophic position, suggesting that higher trophic positions exert more control over lower positions on a biomass specific basis.

Although it seems evident that meiofauna plays a role in nitrogen cycling, this role is almost impossible to evaluate in natural habitats. Inorganic nitrogen, e.g. nitrate, does not accumulate in nature as it can in microcosms and mesocosms. The concentrations of nutrients are naturally low and most often limit the growth of benthos (Heip *et al.* 1985). In addition, it is very difficult to isolate bacterial activity or to monitor the fate of a labelled product at the meiofaunal scale unless part of the benthos is enclosed and some faunal groups are manipulated, both of which are far too expensive and impractical to carry out in the filed.

Few laboratory studies have been done on the role of meiofauna in nitrogen cycling. Most of them concentrate on the mineralization of organic matter. Tenore *et al.* (1977) observed that eelgrass (*Zostera* sp.) detritus labelled with ^{14}C accumulated twice as fast in the polychaete worm *Nepthys* when meiofauna was present in the culture flasks. Tietjen (1980) noted that nematodes increase orthophosphate regeneration, which impiies that nematodes enhance mineralization of detritus. Findlay and Tenore (1982) found that nematodes increased the hourly rate of mineralization of the red algae *Gracilaria* by up to three times, and produced 71% more inorganic carbon after one week. However, it should be noted that the latter figure represents only 4% of the initial organic carbon.

Nematodes, as many benthic detritus feeders, cannot directly assimilate most detrital sources because they are deficient in digestive carbohydrases to hydrolyse the cellulose, xylan, and other structural carbohydrates that comprise the bulk of detritus (Hylleberg Kristensen 1972). They must instead stimulate microbial (both bacterial and fungal) degradation through grazing, bactericulture, bioturbation, fragmentation or any combination of these factors. According to Alkemade *et al.* (1992), nematodes increase mineralization mainly through bioturbation.

As for the role of meiofauna in nitrification, recent studies done in freshwater trickling filters have shown that microfauna and macrofauna, such as fly larvae, snails and worms, reduce nitrification (Andersson *et al.* 1994; Parker *et al.* 1989; Lee and Welander 1994). It also seems that meiofauna such as rotifers have a strong negative impact on nitrification in aerobic biofilm processes, at least in systems not limited by oxygen or ammonium transfer. The general explanation for these results is that grazing has a more negative effect on the slow-growing autotroph nitrifiers than on the fast-growing heterotrophs (Lee and Welander 1994).

Whatever process is used to biologically treat polluted waters, activated sludge or biofilms, it comprises not only bacteria and meiofauna but also protozoa. More and more, bacteria and protozoa are viewed as indissociable units of the microbial loop (Clarholm, 1994). Bott and Kaplan (1990) found potential for protozoa to graze on bacteria in streambed sediments. Vorticellidae were found to metabolize only free-living bacteria (Sudo and Aiba 1975) and to break down bacterial phosphorus removal in a sludge system (Cech *et al.* 1994). Verhagen and Laanbroek (1992) found that grazing by flagellates affects competition for ammonium between nitrifying and heterotrophic bacteria in chemostats. In some terrestrial ecosystems, protozoa are responsible for up to 20% of the total net nitrogen mineralization (Griffiths 1994). Therefore, protozoa have to be taken into consideration when setting up experiments involving bacteria and meiofauna.

The St. Lawrence marine mesocosm

The St. Lawrence marine mesocosm of the Montreal Biodome is a closed system which holds about 3 million litres of cold (10°C) artificial seawater. It consists of four pools: the main pool, the medical pool, the holding pool and the tidepool.

The main pool is kidney-shaped and roughly 550 m^2 in size. It is 5.5 m deep and contains 2.4 million litres of water. Half of the bottom is covered with 3 to 30 cm granitic river gravel 7-12 mm in diameter. At one end, a channel leads to the watertight door of the medical pool. This pool is rectangular, 1.5 m deep, and contains 26 000 litres of water. A second watertight door leads to the holding pool which is circular and holds 225 000 litres of water. It is 10.7 m in diameter and 2.5 m deep. The tidepool is connected to the main pool and contains 25 000 litres of water.

There are two independent life support systems: one for the main pool and the tidepool, and another for the holding pool. The medical pool can be filtered by either system. Both systems treat water with high-pressure sand filters, and then sterilize it with an ozone contactor. The main pool's filtered water passes through a trickling filter, a 200 m^3 percolator filled with bioballs (Lanpac® plastic packing 9 cm in diameter), before entering the ozone contactor.

The data for the main pool life support system are the following: 20 800 $L \cdot min^{-1}$ flow rate, 144 min turnover rate, 5 min contact time with ozone, 192 $L \cdot m^{-2} \cdot min^{-1}$ percolator flow rate and 110 kg biological load tolerance. In addition, there are three 150 000 L reservoirs which are used to prepare and recycle seawater. Due to annual losses through backwashes, treatments, etc., the turnover rate of prepared seawater is expected to be 7 years.

Bacterial filtration of the mesocosm is achieved by forcing water through filter beds: sand filters, gravel on the bottom of the tank, and bioballs (Lanpac® plastic packing 9 cm in diameter) in the trickling filter. The goal was to have a bacterial biomass which was as large as possible.

What happened when meiofauna appeared in the mesocosm? Most likely an increase in the mineralization rate and a decrease in the nitrification rate. For a given meiofaunal biomass, the mineralization rate may be higher than in nature for two reasons:

1. Mesocosm detritus are not the complex structural carbohydrates of *Zostera* and *Spartina* but rather more labile green algae and fauna products such as uneaten food (fish, shrimp, etc.) and animal secreta (feces, ammonia, etc.);

2. Refractory organic matter is broken down by ozone in the contactor, especially when redox levels are maintained above 800 mV (Spotte 1979b).

The presence of meiofauna in the St. Lawrence mesocosm may also have beneficial side effects. Through grazing, meiofauna may help in reducing levels of infectious bacteria such as *Vibrio* – some copepods actively seek these bacteria (Hicks 1983) – and opportunistic protozoan parasites such as *Trichodina* and *Cryptocaryon*.

Lastly, the St. Lawrence mesocosm may contain a very peculiar meiofauna because it is highly enriched with non-phytoplanktonic particulate material. Warwick *et al.* (1986) found that natural communities living in such habitats exhibited three specific characteristics:

1. Each locality only accommodates a small number of meiofaunal species (less than 20 vs. 179 at a "normal" unpolluted and unenriched station), with each species being present in very large numbers.

2. These species are "pollution indicator species". They are relatively rare in unenriched communities and are opportunistic rather than pollution tolerant.

3. The idealized "enrichment community", made using samples from the Celtic Sea, has a species size distribution occupying the trough in normal benthic communities.

Warwick *et al.* (1986) suggest that the convergence of macrofauna and meiofauna to a single optimum size can be explained by the absence of macrofaunal coupling with the pelagic system (large macrofaunal species have planktonic larvae feeding on phytoplankton) and the more generalist feeding regime of large meiofaunal species. As the St. Lawrence mesocosm has no phytoplankton and is heavily "polluted", its meiofauna is expected to be similar to Warwick's "enrichment community" in number of species, type of taxa and species size distribution.

Objectives

The overall objective of this study was to quantify the role of meiofauna in the nitrogen cycle of a cold marine mesocosm. Preliminary work showed that the meiofauna is very abundant in different areas of the St. Lawrence mesocosm, such as the gravel beds of the main pool and the tidepool, the *Cladophora* grasses of the walls and rocks of the main pool, and the sand in the sand filters. The highest abundance was found in the sand filters. It was therefore decided to set up microcosms with sand and to use the meiofauna from the sand filters to quantify the role of meiofauna in the nitrogen cycle. Three specific objectives were fixed and are developed in the following chapters.

The first specific objective was to measure the bacterial mineralization and nitrification processes in the St. Lawrence mesocosm. These two processes have to be significant in the cycling of nitrogen in the St. Lawrence mesocosm for meiofauna to play a major role in the nitrogen cycle. A nitrogen budget for 1995 was prepared: inputs, outputs and changes in nitrogen pools were independently measured, and the role of each process was assessed. The results are presented and discussed in Chapter One.

The second specific objective was to study the role of meiofauna in the mineralization of organic matter in the sand filters of a cold marine mesocosm. The hypothesis was that, as observed in natural marine habitats (Tenore *et al.* 1977; Tietjan 1980; Findlay and Tenore 1982),

meiofauna living in sand filters increase the mineralization rate of organic matter. Three 30-day experiments were conducted under controlled conditions in microcosms with natural populations of meiofauna taken from the sand filters of the St. Lawrence mesocosm, using tryptone, detritus and fishmeal as organic substrates. Particle size and C:N ratio were examined to see if they affected the impact of meiofauna. The results of these experiments are set out and discussed in Chapter Two.

The third specific objective was to study the role of meiofauna in the nitrification process in the sand filters of a cold marine mesocosm. The hypothesis was that, as observed in freshwater trickling filters (Andersson *et al.* 1994; Parker *et al.* 1989; Lee and Welander 1994), the meiofauna living in the sand filters reduce the nitrification of ammonia into nitrate. Since nitrification is a two-step process – conversion of ammonia into nitrite through nitrition and conversion of nitrite into nitrate through nitration – each step was tested. Two nitrition experiments, with two replicates of each, and four nitration experiments were done. The results of these experiments are given and discussed in Chapter Three.

The long term objective of this research is to expand our knowledge of meiofaunal ecology and to develop better wastewater treatment systems incorporating this knowledge.

REFERENCES

Alkemade, R.A., A. Wielemaker, S.A. de Jong and A.J.J. Sandee. 1992. Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella dievengatensis* in stimulating the mineralization of *Spartina anglica* detritus. Mar. Ecol. Prog. Ser. 90: 149-155.

Aller, R.C. and J.Y. Aller. 1992. Meiofauna and solute transport in marine muds. Limnol. Oceanogr. 37(5): 1018-1033.

Andersson, B., H. Aspegren, D.S. Parker and M.P. Lutz. 1994. High rate nitrifying trickling filters. Wat. Sci. Tech. 29(10-11): 47-52.

Bott, T.L. and L.A. Kaplan. 1990. Potential for protozoan grazing in stream-bed sediments. J. N. Am. Benthol. Soc. 9(4): 336-345.

Cech, J.S., P. Hartman and M. Macek. 1994. Bacteria and protozoa population dynamics in biological phosphate removal systems. Wat. Sci. Tech. 29(7): 109-117.

Clarholm, M. 1994. The microbial loop in soil. In: K. Ritz, J. Dighton and K.E. Giller (Ed.). Beyond the biomass. British Society of Soil Science. London: Wiley-Sayce. pp. 221-230.

Epstein, S.S. and M.P. Shiaris. 1992. Rates of microbenthic and meiobenthic bactivory in a temperate muddy tidal flat community. Appl. Environ. Microbiology 58: 2426-2431.

Escaravage, V., M.E. Garcia and J. Castel. 1989. The distribution of meiofauna and its contribution to detritic pathways in tidal flats (Archachon, France). Sci. Mar. 53: 551-559.

Findlay, S.E.G. and K.R. Tenore. 1982. Effect of the free-living marine nematode *Diplolaimella chitwoodi* on detrital carbon mineralization. Mar. Ecol. Prog. Ser. 8: 161-166.

Fuller, W.A. 1987. Measurement Error Models. John Wiley & Sons, Toronto. 476 pages.

Gerlach, S.A. 1978. Food-chain relationships in subtidal silty-sand marine sediments and the role of meiofauna in stimulating bacterial growth. Oecologia (Berlin) 33: 55-69.

Gerlach, S.A., A.E. Hahn and M. Schrage. 1985. Size spectra of benthic biomass and metabolism. Mar. Ecol. Prog. Ser. 26: 161-173.

Griffiths, B.S. 1994. Microbial-feeding nematodes and protozoa in soil: their effects on microbial activity and nitrogen mineralization in decomposition hotspots and the rhisosphere. Plant and Soil 164: 25-33.

Heip, C., M. Vincx and G. Vranken. 1985. The ecology of marine nematodes. Oceanogr. Mar. Biol. Ann. Rev. 23: 399-489.

Hicks, G.R.F. 1983. The ecology of marine meiobenthic harpacticoid copepods. Oceanogr. Mar. Biol. Ann. Rev. 21: 67-175.

Hicks, G.R.F. and J. Grahame. 1979. Mucus production and its role in the feeding behaviour of *Diarthrodes nobilis* (Copepoda: Harpacticoida). J. mar. Biol. Ass. U.K. 59: 321-330.

Higgins, R.P. and H. Thiel (Ed.). 1988. Introduction to the study of meiofauna. Washington: Smithsonian Institution Press. 488 p.

Hylleberg Kristensen, J. 1972. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. Mar. Biol. 14: 130-142.

Kuipers, B.R., P.A.W.J. de Wilde and F. Creutzberg. 1981. Energy flow in a tidal flat ecosystem. Mar. Ecol. Prog. Ser. 5: 215-221.

Lebrun, P. 1987. Quelques réflexions sur les rôles exercés par la faune édaphique. Revue d'écologie et de biologie des sols 4: 495-502.

Lee, N.M. and T. Welander. 1994. Influence of predators in aerobic biofilm processes. Wat. Sci. Tech. 29(7): 355-363.

Meyer-Reil, L.A. and A. Faubel. 1980. Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. Mar. Ecol. Prog. Ser. 3: 251-256.

Montagna, P.A. 1983. Live controls for radioisotope food chain experiments using meiofauna. Mar. Ecol. Prog. Ser. 12: 43-46.

Montagna, P.A. 1984. In situ measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. Mar. Ecol. Prog. Ser. 18: 119-130.

Montagna, P.A. 1995. Rates of metazoan meiofaunal microbivory. Vie Milieu 45(1): 1-9.

Moore, J.C., P.C. DeRuiter and H.W. Hunt. 1993. Soil invertebrate/micro-invertebrate interactions: disproportionate effects of species on food web structure and function. Veterinary Parasitology 48: 247-260.

Nilsson, P., B. Jönsson, I. Lindström Swanberg and K. Sundbäck. 1991. Response of a marine shallow-water sediment system to an increased load of inorganic nutrients. Mar. Ecol. Prog. Ser. 71: 275-290.

Parker, D., M. Lutz, R. Dahl and S. Bernkopf. 1989. Enhancing reaction rates in nitrifying trickling filters through biofilm control. J. WPCF 61(5): 618-631.

Platt, H.M. 1981. Meiofaunal dynamics and the origin of the metazoa. In: P.H. Greenwood (Ed.). The evolving biosphere. Cambridge: Cambridge University Press. pp. 207-216.

Remane, A. 1933. Verteilung und Organisation der benthonischen Mikrofauna der Kieler Bucht (Benthic microfauna of Kiel Bight: description and organisation). Wiss Merresunters, Abt Kiel, NF 21, pp. 161-221.

Rieman, F. and M. Schrage. 1978. The mucus-trap hypothesis on feeding of aquatic nematodes and implications for biodegradation and sediment texture. Oecologia (Berlin) 34: 75-88.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry. W.H. Fremman and Cie, New York. 887 pages.

Schwinghamer, P. 1981. Characteristic size distributions of integral benthic communities. Can. J. Fish. Aquat. Sci. 38: 1255-1263.

Spotte, S. 1973. Marine aquarium keeping: the science, animals and art. Toronto: John Wiley & Sons. 171 p.

Spotte, S. 1979a. Seawater aquariums. The captive environment. Toronto: John Wiley & Sons. 413 p.

Spotte, S. 1979b. Fish and invertebrate culture. New York: John Wiley & Sons. 179 p.

Spotte, S. 1991. Captive seawater fishes: science and technology. New York: John Wiley & Sons. 942 p.

Sudo, R. and S. Aiba. 1975. Role and function of protozoa in the biological treatment of polluted waters. Adv. Biochem. Eng. Biotechnol. 29: 118-141.

Tenore, K.R. and D.L. Rice. 1980. A review of trophic factors affecting secondary production of deposit-feeders. In: K.R. Tenore and B.C Coull (Ed.). Marine benthic dynamics. Belle W. Baruch Libr. Mar. Sci. 11. Columbia (SC): University of South Carolina Press. pp. 325-340.

Tenore, K.R., J.H. Tietjen and J.J. Lee. 1977. Effect of meiofauna on incorporation of aged eelgrass, *Zostera marina*, detritus by the polychaete *Nephthys incisa*. J. Fish. Res. Board Can. 34: 563-567.

Tietjen, J.H. 1980. Microbial-meiofaunal interrelationships: a review. In: American Soc. for Microbiology. Microbiology 1960. pp. 135-138.

Verhagen, F.J.M. and H.J. Laanbroek. 1992. Effects of grazing by flagellates on competition for ammonium between nitrifying and heterotrophic bacteria in chemostats. Appl. Environ. Microbiol. 58(6): 1962-1969.

Warwick, R.M. 1984. Species size distribution in marine benthic communities. Oecologia (Berlin) 61: 32-41.

Warwick, R.M., N.R. Collins, J.M. Gee and C.L. George. 1986. Species size distributions of benthic and pelagic metazoa. Mar. Ecol. Prog. Ser. 34: 63-68.

Yingst, J.Y. and D.C. Rhoads. 1980. The role of bioturbation in the enhancement of bacterial growth in marine sediments. In: K.R. Tenore and B.C Coull (Ed.). Marine benthic dynamics. Belle W. Baruch Libr. Mar. Sci. 11. Columbia (SC): University of South Carolina Press. pp. 407-421.

CHAPTER I

N BUDGET AS WATER QUALITY MANAGEMENT TOOL IN CLOSED AQUATIC MESOCOSMS

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ABSTRACT

Following a high rise in nitrate levels in St. Lawrence mesocosm of the Montreal Biodome in 1995, inputs, outputs and changes in N pools were independently measured to estimate the N budget of the system. The resulting budget had only a 8% difference between the inputs (CV = 11.1%) and the sum of the outputs and changes in N pools (CV = 9.6%). N inputs amounted to 342 kg for 1995; 58% was contributed by the food for fish and invertebrates and 35% by the seabird guano. Filtration and general cleaning removed 20% of the inputs. The pool vacuum cleaner designed by the Biodome staff proved to be an important addition to the system since it increased by 30% the efficacy of the life support systems (LSS) at removing N, and removed detritus glued to surfaces or trapped in sediments that could not be removed by the LSS. 65% of N accumulated in mesocosm mainly as NO_3 (92%), but also as fish and invertebrate biomass (8%). Assuming 21 mM as the maximum NO_3 concentration for survival of the animals on display, the N budget showed that, without integrated dissimilation or assimilation processes, marine aquaria similar in design and operation to the SLM must be opened after 5 to 7 years of operation unless N inputs can be reduced or N outputs increased. As suggested by the budget, the removal of one seabird species (gannets) and an increase in partial water changes (from 9 to 16% per year) in 1996 have, since then, stabilized the level of NO_3 to 12-13 mM. The N budget was effective in identifying the role of each component in the system, suggesting changes in the operating practices, and predicting the impact of these changes. It is a useful water quality management tool for artificial environments, such as closed aquatic mesocosms.

Key words: **Nitrogen budget, marine mesocosm, management tool**

RÉSUMÉ

À la suite d'une hausse rapide des nitrates dans le mésocosme du Saint-Laurent marin du Biodôme de Montréal en 1995, le budget de l'azote du système a été calculé en mesurant indépendamment tous les intrants, les extrants et les changements dans les pools d'azote. Une différence de 8% seulement a été observée entre les intrants (CV = 11.1%) et la somme des extrants et des changements dans les pools d'azote (CV = 9.6%). Les intrants d'azote ont totalisé 342 kg dont 58% provenaient de la nourriture des poissons et des invertébrés et 35% du guano des oiseaux marins. La filtration et l'entretien général des bassins ont enlevé 20% des intrants. L'aspirateur de piscine mis au point par le personnel du Biodôme a constitué un apport important au système en augmentant de 30% l'efficacité des filières de traitement de l'eau et en enlevant du mésocosme les déchets irrécupérables par les filières parce que collés aux surfaces ou piégés dans les sédiments. 65% de l'azote s'est accumulé dans le mésocosme, surtout sous forme de nitrates (92%) mais aussi de production secondaire (8%). Sur la base d'un niveau maximal tolérable par les animaux en exposition de 21 mM de nitrates, le budget de l'azote montre que, sans dissimilation ou dénitrification, les aquariums marins aménagés et opérés tel le SLM doivent être ouverts après 5 à 7 ans d'activité. Tel que prédit par le budget, l'enlèvement des fous de Bassan et l'accroissement des changements d'eau partiels (de 9% à 16%) en 1996, ont depuis stabilisé le niveau des nitrates à 12-13 mM. Le budget a permis d'identifier les composantes azotées du système, de suggérer des changements opératoires et de prédire l'impact de ces changements. C'est un outil très utile pour la gestion de la qualité de l'eau des habitats aquatiques artificiels, mésocosmes ou autres, fonctionnant en circuit fermé.

1.1 Introduction

The Montreal Biodome is a living museum that exhibits reproductions of natural ecosystems. One of the ecosystems on display is the Gulf of St. Lawrence, represented by a large (1 600 m^2) closed aquatic mesocosm, i.e. an aquarium containing a limited influx of new seawater (Spotte 1991). It is called the St. Lawrence Mesocosm (SLM) and is home to a large community of fish, invertebrates and seabirds since its start-up in April 1992.

In 1995, after less than three years of operation, NO_3 levels in the mesocosm had increased from 0 to 10.7 mM (Fig. 1.1). The average daily NO_3 increase (9.1 $\mu M \cdot d^{-1}$) was 2.4 times faster than a similarly operated 23 million L tropical mesocosm (Grguric and Coston 1998). This rapid increase was apparently responsible for invertebrate deaths and fish health problems, such as high levels of parasitism, bacterial infections and mortalities (Dancosse 1998). Concerns about chronic and acute toxicity of NO_3 in the SLM prompted a review of the mode of operation to determine ways to stabilize or reduce the NO_3 concentration in the SLM.

Many other public aquaria that exhibit marine animals in closed systems have also experienced rapidly rising nitrate concentrations (Grguric and Coston 1998; Spotte 1991), but none to our knowledge has computed a N budget in order to examine how the increase could be attenuated or stopped. It was therefore decided to study the annual N budget of the SLM so as to identify and quantify the role of each component in the system, to suggest changes in the operating practices and to predict the impact of these changes on the water quality of the mesocosm.

Data on the physics, chemistry and biology of the system were collected for three years to prepare the nitrogen budget of the SLM. Changes in the standing stocks of various pools and inputs and outputs of organic and inorganic N were measured or estimated to produce a budget

for 1995. Finally, the budget was used to suggest management and operating options for improving water quality, some of which were implemented in 1996.

1.2 Materials and Methods

1.2.1 St. Lawrence mesocosm

The SLM contains 3.25 million L of cold (10°C) artificial seawater (28-30‰). It is composed of two life support systems (LSS) and four pools (Fig. 1.2): an isolation pool (222 kL), the bay (2 400 kL), the rocky shore (23 kL) and the medical pool (26 kL). The isolation pool has its own LSS consisting of three 1.8 m diameter rapid sand filters and an ozone contactor (ozonator). The three other pools are interconnected and treated by the main LSS composed of six 3.7 m diameter rapid sand filters, a 200 m^3 trickling filter and an ozone contactor. In addition, both the bay and the rocky shore pools have a reversed flow undergravel filter. Three 150 kL reservoirs help maintain closed circuit of the system. One of them is used to recover and purify the backwash water from filters, the second is used to stock purified or new seawater and to maintain the water level in the system, and the third is used to prepare seawater (Fig. 1.2). The filtration rate of the isolation pool is 3 300 $L \cdot min^{-1}$ and the turnover time is 70 min. The filtration rate of the main subsystem is 21 600 $L \cdot min^{-1}$ and the turnover time is 140 min. About 7-9% of the water was replaced with fresh seawater every year since 1992.

1.2.2 Inputs

There were five inputs of N into the system in 1995: the food provided to the fish and invertebrates, guano produced by seabirds, invertebrates and fish added to the system and inorganic N introduced with new water. N fixation was not considered an input because NO_3 were abundant in the SLM and sunlight was dimmed.

The N content of each food item used in the system was analyzed on a CHN Analyzer (Table 1.1). The amount of each food item fed to fishes and invertebrates was recorded everyday and data were transformed into N mass (kg N). Similar procedures were adapted for Northern

Gannets (n = 20, *Sula bassanus*) and Black-legged Kittiwakes (n = 13, *Rissa tridactyla*). As these were adult birds, it was assumed that all ingested N was excreted (Welty 1979). Bird keepers were asked to estimate the proportion of guano dropped into the mesocosm or on land (ledges and islands) by each bird species. They observed the birds twice a day, 20 min each time, for 14 days and noted the number of birds defecating in water or on land.

Fresh weights of invertebrates added to the system were recorded. As most of them were hard-shelled, their resulting mass was converted into N based on findings on clams, i.e. 1.15% total body weight (Table 1.1). Fresh weights of fish added to the system were also recorded and the resulting mass was converted into N as 2.6% N as for herring and smelt (Table 1.1).

Mean annual content in NO_3 of the municipal tap water (0.275 mg $N \cdot L^{-1}$) was used to estimate the input of N through water addition. Tap water was added regularly to the system to make up for evaporation. Fresh seawater, prepared with nitrate-free Instant Ocean® and tap water, was added in August and November 1995.

1.2.3 Outputs

Four outputs were identified in the SLM: fish removed from the system, filtration, general cleaning, and water loss. All fish removed from the system either dead or alive were weighed and their mass was converted into N as 2.6% N (Table 1.1).

Amount of N removed in 1995 by both LSS sand filters was estimated in three steps. First, data on the filter operations were collected for three years and the total number of backwashes per year was counted. Second, cores were taken in five filters before and after backwashing, and the mean amount of organic matter removed per backwash was calculated. Third, N content of the detritus was determined assuming the same detritus composition as in the pool vacuum strainer (see below).

General cleaning of the mesocosm was accomplished three times a week mainly through underwater vacuum cleaning. A special pool-vacuum (designed and built by the aquarists and the engineering staff of the Montreal Biodome) was put into use in 1995. It consisted of standard 63 mm diameter swimming pool tubing, a 2 HP pump and a PVC, fiberglass reinforced, prefilter containing a stainless steel strainer with 5 mm diameter holes. Large particles were retained by the strainer, while small particles were transported with the return water directly to the main LSS filters. Extra backwashes of the main LSS filters were then performed to remove the small particles collected by vacuuming. Detritus collected by the strainer were sampled, sorted and weighed two or three times a week for three months (n = 26). Fresh weights were converted into N using Table 1.1. The sum of the detritus collected in the filters by the extra backwashes plus those collected by the strainer gave the total nitrogen removed by the general cleaning. Before 1995, detritus were collected manually by the divers using handnets.

Water losses (leaks, discharges to the sewer) were monitored everyday by electronic sensors and a PC logger. They were converted into N using the monthly measurements of NO_3 and assuming that NO_3 was lost along with the water. Water losses were regular throughout the year.

1.2.4 Changes in N pools

Based on the N cycle in estuarine systems (DeAngelis 1992), changes in standing stock of all forms of N likely to vary in the SLM were examined: inorganic N resulting from the mineralization (NH_4) and subsequent nitrification (NO_2 and NO_3) processes, dissolved organic N (DON), particulate organic N (PON) in suspension or in sediments, primary producer biomass, and secondary producer biomass. No denitrification was assumed because all sediment pore water was well oxygenated and sediments were frequently cleaned either by backwashing (filter sand) or pool vacuuming (gravel and sand beds).

Inorganic N was measured according to standard procedures (APHA *et al.* 1992). NH_4 and NO_2 were measured every week using an NH_4-selective electrode and colorimetric methods. NO_3 was measured twice a month in triplicates on a Tecator FIAstar, Model 5010, using the automated Cd reduction method. The net increase of N in the form of NO_3 was calculated by comparing the total volume of water and NO_3 concentrations on January 1 and December 31, 1995.

Dissolved organic C (DOC) and suspended particulate organic C (POC) were used as proxies for dissolved organic nitrogen (DON) and particulate organic nitrogen (PON). Both have been measured regularly since 1992 in triplicates on a Dohrman DC-180 using the persulfate-ultraviolet oxidation method (APHA *et al.* 1992). No accumulation in DOC and POC, and therefore in DON and PON, was expected since it would have signalled a malfunction in the filtration or ozonation processes.

Organic N of sediments and primary producer biomass were not measured on the assumption that their standing stock did not vary in 1995. During six years of operation, the algal biomass has not changed and, due to filtration and general cleaning of the pools, there has been no visible accumulation of detritus on the bottom of the pools.

The secondary producer biomass was divided into three groups: meiofauna, macroinvertebrates and fish. Most of the meiofauna was found in the rapid sand filters and in the gravel beds of the mesocosm. It has been regularly sampled and weighed since 1992 (S. Parent, unpublished data). Estimation of the macroinvertebrate biomass was performed in four steps: first, inventories were taken in the winter and fall of 1995; second, fresh weight of each invertebrate species was measured and invertebrate mass was estimated by means of stratified sampling methods (Cochran 1977); third, as most animals were hard-shelled, N biomass was assumed at 1.15% N content (Table 1.1); and fourth, change in macroinvertebrate mass was estimated at an assumed increase equivalent to P:B ratio of macrobenthos in temperate estuarine habitats, i.e. 1.0 per year (Kuipers *et al.* 1981, Ankar and Elmgren 1976).

Variations of N sequestered in fish mass were estimated in a similar fashion and one inventory was made on March 31st, 1995. Fish mass was converted into N as 2.6% N (Table 1.1). An annual growth rate of 5% was assumed for fish species with adult specimens only. Natural growth rates, as reported from the Gulf of St. Lawrence, were assumed for fish species with young specimens, such as Atlantic Sturgeon, Atlantic Cod, Pollock and Striped Bass (Table 1.2). For Atlantic Salmon, an annual growth rate of 20% was determined from a tagging experiment in the SLM (Pépin 1998). We assumed that the March 31st inventory reflected the mean number of fish in the SLM in 1995 (Table 1.2).

1.2.5 Statistical analysis

Coefficients of variation (CV) were estimated for all inputs, outputs and changes in N pools. For products of two variables, the following formula was used:

$$CV_{AB} = \sqrt{CV_A^2 + CV_B^2 + (CV_A^2 * CV_B^2)}$$

As shown by Bohrnstedt and Goldberger (1969), the conjunction of expectation- and variance-independence between A and B is an unnecessary assumption of this equation. For sums of two or more variables, a liberal CV was derived by maximizing the covariance term used (0 for correlations $r \leq 0$, and the product of the standard deviations ($S_A * S_B$) for correlations $r > 0$).

1.2.6 Budget

Each of the inputs, outputs and changes in the various N pools in the SLM was estimated independently, and then summed up to express the N budget of the mesocosm as:

$$\textbf{Inputs} = \textbf{Outputs} + \Delta\ \textbf{Pools} + \varepsilon$$

where ε is the error for the budget estimate. A corrected budget was then devised by distributing the error equally (ε/2) among both sides of the equation, and then proportionally to the amount of N in each component. The corrected budget was used to predict the monthly NO_3 levels in the SLM during 1995-1998 on the assumption that SLM water volume remained constant and that mineralization of organic matter was not delayed. The NO_3 level at the end of each month was equalled to the NO_3 level at the end of the preceding month plus the corrected known inputs for the month minus 1/12 of the corrected annual outputs and animal growth.

1.3 Results

1.3.1 Inputs

The annual input of N in the form of food for fish and invertebrates totalled 191 kg N (Table 1.3). CV of this input was 14.2% on the assumption of a 10% error in both the fresh weight and mean N content of the food items (Table 1.1). The intake of N by seabirds amounted to 137 kg N. Bird keepers estimated that 7/8 of the gannet guano and 5/8 of the kittywake guano entered the mesocosm. Net annual input of N from seabirds was 117 kg N per year (Table 1.3). CV of the net annual input of N from seabirds was 18.9% on the assumption of a 10% error in both the wet weight and mean N content of the food items, and an error of ±1/8 in each estimate of guano entering the SLM.

The biomass of invertebrates added to the system totalled 495 kg wet weight or 5.7 kg N in 1995 (CV = 16.6%) (Table 1.4). Most of these invertebrates were periwinkles (*Littorina littorea*) and sea urchins (*Strongylocentrotus droehbrachiensis*). The biomass of fish added in 1995 totalled 585 kg wet weight or 15.2 kg N (CV = 18.4%) (Table 1.2). New seawater introduced 0.2 kg N in the system (CV = 14.2%).

The total input of nitrogen into the SLM was therefore 328 kg N in 1995 (Table 1.5). CV of the total input was 11.1% on the assumption of a positive correlation between added animals and food for fish and invertebrates.

1.3.2 Outputs

In 1995, 188 fish were removed from the system. Their biomass was 512 kg wet weight or 13.3 kg N (Table 1.2). CV of this output was no more than 10% because all fish were counted and most of them were individually weighed.

The net amount of organic N removed by the normal operation of both life support systems (LSS) was 54.9 kg N (CV = 13.4%) (Table 1.6). The composition of the detritus was 39% algae, 50% food, 10% feces and dead invertebrates, and 1% exogenous materials (twigs, feathers, plastic, etc.). Using the detritus composition and N content of each component (Table 1.1), the mean N content of detritus was 6.56% dry weight (CV = 14.2%).

General cleaning of the mesocosm in 1995 removed a total of 16.7 kg N. The extra 122 backwashes of the main LSS by pool vacuum system removed 14.5 kg N, whereas the strainer in the pool vacuum retained only 2.2 kg N (Table 1.6). CV of the general cleaning was 14.8% on the assumption of a positive correlation between the extra backwashes and the pool vacuum strainer.

A total of 39 kg of nitrogen left the mesocosm through water loss, which reached 300 000 L in 1995, or 9.2% of the total content of the mesocosm. CV of this output is no more than 10% because all water losses were logged during 1995.

The sum of the four outputs amounted to 123.7 kg N per year (Table 1.5). CV of the total output was 7.1% on the assumption of no positive correlation between the various outputs.

1.3.3 Changes in N pools

Since NH_4 and NO_2 contents of the system remained below detection limit during 1995, we assumed that there was no change in NH_4 and NO_2 pools. The net increase of inorganic N in the form of NO_3 was 4.7 mM or 214.5 kg N in 1995. CV of the NO_3 increase was 14.2% on the assumption of a 10% error estimate in both water volume and NO_3 content.

There was no accumulation of dissolved organic N or particulate organic N in the system. DOC levels fluctuated between 2 to 5 $mg \cdot L^{-1}$ and POC levels between 10 to 35 $mg \cdot L^{-1}$ since 1992. Meiofaunal mass was 0.525 kg N in 1995. The standing stock of meiofauna has fluctuated since 1992, but there was no net increase or decrease.

The increase in macroinvertebrate biomass was 13 kg N in 1995 (Table 1.4). Inventories showed that 38 745 specimens were living in the SLM in 1995. Their total biomass was 1 122 kg wet weight or 12.9 kg N. (Table 1.4). Since the rate of increase has been grossly estimated, the CV of this change in the N pool was liberally set at 100%.

The increase in fish mass was 5.3 kg N in 1995 (CV = 41.9%). The inventory showed that 366 fish were living in the SLM in 1995 (Table 1.2). Their total biomass was 1 520 kg wet weight or 39.5 kg N (CV = 18.4%) and the population growth rate was 13.5% (CV = 37%).

The total change in N pools for 1995 was 232.7 kg (Table 1.5). CV was 14.2% on the assumption of no positive correlations between the different changes in N pools. The sum of the outputs and changes in N pools amounted to 356.4 kg N (CV = 9.6%).

1.3.4 Budget

The uncorrected N budget of the St. Lawrence mesocosm for 1995 was the following:

328.4 kg N (Inputs) = 123.7 kg N (Outputs) + 232.7 kg N (Δ Pools) – 28.0 kg N (ε)

The error (ε) was 8%, similar to the precision for the inputs (CV = 11.1%) and the sum of the outputs and changes in N pools (CV = 9.6%). The corrected budget was the following:

342.4 kg N (Inputs) = 118.9 kg N (Outputs) + 223.6 kg N (Δ Pools)

Details of both budgets are provided in Table 1.5. Figure 1.3 shows for the 1995-1998 period the measured values of NO_3 in the SLM, the values predicted by the budget and the modified predicted values, which accounted for an increase in partial water change since 1996 and the removal of the gannets in June 1996.

1.4 Discussion

1.4.1 Inputs, outputs and changes in N pools

Food for fish and invertebrates was the primary input of N (58%) into the mesocosm (Table 1.5). The second most important input (35.5%) was seabird guano. Removing the gannets or replacing them with seashore bird species meant a 33% reduction in the input of N. It is suspected that seabird species, such as cormorans and gulls, also contribute significantly to the system N budget in other marine mesocosms.

Daily fish ration was 1.25% body weight in the SLM which is lower than values of 2 to 6% usually found in pisciculture (Handy and Poxton 1993). This confirms that fish were kept on a maintenance diet and that food could not be reduced without a decrease in the fish load of the SLM.

Only 34.7% of the input N was removed from the system in 1995. Both LSS removed 15.4% of the input N, cleaning removed a further 4.7% and 10.9% was removed by water loss (Table 1.5). Given all of the efforts made to keep the mesocosm clean, it is probable that 20% represents the maximum percentage of N input that could be removed by filtration and cleaning. About 86% of N sequestered in detritus collected by the vacuum cleaner was sent directly to the filters of the main LSS. The pool vacuum accomplished 30% of the work done by the two LSS (Table 1.6). Moreover, the pool vacuum removed organic particles glued to surfaces or trapped in the sediments that could not be removed by the LSS. It therefore reduced mineralization and prevented more NO_3 production. Its high efficiency and low cost make the pool vacuum an important adjunct to the LSS of any mesocosm.

We believe denitrification was not significant in the St. Lawrence mesocosm in 1995, even though some authors have suggested that anaerobic dissimilation does occur in marine aquaria

(Spotte 1991, Kawai *et al.* 1964, Mulbarger 1971). All sediments in the mesocosm were kept at maximum O_2 level by the rapid flow of water in the filters or underneath the gravel beds of the pools. Furthermore, sand and gravel were regularly turned over through backwashing or pool vacuuming. If any denitrification took place in the SLM in 1995, it involved mostly the conversion of nitrate into nitrite because of the high level of nitrate, and was therefore reversible. Hall *et al.* (1992) showed that only 0.01% of the N entering low oxygenated sediments under an intensive marine culture of rainbow trout was in fact denitrified. We also believe that no ammonia degassing was occurring in the SLM since ammonia was quickly transformed into nitrates and only 1.5 to 4% of total NH4-N in seawater was in the form of ammonia (Spotte 1991).

N accumulated in the SLM in three forms: NO_3 (92%), macroinvertebrate biomass (5.5%) and fish biomass (2.3%) (Table 1.6). Very little N was sequestered in live biomass, even though we probably overestimated the true rate of increase of macroinvertebrate biomass by using the P:B ratio. Only few species, such as anemones, grow and reproduce in the mesocosm. Most of them are short-lived and exhibit no significant growth.

It was expected that NH_4 and NO_2 concentrations would be below detection limits and probably nil in the SLM in 1995 because these N forms are transient in the N cycle. They are quickly transformed into NO_3 by the nitrification process and, on a much smaller scale, through chemical oxidation by O_3 (Honn and Chavin 1976).

1.4.2 Budget

Budget presented here is a good estimate of the N flow in the St. Lawrence mesocosm in 1995. Given the CV of the inputs, outputs and changes in N pools, both terms of the equation are equal. Moreover, the predicted curve fits reasonably well with observed values in 1995 ($t = 0.585$, $p = 0.57$, df = 11) (Fig. 1.3). The curves do not fit perfectly because new seawater was not added

as and when it was needed, and annual predictors for outputs and changes in N pools were used instead of seasonal monthly values.

N budget developed for 1995 was also a good predictor of the NO_3 level in the SLM since that year. In Figure 1.3, the modified predicted values curve is consistently 8-16% above the observed curve. We suspect that if true confidence intervals could be generated, the observed values would lie well within this region.

Without denitrification by anaerobic bacteria or nitrogen assimilation by cyanobacteria and algae, mesocosms containing an average biomass of fish and invertebrates such as the SLM must be opened after a few years, i.e. their water must be changed. The closure-period depends on the design and operation of the mesocosm, and the acceptable NO_3 level for animals.

There is no clear acceptable NO_3 level for a healthy population of fish and invertebrates. Even though one knows that NO_3 is the least toxic of the inorganic N forms for aquatic animals, its long-term effects on the immune system of invertebrates and fish are not clear. Until recently, it was believed that even high loads of NO_3 were not toxic to marine fish, as 96-h LC_{50} values invariably exceeded 714 mM of NO_3 (Colt and Armstrong 1981). In addition, many public aquaria reported NO_3 concentrations of up to 21 mM without any problems for their specimens (de Graaf 1975, Spotte 1979, Keafabber *et al.* 1995). However, there were reports of growth problems in marine fishes maintained at 0.7 mM of nitrate (Spotte 1991). In the present case, older resident fish exhibited various health problems, suggesting a depressed immune system, while new fish had abnormal quarantine mortality (Dancosse 1998). It also seems that marine invertebrates cannot withstand NO_3 concentrations above 3.2 mM, as the level is lethal to some species (de Graaf 1975). NO_3 concentrations of less than 1.4 mM is known to be toxic to some marine invertebrates in closed systems (Muir *et al.* 1991). Nowadays, many aquarium authorities consider 3.5 mM of nitrate as the safe upper limit for marine fish and invertebrates (Spotte 1991, Vast 1991, Stoskopf 1993). The St. Lawrence mesocosm reached this value within 16 months.

But let us assume for a moment that the maximum NO_3 concentration for survival of the animals on display is 21 mM, i.e. the maximum value currently found in public aquaria. Figure 1.3 shows that had all N parameters remained the same in the mesocosm, this limit would have been reached in June 1998, i.e. six years after the mesocosm start-up. It is probable that marine mesocosms similar in design and operation to the SLM must also be opened 5 to 7 years after start-up.

Figure 1.3 also shows that NO_3 level in the St. Lawrence mesocosm has stabilized to 12 mM at the end of 1996. This plateau was achieved by removing the gannets from the system in June 1996 and by increasing the annual partial water change to 16% since 1996. This temporary solution was applied until a more permanent one was chosen and implemented. These decisions were based on the N budget produced.

The two solutions to reducing a high nitrate level in a mesocosm are the algae filter ("Algal Turf Scrubber") and anaerobic denitrification. The first solution is usually implemented in aquaria such as coral reef aquaria that require nitrate levels less than 71 μM (Adey and Loveland 1991). However, it necessitates ample space, much more lighting and a higher maintenance. The Montreal Biodome has decided to install an anaerobic system equipped with fluidized biobeds. It will soon be in operation and will maintain the NO_3 level at values less than 2.8 mM.

1.4.3 Mineralization and nitrification rates

A flow diagram of nitrogen in the St. Lawrence mesocosm in 1995 was developed using Table 1.5 to estimate the bacterial mineralization and nitrification rates (Fig. 1.4). Input of 100 g N consisted of animals (6.3 g N), water (0.1 g N) and food (93.6 g N). For reasons of simplicity, it was assumed that all food was eaten and that the biological load consisted entirely of fish. The excretion rate of the biological load was therefore assumed to be the same as for cold marine fishes, i.e. between 43 and 60% of ingested N or 40-50 g N (Handy and Poxton 1993). The

biological load retained 5% of the ingested N as mass and released 35 to 53% of it, i.e. 32-48 g N, as feces. The feces added to dead animals for a total PON of 34.6-50.6 g N (Fig. 1.4). As shown in Table 1.6, 20% of N inputs were removed by the filtration and cleaning systems, i.e. 20 g of PON. This left 15-31 g N of organic N being mineralized by heterotrophic bacteria. By combining bacterial mineralization with biological load mineralization, it was calculated that 71 g out of every 100 g of N input was converted into NO_3 by the nitrifying bacteria. This figure corresponds to the total NO_3 added to the system plus the N removed by water loss (Table 1.6).

Given a total of 342 kg of N input in the St. Lawrence mesocosm, it was estimated that bacterial mineralization rate was between 51 and 105 kg N per year, or 0.14 to 0.29 kg N per day, and the bacterial nitrification rate 243 kg N per year or 0.67 kg N per day. Besides mineralization of food by the biological load of the mesocosm, bacterial mineralization and nitrification are the two most important processes taking place in the N cycle of the SLM. Detritus removal and biomass increase lie far behind them in terms of importance.

The role of meiofauna in both bacterial processes was studied elsewhere (Parent and Morin 1999a, b). In experimental microcosms, meiofauna found in sand filters reduced significantly the mineralization rate of organic matter and greatly increased the nitrification rate. Meiofauna may therefore affect significantly the balance between the removal of particulate organic N by the filtration and cleaning systems, and the production of NO_3.

DeAngelis (1991) stated that ecological models serve three important purposes: to describe, explain and predict. The N budget developed here was used to fulfill those functions, but it was also instrumental in changing how the mesocosm was operated. It was used to significantly reduce the inputs of N, to stabilize the NO_3 concentration, to evaluate the filtration and cleaning operations, and to determine that a denitrifier was needed to reduce the NO_3 concentration. We suggest that the N budget can be used to advantage for the purpose of managing water quality in artificial environments, such as closed aquatic mesocosms.

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REFERENCES

APHA, AWWA and WEF 1992. Standard Methods for the Examination of Water and Wastewater. 18th ed., American Public Health Association, Washington DC, page 4-91.

Adey W.H. and K. Loveland. 1991. Dynamic Aquaria. Building Living Ecosystems. Academic press: Toronto. 643 pages.

Ankar, S. and R. Elmgren. 1976. The benthic macro- and meiofauna of the Askö-Landsort area (northern Baltic proper). A stratified sampling survey. Contr. Askö Lab. Univ. Stockholm, Sweden 11, 115 p.

Cochran W.G. 1977. Sampling Techniques. Toronto: John Wiley & Sons, 428 pages.

Colt J. and D.A. Armstrong. 1981. Nitrogen toxicity to crustaceans, fish, and molluscs. In : Allen, L .J. and E.C. Kinney (Eds.). Bio-engineering Symposium for Fish Culture. American Fisheries Society, Bethesda, Maryland, pp. 37-47.

Dancosse J. 1998. Jacques Dancosse, D.M.V., personal communication. Biodôme de Montréal, 4777 Pierre-De Coubertin, Montréal, Canada H1V 1B3.

DeAngelis D.L. 1992. Dynamics of Nutrient Cycling and Food Webs. Population and Community Biology Series 9. Chapman & Hall, New York, 270 pages.

De Graaf F. 1975. L'aquarium marin tropical. Elsevier Séquoia, Paris. 295 pages.

Fréchet A. and P. Schwab. 1995. Review of the status of the Northern Gulf of St. Lawrence cod stock (3Pn, 4RS) in 1994. Mont-Joli (Québec), DFO Atlantic Fisheries, Research Document 95/53, 20 pages.

Fuller R.J. and R.M. Warwick. 1988. Energetics. In: Higgins, R.P. and H. Thiel (Eds). Introduction to the Study of Meiofauna. Smithsonian Institution, Washington, pp. 181-196.

Geraci, J.R. 1986. Marine Mammals (Cetacea, Pinnipedia, and Sirenia). Nutrition and nutritional disorders. In: Fowler, M.E. (Ed.). Zoo and Wild Animal Medecine. W.B. Saunders, Toronto, pp. 760-764.

Grguric G. and C.J. Coston. 1998. Modeling of nitrate and bromate in a seawater aquarium. Wat. Res. **32**(6): 1759-1768.

Hall, P.O.J., O. Holby, S. Kollberg and M.O. Samuelsson. 1992. Chemical fluxes and mass balances in a marine cage farm. IV. Nitrogen. Mar. Ecol. Prog. Ser. **89**: 81-91.

Handy R.D. and M. G. Poxton. 1993. Nitrogen pollution in mariculture : toxicity and excretion of nitrogenous compounds by marine fish. Rev. Fish Biol. Fish. **3**: 205-241.

Honn K.V. and W. Chavin. 1976. Utility of ozone treatment in the maintenance of water quality in a closed marine system. Mar. Biol. **34**: 201-209.

Kawai A., Yoshida Y. and Kimata M. 1964. Biochemical studies on the bacteria in aquarium with circulating system - 1. Changes of the qualities of breeding water and bacterial population of the aquarium during fish cultivation. Bull. Jap. Soc. Sci. Fish. **30**: 55-62. (Japanese with English summary and legends)

Keafabber J.J., Coston C.J., Sherrill A., Grguric G., Tefry J.H. and Windsor J. 1995. A current perspective on ozone chemistry in aquarium systems. In : Barrett, C. (Ed.). Proceedings of the Third International Aquarium Congress April 25-29, 1993. New England Aquarium, Boston. pp. 360-371.

Kuipers, B.R., P.A.W.J. de Wilde and F. Creutzberg. 1981. Energy flow in a tidal flat ecosystem. Mar. Ecol. Prog. Ser. **5**: 215-221.

Lefort J. 1990. Les merveilleux secrets de la Spiruline. Les Éditions Wénaël, Victoriaville, Québec, 24 pages.

Magnin E. (1962). Recherches sur la systématique et la biologie des Acipenséridés : *Acipenser sturio* L., *Acipenser oxyrhynchus* Mitchill et *Acipenser fulvescens* Raf.. Annal. St. Cent. Hydrobiol. Appliq. 9, 242 pages.

Magnin E. and Beaulieu G. (1967). Le bar, *Roccus saxatilis* (Walbaum), du fleuve Saint-Laurent. Natur. Can. **94**: 539-555.

Mulbarger M.C. 1971. Nitrification and denitrification in activated sludge systems. J. Water Poll. Contrib. Fed. **43**: 2059-2070.

Muir, P.R., Sutton C.D. and L. Owens. 1991. Nitrate toxicity to *Penaeus monodon* protozoea. Mar. Biol.. **108**: 67-71.

Parent S. and A. Morin. 1999a. The role of copepod-dominated meiofauna in the mineralization of organic matter in a cold marine mesocosm. Can. J. Fish. Aquat. Sci. (In press).

Parent S. and A. Morin. 1999b. The role of copepod-dominated meiofauna in the nitrification process of a cold marine mesocosm. Can. J. Fish. Aquat. Sci. (In press).

Pépin S. 1998. Serge Pépin, M.Sc. Biol., personal communication. Biodôme de Montréal, 4777 Pierre-De Coubertin, Montréal, Canada H1V 1B3.

Spotte, S. 1979. Seawater Aquariums. The Captive Environment. John Wiley & Sons, Toronto, 413p.

Spotte S. 1991. Captive Seawater Fishes. Science and Technology. John Wiley & Sons, Toronto, 942 pages.

Stoskopf M.K. 1993. Environmental Requirements of Marine Tropical Fishes. In : Stoskopf, M.K. (Ed.). Fish Medecine. W.B. Saunders, Montréal. pp. 618-625.

Tucker C.S. 1993. Water Analysis. In : Stoskopf, M.K. (Ed.). Fish Medecine. W.B. Saunders, Montréal. pp. 166-197.

Vast C. 1991. ABC. Technique de l'aquarium marin. Editions Arnaud Daniel, Limoges (France). 183 pages.

Welty J.C. 1979. The life of birds. Saunders College Publishing, Philadelphia, 623 pages.

Table 1.1 N content (expressed as a percentage of the wet weight) of food items fed to invertebrates, fishes and seabirds in the St. Lawrence mesocosm. Except for shrimp, the coefficient of variation (CV) of each measurement was taken from literature or provided by the supplier.

Food item	N (%)	CV (%)	Reference
Herring *(Clupea harengus)*	2.6	9.7	CV from Geraci (1986)
Smelt *(Osmerus mordax)*	2.6	7.7	CV from Geraci (1986)
Capelin *(Mallotus villosus)*	2.2	3.6	CV from Geraci (1986)
Maquerel *(Scomber scombrus)*	3.1	16.1	CV from Geraci (1986)
Squid *(Loligo brevis)*	2.3	10.2	CV from Geraci (1986)
Shrimp (whole) *(Pandalus borealis)*	2.6	15.2	n = 4
Shrimp (shelled) *(Pandalus spp.)*	2.3	9.0	n = 4
Krill (Euphausiacae spp.)	2.6	17.9	CV provided by the supplier
Clams (Pelecypoda spp.)	1.2	10.8	Based on shrimp and squid content
Amphipods (Gammaridea spp.)	1.4	8.6	CV provided by the supplier
Egg yolk	5.0	no data	% N provided by the supplier
Spirulina (*Spirulina* spp.)	10.0	4.4	Lefort (1990)
Brine schrimp *(Artemia salina)*	1.0	no data	% N provided by the supplier
Trout chow	6.4	10.0	Values provided by the supplier
Prepared food (gelatin + trout chow)	3.4	10.0	Based on trout chow values

Table 1.2 Fresh wt. (in kg), N mass (in kg), and annual growth rate of fish in the SLM in 1995. Number and biomass of fish removed from the SLM in 1995 are also given. Fresh weights were converted into N mass using a 2.6% conversion factor factor (see Table 1.1).

Common Name	Species	Number of fish weighed	Mean individual weight	As on 03-31-99 Number of fish	As on 03-31-99 Species mass	Growth in 1995 Individual growth rate	Growth in 1995 Partial pop. growth rate	Added in 1995 Number of fish	Added in 1995 Mass of fish	Removed in 1995 Number of fish	Removed in 1995 Mass of fish
Spiny Dogfish	*Squalus acanthias*	4	2.7	4	10.9	5%	0.04%	-	-	-	-
Skates	*Raja* spp.	3	1.5	4	6.2	5%	0.02%	2	3	11	17
Atlantic Sturgeon	*Acipenser oxyrhynchus*	4	26.0	10	259.8	10% [a]	1.71%	-	-	-	-
Atlantic Salmon	*Salmo salar*	13	11.1	13	143.9	20% [b]	1.89%	30	332	9	100
Brook Trout	*Salvelinus fontinalis*	11	2.3	12	27.5	5%	0.09%	37	85	24	55
Atlantic Cod	*Gadus morhua*	29	6.8	83	563.6	18% [c]	6.49%	10	68	27	183
Greenland Cod	*Gadus ogac*	2	2.1	6	12.3	5%	0.04%	3	6	5	10
Pollock	*Pollachius virens*	3	9.0	15	134.5	18%	1.55%	3	27	2	18
Striped Bass	*Morone saxatilis*	6	2.8	56	154.1	10% [d]	1.01%	-	-	9	25
Ocean pout	*Macrozoarces americanus*	3	2.6	5	12.8	5%	0.04%	7	18	5	13
Atlantic Wolffish	*Anarhichas lupus*	1	4.0	1	4.0	5%	0.01%	4	16	1	4
Sea Raven	*Hemitripterus americanus*	10	3.4	15	50.7	5%	0.17%	2	7	3	10
Grubby	*Myoxocephalus aenaeus*	5	0.5	10	4.9	5%	0.02%	-	-	-	-
Longhorn Sculpin	*M. octodecemspinosus*	10	0.8	17	13.5	5%	0.04%	-	-	9	7
Shorthorn Sculpin	*M. scorpius*	10	1.1	25	27.0	5%	0.09%	8	9	13	14
American Plaice	*Hippoglossoides platessoides*	4	0.6	8	4.9	5%	0.02%	5	5	36	22
Atlantic Halibut	*Hippoglossus hippoglossus*	2	4.4	2	8.8	5%	0.03%	1	4	-	-
Winter Flounder	*Pseudopleuronectes americanus*	11	1.0	80	80.5	5%	0.26%	5	5	34	34
TOTAL		**131**		**366**	**1 520**		**13.5%**	**117**	**585**	**188**	**512**
Total N mass					39.5		5.3		15.2		13.3
Coefficient of variation (CV)					18.4%	37.0%	41.9%		18.4%		10.0%

[a] After Magnin 1962
[b] After Pépin, personnal communication
[c] After Fréchet and Schwab 1995
[d] After Magnin and Beaulieu 1967

Table 1.3 Monthly input of N (in kg per month) as food for fish and invertebrates and as seabird feces in the SLM in 1995.

Month	Fish and Invertebrates	Gannets	Kittiwakes
January	14.8	11.5	1.1
February	13.4	10.4	1.0
March	13.9	10.8	1.1
April	10.9	10.3	1.1
May	12.7	10.7	1.1
June	12.5	8.8	1.0
July	19.8	9.1	1.0
August	21.2	11.5	1.0
September	19.9	11.0	1.0
October	17.3	10.7	1.1
November	15.7	9.5	1.1
December	18.6	9.8	1.3
TOTAL	**190.8**	**123.9**	**13.1**
Proportion of feces entering the SLM		7/8	5/8
Net N input in the SLM	190.8	108.4	8.2
Coefficient of variation (%)	14.2%	20.0%	25.0%

Table 1.4 Number, fresh weight (in kg) and N mass (in kg) of live and dead invertebrates in the SLM in 1995. Fresh weights were converted into N mass as 1.15% N (see text).

Common Name	Scientific Name	Number of specimens	Number weighed	Mean weight	Species mass	Number added	Addition mass	Deaths	Dead mass
Anemones	Hexacorallia spp.	3 000	11	0.085	255	106	9	100	9
Red Soft Coral	*Gersemia carnea*	200	4	0.022	4	200	9	359	8
Common Periwinkle	*Littorina littorea*	20 000	150	0.008	152	21 000	159	18 000	136
Waved Whelk	*Buccinum undatum*	400	20	0.051	20	200	2	417	21
Toad Crab	*Hyas araneus*	40	15	0.217	9	15	3	20	4
Rock Crab	*Cancer irroratus*	500	12	0.245	123	84	21	100	25
Northern Lobster (large)	*Homarus americanus*	11	2	1.350	15	2	3	3	4
Northern Lobster (small)	*Homarus americanus*	9	2	0.395	4	-	-	-	0
Polar Starfish	*Leptasterias polaris*	250	13	0.141	35	152	21	250	35
Sun Starfishes	Solasteridae spp.	10	15	0.199	2	-	-	5	1
Green Sea Urchin	*Strongylocentrotus droebachiensis*	14 000	108	0.031	430	8 000	246	9 500	292
Northern Sea Cucumber	*Cucumaria frondosa*	250	11	0.281	70	80	22	150	42
Scarlet Psolus	*Psolus fabricii*	25	15	0.057	1	-	-	20	1
Sea Peach	*Halocynthia pyriformis*	50	2	0.039	2	-	-	50	2
TOTAL		**38745**	**380**		**1122**	**29839**	**495**	**28974**	**580**
Total N mass					12.91		5.69		6.67
Coefficient of variation (%)					16.6%		16.6%		16.1%

Table 1.5. The raw and corrected N budgets (in kg N) in the St. Lawrence mesocom for 1995 (CV = coefficient of variation).

	Raw Budget	CV	Corrected Budget	% of Total	% of Sub-total
Inputs					
Food	190.8	14.2%	198.9	58.1%	
Gannets guano	108.4	20.0%	113.0	33.0%	
Kittiwakes guano	8.2	25.0%	8.5	2.5%	
Invertebrates added	5.7	16.7%	5.9	1.7%	
Fish added	15.2	18.4%	15.8	4.6%	
Nitrogen in new water	0.2	14.2%	0.2	0.1%	
Total	**328.4**	**11.1%**	**342.4**	**100.0%**	
Outputs					
Filtration	54.8	13.4%	52.6	15.4%	44.3%
Cleaning	16.6	14.8%	15.9	4.7%	13.4%
Fish removed	13.3	10.0%	12.8	3.7%	10.8%
Water losses	39.0	10.0%	37.5	10.9%	31.5%
Subtotal	**123.7**	**7.1%**	**118.9**	**34.7%**	**100.0%**
Changes in nitrogen pools					
Nitrates	214.5	14.2%	206.1	60.2%	92.2%
Invertebrate growth	12.9	100.0%	12.4	3.6%	5.5%
Fish growth	5.3	41.9%	5.1	1.5%	2.3%
Subtotal	**232.7**	**14.2%**	**223.6**	**65.3%**	**100.0%**
Total	**356.4**	**9.6%**	**342.4**	**100.0%**	
Error	**(28.0)**		-		

Table 1.6 Amounts of organic matter (kg DW) and N (kg) removed by the filters of the two life support systems (LSS) and the general cleaning of the SLM in 1995. The mean N content of organic matter was 6.56% DW (CV = 14.2%). A positive covariance (r = 1) was assumed between the extra backwashes and the pool vacuum strainer (CV = coefficient of variation).

	Main LSS		Auxiliary LSS	
	Value	CV	Value	CV
Detritus collected through filtration				
Number of backwashes	414	0.0%	189	0.0%
Organic matter removed by one backwash	1.8	4.1%	0.4	4.0%
Organic matter removed by the LSS	752.5	4.1%	84.0	4.0%
N removed by the LSS	49.4	14.8%	5.5	14.8%
Total N removed by both LSS	54.9	13.4%		
Detritus collected through general cleaning				
Number of extra backwashes	122	0.0%		
Organic matter removed by the filters of the main LSS	220.5	4.1%		
Organic matter removed by the pool vacuum strainer	33.5	4.0%		
Total N removed through general cleaning (extra backwashes + pool vacuum strainer)	16.7	14.8%		
Total N removed through filtration and general cleaning	71.5	14.1%		

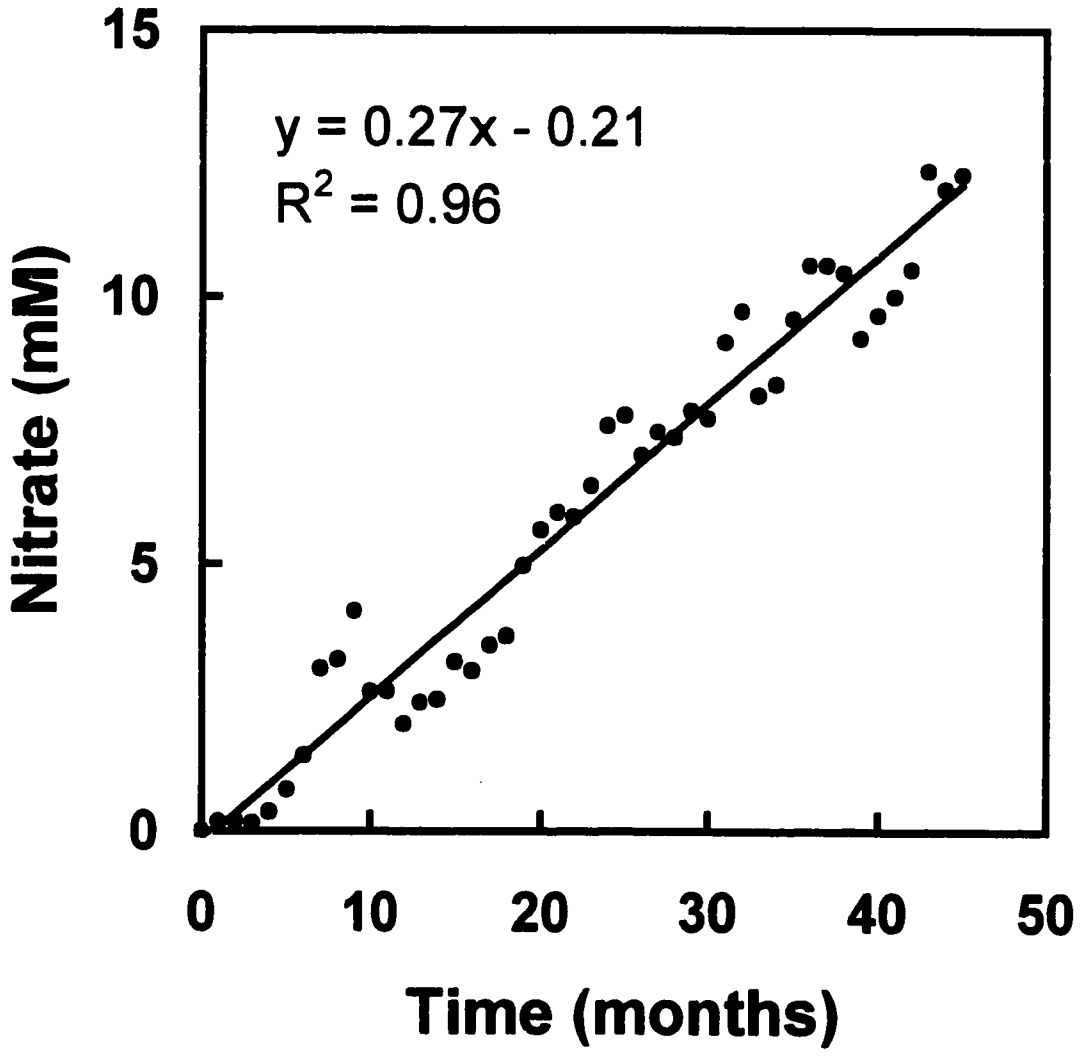

Figure 1.1 Monthly NO_3 measurements in the St. Lawrence mesocosm between 15 April 1992 and 15 January 1996.

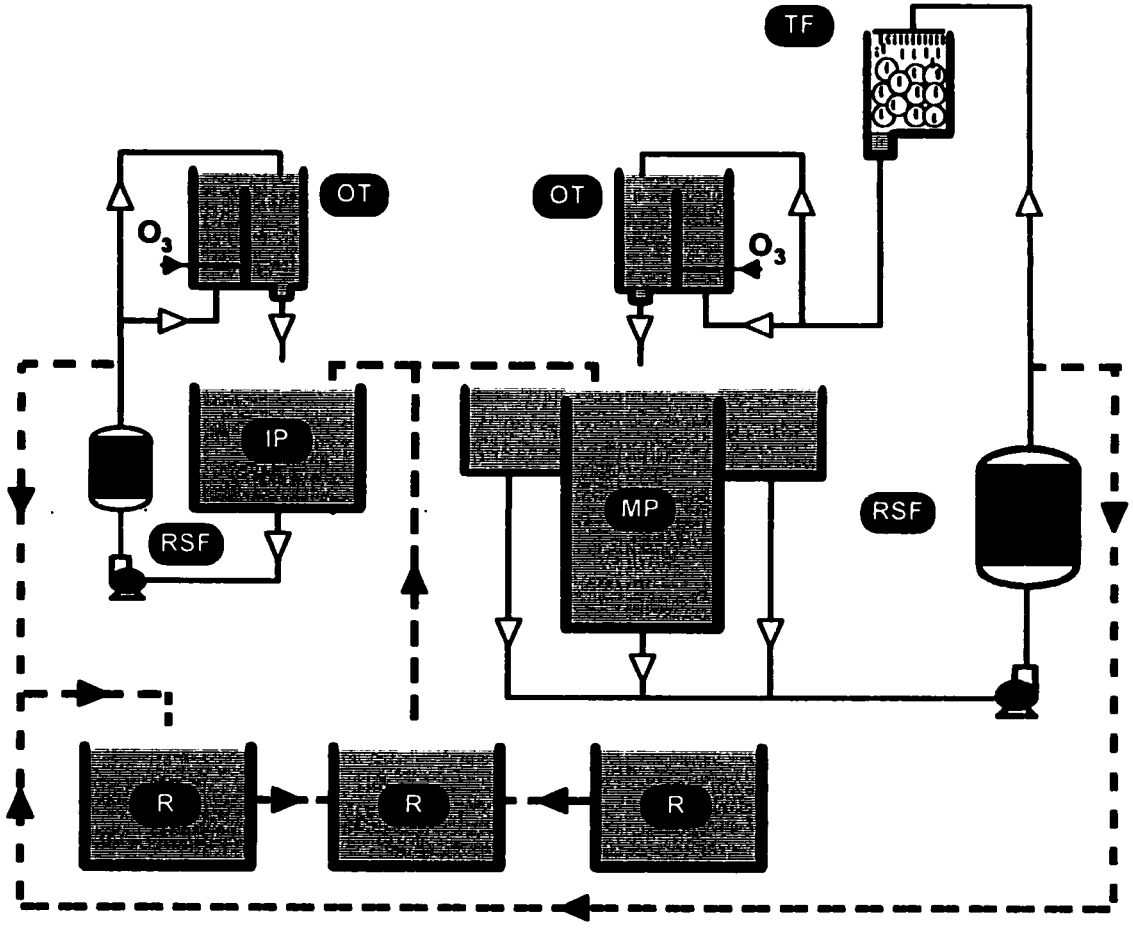

Figure 1.2 Hydraulic diagram of the St. Lawrence mesocosm. Legend : MP) bay pool, medical pool and rocky shore; IP) isolation pool; RSF) rapid sand filters; TF) trickling filter; OT) ozone tower; R) reservoirs. Solid line : normal water circulation. Dotted line: path for the filter backwashes and partial water changes.

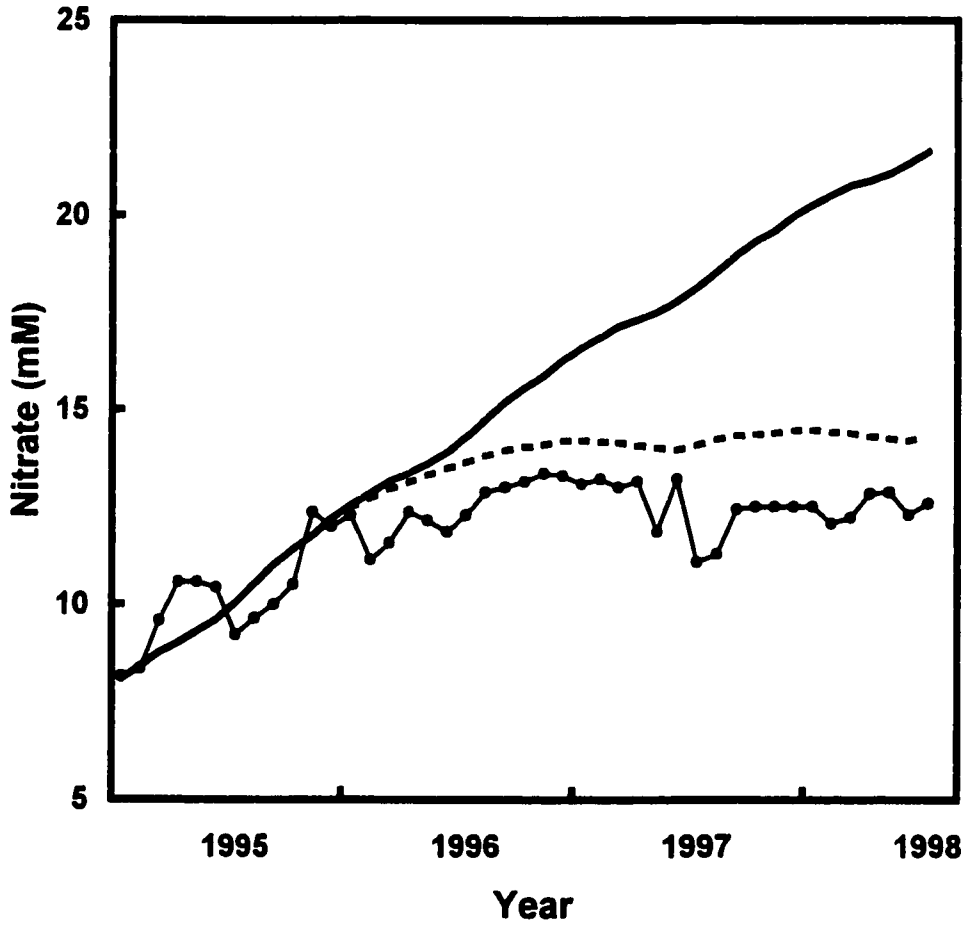

Figure 1.3 NO_3 in the St. Lawrence mesocosm during 1995 - 1998: (-•-) observed values, (____) values predicted by the 1995 equation, and (- - -) predicted values modified for the increase in partial water change and removal of the gannets in 1996.

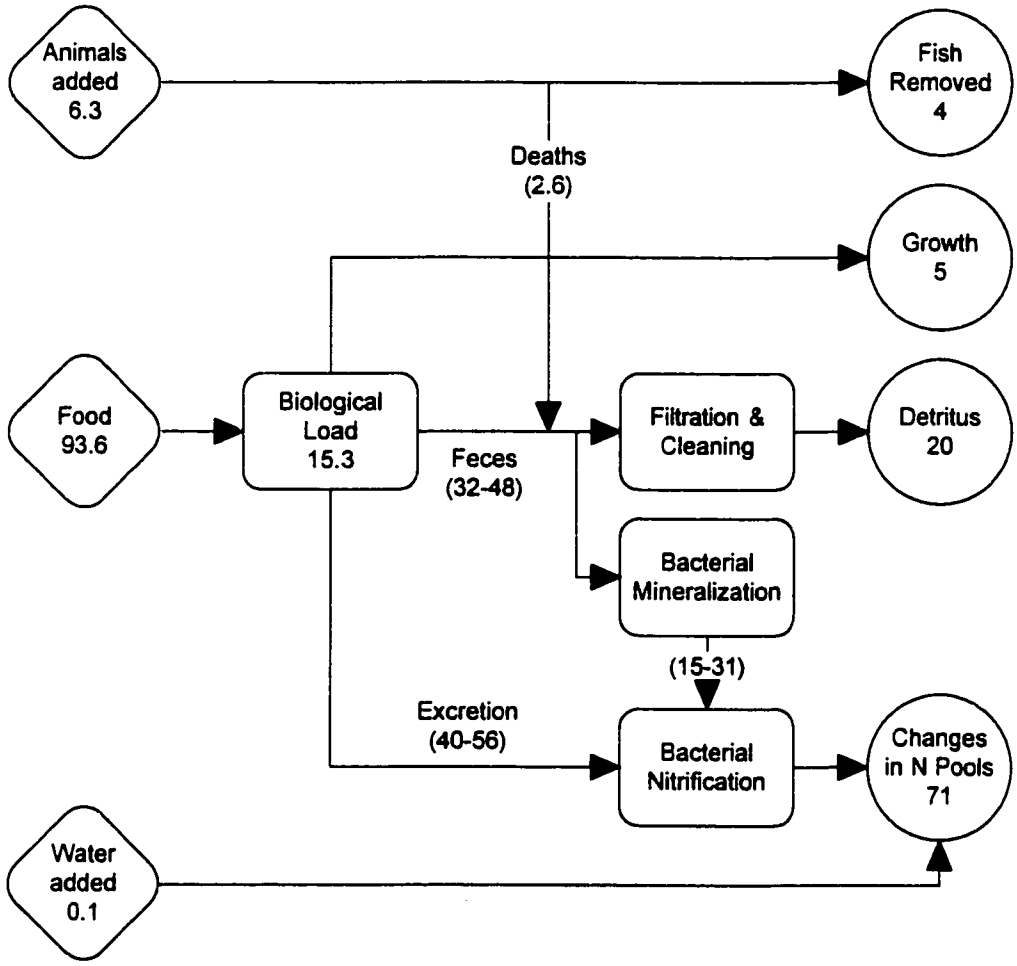

Figure 1.4 Flow chart of N in the St. Lawrence mesocosm in 1995. Data are expressed in g $N \cdot y^{-1}$, except for the biological load (g). Inputs are shown in diamonds, processes in boxes and outputs and changes in nitrogen pools in circles.

CHAPTER II

THE ROLE OF COPEPOD-DOMINATED MEIOFAUNA IN THE MINERALIZATION OF ORGANIC MATTER IN A COLD MARINE MESOCOSM

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ABSTRACT

Large populations of meiofauna are found in the sand filters of the St. Lawrence mesocosm at the Montreal Biodome. Three 30 d experiments were conducted in heterotrophic microcosms to quantify how populations of micro- and meiofaunal organisms affect mineralization using the Apparent Mineralization Rate (AMR), i.e. nitrate production, as a proxy. Tryptone, mesocosm detritus and fishmeal were used as organic substrates (C:N ratios 4-8). Harpacticoid copepods dominated the meiofauna in numbers (87%) and biomass (90%). AMR was inversely related to meiofaunal mass, and not related to ciliate density. Through grazing, 1 g dry mass·m^{-2} of meiofauna reduced the AMR of tryptone by 42%, that of detritus by 9.4% and that of fishmeal by 2.7%. The effect of meiofauna is correlated to particle size whereas the AMR is correlated to the C:N ratio. The scarcity of nematodes, which are known to stimulate mineralization, may explain these results. Copepod-dominated meiofauna decrease nutrient regeneration rates in heterotrophic habitats when C:N ratios are low.

RÉSUMÉ

Les filtres à sable du mésocosme du Saint-Laurent marin du Biodôme de Montréal renferment d'importantes populations de méiofaune. Trois expériences de 30 jours ont été menées dans des microcosmes hétérotrophiques pour mesurer l'effet de cette méiofaune et de la microfaune sur la minéralisation en utilisant le Taux Apparent de Minéralisation (TAM), c.-à-d. la production des nitrates, comme indicateur. Du tryptone, des détritus du mésocosme et de la farine de poisson ont été utilisés comme substrats organiques (rapport C:N de 4-8). Les copépodes harpacticoïdes dominent la méiofaune en nombre (87%) et en biomasse (90%). Le TAM a été inversement corrélé à la masse de méiofaune mais non à la densité des ciliés. Par le broutage, 1 g masse sèche·m^{-2} de méiofaune réduit le TAM du tryptone de 42%, celui des détritus de 9.4% et celui de la farine de poisson de 2.7%. L'effet de la méiofaune est corrélé à la taille des particules tandis que TAM est corrélé au rapport C:N du substrat. La rareté des nématodes, connus pour stimuler la minéralisation, explique peut-être ces résultats. Une méiofaune dominée par les copépodes diminue le taux de régénération des nutriments dans les habitats hétérotrophiques lorsque le rapport C:N est faible.

2.1 Introduction

The mineralization of organic matter is an important process in all aquatic habitats, but is of utmost concern in closed systems such as aquaria and mesocosms. In these aquatic systems, impairment of the mineralization process, i.e. dead fish or accumulation of detritus, generally results in a build-up of ammonia, the toxicity of which leads to the death of the fish and invertebrates on display. In a healthy system, ammonia produced by the digestion of the organic material by heterotrophic bacteria is quickly nitrified into much less toxic nitrate by chemoautotrophic bacteria. Biological filtration systems for large marine aquaria, such as the St. Lawrence mesocosm of the Montreal Biodome depend on this bacterial activity to maintain life support.

The rate at which organic matter is mineralized into ammonia is directly related to bacterial biomass and metabolic rate. However, it is also affected by animals that graze these bacteria, bioturbate sediments or fragment organic matter. Direct and indirect evidence suggest marine meiofauna increases the mineralization rate of organic matter. Direct evidence comes mainly from short-term laboratory experiments done with nematodes (Tenore *et al.* 1977; Findlay and Tenore 1982; Alkemade *et al.* 1992). Indirect evidence is provided by reports that nematodes and copepods increase bacterial biomass (and presumably mineralization) through bactericulture (Heip *et al.* 1985; Hicks and Coull 1983), symbiosis (Heip *et al.* 1985), bioturbation (Aller and Aller 1992), fragmentation (Tenore *et al.* 1977), and even through grazing (Montagna 1984; Epstein and Shiaris 1992). Grazing by meiofauna eliminates senescent cells, and maintains colonies in a state of exponential growth (Yingst and Rhoads 1980; Meyer-Reil and Faubel 1980) and therefore leads to an increase of bacterial turnover.

The present study was carried out to test whether the meiofauna living in the sand filters of the St. Lawrence mesocosm (SLM) positively affect mineralization. Experiments were conducted in laboratory model systems (heterotrophic microcosms) containing synthetic seawater and fixed

quantities of three organic substrates differing in particle size and C:N ratios. Inhibitors specific to eukaryotic cells were used to selectively control microfaunal and meiofaunal abundances. Nitrate production was measured as a proxy for mineralization because ammonia, the end product of mineralization, is transient and quickly transformed into nitrate by the nitrification process. Since the rate of nitrate production is a composite of the mineralization rate ($R\text{-}NH_2 \rightarrow NH_3$), the nitrification rate ($NH_3 \rightarrow NO_3$) and the faunal metabolic rates ($NH_3 \leftrightarrow R\text{-}NH_2$) within the system, the experiments produced Apparent Mineralization Rate (AMR) (see Parker *et al.* 1989). Nitrate concentrations were measured at regular time intervals to estimate the AMR which were then correlated with protozoan and meiofaunal abundances.

2.2 Materials and Methods

2.2.1 The St. Lawrence mesocosm

The St. Lawrence marine mesocosm is a closed system that holds 3 x 10^6 L of cold (10°C) artificial seawater (28-30‰). It exhibits fish, invertebrates and seabirds from the Gulf of St. Lawrence. The water treatment system consists of six sand filters, a 200 m^3 trickling filter filled with plastic bioballs, and an ozonation system (Fig. 2.1). The sand filters are 3.6 m in diameter and contain 65 cm of fine sand (250 µm to 300 µm effective particle size). The water rate through the filters is 6 $L \cdot m^{-2} \cdot s^{-1}$. The filters are backwashed approximately every 5 d. All detritus ends up in the sand filters where most of the meiofauna is located (S. Parent unpublished data). Pre-test analyses showed that the top 2.5 cm of sand contain 276-314 g dry $mass \cdot m^{-2}$ of organic matter before backwash (Table 2.1). The organic matter contains by weight about 2% nitrogen (N) and has a C:N ratio of 6.1-6.5. Water temperature is maintained throughout the year at 10°C. Nitrate levels in the mesocosm water were above 100 mg $NO_3\text{-}N \cdot L^{-1}$ at the time of the experiments.

2.2.2 Experimental setup

Three experiments were conducted under controlled conditions using tryptone, mesocosm detritus from the sand filters and fishmeal as organic substrates. Tryptone and fishmeal, differing from detritus in particle size or C:N ratio, were used to test whether these factors influenced meiofaunal effects on the mineralization rate of detritus.

Two sets of eight heterotrophic microcosms were used to conduct the experiments. Each microcosm consisted of a 20-L aquarium (778 cm^2) containing 13.5 L of water and an undergravel filter with two airlifts (filtration rate 0.7-1.4 $L \cdot m^{-2} \cdot s^{-1}$, 2-4 min turnover rate). The bottom of each microcosm was covered with 4 cm of filter sand. A shallow sand bed and a uniform yet rapid flow of oxygen saturated water from top to bottom of the sand bed insured

constant oxygen saturation in the sediment pore water. Seawater (28-30‰) was prepared using Instant Ocean® salts. The microcosms were maintained at 10°C in the dark to prevent algal growth.

The experiment with tryptone was run in April 1996 (day 0 = start of the experiment) with one set of microcosms (Fig. 2.2). Eight months before (day –250), meiofauna were extracted from the sand of one of the mesocosm filters by decantation (Pfannkuche and Thiel 1988) and put in the microcosms. Meiofauna was then maintained on tryptone until day 0. On day –204, meiofauna was suppressed in three microcosms with one dose of 10 ppm nystatin and cycloheximide (Sigma Chemical Co., St. Louis, MO. USA 63178), two substances inhibitory to eukaryotic organisms (Lee and Welander 1994). On day –20, 70% of the water was replaced and on day –1, water levels were adjusted. On day 0, 4 g of tryptone were quickly dissolved in each microcosm. Nitrate levels on day 0 were between 13.7 and 17.5 mg NO_3-N·L^{-1}.

The tryptone used was soybean-casein digest broth (Difco 1984). It quickly dissolved when put in seawater. It contained 8.7% nitrogen and had a C:N ratio of 4.4 (Table 2.1). By adding 4 g of tryptone to each tank, we were adding about the same amount of nitrogen (4.5 g N·m^{-2}) but less carbon (17.1 g C·m^{-2}) than what is found in the upper 2.5 cm layer of a sand filter. Given the small amounts of tryptone added before the experiment, there was probably little organic nitrogen in the sand on day 0.

The experiment with detritus was run in June 1996 with the other set of eight microcosms (Fig. 2.2). These were set up 87 days before the start of the experiment (day -87). A mesocosm sand filter clogged with detritus was accessed and 4 cm of wet sand was put in each microcosm. From day –82 to day –68, meiofauna were suppressed in four microcosms using repeated dosages of 10 ppm nystatin and cycloheximide. On day –2, 75% of the water was replaced. Nitrate levels on day 0 were between 26 and 35 mg NO_3-N·L^{-1}. All microcosms had organic matter, N and C contents within the range of values in the sand filters (Table 2.1). On day –17, the organic matter

content of the sand was 281 $g \cdot m^{-2}$. On day –2, the organic matter contained 2.2% nitrogen and had a C:N ratio of 7.9.

To determine particle size, detritus was collected in a sand filter, dried at 60-80°C and put through a geometric series of test sieves with 1.0 phi (Ø) intervals. The median particle diameter was 400 µm (Table 2.1). Over 35% of the particles were larger than 1 mm; 4.4% were less than 300 µm.

The eight microcosms used for the tryptone experiment were used again for the fishmeal experiment and were fed fishmeal in May 1996 (Fig. 2.2). One trial was done in July, and another in October 1996. Before the start of each trial (day –44), meiofauna was depressed in 2 or 3 microcosms using repeated dosages of 10 ppm nystatin and cycloheximide. This caused the loss of three microcosms in the second trial. One g of fishmeal was added to each microcosm on day -14 and 4 g more on day –7. On day –3, 75% of the water was changed. Nitrate levels on day 0 were between 15 and 24 mg NO_3-$N \cdot L^{-1}$.

Redfish (*Sebastes marinus*) fishmeal, produced locally, was used in the experiments. It contained 11.5% nitrogen and had a C:N ratio of 4.6 (Table 2.1). The fishmeal particle size was between 100 and 150 µm. Before adding fishmeal, each microcosm contained about 0.6 g $N \cdot m^{-2}$ and 6.3 g $C \cdot m^{-2}$. Through the addition of 5 g of fishmeal, each microcosm contained the same amount of N and C as in the upper 2.5 cm layer of a sand filter (Table 2.1).

Water sampling in all experiments began on day 0, and occurred every three days for 30 days (Fig. 2.2). Four water samples (28 ml total) were taken from each microcosm and quickly frozen at –20°C. Water losses through sampling and evaporation amounted to 5-10% during the experiments. No water was added during the experiment and no corrections for water loss were made in data analyses.

2.2.3 Biological determinations

Microfauna and meiofauna were generally measured no more than 10 days before and after each experiment. Since meiofauna are patchy in their distribution (Findlay 1981), the sand was combed before each sampling to obtain a better estimate of the meiofaunal abundance with the least effort. Five to ten samples of the 0-2.5 cm layer were randomly taken with a 10 cc syringe (1.63 cm^2). Meiofauna and microfauna were separated in a 50 cc cylinder using the decantation method (Pfannkuche and Thiel 1988) and then collected on a 20 µm sieve. By closely examining 1 cc of residue from randomly chosen samples, decantation was estimated to be at least 95% effective in extracting meiofauna. All animals were counted live. A different set of samples was taken to assess the standard weight of each taxon: length and width of specimens were measured and then converted into weights using conversion factors (Feller and Warwick 1988). These standard weights were then combined with the abundance results to establish the meiofaunal mass of each sample. The sand of all samples was put back in the microcosms but not the meiofauna. Sampling removed an insignificant amount of meiofauna (between 1 and 2%) before each experiment.

Three groups of protozoans were observed: ciliates, foraminiferans and heliozoans. Only ciliates were quantified. Ciliate populations were found by Fenchel (1978) to outnumber all other fauna in well mixed (100-300 µm) medium sands with little interstitial silt and clay, as in the case here. They were therefore used as an index of protozoan abundance.

2.2.4 Chemical analysis

Nitrate was measured on a Tecator FIAstar, Model 5010, using the automated cadmium reduction method (APHA *et al.* 1992) and the analysis protocol for 2-200 mg NO_3-N·L^{-1}. AMR results were then converted to mg NO_3-N·m^{-2}·d^{-1} using the water volume and sand surface of each

microcosm. Carbon and nitrogen analyses were performed on a Carlo-Erba CHN Analyzer. Organic matter content was determined by loss on ignition at 400-450°C.

2.2.5 Statistical analysis

The relationships between AMR and microfaunal number (ind·10 cm^{-2}) or meiofaunal biomass (g dry mass·m^{-2}) were quantified by linear regression analysis. For each microcosm, the biomass was set as the arithmetic mean of the biomasses before and after the experiment. Regression models for each of the three organic substrates were compared by covariance analysis. Detailed analysis of mineralization was done by looking at the AMR_t, i.e. AMR on days t = 6, 9, 12, ..., 24, during each experiment. AMR_t of each microcosm was calculated by regressing five nitrate concentrations (t–6, t–3, t, t+3 and t+6) over time. Each set of AMR_t was then regressed against meiofaunal biomass. The resulting Y-intercept (AMR_o), regression coefficient (effect of meiofauna) and R^2 of each regression were plotted against time to describe the phases of each experiment.

2.3 Results

2.3.1 Meiofauna composition and abundance

Meiofauna was composed of seven taxa: harpacticoid copepods, nematodes, rotifers, polychaetes, turbellarians, mites (Acari) and gastrotrichs (Table 2.2). The harpacticoids dominated in both number (87%) and biomass (98%), with three species present: *Tisbe* (*furcata*?), *Pseudonychocamptus proximus* and *Paramphiascella* (*fulvofasciata*?). *Pseudonychocamptus* largely dominated the group with over 90% of the specimens. Nematodes and rotifers accounted for only 4.6% (8%) of the specimens and 0.8% (0.5%) of the biomass; other taxa represented 1% of the specimens and the biomass. The composition of the sand filter meiofauna was the same, except for a few hydrozoan polyps which were absent in the microcosms.

Meiofaunal biomass in the 0-2.5 cm layer of the sand filters varied from 0.4 to 40 g dry mass·m^{-2} (1992-1996) with values of 20-40 g dry mass·m^{-2} during the year the experiments took place (1996). Later sampling (July 1997) revealed that meiofauna were abundant throughout the sand column with values close to 1 g·m^{-2}, in core increments of 2.5 cm, at least down to 25 cm (S. Parent unpublished data).

Meiofaunal abundance in the tryptone microcosms varied from 0 to 1.10 g dry mass·m^{-2}. One microcosm was excluded from analyses, on the basis of unusually high mortality rate (50%) of the inoculated meiofauna. The mortality in that microcosm continued for at least one month after the experiment. Preliminary statistical analysis also revealed this microcosm to be an outlier (Studentized Residual = 4.28).

Meiofaunal biomass in the detritus microcosms varied from 0 to 1.01 g dry mass·m^{-2}. Four microcosms had little meiofauna throughout while, in the four other microcosms, the meiofaunal

biomass decreased at some point during the experiment from 1.5-1.8 g dry mass·m^{-2} to 0.13-0.24 g dry mass·m^{-2}.

Meiofaunal biomass in the fishmeal microcosms varied from 0.2 to 6.3 g dry mass·m^{-2}. We compared the meiofaunal biomasses of the five microcosms that were used for both trials and no correlation was found between them. This lack of correlation and the long interval between the two trials (98 days) imply that the two trials constituted 13 independent replicates of the experiment.

2.3.2 Apparent Mineralization Rate (AMR)

In the tryptone experiment, the production of nitrate during the 30 d period varied from 19 to 23 mg NO_3-N·L^{-1}. This represents 73-89% of the organic nitrogen present at the start. In contrast to the two other experiments, the production rate of nitrate during the experiment was clearly biphasic (Fig. 2.3). In all eight microcosms there was a rapid increase in nitrates from day 0 to day 6, followed by a slow and steady production from day 6 to day 30. The AMR were measured for the 6-30 d period through linear regression. The standard error of the regression slopes was between 8% and 18% of the slope value.

In the detritus experiment, the production of nitrate during the 30 d period varied from 10 to 12 mg NO_3-N·L^{-1}. This represents 28.7-34.1% of the organic nitrogen present at the start. The production rate of nitrate was steady throughout the experiment (Fig. 2.3). The AMR could therefore be estimated by linear regression. The standard error of the regression slopes was between 5% and 20% of the slope value.

In the fishmeal experiment, the production of nitrates during the 30 d period of each trial varied from 12 to 16 mg NO_3-N·L^{-1}. This represents 27-36% of the organic nitrogen present at the start. The rate of nitrate production was slightly curvilinear during each trial (Fig. 2.3) but the

AMR could still be estimated by linear regression with enough accuracy. The standard error of the regression slopes was between 6% and 11% of the slope value.

Water loss due to sampling was constant at 0.7% whereas water loss due to evaporation varied from 4% to 9% from one experiment to another. Water loss was the same in all the microcosms of a given experiment, so no correction was needed to estimate the effect of meiofauna on AMR although it resulted in slight overestimations of AMR.

2.3.3 Linear regression models

In all three experiments, the AMR was not related to ciliate density, but was inversely related to meiofaunal biomass (Table 2.3). Ciliate densities were stable throughout the tryptone and fishmeal experiments, but dropped from 210 000 ind·m^{-2} to less than 15 000 ind·cm^{-2} in the detritus experiment (Table 2.3). The ciliate density in the 0-2.5 cm layer of the sand filters was 192 000 ± 92 630 ind·m^{-2} (mean ± SEM, n = 8).

There was a negative relationship between AMR and meiofaunal biomass (Fig. 2.4). In each experiment, the relationship was significant and meiofaunal biomass explained between 44% and 80% of the variation in AMR (Table 2.4). The effect of meiofaunal biomass varied among the organic substrates: 1 gram of meiofauna reduced the AMR of tryptone by 42%, that of mesocosm detritus by 9.4% and that of fishmeal by 2.7% (Table 2.4).

Covariance analysis showed that the effects of meiofauna in the detritus and the fishmeal experiments were similar ($\alpha F_{1,18}$ = 1.42, p = 0.25) but that the AMR_0 were different ($\alpha F_{1,18}$ = 99.8, $p < 0.001$). It also showed that the effects of meiofauna in the fishmeal and tryptone experiments were different ($\alpha F_{1,18}$ = 11.1, p = 0.004) and therefore the AMR_0 could not be tested. The detritus and tryptone models were not compared because of unequal residual variances.

2.3.4 Phases of the experiments

In the tryptone experiment, AMR_0, i.e. the AMR when there is no meiofauna, did not vary much and meiofauna always had a strong negative effect on AMR (Fig. 2.5). About 30% of the tryptone had been mineralized on day 12. The R^2 was above 0.50 on days 18 and 21. No data are available for days 6 and 9.

In the detritus experiment, AMR_0 increased until day 18, and then decreased slightly. Meiofauna had a positive effect on days 6 and 9. Its negative effect appeared strongly on day 12, was maintained through day 15 but was then quickly reduced to near zero on days 18 and 21. On days 12 and 15, 12 to 15% of the detritus had been mineralized and the R^2 were above 0.20 (Fig. 2.5).

In the fishmeal experiment, AMR_0 decreased from 120 to less than 60 mg $NO3\text{-}N \cdot m^{-2} \cdot d^{-1}$ throughout the experiment. Meiofauna had no significant effect on day 6. Its negative effect appeared on day 9, increased until day 18 and then decreased. About 15 to 18% of the fishmeal had been mineralized by days 15 and 18. The R^2 was above 0.20 only on days 18 and 21 (Fig. 2.5).

AMR rates in all three experiments can be divided into three phases: an early phase (days 6 and 9), a middle phase (days 12, 15 and 18), and a late phase (days 21 and 24). During the early phase, the effects of meiofauna were either null or slightly positive, the R^2 were less than 0.10, and the AMR_0 values were either low or high. During the middle phase, the effects of meiofauna became strongly negative, the R^2 reached values as high as 0.83, and AMR_0 values were high. Finally, during the late phase, the effects of meiofauna became less and less negative, the R^2 reached low values again, and the Y-intercepts (AMR_0) decreased.

2.4 Discussion

2.4.1 Meiofauna composition and abundance

The microcosm meiofauna was similar in abundance to natural marine meiofauna but differed from it in composition. In marine subtidal sands, the meiofaunal biomass amounts to 1-2 g dry mass·m^{-2} and nematodes regularly dominate the meiofauna comprising 50% of the total meiofaunal abundance, while copepods comprise only 7-12 % (Coull 1988).

The meiofauna in both the microcosms and the sand filters were composed mainly of prehensile epibenthic species, such as the harpacticoid *Pseudonychocamptus*, and of strongly adhesive species, such as the nematode *Anticoma*. *Pseudonychocamptus proximus*, accounted for 79% of all specimens and 88% of the meiofaunal mass. There were only a few mesopsammic species such as the harpacticoid *Paramphisciella*. Sand in the sand filters is strongly backwashed every 5 days or so, and may have prevented the colonization by mesopsammic species. In addition, water is forced through the sand in both the microcosms and the sand filters, possibly favoring epibenthic species at the expense of mesopsammic ones. Hence the observed scarcity of nematodes which may explain the results obtained in our experiments. Nematodes were shown to be largely responsible for increasing the mineralization rate of organic matter (Tenore *et al.* 1977; Alkemade *et al.* 1992).

Most meiofaunal species found here eat diatoms, microalgae or bacteria. *Anticoma*, for example, is a selective deposit feeder (Wieser 1959). *Pseudonychocamptus* feeds not only on microalgae and diatoms, but also on bacterial exopolymer (Decho and Moriarty 1990). *Tisbe furcata* prefers bacteria (Vanden Berghe & Bergmans 1981). Our observations indicate that hydrozoans live in the pools and are only transients in the sand filters.

2.4.2 Conditions of the experiments

A significant amount of the organic nitrogen was mineralized in all three experiments: 29% of the fishmeal, 32% of the detritus and 80% of the tryptone. The measured AMR was probably not dependent on the amount of mineralizable substrate because the production rate of nitrate was relatively constant in the experiments. Organic nitrogen mineralization usually follows first order kinetics (Stanford and Smith 1972) and the typical curve for net nitrogen mineralization has a parabolic shape (Campbell *et al.* 1993). We did not observe this phenomenon here, mostly because the experiment was not long enough for the AMR to become dependent on the available substrate.

The observed variability of AMR was not correlated to protozoan density. Protozoans are known to affect mineralization (Pomeroy 1970; Barsdate *et al.* 1974), but either they equally affected all microcosms, or their effect was too small compared to that of meiofauna.

2.4.3 Linear regression models

Linear regressions were used to describe the effect of meiofauna on AMR because we postulated grazing was the main biological mechanism involved in our experiments and grazing rate per capita was probably constant over a wide range of meiofaunal masses. Other processes, e.g. bactericulture and bioturbation, were not considered important because water was forced through the sand. Fragmentation of food by meiofauna was considered negligible because of the detritus small particle size.

All three models show that meiofauna in the sand filters decreased the AMR in our microcosms where C:N ratios ranged from 4.4 to 8 (Fig. 2.4). These results confirm the idea that meiofauna feed little on detritus and extensively on bacteria (Meyer-Reil and Faubel 1980; Montagna 1995). Meiofauna also feed on diatoms, but none were present in the heterotrophic microcosms. There are therefore situations where marine meiofauna do not increase the

mineralization rate of organic matter. This probably also occurs in streams, since common stream-dwelling copepods reduce the density of detritally associated bacteria by as much as 58% (Perlmutter and Meyer 1991).

The AMR_0 for detritus (66 mg NO_3-N·m^{-2}·d^{-1}) is significantly lower than the AMR_0 for fishmeal (86 mg NO_3-N·m^{-2}·d^{-1}), even though more organic matter was present at the start of the detritus experiment. We think there are three reasons for this. First, detritus was more variable and slightly larger in size than fishmeal. Second, at the start of the experiment, the C:N ratio of detritus was higher than the C:N ratio of fishmeal (Table 2.1). At C:N ratio less than 25, mineralization increases exponentially as C:N decreases (Parnas 1975). DeAngelis (1992) estimated that mineralization increases three-fold when the C:N ratio decreases from 8 to 4. Third, fishmeal is more labile than detritus. Fresh fishmeal was used in the microcosms whereas detritus was comprised of unused food, unabsorbed food and refractory objects such as scales and feathers that were left in the microcosms for almost three months before the start of the experiment.

Results of the covariance analysis suggest that the effect of meiofauna is independent of the C:N ratio of organic matter, but may be dependent on particle size (Table 2.1, Fig. 2.4). Meiofauna had the same effect on AMR in the fishmeal and detritus experiments, which shared similar particle sizes but different C:N ratios. Meiofauna had a significantly different effect on AMR in the fishmeal and tryptone experiments which shared similar C:N ratios but different particle sizes.

Results of the covariance analysis also suggest that the C:N ratio of organic matter is significantly correlated to AMR_0 (Table 2.1, Fig. 2.4). Bacteria mineralize organic matter with a low C:N ratio faster than that with a high C:N ratio and labile organic matter more easily than refractory organic matter (Parnas 1975).

Harpacticoid copepods, especially *Pseudonychocamptus proximus* that accounts for 98% of the meiofaunal biomass in our system, are mainly responsible for the observed effect of meiofauna on AMR. They comprise up to 98% of the meiofaunal biomass in our system. Other taxa such as rotifers are known to graze on bacteria (Thane-Fenchel 1968), but we suspect that in this case they played a minor role or no role at all because of their scarcity. Indeed, AMR was not related to the residual meiofaunal biomass (meiofaunal biomass minus harpacticoid biomass) in the tryptone experiment ($R^2 = 0.026$, $F = 0.13$, $p = 0.73$).

The linear regression models show that the effect of meiofauna varied with the substrate (Table 2.4). One g dry mass·m^{-2} of meiofauna reduced the AMR_0 of the fishmeal by 2.7%, the AMR_0 of detritus by 9.4% and the AMR_0 of tryptone by as much as 42%. The AMR_0 of the experiments, which reflects bacterial activity in the absence of meiofauna, explains the observed variability in the effect of meiofauna. The AMR_0 of fishmeal (86 mg NO3-N·m^{-2}·d^{-1}) was significantly higher than the AMR_0 of detritus (65 NO_3-N·m^{-2}·d^{-1}). Mineralizing bacteria being more productive in the fishmeal experiment, they better resist the grazing effect of meiofauna. The same explanation can be given in part for the tryptone experiment. The AMR_0 of tryptone (80 NO_3-N·m^{-2}·d^{-1}) was lower than that of fishmeal, but higher than that of detritus. We suspect that the grazing rate of meiofauna was higher in the tryptone experiment because meiofauna could feed on nothing else but bacteria. Harpacticoids are capable of direct assimilation of small organic compounds such as glucose, but probably not of complex dissolved organic matter such as tryptone (Hicks and Coull 1983).

2.4.4 Phases of the experiments

Through grazing, copepod-dominated meiofauna better prepared bacterial colonies for an influx of organic matter, but then took a heavier toll on their production (Fig. 2.5). Meiofauna had a small positive effect on the AMR during the early phase of the detritus and fishmeal

experiments when the reaction was starting: the substrate was plentiful with a low C:N ratio, but mineralizing bacteria were not yet taking full advantage of it. Afterwards, during the middle phase, the negative effect of meiofauna was strongest when AMR_0 was at its highest and the C:N ratio still low. Finally, as the C:N ratio increased, the AMR_0 decreased and meiofauna had a diminishing effect on AMR_0. The fact that meiofauna had less effect on mineralization during the late phase is probably an artifact produced by the differences in mineralization rates in the microcosms. The more meiofauna there was in a microcosm, the less substrate was mineralized during the middle phase and the more substrate with a lesser C:N ratio was available in the late phase. Consequently, more mineralization took place during the late phase in microcosms having more meiofauna, which resulted in an apparent reduced or, in the case of detritus, an apparent positive effect by the meiofauna.

Meiofauna exerted its negative effect even when very little substrate had been mineralized (Fig. 2.5). In the detritus experiment, the negative effect of meiofauna appeared on day 12 when only 12% of the organic nitrogen had been mineralized and, in the fishmeal experiment, the negative effect appeared on day 9 when only 10% of the organic nitrogen had been mineralized.

Changes in the impact of meiofauna on mineralization during the three phases are easy to understand for the tryptone and fishmeal experiments because these substrates were added just prior to the experiment. However, since detritus were added 87 days prior to the detritus experiment, we would have expected a constant AMR_0 and a constant meiofauna effect throughout the experiment. We suggest that changing 70% of the water before each experiment was very important in stimulating mineralization in the microcosms. The microcosms were in closed circuit and no physico-chemical filtration, such as granular activated carbon or ozone, was used. Seawater was yellow before it was changed and colorless afterwards. Refractory by-products of mineralization, such as phenols and humus, are known to build up in aquaria and to impart the yellow coloration to old aquarium seawater (Spotte 1991). They were also possibly reducing the AMR before each experiment.

The results of the early phase support the suggestion that meiofauna grazing is in equilibrium with microbial production (Montagna 1984, 1995). The observed null or slightly positive effect of meiofauna means that bacterial production increased in microcosms with large meiofaunal biomass and adjusted to a larger grazing pressure. However, the results of the middle phase show that the influx of organic matter ruptured the equilibrium between microbial production and meiofaunal grazing. Bacterial productivity decreased in microcosms with large meiofaunal biomass and this resulted in a net negative effect of meiofauna on AMR (Fig. 2.5). The results of the late phase suggest a return to equilibrium as the added organic substrate became depleted. It therefore seems that the equilibrium between meiofaunal grazing and bacterial production is produced rather by a steady influx of organic matter than by a variable grazing rate per meiofaunal capita.

The strongest short-term effect of meiofauna was –50 mg NO_3-N·m^{-2}·d^{-1} per g dry mass·m^{-2} of meiofauna for tryptone, –29 for detritus and –7.3 for fishmeal (Fig. 2.5). These effects were observed when only 12% of the detritus and 10% of the fishmeal had been mineralized. Our results therefore suggest that when the substrate is plentiful and the water is relatively clean, meiofauna actually has a very strong impact on AMR. The strongest significant effect of 1 g dry mass·m^{-2} meiofauna, as measured in the middle phase, was 9% reduction in AMR for fishmeal (R^2 = 0.58), 36% reduction for detritus (R^2 = 0.56) and 55% for tryptone (R^2 = 0.83). This suggests that less than 3 g dry mass·m^{-2} of meiofauna would have completely stopped the mineralization in the microcosms.

2.4.5 Microcosms compared with the sand filters

The regression model of the detritus experiment showed the effect of 1 g dry mass·m^{-2} meiofauna on the mineralization of detritus to be –9.4% (Table 2.4). Meiofauna biomasses of 20 to 40 g dry mass·m^{-2} have been found in the top 2.5 cm of sand in the sand filters of the St.

Lawrence mesocosm in 1996. If our results in the microcosms were extrapolated to the sand filters, we would conclude that little or no mineralization of organic matter occurred there.

However, it is impossible to simply extrapolate our results to the sand filters because the conditions are different. The AMR_0 is probably higher in the sand filters than it was in the microcosms. Water in the mesocosm is ozone treated, there is a constant influx of fresh organic matter in the sand filters and the filters are frequently backwashed. As a result, the organic matter is kept at its maximum lability, which permits the highest AMR_0. Moreover, there is a constant influx of algae in the sand filters, an ingredient which was absent from the detritus experiment. Harpacticoid copepods, especially *Pseudonychocamptus*, are known to prefer feeding on live or recently dead algae and therefore exert less pressure on mineralizing bacteria (Rudnick 1989; Decho and Moriarty 1990).

Consequently, we cannot conclude that meiofauna exerts a strong negative impact on AMR in the St. Lawrence mesocosm. To examine this question, *in situ* experiments would be required, especially in the deeper parts of the filter sand, where meiofauna is abundant and less algae and more refractory organic matter are probably present.

One reason why meiofauna did not have a positive effect in the microcosms is that all beneficial action from meiofauna may have been eliminated in the microcosms and in the sand filters: there are few nematodes, water flow through the sand leaves no role for bioturbation, which is thought to be the main mechanism by which meiofauna exerts its positive action (Alkemade *et al.* 1992), and there can be little bactericulture because the sand is frequently backwashed. All that is left is possibly fragmentation and a fecal pellet effect by the copepods. These effects might be negligible compared to the grazing action of meiofauna.

Meiofauna therefore does not always increase the mineralization rate of organic matter. Copepod-dominated meiofauna decrease nutrient regeneration rates in heterotrophic habitats when the C:N ratios are low. The effect of meiofauna on the mineralization rate of organic matter

can be seen as a delicate balance between negative and positive processes, such as grazing, bioturbation, bactericulture and fragmentation. The net result of these processes, an increase or a decrease in the mineralization rate of organic matter, depends on environmental factors, such as filtration and heterotrophy in closed systems, and faunal composition: two sets of factors that cannot easily be separated one from each other.

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REFERENCES

Alkemade, R., Wielemaker, A., de Jong, S.A., and Sandee, A.J.J. 1992. Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella dievengatensis* in stimulating the mineralization of *Spartina angli*. Mar. Ecol. Prog. Ser. **90**:149-155.

Aller, R. C., and Aller, Y.C. 1992. Meiofauna and solute transport in marine muds. Limnol. Oceanogr., **37**:1018-1033.

APHA, AWWA and WEF. 1992. Standard methods for the examination of water and wastewater. 18th ed., American Public Health Association, Washington DC, page 4-91.

Barsdate, R.J., Prentki, R.T., and Fenchel, T. 1974. Phosphorus cycle of model ecosystems: significance for decomposer food chains and effect of bacteria grazers. Oikos **25**: 239-251.

Campbell, C.A., Ellert, B.H., and Jame, Y.W. 1993. Nitrogen mineralization potential in soils. *In*: Soil sampling and methods of analysis. Chapt. 33. *Edited by* Carter, M.R. Lewis Publishers, London, pp. 341-349.

Coull, B.C. 1988. Ecology of the marine meiofauna. *In* Introduction to the study of meiofauna. Chapt. 3. *Edited by* Higgins, R.P., and Thiel, H. Smithsonian Institution Press, Washington, pp. 18-38.

DeAngelis, D.L., 1992. Dynamics of nutrient cycling and food webs. Chapman & Hall, London. 270 p.

Decho, A.W., and Moriarty, D.J.W. 1990. Bacterial exopolymer utilization by a harpacticoid copepod: a methodology and results. Limnol. Oceanogr. **35**:1039-1049.

Difco 1984. Difco Manual. Dehydrated culture media and reagents for microbiology. 10th ed., Difco Laboratories, Detroit, Michigan, p. 1027.

Epstein, S.S., and Shiaris, M.P. 1992. Rates of microbenthic and meiobenthic bactivory in a temperate muddy tidal flat community. App. Environ. Microbiol. **58**: 2426-2431.

Feller, R.J., and Warwick, R.M. 1988. Energetics. *In* Introduction to the study of meiofauna. Chapt. 13. *Edited by* Higgins, R.P., and Thiel, H. Smithsonian Institution Press, Washington, pp. 181-196.

Fenchel, T. 1978. The ecology of micro- and meiobenthos. Annual Review of Ecology and Systematics **9**: 99-121.

Findlay, S.E.G. 1981. Small scale spatial distribution of meiofauna on a mud- and sandflat. Estuarine and Coastal Shelf Science, **7**: 255-268.

Findlay, S, and Tenore, K.R. 1982. Effect of a free-living marine nematode (*Diplolaimella chitwoodi*) on detrital carbon mineralization. Mar. Ecol. Prog. Ser. **8**:161-166.

Heip, C., Vincx, M., and Vranken, G. 1985. The ecology of marine nematodes. Oceanogr. Mar. Biol. Ann. Rev. **23**: 399-489.

Hicks, G.R.F., and Coull, B.C. 1983. The ecology of marine meiobenthic harpacticoid copepods. Oceanogr. Mar. Biol. Ann. Rev. **21**: 67-175.

Lee, N. M., and Welander, T. 1994. Influence of predators on nitrification in aerobic biofilm processes. Wat. Sci. Tech., **29**:355-363.

Meyer-Reil, L.-A., and Faubel, A. 1980. Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. Mar. Ecol. Prog. Ser. **3**: 251-256.

Montagna, P.A. 1984. *In situ* measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. Mar. Ecol. Prog. Ser. **18**:119-130.

Montagna, P.A. 1995. Rates of metazoan meiofaunal microbivory: a review. Vie Milieu **45**: 1-9.

Parker, D., Lutz, M., Dahl, R., and Bernkopf, S. 1989. Enhancing reaction rates in nitrifying trickling filters through biofilm control. J. Water Pollut. Control Fed. **61**:618-631.

Parnas, H. 1975. Model for decomposition of organic material by microorganisms. Biol. Biochem. **7**: 161-169.

Perlmutter, D.G., and Meyer, J.L. 1991. The impact of a stream-dwelling harpacticoid copepod upon detritally associated bacteria. Ecology **72**:2170-2180.

Pfannkuche, O., and Thiel, H. 1988. Sample processing. *In* Introduction to the study of meiofauna. Chapt. 9. *Edited by* Higgins, R.P., and Thiel, H. Smithsonian Institution Press, Washington, pp. 134-145.

Pomeroy, L.R. 1970. The strategy of mineral cycling. Ann. Rev. Ecol. Systems **1**: 171-190.

Rudnick, D.T. 1989. Time lags between the deposition and meiobenthic assimilation of phytodetritus. Mar. Ecol. Prog. Ser. **50**: 231-240.

Spotte, S. 1991. Captive Seawater Fishes. Science and Technology. John Wiley & Sons, Toronto, 942 pages.

Stanford, G., and Smith, S.J. 1972. Nitrogen mineralization potentials of soils. Soil. Sci. Soc. Am. Proc. **36**: 465-472.

Thane-Fenchel, A. 1968. Distribution and ecology of non-planktonic brackish-water rotifers from scandinavian waters. Ophelia **5**: 273-297.

Tenore, K.R., Tietjen, J.H., and Lee, J.J. 1977. Effect of meiofauna on incorporation of aged Eelgrass, *Zostera mari*na, detritus by the polychaete *Nephthys incisa*. J. Fish. Res. Board Can. **34**:563-567.

Vanden Berghe, W., and Bergmans, M. 1981. Differential food preferences in three co-occurring species of *Tisbe* (Copepoda, harpacticoida). Mar. Ecol. Prog. Ser. **4**: 213-219.

Wieser, W. 1959. Free-living marine nematodes - IV. General Part. Lunds Universitets Arsskrift, Ny Foljd Avdelning2, **55**: 1-111.

Yingst, J.Y., and Rhoads, D.C. 1980. The role of bioturbation in the enhancement of bacterial growth in marine sediments. *In* Marine Benthic Dynamics. *Edited by* Tenore, K. R., and Coull, B.C. University of South Carolina Press, Columbia, SC, pp. pp. 407-421. (Belle W. Baruch Libr. Mar. Sci.; v. 11).

Table 2.1 Particle size, content in organic matter, organic nitrogen and organic carbon, and C:N ratio of the detritus in sand filters and of the three organic substrates added to the microcosms (mean ± SEM).

	Particle size µm	Organic content $g \cdot m^{-2}$	Nitrogen content $g\ N \cdot m^{-2}$	Carbon content $g\ C \cdot m^{-2}$	C:N ratio
Sand filters	400	295 ± 18.7	5.9 ± 0.31	31.9 ± 3.66	6.3 ± 0.18
Microcosms :					
4 g Tryptone	< 0.45	51	4.5 ± 0.01	17.1 ± 0.03	4.4 ± 0.08
22 g Detritus	400	281	6.0 ± 0.01	40.9 ± 0.05	7.9 ± 0.09
5 g Fishmeal	100 - 150	64	7.4 ± 0.02	29.4 ± 0.18	4.6 ± 0.11

Table 2.2 Relative abundances (individuals $\cdot 10^3$ specimens) of meiofauna in the experimental microcosms and sand filters of the St. Lawrence mesocosm.

Taxon	Microcosms	Sand filters
Copepoda harpacticoida	871	911
Pseudonychocamptus proximus		
Tisbe (furcata?)		
Paramphisciella (fulvofasciata?)		
Nematoda	81	76
Anticoma sp.		
Chomadoridae sp.		
Monhystera sp.		
Rotifera	35	7
Colurella (colurus?)		
Dicranophoridae sp.		
Turbellaria	3.5	2.3
Species A		
Species B		
Species C		
Species D		
Polychaeta	5	0.4
Dinophilus (gyrociliatus?)		
Nerilla sp.		
Gastrotricha	4	2.0
Chaetonotida sp.		
Acari	1	0.3
Thalassarachna basteri		
Isobactrus setosus		
Copidognathus biodomus nov. sp.		
Hydrozoa	-	1
Corynidae sp.		
Clavidae sp.		
Pandeidae sp.		
Hydractinia echinata		

Table 2.3 Mean ciliate densities in experimental microcosms and results of the Pearson correlations between AMR and ciliate density or meiofaunal biomass.

Experiment	Ciliate densities ($ind \cdot m^{-2}$)	Ciliates		Meiofaunal mass	
		r	p	r	p
Tryptone	2500 – 42000	-0.65	0.12	-0.89	0.007 **
Detritus	0 – 210 000	-0.34	0.40	-0.79	0.02 *
Fishmeal	5000 – 371000	-0.43	0.14	-0.64	0.02 *

* $0.01 < p \leq 0.05$ ** $p \leq 0.01$

Table 2.4 Linear regression models of the mineralization experiments (AMR in mg NO_3-N·m^{-2}·day^{-1} and meiofaunal biomasses in g dry mass·m^{-2}).

Experiment	Model	n	F	p	R^2	95% confidence interval of the slope
Tryptone	AMR = 79.6 – 33.3 Biomass	7	19.5	0.01	0.80	$-52.7 < \beta_1 < -13.9$
Detritus	AMR = 65.5 – 6.2 Biomass	8	10.4	0.02	0.63	$-10.9 < \beta_1 < -1.48$
Fishmeal	AMR = 86.4 – 2.3 Biomass	13	8.5	0.01	0.44	$-4.06 < \beta_1 < -0.57$

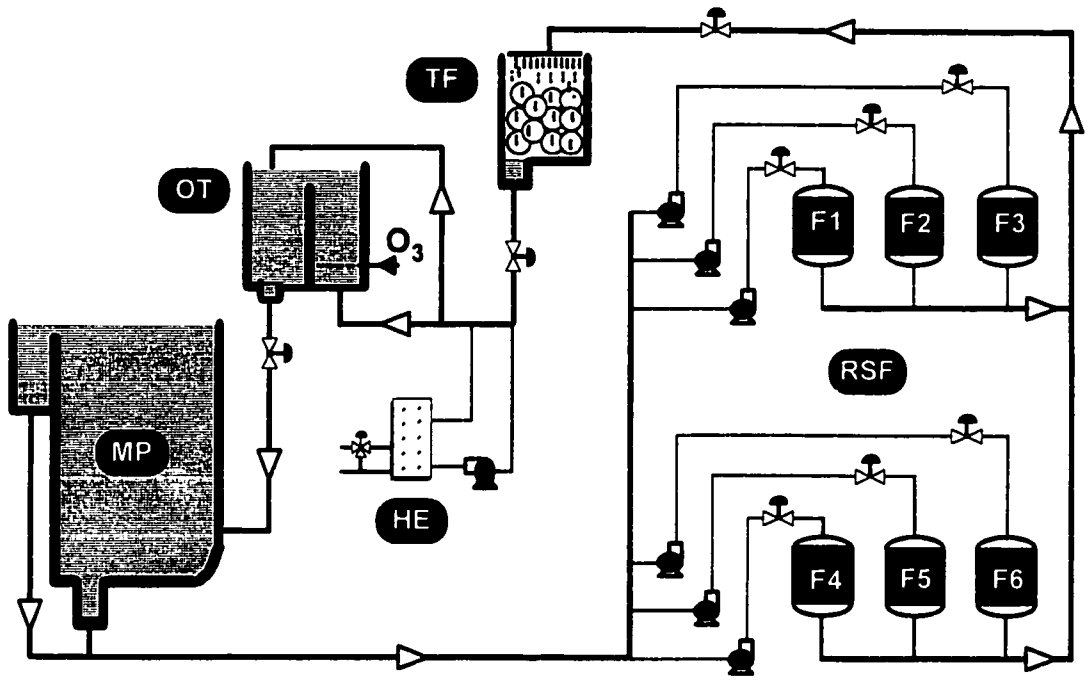

Figure 2.1 Hydraulic diagram of the St. Lawrence mesocosm. Legend: MP, main pool, medical pool and tidepool; RSF, rapid sand filters (F1-F6); TF, trickling filter; OT) ozone tower; HE) heat exchangers.

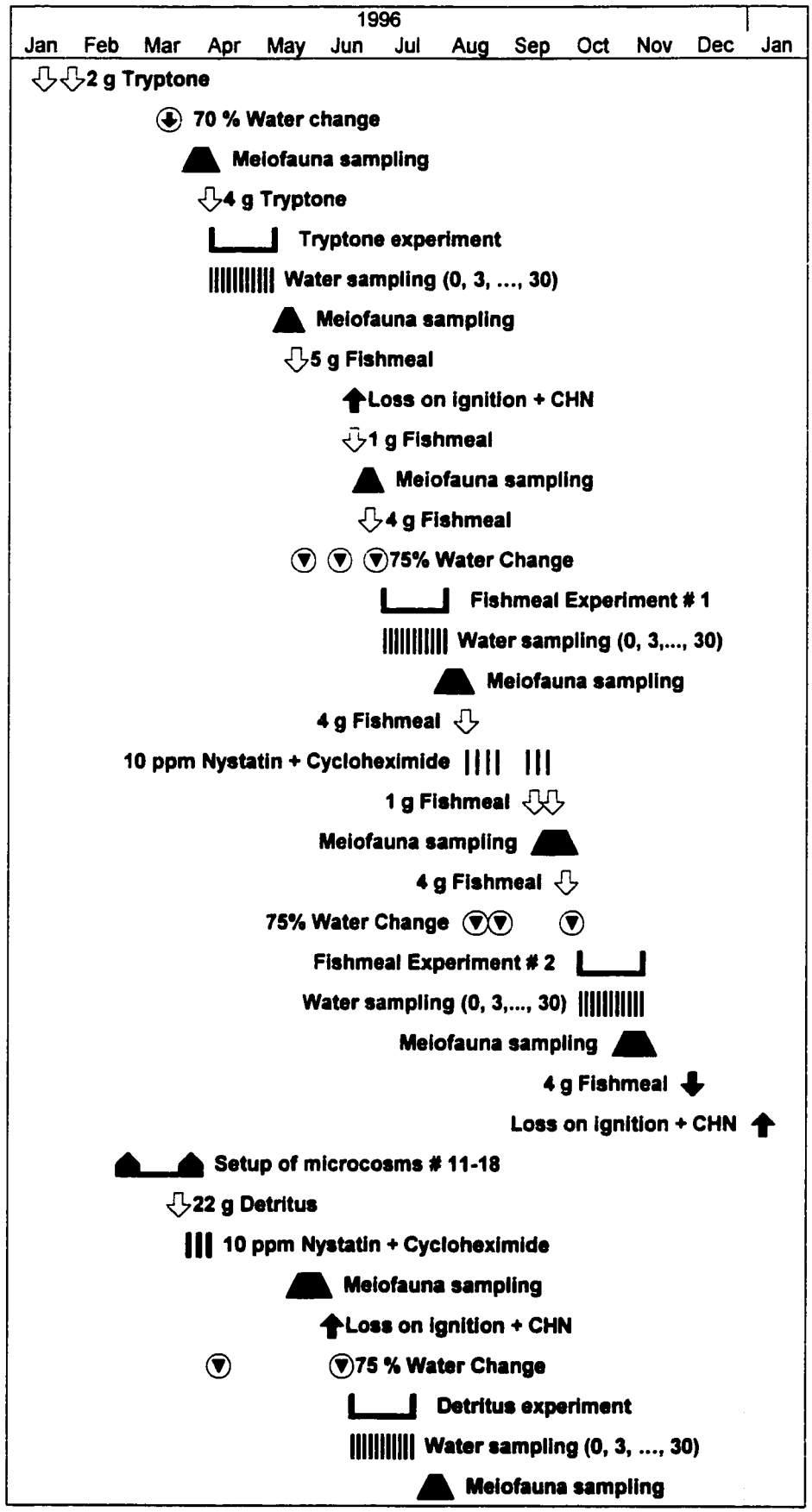

Figure 2.2 Gantt chart of the three experiments. The tryptone and the fishmeal experiments were done using microcosms #1-8 whereas the detritus experiment was done using microcosms #11-18.

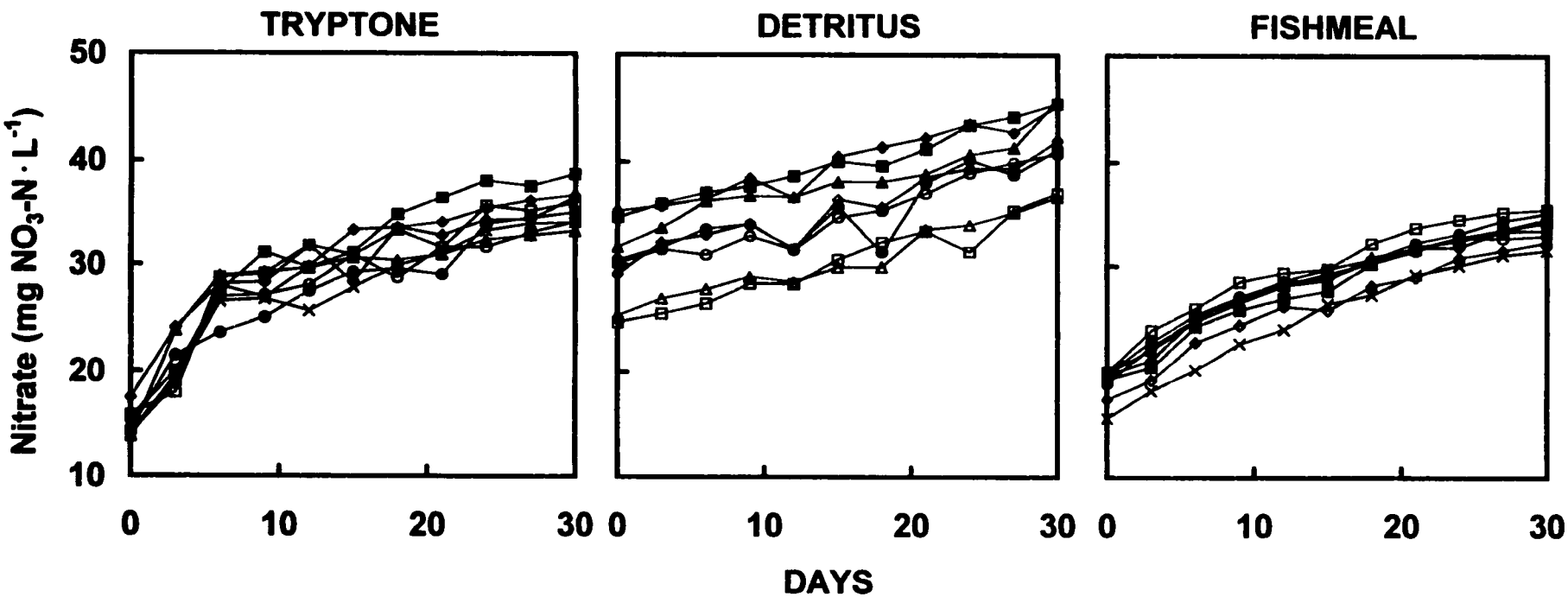

Figure 2.3 Production of nitrate during the tryptone, detritus and fishmeal experiments. Differents symbols represent each of the microcosms in the experiment.

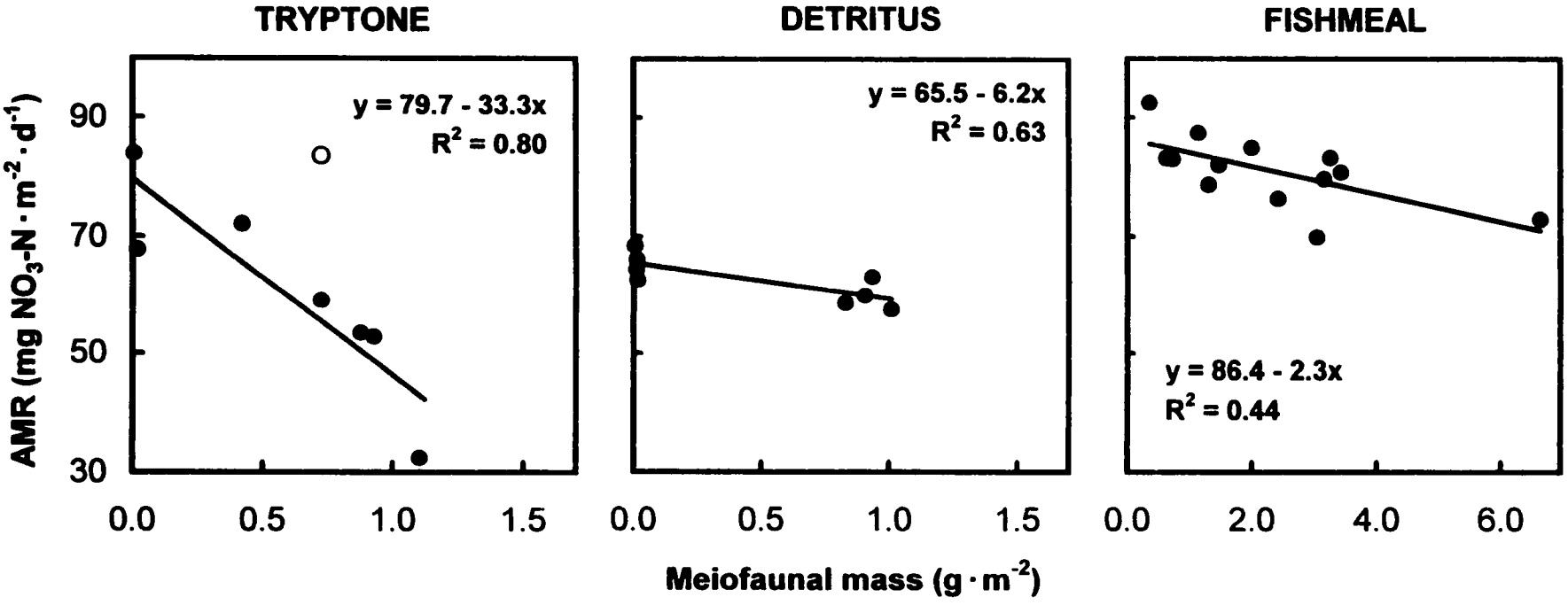

Figure 2.4 Linear regression models of Apparent Mineralization Rate (AMR) and meiofaunal biomass for the tryptone, detritus and fishmeal experiments. Open circle in tryptone represents the microcosm excluded from analyses on the basis on unusually high mortality rate.

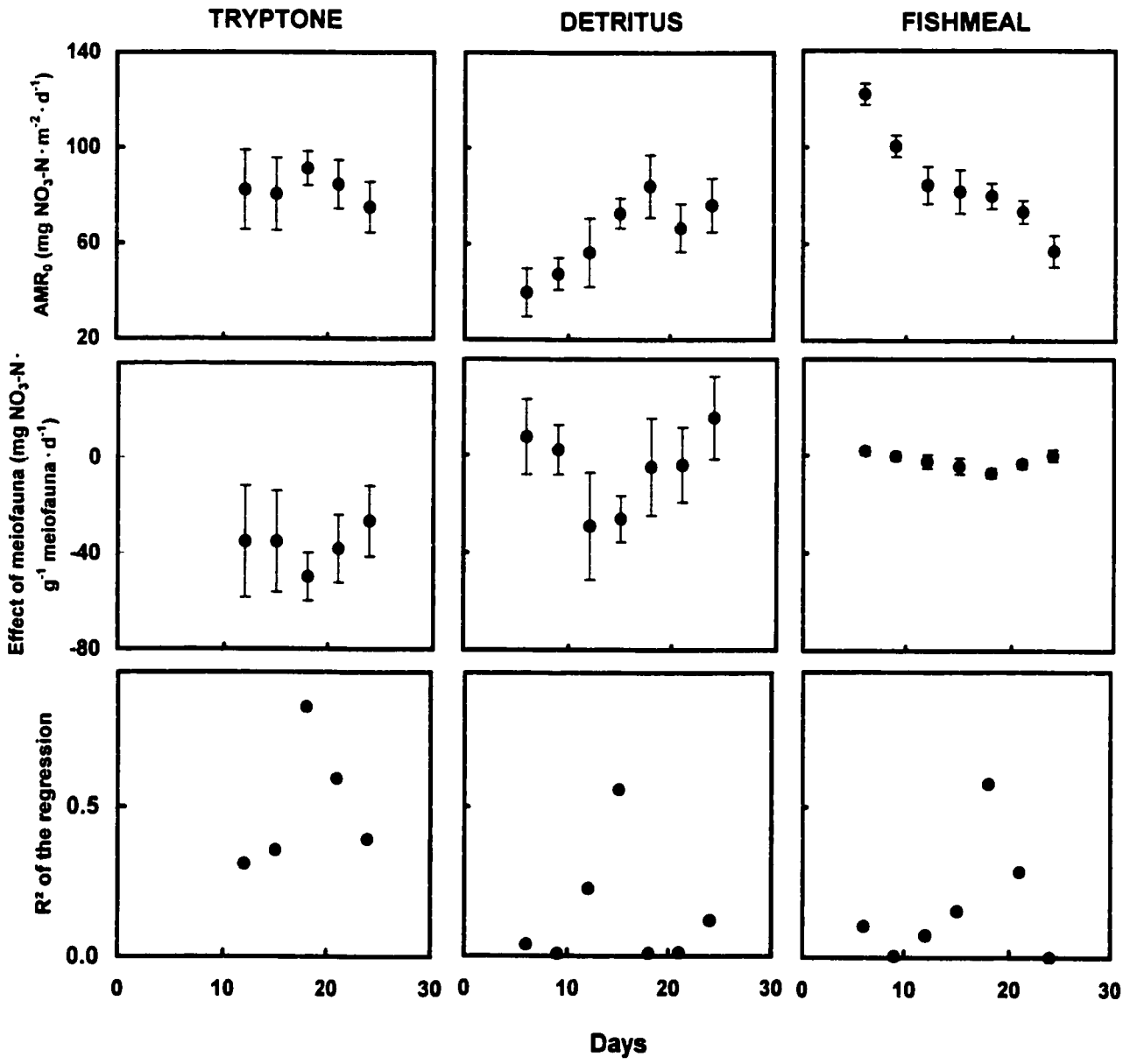

Figure 2.5 Detailed analysis of the experiments showing the AMR_0 (mean ± SE), the effect of meiofauna (mean ± SE) and the R^2 on days 6, 9, 12, ..., and 24 for each experiment. AMRt of each microcosm, i.e. AMR on days t = 6, 9, 12, ..., 24, were calculated by regressing five nitrate concentrations (t-6, t-3, t, t+3 and t+6) over time. Each set of AMRt was then regressed against meiofaunal biomass.

CHAPTER III

THE ROLE OF COPEPOD-DOMINATED MEIOFAUNA IN THE NITRIFICATION PROCESS OF A COLD MARINE MESOCOSM

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ABSTRACT

Large populations of copepod-dominated meiofauna are found in the sand filters of the St. Lawrence marine mesocosm at the Montreal Biodome. Experiments were conducted in heterotrophic microcosms to quantify how populations of micro- and meiofaunal organisms affect ammonia oxidation (nitrition) and nitrite oxidation (nitration), using Apparent Nitrition Rate (ANiR) and Apparent Nitration Rate (ANaR) as proxies. ANiR and ANaR were not related to ciliate density. Meiofauna had no effect on ANiR, but a significant relationship between the ANaR and meiofaunal biomass was observed which varied with the particulate organic nitrogen (PON) content of the sediment. The relationship was negative at low PON and positive at high PON. These results suggest a direct negative action by predation on nitrifying bacteria and an indirect positive action by reducing competitors through grazing of heterotrophic bacteria. The negative effect of 1 $g \cdot m^{-2}$ of meiofauna at low PON (–20% of $ANaR_0$, i.e. ANaR without meiofauna) is much smaller than its positive effect at high PON (+172% to 571% of $AnaR_0$). Copepod-dominated meiofaunal biomasses less than 0.16 $g \cdot m^{-2}$ increase 2 to 5 times the nitrification rate in heterotrophic habitats rich in PON.

RÉSUMÉ

Les filtres à sable du mésocosme du Saint-Laurent marin du Biodôme de Montréal renferment d'importantes populations de méiofaune dominées par les copépodes. Des expériences ont été menées dans des microcosmes hétérotrophiques pour mesurer l'effet de cette méiofaune et de la microfaune sur l'oxydation de l'ammoniaque (nitrition) et des nitrites (nitration) via le Taux Apparent de Nitrition (TANi) et le Taux Apparent de Nitration (TANa). Le TANi et le TANa n'ont pas été corrélés à l'abondance des ciliés. La méiofaune n'a pas eu d'effet sur le TANi mais il existe une relation entre la méiofaune et le TANa. Cette relation a varié selon la teneur en azote organique particulaire (APO) des sédiments: elle a été négative pour un APO faible et positive pour un APO élevé. Les résultats suggèrent une action directe négative de prédation des bactéries autotrophes conjugée à une action indirecte positive de réduction des compétiteurs par le broutage des bactéries hétérotrophes. L'effet négatif de 1 $g \cdot m^{-2}$ de méiofaune à des AOP faibles (–20% de $TANa_0$, c.-à-d. le TANa en l'absence de méiofaune) est plus faible que son effet positif à des AOP élevés (+172% à 571% de $TANa_0$). À moins de 0.16 $g \cdot m^{-2}$, une méiofaune dominée par les copépodes accroît de 2 à 5 fois le taux de nitrification dans les habitats hétérotrophiques riches en AOP.

3.1 Introduction

Nitrification, the process by which ammonia is converted into nitrate, is of utmost concern in closed systems, such as public aquaria and mesocosms (Stoskopf 1993). In these managed environments, impairment of nitrification usually results in a build-up of ammonia that threatens the health of the fish and invertebrates on display. Ammonia is produced by the digestion of the organic matter by heterotrophic bacteria. It is further oxidized to nitrite, and then into much less toxic nitrate, by chemoautotrophic bacteria (Spotte 1991). Biological filtration systems for large marine aquaria, such as the St. Lawrence mesocosm of the Montreal Biodome, depend on this bacterial activity to maintain life support.

The oxidation of ammonia into nitrite (nitrition) is mainly performed by *Nitrosomonas*, whereas *Nitrobacter*, *Nitrococcus* and *Nitrospina* (Watson *et al.* 1989; Spotte 1991) are largely responsible for the oxidation of nitrite into nitrate (nitration). Nitrifying bacteria are not attached to the substrate as individuals cells, but exist as colonies within self-secreted layers of biological films that coat sand and gravel (Spotte 1991). The nitrification capacity of an aerobic biofilm is determined by the number and activity of nitrifying bacteria in it (Lee and Welander 1994).

Bacteria are grazed by a large variety of organisms such as flagellates, ciliates, meiofauna (benthos smaller than 1 mm such as rotifers, nematodes, etc.) and macrofauna (oligochetes, snails, etc.). While many studies have shown that the mineralization rate of organic matter is affected by protozoans (Johannes 1965; Pomeroy 1970 Barsdate *et al.* 1974) and by meiofauna (Tenore *et al.* 1977; Findlay and Tenore 1982; Alkemade *et al.* 1992), the effects of protozoans and meiofauna on nitrification have received comparatively little attention (Lee and Welander 1994). Verhagen and Laanbroek (1992) found that flagellates preyed only upon free nitrifying cells and that, in presence of flagellates, the number of nitrifying bacteria declined but not the rate of nitrate production. However, the effect of grazing by protozoans on biofilms containing attached nitrifying bacteria is still unclear. Many phagotrophs graze on only a few bacterial

species (Curds 1975; Sudo and Aiba 1984). Griffiths (1989) did not find any protozoan that would graze specifically on nitrifiers, and attributed this to the large agglomerative and strongly adhesive growth form of nitrifiers that makes them more difficult to ingest than single cells.

Recent studies on freshwater trickling filters have shown that meiofauna reduces nitrification (Andersson *et al.* 1994; Parker *et al.* 1989; Lee and Welander 1994). Predators, such as rotifers, may have a strong negative impact on nitrification in aerobic biofilm processes, at least in systems not limited by oxygen or ammonium transfer. This is probably because grazing by microfauna has a greater negative effect on the slow-growing chemoautotrophs than on the fast-growing heterotrophs (Lee and Welander 1994).

The present study was carried out to test whether the meiofauna living in the sand filters of the St. Lawrence mesocosm (SLM) negatively affect nitrification. The SLM is a closed aquatic system that holds 3 x 10^6 L of cold (10°C) artificial seawater (28-30‰). It exhibits fish, invertebrates and seabirds from the Gulf of St. Lawrence. The water treatment system consists of six sand filters 3.6 m in diameter, a 200 m^3 trickling filter, and an ozonation system. All detritus ends up in the sand filters where most of the meiofauna is located (S. Parent unpublished data). Experiments were conducted in laboratory model systems (heterotrophic microcosms) containing synthetic seawater and fixed quantities of organic substrates. Protozoan and meiofaunal abundances were manipulated prior to addition of ammonia or nitrite. Known quantities of ammonia and nitrite were added and residual concentrations were measured at regular time intervals. Rates of nitrition and nitration were then estimated and correlated with protozoan numbers and meiofaunal biomasses.

3.2 Materials and Methods

3.2.1 Experimental setup

The experiments were performed in heterotrophic microcosms. These were 20 liter all-glass aquaria (778 cm^2) containing 13.5 liters of water. Each microcosm was equipped with an undergravel filter and two airlifts (filtration rate 41-82 $L \cdot m^{-2} \cdot min^{-1}$, turnover rate about 2-4 min). The bottom of each microcosm was covered with 4 cm of filter sand. A shallow sand bed and a uniform yet rapid flow of oxygen saturated water from top to bottom of the sand bed insured constant oxygen saturation in the pore water of the sediment. Seawater 28-30 ‰ was prepared using Instant Ocean® sea salts. The microcosms were maintained in a cold room at 10°C and in the dark to prevent algal growth.

3.2.2 Nitrition experiments

Two nitrition experiments were carried out by adding 1 mg NH_3-$N \cdot L^{-1}$ to selected microcosms (Table 3.1). The source of organic matter for heterotrophic bacteria, ciliates and meiofauna was tryptone or detritus. Tryptone and detritus, differing in particle size and nitrogen content, were used to generate different nitrifying activities and to observe the effect of meiofauna on different nitrition rates. Ammonia and nitrite levels were zero at the start of each experiment, and pH were similar (8.07-8.45).

Eight microcosms (denoted NH_3Try) were given tryptone for four months before the experiment. The tryptone used was Tryp-Soy broth (soybean-casein digest broth, Difco 1984). When put in seawater, tryptone quickly dissolves and becomes dissolved organic matter. It contains 8.7 % Nitrogen and 33.2 % Carbon, for a C:N ratio of 4.4 (Table 3.1). Nitrition was stimulated with two dosages of 1 mg NH_4Cl-$N \cdot L^{-1}$ 32 and 23 days prior to the experiment.

A second set of eight microcosms (denoted NH_3Det) were set up 73 days before the start of the experiment. One sand filter clogged with detritus from the SLM was opened and, for each microcosm, 5.7 kg of the top 2.5 cm of wet sand was collected using a wide aluminium pan with one end sectioned. The top 2.5 cm of sand contained 281 g dry mass·m^{-2} of detritus (Table 3.1). The detritus had a mean particle size of 400 µm and contained 2.2% nitrogen and 14.6% carbon, for a C:N ratio of 7.9 (Table 3.1). The weight of sand corresponded to 4 cm of sand in the experimental microcosms. From day –68 to day –54, meiofauna was depressed in four microcosms using three doses of 10 ppm nystatin and 10 ppm cycloheximide. These two substances are inhibitory to eukaryotic organisms (Lee and Welander 1994). Nitrition was stimulated with one dose of 1 mg NH_3-N·L^{-1} 6 days prior to the experiment.

In all experiments, 27 ml of NH_4Cl 0.0355 M (≅ 1 mg NH_4Cl-N·L^{-1}) were added to each microcosm at t = 0, and residual ammonia concentrations were measured at regular time intervals. It is considered that no significant ammonia degassing occurred during the experiments since only 1.5 to 4.0% of total NH_4-N in seawater was in the form of ammonia (Spotte 1991).

3.2.3 Nitration experiments

Four nitration experiments were carried out by adding 1 mg NO_2-N·L^{-1} in selected microcosms. The source of organic matter for heterotrophic bacteria, ciliates and meiofauna was tryptone in the first experiment (NO_2Try) and fishmeal in the other three (NO_2Fis 2 to 4). Source of organic matter and stimulation of nitrification were varied among experiments to generate different nitrifying activities and to observe the effect of meiofauna on different nitration rates. Ciliates were controlled with doses of 10 ppm nystatin and 10 ppm cycloheximide given about one month before each experiment. Ammonia and nitrite levels were below detection levels at the start of each experiment, and pH were similar (8.13-8.35).

NO_2Try was done using eight microcosms, and nitrification was stimulated with two doses of 1 mg NH_3-N·L^{-1}, 12 and 3 days before the experiment (Table 3.1). NO_2Fis2, 3 and 4 were conducted using 7, 10 and 16 microcosms respectively. All microcosms were given tryptone or fishmeal for at least 4 months. The fishmeal used was redfish (*Sebastes marinus*), produced locally, with a particle size 100-150 µm. It contained 11.5 % nitrogen and 45.7 % carbon (Table 3.1). Nitrification was not stimulated in NO_2Fis2, but it was in NO_2Fis3 and NO_2Fis4 with weekly doses of 0.5 or 1.0 mg NO_2-N·L^{-1}.

Meiofauna was not controlled in the tryptone experiment whereas it was enriched in one or more of the microcosms two weeks before each fishmeal experiment (Table 3.1). Meiofaunal abundances were manipulated through additions of meiofauna from the sand filters. Meiofauna from the mesocosm sand filters was collected at the time of backwash using a Wisconsin style plankton net with a 80 µm mesh. Small floating 300 µm sieves covered with ice were then used to extract meiofauna from detritus.

In all experiments, 10 ml of $NaNO_2$ 0.1 M (≅ 1 mg $NaNO_2$-N·L^{-1}) were added to each microcosm at t = 0, and residual nitrite concentrations were measured at regular time intervals.

3.2.4 Biological determinations

Organisms could not be sampled during the experiments without disturbing the microcosms, so microfauna and meiofauna were measured no more than 10 days prior to or after each experiment.

Since meiofauna are patchy in their distribution (Findlay 1981), the sand was raked before each sampling so as to obtain a better estimate of the meiofaunal abundance with the least effort. Usually five samples – but sometimes up to eight – of the 0-2.5 cm layer were randomly taken using a 10 ml syringe (1.63 cm^2, 4 cm^3 of sand). Meiofauna and microfauna were separated in a

50 ml cylinder using the decantation method described by Pfannkuche and Thiel (1988) and then collected on a 20 μm sieve. By closely examining 1 cm^3 of residue of randomly chosen samples, decantation was estimated to be at least 95% effective in extracting meiofauna. All animals were counted alive. A different set of samples was taken to assess the standard weight of each taxon: length and width of specimens were taken and then converted into weights using conversion factors provided by Feller and Warwick (1988). These standard weights were then combined with the abundance results to establish the meiofaunal biomass in each sample. The sand of all samples, but not the meiofauna, was put back in the microcosms. Less than 1 % of the meiofauna was so removed before or after each experiment.

Three groups of protozoans were observed: ciliates, foraminiferans and heliozoans. Only ciliates were quantified. Ciliate populations were found by Fenchel (1967, 1978) to outnumber all other fauna in well-mixed medium (100-300 μm) sands with little interstitial silt and clay, as is the case here. They were therefore used as an index of protozoan abundance.

3.2.5 Chemical analysis

Total ammoniacal nitrogen (TAN) was measured using the ammonia-selective electrode method and nitrite were measured using the colorimetric method (APHA *et al.* 1992). Detection levels of these methods are 0.03 mg NH_3-N·L^{-1} and 0.02 mg NO_2-N·L^{-1}. The levels of TAN or nitrite of each microcosm were then plotted against time. Levels of TAN higher than 0.2 mg NH_3-N·L^{-1} and levels of nitrite higher than 0.1 mg NO_2-N·L^{-1} were found to decrease linearly with time. Nitrition and nitration rates were estimated by regressing those levels against time. Total carbon and nitrogen content of organic matter were analyzed on a Carlo-Erba CHN Analyser. Organic matter content was determined by loss on ignition at 400-450°C.

Experiments in which measurements of residual concentrations in the water are used to estimate process rates generate "apparent" reaction rates because the rates so obtained are a

composite of all reactions rates occurring within the microcosm (Parker *et al.* 1989), including nitrification by chemoautotrophic bacteria, assimilation by heterotrophic bacteria, and mineralization of organic matter (Verhagen and Laanbroek 1992). We therefore used the expressions Apparent Nitrition Rate (ANiR) and Apparent Nitration Rate (ANaR) (see Parker *et al.* 1989, Andersson *et al.* 1994).

3.2.6 Statistical analysis

The relationships between ANiR or ANaR, and microfaunal abundance ($N \cdot 10\ cm^{-2}$) or meiofaunal biomass ($g \cdot m^{-2}$) were quantified by linear regression analysis. For each microcosm, the biomass was set as the arithmetic mean of the readings (generally 5 readings each time, but sometimes up to 8). Regression analyses between ANR and its standard error showed ANiR and ANaR to be homoscedastic.

3.3 Results

3.3.1 Meiofauna composition and abundance

3.3.1.1 Microcosms

The meiofauna was composed of seven taxa: harpacticoid copepods, nematodes, rotifers, polychaetes, turbellarians, mites (Acari) and gastrotrichs. The harpacticoid copepods dominated both in number (87%) and biomass (98%) with three species present: *Pseudonychocamptus proximus*, *Tisbe* sp. (*furcata*?) and *Paramphiascella* sp. (*fulvofasciata*?). *Pseudonychocamptus* largely dominated the group with over 90% of the specimens. Nematodes and rotifers accounted for only 4.6% (8%) of the specimens and 0.8% (0.5%) of the biomass respectively, while other taxa accounted for less than 1% of the specimens and the biomass. Meiofaunal biomass varied from 0 to 1.84 $g \cdot m^{-2}$ in the nitrition experiments and from 0 to 2.31 $g \cdot m^{-2}$ in the nitration experiments.

3.3.1.2 Sand filters

The composition of the sand filter meiofauna is the same as in the microcosms, except for a few hydrozoan polyps which were absent in the microcosms. Meiofaunal biomass in the 0-2.5 cm layer of the sand filters varied from 20 to 40 $g \cdot m^{-2}$ during the experiments. Recent sampling (July 1997) has revealed that meiofauna is quite abundant throughout the sand column with values close to 1 $g \cdot m^{-2}$, in core increments of 2.5 cm (or g per 25 dm^3), at least down to 25 cm (S. Parent unpublished data). Meiofaunal biomasses in the microcosms were therefore much less than what was found in the sand filters.

3.3.2 Amount and C:N ratio of the organic matter in the substrate

For NH_3Try and NO_2Try, no sampling was done at the time of the experiment for the organic matter content of the substrate and its C:N ratio. Organic matter content was measured 3 months later. Based on the additions of tryptone 35 days before and the mineralization rates of tryptone (Parent and Morin 1999), the organic content of the substrate was estimated to be between 112 and 120 $g \cdot m^{-2}$ and the C:N ratio to be between 4.4 and 12 during the experiment (Table 3.1).

For the NH_3Det experiment, organic matter content was measured three days before the experiment and the C:N ratio one week after. The organic matter content was 281 $g \cdot m^{-2}$ (SE = 18.2, n = 8) and the C:N ratio 7.9 (SE = 0.25, n = 8) (Table 3.1). For the three fishmeal nitration experiments, both parameters were measured before NO_2Fis2, NO_2Fis3 and NO_2Fis4. The organic matter content was 265 $g \cdot m^{-2}$ (SE = 19.9, n = 8) for NO_2Fis2 and NO_2Fis3 and 204 g m^{-2} (SE = 34.5, n = 16) for NO_2Fis4. The C:N ratio was 8.2 (SE = 0.40, n = 8) for NO_2Fis2 and NO_2Fis3 and 7.6 (SE = 0.52, n = 16) for NO_2Fis4 (Table 3.1).

3.3.3 Apparent Nitrition Rate (ANiR)

Ammonia concentrations declined linearly between 1.0 ppm and 0.20 ppm in the NH_3Det microcosms (Fig. 3.1a) and in the NH_3Try microcosms. Only those results were used to calculate the ANiR of each microcosm through linear regression. Standard error of regression slopes was 4% to 18% of the slope. It took 8 to 21 h to reduce the ammonia level from 1.0 to 0.20 ppm in the tryptone experiment and ANiR varied from 7 to 22 $mg \cdot m^{-2} \cdot h^{-1}$ (Fig. 3.2). In the detritus experiment, it took 3 to 5 h to reduce the ammonia level from 1 to 0.2 ppm (Fig. 3.1a) and ANiR varied from 58 to 90 $mg \cdot m^{-2} \cdot h^{-1}$ (Fig. 3.2).

3.3.4 Apparent Nitration Rate (ANaR)

Nitrite concentrations declined linearly between 1.0 and 0.10 ppm in the NO_2Try microcosms (Fig. 3.1b) and in the microcosms of the three fishmeal experiments. Only those results were used to calculate the ANaR of each microcosm through linear regression. Standard error of regression slopes was 0.5% to 6% of the slope. It took 12 to 24 h to reduce nitrite from 1.0 to 0.10 ppm in the tryptone experiment and ANaR varied from 7.0 to 13.2 $mg \cdot m^{-2} \cdot h^{-1}$(Fig. 3.3). In the three fishmeal experiments, nitrite was reduced from 1.0 to 0.1 ppm over a period of 4-13 h, and ANaR varied from 10.1 to 33.2 $mg \cdot m^{-2} \cdot h^{-1}$ (Fig. 3.3).

3.3.5 Effect of ciliate density and meiofaunal density on ANR

The ANiR was not correlated with ciliate density nor with meiofaunal biomass (Table 3.2). In all four nitration experiments, ANaR was not correlated with ciliate density. No significant relationship was found between ANaR and meiofaunal biomass in NO_2Fis4, but a significant relation was found in NO_2Try and in NO_2Fis2 and NO_2Fis3. Correlation tests were also done for subsets of NO_2Fis3 and NO_2Fis4 (these subsets are described below): no relationship was found between ciliate density and ANaR (Table 3.2).

3.3.6 Linear regression models

No correlation was found between ANiR and meiofaunal biomass but the relationship between ANaR and meiofaunal biomass varied: it was significantly negative in the NO_2Try experiment and significantly positive in NO_2Fis2 and NO_2Fis3 (Table 3.3). Meiofaunal biomass explained between 41% and 85% of the variation in ANaR in these nitration experiments (Fig. 3.3). The models show that 1 $g \cdot m^{-2}$ of meiofauna reduced the $ANaR_0$, i.e. the ANaR when there is no meiofauna, by 20% in the tryptone experiment, but increased it by 456% in NO_2Fis2 and by 64% in NO_2Fis3. The apparent effect was nil in NO_2Fis4.

The effect of biomass was seven times stronger in NO_2Fis2 than in NO_2Fis3 in similar conditions (Table 3.1), but over a different range of meiofaunal biomasses (Fig. 3.3). Data from NO_2Fis3 were reanalyzed separately for biomasses similar to those of NO_2Fis2 ($\leq$ 0.16 g·m^{-2}, n = 4) and larger than 0.16 g m^{-2} (n = 6). The regression for the subset of biomasses similar to those of NO_2Fis2 was significant and showed an increase in $ANaR_0$ (571%) similar to the one observed in NO_2Fis2 (456%, Table 3.3). Similarly, NO_2Fis4 data were also subdivided into two subsets, but with different biomasses ranges to account for a poorer organic substrate (Tables 3.1 and 3.3). The subset for biomasses less than 0.12 g·m^{-2} was significant and also showed a strong increase in $ANaR_0$ (970%).

To better understand what controls the variation in ANaR, all results from the four nitration experiments (n = 41) were pooled together and ANaR was regressed against meiofaunal mass, ciliates, organic matter content of the sediment (ORG), nitrate, percent of nitrogen (%N) and C:N ratio of organic matter. ORG and %N were chosen because heterotrophic bacterial activity is related to the amount of organic matter in the sediment and to its nitrogen content (DeAngelis 1992). The square root of ANaR and of meiofaunal biomass were used for the regressions so as to linearize the relationship, to stabilize the variance of the residuals, and to reduce the influence of the few most extreme observations (Table 3.4). ANaR was not correlated with ciliates (R^2 = 0.07) nor with C:N ratio (R^2 = 0.07), but it was correlated with nitrate (R^2 = 0.22), ORG (R^2 = 0.53) and %N (R^2 = 0.57), all of which are multicolinear (0.71 < r < 0.90). ANaR was most strongly correlated with the %N*ORG, i.e. with particulate organic nitrogen (PON) and the meiofaunal biomass (Table 3.4). Moreover, the effect of meiofauna varied significantly with PON. Figure 3.4 shows the plot of the measured data versus curves predicted by the multiple regression model at PON levels equal to 0.65, 3.25 and 6.5 g·m^{-2}. These levels correspond to the PON levels in NO_2Try, NO_2Fis2+NO_2Fis3 and NO_2Fis4 respectively (Table 3.1).

3.4 Discussion

3.4.1 Meiofauna composition and abundance

The meiofauna in the heterotrophic microcosms and sand filters is very different from the meiofauna occurring in natural marine sands or freshwater trickling filters. It is copepod-dominated whereas marine sand meiofauna is nematode-dominated (Higgins and Thiel 1988) and freshwater trickling filter meiofauna is fly larvae- and rotifer-dominated (Andersson *et al.* 1994; Lee and Welander 1994).

The meiofauna of both the microcosms and sand filters is mainly composed of prehensile epibenthic species, such as the harpacticoids *Pseudonychocamptus*, or strongly adhesive species, such as the nematode *Anticoma*. There are only a few rare mesopsammic species (e.g. *Paramphisciella*). The fact that the sand filters are regularly backwashed may prevent the installation of mesopsammic species. Also, the fact that water is forced through the sand in both the microcosms and the sand filters could favor epibenthic species at the expense of mesopsammic ones. Hence the observed paucity of nematodes and rotifers which are known to reduce nitrification in freshwater trickling filters (Lee and Welander 1994).

Most of the meiofaunal species found here generally eat diatoms, microalgae or bacteria. *Anticoma*, for example, is a known selective deposit feeder (Wieser 1959). *Pseudonychocamptus* feeds not only on microalgae and diatoms (Rudnick 1989), but also on bacterial exopolymer (Decho and Moriarty 1990). *Tisbe furcata* has a clear preference for bacteria (Vanden Berghe and Bergmans 1981), and *Paramphiascella* for specific strains of gram-negative bacteria (Rieper 1982). In our experiments, most meiofaunal species had to feed on bacteria since the microcosms were kept in the dark and were devoid of algae.

3.4.2 Conditions of the nitrification experiments

Nitrate levels were higher in the tryptone nitration experiment than in the three fishmeal experiments (Table 3.1). The effect of this difference is unknown, but Spotte (1991) indicates that nitration is not affected by nitrate. He states that nitrification is normal in seawater with 200 mg NO_3-N·L^{-1} or more.

The tryptone experiment also differed from the three fishmeal experiments in the type and amount of organic matter, suggesting that the biomass of nitrate bacteria was either lower or less active than in the fishmeal experiments. Tryptone is dissolved organic matter whereas fishmeal is particulate organic matter. The organic matter content of the sand in the tryptone experiment was only 50% of that found in the fishmeal experiments (Table 3.1). Although nitration in the tryptone experiment was stimulated before NO_2Try, nitration in the absence of meiofauna (as indicated by the intercept of the regressions) was slower than in the three fishmeal experiments (Fig. 3.2).

The correlation tests between ciliates and ANR showed that protozoan densities could not account for the observed variability in ANiR and ANaR in our nitrification experiments. Protozoans, especially ciliates, are known to affect nitrification (Johannes 1965; Pomeroy 1970; Barsdate *et al.* 1974), but either they equally affected all microcosms, or their effect was too small compared to that of meiofauna in the microcosms.

3.4.3 Regression models of the nitration experiments

The results of the nitration experiments showed that the variability in ANaR is best explained by the meiofaunal biomass and the PON content of the sediment. There is a relationship between the ANaR and the meiofaunal biomass, but it can be masked under certain conditions. Depending upon the PON content of the sediment, this relationship is either negative or positive. At PON

loads larger than 3.25 $g \cdot m^{-2}$, meiofaunal masses less than 1.0 $g \cdot m^{-2}$ can increase nitration by up to 5 fold.

A simple way to explain the variability of the effect of meiofauna on ANaR is to say, as for mineralizing bacteria (Yingst and Rhoads 1980; Meyer-Reil and Faubel 1980), that low meiofaunal masses stimulate nitrate bacteria by removing senescent cells and by keeping colonies in exponential growth, whereas high masses reduce their number through grazing. The *y*-intercepts of the regression models in Table 3.3 give an indication of the nitrate bacteria abundance. When autotrophic density is low as in NO_2Try, meiofauna would have a negative effect, and when autotrophic density is high as in NO_2Fis2 and NO_2Fis3, meiofauna would have a positive effect. Also, this would explain why, within a given experiment such as NO_2Fis3, the relationship between meiofauna and ANaR varies with meiofaunal mass. This cannot be the whole explanation, however. First, autotrophic metabolism is at least 24 times slower than heterotrophic metabolism (Lee and Welander 1994). Second, heterotrophic bacteria are 20 to 100 times more abundant than nitrifying bacteria in the filter bed of a seawater aquarium (Yoshida 1967). And third, heterotrophic bacteria are more palatable and easier to graze than nitrifiers (Griffiths 1989). We thus believe that the simple explanation for the relationship between meiofauna and nitrating bacteria given above is not complete. We agree with Lee and Welander (1994) that, in fact, the relationship between meiofauna and ANaR is far more complex than it seems.

The meiofauna in our experiments was mainly composed of harpacticoid copepods (98% biomass). We know from our mineralization experiments in the same heterotrophic microcosms that this meiofauna feeds heavily on mineralizing bacteria: 1 $g \cdot m^{-2}$ of meiofauna may reduce mineralization by up to 42% (Parent and Morin 1999). Perlmutter and Meyer (1991) have shown that stream-dwelling harpacticoid copepods reduce the biomass of detritally associated bacteria (i.e. heterotrophs) by as much as 45%, We also know that harpacticoid copepods are selective feeders. They are specialists with respect to nutritional requirements, nutritional preferences, and

ability to utilize specific food species. Rieper (1982) has shown that four common harpacticoid genus do prefer gram-negative bacteria and that each genus has its own food preference: *Vibrio* sp. for *Tisbe*, *Flavobacterium* sp. for *Paramphiascella*, etc. Vanden Berghe and Bergmans (1981) found that *Tisbe furcata* had a clear preference for rod-shaped bacteria. More recently, Perlmutter and Meyer (1991) have shown that the stream-dwelling *Attheyella* selectively removed larger rod-shaped bacteria. Unfortunately, nitrifying bacteria were not included in these food selectiveness experiments nor were they studied in the field. We suspect that harpacticoid copepods have no clear preferences for nitrifiers because these grow in large agglomerates (also known as zoogleas) which are more difficult to ingest than single cells. Moreover, nitrifiers are not surrounded by a mixture of enzymes and labile organic detritus that make mineralizing bacteria so palatable to harpacticoids such as *Pseudonychocamptus* (Decho and Moriarty 1990).

Moreover, heterotrophic and autotrophic bacteria compete for space (Spotte 1991) and oxygen (Andersson *et al.* 1994), as well as for ammonium: for limiting amounts of ammonium, the numbers of nitrifying bacteria decreased with increasing glucose concentrations and the numbers of heterotrophic bacteria rose (Verhagen and Laanbroek 1992). Since harpacticoids are known to eat an important fraction of the mineralizing bacterial biomass and since these are known to compete strongly with autotrophs, meiofauna may have indirectly influenced the ANaR of our experiments by removing heterotrophic bacteria. Meiofauna may therefore exert two opposite effects: a direct negative action by predation on nitrifiers and an indirect positive action by reducing competitors as they grazed on heterotrophic bacteria.

Because bacterial masses were not measured, we do not know which effect was more important. However, we suspect that the reduction of competitors may be the main factor by which meiofauna had an effect on ANaR in the nitration experiments, due to the observed asymmetry in the effect of meiofauna (–20% per gram of meiofauna in the tryptone experiment versus +172% to 571% in the fishmeal experiments) and to the absence in our meiofauna of most of the known grazers of nitrifiers, such as filter fly larvae (Psychodidae spp.), snails (*Physa* spp.),

worms (Naididae spp.), rotifers (*Philodina* spp.) and nematodes (*Diplogaster* spp.) (Boller and Gujer 1986; Parker *et al.* 1989; Andersson *et al.* 1994; Lee and Welander 1994). All of these groups live in freshwater and were found in trickling filters consuming the biofilm on the trickling media. A second reason is that species of rotifers and nematodes present in our meiofauna, had very low densities. Moreover, these species apparently do not feed on nitrifiers (Lee and Welander 1994). We found no mention of harpacticoid copepods selectively feeding on nitrifiers.

We therefore suggest the following explanation for the variability of meiofaunal effect on nitrate bacteria with the PON content of the sediment, assuming that the *y*-intercepts of the regression models (Table 3.3) and the PON levels (Table 3.1) are good indications of autotroph and heterotroph abundances respectively. When organic matter is low, as in NO_2Try, heterotrophs are not abundant. Even low densities of harpacticoids need to feed not only on heterotrophs but also on autotrophs to sustain themselves, resulting in a negative effect of meiofauna on nitration. Conversely, when organic matter is abundant, as in NO_2Fis2 and NO_2Fis3, heterotrophs are also abundant. Low densities of harpacticoids need to feed only on heterotrophs to sustain themselves, resulting in an increase in the number of autotrophs and in a strong positive effect of meiofauna on nitration. High densities of harpacticoids will also have to feed on autotrophs thus reducing the abundance of autotrophs as well as its positive effect on ANaR.

NO_2Fis4 is found in between these two situations. At low biomass, meiofauna probably fed only on heterotrophs whereas at high biomass meiofauna ate some autotrophs as well. There was a positive effect of the meiofauna on ANaR which occurred at lower biomasses than for NO_2Fis2 and NO_2Fis3 because of a lower PON content (Table 3.3 and Fig. 3.4). It was probably followed by a negative effect of meiofauna which could not be observed directly, but which resulted in an apparent non effect of meiofauna on nitration.

The positive effect of meiofauna at medium and high PON levels in marine microcosms contradicts the findings in freshwater trickling filters where freshwater organisms always decreased or reduced to nil the ANaR of the system (Boller and Gujer 1986; Parker *et al.* 1989; Andersson *et al.* 1994; Lee and Welander 1994). Our findings suggest that the effect of meiofauna on nitration varies with the composition of the meiofauna which is determined, among other things, by water type and substrate type.

3.4.4 Nitrition experiments

The nitrition experiments exhibited a wide range of $ANiR_0$: from 7 to 90 $mg \cdot m^{-2} \cdot h^{-1}$ (Fig. 3.2). Nowhere within that range has meiofauna had any effect on ANiR with biomasses of up to 1.84 $g \cdot m^{-2}$. Contrarily to nitration, nitrition is a situation where heterotrophs strongly compete with nitrifiers for the ammonium (Verhagen and Laanbroek 1992). If meiofauna was feeding only on heterotrophs, we would probably have observed a positive effect of meiofauna on nitrition. Conversely, if meiofauna was feeding only on nitrifiers, we would probably have observed a negative effect of meiofauna on nitrition. We therefore hypothetize that, as for the nitration experiments, meiofauna exerts a dual action on nitrition.

When at low masses, meiofauna would graze only on heterotrophs, the decrease in ammonium intake by heterotrophs would be counterbalanced by an increase in ammonium intake by nitrifiers, through either an increase in biomass or in metabolic activity. There would be an increase in nitrition but it would be masked by a decrease in assimilation by heterotrophs, resulting in no effect of meiofauna on ANiR as observed here.

At high masses, meiofauna would graze on both heterotrophs and nitrifiers, and we would expect a negative effect to show up. Our meiofaunal masses were probably not large enough (less than 2.0 $g \cdot m^{-2}$) to show a significant negative effect as in the nitration experiments. The intake of ammonium by heterotrophs probably masks this negative effect over a wide range of meiofaunal

masses. It would therefore appear that, as for nitration, the results are best explained by a dual action of meiofauna on bacteria.

Copepod-dominated meiofauna therefore has no apparent effect on nitrition at biomass levels less than 1.8 $g \cdot m^{-2}$, but, through nitration, it has a variable effect on the nitrification rate in heterotrophic marine habitats. At low PON levels, meiofaunal biomasses decrease nitrification and, at high PON levels, biomasses less than 0.16 $g \cdot m^{-2}$ increase its rate 2 to 5 times. These results suggest that copepod-dominated meiofauna exert a direct negative action by predation on nitrifiers and an indirect positive action by reducing competitors as they grazed on heterotrophic bacteria.

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REFERENCES

Alkemade, R., Wielemaker, A., de Jong, S.A., and Sandee, A.J.J. 1992. Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella dievengatensis* in stimulating the mineralization of *Spartina angli*. Mar. Ecol. Prog. Ser. **90**:149-155.

Andersson, B., Aspegren, H., Parker, D.S., and Lutz, M.P. 1994. High rate nitrifying trickling filters. Wat. Sci. Tech. **29** (10-11): 47-52.

APHA, AWWA, and WEF. 1992. Standard Methods for the Examination of Water and Wastewater. 18th ed., American Public Health Association, Washington, D.C., p. 4-91.

Barsdate, R.J., Fenchel, T., and Prentki, R.T. 1974. Phosphorus cycle of model ecosystems: Significance for decomposer food chains and effects of bacterial grazers. Oikos **25**: 239-251.

Boller, M., and Gujer, W. 1986. Nitrification in tertiary trickling filters followed by deep-bed filters. Wat. Res. **20**(11): 1363-1373.

Curds, C.R. 1975. Protozoa. The organisms and their ecology. *In* Ecological Aspects of Used Water Treatment. Vol.1. Edited by C.R. Curds and H.A. Hawkes. Academic Press, London pp. 203-268.

DeAngelis, D.L., 1992. Dynamics of nutrient cycling and food webs. Chapman & Hall, London. 270 p.

Decho, A.W., and Moriarty, D.J.W. 1990. Bacterial exopolymer utilization by a harpacticoid copepod: a methodology and results. Limnol. Oceanogr. **35**(5):1039-1049.

Difco, 1984. Difco Manual. Dehydrated Culture Media and Reagents for Microbiology. 10th ed., Difco Laboratories, Detroit, Michigan, p. 1027.

Feller, R.J., and Warwick, R.M. 1988. 13. Energetics. *In* Introduction to the Study of Meiofauna. Edited by R.P. Higgins and H. Thiel. Smithsonian Institution Press, Washington, D.C. pp. 181-196.

Fenchel, T. 1967. The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with Metazoans in various types of sediments. Ophelia **4**: 121-137.

Fenchel, T. 1978. The ecology of micro- and meiobenthos. Ann. Rev. Ecol. Syst. **9**: 99-121.

Findlay, S.E.G. 1981. Small scale spatial distribution of meiofauna on a mud- and sandflat. Estuarine and Coastal Shelf Science **7**: 255-268.

Findlay, S., and Tenore, K.R. 1982. Effect of a free-living marine nematode (*Diplolaimella chitwoodi*) on detrital carbon mineralization. Mar. Ecol. Prog. Ser. **8**:161-166.

Griffiths, B.S. 1989. The effect of protozoan grazing on nitrification – implications from the application of organic wastes applied to soils. *In* Nitrogen in Organic Wastes Applied to Soils. Edited by J.A.A. Hansen, and K. Henriksen. Academic Press, London pp. 37-46.

Higgins, R.P. and H. Thiel. 1988. Introduction to the Study of Meiofauna. Smithsonian Institution Press, Washington D.C., 488 pages.

Johannes, R.E. 1965. Influence of marine protozoa on nutrient regeneration. Limnol. Oceanogr. **10**: 434-442.

Lee, N.M. and Welander, T. 1994. Influence of predators on nitrification in aerobic biofilm processes. Wat. Sci. Tech. **29**(7):355-363.

Meyer-Reil, L.-A., and Faubel, A. 1980. Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. Mar. Ecol. Prog. Ser. **3**: 251-256.

Parent, S., and Morin, A. 1999. The role of copepod-dominated meiofauna in the mineralization of organic matter in a cold marine mesocosm. Can. J. Fish. Aq. Sci. **56**: In press.

Parker, D., Lutz, M., Dahl, R., and Bernkopf, S. 1989. Enhancing reaction rates in nitrifying trickling filters through biofilm control. J. Water Pollut. Control Fed. **61**(5):618-631.

Perlmutter, D.G., and Meyer, J.L. 1991. The impact of a stream-dwelling harpacticoid copepod upon detritally associated bacteria. Ecology **72**(6):2170-2180.

Pfannkuche, O., and Thiel, H. 1988. 9. Sample processing. *In* Introduction to the study of meiofauna. Edited by R.P. Higgins and H. Thiel. Smithsonian Institution Press, Washington, D.C. pp. 134-145.

Pomeroy, L.R. 1970. The strategy of mineral cycling. Annual Review of Ecological Systems **1**: 171-190.

Rieper, M. 1982. Feeding preferences of marine harpacticoid copepods for various species of bacteria. Mar. Ecol. Prog. Ser. **7**: 303-307.

Rudnick, D.T. 1989. Time lags between the deposition and meiobenthic assimilation of phytodetritus. Mar. Ecol. Prog. Ser. **50**: 231-240.

Spotte, S. 1991. Captive Seawater Fishes. Science and Technology. John Wiley & Sons, Toronto 942 pages.

Stoskopf, M.K. 1993. Fish Medecine. W.B. Saunders, Montreal, 882 pages.

Sudo, R., and Aiba, S. 1984. Role and function of protozoa in the biological treatment of polluted waters. *In* Adv. Biochem. Eng./Biotechnol. Edited by A. Fiechter. Springer Verlag, Berlin pp. 117-141.

Tenore, K.R., Tietjen, J.H., and Lee, J.J. 1977. Effect of meiofauna on incorporation of aged Eelgrass, *Zostera mari*na, detritus by the polychaete *Nephthys incisa*. J. Fish. Res. Board Can. **34**:563-567.

Vanden Berghe, W., and Bergmans, M. 1981. Differential food preferences in three co-occurring species of *Tisbe* (Copepoda, Harpacticoida). Mar. Ecol. Prog. Ser. **4**: 213-219.

Verhagen, F.J.M., and Laanboek, H.J. 1992. Effects of grazing by flagellates on competition between nitrifying and heterotrophic bacteria in chemostats. Appl. Environ. Microbiol. **58**: 1962-1969.

Watson, S.W., Bock, E., Harms, H., Koops, H.-P., and Hooper, A.B. 1989. *In* Bergey's Manual of systematic microbiology. Volume 3: Nitrifying bacteria. Edited by J.G. Holt. Williams & Wilkins, Baltimore pp. 1808-1834.

Wieser, W. 1959. Free-living marine nematodes - IV. General Part. Lunds Universitets Arsskrift, Ny Foljd Avdelning 2, 55: 1-111.

Yingst, J.Y., and Rhoads, D.C. 1980. The role of bioturbation in the enhancement of bacterial growth in marine sediments. *In* Marine Benthic Dynamics. Edited by K.R. Tenore and B.C. Coull. University of South Carolina Press, Columbia, SC, pp. 407-421. (Belle W. Baruch Libr. Mar. Sci.; v. 11).

Yoshida, Y. 1967. Studies on the marine nitrifying bacteria, with special reference to characteristics and nitrite formation of marine nitrite formers. Bull. Misaki Mar. Biol. Inst., Kyoto Univ. (11): 1-58.

Table 3.1 Summary of the nitrification experiments and organic matter content of the mesocosm sand filters.

Nitrition experiments	**NH_3TRY**	**NH_3DET**		**Sand Filter**
Number of microcosms	8	8		
Organic substrate	Tryptone	Detritus		
Stimulation of nitrition	Yes	Yes		
Impoverishment in meiofauna	No	Yes		
Organic content ($g \cdot m^{-2}$)	112 - 120	281		231 – 332
C:N ratio	4.4 – 12	7.9		6.3
% N	0.38 - 0.62	2.15		2.00
PON content ($g \cdot m^{-2}$)	0.46 - 0.78	6.0		4.6 – 6.6
Nitrates (mg NO_3-$N \cdot L^{-1}$)	26 – 40	20 – 40		> 100
Nitration experiments	**NO_2TRY1**	**NO_2FIS2**	**NO_2FIS3**	**NO_2FIS4**
Number of microcosms	8	7	10	16
Organic substrate	Tryptone	Fishmeal	Fishmeal	Fishmeal
Stimulation of nitrification	Yes	No	Yes	Yes
Meiofauna enrichment	No	Yes	Yes	Yes
Organic content ($g \cdot m^{-2}$)	112 – 120	265	265	204
C:N ratio	4.4 - 12	8.2	8.2	7.6
% N	0.38 - 0.62	2.40	2.40	1.31
PON content ($g \cdot m^{-2}$)	0.46 - 0.78	5.2 – 7.8	5.2 - 7.8	1.8 - 4.6
Nitrate (mg NO_3-N $mg \cdot L^{-1}$)	26 - 40	9 – 22	9 - 22	5 - 19

Table 3.2 Pearson correlations between ANR and ciliate density or meiofaunal biomass.

Experiment	Ciliates		Meiofaunal mass		
	r	p	r	p	df
Nitrition					
NH_3TRY	-0.15	0.72	-0.35	0.40	6
NH_3DET	-0.10	0.81	0.01	0.98	6
Nitration					
NO_2TRY	0.01	0.98	-0.89	0.003	6
NO_2FIS2	0.27	0.56	0.81	0.03	5
NO_2FIS3	0.39	0.27	0.64	0.05	8
NO_2FIS4	0.20	0.45	0.20	0.46	14
Subsets of NO_2FIS3					
$B \leq 0.16$ g m^{-2}	0.26	0.74	0.94	0.05	2
$B > 0.16$ g m^{-2}	0.19	0.72	0.70	0.12	4
Subsets of NO_2FIS4					
$B \leq 0.12$ g m^{-2}	0.28	0.48	0.77	0.02	7
$B \geq 0.20$ g m^{-2}	0.74	0.15	-0.46	0.44	3

Table 3.3 Linear regression models and submodels of the nitrification experiments

Experiment	Model	n	F	p	R^2	95% confidence interval of the slope
Nitrition						
NH_3TRY	ANiR = 14.0 – 3.8 Biomass	8	0.82	0.40	0.12	$-14.0 < ß_1 < 6.44$
NH_3DET	ANiR = 79.2 + 0.15 Biomass	8	6.65	0.05	0.002	$-11.7 < ß_1 < 12.0$
Nitration						
NO_2TRY	ANaR = 12.0 – 2.56 Biomass	8	32.7	0.001	0.85	$-3.66 < ß_1 < -1.47$
NO_2FIS2	ANaR = 17.5 + 79.7 Biomass	7	8.2	0.03	0.62	$8.03 < ß_1 < 151.3$
NO_2FIS3	ANaR = 18.2 + 11.6 Biomass	10	5.5	0.05	0.41	$0.15 < ß_1 < 23.0$
$NO_2FIS3 \leq 0.16\ g \cdot m^{-2}$	ANaR = 14.7 + 84.0 Biomass	4	16.8	0.05	0.89	$0.05 < ß_1 < 172.2$
$NO_2FIS3 > 0.16\ g \cdot m^{-2}$	ANaR = 10.9 + 22.6 Biomass	6	4.1	0.11	0.51	$-8.35 < ß_1 < 153.4$
NO_2FIS4	ANaR = 16.0 – 0.77 Biomass	16	0.04	0.84	0.003	$-8.87 < ß_1 < 7.33$
$NO_2FIS4 \leq 0.12\ g \cdot m^{-2}$	ANaR = 12.0 + 97.4 Biomass	9	10.1	0.02	0.59	$24.8 < ß_1 < 169.9$
$NO_2FIS4 > 0.20\ g \cdot m^{-2}$	ANaR = 18.1 – 4.24 Biomass	5	0.54	0.52	0.21	$-49.6 < ß_1 < 31.0$

Table 3.4 Details of the multiple regression models of the square root of ANaR ($mg^{0.5}$ NO_2-N·m^{-1}·$h^{-0.5}$) over the organic matter content of the sediment (ORG, (g·m^{-2})), its %N, PON (= ORG * %N, g·m^{-2}) and meiofaunal biomass (SQMEIO, $g^{0.5}$·m^{-1}) (n = 41).

Model with ORG	R^2 = 0.534	RMS = 0.307	F-ratio = 44.65	p = 0.000
Effect	Coefficient	Partial R^2	Std Error	P (2 tail)
Constant	2.05		0.32	0.000
ORG	0.01		0.001	0.000
Model with %N	R^2 = 0.668	RMS = 0.259	F-ratio = 24.81	p = 0.000
Effect	Coefficient	Partial R^2	Std Error	P (2 tail)
Constant	3.35		0.29	0.000
%N	36.20	0.040	17.12	0.041
SQMEIO	-0.96	0.074	0.36	0.011
SQMEIO*%N	90.15	0.103	26.60	0.002
Model with PON	R^2 = 0.674	RMS = 0.226	F-ratio = 25.52	p = 0.000
Effect	Coefficient	Partial R^2	Std Error	P (2 tail)
Constant	3.46		0.18	0.000
PON	0.11	0.040	0.05	0.036
SQMEIO	-0.53	0.042	0.19	0.007
SQMEIO*PON	0.27	0.089	0.08	0.001

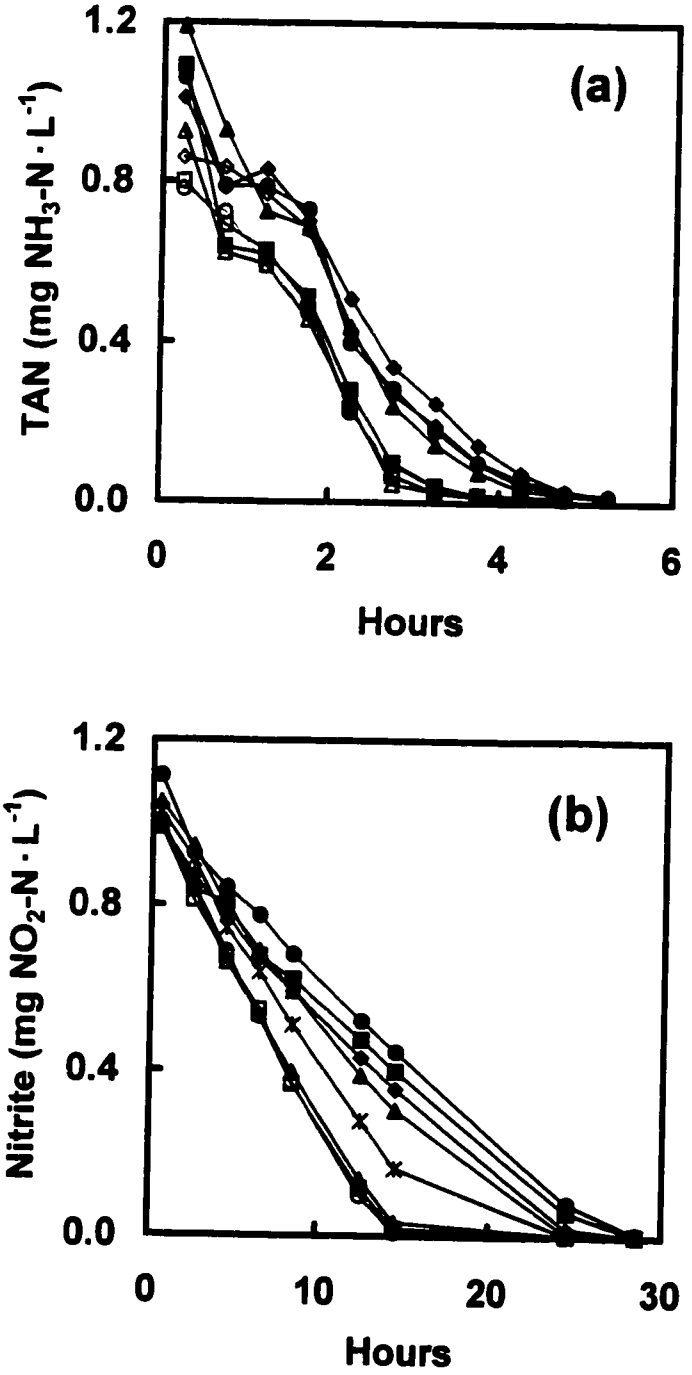

Figure 3.1 Reduction of (a) total ammoniacal nitrogen level (TAN) during NH_3DET experiment, and of (b) nitrite level during NO_2TRY experiment. Linear regression adjusted well the data between 0.2 and 1.0 mg NH_3-N $\cdot L^{-1}$, and between 0.1 and 1.0 mg NO_2-N $\cdot L^{-1}$. Different symbols represent the various microcosms used for each experiment.

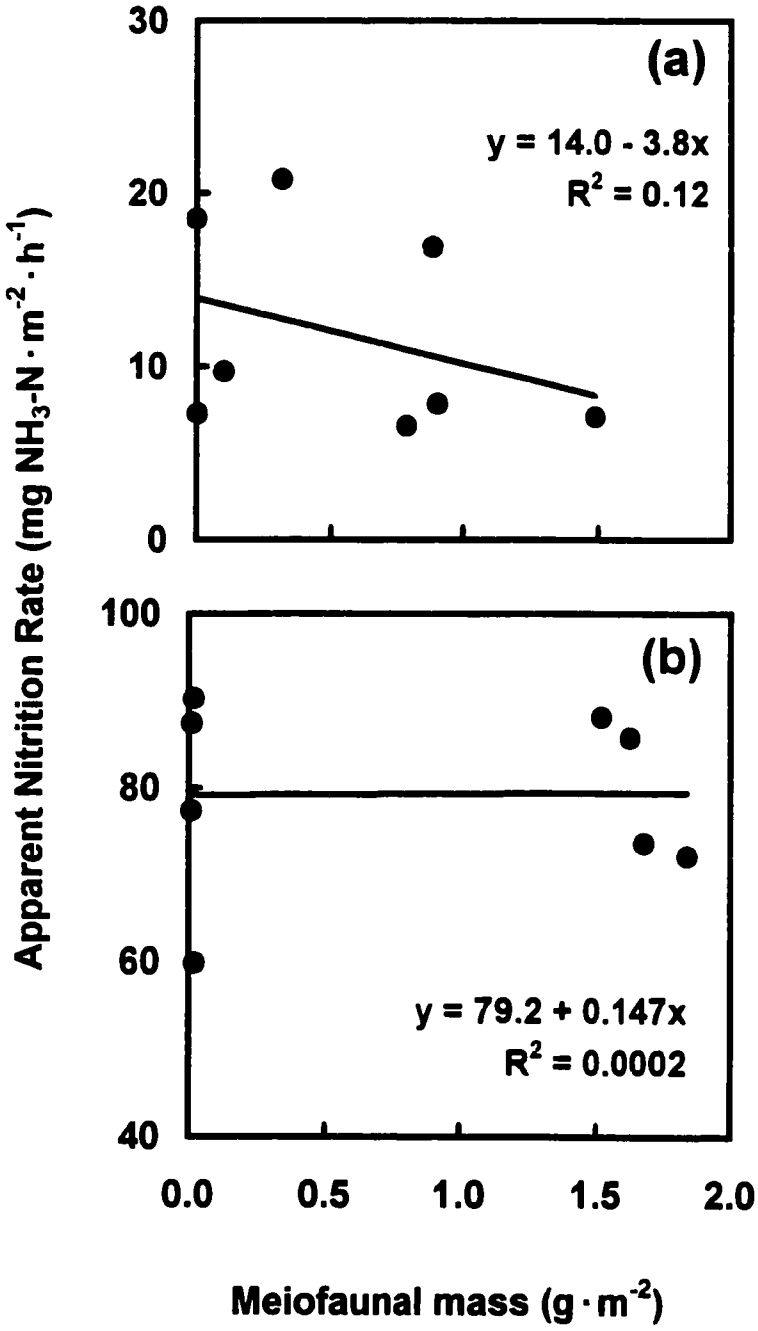

Figure 3.2 Plot of the Apparent Nitrition Rate (ANiR) versus meiofaunal biomass in the (a) tryptone experiment and (b) detritus experiment. There are no correlations between ANiR and meiofaunal biomasses.

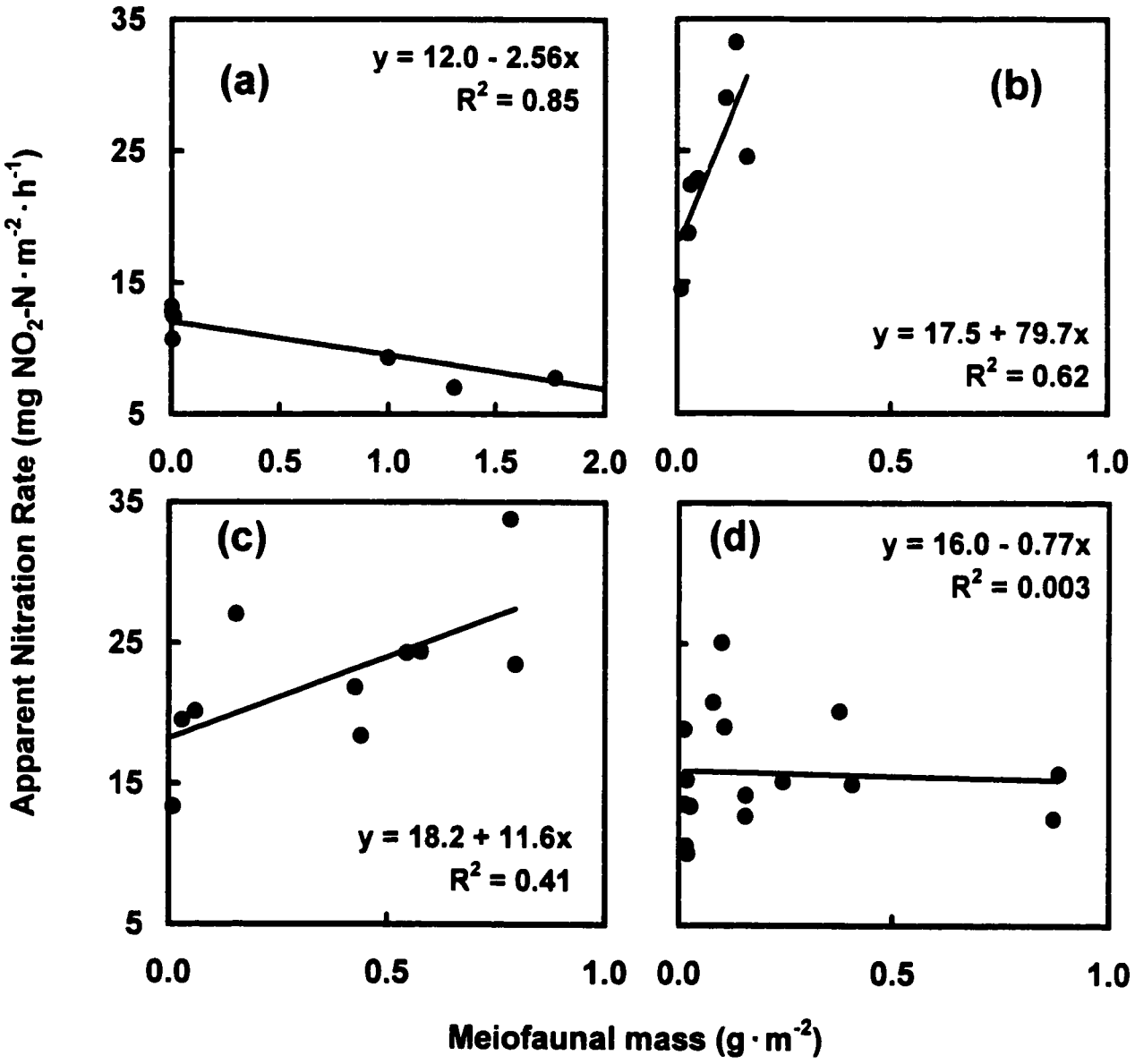

Figure 3.3 Regression models between the Apparent Nitration Rate (ANaR) and meiofaunal biomass for the (a) tryptone experiment, and (b, c, d) fishmeal experiments 2, 3 and 4.

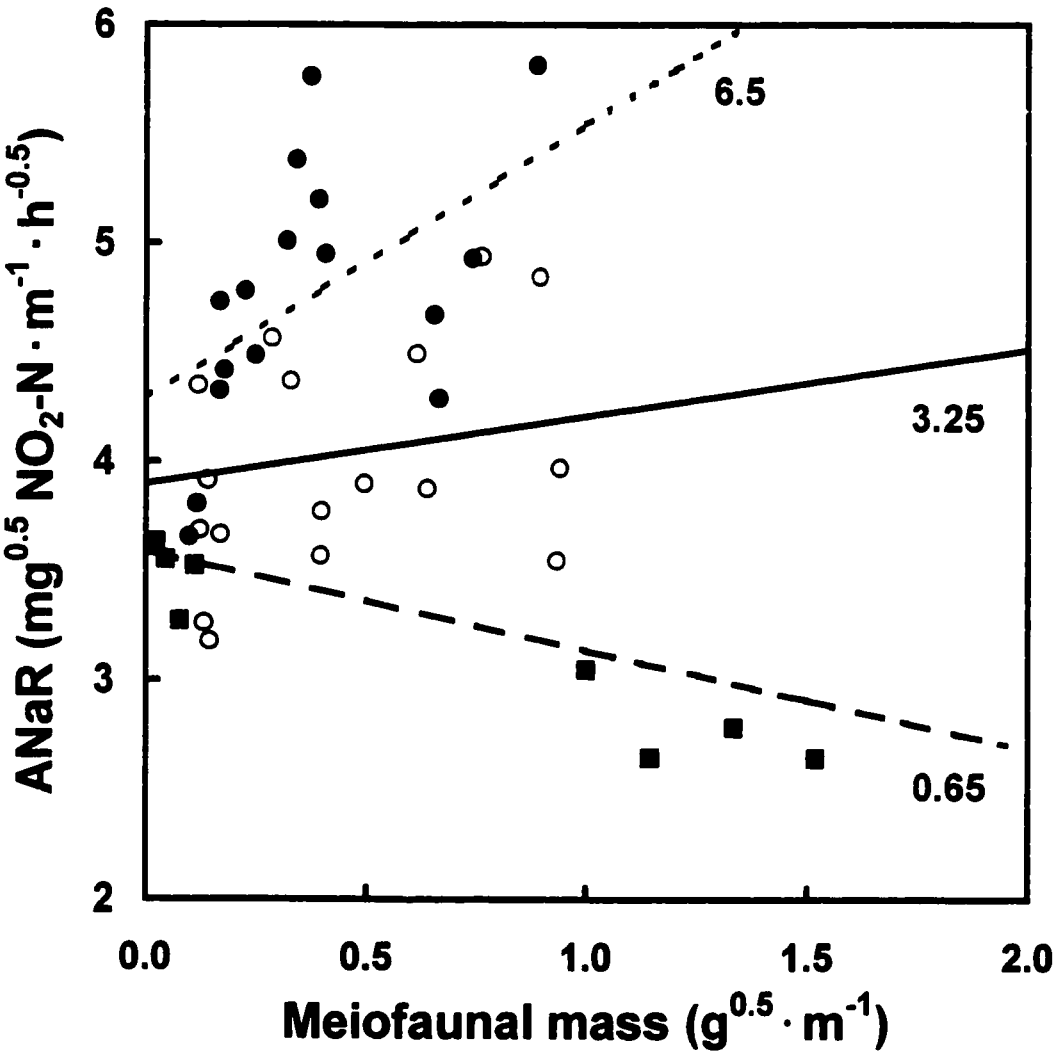

Figure 3.4 Plot of all nitration results grouped into PON levels, and of the corresponding PON curves (0.65, 3.25 and 6.5 g m-2) predicted by the multiple regression of the square root of ANaR over PON and the square root of meiofaunal biomass (see Table 4 for details). PON levels (in g m-2): 0.5 – 0.8 (squares), 1.8 – 4.6 (open circles), and 5.2 – 7.8 (circles).

CONCLUSION

It was demonstrated in the first chapter that a nitrogen budget could be prepared for a cold marine mesocosm, and that its components, inputs, outputs and changes in nitrogen pools, could be independently measured. The resulting budget for the St. Lawrence mesocosm (SLM) in 1995 had only a 8% difference between the inputs (CV = 11.1%) and the sum of outputs and changes in nitrogen pools (CV = 9.6%). Moreover, the resulting nitrogen budget was successful at identifying the role of each component and at predicting the impact of changes in the operation practices. Fish and invertebrates were kept on a maintenance diet and little more particulate organic nitrogen could be removed by the filtration and the general cleaning systems. Assuming 21 mM as the maximum nitrate concentration for survival of the animals on display, the nitrogen budget showed that, without integrated dissimilation or assimilation processes, marine aquaria similar in design and operation to the SLM must be opened after 5 to 7 years of operation unless nitrogen inputs can be reduced or nitrogen outputs increased. In 1996, the Northern gannets (*Sula bassanus*) were removed and partial water changes were increased to 16%. As predicted by the nitrogen budget, these actions have, since then, stabilized the nitrate level to 12-13 mM.

The nitrogen budget showed that the main inputs in the St. Lawrence mesocosm were food for fish and invertebrates (58%) and seabird guano (35%). The nitrogen inputs totalled 342 kg in 1995. Only 34.7% of the input nitrogen was removed from the system (119 kg, CV = 7.1%), either by the two life support systems (LSS, 52.6 kg, CV = 13.4%), the general cleaning of the mesocosm (15.9 kg, CV = 14.8%), fish removal (12.8 kg, CV = 10.0%) or by water losses (37.5 kg, CV = 10.0%). The rest of the nitrogen inputs were stored in pools of nitrogen (224 kg, CV = 9.6%), mainly in the form of nitrate (206 kg, CV = 14.2%), but also in fish biomass (5.1 kg, CV = 41.9%) and macroinvertebrate biomass (12.4 kg, CV = 12.9%).

Given all of the efforts made to keep the mesocosm very clean, it is probable that 20% represents the maximum amount of the nitrogen input that can be removed by filtration and

general cleaning in any mesocosm that is used to keep fish and invertebrates. The pool vacuum cleaner designed by the Biodome staff proved to be an important addition to the system since it increased by 30% the efficacy of the LSS at removing nitrogen, and removed detritus glued to surfaces or trapped in sediments that could not be removed by the LSS.

A flow diagram of nitrogen in the St. Lawrence mesocosm showed that bacterial nitrification is the main process in the nitrogen cycle of a cold marine mesocosm. 71% of the input nitrogen went through the nitrification process and was transformed into nitrate, i.e. 243 kg per year or 0.67 kg per day. Bacterial mineralization is also an important process in the nitrogen cycle, transforming 15% to 31% of the input nitrogen into ammonia. It is second only to the mineralization process by the biological load of the mesocosm, i.e. invertebrates, fishes and seabirds. If meiofauna has a significant effect on either the mineralization rate of organic matter or the nitrification rate of ammonia into nitrate, then it may also significantly affect the balance between the removal of particulate organic nitrogen by the filtration and general cleaning, and the production of nitrate in a cold marine mesocosm.

Ecological models serve three important purposes: to describe, to explain and to predict. The nitrogen budget developed here was used for those purposes, but also to change the operation practices of the mesocosm. It was used to significantly reduce the inputs of nitrogen, stabilize the nitrate concentration, evaluate the filtration and cleaning operations, and determine that a denitrifier was needed to reduce the nitrate concentration to an healthier level. The nitrogen budget is a useful water quality management tool for artificial environments, such as closed aquatic mesocosms.

Preliminary work showed that the sand filters of the St. Lawrence mesocosm contained very large populations of meiofauna, reaching 20-40 $g \cdot m^{-2}$ in the top 2.5 cm of sand.

The second chapter showed that the meiofauna in the heterotrophic microcosms used for the mineralization and nitrification experiments, as well as the meiofauna in the sand filters, were

almost pure cultures of harpacticoid copepods. These largely dominated both in number (87%) and biomass (98%). One species, *Pseudonychocamptus proximus*, accounted for 79% of all specimens and 88% of the meiofaunal mass. The nematodes accounted for only 4.6% of the specimens and 0.8% of the biomass. The rotifers amounted to 8% of the specimens but only 0.5% of the biomass. Polychaetes, turbellarians, mites (Acari) and gastrotrichs accounted for less than 1% of the specimens and biomass. The meiofauna of both the microcosms and the sand filters were mainly composed of prehensile epibenthic species and strongly adhesive species. There were few mesopsammic species. Most meiofaunal species eat diatoms, microalgae and bacteria, including bacterial exopolymers.

This copepod-dominated meiofauna reduced the Apparent Mineralization Rate (AMR) of tryptone, fishmeal and detritus in the microcosms. Over a 30 day period, 1 $g \cdot m^{-2}$ reduced the AMR of tryptone by 42%, the AMR of fishmeal by 2.7% and the AMR of detritus by 9.4%. The effect of meiofauna varied with particle size, but not with the C:N ratio which affected the AMR_0. Mineralizing bacteria were more productive in the fishmeal experiment than in the detritus experiment, and are therefore more resistant to the grazing effect of meiofauna. We suspect that the grazing rate of meiofauna was higher in the tryptone experiment because meiofauna had nothing to feed on except bacteria. Harpacticoid copepods are known to feed on particulate organic matter but they cannot directly assimilate complex dissolved organic matter such as tryptone.

Detailed analysis of the mineralization experiments showed that each experiment could be divided into three phases. During the early phase (days 0 to 9), the effect of meiofauna was nil or slightly positive. During the middle phase (days 12 to 18), the effect of meiofauna was strongly negative. Finally, during the late phase (days 21 to 30), the effect of meiofauna tended to be nil again. The results of the early phase support the suggestion that meiofauna grazing is in equilibrium with microbial production. The results also suggest that, through grazing, meiofauna better prepare bacterial colonies to an influx of organic matter, but then take a heavier toll on

bacterial production. The fact that the meiofauna had less impact on mineralization during the late phase is probably an artifact produced by the differences in mineralization rates in the microcosms. Detailed analysis also showed that 1 $g \cdot m^{-2}$ of meiofauna can reduce the AMR of detritus by as much as 36%.

Meiofauna is known to influence bacterial activity through different mechanisms: fragmentation or organic matter, bioturbation of the sediment, bactericulture and symbiosis, and grazing. All of these mechanisms except grazing lead to an increase in AMR. Meiofauna of the St. Lawrence mesocosm exerted its action on AMR mainly through the grazing of mineralizing bacteria.

The results of these mineralization experiments are the opposite of what has been observed in natural marine habitats. There are two possible reasons for this: meiofauna composition and meiofaunal action on bacteria. Nematodes usually dominate in natural habitats. They lack many of the enzymes necessary to digest detritus and must therefore rely more on bacteria than copepods. Nematodes stimulate mineralization mainly through bioturbation. In the St. Lawrence mesocosm, harpacticoid copepods dominate largely. Also, water was forced through the sand in the microcosms, as it is in the sand filters, which reduced the impact of bioturbation in favor of grazing.

Given its abundance in the sand filters of the mesocosm, meiofauna could exert a strong negative impact on AMR in marine mesocosms. This could particularly be true in the deeper parts of the sand where meiofauna is still abundant but less algae are probably found and more refractory organic matter accumulates. However, our results in microcosms cannot be directly translated to the sand filters because meiofauna was far more abundant in the sand filters than in the microcosms and because other food sources, such as bacterioplankton and algae, are available to the meiofauna in sand filters. Evidence for the negative impact of meiofauna in the sand filters

will have to be collected through direct *in situ* experiments or through indirect measurements, such as the P:B ratio of meiofauna in the sand filters.

Meiofauna therefore does not always increase the mineralization rate of organic matter. Copepod-dominated meiofauna decrease nutrient regeneration rates in heterotrophic habitats when the C:N ratios are low. The effect of meiofauna on the mineralization rate of organic matter can be seen as a delicate balance between negative and positive processes, such as grazing, bioturbation, bactericulture and fragmentation. The net result of these processes, an increase or a decrease in the mineralization rate of organic matter, depends on environmental factors, such as filtration and heterotrophy in closed systems, and faunal composition: two sets of factors that cannot easily be separated one from each other.

In the third chapter, it was demonstrated that the relationship between meiofauna and nitrifying bacteria is more complex than originally thought and that the presence of meiofauna does not always lead to a decrease in nitrification.

At biomasses of less than 2 g m^{-2}, copepod-dominated meiofauna had no apparent effect on nitrition over a range of apparent nitrition rates (ANiR) from 10 to 90 $mg \cdot m^{-2} \cdot h^{-1}$. However, a negative effect is expected at higher meiofaunal masses and at low ANiR values. These results are best explained by a direct negative action by predation on nitrifying bacteria and an indirect action by reducing competitors through grazing of heterotrophic bacteria.

A relationship exists between the apparent nitration rate (ANaR) and the meiofaunal mass, but it may not be apparent under certain conditions. A multiple regression analysis showed that ANaR was most strongly correlated with meiofaunal biomass and the particulate organic nitrogen (PON) content of the microcosms. The resulting model showed that meiofaunal biomass and PON accounted for 67% of the variation in ANaR. Depending on the amount of particulate organic nitrogen (PON), the relationship between meiofaunal biomass and ANaR was either negative, nil or positive. In addition, for a given PON, the relationship is not linear but varies with

the meiofaunal mass: low meiofaunal masses appear to have a stronger positive effect than high meiofaunal masses. At PON loads larger than 3.25 $g \cdot m^2$, meiofaunal biomasses less than 1.0 $g \cdot m^2$ can increase nitration by up to 5 fold. Again, the results are best explained by the dual action of meiofauna on nitrition: a direct action of preying on nitrifiers and an indirect action of reducing competition by grazing on heterotrophs.

The very high positive effect of low meiofaunal biomasses in a high PON situation (+172% to 571%) suggests that the impact of grazing on heterotrophs is stronger than the impact of grazing on nitrifiers. There are two reasons for this: 1) most of the metazoan groups which are known to prey upon nitrifying bacteria, such as fly larvae, snails and worms, were absent from the meiofauna in our system; and 2) rotifers and nematodes, two groups which are known to graze on nitrifying bacteria, were present in very low densities in the microcosms.

The positive effect of meiofauna at medium and high PON levels in marine microcosms contradicts the findings in freshwater nitrifying trickling filters where meiofauna (rotifers and nematodes) or macrofauna (fly larvae, snails and worms): always decreased or reduced to nil the ANaR of the system. The findings suggest that the effect of meiofauna on nitration varies with the composition of the meiofauna which is determined, among other things, by water type and substrate type.

Copepod-dominated meiofauna therefore has a variable effect on the nitrification rate in heterotrophic marine habitats. Most of the marine benthic systems are heterotrophic. However, they are usually nematode-dominated (70-90% in numbers). Copepod-dominated meiofauna are expected in estuarine areas with coarse sand or fine gravel beds, or in neritic zones with a high organic enrichment. Such zones, i.e. the Celtic Sea, have been shown to exhibit a high copepod/nematode ratio.

The role of meiofauna in the nitrogen cycle of a cold marine mesocosm has been established and quantified in microcosms. The meiofauna of the mesocosm reduced the AMR and increased

the ANR in microcosms at densities less than 1 $g \cdot m^{-2}$ and at PON levels higher than 2.3 $g \cdot m^{-2}$. The results showed that meiofauna does not have the same effect on mineralization and nitrification in all habitats. Moreover, this effect is not always linear and may be negative or positive depending on the PON content of the sediments. Does meiofauna have the same effects in the sand filters of the St. Lawrence mesocosm? We have no answer to this question because our results cannot be extrapolated directly to the sand filters. First, meiofauna is far more abundant in the sand filters than in the microcosms. Second, other food sources, such as bacterioplankton and algae, are available in the sand filters. To determine the exact effect of meiofauna in the sand filters, direct experiments would be necessary. Filters would have to be put in a closed circuit and known quantities of substrates injected. However such *in situ* experiments are not feasible. Indirect evidence could be gained by examining the P:B ratio of the meiofauna in the sand filters and measuring the amounts of available bacterioplankton and algae. It is also possible that the meiofauna in the sand filters is a virtual reality due to filtration and has no effect on AMR and ANR in the sand filters. The really active meiofauna could in fact be living in the gravel and the periphyton of the display pools, in which case experiments could be repeated with these substrates.

When it was found out that freshwater meiofauna was greatly reducing nitrification in freshwater trickling filters, filtration systems and their operation were modified in order to constantly eliminate meiofaunal invaders. Since meiofauna has a double positive action in marine mesocosms (reduction of the mineralization rate and increase of the nitrification rate) at biomass levels less than 1.0 $g \cdot m^2$, it is recommended that marine filtration systems be modified so as to include meiofauna in the process. Quick bioessays could be designed to monitor meiofaunal abundance in biological beds and simple techniques, i.e. freshwater backwashes of the filters, could be used to keep their biomass at a low level. A quick bioessay was found here but was not included in the thesis: copepodids and adults copepods are an excellent index of meiofaunal biomass. Their biomass is strongly correlated to meiofaunal biomass ($R^2 = 0.99$, $n = 28$) and the

organisms are easy to count because of their large size and spherical aspect. Further studies need to be done, but a good working hypothesis is that meiofauna also reduces the numbers of opportunistic protozoan ectoparasites that are the main threat for fish in captivity.

The contribution of true nitrition to the loss of NH_3 was not determined in this study and therefore the method used to measure the nitrition rates are only indirect estimates of the true nitrition rates. It is only suggested that it was important since the microcosms were heterotrophic. This method was chosen because it is commonly used in biofilter/wastewater studies. It is used not only to determine the effectiveness of a system at removing ammonia from some water source, but it is also used to measure the impact of meiofauna on nitrification in freshwater treatment systems. In fact, as for all research done on the "small food web" in life support systems or wastewater plants, this method was chosen for lack of a better one. Many actors were staged in the nitrification experiments: autotrophic bacteria, heterotrophic bacteria, ciliates and meiofauna (algae being the only actor that could be removed by keeping the microcosms in the dark for months). The effect of the ciliates was controlled through the use of nystatin and cycloheximide, but little else could be done. Nitrification is achieved by one group of bacteria (nitrifiers), bacteria in general are grazed by both ciliates and meiofauna, and ciliates are preyed upon by meiofauna. The only available way to measure the effect of meiofauna on nitrition was to compare meiofaunal densities with the net observable effect (NH_3 loss) and then to interpret the results. The use of NH_3 loss is probably not a good proxy for ammonia oxidation, unless assimilation is controlled. However, the method used was good at suggesting that meiofauna interacts differently with heterotrophic and autotrophic bacteria and that meiofauna, at low densities, feeds only on heterotrophic bacteria, its preferred food according to numerous food preferences studies and the results with mineralization experiments (Chapter Two). At observed densities, meiofauna had little impact on ammonia oxidation because it feeds much on heterotrophs and little on autotrophs, and that these two preys compete with each other for available ammonia. The same complex interaction between meiofauna and bacteria was found

with the use of the NO_2 loss method. As for nitrite oxidation, the net NO_2 loss is a very good proxy for its measurement since there is much less assimilation of NO_2 than NH_3 by heterotrophic bacteria because NO_2 assimilation requires energy to further reduce NO_2 to NH_3 and because heterotrophs outcompete autotrophs for ammonia when organic matter is abundant, as it was the case here. In conclusion, the method used is the best known one to measure the effect of metazoans on nitrition rates in biofilter/wastewater studies. This method was also good at showing the complexity of the relationship between meiofauna and nitrifying bacteria. As for the other considerations, it must be remembered that the all experiments were done at a pH higher than 8 where ammonia is mostly found in the form of ammonium (it was also added as ammonium), that the nitration experiments last only six hours which left little time for other events to occur, that there were no algae, that no significant nitrite reduction was expected in such high oxic-rich ammonium microcosms, and that excretion by faunas is not likely to have significantly affect the results since we were dealing with very high concentrations of ammonia (0.2 to 1.0 ppm).

As for future work, two avenues are of great interest. First, there is the estimation of both heterotrophic and autotrophic biomasses in the nitrification experiments with antigenic techniques so as to prove the dual action of meiofauna on bacteria. The quantification of bacterial biomasses would also be useful to test the possibility that the effect of meiofauna is not linear as assessed in this study with the linear regression. The results of such experiments could then be tested against the "Herbivory Optimization Curves" theory. Second, the true nitrification rates could be measured by using the stable isotopic $\delta 18$ nitrogen in the ammonia and nitrite added to the microcosms. Conversion into nitrate could then be assessed as well as assimilation of nitrogen by heterotrophic bacteria.

VITA

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EDUCATION

1994-1999	Ph. D. Department of Biology, University of Ottawa, Ottawa, Canada
1984	Diploma in Mariculture Marine Biological Laboratory Woods Hole Institute of Oceanography
1984	Aquavet II : Health management in confined populations of invertebrates and fish College of Veterinary Medecine Cornell University, New York.
1972-1975	Ph. D. scolarity Institute of Oceanography Dalhousie University, Halifax, Nova Scotia
1969-1972	B. Sc. in Biology Département des Sciences biologiques Université de Montréal

PROFESSIONAL EXPERIENCE

1992-	Scientific Advisor for the Research and Scientific Development Division Biodôme de Montréal, Ville de Montréal
1988-1992	Member of the Concept Team of the Biodôme de Montréal (responsible for the

	conception of aquatic mesocosms and hydraulic systems) Ville de Montréal
1977-1989	Biologist, Aquarium de Montréal Ville de Montréal
1976	Course Instructor / Lower Invertebrates Université de Montréal, Montréal
1975	Course Instructor / Marine Ecology Université Laval, Québec

SCHOLARSHIPS AND DISTINCTIONS

1992	Achievement Award by the City of Montréal in recognition of the exceptional contribution to the concept development and construction of the Biodôme de Montréal.
1987	Award of Merit from the Canadian Association of Zoological Parks and Aquariums (CAZPA)
1972	Honorific Scholarship from the Québec Education Department
1969-1972	1967 – Science Graduate Scholarship from the National Research Council of Canada.

PUBLICATIONS

Refereed papers

1. Parent, S. and A. Morin. 1999. N budget as water quality management tool in closed aquatic mesocosms. Water Research, 33: 0000 - 0000 (In Press).

2. Parent, S. and A. Morin. 1999. The role of copepod-dominated meiofauna in the mineralization of organic matter in a cold marine mesocosm. Can. J. Fish. Aquat. Sci., 56(10): 1938-1948.

3. Parent, S. and A. Morin. 1999. Role of copepod-dominated meiofauna in the nitrification process of a cold marine mesocosm. Can. J. Fish. Aquat. Sci., 56(9): 1639-1648.

4. Parent, S. and L. Schriml, 1995. A model for the determination of fish species at risk based upon life-history traits and ecological data. Can. J. Fish. Aquat. Sci., 52(8) : 1767-1781.

5. Parent, S. and P. Brunel, 1976. Aires et périodes de fraye du Capelan (*Mallotus villosus*) dans l'estuaire et le golfe du Saint-Laurent. Ministère de l'Industrie et du Commerce, Travaux de recherche sur les pêcheries no. 45, 46 p.

6. Parent, S. and J. Laurin, 1975. Première mention du Loup à tête large, *Anarhichas denticulatus* (Pisces : Blennioidea), pour l'estuaire du Saint-Laurent. Naturaliste canadien, 102: 363-365.

Abstracts

1. Parent, S. and A. Morin, 1997. Does marine meiofauna really increase the mineralization rate of organic matter? North American Benthological Society 44th Annual Meeting, San Marcos Texas. Bull. NABS 14(1) : 79.

2. Parent, S. and A. Morin, 1996. Le budget de l'azote dans un mésocosme marin froid. Procès-verbaux du 64^{e} Congrès de l'Association Canadienne-Française pour l'Avancement des Sciences (ACFAS), Montréal, p. 76.

3. Parent, S. and G. Desaulniers, 1993. The Biodôme de Montréal : a shift in our attitude towards nature and a concern for system integrity. International Congress on environmental consciousness and mass media, Dresden (Germany), October 14-17, 1993.

Other significant achievements or contributions

1. Sauvé, I., Y. Comeau and S. Parent. Dénitrification biologique des grands aquariums marins: synthèse (Biological denitrification of large marine aquariums: a review). Revue des Sciences de l'Eau (In preparation).

2. Boyer, J.-F. 1999. The estimation of the P:B ratio for harpacticoid copepods in the sand filters of a cold marine mesocosm. Honours Thesis, Dalhousie University, Halifax, Nova Scotia, 37 p.

3. Bartsch, Ilse, 1997. *Copidognathus biodomus* (Halacaridae : Acari), a new species from Eatern Canada. Mitt. Hamb. Zool. Mus. Inst. 94 : 153-159.

4. Bérubé, Y., F.G. Brière, R. Hausler and S. Parent. 1997. Élaboration de deux unités pilotes de simulation du Saint-Laurent marin du Biodôme de Montréal. 65th Annual Conference of the Association Canadienne-Française pour l'Avancement des Sciences (ACFAS), Trois-Rivières, 12 – 16 May 1997.

5. Parent, S. and G. Desaulniers, 1995. Der Biodôme de Montréal : Makroskop des Lebenden (The Montreal Biodome: A macroscope of the living). In: de Hoan, G. (Ed.). Umweltbewußtsein und Massenmedien : Perspektiven ökologisches Kommunikation (Environment and the mass media : perspectives for ecological communication). Berlin: Akademie Verlag, pp. 279-288.

6. L'arche de verre. 1992. A movie produced by the National Film Board of Canada and realized by Bernard Gosselin.

SERVICE TO THE COMMUNITY

1995-	Reviewer for the Journal of the North American Benthological Society.
1992-1997	Québec Marine Mammals Rescue Centre (Treasurer)
1992-	Société des Musées québécois (SMQ) (Consulting member)
1992-1996	Canadian Heritage Information Network (CHIN) (Biodome's representative)
1990-1996	International Species Information System (ISIS) (Biodome's representative).

PROFESSIONAL SOCIETIES

- International Association on Water Quality
- International Association of Meiobentlogists
- North American Benthological Society
- American Fisheries Society
- American Society of Limnology and Oceanography
- Canadian Association of Zoos and Aquariums (CAZA)

APPENDIX A: Raw data of the mineralization experiments

Tryptone3	AMR 6-30	Meiofauna (g/m²)		Ciliates (Ind./m²)		OM [a]	%N	C:N	PON [b]	Nitrates (mg/L)	
09-04-96	mg/m²/j	Day 0	Day 30	Day 0	Day 30	Day 0	Day 0	Day 0	Day 0	Day 0	Day 30
1	59.054	0.888	0.563	9225	9840	51	8.82	4.4	0.0	17.50	36.53
2 (Reject)	83.531	1.489	0.758	6150	2460	51	8.82	4.4	0.0	15.58	38.73
3	32.431	0.319	1.889	6150	41820	51	8.82	4.4	0.0	13.73	33.23
4	52.780	0.906	0.949	26138	2460	51	8.82	4.4	0.0	14.05	34.13
5	72.148	0.103	0.740	9225	9840	51	8.82	4.4	0.0	14.08	36.70
6	67.866	0.004	0.034	6150	4920	51	8.82	4.4	0.0	13.93	34.05
7	83.917	0.000	0.011	10250	7688	51	8.82	4.4	0.0	14.45	35.05
8	53.438	0.790	0.965	0	6150	51	8.82	4.4	0.0	15.80	35.95

Detritus1	AMR	Meiofauna (g/m²)		Ciliates (Ind./m²)		OM [a]	%N	C:N	PON [b]	Nitrates (mg/L)	
20-06-96	mg/m²/j	Day 0	Day 30	Day 0	Day 30	Day 0	18-06-96	Day 0	Day 0	Day 0	Day 30
11	58.616	1.519	0.139	147601	14760	281	2.14	7.9	6.0	35.28	45.23
12	68.515	0.009	0.003	210332	1230	281	2.14	7.9	6.0	24.63	37.00
13	63.191	1.629	0.239	145141	8610	281	2.14	7.9	6.0	31.73	45.78
14	64.479	0.011	0.010	9840	2460	281	2.14	7.9	6.0	30.13	40.78
15	59.931	1.680	0.132	184502	7380	281	2.14	7.9	6.0	34.63	45.43
16	66.162	0.021	0.006	8610	0	281	2.14	7.9	6.0	29.15	42.00
17	57.538	1.839	0.179	201722	8610	281	2.14	7.9	6.0	30.48	41.18
18	62.608	0.019	0.012	6150	0	281	2.14	7.9	6.0	25.23	36.60

Fishmeal	AMR	Meiofauna (g/m²)		Ciliates (Ind./m²)		OM [a]	%N	C:N	PON [b]	Nitrates (mg/L)	
7/96+10/96	mg/m²/j	Day 0	Day 30	Day 0	Day 30	Day 0	18-06-96	Day 0	Day 0	Day 0	Day 30
1.1	78.690	0.740	1.867	55351	108241	64	11.56	4.6	7.4	19.75	33.45
1.2	87.431	0.654	1.625	34440	18450	64	11.56	4.6	7.4	19.33	34.25
1.3	81.963	0.888	2.039	11070	20910	64	11.56	4.6	7.4	19.90	34.58
1.4	76.310	1.030	3.802	20910	7380	64	11.56	4.6	7.4	18.98	32.95
1.5	83.120	0.087	1.157	172202	93481	64	11.56	4.6	7.4	17.45	32.30
1.6	92.611	0.041	0.674	688807	3690	64	11.56	4.6	7.4	15.63	31.50
1.7	82.988	0.087	1.353	9840	20910	64	11.56	4.6	7.4	19.95	35.13
1.8	84.881	1.228	2.765	116851	15990	64	11.56	4.6	7.4	20.03	35.50
2.1	72.879	5.184	8.079	60271	81181	64	11.56	4.6	7.4	21.13	35.00
2.2	83.177	3.996	2.505	4920	17220	64	11.56	4.6	7.4	23.93	39.00
2.3	80.740	4.733	2.108	84871	371464	64	11.56	4.6	7.4	23.93	38.65
2.4	79.632	4.179	2.122	77491	97171	64	11.56	4.6	7.4	21.10	35.38
2.8	69.904	3.719	2.357	8610	98401	64	11.56	4.6	7.4	18.50	32.48

[a] OM = Organic Matter (in g/m²)

[b] PON = Particulate Organic Nitrogen (in g/m²)

APPENDIX B: Raw data of the nitrition experiments

NH_3TRY	ANR	Meiofauna	Ciliates	OM [a]	%N	C:N	PON [b]	Nitrates
05-04-96	mg/m²/h	g/m²	ind/m²	g/m²	20-09-97	ratio	g/m²	mg/L
1	16.882	0.888	9840	120.51	0.38	4.4 - 12	0.46	17.50
2	7.097	1.489	6150	117.48	0.40	4.4 - 12	0.47	15.58
3	20.790	0.319	6150	115.66	0.45	4.4 - 12	0.52	13.73
4	7.831	0.906	26138	124.14	0.50	4.4 - 12	0.62	14.05
5	9.678	0.103	9840	130.19	0.60	4.4 - 12	0.78	14.08
6	7.254	0.004	6150	115.66	0.59	4.4 - 12	0.68	13.93
7	18.504	0.000	10250	112.63	0.57	4.4 - 12	0.64	14.45
8	6.552	0.790	6150	121.11	0.62	4.4 - 12	0.75	15.80

NH_3DET	ANR	Meiofauna	Ciliates	OM [a]	%N	C:N	PON [b]	Nitrates
06-06-96	mg/m²/h	g/m²	ind/m²	g/m²	18-06-96	ratio	g/m²	mg/L
11	88.099	1.519	147601	300.18	2.04	7.83	6.12	35.28
12	77.409	0.009	210332	285.71	2.14	7.76	6.11	24.63
13	85.689	1.629	145141	276.16	2.29	7.99	6.32	31.73
14	87.469	0.011	9840	284.51	2.33	7.57	6.63	30.13
15	73.614	1.680	184502	291.74	2.01	7.76	5.86	34.63
16	59.820	0.021	8610	295.96	2.20	8.20	6.51	29.15
17	72.098	1.839	201722	242.31	2.20	7.89	5.33	30.48
18	90.231	0.019	6150	273.06	1.97	8.32	5.38	25.23

[a] OM = Organic Matter

[b] PON = Particulate Organic Nitrogen

APPENDIX C: Raw data of the nitration experiments

NO2TRY1	ANR	Meiofauna	Ciliates	OM [a]	%N	C:N	PON [b]	Nitrates
09-03-96	mg/m²/h	g/m²	ind/m²	g/m²		ratio	g/m²	mg/L
1	7.746	1.775	9225	120.51	0.38	4.4 - 12	0.46	38.30
2	6.999	1.306	6150	117.48	0.40	4.4 - 12	0.47	33.47
3	12.412	0.012	16913	115.66	0.45	4.4 - 12	0.52	30.08
4	6.983	2.309	26138	124.14	0.50	4.4 - 12	0.62	39.00
5	12.611	0.002	9225	130.19	0.60	4.4 - 12	0.78	31.55
6	10.704	0.006	127921	115.66	0.59	4.4 - 12	0.68	23.85
7	13.199	0.001	10250	112.63	0.57	4.4 - 12	0.64	36.99
8	9.280	1.000	76876	121.11	0.62	4.4 - 12	0.75	31.69

NO2FIS2	ANR	Meiofauna	Ciliates	OM [a]	%N	C:N	PON [b]	Nitrates
28-08-97	mg/m²/h	g/m²	ind/m²	g/m²		ratio	g/m²	mg/L
11	33.224	0.138	14350	299.75	2.61	7.71	7.82	18.10
12	18.714	0.027	102091	271.89	2.24	8.08	6.09	8.76
13	28.967	0.115	1757	276.74	2.12	8.38	5.87	10.30
14	24.506	0.165	3075	274.92	2.46	8.34	6.76	11.10
16	14.500	0.010	1230	247.07	2.33	8.48	5.76	9.23
17	22.395	0.033	4510	247.07	2.12	8.94	5.24	9.02
18	22.864	0.049	6150	239.80	2.73	8.01	6.55	11.20

NO2FIS3	ANR	Meiofauna	Ciliates	OM [a]	%N	C:N	PON [b]	Nitrates
18-09-97	mg/m²/h	g/m²	ind/m²	g/m²		ratio	g/m²	mg/L
7	24.386	0.578	100861	162.29	1.68	6.38	2.73	14.70
8	23.477	0.797	86101	179.85	2.17	6.24	3.90	17.00
11	33.794	0.785	43050	299.75	2.61	7.71	7.82	18.10
12	19.521	0.031	44280	271.89	2.24	8.08	6.09	8.76
13	27.033	0.152	17220	276.74	2.12	8.38	5.87	10.30
14	24.290	0.545	44280	274.92	2.46	8.34	6.76	11.10
15	18.386	0.441	7380	266.44	2.55	7.99	6.79	20.70
16	13.368	0.010	2460	247.07	2.33	8.48	5.76	9.23
17	20.133	0.060	4920	247.07	2.12	8.94	5.24	9.02
18	21.823	0.427	49200	239.80	2.73	8.01	6.55	11.20

NO2FIS4	ANR	Meiofauna	Ciliates	OM [a]	%N	C:N	PON [b]	Nitrates
17-12-97	mg/m²/h	g/m²	ind/m²	g/m²		ratio	g/m²	mg/L
1	20.199	0.377	28115	164.11	1.14	6.81	1.87	11.90
2	14.244	0.157	9225	156.84	1.16	6.73	1.82	15.30
3	15.761	0.883	21086	182.88	1.29	7.37	2.36	11.30
4	15.202	0.244	24600	164.71	1.32	7.15	2.17	15.50
5	20.842	0.081	51661	187.72	1.16	7.29	2.18	13.50
6	12.573	0.871	15814	182.88	1.14	7.48	2.08	19.40
7	12.747	0.157	31629	185.30	1.27	7.55	2.35	11.80
8	13.597	0.015	17220	166.53	1.67	6.86	2.78	10.30
11	25.089	0.101	3075	260.39	2.45	7.45	6.38	7.89
12	10.642	0.017	12300	227.08	1.19	8.35	2.70	4.72
13	18.913	0.014	0	247.07	1.64	7.92	4.05	5.71
14	19.076	0.107	15375	237.38	1.37	8.17	3.25	5.94
15	15.029	0.407	12300	227.08	1.65	8.11	3.75	8.18
16	10.106	0.020	0	200.74	1.88	7.93	3.77	5.38
17	15.328	0.019	0	239.19	1.79	7.98	4.28	5.96
18	13.452	0.028	0	237.38	1.95	8.02	4.63	6.18

[a] OM = Organic Matter

[b] PON = Particulate Organic Nitrogen