HENRICE M JANSEN

# BIVALVE NUTRIENT CYCLING

NUTRIENT TURNOVER BY
SUSPENDED MUSSEL COMMUNITIES
IN OLIGOTROPHIC FJORDS

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## Nutrient turnover by suspended mussel communities in oligotrophic fjords

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# Abstract

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This study examined a range of eco-physiological processes (i.e filtration, growth, excretion, faeces production) and feedback mechanisms with the aim to investigate the contribution of suspended mussel *Mytilus edulis* communities to nutrient cycling in oligotrophic fjords. Previous work has shown that bivalves have the potential to play an important role in coastal nutrient cycling. Understanding bivalve nutrient dynamics is particularly essential in oligotrophic environments, where bivalve communities potentially have a higher influence as a consequence of low background nutrient levels.

The eco-physiological response of individual mussels to oligotrophic conditions indicated that clearance and biodeposition rates were related to food/nutrient availability and were therefore respectively higher and lower compared to rates determined for eutrophic conditions. No specific responses to oligotrophic conditions were observed for excretion of inorganic metabolites or nutrient storage in tissue. However, *in situ* methods that determined nutrient dynamics along suspended communities (ropes) demonstrated that rates under field conditions may differ from what can be expected from extrapolation of rates measured in the laboratory for individual mussels. Clearance rates were lower for communities while nutrient regeneration was higher, specifically during periods with high fouling activity of ascidians. This study thereby highlights the need to consider community specific processes while evaluating bivalve-ecosystem interactions.

Biodeposition is an important pathway in bivalve nutrient cycling and represented up to 47% of ingested nutrients under oligotrophic conditions. Nutrient releases from decomposing biodeposits were high for all nutrients (C-N-P-Si), and approximately 24% of carbon and 17% of nitrogen in the biodeposits were mineralized with enhanced temperatures resulting in faster decomposition ($Q_{10}$=2-3).

Combining mussel physiology with physical conditions of the systems showed that the fraction of ingested nutrients allocated to either nutrient regeneration (source) or nutrient removal (sink) was similar between oligotrophic fjords and eutrophic bays. Nutrient regeneration was imbalanced for each of the elements and differed from ratios observed in the ambient water. Mussel cultures thereby have the potential to influence phytoplankton community composition. However, positive and negative feedback estimates indicated that present mussel aquaculture in Norwegian fjord systems has low influence on nutrient cycling due to the low bivalve densities and physical characteristics of the fjords.

This thesis provided insights in the pathways in which mussels interact with nutrient cycling, with special reference to oligotrophic conditions. The empirical data collected in this study can be applied to optimize models that simulate bivalve-ecosystem interactions, and thereby help to understand and predict the exploitation and management of coastal zones.

# Preface

The moment I am writing the current preface almost four years have passed since I moved to Norway and joined the CANO-project (Carrying Capacity in Norwegian Aquaculture) to start a PhD on nutrient dynamics in bivalve culture. Working on sustainability issues in aquaculture always had my strong interest and moving to Scandinavia was a long-awaited wish come true. It turned out to be the right decision, and the past years were a fantastic and unforgettable period. I am thankful to both IMARES and IMR providing me the opportunity for this collaboration. I realise this has been an unusual privilege.

And now it has come to its end. Numerous pilot studies, experiments, field work, lab work, data analysis, and writing of manuscripts have resulted in the current thesis. This work would, however, not have been possible without the support of a number of people.

First and foremost I would like to thank my supervisors Øivind Strand, Aad Smaal and Marc Verdegem who guided me through the whole process. Our meetings at Austevoll, "the castle" in Moermond and Wageningen were a good mix of scientific discussions, technical details, evaluation of the progress and took place within an excellent ambiance. These meetings formed the basis for a smooth cooperation. Øivind, your critical mind and broad overview often pushed me in the right direction. Giving me the freedom to find my own way made the project become *mine*, which I appreciated. But more than the technical and scientific support I'd like to thank you for your motivation, encouragement and patience, which pulled me through the periods when I was struggling most. Your everlasting positivity made it a joy to work together. Aad, I would like to thank you for introducing me to the CANO-project, for having confidence in me and for taking me in as your first PhD student. I have learned much from your extensive knowledge on bivalve eco-physiology and bivalve-ecosystem interactions. Our discussions and your critical comments on manuscripts were essential. Marc, being a slight outsider in the bivalve world, but with extensive knowledge on aquatic nutrient dynamics, you often gave a slightly different perspective on the approaches and interpretation of results which was very valuable. The nearly 1000 km between Bergen and Yerseke/Wageningen have not led to any of the typical long-distance student-supervisor troubles, and I simply like to say it was a pleasure and privilege to work together with you.

I also specially like to thank Tore Strohmeier and Cathinka Krogness. Tore, your advice, practical assistance and critical mind have been invaluable. Together with Øivind, you got me introduced, indulged and enthusiastic for bivalve eco-physiology and taught me all about the special features of Norwegian fjord systems. The fieldwork we carried out together was an experience I never would have wanted to miss, and definitely will miss in the coming future. Cathinka, without you the time at Austevoll would not have been the same. Thank you for your technical help, mental support, the fun moments during the samplings and for the numerous dinners at your place.

Being part of the CANO-team introduced me to a number of inspiring and interesting people. Thanks to all of you for the motivating and dynamic meetings. A nice spin-off from CANO has been the modelling work with Ramon Filgueira. Unfortunately I did not obtain the funding to spend some months in Canada to learn all about your models, our long-distance collaboration has been a fruitful -and enjoyable- one anyway. Many thanks.

I am grateful for the link to the industry we had through Åfjord Skjell AS, and in particular with Roar Olson. I enjoyed the days out on Åfjord and learned a lot from the discussions on fjord ecology and Norwegian mussel industry.

# Contents

# Chapter 1

General Introduction

## Background

Suspension feeding bivalves have the potential to influence ecosystem functioning due to their role in nutrient cycling (Dame 1996). Yet, a thorough understanding of the interactions between bivalve communities and their ecosystems is lacking due to its spatial and temporal complexity. Bivalves have a wide global distribution across ecosystems ranging from nutrient-rich (eutrophic) to nutrient-poor (oligotrophic) conditions (Gosling 2003). However, most bivalve cultivation sites are situated in eutrophic coastal areas that are influenced by nutrient-rich river run-off, thereby taking advantage of high food production to realize fast growth (Saxby 2002). As a consequence most studies on bivalve eco-physiology have been performed in those areas, whereas less is known about oligotrophic environments, such as Norwegian fjord systems (Strohmeier et al. 2009). In general there is a need to understand more about bivalve-ecosystem interactions as a function of the physical, chemical and biological properties of a site, and its relation to the type of culture applied. The current thesis investigates the role of bivalves in nutrient dynamics of oligotrophic systems, using suspended culture of the blue mussel *Mytilus edulis* in Norwegian fjord systems as a case study.

*Eco-physiology of the mussel*

The mussel *Mytilus edulis* is one of the most studied bivalves in terms of its eco-physiological responses (Bayne 1998, Gosling 2003, Shumway 2011). These studies have shown that mussels tolerate a wide range of environmental conditions, facilitated by a remarkable plasticity of physiological response. This physiological plasticity can vary between populations, among individuals of the same population, and due to seasonal changes in the natural environment (Hawkins & Bayne 1992, Shumway 2011). Strohmeier (2009) recently demonstrated that mussels can display high feeding rates and high net absorption efficiencies under oligotrophic and low seston conditions despite contradicting feeding paradigms for mussels. This suggests that the physiological response of mussels under oligotrophic conditions may vary from other areas with higher nutrient and food concentration.

*Classification of mussel aquaculture*

Particularly in areas with dense mussel communities, mussels may significantly influence the functioning of the ecosystem (Smaal & Prins 1993, Dame & Prins 1998). Dense mussel communities are often found in aquaculture settings. The culture of mussels can be classified into two categories; either on-bottom or off-bottom cultivation (Hickman 1992). The type of culture has implications for the mechanisms of how mussel communities interact with their ecosystem. On-bottom cultivation or seabed cultivation is based on the principle of transferring mussels (seed) from areas where they have settled in great abundance, to culture plots where they can realise a fast grow-out phase. Off-bottom cultivation comprises various culture methods ranging from grow out on stakes or poles set into the seabed, to methods utilizing ropes suspended from rafts or longlines deployed at the sea surface. Off-bottom cultivation adds a third dimension to the essentially two dimensional on-bottom culture and thereby utilizes a greater proportion of the water column. As a result of the large depths in most fjords, suspended mussel culture situated high above the seabed is the main culture practice applied by the Norwegian mussel industry.

*Mussel communities as intermediates in nutrient cycling*

Mussels have the potential to clear considerable quantities of particulate matter from the water column (Dame & Prins 1998). Nutrients absorbed from the ingested food are used for immediate metabolic needs, or to increase somatic and reproductive tissue. Ingested food particles are digested in the mussel's digestive system (stomach, digestive diverticula, gut) and the undigested particles, together with some metabolic waste products, are expelled as faeces. Excess and low quality food particles are, after pre-ingestive sorting and selection processes, rejected as pseudofaeces (Ward & Shumway 2004). Most metabolic waste products are excreted in a dissolved inorganic form with gaseous exchange of carbon through the gills, and nitrogen and phosphorus ions excreted through the kidneys and leave the body via the renopore. All these processes show seasonal variability, reflecting fluctuations in environmental conditions (e.g. temperature and food quantity and quality) and endogenous requirements (e.g. partitioning between gametogenesis and somatic growth) (Smaal & Vonck 1997, Cranford & Hill 1999). Although insights in the eco-physiological functioning of mussels under eutrophic conditions are well developed, validation of specific rates and interactions under oligotrophic conditions requires further investigation.

Biodeposits (faeces + pseudofaeces) produced by suspended mussel cultures are subject to microbial degradation which may occur at three sites; (i) decomposition of biodeposits trapped in between the mussel-matrix of suspended cultures creates a benthic compartment in the pelagic water column (Richard et al. 2006), (ii) descending biodeposits may decompose in the pelagic phase of the water column (Carlsson et al. 2010), and (iii) biodeposits reaching the seafloor will
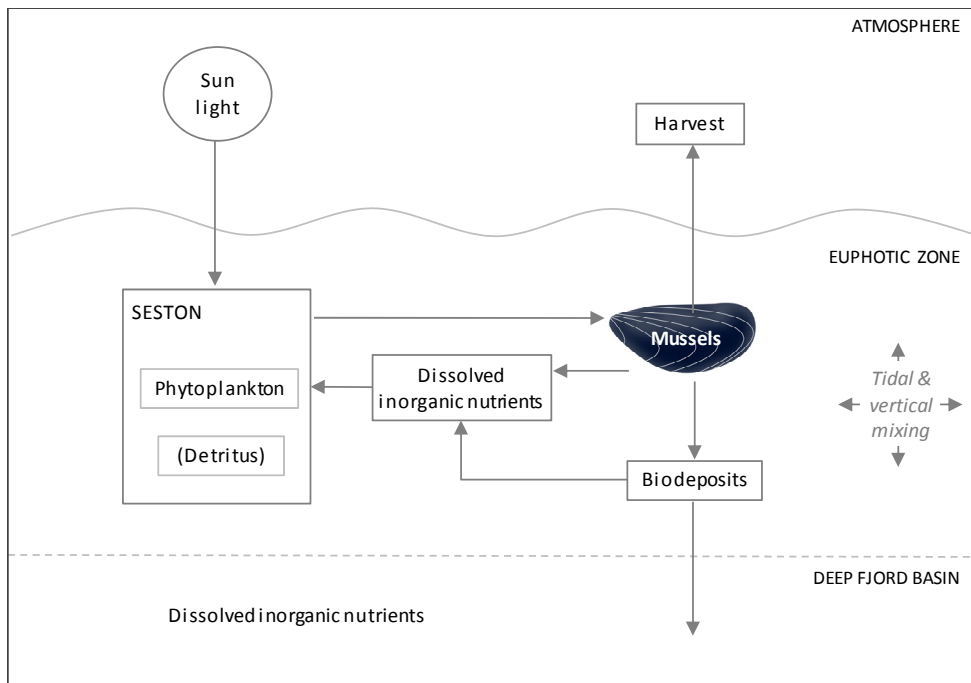


**Figure 1.1**
Diagram of mussel-ecosystem interactions

either be buried in the sediment or be decomposed by benthic processes (Baudinet et al. 1990, Hatcher et al. 1994). The benthic system comprises a complex suite of processes, influenced by oxygen concentration, redox potential and activity of benthic fauna. Biodeposit decomposition leads to nitrogen, phosphorus and silicon regeneration into the water column. Although the importance of biodeposition and remineralization in nutrient cycling is recognized (Fabiano et al. 1994, Cranford et al. 2007), relatively little information is available on decomposition dynamics specific for mussel biodeposits.

Processes at the organism level, such as clearance, ingestion, absorption, respiration and excretion have frequently been studied (among others Bayne & Widdows 1978, Hawkins 1985, Smaal & Vonck 1997, Bayne 1998, Cranford et al. 2011). Conventionally, these organismic rate processes have been extrapolated to yield population estimates (Dame 1996, Gosling 2003). The same approach is used in carrying capacity models. Although these models have addressed processes from farm to coastal ecosystem scales, they are principally constructed by extrapolating an "average" individual rate to a whole population (Beadman et al. 2002, Duarte et al. 2010, Guyondet et al. 2010). However, dense mussel populations consist of a complex community, incorporating mussels, bacteria, epifauna and biodeposits, each contributing to nutrient uptake and release. Approaches that extrapolate individual rates may not accurately reflect community scale processes as community specific effects, such as metabolic activity of associated fauna and decomposing organic material on mussel ropes (AFOM), are neglected. Insight is lacking on the composition and dynamics of mussel rope communities. Furthermore, information on how to link individual rates to community and ecosystem level is missing.

*Feedback mechanisms between mussel communities and the ecosystem*
The biological processes related to mussel communities interact with nutrient cycling in coastal ecosystems through various feedback systems (see reviews by Prins et al. 1998, Newell 2004 and Figure 1.1). The impact of mussels on nutrient cycling at ecosystem level potentially influences primary production (Prins et al. 1995), and consequently the carrying capacity of the ecosystem (Smaal & Heral 1998). The feeding activity of mussel communities may influence the abundance of phytoplankton and thereby exert a 'top-down' control on primary production (negative feedback). Meanwhile, mussel excretion and decomposition of biodeposits exert a 'bottom-up' control on primary production by the regeneration of inorganic nutrients (positive feedback). Given the fact that phytoplankton require nutrients in fixed ratios (Redfield ratio; Redfield et al. 1963), the 'bottom-up' control is influenced by both nutrient availability and stoichiometry of the regenerated nutrients. 'Top-down' and 'bottom-up' control occur simultaneously, and the extent and effect on the coastal ecosystem are situation specific and are determined by physical and environmental conditions of the area (Newell et al. 2005).

## Scope and objective of the thesis

The main objective of this thesis was to investigate the contribution of suspended mussel communities in nutrient cycling of oligotrophic systems. We investigated nutrient dynamics in mussel rope cultures using an array of experimental tools, ranging from individual respiration chambers to *in situ* pelagic chambers measurements. The different chapters in the thesis follow the spatial order from individual (Chapter 2 & 3) over community (Chapter 4 & 5) to farm/ecosystem scale (Chapter 6). Each chapter describes a specific aspect of nutrient dynamics related to mussel activity. Combing the insights gained throughout the chapters eventually

elucidate the main objective. To allow integration and considering the fact that plankton dynamics are influenced both by nutrient availability and stoichiometry (Redfield ratio; Redfield et al. 1963) a multiple-element approach (C-N-P-Si) was applied throughout the thesis.

*Chapter 2: Nutrient allocation in individual mussels*

The intra-annual variability in tissue nutrient concentrations, egestion, respiration and inorganic excretion were determined for individual mussels. Allocation of carbon, nitrogen, phosphorus and silicon to the various metabolic processes was investigated and related to endogenous requirements (gamotegenesis and somatic growth) and exogenous factors (temperature, food availability).

*Chapter 3: Biodeposit mineralization*

Mineralization dynamics for decomposing mussel biodeposits were determined during different seasons. Fluctuations in mineralization rates could subsequently be related to changes in temperature and biodeposit nutrient composition.

*Chapter 4: Nutrient regeneration by mussel communities*

Nutrient regeneration from mussel communities is defined by the integration of mussel metabolism and dynamics of the 'associated fauna and organic matter' (AFOM) complex. Nutrient release rates from mussel communities (suspended mussel ropes) were quantified *in situ* with pelagic chambers during an annual cycle, and related to mussel growth and dynamics of AFOM complex.

*Chapter 5: Scaling from individual mussels to communities*

The question is whether eco-physiological rates determined for individual mussels can be summed to yield community estimates. We compared individual (laboratory) and community (*in situ*) based estimates for clearance, respiration and nutrient release with the aim to determine which part of the overall community estimates could be attributed to physiological activity of the mussels and which part could be related to community specific processes.

*Chapter 6: General Discussion*

Chapters 2 to 5 each provided more insight in specific nutrient pathways, their seasonal variability and driving forces. In the 'General Discussion' data from previous chapters have been collated, and the feedback from mussel communities to primary producers have been considered at ecosystem level. To understand the effect of trophic conditions and site-specific interactions, results of the present study were placed into a broader context based on a literature overview describing effects of mussel cultures across cultivation areas around the world. This allowed us to answer the overall objective of this study on the bivalve-environment interactions in oligotrophic systems.

# Chapter 2

## Nutrient allocation by the blue mussel

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## ABSTRACT

To evaluate the potential role of mussels in nutrient cycling in oligotrophic fjord ecosystems, we applied a multiple-element (C-N-P-Si) approach considering several physiological processes (excretion, tissue composition, biodeposition) simultaneously. The study covered one annual cycle, reflecting the effect of endogenous and exogenous factors. Respiration (6 - 29 $\mu$mol C g$^{-1}$ hour$^{-1}$), ammonia (0.3 - 2.2 $\mu$mol N g$^{-1}$ hour$^{-1}$) and phosphate (< 0.3 $\mu$mol P g$^{-1}$ hour$^{-1}$) excretion were correlated to food (POC) and temperature. Tissue nitrogen (127 − 167 mg N g$^{-1}$), carbon (547 − 629 mg C g$^{-1}$) and phosphorus content (547 − 629 mg P g$^{-1}$) seemed related to reproductive processes. Nutrient turnover showed a seasonal pattern with fast turnover in summer and slow turnover in winter. On an annual basis, nitrogen turnover (643 days) was slower compared to phosphorus (290 days) and carbon (181 days). Fluctuations in biodeposition rates (11 - 72 mg g$^{-1}$) were correlated to food parameters. Although the food concentration in our study area was low (Chl $a$ < 2 $\mu$g l$^{-1}$; POC < 546 $\mu$g l$^{-1}$) and food quality high (CN ~9), physiological rates, except biodeposition, were similar to rates reported in other cultivation areas. The relative importance of each of the processes determines how mussels contribute to nutrient cycling in coastal ecosystems. Respiration and excretion of dissolved metabolites (1639 mg C, 92 mg N, 23 mg P g$^{-1}$ y$^{-1}$) is regarded as a source of regenerated nutrients and may enhance nutrient availability in the euphotic zone with potential consequences for phytoplankton dynamics. Nutrient accumulation in tissue material (560 mg C, 168 mg N, 12 mg P g$^{-1}$ y$^{-1}$) is regarded as a sink of nutrients given that mussels will be harvested. Biodeposition (981 mg C, 113 mg N, 9 mg P g$^{-1}$ y$^{-1}$) is regarded as another nutrient sink in fjord ecosystems, although a fraction is regenerated in the euphotic zone. This shows a dissimilar allocation of elements to each of the physiological processes, resulting in relatively more nitrogen being accumulated in tissue material, and relatively more phosphorus being regenerated. The relatively high fraction of regenerated nutrients (25 - 53%) and the low fraction of nutrients allocated to biodeposition (20 - 31%) indicates that the potential role of mussels in nutrient cycling in oligotrophic fjord systems is different from shallow eutrophic areas.

## Introduction

Mussels have the potential to optimize their nutrient balance by regulating food uptake quantitatively as well as qualitatively, and are capable of recycling endogenous nutrient reserves through a complex suite of metabolic processes (Kreeger et al. 1995). In doing so, mussels act as a sink or source of nutrients and thereby interact with nutrient cycling in coastal ecosystems, particularly in areas with dense mussel communities (see reviews by Prins et al. 1998, Newell 2004). The major pathways in which mussels interact with coastal nutrient cycling are; (*i*) filtration of seston from the water column, (*ii*) nutrient storage and growth of mussel tissue, (*iii*) excretion of faecal material, and (*iv*) excretion of inorganic metabolic waste products (Prins et al. 1998, Newell 2004). These processes show seasonal variability, reflecting environmental fluctuations in temperature and food supply and endogenous metabolic requirements of the mussels such as gametogenesis and somatic growth (Smaal & Vonck 1997, Cranford & Hill 1999). The ecological importance of the interaction between mussels and nutrient cycling is the potential effect on primary production (Prins et al. 1995), and consequently on the carrying capacity of the ecosystem (Smaal & Heral 1998).

Numerous studies have focused on one aspect of the nutrient cycle, e.g. excretion of dissolved metabolites (Bayne & Widdows 1978, Hatcher et al. 1997), biodeposit production and decomposition (Kautsky & Evans 1987, Baudinet et al. 1990, Callier et al. 2006), or use a single-element approach (e.g. nitrogen by Cranford et al. 2007). There are only a few studies combining different components of nutrient cycling. Hawkins and Bayne (1985) and Kreeger et al. (1995) studied the allocation of carbon and nitrogen towards different metabolic processes, and showed that most of the ingested carbon and nitrogen were expelled with faeces. Both studies also demonstrate that the relative importance of each of the metabolic processes varies seasonally since nutrient allocation is influenced by temporal dynamics of the endogenous and exogenous processes. They also identified that nitrogen was more efficiently retained than carbon. The role of mussels in phosphorus cycling is less well understood than for carbon and nitrogen (Newell 2004). Smaal and Vonck (1997) were the first ones to study simultaneously carbon, nitrogen and phosphorus budgets in *Mytilus edulis*. They showed that nitrogen is better retained than phosphorus and carbon. This result was opposite from Hatcher (1994) who showed that phosphorus turnover exceeded nitrogen turnover for various benthic invertebrates. The studies presented by Hawkins and Bayne (1985), Kreeger et al. (1995) and Smaal and Vonck (1997) demonstrate that to understand the contribution of mussels to nutrient dynamics in coastal areas it is essential to consider several aspects of the nutrient cycle simultaneously. Given the fact that phytoplankton dynamics are influenced both by nutrient availability and stoichiometry (Redfield ratio; Redfield et al. 1963) it is required to apply a multiple-element (C-N-P-Si) approach. By doing so, we will be able to evaluate the potential role of mussels in nutrient cycling in coastal ecosystems. The above mentioned studies were all carried out in areas with higher phytoplankton biomass and sediment resuspension, whereas less is known about oligotrophic and low seston environments. Particularly under oligotrophic conditions the interaction of mussels with coastal nutrient cycling might be a significant factor in understanding the system's productivity.

The objectives of this study were (*i*) to evaluate how nutrient turnover of the different elements is regulated by the blue mussel under oligotrophic conditions throughout one annual cycle, and (*ii*) to quantify accumulation and release of nutrients by mussels (*Mytilus edulis*) in order to determine potential sinks and/or sources in nutrient cycling in oligotrophic fjord systems. The study was carried out in South-West Norway which is classified as an oligotrophic area (Chapter 4). The euphotic zone is nutrient limited for extended periods of the year (Paasche

& Erga 1988, Sætre 2007) generally resulting in low chlorophyll *a* (Chl *a*) levels. Seston in these areas is mainly composed of phytoplankton (Erga 1989a, Erga et al. 2005, Strohmeier et al. 2009), and hence food availability for mussels in Norwegian fjords is low while the quality of the food is relatively high (Strohmeier et al. 2009). The physiological responses of mussels in Norwegian fjords may therefore differ from other culture areas. In this paper we explore the hypothesis that uptake and release of nutrients by mussels depends on trophic conditions of the system.

## Materials and Methods

Intra-annual variability in nutrient tissue composition, egestion rates and nutrient excretion was determined for mussels (*Mytilus edulis*) kept under oligotrophic conditions. The experimental period started in January 2009 and lasted until January 2010.

### Experimental facilities

The study was carried out at Austevoll Research Station which is situated in a fjord area in South-West Norway (N60º05´, E005º16´). The laboratory facilities received unfiltered seawater from 1.5 m depth at the research station. Water was pumped into two header tanks (400 L) and excess water left the tanks by means of an overflow outlet. The estimated resident time in the header tanks was less than 0.2 hours, and hence environmental conditions were assumed to be similar to natural conditions in the ambient water.

### Environmental conditions

Measurements for environmental conditions were performed next to the intake point for water used in the laboratory. Fluorescence, temperature and salinity were simultaneously measured at 30 minutes intervals using a STD/CTD 204 (SAIV A/S, Norway). Water samples (250-500 ml) for analysis of food quantity and quality were filtered onto 0.7 µm Whatmann GF/F filters at weekly intervals (twice a week during the spring bloom). Particulate organic carbon (POC) and nitrogen (PON) concentrations were determined using a Thermo Finnigan Flash EA 1112 NC Analyzer after drying and fluming the filters over concentrated HCL for 0.5 h in a closed container to remove inorganic carbon (Ehrhardt 1983). Chlorophyll *a* (Chl *a*) and phaeopigment (Phaeop.) concentrations were analyzed after extraction with 90% acetone using the fluorescence method with correction for acidified measurements (Strickland & Parsons 1968). The fluorometer (Turner Designs Model 10-AU) was calibrated with known concentrations of Chl *a* (Sigma Chemicals, St. Louis, Mo., USA) and measured spectrophotometrically. Fluorescent data (F in $\mu g \, l^{-1}$) were calibrated against Chl *a* ($\mu g \, l^{-1}$) concentrations using the equation

Chl $a = 0.75 \, ^* \text{florescence} - 0.06$ ($r^2 = 0.83$, n = 55)

Once a month water samples were collected for determination of phytoplankton community composition and abundance according to Utermöhl (1931) using Lugol as preservative.

### Experimental design and mussel stock

Mussels used in the experiments were cultured on mussel ropes and kept at the same location where water was taken in to the laboratory (0.5-1.5m depth). Mussel ropes were transferred from a commercial mussel farm (Åfjord, N63º55´, E0010º11´) to the study site in November 2008. The mussels (age 1.5 year at start of the study) settled in 2007 and were re-socked in February 2008. In May 2009, the mussel stock was lost due to predation. Therefore new mussel ropes were

transferred from the commercial farm to the study site by the end of May. These mussels originated from the same cohort and farming site as the initial batch.

Each month 50 mussels were collected from the mussel ropes. 15 mussels were directly frozen until analysis for nutrient tissue composition. The remaining mussels were placed in a 50 litre flow-through tank for a 2 hour acclimatization period before starting the biodeposition and metabolic experiments.

### Mussel biomass and nutrient composition of tissue material

Individual mussel length was determined with a digital calliper (± 0.01mm). Mussels were thawed in aluminium trays to retain leached water and tissue was removed from the shells. Tissue samples were dried at 60 °C for at least 72 hours to determine dry weight (DW), and combusted at 450 °C for 6 hours to determine ash free dry weights (AFDW).

From each sampling, tissue of 15 mussels was homogenized and samples from five individuals were pooled, resulting in three groups. A subsample of the tissue material was analyzed using a Thermo Finnigan Flash EA 1112 NC Analyzer to determine organic carbon and nitrogen content in mussel tissue. To determine organic phosphorus in the tissue material a subsample was analyzed using spectrophotometric methods as described by Grasshoff et al. (1999).

### Biodeposition measurements

Measurements on individual egestion rates of mussels were performed by transferring 10 mussels from the acclimatization tank to individual flow-through chambers. The internal dimensions of those chambers were 3.8 cm (width) x 19.5 cm (length) x 8.1 cm (height), and the chambers received a continuous flow of seawater (50 ml min$^{-1}$). This chamber design restrains internal recirculation and helps to prevent refiltration of the water by the mussels (Strohmeier et al. 2009). The mussels were removed from the chambers after 24 hours and biodeposits of the individual mussels were collected with syringes. Quantity (total particulate material - TPM; Particulate organic matter - POM) and quality (fraction organic material - %OM) of the biodeposits was determined by filtering the egested material onto pre-combusted and weighed 1.2 μm filters (Whatman GF/C). Salt was expelled by rinsing each filter with deionised water. Filters were dried at 60 °C over night to determine TPM values, and combusted at 450 °C for 6 hours to determine the inorganic fraction of the biodeposits.

Additionally, mussel biodeposits were collected within a study on decomposition rates of biodeposits (Chapter 3). The mussels had the same origin, and were kept in the acclimatization tanks and thus can be considered similar to the mussels used in this study. Deposits were collected on a daily basis during defined periods, with five overlapping samplings dates to the current study (6 & 16 March, 15 April, 9 September, 18 November). To obtain more information on biodeposit nutrient content, we analyzed the biodeposits collected on these dates for organic carbon, nitrogen and phosphorus concentration by methods described in Section 'Mussel biomass and nutrient composition of tissue material'.

### Respiration and inorganic nutrient excretion measurements

Respiration and dissolved inorganic nutrient excretion measurements of individual mussels were performed using 1.2 liter incubation chambers. In total we used five experimental chambers each containing five mussels, and two control chambers without mussels. The incubation chambers were placed in a water bath with running seawater to maintain ambient temperature during the incubations. Prior to the incubations, a water flow (>0.5 l h$^{-1}$)was

supplied through the chambers for at least 30 minutes to let the mussels acclimatize. After this period it was checked whether the valves of the mussels were open, and incubation was started by shutting down the water flow. Incubations were terminated when the oxygen concentration had decreased 10% compared to initial values, resulting in incubation times ranging from 0.5 to 3.5 hours, depending on the season. All incubations were performed in the dark to exclude absorption of nutrients by phytoplankton. Oxygen measurements (optode no. 4835, Aanderaa) and water samples for dissolved inorganic nutrient concentrations were taken in all chambers at the start and end of each incubation. Samples (total 20 ml) for nitrite, nitrate, phosphate and silicate were preserved with chloroform and stored in a cool and dark place until analysis. Those samples were analyzed according to standard methods (Parson et al. 1992) adapted for an auto-analyzer. Total Ammonia Nitrogen (TAN) samples (20 ml) were directly frozen until analysis. TAN concentrations were analyzed by means of fluorometric analysis (Kerouel & Aminot 1997, Holmes et al. 1999). Following each incubation mussels were frozen for biomass analysis.

Respiration and nutrient excretion rates were calculated as the difference between the start and the end values, multiplied by the chamber volume and standardised to one hour. Rates were corrected for the control measurements, although fluxes in the control chambers fluctuated around zero for the majority of the measurements.

*Data standardization and statistical analysis*

To standardize for changes in body weight throughout the study period respiration and excretion rates were transformed to an equivalent mean individual of 1 g tissue AFDW using:

$$Y_s = (\frac{W_s}{W_e})^b \times Y_e$$ 

(Equation 2.1)

where $Y_s$ is the standardized parameter, $W_s$ is the standardized weight (1 g), $W_e$ is the mean weight of the experimental animals, $Y_e$ is the mean measured rate and $b$ is a mean weight exponent. A $b$ value of 0.7 (Smaal et al. 1997) was chosen for all variables. Nutrient tissue content and biodeposition rates were standardized to a mean individual (1 g) by linear correction for body weight of the measured individual.

Ingested nutrients are allocated to growth, metabolic requirements or are rejected with the faeces. To estimate the scale of each of those processes a nutrient budget was established for annual rates and for each season (Jan-Mar, Apr-Jun, Jul-Sept, Oct-Jan). Excretion, biodeposition and accumulation rates have therefore first been calculated for per individual mussel, and subsequently been standardized to a mean individual (1 g) by correcting for average body weight during the different seasons.

Elemental turnover (C-N-P) is defined as the time needed to excrete an amount of an element equivalent to the amount contained in the tissue (Hatcher 1994), and is calculated using:

$$ET = \frac{TC}{E}$$ 

(Equation 2.2)

where $ET$ is element turnover in days, $TC$ is the element content in tissue material, and $E$ is excretion per day. Oxygen consumption (respiration) was converted to C-excretion based on a mean Respiratory Quotient (RQ) of 0.85 (Hawkins & Bayne 1985).

Prior to statistical analysis, all data were checked for homogeneity and normality of variance assumptions by (*i*) visually examining standardised residuals versus predicted values plots and Q-Q plots of residuals, (*ii*) Shapiro-Wilk tests and (*iii*) Levene tests (Quinn & Keough 2002). One-way Analysis of Variances (ANOVA) tests were used to test temporal variability in the following variables separately: mussel weight, mussel length, total and organic biodeposition rates,
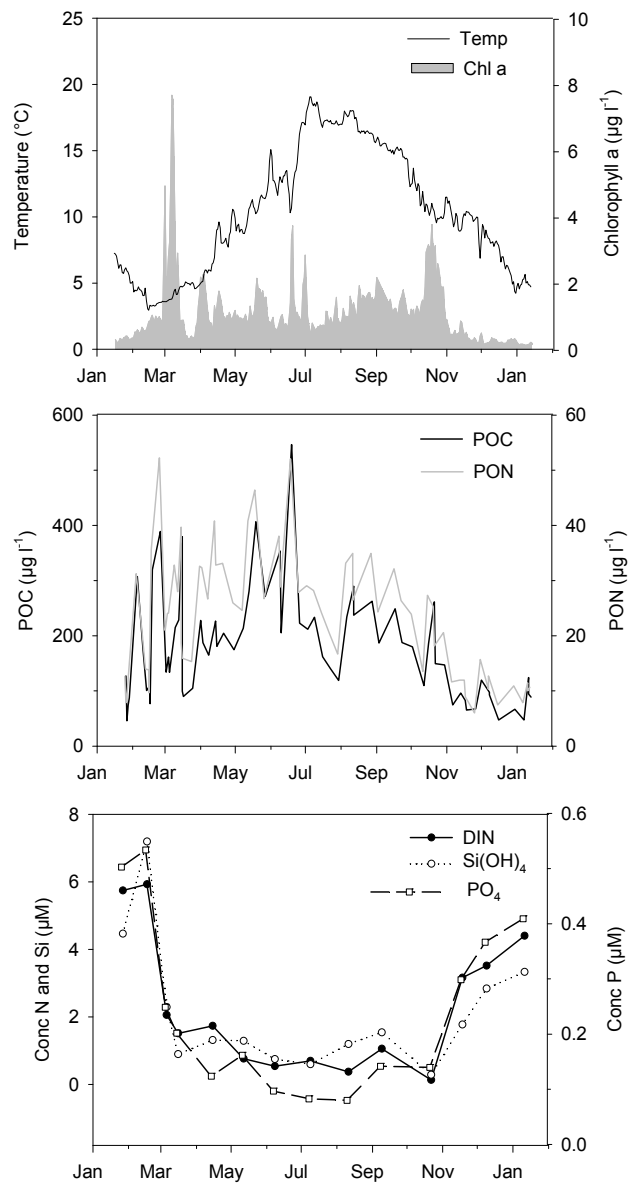
**Figure 2.1**

Seasonal variability in environmental conditions at the study site. (A) Temperature and Chlorophyll *a* concentrations, (B) Particulate organic carbon (POC) and nitrogen (PON), (C) Dissolved inorganic nitrogen, phosphate and silicate

respiration, TAN excretion, phosphate and silicate excretion. In case of significant results Tukey's HSD post hoc multiple comparison tests were used to determine which of the sampling months were significantly different from each other. In case of the parameter 'O:N excretion ratios' the assumptions of homogeneity and normality of variance were violated, even after transformations of the data. Therefore non-parametric Kruskal-Wallis tests, followed by pairwise comparisons with Mann-Withney $U$ tests, were used. To explore the relation between inorganic excretion and biodeposition rates and environmental parameters, Pearson correlation analysis were performed using Bonferroni-adjusted probabilities. All statistical analyses were performed using SAS 9.1, and data are presented as mean ± standard error (SE), unless stated otherwise.

## Results

*Environmental factors*

Average daily water temperature ranged between 3ºC in February to 19ºC in July (Figure 2.1a) and salinity was stable throughout the study period with average daily values of 29.7 ± 1.6 ppt (mean ± SD). The spring bloom started in late February and lasted for approximately 2 weeks (Figure 2.1a), and primarily consisted of an increase in diatoms (3250 cells ml$^{-1}$ measured on the 4$^{th}$ of March, Figure 2.2). Maximum Chl *a* values (7.7 µg l$^{-1}$) were recorded on the 7$^{th}$ of March. Chl *a* concentrations varied between 1-2 µg l$^{-1}$ from mid-March to October, followed by an autumn bloom in October (max. 3.8 µg l$^{-1}$). From November to January the Chl *a* concentrations were below 0.5 µg l$^{-1}$. Flagellates, especially the ones smaller than 5µm, were the most numerous phytoplankton group present throughout the year and varied between 500 and 1980 cells ml$^{-1}$. There were three distinct diatom blooms; during the spring and autumn bloom *Skeletomena* species dominated the diatom abundance and in May *Leptocylindrus danicus*, *Pseudo-nitzschia calliantha* and *Pseudo-nitzschia seriata* were the most abundant diatom species. During those three periods, diatoms comprised 50-70% of the total phytoplankton abundance. Dinoflagellates represented 0.2% to 6% and ciliates <0.2% of the total phytoplankton abundance.
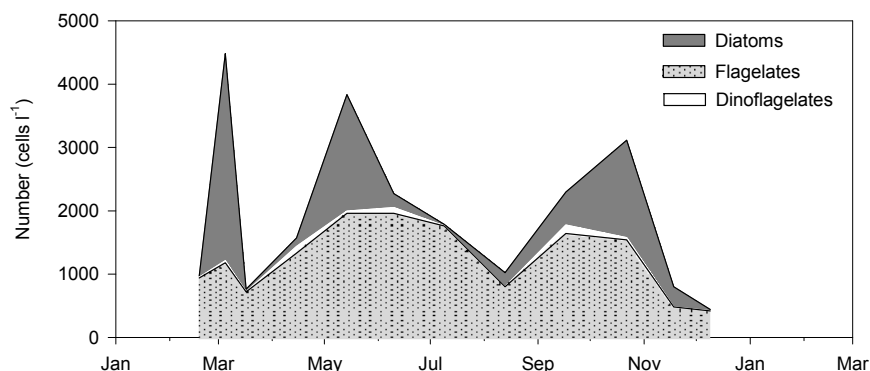


**Figure 2.2**
Phytoplankton community composition from February to December 2009

Particulate organic carbon (POC) ranged between 45 - 546 µg l$^{-1}$ and nitrogen (PON) between 6 - 52 µg l$^{-1}$ (Figure 2.1b). There was a strong correlation between POC and PON concentrations POC = 7.77·PON; $r^2$ = 0.86; n = 67), with highest concentrations in spring and summer, decreasing concentrations during autumn, and lowest concentrations in winter. The average annual Carbon:Nitrogen (CN) molar ratio was 8.9 ± 1.6 (mean ±SD). POC and Chl $a$ concentrations did not correlate ($r^2$ = 0.047; n = 50).

Dissolved inorganic nutrient concentrations (N-P-Si) were high in winter, dropped sharply during spring and remained at low levels throughout summer until they increased again in late autumn (Figure 2.1c). Total inorganic nitrogen was calculated as the sum of total ammonia nitrogen (TAN), nitrate and nitrite. In particular during winter conditions, the total nitrogen concentration was highly dominated by nitrate (> 90%).

*Mussel growth*
Individual length increased from 34.1 ± 0.9 mm to 51.0 ± 1.0 mm from January 2009 to January 2010 (Figure 2.3). Mussel tissue weight (AFDW) increased from 197 ± 17 to 822 ± 65 mg indiv$^{-1}$ between January and November 2009, but decreased to approximately 540 mg indiv$^{-1}$ during the two subsequent months (December 2009 and January 2010). Tissue weight estimates in December and January (2010) were significantly different from the two previous months (October-November), whereas length estimates were not significantly different between those periods, indicates that the weight decrease was caused by a decrease in tissue material and not due to sampling of smaller individuals. The relationship between tissue dry weight (DW) and ash free dry weight (AFDW) can be described by the following equation:
AFDW = DW $^*$ 0.74 ($r^2$ = 0.96; n = 350).

*Nutrient composition in tissue material*
Organic carbon in mussel tissue varied between 547 and 629 mg C g$^{-1}$ AFDW, organic nitrogen varied between 127 and 167 mg N g$^{-1}$ AFDW and organic phosphorus varied between 6.5 and 14.4 mg P g$^{-1}$ AFDW (Figure 2.4). Minimum values were recorded in June for carbon as well as for nitrogen. Highest phosphorus concentrations were recorded in the period January-June, with maximum values in April. Average annual CN ratio was 4.9 ± 0.1, with significant higher ratios observed in summer compared to winter. Average NP ratio was 35.4 ± 1.4, with values below the average during the period January-June and values above the annual average during the period August-December. The average annual CP ratio was 172.7 ± 7.5.

*Biodeposition*
Production of pseudofaeces was only observed during the spring bloom (early March 2009), which was also the time when maximum biodeposition rates were recorded (71.5 ± 5.4mg g$^{-1}$ d$^{-1}$, Figure 2.5). Although biodeposition rates varied significantly throughout the year, there was no clear seasonal pattern. Biodeposition rates were high during the spring bloom and in May, September and December. The fraction of organic material (OM) in the faeces varied between 22% and 53% (average 36%), with lowest fractions in winter (January, February, beginning of March; < 30%) and highest in summer (July, August; > 45%). Seasonal variations in biodeposition rates correlated with food quantity parameters (Table 2.1). Phytoplankton counts showed strong correlation to both total ($p$ < 0.001) and organic ($p$ < 0.001) biodeposition. Looking in more detail to the total phytoplankton counts we see that biodeposition rates are particularly correlated to the number of diatoms (r = 0.62; $p$ < 0.0001) and not to the number of flagellates (r = 0.09;
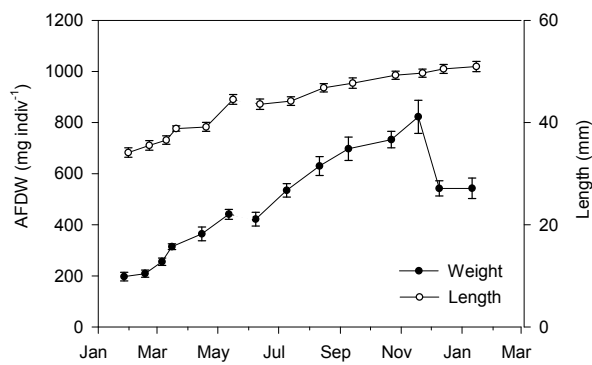
**Figure 2.3**
Mussel (*Mytilus edulis*) tissue weight (mg AFDW indiv$^{-1}$) and shell length (mm). Data are expressed as average (± standard error). Gap between May and June sampling specifies the time of mussel batch replacement.
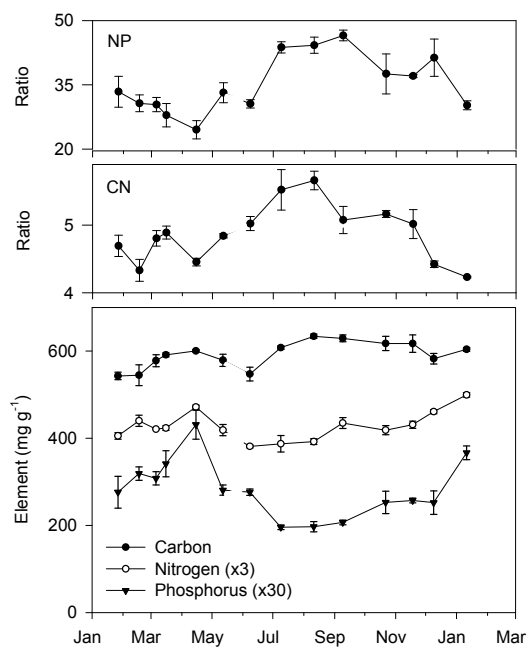


**Figure 2.4**
Nutrient composition (mg C, N, P g$^{-1}$ tissue AFDW) and nutrient molar ratios (CN / NP) in mussel (*Mytilus edulis*) tissue. Data are expressed as average (± standard error), where concentrations of phosphorus (P) and nitrogen (N) are scaled to carbon (C) by means of multiplication by a factor 3 and 30, respectively. Gap between May and June sampling specifies the time of mussel batch replacement.

$p$ = 0.10). Carbon, nitrogen and phosphorus concentrations in the mussel biodeposits were 88.2 ± 18.4, 10.4 ± 2.2 and 1.0 ± 0.1 mg per g$^{-1}$ DW, respectively, and showed large seasonal variation with lowest values in March and high values in April and September (Table 2.2).

*Respiration and excretion of dissolved inorganic nutrients*
Respiration increased significantly during the spring bloom with elevated levels in summer which were double the spring bloom values, and decreased in autumn (Figure 2.6a). Respiration rates varied between 7 µmol g$^{-1}$ hour$^{-1}$ in January (2009 & 2010) and 34 µmol g$^{-1}$ h$^{-1}$ in June, and summer respiration rates (May-August) were on average 2.5 times higher compared to the rest of the year. Total ammonia nitrogen (TAN) was the main component of the total released inorganic nitrogen (DIN), as nitrate and nitrite fluxes were negligible for the majority of the sampling points (<0.01 µmol g$^{-1}$ h$^{-1}$). TAN release varied significantly between 0.33 ± 0.02 and 2.16 ± 0.26 µmol g$^{-1}$ h$^{-1}$ with highest values in summer (Figure 2.6b). We believe that the high TAN release rates during the May sampling are either caused by a sampling error or spawning was induced due to handling of the mussels during the experiment, resulting in contaminated samples. ON ratios varied between 13 and 35 for most of the sampling points, with an outlying value of 59.4 ± 9.6 in June (Figure 2.6c). Besides the high phosphate flux observed during the spring bloom (0.29 ± 0.05 µmol g$^{-1}$ h$^{-1}$), the seasonal dynamics in phosphate fluxes followed a similar pattern as for oxygen and TAN fluxes; low values in winter and elevated values in summer (Figure 2.6d). Silicate uptake was observed during the spring bloom (0.21 ± 0.01 µmol g$^{-1}$ h$^{-1}$), while there was no apparent net flux of silicate for any of the other sampling points (Figure 2.6e).

Seasonal variations in oxygen, TAN and phosphate fluxes were positively correlated to POC levels in the food ($p$ < 0.001; Table 2.1). The correlation between respiration and POC was higher than 0.9, representing a strong correlation. As POC and PON concentrations in the seston are interrelated, PON concentrations also showed positive correlations to the inorganic nutrient fluxes ($p$ < 0.001). Chl $a$ only correlated with phosphate excretion ($p$ < 0.001; Table 2.1), but this correlation was merely based on the high excretion during the spring bloom, as the correlation was insignificant ($p$ = 0.16) when spring bloom values were excluded from analysis. Respiration and TAN excretion also correlated to variations in water temperature (Table 2.1).

*Nutrient turnover time*
Element turnover showed a seasonal pattern with low turnover times in summer and higher values in winter (Figure 2.7). Carbon turnover varied between 65 and 331 days, nitrogen turnover between 191 and 1296 days and phosphorus between 48 and 819 days. Increase in release rates of carbon, nitrogen and phosphorus during the spring bloom are represented by a drop in turnover rates during this period. On an annual basis, nitrogen turnover (643 ± 88 days) was slower compared to phosphorus (290 ± 79 days) and carbon (181 ± 24 days). The low phosphate excretion in January (2009 and 2010) (Figure 2.6) resulted in non-representative phosphorus turnover rates, which are for this reason not incorporated in average values.

*Nutrient accumulation and release*
Nutrients accumulated in tissue material are represented by tissue growth. Table 2.3 presents nutrient accumulation and release (excretion and biodeposition) rates during different seasons. On an annual basis 1639 mg C, 92 mg N and 23 mg P are regenerated per gram tissue AFDW, tissue growth accounted for 560 mg C, 168 mg N and 12 mg P g$^{-1}$ y$^{-1}$ and biodeposition resulted in 981 mg C, 113 mg N and 9 mg P g$^{-1}$ y$^{-1}$ (Table 2.3). These annual averages show that release of carbon dioxide (52%) is in the same scale compared to the sum of tissue growth (18%) and

**Table 2.1**
Correlation matrix of biodeposition, respiration and inorganic nutrient release rates against environmental variables (temperature and food).

|  | Temp. (ºC) | Chl $a$ (µg l$^{-1}$) | POC (µg l$^{-1}$) | PON (µg l$^{-1}$) | Cell count (no. ml) |
|---|---|---|---|---|---|
| *Biodeposition rates (mg g$^{-1}$ d$^{-1}$)* |  |  |  |  |  |
| Total | -0.36[*] | 0.54[*] | -0.06 | 0.08 | 0.53[*] |
| Organic | -0.03 | 0.32[*] | 0.23[*] | 0.37[*] | 0.38[*] |
| *Inorganic nutrient release (µmol g$^{-1}$ h$^{-1}$)* |  |  |  |  |  |
| Respiration | 0.72[*] | 0.05 | 0.91[*] | 0.82[*] | 0.31 |
| TAN excretion | 0.40[*] | 0.00 | 0.47[*] | 0.54[*] | 0.28 |
| Phosphate excretion | 0.26 | 0.61[*] | 0.51[*] | 0.61[*] | 0.68[*] |

*\* Bonferroni corrected significant correlations (p < 0.01)*

**Table 2.2**
Nutrient composition in mussel (*Mytilus edulis*) biodeposits. Data are expressed in mg C, N or P g$^{-1}$ DW

|  | Carbon (mg g$^{-1}$) | Nitrogen (mg g$^{-1}$) | Phosphorus (mg g$^{-1}$) |
|---|---|---|---|
| 6 March | 34.1 | 4.7 | 0.90 |
| 16 March | 56.0 | 6.4 | 0.61 |
| 15 April | 112.4 | 15.7 | 1.28 |
| 9 September | 132.0 | 14.4 | 1.25 |
| 18 November | 106.5 | 10.7 | 0.99 |
| Average | 88.2 ± 18.4 | 10.4 ± 2.2 | 1.0 ± 0.1 |
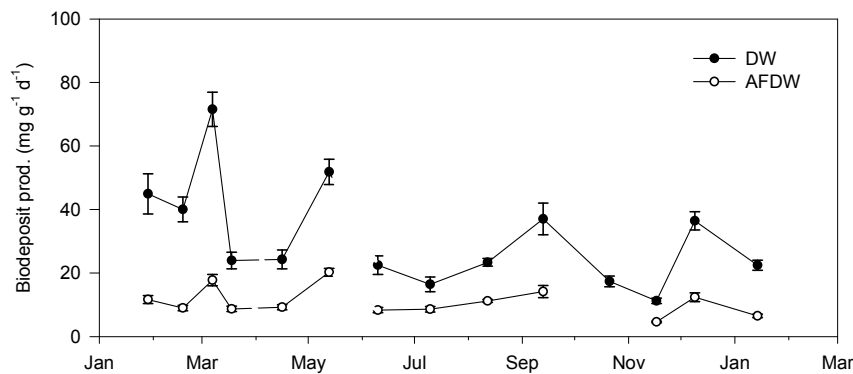


**Figure 2.5**
Biodeposit production by individual mussels (*Mytilus edulis*). Data are expressed as average (± standard error) dry weight (DW) and ash free dry weight (AFDW), and standardized to 1g tissue weight (AFDW). Gap between May and June sampling specifies the time of mussel batch replacement.
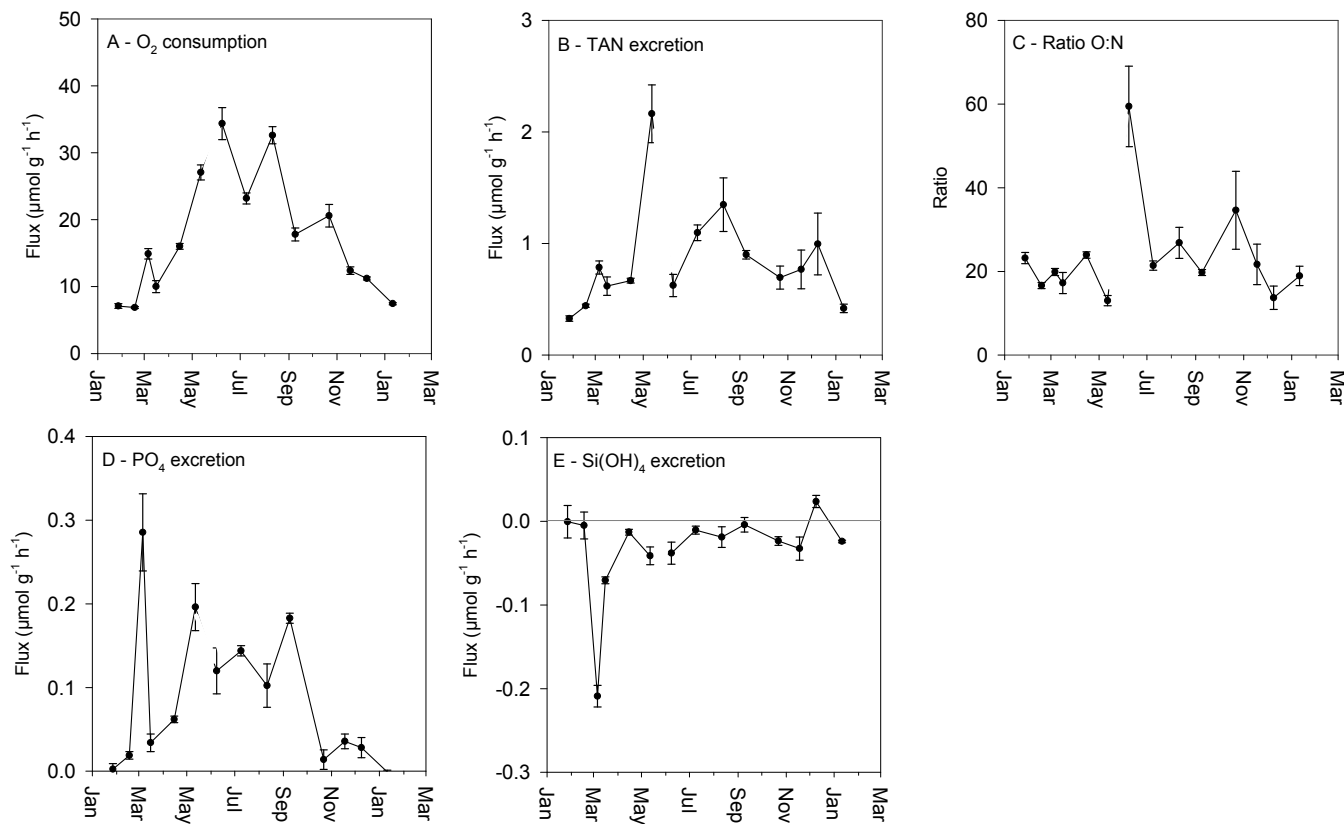
**Figure 2.6**
(A) Respiration, (B) Total Ammonia Nitrogen (TAN), (D) Phosphate and (E) Silicate excretion by mussels (*Mytilus edulis*). (C) ON ratios based on respiration and TAN release. Data are expressed as average (± standard error) fluxes, standardized to 1g tissue weight (AFDW). Gap between May and June sampling specifies the time of mussel batch replacement.
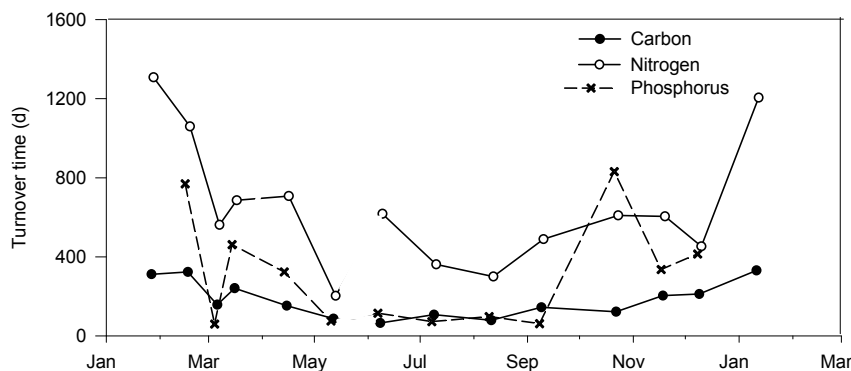
**Figure 2.7**
Seasonal changes in turnover times of carbon, nitrogen and phosphorus by mussels (*Mytilus edulis*). Data are expressed as average number of days. Gap between May and June sampling specifies the time of mussel batch replacement.

biodeposition (31%). Approximately half of the nitrogen was allocated to tissue growth (45%) followed by biodeposition (30%) and inorganic excretion (25%). Phosphorus allocation is similar to carbon, and inorganic excretion (53%) is higher than tissue growth (28%) or biodeposition (20%). The loss in tissue weight from November to December (Figure 2.3) resulted in negative values for tissue growth for all elements in the period October 2009 to January 2010. CN ratios were always higher for excretion than for biodeposition and growth of tissue material ($CN_{excr}$ >$CN_{biodep}$ > $CN_{tissue\ growth}$). The high CN ratio for excretion in the period April-June was primarily caused by the outlier during the June sampling (presented as ON ratio in Figure 2.5); when June sampling is removed from analysis CN ratios are similar to the other seasons (16.4). NP ratios showed a different pattern than the CN ratios with highest ratios observed for biodeposition and lowest ratios observed for tissue growth ($NP_{biodep}$ > $NP_{excr}$ > $NP_{tissue\ growth}$). $NP_{tissue\ growth}$ ratios varied considerably due to the relatively low phosphorus concentrations and subsequently low tissue increase in phosphorus.

## Discussion

This study presents data on intra-annual variability in accumulation and release of nutrients (C-N-P-Si) by blue mussels (*M. edulis*) under oligotrophic conditions. Firstly, the regulation of nutrient allocation to various metabolic processes is discussed. Next, potential nutrient sinks and nutrient sources for mussel cultures in oligotrophic fjord systems are reviewed.

*Regulation of nutrient allocation by mussels*
Nutrient turnover is the result of catabolic and anabolic requirements, influenced by endogenous and exogenous driven processes. The average annual turnover times in our study (181, 643, 290 days for C, N and P respectively) demonstrate that nitrogen was longer retained than phosphorus and carbon. Carbon turnover was similar, nitrogen turnover was higher and phosphorus turnover was lower compared to Smaal and Vonck (1997), who reported turnover

**Table 2.3**
Average nutrient accumulation (tissue growth), and inorganic (excretion, respiration) and organic (biodeposition) release of nutrients by individual mussels. Data are presented as average values (mg element) and standardized to a mean individual of 1g tissue AFDW, for each season and annually.

| | Jan-Feb -Mar | Apr-May -June | July-Aug -Sept | Oct-Nov Dec-Jan | Annual | |
|---|---|---|---|---|---|---|
| | $mg\ g^{-1}\ d^{-1}$ | | | | $mg\ g^{-1}\ d^{-1}$ | $mg\ g^{-1}\ y^{-1}$ |
| *Carbon* | | | | | | |
| Tissue growth | 8.40 | 3.00 | 3.57 | -2.43 | 1.53 | 560 |
| Respiration | 2.46 | 6.44 | 5.97 | 3.29 | 4.49 | 1639 |
| Biodeposition[*] | 2.14 | 2.43 | 5.49 | 1.49 | 2.69 | 981 |
| *Nitrogen* | | | | | | |
| Tissue growth | 1.85 | 0.72 | 0.83 | -0.32 | 0.46 | 168 |
| Excretion | 0.19 | 0.14 | 0.37 | 0.24 | 0.25 | 92 |
| Biodeposition[*] | 0.28 | 0.34 | 0.60 | 0.15 | 0.31 | 113 |
| *Phosphorus* | | | | | | |
| Tissue growth | 0.19 | 0.02 | 0.02 | 0.00 | 0.03 | 12 |
| Excretion | 0.07 | 0.10 | 0.11 | 0.02 | 0.06 | 23 |
| Biodeposition[*] | 0.01 | 0.01 | 0.05 | 0.02 | 0.02 | 9 |
| *Ratio CN* | | | | | | |
| Tissue growth | 4.9 | 6.5 | 5.4 | 6.5 | 5.8 | |
| Excretion | 15.4 | 26.0 | 18.5 | 15.9 | 18.5 | |
| Biodeposition[*] | 9.3 | 8.4 | 10.7 | 11.6 | 9.9 | |
| *Ratio NP* | | | | | | |
| Tissue growth | 23.4 | 4.4 | 7.4 | 20.2 | 15.2 | |
| Excretion | 9.0 | 9.0 | 8.6 | 35.8 | 17.0 | |
| Biodeposition[*] | 17.4 | 27.3 | 25.5 | 24.0 | 22.3 | |

[*] *Biodeposition values were based on the sampling points provided in Table 2.2 and thus do not cover all sampling points*

times of 189, 485, 380 days respectively. The higher nitrogen turnover observed in our study is influenced by a relatively high nitrogen tissue content. It is generally assumed that proteins contain 16% nitrogen, which indicates that the theoretical maximum concentration in tissue material is 160 mg N $g^{-1}$ AFDW if tissue material would be composed of proteins only. However, more likely a maximum concentration of 145 mg N $g^{-1}$ AFDW can be expected if we assume that 90% of the tissue AFDW is composed of proteins (recalculated from FAO 1989, Rosland et al. 2009). During three sampling occasion nitrogen content exceeded the latter value and during one occasion the nitrogen content even exceeded the theoretical value of 160 mg N $g^{-1}$ AFDW, indicating that nitrogen tissue content reported in our study might be slightly overestimated. Nutrient turnover time per element was relative short in summer, which indicates a great loss of elements in comparison with tissue content, and is in accordance to Smaal and Vonck (1997). This pattern was induced by high excretion (C-N-P) rates as well as low tissue content (N-P) in summer. However, one should be aware that release of carbon dioxide is converted from respiration rates calculated based on a constant Respiratory Quotient (RQ). This might induce an error since the RQ is dependent on catabolism of carbohydrates, lipids and proteins (Hatcher

1989) which may vary seasonally. Furthermore, respiration and excretion rates were standardized to a mean individual of 1 g tissue using allometric scaling functions using a value of 0.7 for the exponent $b$ (based on Smaal et al. 1997). However, the exponent $b$ may vary between environmental conditions and seasons and values for bivalves can range from 0.2 to 1.0 for respiration, although the majority fall within the range of 0.6 to 0.8 (reviewed in Bayne et al. 1976). The use of a theoretical $b$ exponent instead of empirical values specifically determined for our experimental conditions, may introduce bias. Recalculation of the data showed on average 1.6 times lower rates when a minimum $b$ value of 0.2 was chosen, and 1.3 times higher rates with a maximum $b$ value of 1.

Seasonal changes in metabolic requirements are known to vary with reproductive activity (Bayne & Widdows 1978, Hatcher et al. 1997, Cranford & Hill 1999). Gametogenesis in *Mytilus edulis* in Western Norway is generally initiated during late autumn, ripening of the gonads take place during winter, and spawning starts in April and can continue until June (Duinker et al. 2008). However, these authors also report deviations from this pattern, indicating a scattered spawning pattern throughout spring and autumn. Release of gametes during the spawning period can usually be detected by a loss in weight of individual mussels (Rodhouse et al. 1984). However, no significant weight loss was observed during the presumed spawning period in April-June. The sampling interval of one month may have been too long to pick-up a spawning signal or the spawning period may have been more scattered throughout a longer period, thereby masking a decrease in tissue weight. High Carbon:Nitrogen (CN) tissue ratios observed during summer in our study revealed that nitrogen content in tissue was low during late spring and early summer. Nitrogen content increased during late summer and autumn which coincides with the period of germinal quiescence when glycogen storage generally takes place (Bayne et al. 1982). Seasonal fluctuations in tissue CN content observed in our study were comparable to Smaal and Vonck (1997), and absolute values of CN ratios were in the same range as presented by Hawkins et al. (1985), slightly lower than Smaal and Vonck (1997), but higher compared to Vink and Atkinson (1985). NP ratios in tissue showed similar seasonality as CN ratios, which is in accordance with seasonal patterns as described by Smaal and Vonck (1997). High phosphorus concentrations in tissue material during the presumed spawning period, as observed in this study, have previously been reported by Kuenzler (1961) who presumed that tissue phosphorus was converted to phosphorus-rich gametes prior to spawning. High metabolic requirements of reproduction usually result in high respiration rates during spawning periods (Bayne & Widdows 1978, Thompson 1984, Hatcher et al. 1997, Smaal et al. 1997). Elevated respiration levels were indeed observed during the presumed spawning period (May-June) in comparison to the preceding months, however, respiration remained high throughout summer. Nitrogen (TAN) excretion is generally highest when glycogen reserves are depleted (Smaal & Vonck 1997). In our study TAN release rates showed elevated levels during summer. TAN release rates in June were relatively low compared to respiration rates as is demonstrated by a high ON ratio. ON ratios provide information on the physiological status of the mussel (Schluter & Josefsen 1994, Hatcher et al. 1997) and can also be linked to reproductive status. Pre-spawning periods are generally characterized by low and post-spawning periods by high ON ratios (Devooys 1976, Bayne et al. 1977, Bayne & Widdows 1978, Widdows 1978, Hawkins & Bayne 1985, Smaal et al. 1997). The high ON ratio in June might therefore be explained by spawning events after the transport of the second mussel batch in the end of May (2 weeks prior to the June sampling). The average annual ON ratio was 24, which is in the same range as reported by Smaal and Vonck (1997) and Strohmeier (2009), but several fold lower compared to Bayne and Widdows (1978) and Barbarro et al.(2000). However, some authors pointed out that ON ratios are dependent on the quality

(Hatcher et al. 1997) and quantity (Bayne & Widdows 1978, Kreeger 1993) of the food source and temporal variability is therefore more informative than its absolute value.

Seasonal patterns in respiration, TAN and phosphate excretion rates correlated with environmental variations in food and temperature. Mussel metabolism is known to correlate with temperature (Widdows & Bayne 1971, Devooys 1976) and food (Bayne et al. 1989b, Bayne et al. 1993), although direct correlation with any of those factors is often difficult due to the interrelationship between food and temperature (Grant 1996, Smaal et al. 1997). Elevated phytoplankton concentrations during the spring bloom lead to increased respiration, TAN and phosphate excretion rates although these increases were relatively small compared to the annual variability. Although food quantity is often described in terms of Chl *a* concentrations, we found a much better fit between excretion rates of inorganic nutrients and POC and PON than with Chl *a* concentrations. Strohmeier (2009) has also shown a high correlation between respiration and POC concentrations for mussels kept under oligotrophic conditions. A sharp drop in tissue weight was observed from November to December/January which coincided with a period of low food availability. Weight decrease of mussels in autumn and winter is a normal phenomenon in temperate areas, and is attributed to food scarcity (Zandee et al. 1980). However, the decrease observed in our study was steep compared to a study performed in the same area (Strohmeier 2009). A second hypothesis for loss of tissue weight in mussels is spawning activity (Rodhouse et al. 1984), although late autumn is not characterized as a period with massive spawning events in Norwegian waters (Duinker et al. 2008) and respiration and nitrogen release rates do not point towards spawning activity during this period. Fluctuations in food quantity and quality (cell counts) were positively correlated with biodeposit production rates, which is in accordance with other studies (Navarro & Thompson 1997, Ren et al. 2006). Positive correlations between biodeposition and temperature have been reported (Tsuchiya 1980, Kautsky & Evans 1987), but were not observed in our study. The feeding response to low food concentrations, rather than temperature, are likely the determining factor for total ingestion (Strohmeier et al. 2009) and consequently biodeposition rates in our study area. The fraction of organic content in the faeces (22 - 48%) was high compared to other studies which report values between 10 - 27% (Navarro & Thompson 1997, Callier et al. 2006, Giles & Pilditch 2006), which might be explained by a high fraction organic material in the food source in our study area (73%; Strohmeier et al. 2009).

*Sinks and sources in nutrient cycling by mussels*

The fluctuations in metabolic processes, as discussed in the previous section, determine the pathways in which mussels may contribute to nutrient cycling in coastal ecosystems. Mussels regenerate nutrients by means of the respiration ($CO_2$ release) and excretion of inorganic metabolic waste products, and in this way mussel cultures can be regarded as a source of recycled nutrients to the system. Hence mussel cultures do not only filter phytoplankton from the system but can also support phytoplankton production through regenerated nutrients (Prins et al. 1998, Newell 2004). However, one should keep in mind that the phytoplankton stock sustained by regenerated nutrients can never exceed the level that can be sustained by ambient nutrients. TAN excretion and phosphate excretion observed in this study are comparable to previously reported values (Hawkins & Bayne 1985, Smaal & Vonck 1997, Smaal et al. 1997). No silicate release was observed in our study, which is in accordance to Prins and Smaal (1994), but is conflicting with Asmus et al. (1990) who showed that silicate excretion rates were positively correlated with respiration rates.

Nutrients accumulated in the tissue represent a loss to the system when the mussels are harvested. Growth rates observed in our study were at the same scale as reported by Strohmeier

(2009) for mussels cultured in the same area in 2006/2007. However, the organic fraction of tissue material in our study (0.74) was lower compared to a literature overview reported by Ricciardi and Bourget (1998), who showed average conversion factors of AFDW/DW above 0.80. Additional to tissue growth, mussels store nutrients in the shell and byssus threads. Hawkins and Bayne (1985) showed that carbon and nitrogen production by the formation of byssus might be significant. Our estimates therefore represent an underestimation of total nutrient loss through harvest, as growth of shell and formation of byssus threads was not incorporated in our estimates.

Nutrients regenerated from decomposition of mussel biodeposits are generally considered to contribute to the pool of regenerated nutrients (= nutrient source) due to a well mixed water column and subsequent benthic-pelagic coupling (Asmus & Asmus 1991, Dame & Libes 1993, Prins et al. 1998). However, suspended mussel farming in Norway is preferably located in the deeper parts of fjords (Ervik et al. 2008), which have stratified water columns during spring and summer (Aure et al. 1996, Asplin et al. 1999). Particularly in cases of profound vertical stratification, nutrients released from benthic decomposed biodeposits do not contribute to the nutrient pool in the euphotic zone, and biodeposition can therefore be regarded as a nutrient sink to the system. Nevertheless, not all biodeposits sink to the bottom and a fraction is trapped in the culture structures (Chapter 4; Richard et al. 2006). The fraction of biodeposits trapped in the culture structures (euphotic zone) relative to the fraction sinking to the bottom is unknown. Egestion rates observed in our study were comparable to rates reported for *Modiolus modiolus* in areas with similar Chl *a* concentrations (Navarro & Thompson 1997) but lower compared to rates observed for *Mytilus edulis* in areas with higher food concentrations (Callier et al. 2006).

The average annual values for nutrient accumulation and release (Table 2.3) indicate that regeneration of carbon and phosphorus was approximately in the same scale compared to the sum of the loss by harvest and biodeposition. Most nitrogen was allocated to storage in tissue material, but regeneration still represented 25% of the total losses. Regeneration of carbon and nitrogen in our study was thereby of relative higher importance compared to results presented by Hawkins and Bayne (1985). Additionally, nitrogen regeneration in our study was also relatively higher compared to Cranford et al. (2007) who showed that excretion was 12-15% from the sum of harvest and biodeposition in Tracadie Bay (Canada). The higher contribution of carbon and nitrogen regeneration relative to the other metabolic processes found in our study is primarily caused by the fact that the biodeposit contribution was higher in the aforementioned studies, likely induced by high food concentrations in those study areas. The relative contribution of regeneration in phosphorus dynamics was also higher compared to other studies. Kuenzler (1961) showed that phosphorus regeneration was half the amount of biodeposition in a *Modulus demissus* population. Silicate dynamics were only investigated by dissolved inorganic silicate fluxes. Nevertheless, the fact that no release of inorganic silicate was observed indicates that all ingested silicate is expelled with the faeces as silicate is not incorporated in mussel tissue.

The elemental ratios demonstrate that the rates per element (C-N-P-Si) are specific for each of the metabolic processes. CN ratios of the inorganic release rates (18.5) were higher compared to CN ratios in biodeposits (9.9), seston (8.9) and tissue (growth = 5.8; content = 4.8). This implies that relatively more carbon is respired, while nitrogen accumulates in mussel tissue and will be removed from the system by harvest. The relation between NP ratios was less clear due to large seasonal variability. Annual NP ratios in biodeposits (22.3) were relatively high compared to ratios in excretion rates and tissue growth, and were also 2-3 times higher compared to ratios reported by Kautsky and Evans (1987). The high NP ratios in biodeposits imply a relative loss of

nitrogen towards the seabed. In most of the seasons, except from the last (October-January), NP ratios of the excretion rates were lower compared to Redfield's ratio (16) as well as NP ratios in natural seston (21) reported along the coastal zone of Western Norway during summer conditions (Anonymous 2010). The CN and NP ratios show that mussels through regeneration of nutrients may not only influence the absolute nutrient concentrations and thus primary production rates but also have the potential, especially during periods of nutrient limitation, of changing nutrient stoichiometry of the inorganic nutrient pool with cascading effects towards phytoplankton community composition (Dame & Libes 1993, Prins et al. 1995).

## Conclusions and implications

Food concentration in our study area was considerably lower while food quality was higher compared to other bivalve cultivation sites (for overview see Strohmeier et al. 2009). However, despite the differences in food conditions, excretion rates and nutrient tissue composition were approximately at the same scale as values reported for mussels cultured in other areas. Biodeposition rates were lower compared to areas with higher food concentrations. Environmental changes (food and temperature) were correlated to excretion rates and to a lower extent to biodeposition rates. Tissue nitrogen content seemed to follow reproductive processes and phosphorus content was highest during the presumed spawning period. Subsequently, nutrient turnover showed a seasonal pattern with fast turnover in summer and slow turnover in winter. On an annual basis, nitrogen turnover was slower compared to phosphorus and carbon (N > P > C).

The allocation of the different elements to each of the metabolic processes was dissimilar, with $C_{excr.} > C_{biodep} > C_{tissue\ growth}$ and $N_{tissue\ growth} > N_{biodep.} > N_{excr}$ and $P_{excr.} > P_{tissue\ growth} > P_{biodep}$ for annual averages, although seasonal differences were observed. Nutrient allocation determines the pathways in which mussels contribute to nutrient cycling. Mussel cultures act as a source of recycled nutrients to the system through excretion of dissolved metabolites, while harvest of the mussels results in removal of nutrients and thus represents a sink to the system. Additionally, nutrients are lost through biodeposition to the deep water fjord basin, although an unknown fraction of the biodeposits is also regenerated in the euphotic zone. Consequently relatively more nitrogen was accumulated in tissue material and biodeposits (= sink) and relatively more carbon and phosphorus were regenerated (= source). Silicate excretion was absent, indicating that all ingested silicate is expelled with the biodeposits (= sink).

Results of this study illustrate that the potential role of mussels in nutrient dynamics in oligotrophic fjord systems is different from shallow eutrophic areas. In oligotrophic fjord systems a lower fraction of nutrients is allocated to defecation processes, likely caused by the low food concentrations. Lower importance of the biodeposition reflects higher relative importance in the other processes reciprocally. Excretion of dissolved metabolites is therefore relatively more important in oligotrophic systems compared to eutrophic areas. Primary production rates in Norwegian fjords (100 g C $m^{-2}$ $y^{-1}$; Erga 1989a, Aure et al. 2007a) are considerably lower than for example in the Oosterschelde estuary in the Netherlands (350 g C $m^{-2}$ $y^{-1}$; Smaal et al. 2001) or Thau lagoon in France (400 g C $m^{-2}$ $y^{-1}$; estimated by Vaguer and published by Plus et al. 2006). The relatively large fraction of direct excretion combined with the low ambient nutrient concentrations, indicates that the potential net effect of mussel aquaculture on primary production can be considerable in oligotrophic systems. Conversely, the absolute pool of regenerated nutrients might be larger in shallow eutrophic areas due to the contribution of

remineralized biodeposits, its net effect on primary production may still be lower. The current study solely focused on the potential effects of mussels on nutrient dynamics by studying processes at individual level. However, evaluating the effect of mussel aquaculture at ecosystem scale, population specific (e.g. mortality), culture specific (e.g. culture density, management or associated fauna community), and ecosystem specific (e.g. hydrodynamics, water resident times and ambient nutrient concentrations) processes determine to what extent mussel aquaculture contributes to the system's overall productivity.
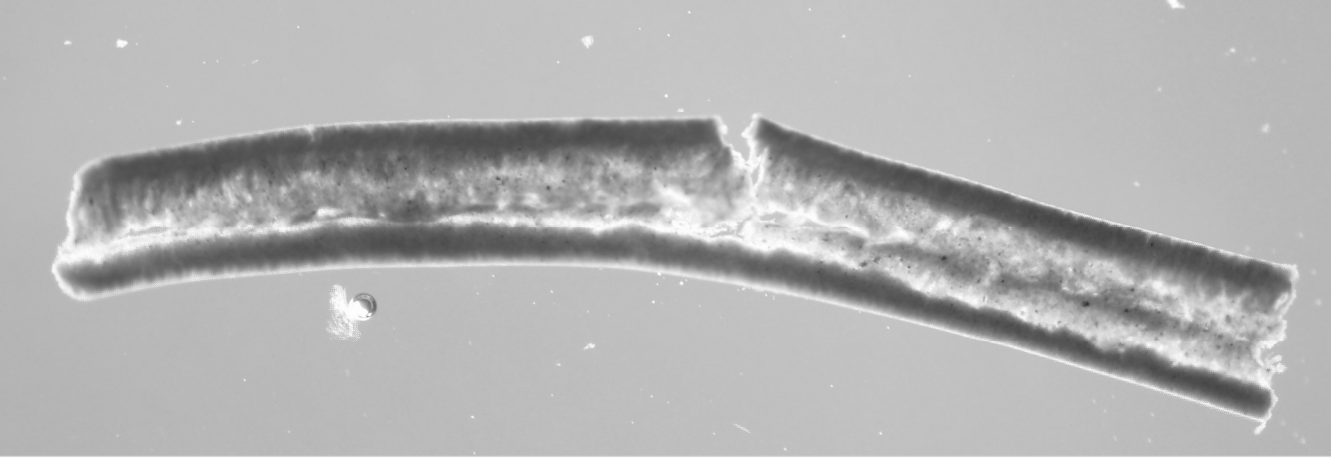
# Chapter 3

Biodeposit remineralization

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**ABSTRACT**

To determine seasonal variability in mineralization dynamics of mussel biodeposits, we applied a multiple-element approach measuring mineralization rates of carbon (C), nitrogen (N), phosphorus (P) and silicate (Si) during three periods (March, August and November). The results of this study showed that mineralization rates vary between seasons and between elements, and that mineralization dynamics were influenced by both temperature and biodeposit nutrient composition. Mineralization rates were 3.2 ± 0.4 mmol C, 0.17 ± 0.04 mmol N, 0.06 ± 0.02 mmol P and 3.91 ± 3.75 mmol Si per gram biodeposit (DW) per day, which represented 24% of the particulate organic C and 17% of the particulate organic N in mussel biodeposits. Seasonal variability was largest for Si mineralization with 60-80-fold higher rates measured in March compared to August and November. This difference is most likely related to the difference in biodeposit nutrient composition. It was furthermore shown that the labile fraction of biodeposits became mineralized after respectively 18, 9 and 13 days during the experimental periods in March, August and November. This indicates that temperature enhances biodeposit decomposition with approximately 2-3 times faster turnover at a 10 °C temperature interval ($Q_{10}$).

## Introduction

Suspension feeding mussels have the potential to filter considerable quantities of particulate matter from the water column (Cranford et al. 2011). Ingested food particles are digested and the remnants, together with some metabolic waste products, are expelled as faeces (Hawkins and Bayne 1985). During periods of excess food availability, mussels reject part of the filtered material before ingestion and expel it from the inhalant siphon as pseudofaeces (Ward and Shumway 2004). Faeces and pseudofaeces are collectively called biodeposits. Mussel biodeposits are rich in organic nutrients (Kaspar et al. 1985, Grenz et al. 1990) and show relatively high decay rates compared to decomposing phytoplankton or macroalgae (Giles and Pilditch 2006). Although measurements on biodeposit degradation are essential to understand and quantify bivalve-ecosystem interactions, relatively little has been published on bivalve biodeposit quality and specific mineralization and decay rates (see review by McKindsey et al. 2011). The ecological importance of biodeposit mineralization is the availability of regenerated nutrients for primary producers (Prins et al. 1998).

Decomposition of organic material (including mussel biodeposits) is often described by first-order (G) or multi-G exponential models (Canfield et al. 2005), which roughly can be divided into two phases; 1) a steep decrease in decomposition rates during the first 4-8 days after deposition indicating that the labile nutrient pool in biodeposits becomes depleted (Giles and Pilditch 2006, Carlsson et al. 2010), and 2) a period with very low decomposition rates representing the refractory material which decomposes on much longer time scales. Yet, all organic material (OM) will eventually be mineralized (Meyerreil 1994). It is suggested that high mineralization rates of biodeposits are related to presence of resident gut bacteria that are, in connection with the faecal pellets, expelled from the mussel's digestive system (Harris 1993). However, carbon mineralization rates of fresh biodeposits increase considerably after an initial lag phase of one day (Carlsson et al. 2010), suggesting bacterial growth and/or colonalization by outside bacteria during the lag phase (Canfield et al. 2005). Indeed, Fabiano et al. (1994) showed that bacterial numbers increased during the first two days of biodeposit decomposition. However, when biodeposits were added to benthic substrates maximum levels of carbon mineralization were observed immediately after addition (Giles and Pilditch 2006, Carlsson et al. 2010), presumably as a consequence of high microbial biomass in sediments facilitating rapid colonalization of the biodeposits. Bacterial colonization, and thus mineralization dynamics, seem to vary between benthic (with substrate) and pelagic (without substrate) systems. Bacterial growth is also dependent on nutrient composition of the biodeposits (del Giorgio and Cole 1998), which in turn depends on the concentration and type of diet of the mussels (Miller et al. 2002, Giles and Pilditch 2006). Furthermore, bacterial growth rates and organic matter decomposition are positively correlated to temperature (White et al. 1991, Katterer et al. 1998). Due to variations in biodeposit composition and fluctuating water temperatures, mineralization rates of decomposing biodeposits may vary over temporal and spatial scales.

The few studies defining mineralization rates for mussel biodeposits did not include temporal or spatial variability (Giles and Pilditch 2006, Carlsson et al. 2010) nor has any study defined the specific effects of temperature or biodeposit composition on biodeposit mineralization rates. The variability in specific mineralization rates is therefore unknown. The current study investigates the difference in mineralization rates of mussel biodeposits during three seasons (March, August and November). Given the fact that phytoplankton dynamics are influenced both by nutrient availability and stoichiometry (Redfield ratio; Redfield et al. 1963) a multiple-element approach was applied to the present study and mineralization rates were determined for carbon (C), nitrogen (N), phosphorus (P) and silicate (Si). The study was carried out

in a fjord area in South-West Norway, which is characterized by oligotrophic conditions (Chapter 4) and relatively low water temperatures (between 4 and 18°C on an annual basis; Sætre 2007). The oligotrophic conditions in our study area were different from the studies by Carlsson et al. (2010) and Giles and Pilditch (2006), who investigated more eutrophic conditions. As the trophic conditions determine the food quality and quantity for the mussels, it might also influence the nutrient composition of the produced biodeposits and thus remineralization rates. The physical conditions of most fjord systems (deep stratified water columns) inhibit the contribution of benthic remineralized nutrients to the nutrient pool in the euphotic zone (Aure et al. 1996, Asplin et al. 1999). Biodeposit mineralization in the pelagic phase is therefore the most important decomposition site in the context of fjord-mussel culture interactions (Chapter 2). Biodeposits produced by the mussels are partly trapped in between the mussel matrix of suspended ropes (Chapter 4) and its decomposition contributes to nutrient regeneration in the pelagic zone (Richard et al. 2006). To mimic continuous deposition on suspended ropes, biodeposits were added to incubation chambers on a daily basis during an experimental period of 3 weeks. Since the labile nutrient pool in biodeposits becomes depleted within several days (Giles and Pilditch 2006, Carlsson et al. 2010), we expected to observe stable nutrient release rates towards the end of the experimental period. The relatively long experimental period was chosen so that a stable microbial community could develop, as is the case for *in situ* ropes, and to cover variability in stable state estimates reflecting fluctuations in environmental conditions. This experimental approach allowed us to test the following hypothesizes: (*i*) higher biodeposit nutrient composition will lead to higher mineralization rates; (*ii*) mineralization rates expressed as fraction of the available organic nutrient in the biodeposits (e.g. $CO_2$ released/POC in biodeposit) will be similar between temperatures; (*iii*) respiration and nutrient releases will reach stable state conditions more rapidly at higher temperatures.

## Materials and Methods

*Experimental design*

The study was carried out at Austevoll Research Station which is situated in a fjord area in South-West Norway (N60°05´, E005°16´). The laboratory facilities received unfiltered seawater (1.5 m depth) which was pumped into two header tanks (400 l). The estimated resident time in the header tanks was less than 0.2 hours, and hence environmental conditions were assumed to be similar to natural conditions..

Respiration and nutrient release rates from decomposing biodeposits were quantified for three seasons with varying food concentrations and water temperatures; spring (March), summer (August), autumn (November). During each of the seasons, freshly collected mussel biodeposits were added daily to incubation chambers during an experimental period of 3 weeks. Respiration and nutrient release rates from decomposing biodeposits were determined every $2^{nd}$ day throughout the experimental periods.

*Biodeposit collection*

The approximately 750 mussels *Mytilus edulis* (1-2 year old) used for the experiments were kept outdoor in cages (1.5 m depth) in between the experimental periods. Mussels were acclimatized to the laboratory conditions one week prior to the first biodeposit collections. The mussels were randomly divided over three biodeposit collection tanks (V = 50 l; Ø = 50 cm; H = 25 cm), in which they were placed on a tray at mid water depth. The tanks received seawater through an

inflow opening at the bottom of the tank (15 l min$^{-1}$), and a drain along the entire top of the tank served as the outflow. This upwelling design assured a well mixed water column providing food and oxygen to the mussels while the biodeposits sank to the bottom of the tank. Water quality parameters describing mussel food quantity and quality are presented in (Chapter 2 & 4). Biodeposits were collected once a day with syringes and remaining biodeposits were removed after each collection, assuring that all collected biodeposits were not older than 24 hours.

Biodeposits were collected in excess and were used for the following three purposes: (*i*) for measurements on mineralization rates, (*ii*) to monitor the amount of biodeposits added to each chamber, and (*iii*) to determine the quality of the biodeposits. A volumetric approach was used for standardizing the amount of biodeposits added to the incubation chambers. To monitor the amount of biodeposits added to the incubation chambers triplicate samples, with a similar volume as added to the chambers, were filtered onto pre-weight filters (Whatman GF/A) and salt was expelled by rinsing each filter with deionised water. Filters were dried at 60 °C for at least 12 hours to determine dry weight (DW), and combusted at 450 °C for 6 hours to determine ash free dry weights (AFDW). Another biodeposit sample was collected for determination of biodeposit quality. These samples were pooled into weekly samples, resulting in 3 samples (week I, II, III) per experimental period. Following the experimental period of 3 weeks, the remaining biodeposits were collected from the incubation chambers. Both the fresh biodeposit samples and post- mineralization samples were dried and homogenized, and a subsample was analyzed using a Thermo Finnigan Flash EA 1112 NC Analyzer to determine organic carbon and nitrogen composition. To determine organic phosphorus a subsample was analyzed using spectrophotometric methods as described by Grasshoff et al. (1999).

*Mineralization measurements*

The experimental unit for mineralization measurements consisted of 6 incubation chambers placed in a water-bath with running seawater ensuring that temperatures were similar to ambient values. Biodeposits were added to 4 of the incubation chambers, and the remaining 2 chambers were used as a control. The incubation chambers consisted of 1.2 l sealed tanks (Ø = 10 cm; H = 13 cm) with the water inflow tap located at mid-water depth and a water outflow tap positioned at the top of the chamber. Water flow through the chambers was set to 180-200 ml min$^{-1}$ and incubations were performed by closing the in- and outflow taps. Magnetically driven stirring bars fitted into the inside of the lids mixed the water in the chambers during the incubations. A dye test confirmed that the water column was well-mixed during both the incubations and flow periods, while biodeposit resuspension was prevented. Incubations were terminated when the oxygen concentration had decreased approximately 10% compared to initial values, resulting in incubation times ranging from 2 to 12 hours. A linear oxygen decline through time was confirmed by a pilot study (data not shown), where an oxygen optode with a measurement interval of 1 second was mounted inside a chamber during the incubation period. All incubations were performed in the dark to limit absorption of nutrients by phytoplankton. Oxygen measurements (optode no. 4835, Aanderaa) and water samples for dissolved inorganic nutrient concentrations were taken in all chambers at the start and end of each incubation. Samples (total 20 ml) for nitrite, nitrate, phosphate and silicate were preserved with chloroform and stored in a cool and dark place until analysis. Those samples were analyzed according to standard methods (Parson et al. 1992) adapted for an auto-analyzer. Total Ammonia Nitrogen (TAN) samples (20 ml) were directly frozen until analysis. TAN concentrations were analyzed by means of fluorometric analysis (Kerouel and Aminot 1997, Holmes et al. 1999).

*Data standardization and statistical analysis*

Prior to statistical analysis, all data were checked for homogeneity and normality of variance assumptions by (*i*) visually examining standardised residuals versus predicted values plots and Q-Q plots of residuals, (*ii*) Shapiro-Wilk tests and (*iii*) Levene tests (Quinn and Keough 2002). All statistical analyses were performed using SAS 9.2, and data are presented as mean ± standard error (SE), unless stated otherwise.

One-way Analysis of Variances (ANOVA) tests were used to test variability in biodeposit quality (OM, POC, PON, POP) between the three experimental periods for both freshly collected biodeposits and biodeposit remnants from the incubation chambers at the end of the experimental period. In case of significant results Tukey's HSD post hoc multiple comparison tests were used to determine which of the experimental periods were significantly different from each other.

Oxygen concentrations were recalculated into carbon ($CO_2$) concentrations assuming a respiratory quotient (RQ) of 1 (Findlay et al. 1986, Hatcher et al. 1994, Tornblom and Bostrom 1995). Nutrient release rates of decomposing biodeposits were calculated according to the following equation:

$$N = \frac{(NC_{end} - NC_{start}) \cdot V}{t} \qquad \text{(Equation 3.1)}$$

where $N$ is nutrient release rate ($\mu$mol d$^{-1}$ for $CO_2$, TAN, $PO_4$, Si(OH)$_4$), $NC$ is nutrient concentration at start and end of each incubation ($\mu$mol l$^{-1}$), $V$ is the total volume of the incubation chamber (1.2 l) and $t$ is incubation time (d). Rates were corrected for the control measurements, although fluxes in the control chambers fluctuated around zero for the majority of the measurements. Broken line analysis was used to estimate timing and amplitude of the stable state conditions for respiration and nutrient releases (Koops and Grossman 1993, Robbins et al. 2005).
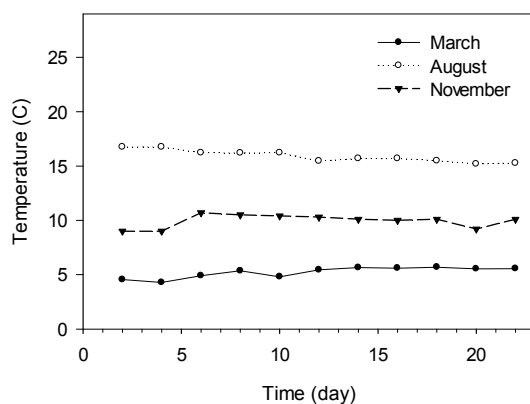


**Figure 3.1**
Temperature profiles measured in the incubation chambers during the three experimental periods (March, August, November).

The analysis was performed with the non-linear regressions (PROC NLIN) using the following model statement:

$$N = A - S \cdot Z \qquad \text{(Equation 3.2)}$$

This model fits a diphasic function with a one slope linear function and an upper asymptote, where $N$ represents the nutrient flux (in $\mu mol\ d^{-1}$ for $CO_2$, TAN, $PO_4$ or Si), $A$ is the asymptote representing the stable state conditions, $S$ is the slope of the linear function, and $Z$ was defined as $((day < B)\ ^{*}\ (B - day))$ which indicates that $(B - day)$ is defined as zero if $(day > B)$. This allows us to estimate the inflection point $(B)$ which represents the day when the stable state conditions were reached. Estimates of $B$ were restricted by bound statements which were set to the duration of the experimental period ($B < 20$ for March; $B < 22$ for August and November). Subsequently estimates for $A$, $S$ and $B$ were obtained by iterative processes. One-way ANOVA was performed to test the difference in $B$ values between experimental periods.

Nutrient stoichiometry of C:N, N:P and N:Si for release rates were calculated in atomic equivalents. Two-way multivariate analysis of variance (MANOVA) was used to test the effect of day and experimental period on the complete dataset.

## Results

*Temperature profiles*
The experimental periods were significantly different from each other (Tukey HSD; $p < 0.05$) with approximately 5°C differences between them (Figure 3.1). Lowest temperatures were observed in March (5.2 ± 0.5 °C), highest temperatures in August (15.9 ± 0.6 °C), and intermediate temperatures in November (9.9 ± 0.6 °C).

*Biodeposit characteristics*
Variations in the quantity of biodeposits added to the incubation chambers were observed over both daily and seasonal time scales (Figure 3.2). On average 95.3 ± 6.0 mg, 118.5 ± 3.3 mg and 107.9 ± 4.6 mg biodeposits were daily added to the incubations chambers in March, August and November respectively. The organic fraction of the biodeposits varied considerable during the March experiment, from 19% in the first days of the experiment to >45% during the last days. The overall organic fraction was thereby significantly lower for the March experiment (28.8 ± 2.1 %) compared to August (54.2 ± 1.5 %) and November (36.5 ± 0.7 %) (Tukey HSD; $p < 0.05$; Table 3.1). Pseudofaeces was only produced during the first days of the March experiment (visual observations), which coincided with the spring bloom. Carbon and nitrogen concentration were highest in August and lowest in March, although there were no statistical significant differences between the experimental periods (ANOVA; $F_{2,8} = 1.83$, $p = 0.240$ for carbon and $F_{2,8} = 1.93$, $p = 0.226$ for nitrogen). Phosphorus concentrations were highest in March and lowest in November (ANOVA; $F_{2,8} = 5.56$, $p = 0.043$). CN ratios were stable both on weekly as well as seasonal time scales (CN = 10.6), whereas NP ratios showed more variation (from 11.4 to 41.7) with significantly different values between November and March (Tukey HSD; $p < 0.05$).

Carbon and nitrogen concentrations were 2-4 times lower at the end of the experiments compared to the fresh biodeposits (Table 3.2). However, phosphorus concentration of biodeposits present in the chambers at the end of the experiment decreased less than for carbon and nitrogen, and was only 1.1 to 1.7 times lower compared to POP concentrations in fresh biodeposits. CN ratios in March and August decreased during decomposition, while elevated
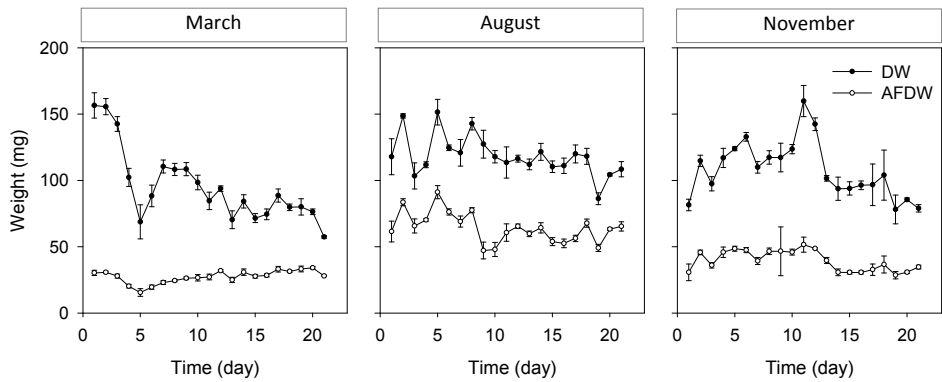
**Figure 3.2**
Average daily biodeposit quantity, in dry weight (DW) and ash free dry weight (AFDW), added to the incubation chambers during the three experimental periods (March, August, November).

**Table 3.1**
Quality characteristics of freshly deposited biodeposits. Data is expressed as the organic fraction (OM; %), particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP) composition (mg element $g^{-1}$ DW) and molar CN and NP ratios

| Exp. period | Week | OM | POC | PON | POP | CN | NP |
|---|---|---|---|---|---|---|---|
| March | Week I | 20 | 62.0 | 6.9 | 1.3 | 10.4 | 11.4 |
| | Week II | 30 | 104.1 | 11.3 | 1.6 | 10.7 | 16.0 |
| | Week III | 41 | 139.1 | 15.1 | 1.6 | 10.7 | 20.8 |
| | Average | 29 ± 2 [a] | 101.7 ± 22.3 [a] | 11.1 ± 2.4 [a] | 1.5 ± 0.1 [a] | 10.6 ± 0.1 [a] | 16.1 ± 2.7 [a] |
| August | Week I | 59 | 101.1 | 10.8 | 1.1 | 10.9 | 21.3 |
| | Week II | 50 | 189.0 | 20.4 | 1.3 | 10.8 | 33.6 |
| | Week III | 54 | 194.0 | 22.9 | 1.7 | 9.9 | 30.6 |
| | Average | 54 ± 2 [b] | 161.4 ± 30.2 [a] | 18.0 ± 3.7 [a] | 1.4 ± 0.2 [ab] | 10.5 ± 0.3 [a] | 28.5 ± 3.7 [ab] |
| November | Week I | 38 | 125.6 | 15.5 | 1.1 | 9.4 | 30.3 |
| | Week II | 36 | 140.7 | 15.5 | 0.8 | 10.6 | 41.7 |
| | Week III | 36 | 166.1 | 16.6 | 1.0 | 11.7 | 36.1 |
| | Average | 37 ± 1 [c] | 144.1 ± 11.8 [a] | 15.9 ± 0.4 [a] | 1.0 ± 0.1 [b] | 10.6 ± 0.6 [a] | 36.0 ± 3.3 [b] |

**Table 3.2**
Quality characteristics of partly decomposed biodeposits at the end of the experimental period. Data is expressed as the average (± SE) particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP) composition (mg element $g^{-1}$ DW) and molar CN and NP ratios.

| | POC | PON | POP | CN | NP |
|---|---|---|---|---|---|
| March | 41.5 ± 3.9 | 5.4 ± 0.5 | 0.88 ± 0.06 | 9.0 ± 0.1 | 13.7 ±1.5 |
| August | 59.1 ± 6.7 | 7.2 ± 0.9 | 1.32 ± 0.13 | 9.6 ± 0.2 | 12.6 ± 2.3 |
| November | 37.3 ± 1.7 | 3.6 ± 0.2 | 0.78 ± 0.02 | 12.1 ± 0.3 | 10.3 ± 0.5 |

values were observed in November. NP ratios in the biodeposit remnants were substantially lower compared to ratios in fresh biodeposits for all three experimental periods.

*$CO_2$ and nutrient release rates*

During all three experimental periods a steep increase of $CO_2$ TAN, $PO_4$ and $Si(OH)_4$ releases was observed from the total amount of biodeposits present in the mineralization chambers (Figure 3.3). The steep increases were followed by a stable state condition where releases were relatively constant indicating that the labile nutrient pool of biodeposits added to the chambers at day 1 becomes depleted. The timing when stable state conditions were reached (inflection point *B*) varied between experimental periods (Table 3; ANOVA; $F_{2,9}$ = 6.98; $p < 0.05$). Overall stable state conditions were reached after 18.3 days in March, 8.8 days in August and 12.9 days in November. During all three periods $CO_2$ reached stable state conditions before the other nutrients (TAN, $PO_4$, $Si(OH)_4$). Releases during stable state, indicated by the asymptote (*A*), also varied between experimental periods (Table 3.3). The most striking difference was found for the silicate releases in March, which were 60-fold higher than for August and November measurements. Nitrogen release was dominated by TAN releases, and nitrate and nitrite concentrations or fluxes were below the detection or sensitivity limits of the instrument for many sampling points (data not shown). Copepods settled in the incubation chambers during the last two days of the March experiment, which resulted in high oxygen consumption and TAN excretion rates which could not be related to biodeposit composition but rather were an effect of copepod metabolism. Results of the last sampling in March (day = 22) were therefore not included into the analysis.

Stoichiometric comparisons (CN − NP − NSi release ratios) varied both within as between experimental periods (MANOVA; $p < 0.05$). Likewise, overall the nutrient stoichiometry was different between the three experimental periods during the stable state conditions (Figure 3.4). CN ratios in August (~23) were higher compared the CN values in March and November (~17), NP ratios in November (~11) were higher than March and August (~2.5). NSi ratios were highest in August (1.3), mediocre in November (0.6) and lowest in March (0.02). For each experimental period we observed CN flux ratios which were higher compared to CN ratios in the biodeposits, and NP flux ratios which were lower compared to NP ratios in the mussel biodeposits.

*Mineralization rates*

Mineralization rates were determined by correcting the stable state estimates for $CO_2$, TAN, $PO_4$, $Si(OH)_4$ (defined by the asymptote *A* in the broken line analysis, Table 3.3) for average daily biodeposit quantity (DW) to obtain weight standardized mineralization rates (upper section Table 3.4). Subsequently, mineralization were standardized to organic nutrient concentrations in the biodeposits (POC, PON, POP) to obtain mineralization rates corrected for biodeposit quality (lower section Table 3.4) .However, phosphate mineralization rates in March and August exceeded potentially feasible values based on biodeposit nutrient composition, indicated by fractions >100% when mineralization rates were standardized to the fraction of initial nutrient composition (lower part Table 3.4; values within parentheses). High silicate releases in March lead to mineralization rates which were almost 60-90 fold the values in August and November. Due to the absence of estimates for biogenic silicon in mussel biodeposits, it was not possible to derive Si mineralization rates standardized to element quantity in the biodeposits. On average 24 ± 5 % of the carbon and 17 ± 3 % of the nitrogen initially available in mussel biodeposits is mineralized, with lower values observed in November compared to March and August.
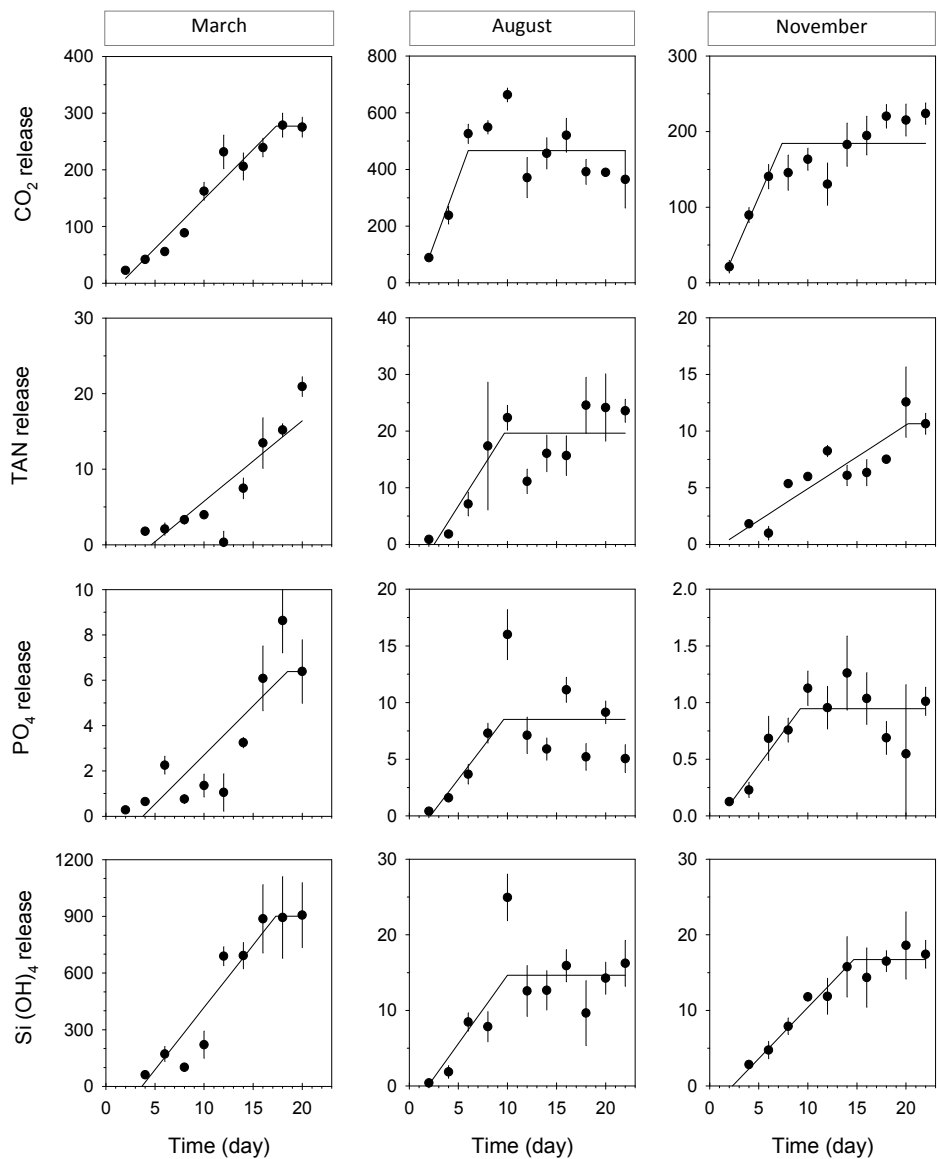
**Figure 3.3**
Measured (dots; mean ± SE) and modeled (solid line) release of carbon dioxide (A), total ammonia nitrogen (B), phosphate (C) and silicate (D) from decomposing mussel biodeposits during the three experimental periods (March, August, November). Modeled values are based on the broken-line analysis (see also Table 3.3). Values are expressed in $\mu mol\ d^{-1}$.

Total nutrient degradation of the biodeposits added to the incubation chambers was quantified in two ways. In the first approach (I) release rates of the inorganic nutrients were integrated from day 1 to day 22, which provided total release rates for the entire experimental period, and were compared to the total amount of organic nutrients added -with biodeposits- to the incubation chambers. This indicated that on average 22 ± 5% carbon and 11 ± 3% nitrogen was mineralized during the entire experimental period (Table 3.5). Phosphorus mineralization showed large fluctuations between the three experimental periods, with flux estimates outreaching the potential values based on biodeposit phosphorus concentration in August (>100%). In the second approach (II) decomposition estimates were based on nutrient composition in fresh biodeposits and biodeposit remnants present in chambers at the end of the experimental period.
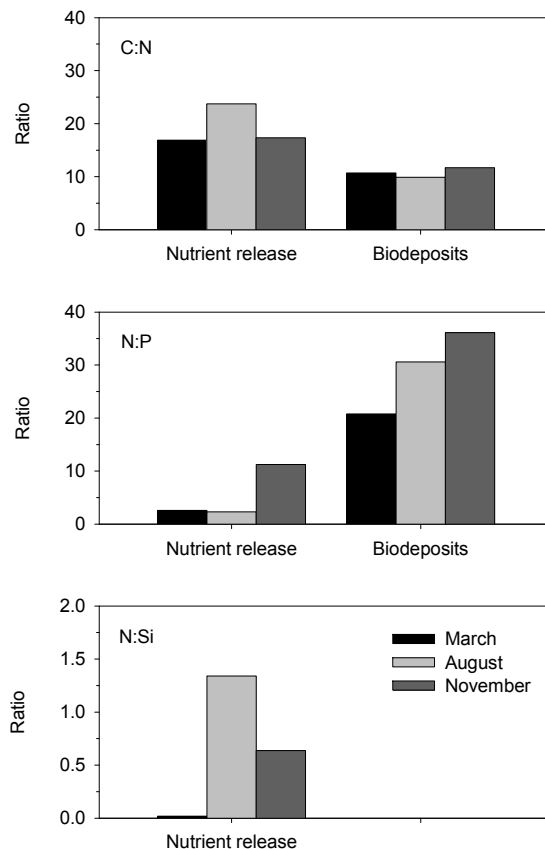


**Figure 3.4**
Stoichiometric comparisons (CN − NP − NSi) of nutrient release rates from decomposing mussel biodeposits and for organic nutrient composition in fresh mussel biodeposits for the three experimental periods (■ March; ▪ August; ■ November) during stable state conditions.

Initial concentrations showed 3-5 times higher values, indicating that on average 66 ± 4% carbon and 63 ± 8% nitrogen was removed from the biodeposits during the experimental period. Based on inorganic nutrient fluxes (approach I) lower degradation estimates were observed for carbon an nitrogen compared to degradation based on organic nutrient composition in biodeposit remnants (approach II). Patterns observed for phosphorus were opposite from carbon and nitrogen, with higher degradation estimates based on approach I.

## Discussion

This study presents mineralization rates for biodeposits trapped in the pelagic phase, for example in between the mussel matrix of suspended cultures (Richard et al 2006, Chapter 4), and we showed that up to one-third of the organic nutrients in biodeposits can be mineralized and thereby become available for primary producers. Although it is well established that degradation of mussel biodeposits leads to enhanced respiration and release of inorganic nutrients, little has been published on biodeposit quality and their specific mineralization rates (reviewed by McKindsey et al. 2011). Hence, our study contributes with new data and insights to this field. Mineralization rates specific for biodeposits are a necessity for optimizing models describing the contribution of bivalves in nutrient cycling of coastal ecosystems. Currently, there is a need for more accurate representation of the effects of biodeposit mineralization in existing carrying capacity models (Henderson et al. 2001).

### Mineralization rates

The present study showed that 24% of the POC and 17% of the PON in mussel biodeposits is mineralized. This is in the same range as reported by Carlsson et al. (2010) who showed that respectively 25% and 38% of the POC in biodeposits are mineralized during decomposition with and without benthic substrate. Giles and Pilditch (2004) indicated that 40% and 18% of respectively the POC and PON composition in biodeposits became mineralized into $CO_2$ and TAN. These authors also suggested that additionally 34% of the PON might have been removed by

**Table 3.3**
Results of the broken line analysis for nutrient release rates (N) of decomposing biodeposits during the three experimental periods. Estimates (± SE) of the asymptote (A), slope (S) and inflection point (B), and $F$ and $p$ statistics for the diphasic function.

| Experimental period | Variable (N) | A | S | B | F | p |
|---|---|---|---|---|---|---|
| March | $CO_2$ | 276.9 ± 13.0 | 17.5 ± 1.4 | 17.3 ± 1.1 | 125.7 | <0.0001 |
| | TAN | 16.4 ± 1.2 | 1.1 ± 0.1 | 19.3 ± 7.1 | 90.0 | <0.0001 |
| | $PO_4$ | 6.4 ± 1.1 | 0.4 ± 0.1 | 18.5 ± 2.9 | 25.1 | <0.0001 |
| | $Si(OH)_4$ | 900.0 ± 80.6 | 66.1 ± 8.8 | 17.3 ± 1.8 | 46.7 | <0.0001 |
| August | $CO_2$ | 466.1 ± 21.2 | 94.7 ± 44.0 | 6.0 ± 1.5 | 20.2 | <0.0001 |
| | TAN | 19.6 ± 1.7 | 2.7 ± 1.0 | 9.7 ± 2.1 | 13.4 | <0.0001 |
| | $PO_4$ | 8.5 ± 0.7 | 1.1 ± 0.4 | 9.6 ± 2.0 | 13.9 | <0.0001 |
| | $Si(OH)_4$ | 14.7 ± 1.1 | 1.8 ± 0.7 | 10.0 ± 2.1 | 17.5 | <0.0001 |
| November | $CO_2$ | 184.5 ± 8.2 | 29.9 ± 8.2 | 7.4 ± 1.1 | 27.4 | <0.0001 |
| | TAN | 10.6 ± 1.3 | 0.6 ± 0.1 | 20.2 ± 2.6 | 40.3 | <0.0001 |
| | $PO_4$ | 1.0 ± 0.1 | 0.1 ± 0.1 | 9.2 ± 2.4 | 7.6 | <0.01 |
| | $Si(OH)_4$ | 16.7 ± 1.1 | 1.4 ± 0.2 | 14.7 ± 1.5 | 40.5 | <0.0001 |

*N = A − S * Z   (with Z = (day < B) - (B − day))*

bacterial assimilation and/or nitrification-denitrification processes and $N_2$ production. As our systems did not include sediment and anoxic conditions were prevented, we assume that in this study $N_2$ production was insignificant. However, anoxic microsites within biodeposits, as suggested by Gowing and Silver (1983), could allow denitrification to occur. The above mentioned studies (Giles and Pilditch 2006, Carlsson et al. 2010) evaluated respiration and nutrient release rates through time (4-10 days) after a single addition of mussel biodeposits. This is different from our approach with multiple additions of biodeposits, and we were therefore also able to quantify mineralization rates under steady state conditions (see Table 4), which represent $CO_2$ and nutrient releases under natural conditions (e.g. within a farm) where continuous deposition of biodeposits occurs. An approach with continuous biodeposition was also chosen by Callier et al. (2009) who performed an *in situ* benthocosm experiment where mussels were placed on top of sediment cores and sediment respiration and nutrient release rates were measured after a 50 day enrichment period (summer). Recalculating nutrient releases from Callier et al. (2009) based on estimated biodeposition rates, results in maximum mineralization rates of 4.5 mmol $CO_2$, 0.3 mmol TAN, 0.02 mmol $PO_4$ and 1.0 mmol $Si(OH)_4$ per gram biodeposit (DW) per day. $CO_2$ mineralization rates were thereby in the same scale as reported for the August results in our study, while TAN and phosphate releases were lower compared to our results. Phosphate releases from sediments underneath suspended bivalve cultures show incoherent results, with positive fluxes measured in some cases (Baudinet et al. 1990, Souchu et al. 2001, Richard et al. 2007b) and neutral or negative fluxes in others (Hatcher et al. 1994, Mazouni et al. 1996, Giles and Pilditch 2006). Low or negative phosphate fluxes are often explained by the absorption of phosphate by iron hydroxides or calcite present in oxidized surface layer of marine sediments (Sundby et al. 1992). Silicate mineralization rates in our study varied between 0.1 and 11.4 mmol $g^{-1}$ $d^{-1}$, which includes the rates reported by Callier et al. (2009). Silicate releases from mussel biodeposit enriched sediments have reported to be in the same order of magnitude or higher than ammonia releases (Baudinet et al. 1990, Richard et al. 2007a, Richard et al. 2007b). Smaal and Prins (1993) also reported relatively high silicate releases from decomposing biodeposits, while no silicate releases from decomposing mussel biodeposits were observed by Fabiano et al. (1994).

**Table 3.4**
Mineralization rates derived from the stable state measurements during the three experimental periods (March, August, November).

| | $CO_2$ | TAN | $PO_4$ | $Si(OH)_4$ |
|---|---|---|---|---|
| *Mineralization (mmol $g^{-1}$ DW biodeposit $d^{-1}$)* | | | | |
| March | 3.53 | 0.21 | 0.08 | 11.47 |
| August | 4.30 | 0.18 | 0.08 | 0.14 |
| November | 2.04 | 0.12 | 0.01 | 0.18 |
| Annual average | 3.29 ± 0.66 | 0.17 ± 0.03 | 0.06 ± 0.02 | 3.93 ± 3.77 |
| *Mineralization (% of the organic nutrient composition in biodeposits)* | | | | |
| March | 31 | 20 | (157) | |
| August | 27 | 11 | (147) | |
| November | 15 | 10 | 32 | |
| Annual average | 24 ± 5 | 17 ± 3 | | |

Total nutrient mineralization throughout the experimental periods was 3-5 times lower for carbon and nitrogen based on flux measurements compared to biodeposits nutrient composition measurements, indicating that more nutrients were released from the biodeposits than was estimated based on the measured inorganic nutrient fluxes. This may partly be due to the fact that biodeposits were added to the chambers after the incubations and hence mineralization during the first day was not included in the measurements. Potentially, this has led to an underestimation of the nutrient fluxes and mineralization rates. However, similar mismatches between inorganic nutrient releases and biodeposit nutrient composition were observed by Carlsson et al. (2010) who related the relatively low nutrient composition in decomposed faeces to the release of dissolved organic material. They demonstrated that only 53% of the organic carbon (POC) degradation in biodeposits could be attributed to mineralization related processes ($CO_2$ release), whereas 47% was related to leaking of labile dissolved organic carbon (DOC) from the faecal pellets. Dissolved organic nutrients might have been removed from our experimental chambers during the periods in between the incubations when the chambers functioned as a flow-through system, hence carbon and nitrogen mineralization rates reported for our study may represent an underestimation. Phosphorus results in our study showed an opposite pattern to carbon and nitrogen, as higher degradation values were observed based on the flux measurements compared to biodeposits nutrient composition measurements, indicating that more phosphate was released from decomposing biodeposits than predicted from nutrient composition in the biodeposit remnants at the end of the experimental period. This was particularly clear in August when flux estimates exceeded the theoretical value of 100% and POP composition in the biodeposit remnants was comparable to the composition in fresh biodeposits. This suggests that potentially there has been an external source of phosphorus accumulating onto, or binding with, the biodeposits throughout the experimental period, which partially reversed during the incubations. Further research is required to determine the potential mechanisms that underlie to these processes.

*Mineralization kinetics and seasonal variability*

Mineralization kinetics vary between the different elements and are influenced by seasonal changes in temperature and biodeposit nutrient composition. Carbon, nitrogen and phosphorus are mineralized by microbial activity, whereas silicate mineralization is essentially different as it relies on dissolution processes rather than biological activity (Canfield et al. 2005). Carbon mineralization was faster compared to the other elements, indicated by lowest inflection points (B) during all experimental periods. This is in agreement with Giles and Pilditch (2006) who

**Table 3.5**
Nutrient degradation as a fraction (%) of initial nutrient composition based on two complementary approaches: (I) integrated nutrient fluxes related to the total amount of nutrients added to the incubation chambers during the whole experimental period, (II) nutrient composition in the biodeposit remnants in the incubation chambers following the experimental period related to nutrient composition in fresh biodeposits

| | Carbon | | Nitrogen | | Phosphorus | |
|---|---|---|---|---|---|---|
| | Flux | Composition | Flux | Composition | Flux | Composition |
| March | 25 | 59 | 15 | 52 | 82 | 41 |
| August | 28 | 63 | 10 | 60 | 133 | 4 |
| November | 13 | 74 | 5 | 77 | 23 | 21 |

showed that maximum respiration ($CO_2$ release) occurred before maximum TAN releases. It is generally assumed that silicon in biodeposits is mineralized on long time scales (Baudinet et al. 1990, Nelson et al. 1995). However, during all three experimental periods we observed an immediate response in silicate releases. Although underlying kinetics may vary between the different elements, mineralization processes of all four elements (C-N-P-Si) should be positively correlated to temperature as both bacterial growth and dissolution processes are enhanced by increasing water temperatures (Lerat et al. 1990, White et al. 1991, Katterer et al. 1998). Indeed we observed faster mineralization at increasing temperatures, with a 2-3 times higher inflection point ($B$) in spring compared to summer. This fits with $Q_{10}$ values which are typically assumed to vary between 2 and 3 (Katterer et al. 1998, Thamdrup and Fleischer 1998), indicating that organic matter decomposition increases by a factor 2-3 with a 10 °C increase in temperature.

Biodeposits are rich in nutrients and organic nutrient concentrations and stoichiometry are essential rate-controlling parameters for mineralization (del Giorgio and Cole 1998, Canfield et al. 2005). Nutrient composition varies on both temporal and spatial scales (Kautsky and Evans 1987, Jaramillo et al. 1992, Giles and Pilditch 2004) and is influenced by the quantity and quality of food the mussels feed on (Miller et al. 2002, Giles and Pilditch 2006). Our study area is characterized by low food conditions throughout most of the year, with exception of phytoplankton blooms in spring and occasionally in autumn (Chapter 2, Strohmeier et al. 2009). Pseudofaeces production is rare in areas with low seston conditions (Strohmeier 2009), and was only observed in the first week of the March experiment during the spring bloom. The biodeposit nutrient composition was relatively low during the first week of March, which is in agreement with Smaal and Prins (1993) who showed that nutrient composition of faeces is higher than for pseudofaeces. However, this does not seem to be a consisted pattern as other studies reported the opposite with higher nutrient composition for pseudofaeces compared to faeces (Navarro and Thompson 1997, Giles et al. 2006). In accordance with the lower nutrient composition of pseudofaeces Smaal and Prins (1993) also demonstrated that mineralization rates of pseudofaeces were lower compared to faeces. Biodeposit mineralization rates measured in our study were an integrated result of faeces and pseudofaeces mineralization. Indeed we observed relatively slow increase in TAN and $PO_4$ releases during the first week of the March experiment which might be the result of the presence of pseudofaeces. Although the average carbon and nitrogen composition in biodeposits did not vary between seasons, mineralization rates in spring were almost double the rates measured in autumn, suggesting that biodeposits consisted of more labile material during spring season. Additionally, low availability of micronutrients might be limiting mineralization processes in autumn (Dixon 2008), while the abundance of micronutrients is generally high following the winter season. Nutrient concentrations measured in our study were in the higher range of values reported for other areas which ranged between 25-130 and 3-11 mg $g^{-1}$ for POC and PON, respectively (Kautsky and Evans 1987, Jaramillo et al. 1992, Hatcher et al. 1994, Navarro and Thompson 1997, Giles and Pilditch 2004, 2006), indicating that weight (DW) standardized mineralization rates measured in our study will likely be higher from other areas. In order to determine mineralization rates standardized to biodeposit quality it is necessary to know the biodeposit nutrient composition. However, in general there is little information available about phosphorus and silicon composition in bivalve biodeposits. Phosphorus composition as measured in our study varied between seasons, and the overall values were lower compared to values reported by Kautsky and Evans (1987). The relatively high phosphate mineralization rates measured in spring and summer compared to autumn are likely the cause of a combination of biodeposit composition and release of phosphate by and external source (see previous section), as standardization to biodeposit quality

showed that mineralization rates exceeded potential rates based on phosphorus composition in the biodeposits during spring and summer. Silicate mineralization rates were 60-80 times higher in spring compared to summer and autumn measurements, which might be induced by higher concentrations of biogenic silicon in the biodeposits during spring. Although direct estimates were not available it is likely that silicon composition of the biodeposits was higher in spring as the phytoplankton population was characterized by high numbers of the diatom species *Skeletomena* during the spring bloom. During summer and autumn diatoms were less abundant and the phytoplankton population consisted predominantly of small flagellates (Jansen et al. in press). As diatoms contain high concentrations of silicon while mussels have minimal requirements for this element, nearly all ingested silicon will be expelled with the biodeposits (Wikfors 2011). There is one study providing estimates of biogenic silicon concentrations in mussel (*Modiolus modiolus*) biodeposits (Navarro and Thompson 1997). For this study conditions were similar to spring bloom conditions at our study site in terms of chlorophyll *a* concentrations, phytoplankton composition (high number of diatoms), and biodeposit carbon and nitrogen composition (Navarro and Thompson 1997, Chapter 2). They reported a biodeposit silicon concentration which was approximately 3 times higher than the carbon concentration. Although it is unknown whether biodeposit composition and mineralization rates can be directly linked it is noteworthy that the C:Si ratio in biodeposits reported by Navarro and Thompson (1997) was similar to the ratio of $CO_2$:$Si(OH)_4$ in mineralization rates observed in our spring experiment.

*Implications*

Through production and mineralization of biodeposits mussel populations are efficient mediators in nutrient recycling. Biodeposits produced by suspended mussel cultures may decompose at three sites; (*i*) biodeposits trapped in between the mussel-matrix on the suspended mussel ropes decompose in the euphotic zone of the water column where the culture structure is located (Richard et al. 2006), (*ii*) while descending to the seafloor, biodeposits may decompose in the pelagic phase of the water column (Carlsson et al., 2010), and (*iii*) biodeposits reaching the seafloor will either be buried in the sediment or be decomposed by benthic processes (Baudinet et al. 1990, Hatcher et al. 1994). The relative contribution of each of the decomposition-sites is situation specific and determined by physical and environmental conditions of the bivalve cultivation area (Newell et al. 2005). In bottom cultures pelagic decomposition is limited and it is suggested that benthic biodeposit decomposition contributes significantly to total nutrient regeneration from mussel beds (Asmus et al. 1990, Prins and Smaal 1994). In suspended bivalve cultures benthic decomposition sites are spatially decoupled from the pelagic culture units (Newell 2004), yet both benthic and pelagic decomposition sites contribute to nutrient regeneration in shallow culture areas due to a strong benthic-pelagic coupling (Prins et al. 1998). However, when benthic-pelagic coupling is limited, as in deep fjord systems, only the decomposition which takes place in the pelagic/euphotic zone is relevant for primary producers.

It is unknown which fraction of the total produced biodeposits is trapped within the mussel matrix and which fraction is transported to the benthic system. The total amount of organic material associated with mussels rope sections of 1m length was determined in Chapter 4 and showed that this was approximately 3 times the total daily biodeposit production per meter rope (recalculated from Chapter 2). The amount of organic material associated with the mussel ropes was equal between culture sites that were comparable in terms of mussel size and density (Jansen unpublished data), indicating that settling of organic material (biodeposits) on the ropes is space limited. Integrating total organic material on ropes (Chapter 4) with mineralization rates

determined in the current study, showed that maximum release rates per meter suspended rope ($m^{-1}$) were in the same order of magnitude with maximum release rates from sediment ($m^{-2}$) underneath suspended mussel farms (Baudinet et al. 1990, Hatcher et al. 1994, Giles et al. 2006, Richard et al. 2007b). However, the pelagic regeneration for suspended ropes is slightly overestimated by this approach as the fraction of inorganic material was lower for mussel biodeposits (Tabel 3.1) compared to the material associated with the ropes (Chapter 4). This suggests that the material on the ropes consists of more refractory material, subsequently leading to lower potential mineralization rates. Lower minimum TAN and phosphate release rates for sediments underneath farms ($m^{-2}$) (Baudinet et al. 1990, Hatcher et al. 1994, Giles et al. 2006, Richard et al. 2007b) compared to estimates from the present study for suspended ropes ($m^{-1}$) can partly be related to differences in nutrient release kinetics between benthic and pelagic decomposition sites. Marine sediments may bind phosphate (Sundby et al. 1992) and TAN-nitrogen may be transformed into other nitrogenous forms (nitrate, nitrite or nitrogen gas) by nitrification-denitrification processes that occur in oxic/anoxic sediment layer (Canfield et al. 2005, Torres-Beristain et al. 2006) resulting in lower benthic fluxes.

Regenerated nutrients originating from decomposition of biodeposits may enhance the availability of nutrients for primary producers (Prins et al. 1998) but may also affect stoichiometric relations between the elements which potentially can lead to a shift in phytoplankton population composition (Prins et al. 1995). This study showed that mineralization of carbon and phosphorus was preferred over nitrogen, as indicated by higher CN ratios and lower NP ratios for the inorganic nutrient releases compared to the organic nutrient composition of the biodeposits. The relative nitrogen releases (NP and NSi) were below Redfield's ratios (Redfield et al. 1963) and below ratios measured in the ambient water (Jansen et al. 2011), indicating that regenerated nutrients indeed have different stoichiometric characteristics compared to inorganic nutrient pools available in the natural environment.

*Conclusions*

This study has shown that mineralization rates of mussel biodeposits vary seasonally. Variations were induced by the concentrations of (macro-)nutrients (C-N-P-Si) in biodeposits, whereas mineralization rates standardized to biodeposit nutrient composition suggested that the proportion of labile material in the biodeposits or availability of micronutrients also regulate mineralization processes. Increased temperatures enhance biodeposit mineralization with approximately 2-3 times faster turnover at a 10ºC temperature interval ($Q^{10}$). Furthermore, mineralization dynamics varied between nutrients, with mineralization of carbon and phosphorus being preferred over nitrogen. This resulted in different stoichiometric characteristics of the regenerated nutrients compared to inorganic nutrient pools in the natural environment. Data presented by the current study can be used to optimize the biodeposition compartment of models describing the contribution of bivalves in nutrient cycling of coastal ecosystems.

# Chapter 4

Nutrient regeneration by suspended
mussel cultures

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## ABSTRACT

Mussel cultures (*Mytilus edulis*) contribute to nutrient cycling in coastal ecosystems. Mussel populations filter particulate nutrients from the water-column and inorganic nutrients are regenerated by excretion of metabolic wastes and decomposition of (pseudo)faeces. The objective of this study was to determine the intra-annual variability in nutrient regeneration by mussel rope cultures in oligotrophic fjord systems. In situ respiration and nutrient uptake and release rates of mussel ropes (1m) were measured using pelagic chambers (250L). There was a 20-fold difference between winter and summer respiration and nutrient release rates. Inorganic nitrogen release ranged from 50 to 1000 µmol hour-1 per meter rope. These variations were mainly related to mussel growth but also to changes in water temperature as well as biofouling biomass (organisms that colonized the mussel ropes). In total 24 genera of fouling organisms were observed, diversity increased over time (ranging between 2-12 genera $m^{-1}$), and fouling biomass was mainly determined by ascidians (max. 37 ± 14 g $m^{-1}$). However, mussels dominated the culture ropes, representing >90% of total fauna biomass. The amount of organic material associated with the ropes was stable (6.9 ± 0.3 g $m^{-1}$). At the scale of one mussel farm, nutrient regeneration by mussel rope cultures increase inorganic nitrogen concentrations by 20% and inorganic phosphorus concentrations by 5% during summer conditions. During winter, there is no significant effect of mussel cultures on the inorganic nutrient pools. Nutrient regeneration by mussel cultures also affect stoichiometry as nutrients were excreted in dissimilar proportions (N>P>Si). The increased nutrient availability may contribute to primary production, especially in nutrient limited (oligotrophic) fjord ecosystems. However, fjord-scale effects are largely dependent on hydrographic conditions of the fjord system.

## Introduction

Suspension feeding bivalves influence the ecosystem by filtration of particulate matter, biodeposition and decomposition of (pseudo)faeces, and excretion of dissolved metabolites (Prins et al. 1998, Newell 2004, Cranford et al. 2007). It has been argued that, under nutrient limiting conditions, inorganic nutrients released by bivalve populations and decomposed (pseudo)faeces may stimulate primary production (Asmus & Asmus 1991, Dame & Libes 1993, Prins et al. 1998). Indeed, several studies confirmed nutrient release by benthic mineralization underneath mussel farms (Baudinet et al. 1990, Giles et al. 2006, Richard et al. 2007b). However, the contribution of benthic mineralization to nutrient availability for pelagic primary producers is largely dependent on the mixing between benthic and euphotic water layers. In shallow areas, the water column is well mixed and benthic released nutrients are available to the pelagic primary producers (Prins & Smaal 1990, Hatcher et al. 1994). In contrast, in deep culture areas, regenerated nutrients are not available to pelagic primary producers, particularly not in cases of profound vertical stratification of the water column. In deep coastal ecosystems, nutrient regeneration in the euphotic zone is therefore most important in the feedback loop from suspended mussel cultures to primary production.

Norwegian fjords are characterized by a deep water column (100 - 1000 m), and in spring and summer fjords are stratified as a result of calm wind conditions that restrict vertical mixing of nutrients into the euphotic layer (Aure et al. 1996, Asplin et al. 1999). Nutrients derived from freshwater runoff are generally less important than the vertical mixing of nutrients in Norwegian fjord systems (Aksnes et al. 1989). Consequently, the euphotic zone is nutrient limited for extended periods of the year (Paasche & Erga 1988, Sætre 2007) resulting in chlorophyll $a$ (Chl $a$) concentrations of generally less than 2 µg l$^{-1}$ (Erga 1989a, Aure et al. 2007b). Phytoplankton is the major component of seston along the Norwegian coast (Erga 1989a, Erga et al. 2005, Strohmeier et al. 2009). Primary production rates are typically 100-140 g C m$^{-2}$ yr$^{-1}$ (Aure et al. 2007a) of which a ratio of 0.4 is regarded as new primary production (Wassmann 1990), resulting in a new carbon supply of 40-56 g C m$^{-2}$ yr$^{-1}$. Norwegian fjords are thereby classified as oligotrophic within the trophic classification of marine systems by Nixon (1995). In this kind of oligotrophic environment, it is essential to understand nutrient regeneration, especially in bivalve culture areas where bivalve growth depends on the availability of phytoplankton.

Studies on nutrient cycling in suspended cultures often focus on (*i*) benthic mineralization of biodeposits (Baudinet et al. 1990, Giles et al. 2006, Richard et al. 2007b), or (*ii*) biochemical processes of the culture itself considering only the mussel biomass (Dowd 2005, Cranford et al. 2007, Brigolin et al. 2009) rather than the whole biological community on the ropes. Besides mussels, suspended mussel cultures comprise of a complex habitat of bacteria, epifauna, epiflora, and trapped biodeposits, each contributing to the uptake and release of nutrients (Richard et al. 2006, 2007). The abundance and composition of epifauna related to mussel cultures include ascidians, barnacles, bryozoans, polychaetes, amphipods and gastropods, and vary seasonally (Cayer et al. 1999, Khalaman 2001, Richard et al. 2006, Lutz-Collins et al. 2009). Biodeposits partially accumulate in spaces between mussels on ropes, and create a sediment compartment in the water column (Mazouni 2004, Richard et al. 2006). The combined fauna and biodeposit compartment will hereafter be referred to as the 'associated fauna and organic matter' (AFOM) complex (see also Richard et al. 2006).

Nutrient dynamics of complete bivalve culture units indicate that associated fauna contributes to the total nutrient release (LeBlanc et al. 2007), and that AFOM complexes contribute to nitrate, nitrite and silicate fluxes, while the cultivated species (*Mytilus edulis*)

primarily contribute to ammonia, phosphate and oxygen fluxes (Richard et al. 2006). By investigating two year classes and sampling both in August and September, Richard et al. (2006) showed that the relative contribution of the AFOM complex to total nutrient fluxes depends on its composition and thus on farming cycle and season. Furthermore, seasonal variation in AFOM biomass and its composition also influenced nutrient dynamics in oyster culture (Mazouni et al. 2001). Previous mentioned studies were all performed in shallow ecosystems but in order to get a better understanding in nutrient regeneration by mussel suspended cultures there is a need for studies in contrasting environments, such as deep oligotrophic fjords, as well as studies covering a full annual cycle.

The objective of this study was to explore the hypothesis of intra-annual variability in nutrient regeneration by mussel (*Mytilus edulis*) rope culture in the euphotic zone of Norwegian fjord systems. During the course of one year, two *in situ* experiments were conducted simultaneously: (*i*) an AFOM experiment which focussed on quantifying temporal changes in mussel density and, fauna and organic material associated with mussel ropes and (*ii*) a nutrient flux experiment which aimed at quantifying temporal variation in oxygen, nitrogen, phosphate and silicate fluxes along mussel ropes using large pelagic chambers.

## Material and methods

### Environmental monitoring

Fluorescence, turbidity, temperature and salinity were simultaneously measured at 30 minutes intervals at 1.5 meter depth using a STD/CTD 204 (SAIV A/S, Norway). Water samples for analysis of seston quantity and quality were taken at 1.5 meter depth at weekly intervals (twice a week during the spring bloom). Particulate organic carbon (POC) and nitrogen (PON), chlorophyll *a* (Chl *a*) and phaeopigment (Phaeop.) concentrations were determined by filtering 250-500 ml onto a 1.2 µm filter (Whatman GF/F). Chl *a* and Phaeop. were analyzed after extraction with 90% acetone using the fluorescence method with correction for acidified measurements (Strickland & Parsons 1968). The fluorometer (Turner Designs Model 10-AU) was calibrated with known concentrations of Chl *a* (Sigma Chemicals, St. Louis, Mo., USA) and measured spectrophotometrically.

Fluorescent measurements were converted to Chl *a* concentrations ($\mu g \, l^{-1}$), to obtain high-frequent Chl *a* time series, using the equation:

Chl *a* = 0.75 * fluorescence - 0.06      ($r^2$ = 0.83, n = 55).

POC/PON concentrations were determined using a Thermo Finnigan Flash EA 1112 NC Analyzer after drying and fluming the filters over concentrated HCL for 0.5 h in a closed container to remove inorganic carbon (Ehrhardt 1983).

### Mussel ropes

Mussels used in this study settled in 2007 and were re-socked in February 2008 at a commercial mussel farm (Åfjord, N63º55´, E0010º11´). Mussel ropes consisted of extruded polypropylene rope material (Christmas Tree), attached mussels (*Mytilus edulis*) and the AFOM complex. Forty sections of 1 meter rope were transferred to the study site (Austevoll, N60º05´, E005º16´) in November 2008 and deployed vertically in the water column from approximately 30cm depth. Ten of these sections were used in the nutrient flux study; the remaining 30 sections were used for the AFOM study (see paragraph 'experimental design'). In May 2009, most mussels were lost

due to predation. New mussel rope sections (1m) were transferred from the commercial farm to the study site by the end of May 2009. These mussels originated from the same cohort and farming site as the first batch. Ten sections were assigned to the nutrient flux experiments, and 20 sections to the AFOM study.

*Experimental design*

Two studies were performed from February 2009 to January 2010; (*i*) an AFOM study, and (*ii*) a nutrient flux study. Within the AFOM study, four mussel ropes were bimonthly collected by scuba diving. Within the nutrient flux study repeated measurements on ten mussel ropes were conducted monthly, with one additional measurement during the spring bloom. Due to the loss of mussels in May 2009, only five ropes were included in the May sampling. To compare biomass estimates in both studies, AFOM determinations were also performed on the ropes used for the nutrient flux study (n = 10) following the last sampling in January 2010. Extrapolation of AFOM results to the corresponding oxygen and nutrient fluxes specifies the relative contribution of the mussels and AFOM complexes.

*AFOM (associated fauna and organic material) study*

To determine mussel and AFOM biomass, mussel ropes were collected by scuba diving. A soft polyethylene plastic enclosure (diameter 30 cm, height 100 cm) was gently raised around the mussel rope. Hence, all organic material associated with a mussel rope was collected within the plastic enclosure. The enclosures, including the mussel ropes and water, were transferred to the laboratory where the mussels were removed from the ropes and organic material was resuspended. The mussels were frozen until biomass analysis. The suspension was sieved through a 1 mm sieve and the fauna were collected and preserved with formalin (4%). The total amount of Suspended Particulate Material (SPM) was determined by filtering subsamples in triplicate onto pre-combusted and weighed filters (Whatman GF/C). Salt was removed from the filters by rinsing with deionised water. Filters were dried at 60 °C over night and weighed resulting in SPM values. The filters were combusted at 450 °C for 6 hours to determine organic (POM) and inorganic (PIM) fractions. Additional subsamples were taken to determine the quality of the organic material; POC, PON, Chl *a* and Phaeop (see section "Environmental monitoring" for description analyses). Mussel samples were subsampled to determine the total number of mussels on a rope and to determine individual length and weight. Individual length was measured with a digital calliper (± 0.01 mm), and tissue was removed from the shells and dried for at least 72 hours at 60 °C to determine dry weight (DW). These tissue samples were also combusted at 450 °C for 6 hours to determine ash free dry weight (AFDW). Fauna determinations were performed to genus level, since we are mainly interested in the functional role of the associated organisms. A similar protocol for DW and AFDW estimation as described above for mussels was applied to the fauna samples.

*In situ nutrient flux study*

Incubations with pelagic chambers were conducted to determine oxygen and nutrient fluxes along the mussel rope interface. The pelagic chambers consisted of 250 l rigid white polyethylene tanks (diameter = 50 cm, height = 150 cm with conical shaped bottom) which could be sealed off from the bottom and the top (Figure 4.1). A pump was mounted inside each chamber to mix the water and flow was regulated (10 l min$^{-1}$) in order to minimize resuspension of organic material. Homogenously mixing was confirmed by measuring nutrient concentrations simultaneously at 16 positions within the chamber. An oxygen optode (no. 4835, Aanderaa) and

STD/CTD were mounted into one of the chambers to record oxygen and fluorescence concentrations at two second intervals during the incubations.

In total six pelagic chambers were used, allowing simultaneous sampling of five ropes and one control. The control consisted of a pelagic chamber filled with water but without a mussel rope, and was used to correct for fluxes other than mussel rope metabolism. To sample all ten ropes, two sets of incubations were performed during one day or two subsequent days, depending on incubation time. When deploying the chambers, both bottom and top were open and the chambers were gently raised around the mussel ropes, minimizing water movement around the ropes. Incubations started when both the bottom and top were sealed off, and ended when the oxygen concentration had decreased 10% compared to initial values. Total incubation time varied between 0.5 and 6 hours, depending on the season. Oxygen measurements and water samples for dissolved inorganic nutrient concentrations were taken in all chambers at the start and end of each incubation. Dissolved inorganic nutrient samples (20 ml), other than Total Ammonia Nitrogen (TAN), were preserved with chloroform and stored in a cool and dark place until analysis. Those samples were analyzed according to standard methods (Parson et al. 1992) adapted for an auto-analyzer. TAN samples (20 ml) were directly frozen until analysis (Holmes et al. 1999). TAN concentrations were analyzed by means of fluorometric analysis (Kerouel & Aminot 1997, Holmes et al. 1999).

Fluxes were determined by the difference between the start and the end values, and multiplied by the chamber volume. Linear decline in oxygen concentration was confirmed by the continuous oxygen measurements in one of the pelagic chambers during each incubation. Finally, fluxes were corrected for fluxes measured in the control chamber, even though the control fluxes were negligible small compared to fluxes measured on the mussel ropes.
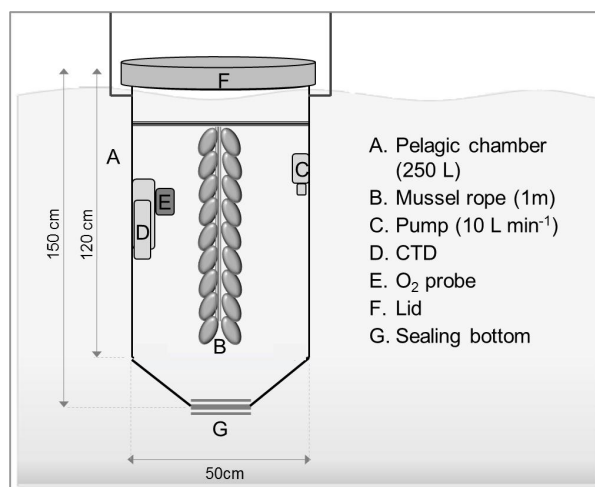


**Figure 4.1**
Schematic representation of the pelagic chamber used for the *in situ* oxygen and nutrient flux measurements of mussel ropes

*Statistical analysis*

All data were checked for homogeneity and normality of variance assumptions by i) visually examining standardised residuals versus predicted values plots and Q-Q plots of residuals, ii) Shapiro-Wilk tests and iii) Levene tests (Quinn & Keough 2002). When one of the assumptions was violated, appropriate data transformations were performed. In case transformations did not lead to acceptance of the assumptions, non-parametric tests were performed. Statistical analyses were performed using SAS 9.1, and all data are presented as mean ± SE, unless stated otherwise.

One-way Analysis of Variances (ANOVA) were used to test the temporal variation in mussel and AFOM parameters in the AFOM study. The effect of time (sampling month) was tested for the following variables separately: mussel density, mussel weight, mussel length, fauna biomass (sqrt transformations), amount (POM) and quality of organic material (POC and PON log transformations). In case of significant results, Tukey's HSD post hoc multiple comparison tests were used to determine which of the sampling months were significantly different from each other. A nonparametric Kruskal-Wallis test followed by pairwise comparisons with Mann-Withney *U* tests were used to test the temporal variation for the parameter 'number of fauna genera'.

We used t-tests to test whether mussel ropes used in the AFOM and the nutrient study were similar. For the following variables, separate t-tests were used to test for variation in: mussel density, mussel weight, mussel length, fauna biomass, number of fauna genera, amount of organic material (POM) and quality of organic material (POC, PON, Chla, phaeopigments). A non-parametric Kruskal-Wallis test was performed for the C:N ratio of organic matter.

Pearson correlation analysis was used to identify whether oxygen consumption and nutrient release rates were significantly related to mussel biomass.
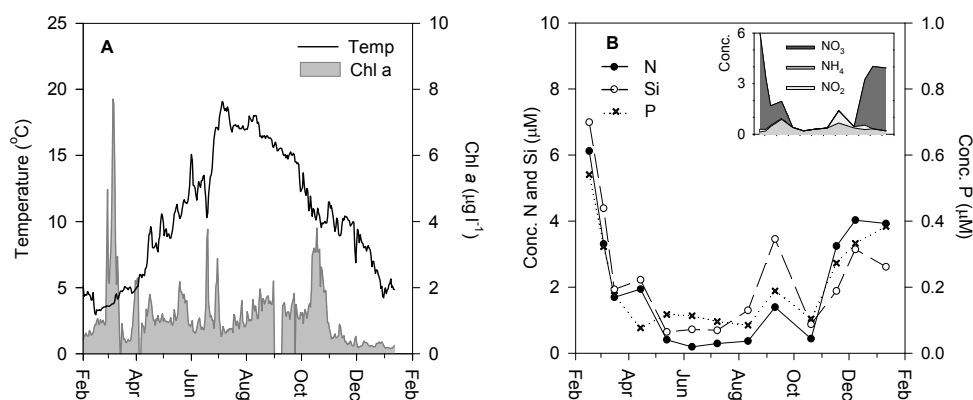


**Figure 4.2**
Changes in environmental conditions measured in the water column at the study site from February 2009 to January 2010. A: Daily averages of phytoplankton (Chl *a*) and temperature. B: monthly measurements of dissolved inorganic nitrogen (N), phosphate (P) and silicate (Si) concentrations. Inset represents fractionation of nitrogen flux into nitrate ($NO_3$), nitrite ($NO_2$) and total ammonia nitrogen (TAN)

Stoichiometric comparisons (N:P, in atomic equivalents) were made for the ambient water and nutrient fluxes by performing repeated measures ANOVA's. The sampling months (time) comprised the within factor treatment, and the two stoichiometric variables (ambient and flux) comprised the between factor treatment. In addition, statistical significance was estimated from the Greenhouse-Geisser adjusted probability to avoid violating the assumption of sphericity of the within-subject (time) variance-covariance matrix (Field 2005).

## Results

### Environmental conditions

Temperature ranged from 3ºC in February to 19ºC in the beginning of July (Figure 4.2a). The spring bloom started in late February and lasted for approximately 2 weeks (Figure 4.2a). Maximum Chl $a$ values were recorded in the first week of March (7.7 µg l$^{-1}$). Chl $a$ concentration varied between 1-2 µg l$^{-1}$ from mid-March to October, followed by an autumn bloom in October (maximum 3.8 µg l$^{-1}$). From November to January Chl $a$ concentration was below 0.5 µg l$^{-1}$. Salinity was 29.7 ± 1.6 ppt (mean ± SD) throughout the study period. Dissolved phosphate, silicate and inorganic nitrogen concentrations were highest in winter and lowest in summer (Figure 4.2b). Total inorganic nitrogen was calculated as the sum of TAN, nitrate and nitrite. Nitrate values, however, dominated the pattern for total nitrogen concentrations (see inset Fig 2b). During the study, concentrations of particulate organic carbon (POC) and nitrogen (PON) ranged between 45-546 µg l$^{-1}$, and 6-52 µg l$^{-1}$, respectively. Highest values were recorded in spring and summer, decreased during autumn, and the lowest values were recorded in winter. Seasonal changes in POC and PON followed a parallel pattern and hence C:N ratios showed little variation (10.2 ± 1.9; mean ± SD).

### Mussel biomass

Individual mussel weight (AFDW) doubled from March 2009 to January 2010 (Figure 4.3), and although weight decreased from October to January, this was not significant (Tukey; $p < 0.05$).
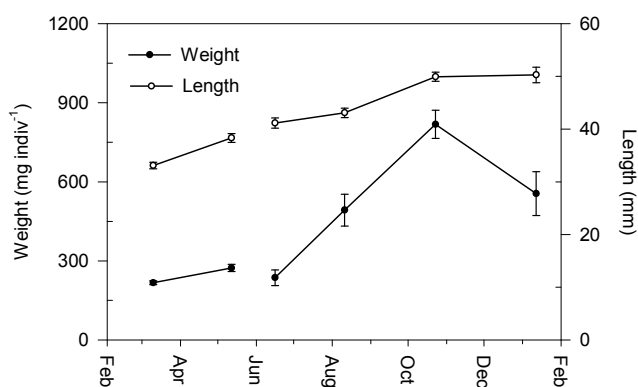


**Figure 4.3**
Individual mussel (*Mytilus edulis*) length and weight (AFDW), from February 2009 to January 2010. Data is presented as average (± SE) and dotted lines between the May and June sampling specifies the two separate mussel batches used in the study

Average length increased from 33 ± 1 to 50 ± 2 mm (Figure 4.3). Interpolating individual mussel weight, to mussel density on the ropes used in the flux study (543 ± 23 m$^{-1}$) resulted in average biomass estimates of 118 g m$^{-1}$ in February 2009 and reached 301 g m$^{-1}$ in January one year later. There were no significant differences in density (t-test; $F_{1,12}$ = 3.31; $p$ = 0.094), length (t-test; $F_{1,12}$ = 3.70; $p$ = 0.079) and weight (t-test; $F_{1,12}$ = 0.76; $p$ = 0.400) between mussel ropes used in the AFOM or in the nutrient flux study in January 2010.

*AFOM composition and biomass*

The biomass and composition of fauna associated with the mussel ropes increased significantly during the study (Table 4.1). The total fauna biomass (AFDW), excluding mussels, varied from 0.10 ± 0.03 g m$^{-1}$ in June to 37.66 ± 14.07 g m$^{-1}$ in October. The difference in fauna biomass of the second batch (mussel ropes obtained in June) compared to the first batch (March-May) was considered to be negligible in the context of biomass increases observed later during the study period. Additionally, similar fauna genera were observed between the two batches, indicating that the two batches were comparable in terms of fauna type and biomass. At the end of the study, in January, no statistical difference in fauna biomass was found between the ropes used in the AFOM study and the ropes used in nutrient flux study (t-test; $F_{1,12}$ = 0.83; $p$ = 0.380). In total, 24 genera were identified, with a maximum mean number of approximately 12 genera observed per meter rope. The fauna composition consisted both of sedentary as well as errant species. Sedentary fauna accounted for most of the faunal biomass and was predominantly represented by the ascidian *Ciona intestinalis*. Settlement of juvenile *C. intestinalis* was observed in August followed by a rapid increase in biomass. Maximum biomass was measured in October, however, it is assumed that this biomass was already reached in September (visual observations). Towards the end of the study (Oct-Jan) >90% of the total fauna biomass was represented by ascidians. Abundance of bryozoans and the ascidian *Botryllus* also increased over time (visual observations). The polychaetes *Harmothoe* and *Nereis* were the most abundant errant species. In January, the average biomass was 0.44 ± 0.09 and 0.68 ± 0.25 g m$^{-1}$, for those two genera respectively, which corresponded to 10-12 individuals m$^{-1}$. Not all individuals were identified to species level, but *Nereis diversicolor* attributed primarily to the *Nereis* genus.

The average amount (AFDW) of organic material associated with mussel ropes was 6.9 ± 0.3 g m$^{-1}$ (Table 4.2) and did not show significant differences between sample dates (ANOVA; $F_{5,18}$ = 2.53; $p$ = 0.067). The C:N ratio of the organic material did not differ between sample dates (Kruskal-Wallis; $p$=0.071), while the Pheo:Chl $a$ ratio varied between sampling dates (ANOVA; $F_{4,15}$ = 6.05; $p$ = 0.004), where the October showed significantly higher values compared to March, May and June (Tukey; $p$ < 0.05). The above indicates that there was no difference between the two mussel batches (March-May vs June-January) in terms of quantity and quality of the organic material associated to the ropes. Almost twice as much organic material was found on the ropes used in the flux study compared to the AFOM study (t-test; $F_{1,12}$ = 11.69; $p$ = 0.005), C:N ratios were significantly different (Kruskal-Wallis; $p$ = 0.034), while Pheo:Chl $a$ ratios were not significantly different (t-test; $F_{1,12}$ = 0.40; $p$=0.538) between the nutrient flux and AFOM study.

**Table 4.1**

Biomass of fauna associated with mussel ropes (*Mytilus edulis*) for the ropes used in the AFOM study (n=4 ropes per sampling point) and nutrient flux study (n=10 ropes). Data is presented as average (± SE) AFDW (g) and standardized to mussel ropes of 1m length. Standard error is only provided when a fauna genus was found on more than one replicate rope.

| | AFOM study | | | | | | Flux study |
|---|---|---|---|---|---|---|---|
| | March | May | June | August | October | January | January |
| *Sedentary fauna* | | | | | | | |
| Anthozoa, indet[***] | | | | 0.01 | | | 0.05 ± 0.02 |
| Ascidiacea, *Ascidiella*[*] | | | | | 0.09 ± 0.07 | 0.19 ± 0.12 | 0.04 ± 0.03 |
| Ascidiacea, *Botryllus*[*l] | | | | present | present | present | present |
| Ascidiacea, *Ciona*[*] | 0.45±0.39 | 0.67 ± 0.36 | 0.02 ± 0.01 | 1.15 ± 0.41 | 36.50 ± 14.00 | 21.24 ± 9.43 | 30.29 ± 5.34 |
| Ascidiacea, *Styela*[*] | 0.15 | | | | | | 0.01 |
| Bivalvia, *Hiatella*[*] | | 0.13 ± 0.05 | | | | | |
| Echinoidea, *Psammachinus*[*] | | | | 0.01 | 0.09 | 0.03 | 0.10 ± 0.06 |
| Gymnolaemata, *Electra*[*l] | present | present | | present | present | present | present |
| Gymnolaemata, *indent.*[***l] | | | | present | present | present | present |
| Polychaeta, *Capitella*[*] | | | | | | | <0.01 |
| Polychaeta, *Neoamphitrite*[*] | | | | | 0.09±0.05 | <0.01 | 0.09 ± 0.03 |
| Total sedentary fauna | 0.60 ± 0.54 | 0.80 ± 0.33 | 0.02 ± 0.01 | 1.18 ± 0.41 | 36.76 ± 13.98 | 21.46 ± 9.33 | 30.60 ± 5.36 |

**Table 4.1**  Continued

| | AFOM study | | | | | | Flux study |
|---|---|---|---|---|---|---|---|
| | March | May | June | August | October | January | January |
| *Errant fauna* | | | | | | | |
| Anopla, *Longissimus*[*] | | | | 0.02 | | | 0.02 |
| Crustacea, *Gammarus*[*] | <0.01 | <0.01 | <0.01 | 0.01 | <0.01 | | |
| Crustacea, *Brachyura*[**] | | | | | 0.01 | 0.01 ± 0.01 | 0.03 ± 0.02 |
| Crustacea, *Galatheidae*[**] | | | | <0.01 | 0.10 ± 0.03 | 0.19 ± 0.06 | 0.07 ± 0.02 |
| Crustacea, *Idoteidae*[*] | <0.01 | | | | <0.01 | 0.01 ± 0.01 | <0.01 |
| Crustacea, *Porcellanidae*[**] | | | | | | 0.10 ± 0.03 | 0.07 ± 0.02 |
| Gastropoda *Facelina*[*] | | | | | | | |
| Polychaeta, *Kefersteinia*[*] | | | | | | | 0.03 ± 0.01 |
| Polychaeta, *Syllidia*[*] | | | | | | | 0.01 ± 0.00 |
| Polychaeta, *Eunoe*[*] | | <0.01 | | | | 0.01 | |
| Polychaeta, *Lepidonotus*[*] | 0.01 ± 0.01 | | | | 0.01 | | |
| Polychaeta, *Harmothoe*[*] | 0.02 ± 0.02 | 0.12 ± 0.04 | 0.05 ± 0.02 | 0.12 ± 0.02 | 0.38 ± 0.13 | 0.44 ± 0.09 | 0.73 ± 0.07 |
| Polychaeta, *Nereis*[*] | 0.05 ± 0.03 | 0.04 | 0.02 | 0.20 ± 0.07 | 0.40 ± 0.09 | 0.68 ± 0.25 | 0.89 ± 0.16 |
| Total errant fauna | 0.09 ± 0.05 | 0.17 ± 0.03 | 0.07 ± 0.02 | 0.35 ± 0.08 | 0.90 ± 0.14 | 1.44 ± 0.29 | 1.85 ± 0.16 |
| | | | | | | | |
| *Total biomass associated fauna*[II] | *0.69 ± 0.55$^b$* | *0.97 ± 0.36$^b$* | *0.10 ± 0.03$^a$* | *1.53 ± 0.36$^b$* | *37.66 ± 14.07$^c$* | *22.90 ± 9.50$^c$* | *32.44 ± 5.44$^c$* |
| *Total number of associated fauna genera*[II] | *3.75 ± 1.03$^a$* | *3.25 ± 0.63$^a$* | *2.00 ± 0.41$^a$* | *7.25 ± 0.48$^b$* | *9.75 ± 0.25$^c$* | *10.25 ± 0.48$^c$* | *12.10 ± 0.64$^c$* |
| *Biomass fauna / biomass mussels* | *0.5%* | *0.4%* | *0%* | *0.4%* | *8.3%* | *7.1%* | *8.2%* |

*Identified to: * genus, ** order, *** class*
[I] *Only qualitative data recorded: absence or presence.*
[II] *Values with different letters are significantly different (p < 0.05)*

**Table 4.2**
Quantity and quality of organic material associated with mussel ropes (*Mytilus edulis*) used in the AFOM study (n=4 ropes per sampling point) and nutrient flux study (n=10 ropes). Data is presented as average (± SE) and different letters indicate significant differences ($p < 0.05$)

| Date | POM (g m$^{-1}$) | OM (%) | POC (mg g$^{-1}$) | PON (mg g$^{-1}$) | C:N | Chl $a$ (mg g$^{-1}$) | Phaeop. (mg g$^{-1}$) | Phaeop: Chl $a$ |
|---|---|---|---|---|---|---|---|---|
| *AFOM study* | | | | | | | | |
| March | 6.1 ± 0.3 [a] | 21% | 257 ± 13[a] | 32 ± 1[abc] | 9.3 [a] | 1.2 ± 0.4 [a] | 2.3 ± 0.4[b] | 2.1[a] |
| May | 8.8 ± 0.4 [a] | 29% | 120 ± 37[b] | 18 ± 5[a] | 7.8 [a] | 0.8 ± 0.1 [a] | 0.8 ± 0.1[a] | 0.9 [a] |
| June | 8.4 ± 0.3 [a] | 26% | 420 ± 54 [a] | 50 ± 9[dc] | 10.4 [a] | 1.2 ± 0.7 [a] | 1.9 ± 0.3[ab] | 1.9 [a] |
| August | 6.6 ± 0.6 [a] | 32% | 113 ± 15 [b] | 18 ± 3[ba] | 7.6 [a] | | | |
| October | 5.3 ± 0.8 [a] | 21% | 587 ± 107 [a] | 82 ± 16[d] | 8.5 [a] | 0.7 ± 0.3 [a] | 4.4 ± 0.3[c] | 6.8 [b] |
| January | 5.9 ± 0.4 [a] | 21% | 319 ± 60 [a] | 42 ± 8[bcd] | 8.9 [a] | 0.3 ± 0.2 [a] | 1.0 ± 0.3[ab] | 4.9[ab] |
| *Nutrient flux study* | | | | | | | | |
| January | 12.7 ± 0.7 | 35% | 461 ± 29 | 66 ± 4 | 8.1 | 0.5 ± 0.0 | 1.7 ± 0.2 | 3.9 |

*POM=Particulate Organic Material; OM=Organic Material (relative to total particulate material); POC/PON=Particulate Organic Carbon/Nitrogen; Chl a= chlorophyll a; Phaeop=phaeopigments*

*Oxygen and nutrient fluxes*

Continuous oxygen measurements within the pelagic chambers confirmed a linear decrease throughout the incubations. Oxygen consumption was lowest in February (0.6 ± 0.1 mmol hour$^{-1}$), increased until maximum values in October (12.8 ± 0.8 mmol hour$^{-1}$), and decreased again during the winter months to 2.4 ± 1.3 mmol hour$^{-1}$ in January (Figure 4.4a).

As for oxygen consumption, the general pattern in nutrient release, except for silicate, showed a significant increase during the spring bloom, followed by an increase until late summer and a decrease during the subsequent months (Figure 4.4b). Total Ammonia Nitrogen (TAN) release increased in August and September to maximum values of 946.7 ± 105.2 µmol hour$^{-1}$. TAN releases completely dominated the total dissolved nitrogen fluxes as nitrite and nitrate fluxes were low, often below the detection limit of the instruments (0.5 and 0.05 µmol l$^{-1}$ for nitrate and nitrite, respectively). As for oxygen and nitrogen, an increase in phosphate release was observed during the spring bloom. Oxygen consumption and TAN release remained at similar levels in the months following the spring bloom while phosphate release decreased (Figure 4.4c). Highest phosphate release rates were observed in summer with maximum values in September (62.7 ± 7.7 µmol hour$^{-1}$). No apparent seasonal pattern in silicate fluxes was observed (Figure 4.4d). Uptake of silicate was observed in February and beginning of March. After that, silicate fluxes varied around zero except for some outlying values in September and October.

Variation in oxygen consumption and ammonia releases were related to mussel biomass as significant positive correlation coefficients were observed between mussel biomass and oxygen (r = 0.68; $p = 0.010$) or TAN (r = 0.66; $p = 0.014$) fluxes.

N:P ratios varied significantly both throughout the season (Repeated measures ANOVA; $p < 0.0001$) and between ambient water and flux measurements (Repeated measures ANOVA; $p = 0.0008$) (Figure 4.5). On average, the N:P flux ratios were 3 times higher compared to ambient values. As no silicate fluxes were observed (Figure 4.4), it was not possible to calculate N:Si and P:Si ratios. However, it is evident that nitrogen and phosphate were excreted in higher amounts than silicate.

## Discussion

This study presents new data on seasonal dynamics of nutrient release rates from suspended mussel culture in relation to succession of mussel biomass, species composition and abundance of fauna, and organic material associated with mussel rope cultures in an oligotrophic fjord environment.

### AFOM succession

The settlement of different ascidian, polychaete and crustacean genera, reflected a significant increase in taxonomic richness throughout the study period. This is in agreement with Taylor et al. (1997), Richard et al. (2006) and Lutz-Collins et al. (2009) who showed that number and
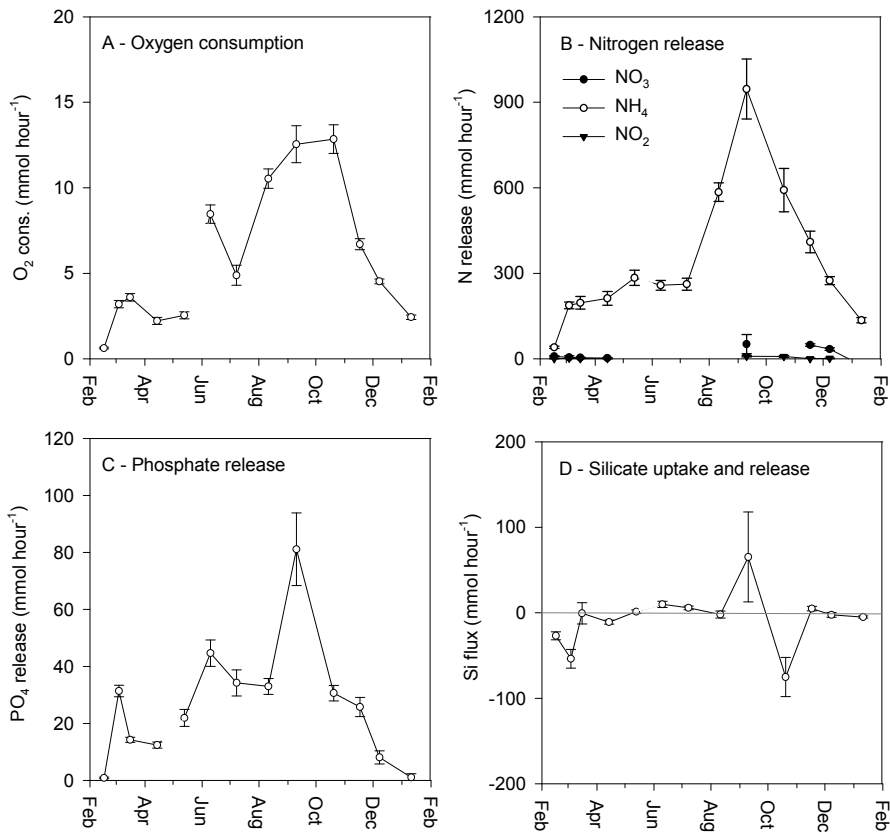


**Figure 4.4**
Oxygen consumption (A) and, nitrate, nitrite, ammonia (B), phosphate (C) and silicate (D) release from mussel ropes (*Mytilus edulis*) measured *in situ* with pelagic chambers from February 2009 to January 2010 (n=10 ropes per sampling point). Data is presented as average (± SE) and is standardized to ropes of 1m length. Dotted lines between the May and June sampling specifies the two separate mussel batches used in the study.

composition of fauna associated to bivalve cultures is dependent on culture duration. Intra annual variation in associated fauna abundance in suspended oyster culture showed similar patterns as observed in our study (Mazouni et al. 2001), but they observed summer mortality of fouling ascidians due to anoxic (bottom) conditions. The average number of fauna genera associated with mussel ropes found in our study (6.9 ± 1.5 m$^{-1}$) was comparable to average values of 7-10 genera per 25cm rope found by Richard et al. (2006). However, the four times longer mussel rope sections used in our study may have resulted in higher fauna diversity, as diversity is spatially dependent. Biomass estimates for associated fauna were approximately 60-fold higher in our study compared to Richard et al. (2006), and were still 4-fold higher when biomass of *C. intestinalis* was excluded from our estimates. The proportion of the fouling biomass relative to mussel biomass found in our study (0-8%) was at the same magnitude as reported for commercial mussel farming in Canada (LeBlanc et al. 2007). Although taxonomic richness increased with time, abundance was dominated by the ascidian *C. intestinalis* (>90%). Ascidians, and especially *C. intestinalis*, are well known fouling organisms in bivalve cultures (see McKindsey et al. 2009 for review).

The presence of the deposit-feeding polychaetes *Capitella* and *Neoamphitrite* indicate that mussel ropes contain a large amount organic material (Grassle & Grassle 1974, Pearson & Rosenberg 1978). This study confirmed that suspended bivalve cultures form a sediment compartment in the water column (Arakawa 1990, Mazouni et al. 2001, Richard et al. 2006). However, it is the first study to quantify the amount of organic material associated with mussel ropes. The organic material associated with mussel ropes was stable throughout the study period (6.9 ± 0.3 g m$^{-1}$). As biomass of mussels, and subsequently faeces production increased during the culture cycle, settlement of faeces fragments on mussel ropes seems space limited and hence a high fraction of the total produced faeces will sink towards the seabed, as observed in shallow systems (Grant et al. 2005, Callier et al. 2006, Giles et al. 2009, Weise et al. 2009). Fouling
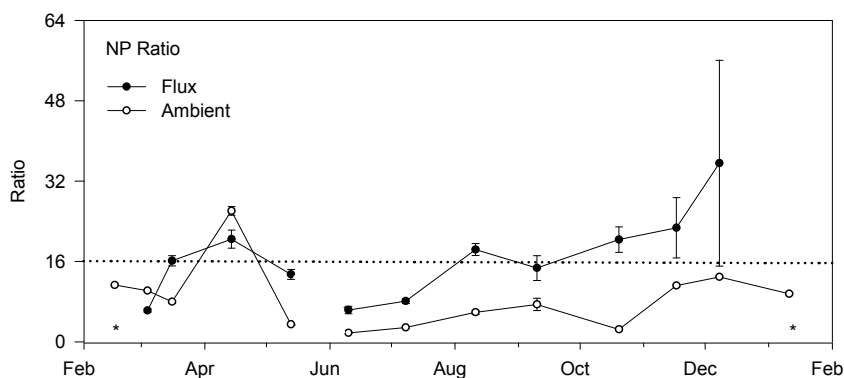


**Figure 4.5**
N:P ratio of the ambient water (see Figure 4.2b) and of nutrient release rates per hour by mussel ropes (see Figure 4.4) measured from February 2009 to January 2010 (n=10 per sampling point). Data is presented as average (± SE). The sampling dates for which phosphate release rates were too low to calculate representative N:P ratios are indicated by an asterisk. Dotted lines between May and June sampling specifies the two separate mussel batches used in the study. Horizontal grey line specifies Redfield's elemental ratio for N:P (16).

ascidians may significantly enhance sedimentation from suspended mussel farms (McKindsey et al. 2009), however, the development of ascidian populations from August onwards did not increase organic loading on the mussel ropes itself in our study, further, indicating that settlement of organic material on mussel ropes is space limited.

*Oxygen consumption and nutrient release*

This study showed significant temporal variation in oxygen consumption and nutrient release rates along the mussel rope interface during one annual cycle. Differences between minimum and maximum rates varied up to 20 times. The *in situ* method using pelagic chambers was chosen to minimize movement of the mussel and water displacement around the ropes, prevent resuspension of trapped organic matter and minimized changes in mussel behaviour, thus allowing us to study intact mussel ropes under natural conditions. The length of the chambers (1.5m) developed for this study allowed us to study mussel rope sections of 1m length, which is at least four fold larger than previously reported studies on biochemical processes along mussel ropes (Richard et al. 2006, 2007) and permitted us to better integrate spatial variability in AFOM biomass and diversity.

Linking oxygen consumption and nutrient release to temporal changes in environmental conditions, mussel growth, and succession of associated fauna and organic material (AFOM) complexes provides insight in the relative contribution of each compartment to the oxygen consumption and nutrient uptake or release from the mussel rope interface. Although biomass of the fouling organisms increased significantly throughout the study period, mussels were always the dominating species on the ropes. Even during maximum ascidian abundance more than 90% of the fauna biomass (epifauna + mussels) was represented by mussels.

Metabolic processes in mussels are weight dependent (Smaal et al. 1997), and part of the annual variation in respiration and TAN release rates were explained by growth of the mussels throughout the year. Mussel metabolism is also correlated to temperature (Widdows & Bayne 1971, Devooys 1976) and food (Bayne et al. 1989a, 1993), although direct correlation to any of those factors is often difficult due to the interrelationship between food and temperature (Grant 1996, Smaal et al. 1997). The increased release rates during the spring bloom, maximum levels in September followed by a gradual decrease in the subsequent months as observed in our study is in accordance with Strohmeier (2009). They showed that temperature and respiration were positively correlated for individual mussels grown in oligotrophic areas during autumn and winter, but these parameters were uncoupled during spring when food concentrations suddenly increased due to the spring bloom of phytoplankton. In our study, maximum respiration and nutrient release rates were observed in September, which did not coincide with maximum temperature (August) or elevated food levels. However, biomass of associated fauna (ascidians) was high from September onwards. The ecological functioning of ascidians is similar to mussels (Petersen 2007) and the high abundance of fouling ascidians indicates a source of nutrients. As for mussels, ascidian metabolism is temperature dependent (Goodbody 1974), although little quantitative data is available. Nevertheless, lower nutrient release rates are expected with decreasing temperatures (e.g. from September to October).

The maximum mussel rope oxygen consumption (standardised to g AFDW), measured in September in our study, were lower than the rates of mussel ropes in Canada during the same season (39 µmol h$^{-1}$ g$^{-1}$ in our study compared to 55-75 µmol h$^{-1}$ g$^{-1}$ AFDW reported by Richard et al. 2006). Hence, lower values were observed despite the fact that considerably more fauna were

associated with the ropes in our study. TAN release measured in our study (2.5 µmol h$^{-1}$ g$^{-1}$; September) was comparable to values reported by Richard et al. (2006) in September (2.5 µmol h$^{-1}$ g$^{-1}$), while release rates measured in August in their study were >5 times higher (14 µmol h$^{-1}$ g$^{-1}$). These high TAN fluxes were explained by protein catabolism of the mussels which were in poor post-spawning condition and by decomposition of dead mussels (Richard et al. 2006). Almost no dying mussels were observed during our study and the main spawning events in Norway occur in May-June (Duinker et al. 2008) and therefore did not influence the summer values used for comparison here. Phosphate release rates were comparable between our study and Richard et al. (2006; 0.2 µmol h$^{-1}$ g$^{-1}$ AFDW). Population based estimates were also carried out by Prins & Smaal (1990, 1994) on mussel beds (including AFOM complexes) in the Oosterschelde estuary in the Netherlands. When TAN and phosphate fluxes are standardized to mussel biomass similar to our ropes, release rates of the mussel beds were 6.0 and 0.3 µmol h$^{-1}$ g$^{-1}$ for TAN and phosphate, respectively. These rates are 3-5 fold higher than yearly averages observed in our study (1.2 and 0.1 µmol h$^{-1}$ g$^{-1}$ for TAN and phosphate). Enhanced release rates observed in the mussel beds might be induced by higher food availability in the Oosterschelde and related enhanced metabolic activity or by decomposition of organic material which is more abundant in mussel beds compared to mussel ropes. Richard et al. (2006) concluded that the AFOM complex on mussel ropes, and specifically the decomposition of organic material, contributes most to nitrate, nitrite and silicate fluxes. Prins & Smaal (1990) also measured high nitrate, nitrite and silicate release rates in mussel beds. Low, but comparable to Richard et al. (2006), nitrate and nitrite fluxes were observed in our study (<10% of total DIN flux). Those rates are considerable lower than nitrate and nitrite rates measured on mussel beds (>20% of total DIN flux, Prins & Smaal 1990). No major anoxic areas were observed within our ropes (unpublished data) and it is therefore unlikely that nitrate and nitrite were directly removed by denitrification but rather that less organic material was present on the ropes compared to mussel beds. Absence of silicate release by bivalve cultures as observed in our study is in disagreement with Richard et al. (2006, 2007b) and Prins & Smaal (1990). Silicate uptake in March can be attributed to uptake by diatoms, which were abundantly present during the spring bloom (unpublished data). Silicate release could originate from the dissolution and microbial degradation of siliceous diatom tests (Canfield et al. 2005) that could be trapped in mussel biodeposits (Callier et al. 2009). However, as silicate concentrations were low during large parts of the year (Figure 4.2b) and diatoms were only present during the spring and autumn bloom (http://algeinfo.imr.no), few diatom tests might have been present in the mussel biodeposits during large parts of the year. This may explain the lack of silicate fluxes measured along the mussel rope interface.

*Interactions between suspended mussel culture and its environment*

The ecological importance of nutrient regeneration is an enhanced nutrient availability for phytoplankton, resulting in increased primary production rates (Smaal 1991). Average nutrient ratios for phytoplankton growth are 16:16:1 for Si:N:P (Redfield et al. 1963), and deviations from this ratio, in combination with low absolute nutrient concentrations, may result in nutrient limitation for phytoplankton growth (Goldman et al. 1979). Subsequently, changes of nutrient ratios in the water column can lead to a shift in phytoplankton community composition (Dame & Libes 1993, Prins et al. 1995). This study showed low ambient nutrient concentrations in spring and summer, and N:P ratios below 16 and N:Si ratios below 1 during extended periods of the year (see Figure 4.2b and Figure 4.5). This points towards a nitrogen limited system, which is commonly observed in marine environments (Nixon et al. 1996). Mussel ropes release inorganic nitrogen and phosphorus to the water column (Figure 4.4) and may thereby favour

**Table 4.3**
Contribution of suspended mussel farming to dissolved nutrient pools in the euphotic zone of oligotrophic systems. Nutrient release by a mussel farm and background nutrient flux in the ambient water. Annual average, maximum (August) and minimum (February) scenarios are presented.

| | Nutrient flux released by a mussel farm (kg day$^{-1}$) | Background nutrient flux in ambient water (kg day$^{-1}$) | Nutrients released by a mussel farm relative to background nutrient flux (%) |
|---|---|---|---|
| *Nitrogen* | | | |
| Average | 1.4 | 72.8 | 2.0 |
| Maximum (Aug) | 2.4 | 12.8 | 18.9 |
| Minimum (Feb) | 0.2 | 211.8 | 0.1 |
| | | | |
| *Phosphate* | | | |
| Average | 0.2 | 16.6 | 1.4 |
| Maximum (Aug) | 0.3 | 6.5 | 4.6 |
| Minimum (Feb) | 0.01 | 41.4 | 0.0 |

phytoplankton growth. However, net release was not according to Redfield's ratio, as proportionally more nitrogen was produced than phosphorus and silicate (N>P>Si). During part of the nutrient limited period (May-July) N:P flux ratios did not exceed Redfield's ratio of 16 (Figure 4.5). This indicates that although mussel culture releases more nitrogen than phosphorus, it does not eliminate nitrogen limitation. However, the absence of silicate release by mussel ropes might lead to silicate rather than nitrogen limitation. Mussel cultures thereby have the potential to suppress the development of siliceous phytoplankton such as diatoms (Turner et al. 1998), and favour development of non-siliceous phytoplankton such as flagellates and dinoflagellates. Phytoplankton composition is not solely dependent on nutrient ratios but absolute nutrient concentrations may also play a role. It has been shown that silicate concentrations affect the phytoplankton community structure (Egge & Aksnes 1992, Fouillaron et al. 2007), and diatom dominated systems are stimulated if silicate concentrations exceeded 2 µM (Egge & Aksnes 1992). Effects of silicate limitation would therefore be more profound in summer periods when silicate concentrations are <2 µM.

Nutrient release rates of mussel ropes measured in this study, may be extrapolated to farm scale level to evaluate the impact of mussel farming on inorganic nutrient pools. Combining ambient nutrient concentrations (Figure 4.2b) and flux estimates (Figure 4.4) with farm characteristics (length and width farm, current velocities, length longlines) as described in Strohmeier et al. (2008), allow us to estimate the amount of nutrients excreted by a mussel farm relative to the amount of nutrients naturally present in the water column (Table 4.3). Number of longlines (mussel biomass) provided by Strohmeier et al. (2008) were standardized to a regular mussel farm, which has approximately 5 times fewer longlines. In winter (February) when release by mussel cultures is low and ambient nutrient concentrations are high, the impact of mussel farming on the total nutrient pool is insignificant. On the other hand, in summer (August) mussel farming can increase the amount of dissolved inorganic nitrogen by 20% and the amount of dissolved inorganic phosphorus by 5%. As fjord systems in Norway are nutrient limited (Paasche & Erga 1988, Erga 1989a, b, Sætre 2007), it can be postulated that the enhanced nutrient availability stimulates primary production. The farm scale estimates above are restricted to a single mussel farm, however, while evaluating the effects of nutrient regeneration on fjord ecosystem level, dilution and dispersion processes should be taken into account.

Concluding, this study has shown (*i*) an increase of species richness and biomass of fauna associates with mussel cultures through time, (*ii*) a stable amount of organic material associated with mussel ropes through time, (*iii*) a dominating role of mussels in nutrient releases in comparison to the AFOM complex, (*iv*) seasonal fluctuations in nutrient release rates by mussel ropes, (*v*) a dissimilar release of different elements (N>P>Si), and (*vi*) the substantial effect of mussel cultures on nutrient regeneration, especially in summer when the pool of natural available nutrients is low and release rates by mussel cultures are high. The temporal fluctuations involved in these processes are relevant in management advice.
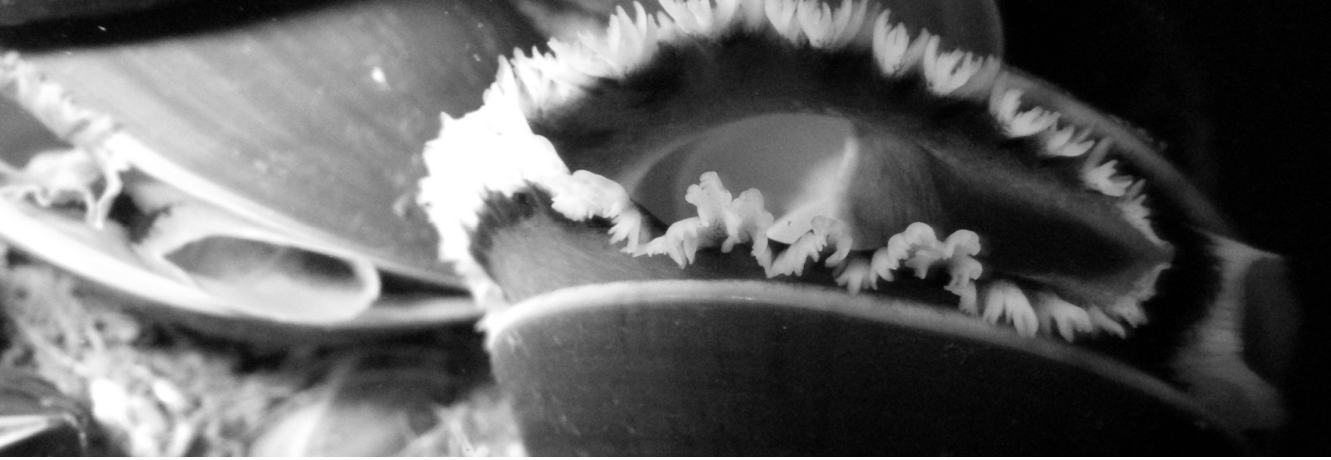
# Chapter 5

Scaling individual physiological rates to community level

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## ABSTRACT

This study compares feeding (clearance), respiration and nutrient release rates of individual and communities of mussels *Mytilus edulis.* The aim of the study was to determine which part of the overall community estimates could be attributed to physiological activity of the mussels and which part could be related to community specific processes. A range of methods was used including *in situ* and laboratory experiments, and evaluation of two comprehensive datasets. A modelling approach was applied to investigate the dynamics between mussel physiology, nutrient availability and phytoplankton in terms of nitrogen fluxes. The results demonstrated that physiological measurements on individual mussels, in certain cases, are different from rates measured in suspended mussel communities (culture). It is assumed that differences are induced by interactions between individual mussels (crowding, flow alteration, refiltration) or by the contribution of fauna and decomposition of organic material associated with mussel communities. Largest differences were observed for clearance rates, with 2-4 times higher rates recorded for individuals compared to communities. In general there was a good agreement between nutrient release rates from individuals and from the community, with correlation coefficients of 0.83, 0.99 and 0.77 for respiration, nitrogen and phosphorus release respectively. This indicates that the mussels are the main contributors to nutrient release in mussel cultures, which was confirmed by the output of the dynamic model showing that for most occasions mussels contributed for 99% to the total nitrogen fluxes of communities. However, during periods when the ascidian *Ciona intestinalis* was highly abundant, respiration and nitrogen release rates of the community could almost double the rates expected from linear extrapolation of individual rates. The present findings imply that extrapolation of individual rates to community level is not always sufficient for understanding the interaction between mussel cultures and phytoplankton populations. We have shown that feeding rates under field conditions are generally lower while nutrient release rates might be higher owing to the role of fauna and organic material associated with mussel communities.

## Introduction

Blue mussels *Mytilus edulis* have the potential to influence the functioning of coastal ecosystems (Dame 1996, Smaal et al. 2001, Newell 2004). However, understanding the interaction between mussel communities and their environment is complex, and requires an approach that integrates different spatial scales (Levin 1992, Dame 1996). Processes at the organism level, such as clearance, ingestion, absorption, respiration and excretion have been extensively studied (among others Bayne & Widdows 1978, Hawkins 1985, Smaal et al. 1997, Cranford et al. in press). Conventionally, these organismic rate processes have been extrapolated to yield population estimates (Dame 1996, Gosling 2003). Population scale data are needed for examining the functional role of mussels at ecosystem level, to assess the ecological services provided by mussels, to assess the environmental impacts of mussel farming and for industry optimization. Although many carrying capacity models have addressed processes from farm to coastal ecosystem scales, they are principally constructed by extrapolating an "average" individual rate to a whole population (Beadman et al. 2002, Duarte et al. 2010, Guyondet et al. 2010). Such approaches do not address community specific effects, which may contribute to the feedbacks between bivalve communities and the ecosystem as well. Studies have been performed at both individual (Bayne & Widdows 1978, Hawkins 1985, Smaal et al. 1997, Cranford et al. in press) and community scale (Dame & Libes 1993, Prins et al. 1996, Newell et al. 2005, Richard et al. 2006), however, quantitative relationships between extrapolation of individuals and community scale measurements are lacking.

That linear extrapolation of organismic rates not always results in accurate estimates for community scale was shown for clearance rate estimates in Norwegian mussel culture, as clearance of individual mussels (Strohmeier et al. 2009) overestimated mussel culture estimates (Aure et al. 2007b). This is supported by Cranford, Ward & Shumway (in press) in a review of bivalve feeding, who stated that clearance measurements performed on individual bivalves and with populations may not be equal due to interactions between individuals. In addition, these authors noted that average responses obtained using instantaneous measurements of clearance rates are less accurate than similar methodologies that utilize populations or methodologies that integrate responses over time due to the large short-term variability within and between individual bivalves. Local phytoplankton depletion within suspended mussel farms is an example where competition for food between individuals exists (Strohmeier *et al.* 2005). Reduced food availability within a population may result in a lower energy uptake by individual mussels, resulting in lower growth and potential alternations in metabolic processes.

Mussel cultures can act as structural habitats for a taxonomically diverse assemblage of fauna and thereby create local communities (Chapter 4; Richard et al. 2006). In addition to the associated fauna the cultures also incorporate bacteria and decomposing biodeposits, and hence mussel cultures can be regarded as complex community structures in which various components contribute to nutrient uptake and release. Higher nutrient release rates have been observed for mussel beds (community) compared to individual mussels that were scaled to the same population size (Smaal & Prins 1993, Prins & Smaal 1994), demonstrating the contribution of nutrient release by associate fauna and mineralization of biodeposits. Similarly, 'associated fauna and organic material' (AFOM) complexes with suspended mussel communities on ropes have shown to contribute to nutrient regeneration (Richard *et al.* 2006).

Within this study we tested the hypothesis that clearance, respiration and nutrient release rates from suspended mussel communities are different from individual rates which are extrapolated to similar mussel population densities by using linear scaling functions. To test this hypothesis

and to quantify the relationship different methods were used, including *in situ* and laboratory experiments, a modelling approach and evaluation of two comprehensive datasets from related studies.

## Materials and Methods

*Study set-up*

Two field studies were performed; 1) a short term field study carried out in September 2010 in a fjord in Midwest Norway comparing individual and community feeding and respiration rates, and 2) a one-year field study from February 2009 to January 2010 in Western Norway comparing individual and community nutrient release and respiration rates. Results of this second study have been published previously to address separate objectives (Chapter 2 & 4); Chapter 4 addressed the role of nutrient regeneration by suspended mussel cultures in oligotrophic fjord systems, and Chapter 2 determined the allocation of each nutrient to different metabolic processes (excretion, tissue growth, biodeposition) in relation to endogenous requirements and environmental factors. In addition to measurements on mussel physiology, nutrient release and respiration rates of fauna and organic material associated with mussel communities were obtained during study 2. A modelling exercise was performed to estimate nitrogen release rates at the individual and community levels. The modelling approach gave us the opportunity to investigate the dynamic processes between mussel physiology, nutrient availability and phytoplankton dynamics and thereby allows us to quantify the contribution of each of the processes occurring within mussel culture communities.

*Environmental conditions*

Environmental conditions in both field studies were measured by the same methods, which are described in full detail in Chapter 4. Fluorescence, temperature and salinity of the ambient water were simultaneously measured using a STD/CTD 204 (SAIV A/S, Norway). Particulate organic carbon (POC) and nitrogen (PON) in the seston was analysed with a Thermo Finnigan Flash EA 1112 NC Analyzer (Ehrhardt 1983), and chlorophyll a (Chl *a*) concentrations were analysed by means of fluorometric analysis (Strickland & Parsons 1968).

*Clearance and respiration rates: short term field study (1)*

The community scale measurements were performed with one mussel rope section (0.68 m length; 361 individual mussels) and these were compared with measurements on 20 individual mussels. All mussels were collected from a commercial suspended mussel farm in Åfjord (Midwest Norway). The measurements were carried out on board of a research vessel anchored in the vicinity of the farm, and received seawater that was pumped from 2.5m depth. For four subsequent days, clearance rates (CR) and respiration rates were determined one to three times per day for community and individual measurements. Clearance rate measurements on individual mussels were conducted using the flow-through method (Petersen *et al.* 2004). A detailed description of the feeding chambers and protocol used for determining individual feeding rates is reported by Strohmeier et al. (2009). In brief, this method is based on determination of the flow rate and particle concentration in water from the outlet of experimental (n = 20) and control (empty; n = 2) chambers. Particle concentration and size-distribution were determined in triplicate using a Pamas particle analyzer (Model S4031GO).

Individual CR (l h$^{-1}$) were calculated from mean retained particle sizes of particles ranging from 4 to 30 µm according to equation 5.1:

$$CR_{indiv} = (1 - (\frac{P_{exp}}{P_{control}})) \cdot F \cdot D \qquad \text{(Equation 5.1)}$$

where $P_{exp}$ and $P_{control}$ are the particle counts in the experimental and control chamber respectively, $F$ is the flow rate (l h$^{-1}$) measured at the outlet of each chamber (Coughlan 1969), individual rates were scaled to a population density similar to the community scale measurements by multiplying with mussel densities ($D$) on 1 meter suspended mussel rope (531 mussels m$^{-1}$). Respiration rates of individuals (n = 10) were conducted using the static (closed chamber incubation) method as described in Chapter 2 (see also Equation 5.4), except that smaller respiration chambers (400 ml) were used and only one individual was placed in each chamber.

The clearance rate measurements for the mussel community were conducted using the static method (Coughlan 1969). To determine community scale clearance rates, we temporally transferred the mussel rope section to a chamber (210 l) which was placed on deck of the research vessel. The intake hose of the Pamas particle analyzer was mounted inside the chamber and mixing of water was supported by creating a circular flow in the chamber using a pump (Chapter 4). The decline in particle concentration through time was followed by means of rapid sampling (every 90 seconds) with the Pamas particle counter. Clearance rates were calculated by summing particle counts for all size classes between 4 and 30 µm during the period of exponential decrease of particles using the formula (Coughlan 1969):

$$CR_{population} = \frac{V}{t \cdot N} \cdot \ln(\frac{P_0}{P_t}) \cdot D \qquad \text{(Equation 5.2)}$$

where $V$ is the volume of the chamber, $t$ is time in hours, $N$ is the total number of mussels (361), and $P_0$ and $P_t$ are the particle concentrations at time 0 and t, respectively, and $D$ is the mussel density on 1 meter suspended mussel rope (531 mussels m$^{-1}$). Calculations were performed for the period in which the exponential decrease was linear in a semi-log plot (R$^2$ values > 0.97), and $P_t$ did not decrease further than 40% of initial values ($P_0$). Community respiration rates were measured simultaneously with the clearance by measuring oxygen concentrations (O$_2$-optode, Aanderaa) at the start and end of each incubation. Mussels were frozen for biomass estimates after the experimental period. Length and tissue weight (AFDW) were determined for each mussel used in the individual scale measurements. Furthermore, length was determined for all 361 mussels present on the mussel rope used in the community scale experiments and individual tissue weight (AFDW) was determined for a subsample of 30 mussels. Separate student's $t$ Tests were performed to test whether there was a difference between individual and community scale measurements for clearance, respiration, and mussel weight and length.

*Nutrient release, respiration and clearance rates: long-term field study (2)*

The experimental set-up of this study has been reported by in Chapter 2 & 4, and is therefore summarized in the following section. The individual scale experiments were carried out in an indoor laboratory receiving unfiltered seawater from 1.5 m depth at the study site. Individual and community based experiments were conducted during the same day assuring equal environmental conditions. The experiments were repeated at monthly intervals, including one additional measurement during the spring bloom (early March). Each sampling date, 50 mussels were collected from mussel ropes. Half of them (n = 25) were transferred to incubation chambers (1.2 l) to determine respiration and nutrient release rates of individual mussels. A total of five

experimental chambers each containing five mussels, and two control chambers without mussels, were used. After the respiration and excretion measurements all 50 mussels were frozen for biomass and length measurements. The community scale respiration and nutrient release rates were determined for mussel rope sections of 1m length using *in situ* incubations with the pelagic chambers (250 l). Repeated measurements on the same 10 ropes were performed throughout the study. Roughly every second month, four additional ropes were collected for AFOM determinations. In both the individual and the community scale experiments, oxygen concentration was measured with an optode (Aanderaa), phosphate concentrations were analyzed according to Parson et al. (1992), and total ammonia nitrogen (TAN) concentrations were analyzed by means of fluorometric analysis (Kerouel & Aminot 1997, Holmes et al. 1999). Respiration and nutrient release rates were calculated as:

$$N = \frac{(NC_{end} - NC_{start}) \cdot V}{t} \qquad\qquad \text{(Equation 5.3)}$$

$$R = \frac{(OC_{start} - OC_{end}) \cdot V}{t} \qquad\qquad \text{(Equation 5.4)}$$

where $N$ is nutrient release rate ($\mu$mol indiv$^{-1}$ h$^{-1}$), $R$ is respiration rate ($\mu$mol indiv$^{-1}$ h$^{-1}$), $NC$ is nutrient concentration and $OC$ is oxygen concentration at start and end of each incubation ($\mu$mol l$^{-1}$), $V$ is the total volume of the incubation chamber (l) and $t$ is incubation time (h). $N$ and $R$ were corrected for mean rates measured in the control chambers. Individual respiration and nutrient release rates were extrapolated — using the same approach as in Study 1 - to mussel densities on ropes of 1m length to yield population estimates. Student's $t$ Tests were performed to assess the difference in physiological rates (respiration, TAN and phosphate release) between individual and community scale measurements for (*i*) average annual rates, and (*ii*) separately for each month.

Community CR was determined for 1 or 2 ropes per sampling date. The decline in fluorescence was measured every second with a STD/CTD that was mounted inside one of the pelagic chambers, and CR was calculated according to Equation 5.2 for the period in which the exponential decrease was linear on a semi-log plot ($R^2 > 0.60$). Measurements in the control chamber confirmed that particle concentrations at start and end of the incubations chamber did not change significantly ($t$ Test; $T_{1,20} = 0.28$; $p = 0.785$), and hence particle depletion in the experimental chambers were related to mussel grazing activity.

The individual mussel and community scale experiments were supplemented with specific measurements on respiration and nutrient release rates of fauna and organic material associated to mussel ropes. Individual ascidians were carefully collected from mussel ropes in August, October and January. For each sampling point, total dry weight ($DW_{total}$), tunic dry weight ($DW_{tunic}$) and body or tissue dry weight ($DW_{body}$) were determined for twenty individuals and condition indexes were calculated by dividing $DW_{body}$ by $DW_{total}$ (Petersen et al. 1995). One-way ANOVA's followed by Tukey's post hoc tests were performed to test whether there was a difference in biomass and condition index between the three sampling months. Respiration, TAN and phosphate release rates of ascidians were determined in October and January according to the experimental design for individual mussel flux measurements (see Chapter 2); after an acclimatization period of 1 day approx. five ascidians were placed in each incubation chamber ($N_{exp.} = 5$ and $N_{control} = 2$), and oxygen and nutrient concentrations were measured at the start and end of each incubation. Respiration and excretion rates were calculated as described by 5.3 and 5.4, and rates were standardized to 1 gram ascidian dry weight ($DW_{total}$) in order to be able scale to population density present on mussel cultures. Student's $t$ Tests were performed to test

whether there was a difference between the physiological rates measured in October and January.

To determine decomposition rates of organic material associated with mussel ropes, subsamples of the organic material collected during the AFOM samplings were incubated. Incubation chambers ($N_{exp.}$ = 6 and $N_{control}$ = 1) similar to the respiration chambers used for mussel and ascidian physiological measurements were used, with the addition of a magnetic stir in the lid assuring a homogeneous water column. Respiration and excretion rates were calculated as described by equation 5.3 and 5.4, and standardized to 1 gram organic material (POM). One-way analysis of variance (ANOVA) followed by Tukey's post hoc tests were performed to test whether there was a difference in respiration rates between the sampling months.

A model originally developed to examine carrying capacity for mussel aquaculture (Filgueira & Grant 2009, Filgueira et al. 2010b) was applied to simulate the nitrogen releases from individuals and communities. The original model includes mussel (M), phytoplankton (P), zooplankton (Z), nutrient (N), suspended detritus (SD) and bottom detritus (BD) submodels (Filgueira & Grant 2009). In the context of the current study we added a submodel describing the detritus captured within the mussel rope matrix (CD), whereas the zooplankton submodel was turned off given the lower impact of this submodel on the results (Filgueira et al. 2010b). Moreover, the model does not include fauna submodels as data on ascidian physiology required for dynamic modelling is limited. The model was developed with GUI-based software (Simile v5.3), and the differential equations that define the submodels are based on Kremer & Nixon (1978), Grant et al. (1993, 2007, 2008) and Dowd (1997, 2005) and are presented in Box 5.1. Specific modifications for Norwegian waters are described by Filgueira et al. (in press). Boundary conditions including temperature, POC, Chl *a* and dissolved inorganic nitrogen (DIN)

**Box 5.1**
Brief description of differential equations that define the submodels in the nitrogen release model (study 2) based on Filgueira & Grant (2009) and Filgueira et al. (2010b). All submodels are characterized in terms of mg C m$^{-3}$, with exception of the dissolved nutrients which are characterized in terms of mg N m$^{-3}$.

| Submodel | Equation |
|---|---|
| P - Phytoplankton | $\dfrac{dP}{dt} = +P_{growth} - P_{mortality} - M_{grazing} \pm P_{mixing}$ |
| N - Nutrients | $\dfrac{dN}{dt} = +M_{excretion} + CD_{decomp} + SD_{decomp} + BD_{decomp} - P_{uptake} \pm N_{mixing}$ |
| CD – Captured Detritus | $\dfrac{dCD}{dt} = +OM_{rope} - CD_{sinking} - CD_{decomp}$ |
| SD – Suspended Detritus | $\dfrac{dSD}{dt} = +OM_{rope} - SD_{sinking} - SD_{decomp} + P_{mortality} - M_{grazing} \pm SD_{mixing}$ |
| BD – Bottom Detritus | $\dfrac{dBT}{dt} = +CD_{sinking} - BD_{decomp} + SD_{sinking}$ |
| M - Mussel | $\dfrac{dM}{dt} = M_{growth} = 0$ |

$OM_{rope}$: organic material associated with mussel ropes (see Table 5.2); The mussel submodel was based on a Scope For Growth (SFG) approach.

concentrations were obtained from direct measurements (Table 5.1). It is assumed that 10% of organic material associated with the mussel ropes (CD compartment; Table 5.2) is brought in suspension by water movement and channelled to the suspended detritus (SD) compartment. The mussel compartment was not affected by mortality, seeding or harvesting because the time span of each simulation was equal to incubation time (0.5 - 6 hours). The focus of the model was to simulate nitrogen release rates and not bivalve growth, and for that reason mussel biomass was introduced as a constant parameter for each sampling month separately, which implies that the mussel submodel interacts with the global model as a forcing function (Dowd 2005).

As the model was originally developed to simulate mussel aquaculture dynamics at ecosystem level (Filgueira & Grant 2009, Filgueira et al. 2010b), the temporal and spatial resolution of the original model were modified to simulate the individual chamber and the pelagic chamber experiments. The individual chamber experiments were used to calibrate and validate the nitrogen fluxes provided by the biogeochemical submodel. For this purpose, the model was configured as a 0-D model and scaled to the volume of the individual chamber (1.2 l). The calibration was performed using the June dataset and tuning the remineralization rate to 0.02 for suspended (SD) and captured (CD) detritus and 0.01 for detritus that sank to the bottom of the tank (BD), which fit into the described range of values for these rates (Dowd 2005). The validation was performed by comparison of the modelled values with the observed ones in the remaining datasets using this individual chamber set-up. In order to study the pelagic chamber (250 l) performance, the model was configured as a 3-D model with a grid size of 1 cm$^3$, a spatial resolution that is required to provide detailed information about micro-environments and interactions between mussels (e.g. refiltration) within the community structure. The water exchange between adjacent cells was averaged according to pump flow placed inside the chamber (Chapter 4). The sensitivity of the water exchange coefficients was analyzed in terms of averaged nitrogen fluxes and spatial variability of nitrogen compounds in one scenario (June). A variation of +10% / -10% in the estimated water exchange coefficients did not cause statistically significant changes in the average nitrogen fluxes. The same sensitivity test caused only a small change (+0.08% / -0.09%) in the spatial distribution of nitrogen compounds in the chamber, which is in good agreement with the well-mixed performance of the pelagic chamber (Chapter 4). The overall correspondence between observed and modelled nitrogen release values for both the individuals and communities was analyzed with regression analysis following a protocol similar to that of Duarte et al (2003). ANOVA was used to test the significance of the regression. Comparisons of slopes and intercepts with the theoretical values of 1 and 0 respectively, were carried out following Zar (1984).

## Results

*Environmental conditions*

Summarized results of the environmental conditions prevailing at the study sites are presented in Table 5.1.Temperature, Chl *a* and POC concentrations for study 1 were in the range observed during the annual study (study 2), whereas particulate organic C:N ratios were considerably lower in study 1.

*Mussel and community descriptions*

In study 1, mussels used in the individual experiments (40 ± 1 mm) were bigger than the mussels on the rope used in the community based experiment (37 ± 0.8 mm) (*t* Test; $T_{1,48}$ = 2.49, $p$ < 0.05). Likewise, mussel weight was also significantly higher for the mussels used in the individual based experiments (426 ± 25 mg AFDW indiv$^{-1}$) compared to the ones growing on the rope (342 ± 18 mg AFDW indiv$^{-1}$) (*t* Test; $T_{1,48}$ = 2.81, $p$ < 0.01). Examination of the ropes showed that only a few amphipods represented the fauna compartment in the community scale experiment. Organic material associated with the mussel rope was 4.8 ± 0.2 g m$^{-1}$.

Length of mussels in study 2 increased from 36 ± 0.7 mm in February 2009 to 51 ± 1.0 mm in January 2010, and weight increased from 225 ± 17 mg to 522 ± 31 mg AFDW indiv$^{-1}$. As the ropes used in the community based experiments were repeatedly measured through time we assume that mussels were similar to the ones used in the individual experiments. This assumption seems valid as mussels were statistically similar (*t* Test; $T_{1,53}$ = 1.93, $p$ = 0.60) for the individual and the community scale experiments at the end of the study (January 2010). Mussel mortality on the ropes was assumed to be minimal as only few shells were collected from cages placed direct underneath the ropes. Fauna associated with the mussel ropes increased both in total weight (Table 5.2) and diversity through time (Chapter 4). The ascidian *Ciona intestinalis* was the dominant fouling species. Organic material associated with the mussel ropes was stable through time (6.9 ± 0.3 g m$^{-1}$).

**Table 5.1**
Summarized results of environmental conditions.

| Date | Temperature (ºC) | Chl *a* (µg l$^{-1}$) | POC (µg l$^{-1}$) | CN |
|---|---|---|---|---|
| *Study 1* | | | | |
| 19 Sept | 11.8 | 1.3 | 140.6 | 5.2 |
| 20 Sept | 12.0 | 0.9 | 59.1 | 4.3 |
| 21 Sept | 11.8 | 1.0 | 95.3 | 4.8 |
| 22 Sept | 11.6 | 0.5 | 83.1 | 4.7 |
| Average (± SE) | 11.8 ± 0.1 | 0.9 ± 0.2 | 95.5 ± 17.1 | 4.8 ± 0.2 |
| *Study 2* | | | | |
| February | 3.3 | 1.0 | 110.3 | 6.8 |
| March (spring bloom) | 3.8 | 7.7 | 133.9 | 9.3 |
| March | 4.7 | 0.9 | 99.5 | 6.5 |
| April | 9.0 | 1.5 | 180.8 | 6.2 |
| May | 9.9 | 0.9 | 280.7 | 6.4 |
| June | 12.5 | 0.9 | 353.6 | 8.0 |
| July | 18.5 | 0.6 | 233.5 | 10.8 |
| August | 18.0 | 1.4 | 289.7 | 9.7 |
| September | 15.5 | 1.1 | 249.0 | 9.0 |
| October | 10.0 | 2.0 | 149.3 | 9.6 |
| November | 10.4 | 0.3 | 65.4 | 8.6 |
| December | 8.0 | 0.3 | 93.0 | 8.5 |
| January | 4.9 | 0.2 | 94.3 | 11.0 |

*Feeding rates*

Average CR based on individual scale measurements in study 1 and extrapolated to a meter rope with 531 indiv m$^{-1}$ was 1970 ± 222 l m$^{-1}$ h$^{-1}$, and was thereby approximately 4 times higher ($t$ Test; $T_{1,12}$ = 5.20 , $p$ < 0.001) compared to average CR of 514 ± 65 l m$^{-1}$ h$^{-1}$ based on community scale measurements (Figure 5.1a). Correction for lower tissue weight of the mussels used in the community scale experiment, by standardizing CR to an equivalent individual of 1 g tissue AFDW based on allometric scaling functions given by Smaal et al. (1997; Table 5.3), did not explain the differences between individual and community based CR. In study 2 we only determined CR for the communities. Rates ranged between 551 and 2453 with an average of 1132 ± 143 l m$^{-1}$ h$^{-1}$ (Figure 5.1b). A seasonal study by Strohmeier *et al.* (2009) performed at the same field station (Austevoll, study 2) has determined CR estimates for individual mussels (Table 5.3).

*Respiration rates*

In study 1, average respiration rates based on individual scale measurements were 16.0 ± 1.1 mmol m$^{-1}$ h$^{-1}$, and were approximately 3 times higher ($t$ Test; $T_{1,13}$ = 13.71, $p$ < 0.0001) compared to respiration based on community scale measurements which were 4.7 ± 0.4 mmol m$^{-1}$ h$^{-1}$ (Figure 5.2a). Weight standardized respiration rates (Table 5.3) did not explain the difference in individual and community scale rates.

Although average annual respiration rates measured in study 2 were similar between the individual and community scale ($t$ Test; $T_{1,248}$= 0.08 , $p$ = 0.777), large seasonal variations were

**Table 5.2**
Summarized results on community composition of mussel *M. edulis* rope interfaces for both studies. Data presented as average (± SE) gram AFDW and standardized to 1m mussel rope

| | Mussel | | Total fauna | Ascidians | Organic material |
|---|---|---|---|---|---|
| | number | weight | weight | | weight |
| *Study 1* | | | | | |
| Average | 531 | 182 | - | - | 4.8 ± 0.2 |
| *Study 2* | | | | | |
| February | 516[*] | 116 | | | |
| March (spring bloom) | 516[*] | 133 | | | |
| March | 516[*] | 164 | 0.7 ± 0.6 | 0.5 ± 0.4 | 6.1 ± 0.3 |
| April | 516[*] | 189 | | | |
| May | 516[*] | 215 | 1.0 ± 0.4 | 0.7 ± 0.4 | 8.8 ± 0.4 |
| June | 543 ± 23 | 218 | 0.1 ± 0.0 | < 0.1 | 8.4 ± 0.3 |
| July | 543 ± 23 | 286 | | | |
| August | 543 ± 23 | 315 | 1.5 ± 0.4 | 1.2 ± 0.4 | 6.6 ± 0.6 |
| September | 543 ± 23 | 386 | | | |
| October | 543 ± 23 | 400 | 37.7 ± 14.1 | 36.5 ± 14.0 | 5.3 ± 0.8 |
| November | 543 ± 23 | 445 | | | |
| December | 543 ± 23 | 287 | | | |
| January | 543 ± 23 | 283 | 22.9 ± 9.9 | 21.2 ± 9.4 | 5.9 ± 0.4 |

[*] *Mussel stock was lost due to predation in May 2009, Mussel density for the period February-May is based on estimates*

observed (Figure 5.2b). Community based respiration rates in September and October were much higher than estimates based on individual measurements, while community scale measurements in the remaining months were somewhat lower compared to individual based rates. Correlations between community and individual based respiration rates, excluding September and October results, can be described by the following equation:

$$C_{Resp} = 0.83 \cdot I_{Resp} \quad (R^2 = 0.84; F_{1,9} = 48.26; p < 0.0001)$$

where $C$ represents the estimates based on *in situ* community scale measurements and $I$ represents the estimates based on individual measurements.

*Nutrient release rates*

Average annual TAN release rates were significantly higher (17%) for the community scale measurements compared to individual based measurements ($t$ Test; $T_{1,248} = 4.15$ , $p = 0.043$), while average annual phosphate release rates of individuals were similar to rates for mussel communities ($t$ Test; $T_{1,248} = 0.43$ , $p = 0.513$). However, similar to respiration patterns, large seasonal variations were observed (Figure 5.3ac), and significant differences were found between individual and community based flux estimates for most sampling dates ($t$ Test; $p < 0.05$). During September and October the TAN release rates for the community were double compared to the individual estimates. Phosphate release rates in September were similar ($t$ Test; $T_{1,18} = 0.16$, $p = 0.873$), whereas values in October were five times higher for the community compared to individual based measurements ($t$ Test; $T_{1,18} = 8.64$ , $p < 0.0001$). Correlation between community and individual rates (Figure 5.3bd) demonstrates a good fit for most of the sampling months, except for September and October. Correlations between community and individual rates, excluding September and October results, can be described by the following equations:

$$C_{TAN} = 0.99 \cdot I_{TAN} \; (R^2 = 0.78; F_{1,9} = 30.61; p < 0.001) \text{ for TAN release}$$

$$C_{PO4} = 0.77 \cdot I_{PO4} \; (R^2 = 0.69; F_{1,9} = 23.76; p < 0.01) \text{ for phosphate release}$$
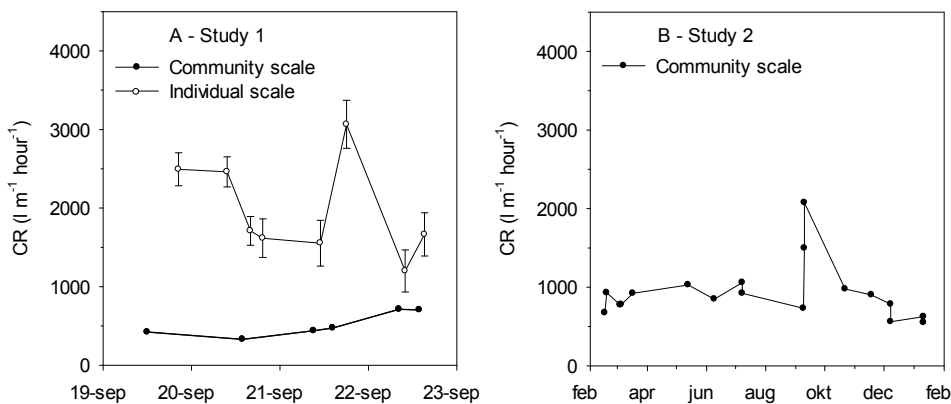


**Figure 5.1**
Clearance rates based on individual (open symbols) and community (solid lsymbols) scale measurements for mussel (*M. edulis*) cultures (A: Study 1; B: Study 2). Data for individual measurements are expressed as average (± SE) and standardized to 1m mussel rope. The community scale measurements represent sampling of one rope section for each data point.
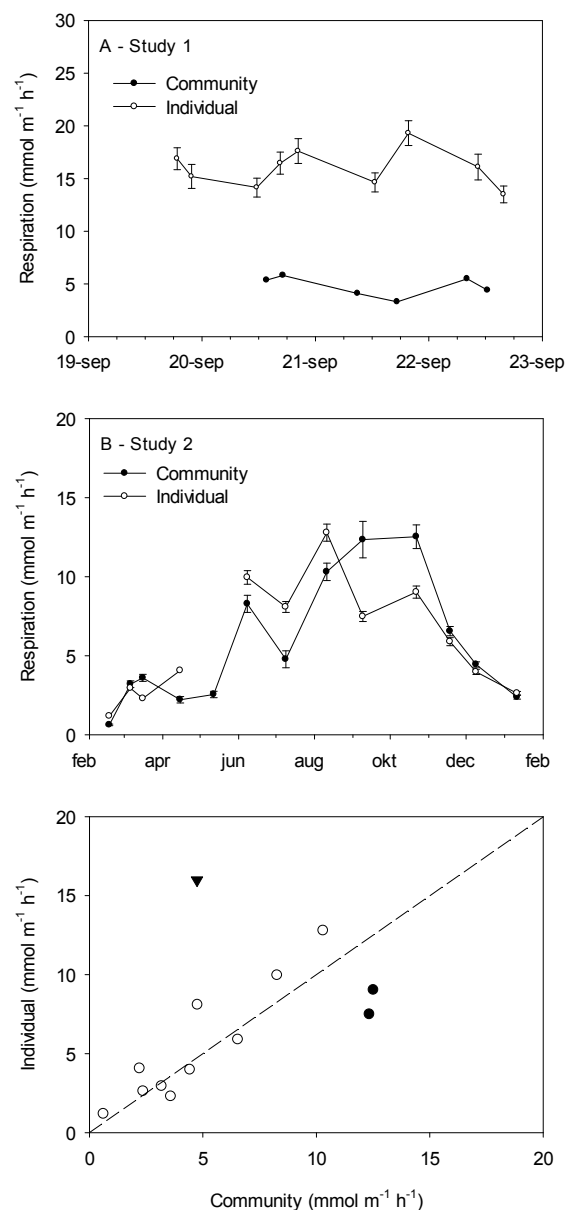
**Figure 5.2**

Individual (open circles) and community (closed circles) based respiration rates for *M. edulis* cultures (panel A: Study 1; Panel B: Study 2). Data are expressed as average (± SE) and standardized to 1m mussel rope. Panel C shows the correlation between the individual (y-axis) and community (x-axis) scale estimates. Different symbols are used to indicate the two studies (Triangle represents average value Study 1; Circles represent Study 2, with closed circles for September and October results).

*Respiration and nutrient release rates of AFOM compartments*

Individual weight (DW$_{total}$) of the ascidians increased significantly from 32 to 374 mg per individual between August 2009 and January 2010 (ANOVA; $F_{2,57}$= 224.76 , $p$ < 0.001), with the largest growth realized from August to October (Table 5.4). A condition index of 0.37 was observed in August, increased significantly to 0.45 in October and remained at that level in January (ANOVA; $F_{2,57}$= 5.30, $p$ < 0.01). Respiration and nutrient release rates of ascidians in January were 3-5 times lower compared to rates measured in October (Table 5.4; $t$ Test $T_{1,5}$= 11.72, $p$ < 0.05 for respiration, $T_{1,5}$= 33.87, $p$ < 0.01 for TAN release and, $T_{1,5}$ = 33.42, $p$ < 0.05 for phosphate release). Physiological rates, standardized to one gram tissue weight (DW $_{body}$), were higher for ascidians compared to individual mussels in October, while physiological rates of the two species were in similar in January.
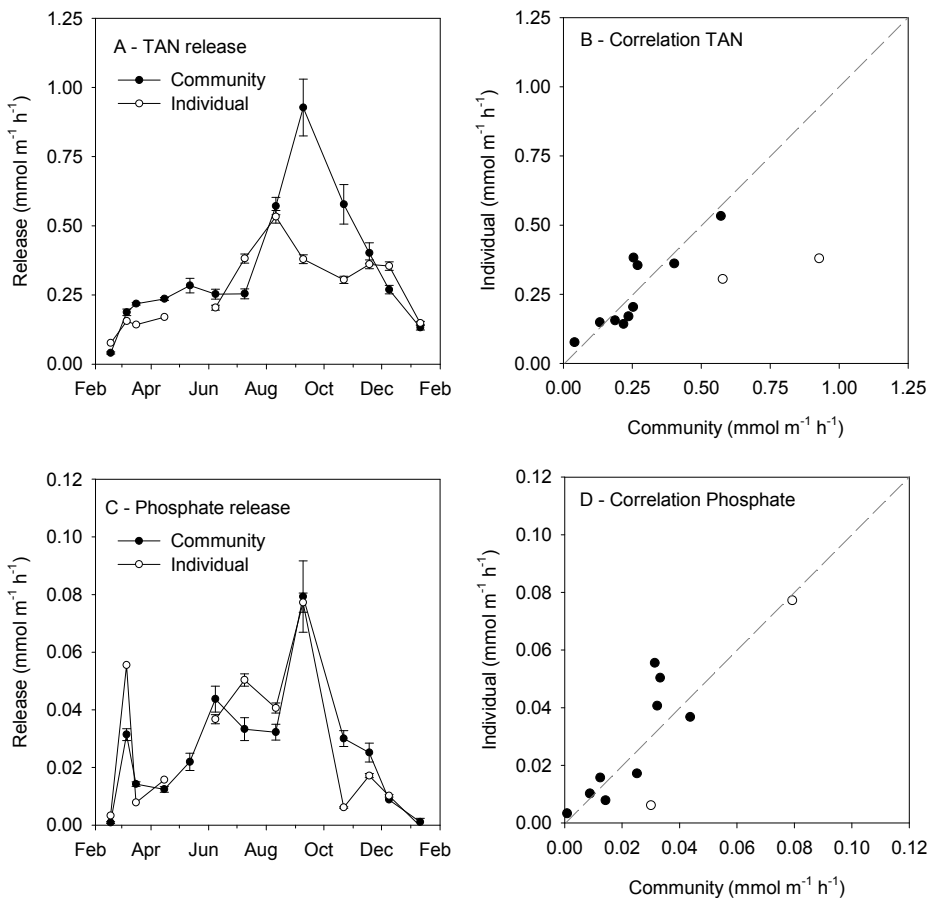


**Figure 5.3**
Total ammonia nitrogen release (A) and phosphate release (C) based on individual (dotted line) and community (solid line) scale measurements for mussel (*M. edulis*) cultures throughout one annual cycle (study 2). Data are expressed as average (± SE) and standardized to 1m mussel rope. Right panels show the correlations between the individual (y-axis) and community (x-axis) scale estimates (B, D), where open symbols represent the September and October results.

Respiration rates of decomposing organic material collected from mussel ropes varied between sampling months (ANOVA; $F_{5,30}$ = 40.52; $p$ < 0.0001), with maximum values recorded in May (110 μmol g OM$^{-1}$ h$^{-1}$) and minimum values in March (20 μmol g$^{-1}$ OM h$^{-1}$) (Table 5.5). The organic fraction also varied significantly between sampling months (Kruskal-Wallis; $\chi^2$ = 25.01; $p$ < 0.0001), while the CN ratio of the organic material was stable through time (Kruskal-Wallis; $\chi^2$ = 10.17; $p$ = 0.071).

**Table 5.3**
Average (± SE) weight standardized physiological rates for individual and community scale measurements for both studies. Data has been weight standardized according to allometric scaling functions given by Smaal et al. (1997).

| | Study 1 | | Study 2 | |
|---|---|---|---|---|
| | Individual | Community | Individual | Community |
| Clearance rates (l h$^{-1}$ g$^{-1}$ AFDW) | 5.8 ± 0.2 | 1.7 ± 0.7 | 7.8 ± 0.4[*] | 2.3 ± 0.2 |
| Respiration rates (μmol h$^{-1}$ g$^{-1}$ AFDW) | 52.8 ± 2.0 | 18.9 ± 1.6 | 17.3 ± 2.5 | 20.0 ± 3.0 |
| TAN release rates (μmol h$^{-1}$ g$^{-1}$ AFDW) | | 1.21 ± 0.13[**] | 0.85 ± 0.13 | 0.97 ± 0.14 |
| Phosphate release rates (μmol h$^{-1}$ g$^{-1}$ AFDW) | | 0.12 ± 0.01[**] | 0.09 ± 0.02 | 0.08 ± 0.02 |

[*] *Strohmeier et al. (2009);* [**] *Jansen (Unpublished data)*

**Table 5.4**
Individual dry weight, respiration and nutrient release by ascidians *Ciona intestinalis* (study 2). Data is presented as average (± SE), and respiration and nutrient release rates are standardized to 1g total dry weight (DW$_{total}$). Different letters indicate significant differences (Tukey post hoc test; $p$ < 0.05).

| | Body weight[*] | Tunic weight[*] | Total weight[*] | Respiration | TAN release | Phosphate release |
|---|---|---|---|---|---|---|
| | (mg indiv$^{-1}$) | | | (μmol g$^{-1}$ DW$_{total}$ h$^{-1}$) | | |
| August | 11.9 ± 1.2 [a] | 19.9 ± 1.4 [a] | 31.8 ± 2.4 [a] | | | |
| October | 124.5 ± 17.1 [b] | 141.1 ± 11.6 [b] | 265.6 ± 25.7 [b] | 16.22 ± 3.03 [a] | 1.33 ± 0.14 [a] | 0.26 ± 0.05 [a] |
| January | 159.1 ± 14.8 [c] | 214.6 ± 27.9 [c] | 373.6 ± 38.8 [b] | 3.84 ± 0.09 [b] | 0.27 ± 0.03 [b] | 0.08 ± 0.02 [b] |

*\* Data log-transformed prior to statistical analysis to meet the assumption on normality of variances*

**Table 5.5**
Decomposition and quality of organic material associated with the mussel *M. edulis* cultures used in study 2. Data is presented as average (± SE), and different letters indicate significant differences (Tukey post hoc test; $p$ < 0.05).

| | Respiration (μmol g$^{-1}$ h$^{-1}$) | Organic fraction (%) | CN ratio |
|---|---|---|---|
| March | 20.4 ± 2.8 [a] | 20 [a] | 10.6 [a] |
| May | 109.7 ± 8.1 [e] | 30 [bc] | 9.0 [a] |
| June | 43.8 ± 4.8 [bc] | 28 [bc] | 11.9 [a] |
| August | 75.4 ± 2.8 [d] | 36 [d] | 8.7 [a] |
| October | 51.5 ± 6.9 [c] | 25 [b] | 9.7 [a] |
| January | 28.5 ± 3.6 [ab] | 32 [cd] | 10.2 [a] |

*Modelled nitrogen release*

The regression between the observed and modelled nitrogen release rates for individual mussels can be described by the equation:

$N_{obs} = 0.95 \cdot N_{model} + 0.03$ ($R^2 = 0.91$; $F_{1,9} = 93.38$; $p < 0.0001$; see also Figure 5.4a)

where $N_{obs}$ represents the observed and $N_{model}$ the modelled DIN release. This indicates that the mussel submodel represents the mussel physiology well and provides sound predictions for DIN release rates of individual mussels as the slope is similar to 1 ($p = 0.632$), the intercept is not different from zero ($p = 0.539$) and the regression shows a high correlation coefficient. The regression between the observed and modelled nitrogen release rates for community basedmeasurements (excluding September and October) can be described by the following equation (see also Figure 5.4b):

$N_{obs} = 0.84 \cdot N_{model} + 0.18$ ($R^2 = 0.57$; $F_{1,11} = 13.28$; $p < 0.01$)

The slope of this regression was similar to 1 ($p = 0.537$) but the intercept excluded the origin ($p < 0.05$), suggesting that the community scale simulations underestimate the DIN release.

Partitioning of the nitrogen flux into the different compartments by means of the current model output demonstrated that mussels contributed for >99% to the total nitrogen flux and decomposition of detritus (SD, CD and BD compartments combined) contributed to 0.2% of the total nitrogen flux.
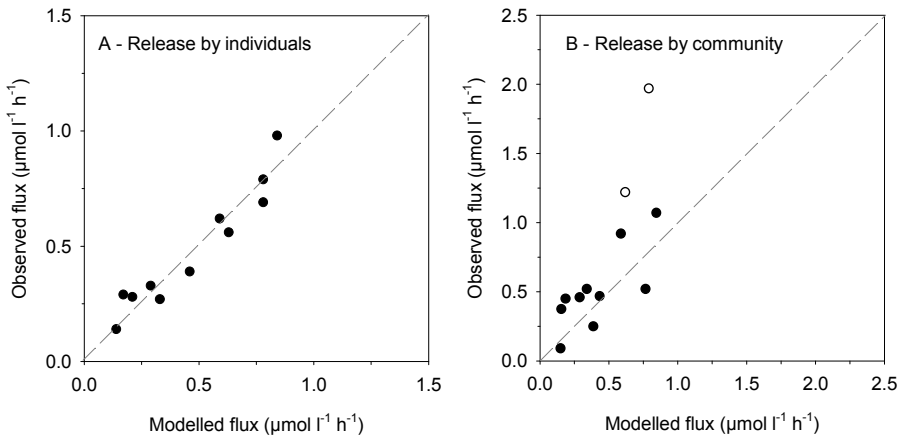


**Figure 5.4**
Modeled (X-axis) versus observed (Y-axis) nitrogen release based on individual (A) and community (B) scale measurements. Each data point represents average values of one sampling point (month). Open symbols in panel B represent the September and October results for the community scale measurements. Dotted diagonal line represents the theoretical correlation of 1.

## Discussion

This study compares feeding (clearance), respiration and nutrient release rates of individual and communities of mussels *Mytilus edulis* to determine which part of the overall community estimates can be attributed to physiological activity of the mussels and to what extent community specific processes play a role. The results show that laboratory measurements on individual mussels, in certain cases, are not sufficient for field relevant conclusions as linear extrapolation did not always result in values measured *in situ* on mussel cultures due to other processes that play a role at community scale. These results are confirmed by other *in situ* field studies which also indicated that community specific processes affect overall rates (Prins & Smaal 1994, Prins et al. 1996, Richard et al. 2006). Bivalve-ecosystem interactions are often characterized by the feedback systems between bivalves and phytoplankton populations (Prins et al. 1998, Newell 2004). The present findings imply that community specific processes also contribute to the feedback mechanisms, potentially resulting in a lower pressure on phytoplankton populations than might be expected when the responses of a few animals are linearly extrapolated to bivalve culture densities.

*Interactions between individuals*
Largest differences between individuals and communities were detected for clearance rates (CR). On most occasions linear extrapolation from individual measurements resulted in an overestimation of clearance measured for whole communities. Both studies showed a similar pattern with 2-4 times higher estimates based on individuals compared to community scale experiments. Weight standardized clearance rates based on the individual measurements were relatively high compared to the overview reported by Cranford et al. (in press), but were in the same order of magnitude as previously reported for oligotrophic conditions (Strohmeier et al. 2009; Table 5.3). The 2-4 fold difference between individual and community scale clearance rates is in the same range as predicted by combining information on individual and farm scale measurements (Aure et al. 2007b, Strohmeier et al. 2009). Prins et al. (1996) also showed that clearance rates observed for mussel beds (= community) were slightly lower compared to rates observed for individuals, although the differences were smaller compared to our results. We used different methods for estimating community (static) and individual (flow-through) clearance rates. Although the accuracy of various methods have been subject of debate (Riisgard 2001, Bayne 2004, Petersen 2004, Petersen et al. 2004, Riisgard 2004, Cranford et al. in press), inter-calibration and meta-analysis studies by Petersen et al. (2004) and Cranford et al. (in press) showed that clearance rate measurements with individuals based on flow-through methods are not statistically different from similar measurements based on static methods. In study 2 we used fluorescence depletion for calculations of community clearance rates. Fluorescence measurements provides an integrated measure of particle concentrations over all particle sizes, including sizes which are too small or too large to be captured by mussels. The static method assumes that all particles can be captured by bivalves (Coughlan 1969) and in cases the available phytoplankton is not 100% retained by the mussels the fluorescence technique will result in underestimations of clearance rate. Additionally, fluorescence measurements are criticized as they do not represent the total seston availability and exclude e.g. detritus particles. However, seston in Norwegian fjord areas is mainly composed of phytoplankton (Erga 1989a, Erga et al. 2005, Strohmeier et al. 2009). Moreover, study 1 (particle counts on different size classes) and study 2 (fluorescence) both showed similar patterns (Table 5.3). Considering the methodological constrains, our results suggest that interactions between animals growing in dense mussel communities lower the clearance rates as would be expected from measurements on individuals.

Interactions between individuals growing in cultures might affect their physiological responses and potentially result in altered rates. Potential mechanisms explaining reduced clearance by communities are crowding, flow alteration and refiltration. Shells located at the inner part of the mussel matrix might encounter physical hindrance when cultured at high densities. External pressure on mussel shells will impair shell opening, which is a factor in controlling feeding rates (Frechette et al. 1992). Crowding might therefore lead to lower overall clearance rates. Bivalves themselves as well as culture structures (ropes) may change hydrodynamic flow patterns and thereby food availability for mussels (Lassen et al. 2006, Aure et al. 2007b, Petersen et al. 2008). Turbulence around culture structures increases the possibility of re-filtrating water which has earlier been filtered by another individual, and hence refiltration within communities may lead to lower overall clearance rates.

Similar to clearance, a 3-fold difference was observed between individual and community scale respiration estimates in study 1. These results were, however, not confirmed by the second study, where a good fit between individual and community measurements was observed (with exception from September and October measurements). Weight standardized respiration rates for individuals measured in study 1 were three times higher than the average of study 2, and were in the higher scale of respiration rates reported in literature (Bayne & Widdows 1978, Hawkins & Bayne 1985, Smaal & Vonck 1997, Smaal et al. 1997, Strohmeier 2009).

*The contribution of AFOM*

Mussel cultures consist of a community structure including the mussel matrix and AFOM complex (associated fauna and organic material). Each of those components may contribute to the physiological response of the mussel community. As very few associated fauna was observed in study 1 we will only discuss the contribution of AFOM in terms of nutrient dynamics and respiration as observed in study 2. In general there was a good agreement between the nutrient release rates from individuals and from the community, indicating that the mussels are the main contributors to nutrient release in mussel cultures. This was also confirmed by the output of the nitrogen release model. However, the statistical differences in the intercept of model vs observed regression suggests that the model underestimates the nitrogen flux. As the model is precise for the mussel compartment, underestimations are likely induced by the detritus compartment or by the absence of fauna submodels. Additionally, September and October were excluded from the regression due to the large discrepancies between individual and community scale measurements which could be as much as 40%, 60% and 80% for respiration, TAN and phosphate release, respectively. In bottom cultures, discrepancies of 10 - 80% have been reported between individual and community based TAN and phosphate release rates, although the difference in phosphate releases was less clear as phosphate was also fixed within the mussel bed (Asmus et al. 1990, Prins & Smaal 1994). These authors attribute the higher community estimates primarily to decomposition of mussel biodeposits. The importance of the organic matter compartment may, however, vary between culture types. Bottom cultures are essentially two dimensional and, although dependent on local physical conditions, the majority of biodeposits accumulate within the mussel beds itself. Suspended culture adds a third dimension (depth) and biodeposits sink to the seafloor resulting in a reduced biodeposit compartment on the mussel culture itself. However, biodeposits also partially accumulate in spaces between mussels on ropes (Chapter 4, Mazouni 2004, Richard et al. 2006). The amount of organic material associated to mussel ropes was $6.9 \pm 0.3$ g m$^{-1}$ and seemed space limited as biodeposition rates vary between $0.6 - 4.6$ g$^{-1}$ m$^{-1}$ (recalculated from Chapter 2), and decomposition rates of biodeposits are considerable slower (Giles & Pilditch 2006, Carlsson et al. 2010). Decomposition

of organic material collected from mussels ropes resulted in oxygen consumption rates of 0.02 - 0.1 mmol h$^{-1}$ m$^{-1}$. This represents 2 to 7 % of the rope respiration, indeed demonstrating the low contribution of the detritus compartment. This was also confirmed by the output of the nitrogen model.

Fauna associated with mussel ropes may interact with nutrient dynamics of suspended cultures. During the nutrient release study biomass and diversity increased throughout the year, and the rope community changed considerably with the settlement of juvenile ascidians *Ciona intestinalis* during summer (Chapter 4). *C. intestinalis* is a suspension feeding organism which has a similar ecological functioning to mussels (Petersen 2007) and competition for food between the cultured mussels and fouling ascidians might exist (Lesser et al. 1992, Petersen 2007). Respiration rates of ascidians were relatively low in comparison to other studies (Shumway 1978, Petersen et al. 1995). Weight standardized rates were lower in January compared to October, likely induced by fluctuations in temperature and food availability (Goodbody 1974), and by a higher body weight (Shumway *et al.* 1985). Scaling the individual rates to ascidian densities present on mussel cultures results in respiration rates of 0.05 − 1.2 mmol m$^{-1}$ h$^{-1}$, TAN release rates of 0.004 − 0.1 mmol m$^{-1}$ h$^{-1}$, and phosphate release rates of <0.001 - 0.02 mmol m$^{-1}$ h$^{-1}$.

The question is whether the discrepancies in September and October can be explained by dynamics of the other compartments present within mussel cultures (fauna and organic material). Budget analysis of respiration rates for October, results in rates of 9.0 mmol m$^{-1}$ h$^{-1}$ for mussels, 1.2 mmol m$^{-1}$ h$^{-1}$ for ascidians and 0.3 mmol m$^{-1}$ h$^{-1}$ for decomposing organic material. Respiration rates measured for the complete community were 12.5 mmol m$^{-1}$ h$^{-1}$, which is still higher than the sum of the separate compartments. The same applies for TAN release rates, indicating that the contribution of fauna and decomposing organic material only partly explain the discrepancies between individual and community scale respiration and nitrogen release rates. The model results showed a good fit for the individual estimates for all sampling points, including September and October, indicating that the nitrogen release by mussels can be well simulated. Community model has also provided good results all over the year with the exception of September and October, which did not match the observed data. This suggests that the differences between individual and community scale fluxes during those two months are caused by external factors and not by underestimations for the individual measurements.

*Implications for ecosystem dynamics*

The difference between individual and community based estimates is influenced by temporal and spatial dynamics. The effect of environmental fluctuations on mussel physiology is well studied and can be predicted with the mussel submodel. Fauna diversity and biomass fluctuate through time (Chapter 4). Especially the colonization of ascidians may add a distinct seasonal component to mussel culture dynamics as *C. intestinalis* is abundant in mussel aquaculture (Lesser et al. 1992, Grant et al. 1998, McKindsey et al. 2009) and has an annual cycle, with drastic seasonal declines in population biomass and high reproductive investment (Svane 1983). Fauna composition is also dependent on farming location and management (Cayer et al. 1999, LeBlanc et al. 2007), introducing a spatial component to the mussel culture dynamics. Large variations in composition and abundance of fauna associated with mussel cultures are in accordance with previous studies (Cayer et al. 1999, Khalaman 2001, Richard et al. 2006, Lutz-Collins et al. 2009). Presently insufficient insight is available on the temporal and spatial variability in fauna abundance and composition, yet has to be taken into account to improve generic predictions.

Although during most occasions individual and community based measurements resulted in comparable estimates, some of the differences were to an extent which can be significant for predictions at ecosystem scale. Mussel-ecosystem interactions are frequently described by the effects on phytoplankton populations; mussels diminish phytoplankton populations by their grazing activity, while simultaneously support primary production by excretion of nutrients (dissolved metabolites) and decomposition of biodeposits. The first process is known as "top-down" control while the latter represents "bottom-up" control on phytoplankton populations. The current study demonstrated that the top-down control on phytoplankton might be lower when we consider mussel communities (culture) as a whole instead of extrapolating values obtained from measurements on individual mussels. On the other hand, the AFOM complex increase nutrient regeneration and might thereby enhance the bottom-up control on phytoplankton production. Overall, our results indicate that not only mussel activity but also community specific processes may contribute to the net impact of mussel cultures on phytoplankton populations. Especially, in areas with high faunal biomass associated with suspended bivalve cultures it is therefore important to carefully consider relationships between individual and community scale estimates.

# Chapter 6

General Discussion

## Introduction

This thesis discusses the contribution of suspended mussel communities to nutrient cycling, with special reference to oligotrophic systems. To provide an integrated view onto bivalve nutrient cycling in oligotrophic fjords, the results presented in the previous chapters are discussed and placed in a broader context by means of a literature review on bivalve nutrient cycling in mussel cultivation areas around the world.

The following sections describe *(i)* bivalve nutrient cycling by means of budget analysis and a literature review for eco-physiological rates comparing responses of mussels under oligotrophic and eutrophic conditions, and *(ii)* mussel-ecosystem interactions for both oligotrophic Norwegian fjords and eutrophic coastal cultivation areas. The current chapter ends with *(iii)* concluding remarks and *(iv)* perspectives on how knowledge on bivalve nutrient cycling can be applied.

## Mussel communities as intermediates in nutrient cycling

The current study provided information on interrelationships between different physiological processes in relation to the four macro-elements (C-N-P-Si) within a seasonal context. This kind of information is infrequently available as most studies report single-element (Cranford et al. 2007), single-process (Hatcher et al. 1997) or a single point in time (Richard et al. 2006) approaches. However, the few studies combining different aspects (this thesis; Hawkins & Bayne 1985, Prins & Smaal 1994, Kreeger et al. 1995, Smaal & Vonck 1997) underline the importance to study interdependence and coherence of processes related to nutrient cycling in order to be able to accurately evaluate bivalve-ecosystem interactions. The major pathways in which mussels interact with coastal nutrient cycling are; *(i)* filtration of seston (particulate nutrients) from the water column, *(ii)* nutrient storage and growth of mussel tissue, *(iii)* excretion of inorganic metabolic waste products, and *(iv)* excretion and mineralization of biodeposits (reviews by Prins et al. 1998, Newell 2004). These eco-physiological processes were determined simultaneously during our study and can therefore easily be collated to provide an integrated overview of fluxes involved in nutrient cycling by mussel cultures under oligotrophic conditions. Fluxes were determined for individual mussels along with a selection of fluxes determined for mussel communities. Flow diagrams are presented for each element (C-N-P-Si) separately (Figures 6.1-6.4), where silicon dynamics was only determined for the inorganic nutrient fluxes (silicate) and organic fluxes are therefore lacking in Figure 6.4. The flow diagrams did not have the aim to present entirely closed budgets but rather had the aim to quantify and compare different fluxes (see also Tables 6.1 and 6.2).

*Feeding*
Bivalve feeding has extensively been studied for individuals (see review by Cranford et al. 2011) while studies on feeding responses at community level are sparse (Prins & Smaal 1994, Prins et al. 1996, Smaal & Zurburg 1997). The flow diagrams (Figures 6.1-6.3) present two values for uptake of particulate nutrients based on respectively individual and community scale clearance rates measurements. Clearance rates measured for individual mussels (Chapter 5) were comparable to rates reported by Strohmeier et al. (2009) for the same study area. Table 6.3 and a review by Cranford et al. (2011) showed that clearance rates reported for individual mussels under oligotrophic conditions in Norway were among the highest reported for this species.
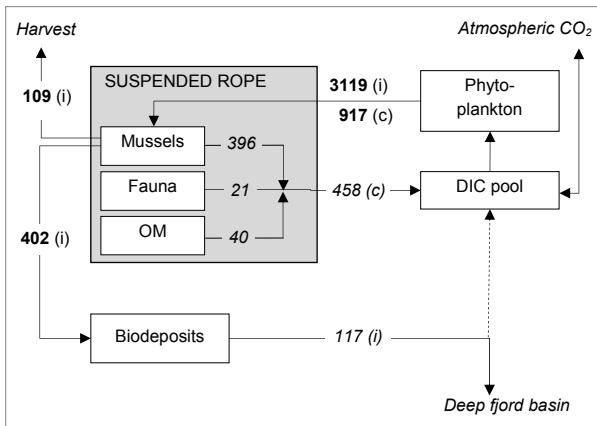
**Figure 6.1 - Carbon**

Carbon flow through suspended mussel cultures in oligotrophic fjord systems. Data is expressed in annual averages for a mussel rope (1m length) with a mussel density of 256 g AFDW (543 individuals). Fluxes are expressed in g C m$^{-1}$ y$^{-1}$. Organic fluxes in bold, inorganic fluxes in italics, (i) for individual and (c) for community based measurements. Note: fluxes are based on direct measurements and the diagram therefore not represent an entirely closed budget.
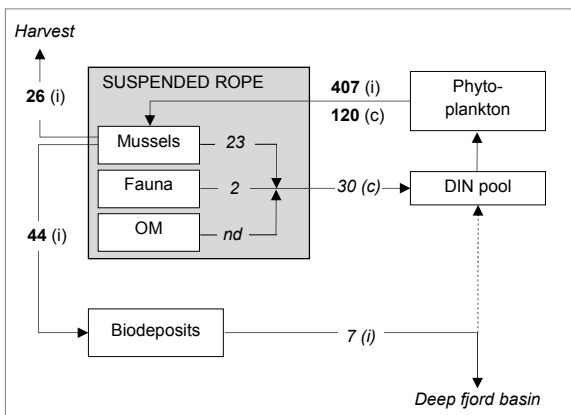


**Figure 6.2 - Nitrogen**

Nitrogen flow through suspended mussel cultures in oligotrophic fjord systems. Data is expressed in annual averages for a mussel rope (1m length) with a mussel density of 256 g AFDW (543 individuals). Fluxes are expressed in g N m$^{-1}$ y$^{-1}$. Organic fluxes in bold, inorganic fluxes in italics, (i) for individual and (c) for community based measurements. Note: fluxes are based on direct measurements and the diagram therefore not represent an entirely closed budget.
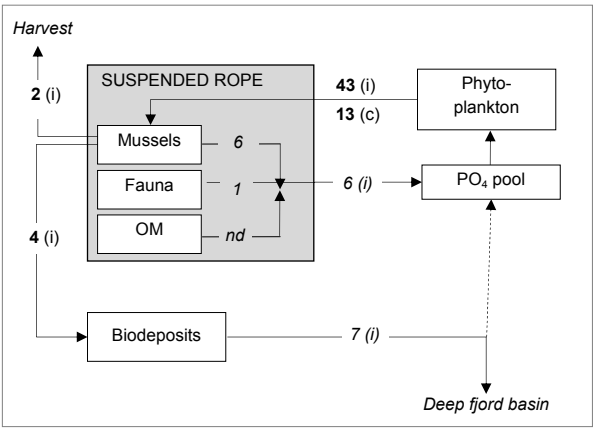
**Figure 6.3 - Phosphorus**
Phosphorus flow through suspended mussel cultures in oligotrophic fjord systems. Data is expressed in annual averages for a mussel rope (1m length) with a mussel density of 256 g AFDW (543 individuals). Fluxes are expressed in g P m$^{-1}$ y$^{-1}$. Organic fluxes in bold, inorganic fluxes in italics, (i) for individual and (c) for community based measurements. Note: fluxes are based on direct measurements and the diagram therefore not represent an entirely closed budget.
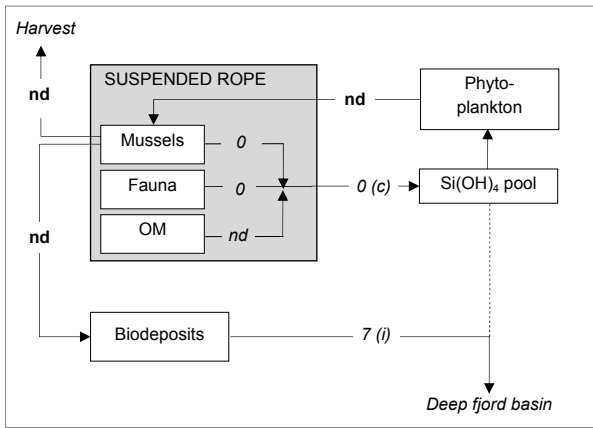


**Figure 6.4 - Silicon**
Silicon flow through suspended mussel cultures in oligotrophic fjord systems. Data is expressed in annual averages for a mussel rope (1m length) with a mussel density of 256 g AFDW (543 individuals). Fluxes are expressed in g Si m$^{-1}$ y$^{-1}$ and were constructed by applying high mineralization rates for diatom dominated periods (⅓ of the year) and low mineralization rates for flagellate dominated periods (⅔ of the year; Chapter 2). Organic fluxes were not determined, inorganic fluxes in italics, (i) for individual and (c) for community based measurements. Note: fluxes are based on direct measurements and the diagram therefore not represent an entirely closed budget.

However, in Chapter 5 it was shown that feeding rates under field conditions (community scale) were 2-3 times lower compared to feeding rates measured on individuals and scaled to community densities (Table 6.1, Chapter 5). This was supported by a comparison between individual (Strohmeier et al. 2009) and farm scale (Aure et al. 2007b) clearance estimates suggesting that measurements on individuals overestimate culture filtration activity by a factor 2-4 (Chapter 5). Another study directly comparing individual and community scale feeding rates, indicated that estimates for mussel beds (=benthic community) were also lower compared to measurements on individuals (Prins et al. 1996), although the differences were not as profound as in our study. It is hypothesized that lower community scale clearance rates could be related to crowding causing flow alteration or refiltration (Frechette et al. 1992, Cranford et al. 2011). One should note that direct comparison of individual and community scale clearance was only determined during the study in Åfjord (Sept 2010), and further investigations should elucidate the difference in feeding responses between individuals and communities. Methods for determination of clearance rates for individuals have been subject of debate during the last decade (Riisgard 2001, Petersen 2004, Petersen et al. 2004, Riisgard 2004, Cranford et al. 2011), while current results imply that focus should also be given to elucidation of the relation between individual and community scale clearance rates.

**Table 6.1**
Budget analysis for nutrient uptake based on clearances rates and based on nutrient allocation to respiration/excretion, growth (harvest) and biodeposition. Values are presented as annual averages standardised to an average rope. Percentages in brackets relate clearance rate estimates to the sum of three processes. A value of 100% indicates a good match between the two approaches, a value >100% indicates a loss in the nutrient budget.

| Process | Scale | Chapter | Carbon [g m$^{-1}$ y$^{-1}$] | Nitrogen [g m$^{-1}$ y$^{-1}$] | Phosphorus [g m$^{-1}$ y$^{-1}$] |
|---|---|---|---|---|---|
| Respiration/Excretion (E) | Individual | 2 | 396 | 23 | 6 |
| Tissue growth (T) | Individual | 2 | 109 | 26 | 2 |
| Biodeposition (B) | Individual | 2 | 402 | 44 | 4 |
| *Sum (E+T+B)* | | | *908* | *94* | *12* |
| Clearance rates individuals | Individual | 5 | 3119 (344%) | 407 (432%) | 43 (358%) |
| Clearance rates communities | Community | 5 | 917 (102%) | 120 (127%) | 13 (108%) |

**Table 6.2**
Budget analysis for nutrient release rates from a mussel community (rope) and for the sum of components (mussels, fauna and organic material). Values are presented as annual averages standardised to an average rope (see Figures 6.1-6.3). Percentages in brackets relate the sum of the separate components to the *in situ* release rates of the community. A value of 100% indicates a good match between the two approaches.

| Process | Scale | Chapter | Carbon [g m$^{-1}$ y$^{-1}$] | Nitrogen [g m$^{-1}$ y$^{-1}$] | Phosphorus [g m$^{-1}$ y$^{-1}$] |
|---|---|---|---|---|---|
| Mussel excretion | Individual | 2 | 396 | 23 | 6 |
| Fauna excretion | Individual | 5 | 21 | 2 | 1 |
| Mineralization organic material | Individual | 3 | 40 | nd | nd |
| *Sum* | | | *457* | *26* | *7* |
| Release community (rope) | Community | 4 | 458 (100%) | 30 (119%) | 6 (78%) |

## Nutrient storage and growth of mussel tissue

The storage of nutrients in tissue material is indicated by the 'harvest' flux in Figures 6.1-6.4. In this context 'harvest' is defined as a production estimate rather than the actual material removed from the water. Relatively more nitrogen accumulated in tissue compared to carbon and phosphorus (Chapter 2) and seasonal changes in nutrient composition were related to reproductive cycle in mussels (Chapter 2). These results confirmed patterns described by Smaal & Vonck (1997), Hawkins et al. (1985) and Kuenzler (1961). Average tissue nutrient content in this study was similar to values reported for most other areas (Table 6.4)

## Release of inorganic nutrients

Appraising the growing body of literature describing inorganic nutrient releases at individual (Bayne & Widdows 1978, Hawkins 1985, Smaal et al. 1997) and community level (Dame & Libes 1993, Prins et al. 1996, Newell et al. 2005, Richard et al. 2006), the current study was the first to simultaneously determine individual excretion rates and release rates measured *in situ* for suspended mussel ropes (Chapter 2, 4 & 5).

**Table 6.3**
Comparison of clearance rates across different areas. Data is standardized to l g$^{-1}$ tissue DW h$^{-1}$. Weight conversion factors reported by Riccardi and Bourget (1998) were applied. Values are presented as average (minimum - maximum), and empty cells indicate that rates were not determined. Country codes (also for following tables): NO=Norway, SW=Sweden, DEN=Denmark, GER=Germany, NL=The Netherlands, NIR=Northern Ireland, UK= United Kingdom, FR=France, ESP=Spain, IT=Italy, CA=Canada, USA=United States, AU=Australia, NZ=New Zealand, JP=Japan.

| Area | Country | Species | Food source | Clearance rates [l g$^{-1}$ h$^{-1}$] | Ref |
|---|---|---|---|---|---|
| *Measurements on individuals* | | | | | |
| Åfjord | NO | *M. edulis* | Natural seawater | 5.4 (3.2-8.4) | 1 |
| Austevoll | NO | *M. edulis* | Natural seawater | 6.4 (3.0-9.6) | 2 |
| Oosterschelde | NL | *M. edulis* | Natural seawater | (1.4-2.8) | 3 |
| Oosterschelde | NL | *M. edulis* | Natural + *P tricornutum* | 1.5 (0.3-3.5) | 4 |
| Oosterschelde | NL | *M. edulis* | Natural seawater | 2.6 (1.3-3.5) | 5 |
| Oosterschelde | NL | *M. edulis* | Natural + *S costatum* | (5.0-8.5) | 6 |
| Lynher estuary | UK | *M. edulis* | Natural seawater | (1.0-2.5) | 7 |
| Aiguillon | FR | *M. edulis* | Natural + *S costatum* | (9.6-11.0) | 6 |
| Ria de Arousa | ESP | *M. galloprovincialis* | Mix sediment & *I galbana* | 5.0-5.8 | 8 |
| New Foundland | CA | *M. edulis* | Natural seawater | (1.5-2.0) | 9 |
| Nova Scotia | CA | *M. edulis* | Natural seawater | (1.0-8.0) | 10 |
| New Foundland | CA | *M. edulis* | Natural seawater | (0.2-3.5) | 10 |
| Great Entry Lagoon | CA | *M. edulis* | Algae mix | (3.0-4.5) | 11 |
| Amherst Basin | CA | *M. edulis* | Algae mix | (2.5-4.0) | 11 |
| Beatrix Bay | NZ | *P. canaliculus* | Natural seawater | (0.8-3.9) | 12 |
| *Measurements on communities (benthic mussel beds)* | | | | | |
| Sylt | DEN | *M. edulis* | Natural seawater | 1.1 | 13 |
| Waddensea | NL | *M. edulis* | Natural seawater | 1.5 (0.7-1.9) | 14 |
| Oosterschelde | NL | *M. edulis* | Natural seawater | 2.2 (1.1-4.8) | 5 |
| Marennes-Oleron | FR | *M. edulis* | Natural seawater | 1.8 (1.0-2.9) | 15 |
| *Measurements on communities (suspended ropes)* | | | | | |
| Åfjord | NO | *M. edulis* | Natural seawater | 1.5 (1.0-2.1) | 1 |
| Havre-aux-Maisons | CA | *M. edulis* | Natural seawater | (1.7-6.3) | 16 |

*1 (Chapter 5); 2 (Strohmeier et al. 2009) ; 3 (Smaal & Vonck 1997); 4 (Smaal et al. 1997); 5 (Prins et al. 1996); 6 (Petersen et al. 2004); 7 (Bayne & Widdows 1978); 8 (Filgueira et al. 2008); 9 (Thompson 1984); 10 (MacDonald & Ward 2009); 11 (Tremblay et al. 1998); 12 (James et al. 2001); 13 (Asmus et al. 1990); 14 (Prins et al. 1994); 15 (Smaal & Zurburg 1997); 16 (Trottet et al. 2008b)*

Respiration and nutrient excretion rates measured for individual mussels under oligotrophic conditions (Chapter 2) were within the range, albeit somewhat in the lower end, as reported for other systems (Table 6.5). The slightly lower rates are likely related to the environmental conditions in our study area, as respiration and excretion rates of mussels are influenced by fluctuations in temperature (Widdows & Bayne 1971, Leblanc et al. 2003) and food (Chapter 2; Bayne et al. 1993, Lutz-Collins et al. 2009). Eco-physiological models are often used to integrate mussel responses and (fluctuations in) environmental conditions (Beadman et al. 2002, Dowd 2005). A model was applied to simulate nitrogen and carbon fluxes (see Chapter 5, model based on Filgueira & Grant 2009). The results indicated that excretion rates ($CO_2$, TAN) for individual mussels under oligotrophic conditions could accurately be predicted by the model (Chapter 5, Filgueira & Jansen unpublished data). This indicates that metabolic responses in mussels are comparable between the various cultivation areas as the model is based on generic equations.

Respiration and nitrogen release rates based on communities were generally higher than rates for individuals (Figures 6.1 - 6.3, Table 6.2), indicating that extrapolation of rates determined for individual mussels may not accurately represent community scale processes. However, distinct temporal variation was observed in the (dis)similarity between individual and community rates (Chapter 5); During most of the year individual and community scale rates were in good agreement, except from two months early autumn when respiration and nitrogen release rates were considerable higher for the community. Mussel cultures are complex community structures including the mussel matrix, bacteria, epifauna, epiflora and trapped biodeposits, each contributing to nutrient uptake and release (Richard et al. 2006, Richard et al. 2007b). Comparison of *in situ* community scale estimates with the integrated sum of individual scale measurements for each of the components (mussels, fauna, decomposition organic material) indicates that sum of the different components is similar for carbon, lower for nitrogen and higher for phosphorus compared to excretion measured *in situ* on mussel communities.

The relatively low rates reported for the fauna compartment suggested that decomposition of organic material contributes more to carbon release rates than excretion by associated fauna (Figure 6.1 and Table 6.2). This might, however, be somewhat misleading due to seasonal patterns, and it was shown that the contribution of decomposing organic material to total community respiration rates was low (Chapter 5). Low contribution of decomposing biodeposits to nutrient release rates observed for the suspended cultures (Chapter 5) is different from bottom cultures where high community nutrient release rates were primarily linked to decomposition of

**Table 6.4**
Comparison of nutrient composition in mussel tissue across different areas. Data was standardized to mg element $g^{-1}$ tissue DW. Weight conversion factors by Riccardi and Bourget (1998) were applied. Values are presented as average (*minimum - maximum*), and empty cells indicate that concentrations were not determined. Country codes are similar to table 6.3

| Area | Country | Species | Carbon [mg g$^{-1}$] | Nitrogen [mg g$^{-1}$] | Phosphorus [mg g$^{-1}$] | Ref. |
|---|---|---|---|---|---|---|
| Austevoll | NO | *M. edulis* | 438 *(402-469)* | 106 *(94-123)* | 7 *(5-11)* | 1 |
| Whitsand Bay | UK | *M. edulis* | 440 *(400-470)* | 80 *(55-110)* | | 2 |
| Oosterschelde | NL | *M. edulis* | 448 *(113-623)* | 102 *(68-126)* | 7 *(5-12)* | 3 |
| Ria de Arosa | ESP | *M. galloprovincialis* | 448 | | | 4 |
| Western Australia | AU | *M. edulis* | 333 | 101 | 4 | 5 |
| Mahurangi Harb. | NZ | *A. zelandica* | 396 | 71 | | 6 |

1(Chapter 2); 2 (Hawkins et al. 1985); 3 (Smaal & Vonck 1997); 4 (Tenore et al. 1982); 5 (Vink & Atkinson 1985); 6 (Gibbs et al. 2005)

**Table 6.5**

Comparison of respiration and inorganic nutrient release rates of different species of mussels and culture types across different areas. All data is standardized to rates in µmol g$^{-1}$ tissue dry weight h$^{-1}$. When needed weight conversion factors as presented by Riccardi and Bourget (1998) were used. Values are presented as average (minimum - maximum), and empty cells indicate that rates were not determined. Country codes are similar to table 6.3

| Area | Country | Species | Temperature [°C] | Respiration [µmol g$^{-1}$ h$^{-1}$] | TAN excretion [µmol g$^{-1}$ h$^{-1}$] | PO$_4$ excretion [µmol g$^{-1}$ h$^{-1}$] | Si excretion [µmol g$^{-1}$ h$^{-1}$] | Ref |
|---|---|---|---|---|---|---|---|---|
| *Measurements on individuals* | | | | | | | | |
| Austevoll | NO | *M. edulis* | 3-19 | 14.2 (5.7-27.8) | 0.7 (0.3-1.8) | 0.07 (<0-0.24) | - | 1 |
| Austevoll | NO | *M. edulis* | 5-20 | 25.9 (12.6-48.1) | (1.8-2.6) | | | 2 |
| Åfjord | NO | *M. edulis* | 12 | 48.9 | | | | 3 |
| Waddensea | NL | *M. edulis* | June & Sept | | (0.8-5.0) | (0.02-0.17) | | 4 |
| Waddensea | NL | *M. edulis* | 3-24 | (10.0-70.0) | | | | 5 |
| Oosterschelde | NL | *M. edulis* | 5-18 | 21.3 (10.3-36.0) | 1.0 (0.2-3.1) | 0.07 (0- 0.13) | - | 6 |
| Oosterschelde | NL | *M. edulis* | 1-20 | 26.3 (15.6-53.1) | 1.1 (0.9-1.6) | | | 7 |
| South | UK | *M. edulis* | 8-20 | (22.3-71.5) | (0.1-2.9) | | | 8 |
| Whitsand Bay | UK | *M. edulis* | . | | 0.9 (0.3-2.1) | | | 9 |
| Whitsand Bay | UK | *M. edulis* | 9-15 | 9.8 (3.1-17.2) | 0.7 (0.1-1.2) | | | 10 |
| Whitsand Bay | UK | *M. edulis* | 9-13 | 4.6 (4.2-8.3) | 0.4 (0.2-0.5) | | | 11 |
| Lynher river | UK | *M. edulis* | 11-21 | | (0.3-2.7) | | | 12 |
| Lynher river | UK | *M. edulis* | 8-15 | | (0.4-1.3) | | | 13 |
| Lynher estuary | UK | *M. edulis* | 5-25 | (18.8-34.8) | (0.6-2.8) | | | 14 |
| Swansey Bay | UK | *M. edulis* | . | | (1.6-2.1) | | | 15 |
| Heacam Bay | UK | *M. edulis* | 15 | (17.9-44.7) | (0.1-0.6) | | | 16 |
| Ria de Arosa | ESP | *M. galloprovincialis* | July | | (0.1-0.2) | | | 17 |
| Ria de Arosa | ESP | *M. galloprovincialis* | 14-15 | | (0.4-0.6) | | | 18 |
| New Foundland | CA | *M. edulis* | 0-15 | (8.9-35.7) | (0.1-0.9) | | | 19 |
| Great Entry Lagoon | CA | *M. edulis* | 20 | (44.7-160.8) | (0.7-7.9) | | | 20 |
| Amherst Basin | CA | *M. edulis* | 20 | (35.7-80.4) | (0.7-2.5) | | | 20 |
| Nova Scotia | CA | *M. edulis* | 0-15 | 8.0 (3.3-12.1) | 1.4 (0.5-2.5) | | | 21 |
| Beatrix Bay | NZ | *P. canaliculus* | 11-17 | 31.8 (22.3-38.7) | (1.6-4.4) | | | |
| Western Australia | AU | *M. edulis* | 15-20 | 6.7 | | 0.02 | | 22 |

**Table 6.5** Continued

| Area | Country | Species | Temperature [°C] | Respiration [$\mu mol\ g^{-1}\ h^{-1}$] | TAN excretion [$\mu mol\ g^{-1}\ h^{-1}$] | PO$_4$ excretion [$\mu mol\ g^{-1}\ h^{-1}$] | Si excretion [$\mu mol\ g^{-1}\ h^{-1}$] | Ref |
|---|---|---|---|---|---|---|---|---|
| *Measurements on communities (benthic mussel beds)* | | | | | | | | |
| Baltic | | *M. edulis* | | | (0.1-3.5) | (0.01-0.50) | | 23 |
| Sylt | DEN | *M. edulis* | 13-19 | | 1.2 *(0.02-5.0)* | | | 24 |
| South | DEN | *M. edulis* | 1-18 | (0-12.5) | (0.1-3.2) | (0.10-0.53) | | 25 |
| Waddensea | GER | *M. edulis* | | | 1.2 (0-5.0) | 0.10 (0- 0.60) | 0.6 (<0 -1.4) | 26 |
| Waddensea | NL | *M. edulis* | June & Sept | | (1.7-14.4) | (0.08-0.50) | | 4 |
| Waddensea | NL | *M. edulis* | June-Sept | | 4.4 | | 2.5 | 27 |
| Oosterschelde | NL | *M. edulis* | | | 5.6 | 1.70 | 2.3 | 27 |
| Oosterschelde | NL | *M. edulis* | | | (0.9-15.8) | (0.03-0.68) | (<0 -3.0) | 28 |
| Marennes-Oleron | FR | *M. edulis* | M-O-J-O | | (0 -7.3) | | | 29 |
| Narragansett Bay | USA | *M. edulis* | 15 | | 3.1 | | | 30 |
| *Measurements on communities (suspended ropes)* | | | | | | | | |
| Austevoll | NO | *M. edulis* | 3-19 | 16.2 (3.4-28.7) | 0.8 (0.2-1.8) | 0.06 (0.00-0.15) | ~0 | 3,31 |
| Åfjord | NO | *M. edulis* | 12 | 17.1 | 1.1 | 0.11 | | 3 |
| Sacca di Goro | IT | *M. edulis* | 8-27 | (25.1-26.9) | (3.2-7.6) | | | 32 |
| Great Entry Lagoon | CA | *M. edulis* | 16-19 | (53.0-92.4) | (1.7-11.6) | (0.22-0.34) | (0.0-0.7) | 33 |

*1 (Chapter 2); 2 (Strohmeier 2009); 3 (Chaper 5); 4 (Prins & Smaal 1994); 5 (Devooys 1976); 6 (Smaal & Vonck 1997); 7 (Smaal et al. 1997); 8 (Bayne & Widdows 1978); 9 (Kreeger et al. 1995); 10 (Hawkins et al. 1985); 11 (Hawkins & Bayne 1985); 12 (Bayne & Scullard 1977); 13 (Livingstone et al. 1979); 14 (Widdows 1978); 15 (Bayne et al. 1979); 16 (Gabbott & Bayne 1973); 17 (Lum & Hammen 1964); 18 (Labarta et al. 1997); 19 (Thompson 1984); 20 (Tremblay et al. 1998); 21 (Hatcher et al. 1994); 22 (Vink & Atkinson 1985); 23 (Kautsky & Wallentinus 1980); 24 (Asmus et al. 1990); 25 (Schluter & Josefsen 1994); 26 (Asmus et al. 1990); 27 (Dame et al. 1991); 28(Prins & Smaal 1990); 29 (Smaal & Zurburg 1997); 30 (Nixon et al. 1976); 31 (Jansen et al. 2011); 32 (Nizzoli et al. 2006); 33 (Richard et al. 2006);*

biodeposits (Asmus et al. 1990, Prins & Smaal 1994). Lower importance of the organic matter compartment for suspended cultures seems reasonable as the majority of biodeposits sink to the seafloor resulting in a reduced biodeposit compartment on the mussel culture itself. Nevertheless, Richard et al. (2006, 2007b) showed that nitrate and nitrite fluxes of suspended bivalve cultures can be significant compared to benthic fluxes and related these fluxes to decomposition of organic material trapped in between the bivalve matrices. Nitrate and nitrite fluxes during the *in situ* measurements on suspended ropes in this thesis (Chapter 4) were low or below the detection limit of the methods.

The large dissimilarity between individual and community scale nutrient release rates in early autumn could be linked to the role of fouling ascidians *Ciona intestinalis*. This species has an annual cycle with drastic seasonal changes in population biomass (Chapter 4, Svane 1983), which added a distinct temporal component to the mussel culture dynamics (Chapter 4). During periods of high fouling abundance, ascidian metabolism contributed up to 18% of total nitrogen releases from mussel communities (Chapter 5). The contribution of fauna in nutrient cycling can therefore not be ignored. This was also acknowledged by Tang et al. (2004) who estimated that carbon tissue content of fouling ascidians was approximately 6.4% of the carbon production in scallops in Sungo Bay (China). Abundance and species composition of fauna associated with mussel cultures varies between seasons and farming locations, adding both temporal and spatial components to mussel farming dynamics (Chapter 4 & 5, Jansen unpubl data, Cayer et al. 1999, Khalaman 2001, Richard et al. 2006, Lutz-Collins et al. 2009). Presently insufficient insight is available on fauna dynamics within and across cultivation areas, yet this kind of information is needed to understand nutrient regeneration by mussel cultures.

*Biodeposition*

Biodeposit production represents a significant pathway in bivalve nutrient cycling (Figures 6.1-6.3, Chapter 2, Kuenzler 1961, Prins & Smaal 1994, Cranford et al. 2007). Biodeposition rates under oligotrophic conditions, as measured in the laboratory for individual mussels, were on the lower end from rates reported in other areas, whereas organic content (OM) was relatively high (Table 6.7). The latter is likely related to high OM in the food source (~70%; Strohmeier et al. 2009) and the fact that pseudofaeces production was absent in most parts of the year. Seasonal fluctuations in biodeposition rates were related to changes in food quantity and quality and not to temperature (Chapter 2). This was consistent with Strohmeier et al. (2009) who suggested that the feeding response to low food concentrations (oligotrophic conditions), rather than temperature, is likely the determining factor for total ingestion. Nutrient concentration in biodeposits depends on the concentration and type of diet the mussels feed on (Miller et al. 2002, Giles & Pilditch 2006) and therefore varied between seasons (Chapter 2 & 3). Comparing nutrient concentrations (POC, PON, POP) in mussel biodeposits measured in our study with the few studies available (Table 6.7) indicated that organic nutrients concentrations were relatively high under oligotrophic conditions.

Although measurements of mussel deposits are essential to understand and quantify their contribution to regeneration of nutrients, little has been published on biodeposit quality and their decay rates (reviewed by McKindsey et al. 2011). Our study showed that mineralization of biodeposits resulted in a significant nutrient flux for all elements (Figures 6.1-6.4), and on average respectively 24% of the carbon and 17% of the nitrogen in decomposing mussel biodeposits were mineralized (Chapter 3). These values were in accordance with Carlsson et al. (2010) and Giles & Pilditch (2006) (Table 6.7), but the high concentrations of organic nutrients in

**Table 6.6**

Comparison of biodeposition and biodeposit composition across different areas. Data is standardized to mg biodeposit g$^{-1}$ tissue dry weight d$^{-1}$ (biodeposition rates), percentage organic material in the biodeposits (%), and mg element g$^{-1}$ biodeposit dry weight (organic nutrient content). When needed weight conversion factors as presented by Riccardi and Bourget (1998) were used. Values are presented as average (minimum - maximum), and empty cells indicate that rates were not determined. Country codes are similar to table 6.3

| Area | Country | Species | Biodeposition [mg g$^{-1}$ tissue d$^{-1}$] | OM [%] | Carbon [mg g$^{-1}$ biodep] | Nitrogen [mg g$^{-1}$ biodep] | Phosphorus [mg g$^{-1}$ biodep] | Silicon [mg g$^{-1}$ biodep] | Ref |
|---|---|---|---|---|---|---|---|---|---|
| Austevoll | NO | *M. edulis* | 32 (11-72) | 36 (22-48) | 135 (62-194) | 15 (7-23) | 1.3 (0.8-1.7) | | 1 |
| Askö, Baltic | SW | *M. edulis* | 31 (7-104) | 19 (8-45) | 129 (50-200) | 15 (8-21) | 1.9 (1.0-3.0) | | 2 |
| Bedford Basin | CA | *M. edulis* | (0-20) | (30-70) | | | | | 3 |
| Mahone Bay | CA | *M. edulis* | (0-80) | (10-70) | | | | | 3 |
| Great Entry Lagoon | CA | *M. edulis* | 54 (18-114) | 22 (20-25) | | | | | 4 |
| Logy Bay (NF) | CA | *M. modiolus* | 5 (1-8) | 17 (13-23) | 69 (47-103) | 8 (5-12) | | 205 (100-335) | 5 |
| Queele Estuary | CH | *M. chilensis* | | 21 | 60 | 4 | | | 6 |
| Firth of Thames | NZ | *P. canaliculus* | | 10 | 25 | 3 | | | 7 |
| Mutsu Bay | JP | *M. edulis* | (6-116) | | | | | | 8 |

*1 (Chapter 2, 3); 2 (Kautsky & Evans 1987); 3 (Cranford & Hill 1999); 4 (Callier et al. 2006); 5 (Navarro & Thompson 1997; during springbloom conditions); 6 (Jaramillo et al. 1992); 7 (Giles & Pilditch 2006); 8 (Tsuchiya 1980)*

**Table 6.7**

Comparison of biodeposit remineralization rates across different areas. Data is standardized either to rates in mmol g$^{-1}$ biodeposit dry weight d$^{-1}$ or to fraction of initial nutrient content in the biodeposits (e.g. %=TAN/PON*100). Values are presented as average (minimum - maximum), and empty cells indicate that rates were not determined. Country codes are similar to table 6.3

| Area | Country | Species | Temp (°C) | Unit | $CO_2$ release | TAN release | $PO_4$ release | $Si(OH)_4$ release | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Austevoll | NO | *M. edulis* | 5,10,15 | mmol g$^{-1}$ d$^{-1}$ | 3.3 (2.0-4.3) | 0.17 (0.12-0.21) | 0.06 (0.01-0.08) | 3.9 (0.1-11.5) | 1 |
| | | | | % | 24 (15-31) | 17 (10-20) | | | 1 |
| Great Entry Lagoon | CA | *M. edulis* | June-Aug | mmol g$^{-1}$ d$^{-1}$ | (max 4.5) | (max 0.3) | (max 0.02) | (max 1.0) | 2 |
| Roskilde & Limfjord | DEN | *M. edulis* | 8-10 | % | (25-38) | | | | 3 |
| Firth of Thames | NZ | *P. canaliculus* | 20 | % | 40 | 18 | | | 4 |

*1 (Chapter 3); 2 (recalculated from Callier et al. 2009); 3 (Carlsson et al. 2010); 4 (Giles & Pilditch 2006)*

the mussel biodeposits in our study indicated that nutrient releases per gram biodeposit will be higher under oligotrophic conditions. Giles & Pilditch (2006) suggested that approximately one third of the organic nitrogen in biodeposits might have been removed by bacterial assimilation and/or nitrification-denitrification processes and the production of $N_2$ gas taking place at the sediment interface. Our study mimicked pelagic decomposition and no sediment was added to the experimental chambers providing well oxygenated conditions, minimal losses by formation of $N_2$ gas were therefore assumed. Phosphorus mineralization results provided incoherent patterns, as it seemed that more phosphate was mineralized than theoretically possible from biodeposit phosphorus content (Chapter 3). Giles & Pilditch (2006) reported the opposite as phosphate fluxes were absent in their study, which was related to absorption of phosphate by the sediments (Sundby et al. 1992).

Additional to previous knowledge, our study demonstrated that mineralization rates vary considerably between seasons and elements (Chapter 3). Most profound seasonal differences were observed for silicate mineralization with 60-80 times higher mineralization rates in spring compared to summer and autumn, which was likely related to the amount of diatoms in the mussel food (Navarro & Thompson 1997). Variations in mineralization rates could be related to concentrations of macro-nutrients (C-N-P-Si) in the biodeposits, but the results also suggested that the proportion of labile material in the biodeposits or availability of micronutrients partly regulate mineralization processes. It is generally recognized that decomposition rates of organic material are also related to changes in water temperature (White et al. 1991, del Giorgio & Cole 1998, Katterer et al. 1998). We showed that temperature enhances mussel biodeposit decomposition at approximately 2-3 times faster turnover for all nutrients (C-N-P-Si) at a 10 °C temperature interval ($Q_{10}$=2-3).

Considering the importance of biodeposition in bivalve nutrient cycling in several cultivation areas (Kuenzler 1961, Prins & Smaal 1994, Cranford et al. 2007), the mineralization rates quantified in this thesis provide important information to improve generic predictions/modelling. Although significant phosphorus and silicon release rates were observed from decomposing mussel biodeposits, the observed patterns were either divergent (P) or information on initial biodeposit concentration was lacking (Si). The next steps in research should therefore further elucidate phosphorus and silicon dynamics.

Due to physical characteristics of fjord systems defined by large depths and stratified water column, benthic mineralized nutrients will normally not be available in upper water layers and only the biodeposits trapped in between the mussel matrices (ropes) will contribute to the pool of regenerated nutrients available for primary producers in the euphotic zone of fjord systems (see Chapter 2). Richard et al. (2006) showed that decomposition of organic material on mussel ropes contributes to regeneration of nitrate, nitrite and silicate. However, releases of these elemental forms were insignificant during all occasions in our study (Chapter 4 & 5) and the contribution of decomposing biodeposits to nutrient regeneration in the euphotic zone is therefore limited.

*Budget analysis*

The flow diagrams (Figures 6.1-6.4) provide an integrated overview of fluxes which can be used to evaluate the coherence between measurements on the various eco-physiological processes. This was already demonstrated above for the *in situ* nutrient release rates in relation to measurements on the individual components (mussel, fauna, decomposition organic material) (Table 6.2). Budget analysis for carbon, nitrogen and phosphorus furthermore indicated that the

**Table 6.8**
Ratios for inorganic and organic nutrients in the ambient water and for each of the physiological processes as measured under oligotrophic conditions (see Fig. 6.2). Ratios are expressed in molar; nd = not determined.

| | Ratio C:N | Ratio N:P | Ratio N:Si |
|---|---|---|---|
| *Organic nutrients* | | | |
| Ambient water | 8.9 | (21) | nd |
| Growth mussel tissue | 4.8 | 33.5 | nd |
| Biodeposits | 10.6 | 25.5 | nd |
| OM on ropes | 8.8 | nd | nd |
| *Inorganic nutrients* | | | |
| Ambient water | nd | 9.5 | 0.9 |
| Excretion by individual mussels | 19.9 | 8.5 | ∞ |
| Excretion by ascidians | 13.2 | 4.1 | ∞ |
| Remineralization of biodeposits | 19.3 | 2.8 | 0.04 |
| Release by mussel community | 17.5 | 12.1 | ∞ |

sum of excretion, tissue growth ('harvest') and biodeposition adequately represented the estimated nutrient uptake through feeding at community level (see Table 6.1). Feeding rates measured on individual mussels resulted in higher uptake rates, subsequently leading to a significant unexplained loss in the budget. These results again indicate that focus should be given to determination of clearance rates and food uptake by mussel communities.

*Stoichiometry*
Subsequently stoichiometric characteristics between elements and physiological processes were evaluated (Table 6.8). CN ratios in excretion of inorganic metabolic waste products by mussels were generally higher compared to the organic nutrients in the ambient water, biodeposits and mussel tissue. This indicated that more carbon was regenerated by formation of $CO_2$ (DIC), while nitrogen accumulated in mussel tissue. Low NP ratios in release of inorganic nutrients relative to the organic nutrients indicated that relatively more phosphorus was regenerated, while the high NP ratios in biodeposits and mussel tissue indicated storage of nitrogen. Differences in metabolic processes and their stoichiometry were related the endogenous factors (e.g. gametogenic cycle) as well as environmental factors (e.g. food and temperature), which are all interrelated given that food, temperature and gametogenesis follow annual cycles (Chapter 2 & 4, Smaal & Vonck 1997). Data in Table 6.10 also demonstrate the importance of silicate mineralization in decomposing biodeposits. Furthermore, nutrients released by individual mussels and from mussel communities (C>N>P>>Si), were different from ratios observed in the ambient water.

## Feedbacks to the ecosystem

The previous section demonstrated that mussels contribute to nutrient cycling by translocation, transformation and remineralization of the essential nutrients. These processes determine how and to what extent mussels may contribute to nutrient cycling, and consequently primary production dynamics, in coastal ecosystems. Hence mussels can depress phytoplankton biomass (negative feedback) while promoting higher turnover rates (positive feedback)(Prins et al. 1995). It has been argued that this control on phytoplankton biomass can stabilize ecosystems (Herman & Scholten 1990). The extent of both positive and negative feedbacks is situation specific and determined by physical and environmental conditions of the area (Newell et al. 2005). The current section aims to evaluate the potential pathways and magnitude of the feedback mechanisms. Again, focus was given to oligotrophic fjord systems while differences between systems were addressed by means of a literature review for several bivalve cultivation areas around the world.

*Physical and environmental characteristics of bivalve cultivation areas*

Natural mussel communities have a wide geographic distribution including both sheltered and exposed habitats (Gosling 2003), whereas present shellfish aquaculture sites are predominantly situated in relatively sheltered estuaries, bays or fjord systems. Fjord systems are special compared to "coastal plain estuaries" due to the large depth (100-1000m). Many Norwegian fjords have a sill at the mouth of the fjord which limits renewal of the deep water basin, and results in relatively long residence times for the complete system (Table 6.9). Fjords typically have a stratified water column (Aure et al. 1996, Asplin et al. 1999) resulting in three main layers; the upper brackish water, the intermediate water extending from the top of the pycnocline and down to the sill depth, and the basin water below (Figure 6.5). The water residence time of the upper and intermediate layers (at scale of days) is much shorter compared to the residence time of the deep water basin (at scale of months to years). The majority of other bivalve cultivation areas are shallow mesotidal bays or estuaries. Due to the variation in physical conditions of the shallow bays and estuaries, water residence times vary from 1 day to the scale of several months (Table 6.9).

The stratified water column of Norwegian fjords, in spring and summer, restricts vertical mixing of nutrients into the euphotic layer (Aure et al. 1996, Asplin et al. 1999), which leads to a weak
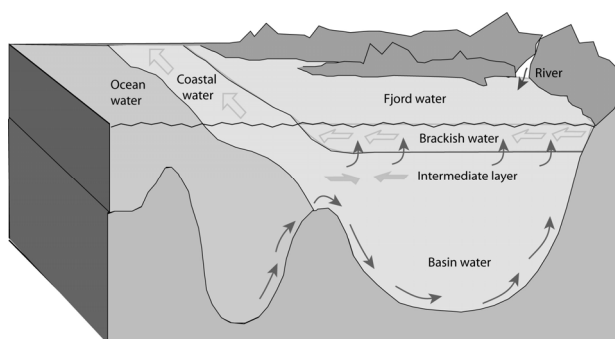


**Figure 6.5**
Water exchange patterns in a typical fjord system (Source: Sætre 2007)

coupling between the benthic and euphotic zone. Phytoplankton growth above the pycnocline therefore relies on regenerated nutrients ('regenerated production') and nutrients supplied by precipitation or from anthropogenic sources through land run-off, whereas phytoplankton growth below the pycnocline relies on nutrients provided from below through mixing or upwelling ('new production') (Erga et al. in prep). Nutrients derived from anthropogenic sources are generally not important in fjord systems (Aksnes et al. 1989), and regenerated nutrients are therefore important for phytoplankton growth. Nutrient regeneration in marine systems takes place through various pathways, such as benthic mineralization, zooplankton activity or by filter feeders. Benthic regeneration does not contribute to the nutrient pools in the euphotic zone during periods of stratified water columns in fjords. Furthermore, biomass of natural mussel stocks is generally low in fjord systems (180 g AFDW m$^{-1}$ shoreline or 2 g AFDW m$^{-2}$ per unit fjord areal; Olafsson & Hoisaeter 1988), since predation prevents mussel survival in the deeper water bodies (below halocline) and consequently only the upper few meters of the steep rocky shoreline is available for mussel growth (Bodvin). Nutrient regeneration therefore primarily relies on zooplankton activity (Wassmann 1991), and it is shown that the euphotic zone is nutrient limited for extended periods of the year (Paasche & Erga 1988, Sætre 2007) resulting in low Chl *a* concentrations (Erga 1989a, Aure et al. 2007). Primary production rates in

**Table 6.9**
Physical characteristics bivalve cultivation areas. Country codes are similar to Table 6.3

| Area | Country | Type | Water depth [m] | Volume system [$10^6$ m$^3$] | Residence time [d] | Ref |
|---|---|---|---|---|---|---|
| Lysefjord- total | NO | Fjord | (460 max) | 9100 | 7 year | 1 |
| above sill | | | 14 | 880 | 11 | |
| Åfjord – total | NO | Fjord | 50 (120 max) | 807 | 150 | 2 |
| above sill | | | 20 | 250 | 5 | |
| Limfjorden | DEN | Estuary with multiple basins | 5 | 7100 | 225 | 3 |
| Sylt | DEN | | 2 | 7 | 0.5 | 4 |
| Oosterschelde | NL | Estuary | 9 | 2740 | 40 (10-150) | 5,6 |
| Wadden Sea | NL | Bay | 3 | 4020 | 10 (5-15) | 6 |
| Carlingford Louch | NIR | Estuary | (35 max) | 460 | 14-26 | 7 |
| Louch Foyle | NIR | Bay | (19 max) | 752 | 4-30 | 7 |
| Bay of Brest | FR | Bay | 10 | 1480 | 17 | 8 |
| Thau Lagoon | FR | Lagoon | 4 | 300 | 90-120 | 9 |
| Marennes-Oleron | FR | | 5 | 675 | 7 | 10 |
| Ria de Arosa | ESP | Bay, upwelling, | 19 | 4335 | 23 | 11 |
| N. Adriatic Sea | IT | Open Sea | 22 | - | - | 12 |
| Tracadie Bay | CA | Bay | 2.5 (6 max) | 41 | 4-10 | 13 |
| Great Entry Lagoon | CA | Two-lagoon system | 6 | 117 | 20-30 | 14 |
| Saldanha Bay | SA | Two-bay system, upwelling | 10 (30 max) | 596 | 6-10 | 15 |
| Firth of Thames | NZ | Estuary | (50 max) | 16500 | 12 | 16 |

*1 (Aure et al. 2001); 2 (Aure pers. comm.); 3 (Wiles et al. 2006, Maar et al. 2010); 4 (in Smaal & Prins 1993); 5 (Smaal et al. 2001); 6 (Dame et al. 1991); 7 (Ferreira et al. 2007); 8 (in Smaal & Prins 1993); 9 (Thouzeau et al. 2007); 10 (in Smaal & Prins 1993); 11(Ferreira et al. 2007); 11 (AlvarezSalgado et al. 1996a, Figueiras et al. 2002); 12 (Brigolin 2007); 13 (Filgueira & Grant 2009); 14 (eastern basin; pers comm. T. Guyondet); 15 (Shannon & Stander 1977, Monteiro et al. 1998); 16 (Zeldis 2005)*

**Table 6.10**

Biochemical characteristics of bivalve cultivation areas. Country codes are similar to table 6.3. Trophic classification according to Nixon (1995). Primary production (PP), Suspended Particulate Material (SPM), Organic Material (OM), Chlorophyll *a* (Chl a), Particulate Organic Carbon and Nitrogen (POC, PON), Dissolved Inorganic Nitrogen (DIN), Phosphate ($PO_4$) and Silicate (Si). Values are presented as average (minimum - maximum).

| Area | Country | Trophic classification | Time | PP [g C m$^{-2}$ y$^{-1}$] | SPM [mg l$^{-1}$] | Chl *a* [µg l$^{-1}$] | POC [mg l$^{-1}$] | PON [mg l$^{-1}$] | DIN [µM] | PO$_4$ [µM] | Si [µM] | Ref |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Austevoll | NO | Oligotrophic | Annual | | 0.4 (0.2-1.1) | 1 (0-8) | 0.2 (0.1-0.6) | 0.02 (0.01-0.05) | 2.1 (0-7) | 0.2 (0-0.6) | 2.4 (0-8.5) | 1 |
| Lysefjord | NO | Oligotrophic | Annual | 100-140 | (0.8-4) | 1-1.5 (0.9-6.5) | (0.15-.05) | (0.01-0.02) | (0-2) | (0.02-0.03) | (1-5) | 2 |
| Åfjord | NO | Mesotrophic | Seasonal | | | 1.2 (0.1-4.0) | 0.3 (0.1-1.5) | 0.04 (0.01-0.2) | 2.4 (0.5-7.3) | 0.3 (0.1-0.5) | 3.2 (0.9-5.5) | 3 |
| Sylt | DEN | | July-Aug | 73 | 30 (17-202) | | 1.0 (0.3-7.5) | 0.15 (0.05-0.6) | | | | 4 |
| Limfjorden | DEN | Eutrophic | Annual | 284 (0-1460) | 5.6 | >10 (0-50) | | | 37 (0-100) | 0.5 (0-9) | | 5 |
| Oosterschelde | NL | Eutrophic | Annual | 200 (115-456) | 6 (5-6) | 5 | 1 | 0.1 | 30 | 1.5 (1-6) | 15 (1-40) | 6 |
| W. Waddenzee | NL | Eutrophic | Annual | 200 | 36 (6-120) | (3-13) | | | 17 (0-64) | (0.5-3) | | 7 |
| | | | Springbl | | | | | | 67 | 0.8 | 16.3 | 8 |
| Carlingford Lough | UK | Eutrophic | Annual | | 7.6 | 2.3 | | | 8.1 | 0.6 | | 9 |
| Lough Foyle | UK | Eutrophic | Annual | | 15.5 | 3.2 | | | 35.1 | 1.1 | | 9 |
| Thau Lagoon | FR | Eutrophic | Annual | 400 | (0.5-5) | (0-20) | 0.3 (0.1-0.7) | 0.04 (0.01-0.13) | 1.8 (1-12) | | | 10 |

**Table 6.10** Continued

| Area | Country | Trophic classification | Time | PP [g C m$^{-2}$ y$^{-1}$] | SPM [mg l$^{-1}$] | Chl $a$ [µg l$^{-1}$] | POC [mg l$^{-1}$] | PON [mg l$^{-1}$] | DIN [µM] | PO$_4$ [µM] | Si [µM] | Ref |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ria de Arousa | ESP | Eutrophic | Seasonal | 99 (0-1351) | 1.1 (0.5-2.6) | 4.6 (0.1-34) | 0.3 | (0.01-0.07) | (2-12) | | | 11 |
| N. Adriatic Sea | IT | - | Annual | | | (0.4-16) | 0.2 | 0.03 (0.02-0.1) | | | | 12 |
| Tracadie Bay | CA | Eutrophic | Seasonal | 318 (18-1204) | 3.3 | 2.9 (1-12) | | 0.1 | 5 (1-14) | 0.3 (0.1-0.6) | 2 (0.2-6) | 13 |
| Great Entry Lagoon | CA | Oligotrophic | Jun-Oct | (50-220) | (4-27) | 1.8 (0.8-3.1) | 0.4 | | 0.3 | 0.3 (0.1-0.5) | 1.1 (0.4-2.5) | 14 |
| New Foundland | CA | | Seasonal | | 4.3 (2.2-6.5) | (0.2-5) | | | | | | 15 |
| Saldanha Bay | SA | Eutrophic | Seasonal | 1240 (581-5875) | 3.6 (Feb) | 8.6 (0.4-5.9) | | | (0-35) | | | 16 |
| Firth of Thames | NZ | Mesotrophic | Annual | 168 (69-384) | (3-10) | | | | 1.5 | 0.3 | | 17 |

1 (Strohmeier et al. 2009; Chapter 2); 2 (Aure et al. 2001, Aure et al. 2007a, Strohmeier unpubl data; values provided for upper 10m); 4 (Asmus et al. 1990, Smaal & Prins 1993); 5 (Olesen 1996, Wiles et al. 2006, Maar et al. 2010) 6 (Smaal & Vonck 1997, Smaal et al. 2001, Wetsteyn et al. 2003); 7 (Dame et al. 1991, Philippart et al. 2007; waterbase NL); 8 (Philippart et al. 2007); 9 (Ferreira et al. 2007) ; 10 (Souchu et al. 2001, Plus et al. 2006); 11 (Smaal & Prins 1993, AlvarezSalgado et al. 1996b, Figueiras et al. 2002, Filgueira et al. 2010a); 12 (Brigolin 2007); 13 (Bates & Strain 2006, Cranford et al. 2007, Cranford unpubl data, Harris unpubl data); 14 (Tremblay et al. 1998, Callier et al. 2006, Trottet et al. 2007); 15 (Thompson 1984, Navarro & Thompson 1997); 16 (Monteiro et al. 1998, Pitcher & Calder 1998, Probyn unpubl data); 17 (Zeldis 2005).

Norwegian fjord systems typically vary between 100-140 g C m$^{-2}$ yr$^{-1}$ (Aure et al. 2007a), and approximately 40% of the total primary production is regarded as new primary production (Wassmann 1990). Consequently this results in a new carbon supply of 40-56 g C m$^{-2}$ yr$^{-1}$ and Norwegian fjords are thereby classified as 'oligotrophic' within the trophic classification of marine systems by Nixon (1995).

Other bivalve areas generally have well mixed water columns, with average annual primary production rates varying between 73 and 1245 g C m$^{-2}$ y$^{-1}$ (Table 6.10). Different from Norwegian fjord systems, background nutrient levels in most areas are influenced by anthropogenic nutrient sources. Wassmann (2005) demonstrated that estuaries and coastal ecosystems are now the most nutrient over-enriched ecosystems in the world, attributed primarily to land-based nutrient sources. Limfjorden (Denmark), for example, receives approximately 20 000 ton N y$^{-1}$ from land-based sources, and the increased nitrogen input during the last decades resulted in high phytoplankton biomass which sustained high densities of bivalves, up to a level causing hypoxia-based mortality (Christiansen et al. 2006). Highest primary production rates were reported in Ria-de-Arousa and Saldahna Bay, which are coastal bays that benefit from upwelling of deep nutrient-rich water. The coastal upwelling along the South African coastline (Belunga system) supplies a flux of approximately 1819 ton $NO_3$-N y$^{-1}$ into Saldanha Bay (Monteiro et al. 1998). Areas that benefit from coastal upwelling are among the most productive and successful mussel farming areas (Figueiras et al. 2002, Saxby 2002). Furthermore, as a consequence of benthic-pelagic coupling in shallow ecosystems, pathways for 'nutrient regeneration' are different for shallow systems compared to the deep fjord systems. Benthic nutrient regeneration can play an important role in coastal ecosystems with well mixed water columns as it may provide up to 80% of nutrients required for primary production (Jensen et al. 1990; Giles, 2006; Zeldis 2005).

*Nutrient sinks and sources*
Through the different physiological processes, ingested nutrients become regenerated, retained or are permanently removed from the system (Newell et al. 2005). In this way, mussel communities may act as a source or a sink of nutrients within the ecosystem. The specific pathways contributing to sinks/sources depend on physical features (depth) of the area and the culture type applied (Table 6.11).

Table 6.12 provides an overview of the relative importance of the physiological processes involved in nutrient cycling by mussel cultures. The processes have been expressed as fractions, with the sum of the three processes (inorganic excretion, biodeposition and tissue growth) resulting in 100%. It is thereby assumed that the sum of three processes equals ingestion (in accordance to Kreeger et al. 1995). Under oligotrophic conditions <50% of the ingested nutrients were expelled with biodeposits, which is lower compared to the other areas where more than half, and in certain cases up to 95%, of the ingested nutrients are expelled with biodeposits (Table 6.12). The relative low contribution of biodeposition under oligotrophic conditions is in accordance with relatively low egestion rates observed earlier (Chapter 2; Table 6.6).

In deep fjord systems regenerated nutrients (= source) are predominantly related to respiration ($CO_2$) and release of metabolic waste products (TAN, $PO_4$) by the suspended mussel communities in the euphotic zone. Approximately half of the ingested carbon and phosphorus, and 25% of nitrogen became regenerated (Table 6.12). Lower regeneration values for nitrogen are related to the fact that relatively more nitrogen accumulates in tissue material (Chapter 2). Mineralization

of biodeposits does not significantly contribute to the source of recycled nutrients as benthic regenerated nutrients are not available in the euphotic zone of fjord systems due to stratification of the water column (see previous section). Mineralization of biodeposits trapped in between the mussel matrix of suspended cultures may theoretically contribute to the pool of regenerated nutrients, however in Chapter 5 we showed that this contribution was low. For bottom and suspended cultivation of mussels in shallow areas it has been shown that benthic biodeposit decomposition significantly contributes to total nutrient regeneration (Asmus et al. 1990, Baudinet et al. 1990, Hatcher et al. 1994, Prins & Smaal 1994, Giles et al. 2006, Richard et al. 2007b). In these areas the pool of regenerated nutrients therefore consists of both the released metabolic waste products as well as nutrients mineralized during biodeposit decomposition (Table 6.11 & 6.12). This results in similar 'source' values for carbon and nitrogen regeneration in oligotrophic fjords compared to the shallow eutrophic areas, while phosphorus regeneration in oligotrophic fjords was relatively high. Low phosphorus regeneration rates for the other two systems are a consequence of low or absent phosphorus excretion reported by Kuenzler (1961) and Brigolin et al. (2009). There are however no indications that phosphorus excretion in oligotrophic systems is higher compared to other systems (see literature overview of phosphorus excretion rates presented previously, Table 6.5). Phosphorus regeneration values ('source') wereunderestimated as mineralization of biodeposits was not included in the estimates due to the conflicting results observed in Chapter 3. Regeneration of benthic communities was

**Table 6.11**
Overview of processes involved the nutrient sink and source kinetics related to different mussel-ecosystems characterized by varying depth profiles and culture types

| Depth system | Culture type | Regeneration (source) | Retention (sink) | Release (sink) |
|---|---|---|---|---|
| Shallow | Bottom | *Benthic*<br>- $CO_2$ (DIC) & $NH_4$ & $PO_4$ excretion mussels & fauna<br>- $CO_2$ (DIC), $NH_4$, $PO_4$ & Si biodeposit mineralization<br>- $NO_2$/$NO_3$ nitrification of $NH_4$ | *Benthic*<br>- $PO_4$ binding to sediment<br>- POC, PON, POP, POSi burial of biodeposits | *Benthic*<br>- $N_2$ from nitrification/ denitrification of $NH_4$<br>- PON, PON, POP harvest mussel tissue |
| Shallow | Suspended | *Pelagic*<br>$CO_2$ (DIC) & $NH_4$ & $PO_4$ excretion mussels & fauna | | *Pelagic*<br>- PON, PON, POP harvest mussel tissue |
| | | *Benthic*<br>- $CO_2$ (DIC), $NH_4$, $PO_4$ & Si biodeposit mineralization<br>- $NO_2$/$NO_3$ nitrification from $NH_4$ | *Benthic*<br>- $PO_4$ binding to sediment<br>- POC, PON, POP, POSi burial of biodeposits | *Benthic*<br>- $N_2$ nitrification/ denitrification from $NH_4$ |
| Deep | Suspended | *Pelagic*<br>- $CO_2$ (DIC) & $NH_4$ & $PO_4$ excretion mussels & fauna | *Benthic (deep fjord basin)*<br>- POC, PON, POP, POSi burial of biodeposits<br>- $CO_2$ (DIC), $NH_4$, $PO_4$ & Si biodeposit mineralization | *Pelagic*<br>- PON, PON, POP harvest mussel tissue |

expressed as the relation between estimates for uptake of organic material with total release of inorganic nutrients determined with benthic tunnel measurements (Table 6.12). High variation was observed with occasionally higher release rates than uptake rates (source >100%), likely induced by mineralization of biodeposits trapped in between the culture structures. An extensive seasonal study on nutrient cycling by oyster *Crassostrea virginica* reefs in the North Inlet estuary (South Carolina; Dame et al. 1989) indicated that 66% of the nitrogen and 8% of the phosphorus taken up by the reef was regenerated as ammonia and phosphate, respectively.

**Table 6.12**
Relative importance of physiological processes across cultivation areas. Physiological processes are indicated with E for excretion/release of inorganic nutrients, B for biodeposition and T for tissue growth (=harvest). Data presented in the table originates from budget analysis studies. 'Source' refers to the fraction of ingested nutrients (calculated as the sum of release, biodeposition and tissue growth) which are regenerated, and 'Sink' refers to the fraction of ingested nutrients retained or permanently lost from the system. Sinks and sources are calculates as:

[I] *Source = Excretion; Sink=Biodeposition + Tissue growth*
[II] *Source = Excretion + remineralization; Sink = Tissue growth + Biodeposition - remineralization*
*(with mineralization rates of 32% for C, 17% for N, and 0% for P; average values presented in Table 6.7)*
[III] *Based on uptake and release rates measured in situ in benthic tunnels*

| | Area | Country | Species | Element | Physiological process (%) | | | | Ecosystem interaction (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | E | B | T | | Source | Sink |
| *Measurements on individuals* | | | | | | | | | | |
| 1a | Austevoll | NO | M. edulis | C | 44 | 44 | 12 | I | 44 | 56 |
| 2 | Whitsand Bay | UK | M. edulis | C | 63/40 | 67/52 | -30/8 | II | 85/25 | 15/75 |
| 3 | Whitsand Bay | UK | M. edulis | C | 2 | 85 | 13 | II | 36 | 64 |
| 4 | NW Adriatic sea | IT | M. galloprovincialis | C | 20 | 65 | 15 | II | 41 | 59 |
| 1a | Austevoll | NO | M. edulis | N | 25 | 47 | 28 | I | 25 | 75 |
| 2 | Whitsand Bay | UK | M. edulis | N | 53/19 | 64/61 | -17/20 | II | 78/44 | 22/66 |
| 3 | Whitsand Bay | UK | M. edulis | N | 1 | 39 | 61 | II | 11 | 89 |
| 4 | NW Adriatic sea | IT | M. galloprovincialis | N | 30 | 55 | 16 | II | 39 | 61 |
| 5 | Tracadie Bay | CA | M. edulis | N | 14 | 81 | 5 | II | 28 | 72 |
| 6 | New England | VS | G. demissa | N | 32 | 60 | 8 | II | 43 | 57 |
| 1a | Austevoll | NO | M. edulis | P | 52 | 33 | 15 | I | 52 | 48 |
| 4 | NW Adriatic sea | IT | M. galloprovincialis | P | 0 | 89 | 11 | II | 0 | 100 |
| 7 | Sapelo estuary | USA | *Modilus demissus* | P | *5* | 95 | 0 | II | 5 | 95 |
| *Measurements on communities (benthic beds)* | | | | | | | | | | |
| 8 | Sylt | DEN | M. edulis | N | | | | III | 37 | 63 |
| 9 | Oosterschelde | NL | M. edulis | N | | | | III | 85/>100 | 15/ - |
| 10 | North Inlet est | USA | C. virginica | N | | | | III | 66 | 44 |
| 9 | Oosterschelde | NL | M. edulis | P | | | | III | 23/>100 | 77/ - |
| 10 | North Inlet est | USA | C. virginia | P | | | | iii | 8 | 92 |
| 9 | Oosterschelde | NL | M. edulis | Si | | | | III | 48/>100 | 52/ - |
| *Measurements on communities (suspended ropes)* | | | | | | | | | | |
| 1b | Austevoll | NO | M. edulis | C | 50 | 39 | 11 | I | 50 | 50 |
| 1b | Austevoll | NO | M. edulis | N | 37 | 40 | 24 | I | 37 | 63 |
| 1b | Austevoll | NO | M. edulis | P | 56 | 31 | 14 | I | 56 | 44 |

*1 (this study see figure 6.2. Release of inorganic nutrients based on (1a) individual and (1b) community rates); 2 (Hawkins & Bayne 1985; March & June results respectively); 3(Kreeger et al. 1995); 4 (Brigolin et al. 2009); 5 (Cranford et al. 2007); 6 (Dame et al. 1989); 7 (Kuenzler 1961); 8 (Asmus et al. 1990); 9 (Prins & Smaal 1994; maximum and minimum values); 10 (Dame et al. 1989)*

Studies performed on benthic cultures (Dame et al. 1989, Asmus et al. 1990, Prins & Smaal 1994) also pointed out that sediment processes may bind, and thus retain, phosphate and can transform TAN into nitrate or nitrite which subsequently may lead to a loss of nitrogen from the system by the formation of nitrogen gas. The influence of bivalve cultures on denitrification rates have not fully been characterised (Newell 2004) and previous studies on the impacts of mussel farms on denitrification rates have been inconsistent, showing both increase (Kaspar et al. 1985, Giles et al. 2006) and decrease (Christensen et al. 2003) in sediments affected by suspended mussel cultures.

Nutrient sinks related to bivalve cultivation are related to harvest and biodeposition (minus fraction remineralized nutrients). As sinks and sources are expressed as the fraction of ingested nutrients (Table 6.12), the sink represents the reverse of the source value. In the current context 'harvest' is again defined as a production estimate rather than the actual material removed from the water. In suspended cultures mussels may die or fall off the cultivation structures before harvest (Frechette in press). 'Fall-off' was not incorporated into the analysis applied in this thesis, and therefore overestimates the production and nutrient removal by 'harvest'. On the other hand, harvest was slightly underestimated as only nutrient storage in the mussel tissue material and not storage in byssus or shell were incorporated in the estimates (Hawkins & Bayne 1985; see also Chapter 2). As mentioned before, in fjord systems all biodeposition is considered as a loss while in shallow systems only a part of the biodeposits are buried in the sediment and another part will be mineralized and contribute to the source of regenerated inorganic nutrients. In Table 6.12 all regenerated carbon is assumed to contribute to the source of recycled nutrients. This assumption is valid for Norwegian fjord systems, whereas in some eutrophic estuaries $CO_2$ might be released to the atmosphere as these systems often have oversaturated $pCO_2$ levels (Frankignoulle et al. 1998). In these estuaries release of $CO_2$ is a sink to the systems, and values presented in Table 6.12 might therefore underestimate the carbon sink.

*Stoichiometry of regenerated nutrients*

The previous section pointed out that mussel communities can act as source of regenerated nutrients to the system. As a consequence of the dissimilar physiological requirements of mussels for the different elements (Chapter 2), regenerated nutrients can have different stoichiometric characteristics compared to the inorganic nutrient pool available in the ambient waters (Table 6.8; Prins et al. 1998). This indicates that the positive feedback mechanism is influenced by both nutrient availability and stoichiometry of the regenerated nutrients.

Ratios between the available inorganic nutrients determine the nature and strength of nutrient limitation in ambient waters of coastal areas. Figure 6.6 shows NP ratios of the ambient water across bivalve cultivation areas with ratios below Redfield's ratio (NP=16) pointing towards nitrogen limited systems, and ratios above 16 indicate phosphorus limitation. Nutrient stoichiometry observed for Norwegian fjord systems (with N:Si:P ratios of 10:11:1 for Austevoll, 13:11:1 for Lysefjord, and 8:11:1 for Åfjord) indicate that these systems are nitrogen limited during extended periods of the year (Table 6.10) which confirmed previous studies for other Norwegian fjords (Paasche & Erga 1988). Nitrogen limitation is commonly observed in marine environments (Nixon et al. 1996). The assumption that phosphorus is generally sufficiently available in coastal waters (Nixon et al. 1996), seems not valid for some of the coastal waters used for shellfish cultivation, as the Waddensea (during springbloom), Lough Foyle and the

Northern Adriatic Sea have been reported to be phosphorus limited (Ferreira et al. 2007, Philippart et al. 2007, Brigolin et al. 2009).

NP ratios of regenerated nutrients observed from individual mussels and mussel communities are presented by broken and solid lines, respectively, in Figure 6.6. In most cases the stoichiometry of the regenerated nutrients (lines) varies from ambient stoichiometric characteristics (bars). Mussel communities at Austevoll released proportionally more nitrogen than individual mussels (Figure 6.6; Chapter 2 & 4), yet this cannot directly be linked to release rates of the AFOM complex as ascidians and decomposition of biodeposits show low NP ratios (Table 6.8; Chapter 3 & 5). Although relatively more nitrogen was released than phosphorus, the net release rates from communities did not exceed Redfield's ratio indicating that mussel activity can not diminish nitrogen limitation in the Austevoll area. NP ratios in the inorganic excreta of individual mussels in the Oosterschelde estuary were higher compared to ratios in the release from mussel beds (Prins & Smaal 1994). Low NP values were also observed for mussel beds in the Sylt area (Asmus et al. 1990). Removal of nitrogen through denitrification processes is generally suggested as a cause of the low net DIN release measured in mussel beds (Asmus et al. 1990, Prins & Smaal 1994). Phosphate release rates from sediments underneath bivalve farms have shown divergent results with positive fluxes measured in some cases (Baudinet et al. 1990, Souchu et al. 2001, Richard et al. 2007b) and neutral or negative fluxes in others (Hatcher et al. 1994, Mazouni et al. 1996, Giles & Pilditch 2006). Asmus et al. (1995) noted that differences in the phosphorus fluxes can be attributed to site specific environmental characteristics. Low or negative phosphate fluxes are often related to the buffering capacity of sediments caused by the absorption of phosphate by iron hydroxides or calcite present in oxidized surface layer of marine sediments (Sundby et al. 1992). This shows that phosphate release kinetics vary between different decomposition-sites. Benthic mineralized phosphate may be trapped in the sediment
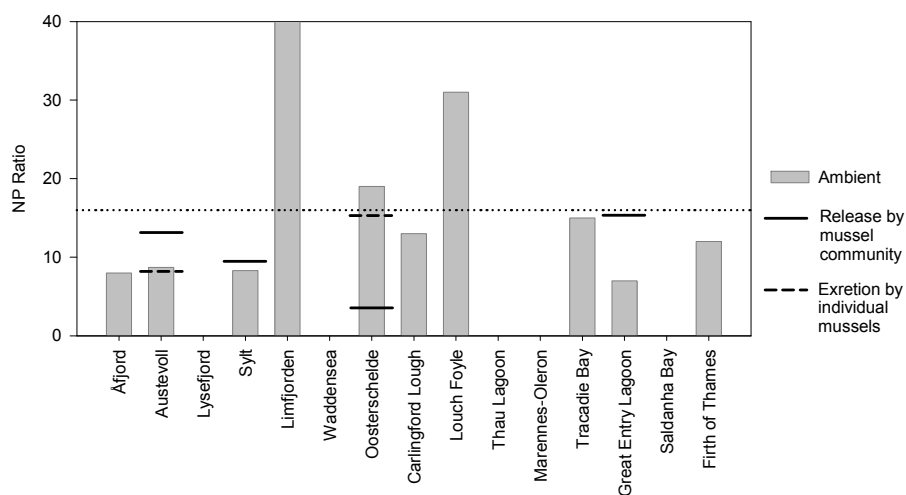


**Figure 6.6**
Annual stoichiometric NP [DIN/DIP] values across bivalve cultivation areas for the ambient water (bar diagram), and measured in release rates of individual mussels (broken line) and mussel communities (solid line). Horizontal dotted line indicates the Redfield ratio (NP=16). For references see tables 6.2 and 6.5.

while pelagic mineralized phosphate is available in the water column. Despite high silicate release rates from decomposing biodeposits during specific times of the year (Chapter 3), no silicate release was observed during the *in situ* measurements on suspended mussel ropes at Austevoll (Chapter 4). This indicates that all ingested silicon is transported to the deep fjord basin by biodeposition and does not become regenerated in the euphotic zone. The absence of silicate regeneration by suspended communities may theoretically lead to silicon removal from the system. In shallow estuaries biodeposit mineralization contributes to the pool of regenerated silicate (Asmus et al. 1990, Prins & Smaal 1994), which reduces the potential of silicate limitation in those areas.

Nutrient availability in the ambient water is a regulating factor in phytoplankton dynamics. It is generally accepted that phytoplankton require nutrients in fixed ratios according to Redfield's ratio (Redfield ratio 106C:16Si:16N:1P; Redfield et al. 1963). This ratio represents an average value though, and each phytoplankton species has specific requirements based on their internal chemical stoichiometry, which may vary from the Redfield ratio (Falkowski 2000). Diatoms require for example silicate while this element is not used by (dino)flagellates (Turner et al. 1998). Inorganic nutrients regenerated by mussel communities may deviate from Redfield's ratio or from the ambient water and can thereby affect phytoplankton growth (Goldman et al. 1979), or potentially lead to a shift in phytoplankton species composition. A mesocosm study by Prins et al. (1995) indeed showed that mussel beds can reduce phytoplankton biomass, enhance phytoplankton growth rates, and change phytoplankton community composition towards a system dominated by fast-growing diatoms. Since mussel communities in deep fjord systems do not regenerate silicate in the euphotic zone they theoretically have the potential to suppress the development of siliceous phytoplankton such as diatoms and favour development of non-siliceous phytoplankton such as flagellates and dinoflagellates in fjord systems.

The above showed the potential influence of bivalve nutrient regeneration on phytoplankton community composition, and is thus related to the positive feedback mechanism. The negative feedback of grazing bivalves diminishes phytoplankton stocks, but may also influence community composition of phytoplankton. Cranford et al. (2009) reported a shift towards a phytoplankton population dominated by picophytoplankton in bays with high bivalve densities cultivation activities.

*Significance at ecosystem scale*
The magnitude of bivalve suspension feeders as regulative mechanisms varies between coastal ecosystems (Dame & Prins 1998). The previous sections discussed the *potential* effects of mussel communities on nutrient cycling across coastal ecosystems, irrespective of mussel abundance or dimensions of the system. The current section addresses consequences of bivalve culture at the scale of entire systems.

In order to be able to evaluate system-wide interactions, estimates for the bivalve standing stock are an essential parameter. Table 6.13 presents data on the standing stock for the different cultivation areas, however, one should keep in mind that the majority of these values have a large uncertainty. Combining total standing stock estimates with dimension of the systems (see Table 6.9) provides areal and volume based biomass estimates. Biomass in the two Norwegian fjord systems was among the lowest ones reported. The Waddensea (NL) and several systems in

France are important mussel cultivation areas in terms of total harvest quantities (Table 6.13).

However, these systems are also characterized by co-culture or co-existence of several bivalve species (e.g. *Crasosstrea gigas* or *Ensis*). The current study focussed on eco-physiological responses specifically for mussels. For this reason systems where mussels comprise a minor proportion of total bivalve biomass were excluded while addressing mussel-ecosystem interactions.

Interactions were first evaluated by the total food uptake relative to the total food available (Figure 6.7a, Smaal & Prins 1993, Dame & Prins 1998), which can also be described as an indicator for the 'top-down control' or 'negative feedback mechanism'. Despite oligotrophic conditions in Åfjord and Lysefjord, clearance times (CT) were longer than water residence times (RT) and primary production times (PPT) indicating that mussel cultures do not influence

**Table 6.13**
Bivalve density across different culture areas. Density is expressed as harvest (ton WW $y^{-1}$), and in standing stock for the whole system (ton DW), per areal unit (g DW $m^{-2}$) and per volume (g DW $m^{-3}$). For the Norwegian fjords only the volume above the sill was used in the calculations. Asterisks (*) indicates that standing stock was reconstructed based on harvest, length of the production cycle and WW/DW conversion factors reported by Riccardi and Bourget (1998). Country codes are similar to table 6.3.

| Area | Country | Species | Culture type | Harvest (WW) [ton $y^{-1}$] | Standing stock (DW) [ton] | [g $m^{-2}$] | [g $m^{-3}$] | Ref |
|---|---|---|---|---|---|---|---|---|
| Lysefjord | NO | *M. edulis* | Rope | | 94 | 2.1 | 0.1 | 1 |
| Åfjord | NO | *M. edulis* | Rope | 1200 | 109* | 7.8 | 0.4 | 2 |
| Limfjorden | DEN | *M. edulis* | Bottom | 90,000 | 2509*' | 1.6 | 0.4 | 3 |
| | | *C. gigas* | | 580 | 6* | 0.0 | 0.0 | |
| Sylt | DEN | *M. edulis* | Bottom | | 189 | | 26.3 | 4 |
| Oosterschelde | NL | *M. edulis* | Bottom | 25,000 | 6061 | 17.3 | 2.2 | 5 |
| | | *C. giga* | Bottom | | 2424 | 2.4 | 0.3 | |
| | | cockles | Bottom | | 848 | 6.9 | 0.9 | |
| Wadden Sea | NL | *M. edulis* | Bottom + rope | | 5018 | 3.6 | 1.3 | 6 |
| | | *M. arenaria* | Natural | | 8419 | 6.0 | 2.1 | |
| | | *Ensis* | Natural | | 12880 | 9.1 | 3.2 | |
| | | Other bivalves | Natural | | 5799 | 4.1 | 1.4 | |
| Carlingford Lough | UK | *M. edulis* | Bottom + rope | 2500 | 209* | 4.3 | 0.5 | 7 |
| | | *C. gigas* | Trestles | 320 | 27* | 0.6 | 0.06 | |
| Belfast Lough | UK | *M. edulis* | Bottom | 15,318 | 1281* | | 1.7 | 8 |
| | | *C. gigas* | Trestles | 50 | 4* | | 0.006 | |
| Bay of Brest | FR | various | | | 13275 | 90 | 8.9 | 9 |
| Thau Lagoon | FR | *C gigas + M. edulis* | | 13,500 | | | | 10 |
| Marennes-Oleron | FR | *M. edulis* | | | 242 | | 0.4 | 11 |
| | | *C. gigas* | | | 2424 | | 3.6 | |
| | | Other bivalves | | | 788 | | 1.2 | |
| Ria de Arosa | SP | *M. galloprovincialis* | Raft | 172,500 | 4809* | 19.6 | 1.1 | 12 |
| Tracadie Bay | CA | *M. edulis* | Rope | 1943 | 261 | 15.9 | 6.4 | 13 |
| Great Entry Lagoon | CA | *M. edulis* | Rope | 180 | 15* | 0.5 | 0.1 | 14 |
| Saldanha Bay | SA | *M. galloprovincialis* | Rope | | | | | |
| Firth of Thames | NZ | *P. canaliculus* | Rope | 9000 | 251* | 0.2 | 0.02 | 15 |

*1 (Strohmeier et al. 2005; pers comm Strohmeier); 2 (pers. comm. M. Hoem & A. Koteng); 3 (Dolmer & Geitner 2004); 4 (in Smaal & Prins 1993); 5 (Smaal et al. 2001); 6 (Philippart et al. 2007, Schellekens et al. 2011); 7 & 8 (Ferreira et al. 2007); 9 (in Smaal & Prins 1993); 10 (Thouzeau et al. 2007); 11 (Smaal & Zurburg 1997); 12 (Figueiras et al. 2002); 13 (Cranford et al. 2007); 14 (Trottet et al. 2008a); 15 (Zeldis 2005)*

food dynamics in these fjord systems. This was different from many other systems where clearance times were shorter than residence times (CT/RT < 1). This confirmed studies by Smaal & Prins (1993) and Dame & Prins (1998) who reported that clearance times are shorter than the residence times for most bivalve cultivation areas. However, for most areas primary production is faster than bivalve feeding (CT/PPT>1) indicating that the food source is renewed faster than filtered. Limfjorden has longest residence times (almost one year), and a high mussel biomass which together resulted in high food uptake relative to residence times (CT/RT<<1) indicating that the system is potentially regulated by bivalve filtration. However, high nutrient loading in this system results in high primary production rates (Maar et al. 2010) which subsequently indicated that bivalves do no overgraze phytoplankton populations (CT/PPT>>1).

Secondly, bivalve-ecosystem interactions were evaluated by nitrogen (DIN) turnover time (Dame 1996) relative to the residence time (Figure 6.7b). Nitrogen turnover time (NTT) in this context refers to system turnover and should not be confused with tissue turnover times described in Chapter 2. This type of indicator can also be described as 'bottom-up control' or the 'positive feedback mechanism'. The total DIN pool in the ambient water was lowest in Åfjord, Lysefjord and the Firth of Thames, potentially leading to a high contribution of regenerated nutrients. However, bivalve density in these areas is low as well (<0.4 g DW m$^{-3}$) resulting in long nitrogen turnover times relative to water residence times (NTT/RT>40) indicating a low impact of bivalves on nutrient cycling. Low DIN concentrations reported for Great Entry Lagoon result in high relative impact on the inorganic nutrient pool (NTT/RT<1). However ambient values were based on the period June-October, thus excluding high winter values, which influences the relative importance as winter values are generally higher than during summer. Relative impact of regeneration processes (NTT) is most pronounced in the Oosterschelde and Tracadie Bay, indicating that bivalves may influence nutrient cycling although NTT/RT values did not drop below 1. These are shallow estuaries/bays with high mussel cultivation activity, which is also indicated by the high relative bivalve density (2-6 g m$^{-3}$, Table 6.12).

Åfjord is a successful mussel cultivation area in Norway due to a combination of good management practice and several environmental advantages; a river transports nutrient rich water from agricultural areas to the fjord and primary production rates are thereby (expected to be) enhanced, frequent pulses of brackish water in the upper water layers reduce the fouling on the mussel ropes, and toxic algal blooms are rare in Åfjord. Preliminary results furthermore indicate that upwelling events may occur in Åfjord (Aure unpublished data), thereby enhancing nutrient availability and thus primary production and bivalve food in the euphotic zone. At this moment bivalve densities in Åfjord are low compared to other bivalve cultivation areas (Table 6.12), resulting in low impact on both feedback mechanisms (Figure 6.7). The question is what happens when bivalve culture expands. Scaling to mussel densities similar to the Oosterschelde (2.2 g m$^{-3}$) or Tracadie Bay (6.4 g m$^{-3}$) demonstrated the significance of physical and environmental conditions of the systems in relation to magnitude of mussels as regulative mechanisms. Both positive and negative feedbacks (all >>1) had less impact in Åfjord than in the Oosterschelde under the same bivalve pressure, which is mostly related to the 8-times longer
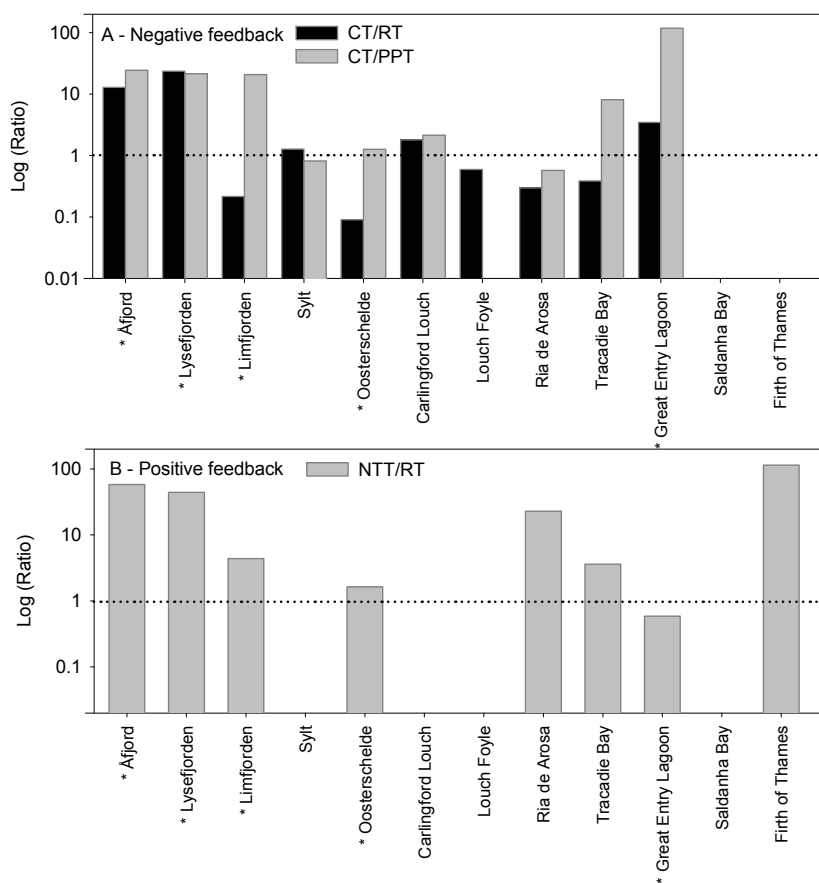
**Figure 6.7**

Mussel-ecosystem interactions expressed by negative and positive feedback indicators, calculated according to Dame & Prins (1998), Smaal & Prins (1993) and Dame (1996) based on the following parameters:

| | |
|---|---|
| *Residence time (RT)* | = *Time to exchange water body (see Table 6.1)* |
| *Clearance Time (CT)* | = *Time to filter the water body* |
| | = *(system volume) / (CR x bivalve biomass)* |
| *Primary production time (PPT)* | = *Time for phytoplankton to double ($B_P/P$)* |
| | = *($POC_{phytopl.}$ x volume system) / (Primary production x Area system)* |
| | *with the assumption: 40 $mgPOC_{phytopl}$ $mgChla^{-1}$* |
| *Nitrogen turnover time (NTT)* | = *Time to renew DIN* |
| | = *(DIN x system volume) / (DIN Release x Bivalve biomass)* |

The magnitude of bivalve populations as regulative mechanisms in ecosystem functioning is evaluated by the ratios between the parameters:

| | |
|---|---|
| CT/RT  >1 : no/minor regulation | CT/RT  <1 : system potentially regulated by bivalve filtration |
| CT/PPT >1 : no/minor regulation | CT/PPT <1 : phytoplankton is overgrazed |
| NTT/RT >1 : no/minor regulation | NTT/RT<1 : bivalves potentially influence nutrient cycling |

For references see Tables 6.3-6.6.10. Asterisk (*) indicates that community scale rates were applied.

residence times in the Oosterschelde estuary compared to the upper water layers in Åfjord (Table 6.9). Up-scaling to mussel densities in Tracadie Bay resulted in a theoretical impact of CT/RT=0.9 (below 1), CT/PPT=2.2 and NTT/RT=3.9 for Åfjord which differs from values reported for Tracadie Bay. Residence times of Tracadie Bay and Åfjord were in the same order (Table 6.9), thereby signifying the role of biological and environmental conditions of the system.

In Åfjord and Lysefjord both the direct impact on food source (negative feedback) and impact on inorganic nutrient pool (positive feedback) is low compared to most other bivalve cultivation areas. However, the results presented in Figures 6.7 provided integrated annual values, wherefore care must be taken in interpreting the findings as most of the parameters are dynamic and fluctuate over temporal scales. In Chapter 4 we demonstrated that at the scale of one mussel farm, contribution of mussels to the inorganic nutrient pool was insignificant during winter conditions but contributed substantially during summer conditions due to the combination of low nutrient concentrations (nutrient limitation) in the ambient water, high metabolic activity of the mussel population and high biomass of fouling organisms. Similar conclusions were drawn by Prins & Smaal (1994) who addressed the importance of seasonality in the contribution of bivalves to nutrient regeneration in the Oosterschelde. These authors demonstrated that mussel beds accounted for almost half of the total DIN regeneration of the system during summer (nutrient limiting) conditions. Yet, the conclusion that nitrogen regeneration by mussel farming has little effect on the DIN pool in Åfjord remains similar when applying ambient DIN concentrations and mussel excretion rates for summer conditions to the calculations for negative feedback (NTT/RT=9.0).

This scaling exercise on bivalve-ecosystem interactions (Figure 6.7) addressed some consequences of bivalves to ecosystem processes but was based on a static approach. However, marine systems are complex, with suspended organic material and inorganic nutrients being dynamic quantities subject to physical, biochemical and eco-physiological processes which fluctuate over both temporal and spatial scales. Simulation models are therefore an important approach in predicting dynamics of bivalve-ecosystem interactions (Grant & Filgueira 2011). One of the aspects restricting current modelling is the availability of empirical field data (FAO 2008, Fulton 2010). The extensive set of empirical data provided in the current thesis can be applied to calibrate, validate and optimize existing models.

## Conclusions

This thesis contributed to knowledge on bivalve nutrient cycling in general and specifically provided insight in the interactions between suspended mussel cultures and nutrient cycling under oligotrophic conditions.

*Physiological response under oligotrophic conditions*

Comparison of our data with a literature review of eco-physiological rates across cultivation areas indicated that clearance and biodeposition rates were related to food/nutrient availability and were respectively higher and lower under oligotrophic conditions. Amplitude and seasonal patterns in respiration, excretion of metabolic waste products ($NH_4$, $PO_4$) and nutrient composition of tissue material were comparable between cultivation areas and no specific response under oligotrophic conditions was observed.

*Individual versus community scale*

This thesis also indicated that eco-physiological rates measured *in situ* on mussel communities (= field conditions) may differ from rates measured for individuals and extrapolated to a similar population size. Clearances rates were lower at community scale, while respiration and TAN excretion were higher for communities during periods of high ascidian fouling. These results imply that lower negative feedback but higher positive feedback from mussel communities to phytoplankton may be expected than based on extrapolations of an "average" individual rate to a whole population. This highlights the need to consider community specific processes while evaluating bivalve-ecosystem interactions.

*Biodeposition & mineralization*

Mineralization experiments and budget analysis demonstrated the importance of biodeposit mineralization to nutrient cycling for all four nutrients (C-N-P-Si). Approximately 24% of carbon and 17% of the nitrogen in mussel biodeposits were mineralized. Temperatures enhanced biodeposit mineralization with approximately 2-3 times faster turnover at a 10°C temperature interval ($Q_{10}$). Mineralization rates varied 2-80 fold between seasons due to fluctuations in biodeposit nutrient content and water temperature.

*Stoichiometry*

This study underlined the importance to study interdependence and coherence of metabolic processes by a multiple-element approach in order to be able to accurately evaluate bivalve-ecosystem interactions. Turnover (tissue/excretion) of nitrogen was slower compared to phosphorus and carbon (N>P>C), indicating that more carbon was regenerated while nitrogen accumulated in tissue material. Mineralization experiments of mussel biodeposits specified the importance of silicate regeneration pathways (C>Si=N>P). Nutrients released by individual mussels and from mussel communities (C>N>P>>Si) were different from ratios observed in the ambient water as well as from Redfield ratio indicating that mussel cultures have the potential to influence phytoplankton community composition through imbalanced regeneration of the different elements.

*System feedbacks*

Approximately 50% of the ingested carbon and phosphorus, and 25% of nitrogen was allocated to nutrient regeneration (source) by mussel communities in oligotrophic fjord systems, and the

inverse can be considered as a loss (sink). The fraction of ingested nutrients divided between nutrient sinks and sources was comparable between systems, indicating that the potential role of mussels in nutrient cycling was relatively similar across systems. In deep fjord systems the pool of regenerated nutrients is only composed of inorganic nutrients excreted by mussels (metabolic waste products), while in shallow areas mineralization of biodeposits is also considered as a contributor to the source of regenerated nutrients.

As a consequence of low background nutrient levels in oligotrophic systems, mussel communities potentially have a larger impact on nutrient dynamics in oligotrophic than in eutrophic systems if corrected for bivalve biomass. Although quantifying nutrient dynamics at ecosystem (fjord) scale was beyond the scope of this thesis, a theoretical scaling exercise demonstrated that present mussel aquaculture in Norwegian fjord systems has low impact due to the low bivalve densities and physical characteristics of the fjords (large volume, short residence times of the upper water layer). Estimates for bivalve-ecosystem interactions were more profound in shallow areas with high mussel biomass, especially in terms of the negative feedback mechanisms (feeding). The significance of the positive feedback mechanism (nutrient regeneration) has a strong seasonal component; at the scale of one mussel farm it was demonstrated that the contribution to inorganic nutrient pools was insignificant during winter conditions (0.1% DIN) but contributed substantially during summer conditions (19% DIN) due to the combination of nutrient limitation in the ambient water, high metabolic activity of the mussel population and high biomass of fouling organisms.

*Implications*

This thesis has provided insights in the pathways in which mussels interact with nutrient cycling, with special reference to oligotrophic conditions, which can be applied to support sustainable exploitation and management of coastal zones. Furthermore, a set of empirical data was provided describing temporal dynamics in nutrient cycling by mussel communities for multiple-elements (C-N-P-Si), which can be applied to calibrate, validate and optimize existing models simulating bivalve-ecosystem interactions.

## Perspectives on bivalve nutrient cycling

Scientific insights in bivalve nutrient cycling can be applied to management advice on the exploitation of shellfish stocks, in integrated coastal zone-management, or for innovative developments in the coastal zone. In the current section a few applications of bivalve nutrient cycling are outlined.

*Carrying capacity*

The concept of 'carrying capacity' is relevant for analysis of bivalve-ecosystem interactions in relation to exploitation of shellfish stocks (Smaal & Heral 1998). Models simulating ecological carrying capacity (McKindsey et al. 2006) basically describe nutrient cycling and the various submodels are often linked together through nitrogen and/or carbon fluxes. Phosphorus, silicon and the interrelationships between the different elements are not (yet) included into present models. The information presented in the current thesis, can be used to optimize carrying capacity models and thereby contributes to efficient management and exploitation of shellfish stocks in coastal ecosystems. Generally carrying capacity models follow the order from individuals to farm and ecosystem scale (Guyondet et al. 2010). This study has also shown that there is a scale between individual and farm scale, namely the community scale, which is important to consider while evaluating nutrient dynamics in bivalve cultures (Chapter 4 & 5). Furthermore, quantification of biodeposit mineralization rates (Chapter 3) may fulfill the need for a more accurate representation of the effects of biodeposit mineralization on ecosystem productivity in the existing carrying capacity models (Henderson et al. 2001).

*Extractive aquaculture*

It has been suggested that bivalve cultivation may help to control the impacts of nutrient enrichment in eutrophic coastal waters by grazing, bivalve growth and subsequent removal of nutrients in the shellfish harvest. Approximately 5-30% of the ingested nutrients are allocated to mussel growth (Table 6.12). Lindal et al. (2005) proposed that shellfish aquaculture can be incorporated into a nutrient trading system as an alternative to nutrient (nitrogen) reduction for improving coastal water quality.

Integrated multi-trophic aquaculture (IMTA) also utilizes the extractive properties of bivalves. The IMTA approach is based on recycling of nutrient waste streams by different species, and aims to increase long term sustainability and profitability per cultivation unit, rather than per species in isolation as is done in monoculture (Neori et al. 2004). A well-known example of marine-IMTA is the combination of salmon farming with organic extractive shellfish (e.g. mussels) and inorganic extractive seaweeds (Neori et al. 2004, Chopin et al. 2005). This thesis presented new and elaborate information on allocation and cycling of nutrients by mussel communities, which may contribute to insights in the nutrient conversion efficiencies of bivalves in IMTA systems.

*Upwelling of nutrient rich water*

Opposite to eutrophic areas where coastal zone management aims at reducing the effects of excess nutrient input (top down control), attempts have been made to improve conditions for bivalve growth in Norwegian fjords by increasing the nutrient concentrations in the euphotic zone (bottom up control) (Aure et al. 2007a). This innovative project is based on a fjord scale experiment and relates to the concept of upwelling of nutrient rich water as occurs naturally in e.g. Ria de Arousa and Saldanha Bay leading to high bivalve production in these areas (see

section 'Physical and environmental characteristics of bivalve cultivation areas' in the current chapter). Controlled upwelling of nutrient rich water from the deeper water layers resulted in inorganic nutrient fluxes to the euphotic zone (450 kg N, 760 kg P and 75 kg Si per day in an influence area of 10 km$^2$) which tripled phytoplankton production in the influence area of upwelling (Aure et al. 2007b). It is hypothesized that the enhanced phytoplankton production (predominantly diatoms) can efficiently be exploited to mitigate the risks of mussel toxicity and increase growth of mussels. Total bivalve biomass will determine which fraction of the upwelled nutrients will be removed (harvest), retained (biodeposition) or regenerated (excretion) from the system. Appraising nutrient cycling by mussel communities (Table 6.12) indicates that approximately 30-50% of the ingested phytoplankton (organic nutrients) will be transported to the deep fjord basin through production of biodeposits and 10-30% of the upwelled nutrients can be removed by harvest. The remaining fraction (25-50%) will be regenerated in the upper water column and thereby stimulates total phytoplankton growth. However, these fractions might slightly shift towards a system where biodeposition is relatively more important as the upwelling alters the characteristics of (a section of) the fjord from oligotrophic to mesotrophic conditions.

*Global carbon cycling*

The role of bivalves in global carbon cycling receives increasing interest as a consequence of recent discussions on climate change and ocean acidification. Bivalve carbon cycling has been described throughout this thesis, and the fate of $CO_2$ production in relation to global carbon cycling is dependent on the features of the system. Chauvaud et al. (2003) indicated that clam *Potamocorbula amurensis* populations in San Francisco Bay were generators of atmospheric $CO_2$. They showed that $CO_2$ production through calcification during shell formation ($2HCO_3 + Ca = CaCO_3 + CO_2 + H_2O$) was approximately half of the $CO_2$ respiration in the clams. Total $CO_2$ production was double the carbon uptake through primary production in San Francisco Bay, resulting in an excess $CO_2$ production. As $p$CO$_2$ concentrations in San Francisco Bay were supersaturated, an (unknown) fraction of the clam regenerated $CO_2$ was transformed to $HCO_3$ and another fraction was released to the atmosphere. On the contrary, Tang et al. (2011) suggested that shellfish mariculture systems in China enhance the absorption of atmospheric $CO_2$ by coastal ecosystems. They reasoned that cultivation of shellfish results in carbon fixation through tissue growth and shell formation, which are both removed from the system during harvest. It should be mentioned though that the systems evaluated in this study combined both shellfish and seaweed production, and $CO_2$ production by shellfish was thus counteracted by uptake of $CO_2$ in seaweeds. The seaweeds were considered as efficient sinks for $CO_2$ (DIC) and reduced the $p$CO$_2$ concentration in the water column. Fjord systems in Norway are generally considered weak absorbers of atmospheric $CO_2$ (pers. comm. SR Erga), and it is concluded that $CO_2$ produced by mussel communities dissolves into the water column and is thus regenerated rather than released to the atmosphere. $CO_2$ production (respiration) by mussel stocks in Åfjord represented <1% of the primary production rates of the system.

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# Summary

English - Dutch - Norwegian

## Summary

Suspension feeding bivalves have the potential to influence ecosystem functioning at all trophic levels due to their role in coastal nutrient cycling. The major pathways of bivalve nutrient cycling are: (*i*) filtration of seston from the water column, (*ii*) nutrient storage and growth of somatic and reproductive tissue, (*iii*) respiration and excretion of inorganic metabolic waste products, and (*iv*) egestion and mineralization of biodeposits. Through these processes bivalves exert a negative feedback on phytoplankton populations (feeding), while simultaneously exert a positive feedback through regeneration of inorganic nutrients thereby stimulating phytoplankton production. The extent and effect of both feedback mechanisms are situation specific and determined by physical and environmental conditions of the area and culture type applied (bottom vs suspended).

There is a general need to understand more about bivalve-ecosystem interactions as a function of physical, chemical and biological properties of an area. The eco-physiology of bivalves has widely been studied for eutrophic conditions, whereas less is known about the specific rates and interactions under oligotrophic conditions. The objective of this thesis was to determine the role of bivalves in nutrient dynamics (C-N-P-Si) under oligotrophic conditions, using suspended culture of the blue mussel *Mytilus edulis* in Norwegian fjord systems as a case study. Different aspects of nutrient cycling were discussed in the consecutive chapters.

Allocation of the elements to different physiological processes in mussels (tissue growth, biodeposition, respiration and excretion) was determined in *chapter 2*. The aim of this study was to validate physiological rates for individual mussels under oligotrophic conditions and to evaluate specific interactions in oligotrophic fjord systems. The study covered an annual cycle, reflecting the effect of endogenous requirements and environmental factors. It was shown that changes in nutrient (C-N-P) content in tissue material were related to reproductive processes, while respiration (C) and excretion (N-P) were mostly correlated to food and temperature. No silicate excretion was observed. Biodeposition rates were correlated to fluctuations in food availability but not to temperature, which was similar to the feeding response of mussels under oligotrophic conditions. Nutrient tissue concentrations and excretion rates were in the same scale as reported for eutrophic conditions, while biodeposition rates were lower under oligotrophic conditions. Nutrient turnover (excretion/tissue content) showed a seasonal pattern with fast turnover in summer and slow turnover in winter. On an annual basis, nitrogen turnover was slower compared to phosphorus and carbon (N>P>C) indicating than more carbon was regenerated, while nitrogen accumulated in tissue material.

Although the importance of biodeposition and mineralization in nutrient cycling is recognized, little information is available on decomposition dynamics specific for mussel biodeposits. In *chapter 3* mineralization rates of decomposing mussel biodeposits were determined during different seasons with the aim to define the effect of temperature and biodeposit nutrient content on mineralization rates. Seasonal variations in mineralization rates were related to concentrations of macro-nutrients (C-N-P-Si) in the biodeposits, whereas mineralization rates standardized to biodeposit nutrient content suggested that the proportion of labile material in the biodeposits or availability of micronutrients also regulate mineralization processes. Temperature enhanced biodeposit mineralization with approximately 2-3 times faster turnover at a 10ºC temperature interval ($Q_{10}$).

A new method to measure *in situ* nutrient regeneration along suspended mussel communities (ropes) was developed of which the results are presented in *chapter 4*. The aim of this study was to quantify dynamics in community structure of suspended mussel ropes and to

determine nutrient regeneration of complete mussel cultivation units. The mussel ropes consisted of a complex community structure including the mussel matrix, associated fauna and organic material (also referred to as 'AFOM' complex), which all may contribute to nutrient uptake and release. The organic material was stable through time while the fauna compartment showed large temporal variation both in biomass as well as species composition. Nutrient regeneration varied significantly throughout the season and dissimilar regeneration rates for each of the elements were observed (N>P>Si).

In *chapter 5* individual and community scale estimates for clearance, respiration and nutrient excretion were compared with the aim to determine which part of the overall community estimates could be attributed to the physiological activity of the mussels and which part could be related to community specific processes. Largest differences were observed for clearance rates, with 2-4 times higher rates recorded for individuals compared to communities. In general there was a good agreement between respiration and nutrient release rates from individuals and from the community, except from periods when the ascidian *Ciona intestinalis* was highly abundant. During those periods respiration and nitrogen release rates of the community could almost double the rates expected from extrapolation of individual rates.

In the general discussion (*chapter 6*) a holistic view onto bivalve nutrient cycling in oligotrophic fjords was provided by comparing data presented in this thesis (oligotrophic conditions) with a literature review on bivalve nutrient cycling in cultivation areas around the world. This indicated that there is a physiological adaptation of mussels to oligotrophic conditions in terms of higher clearance rates and lower biodeposition rates. Allocation of nutrients towards tissue growth (content) and excretion of metabolic waste products was similar to mussels studied under more eutrophic conditions. The physical characteristics (depth) of the systems determine whether or not biodeposit mineralization also contributes to the source of recycled nutrients available for primary producers (= positive feedback). Combining eco-physiological rates and physical properties of cultivation areas indicated that potential nutrient sources & sinks were comparable between systems. An evaluation of positive and negative feedback mechanisms indicated that present mussel aquaculture in Norwegian fjord systems has low impact on nutrient cycling in fjord systems due to the low bivalve densities and physical characteristics (large volume, short residence times of upper water layer) of the fjords.

Concluding, this thesis has shown that:
- Community specific processes imply that extrapolation of individual rates to field conditions is not always sufficient for fully understanding the mussel-ecosystem interactions. Feeding rates under field conditions seemed lower while nutrient release rates were higher owing to the role of fauna and organic material associated with mussel communities.
- Mineralization of biodeposits plays an important role in bivalve nutrient cycling of C-N-P-Si.
- Relatively more carbon and phosphorus was regenerated while nitrogen accumulated in tissue material. Dissimilar regeneration rates for each of the elements indicated that mussel populations have the potential to influence phytoplankton community composition.
- Eco-physiological rates were higher (clearance), similar (tissue nutrient content, excretion), or lower (egestion) for mussels cultured under oligotrophic compared to eutrophic conditions.
- Despite the fact that fewer nutrients were allocated to biodeposition under oligotrophic conditions, potential nutrient sinks and sources were comparable between systems due to physical characteristics (depth) and its relation to biodeposit mineralization.
- Present mussel aquaculture in Norwegian fjords has little impact on positive and negative feedback mechanisms due to low bivalve densities and physical characteristics of the fjords.

## Samenvatting (Dutch)

Schelpdieren spelen een rol in nutriënten recycling waardoor zij ecologische processen in kustzones op verschillende trophische niveaus kunnen beinvloeden. De belangrijkste processen hierbij zijn: *(i)* filtratie van voedsel (fytoplankton — in het water zwevende eencelige algen), *(ii)* opslag van nutriënten voor groei en voortplanting, *(iii)* ademhaling en excretie van metabolische afvalproducten, en *(iv)* productie en mineralizatie van biodepositie (feces). Door filtratie oefenen schelpdieren druk uit op de fytoplankton populaties (negatief terugkoppelingsmechanisme), terwijl zij tegelijkertijd ook de groei van nieuw phytoplankton stimuleren door regeneratie van anorganische nutriënten (postief terugkoppelingsmechanisme). De omvang en effecten van beide terugkoppelings-mechanismen worden bepaald door fysische en biologische eigenschappen van het systeem, waardoor verschillen optreden tussen kweekgebieden en kweektechnieken (hang- versus bodemcultuur).

De eco-physiologie van schelpdieren is uitgebreid bestudeerd voor nutriënt-rijke (eutrofe) gebieden, terwijl minder bekend is over de physiologische aanpassingen onder nutriënt-arme (oligotrofe) omstandigheden. Het doel van dit proefschrift is om de rol van schelpdieren in kringlopen van koolstof (C), stikstof (N), fosfor (P), en silicaat (Si) te bepalen voor oligotrofe omstandigheden. Hierbij is de hangcultuur van mosselen (*Mytilus edulis*) in Noorse fjorden gebruikt als een case studie. Verschillende aspecten van interacties tussen mosselen en nutriënten kringlopen zijn behandeld in de opeenvolgende hoofdstukken van dit proefschrift en worden hieronder samengevat.

De seizoenale patronen in verschillende physiologische processen (groei, biodepositie, ademhaling en excretie van anorganische nutriënten) zijn beschreven in *hoofdstuk 2*. Het doel van deze studie was om deze physiologische processen te kwantificeren voor oligotrofe omstandigheden. De seizoensdynamiek in de physiologische processen is enerzijds gerelateerd aan externe factoren zoals fluctuaties in water temperatuur en voedsel aanwezigheid, en anderzijds aan de nutritionele eisen in de jaarlijkse voortplantingscycus. De omzetting van nutriënten (excretie/weefsel concentratie) was het snelst in de zomer en uit de jaarlijksegemiddelden kon worden geconcludeerd dat de omzetting van stikstof langzamer was dan fosfor en koolstof (N>P>C). Dit heeft als gevolg dat relatief meer stikstof wordt opgeslagen in weefsel (mosselvlees) terwijl meer fosfor en koolstof teruggeleverd worden aan de waterkolom in een anorganische vorm.

Er is weinig bekend over de afbraak- en mineralizatiesnelheden van mossel feces, ondanks dat feces een belangrijke rol speelt in de nutriënten kringloop van schelpdieren. In *hoofdstuk 3* zijn minerlizatiesnelheden bepaald gedurende drie seizoenen met als doel het effect van temparatuur en feces kwaliteit (nutriënten concentraties) op mineralizatie-snelheden te bepalen. Ongeveer 24% van het koolstof en 17% van het stikstof aanwezig in de feces werd afgebroken en omgezet in een anorganische vorm (mineralizatie). De gemeten seizoenale verschillen konden inderdaad worden gerelateerd aan feces kwaliteit, maar de resultaten suggereerden ook dat het aandeel labiel (makkelijk verteerbaar) materiaal en aan de aanwezigheid van micro-nutriënten een rol speelde in de regulatie van feces afbraak. Feces afbraak was 2 tot 3 keer sneller bij een temperatuurstijging van 10ºC ($Q_{10}$=2-3).

Een nieuwe methode is ontwikkeld om *in situ* (in het veld) metingen van nutriënten excretie uit te voeren aan complete mossel touwen (populatie). De resultaten hiervan worden beschreven in *hoofdstuk 4*. De touwen bestonden uit een complexe gemeenschap waaronder de mossel matrix, fauna en organische material, welke allen bij kunnen dragen aan nutriënten opname en excretie. De fractie organische material aanwezig op de touwen was constant, terwijl fauna grote

seizoenale verschillen vertoonde in zowel biomassa als soorten diversiteit. Nutriënten regeneratie (excretie) van de touwen liet significante seizoenale verschillen zien en tevens werd een ongelijke regeneratie van de verschillende nutriënten (N>P>Si) waargenomen. Doordat groei en samenstelling van fytoplankton populaties afhankelijk is van de aanwezigheid van de verschillende anorganische nutriënten, suggereert een ongelijke regeneratie door mussel populaties dat deze de mogelijkheid hebben om soorten samenstelling van fytoplankton te beinvloeden.

In *hoofdstuk 5* werden de individuele en populatie processen met elkaar vergeleken met betrekking tot filtratie, respiratie en excretie. Het doel hiervan was om te bepalen welk deel van de populatieschattingen konden worden gerelateerd aan de physiologische activiteit van individuele mosselen en welk deel kon worden toegeschreven aan processen specifiek voor de gehele populatie. Grootste verschillen werden gevonden voor filtratie snelheden, met hogere waarden voor individuen in vergelijking met de populatie schattingen. Over het algemeen was er een goede overeenkomst tussen respiratie en excretie waardes van individuen en populaties, behalve voor de periodes wanneer de zakpijp *Ciona intestinalis* dominant aanwezig was op de touwen. Tijdens deze periodes was de respiratie en excretie van de populatie bijna dubbel de waarde die verwacht kon worden op basis van extrapolatie van metingen aan individuele mosselen.

In de algemene discussie *(hoofdstuk 6)* wordt een bredere kijk gegeven op de rol van schelpdieren in nutriënten recycling in oligotrofe fjorden. Hierbij werden gegevens uit dit proefschrift (oligtrofe omstandigheden) vergeleken met kweekgebieden uit de hele wereld. Dit liet zien dat individuele mosselen hogere filtratiesnelheden en lagere feces productie realiseren onder oligotrofe omstandigheden. De opslag van nutriënten in het mosselvlees en de excretie van anorganische afvalproducten was echter gelijk voor oligotrofe en eutrofe omstandigheden. De fysische eigenschappen (diepte) van de systemen bepaalt of feces mineralizatie al dan niet bijdraagt aan de totale hoeveelheid geregenereerde anorganische nutriënten die beschikbaar komen voor fytoplankton (het positieve terugkoppelings-mechanisme). Het combineren van eco-physiologische processen en fysische eigenschappen van kweekgebieden liet zien dat potentiele nutriënten 'sinks & sources' vergelijkbaar waren tussen verschillende gebieden. Dit impliceert dat ondanks dat de processen verschillen, mosselen toch een vergelijkbare rol in nutrienten recycling hebben in verschillende kweekgebieden. Schatting van de positieve en negatieve terugkoppelings-mechanismen gaf aan dat de huidige mosselkweek in Noorse fjorden een geringe invloed heeft op nutriënten kringlopen door de combinatie van lage mossel dichthedenen en fysische eigenschappen (groot volume en korte water verblijftijden) van de fjorden.

Dit proefschrift leidt tot de volgende conclusies:
- Eco-physiologische processen waren hoger (filtratie), gelijk (nutriënten concentraties in mosselvlees, excretie) of lager (biodepositie) voor mosselen in oligotrofe gebieden in vergelijking met meer eutrofe omstandigheden
- Extrapolatie van processen gemeten voor individuele mosselen leidt niet altijd tot relevenate waarden voor mossel populaties in het veld. Filtratiesnelheden bleken lager voor populaties en excretie van populaties was hoger in periodes wanneer er veel fauna op de touwen aanwezig was.

- Afbraak van feces speelt een belangrijke rol in de nutriënten kringloop van schelpdieren voor alle elementen (C-N-P-Si). Mineralizatie snelheden waren sterk afhankelijk van feces kwaliteit (en dus van het aanwezige voedsel) en temperatuur ($Q_{10}$=2-3).
- Realtief meer stikstof werd opgeslagen in mosselvlees terwijl meer fosfor en koolstof teruggeleverd werden in een anorganische vorm. Ongelijke regeneratie van de verschillende nutriënten suggereert dat mossel populaties de mogelijkheid hebben om de soorten samenstelling van fytoplankton te beinvloeden.
- Ondanks dat minder van de opgenomen nutriënten in biodepositie terrecht kwamen in oligotrofe systemen bleek dat de potentiele 'sinks & sources' vergelijkbaar waren tussen verschillende kweekgebieden. Mosselen in de verschillende kweekgebieden hebben hierdoor toch een vergelijkbare rol in nutriënten recycling.
- Huidige mosselkweek in Noorse fjorden heeft weinig invloed op de positieve en negatieve terugkoppelingsmechanismen door een combinatie van lage schelpdierdichtheden en fysische eigenschappen van de fjorden.

## Sammendrag (Norwegian)

Skjell som filtrerer og omsetter fødepartikler fra sjøvann kan påvirke økologisk funksjon på ulike trofioske nivå  har derfor mulighet til å innvirke på økologiske prosesser i kystsonen. De viktigste prosessene er: (i) filtrering av mat (planteplankton), (ii) lagring av næringsstoffer for vekst og reproduksjon, (iii) respirasjon og ekskresjon av metabolske avfallsprodukter, og (iv) produksjon og mineralisering av partikulær avføring (faeces). Skjell har dermed innvirkning på planteplankton populasjoner (negativ feedback mekanisme), mens de på samme tid stimulerer vekst av ny planteplankton ved regenerering av uorganiske næringsstoffer (postiv feedback mekanisme). Omfanget og effekter av feedback mekanismer er avhengig av fysiske og biologiske egenskapene av systemet og varierer mellom områder og dyrkingsmetoder (suspensjon- versus bunn kultur).

Fysiologi hos skjell er grundig studert for næringsrike (eutrofe) områder, mens mindre er kjent om de fysiologiske tilpasninger til næringsfattige (oligotrofe) forhold. Målsettingen for denne avhandlingen var å bedre forstå skjellenes rolle for dynamikken i omsetning av næringsstoffer (karbon (C), nitrogen (N), fosfor (P) og silikat (Si)) under oligotrofe forhold. Studiet ble gjort for dyrking av blåskjell (*Mytilus edulis*) i hengende kulturer i norske fjorder. Flere aspekter av interaksjonene mellom blåskjell og dynamikken i omsetning av næringsstoffer er diskutert i påfølgende kapitlene og blir oppsummert nedenfor.

Den sesongmessige dynamikken i ulike fysiologiske prosesser (vekst, avføring, respirasjon og ekskresjon av uorganiske næringsstoffer) er beskrevet i *kapittel 2*. Hensikten med denne studien var å kvantifisere de fysiologiske prosessene under oligotrofe forhold. Den sesongmessige dynamikken i fysiologiske prosesser var både relatert til eksterne faktorer som endringer i vanntemperatur og matkonsentrasjon, og til de ernæringsmessige kravene i den årlige reproduksjonsyklusen. Konverteringen av næringsstoffer (ekskresjon / bløtdel konsentrasjon) var raskest om sommeren og fra et årlig gjennomsnitt kunne det konkluderes at nitrogen transformasjon var saktere enn for fosfor og karbon (N>P>C). Det resulterer i at relativt mer nitrogen ble lagret i bløtdelene, mens fosfor og karbon ble regenerert i uorganisk form.

Lite er kjent om nedbrytning og mineralisering av blåskjell avføring, til tross for at avføring spiller en viktig rolle i omsetning av næring hos skjell. I *kapittel 3* var mineralisering målt over tre sesonger med formål å bestemme effekten av temparatur og avføringens kvalitet (næringsstoff konsentrasjoner) på mineralisering. Omtrent 24% av karbon og 17% av nitrogen i avføringen ble nedbrutt og frigitt i uorganisk form (mineralisering). De sesongmessige forskjeller var relatert til avføringens kvalitet, men resultatene indikerer også at andelen labilt (lett fordøyd) materiale og mikronæringsstoffer spilte en rolle i reguleringen av nedbrytning av avføringen. Mineralisering av avføringen var cirka 2-3 ganger raskere ved temperaturøkning på 10 °C ($Q_{10}$ = 2-3).

En ny metode ble utviklet for å gjøre *in-situ* målinger av ekskresjon fra en blåskjell kultur (populasjon) festet på et hengende tau (anlegg skala). Resultatene er beskrevet i *kapittel 4*. Tauene var et komplekst samfunn som inkluderte blant annet blåskjell, fauna, flora og organisk materiale, som alle kan bidra til næringsopptak og utskillelse. Fraksjonen av organisk materiale på tauene var konstant, mens store sesongmessige forskjeller ble observert i fauna biomasse og artsmangfold. Regenerering av næringsstoffer fra kulturene viste betydelige sesongmessige forskjeller, og det ble observert forskjeller i regenerering for de ulike næringsstoffer (N>P>Si). Vekst og sammensetning av planteplankton populasjoner er avhengig av uorganiske næringsstoffer, og ujevn regenerering av næringhsstoffer i blåskjell har derfor evnen til å påvirke planteplankton artssammensetning.

I *kapittel 5*, er målinger av prosesser på individ og populasjon (kultur) sammenlignet med hensyn til filtrering, respirasjon og ekskresjon. Målet var å fastslå hvor stor andel av estimatene for populasjonen som kan være relatert til den fysiologiske aktiviteten til individuelle blåskjell og hvilken del kunne tilskrives prosesser som er spesifikke for hele populasjon. Størst forskjell ble funnet for filtrering, med høyere verdier til individuelle blåskjell i sammenligning med populasjon. Generelt var det god overensstemmelse mellom verdier for individer og populasjoner for målinger av respirasjon og ekskresjon, bortsett fra perioder når mange ascidian *Ciona intestinalis* var på tauene. I løpet av disse periodene, var respirasjon og ekskresjon i populasjonene nesten dobbelt fra verdiene som kunne forventes basert på ekstrapolering av målinger for individuelle blåskjell.

I den Generelle Diskusjonen (*kapittel 6*) er det gitt et bredt perspektiv på rollen for skjell i næringsstoff resirkulering i oligotrofe fjorder. Mine data fra oligtrofe forhold ble samenlignet med områder med skjelldyrking i andre deler av verden. Dette viste at filtrering fra individuelle blåskjellene var høyere og produksjon av avføring var lavere under oligotrofe forhold. Lagring av næringsstoffer i blåskjellenes bløtdeler og ekskresjon av uorganiske avfallprodukter var like for eutrofe og oligotrofe forhold. De fysiske egenskaper (dybde) av systemet avgjør om avføringens mineralisering bidrar til den totale regenerering av uorganiske næringsstoffer som blir tilgjengelig for planteplankton (den positive feedback mekanisme). Kombinasjon av øko-fysiologiske prosesser med fysiske egenskaper for områdene viste at potensiell næringsstoff 'sink & source' var sammenlignbare mellom områder. Dette innebærer at selv om prosessene er forskjellig, vil blåskjell fremdeles ha en tilsvarende rolle i næringsstoff sykluser i forskjellige områder for skjelldyrking. Beregning av positive og negative feedback mekanismer indikerer at det nåværende nivå av blåskjelldyrking i norske fjorder har liten påvirkning på næringsstoff sykluser på grunn av kombinasjonen av lave blåskjell tettheter og fysiske egenskaper (stort volum og kort oppholdstid for vann) i fjordene.

Denne avhandlingen fører til følgende konklusjoner:
- Øko-fysiologiske prosesser var høyere (filtrering), like (næringsstoff konsentrasjoner i blåskjell bløtdeler, ekskresjon), eller lavere (avføring) for blåskjell i oligotrofe områder sammenlignet med mer eutrofe forhold.
- Ekstrapolering av prosesser målt for individuelle blåskjell representerer ikke alltid relevante verdier for blåskjell populasjoner i felt. Filtrering var lavere for populasjoner, og ekskresjon fra populasjoner var høyere i perioder når mye fauna var på tauene.
- Produksjon og minerlisering av avføring i skjelldyrking spiller en viktig rolle i næringsstoff regenerering for alle næringsstoffer (CNP-Si). Mineraliserings hastighet var sterkt avhengig av avføringens kvalitet (og dermed tilgjengelighet) og temperatur ($Q_{10}$ = 2- 3).
- Relativt mer nitrogen ble lagret i blåskjellenes bløtdeler mens mer fosfor og karbon ble returnert til vannmasse i en uorganisk form. Forskjell i regenerering av ulike næringsstoffer antyder at blåskjell i kultur har mulighet til å påvirke artssammensetningen av planteplankton.
- Selv om mindre næringsstoffer fra blåskjell ble tilført gjennom avføring i oligotrofe systemer, var potensielle 'sinks & sources' sammenlignbare mellom områder. Blåskjell i forskellige dyrkningsområder har derfor fremdeles lignende rolle i næringsresirkulering.
- Nåværende blåskjelldyrking i norske fjorder har liten påvirkning på de positive og negative feedack mekanismer på grunn av en kombinasjon av lave blåskjell tettheter og fysiske egenskapene til fjordene.

# Curriculum Vitae

# List of Publications

# Training & Supervision Plan

## Curriculum vitae

Henrice Maria Jansen was born on the 9th of January 1981 in Egmond-Binnen, The Netherlands. She completed her pre-University education at Jac. P. Thijsse College in 1999, and continued with a MSc in Animal Sciences at Wageningen University (The Netherlands). During the MSc, specializations in both 'Animal Production Systems' and 'Aquaculture & Fisheries' were completed. Internships were performed at the University of Can Tho (Vietnam) and University of New Brunswick (Canada) where sustainability issues in integrated multi-trophic aquaculture (IMTA) were studied. A first MSc-thesis, evaluating the effects of hydropower and fisheries on the migration of eel, was performed at IMARES. The second MSc-thesis provided policy recommendations for the ministry of Agriculture for development of a sustainable aquaculture sector in the Netherlands. Henrice graduated in 2005, and started the same year in a research position at IMARES. She worked as scientist and project leader on a variety of projects in the field of aquaculture and aquatic ecology, until the opportunity to start a PhD project came up in 2008. The PhD study has been carried in Norway in cooperation with the Institute of Marine Research (IMR — Norway), Institute for Marine Resources and Ecosystem Studies (IMARES — The Netherlands) and department of Aquaculture and Fisheries at Wageningen University. The current thesis is the result of this PhD project. From January 2012 Henrice will continue her scientific carreer at the aquaculture department at IMARES.

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Jansen, H.M., 2007. Changes in the commercial fish stocks of lake IJsselmeer: description, causes and predictions for the future. *Visserijnieuws*. (*in Dutch*)

Jansen, H.M., 2004. Towards a sustainable aquaculture; policy recommendations for development of sustainable aquaculture in the Netherlands. *AQUAcultuur* 19 (6), 9-13. (*in Dutch*)

**Training & Supervision Plan**

| *The Basic Package* | *Year* | *ECTS* [*] |
|---|---|---|
| WIAS Introduction Course | 2011 | 1.5 |
| Course on philosophy of science and/or ethics | 2010 | 1.5 |

*Scientific Exposure*

| International conferences | | |
|---|---|---|
| Physiomar conference, Brest, France | 2008 | 1.2 |
| Aquaculture Europe, Trondheim, Norway | 2009 | 1.2 |
| Aquaculture Europe, Porto, Portugal | 2010 | 1.5 |
| World Aquaculture, Natal, Brazil | 2011 | 1.2 |

| Seminars and workshops | | |
|---|---|---|
| Carrying capacity workshop, Bergen/Trondheim, Norway | 2008 | 0.6 |
| Conference on the exploitation of the sea, Trondheim, Norway | 2010 | 0.9 |
| Workshop on seed mussel collectors (SMC), Moermond, The Netherlands | 2010 | 0.4 |
| Conference on Sustainable Fisheries and Aquaculture, Bergen, Norway | 2010 | 0.4 |
| Workshop on modeling IMTA, Bodø, Norway | 2010 | 0.4 |
| Shellfish conference, Bergen, Norway | 2010 | 0.3 |

| Presentations | | |
|---|---|---|
| Oral at the Workshop on carrying capacity | 2008 | 1.0 |
| Poster at the Int. Conf. Physiomar | 2008 | 1.0 |
| Oral at Int. Conf. Aquaculture Europe | 2009 | 1.0 |
| Oral at the Workshop on modelling IMTA | 2010 | 1.0 |
| Oral & Poster at the Int. Conf. Aquaculture Europe | 2010 | 2.0 |
| Poster at the Int. Symp. on International Coastal Zone Management | 2011 | 1.0 |
| Oral at the Int. Conf. World Aquaculture | 2011 | 1.0 |

| *In-Depth Studies* | | |
|---|---|---|
| Aquatic Microbial and Molecular Ecology (PhD summer school), University of Southern Denmark, Odense, Denmark | 2010 | 10.0 |
| Experimental design and data analysis for Biologists, Tjarnø, Sweden | 2008 | 4.0 |

| *Professional Skills Support Courses* | | |
|---|---|---|
| Writing for Academic Publication | 2007 | 3.0 |
| Course on 'How to write a competitive EU Framework-7 proposal' | 2011 | 0.3 |

| *Research Skills Training* | | |
|---|---|---|
| Preparing PhD research proposal | 2008 | 6.0 |

| *Didactic Skills Training* | | |
|---|---|---|
| Supervising MSc thesis (Wageningen University - WUR) | 2009 | 1.5 |
| Supervising BSc thesis (Bergen University - UiB) | 2008 | 1.0 |
| **Total** | | **44** |

[*] *One ECTS represents a load of 28 hours*

**Colophon**