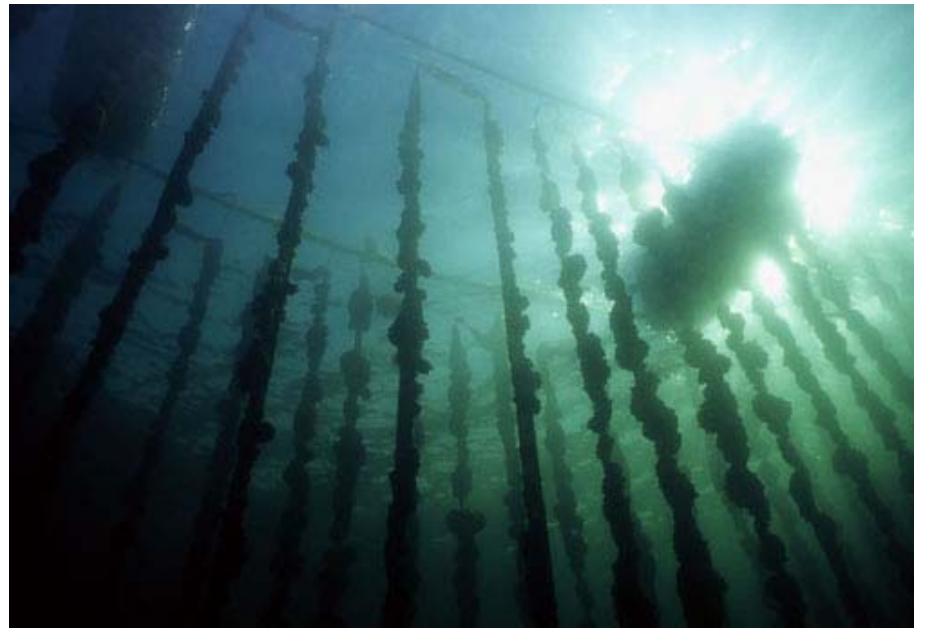




GÖTEBORGS
UNIVERSITET



**Benthic oxygen and nutrient fluxes
beneath long-line mussel farms**

Markus Klingberg

**Examensarbete i marin kemi för
magisterexamen
20 poäng**

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Abstract

Increasing eutrophication along the Swedish west coast has resulted in several environmental actions and regulations which aim to reduce the load of nutrients (i.e. inorganic nitrogen and phosphorus) to coastal waters. Mussel farming might be an alternative, or complement, to expand and increase the capacity of ordinary sewage treatment plants. In May 2005, 17 long-line mussel farms were placed in the archipelago outside Lysekil in order to evaluate the efficiency of mussel farming as a method of removing nutrients from coastal marine waters.

In this study, benthic nitrogen cycling was studied underneath three of the long-line mussel farms. The investigation was performed in September 2005 and included benthic flux incubations, pore water distributions, water current and CTD- measurements. Reference stations were chosen 200 m away from each farm. A similar investigation was performed in May 2005, before the cultivation of mussels was started. Nutrient fluxes in the present study were similar as those measured in the May investigation, and further demonstrated the importance of DON for benthic N-cycling. Calculations indicated that 20-90 % of the total N-flux out of the sediments were due to DON. Rates of oxygen consumption were higher in May ($380-700 \mu\text{mol m}^{-2} \text{h}^{-1}$) than in September ($290-530 \mu\text{mol m}^{-2} \text{h}^{-1}$). There were no obvious differences in oxygen consumption and nutrient flux rates between the farm and the reference sites except for the Ramsvik sampling area. At Ramsvik, bottom water conditions and general sediment characteristics were significantly different between farm and reference sites. Since the mussel farms are relatively young, sediment and infauna characteristics (e.g. organic content and macrofaunal activities) and bottom topography seemed more important for measured flux rates than organic input from the mussel farms.

1. Introduction

1.1 Background and Objectives

This study is part of a larger research project with the aim to investigate long-line mussel farming as a technique to reduce nutrients from coastal marine waters (Lindahl et al. 2005). This thesis focuses on benthic nutrient cycling under the mussel farms, with particular emphasis on fluxes of dissolved organic nitrogen (DON) across the sediment water interface.

There is an intensive debate on what actions should be taken to reduce

nitrogen and phosphorous in coastal Swedish waters. The Swedish Environmental Protection Agency (EPA) has established a national program in order to improve the coastal marine environment (Naturvårdsverket, 2005). Since Sweden became a member of the European Union, the EU-regulation 2000/60/EG is a framework for restoration of coastal marine environments (Naturvårdsverket 2000). The international goal is a 50 % reduction (1985 level) of the nitrogen and phosphorus transport from anthropogenic, land-based sources to the sea. This should be accomplished until 2005. However, observations

indicate that a 50 % reduction will not be reached (Naturvårdsverket, 2003). The Swedish national goal is to reduce the waterborne nitrogen by 40 % (1995 level) before 2025. Locally, the County Board of Västra Götaland (Länsstyrelsen) has established a goal of reducing waterborne nitrogen to the Skagerrak by 18 % (1995 level) by 2010. This reduction equals 2750 tones per year (Lindahl et al. 2005).

An alternative, or complement, to increase the capacity of the sewage treatment plants is to utilize filter-feeding blue mussels (*Mytilus edulis*). Blue mussels feed on particles suspended in the water column, thereby removing large quantities of nitrogen and phosphorous from the water mass. Harvesting these filter-feeding blue mussels may provide a sustainable method for producing food of high nutritional value, while simultaneously recycling nutrients from sea to land (Haamer et al. 1999; Lindahl et al. 2005). For each harvested ton of blue mussels, 6,4-10,2 kg N and 0,4-0,6 kg P will be removed (Loo and Petersen, 2004).

The Lysekil sewage treatment plant presently releases nearly 40 t of nitrogen per year into Swedish west coast waters. In accordance with the EU-regulation, Lysekil community has to remove additional 28 t of this nitrogen (70 %). Removing 28 t of nitrogen corresponds to an annual production and harvest of 2 800 t of blue mussel biomass, or approximately 20 mussel farm units with 140 t in each unit (Lindahl et al. 2005). At present, 17 such units have been established in the archipelago outside Lysekil.

There are two main disadvantages associated with mussel farming. One is the appearance of natural phytoplankton toxins, like diarrhetic shellfish toxins (DST) (Edebo et al.

2000). The other is the increased load of organic material accumulating in sediments beneath the mussel farms due to faeces and pseudofaeces. Large-scale mussel farms are known to produce significant volumes of biodeposits, potentially altering seabed sediment characteristics and benthic community structure (Hartstein, 2005). For example, denitrification, perhaps the most important ecosystem function to remove nitrogen from marine environments (Hulth et al. 2005), may be significantly reduced or even inhibited at high loadings of organic material (Gilbert et al. 2003).

The overall objective of this study was to investigate the importance of benthic fluxes for overall N-cycling in sediments influenced by long-line mussel farms.

1.2 Eutrophication

It is generally considered that nitrogen availability is one of the major factors regulating primary production in temperate coastal marine environments (Cook et al., 2004). Generally, eutrophication means a larger phototropic biomass, which feeds heterotrophic organisms, resulting in a larger biomass as a whole (Herbert, 1999). Overall, increased primary production increases sedimentation, causing larger inputs of organic matter to sediments in eutrophic, compared to oligotrophic, systems. Further, other indications of eutrophication include a reduced light penetration (or water transparency), macro algal mats and hypoxic or anoxic bottom waters in areas of a restricted water circulation. Not only the amount, but also the general composition of deposited material, affect microbial communities and the distribution patterns of solutes they produce in the sediment during mineralization. Hence, eutrophication may not only affect structure of

epibenthic macrofauna but also influence rates and pathways of mineralization (Cloern, 2001; Herbert, 1999).

1.3 Mineralization of organic material and benthic nutrient cycling

Microbial communities in marine sediments are essential to the biogeochemical cycling of nutrients. The energy-gaining oxidation of organic material by microbes releases nutrients to the surrounding environment. Mineralization in sediments often results in sharp concentration gradients between the overlying water and the surface sediment layer. These gradients induce a transport of solutes (i.e. nutrients) across the sediment-water interface to the overlying bottom water. As a direct consequence of mineralization, there is a flux of oxygen from the overlying water to the sediment (Brune et al. 2000).

There is much interest in how natural systems process nitrogen because primary production in coastal marine waters is often limited by nitrogen

(Cook et al. 2004). In coastal environments, benthic remineralisation processes are often significant for nitrogen cycling. Examples of important processes include nitrification and coupled nitrification/denitrification. Denitrification represents a significant sink for nitrogen in estuaries (Cook et al. 2004; Seitzinger et al. 1988; Middelburg et al. 1996). The complex nature of benthic nitrogen cycling is shown schematically in figure 1.

The driving forces for nitrogen mineralisation in sediments are the quantity and overall composition of the organic material being degraded, along with the availability and concentration of oxidants used during the various mineralization pathways. Additionally, important factors controlling the concentrations of inorganic nitrogen species (i.e. NO_3^- and NH_4^+) in the water column of shallow coastal marine ecosystems include inputs rising from fluvial discharges, and those resulting from exchange across the sediment-water interface (Herbert, 1999).

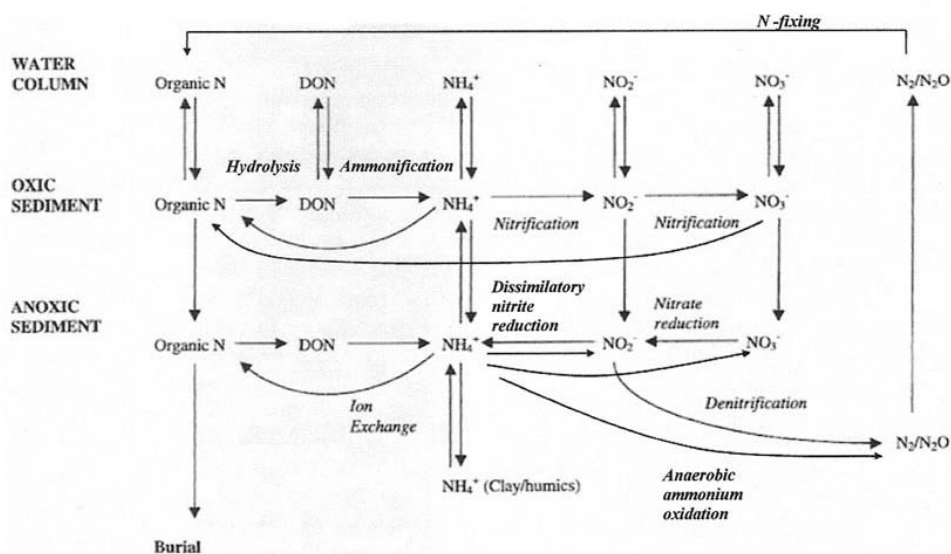


Fig 1. Schematic representation of nitrogen cycling in coastal marine sediments. (Modified from Herbert, 1999 and Hulth et al. 2005).

1.4 The importance of DON for N-cycling

Dissolved organic nitrogen (DON) is a heterogeneous class of organic compounds that ranges from well-defined structures, such as urea or amino acids, to more complex and often poorly characterized compounds such as humic acids and fulvic acids (Burdige and Zheng, 1998). Concentrations of humic-like compounds generally increase with sediment depth and are closely correlated to those of dissolved organic carbon (DOC) (Burdige et al. 2004). Relatively labile compounds such as amino acids are produced as intermediates during remineralisation, whereas more refractory DON may be end-products produced from partial remineralisation or condensation of the sediment organic matter (Burdige and Zheng, 1998). A large fraction of the total pore water DOC and DON is of relatively low molecular weight (Burdige, 2002). A high proportion of heterocyclic nitrogen functionality (i.e. nitrogen in aromatic rings such as five-member pyrrole-like or six-member pyridine-like structures) has been observed in sediment organic matter. The occurrence of this material may be due to either selective utilization of amino nitrogen compounds, or the occurrence of *in situ* rearrangement reactions that produce new heterocyclic compounds from amino-nitrogen compounds (Burdige, 2002).

Since marine phytoplankton, at least occasionally, can use DON as nitrogen source (Antia et al., 1991), there is additional interest in understanding the role of sediment as a source of DON to the water column (Bronk et al. 1994). In part, the significance of benthic DON fluxes for marine nitrogen cycling is similar to that discussed for benthic fluxes of DOC and the marine carbon cycle (Burdige and Zheng, 1998).

Until recently, benthic DON fluxes were assumed to be of only minor importance for overall N-cycling in sediment. Denitrification rates were indirectly used to balance sediment nitrogen budgets, assuming that benthic DON fluxes were insignificant (Burdige, 2002). However, several studies suggest that DON fluxes might be an important component of the general N-cycling, though their results exhibit large variations, both in magnitude and directions (i.e. into or out of the sediments) (Burdige 2002; Burdige et al., 2004).

1.5 Long-line mussel farming

Modern mussel farming has been established in Sweden since 1971 (Edebo et al., 2000). The method most frequently used for mussel farming in Sweden is the long-line system (Fig. 2). Tests have also been initiated with farming from rafts. The farming capacity of an average Swedish long-line farm is about 200 t of mussels during a common farming cycle of two years. Each farm occupies a water surface area of about 2000 m² (Edebo et al., 2000).

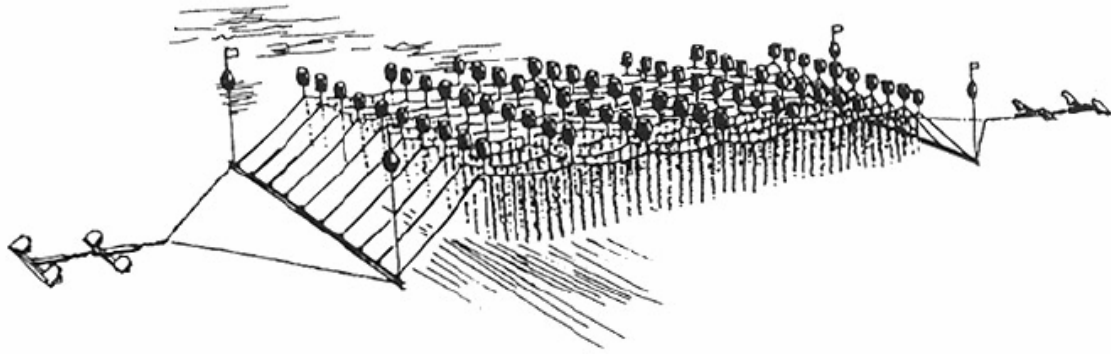


Fig. 2. The Swedish long-line system with typically dimensions 10 x 200 m. The long-lines can carry 20 000 m of farming strips with a production capacity of about 200 t during a cycle of two years. The mussel operation consists of 10 wires each about 200 m long, which are supported by 200 buoys. The mooring is done with two 10 m long rails and four 200 kg anchors. Drawing: Terrence Florell (Edebo et. al.,2000).

2. Material and Methods

2.1 Sampling sites

In September 2005, sediment was sampled underneath three long-line mussel farms (Valboholmen, Högholmen and Ramsvik) northward Lysekil on the Swedish west coast (Fig. 3). Cultivation of mussels in the farms started about six month ago, and a preceding investigation of benthic solute fluxes was performed at the time for starting the farm activities (Engström et al., unpublished). The present study expanded the initial investigation and also included pore water distributions and benthic fluxes of DON.

Overall, the mussel farms are located close to the Lysekil waste-water treatment plant, with water depths of 15 – 20 m. Due to logistic reasons, the sediment samples (n=6) were taken beside the center of each farm. The reference sites were selected approximately 200 m outside the boundary of each farm (Hartstein, 2005). *In situ* environmental conditions are described in chapter 3.1.

2.2 Sediment sampling and benthic flux incubations

Sediment was collected with an Olausen box-corer and plexiglas tubes (\varnothing 10 cm) were inserted into the sediment. The tubes were sealed, removed from the box-corer and transported to a temperature-controlled room at the Kristineberg Marine Research Station. At each station, seven cores were inserted into the sediment for benthic flux incubations (n=6) and pore water solute distributions (n=1). Bottom water was sampled for nutrients (NH_4^+ , NO_3^- , $\text{Si}(\text{OH})_4$, HPO_4^{2-} and dissolved organic nitrogen, DON) and oxygen concentrations. Samples for nutrients were filtered (0,45 μm , cellulose acetate) and stored frozen (-20°C). Samples for oxygen were immediately precipitated directly in the glass vials and analyzed within 24 hours.

At the laboratory, a continuous flow of seawater (directly supplied from the bottom water of the Gullmarsfjord; 15°C , salinity of ~ 30) was applied to each core. After approximately 12 hours of acclimatization, the water flow was stopped and the cores were sealed with plexiglass lids.



Fig. 3 The sediment sampling sites: Valboholmen (A), Högholmen (B) and Ramsvik (C)

The lids were equipped with two valves (one for sample removal, and one for incoming replacement water), and a Teflon-coated magnetic stirring bar. The cores were placed in groups of six, with a central power-driven magnet supporting stirring in each chamber. Samples from the overlying water were withdrawn six times during an incubation period of about 10-14 hours. Each time, approximately 60 ml was withdrawn (exact volume recorded) for benthic oxygen and nutrient (NH_4^+ , NO_3^- , $\text{Si}(\text{OH})_4$, HPO_4^{2-} and DON) fluxes. Exact time period between sampling was determined during separate pre-incubations, and intervals adjusted so that oxygen concentrations in the overlying water did not decrease by more than 20 %

from oxygen saturation. Benthic flux rates were calculated from the initial linear change in the overlying water with time of incubation.

For pore water at each station, the top 10 cm of the surface sediment was vertically sectioned in 0,5 cm intervals. The sediment was transferred to 50 mL centrifuge tubes and centrifuged at 2100 rpm for 30 min. Obtained pore water was filtered (0,45 μm , cellulose acetate), stored frozen and analyzed in the same way as nutrients in the overlying water.

The oxygen and DON samples were stored in 12 ml gas-tight glass vials (Exetainers), and the inorganic nutrient samples were stored in 9 ml polypropylene vials before analysis.

2.3 Diffusive transport of ammonium

To estimate pathways of N mineralization, diffusion rates of ammonium towards the sediment-water interface were calculated from the linear gradient of ammonium in the pore water (0-2 cm) using Fick's first law of diffusion (Liu, 2005):

$$J_{Diff.} = R_{NH_4^+} = -D_0 * \phi^3 * \frac{dC}{dx} \quad (1)$$

D_0 is the molecular diffusion coefficient in free solution corrected for temperature and viscosity, Φ is the porosity and dC/dx is the diffusion gradient in the top 2 cm of the sediment. Since porosity was not available from the actual sampling site, the porosity was set to 0.8 based on previous measurements in similar types of sediments (S. Hulth, pers. comm.). The flux of ammonium within the sediment was assumed to correspond to net rates of ammonium production ($R_{NH_4^+}$) in the surface sediment.

2.4 Chemical analysis and hydrographic measurements

Samples for DON were transferred to 50 ml Scott Duran[®] bottles and analyzed by wet chemical procedures according to (Bronk et al. 2000; Valderrama, 1980). Briefly, 1 mL 5,4 M potassium persulfate was added to 8 mL sample to oxidize organic and inorganic N (total N, TN) to nitrate. Oxidation was performed in a CertoClave (i.e. pressure cooker: Hochdruck-Sterilisator) for 30 min at 120 °C. Samples for background correction (n=3) and internal standards (n=3) for the oxidation yield (glycine) were included in the oxidation procedure. The content of nitrate following oxidation (total nitrogen) was measured with an auto analyzer (TRACCS 800) using standard

colorimetric methods (Strickland and Parsons, 1972). Concentrations of NH_4^+ and NO_3^-/NO_2^- in the samples were measured separately (Strickland and Parsons, 1972). The fraction of dissolved organic nitrogen was obtained from subtracting concentrations of dissolved inorganic N ($NH_4^+ + NO_3^-/NO_2^-$) from concentrations of total dissolved N, i.e. $DON = TN - DIN$.

Measurements of bottom water currents took place between September 12 and 16 at the Högholmen farm site and the Högholmen reference site. Bottom water currents were measured by two current meters (SD-6000 mini current meter) deployed about one meter above the sediment surface. The current meters were programmed to sample every 15 minutes during one week. At the same time, CTD-profiles (i.e. Conductivity, Temperature and Density; Sea-Bird SBE19plus) were recorded when the current meters were deployed and removed (see Chap. 3.2).

3. Results and Discussion

3.1 Sediment and bottom water characteristics

In addition to the September sampling occasion, diving was used to visually inspect the sediment conditions and the settling of each mussel farm. At the Valboholmen farm site, the sediment was mainly composed of clay with typical characteristics of an accumulation type of bottom (i.e. the topography facilitated trapping of sediment particles). Several mussels from the farm were seen also on the sediment surface. Bottom fauna was characterized by crabs, crayfishes, sea stars and flatfishes. At this farm, the settling of mussels was relatively uniformly distributed.

The sediment at the Högholmen farm site revealed similar characteristics of an accumulation type of bottom as was observed at the Valboholmen farm site. At Högholmen, the sediment was significantly perforated by macrofauna and different kinds of digging animals were observed. Overall, the settling of mussels was poor and uneven with significant amounts of algae and sea shells between the mussels. Further, mussels observed on the lines were relatively small (about 2-4 cm).

The Ramsvik farm site differed significantly from the other two mussel farms. Above all, the surface sediment consisted of sand and a mixture of shells and gravel, i.e. Ramsvik was a typical transport bottom type (sediment particles were resuspended and eroded from the surface sediment). The settling of mussels was relatively good, despite partly uneven settling. Several mussel strips with mussels in clumps were observed under the farm, and the density of sea stars and crabs were significant on the sediment surface.

At the reference sites (200 m from the farm sites) overall sediment characteristics was similar (accumulation bottoms) to that observed for the Valboholmen and the Högholmen farm sites. However, the reference site at Ramsvik was also an accumulation type of sediment, i.e. distinctly different from the mussel farm site at Ramsvik.

In situ bottom water salinity and temperature were similar between the sampling sites (Table 1). Since the mussel farms were located relatively close to each other (Fig 3), they were likely affected by the same water masses. However, oxygen concentration of the bottom water at the Ramsvik reference site was significantly lower (91,8 μM) than concentrations

observed at the other sampling sites (Table 1). At the same time, concentrations of nutrients (NH_4^+ , $\text{Si}(\text{OH})_4$, HPO_4^{2-} and DON) were higher than at the other sites. This indicated oxygen consumption and organic matter mineralization of recently deposited organic material in the surface sediment and the bottom water at the Ramsvik reference site. Overall, measured bottom water concentrations of ammonium and nitrite/nitrate were relatively low for Swedish west coast areas (Naturvårdsverket, 1999).

Since information on wind conditions of the sampling area could not be obtained, data from the Göteborg region (~ 70 km south of the mussel farms) was used for comparison (Local Environmental Agency in Gothenburg; Fig 4). During the sampling period, the wind speed varied between 1 and 12 m/s, with overall patterns that correlated well with the current measurements from the sampling sites (Fig. 4). Directly following a progressively increasing wind speed, bottom water currents increased drastically September 14. This event likely mixed the entire water column down to the bottom. As the wind speed was reduced, bottom water currents were also reduced about a day later, although irregular patterns were observed.

During the week September 12 to 16, temperature decreased in the entire water column probably due to wind mixing of the water column and water exchange with surrounding water masses (Fig. 5). As the salinity of the bottom water increased, while the salinity of the surface water was more or less constant, there was likely horizontal as well as vertical mixing of the water column during the sampling period.

Tab. 1 *In Situ* parameters from the bottom water of the six sampling sites \pm SE (n=2)

station	Salinity/Temp (psu/°C)	[O ₂] (μ M)	[NH ₄ ⁺] (μ M)	[NO ₃ ⁻ +NO ₂ ⁻] (μ M)	[Si(OH) ₄] (μ M)	[HPO ₄ ²⁻] (μ M)	[DON] (μ M)
Valboholmen farm	26 / 17,0	209 \pm 2,63	0,79 \pm 0,09	0,65 \pm 0,11	5,49 \pm 0,40	0,68 \pm 0,08	12,1 \pm 0,09
Valboholmen ref.	26 / 17,0	178 \pm 4,63	0,50 \pm 0,02	6,30 \pm 0,05	12,5 \pm 0,10	0,70 \pm 0,01	8,00 \pm 0,33
Högholmen farm	31 / 16,6	240 \pm 4,38	0,21 \pm 0,00	0,52 \pm 0,01	4,98 \pm 0,77	0,36 \pm 0,01	8,71 \pm 0,10
Högholmen ref.	31 / 16,4	155 \pm 4,63	0,53 \pm 0,01	2,67 \pm 0,06	23,4 \pm 7,4	0,79 \pm 0,03	9,04 \pm 0,03
Ramsvik farm	30 / 15,9	193 \pm 5,88	0,29 \pm 0,02	2,84 \pm 0,01	10,1 \pm 0,040	0,56 \pm 0,01	7,53 \pm 0,11
Ramsvik ref.	30 / 15,9	91,8 \pm 3,75	1,13 \pm 0,03	2,82 \pm 0,05	72,7 \pm 0,40	0,97 \pm 0,04	12,9 \pm 0,19

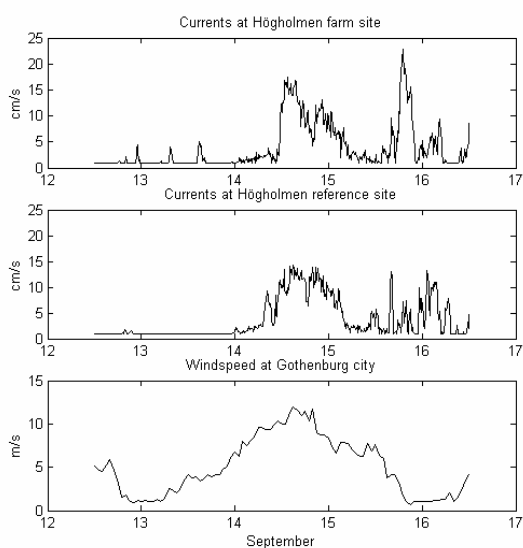


Fig. 4. Current speeds in the bottom water measured at the Högholmen farm (top) and reference (mid) sites. The wind speed recorded in Göteborg (bottom) is shown for comparison.

3.3 Pore water distributions of NH₄⁺ and DON

At all stations investigated, ammonium increased in a more or less linear fashion with depth in the surface sediment (Fig. 6). This indicated a mobilization of ammonium in the pore water during mineralization of organic material, and a diffusive transport of ammonium towards the sediment-water interface. At the Valboholmen reference site, the Högholmen farm and reference sites, and the Ramsvik farm site, a distinctive disturbance in the ammonium gradient was observed at 3-

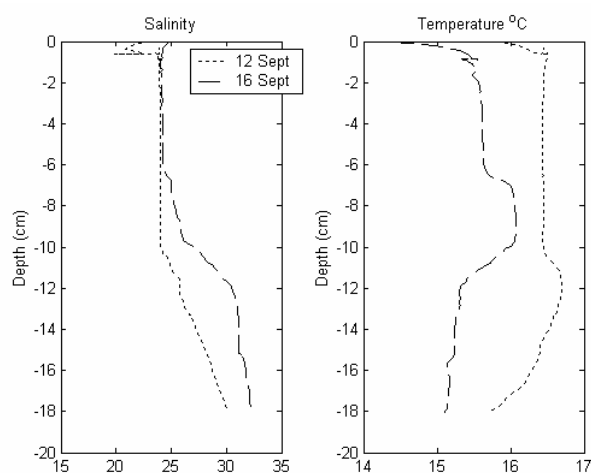


Fig. 5. Vertical profiles of salinity and temperature at the Högholmen farm site measured September 12 (dotted) and September 16 (dashed).

5 cm depth. This could indicate the presence of macrofauna (e.g. the brittle star, *Amphiura filiformis*) at this particular depth interval (Fig. 6).

Moreover, at the Valboholmen farm site, ammonium concentrations were significantly higher (maximum \sim 300 μ M) than at the other sampling stations (maximum \sim 100 μ M). High concentrations of ammonium in pore waters of the anoxic sediments were likely caused by enhanced mineralization at this site. A linear gradient indicated progressive mineralization with depth.

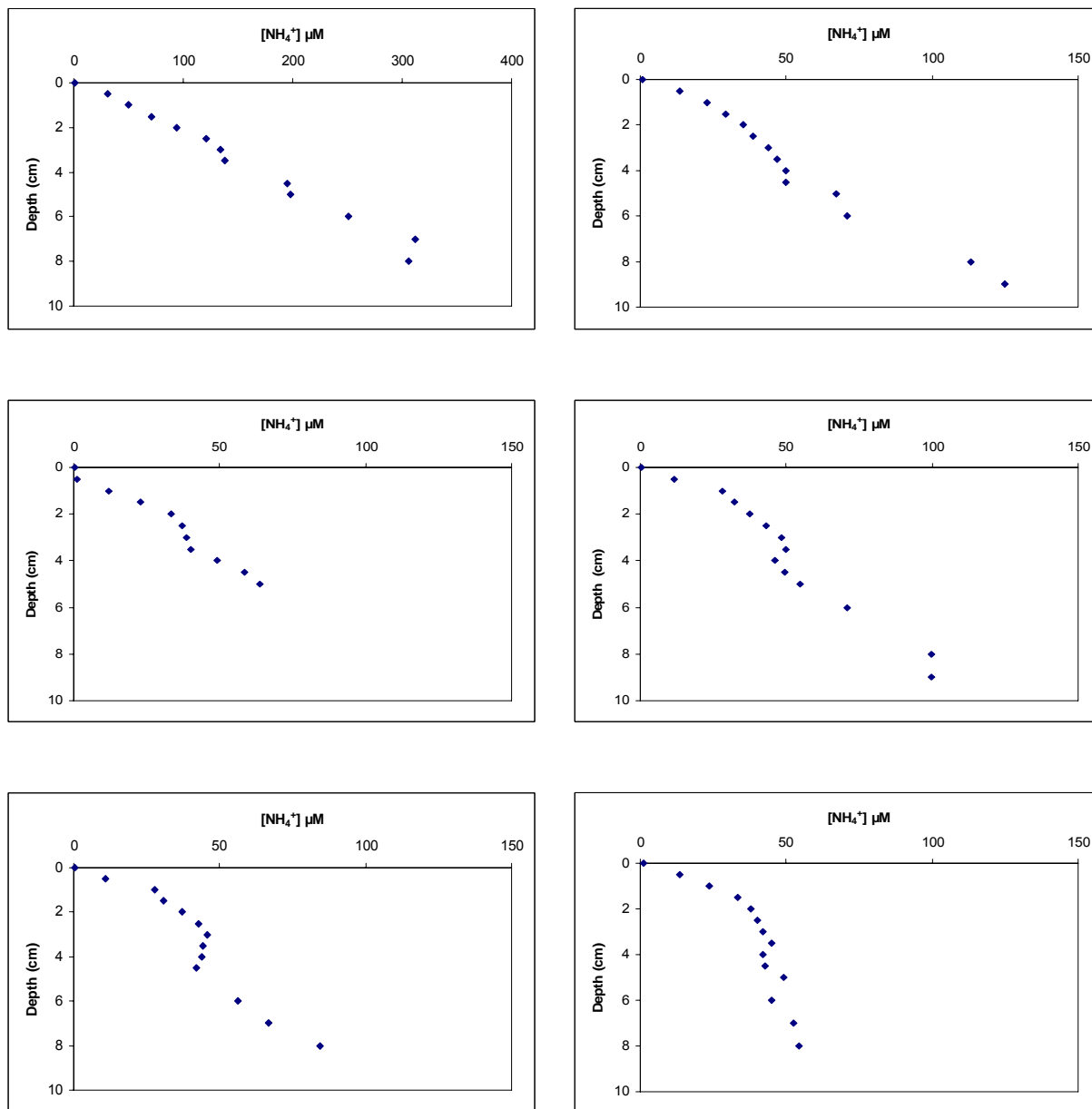


Fig. 6. Distributions of ammonium in the pore water at the Valboholmen (top), the Högholmen (middle), and the Ramsvik (bottom) farm and reference sites. The farm sites are shown to the left, while the reference sites are shown to the right. Note the different concentration scale at the Valboholmen farm site (top left).

In comparison, at the Ramsvik reference station the ammonium gradient decreased rapidly with depth which indicated a distinct transition towards sediment layers with a significantly lower reactivity.

In contrast to the distributions of ammonium, pore water concentrations of DON were not linear with depth (Fig. 7). With the exception of the Valboholmen reference station, there

was no clear trend with depth between stations, or when comparing farm sites with reference sites. In general, concentrations varied between close to or below detection, and $\sim 50 \mu\text{M}$. The indirect measure ($\text{DON} = \text{TN} - \text{DIN}$) of pore water DON indicated shortcomings during the analytical procedure, including e.g. sampling and sample preparation, and the oxidation of organic N to NO_3^- .

Occasionally, measured concentrations of NO_3^- after the wet chemical oxidation

of organic N were even lower than before the oxidation.

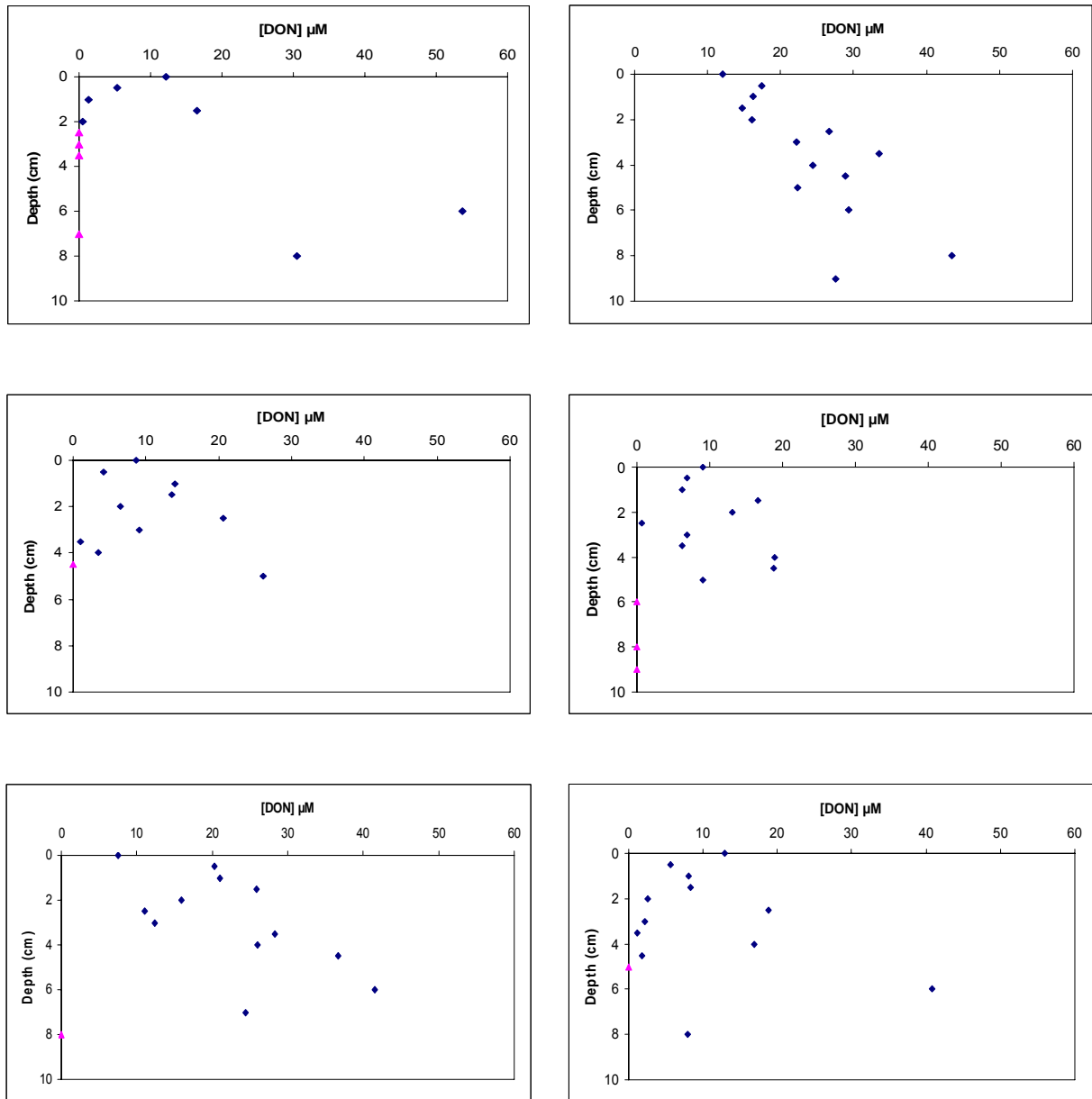


Fig. 7. Distributions of dissolved organic nitrogen (DON) in the pore water at the Valboholmen (top), the Högholmen (middle), and the Ramsvik (bottom) sampling sites. Farm sites are shown to the left, while the reference sites are shown to the right. Sediment depths where the analytical procedure was not working properly are illustrated by triangles.

3.3 Benthic oxygen consumption

Rates of oxygen consumption in the current study (September 2005) were similar for the three mussel farms, and ranged between 290 and 530 $\mu\text{mol m}^{-2} \text{h}^{-1}$. At Ramsvik, however the farm and reference sites were significantly different with higher consumption rates at the reference compared to the farm site (Fig. 8). At both Valboholmen and Högholmen, rates were similar between farm and reference sites. As a response to an additional input of organic material from the mussel farms, sediment oxygen consumption was expected to be higher at the farm compared to the reference sites. However, the general sediment characteristics and hydrographic conditions of the bottom water differed between the three sampling stations. At Ramsvik, for example, visual observations by divers confirmed that sediment particles were resuspended and eroded from the surface sediment (i.e. transport bottom), while sediment particles were trapped at the reference site (i.e. accumulation bottom). A generally higher reactivity of the sediment material, or the result from a recent input of reactive organic material, at the Ramsvik reference site compared to the farm site, was confirmed e.g. by the low bottom water oxygen concentration (92 μM) and the high rates of oxygen consumption (820 $\mu\text{mol m}^{-2} \text{h}^{-1}$) at the reference site.

In general, benthic oxygen fluxes were similar or higher in May than in September (Fig. 9). Higher rates of oxygen consumption in May (380-700 $\mu\text{mol m}^{-2} \text{h}^{-1}$) than in September (290-530 $\mu\text{mol m}^{-2} \text{h}^{-1}$) were recorded despite the significantly higher bottom water temperatures in September (16 °C) than in May (7,5 °C). This is probably a direct consequence of a

distinct input of labile organic material in May compared to in September.

A similar investigation as the current, was performed in March 2005 (L. Ljungqvist, unpublished). Sediment oxygen consumption and ammonium fluxes in farm-associated sediments were 3-7 and 17-200 times higher than at the corresponding reference sites, respectively. These farms were, however, significantly older (~ 10-15 years) than the farms in the present study.

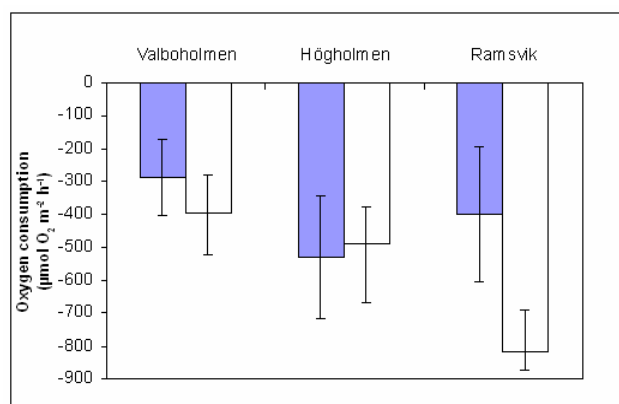


Fig. 8. Rates of oxygen consumption (\pm SD) measured during sediment-water incubations ($n=6$). Filled bars represent farm sites, while open bars denote reference sites.

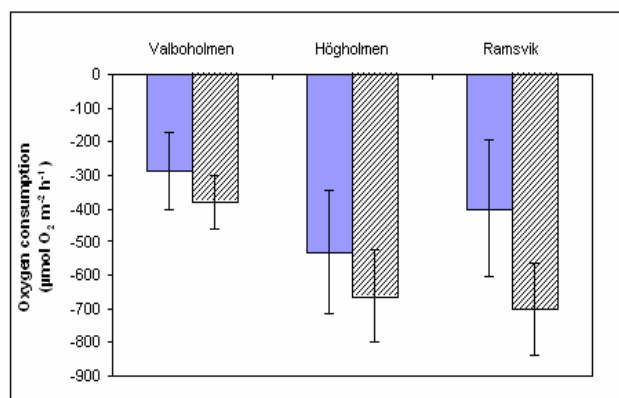


Fig. 9. Comparison of oxygen consumption (\pm SD) between the May (dashed) and the September (filled) sampling occasions ($n=6$).

3.4 DON fluxes and benthic N-cycling

Overall, variations (relative standard deviation) in benthic flux rates between replicate cores were significantly higher for nitrite/nitrate (40-200 %) and DON (30-700 %), compared to those observed for NH_4^+ , HPO_4^{2-} and $\text{Si}(\text{OH})_4$ (10-70 %)(Fig. 10). Possible explanations for a high variability in benthic nutrient fluxes (nitrate in particular) are related to irregular and patchy activities of macrofauna and a heterogeneous sampling site in general. Bioturbating animals alter the multi-dimensional distribution of solutes and particles in surface sediments in patterns related to the function (e.g. feeding behaviour) of the macrofaunal species present. Hence, the availability of oxygen, and thereby rates and pathways of organic material, can be directly coupled to activities and functional biodiversity of macrofauna (Gilbert et al. 2003; Aller et al.2001; Emmerson et al. 2001). A heterogeneous environment under the mussel farms was confirmed by divers (K. Norling, pers. comm.).

There was a negative flux (i.e. from the overlying water to the sediment) of nitrate at Ramsvik (farm and reference) and Valboholmen (farm) (Fig 10). This indicated a pronounced denitrification in these sediments and that additional nitrate was supplied from the overlaying water. At the Ramsvik reference site, benthic NH_4^+ , HPO_4^{2-} , $\text{Si}(\text{OH})_4$ and DON fluxes were comparably high (Fig. 10-11). For example, the DON flux was $53 \mu\text{mol m}^{-2} \text{h}^{-1}$, that is $\sim 2\text{-}10$ times higher than at the other sites (Fig. 11). In conjunction with the high oxygen consumption, high DON fluxes indicated a recent input of labile organic material, a non-steady state situation in the surface sediment layers, and that a large proportion of overall

mineralization took place at the sediment surface.

In a similar a study from Chesapeake Bay (Burdige et. al., 1998), measured DON fluxes from sediments ranged from 3 to $17 \mu\text{mol m}^{-2} \text{h}^{-1}$, and NH_4^+ fluxes ranged from 100 to $400 \mu\text{mol m}^{-2} \text{h}^{-1}$. DON fluxes in the present study were significantly higher ($5\text{-}53 \mu\text{mol m}^{-2} \text{h}^{-1}$) while the NH_4^+ fluxes were significantly lower ($0,5\text{-}10 \mu\text{mol m}^{-2} \text{h}^{-1}$) than observed by Burdige et. al. (1998). In the Chesapeake Bay, DON fluxes were only $\sim 3\text{-}4\%$ of DIN fluxes. In the present study, DON fluxes contributed 20-90% to the total benthic DIN flux (Table 3).

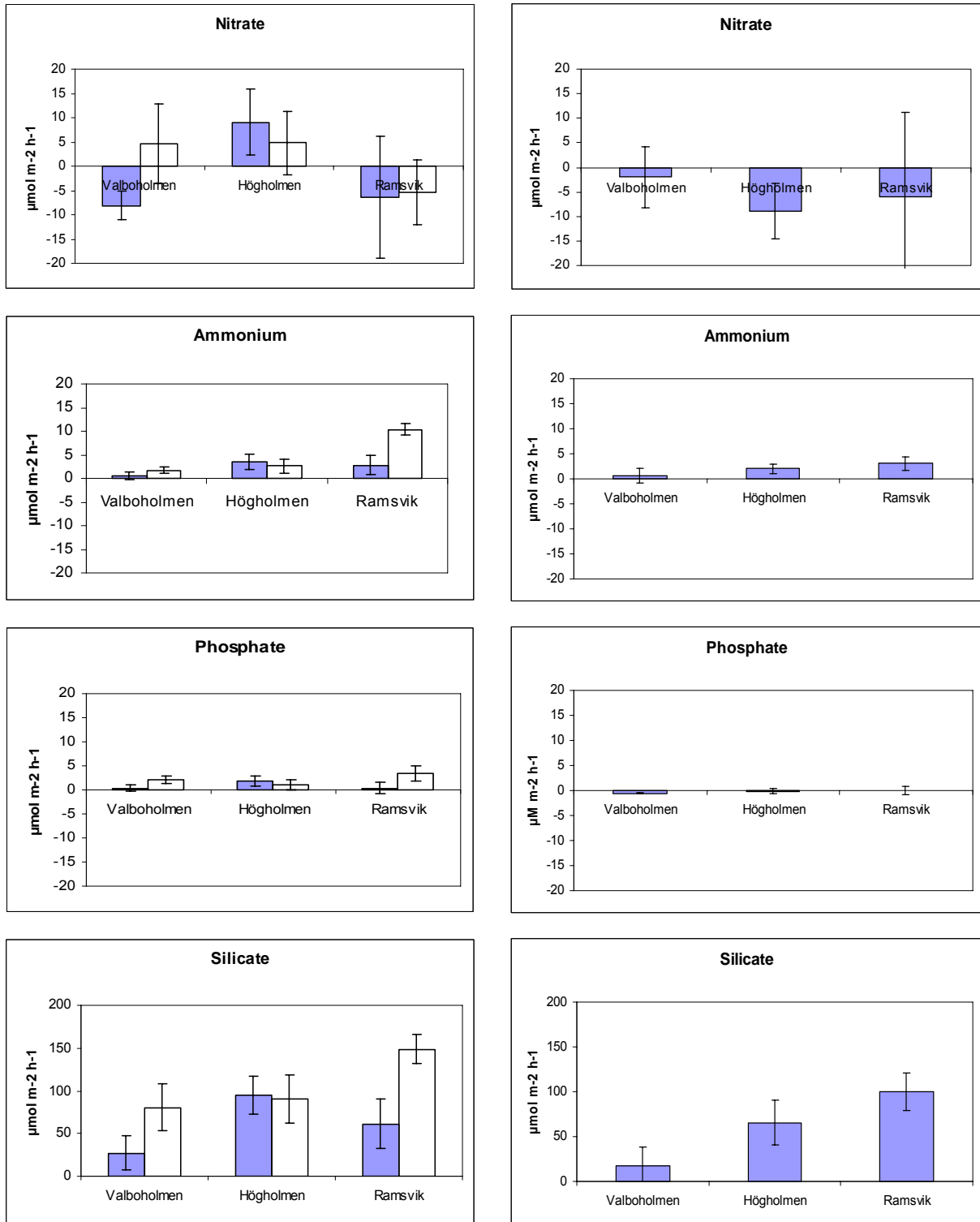


Fig. 10. Benthic nitrate, ammonium, phosphate and silicate fluxes (\pm SD) from September (n=6) and May (n=5) at the Valboholmen, the Högholmen and the Ramsvik sampling sites. The September sampling occasion is shown to the left, while the May sampling occasion is shown to the right. Open bars represent the reference sites (where appropriate), and filled bars denote the farm sites.

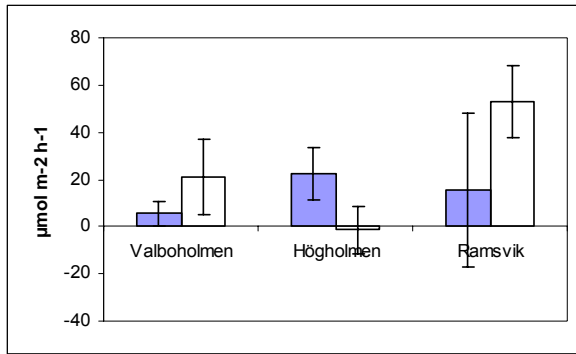


Fig. 11. Rates of benthic DON fluxes (\pm SD) measured during laboratory sediment-water incubations ($n=6$). Filled bars represent farm sites and open bars denote reference sites.

Net rates of ammonium production ($R_{\text{NH}_4^+}$) in the surface sediment was used together with benthic fluxes of ammonium and nitrate to estimate rates and reaction pathways during N-mineralization. Potential nitrification was calculated as $R_{\text{NH}_4^+}$ minus the measured flux of NH_4^+ . Potential denitrification was calculated as the potential nitrification minus the measured NO_3^- flux (Fig. 12). Diffusion gradients of NH_4^+ towards the sediment water interface were calculated from the linear distributions of ammonium in the upper 2 cm of the sediment (Fig 6 and Table 2).

Table 2. NH_4^+ diffusion gradients at the various sampling sites (see Fig 6). The diffusion constants (D_{corr}) were corrected for viscosity and temperature.

Station	NH_4^+ gradient ($\mu\text{M}/\text{cm}$)	D_{corr} (cm^2/s)
Valboholmen farm	45,3	15,2
Valboholmen ref.	17,0	15,2
Högholmen farm	17,7	14,8
Högholmen ref.	18,9	14,8
Ramsvik farm	18,6	14,6
Ramsvik ref.	18,6	14,6

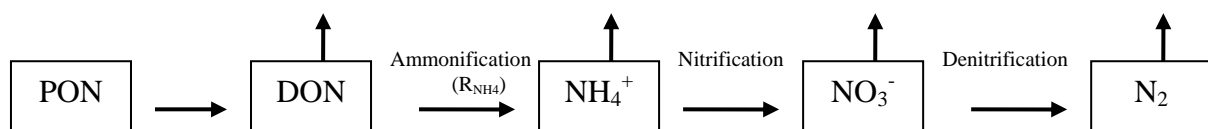


Fig. 12. Simplified illustration of N- mineralization pathways in surface sediments. Relate to Figure 1 for a more detailed presentation of the benthic nitrogen cycling.

Due to the large calculated rate of ammonium production ($13 \mu\text{mol m}^{-2} \text{h}^{-1}$), the comparably low benthic flux of ammonium and the large negative flux of nitrate, potential rates of nitrification and denitrification were significantly higher at the Valboholmen farm site compared to at the other sites (Table 3). Thus, mass balance calculations indicated that a large fraction of N-mineralization proceeded by coupled nitrification/denitrification at the Valboholmen farm site.

At the Ramsvik reference site, ammonium production estimated from the pore water gradient of ammonium was significantly smaller ($5.0 \mu\text{mol m}^{-2} \text{h}^{-1}$) than the benthic flux of NH_4^+ . The negative nitrate flux ($5.4 \mu\text{mol m}^{-2} \text{h}^{-1}$), in combination with the relatively large ammonium and DON fluxes, supported indications of a non-steady state situation and organic matter mineralization at the sediment surface rather than in the sediment. Solute (e.g. NH_4^+) production was therefore not seen as a build-up in the pore water but rather as a concentration change in the overlying water (Fig. 12).

Table 3. Ammonium production, benthic fluxes of ammonium, nitrate and DON, and calculated potential nitrification and denitrification.

Station	$R_{\text{diff.}}(\text{NH}_4^+)$ ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	NH_4^+ flux ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	NO_3^- flux ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	DON flux ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	Potential nitrification ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	Potential denitrification ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	Potential N-deficiency ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	Total N-flux ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	Fraction DON (of total N-flux) %
Valboholmen farm	12,7	0,5	-8,1	5,5	12,2	20,3	0,0	26,3	21,0
Valboholmen ref.	4,7	1,7	4,6	21,1	3,0	insign.	0,0	27,5	76,9
Högholmen farm	4,8	3,5	9,1	22,3	1,3	insign.	0,0	34,9	64,0
Högholmen ref.	5,2	2,6	4,8	-1,4	2,6	insign.	0,0	6,0	-
Ramsvik farm	5,0	2,8	-6,3	15,5	2,3	8,6	0,0	26,9	57,8
Ramsvik ref.	5,0	10,4	-5,4	53,0	0,0	0,0	5,4	58,0	91,3

4. Conclusions

My main conclusions in this thesis are:

-It is not straight-forward to measure reliable concentrations of dissolved organic nitrogen (DON) in complex samples (e.g. pore waters). The “poor” result from the present sampling are probably related to problems with insufficient oxidation of complex organic nitrogen compounds. More research about chemical characterization of DON is therefore required.

-Of the total N-flux in the present study DON fluxes contribute between 20 and 90 %, i.e. occasionally DON fluxes is almost as large as the fluxes of inorganic N.

- There is no obvious difference in oxygen consumption and nutrient flux rates between farm and reference stations (except the Ramsvik farm and reference sites).

Since the mussel farms are relatively young, sediment characteristics, such as bottom type, organic content and benthic activity, seem to benefit more to alterations in flux rates than organic input from the mussel farms.

- The oxygen consumption was higher in May than in September despite higher temperature in the bottom water in September. This is probably a direct

consequence of a high input of labile organic material in May compared to in September.

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