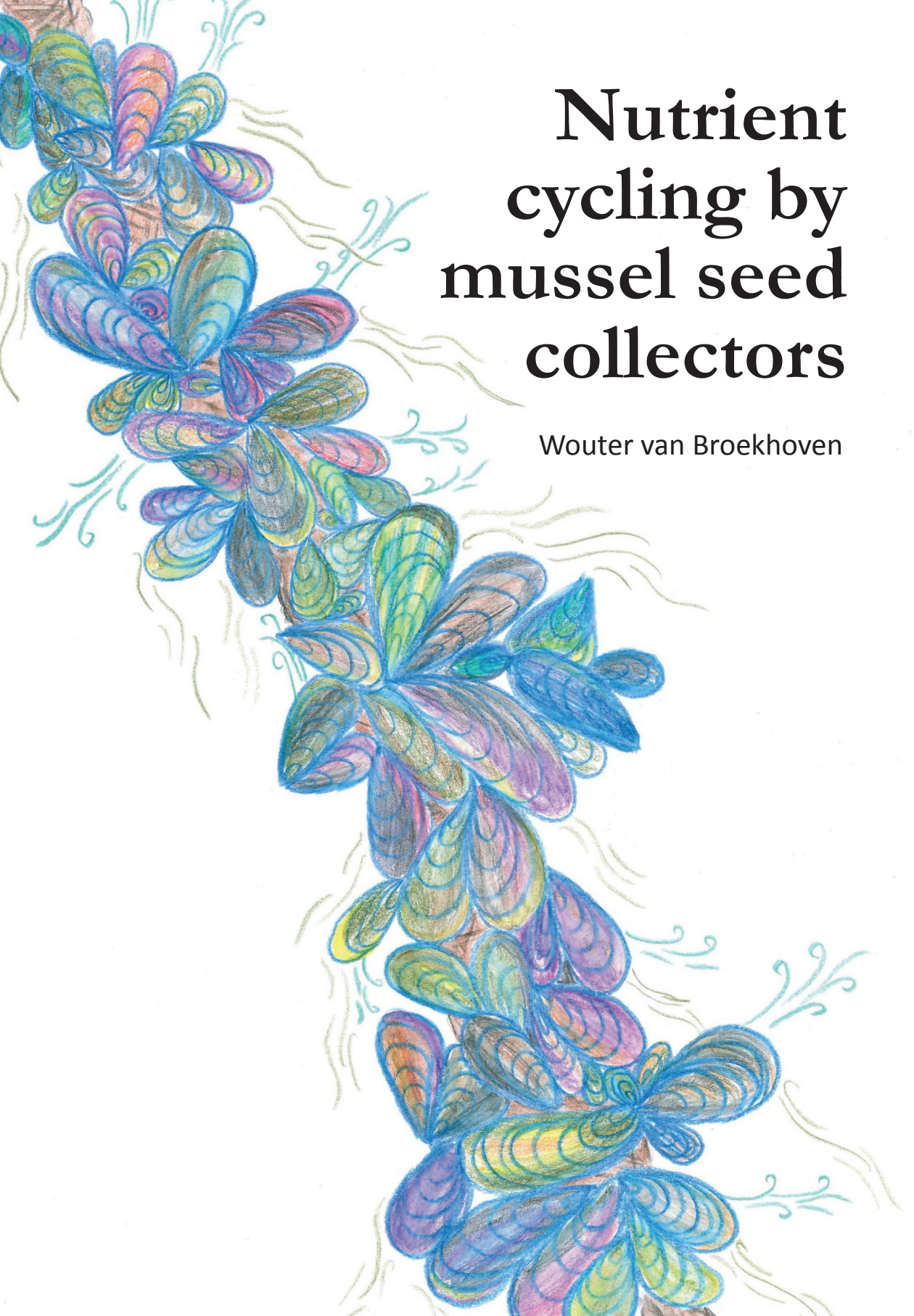
Nutrient cycling by mussel seed collectors

Wouter van Broekhoven



Propositions

1. Determining nutrient limitation status for phytoplankton is crucial for evaluation of ecological impacts of bivalve aquaculture. (this thesis)

2. At the farm scale, seed mussel cultivation in the Oosterschelde bay can be intensified without loss of productivity. (this thesis)

3. Existing espresso making technology is insufficiently capable of controlling the critical parameters required to reliably achieve optimal extraction.

4. Fact and reference checking are insufficiently incentivized in science, limiting the rate of scientific progress.

5. Mental focus is better achieved by removing distractions than by directing attention to a subject.

6. To measure is not to know, but to reduce the error in one's approximation of a quantity.

Propositions belonging to the thesis, entitled

Nutrient cycling by mussel seed collectors

Wouter van Broekhoven
Wageningen, 11 October, 2024

Nutrient cycling by mussel seed collectors

Wouter van Broekhoven

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Nutrient cycling by mussel seed collectors

Wouter van Broekhoven

Thesis

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Carla van Broekhoven

CHAPTER 1

General introduction

Wouter van Broekhoven

1.1. Seed provisioning for bivalve aquaculture

1.1.1. The role of seed collection in bivalve aquaculture

Bivalve molluscs have been consumed by humans for over 100,000 years (Walter et al. 2000, Marean et al. 2007). In the present day, the vast majority of production is aquaculture based. The aquaculture production of molluscs, mostly bivalves, reached 17.7 million tons in 2020, representing 20% of global marine and coastal aquaculture animal production worldwide (FAO 2022). Globally, farming of bivalves is still heavily reliant on supply of seed from wild fisheries. These fisheries are increasingly under pressure because of a growing concern about potential negative effects on the natural environment and conservation goals (Kaiser et al. 1998, Jeffs et al. 1999, Van Hoof 2012, Piñeiro-Corbeira et al. 2018). In the quest for alternative sources of seed, two main developments can be discerned: hatchery production and seed collection on artificial substrates. Although in various locations hatchery production has advanced technologically to such an extent that it can deliver a sufficiently large supply, this is still associated with a significantly higher financial production cost than both wild caught seed and seed collector seed (Kamermans et al. 2013). On the other hand, seed collector systems consisting of artificial substrate are successfully used in mussel aquaculture sectors such as the French bouchot culture, and raft and longline culture around the globe (Kamermans & Capelle 2019). Seed production on seed collectors avoids both the environmental concerns associated with wild seed fishery and the often prohibitive cost of hatchery production.

1.1.2. Seed collection in the Dutch mussel industry

In the blue mussel (*Mytilus edulis*) aquaculture industry in the Netherlands, following exploratory investigations in the early 2000s (Kamermans et al. 2002), a stepwise transition has been under way since 2009. During this transition suspended seed collectors, known as Seed Mussel Collector (SMC) systems, are meant to gradually replace wild seed fishery. This transition is driven by a covenant between the mussel farming industry, government and NGOs. By the year 2022, 50% of the wild seed fishery was replaced by SMCs. SMCs are predominantly deployed in the two main Dutch mussel growing areas: the Wadden Sea and the Oosterschelde bay (Figure 1.1). SMCs carry a 5 to 6 times higher financial cost of production (Van Oostenbrugge et al. 2018), but have been found to constitute a much more stable seed provisioning resource than the wild fishery which depends on often erratic patterns of natural recruitment (Smaal et al. 2021). In the Netherlands, SMCs essentially consist of artificial substrate in the form of ropes or nets placed in the water column around the end of spring prior to the onset of larval settlement, and harvested at the end of summer (Figure 1.2). The seed is then relayed onto bottom culture plots for growing out to commercial size. Since natural spatfall on the bottom of the Oosterschelde bay is very limited (Van Stralen & Dijkema 1994; Troost 2024, pers.comm.), historically the vast majority of seed mussels (if not all) was imported from Wadden Sea fisheries on wild

beds. As part of the transition, seed is now also collected on SMCs in the Oosterschelde bay itself, resulting in a much higher proportion of seed to adult mussels in the system than before. Moreover, the post-seeding mortality rate of 92% (Capelle et al. 2016) means that the production of seed needs to be proportionally high.

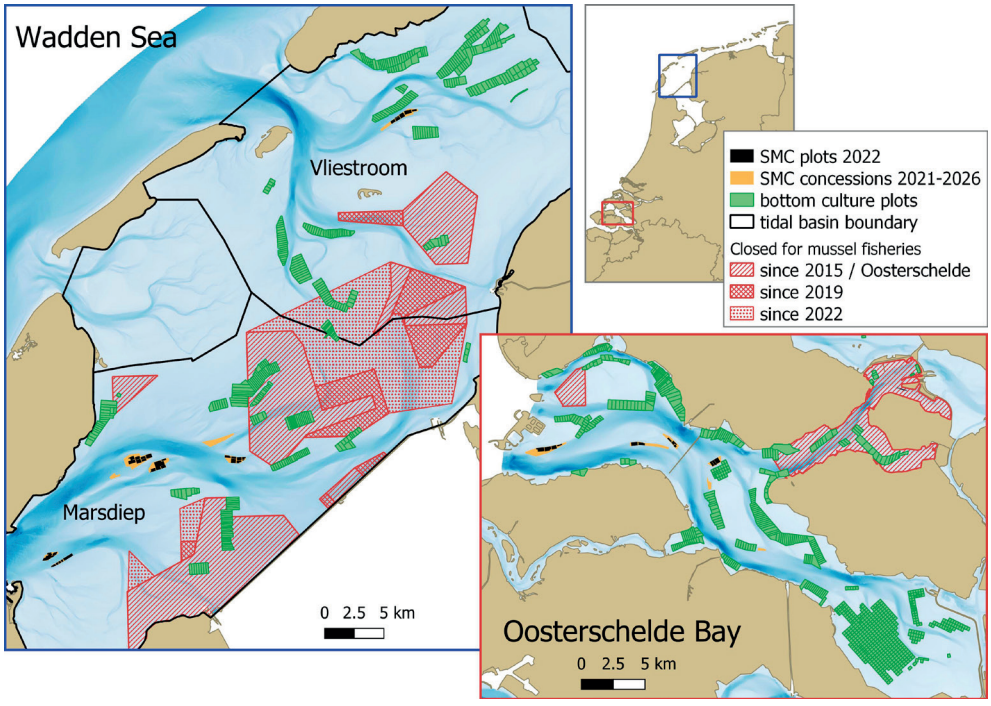

Figure 1.1. The two main mussel growing regions in the Netherlands, the Wadden Sea and the Oosterschelde bay. For SMCs, concession sites and actual use ("SMC plots 2022") are shown. In addition, bottom culture plots for growing mussels to commercial size are shown. The closure of area for bottom fishery of mussel seed is shown by year of closure.

Figure 1.2. Two types of SMC systems currently in use in the Netherlands. Top panel: longline system (photo courtesy Karin Troost). Continuous culture ropes are suspended in loops from a main line. The main lines are held at the water surface by a series of floaters. In the foreground, one mainline is lifted out of the water, exposing the culture rope for inspection. In the background, anchor poles to which the main lines are tethered are visible. Bottom panel: net system (photo courtesy Jacob Capelle). A net is suspended from a floating tube. When not in use, the nets can be bound up to the tube and the system can be left in the water, preventing biological fouling. The tube in the foreground is lifted out of the water exposing the netting for inspection.

1.2. Bivalve seed collection and nutrient cycling

1.2.1. The role of bivalve aquaculture in nutrient cycling

Bivalve suspension feeders exercise top-down (filtration) and bottom-up (nutrient regeneration) control over phytoplankton populations (Newell 2004). Top-down control results from the capacity of bivalve filter feeders to clear large volumes of water from particles including phytoplankton (Cranford et al. 2011). Bottom-up control results from the regeneration of dissolved nutrients which are then accessible for phytoplankton. The two pathways of nutrient regeneration are i) direct excretion due to metabolic activity of the animals, and ii) mineralization of their biodeposits: feces, the waste product egested after internal digestion; and pseudofeces, the part of the filtered material that is rejected before ingestion (Prins et al. 1998). When phytoplankton becomes depleted, top-down filtration control can constitute a negative feedback onto growth rates of the bivalve suspension feeders causing the depletion. In this case the carrying capacity of the system for suspension feeding populations in the system is reduced (Prins et al. 1998, Dame 2012). On the other hand, bottom-up nutrient regeneration control can constitute a positive feedback when phytoplankton productivity is stimulated and promotes growth rates of the bivalve suspension feeders releasing the nutrients (Prins et al. 1995, 1998). The extent of ecological impacts varies with the intensity of production and the characteristics of the ecosystem (reviews by Prins et al. 1998, Newell 2004). Besides enhancing the availability of nutrients for primary producers, bivalve suspension feeders can return nutrients in different ratios than present in the environment (e.g. Jansen et al. 2011) with potential implications for phytoplankton composition (Turner et al. 1998, Philippart et al. 2000, Cloern 2001). These top-down and bottom-up mechanisms can occur simultaneously, and therefore need to be assessed in conjunction when evaluating ecological impacts of bivalve aquaculture, a globally rapidly growing industry (FAO 2022).

Previous studies in relation to nutrient cycling by bivalve suspension feeders investigated either individual mussels from suspended culture systems in isolation (e.g. Smaal & Prins 1993, Cranford et al. 2007, Brigolin et al. 2009), investigated a single direction of control i.e. top-down or bottom-up (e.g. Hatcher et al. 1997), or investigated a single macronutrient (e.g. Cranford et al. 2007, nitrogen). Filtration of particles by bivalve filter feeders including *M. edulis* has been extensively researched, but predominantly on individual specimens (Cranford et al. 2011). A few studies investigated community feeding responses in the main SMC areas the Oosterschelde bay (mussel beds: Prins et al. 1996, Smaal & Haas 1997) and the Western Dutch Wadden Sea (SMCs: Jacobs 2015). Jacobs et al. (2015) argued that high-density SMC mussel aquaculture can result in significant refiltration, meaning that community-level filtration rates would be overestimated when simply extrapolating filtration rates established for individual specimens, which followed conclusions by Jansen (2012) who reported a similar pattern for adult mussel communities. A small number of

investigations combining filtration and nutrient regeneration have been reported, for individual mussels (Hawkins & Bayne 1985, Smaal & Vonck 1997, Smaal et al. 1997b), or intact mussel beds (Prins & Smaal 1994). More recently, a few studies looked at the effects of intact suspended mussel culture communities including the associated fauna, flora, and organic matter (AFFOM) on nutrient regeneration (Richard et al. 2006) or filtration and nutrient regeneration in combination (Nizzoli et al. 2005, Jansen et al. 2011). Richard et al. (2006) and Jansen et al. (2011) showed significant contributions of the associated community to nutrient regeneration, which would be missed when simply extrapolating nutrient regeneration rates established for individual specimens.

1.2.2. The role of bivalve seed collectors in nutrient cycling

Mussel seed exhibit a proportionally high metabolic activity. Metabolic rates scale allometrically with the size of the animal (Smaal et al. 1997b, and review by Cranford et al. 2011). The introduction of SMCs thus has the potential to result in increasing pressures on the ecosystem from the mussels' filtration and metabolic activity. A quantitative understanding of the functioning of mussel seed assemblages has been lacking (e.g. McKindsey et al. 2006). Model studies have suggested that the introduction of SMCs may lead to overgrazing in the Oosterschelde bay (Troost 2013) and the Western Wadden Sea (Brinkman 2013). In these scenarios, competition for food negatively impacts the carrying capacity of the system for filter feeding species. This could lead, for example, to reduction of stocks of the common cockle (*Cerastoderma edule*), an important food source for various bird species of conservation concern. However, more recent insights (Jansen et al. 2019) have contested the view that the Oosterschelde bay was overgrazed (Smaal et al. 2013). Forecasting SMC production levels at which carrying capacity becomes affected has proven difficult. Currently therefore, carrying capacity indicators are monitored and updated regularly as the transition from wild seed fishery to seed collectors progresses (Jansen et al. 2019, Craeymeersch et al. in prep). The top-down control of filtration by SMCs on plankton has been investigated in the Western Wadden Sea (Jacobs 2015), but community-level data on bottom-up control by SMCs to allow an evaluation of the combined effects of SMCs on carrying capacity for filter-feeding populations has been lacking. As the SMC season takes place during summer, this coincides with the period when phytoplankton is nutrient limited (Ihnken & Kromkamp 2011). Regenerated nutrients may therefore directly benefit primary production rates in the system, potentially counteracting the top-down control on phytoplankton.

1.3. Scope and objectives of the thesis

Appraising the importance of community level interactions, the aim of this thesis was to investigate the contribution of suspended mussel seed collector communities, including AFFOM, to nutrient cycling. This was addressed via four objectives:

i) Quantify filtration and nutrient release by SMC communities;
ii) Develop a multi-element (N,P,Si) nutrient budget for an SMC community;
iii) Measure farm scale depletion of seston and accumulation of nutrients under macrotidal conditions;
iv) Explore the top-down and bottom-up regulation of phytoplankton by mussel seed, and evaluate its ecological relevance in the transition of wild seed fishery to SMCs.

Figure 1.3 presents an overview of nutrient pathways investigated as part of the thesis, with reference to chapters presenting empirical data.

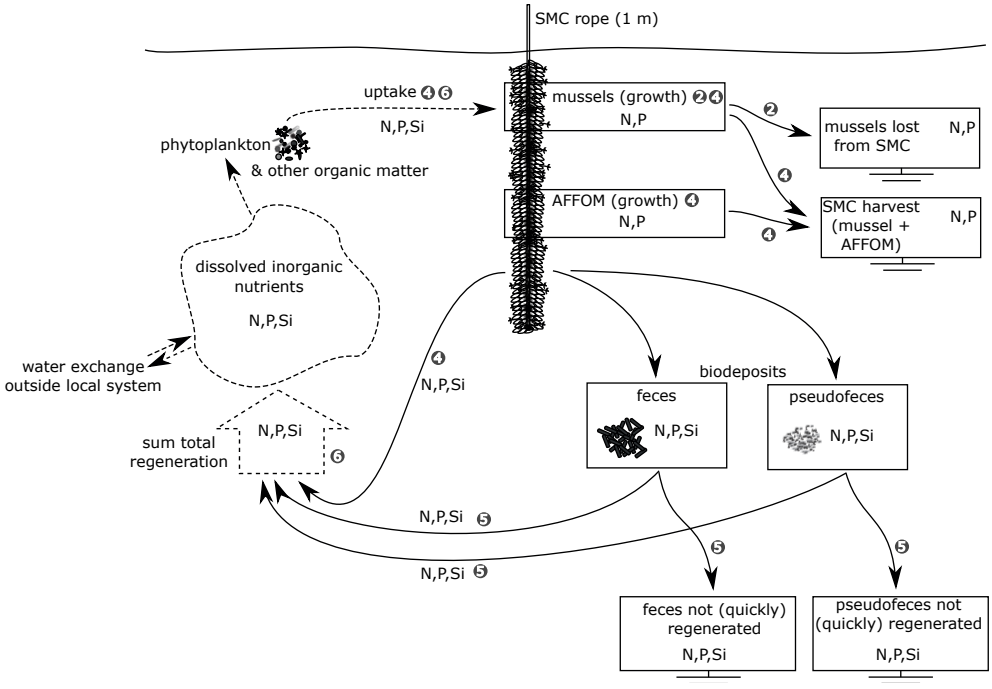

Figure 1.3. Overview of pools, flows and sinks related to nutrient processing by Seed Mussel Collectors (SMCs). N=nitrogen, P=phosphorus, Si=silicon. Chapters 2, 4, 5, 6 report empirical data collected on specific sections of the diagram, and are indicated by the chapter numbers.

The focus of each chapter of the thesis, and how these relate to the objectives, is highlighted next.

Chapter 1: General introduction
This chapter introduces the thesis and its subject matter.

Chapter 2: Growth and production of mussel seed on SMCs
This chapter starts by describing the mussel seed collection culture cycle and the transition from mussel seed fishery to suspended culture seed collection taking place in the Dutch mussel industry. Growth and production data of SMCs were analysed from the start of the transition in 2010 until 2022, exploring factors that might affect the production efficiency of the systems. This chapter contributes to objective iv.

Chapter 3: The role of cultured mussels in nutrient cycling
Here an overview is presented of the role of mussels in nutrient dynamics, outlining and quantifying how mussels exert top-down and bottom-up feedbacks onto phytoplankton communities, thus contributing to objective iv. The review includes a range of cultivation areas worldwide.

The following chapters address nutrient dynamics specifically for mussel seed (communities), with particular focus on bottom-up processes (nutrient regeneration), as top-down processes were evaluated by Jacobs (2015).

Chapter 4: Nutrient regeneration by mussel seed communities
Nutrient dynamics of mussel seed communities (intact SMC sections) were empirically studied using innovative mesocosms (floating chambers) in the Oosterschelde bay. The contribution of an intact section of SMC rope community to nutrient cycling in terms of uptake and release of nutrients is explored, including a discussion on stoichiometry of dissolved nutrients, thus contributing to objectives i and ii.

Chapter 5: Nutrient regeneration from mussel seed biodeposits
Besides the direct excretion as addressed in Chapter 4, another important pathway of nutrient regeneration is through the decomposition of biodeposits. The characteristics and decomposition rates of feces and pseudofeces were studied separately, contributing to objectives i and ii. This chapter provides insight in how both biodeposit fractions differ in their contribution to nutrient recycling and how this influences stoichiometry of dissolved nutrients.

Chapter 6: Farm-scale feedbacks
We investigated the extent to which the top-down (filtration) and bottom-up (nutrient regeneration) control pathways from SMCs were detectable *in situ* at the farm level, addressing objective iii. This was done via a combination of a discrete sampling approach and a towed glider equipped with sensors. Changes in seston and dissolved nutrients were investigated along farm transects, inside and outside four commercial farms.

Chapter 7: Synthesis
Data presented in previous chapters is integrated into a simple model to construct a nutrient budget for mussel seed throughout its production cycle (objective ii). These data are then used to further explore the ecological role of mussel seed in the Oosterschelde bay ecosystem by means of a set of indicators. This allows to evaluate the magnitude of SMC impact on nutrient cycling in the host ecosystem, including the extent to which nutrient regeneration from SMCs may constitute a positive feedback onto primary production (objective iv).

CHAPTER 2

Transitioning from Wild Seed Fishery to Seed Mussel Collectors (SMCs): Reviewing the Efficiency of Collectors for Seed Provisioning in Mussel Bottom Culture

Wouter van Broekhoven | Marnix R. van Stralen | Karin Troost | Jacob J. Capelle

Aquacultural Engineering 105 (2024), 102414

Abstract

The availability of mussel seed is a critical aspect in mussel farming. Since 2009, the Dutch mussel sector has been transitioning from wild seed fishery to suspended seed collectors (Seed Mussel Collectors, or SMCs). Collector systems using either ropes or nets as settlement substrate are placed in Oosterschelde Bay, the Wadden Sea, and the North Sea annually. We analyzed detailed harvest data from 2010 until 2022, to investigate the efficiency of different systems, identify differences between years and areas, and assess how production can be optimized. Additionally, numerical density, biomass, and shell lengths of mussels from 0.375 mm shell length were recorded on SMC ropes at one SMC location during a full growth season to evaluate biomass-density relations and assess the process of self-thinning on the ropes. Total harvest of SMC mussel seed increased over the period 2010–2022, from 8.0×10^6 kg to 21.0×10^6 kg fresh weight. Harvest per unit substrate was remarkably stable over the years across sites, with a lower mean in Oosterschelde Bay (~2.56 kg m^{-1}) than in the Wadden Sea (~3.28 kg m^{-1}). Ropes were found to provide a greater yield per unit area than nets, but nets are less labor-intensive to use. Occurrence of density-dependent growth on the ropes was indicated by the allometric relation between mussel biomass and mussel density. A positive relation between density and growth rate suggested that competition increased with growth rate. In the growth data covering a full SMC season, we first observed a rapid numerical increase as newly settled mussels continued to grow into the measured size range. This was followed by a period of rapid numerical reduction and increasing biomass, indicating self-thinning. Finally numerical reduction stabilized and biomass increase accelerated coupled with comparatively slower shell length increase. The self-thinning occurred between approximately 2.3 mm and 11.6 mm mean shell length. Our analysis of 12 years of production data shows that SMC seed is a robust and annually more reliable alternative to wild capture fishery as a seed provisioning resource for mussel culture. Production per unit substrate does not appear to be easily amenable to further improvement. Production per unit area showed no indication of overstocking on the scale of the SMC plots, suggesting that production gains could be made by increasing substrate density.

2.1. Introduction

2.1.1. Mussel culture and mussel seed provisioning

Mussel culture is an extensive aquaculture practice, relying on natural feed, and requiring mussel seed as an input resource (Smaal et al. 2019). This seed is usually collected from benthic wild mussel seed stocks or from suspended collectors (Kamermans & Capelle 2019). The large year-to-year variability in recruitment success on wild mussel beds in the most important source area in the Netherlands, the Wadden Sea, is not explained well by any single factor (Van Der Meer et al. 2019), and variation in seed availability contributes to the steady decline of EU mussel production (Smaal 2002, Avdelas et al. 2021). Environmental factors that affect variation in seed recruitment include hydrodynamics (Fuentes-Santos & Labarta 2015), predator-population dynamics (Beukema 1982), and food supply (Phillips 2004).

Policy, regulations, and restrictions also affect availability of bivalve seed for aquaculture. Concerns about environmental effects of wild seed fishery have led to policy and regulations that set limits for wild seed harvesting in various areas (Nehls et al. 1997, Kaiser et al. 1998, Piñeiro-Corbeira et al. 2018). The same applies in the Dutch Wadden Sea, where mussel seed fished from wild seed beds has been re-laid on subtidal culture lease plots to grow to commercial size since the 19th century. Until harvest, mussels are generally relocated between one and three times between plots to increase growth rate, reduce density, or reduce losses due to dislodgement. Other active management includes removal of starfish (a major predator of mussels) and removal of silt from the plots after harvest (for details see Jansen et al. 2023). The average ratio between mussel biomass at any given point in the culture cycle, and seeded mussel biomass, is highly variable but on average sits between 1.3 and 2.8, depending on mussel size at seeding (Capelle et al. 2016). In the Dutch Wadden Sea, fishing for mussel seed has been strictly regulated since the 1990s via exclusion from intertidal areas and via the application of food reserve floor levels for birds: shellfish population levels below which the fishery would be reduced or halted. This led to court battles between several environmental NGOs (ENGOs) and the mussel industry. In a 2008 court case brought by ENGOs, the Council of State ruled that mussel seed fishery licensing occurred unlawfully since it conflicted with the European Birds and Habitats Directives. The ruling threatened the existence of the Dutch mussel industry since it depended on mussel seed fishery in the Wadden Sea to obtain mussel seed as its input resource. To prevent further conflict, the Ministry of Agriculture, Nature, and Food Quality, four ENGOs, and the mussel producers' organization jointly set out, in a covenant, a "transition" process from wild seed fishery to alternative means of seed provision. The main points agreed in the covenant are that (i) ENGOs will cease legal challenges against fishery permits, and (ii) the Dutch Wadden Sea will gradually be closed

to mussel seed fishery, under the condition that the mussel industry is given sufficient time to develop alternative means for resource provisioning (Van Hoof 2012).

2.1.2. Seed Mussel Collectors as an alternative seed source

Experiments with seed mussel collectors (SMCs) as an alternative to wild seed started in the Netherlands at the beginning of the 21st century (Kamermans et al. 2002). The aim was to obtain a more reliable seed supply, since the availability of wild mussel seed fluctuated naturally. The covenant accelerated development from 2008, and the use of SMCs increased steadily until about 2015, after which total substrate deployed fluctuated around the same level.

As part of the covenant, the Wadden Sea is closed to wild seed fishery in a stepwise process, matching the pace of development of alternative seed supply. The aim of the covenant is to obtain 100% of the industry's mussel seed demand from SMCs, thus abolishing all seed fishery on wild mussel beds. To date, four steps have been fulfilled: (1) 14% of subtidal areas containing mussel seed beds were closed in 2010, corresponding to a seed supply of 5.5×10^6 kg; (2) this area increased to 28% in 2014 with areas without current mussel beds but historically known to form mussel seed beds, corresponding to a cumulative 11×10^6 kg of seed supply; (3) in 2021 a third step of 7.7% closure (total 35.7%, corresponding to a cumulative seed supply of 14×10^6 kg. And a fourth step in 2022 to a total of 50% closure. Two more steps are foreseen: (1) 65% total closure by 2026, and 2) 100% by 2029, conditional on an assessment to be made in 2026 whether this will be feasible while maintaining a viable mussel industry.

Various studies have compared the performance characteristics of seed from collectors with seed from wild mussel beds. Mussels from suspended collectors have been shown to display more aggregation activity (Christensen et al. 2015). However, Kamermans et al. (2009) found no predation preference by crabs and starfish on mussels from various sources. Comparing performance of mussel seed from collector ropes and from intertidal rocky shores on raft culture in Spanish Rías, Fuentes et al. (1998) and Babarro et al. (2000) found no difference in terms of growth rate and mortality, and condition index, respectively. A monitoring program in the Netherlands found no major differences in overall performance between seed from SMCs and seed from fishery on wild mussel beds (in autumn and spring). The only differences could be related to mussel seed size (Capelle et al. 2016): a smaller seed size when seeding results in a higher overall yield. However, production of mussel seed via SMCs is much more labor-intensive than wild seed fishery, and the cost is estimated as five to six times higher (Van Oostenbrugge et al. 2018). Consequently, there is a demand for significantly higher production efficiency of mussel seed harvested from SMCs.

2.1.3. Aims of this paper

Our aims are: 1) to evaluate the efficiency of the different SMC systems, 2) to identify potential differences between areas and any universal trends observed across SMC systems and areas, and 3) to assess whether production per unit of SMC system (e.g. m of culture rope), or per unit area (e.g. ha of SMC lease plot), can be optimized. The available data sets allowed to assess the influence on production of: deployment area, collector type, mussel seed density-biomass relations, SMC system density, and adverse events.

At the beginning of the covenant, the SMCs were a new technique for the Dutch mussel sector, and development and scaling up were driven by trial and error. Therefore, we expected to find an increase over time in harvest per unit substrate (substrate production efficiency, kg m^{-1}), and in terms of harvest per unit area (plot production efficiency, kg ha^{-1}), since performance was expected to improve. Similarly, we expected to find a reduction of both types of production efficiency at high SMC densities per unit area (rope length or net surface ha^{-1}), as the boundaries of optimal production are explored. To test these hypotheses we investigated if production efficiency is affected by (a) growth rates of mussel seed on SMCs, (b) density of mussel seed on SMCs, and (c) density of SMCs per unit area.

2.2. Material and methods

2.2.1. Dutch mussel industry and SMC deployment

In 2018, the Dutch mussel industry consisted of 88 registered companies, 51 mussel fishing vessels, plus a smaller number of specialized vessels dedicated to rope culture, SMC operation, or facilitating commercial trade handling activities such as shuttling traded mussels between the auction and temporary holding plots (Van Oostenbrugge et al. 2018). Annual mussel production from 2001 to 2021 ranged from 30 x 10^6 kg to 68 x 10^6 kg fresh weight, with an average of 46 x 10^6 kg (https://agrimatie.nl/). Most mussels are grown on bottom lease plots, situated in the south-west of the country in the Oosterschelde Bay (2,250 ha on 319 plots), and in the north in the Wadden Sea (6,884 ha on 458 plots). A smaller surface area is licensed specifically for SMCs (Figure 2.1), divided over three regions: the Wadden Sea (blocks of a total of 708 ha divided into 281 ha of licensed plots, of which 222 ha was used in 2022), the North Sea (a block of 65 ha divided into 27 ha of licensed plots, of which 15 ha was used in 2022), and Oosterschelde Bay (blocks of 316 ha divided into 116 ha of licensed plots, of which 40 ha was used in 2022).

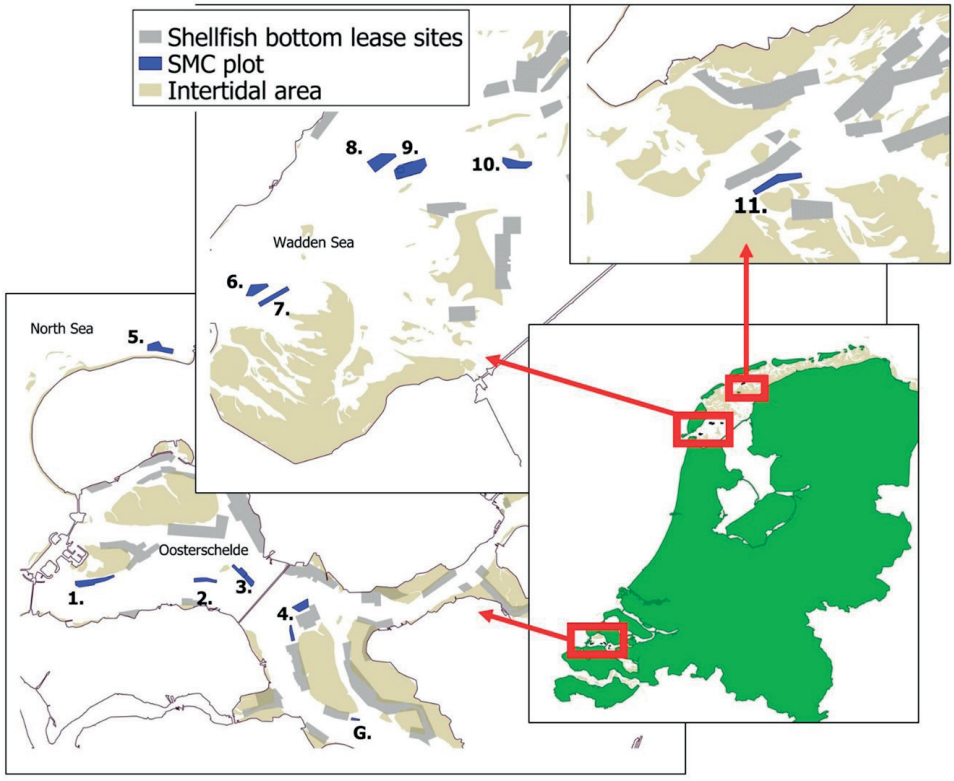

Figure 2.1. SMC sites in use in the Netherlands (2022): 1 = Neeltje Jans (65 ha); 2 = Schaar van Colijnsplaat (28 ha); 3 = Vuilbaard (46 ha); 4 = Vondelingen Noord (40 ha); 5 = BH Gat (North Sea – 60 ha); 6 = Malzwin (55 ha); 7 = Zuidwal (50 ha); 8 = Burgzand (100 ha); 9 = Vogelzand (140 ha); 10 = Gat van Stompe (75 ha); 11 = Zuidmeep 80 ha. G = Galgeplaat: not in use in 2022 but source of self-thinning data in this study. Shellfish bottom lease plots are shown for reference.

2.2.2. SMC systems

The covenant incentivized mussel farmers to invest in SMCs from 2008 onwards. This led to a wide variety of systems and experiments (Poelman & Kamermans). A general distinction can be made between rope-based and net-based systems. Belt collector substrates, such as deployed in Taylor et al. (2019) were never tested. Rope systems use filamentous rope onto which mussels can attach, and can be categorized as (a) wound around rigid structures placed on the seabed, (b) suspended longlines continuously looped from a buoyed main line, (c) suspended horizontally between tubes (for shallow areas). Net-based systems can be categorized as nets suspended (a) under buoys or tubes, and (b) under rafts. Examples are shown in Figure 2.2. Systems are anchored with plough anchors, concrete blocks, anchor piles (ground anchors at sea floor level), or at the water surface on metal poles. From the wide array of experimental systems at the start of the

transition, most disappeared over time due to impracticalities, or company take-overs. By 2022 two systems remained: (a) continuous longlines under a buoyed main line, and (b) suspended nets under tubes (110 m x 3–4 m). Until 2015, a proprietary system with nets suspended under rafts was implemented on a relatively large scale in Oosterschelde Bay by a single mussel farmer. These systems were characterized by a high density of substrate per farmed area. However, they yielded a lower harvest per square meter of substrate compared to other net-based systems (see paragraph 2.3.5).

Figure 2.2. Examples of SMC systems: (a) lines wound around rigid structures placed on the seabed; (b) longlines suspended in continuous vertical loops under buoys; (c) lines suspended horizontally between tubes; (d) suspended nets under rafts; (e) suspended nets under buoys or tubes.

2.2.3. Data collection

SMC production monitoring

The data used to investigate production efficiencies including the effects of density-dependent growth, and differences between SMC systems and areas, were obtained from the production monitoring program. Mussel farmers using SMCs are required to report production statistics annually via two separate registration forms: (1) specification of SMC systems deployed, number of systems, amount of substrate, area, type of anchorage, and hours spent; and (2) SMC harvest (Wet Weight) for each day, with specification of date, number of systems harvested, first or consecutive harvest, hours spent, harvested volume (in the legacy unit "mussel ton" = 100 kg), size of the mussel seed (volumetric index 'bustal' = number of mussels that fit in a 880 ml tin can, converted to grams following Capelle et al. (2016)), presence of starfish, destination (specific bottom or rope plot where the mussel seed will be brought to for growing out further), and any incidents or unusual observations. Mussel density (number) per unit substrate was calculated by dividing the

biomass (g) per unit substrate (m or m^2) by the average mussel weight (g). The most recent results are reported by Capelle (2023).

We calculated the substrate production efficiency as kg harvest per m rope or per m^2 net. In order to compare efficiency between rope and net systems, a Rope Equivalent (REq) index was calculated by dividing the mean annual efficiency of nets (kg m^{-2}) by that of ropes (kg m^{-1}). This unit represents the length (m) of rope needed to obtain a similar harvest as 1 m^2 of net. Over the period 2010–2022, the REq was 12.0 m.

SMC systems are deployed from March until May, with most activity occurring in April. Regulations stipulate that SMCs be removed before November 1st. Harvest of seed takes place from late June until early October. Mussel farmers generally aim to harvest before large aggregations of mussels start to detach and fall off.

SMC growth data

The production data (paragraph 2.3.1) did not permit direct investigation of the effect of self-thinning (Fréchette et al. 2010) on the development of mussel seed density (# m^{-1}) and biomass (g AFDW m^{-1}). Instead, data originally presented by van Broekhoven et al. (2014) were re-evaluated for this purpose. This dataset comprised mussel seed numerical and biomass densities on collector rope sections on six sampling dates (27 June, 11 and 25 July, and 9, 14, and 22 August) spanning the 2012 growing season at Galgeplaat, an SMC location in Oosterschelde Bay (G in Figure 2.1, currently inactive). The rope sections were placed on 4 May, and samples were taken starting from shortly after first observed settlement in large numbers, to the point of harvest which took place within days of last sample. The methods used are described by van Broekhoven et al. (2014).

2.2.4. Data analysis

Data exploration prior to the analysis was carried out following the protocol in Zuur et al. (2010), where appropriate. For each statistical model, histograms of residuals were produced to get an impression of normality. Residual diagnostic plots were used to obtain indications of heteroscedasticity and acf plots were used to detect autocorrelation for the models when appropriate.

2.2.5. SMC Effort and Mussel Seed Production Over Time

We analyzed trends in total harvest per year for each type of system (ropes or nets), with data available from 2011 to 2022, and for each area (Wadden Sea, North Sea, and Oosterschelde Bay), with data available from 2006 to 2022. Additionally, we examined seed collection effort, defined as the amount of substrate used in terms of length of rope (km) for rope substrate and length of Rope Equivalent (km) for net substrate, available from 2010 to 2022.

Given the nonlinear nature of both total harvest per year and effort over the study period, we employed generalized additive modelling (GAM) for analysis. GAMs were constructed in R using the 'mgcv' package (Wood 2011), and model diagnostics were conducted using the 'DHARMa' package (Hartig 2022).

All GAM models utilized cubic spline regression, with parameter estimation performed using Restricted Maximum Likelihood (REML). We modelled total harvest per year using an unpenalized smoother, while for harvest trends over time at different locations, efforts (km rope eq), and types of systems (ropes vs. nets), we employed a penalized smoother. To address heteroscedasticity in the relationship between effort and location, we applied a Tweedie distribution.

Model summaries, diagnostic plots, and partial effects for all GAM models are provided as supplementary material.

SMC mussel growth rates

Average annual growth rate (whole mussel wet weight [WW] per day [d]) on ropes was estimated for each of the last 12 years (2011–2022) by using the exponent of the slope from the log-log relation between size of the mussel at harvest and Julian day number (Figure S10). A distinction was made between the areas (Oosterschelde Bay and Wadden Sea), but not between locations within those areas. The North Sea SMC site was disregarded since it only represented an individual location.

Density-dependent growth

The relationship between the harvest per unit substrate (kg m^{-1}, with REq for nets) and the growth rate of mussels was analyzed using a multiple regression analysis. This analysis incorporated year and area (Wadden Sea or Oosterschelde Bay) as covariates to account for their potential effects. Furthermore, a parallel analysis was conducted using the average density of mussels per unit substrate as the explanatory variable, as mussel growth may exhibit density-dependent dynamics.

The occurrence of density-dependent growth on the ropes was evaluated by the relation between estimated mussel density per unit substrate at harvest (N, # m^{-1}) and harvested biomass per unit substrate (B, kg m^{-1}), where a curvilinear relation would suggest density-dependent growth (Fréchette et al. 2010). For July and August, the number of harvest observations was deemed sufficient (>50, Wilson Van Voorhis & Morgan 2007) for this analysis. An F-test was employed to compare the suitability of a curvilinear model against a linear model using data pooled over the most recent years. Specifically, observations from the years 2016 to 2022 for the months of July and August were assessed separately for a log-log fit using an F-test.

Self-thinning

Specific investigation of self-thinning on ropes over the course of a growth season was based on growth data (density, # m^{-1}, and mean individual biomass, mg AFDW ind^{-1}) from one SMC location (paragraph 0). Hypothetical trajectories of density (# m^{-1}), mean individual biomass (mg AFDW m^{-1}), and biomass per unit culture rope (g AFDW m^{-1}), were calculated by means of fitted mathematical functions. Specifically, a cubic spline was fitted to the density data (calculated in R version 3.5.1 using function interpSpline in package spline), and a power function was fitted to the mean individual biomass data. Biomass per unit culture rope was calculated as the product of these two parameters.

Production efficiencies per SMC location and area

Differences in seed production efficiencies between SMC locations (see Figure 2.1) and area (Wadden Sea, North Sea, and Oosterschelde Bay), were evaluated in terms of total harvest (kg) and harvest per unit substrate (kg m^{-1}, with REq for nets), using a two-way analysis of variance and a *post hoc* Tukey HSD test. Harvest per unit area (kg ha^{-1}) was log-transformed to normalize model residuals. Trends in harvest per unit substrate (kg m^{-1}, with REq for nets), substrate density (km ha^{-1}), and harvest per unit area (kg ha^{-1}) were analyzed per area with a linear model. Substrate density in the North Sea and Oosterschelde Bay, and harvest per unit area in Oosterschelde Bay, were log-transformed to normalize residuals. Also, differences in harvest per unit area between nets and ropes were tested with a linear model for the different areas.

The correlation between harvest per unit substrate (kg m^{-1} (REq)) and substrate density (km (REq) ha^{-1}) for the three production locations with the highest mean annual harvest over the period 2010–2022 (Vogelzand, Zuidmeep, and Gat van Stompe) was analyzed with a Kendall rank correlation procedure, after a Shapiro-Wilk test indicated a non-normal distribution of underlying variables.

2.3. Results

2.3.1. Deployment effort and mussel seed production over time

Seed collection effort, as defined by overall SMC placement (km of rope, or km REq for nets), exhibited a substantial increase from 2,580 km in 2010 to 6,565 km in 2022 (Figure 2.3a), with a significant difference between the areas ($p<0.001$, Table S3). Most of the SMCs were deployed in the Wadden Sea, comprising 81% of the total in 2022. SMC usage in this area exhibited an upward trend until 2016, after which it plateaued (Table S3, Figure S5). In the North Sea, SMC use also demonstrated an overall positive trend over time ($p=0.01$, Table S3, Figure S5). There is also a significant change in production over time in Oosterschelde

Bay (p<0.001, Table S3), but this trend is more complicated, with in increase up to 2013, followed by a decrease until 2018/2019 after which the trend increases again (Figure S5).

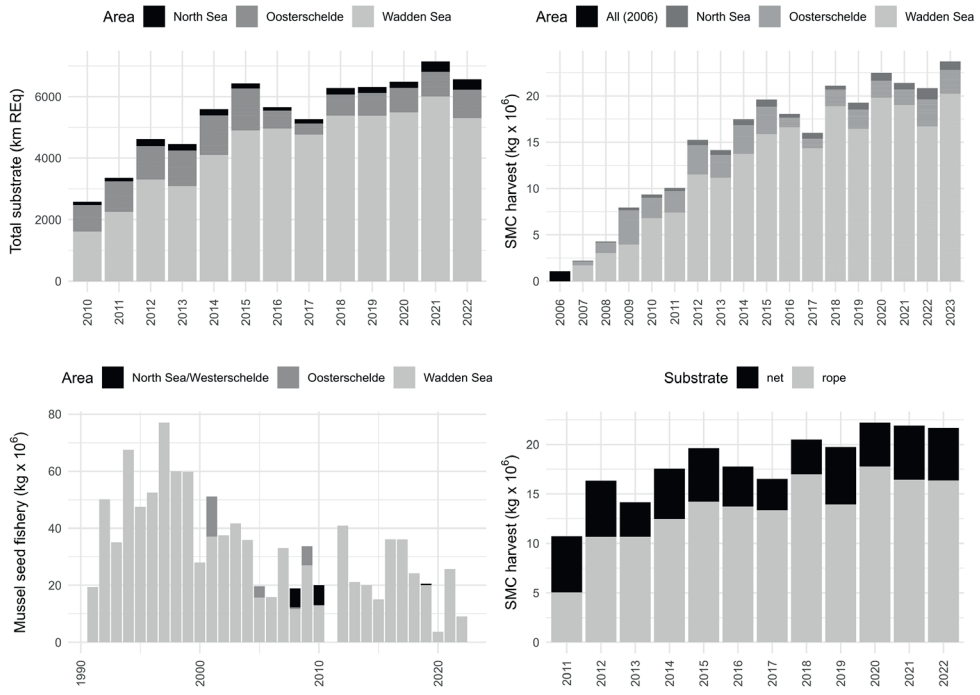

Figure 2.3. (a) Annual amount of substrate deployed (REq in case of nets) in the three SMC areas in the Netherlands; (b) Annual SMC harvest in the same areas since 2006; (c) Annual seed production from seed fisheries in the Netherlands since 1991 in the same areas (Westerschelde included with North Sea); (d) Annual SMC harvest for net and rope systems; data available separately since 2011.

Total annual harvest of SMC mussel seed increased over the period 2006–2022, from 1.0 x 10^6 kg in 2006 to 20.8 x 10^6 kg in 2022 (Figure 2.3b, p<0.001, Table S1). This contrasts with the harvest from mussel seed fisheries (Figure 2.3c), which showed no clear trend since approximately 2006. SMC harvest differs between regions (p<0.001, Table S2): in the Wadden Sea (p<0.001, Table S2) and the North Sea (p<0.001, Table S2) it increased over this period (Figure S3). Harvest from Oosterschelde Bay also changes over time (p<0.001, Table S2), but in a similar pattern as for the amount of substrate: an increase up to 2012/2013, followed by a decrease up to 2018 and since then increasing (Figure S3).

Since 2011 data was available to distinguish between rope systems and net systems in the harvest reports (Figure 2.3d). Harvest from rope systems displayed an increasing trend since 2011 (p<0.001, Table S4), whereas harvest from net systems showed no significant trend (Figure S7).

2.3.2. SMC mussel growth rates

A positive and significant relationship was observed between the estimated growth rates of mussel seed on ropes from the Wadden Sea and from Oosterschelde Bay ($F_{(1,10)}$=5.57, p=0.04, R^2=0.58, Figure S9). This indicates that growth rates followed similar patterns in both regions.

2.3.3. Density-dependent growth

The Pearson correlation coefficient between the estimated growth rate, and the mussel density at harvest, was 0.40 (Figure 2.4a). This relationship was statistically significant (t-test, p<0.001). In the analysis of the relationship between either growth rate or density, and harvest per unit substrate (Table S5 and Table S6), the model based on mussel density yielded the lowest Akaike Information Criterion (AIC) value and was consequently selected, while the other model (based on growth rate) was discarded. In the combined data of Oosterschelde Bay and Wadden Sea (the North Sea location was disregarded in the multiple regression since it was a single site, only consistently used by one farmer with net-based systems), the average harvest per unit substrate was found to be higher when the averaged mussel density was higher at harvest (F(1,22)=30, p<0.001, Figure 2.4b, Table S6). There was no significant difference between the two areas. Moreover, a logarithmic curvilinear relationship between averaged harvest per unit substrate and averaged mussel density provided a significantly better fit (F-test, p<0.001) compared to a linear relationship.

The density-biomass relation was explored in more detail using the data for each reported harvest activity in the more recent period 2016-2022. A power relation between mussel density per unit substrate (N, # m^{-1}) and harvest per unit substrate (B, kg m^{-1}), provided a better fit (July $F_{(1,460)}$=134 p<0.001, August: $F_{(1,789)}$=801, p<0.001, Table S7 and Table S8) for both months than a linear relation (Figure 2.5): July ($F_{(1,460)}$=481.1, p<0.001), August ($F_{(1,789)}$=1139, p<0.001). The power function is remarkably similar between both months (July: B=0.01*$N^{0.61}$; August: B=0.01*$N^{0.62}$). This similarity suggests a comparable density dependence of growth on ropes between months, although size (median July = 0.27 g; August = 0.38 g) and density (median July = 12,174 seed m^{-1}; August = 8,729 seed $^{-1}$) differed.

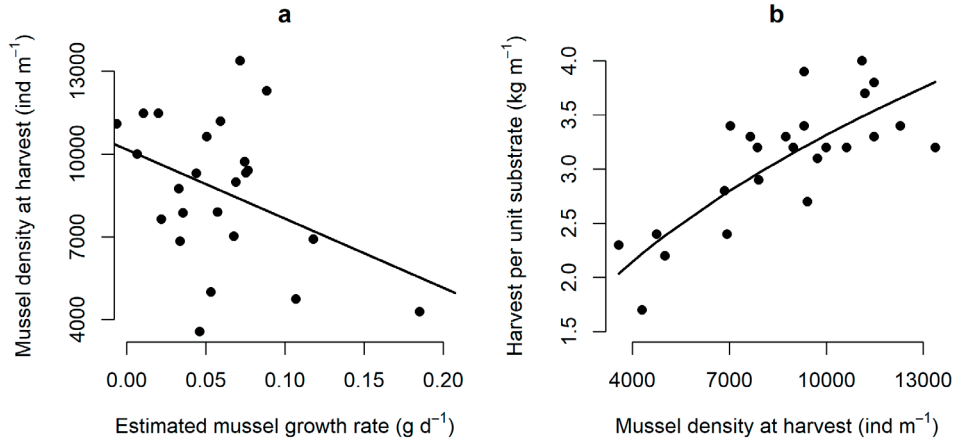

Figure 2.4. (a) Linear relation between estimated mussel growth rate (g WW d^{-1}) and mussel density at harvest (# m^{-1}); (b) Logarithmic curvilinear relation between mussel density at harvest and harvest per unit substrate (kg m^{-1}). Data are only from ropes, not from nets. Each dot represents the mean of a year, Oosterschelde Bay and Wadden Sea areas combined, in the period 2011–2022. Lines indicate a significant relation.

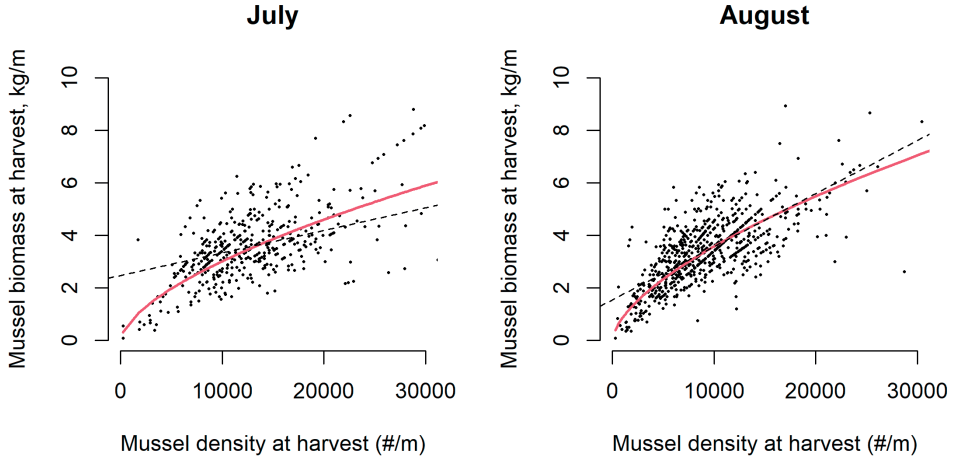

Figure 2.5. Mussel harvest per unit rope substrate (kg m^{-1}) versus mussel density per unit rope substrate (# m^{-1}) for July and August over the years 2016 and 2022. Each dot represents a single harvest event. The red line shows a fitted logarithmic curvilinear relation, and the dotted line shows a fitted linear relation. For both months, the curvilinear relation provided a better fit.

2.3.4. Self-thinning

In the growth data covering a full SMC season at the Galgeplaat SMC (G in Figure 2.1; Figure 2.6), numerical mussel density first increased rapidly and subsequently rapidly reduced, together with an increase of mussel biomass, before finally levelling out together with an accelerating mussel biomass increase. Towards the end of the observation period (from day 97), mean shell length increased more slowly than mean individual biomass.

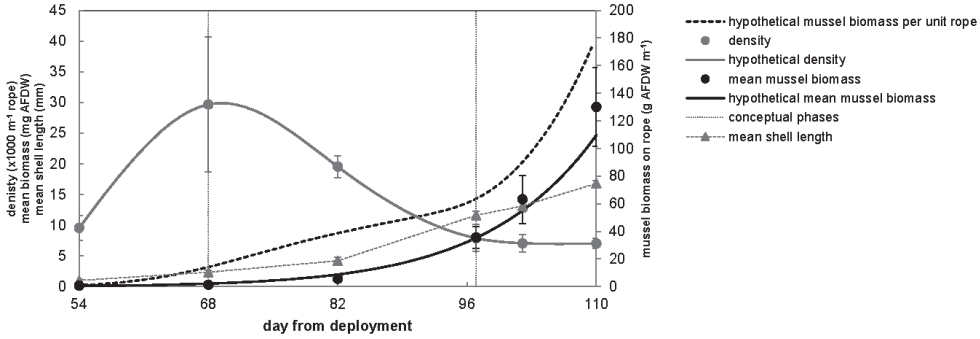

Figure 2.6. Symbols represent mussel density, mean individual tissue mass, and shell length on the Galgeplaat SMC in Oosterschelde Bay during the 2012 season, from 27 June (day 54 from SMC deployment) to 22 August (day 110 from SMC deployment). The curves for density and mean mussel biomass were fitted to the data visualize the hypothetical trajectory over time as an approximation. The dashed line shows the product of these two parameters, indicating the hypothetical tissue biomass per unit rope. Mean shell length observations are connected by straight lines. Error bars indicate SD (n=5 ropes). The vertical dashed lines separate three apparent phases in the density of mussels on the ropes: i) increase, ii) decrease, iii) stabilization accompanied by accelerating biomass increase.

2.3.5. Production efficiencies per SMC location and area

Not all SMC locations (see Figure 2.1) were used intensively. Mean annual production per location from 2015–2022 is summarized in Figure 2.7a. Over this period, the majority of SMC seed (85% since 2015) was produced in the Wadden Sea. Overall, mean annual harvest (kg) and mean annual harvest per unit substrate (kg m^{-1} (REq), Figure 2.7b) differed between locations ($F_{(8,119)}$=17, p<0.001, Table S9 and $F_{(8,119)}$=2, p=0.03, Table S10). Of note is the proprietary system with nets suspended under rafts was implemented on a relatively large scale in Oosterschelde Bay by a single mussel farmer who ceased operations after 2015. These systems were characterized by a high density of substrate per farmed area, but a lower harvest per square meter of substrate compared to other net-based systems. For instance, in Oosterschelde Bay in 2015, the substrate density for rafts with nets was 10,692 m^2 ha^{-1}, whereas other net-based systems had a substrate density of 1,591 m^2 ha^{-1}. However, the harvest from the former was 13.9 kg m^{-2}, whereas the latter yielded 36.5 kg m^2.

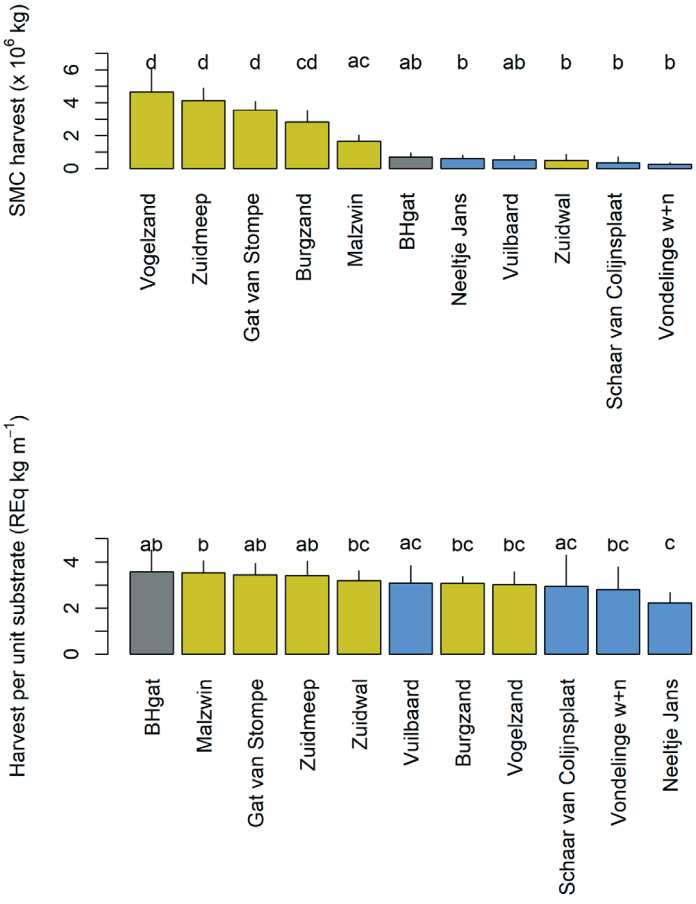

Figure 2.7. (a) mean annual harvest per SMC location, and (b) mean annual harvest per unit substrate (kg m^{-1} (REq)), for the different SMC locations over the years 2015 to 2022 (error bars show standard deviation). Colors indicate SMC production area: yellow represents Wadden Sea, blue Oosterschelde Bay, and grey North Sea, letters indicate significance with an α-level of 0.05.

There was a significant increase in substrate density in all three areas (km (REq) ha^{-1}) (Figure 2.8, left panel set and Table S11). Between 2010 and 2022, an increase in efficiency in SMC harvest per unit substrate (kg m^{-1} (REq)) was only found in the Oosterschelde Bay area (Figure 2.8, right panel set and Table S11). The mean harvest per unit substrate in Oosterschelde Bay was significantly lower ($p<0.001$) than in the Wadden Sea (Table 2.1). Harvest per unit substrate in the North Sea was also higher than in Oosterschelde Bay ($p=0.002$), while there was no significant difference between the Wadden Sea and the North Sea. This difference was caused by a lower density of mussels on ropes (# m^{-1}) in Oosterschelde Bay and not by size differences at harvest (Table 2.1).

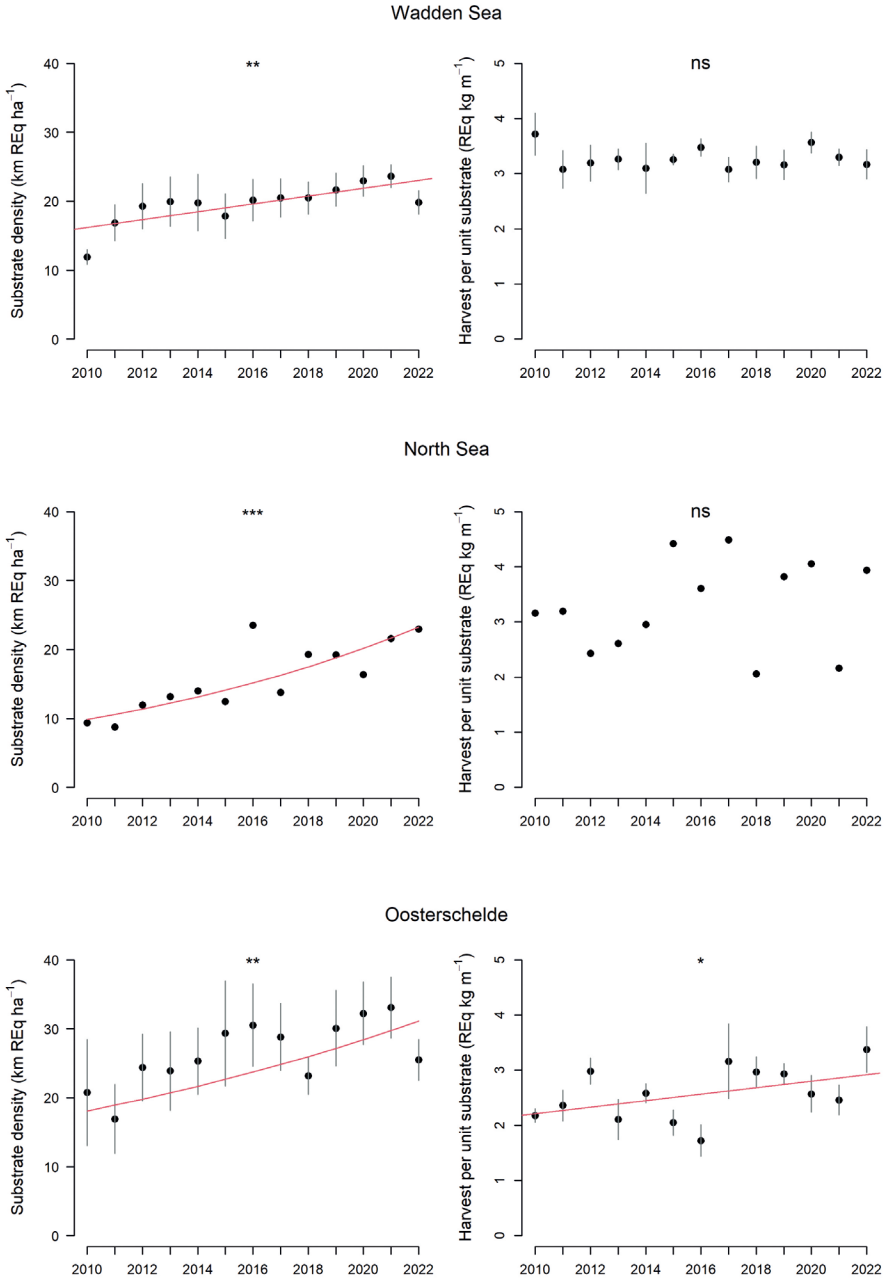

Figure 2.8. Left panels: annual mean substrate density (km (REq) ha^{-1}). Right panels: annual mean SMC harvest per unit substrate (kg m^{-1} (REq)). Error bars show standard error; trend: ^{ns} not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

When considering all the data together, the harvest per unit area (Mg ha^{-1}) showed an overall increase over time ($F_{(12,56)}$=3, p=0.006, Table S11a). There were significant differences in the harvest per unit area between areas ($F_{(2,56)}$=10, p<0.001) and between substrate types ($F_{(1,56)}$=16, p<0.001). Furthermore, there were distinct differences in the trends between areas and substrate types, as indicated by the significant interaction between area and substrate type ($F_{(2,56)}$=13, p<0.001).

Table 2.1. Harvest per unit substrate per area, with mussel density and mean mussel biomass.

Area	Harvest biomass (kg m^{-1} (REq) substrate ± s.e.m.)	Mussel density on substrate (# m^{-1} (REq))	Mean mussel biomass (g)
Oosterschelde Bay	2.57 ± 0.10	7967	0.46
Wadden Sea	3.27 ± 0.07	9646	0.37
North Sea	3.30 ± 0.22	9556	0.46

Specifically, the harvest per hectare in Oosterschelde Bay showed a substantial increase for ropes ($t_{(11)}$=5.4, p<0.001), while it did not change significantly for nets (Figure 2.9). Conversely, in the North Sea ($t_{(11)=}$5.4, p=0.006) and Wadden Sea ($t_{(11)}$=3.7, p=0.003), there was an opposite trend, with nets showing an increase in harvest per hectare, while ropes did not exhibit a significant trend (Table S12). The relation between harvest per unit substrate (kg m^{-1}) and substrate density (km ha^{-1}) was tested for the top-three largest SMC locations with the highest mean annual harvest (all in the Wadden Sea and rope-based): Vogelzand (140 ha), Zuidmeep (80 ha) and Gat van Stompe (75 ha). No significant trend was found (Figure 2.10).

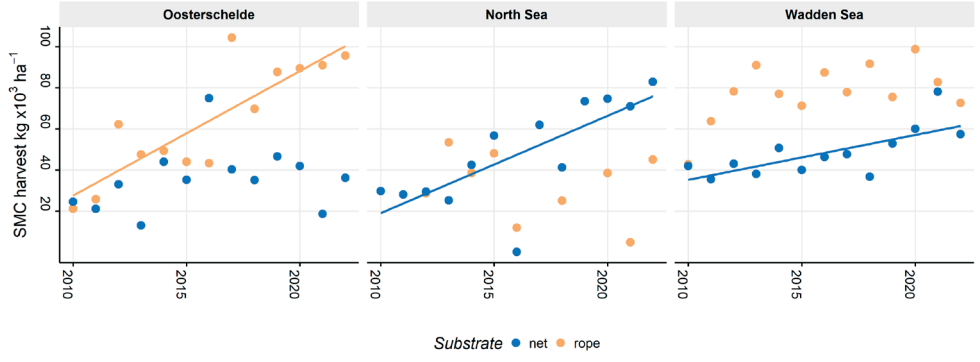

Figure 2.9. Mean harvest of SMC seed per unit area from rope and net-based systems in the three production areas. Lines indicate significant trends.

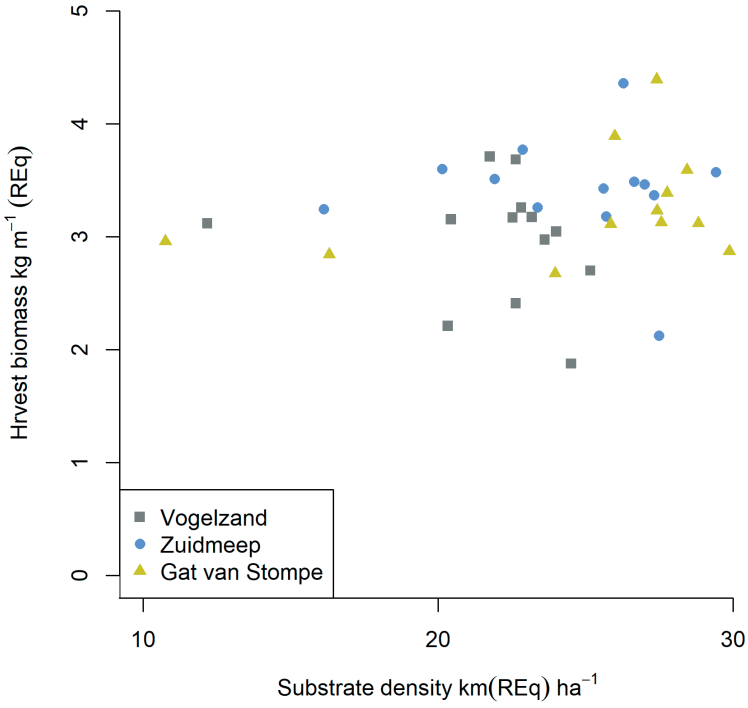

Figure 2.10. Relation between harvest per unit substrate (kg m^{-1} (REq)) and substrate density (km ha^{-1}) for the three SMC areas with the highest mean annual harvest over the period 2010–2022. No significant trend was found.

2.4. Discussion

2.4.1. Production of mussel seed with SMCs

Analysis of 12 years of production data reveals that, from a biological perspective, seed collection is essentially self-regulating. Harvest per unit substrate did not show large inter-annual fluctuations between areas and between years (Figure 2.8), especially when compared to natural recruitment into wild mussel seed beds, which is much more erratic (Van Der Meer et al. 2019). Therefore, the increase in production over time and differences between areas can be explained by the increase in effort.

A variety of adverse events occurred over the years. The most prominent issues that have been reported that negatively affected yields and/or that required a large effort to mitigate, are summarized in Table 2.2. However, the only event that shows up as a lower value in overall production data was fouling by other organisms in Oosterschelde Bay in 2021 and 2022. Fouling on SMCs is often reported to be a problem, especially on nets in Oosterschelde Bay. Problematic fouling species are the ascidians *Ciona intestinalis*, *Molgula*

manhattensis and *Styela clava* and the crustaceans *Monocorophium acherusicum* and *Jassa falcata*. This is considered to be the main reason why, at least for net-based systems, yields are generally lower in Oosterschelde Bay than in the Wadden Sea. The reason that nets are more prone to fouling is unclear, but, speculatively, might be related to nets creating more sheltered conditions than ropes. In 2021 and 2022, massive settlement of the bryozoan *Electra pilosa* on the substrate occurred at most locations. This has caused problems with harvesting and resulted in high tare (non-mussel material) percentages of the harvest. Rope substrate was especially affected, in Oosterschelde Bay and North Sea in 2021. This is reflected in a lower harvest per unit substrate and per unit area (for example, see Figure 2.8 and Figure 2.9). Apart from this recent problem, SMCs have generally proven to be a robust source of mussel seed.

The lack of a reliable seed provisioning source is referred to by studies from all parts of the world as a principal factor that limits mussel production (Fuentes & Molares 1994, Jeffs et al. 1999, Maguire et al. 2007, Laxmilatha et al. 2011, Kamermans & Capelle 2019, Avdelas et al. 2021). The nature of this problem is the dependency of extensive bivalve culture on natural processes, starting with recruitment for the provisioning of seed. In our study area, recruitment of mussel seed on natural mussel beds can show large annual variability (Dankers & Koelemaij 1989, Beukema & Dekker 2007, Van Der Meer et al. 2019). However, the high annual variability in larval abundance and settlement does not appear to translate to recruitment success on SMCs and cannot be used to explain spatial variation (Zhao et al., *submitted*). On natural mussel beds, De Vooys (1999) was unable to find a relation between the adult mussel stock and the number of plantigrades, and between the number of plantigrades and recruitment. This suggests that mussel bed recruitment is limited by other factors, in which habitat availability and predator dynamics seem to be key processes (van der Heide et al. 2014). These factors do not appear to play a role with SMCs. Here, artificial settling material is introduced every year and is therefore sufficiently available. Also, no observations were made of benthic or pelagic predators hampering mussel seed production. The heavy starfish predation in 2011 did not result in lower yields (Table 2.2). In other areas of the world, predation on suspended culture at this stage can be substantial, for example by fish (Peteiro et al. 2010, Šegvić-Bubić et al. 2011), or by waterfowl (Varennes et al. 2013). The overall absence of such major effects on SMCs appeared to result in a predictable harvest with low variation per unit of substrate (kg m^{-1} (REq)) and in a reliable seed supply.

Table 2.2. Most prominent major reported issues that negatively affected SMC production (only *Electra pilosa* fouling) and/or required a large effort to mitigate.

Year	Location	Cause
2011	Oosterschelde Bay and Wadden Sea	Massive starfish (*Asterias rubens*) settlement on SMCs. A significant manual cleaning effort was made but the mussels outgrew the starfish and untreated ropes ultimately produced equal amounts of seed.
2015	Wadden Sea	Summer storm in June damaged and entangled systems. No clear yield reduction followed.
2016	Oosterschelde Bay	Mass mortality event resulted in loss of most mussel seed in parts of the Bay, but overall Oosterschelde Bay production was not clearly affected.
2018	Wadden Sea	Unusual levels of fall-offs from net substrate caused by silt accumulation between mussels and substrate reduced yield. This did not clearly affect production.
2021 2022	All locations Wadden Sea	Heavy fouling with *Electra pilosa* causing problems with harvesting, resulting in reduced harvest per unit substrate and per unit area.

2.4.2. Variation in harvest efficiency

Despite years of development in seed collection techniques, harvest per unit substrate (kg m^{-1} (REq)) did not increase significantly (Figure 2.8) and no indicators were found in our analysis as to how this could be improved. Harvest per unit substrate fluctuated around 3 kg m^{-1} (REq) in all three areas; Oosterschelde Bay reached this level after cessation of the single dominant net-based production location that was producing lower levels of harvest per unit substrate until 2015, using a proprietary multi-net raft system (paragraph 2.2.2 and Figure 2.2d). The lower harvest per unit substrate of the raft systems does show that not all tested substrates have achieved the same level of performance. The disappearance of this system also exemplifies that less productive and less practical SMC systems were phased out by trial and error, to the extent that, after 10 years, only two viable systems remain. At the North Sea production location, high harvest per unit substrate levels of approximately 4.5 kg m^{-1} (REq) were reached in 2015 and 2017, but the mechanism behind this is not clear.

In terms of production per unit area (kg ha^{-1}), no indications of overstocking were found as this would have led to reduction at higher substrate densities (km (REq) ha^{-1}), which was not observed (Figure 2.10). No data are available to determine whether variation within the SMC systems occurs, such as reduced efficiency in the center of SMC clusters resulting from seston depletion (Strohmeier et al. 2005, Cranford et al. 2008, 2014). However, seston depletion is associated with low current speeds, where rope or net systems further reduce the current. Contrary to suspended mussel culture, which is usually found in sheltered

areas to avoid mussel fall-offs (Drapeau et al. 2006, Aure et al. 2007), seed collection for mussel bottom culture using SMCs occurs at exposed sites (maps in Capelle 2023) where current velocities are relatively high and can reach 1 m s^{-1} (Nienhuis & Smaal 1994a, Jiang et al. 2019).

At the substrate level, a lower seed density (# m^{-1} (REq)) resulted in a lower harvest in Oosterschelde Bay than in the Wadden Sea and the North Sea (Table 2.1). This was not due to differences in growth rate, since this was not found to differ between Oosterschelde Bay and Waddenzee. Between years, harvest per unit substrate was remarkably stable. Considering that larval supply and settlement were not limiting (Zhao et al., *submitted*), this stability suggests environmental factors as driving forces of the lower numerical density and thus lower harvest per unit substrate in Oosterschelde Bay. However, it is not clear which environmental factors are responsible. Oosterschelde Bay is a semi-closed off basin, in contrast to the North Sea and Wadden Sea. The Wadden Sea is an open and much more dynamic environment than Oosterschelde Bay, with greater current velocities, greater food concentrations, but lower food quality (Capelle et al. 2021) and the North Sea SMC location is even more physically exposed than the Wadden Sea. In the North Sea we found higher densities of mussels, with similar sizes as in Oosterschelde Bay or the Wadden Sea. Food availability is higher in the North Sea than in Oosterschelde Bay (Smaal & van Stralen 1990) and probably also in the Wadden Sea, which suggests that not only space, but also food availability, modulates density on the SMCs.

2.4.3. Density-dependent growth

The allometric relation between mussel biomass and mussel density (# m^{-1} (REq), Figure 2.5) corresponds with the occurrence of density-dependent growth on the ropes, which is expected, because the availability of substrate is limited (Fréchette et al. 1992, Guiñez 2005). Growth rate was inversely related to density per unit substrate, and density per unit substrate was positively related to harvest per unit substrate (Figure 2.4), suggesting that competition for space and / or for food at the substrate level increases with growth rate.

2.4.4. Self-thinning

We complemented the 2010 to 2022 SMC seed production data set with observations at the Galgeplaat SMC location (Figure 2.6), where we observed mussel growth on culture ropes throughout a full mussel seed growing season. In the available literature, the development of densities, numbers, and biomass are not well described in the early stages of growth. For example, although Lachance-Bernard et al. (2010) argued that smaller mussels should be included in the study of self-thinning processes on mussel longlines because this captures more of the high mortality rates in early life, the authors still implemented a 5 mm shell length cutoff to accommodate methodological constraints in quantifying small specimens. In contrast, we were able to study the process from a much

smaller lower shell length limit of 0.375 mm. Based on the trajectory of mussel density on the ropes we distinguish three apparent developmental phases (indicated by vertical lines in Figure 2.6**)**: a first phase in which density increased, a second phase in which density decreased, and a third phase in which density stabilized, accompanied by an accelerating biomass increase and a comparatively slower shell length growth. Numerical reduction, and thus self-thinning, dominated in the second phase. This phase corresponded to a mean shell length of around 2.3 mm (day 68 from SMC deployment, 11 July) to 11.6 mm (day 97, 9 August) (van Broekhoven et al. 2014). Our data do not permit to distinguish between possible mechanisms of self-thinning. South et al. (2020), investigated *Perna canaliculus* seeded on ropes from a shell length of around 2 mm, a similar size as the start of the second phase in our data. These authors reported a combination of mortality, secondary settlement, and a proportion with an unknown fate. In both studies, the majority of mussels in this small size range were lost: 76% in our study and up to 85% in South et al., (2020). The general sequence observed on ropes can be expected to also take place on nets since these consist of ropes. We do not expect the lack of perpendicular filaments on nets, which are typically abundant on SMC ropes, to result in fundamentally different dynamics. A possible difference might occur if ropes and nets would be left in the water for extended periods of time, and fall-off of aggregations formed by mussels and associated species and organic material starts to occur. On nets, mats can form along a two-dimensional plane which does not exist on ropes. However, harvest of SMCs is typically aimed to take place before substantial fall-offs occur. The self-thinning phase in our study occurred prior to the phase in which most of the biomass was generated. This latter phase presumably contributed most to the observed density-dependent growth (paragraph 4.3). In other words, the outcome of apparent competition for space and / or for food shifted from high mussel seed losses to growth reduction during the course of the second half of the growth season.

2.4.5. Improving commercial production

Van Oostenbrugge et al. (2018) estimated that the production cost of SMC mussel seed is five to six times greater than seed from wild capture fishery. Furthermore, SMCs also require substantially more labor, which has forced a greater degree of cooperation between companies (Van Oostenbrugge et al. 2018). Overall, the ongoing transition from wild capture fishery to SMCs has resulted in higher cost for seed provisioning, which has created a need for cost reduction to maintain a profitable operation.

Since SMC production per unit substrate (kg m^{-1} (REq)) does not appear to be amenable to improvement, and because production per unit area (kg ha^{-1}) did not show signs of overstocking, the logical avenue to increase production would be to increase substrate density per unit area to optimize the use of available space.

Other efficiencies could be sought in reducing processing effort via technical innovations. The main distinction between prevailing SMC implementations is between ropes and nets. Ropes provide a denser substrate than nets and result in greater yield per unit area. However, nets are less labor-intensive to use. Nets are bound up on the floaters when not in use, and when anchored at the start of the SMC season, the nets are simply rolled down. Ropes, on the other hand, are presently manually bound to a main line which is attached to floaters. Pilots using robotics technology have taken place. These pilots consisted of a platform that could move over an SMC system and that automatically installed and harvested the ropes (Brouwer et al. 2015b a). However, thus far this system has not been successful in marine conditions, due to biofouling problems.

Furthermore, to optimize the contribution of SMCs as a seed provisioning service for mussel culture, research should be directed to reducing the large post-seeding mortalities of collector seed, thought to relate to seeding practices, in order to make more efficient use of the resource (Capelle et al. 2016, Jansen et al. 2023).

2.5. Conclusions

Twelve years of production data show that SMCs can be a robust alternative seed provisioning resource for mussel culture compared to wild capture fishery: SMCs are more reliable yearly, and SMC seed quality is comparable to wild-caught seed. Substrate production efficiency (kg m^{-1} (REq)) fluctuated around a similar level between areas, and therefore did not appear to be amenable to further improvement. Plot production efficiency showed no signs of approaching a maximum, which suggests that going forward, production gains could potentially be made by increasing substrate density (kg ha^{-1}) above current levels. In addition, given the greater overall costs associated with SMCs compared to seed fishery, operating cost efficiencies and post-seeding mortality reduction are priority areas for further research.

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CHAPTER 3

Feedbacks from filter feeders: review on the role of mussels in cycling and storage of nutrients

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Abstract

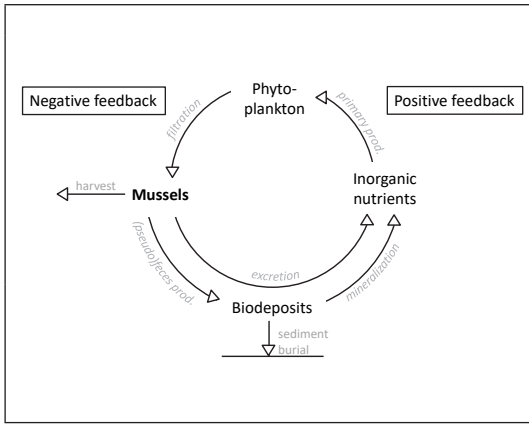
Cultured and wild bivalve stocks provide ecosystem services through regulation of nutrient dynamics; both by regeneration of nutrients that become available again for phytoplankton production (positive feedback), and by extraction of nutrients through filtration and storage in tissue (negative feedback). Consequently, bivalves may fulfil a role in water quality management. The magnitude of regulating services by filter feeding bivalves varies between coastal ecosystems. This review uses the blue mussel as a model species and evaluates how cultured mussel stocks regulate nutrient dynamics for ecosystems that vary in trophic state. We thereby examine (*i*) the eco-physiological response of mussels, and (*ii*) the positive and negative feedback mechanisms between mussel stocks and the surrounding ecosystem. It was found that despite differences in eco-physiological rates, the proportion of nutrients regenerated was similar between (deep) nutrient-poor and (shallow) nutrient-rich areas. Of the filtered nutrients, 40-50% is regenerated and thus made available again for phytoplankton growth, and 10-50% of the filtered nutrients is stored in tissue and could be removed from the system by harvest. A priori, we inferred that as a consequence of low background nutrient levels, mussels would potentially have a larger effect on ecosystem functioning in nutrient-poor systems and/or seasons. However, this review showed that due to the physical characteristics (volume, water residence time) and low mussel densities in nutrient-poor systems, the effects were lower for these sites, while estimates were more profound in shallow nutrient-rich areas with more intensive aquaculture activities, especially in terms of the negative feedback mechanisms (filtration intensity). A specific case is formed by suspended spat collector farms, which result in a high load of mussels during summer. During this season, the potential for a positive feedback via nutrient regeneration warrants particular attention, because phytoplankton may be nutrient limited.

3.1. Introduction

Suspension-feeding bivalves have the potential to influence ecosystem functioning due to their eco-physiological responses and role in nutrient cycling (Dame 1996, Newell 2004). Filtration by bivalves may depress phytoplankton biomass, while at the same time nutrient regeneration by bivalves may stimulate phytoplankton production (Asmus & Asmus 1991, Prins et al. 1995, Cranford et al. 2011). These processes are regarded as the positive and negative feedback mechanisms of bivalves via phytoplankton populations (Dame 1996). The capacity to influence ecosystem functioning is particularly evident in areas with concentrated bivalve communities (Smaal & Prins 1993, Dame & Prins 1998), such as in aquaculture settings. Mussels dominate bivalve production in many regions (see Wijsman et al. 2019), hence this paper uses the blue mussel *Mytilus* spp. as model species to discuss the role of bivalve cultivation in nutrient cycling. Whether the feedback processes contribute to a desirable regulation of the system (service) or results in an undesirable effect (impact) depends on the environmental characteristics of a site and the scale of culture activities (Newell 2004). Most mussel cultivation sites are situated in nutrient-rich coastal areas that are influenced by river run-off, thereby taking advantage of high primary production rates to achieve rapid growth (Saxby 2002), yet commercial mussel cultivation does exist in oligotrophic ecosystems (Strohmeier et al. 2008, Brigolin et al. 2009). Such differences in ecosystem characteristics indicate that the same process in some systems can be regarded as a regulating ecosystem service while in other systems it is rather a negative ecosystem impact (see Figure 3.1). Under excessive nutrient availability, filtration of phytoplankton (negative feedback) may help to prevent or overcome eutrophication problems (particularly when coupled with harvesting of the biomass), wherefore this has been recognized as an ecosystem service of mussel aquaculture (Lindahl et al. 2005, Petersen et al. 2014, Ferreira et al. 2014). At the same time, in oligotrophic (nutrient-poor) systems mussel filtration can impose an ecosystem impact when it leads to depletion of phytoplankton and carrying capacity is exceeded. In these nutrient-poor systems, regeneration of nutrients is considered an ecosystem service as it may boost primary production, and result in higher mussel yields.

This paper aims to evaluate the regulating functions of mussel aquaculture through the two major pathways (filtration, nutrient regeneration) as a function of ecosystem trophic status (from nutrient-poor, to nutrient-rich). The *first section* provides a review of eco-physiological rates and discusses whether and how the functioning of mussels differs between eutrophic and oligotrophic conditions. Specific emphasis is thereby given to differences between measurements on individuals compared to entire communities. Physiological processes are generally studied at the level of the organism (Dame 1996, Gosling 2003), but extrapolating "average" individual rates to yield population estimates neglects community specific effects such as refiltration or metabolic activity of associated

fauna and microbial decomposition of organic material on mussel cultures (Richard et al. 2006, Jansen et al. 2011). The *second section* of this review evaluates interactions between mussel cultivation and the surrounding ecosystem with particular reference to ecosystem services and impacts. To this end, the positive and negative feedback mechanisms of mussel culture on phytoplankton are compared between areas spanning a gradient from nutrient-poor to nutrient-rich. *At last*, perspectives on the role of mussel cultivation on nutrient cycling are provided.



	System nutrient status	
	Rich	**Poor/limited**
Service	Eutrophication control by nutrient removal through filtration and harvest	Stimulation of primary production through nutrient regeneration (may in turn sustain higher filter feeding stock)
Impact	Benthic degradation through biodeposit accumulation	Competition with other filter-feeding species (exceeding carrying capacity)

Figure 3.1. Feedback loop of filter feeder activity on filter feeder growth linked to potential ecosystem services and ecosystem impacts for nutrient-rich and nutrient-poor systems

3.2. Mussels as intermediaries in nutrient cycling (eco-physiology)

The major eco-physiological pathways in which mussels interact with coastal nutrient cycling are; *(i)* filtration of seston (particulate nutrients) from the water column, *(ii)* nutrient storage in mussel tissue (assimilation), and growth, *(iii)* excretion of inorganic metabolic waste products, and *(iv)* production and mineralization of biodeposits (reviews by Prins et al. 1998, Newell 2004). 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 their physiological responses. This physiological plasticity can vary between populations, among individuals of the same population, and due to seasonal changes and variation in the natural environment (Hawkins & Bayne 1992, Shumway 2011). In the following section eco-physiological rates are reviewed for mussels as a function of trophic status of the culture environment, thereby specifically addressing differences between individual and community scale measurements.

3.2.1. Filtration

Bivalve feeding has been extensively studied at the level of individual animals (see review by Cranford et al. 2011). Van Broekhoven et al. (2014; Chapter 4 in this thesis; see Table 3.1) showed that mussel spat in the Oosterschelde bay can display some of the highest clearance rates reported for this species. Strohmeier et al. (Strohmeier et al. 2009, 2015) showed that mussels can also display high feeding rates and high net absorption efficiencies under oligotrophic and low seston conditions despite contradicting feeding paradigms for mussels; Table 3.1 and the review by Cranford et al. (2011) show that clearance rates reported for individual mussels under oligotrophic conditions in Norway were among the highest reported for adults of this species. Jansen (Jansen 2012) confirmed high feeding rates for individual animals under oligotrophic conditions, but also demonstrated that community-scale rates under field conditions were 2 to 3 times lower (Table 3.1). Prins et al. (Prins et al. 1996) showed that community estimates for benthic mussel beds in eutrophic cultivation areas were also lower than measurements on individuals, and Jacobs et al. (2015) concluded that low feeding rates measured on suspended spat collector communities in the Wadden Sea were the result of refiltration within the culture community. Others have also hypothesized that lower community-scale clearance rates could be related to crowding affecting water exchange and/or refiltration (Fréchette et al. 1992, Cranford et al. 2011). While the accuracy of various methods for determination of clearance rates for individuals have been the subject of debate during the last decade (Riisgard 2001, 2004, Petersen et al. 2004, Cranford et al. 2011), there is good evidence for differences in feeding rates between individuals and communities that merit further study.

3.2.2. Nutrient storage in mussel tissue

Surprisingly few studies report on the nutrient composition of mussel tissue, but the concentrations reported seem to correspond between the different cultivation areas and life stages (Table 3.2). These estimates do not account for nutrient storage in byssus or shell (Hawkins & Bayne 1985). Seasonal changes in nutrient composition are primarily driven by endogenous processes, and seasonal nutrient composition as well as metabolic requirements associated with the reproductive cycle are similar for mussels under both nutrient-poor (Jansen et al. 2012a) and nutrient-rich conditions (Kuenzler 1961, Hawkins & Bayne 1985, Smaal & Vonck 1997).

Table 3.1. Clearance rates in mussel cultivation areas. Data were standardized to L g^{-1} tissue DW h^{-1}. Weight conversion factors reported by Ricciardi and Bourget (1998) were applied. Values are presented as mean (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 bay	NL	*M. edulis*	Natural seawater	(1.4-2.8)	3
Oosterschelde bay	NL	*M. edulis*	Natural + *P tricornutum*	1.5 (0.3-3.5)	4
Oosterschelde bay	NL	*M. edulis*	Natural seawater	2.6 (1.3-3.5)	5
Oosterschelde bay	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
Newfoundland	CA	*M. edulis*	Natural seawater	(1.5-2.0)	9
Nova Scotia	CA	*M. edulis*	Natural seawater	(1.0-8.0)	10
Newfoundland	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
Wadden Sea	NL	*M. edulis*	Natural seawater	1.5 (0.7-1.9)	14
Oosterschelde bay	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
Oosterschelde bay	NL	*M. edulis* spat	Natural seawater	(2.4-30.7)	16
Wadden Sea	NL	*M. edulis* spat	Natural seawater	0.8	17
Havre-aux-Maisons	CA	*M. edulis*	Natural seawater	(1.7-6.3)	18

1 (Jansen 2012); 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 (van Broekhoven et al. 2014); 17 (Jacobs et al. 2015) ; 18 (Trottet et al. 2008a)

Table 3.2. Nutrient composition in mussel tissue in mussel cultivation areas. Data were standardized to mg element g^{-1} tissue DW. Weight conversion factors by Ricciardi and Bourget (1998) were applied. Values are presented as mean (minimum - maximum), and empty cells indicate that concentrations were not determined. Country codes given in Table 3.1

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 bay	NL	*M. edulis*	448 *(113-623)*	102 *(68-126)*	7 *(5-12)*	3
Oosterschelde bay	NL	*M. edulis* spat		97 (92-104)	7.5 (6.6-8.4)	4
Ria de Arosa	ESP	*M. galloprovincialis*	448			5
Western Australia	AU	*M. edulis*	333	101	4	6
Mahurangi Harb.	NZ	*A. zelandica*	396	71		7

1 (Jansen et al. 2012a); 2 (Hawkins et al. 1985); 3 (Smaal & Vonck 1997); 4 van Broekhoven (unpublished data); 5 (Tenore et al. 1982); 6 (Vink & Atkinson 1985); 7 (Gibbs et al. 2005)

3.2.3. Excretion of inorganic nutrients

Respiration and nutrient excretion rates of individual mussels measured under nutrient-poor conditions (Table 3.3) are within the range reported for nutrient-rich areas (Table 3.3; see also Burkholder & Shumway 2011), albeit toward the lower end. The slightly lower rates are likely related to the relatively cold and oligotrophic Norwegian fjords, as respiration and excretion rates of mussels are influenced by fluctuations in temperature (Widdows & Bayne 1971, Leblanc et al. 2003) and food supply (Bayne et al. 1993, Lutz-Collins et al. 2009, Jansen et al. 2012a). Eco-physiological models are often used to integrate responses of individual mussels with fluctuations in environmental conditions (Beadman et al. 2002, Dowd 2005). Jansen (Jansen 2012) applied and validated a model normally used to simulate mussel responses in nutrient-rich areas (Filgueira & Grant 2009), and found that the model accurately predicted excretion rates under nutrient-poor conditions. This demonstrates that metabolic responses in mussels are comparable between cultivation areas of different trophic status, as the model is based on generic equations.

Mussel cultures are complex community structures, which besides the mussels include bacteria, epifauna, epiflora, and trapped biodeposits, which also contribute to nutrient exchange rates (Richard et al. 2006, 2007b). The contribution of decomposing biodeposits (see also next section) to community nutrient release rates is particularly evident in the case of bottom cultures, where nearly all egested material is trapped in the community matrix. Indeed, the relatively high release rates for nutrients from bottom cultures are primarily attributed to decomposition of biodeposits (Asmus et al. 1990, Prins & Smaal 1994). Nutrient recycling from the organic matter trapped in suspended cultures is relatively low (Jansen 2012), which seems reasonable as the majority of biodeposits sink to the seafloor resulting in lower biodeposits on suspended mussel culture compared to

benthic mussel cultures. Van Broekhoven et al. (van Broekhoven et al. 2014; Chapter 4 in this thesis) concludes that the combined activity of biodeposit decomposition and fauna on mussel spat collectors are either very small or scaled proportionally with mussel biomass or activity, whilst respiration and nutrient release rates are likely dominated by mussel spat activity. Richard et al. (2006, 2007b), on the other hand, relate the high nitrate and nitrite fluxes of suspended mussel cultures in Canada to decomposition of organic material trapped in the community matrices.

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 (Cayer et al. 1999, Khalaman 2001, Richard et al. 2006, Lutz-Collins et al. 2009, Jansen et al. 2011). Jansen (Jansen 2012) finds that during periods of high fouling abundance, ascidian (*Ciona intestinalis*) metabolism contributes up to 18% of total nitrogen released from suspended mussel culture communities. The contribution of the associated fauna to nutrient cycling cannot be ignored. This is also acknowledged by Tang et al. (2011) who estimate that tissue carbon content of fouling ascidians is approximately 6.4% of the carbon production in scallops in Sungo Bay (China). A full understanding and prediction of nutrient regeneration by mussel culture communities requires more information on faunal growth, abundance, and metabolic dynamics within and across cultivation areas.

3.2.4. Biodeposit release and mineralisation

Biodeposit production represents a significant pathway in bivalve nutrient cycling (Kuenzler 1961, Prins & Smaal 1994, Cranford et al. 2007). Biodeposition rates under oligotrophic conditions, as measured in the laboratory for individual mussels, are in range with, but not at the maximum rates reported for other areas, whereas the organic matter content (OM) is relatively high (Table 3.4). The latter is likely related to high OM in the food source (~60-70%; Strohmeier et al. 2009, 2015) and the fact that pseudofeces production is mostly absent under oligotrophic conditions. Seasonal fluctuations in biodeposition rates seem related to changes in food quantity and quality, rather than to temperature (Jansen et al. 2012b). This is consistent with Strohmeier et al. (2009), who suggest that the feeding response to low food concentrations (i.e. oligotrophic conditions) is likely the determining factor for total ingestion, rather than temperature.

Table 3.3. Respiration and inorganic nutrient release rates of different species of mussels and culture types in mussel cultivation areas. Rates were standardized to µmol g^{-1} tissue DW h^{-1}. Where needed weight conversion factors by Ricciardi and Bourget (1998) were used. Values are presented as mean (minimum - maximum), and empty cells indicate that rates were not determined. Country codes are given in Table 3.1.

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
Wadden Sea	NL	*M. edulis*	3-24	(10.0-70.0)				4
Oosterschelde bay	NL	*M. edulis*	June & Sept		(0.8-5.0)	(0.02-0.17)		5
Oosterschelde bay	NL	*M. edulis*	5-18	21.3 (10.3-36.0)	1.0 (0.2-3.1)	0.07 (0- 0.13)	-	6
Oosterschelde bay	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
Newfoundland	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

Table 3.3. Continued

Amherst Basin	CA	*M. edulis*	20	8.0	(3.3-12.1)	(0.7-2.5)					20
Nova Scotia	CA	*M. edulis*	0-15	31.8	(22.3-38.7)	1.4	(0.5-2.5)				21
Beatrix Bay	NZ	*P. canaliculus*	11-17			(1.6-4.4)					22
Western Australia	AU	*M. edulis*	15-20	6.7				0.02			23
Measurements on communities (benthic mussel beds)											
Baltic		*M. edulis*				(0.1-3.5)		(0.01-0.50)			24
Sylt	DEN	*M. edulis*	13-19			1.2	*(0.02-5.0)*				25
South	DEN	*M. edulis*	1-18		(0-12.5)	(0.1-3.2)		(0.10-0.53)			26
Wadden Sea	GER	*M. edulis*				1.2	(0-5.0)	0.10	(0- 0.60)	0.6 (<0 -1.4)	27
Wadden Sea	NL	*M. edulis*	June-Sept			4.4				2.5	28
Oosterschelde bay	NL	*M. edulis*				5.6		1.70		2.3	28
Oosterschelde bay	NL	*M. edulis*	June & Sept			(1.7-14.4)		(0.08-0.50)			4
Oosterschelde bay	NL	*M. edulis*				(0.9-15.8)		(0.03-0.68)		(<0 -3.0)	29
Marennes-Oleron	FR	*M. edulis*	M-O-J-O			(0 -7.3)					30
Narragansett Bay	USA	*M. edulis*	15			3.1					31
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	3,32
Åfjord	NO	*M. edulis*	12	17.1		1.1		0.11			3
Oosterschelde bay	NL	*M. edulis* spat	18-21		(72-381)	(5-70)		(0.12-6.4)		(0.1-5.3)	33
Sacca di Goro	IT	*M. edulis*	8-27		(25.1-26.9)	(3.2-7.6)					34
Great Entry Lagoon	CA	*M. edulis*	16-19		(53.0-92.4)	(1.7-11.6)		(0.22-0.34)		(0.0-0.7)	35

1 (Jansen et al. 2012a); 2 (Strohmeier 2009); 3(Jansen 2012); 4 (Devooys 1976); 5(Prins & Smaal 1994); 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 (James et al. 2001); 23 (Vink & Atkinson 1985); 24 (Kautsky & Wallentinus 1980); 25 (Asmus et al. 1990); 26 (Schluter & Josefsen 1994); 27 (Asmus et al. 1990); 28 (Dame et al. 1991); 29 (Prins & Smaal 1990); 30 (Smaal & Zurburg 1997); 31 (Nixon et al. 1976); 32 (Jansen et al. 2011); 33 (van Broekhoven et al. 2014); 34 (Nizzoli et al. 2006); 35 (Richard et al. 2006).

Table 3.4. Biodeposition and biodeposit composition in mussel cultivation areas. Data were standardized to mg DW biodeposit g^{-1} tissue DW d^{-1} (biodeposition rates), percentage (organic matter content), and mg element g^{-1} biodeposit DW (organic nutrient content). Where needed weight conversion factors by Ricciardi and Bourget (1998) were used. Values are presented as mean (minimum - maximum), and empty cells indicate that rates were not determined. Country codes are given in Table 3.1

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	135 (62-194)	15 (7-23)	1.3 (0.8-1.7)		1
Askö, Baltic	SW	*M. edulis*	31 (7-104)	19	129 (50-200)	15 (8-21)	1.9 (1.0-3.0)		2
Oosterschelde bay	NL	*M. edulis* spat	Feces	20	52	4.8	1.4	42	3
			Pseudofeces	26	55	5.4	1.4	31	
Bedford Basin	CA	*M. edulis*	(0-20)	(30-70)					4
Mahone Bay	CA	*M. edulis*	(0-80)	(10-70)					4
Great Entry Lagoon	CA	*M. edulis*	54 (18-114)	22 (20-25)					5
Logy Bay (NF)	CA	*M. modiolus*	5 (1-8)	17 (13-23)	69 (47-103)	8 (5-12)		205 (100-335)	6
Queele Estuary	CH	*M. chilensis*		21	60	4			7
Firth of Thames	NZ	*P. canaliculus*		10	25	3			8
Mutsu Bay	JP	*M. edulis*	(6-116)						9

1 (Jansen et al. 2012a, Jansen et al. 2012b); 2 (Kautsky & Evans 1987); 3(van Broekhoven et al. 2015); 4 (Cranford & Hill 1999); 5 (Callier et al. 2006); 6 (Navarro & Thompson 1997; during springbloom conditions); 7 (Jaramillo et al. 1992); 8 (Giles & Pilditch 2006); 9 (Tsuchiya 1980)

Although measurements of mussel biodeposits 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) and more recently reported by Jansen et al. (2012b) and van Broekhoven et al. (2015; Chapter 5 in this thesis). Nutrient concentrations in biodeposits depend on the concentration and type of diet the mussels feed on (Miller et al. 2002, Giles & Pilditch 2006) and therefore varies between seasons (Jansen et al. 2012b) and systems (Table 3.4). It has been suggested that mineralization rates of biodeposits are related to the presence of resident gut bacteria that can be voided from the mussel's digestive system along with the faecal pellets (Harris 1993). However, mineralization rates of fresh biodeposits increase considerably after an initial lag phase of one or two days (Fabiano et al. 1994, Carlsson et al. 2010, van Broekhoven et al. 2015), suggesting that a period of microbial growth may also be due to additional colonization by external microbes during the lag phase (Canfield et al. 2005). Since mineralization rates depend on the presence of microbes on either the benthic or the suspended mussel culture (Giles & Pilditch 2006, Carlsson et al. 2010, Jansen et al. 2012b), decomposition will be more rapid than in the water phase (van Broekhoven et al. 2015). The proportion of carbon and nitrogen decomposed as a function of available (labile) organic nutrients in biodeposits is relatively similar between oligotrophic (Jansen et al. 2012b) and eutrophic environments (Giles & Pilditch 2006, Carlsson et al. 2010, van Broekhoven et al. 2015) (Table 3.5). However, under oligotrophic conditions, the amount of nutrient released per gram biodeposit will be higher due to the higher concentrations of nutrients in the mussel biodeposits (Table 3.4). Phosphorus mineralization patterns are inconclusive among studies, likely as a result of the potential for phosphate to bind to sediment and other organic material (Sundby et al. 1992). Profound seasonal differences (up to a factor 80) are observed for silicon release rates by Jansen et al. (Jansen et al. 2012b), and release is assumed to be high when mussel food contains a large fraction of diatoms (Navarro & Thompson 1997). Proportional silicon mineralization rates are 1.4 times higher for feces than pseudofeces, while proportional nitrogen and phosphate mineralization rates were similar for feces and pseudofeces (van Broekhoven et al. 2015). Hypothesised causes are breakdown of the organic matrix by digestive bacterial activity (Bidle & Azam 1999), selection during the feeding process for less recalcitrant diatom frustules, and fragmentation of diatom frustules during the digestive process (as speculated by Dame et al. 1991). Since the proportion of pseudofeces rises with increasing food concentration above a certain level (Foster-Smith 1975, Tsuchiya 1980), the role of mussels in terms of Si regeneration may be proportionally greater at lower food levels (assuming a similar food composition).

Table 3.5. Biodeposit remineralization rates in mussel cultivation areas. Data were standardized either to release rate per g biodeposit DW per day or to fraction of initial nutrient content in the biodeposits (e.g. %=TAN/PON*100) for feces or pseudofeces ('biodeposit' indicates that it was unknown whether feces or a mix of (pseudo)feces was incubated). Values are presented as mean (minimum - maximum), and empty cells indicate that rates were not determined. Country codes are given in Table 3.1.

Area	Country	Species	Type	Temp (°C)	Unit	CO_2 release	TAN release	PO_4 release	$Si(OH)_4$ release	Ref.
Austevoll	NO	*M. edulis*	feces	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)			
Oosterschelde bay	NL	*M. edulis spat*	feces	20	µmol g^{-1} d^{-1}	2.5				2
			pseudofeces		µmol g^{-1} d^{-1}	2.7				
			feces		%		13.1%	8.7%		
			pseudofeces		%		12.4%	7.9%		
Great Entry Lagoon	CA	*M. edulis*	biodeposit	Jun-Aug	mmol g^{-1} d^{-1}	(max 4.5)	(max 0.3)	(max 0.02)	(max 1.0)	3
Roskilde & Limfjord	DEN	*M. edulis*	biodeposit	8-10	%	(25-38%)				4
Firth of Thames	NZ	*P. canaliculus*	biodeposit	20	%	40%	18%			5

1 (Jansen et al. 2012b); 2 (van Broekhoven et al. 2015); 3 (recalculated from Callier et al. 2009); 4 (Carlsson et al. 2010); 5 (Giles & Pilditch 2006)

3.3. Ecosystem effects of nutrient cycling by mussels

The previous section demonstrated that mussels contribute to nutrient cycling by translocation, transformation and remineralization of nutrients. These processes related to the mussel's physiology interact with nutrient cycling in coastal ecosystems through various feedback systems influencing primary production (see reviews by Prins et al. 1998, Newell 2004). Consequently, intensive cultivation of mussels will affect the ecosystem; for example, by altering the carrying capacity (Smaal & Heral 1998, Grant & Filgueira 2011). The feeding activity of mussel communities may influence the abundance of phytoplankton and thereby inhibit primary production ('top-down' pathway or negative feedback). Furthermore, Cranford et al. (2009) reported a shift towards a phytoplankton population dominated by picophytoplankton in bays with high densities of mussel cultivation and related this to high grazing activity of the cultured stocks. Meanwhile, mussel excretion and mineralisation of biodeposits result in the regeneration of nutrients, which may stimulate primary production ('bottom-up' pathway or positive feedback). Not all ingested nutrients are regenerated in a short cycle; a part is retained by the mussel community or in a non-decomposed fraction of biodeposits, and a part may be permanently removed from the system, e.g. when mussels are harvested. Mussel communities can therefore act as a 'source' and as a 'sink' for nutrients within the ecosystem. The specific pathways contributing to sinks/sources depend on physical features (e.g. depth) of the area and the culture type applied (Table 3.6). Given that phytoplankton use nutrients in specific proportions (Redfield ratio; Redfield et al. 1963), the 'bottom-up' stimulation by bivalve nutrient regeneration is influenced by both nutrient availability and stoichiometry of regenerated nutrients. It has been argued that both feedback control mechanisms on phytoplankton can stabilize ecosystems (Herman & Scholten 1990) with 'top-down' and 'bottom-up' pathways occurring simultaneously. This section evaluates the pathways and magnitude of the feedback mechanisms in different mussel cultivation areas, and assesses if trophic status of the ecosystem is an important driver for defining ecosystem services and ecosystem impacts.

Table 3.6. Nutrient source and sink processes by water depth of the system and mussel culture type.

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

3.3.1. Physical and environmental characteristics of mussel cultivation areas

The extent to which bivalves influence the ecosystem is largely defined by physical and environmental conditions (Newell 2004), which vary considerably among bivalve cultivation areas (Table 3.7). The majority of mussel 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 several months. Oligotrophic fjord systems are exceptional when compared to "coastal plain estuaries" due to the large depths (100-1000 m). Many Norwegian fjords have a sill at the mouth of the fjord which limits renewal of the deepwater basin, resulting in relatively long residence times in terms of months and years for the whole system, whereas residence times are much shorter in terms of days and weeks for the upper and intermediate layers.

Table 3.7. Physical characteristics of mussel cultivation areas. Country codes are given in Table 3.1

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 bay	NL	Estuary	9	2740	40 (10-150)	5,6
Wadden Sea	NL	Bay	3	4020	10 (5-15)	6
Carlingford Louch	IR	Estuary	(35 max)	460	14-26	7
Louch Foyle	IR	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)

Annual primary production rates vary between 73 and 1245 g C m^{-2} y^{-1} for the different mussel cultivation areas, with rates reported for Norwegian fjord systems in the lower region (Table 3.8). Background nutrient levels in most areas are influenced by anthropogenic nutrient sources, with the exception of most Norwegian fjord systems (Aksnes et al. 1989). Wassmann (Wassmann 2005) shows that estuaries and coastal ecosystems are now the most nutrient-enriched ecosystems in the world, which he attributes 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 most recent decades resulted in high phytoplankton biomass levels, sustaining high densities of mussels up to levels causing hypoxia-induced mortality (Christiansen et al. 2006). The highest primary production rates are reported for 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 (Benguela current 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 (Saxby 2002, Figueiras et al. 2002).

The pathways for 'nutrient regeneration' differ between shallow and deep systems as a consequence of depth, stratification, mixing of the water column, and on the resulting presence or absence of benthic-pelagic coupling (see also Table 3.6). Benthic nutrient regeneration can play an important role in shallow coastal ecosystems with well-mixed water columns, as it may provide up to 80% of the nutrients required for primary production (Jensen et al. 1990, Zeldis 2005, Giles 2006). In contrast, benthic regeneration does not contribute to the nutrient pools in the euphotic zone of Norwegian fjords when the water column is stratified (Aure et al. 1996, Asplin et al. 1999). Euphotic zones of fjord systems are nutrient-limited for extended periods of the year (Paasche & Erga 1988, Sætre 2007), resulting in low Chl *a* concentrations (Erga 1989, Aure et al. 2007).

3.3.2. Nutrient sinks and sources

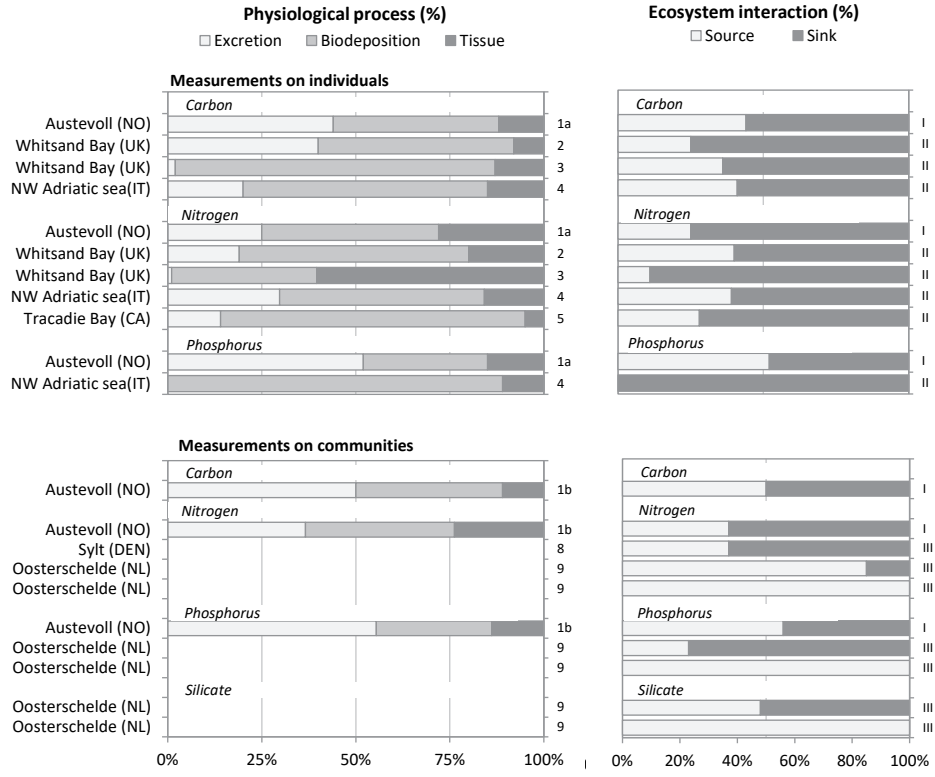
Physiological processes such as inorganic nutrient excretion, biodeposition (and subsequent remineralisation processes), and growth of tissue material (see also previous section) interact with physical features of the area and the culture type applied (Table 3.6) to drive the fraction of ingested nutrients that becomes regenerated, and thus becomes available as a source of nutrients to the ecosystem. Figure 3.2 (left panels) 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 giving 100%. It is thereby assumed that the sum of the three processes equals ingestion (in accordance with Kreeger et al. 1995). Under oligotrophic conditions, less than 50% of the captured nutrients are expelled with biodeposits, which is lower than the other areas where more than 50% and, in certain cases, up to 80% of the ingested nutrients are expelled with biodeposits (Figure 3.2). The right hand panels of Figure 3.2 present the fractions of ingested nutrients either recycled as a source of nutrients, or retained or removed as sinks of nutrients (sum is 100%). Whether remineralisation of biodeposits acts as a source of nutrients available for phytoplankton growth depends on the system (Table 3.6). Excretion of inorganic nutrients always acts as a source, while nutrient removal when mussels are harvested is always considered a sink. Biodeposition can result in both nutrient sources and sinks, depending on interactions with benthic processes: nutrients are either returned to the water column, buried in the sediment, or released in gaseous form (N_2). In deep fjords, biodeposits sink to the seafloor and as a consequence of limited benthic-pelagic coupling it is assumed that remineralized nutrients will not be available for phytoplankton growth. The estimates presented in Figure 3.2 do not account for loss of mussels from the culture structures (Fréchette 2012), nor for nutrient storage in byssus or shell (Hawkins & Bayne 1985); so that harvest values will be either slightly over or underestimated.

Table 3.8. Biochemical characteristics of mussel cultivation areas. 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 mean (minimum - maximum). Country codes are given in Table 3.1.

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 bay	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

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
Newfoundland	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 2009, Jansen et al. 2012a); 2 (Aure et al. 2001, Aure et al. 2007, 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. 2010); 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).



1a (Jansen et al. 2012a); 2 (Hawkins & Bayne 1985) – June; 3 (Kreeger et al. 1995); 4 (Brigolin et al. 2009); 5 (Cranford et al. 2007); 1b (Jansen et al. 2011); 8 (Asmus et al. 1990); 9 (Prins & Smaal 1994)

Figure 3.2. Relative importance of physiological processes (left panels) and ecosystem interactions (right panels) for mussels (Mytilus spp.) across cultivation areas (left panels) for individual and community scale measurements. Data originates from budget analysis studies of which reference numbers are indicated on the secondary vertical axis in the left panels. Ecosystem interactions refer to the fraction of ingested nutrients which is either recycled and available for phytoplankton growth (source), or is permanently lost from the system (sink). The calculation of source and sink fractions takes account of the physical characteristics of the system under consideration (depth, benthic-pelagic coupling) and consequently the fate of remineralized biodeposits. The type of calculation applied to each system is indicated on the secondary vertical axis of the right panels, according to:
[I] Source = Excretion; Sink=Biodeposition + Tissue growth
[II] Source = Excretion + remineralization; Sink = Tissue growth + (Biodeposition – remineralization) (assuming mineralization rates of 32% for C, 17% for N, and 0% for P; see Table 3.5)
[III] Based on in situ measurements of uptake and release rates in benthic tunnels

Firstly, measurements are considered for individual mussels (Figure 3.2, upper panels). For on-bottom and suspended cultivation of mussels in shallow areas, benthic biodeposit decomposition has been shown to significantly contribute to total nutrient regeneration (Baudinet et al. 1990, Asmus et al. 1990, Prins & Smaal 1994, Hatcher et al. 1994, Giles et al. 2006, Richard et al. 2007b). Mineralization of biodeposits does not significantly contribute to the source of recycled nutrients in deep fjord systems, because the majority of nutrients sink to the seafloor and regenerated nutrients are not returned to the euphotic zone of fjord systems within short time intervals due to stratification of the water column. Combining nutrients released by biodeposit remineralisation with those released by direct excretion results in relatively similar 'source' values for carbon and nitrogen regeneration in oligotrophic fjords and shallow eutrophic areas. All regenerated carbon is assumed to contribute to the source of recycled nutrients. This assumption is reasonable for Norwegian fjord systems which are generally considered to be weak absorbers of atmospheric CO_2, whereas in some eutrophic estuaries CO_2 might be released to the atmosphere since these systems often have oversaturated pCO_2 levels (Frankignoulle et al. 1998). In these estuaries, release of CO_2 by eco-physiological processes represents a sink process, and values presented in Figure 3.2 might underestimate the carbon sink for these cases (see also Filgueira et al. 2019).

Secondly, measurements are considered for mussel communities (Figure 3.2, lower panels). Nutrient regeneration rates for suspended cultures are defined in a similar manner as for individuals (see subscript Figure 3.2). Regeneration by benthic communities is defined as the difference between uptake of organic material and release of inorganic nutrients, and has been determined using benthic tunnel measurements in the Oosterschelde bay (Netherlands) and Sylt (Denmark). A high degree of variability between measurements has been observed with occasionally higher release rates than uptake rates (source >100%), likely induced by mineralization of biodeposits or dead mussels trapped within 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), using similar benthic tunnel measurements, indicate that 66% of nitrogen and 8% of phosphorus taken up by the reef is regenerated as ammonia and phosphate, respectively. 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 that denitrification processes may lead to a loss of gaseous nitrogen from the system by the formation of N_2. The effects of bivalve cultures on denitrification rates have not been fully characterised (Newell 2004) and previous studies of sediments underlying suspended mussel cultures have been inconsistent, showing either increase (Kaspar et al. 1985, Giles et al. 2006) or decrease (Christensen et al. 2003).

3.3.3. Stoichiometry of regenerated nutrients

The previous section pointed out that mussel communities can act as a source of regenerated nutrients. The nutrients are regenerated in different proportions (stoichiometry), which may differ to varying degrees from the stoichiometry of the inorganic nutrient pool in the ambient water (Prins et al. 1998, Jansen et al. 2011). On a large scale, the average stoichiometric composition of phytoplankton is described by Redfield's ratio (Redfield et al. 1963; Redfield ratio 106C:16Si:16N:1P). However, the stoichiometric composition of individual phytoplankton species, and therefore their nutrient requirements, may deviate from this ratio (Falkowski 2000). Changes in stoichiometry of available inorganic nutrients may affect phytoplankton growth (Goldman et al. 1979), and in this way could potentially induce a shift in the composition of phytoplankton species.

Figure 3.3 presents dissolved inorganic N:P ratios in the water at various mussel cultivation areas, and for the purposes of this review we assume that ratios below Redfield's ratio (N:P=16) are indicative of more nitrogen-limited systems, whereas ratios above this ratio are indicative of more phosphorus-limited systems. Most of the mussel cultivation areas show a N:P ratio < 16, which is consistent with the common observation of nitrogen limitation in marine environments (Nixon et al. 1996). The assumption that phosphorus is generally sufficiently available in coastal waters (Nixon et al. 1996), does not seem to hold for all of the coastal waters used for shellfish cultivation; the Wadden Sea during spring bloom, 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).

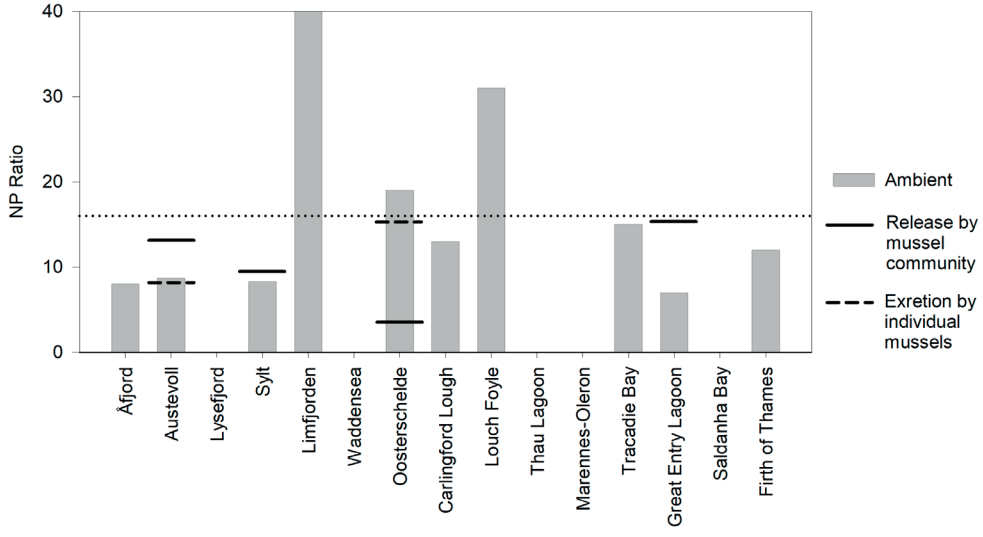


Figure 3.3. Annual N:P [DIN:DIP] stoichiometry in the water at various mussel cultivation areas (bars), with release rates measured for individual mussels (broken lines) and mussel communities (solid lines). Horizontal dotted line indicates the Redfield ratio (N:P=16). References are given in Table 3.3.

N:P ratios of regenerated nutrients determined for individual mussels and for mussel communities are presented in Figure 3.3 by broken and by solid lines, respectively. There are no cases where the N:P ratio of the net release by individual mussels or by mussel communities exceeds Redfield's ratio, indicating that mussel activity is not likely to increase the ratio of N:P in the water. In most cases the N:P ratios of the regenerated nutrients (lines) differ from the ambient water (bars). The N:P ratios of nutrients released by suspended mussel communities (Austevoll, Great Entry Lagoon) are higher than ratios of nutrients released by benthic communities (Oosterschelde bay, Sylt; Figure 3.3). In one case, the Oosterschelde bay in the Netherlands, measurements have been made for both suspended mussel communities and mussel beds. The suspended community released N and P in a ratio of approximately 7, whilst the ratio of N and P released from mussel beds was lower (Figure 3.3). Removal of nitrogen through denitrification processes has been suggested as a cause for the low N:P ratio measured in mussel beds (Asmus et al. 1990, Prins & Smaal 1994).

Measurements of phosphate dynamics over sediments underneath mussel farms have shown release in some cases (Baudinet et al. 1990, Souchu et al. 2001, Richard et al. 2007b), and an apparent balance or an uptake in others (Hatcher et al. 1994, Mazouni et al. 1996, Giles et al. 2006). Asmus et al. (1995) attributed differences in phosphorus fluxes to site-specific environmental characteristics. A balance or an uptake of phosphate can be related to the buffering capacity of sediments, caused by absorption of phosphate by iron hydroxides or calcite occurring in the oxidized surface layer of marine sediments (Sundby et al. 1992). This suggests that phosphate dynamics vary according to the location where decomposition takes place. Benthic mineralized phosphate may become trapped in the sediment, while pelagic mineralized phosphate is likely to become available in the water column.

Silicon does not play a role in physiology of mussels (Prins & Smaal 1994), and, therefore, all ingested silicon is expected to be egested with biodeposits. Decomposing biodeposits show high release rates of silicate (Jansen et al. 2012b, van Broekhoven et al. 2015; see also Table 3.5). In contrast to nitrogen and phosphorus, silicon mineralisation from biodeposits is thought to be driven primarily by chemical dissolution rather than microbial processing (van Broekhoven et al. 2015). In deep stratified systems, biodeposits (including all of the captured silicon, but not all of the carbon, nitrogen, and phosphorus) are transported to the bottom of the basin and regenerated nutrients, including silicon, do not become regenerated in the euphotic zone. This may potentially suppress the development of siliceous phytoplankton diatoms and favour development of non-siliceous phytoplankton such as flagellates and dinoflagellates (Turner et al. 1998). In shallow estuaries, biodeposit remineralization contributes to the pool of regenerated silicate (Asmus et al. 1990, Prins & Smaal 1994), which reduces the potential of silicate limitation in those areas (Prins et al. 1995).

Table 3.9. Bivalve density in mussel cultivation areas. Density is expressed in terms of harvest rate (ton WW y^{-1}), and in standing stock for the whole system (ton tissue DW), per unit area (g tissue DW m^{-2}) and per unit volume (g tissue DW m^{-3}). For the Norwegian fjords, only the water volume above the sill was used in the calculations. Asterisk (*) indicates that standing stock was reconstructed based on harvest, length of the production cycle and WW/DW conversion factors by Ricciardi and Bourget (1998). Country codes are given in Table 3.1.

Area	Country	Species	Culture type	Harvest (WW)	Standing stock (DW)			Ref
				[ton y^{-1}]	[ton]	[g m^{-2}]	[g m^{-3}]	
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 bay	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
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. 2008b); 15 (Zeldis 2005)

3.3.4. Significance at ecosystem scale

The previous sections have discussed the *potential* effects of mussel communities on nutrient cycling in coastal ecosystems, irrespective of mussel abundance or dimensions of the system. In order to be able to evaluate system-wide interactions, estimates for the bivalve standing stock are an essential parameter (Table 3.9); although the majority of these values are associated with a large uncertainty. Combining standing stock estimates with dimensions of the systems (Table 3.7) provides area and volume-based biomass density estimates (Table 3.9). The Wadden Sea (NL) and several systems in France are important mussel cultivation areas in terms of total harvest quantities. However, these systems are also characterized by co-culture or co-existence of several bivalve species (e.g. *Crassostrea gigas* or *Ensis* sp.). As the current review focusses on mussels, systems where mussels comprise a minor proportion of total bivalve biomass were excluded from the analysis of mussel-ecosystem interactions. Mussel biomass density is highest in the eutrophic estuaries in Tracadie Bay (Canada) and the small coastal inlet Sylt (Germany), whereas biomass density in oligotrophic fjord systems is among the lowest reported.

Interactions are firstly evaluated by the total food uptake relative to the total food available (Figure 3.4a; Smaal & Prins 1993, Dame & Prins 1998), which can also be described as an indicator for the 'top-down' influence on phytoplankton or 'negative feedback mechanism'. In the Norwegian fjords (Åfjord and Lysefjord) clearance times (CT) are longer than water residence times (RT) and primary production times (PPT) despite oligotrophic conditions, indicating that mussel cultures do not dominate food dynamics in these fjord systems. This is different from many other systems where clearance times are shorter than residence times (CT/RT <1). This confirms studies by Smaal & Prins (1993) and Dame & Prins (1998) who report that clearance times are shorter than the residence times for most mussel cultivation areas. However, for most areas primary production is faster than mussel feeding (CT/PPT>1) indicating that the food source is renewed faster than it is filtered. Limfjorden has the longest residence times (almost one year), and a high mussel biomass which together result in high food uptake relative to residence times (CT/RT<<1) indicating that the system is potentially regulated by mussel filtration. However, high nutrient loading in this system results in high primary production rates (Maar et al. 2010) which subsequently indicates that mussels do not overgraze phytoplankton populations (CT/PPT>>1).

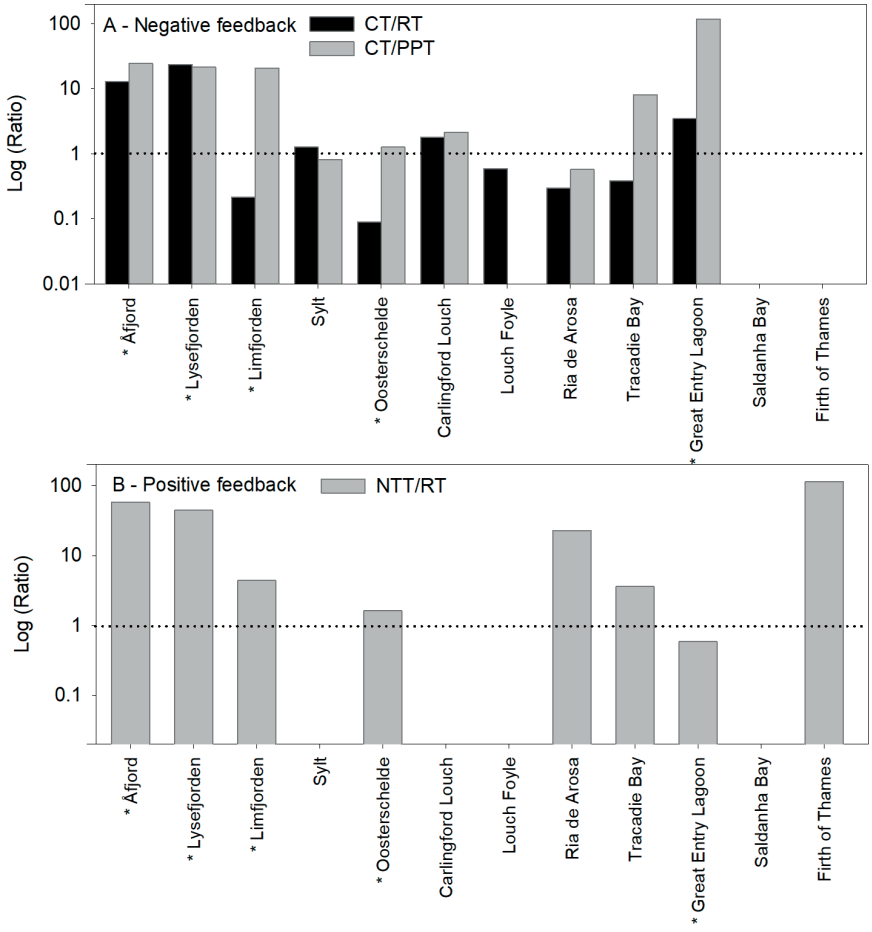


Figure 3.4. Mussel-ecosystem interactions expressed by indicators for negative and positive regulation of primary production, calculated according to Dame & Prins (1998), Smaal & Prins (1993) and Dame (1996) based on the following parameters:

Residence time (RT)	= Time to exchange water body
Clearance time (CT)	= Time to filter the water body
	= (system volume) / (CR x mussel biomass)
Primary production time (PPT)	= Time to renew phytoplankton (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 Mussel biomass)

The extent to which mussel populations have a regulating function in the ecosystem is evaluated by the ratios between the parameters:

CT/RT >1 : no/minor regulation	CT/RT <1 : phytoplankton potentially regulated by mussel filtration
CT/PPT >1 : no/minor regulation	CT/PPT <1 : phytoplankton is overgrazed
NTT/RT >1 : no/minor regulation	NTT/RT<1 : mussels potentially driving nutrient cycling

References are given in Tables 1-9. Asterisk (*) indicates that community-scale rates were applied.

Secondly, mussel-ecosystem interactions were evaluated by nitrogen (DIN) turnover time (Dame 1996) relative to the residence time (Figure 3.4b). This indicator can describe the potential extent of 'bottom-up' stimulation of phytoplankton production or the 'positive feedback mechanism'. The total DIN pool in the ambient water was lowest in Åfjord, Lysefjord and the Firth of Thames, so that a quantity of regenerated nitrogen from mussel cultures could make a proportionally greater contribution to its availability. However, mussel density in these areas is also low (<0.4 g DW m^{-3}). As a result, nitrogen turnover times remain long relative to water residence times (NTT/RT>40), indicating a limited effect of mussels on nutrient cycling. Low DIN concentrations are reported for Great Entry Lagoon resulting in a low NTT value, suggesting a relatively high effect on the DIN pool (NTT/RT<1). However, this outcome may be skewed because ambient values are based on the period June-October, thus excluding the higher winter values. Besides Great Entry Lagoon, the relative effect of regeneration processes (NTT/RT) is most pronounced in the Oosterschelde bay and Tracadie Bay, indicating that mussels may influence nutrient cycling although NTT/RT values did not fall below 1. These are shallow estuaries/bays with high mussel cultivation activity, as indicated by the high relative mussel density (2-6 g DW m^{-3}, Table 3.9).

This analysis of positive and negative feedback mechanisms of mussels acting on phytoplankton growth (Figure 3.4) addresses some consequences of mussel populations for ecosystem functioning, but it is based on a static approach. However, marine systems are complex, and suspended organic matter and inorganic nutrient concentrations are subject to physical, biochemical and eco-physiological processes and fluctuate over both temporal and spatial scales. It should be noted that the literature presented here represents integrated annual values, whereas in fact most of the parameters fluctuate over temporal scales. Prins & Smaal (1994) address the importance of seasonality in terms of the contribution of mussels to nutrient regeneration in the Oosterschelde bay in the Netherlands, demonstrating that mussel beds could account for almost half of the total DIN regeneration of the system, but only during summer when nutrients are limiting. In recent years, the transition from wild seed fishery to Seed Mussel Collectors (SMCs) in the Netherlands (van Broekhoven et al. 2024; Chapter 2 in this thesis) has led to an elevated mussel biomass during summer, which is present temporarily due to high post-seeding mortality in the on-bottom culture practiced here (Capelle et al. 2016). The presence of the SMC mussel seed, which exhibit high weight-specific activity (van Broekhoven et al. 2014) compared to adults (Cranford et al. 2011), coincides with the period of nutrient limitation. This leads to a different dynamic in terms of nutrient circulation mediated by mussels in the system during summer than during the rest of the year, with a much greater potential for positive feedback effects to occur. Similarly, Jansen et al. (2011) demonstrate that at the scale of one mussel farm in a Norwegian fjord, the contribution of mussels to the inorganic nutrient pool is insignificant during winter conditions but substantial during

summer. This is a result of the combination of low nutrient concentrations (nutrient limitation) in the ambient water, high metabolic activity of the mussel population, and high biomass and metabolic activity of fouling organisms.

3.4. Perspective on the regulating services of mussels in nutrient-poor and nutrient-rich cultivation areas

The extent to which bivalve suspension feeders fulfil a regulative role varies between coastal ecosystems (Dame & Prins 1998). Trophic status (nutrient-poor to nutrient-rich) of a system influences the regulating potential for mussels in two ways: 1) the eco-physiological response may vary as a function of ambient nutrient (and thus food) concentrations, and 2) nutrient regeneration has a proportionally greater effect when ambient concentrations are low.

3.4.1. Physiological response

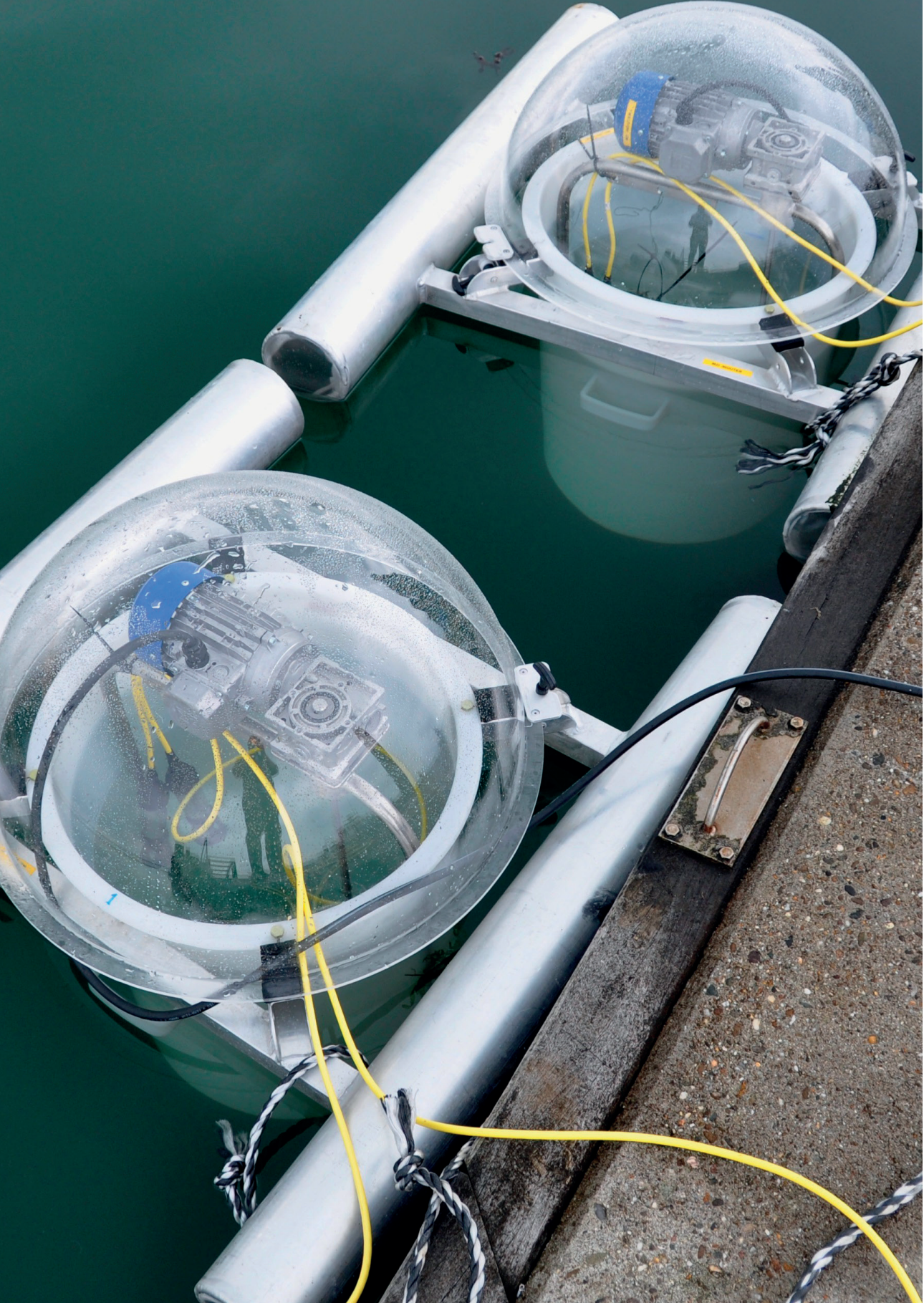
The high feeding rates observed in oligotrophic areas suggest that the physiological response of mussels under low nutrient conditions may differ from areas with higher nutrient concentrations. As model results indicated that metabolic responses are comparable between cultivation areas, this suggests that the slightly lower rates observed for oligotrophic areas are simply a result of low food concentrations rather than a specific response related to the trophic status of the system. Also, nutrient composition of the mussel tissue is similar in oligo- and eutrophic areas, and appears to be endogenously regulated and driven primarily by reproductive processes. Mussels are able to efficiently use the low-concentration but high-quality food sources in oligotrophic systems, resulting in low biodeposit production (in absolute and in relative terms). In eutrophic areas, up to 95% of the filtered nutrients can be expelled with biodeposits in certain cases, which is partly due to pseudofeces production, while in oligotrophic areas less than 50% of all ingested nutrients is expelled with fecal material.

3.4.2. System feedbacks

Differences in eco-physiological rates under oligotrophic as compared to eutrophic conditions (higher clearance, lower egestion, approximately similar excretion, and similar storage in tissue) may lead to distinct mussel-ecosystem interactions. Proportionally more nutrients are excreted as metabolic waste products under oligotrophic conditions (e.g. NH_4), potentially resulting in a higher positive feedback and thus enhanced primary production. In deep stratified systems, the pool of nutrients available for phytoplankton growth is only supplied by directly excreted inorganic metabolic waste products, while in shallow areas remineralization of biodeposits may also contribute to the pool. Ecosystem interactions are here defined as the fraction of ingested nutrients either recycled and

again available for primary production (source) or permanently removed from the system (sink). The current review showed that through these mechanisms the ecosystem interactions are comparable between deep oligotrophic and shallow eutrophic systems. This indicates that the theoretical role of mussels in nutrient cycling and positive feedback processes is relatively similar across mussel cultivation areas. Furthermore, stoichiometry of regenerated nutrients (C>N>P) is generally different from that observed in the ambient water and from the Redfield ratio. This indicates that mussel cultures have the potential to influence phytoplankton community composition by causing shifts in the proportional availability of C, N, P, and Si. The oligotrophic fjord systems are examples where silicate limitation, potentially induced by mussel activity, may suppress diatoms while favouring (dino)flagellate development, while in shallow estuaries this phenomenon is expected to be of less importance due to the contribution of regenerated silicate through biodeposit decomposition.

Evaluation of the regulating potential of mussel cultures at the ecosystem level is based on indicators for negative (CT/RT and CT/PPT) and positive (NTT/RT) feedback processes on primary production. These indicators for mussel-ecosystem interaction demonstrate that estimates for mussel-ecosystem interactions are more profound in shallow nutrient-rich areas with high mussel biomass, especially in terms of the negative feedback mechanisms through filtration of phytoplankton. In contrast, despite low background nutrient levels, mussel aquaculture in oligotrophic fjord systems at present has limited effects owing to low mussel densities and physical characteristics of the fjords (large volume, short residence times of the upper water layer). The significance of the positive feedback mechanism (nutrient regeneration) has a strong seasonal component as many mussel cultivation systems are nitrogen-limited during summer periods when mussel activity is high. The temporary presence of a supplement of highly active mussel spat during summer in SMC culture in the Netherlands creates a particularly high potential for a positive feedback in this situation. These comparisons between cultivation areas suggest that physical characteristics of the site in combination with mussel density and activity better define the feedback to the ecosystem, and hence the regulating potential of mussel cultures, rather than trophic state.



CHAPTER 4

Nutrient regeneration by mussel *Mytilus edulis* spat assemblages in a macrotidal system

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Abstract

Besides exercising grazing control over phytoplankton populations, suspension-feeding bivalves can also stimulate carrying capacity by regeneration of nutrients. This study provides new data on nutrient uptake and release dynamics, and potential implications for availability and stoichiometry of nutrients, for *Mytilus edulis* spat collectors in the Netherlands. Uptake and release rates were measured *in situ* on intact spat collector ropes in a eutrophic macrotidal system in relation to development of ropes in terms of mussel biomass and associated components (fauna, flora, organic material).

There was a good fit between uptake/release rates and mussel weight based on allometric scaling functions, despite the occurrence of a substantial biomass of associated fauna, flora and organic matter on ropes. On a unit biomass basis, nutrient release rates were much higher than reported in other studies, which we attribute to greater activity of small mussels. Accounting for greater weight-specific activity of small mussels, spat collectors released more P than reported for other systems. We show that spat collectors can affect relative availabilities of N, P and Si, and we show that SMCs likely stimulated phytoplankton production through regeneration of N and of Si, which were at limiting concentrations at different points in time. In the case of Si, stimulation would be restricted to diatoms. We conclude that SMCs are able to stimulate phytoplankton production rates, and thereby carrying capacity, and are able to influence phytoplankton composition.

4.1. Introduction

Suspension-feeding bivalves can play a key role in aquatic ecosystems through their filtration of seston, excretion of dissolved nutrients, and production of feces and pseudofeces (Prins et al. 1998, Newell 2004). Hence, suspension-feeding bivalves exert negative and positive feedbacks on carrying capacity (Prins et al. 1998), which can result in potential cascading effects through the food web. The term 'carrying capacity' refers to the optimum or maximum population levels that a natural system is able to support, and has been ascribed various definitions (Inglis et al. 2000, Gibbs 2009, Smaal et al. 2013). This study considers carrying capacity as the maximum population size of one or more species that is supported by the food availability in a given ecosystem (Smaal et al. 1997a). Carrying capacity can be depressed by negative feedbacks; such as grazing control over phytoplankton biomass and composition; and as a result of nutrient removal from a system through denitrification, harvest and burial (Kaspar et al. 1985, Newell 2004, Smaal et al. 2013). Conversely, carrying capacity can be stimulated as a result of positive feedbacks through nutrient regeneration as a result of direct excretion or from the decomposition of feces and pseudofeces. Under nutrient-limited conditions, phytoplankton growth rates can be enhanced by increased nutrient turnover rates and reduced storage in algal biomass; leading to increased food availability for suspension-feeders and thereby enhancing carrying capacity (Asmus & Asmus 1991, Prins et al. 1998, Newell 2004). Phytoplankton composition can be altered when nutrients are regenerated in proportions that differ from the ambient ratios usually present in a given aquatic system (Prins & Smaal 1994, Turner et al. 1998, Philippart et al. 2000, Cloern 2001, Richard et al. 2006, Jansen et al. 2011). Impact evaluations of shellfish culture – a rapidly growing industry worldwide (FAO 2013) – will need to take these various feedbacks between bivalves and their surrounding system into account in an integrated way (Prins et al. 1998, Dame 2012), such as applying an ecosystem modelling approach.

Quantitative data for the parameterisation of carrying capacity models is often incomplete. Much of the literature deals with suspension-feeding by bivalves (see Cranford et al. 2011 for review), and less attention is given to bivalve nutrient regeneration rates. Previous studies of suspension-feeding and nutrient regeneration often consider mussel biomass in isolation from the associated community present both in and on culture systems (e.g. Smaal & Prins 1993, Cranford et al. 2007, Brigolin et al. 2009). Quantitative information on whole-community regeneration rates is needed, as bivalve communities can differ from individual bivalves, in terms of magnitude of nutrient release rates, and nutrient stoichiometry. Only a limited number of studies have investigated intact suspended mussel culture (Nizzoli et al. 2005, Richard et al. 2006, Jansen et al. 2011), and have shown that the biological community and the organic material that is associated with the mussel population can significantly contribute to nutrient exchange rates. Furthermore,

the cultures that were the subject of these studies were fully developed and dominated by adult mussels. Quantitative information on the nutrient regeneration rates of mussel spat is generally lacking (McKindsey et al. 2006). Whereas, small sized mussels are known to display high weight-specific metabolic activity (Smaal et al. 1997b), so that SMCs are expected to have a relatively high impact, compared to adult mussel assemblages. This lack of information is currently of concern in the Netherlands, where suspended Seed Mussel Collectors (SMCs; *Mytilus edulis*) are being increasingly used in spat collection.

SMCs were developed to relieve fishing pressure on natural seed beds and to stabilise seed supply in the context of variable spatfall on natural beds. SMCs consist of ropes or nets that are suspended in the water column, providing a substrate for settlement by mussel larvae. In the Oosterschelde estuary and Western Wadden Sea SMCs are deployed between March and April and harvested August to October. Seed is harvested from SMCs and placed on bottom culture lots at ca. 2 cm shell length. This seed collection technique is currently undergoing upscaling, with the aim of replacing fisheries and supplying $40x10^6$ kg mussel spat per year by 2020 (Kamermans et al. 2002, Meijer 2010). The mussel biomass on the SMCs consists entirely of juveniles and is additional to existing stocks. A complex of Associated Fauna, Flora, and Organic Matter including trapped feces and pseudofeces (hereafter termed AFFOM cf. Richard et al. (2006); but with an F added to indicate the addition of flora) is associated with the culture structures, and may contribute to nutrient exchange.

The Oosterschelde estuary is a shallow, macrotidal and mesotrophic (classification of Nixon 1995) area in the Netherlands that is dominated by mussel culture. Prins and Smaal (1994) demonstrated that nutrient regeneration by filter feeders was important in maintaining primary production after dramatic changes in hydrodynamics, turbidity and water residence times, due to a coastal engineering project (see also Nienhuis & Smaal 1994b). Filtration, biodeposition and nutrient regeneration through mussels increased phytoplankton turnover. Meanwhile an expansion of invasive wild oysters (*Crassostrea gigas*) may have led to overgrazing in some parts of the Oosterschelde (Smaal et al. 2013). Expansion of mussel seed stocks through seed collectors may contribute to further decrease of primary production, hence overgrazing, but their capacity for nutrient regeneration may also enhance nutrient availability for phytoplankton production, particularly as the collectors are deployed in the water column. The impact also depends on the spatial and temporal arrangements of the SMC's in the system. Nutrient concentrations have minimum values in the Oosterschelde in the late spring and summer period (Kromkamp & Ihnken 2012), when the seed mussels are active.

This study investigates nutrient cycling of SMCs in the Oosterschelde estuary. We hypothesized that suspension-feeding bivalves can stimulate phytoplankton production through regeneration of nutrients. We studied nutrient exchange rates across the SMC-water interface during one summer growth season with the aim to 1) quantify uptake and regeneration rates, and 2) assess the potential effects on nutrient stoichiometry in the surrounding water.

4.2. Materials & Methods

Intact sections of SMC rope were incubated *in situ* in pelagic chambers, allowing measurement of nutrient uptake and release rates.

4.2.1. Study area and SMC rope collection

The Oosterschelde estuary is a productive macrotidal system with a total volume of $2750x10^6$ m^3, a mean tidal volume of $880x10^6$ m^3, and a maximum current velocity of 1.0 m s^{-1} (Nienhuis & Smaal 1994b). Salinity throughout 2012 was between 29 and 33 PSU (Rijkswaterstaat, www.waterbase.nl).

As substrate for spat settlement, commercial 'Xmas Tree Rope' (Donaghys) was used. This rope is thickly set with filaments to facilitate spat settlement and weighted down by a lead core. Ropes of 6 m length were attached to rafts in a commercial mussel farm (51°55'N, 3°96'E) in the Oosterschelde estuary in the Netherlands on 4 May, 2012. Loops were formed by attaching both ends spaced approximately 1 m apart to main farm lines resulting in a maximum submersion depth of 3 m. Ropes were collected the day before each incubation and transported immediately by car to the experimental study site (51°62'N, 3°69'E) within the same basin where they were cut into shorter sections, placed suspended in the water column and left overnight to acclimatise.

4.2.2. Experimental design

Nutrient uptake and release rates were determined for intact SMC rope sections using pelagic chambers. Incubations were conducted on 27 June, 11 and 25 July, and 9, 14, and 22 August (hereafter referred to as Julian days: day 178, 192, 206, 221, 226, 234, respectively), spanning the period from 1 mm average shell length until harvest at ca. 2 cm shell length. Pelagic chambers consisted of white rigid polyethylene floating enclosures containing 86 L of water fitted with stirring propellers to keep the water mixed (Figure 4.1). Homogeneous mixing was verified by oxygen probe, by simultaneous triplicate nutrient measurements at six different points within a chamber, and by observation of the path of suspended particles. Although the water surface was exposed to air, oxygen concentrations declined linearly (R^2>0.99), suggesting that diffusion across the water-air

interface was negligible (as described by Jansen et al. 2011). Irradiation of sunlight was reduced to 20% of incident light (Li-Cor LI193 sensor) using darkened covering domes, to counteract prolonged exposure of the water contained in the chambers relative to the surrounding water that is mixed across a larger vertical gradient. Water temperature was stable in chambers during incubations (max. variation 0.2°C).

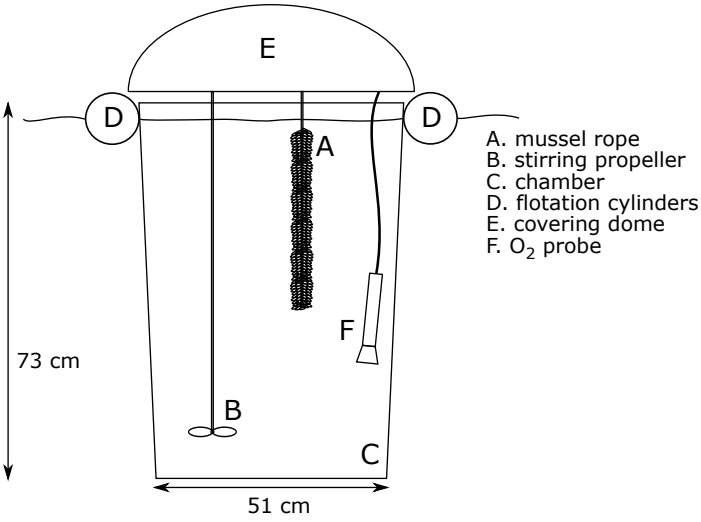


Figure 4.1. Schematic drawing of a pelagic chamber, side view.

In total seven identical pelagic chambers were used during each incubation; five chambers holding sections of SMC rope and two chambers without ropes serving as controls. Results of one mussel chamber were excluded on days 226 and 234 because of propeller malfunction. Total rope length added to chambers varied (range: 18-200cm) in order to keep biomass similar. Nevertheless biomass was lower during the first two incubations because mussels were still very small. Incubations started when mussel ropes were placed in the chambers and were terminated after 75 min, or earlier if oxygen concentrations had decreased by 15-20% compared to start concentrations (Richard et al. 2006). This resulted in 55 – 75 min incubations except on day 178 (240 min) because of very low mussel biomass. After incubations, ropes were carefully removed from chambers and transported cooled to the laboratory.

4.2.3. Mussel & AFFOM abundance and biomass

All ropes were processed in the laboratory immediately after each incubation. Mussels and AFFOM were stripped from ropes by hand and size-fractionated using a 1000 µm mesh size sieve positioned on top of a 250 µm mesh size sieve. Fractions retained on sieves were stored at -18°C until analysis, and water containing any material that passed through both sieves was stirred and sampled in triplicate for inorganic and organic content analysis.

Water samples containing <250 µm material were filtered the same day onto pre-ashed and pre-weighed Whatman GF/C filters, dried for at least 24 h at 70°C, and subsequently ashed for 4 h at 520°C to determine organic matter content. At a later date, mussels and remaining AFFOM retained on sieves were separated by hand after thawing. The 250-1000 µm fraction was only quantified for incubations on days 1, 192 and 206 because its biomass became negligible compared to that of the >1000 µm fraction (on day 192 the 250-1000 µm fraction comprised 7% of mussel biomass, on day 206 this was < 2%). The number of mussels was determined from a subsample, using a binocular microscope. Bias resulting from mussels smaller than the mesh size being retained on the sieve or mussels with a greater shell length than the mesh size passing through was avoided by excluding all individuals smaller than 375 µm from analysis (exclusion of 3.6% of individuals in the 250-1000 µm fraction on day 178; 4.4% on day 192; 0.0% on day 206). Shell length was measured for 100 mussels per chamber using a digital camera (Leica DFC280) attached to a binocular microscope and software ImageJ (version 1.44P). The procedure for the > 1000 µm fraction was identical, and was performed for all incubations but without optical magnification. In addition, ash-free dry weight (AFDW, tissue + shell) was determined for at least 29 randomly selected mussels per incubation from this fraction using an automated drying (70°C) and ashing (520°C) system (Prepash 340). AFFOM AFDW density was quantified by pooling AFFOM from both size fractions (for incubations in which both size fractions were processed), by drying for > 48 h at 70°C and ashing for 4 h at 520°C.

4.2.4. Water parameters

Fluorescence was logged during incubations at 10 s intervals in one of the mussel chambers using a submersible fluorescence probe (Cyclops-7, Turner Designs).

To determine ambient Chl *a* concentrations before the start of each incubation, fluorometric Chl *a* acetone extraction determinations using glass bead homogenisation were performed on at least one 1000 ml water sample. Samples were filtered on Whatman GF/F filters and stored in darkness at -80°C. Spinach extract (Sigma) was used as reference, and correction for phaeophytin after acid addition was applied.

Water samples for particle concentrations (40 ml) were taken before rope insertion, at the start, and then at 10 min intervals during the first 30 min of incubations. Samples were quickly analysed in the field using a portable particle analyser (Pamas S4031 GO fitted with a HCB-LD-15/25 sensor) logging 32 size bins between 1 and 100 µm (the counter's range limits).

Oxygen concentrations were logged at 1 min intervals throughout incubations in four mussel chambers and both control chambers (Hach-Lange HQ40D equipped with LDO

sensor). Carbon excretion was calculated from oxygen consumption using a mean Respiratory Quotient (RQ) of 0.85 (Hawkins & Bayne 1985).

Water samples for dissolved inorganic nutrients were taken at the start and end of the incubations. Samples were immediately filtered on pre-washed Whatman cellulose acetate membrane filters with 0.45 µm pore size, and kept on ice in the dark until storage later the same day at -18°C (14 ml; total ammonia nitrogen (TAN), nitrate, nitrite, phosphate) or 4°C (20 ml; silicate). Dissolved inorganic nitrogen was calculated as the sum of TAN, nitrate and nitrite. Samples were analysed within two months on a Skalar San++ autoanalyser according to the manufacturer's directions.

Water samples (1 L) for ambient particulate organic matter (POM) and for C and N (POC and PON) determination were taken from ambient water at the start of incubations and filtered over pre-combusted and pre-weighed Whatman GF/C filters. Filters were dried for at least 24 h at 70°C. For POM analysis, filters were then ashed for 4 h at 520°C. For POC and PON analysis, filters were stored in sealed containers at -18°C until analysis, within 8 months using a model NC2500 element analyser (CE instruments). Particulate organic P (POP) was obtained from the nearest routine monitoring station (51°36'N, 3°43'E) sampled fortnightly by Rijkswaterstaat of the Ministry of Infrastructure and the Environment (www.waterbase.nl).

4.2.5. Calculation of exchange rates

All exchange rates were calculated from concentration differences measured in chambers over time. Because exchange rates occurring in control chambers cannot be considered fully representative of background rates in mussel chambers due to the depletion of plankton by mussel grazing, rates determined for control chambers were not subtracted from rates measured in mussel chambers but are presented separately.

Cleara

Clearance rates (L h^{-1} m^{-1} rope) were calculated per chamber for each incubation based on the fluorescence signal and on particle concentrations separately, using the equation (modified from Coughlan 1969):

$$CR = \left(\frac{V*D}{N}\right) * \frac{ln\left(\frac{P_0}{P_t}\right)}{t} \qquad \text{Equation 1}$$

Where V is the chamber volume (L), D is the number of mussels per m rope, N is the number of mussels in a chamber, P_0 and P_t are the particle concentration or fluorescence signal at time 0 and t, respectively, and t indicates the time interval (h). The term $\frac{ln\left(\frac{P_0}{P_t}\right)}{t}$ is given by the negative of the slope (h^{-1}) of a linear regression fitted to natural log-transformed concentration or fluorescence series over time for the period during which

the series progressed in a linear way. Some particle concentration time series showed erratic patterns; such series were excluded from further analysis (10% of cases).

Particulate nutrient uptake

Uptake of particulate C, N and P was determined by multiplying clearance rates with particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP) concentrations. The Whatman GF/C filters used to determine these concentrations have effective mesh size 1.2 µm. Particles < 4 µm, however, are considered to be retained incompletely by bivalves (Mohlenberg & Riisgard 1978). Indeed, particles in the 1-4 µm size range were cleared at 70-100% of the rates measured for particles > 4 µm (where differences were significant; Student's t-test, $p<0.05$, $n=5$), except on day 178 (13%). Particles smaller and larger than 4 µm were assumed to have the same nutrient composition. Therefore, clearance rates were calculated for size fractions 1-4 µm and > 4 µm separately, and multiplied by the proportion of total particle volume within that fraction, assuming spherical particles (the 1-4 µm size range made up on average 4.8% of total particle volume in the 1-100 µm size range covered by the particle analyser).

Dissolved oxygen and inorganic nutrient release rates

Oxygen and dissolved inorganic nutrient release rates (µmol m^{-1} rope h^{-1}) were calculated from the difference between start and end concentrations for each incubation, using the equation:

$$F = \frac{(C_t - C_0) * V}{t * L} \qquad \text{Equation 2}$$

where C_t and C_0 are end and start concentrations (µmol L^{-1}), respectively, V is the chamber volume (L), t is the incubation time (h), and L is rope length per chamber (m). Where a single mussel chamber showed opposite directionality (e.g. uptake versus release) to all others in the same session, the single measurement was considered unreliable and was excluded from further analysis.

Data standardisation

For literature comparisons, uptake and release rates were standardised to unit mussel weight using two different methods. Firstly, a linear standardization was performed by dividing rates by mussel weight (g AFDW). Secondly, to enable more direct comparison with other studies, rates were standardized based on allometric scaling of rates with mussel weight (Bayne et al. 1976a b). Rates were standardised to unit mussel weight (giving µmol g^{-1} AFDW h^{-1}) using the equation (Prins & Smaal 1994):

$$R = \frac{F}{\sum n_i W_i^b} \qquad \text{Equation 3}$$

where *F* is the measured *in situ* rate (µmol h^{-1}), *n* is the number of mussels in size class *i*, with individual ash-free dry weight *W*, and *b* is the weight-exponent. Exponent *b* used in the literature varied, and standardisations were adapted for different literature comparisons accordingly. Exponents used are given in Table 4.3B, where literature comparisons are made. The value for exponent *b* used in literature for TAN excretion was also used for calculations involving phosphate, nitrite, nitrate, and silicate, because no empirical values were found.

4.2.6. Statistical analysis

Linear regression functions were tested for statistical significance using ANOVA tests. Normality of data was visually checked using a Q-Q plot, and equality of variances using studentised residuals plotted against predicted values. All statistical tests were performed using software R (version 2.15.1). All values are presented as averages ± standard error, unless stated otherwise.

4.3. Results

4.3.1. Rope composition and start conditions

Average mussel weight and shell length increased throughout the season from 0.165 mg AFDW and 1.1 mm in late June to 29.0 mg AFDW and 16.8 mm in late august (Figure 4.2). Exponential functions produced a good fit with mussel growth in terms of average weight (R^2>0.98, p<0.0001) and average shell length (R^2>0.99, p<0.0001). Shell length correlated well with mussel AFDW (R^2>0.97, p<0.0001) except on the first sampling day, when weights were near the detection limit. The length-weight relation of all analysed mussels together was described by the regression function: $W = 0.04279 * L^{2.140}$ ($R^2 = 0.92$, $p < 0.0001$), where *W* is mussel weight (mg AFDW) and *L* is shell length (mm).

AFFOM biomass was considerable, rising to 62.8 g AFDW m^{-1} on day 234 (Table 4.1). The fraction retained on a 250 µm sieve consisted primarily of green filamentous algae on days 1 and 15, of a mixture of green filamentous algae and hydroid polyps on days 29 and 44, and of hydroid polyps on subsequent dates. This fraction made up more than half of the total AFFOM biomass, except on day 221. The contribution of AFFOM to total rope AFDW declined linearly (R^2>0.99; p<0.0001). Mussels became the dominant component around day 206.

Water temperature measured on incubation days increased during the study period from 17.6°C to 20.7°C (Table 4.1). Chl *a* concentrations ranged from 1.4 to 8.4 µg L^{-1}. At the study site during the study period, ambient inorganic nutrient concentrations fluctuated (Figure 4.3A). Particulate organic C, N and P covaried (Figure 4.3B).

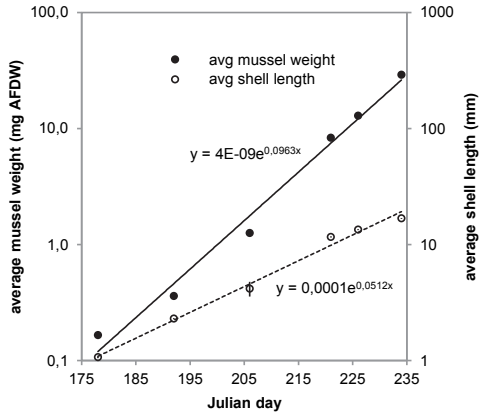


Figure 4.2. Average mussel weight (n=29) and shell length (n=500). Lines are fitted exponential regression functions.

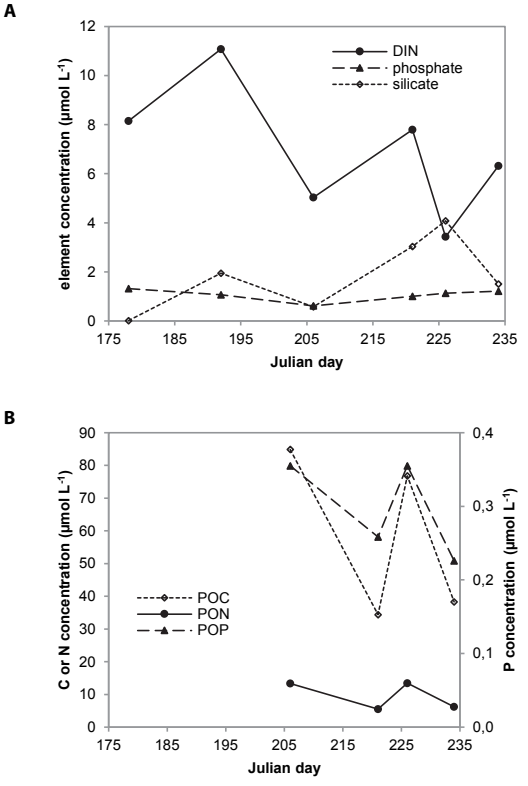


Figure 4.3. Dissolved inorganic (A) and particulate organic (B) nutrient concentrations at the study site measured at the start of incubations. DIN: Dissolved Inorganic Nitrogen; POC/N/P: Particulate Organic Carbon/Nitrogen/Phosphorus.

Table 4.1. Environmental conditions and mussel/AFFOM abundance and biomass throughout the study period.Number (No) and weight (AFDW) are presented both in absolute value per chamber and scaled per meter SMC rope, and the difference between the small AFFOM fraction (<250 µm) and the macro AFFOM fraction (>250 µm) is presented as the ratio between the two.

		temp.	[Chl *a*]	mussels				AFFOM		
date	Julian day	°C	µg L^{-1}	No. m^{-1}	No. $chamber^{-1}$	g AFDW m^{-1}	g AFDW $chamber^{-1}$	g AFDW m^{-1}	g AFDW $chamber^{-1}$	ratio small:macro
27 Jun	178	17,6	2,5	9781 ± 915	17192 ± 6563	1,6 ± 0,0	4,0 ± 0,1	3,3 ± 0,4	7,1 ± 0,8	0,64
11 Jul	192	18,0	1,4	29621 ± 4919	39495 ± 6559	8,9 ± 0,1	11,9 ± 0,1	13,3 ± 1,5	17,8 ± 2,0	0,82
25 Jul	206	19,5	8,4	19553 ± 810	14665 ± 607	24,9 ± 0,4	18,7 ± 0,3	22,7 ± 3,4	17,0 ± 2,6	0,72
9 Aug	221	19,2	4,4	7889 ± 984	2761 ± 344	63,2 ± 0,6	22,1 ± 0,2	36,0 ± 2,4	12,6 ± 0,8	0,18
14 Aug	226	20,6	1,4	6431 ± 270	1608 ± 68	82,9 ± 0,4	20,7 ± 0,1	38,2 ± 3,3	10,1 ± 1,1	0,51
22 Aug	234	20,7	5,6	7031 ± 386	1266 ± 70	205,8 ± 2,0	37,0 ± 0,4	62,8 ± 4,9	11,3 ± 1,6	0,52

4.3.2. Particle clearance rates

Clearance rates calculated from particle (>4 µm; n=5 chambers per incubation date) concentrations showed the same increasing general trend as clearance rates calculated from Chl *a* fluorescence (n=1 chamber per incubation date), but were lower on days 221 and 234 (Figure 4.4). Particle depletion was rapid with >60% reduction after half the incubation time. Clearance rates did not correlate with initial Chl *a* concentrations. Particle concentrations in control chambers decreased during the first three sampling dates, whereas they increased during the last three sampling dates (Table 4.2). On day 178, biomass was considered too low for reliable measurements, which is reflected in minimal difference between particle depletion in mussel and control chambers.

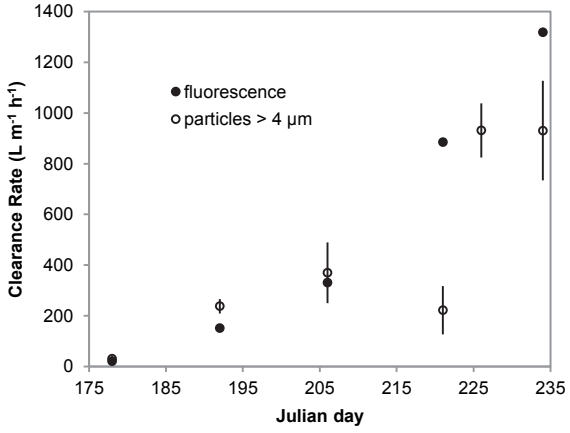


Figure 4.4. Clearance rates calculated from fluorescence signal (solid symbols), and from particle (> 4 µm) concentrations (open symbols), standardised to 1 m rope.

Table 4.2. Comparison of per cent change relative to start concentrations in particle and nutrient concentrations between mussel (n=5) and control (n=2) chambers. For particle concentrations, the table represents only the intervals considered for clearance rate calculations. For nutrient concentrations, the table represents the full incubation period.

	particles		DIN		P		Si	
Julian day	mussel	control	mussel	control	mussel	control	mussel	control
178	-23%	-20%	49%	-3%	11%	-31%	140%	-138%
192	-69%	-20%	98%	8%	90%	-3%	19%	10%
206	-69%	-39%	145%	19%	185%	13%	41%	-25%
221	-23%	20%	50%	-4%	75%	0%	8%	-2%
226	-69%	16%	127%	-49%	90%	-7%	76%	5%
234	-56%	13%	67%	-37%	55%	-3%	19%	-2%

4.3.3. Oxygen and nutrient uptake and release

Oxygen uptake and nutrient release rates generally increased over the season (Figure 4.5). Nutrient uptake rates showed some variation, a result of variation in particulate nutrient levels (Figure 4.3). On day 226 there was as a peak in Si release rate, which was not reflected in the other nutrients.

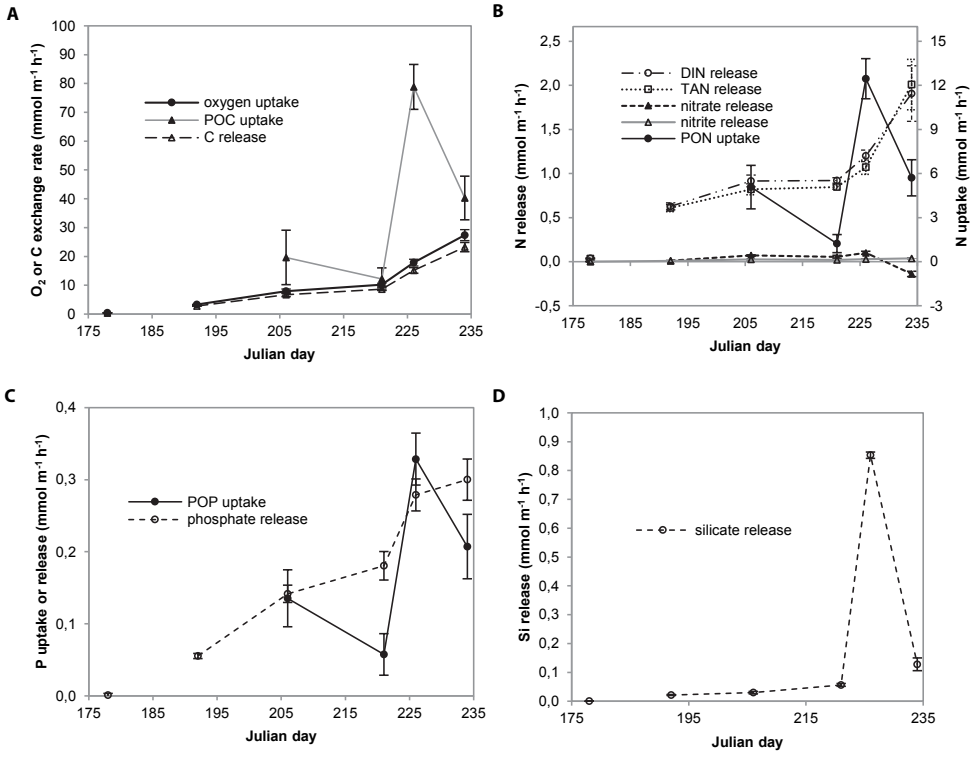
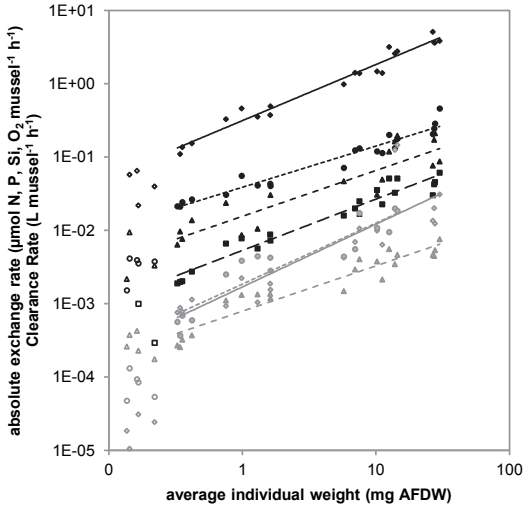


Figure 4.5. Average (n=5) element exchange rates standardised to 1 m SMC rope. Uptake of O_2, particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP), and release of dissolved inorganic carbon (CO2), nitrogen (DIN) and phosphorus (PO4). Dissolved inorganic nitrogen (DIN) represents the sum of total ammonia nitrogen (TAN), nitrate and nitrite. No lines shown between the first incubation and other dates because mussel biomass was considered too low on the first incubation for reliable measurements.

Changes in oxygen concentration in control chambers were < 3% compared to initial values. Nutrient exchange rates in control chambers were mostly small compared to those in mussel chambers (Table 4.2). This was not the case for phosphate and silicate release rates during the first incubation on day 178, when biomass in chambers was very low. Therefore, this incubation was excluded from further analysis. On days 226 and 234, DIN levels in control chambers decreased during incubations.

Clearance rate, oxygen uptake, and nutrient release rates averaged per individual mussel increased with average mussel weight, and good fits (R^2>0.75; p<0.0001) were obtained with allometric scaling functions ($y=ax^b$) (Figure 4.6). The fit for silicate was strongly influenced by the peak in silicate release on day 226. Removing this date improved the fit to R^2 = 0.94. For nitrate, the last sampling day, 234, was removed from this analysis because uptake rather than release was measured (Figure 4.5B).



parameter	symbols	line	a	b	R2
O2 consumption	◆ ◇	———	0.315	0.765	0.97
TAN release	● ○	- - - - - - - - -	0.039	0.562	0.94
CR (> 4 µm)	▲ △	- - - - -	0.016	0.625	0.78
phosphate release	■ □	— — —	0.005	0.700	0.95
silicate release	◈ ◇	———	0.002	0.856	0.75
nitrate release	● ○	- - - - - - - -	0.001	0.618	0.90
nitrite release	△ △	- - - - -	0.002	0.832	0.86

Figure 4.6. Oxygen consumption, clearance rate (CR), and nutrient release rates (TAN = total ammonia N) standardised to one individual mussel as a function of average mussel weight. Each data point represents one individual chamber. Lines are fitted regression functions with the equation $y=ax^b$; symbols, coefficients, and R^2 values are provided in the legend table. All regression fits were significant (p<0.0001). Open symbols represents results obtained at the first incubation, which were excluded from analysis because mussel biomass was considered too low for reliable estimates. For nitrate, day 234 was excluded because rates were negative.

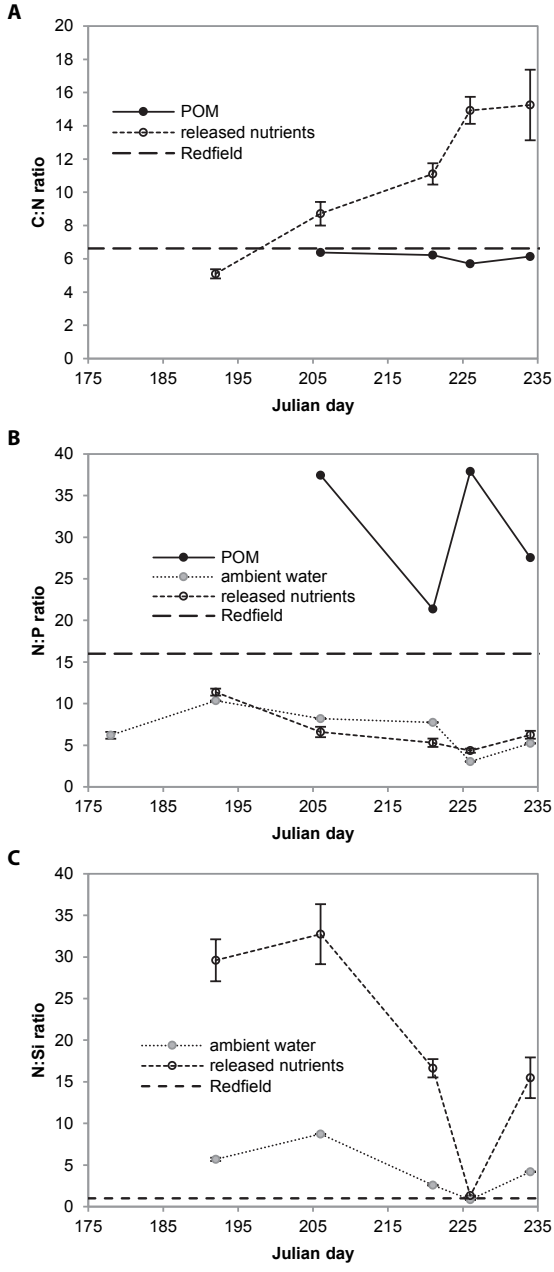


Figure 4.7. Molar elemental nutrient ratios of the particulate organic matter (POM) mussel food source at the study site (n=1), of dissolved inorganic nutrients in ambient water at the study site (n=3), and of released dissolved inorganic nutrients (n=5). A) C:N ratios, B) N:P ratios, C) N:Si ratios. Horizontal dashed lines represent Redfield ratios. No lines shown between the first incubation and other dates for released nutrients because mussel biomass was considered too low on the first incubation for reliable measurements. N:Si ratio of ambient water not shown for the first incubation because a very large value (611.6) resulted from low ambient Si concentration.

4.3.4. Nutrient stoichiometry

C:N ratio of particulate organic material (POM) (6.1 ± 0.1), was close to Redfield's ratio (Figure 4.7A), while the N:P ratio (31.1 ± 4.0) was higher than Redfield's ratio (Figure 4.7B). N:P ratio of dissolved inorganic nutrients released by SMC ropes (6.8 ± 1.2) was comparable to that of dissolved inorganic nutrients in ambient water (6.9 ± 1.3), and both were lower than Redfield's ratio (Figure 4.7B). N:Si ratio of dissolved inorganic nutrients released by SMC ropes (19.2 ± 5.6) was higher than that of dissolved inorganic nutrients in ambient water (4.4 ± 1.4), and both were higher than Redfield's ratio (Figure 4.7C). Ratios of N:P and N:Si of dissolved nutrients in the surrounding water correlated with the ratios of released nutrients (N:P R^2=0.54, p<0.0001; N:Si R^2=0.67, p<0.0001).

4.4. Discussion

This study provides new data on nutrient uptake and release rates, and nutrient stoichiometry, measured *in situ* on intact mussel spat collector ropes in a eutrophic macrotidal system, in relation to biomass development on the ropes.

4.4.1. Nutrient limitation and stoichiometry

To determine whether nutrient regeneration through SMCs could stimulate primary production in the Oosterschelde estuary, the timing of nutrient regeneration as established in this study must be combined with information regarding the occurrence of nutrient limitation.

Potential N-limitation for phytoplankton primary production was suggested by relatively low N:P ratios at the field site compared to Redfield's ratio indicated. In fact, minimum DIN concentration in the Oosterschelde estuary averaged over all three routine monitoring stations was lower than detected at the field site, reaching 1.75 µmol L^{-1} in late August (Rijkswaterstaat, www.waterbase.nl). This is below the half-saturation coefficient of 2 µmol L^{-1} used by Philippart et al. (2007). Depletion of DIN in control chambers on days 226 and 234 also suggests rapid uptake by plankton, further indication of a nitrogen shortage in this period. It has been argued that DIN is likely not limiting for primary production in the Oosterschelde estuary in summer (Kromkamp & Ihnken 2012). However, the analysis of these authors was based on the previous year, 2011. In late August, DIN regeneration rates by SMCs were high, and we conclude that nitrogen regenerated by SMCs likely stimulated primary production in this period.

A potential for Si to be more limiting than N or P, for primary producers that are dependent on this element, was suggested by high N:Si combined with low N:P ratios compared to Redfield's ratio at the field site. Furthermore, the difference between ratios of dissolved

inorganic nutrients between ambient water and released nutrients was greater for N:Si than for N:P, possibly reflecting faster uptake of Si than of P. Kromkamp and Ihnken (2012) argue that Si is likely limiting for diatom growth in the Oosterschelde estuary in summer. In the summer of 2012, silicate concentrations below the half-saturation coefficient of 2 µmol L^{-1} used by Philippart et al. (2007) were not observed system-wide, but were measured in mid-July at the field site (0.58 µmol L^{-1}) and at the most easterly routine monitoring station (1.50 µmol L^{-1}, Rijkswaterstaat, www.waterbase.nl). We therefore conclude that Si regenerated by SMCs likely stimulated primary production by diatoms in this period in certain areas.

Finally, phosphorus concentrations below the half-saturation coefficient of 0.2 µmol L^{-1} used by Philippart et al. (2007) were not detected after late May (Rijkswaterstaat, www.waterbase.nl), so that phosphorus regeneration through SMCs was not likely to substantially stimulate phytoplankton production.

In addition to the implications of regeneration of each separate nutrient, their relative availabilities may change. Proportionally more N than Si was released by SMCs than was present in surrounding water. If N and Si limitation of phytoplankton production were to occur at the same time, such a balance could suppress diatom growth in favour of non-siliceous phytoplankton (Turner et al. 1998). This would counteract a general trend of increasing riverine Si loads and concentrations in continental coastal waters in the Southern Bight of the North Sea (Prins et al. 2012). In terms of the N:P ratio, there was little difference between nutrients released by SMCs and nutrients in the surrounding water. Substantial variation in N:P ratios of nutrients released by mussel assemblages has been reported in literature. Compared to a range of studies on individual mussels or mussel beds in the wider region, and of suspended culture ropes in Canada, Norway and Italy (Asmus et al. 1990, Dame et al. 1991, Prins et al. 1994, Schlüter & Josefsen 1994, Nizzoli et al. 2005, Richard et al. 2006, Jansen et al. 2011, 2012a b), N:P ratio of released nutrients was the lowest in the current study, except for mussel beds reported by Dame et al. (Dame et al. 1991). Among these studies, no uniform tendency was found in the dissolved inorganic N:P ratios of released nutrients being either higher or lower than in surrounding water, although in the studies of isolated intact culture rope sections by Jansen et al. (2011) and Richard et al. (2006), they were higher. But in the case of N:Si ratios there was agreement: greater N release relative to Si was reported in all of the studies considered previously that also measured Si (Asmus et al. 1990, Dame et al. 1991, Prins et al. 1994, Richard et al. 2006, Jansen et al. 2011).

Furthermore, correlations of dissolved inorganic N:P and N:Si ratios of ambient water with those of nutrients released by the mussel ropes were evident, despite the absence of SMC installations in the immediate vicinity of the field site. However, the presence of

hard substrate at the field site may have facilitated substantial populations of mussels and oysters (personal obs.). Assuming that these populations would exhibit similar nutrient cycling activity in terms of stoichiometry as the SMC ropes, this finding would lend further support to the theory that bivalve suspension feeders play an important role in nutrient circulation in the Oosterschelde estuary (Smaal et al. 2013), and can potentially change nutrient stoichiometry.

4.4.2. Nutrient and oxygen exchange rates over the growth season

Increasing oxygen uptake rates per m SMC rope indicated an increase in SMC activity over the season. Increasing clearance rates over the season resulted in a rise in nutrient uptake rates. The increasing release rates of TAN and phosphate over the season were expected because these compounds are excreted by mussels (Prins & Smaal 1994).

However, nitrate, nitrite and silicate release rates also increased over the season, although these compounds are not excreted by mussels. Nitrification likely occurred through decomposition of organic material on SMC ropes (e.g. Richard et al. 2006), but may also be caused by bacteria colonising the mussels (Welsh & Castadelli 2004). Nitrate, nitrite and silicate release (Asmus et al. 1990, Prins & Smaal 1994, Jansen et al. 2012a) does not appear to originate directly from the mussels (e.g. Prins & Smaal 1994, Richard et al. 2006, Jansen et al. 2012a) – although Asmus et al. (1990) argued that they observed silicate release from individuals. Silicate differs from the other elements as its regeneration relies on dissolution processes rather than biological activity. Dissolution rates from materials such as diatom frustules may be enhanced as a result of fragmentation due to bivalve grazing (Paasche 1980). On SMCs, accumulating mussel feces could therefore contribute to increasing silicate release rates during the summer, as observed in the current study. This reinforces the relevance of studying community scale processes, as performed here using intact sections of rope.

Good regression fits were found for clearance rate, oxygen consumption and nutrient release rates per mussel as a function of average mussel weight. Functions of the form $y = ax^b$ were chosen because such allometric relations with individual mussel weight have been described for clearance rate, oxygen consumption, and TAN release (Bayne et al. 1976a b). Fits were remarkably good, considering that allometric relations are generally determined under controlled conditions, and that large seasonal variability is known to occur (e.g. Bayne & Scullard 1977). This suggests that AFFOM activity was either very small or scaled proportionally with mussel biomass or activity. Previous studies have shown that the contribution of AFFOM to nutrient regeneration rates can in fact be substantial on mussel culture structures (Nizzoli et al. 2005, Richard et al. 2006, 2007b) and mussel beds (Prins & Smaal 1994), depending on its abundance and composition (Richard et al. 2006, Jansen et al. 2011, 2012b). Two studies of suspended mussel culture argued that AFFOM

was active in nutrient turnover, while reporting lower AFFOM biomass than the current study: Jansen et al. (2011) reported 0.1-1.53 g AFDW m^{-1} fauna > 1 mm, and Richard et al. (2006) reported 0.01-0.25 g AFDW m^{-1} fauna > 0.5 mm, while the current study found 19.5 g AFDW m^{-1} AFFOM > 1 mm. Neither authors measured any flora. Furthermore, the current study showed >3 times higher organic material per m of rope retained on Whatman GF/C filters than Jansen et al. (2011) (this fraction was not reported by Richard et al. 2006). Similar to the contribution of AFFOM, any variation caused by environmental parameters was either very small or was captured within the allometric relations. A potential temperature effect on the trend of mussel activity cannot be separated from a size effect since incubations were carried out under ambient conditions and both temperature and size increased over time. However, any temperature effect is likely to be relatively small given the limited range of temperatures the SMC's were exposed to over the course of this study (17.6-20.7°C). Furthermore, as Smaal et al. (1997b) argue, effective adaptation of feeding activity takes place over a much wider range of temperatures. The peak in silicate release on day 226 could not be ascribed to one of these factors. Despite the uncertainties, knowledge of only mussel density and biomass allows prediction of exchange rates for the SMC growth season in our study area.

Although determined on intact communities under field conditions, allometric parameters were of similar magnitude to those reported in literature for individual animals, and we provide some here for reference. The allometric exponent of 0.63 for clearance rate was higher than the 0.5 reported by Smaal et al. (1997b) for adult mussels in the Oosterschelde estuary, but closer to the average value of 0.58 reported by Cranford et al. (2011) for a range of shellfish species worldwide. The exponent for respiration rate of 0.77 was higher than the 0.65 reported by Smaal et al. (1997b), and the exponent for TAN release of 0.56 was lower than the 0.66 reported by Smaal et al. (1997b).

Nutrient exchange rates represent the net effect of uptake and release by the rope community, as well as the plankton population present in the incubation chambers. Dynamics measured in control chambers can thereby be considered an extreme reflection of the contribution of the plankton, while it is likely that plankton activity was considerably smaller in rope chambers due to rapid plankton removal by mussel grazing. Particle concentrations in control chambers were not stable, showing a decline during the first three sampling dates and an increase during the last three sampling dates, indicating active plankton processes. Pilot studies performed in early spring indicated stable particle concentrations throughout extended incubations (> 3 h) in control chambers, both by using a model Z2 Beckman Coulter Counter and by SPM measurements using Whatman GF/C filters (data not shown), suggesting that the plankton was more active in summer. The peaks in nutrient uptake rates on day 226 were produced by relatively high POC, PON and POP concentrations, coupled with high clearance rates. Chl *a* levels at the field

site varied, but were high enough to sustain feeding (Cranford et al. 2011), which was confirmed by a lack of correlation of clearance rates with initial Chl *a* concentrations.

4.4.3. Literature comparison of exchange rates

Nutrient release, oxygen uptake, and clearance rates were compared with rates reported in literature in two ways. Firstly, rates expressed per individual animal were simply divided by the average individual ash-free dry weight. These rates were an order of magnitude greater than reported in literature for individual mussels, as well as for natural and cultured assemblages, in summer (July-September) (Table 4.3A). The only results in the same order of magnitude were reported for suspended culture in Italy by Nizzoli et al. (2005), but these authors incubated ropes together with underlying sediment. This exceptionally high activity of SMCs needs to be taken into account when estimating ecological implications. Small individuals are known to exhibit greater weight-specific activity, and clearance, oxygen consumption and release rates of TAN and phosphate scale allometrically with body weight (Bayne et al. 1976a b). The mussel assemblage on SMC ropes consisted solely of juveniles, whereas assemblages considered in other studies were dominated by adults. To illustrate the difference, even at the latest sampling date there were 7031 ± 863 indiv m^{-1} on the ropes with a combined mass of 206 g AFDW. Whereas, Jansen et al. (2011) reported 543 indiv m^{-1} with a mass of 383 g AFDW for suspended rope culture, and Richard et al. (2006) reported 382 adults m^{-1} with a mass of 220 g AFDW (an additional maximum 50040 spat m^{-1} in their September measurements contributed only 0.1% to mussel biomass).

Secondly, a size-independent comparison between studies was made by accounting for the allometric relation in the manner described by Prins and Smaal (1994), standardising all rates to an animal of 1 g AFDW. This comparison (Table 4.3B) produced lower rates per unit AFDW, generally in the same order of magnitude as studies performed in different areas and with a large size range of mussels (20 – 80 mm average shell length). Allometric exponents used in the other studies varied, and data from the current study were recalculated to match the other studies. Clearance rate observed in our study was somewhat lower than in studies of suspended culture and individual mussels, but higher than in a study of an artificial mussel bed. Oxygen consumption rate was in the range reported by other studies. TAN release rates were higher than reported by studies of suspended culture and individual mussels, but a mussel bed showed higher TAN as well as nitrate release rates (Prins et al. 1994). Phosphate release rates were higher than reported by the other studies including the mussel bed.

Table 4.3. Overview of clearance rate (CR), oxygen consumption, and nutrient release rates in several studies, using A) linear standardisation, by dividing observed rates by total mussel biomass (g AFDW); B) allometric standardisation, taking into account the allometric relation of release rates with individual mussel weight. Allometric exponents used to standardise values from the current study were matched to those used in the other studies, and are provided. Values represent averages for the period July – September, and are given as minimum – maximum where multiple studies were included; empty cells indicate that rates were not were reported. TAN = total ammonia Nitrogen; DIN = dissolved inorganic Nitrogen; susp. cul.t incl. sed.: suspended culture including underlying sediment.

	type				CR (L g-1 AFDW h-1)	oxygen (µmol g-1 AFDW h-1)	TAN (µmol g-1 AFDW h-1)	nitrite (µmol g-1 AFDW h-1)	nitrate (µmol g-1 AFDW h-1)	DIN (µmol g-1 AFDW h-1)	phosphate (µmol g-1 AFDW h-1)	silicate (µmol g-1 AFDW h-1)	references [a]
A	SMCs				14,31	251,98	26,58	0,56	1,31	28,45	3,36	2,52	this study
	suspended culture					22,13 - 39,99	1,26 - 7,35	0,03	0,09	7,47	0,12 - 0,22	0,05 - 0,22	1,2
	susp.cult. incl. sed.					80,27	19,39	0,60	-3,77	16,22	0,51		3
	mussel bed					53,24	1,70 - 6,81	0,00 - 0,05	0,00 - 0,10	1,86 - 6,81	0,00 - 2,06	0,30 - 1,56	4,5,6,7
	individuals					29,89	1,32				0,09		8
		allometric exponents used											
	type	CR	oxygen	nutrients									
B	SMCs				1,09	32,22	2,88	0,06	0,19	3,12	0,48	0,37	this study
	suspended culture	0,509	0,651	0,655	1,70	18,90	1,21	0,00	0,02	1,23	0,12	0,17	10
	individuals				5,80	52,80							10
	individuals				2,36	18,91	1,54						12
	SMCs					41,41	3,70	0,08	0,23	4,01	0,61	0,46	this study
	suspended culture		0,7	0,7		27,69	1,48	0,01	0,04	1,52	0,12	0,05	9
	individuals					24,53	1,11	0,00	0,00	1,12	0,14	-0,01	11

SMCs	0,67		2,62							this study
artificial mussel bed			1,70							13
SMCs				3,31	0,07	0,21	3,59	0,55	0,42	this study
mussel bed		0,68		5,86		3,10		0,27		5
individuals				1,52		0,00		0,07		5

[a] 1 (Jansen et al., 2011); 2 (Richard et al., 2006); 3 (Nizzoli et al., 2005); 4 (Dame et al., 1991); 5 (Prins and Smaal, 1994); 6 (Asmus et al., 1990); 7 (Schlüter and Josefsen, 1994); 8 (Smaal and Vonck, 1997); 9 (modified from Jansen et al., 2011); 10 (Jansen, 2012b); 11 (Jansen et al., 2012a); 12 (Smaal et al., 1997); 13 (Prins et al., 1994)

An obvious difference between mussel beds and suspended culture structures is the greatly increased accumulation of deposited material on the beds, including feces and pseudofeces (Newell 2004), which on SMCs are by and large transported away by water currents and decompose underneath (Hatcher et al. 1994, Callier et al. 2006) or some distance away from culture structures (Giles et al. 2009). The high DIN regeneration rates reported for a mussel bed by Prins and Smaal (1994) may be due to the decomposition of this material. A greater role of decomposition of deposited material on the mussel bed is also suggested by a high contribution of nitrate and nitrite to total DIN release rates (>20%; Prins et al. 1994) in contrast to the current study and Jansen et al. (2011) and Richard et al. (2006) (all <12%). This highlights the need to quantify nutrient regeneration from decomposition of feces and pseudofeces from SMCs in order to assess nutrient regeneration at an ecosystem scale. Moreover, ratios of N:P:Si from decomposing feces and pseudofeces may differ from ratios of nutrients released directly from culture structures (e.g. Jansen et al. 2011, 2012b). Nutrient regeneration from decomposition of feces and pseudofeces from SMCs was beyond the scope of the current study but will be addressed in a separate study (Chapter 5 in this thesis).

4.5. Conclusions

Nutrient uptake and release rates from SMCs were quantified during a growth season (spring and summer), starting at 1 mm average shell length. Uptake and release rates were described well by allometric functions of average mussel weight, despite the presence of substantial AFFOM on ropes. Weight-specific nutrient release rates were much higher than reported for other systems, which was attributed to enhanced activity of small specimens. The high specific activity of SMCs needs to be taken into account when considering ecological implications and environmental impacts. Accounting for high weight-specific activity of small mussels, SMCs released more phosphate than reported in other studies.

This study showed that SMCs can affect the relative availability of N, P and Si, releasing more N relative to Si than was present in ambient water. Furthermore, this study showed that in the summer of 2012, SMCs likely stimulated phytoplankton primary production through regeneration of both N and Si at different points in time. In the latter case, stimulation would be restricted to diatoms. We conclude that SMCs thus are able to stimulate phytoplankton production rates, and thereby carrying capacity, and are also able to influence phytoplankton composition.

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CHAPTER 5

Nutrient regeneration from feces and pseudofeces of mussel spat *(Mytilus edulis)*

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Abstract

Suspension-feeding mussels exert top-down grazing control on primary producers, and provide bottom-up feedback of regenerated nutrients. Besides direct excretion, an important pathway of nutrient regeneration is through the decomposition of feces and pseudofeces, of which mussels can produce large quantities. Information on their quality and nutrient regeneration rates is scarce. Feces and pseudofeces, produced in varying proportions, are commonly treated as one pool. We determined nutrient regeneration rates of feces and pseudofeces decomposition in incubations using natural seawater and juvenile *Mytilus edulis* from spat collectors. Besides one 1993 trial, our results are the first to present nutrient regeneration dynamics of feces and pseudofeces separately. Dissolved inorganic nitrogen (DIN) and phosphate regeneration continued at stable rates for approximately three weeks, after which 13.1% and 12.4% of the available N and 8.7% and 7.9% of the available P was regenerated from feces and pseudofeces, respectively. Rates of silicate regeneration declined continuously, which we attribute to its accumulation in the experimental setup. Coinciding potentially limiting environmental levels of DIN and silicate indicate the potential ecological relevance of biodeposit decomposition. Overall DIN regeneration rates were similar between feces and pseudofeces, but depletion of ammonia was initially more rapid for pseudofeces due to stronger nitrification. Phosphate regeneration rates were 1.1 times greater from feces than pseudofeces, and silicate regeneration rates 1.4 times. Future research should clarify the role of bivalve suspension feeders in controlling Si and P availability in coastal ecosystems as relating to the proportion of pseudofeces generated, which depends on food concentration.

5.1. Introduction

Suspension-feeding mussels have a large filtration capacity, extracting important quantities of phytoplankton and other suspended matter from the water column (Cranford et al. 2011). Concurrently, metabolic losses excreted by mussels as dissolved inorganic nutrients constitute a feedback to primary producers (Prins et al. 1998, van Broekhoven et al. 2014). A second pathway of nutrient feedback is the decomposition of feces and pseudofeces (Giles & Pilditch 2006, Jansen et al. 2012b), together called biodeposits, of which mussels produce substantial quantities (Tsuchiya 1980, Smaal et al. 1986). Pseudofeces is the portion of filtered matter rejected during pre-ingestive selection, expelled in loosely mucus-bound form, and feces is the portion of ingested filtered matter egested after food processing in the digestive system (Gosling 2003). Biodeposition represents a significant pathway in bivalve nutrient cycling. For example, 40-80% of N filtered from the water can be expelled with biodeposits (Cranford et al. 2007, Jansen et al. 2012a). Biodeposits can also contain substantial amounts of P, and of silica of biogenic origin (Navarro & Thompson 1997). During biodeposit decomposition ammonia (which may then be transformed into NO_x as a result of bacterial nitrification), phosphate, and silicate are released (Giles & Pilditch 2006, Callier et al. 2009, Jansen et al. 2012b). N and P regeneration are biologically mediated, but Si primarily relies on chemical dissolution (Paasche 1980). A substantial portion of the biodeposits is decomposed within days to weeks (Giles & Pilditch 2006, Carlsson et al. 2010, Jansen et al. 2012b), so that nutrient feedback to primary producers is relevant on the short term. On average, biodeposits decompose more rapidly than phytoplankton or macroalgae (Giles & Pilditch 2006). Not all material digested by mussels is fully decomposed, with, for instance, diatoms surviving after ingestion and gut passage (Barillé & Cognie 2000).

Recently, some studies published results on mussel biodeposit decomposition (e.g. Fabiano et al. 1994, Giles & Pilditch 2006, Carlsson et al. 2010, Jansen et al. 2012b) but information on mussel biodeposit quality and nutrient regeneration rates is still scarce (McKindsey et al. 2011). So far, none of the decomposition studies have made a distinction between feces and pseudofeces decomposition patterns, which is reflected in ecosystem modelling studies (e.g. Dabrowski et al. 2013). It has been suggested that feces may decompose more rapidly than pseudofeces due to loading with bacteria from the animal's digestive system (Harris 1993, Fabiano et al. 1994). One preliminary experiment described in Smaal & Prins (1993) suggested that feces may indeed decompose more rapidly than pseudofeces, indicating the importance of studying decomposition dynamics of the two biodeposit products separately. Given the variability in the proportional contribution of pseudofeces to biodeposits in response to variability in food source and concentration (pseudofeces contribution ranging from 0-90% in Foster-Smith 1975, and a similarly large range in Tsuchiya 1980), lack of knowledge of differential nutrient regeneration rates

leads to potential errors of unknown magnitude in our understanding and quantitative estimates of nutrient regeneration rates from decomposing biodeposits. The present study addresses this gap using replicated, separate incubations of feces and pseudofeces.

The study was conducted in the Oosterschelde estuary in the Netherlands, where large stocks of bivalve suspension feeders are present, possibly reaching the carrying capacity of the system (Smaal et al. 2013). In addition to the natural and cultured benthic bivalve populations, a recent development in the study area is the introduction of Seed Mussel Collector (SMC) systems (Kamermans et al. 2002). This results in additional mussel *Mytilus edulis* stocks during the summer SMC season. In this period dissolved inorganic nutrient concentrations, particularly Si and N, are periodically at limiting levels for primary production (van Broekhoven et al. 2014). Policy decisions regarding future expansion of SMCs are informed by ecosystem model predictions of impacts on other suspension feeding bivalve populations (Meijer 2010, Kamermans et al. 2014b). It has previously been shown that nutrient regeneration by bivalves can enhance primary production rates in the Oosterschelde (Prins & Smaal 1994), so that nutrient feedbacks need to be taken into account for such a model to accurately reflect the carrying capacity of the system.

The aim of the present study is twofold. Firstly, to quantify rates and proportions of nutrient regeneration from decomposing *M. edulis* spat feces and pseudofeces. The hypothesis is that measurable proportions of particulate organic nitrogen (PON), biogenic silica (BSi), and particulate organic phosphorus (POP) contained in feces and pseudofeces are regenerated within days to weeks, thereby constituting a relatively quick feedback to primary producers. Secondly, to compare release rates of N, P and Si from feces and pseudofeces. Rates are expected to be higher for feces than for pseudofeces. The study is performed under controlled conditions using replicated incubations.

5.2. Materials & Methods

5.2.1. Mussels

Mussels were collected from a commercial SMC in the central part of the Oosterschelde estuary (51°55'N, 3°96'E) on 5 August 2013. Shell length was determined to 0.01 mm for 387 randomly selected individuals using a digital calliper. These data were combined with length-weight (tissue plus shell) relations (R^2=0.99) established for 58 randomly selected individuals from the sample using an automatic drying (70 °C) and ashing (520 °C) apparatus (Prepash 340). Mean shell length was 15.11 (±3.52) mm, mean dry weight was 145 (±84) mg and mean ash-free dry weight was 22 (±13) mg.

5.2.2. Biodeposit production

Approximately 711 g (wet weight) of mussels was distributed over six cylinders of 25 cm diameter fitted with mesh bottoms (150 µm mesh size), held in a water tank (Figure 5.1). Unfiltered water was fed to the tank, pumped freshly from the Oosterschelde estuary at the field station (51°59'N, 3°87'E), and entering the cylinders through the mesh bottoms. Water left the setup through tubes installed at the tops of the cylinders. Water flow was regulated by visual inspection to just below the rate where biodeposit particles were occasionally observed to be transported on the upward flow and out of the setup. This way all biodeposits settled on the mesh bottoms, but supply of food to the mussels was ensured.

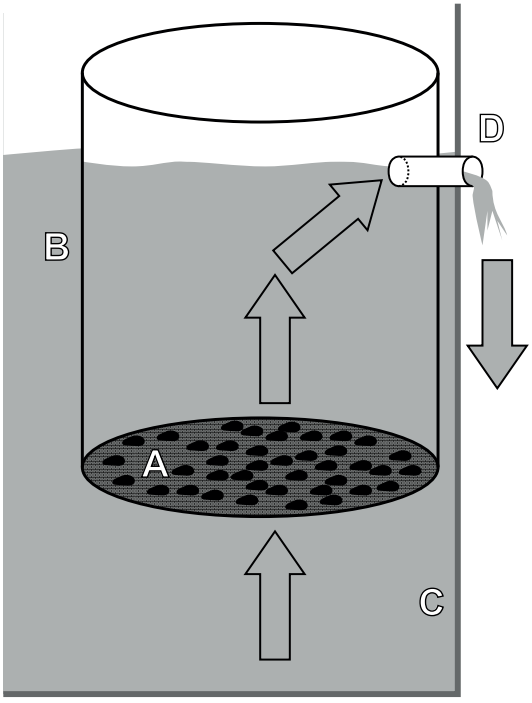

Figure 5.1. Schematic of part of biodeposit production setup (not to scale); side view of one of the six cylinders showing tank section. A: mesh with mussels and accumulating biodeposits; B: cylinder; C: tank wall; D: water exit tube, penetrating tank wall and tightly fit. Water is pumped into the tank; arrows indicate direction of water flow.

Suspended particulate matter (SPM) content of the water fed to the mussels was determined by averaging daily triplicate measurements on the four days leading up to and including the acclimatisation and biodeposit production periods. Samples of 1 L were transported cooled and in darkness to the laboratory for immediate filtration on Whatman GF/F filters, with salt expelled using an ammonium formiate solution. Filters were dried at 103°C for gravimetric determination as dry weight (DW) followed by combustion at 550°C

for ash-free dry weight (AFDW) determination. A separate set of duplicate Whatman GF/F filters produced on the same days in the same way, but with salt expelled using demi water instead of the ammonium formate solution, was kept dry and in darkness and analysed for particulate nutrients within five months.

After placement in the biodeposit production setup, mussels were allowed to acclimatise for 48 h. The setup was cleaned daily by removing accumulated material through a small flexible tube by force of gravity. Biodeposits produced during the following 24 h were collected through a small flexible tube in the same manner, and were used as the start material for incubations.

Feces were separated from all other material, including pseudofeces, by repeated decanting. Visual inspection confirmed the absence of feces particles from the remaining fraction. No further separation between pseudofeces and other material deposited in the production setup could be made. The contribution of non-biodeposit material to the pseudofeces fraction was estimated in a series of trials. Material deposited in a control cylinder amounted to 10% of the DW (analysis described below) and 10% of the AFDW of the total material deposited in a mussel cylinder (feces + pseudofeces + the other deposited material). In the mussel cylinder, feces made up 47% in DW and 41% in AFDW. This means that of the non-feces material, 81% of the DW and 84% of the AFDW was in fact pseudofeces. This method of biodeposit production yielded large enough quantities to enable replicated incubations in relatively large water volumes, but a limitation is that leakage of dissolved material during the production period (e.g. Carlsson et al. 2010) is not captured.

5.2.3. Incubations

Incubations were performed in cylindrical 520 ml transparent polypropylene containers of 95 mm height, closed with lids of the same material. To include the various constituents of the microbial loop from seawater that can contribute to nutrient regeneration rates (Azam et al. 1983, Jacobsen & Azam 1984, Poulsen & Iversen 2008) untreated seawater was selected as the incubation medium. Incubations were conducted without sediment. Regeneration from feces and pseudofeces was thereby described by the net result of decomposition and incorporation by decomposers. Three treatments were prepared: feces, pseudofeces, and untreated seawater as the control. Chambers were placed on a table in a grid pattern, with treatments assigned to chambers at random.

The incubation chambers were pre-filled with untreated seawater pumped from the Oosterschelde estuary. Biodeposits were added by transferring 30 ml from single stirred feces or pseudofeces master stock suspensions by pipette, to give a total volume of 381 ml per chamber. To determine pre-incubation composition, five 30 ml aliquots of the

feces and five of the pseudofeces stocks were filtered onto Whatman GF/F filters, with salt expelled using an ammonium formiate solution. Filters were dried at 103°C for gravimetric DW determination followed by combustion at 550°C for AFDW determination. Seawater control chambers contained the total 381 ml of untreated seawater.

Chambers were gently aerated to ensure oxic conditions representing the well-oxygenated waters of the Oosterschelde estuary (Rijkswaterstaat, www.waterbase.nl), using plastic tubes fitted through the lids, in such a way that the water kept moving, but biodeposits remained settled on the chamber floors. The chambers were kept in a climate-controlled room at 20°C in continued darkness except for brief visits for sampling and checking.

5.2.4. Sampling of incubation chambers

Samples were analysed on days 1, 5, 7, 13, 18, 22, and 28. Incubations were continued until day 36 but most samples could not be processed due to mucous formation; this day was not analysed. On each sampling day, three replicate chambers of each treatment were selected at random and sacrificed. After uncoupling from aeration, chambers were gently swirled. From control chambers, 15 ml samples were directly transferred to 20 ml HDPE containers and stored at -18°C for total nitrogen (TN) and total phosphorus (TP) analysis within three months. Samples from day 1 were lost. Chambers were left to stand for 10 min to allow most of the particulates to settle, before further sampling. Most of the water from the chambers was filtered through a 90 mm diameter 0.8 µm pore size cellulose acetate membrane filter (Sartorius) using a vacuum pump. Filtered water was divided into 20 ml HDPE containers and analysed within three months (Strickland & Parsons 1968, Avanzino & Kennedy 1993, Kotlash & Chessman 1998) for silicate (15 ml, stored at 4°C); total ammonia nitrogen (TAN), nitrate and nitrite, phosphate (all 15 ml, stored at -18°C), and dissolved organic nitrogen (DON, 15 ml, acidified using H_2SO_4, stored at -18°C); dissolved organic carbon (DOC, 15 ml, acidified using HCl, stored at -18°C); and dissolved organic phosphorus (DOP, 15 ml, stored at -18°C). Remaining material in chambers was subsequently mixed and acidified to pH < 3 using H_2SO_4, and subsequently transferred onto the filter by flushing with demi water, and using a spoon to remove material from chamber walls. Salt was expelled by flushing with 150 ml demi water. All particulate material was transferred from the filter to pre-weighed porcelain crucibles, followed by gravimetric DW determination (103°C) and AFDW determination (550°C). For nutrient content analysis the material from biodeposit chambers was subsequently powdered using a pestle, and samples were analysed within three months.

5.2.5. Nutrient analysis

Amounts and concentrations of Si, N or P containing compounds are quantified in terms of their constituent element Si, N, or P. Silicate concentrations were determined by Seal QuAAtro segmented flow analyser (Jodo et al. 1992, Aminot et al. 2009). Ammonia,

phosphate, and NO_x (determined as nitrate plus nitrite), were determined using a Skalar San++ segmented flow analyser (Aminot et al. 2009). Dissolved inorganic nitrogen (DIN) was calculated as the sum of ammonia and NO_x. Total dissolved nitrogen (TDN) was determined from filtered water samples as NO_x following persulfate and subsequent UV digestion (Kroon 1993, Eaton et al. 1999), and dissolved organic nitrogen (DON) was determined by subtraction of DIN. Total nitrogen (TN) and total phosphorus (TP) were determined as NO_x and phosphate after 30 min oxidation at 120°C in stopped volumetric flasks each containing 25 ml of unfiltered water sample and reagent (50 g L^{-1} potassium peroxodisulfate, 7.5 g L^{-1} sodium hydroxide), and filled up to 100 ml (Valderrama 1981). Dissolved organic phosphorus (DOP) was determined using the same procedure applied to filtered water samples, and by subtraction of phosphate. Dissolved organic carbon (DOC) was determined from filtered water samples using a Skalar San++ segmented flow analyser using photochemical conversion to CO_2 and infrared detection (e.g. Collins & Williams 1977; according to NEN-EN 1484). Particulate organic N (PON) and P (POP) from incubation chambers were determined using a Skalar San++ segmented flow analyser after digestion using a H_2SO_4/Se/salicylic acid/H_2O_2 solution (Temminghoff & Houba 2004). Biogenic Si (BSi) was determined after a 3 h extraction of 25 mg of feces in 25 mL of 0.5M NaOH solution and subsequent analysis of extracted silicate concentration on a Skalar San++ segmented flow analyser (adapted from DeMaster 1981, see Barão et al. 2015). Particulate organic C (POC) from incubation chambers was determined using a Thermospectronic Aquamate spectrophotometer following oxidation at 135°C in a solution of H_2SO_4 and $K_2Cr_2O_7$ (Walinga et al. 1992). C and N content of the material collected on filters from the water supply were freeze-dried and ground to a fine powder for analysis on a Interscience Flash 2000 organic element analyser (Nieuwenhuize et al. 1994). P content of this material was analysed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, ThermoFisher iCAP6500) after digestion with HNO_3 at 200°C using Ytterbium as internal reference standard (Poussel et al. 1993).

5.3.6. Calculations & statistics

Throughout the text error values and error bars represent standard deviations unless otherwise specified. The term nutrient "release" is used in this study to refer to the net balance of underlying nutrient uptake and release processes, where the balance represents accumulation of a nutrient.

PON and POP were determined as N and P content of particulate dry mass. Amounts of ammonia, NO_x, DON, phosphate, DOP, DOC, and silicate in chambers were normalised to one gram of feces or pseudofeces. Concentrations were multiplied by the water volume in the chamber, and divided by the dry particulate mass in the chamber, which was calculated as particulate plus dissolved matter present on the sampling day minus mean dissolved matter in chambers sampled on day 1. This accounts for the loss of water due to

aeration, amounting to 2.3 ml d^{-1} on average. This method assumes that the total amount of material (particulate plus dissolved) did not change throughout the experiment. Linear regression analysis of the mass of total material over time confirmed that there was no significant change in the total material throughout the experimental period. To allow comparison, control concentrations (after multiplication by the water volume in the chamber) were standardised by scaling fluxes using the average dry particulate mass used to standardise the experimental chambers to 1 g biodeposit (0.5175 g).

Trends in nitrogen and phosphorus variables were calculated after subtraction of controls and tested using linear regression through the origin for the first 18 days, marking the cessation of accumulation of DIN, which was followed by decreasing concentrations indicating a switch to predominant removal of nutrients. Accumulation of phosphate ceased later, and accumulation of silicate did not cease at all, but all parameters were analysed over the same period for comparability. A coinciding formation of increasing quantities of slimy material observable on chamber walls after the period of accumulation might be an artefact of the experimental setup or the use of incubation vessels; the analyses do not include this period. Trends in silicate were tested using power regression since chemical dissolution leading to increasing concentrations of silicate is expected to lead to diminishing release rates (e.g. Struyf et al. 2007). Regressions were considered significant when $p<0.05$. Power regressions for DIN and phosphate did not indicate suppression of accumulation by approaching equilibrium. Hence, the linear regressions were used for further calculations. Release rates were compared between biodeposit types and between nutrient parameters per biodeposit type using analysis of covariance with either biodeposit type or nutrient parameter as the categorical variable, with results considered significant when $p<0.05$. Initial release rates were calculated by derivative from regression equations, and expressed as daily release in per cent of the particulate amount present on day 1. Stoichiometric ratios of N:P, N:Si and Si:P were calculated on a molar basis. Ratios in released nutrients were based on the derivative from regression equations on day 1.

5.3. Results

5.3.1. Initial conditions

On the four days leading up to and including the day of biodeposit production the water supply feeding the mussels contained 9.6±1.4 mg L^{-1} SPM (DW), of which 37.5% was organic matter. In the SPM, 0.66±0.10 mg C L^{-1}, 0.06±0.01 mg N L^{-1}, and 0.014±0.002 mg P L^{-1} was present.

Slightly more dry mass of pseudofeces was added to chambers than of feces at the start of incubations (not intentional; Table 5.1). Pseudofeces contained more organic matter, C and N than feces, and feces contained more Si than pseudofeces, whereas P content was similar in both compartments. Control chambers contained 1.0±0.8 mg dry mass of particulate material on day 1.

Table 5.1. Dry mass and organic content (day 0; n=5) and nutrient content (day 1; n=3; but for Si n=2) of initial particulate material added per chamber. *: feces and pseudofeces significantly different.

	particulate material added		nutrient content (mg g^{-1} DW)			
biodeposit type	dry mass (mg)*	organic content* (%)	C*	N*	P	Si*
feces	485.6 ± 60.4	20.3% ± 2.2%	51.8 ± 0.3	4.8 ± 0.2	1.38 ± 0.06	42.0 ± 1.5
pseudofeces	549.5 ± 43.1	25.9% ± 0.1%	54.9 ± 1.4	5.4 ± 0.0	1.42 ± 0.04	31.2 ± 0.3

5.3.2. Dissolved nutrients

DIN and phosphate declined after day 22 and 18, respectively. During the first 18 days, the average DIN release rates from feces and pseudofeces after subtraction of controls were 0.035 and 0.037 mg g^{-1} DW d^{-1}, respectively (Table 5.2), which was not significantly different. Expressed per unit initial N, 0.73 and 0.69% d^{-1} were regenerated from feces and pseudofeces, respectively. During the same period, the phosphate release rate from feces after subtraction of controls was significantly higher (0.007 mg g^{-1} DW d^{-1}) than from pseudofeces (0.006 mg g^{-1} DW d^{-1}). Expressed per unit initial P, 0.48 and 0.44% d^{-1} were regenerated from feces and pseudofeces, respectively (Table 5.2). The elemental release rate of N was significantly greater than that of P, and the fraction of initial feces and pseudofeces N released daily was approximately twice that of P. The first 18 days represented the release of 13.1% and 12.4% of initial N in feces and pseudofeces, respectively, and 8.7% and 7.9%, respectively, of initial P.

In the first week, ammonia accumulated in feces chambers, while in pseudofeces chambers there was a much lower accumulation which peaked on day 5 (Figure 5.2). The accumulation in feces chambers was similar to that in the controls. At the same time, NO_x was released faster in pseudofeces chambers than in feces chambers at the start of the study, and appeared to accelerate slightly after the first week (Figure 5.2). In feces chambers NO_x release started more slowly and accelerated more after the first week, catching up with pseudofeces chambers after three weeks.

Silicate release followed power functions throughout the incubation period, with release rates diminishing progressively (Figure 5.3; Table 5.2). There was a very small but significant decrease of silicate in the controls. Release rates after subtraction of controls were significantly greater from feces than from pseudofeces, with 1.21% d^{-1} regenerated

on day 1 from feces and 0.84% d^{-1} from pseudofeces. The first 18 days represented the release of 11.0% (feces) and 6.8% (pseudofeces) of initial BSi.

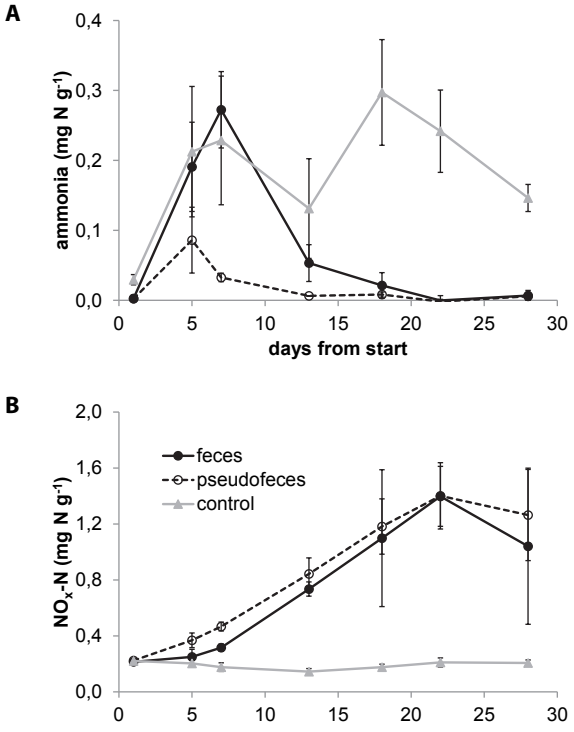

Figure 5.2. Detail of the constituent parts of DIN: quantities of ammonia (panel A) and NO_x (panel B) during the incubation period (element mass standardised to dry mass of start material in chamber; error bars indicate SD; n=3 for each treatment). Data points are connected by straight lines.

Table 5.2. Regression equations describing release of DIN, phosphate and silicate, standardised to 1 g dry masof start material, after correction for controls and fitted through the origin. Nutrient release rates are calculated by derivative on day 1 and expressed as daily release in per cent of initial amounts.

parameter	feces		pseudofeces	
	(mg element g^{-1} DW)	(% d^{-1})	(mg element g^{-1} DW)	(% d^{-1})
DIN	0.035*day	0.73	0.037*day	0.69
phosphate	0.007*day	0.48	0.006*day	0.44
silicate	$0.572*day^{0.886}$	1.21	$0.283*day^{0.930}$	0.84

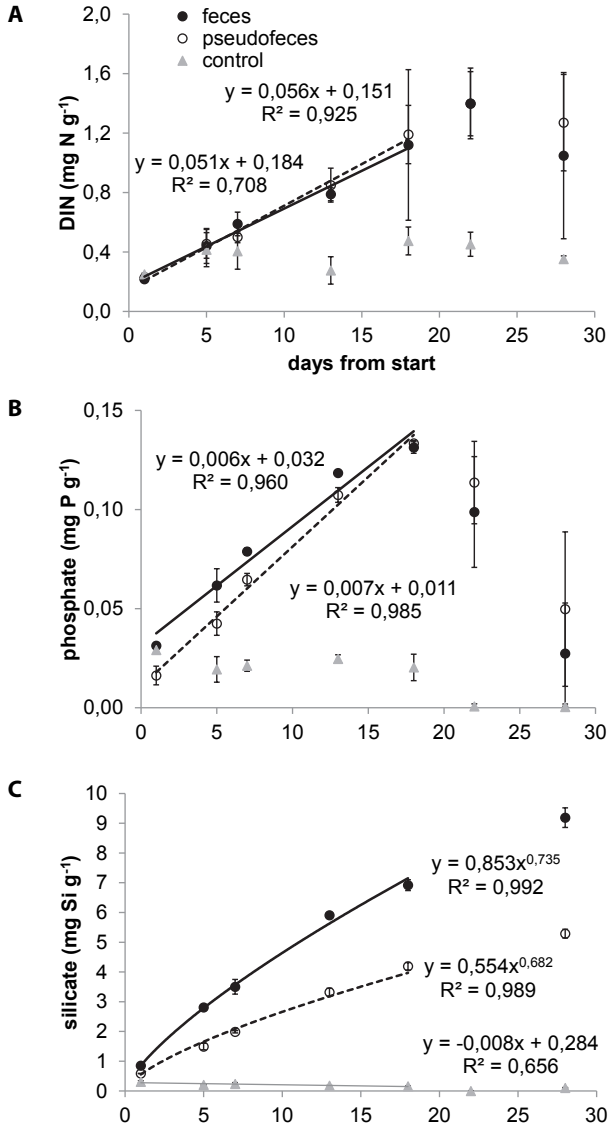

Figure 5.3. Dissolved inorganic nutrient quantities throughout the incubation period (element mass standardised to dry mass of start material in chamber; error bars indicate SD; n=3 for each treatment). Panel A: DIN; B: phosphate; C: silicate. Lines (unbroken: feces; broken: pseudofeces; grey: control) represent all significant regressions over the first 18 days (equations and R^2 shaded for pseudofeces in panel A); linear for DIN and phosphate and power for silicate.

Concentrations of DON and DOP were low compared to their dissolved inorganic forms, and concentrations in controls were similar to feces and pseudofeces chambers. There were significant trends of reduction of DOP and DOC in pseudofeces during the first 18 days, but there were no trends in DON, feces, or controls (Figure 5.4). After subtraction of controls, a statistically significant reduction of DON was found, in feces, which was small compared to the DIN accumulation (DON: 0.05% d^{-1}; DIN: 0.73% d^{-1}). Similarly, a statistically significant reduction of DOP was found, in pseudofeces, which was small compared to the phosphate accumulation (DOP: -0.03% d^{-1}; phosphate: 0.44% d^{-1}). DOC could not be calculated in this way as it was not measured separately in controls. After day 18 there was a sudden increase in DOP in all treatments, which can also be observed in DOC to a lesser extent.

5.3.3. Particulates and nutrient balance

Near the end of the incubation period increasing amounts of mucous material were observed in biodeposit chambers, and filtration on the 90 mm ø, 0.8 µm pore size membrane filters became increasingly difficult. However, there were no significant trends in recovered dry mass of total particulate material over the incubation period.

There were no significant trends in PON for feces and pseudofeces, and for POP there was only a trend in feces (Figure 5.5), of -0.67% d^{-1} after subtraction of controls. The total amounts of N and P per chamber over the incubation period were examined for trends over the first 18 days. Dissolved inorganic, dissolved organic, and particulate organic constituents were summed to estimate total amounts of N and P on each sampling day. After subtraction of controls, the summed N in feces pseudofeces chambers showed a significant increase over time of 0.61% d^{-1}, which was not significantly different from the increase of DIN (Table 5.2; 0.73% d^{-1}). There was no trend in the controls. The summed P did not show any significant trends.

5.3.4. Nutrient stoichiometry

The difference between feces and pseudofeces in N:P ratio of regenerated nutrients was limited, whereas feces released proportionally more Si than pseudofeces (Table 5.3).

Biodeposit decomposition influenced the stoichiometry of dissolved inorganic nutrients in the surrounding water. The N:P ratio of dissolved inorganic regenerated nutrients was lower than Redfield's ratio, but was higher than that of the particulate material on day 1, which in turn was more than double that of dissolved inorganic nutrients in the Oosterschelde water. Availability of N was thus promoted relative to P. Additionally, a considerable surplus of Si relative to N and P in the regenerated nutrients was evident, since N:Si was lower, and Si:P was considerably higher, than both the Oosterschelde water and Redfield's ratio.

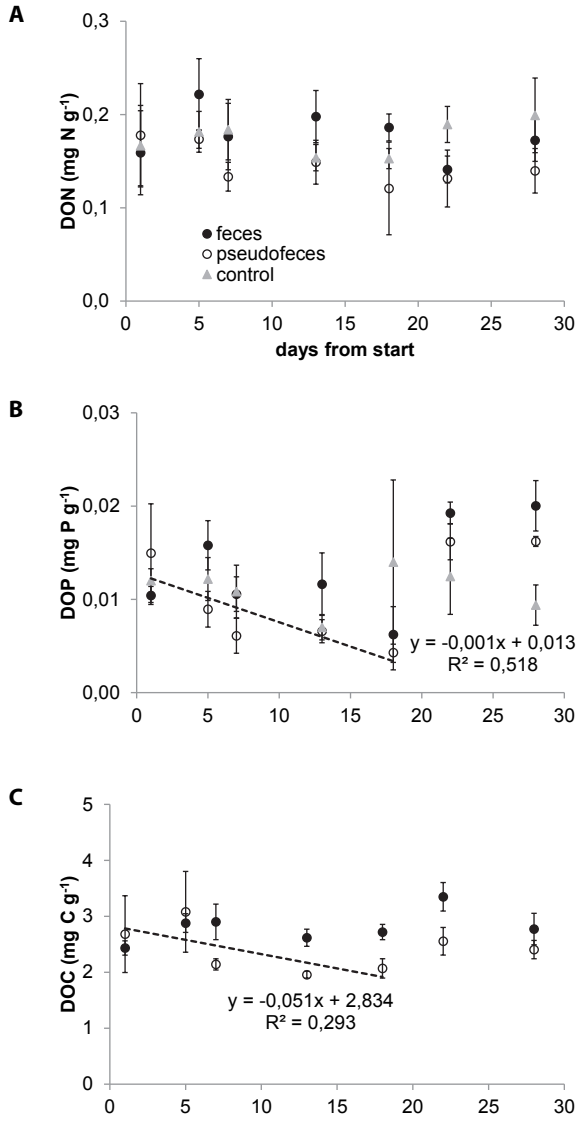

Figure 5.4. Dissolved organic nutrient quantity throughout the incubation period (element mass standardised to dry mass of start material in chamber; error bars indicate SD; n=3 for each treatment). Panel A: DON; B: DOP; C: DOC. DOC was not measured in control chambers. Straight lines represent significant linear regression trends for pseudofeces over the first 18 days (non-significant trends omitted).

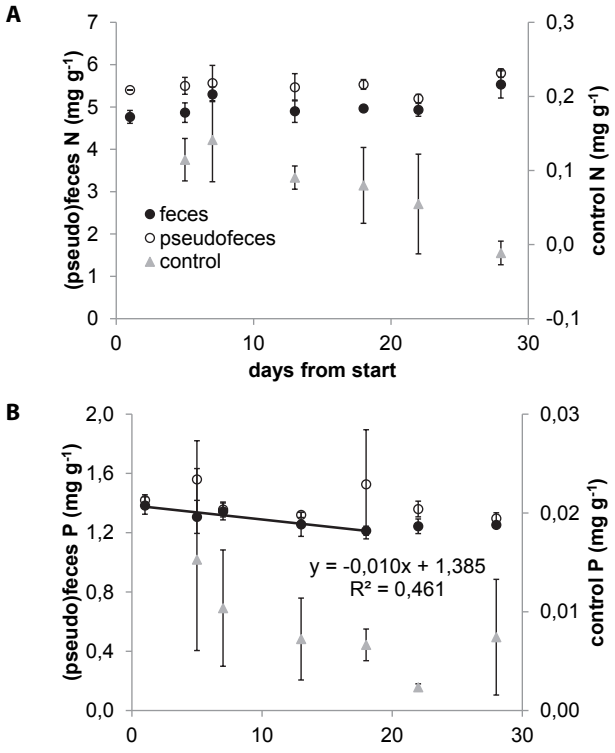

Figure 5.5. Particulate organic N (panel A) and P (panel B) quantities (element mass standardised to dry mass of start material in chamber; error bars indicate SD; n=3 for all treatments). Straight line represents significant linear regression trend for feces P over the first 18 days (non-significant trends omitted). Control data not available for day 1.

Table 5.3. Comparison of stoichiometric ratios of nutrients: in feces and pseudofeces chambers on day 1 of incubations; regenerated from biodeposits on day 1; in the Oosterschelde estuary ecosystem at the time of the experiment; and Redfield's ratios.

		N:P	N:Si	Si:P
feces/pseudofeces day 1	feces	7.6	0.22	34.8
	pseudofeces	8.4	0.30	28.4
regenerated nutrients (dissolved inorganic)	feces	11.4	0.14	83.4
	pseudofeces	13.3	0.26	43.4
Oosterschelde average (dissolved inorganic)		3.2	0.70	4.8
Redfield ratio		16	1.07	15

5.4. Discussion

5.4.1. Mineralisation rates

Processes

The rates as determined in this study represent the net balance of underlying processes. During organic matter decomposition, nutrients are released to the environment in various forms contributing to the dissolved inorganic or organic nutrients, while another part is incorporated by bacteria (Horrigan et al. 1988, Canfield et al. 2005) or by other constituents of the heterotrophic food web (Azam et al. 1983, Fabiano et al. 1994). During the incubations, the period of linear accumulation of DIN and phosphate was followed by decreasing concentrations after days 22 and 18, respectively, indicating a switch to predominant removal of nutrients. This coincided with formation of increasing quantities of slimy material, a mix of microorganisms and trapped organic matter, on chamber walls. This might be an artefact of the experimental setup or the use of incubation vessels, and therefore calculations and comparisons are only based on the period up to this point.

The balance of regeneration and incorporation depends on the proportional nutrient composition of the substrate (Goldman et al. 1987, Tezuka 1990, Canfield et al. 2005). The nutrient composition of the biodeposits in the present study (feces C:N 12.7; pseudofeces C:N 11.9; feces N:P 7.6, pseudofeces N:P 8.4) suggests that the amount of regeneration may be expected to be low and accompanied by high levels of incorporation. C:N content was not much higher than reported for natural marine bacterial assemblages by Goldman et al. (1987), who found little or no ammonia release during the exponential growth phase at a low C:N value of 10. These authors reported that some ammonia release did occur during the subsequent stationary phase when endogenous metabolism and cell death became dominant processes. Tezuka (1990) described interactions between substrate C:N and N:P ratios for freshwater bacterial communities, observing that both ammonium and phosphate were regenerated when both N and P content were high enough ($C:N \leq 10$ and $N:P \leq 16$), but that neither was regenerated when N and P content were at low levels ($C:N \geq 15$ and $N:P \geq 5$). Feces and pseudofeces in the present study lay between these combinations. Several factors may modify these relationships in the context of the present study. Firstly, the nutrient ratios of the complex substrates investigated in the present study may not necessarily correspond to nutrient ratios of the portion of the substrate actually undergoing decomposition (Tezuka 1989, 1990, Canfield et al. 2005). Secondly, the involvement of other organisms present in the untreated seawater used in the current study, for instance primary and secondary consumers of bacteria such as flagellates and microzooplankton (Azam et al. 1983, Jacobsen & Azam 1984), or dinoflagellates(Poulsen et al. 2011), may influence regeneration rates and dynamics. Finally, in making the translation to natural situations where sunlight is available to support primary production, the share of nutrients captured by heterotrophic microbes may be reduced due to competition with

primary producers (e.g. Fuhrman et al. 1988, Danovaro 1998), which would effectively increase the efficiency of the nutrient feedback as more nutrients might be available for primary producers than expected based on the outcomes of the current study.

Dissolved inorganic nutrients

DIN and phosphate accumulated until sampling days 22 and 18, respectively. A comparison can be made in terms of the overall fraction of organic start material which is regenerated into the dissolved inorganic phase in the first 18 days. Values were compared to literature describing biodeposits produced by adult mussels; the use of mussel spat in the present study should be kept in mind. The 12-13% of initial PON released as DIN during this period was lower than results reported by Jansen et al. (2012b), who created stable state conditions analogous to a bioreactor by adding fresh biodeposits daily, and found that overall 17% of PON was released to the environment as ammonia. A similar value of 18% release of PON as ammonia was estimated by Giles & Pilditch (2006) for biodeposit decomposition on sediment cores over 10 days. Another perspective is provided by comparing rates of nutrient regeneration, which shows that daily release of DIN expressed as per cent of initial PON (feces: 0.73% d^{-1}; pseudofeces: 0.69% d^{-1}) was less than half of values reported by Giles & Pilditch (1.8% per day; 2006).

Smaal & Prins (1993) estimated very high rates of 4.6% d^{-1} PON regeneration for feces, and 1.6% d^{-1} for pseudofeces. In further contrast, Fabiano et al. (1994) reported 87% decomposition of organic matter from mussel fecal material within 3 d; part of this material constituted inorganic nutrient regeneration. However, proportional nutrient regeneration rates could not be compared as quantitative information regarding the start material was not provided by these authors.

A comparison as described above for PON could not be carried out for POP, since we did not find studies in literature combining reliable estimates for initial POP content of bivalve biodeposits with release rates of phosphate. Similarly, no studies were found giving initial BSi content. However, previous research can be compared in terms of stoichiometric proportions of released nutrients. N:P ratios (feces 17, pseudofeces 15) were within the range reported in literature, being higher than the range given by Jansen et al. (2-12; 2012b), but lower than reported for benthic biodeposit decomposition by Callier et al. (27; 2009) and reported for sediment core biodeposit decomposition by Giles & Pilditch (elevated by 27; 2006). Si:P ratios (feces 84, pseudofeces 45) were within the range reported by Jansen et al. (2-143; 2012b), and higher than reported by Callier et al. (36; 2009). N:Si ratios (feces 0.2, pseudofeces 0.4) were within the range given by Jansen et al. (0.0-1.3; 2012b), but this was lower than the value reported by Callier et al. (0.7; 2009). This indicates that relatively more P and Si regeneration was observed in the present study

than reported by Callier et al. (2009) (2009), and relatively more P than reported by Giles & Pilditch (2006).

The studies by Jansen et al. (2012b) and Giles & Pilditch (2006) both reported that DIN release was dominated by ammonia, whereas in the present study NO_x was the dominant form. Giles & Pilditch (2006) hypothesised that coupled nitrification-denitrification could have removed ammonia, and this might account for part of an additional estimated 34% PON regeneration that was not detected as ammonia. We do not expect meaningful levels of denitrification and N_2 production in the present study since a well oxygenated system was applied. However, nitrification was likely an important process, as accumulation of TAN in feces and control treatments early on during incubations was followed by its removal and concurrently rising NO_x concentrations. This likely reflects the development of a nitrifying microbial community reaching considerable nitrifying capacity only after several days. Ammonia accumulated initially since the oxidation of this compound is considered to be the rate-limiting step in nitrification (Kaplan 1983, Canfield et al. 2005). In the pseudofeces treatment, a shorter period and lower levels of ammonia accumulation, and more rapid NO_x accumulation, point to a more rapid establishment of nitrifying capacity, suggesting that the associated microbes were more present or more active in pseudofeces than in feces and in (control) seawater.

Regeneration of BSi differs fundamentally from PON and POP because it relies on chemical dissolution rather than biological processes (Paasche 1980). The diminishing rate of Si accumulation over time, with accumulation following a power law, could be indicative of the substrate becoming less degradable during chemical dissolution. However, earlier experiments with dissolved Si release from litter of *Phragmites australis* (common reed), indicated that rather than the substrate becoming less degradable, dissolution is impacted by decreasing rates due to accumulation of dissolved material, and an equilibrium concentration is reached (Struyf et al. 2007). In our experiment, dissolved silicate concentrations at the end of the experiment on day 28 were 475.8±35.9 µmol L^{-1} for feces and 325.1±17.5 µmol L^{-1} for pseudofeces, which is not as high as equilibrium concentrations reached during the reed decomposition experiment (approximately 1500 µmol L^{-1}), but the power function still indicates a reducing release rate during the experiment. Solid (as total amount of incubated BSi)-solution rates in the beginning of our experiment were about one order of magnitude lower than in the experiment described in Struyf et al. (2007), and initial BSi content in the litter of reed (6% BSi) was higher compared to feces (4,2% BSi) and pseudofeces (3.1% BSi). This could explain why saturation was attained in the reed experiment after 30 days, but not yet in our experiment. Due to the different initial conditions, a quantitative comparison of the release rates between both experiments is difficult. At the end of the experiment, 22% of the incubated BSi had been dissolved from the feces, and 17% had been dissolved from the pseudofeces. This

indicates that not only did feces contain relatively more BSi compared to pseudofeces; feces released a larger part of the BSi over the same time period, emphasising that the initial BSi content alone cannot explain the faster release from the feces.

The relative importance of Si release relative to N and P was likely underestimated for two reasons. Firstly, since silicate concentrations were higher than encountered environmentally and thus suppression of BSi release was elevated. Concentrations of silicate on day 1 (feces chambers: 1.06 mg L^{-1}; pseudofeces: 0.78 mg L^{-1}) were already elevated compared to maximum environmental concentrations during the main SMC season in 2013 of June-August (0.25 mg L^{-1}). Secondly, since release rates were calculated for day 1 rather than approaching the very start of the incubations in order to avoid extrapolation outside of the measured range.

Nutrient balance

The low concentrations and stability or relatively small absolute changes of dissolved organic matter concentrations throughout the incubation period suggest that either limited production of dissolved organic matter occurred during incubations, or that production was matched by loss due to processing rates. Since biodeposit production took place over a 24 h period, loss of labile material may have occurred before the start of incubations, with concentrations in chambers having stabilised before measurements started. This may have led to an underestimation of overall nutrient regeneration. In literature, rapid leakage of dissolved organic matter from feces has been argued to occur in the first hours after production. Carlsson (2010) estimated that 2% POC h^{-1} was lost from mussel fecal pellets during the first 24 hours, a large part of which was not regenerated, and speculated that this part of removal represented leakage of DOC. But it is also possible that this part consisted of particles, microbes or other larger compounds (e.g. Jacobsen & Azam 1984). Fabiano et al. (1994) reported that mussel fecal material decomposed into dissolved inorganic nutrients with little change in dissolved organic matter concentrations. Møller (2003) reported DOC leakage rates from copepod fecal pellets exceeding 20% of POC within the first hour after production, with the rate of leakage already rapidly levelling off during this period. Using the estimate of Carlsson et al. (2010), under the assumption that feces and pseudofeces lose POC at a similar rate, approximately 21% of POC could have been lost during the production setup.

Along with the release of dissolved inorganic N and P, and considering that there was little change in the dissolved organic phase, a reduction of PON and POP is expected. However, a reduction was only detected for POP, and only for feces. The rate of decrease of 0.67% d^{-1} was not significantly different from the negative of the rate of increase in phosphate of 0.48% d^{-1}. Slimy material formed on chamber walls throughout the experiment, but it cannot be verified whether inconsistent completeness of recovery of this material could

have contributed to variability and hindered detection of reduction of PON. The sum totals of N and of P were expected to remain constant during incubations. In the case of P this was verified, but the sum total of N increased for both feces and pseudofeces. We expect the analytical methodology to be robust to structural and chemical changes occurring in particulate material over the course of the incubations. However, concentrations were relatively low, increasing the likelihood of not detecting changes. Further research is needed to verify N dynamics in the different compartments and should focus on the methodology.

5.4.2. Comparison of feces and pseudofeces

In contrast to the proposition that bacteria contained in feces may accelerate mineralisation (Gowing & Silver 1983, Harris 1993, Fabiano et al. 1994), our results showed similar N regeneration per unit mass from feces and pseudofeces, despite different dynamics of ammonia and NO_x, and a higher release rate of P regeneration from pseudofeces. It is possible that bacterial colonisation after egestion, which can be very rapid (Stuart et al. 1982, Jacobsen & Azam 1984), and which could potentially be more so due to greater surface:volume ratios in biodeposits produced by juvenile mussels, may have overshadowed any "head start" of the feces. Possibly, the bacterial community promoted by the bivalve enteric environment (e.g. denitrifiers, Stief et al. 2009, Svenningsen et al. 2012) does not perform very effectively in an oxic environment after egestion. In fact, it appears that pseudofeces experienced a "head start" with regard to nitrification, with the formation of nitrifying capacity requiring a shorter lag phase than for feces. It should be noted that nutrient regeneration rates from pseudofeces should be interpreted as an approximation since part of the material was natural sedimented material that was deposited in the production setup; this also resulted in slight organic enrichment of the pseudofeces material (the sedimented material contributed 23% to pseudofeces DW but 28% to AFDW).

Si dissolution rates were higher for feces per unit initial biodeposit DW, and 1.4 times higher per unit initial BSi. We here hypothesise that two processes cause the difference between feces and pseudofeces. Firstly, we suggest that the organic matrix surrounding the BSi is broken down more strongly in the feces, which is reflected in the higher dissolution rate. A similar observation has been found in cattle, where grass BSi dissolved much quicker after digestion, as digestion removed the organic matrix surrounding the BSi (Vandevenne et al. 2013). In cattle feces, a stronger digestion of organic matrices can be expected compared to pseudofeces, which would explain the stronger dissolution. Bidle & Azam (1999) also observed that bacterial activity can accelerate silica dissolution by breaking down the organic matrix protecting diatom frustules. In the *P. australis* decomposition experiment by Struyf et al. (2007), suppression of bacterial activity also slightly decreased Si release rates. Secondly, Dame et al. (1991) speculated that dissolution rates of diatom frustules

can be accelerated by fragmentation during digestion. If diatoms are more fragmented in feces then pseudofeces, this could further explain the difference in dissolution rates. Given the rising proportion of pseudofeces with increasing food concentration beyond a certain level (Foster-Smith 1975, Tsuchiya 1980), the role of bivalve suspension feeders in terms of Si regeneration could be relatively greater at lower food concentrations, assuming food composition does not change. As an alternative hypothesis, mussels could also potentially actively select for ingestion of least recalcitrant BSi, causing increased solubility of feces BSi. Biogenic Si can differ in solubility due to several factors, including specific surface and aluminum content (Van Cappellen et al. 2002).

5.4.3. Nutrient feedback and limitation

During the SMC growth seasons of 2012 and 2013, N and Si concentrations, but not P concentrations, in the study area were at times below the half-saturation coefficient for phytoplankton uptake (N and Si: 2 µmol L^{-1}; P: 0.2 µmol L^{-1}) (Rijkswaterstaat, www.waterbase.nl), suggesting that N and/or Si, and not P, availability was likely limiting primary production at those times (Philippart et al. 2007, Kromkamp et al. 2013; for discussion on limiting nutrients see also van Broekhoven et al. 2014). In the present study N and Si were released during biodeposit decomposition, and this lead to N and Si enrichment relative to P. Furthermore, Si was regenerated at a faster relative rate than N. In a context of N and Si limitation, regeneration of nutrients through decomposition of mussel biodeposits thus has the potential to stimulate primary productivity.

Wikfors (2011) argued that entrapment of diatom frustules in biodeposits might promote a non-diatom algal community – potentially containing harmful species – through preferential N and P recycling relative to Si. Our research, however, suggests that SMCs actually contribute to reduction of Si limitation through preferential recycling of Si compared to N and P, and thus has the potential to stimulate growth of diatoms. In the Bay of Brest, recycling of BSi by the invasive suspension feeder *Crepidula fornicata* was considered an important factor for the avoidance of harmful algal blooms in summer (Ragueneau et al. 2002).

5.4. Conclusions

Substantial regeneration of N, P and Si from decomposing mussel biodeposits was measured. There was no significant difference between feces and pseudofeces in terms of overall DIN (feces: 0.73% d^{-1}; pseudofeces: 0.69% d^{-1}) regeneration rates, but early DIN dynamics were different in terms of more rapid depletion of ammonia due to nitrification in pseudofeces. Regeneration rates of phosphate were 1.11 times higher from feces (0.48% d^{-1}) than from pseudofeces (0.44% d^{-1}). Silicate regeneration rates were 1.43 times higher

from feces (1.21% d^{-1}) than from pseudofeces (0.84% d^{-1}). During the summer season when SMCs are deployed, shortages of N and Si in the study system, the Oosterschelde, indicate that nutrient regeneration from biodeposit decomposition constitutes an important feedback pathway that needs to be quantified in order to assess aquaculture impacts. Our results add to the growing evidence that, besides having the capacity to control N and P circulation in ecosystems, producer-consumer interactions can also play an important role in the regulation of the global Si cycle.

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CHAPTER 6

In situ evidence for bottom-up and top-down regulation of phytoplankton by suspended mussel (*Mytilus edulis*) farms under macrotidal conditions

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Abstract

Suspension-feeding bivalves filter large quantities of particulate matter from the water column, while at the same time returning inorganic nutrients to the environment. However, *in situ* observations for these processes are scarce. In this study we aimed to demonstrate both top-down (filtration) and bottom-up (nutrient regeneration) effects on nutrient cycling in suspended mussel farms in the Oosterschelde bay in the Netherlands. Measurements were carried out *in situ* at three Mussel Seed Collector (SMC) farms and one suspended grow-out mussel farm in the macrotidal Oosterschelde bay in the Netherlands. A range of parameters (TPM, Chl *a*, particle concentration, POC, PON, DIN, DIP) was measured from water samples taken throughout the farms. This was complemented by continuous measurements of TPM and Chl *a* with a towed glider, that rendered a high-resolution spatial map. A reduction in seston concentrations (up to 65%, POC) and an increase of overall nutrient concentrations (up to 208%, DIN) were observed. Variability between farms and parameters was high, likely due to differences in hydrodynamic circumstances and patchy water mass contents. This study is one of the first to *in situ* simultaneously show top-down and bottom-up processes for suspended bivalve aquaculture.

6.1. Introduction

Suspension-feeding bivalves can play a key role in the ecosystem, involving both top-down and bottom-up mechanisms (Dame et al. 1991, Dame & Prins 1998, Prins et al. 1998, Newell 2004, Hulot et al. 2020). Top-down regulation here refers to bivalve filtration exerting control over phytoplankton populations. Suspension-feeding bivalves are efficient filter feeders, able to clear significant water volumes of particles on a daily basis (Cranford et al. 2011). Bottom-up regulation refers to nutrient regeneration by bivalves fertilising their own food source (e.g. Smaal et al. 2001). The animals return nutrients to the environment via two principal routes: excretion and biodeposition. In bivalves such as the blue mussel *Mytilus edulis*, particle selection results in two forms of biodeposits: feces (ingested) and pseudofeces (rejected prior to ingestion). Excreted nutrients are returned to the water column directly in a short-term process (van Broekhoven et al. 2014), whereas release of inorganic nutrients through remineralization of biodeposits takes place on longer timescales (Jansen et al. 2012b, van Broekhoven et al. 2015).

At high bivalve culture densities, local food depletion due to intra-specific competition has been observed inside mussel farms (Heasman et al. 1998, Fuentes et al. 2000, Strohmeier et al. 2008, Maar et al. 2008, Petersen et al. 2008, Cranford et al. 2014, Kamermans et al. 2014a). Strohmeier et al. (2008) further reported reduced meat ratio and biomass of mussels at the center of a suspended mussel farm, Kamermans et al. (2014a) reported reduced mussel biomass at the center of a suspended mussel seed collector farm, and Heasman et al. (1998) reported reduced growth rates inside mussel rafts, all indicating that growth reduction was a result of reduced food availability within the farms. Bottom-up effects inside suspended bivalve farms have been reported for two locations: Zuñiga (2013) reported elevated nutrient concentrations, particularly of ammonia, in a mussel raft in the Ría de Vigo in Spain, and Trottet et al. (2008) reported increased primary productivity of phytoplankton inside a mussel farm in Grande-Entrée Lagoon in Canada. In general, the extent to which top-down and bottom-up regulation in suspended bivalve culture influence the composition of seston and nutrients in the water column is a function of the activity level of the animals, in combination with local environmental conditions, and against background concentrations. The activity level of the culture depends on the density and the individual metabolic activity of the animals. Furthermore, local hydrodynamic conditions will determine the extent of dilution of the effects of the bivalves' metabolic activity. Water flushing rates are a result of environmental water currents and drag resulting from the configuration, density, and rugosity characteristics of the farm. However, at low water current velocities when the water layer close to the mussels' inhalant and exhalant syphons is not rapidly refreshed, refiltration becomes more important and limits the degree of food depletion (Cranford et al. 2014, Cranford 2019).

Since 2010, Seed Mussel Collectors (SMCs) consisting of nets and ropes have been increasingly deployed in the Netherlands in the spring and summer period (van Broekhoven et al., submitted). After settlement and growth on the collectors, the juvenile mussels are harvested at a shell length of 5 to 20 mm and relayed on bottom culture plots. Reduced mussel condition was reported in the centre of SMCs compared to the edge at one out of five studied farms in the Netherlands. (Kamermans et al. 2014a). This points to the possible occurrence of food competition between mussel seed on SMCs at the scale of the farm. Metabolic rates in SMCs are particularly high since the weight-specific activity level of suspension-feeding bivalve specimens decreases with increasing size of the animal according to an allometric relation (Cranford et al. 2011, van Broekhoven et al. 2014, Jacobs et al. 2015). Seed collector farms will thus have greater impact per unit biomass than adult farms. In the Oosterschelde bay, Western Wadden Sea, and near-shore North Sea (van Broekhoven et al., submitted) SMCs experience macrotidal conditions. As a result, the level to which seston depletion or nutrient accumulation are able to build up in the water column inside a farm could be counteracted by a short residence time and dynamic water currents.

In this study, we combine two approaches to investigate farm-scale processes in mussel (*M. edulis*) farms *in situ*, including a total three SMC farms and one suspended grow-out mussel farm. The aim was to evaluate if reduction of seston and enhancement of nutrient concentrations in the water column could be demonstrated *in situ* for suspended mussel farms in a highly dynamic bay. This information is useful to better understand the potential for food limitation, and the build-up of nutrients with potential implications for primary productivity, at the farm scale under natural conditions. Firstly, a range of seston and nutrient parameters were measured via a discrete sampling setup. Secondly, continuous high-resolution measurements of Chl *a* and total particulate matter (TPM) were taken using an undulating towed glider.

6.2. Materials & Methods

6.2.1. Study sites

The macrotidal and productive Oosterschelde bay in the south-west of the Netherlands is connected to the North Sea on the western side by a closable storm surge barrier, and on the eastern side fresh water carrying nutrients enters in small volumes through water locks (Figure 6.1). It is classified as a eutrophic system (cf. Nixon 2013). Nutrient and TPM concentrations show a gradient with higher concentrations on the western side (Nienhuis & Smaal 1994a). Water residence times increase from west to east (Jiang et al. 2019). Table 6.1 provides basic physical, environmental and biological data.

Table 6.1. Physical, environmental and biological characteristics of the Oosterschelde bay.

Parameter	Quantity	Source
surface area at mean water level NAP (Amsterdam gauge)	304 km^2	1
water volume at mean water level NAP	$274*10^7$ m^3	1
mean depth	9 m	1
mean tidal range	3.25 m	2
mean tidal volume	$880*10^6$ m^3	2
maximum current velocity	1.0 m s^{-1}	2
(mean) water residence time	0 – 150 (40) d	1,3
SMC *M. edulis* harvest, 2012	$3.2*10^6$ kg	5
Wild and culture plots *M. edulis* biomass, 2012	$44.7*10^6$ kg	5
Other bivalve filter feeder biomass, 2012	$66.6*10^6$ kg	5
[DIN-N] range Jun-26 – Aug-21 2012	1.6 – 8.2 (µmol L^{-1})	6
[phosphate-P] range Jun-26 – Aug-21 2012	0.6 – 1.1 (µmol L^{-1})	6
[silicate-Si] range Jun-26 – Aug-21 2012	2.1 – 7.7 (µmol L^{-1})	6

Sources: 1: (Smaal & Boeije 1991a); 2: Nienhuis and Smaal (1994a); 3: Jiang et al. (2019); 5: Jansen et al. (2019); 6: biweekly routine monitoring programme, www.waterbase.nl. DIN = Dissolved Inorganic Nitrogen (sum of ammonia, nitrate, and nitrite).

Four farms were studied (Figure 6.1; Figure 6.2; Table 6.2). The farm at Slaak was located at a sheltered site and was therefore expected to be most likely to be able to detect the influence of seed collector mussels on water column composition *in situ*. The farm at Krammer was more exposed, but its position at the end of the bay provided some shelter from tidal currents and waves. The farms at Neeltje Jans and Vuilbaard were exposed to tidal currents and longer wave fetch distances, and were included to test the possibility of detecting the effects at exposed locations. The Neeltje Jans and Vuilbaard farms consisted of mussel seed collectors only (Kamermans et al. 2002), the Slaak farm predominantly consisted of mussel seed (80%) with some adult mussels (20%), and the Krammer farm consisted of 1-year old (adult) mussels.. The discrete sampling campaign took place at the Slaak, Neeltje Jans, and Vuilbaard farms, and the towed glider campaign at the Krammer, Slaak, and Vuilbaard farms.

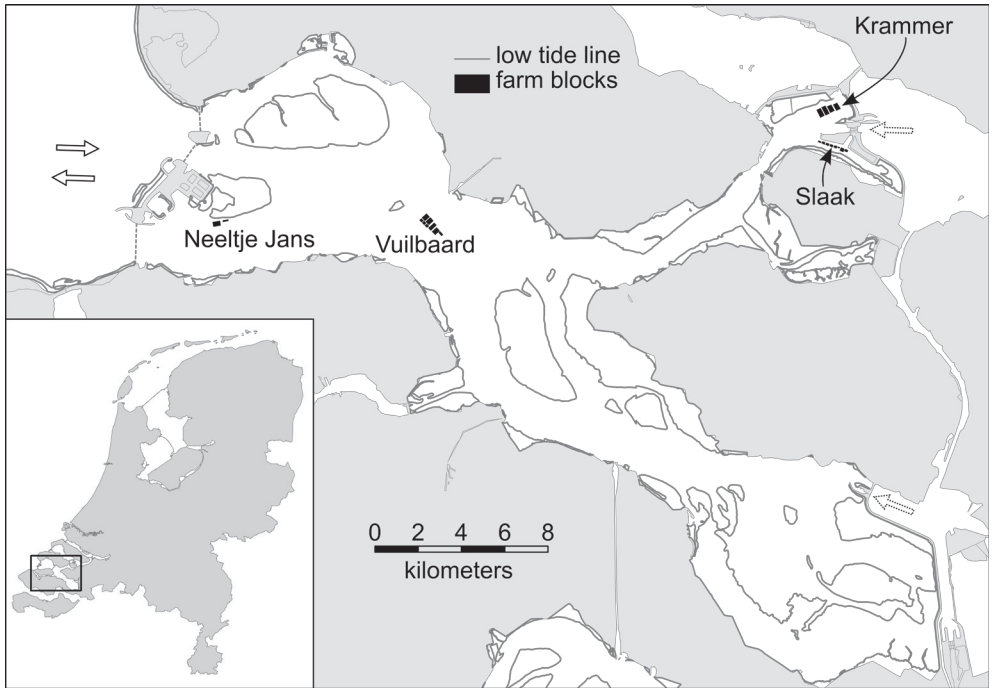

Figure 6.1. Seed Mussel Collector (SMC) sites in the Oosterschelde bay included in this study; inset shows location in the Netherlands. Land is shown in grey, and mean low water level as lines. Blocks of seed collectors are represented by solid black shapes. Solid arrows: tidal exchange through storm surge barrier (indicated by dotted lines); dashed arrows: fresh water inflow through water locks.

The farms were configured into distinct sections, hereafter "blocks" (see Figure 6.2 for detailed maps per farm), consisting of parallel rows of ropes or net substrate. The farm at Neeltje Jans near the mouth of the Oosterschelde bay consisted of two blocks spaced 80 m apart, south from a tidal flat. Samples were taken from the easternmost block (140 x 80 m). The large farm at Vuilbaard, in the mid-section of the Oosterschelde bay, consisted of blocks of varying sizes. Samples were taken from the block on the northern corner (190 x 190 m). The farm at Slaak in the northern branch of the Oosterschelde bay was located in an elongated channel enclosed by land on both sides. The farm consisted of eight sequential blocks of on average approximately 110 m length spaced on average approximately 60 m apart. Samples were taken across the farm's full length. Directly to the east, a series of adult mussel farm blocks in a similar layout extended further into the channel. The farm at Krammer consisted of four 400–500 m wide blocks of approximately 260 m length along the tidal current direction, spaced 115 m, 55 m, and 55 m apart. No samples were taken there.

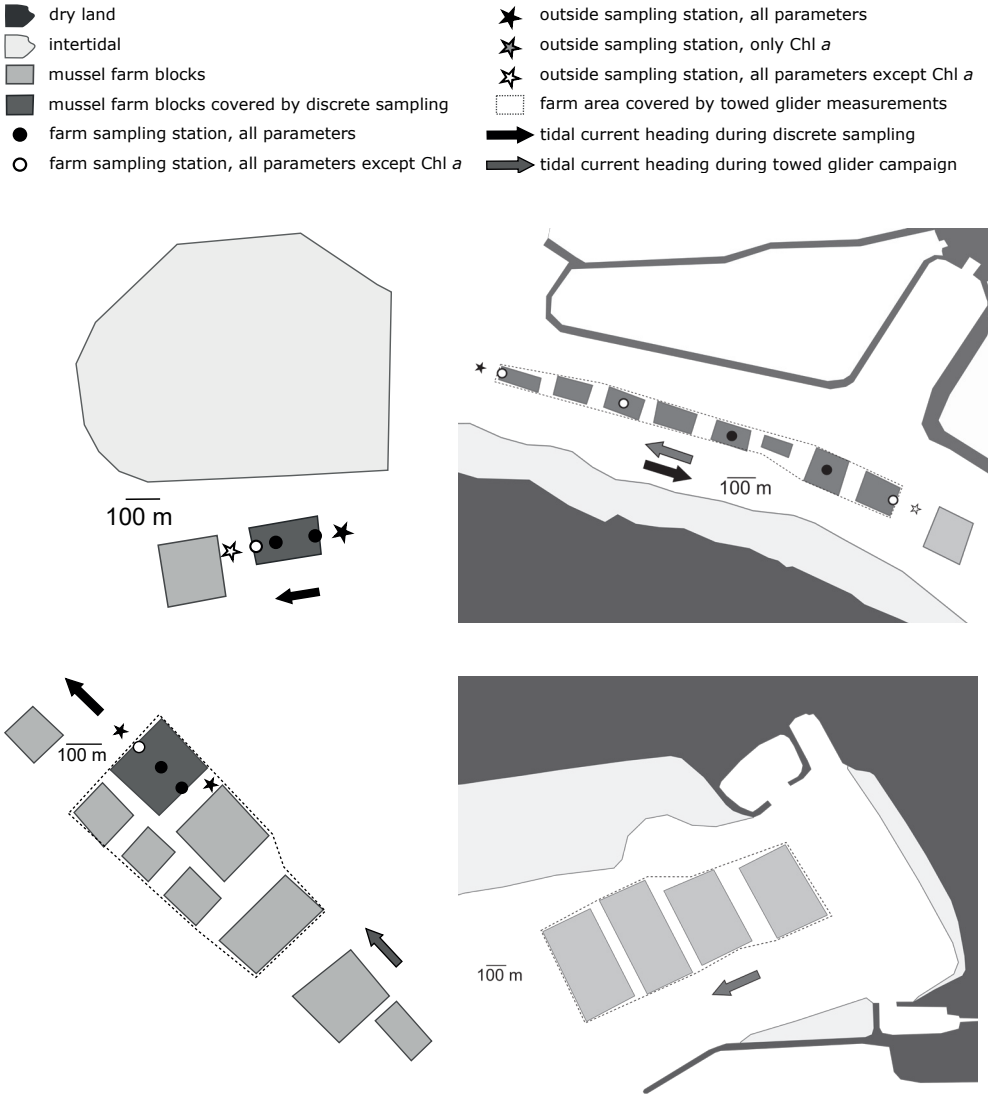

Figure 6.2. Detail of mussel farm blocks and coverage of the discrete sampling and towed glider campaign measurements.

Table 6.2. Characteristics of the three Seed Mussel Collector farms (SMCs). Data from Krammer farm was not available.

	Neeltje Jans	Vuilbaard	Slaak
water depth (m)	8–10	5–7	6 to 8
total seed collector area at site(ha)*	4.2	25.3	5.7
sampled block(s) area (ha)	1.2	3.7	5.7
density at harvest (kg ha^{-1})*	40,200	58,600	51,100
number of substrate rows	5	10	3–8
substrate type	ropes	ropes	nets
substrate max depth (m)	6	4	3
mussel shell length (mm)	10.7±0.8 (n=154)	13.4±1.4 (n=151)	12.6±0.5 (n=179)#

* van Stralen (2013). Densities represent time of harvest, several weeks after sampling campaign
# adult mussels not sampled

6.2.2. Discrete measurements campaign

Sample collection

Farm-scale top-down and bottom-up impacts on water column composition were investigated at the Slaak, Neeltje Jans, and Vuilbaard farms. These farms were sampled at successive days in the period 13–15 August 2012. Water temperature was ~20°C at all three sites. Current velocity and direction were determined at 1 m vertical resolution and at 10 s intervals using a pair of Aquadopp (Nortek) acoustic current profilers, positioned on the seafloor up-current and down-current of the farms (only up-current at Neeltje Jans). Values were integrated over the depth interval occupied by the seed collector substrate (top 6 m at Neeltje Jans; top 4 m at Vuilbaard; top 3 m at Slaak).

Water samples were collected along farm transects; in the case of Slaak the transect covered 8 SMC blocks spanning a distance of almost 1400 m, compared to transects of approximately 200 m at Neeltje Jans and at Vuilbaard. Samples were taken inside and outside of the farms; the spatial configuration of sampling stations is given in Figure 6.2. The Slaak farm was sampled during flood tide, Vuilbaard and Neeltje Jans were sampled during ebb tide. All stations were sampled for particulate material, dissolved nutrients, particles, and fluorescence. Fluorescence was not measured at Vuilbaard. Chlorophyll *a* (Chl *a*) concentration was determined for selected stations (Figure 6.2). Water sampling was completed for all stations within a timeframe of 20–30 min, in the same direction as the water flow. A manual diaphragm pump was used to collect water, fed by a tube submerged to a depth of 2 m. Samples were transported in 10 L jerry cans to a ship-borne laboratory (transfer times < 10 min) where samples were processed, stored, or analysed. Fluorescence was measured at Slaak and Neeltje Jans *in situ* within 40 min after initial samples were taken. Fluorescence was measured using a fluorescence probe (Cyclops-3, Turner Designs) at 2 m depth for 15 repetitive measurements at 2 s intervals (data present mean), and is reported in relative fluorescence units.

Laboratory analysis

Water samples (10 L) were divided into subsamples. TPM concentrations, reported as dry weight (DW), were determined in duplicate gravimetrically, after filtration of 1500 ml sample onto pre-weighed GF/F (Whatman) filters and drying at 50°C for at least 12 h. To characterise TPM quality, POC and PON concentrations were quantified from the duplicate filters using a NC2500 element analyser (ThermoQuest, Rodano, Italy). Particle concentrations were measured in triplicate using a portable particle analyser (Pamas S4031 GO using a HCB-LD-15/25 sensor; detection range 1–100 µm). For pragmatic reasons, particles > 4 µm were included in the analysis to exclude the smallest sizes that are often not efficiently retained by mussels (Cranford et al. 2011). The concentration of Chl *a* was determined spectrophotometrically after extraction from GF/F (Whatman) filters containing the filtrate from 1500 ml seawater, using 10 ml 90% acetone and 20 s glass bead-beating homogenisation, according to Jeffrey and Humphrey (1975).

Samples for dissolved nutrient concentrations were preserved, after filtration in the field using GF/F filters (Whatman), by storage at -20℃ until analysis. All samples were analysed within three months. Before analysis an additional filtration was performed at the laboratory using 0.45 µm pore size cellulose ester membrane filters (Millex-HA SLHA02510, Millipore). Colorimetric analysis of ammonia, nitrate, nitrite, and phosphate concentrations was performed using a QuAAtro autoanalyser (SEAL Analytical, United Kingdom). Urea concentration was analysed colorimetrically using an autoanalyser (Skalar, the Netherlands).

Data analysis

Besides actual concentrations along the transects, data are also presented in the form of trends. Trends were determined in a stepwise approach (Table 6.3) for all parameters. The first step that reached a conclusion was reported. The order of the steps was:

1) Linear regression with distance through the farm. This step was only performed for the SMC farm at Slaak. At this elongated farm (Figure 6.2), five stations were sampled inside the farm, allowing trend analysis by linear regression (α=0.05). Distance was calculated by summing the total distance occupied by seed collector blocks. Unused spaces in between were excluded since trends were hypothesised to result from seed collector activity.
2) Consistency of increase or decrease (same direction of change for each subsequent station pair).
3) The direction of change from the station at the up-current edge of the farm to the mid station, as the down-current section of the seed collectors was considered to be more affected by water intrusion.

Trends were expressed as percentage change over the full length of the sampled farm section, relative to the measured (or where not measured: extrapolated) value at the up-current edge of the sampled farm block. Stations outside the farms up and downstream were not included in the trend analysis.

For Chl *a* concentration, a reduced number of stations was sampled in each of the seed collectors (Figure 6.2, closed symbols), hence only the change between two stations could be reported. At Vuilbaard, no the trend for Chl *a* concentration could be defined because of the lack of information from the up-current station.

Table 6.3. Overview of the three trend analysis steps for the discrete sampling campaign

order	trend	symbol (Table 6.4)	locations	Parameters	nr of stations
1	statistically significant linear regression trend	---/+++	SL	all except Chl *a*	5
2	consistent down/up over all farm stations	--/++	SL, NJ, VB	all except Chl *a*	3
3	down/up up-current to mid farm stations*	-/+	SL, NJ, VB	all	2

*at Slaak Chl *a* concentration was obtained at a reduced number of stations (Figure 6.2)

6.2.3. Towed glider campaign

Farm-scale food reduction by mussels was investigated on 17 and 18 August of 2010 inside the farms at Slaak, Vuilbaard, and Krammer (Figure 6.1, Figure 6.2). Water temperature was 19–20°C at all three sites. 3D spatial surveys of TPM and Chl *a* were conducted using *in situ* electronic sensors mounted on an undulating tow vehicle (Acrobat LTV-50, Sea Sciences Inc., Arlington, Mass., USA). The sensor payload consisted of a CTD (AML Oceanographic MicroCTD, Sidney, Canada), a Chl *a* fluorometer (Seapoint Sensors, Inc., Kingston, NH, USA) and a transmissometer with a 25 cm optical path length (c-Rover CRV5, WET Labs, Philomath, OR, USA). The towed vehicle was programmed to continuously undulate between set depths (see below) at each farm. Measurements were made at a sampling frequency of 1 Hz. Tow speed was maintained at approximately 2 m sec^{-1} and the sensor data stream was combined in real time with simultaneous GPS and water depth readings (Garmin Model GPSMAP 530s) using Windmill 7 data acquisition and visualization software (Windmill Software Ltd., Manchester, UK). Towed vehicle surveys were conducted on 17 August, 2010 at Krammer (0.5–10 m undulation depth range) and Slaak (0.5–2 m) and on 18 August at Vuilbaard (0.5–3 m). Tow paths are shown in Figure 6.5. Data were collected inside the farm (tows between mussel lines) and in reference areas surrounding each farm. All tow surveys were completed within a one to two hour period, at ebb tide.

In situ calibrations of the towed vehicle fluorometer and transmissometer were conducted using seawater sampled at vehicle operating depth. Water samples were filtered in the laboratory the same day. Filters used were pre-washed Advantec GC-50 glass fibre filters (0.5 µm porosity; n=1 for Chl *a* and n=3 for TPM analysis). The concentration of Chl *a* was determined spectrophotometrically after extraction using 10 ml 90% acetone and 20 s glass bead-beating homogenisation, according to Jeffrey and Humphrey (1975), and was correlated to fluorescence voltage (mV) using:

$$Chl\ \mathrm{a} = 11.632 * mV + 0.086\ (n = 27; R^2 = 0.60)$$

TPM filters were rinsed under vacuum with isotonic ammonium formate to remove salt, dried at 60°C and weighed to the nearest 0.01 mg. A narrow TPM concentration range was observed so a laboratory calibration was conducted using a standard series prepared from a stock silt/clay mixture that provided the following calibration equation for the c-Rover transmissometer:

$$TPM = -11.863 * \ln(counts) + 17.883\ (n = 101;\ R^2 = 0.84)$$

Vertical profile plots and contour maps of water density (Sigma-t; σ_T), Chl *a,* and TPM concentrations were used to summarize and observe spatial patterns in each parameter measured during each towed vehicle survey. The depth range exhibiting low vertical variation in the measured parameter was selected for contour mapping using the Ordinary Kriging interpolation method in Surfer 9 (Golden Software, Inc., USA), thus a depth of 2–7 m (Krammer, n=3806 measurements), 1.5–3 m (Vuilbaard, n=1839), or 1–2 m (Slaak, n=2615). The average percentage food reduction within the farm volume (defined by the corner navigation buoys and the sampling depth range) was calculated as:

$$\%\ depletion = \frac{(R - F)}{R} * 100$$

where *F* and *R* are average Chl *a* or TPM concentrations within the farm and reference areas, respectively. The reference area included all data collected outside the farm boundaries.

6.3. Results

6.3.1. Discrete measurements campaign

Water current headings measured at the up- and down-current sides of the sampled farm blocks were in line with the prevailing tidal current at the time of sampling, except for a perpendicular heading at the down-current side of the Slaak farm (Figure 6.3). TPM concentrations showed a decrease with water passage through the farms at all farms

(Fig. 3). Particle and Chl *a* concentrations also generally trended downward with water passage through the farms. The fluorescence measurements corresponded with the Chl *a* measurements, but at the Slaak farm the trend assessment of fluorescence resulted in a minor increase, of 7%. Similarly, POC and PON at Slaak also apparently increased, by a relatively small margin of 6–13%. In both cases, the first (POC and PON) or the first two (fluorescence) stations appeared to be out of line with the remaining four farm stations (Figure 6.4). For POC and PON, the remaining four sampling stations showed a sustained and consistent decrease which tested as statistically significant (difference scaled to SMC length: POC, -39.1% or -0.50 mg L^{-1}; PON, -44.3% or -0.11 mg L^{-1}). However, since TPM and particles showed no corresponding patterns, it was not considered justified to deviate from the standard analysis procedure (paragraph 0) in Table 6.4.

All measured nutrient concentrations increased substantially through the Slaak and Neeltje Jans farms, with only two exceptions: NH_4-N at Slaak (-4%) and PO_4-P (no change) (Table 6.4). Five out of eight dissolved nutrient parameters at Slaak showed a statistically significant increase with distance through the farm. At Vuilbaard, relatively little difference was detected with differences ranging from -4–6%. The greater increase in DIN at Neeltje Jans than at Slaak (208% and 76%, respectively) was largely driven by the aforementioned difference in NH_4-N. Approximately equal amounts of dissolved inorganic nitrogen and urea accumulation were observed at Slaak. At Neeltje Jans, urea contributed an order of magnitude less than dissolved inorganic nitrogen. For NO_x, the maximum relative nutrient accumulations, observed at Slaak, represented a more than five-fold increase. In absolute quantities, all changes in dissolved nutrient concentrations except PO_4-P were larger at Neeltje Jans than at Slaak.

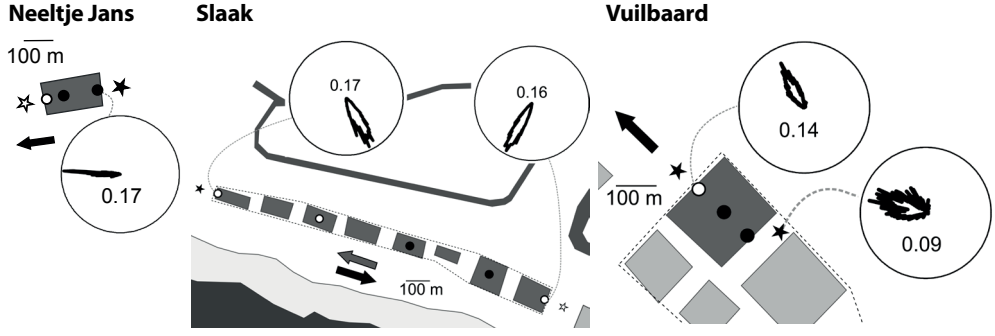

Figure 6.3. Mean current velocity (m s^{-1}) and direction determined by current profiler during the discrete measurements campaign, averaged over the depth range occupied by SMC substrate at each location, shown by rose charts for each profiler station. Arrows indicate overall tidal direction of travel as observed visually at the surface during the measurements. See also the legend to Figure 6.2.

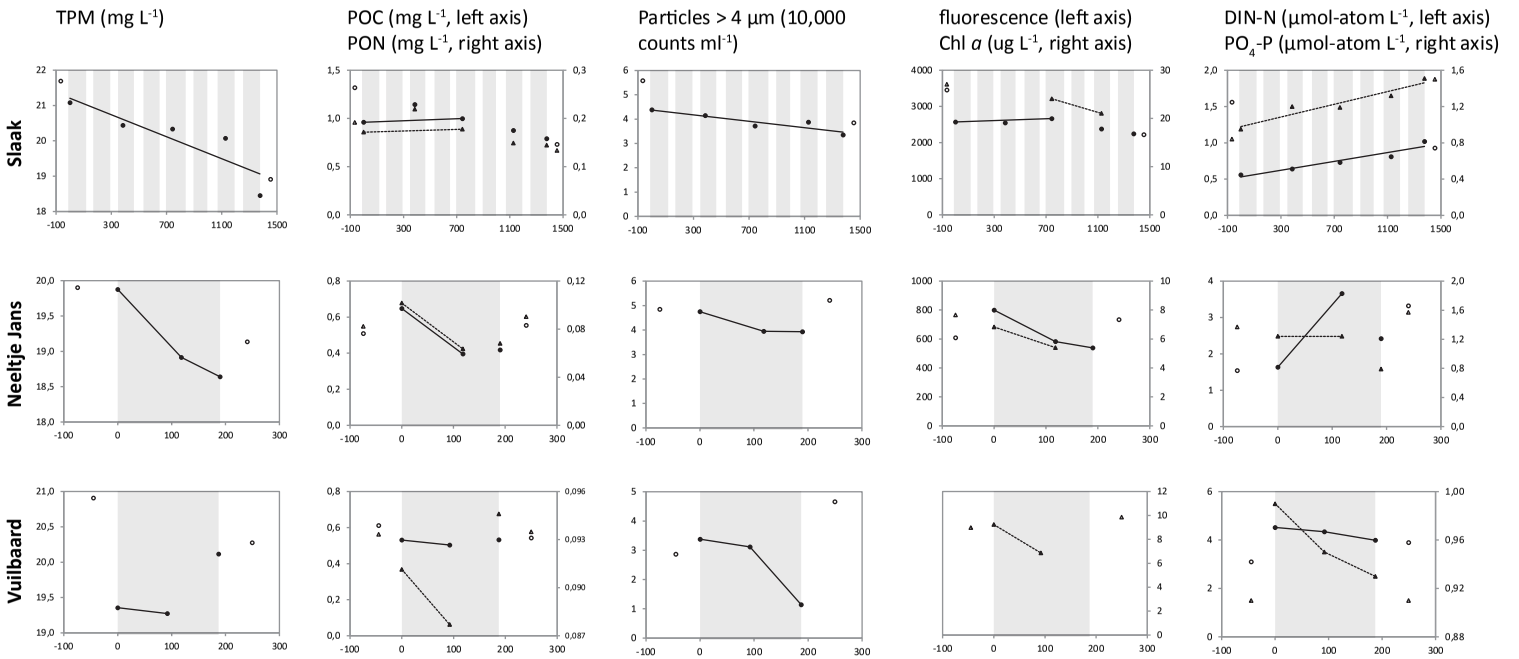

Figure 6.4. Main parameters observed along transects inside SMCs (open symbols; SMC blocks represented by grey shaded sections) and at reference stations (closed symbols). Tidal current direction was left to right. Lines are shown according to the type of trend observed (paragraph 0; Table 6.3)

Table 6.4. Summary of observations along SMC transects, with trends reported according to paragraph 0; Table 6.3. SL: Slaak; NJ: Neeltje Jans; VB: Vuilbaard.

			observed trend				change (scaled to farm length)			% change (scaled to farm length)			start value (first station in farm)		
			SL		NJ	VB									
category	parameter	unit	effect	R^2	effect	effect	SL	NJ	VB	SL	NJ	VB	SL	NJ	VB
suspended material	TPM	mg L^{-1}	---	0,79	--	-	-2,20	-1,24	-0,16	-10%	-6%	-1%	21,1	19,9	19,4
	POC	mg L^{-1}	+		-	-	0,07	-0,42	-0,05	7%	-65%	-10%	0,96	0,65	0,57
	PON	mg L^{-1}	+		-	-	0,01	-0,06	-0,01	6%	-63%	-7%	0,17	0,10	0,09
	POC	%	+		-	-	0,67	-1,95	-0,26	13%	-60%	-10%	4,6	3,3	2,9
	PON	%	+		-	-	0,11	-0,29	-0,03	12%	-57%	-7%	0,8	0,5	0,5
	particles	1000 ml^{-1}	---	0,84	--	-	-61	-174	-5	-36%	-31%	-15%	167	556	51
	fluorescence	-	+		--	nd	174	-261	nd	7%	-33%	nd	2570	800	nd
	Chl *a*	µg L^{-1}	-		-	-	-10,69	-2,32	-4,83	-40%	-34%	-52%	24,09	6,83	9,24
dissolved inorganic nutrients	NH_4-N	µmol-atom L^{-1}	-		+	--	-0,02	2,51	-0,45	-4%	253%	-15%	0,4	1,0	4,0
	NO_2-N		++		+	0	0,06	0,13	0	120%	70%	0	0,1	0,2	0,4
	NO_3-N		+++	0,91	+	--	0,39	0,75	-0,08	554%	167%	-7%	0,1	0,5	1,3
	NO_x-N		+++	0,91	+	--	0,43	0,89	-0,08	359%	138%	-5%	0,1	0,6	1,7
	DIN-N		+++	0,92	+	--	0,43	3,39	-0,53	76%	208%	-12%	0,6	1,6	5,7
	urea-N		++		+	--	0,43	0,50	-0,11	29%	27%	-5%	1,5	1,9	2,8
	DIN&urea-N		+++	0,94	+	--	0,82	3,90	-0,64	40%	112%	-9%	2,0	3,5	8,5
	PO_4-P		+++	0,93	0	--	0,50	0,00	-0,06	52%	0%	-6%	1,0	1,2	1,1

6.3.2. Towed glider campaign

At Slaak and Krammer, depletion of both seston parameters was observed along the SMC, whereas at Vuilbaard no clear patterns were evident (Fig. 5). Below, observations from each farm are presented in turn. At Slaak, the spatial distribution of TPM concentrations indicated reduction of seston along the farm. This was also detected in an inside versus outside comparison without regard for current direction: TPM levels in farm and reference areas averaged 4.78 mg L^{-1} (SD = 0.38; n= 844) and 5.18 mg L^{-1} (SD = 0.38; n= 906), respectively, indicating an average TPM reduction of 7.6%. However, there was no clear reduction in Chl *a* concentrations in the farm compared to the reference areas. TPM and Chl *a* both decreased from east to west (along current direction) within the SMC, but also in the reference area outside the farm boundaries, in particular on the southern side.

At Krammer, Chl *a* concentrations in the farm decreased along the current direction, but also did so in part of the reference area near to the farm. In contrast, a maximum TPM reduction of approximately 50% was observed over the full width of the farm in its centre, but approaching the down-current sides, levels returned to those observed in the surrounding water. Chl *a* and TPM were thus both reduced in or near to the farm, but did not appear to be coupled. On average, TPM was lower within the farm boundaries (3.65 mg L^{-1}; SD = 0.54; n = 2376) than in the reference area (4.49 mg L^{-1}; SD = 0.76; n = 4831), giving an average level of seston reduction of 18%.

At Vuilbaard, Chl *a* concentrations in the reference area were lower than in the reference areas of Krammer and Slaak, while TPM was relatively high. TPM concentrations at Vuilbaard were patchy, averaging 6.51 mg L^{-1} (SD = 0.42; n = 1549) in the reference area and 6.47 mg L^{-1} (SD = 0.54; n = 1316) inside the farm boundaries. No reduction of TPM or Chl *a* was observed.

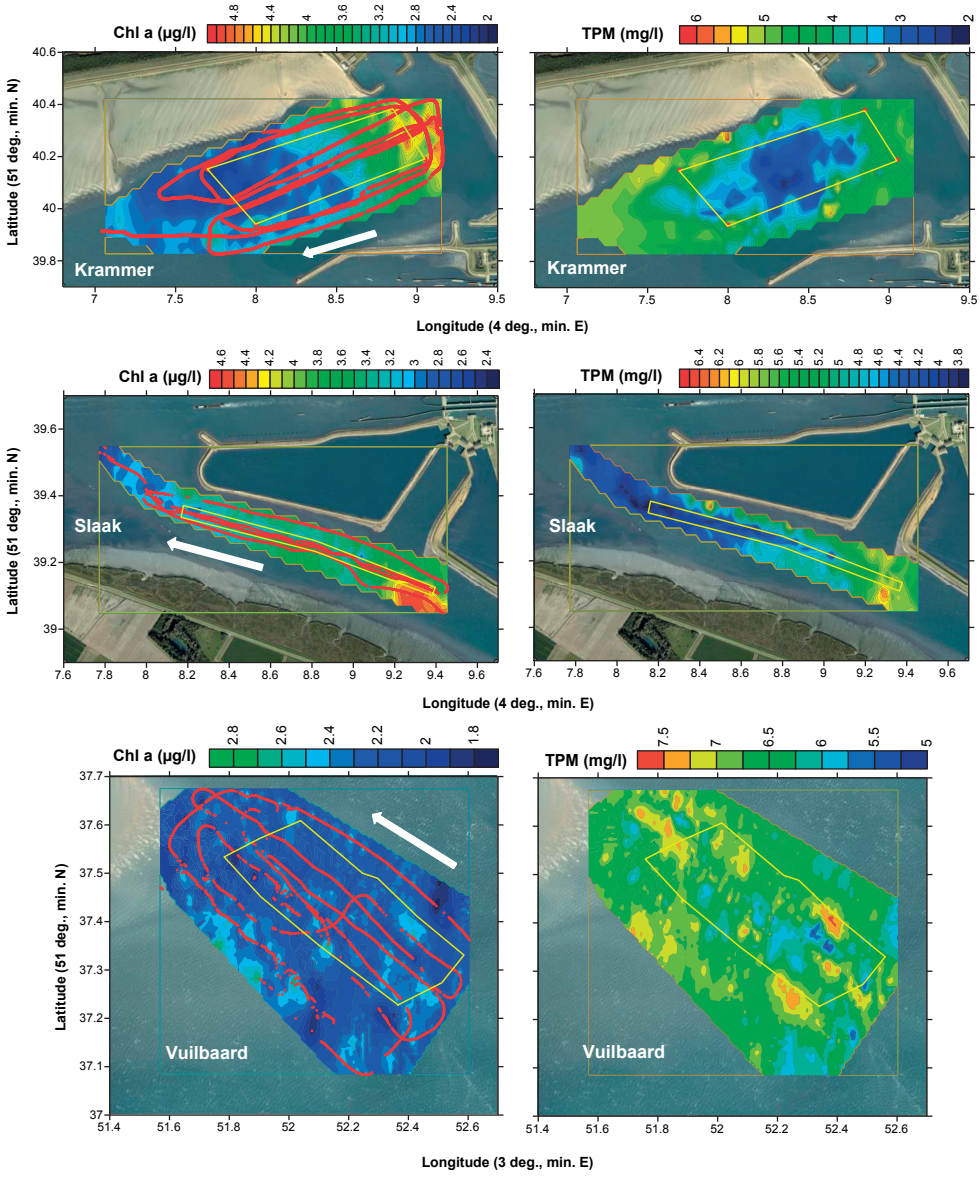

Figure 6.5. Contour plots of Chl a concentration (left) and total particulate matter (TPM; right) at study sites Slaak, Vuilbaard, and Krammer, during the towed vehicle campaign in August 2010 (Krammer and Slaak plots reproduced from Cranford (2019); Vuilbaard plot reprocessed from Kamermans et al. (2010)). Tow tracks (sampling locations) are shown as red dots in the left panels. Yellow polygons show the boundaries of the farms; the remainder of the plotted surfaces represent the reference areas. Arrows in the left panels indicate tidal current direction during sampling.

6.4. Discussion

In situ evidence for reduction of seston and enhanced nutrient concentrations is presented in this study for mussel farms located in a highly dynamic bay in the Netherlands. There was variability between farms and parameters, leading to a few cases where reversed trends were observed. This reflected potential hydrodynamic effects such as water intrusion due to body drag of the farms, and patchy water mass contents as indicated by the towed glider measurements.

6.4.1. Top-down regulation: Seston

Reduction of seston concentrations was observed in most, but not conclusively in all, cases under study in both the discrete and towed glider campaign. Maximum reductions of seston observed in the present study were between 40% and 65%. Nielsen et al. (2016) reported maximum reduction in some sites within of a mussel farm of >50%. In mussel rafts, maximum reductions of phytoplankton were reported of 55% (Petersen et al. 2008) and of 72% (Cranford et al. 2014), with reductions of zooplankton reaching 77% (Maar et al. 2008). The greatest proportional reductions of seston concentrations in mussel farms of > 90% were reported by Strohmeier et al. (2008) and Heasman et al. (1998), who also noted reduced mussel growth or condition in the centre of the farms. Culture densities were respectively > 4 times and > 82 times higher in these latter studies compared to the present study. The proportional depletion of seston depends on mussel density and activity, but also on background concentrations, current speed and mixing (next paragraph), and refiltration. Background seston concentrations were higher in the present study, with for example Chl *a* at 6 µg–25 µg L^{-1} compared to 0.7–2.4 µg L^{-1} reported by Strohmeier et al. (2008), and approximately 3–5 µg L^{-1} by Heasman et al. (1998). A correspondingly greater level of filtration activity would be needed to achieve the same proportional depletion. In general, refiltration of water within a mussel farm is considered to be an important mechanism constraining reduction of seston concentrations (Cranford 2019). In the Norwegian case (Strohmeier et al. 2008), the extreme depletion values may be linked to the mussels' continued feeding at high rates despite depletion of particles, as a possible adaptation to oligotrophic conditions (Strohmeier et al. 2009). On a unit weight basis, the mussel population on SMCs is likely to be metabolically more active than adult mussels since it is entirely made up of seed, and smaller specimens exhibit higher weight-specific activity than larger ones (Smaal & Vonck 1997, Cranford et al. 2011). An indication of the magnitude of this difference can be obtained via the allometric relation based on a meta-analysis reported by the latter authors: clearance rate (L g^{-1} h^{-1}) = $2,5*X^{0,58}$, where X is the individual tissue dry weight (g). Per unit weight, mussels of 12 mm shell length, representative of the SMC mussels sampled in the present study, clear almost 8 times more water (19,4 L g^{-1} h^{-1} compared to 2,5 L g^{-1} h^{-1}) than adult mussels of 1 g tissue dry weight or 60 mm shell length. The macrotidal conditions in the Oosterschelde bay may

offer an explanation for why this high activity did not translate to especially high levels of seston depletion compared to other studies. Mean current velocities were 9–17 cm s^{-1} in the present study, compared to 3 - 6 cm s^{-1} in a Norwegian fjord reported by Strohmeier et al. (2008), and 1–8 cm s^{-1} in a South African bay reported by Heasman et al. (1998). With current velocities reaching 100 cm s^{-1} in the Oosterschelde bay (Nienhuis & Smaal 1994a), the extent of competition for food within mussel farms may be comparatively limited. For SMCs in the Netherlands, indeed there appears to be room for further upscaling of production per unit area within the lease plots (van Broekhoven et al. 2024). The most significant competition for food in Dutch SMCs is likely to occur during the last weeks before harvest, given the rapid growth of mussel biomass during this period. At the Oosterschelde bay SMCs described by van Broekhoven et al. (submitted), harvest took place just over one week after the discrete measurements of the current study in the same year, and during this period the mussel biomass more than doubled.

Selective removal of phytoplankton (cf. Cranford et al. 2014), or POC and PON, versus TPM was not consistenly evident. It is possible that such effects (Ward & Shumway 2004, Newell 2004) were masked by hydrodynamic effects with water passage through the farms, resulting in variability in the measurements. Several observations indicated that the water mass travelling through the farm was not homogeneous. This might also explain the apparently out of line measurements at the first (POC and PON), and first and second (fluorescence) stations at Slaak. Firstly, the contour plots of the glider campaign showed a patchy distribution of both Chl *a* and TPM. Secondly, the down-current profiler station at Slaak showed water flow perpendicular to the visually confirmed general tidal current direction of travel. This may be related to the depth topography, to the presence of a channel and lock to the north, or to outflow turbulence (cf. Petersen et al. 2008). Third, the towed glider measurements at Krammer showed a clear reduction of TPM in the center of the SMC, but concentrations returned to values observed in the reference area towards the down-current corners of the farm. This pattern suggests a possible local source of additional TPM, for instance turbulent resuspension from the seafloor in this shallow embayment. Alternatively, the observed reduction of TPM concentration in the centre of the farm may to some extent be caused by sedimentation resulting from reduced flow velocity due to the presence of the farm structures and the suspended mussel community. Intrusion of water carrying higher TPM concentrations from the sides appears less likely as an explanation because the same pattern was not observed for Chl *a*. However, tidal current disturbance might help explain the observations on various seston components at Neeltje Jans and Vuilbaard where the station at the down-current edge of the SMC showed a higher value than the mid station, while the mid station showed the expected reduced value compared to the up-current edge station. Tidal current disturbance such as lateral flows which interfere with the downstream propagation of a signal can occur as a result of reduced current velocities resulting from farm drag (O'Donncha et al. 2013). Reduced

current velocities have been documented inside farms (Cranford 2019, Zhong et al. 2022), inside assemblages of multiple culture structures (Grant & Bacher 2001, Strohmeier et al. 2005), and inside mussel rafts (Blanco et al. 1996, Petersen et al. 2008, Cranford et al. 2014). In any case, these observations imply that discrete samples taken from the passing water mass over the down-current part of the farms might not necessarily be reflective of the action of the mussels on seston and dissolved nutrients contained within it due to patchiness and other hydrodynamic effects (Nielsen et al. 2016).

6.4.2. Bottom-up regulation: Dissolved nutrients

In line with expectations, nutrient concentrations increased along the length of the farms, and was particularly evident at the most sheltered Slaak location. However, at Vuilbaard an opposite pattern was observed showing a decrease of nutrient concentrations along the length of the farm block. We could not ascertain from our data what caused this reversed pattern. There may be a relation with patchiness of the water mass contents or other hydrodynamic effects, as discussed in the previous section, particularly considering the multitude of other farm blocks up-current and in close proximity at this farm. Elevated nutrient concentrations within a mussel farm as observed at Slaak and Neeltje Jans were expected to result from nutrient regeneration by mussels on farm ropes (Richard et al. 2006, van Broekhoven et al. 2014). Zúñiga et al. (2013) also observed elevated nutrient concentrations inside a mussel farm, and Trottet et al. (2008) observed increased primary productivity inside a mussel farm, which the authors inferred was likely due to nutrient subsidy from the activity of the mussels. In the present study, relative increases of dissolved nutrient concentrations in the water column were large at Slaak. Note that the percentage changes are influenced by farm length (the Slaak farm being the longest) and ambient nutrient concentrations (substantially higher at Vuilbaard). Urea, a metabolic waste product excreted by animals including mussels, is not commonly included in studies into the role of shellfish in nutrient cycling (see chapter 3), but followed the same pattern as the other nutrients that were measured. Urea has been found to have relevance to phytoplankton production (Moschonas et al. 2017), and is even added to Manila clam (*Ruditapes philippinarum*) production ponds to promote phytoplankton growth (Mao et al. 2019). Our *in situ* observations suggest that adding urea to the selection of nutrients measured might improve our insight into the role of shellfish in nutrient cycling in relation with primary production. The large relative NO_x accumulations, observed at Slaak, contrast with the general view that NO_x-dynamics related to mussel farms often play a minor role (Richard et al. 2006, Jansen 2012), and with previous observations in the Oosterschelde bay where seed collector ropes were also found to excrete little NO_x (van Broekhoven et al. 2014). However, in those studies, which involved incubations of culture rope sections, the suspended farms were decoupled from the decomposition of biodeposits, which prior to experimental incubation would have largely been transported away from the *in situ* culture ropes. Decomposition of biodeposits can be accompanied by NO_x production (van

Broekhoven et al. 2015). A possible source of NO_x in our study is decomposition of organic material such as biodeposits (van Broekhoven et al. 2015) or dead mussels on the seafloor below the farms (Newell 2004).

The reduction of ammonia observed at the Slaak and Vuilbaard farms contrasts with expectations, considering that ammonia is the predominant form of DIN release by seed mussel ropes (van Broekhoven et al. 2014), mussel culture rafts (Zúñiga et al. 2013), individual mussel beds (Prins & Smaal 1990, Asmus et al. 1990), and other bivalve aggregations such as oyster beds (Boucher & Boucher-Rodoni 1988). As noted in the previous paragraph, the effect at Vuilbaard may be related to patchy water mass contents or other hydrodynamic effects, but this did not appear to be the case at Slaak. Phytoplankton absorb ammonia, especially when cells are nitrogen-starved, and prefer ammonia over nitrate (Dortch 1990, Middelburg & Nieuwenhuize 2000). Phytoplankton at Slaak were likely nitrogen limited at the time of the measurements, as DIN concentrations were below the half-saturation constant of 2 µmol L^{-1} used for phytoplankton in Dutch coastal waters (Philippart et al. 2007). Furthermore, sediments can act as an ammonia sink (Boucher & Boucher-Rodoni 1988). Both processes can affect measurements of free ammonia and mask ammonia release by mussels, and are potential explanations for the lack of ammonia accumulation observed at Slaak.

6.5. Conclusions

The combined approach of discrete sampling and a towed glider proved valuable, because it allowed to combine measurement of a wide range of parameters at low resolution with a limited number of parameters at high resolution. We observed farm impacts on water column composition, simultaneously reducing seston concentrations and increasing dissolved inorganic nutrient concentrations. This was observed at the elongated Slaak farm located at a sheltered site, the large and relatively sheltered Krammer farm, and also inside the smaller and exposed Neeltje Jans farm, indicating that the high activity of the (SMC) mussels can result in measurable impacts on water column composition even under macrotidal conditions. However, the expected effects were not confirmed at the other exposed farm at Vuilbaard, and not all deviations from the expected patterns could be conclusively explained with the available data. Future studies could address this by focussing on realising higher spatial and temporal resolution in measuring nutrients, aided by developments in sensor technology and applicability (Daniel et al. 2020). Our findings add to the growing body of primarily theoretical and indirect evidence of the simultaneous top-down and bottom-up control of suspended bivalve culture on phytoplankton (Smaal et al. 1997a, Newell 2004, Jansen 2012, van Broekhoven et al. 2014). To our knowledge, our study is one of the first (but see Trottet et al. 2008, Zúñiga et al.

2013) to simultaneously show both top-down control and bottom-up feedback processes *in situ* for suspended bivalve culture.

Acknowledgements

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CHAPTER 7

Synthesis

Wouter van Broekhoven

In this thesis I aimed to investigate the contribution of suspended Seed Mussel Collector (SMC) communities to nutrient cycling during the summer growing cycle, and to explore its ecological relevance. This was addressed via four objectives:

i. Quantify filtration and nutrient release by SMC communities;
ii. Develop a multi-element (N,P,Si) nutrient budget for an SMC community;
iii. Measure farm scale depletion of seston and accumulation of nutrients under macrotidal conditions;
iv. Explore the top-down and bottom-up regulation of phytoplankton by mussel seed, and evaluate its ecological relevance in the transition of wild seed fishery to SMCs.

The previous chapters provided insights into specific nutrient cycling pathways at individual, community (objective i), and/or farm levels (objective iii), using a multi-element (N-P-Si) approach. In this final chapter, I start by summarising the role that the culture of bivalve spat plays in mediating nutrient cycling in an ecosystem. Then, I integrate the findings from the previous chapters to construct a (cumulative) nutrient budget over the summer growing season (section 7.2; objective ii). This allows an assessment of the magnitude of impact of suspended mussel spat collectors on nutrient cycling in the host ecosystem. Subsequently, building on previously presented indicators (Chapter 3), in combination with a new indicator specifically aimed at evaluation of bottom-up control and positive feedback, I evaluate the (potential) level of ecological influence exerted by SMCs (7.3, objective iv). The thesis finishes with concluding remarks in section 7.4.

7.1. The role of bivalve spat culture practices in nutrient feedbacks

This paragraph outlines how bivalve spat culture practices can mediate nutrient cycling in an ecosystem. The bivalves interact with the system via consumption of phytoplankton and regeneration of nutrients, both of which are also processed locally via other pathways, and exchanged with external locations (Figure 7.1). Removal by filtration represents a top-down control on phytoplankton populations, and stimulation of growth by regeneration of nutrients represents a bottom-up control. The top-down control can result in a negative feedback when the bivalves' filtration pressure reduces food availability to the extent that bivalve growth is affected. In contrast, when nutrients recycled by bivalves – either in the form of directly excreted products or through the decomposition of released biodeposits – are taken up by primary producers under nutrient-limited conditions, a fertilisation of the mussels' food source takes place. When this primary production is subsequently consumed by bivalves and other filter feeding organisms, a positive feedback occurs. Furthermore, the top-down and bottom-up forces can interact, and both need to be understood and quantified to adequately assess the influence of a bivalve population on carrying capacity for filter feeders in a system (Prins et al. 1998).

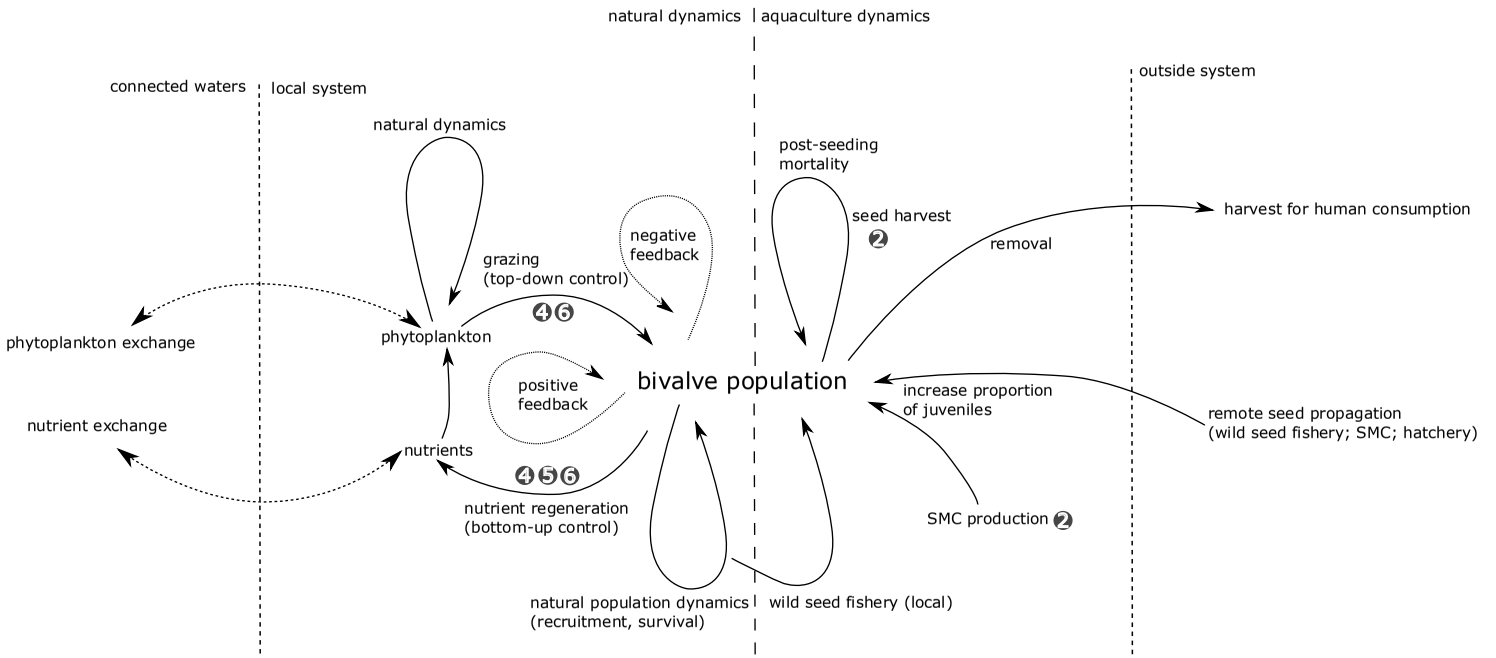

Figure 7.1. Factors influencing bivalve populations, distinguishing between aquaculture and natural dynamics, and between local and external sources and sinks. Numbers indicate chapters in this thesis in which original data for the parameter is presented for evaluation of the role of SMCs in nutrient cycling in the Oosterschelde bay.

Aquaculture activities have an effect on the total bivalve stock present in the ecosystem, which may lead to changes in top-down and bottom-up processes acting on the phytoplankton. Figure 7.1 summarizes the natural and aquaculture-driven factors controlling a population of bivalve shellfish in an ecosystem. The bivalve population is subject to natural population dynamics and is also influenced by aquaculture interventions such as harvest or seed provisioning. Spat for bivalve aquaculture is provisioned either from inside the local ecosystem within which the adults are grown to commercial size or from an external source (Figure 7.1). Within the system, apart from wild capture fishery, a common method is the placement of settlement substrate such as lines in the water column, often followed by thinning practices (e.g. socking) to improve yield. Common external sources are hatcheries or wild capture fishery import from another ecosystem. All these routes may increase the proportion of juveniles in the system compared to the situation without active measures aimed at spat production.

This is exemplified in the case of the Oosterschelde bay. Here, Seed Mussel Collectors (SMCs) represent a change from the traditional culture practice, where wild seed was collected primarily from the Wadden Sea (Chapter 2; Capelle 2017). The change is twofold: i) the seed grows in the local system during the period of nutrient limitation of primary producers whereas imported seed is already half-grown when introduced in spring and autumn, and ii) the post-harvest mortality of imported wild-caught seed is much lower at 54% compared to 92% for SMC mussel seed (Capelle et al. 2016). Both factors imply an increased bivalve stock size in the Oosterschelde bay, particularly in summer. Overall mussel recruitment in the bay is also raised by SMCs: natural recruitment of mussels on the bottom of the Oosterschelde bay (in contrast to SMCs) is very limited (Troost, 2023, pers. comm.). Large numbers of mussel seed are lost from the SMC during their development (Chapter 2), but to date no evidence has been found of substantial secondary settlement of these mussels (e.g. South et al. 2021) leading to the establishment of mussel beds (Karin Troost, 2023, pers.comm. based on long-term annual monitoring in the western Dutch Wadden Sea). The key intervention of SMCs is the provision of substrate, thus allowing the growth of a significant quantity of mussel seed that would not otherwise be able to establish itself. Furthermore, mussel spat exhibit a proportionally high metabolic activity, levels of which scale allometrically with animal body size (Smaal et al. 1997b, and review by Cranford et al. 2011). This timing and high activity level means that the change in seed provisioning practice is likely to influence nutrient cycling in the system during the summer. Insight into the regeneration of nutrients by the spat cohort is crucial for a quantitative understanding of the magnitude of the impacts.

7.2. Nutrient cycling by bivalve spat

7.2.1. Nutrient budget of a SMC rope section

I constructed a full cumulative nutrient budget, spanning the SMC growing season from the first observations of settled mussel spat until harvest on day 234, including post-harvest remineralisation of biodeposits (Figure 7.2). To do so, data from previous chapters was supplemented with unpublished data and environmental data from national monitoring (sources given in Box 1).

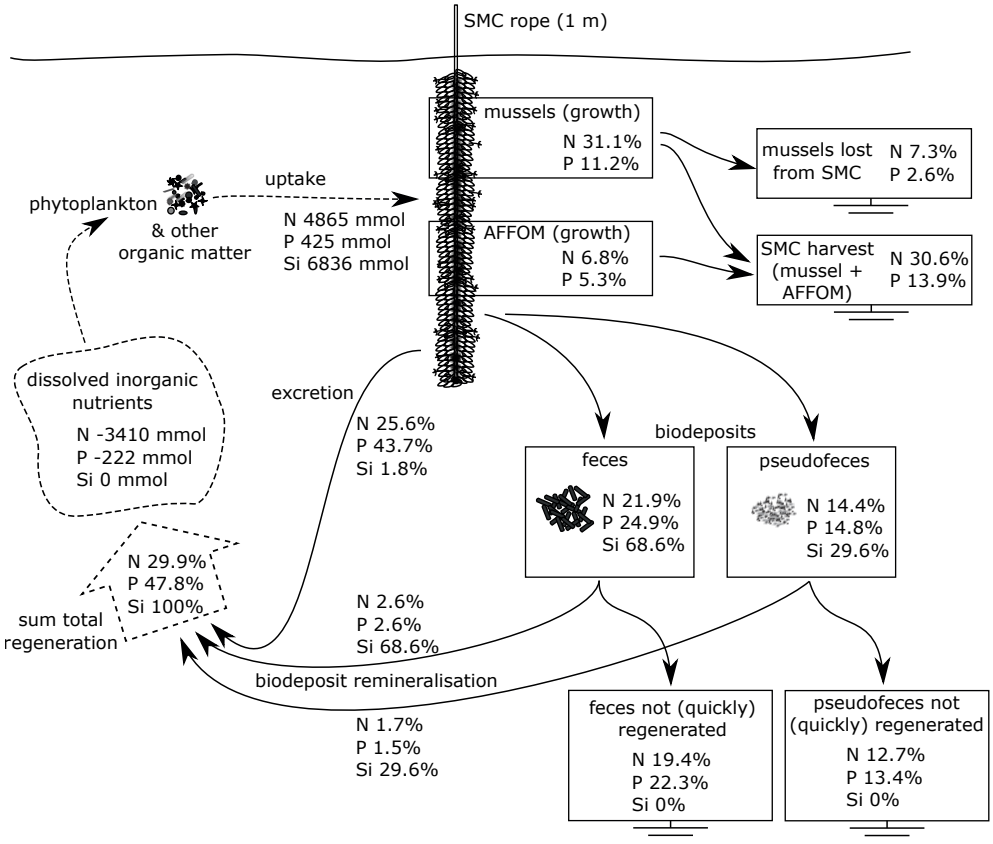

Figure 7.2. Integrated nutrient budget calculated for a 1 m section of SMC rope over the full 2012 SMC growing season, including post-harvest remineralisation of labile fractions of biodeposits. Boxes indicate pools and arrows indicate flows. Uptake and dissolved inorganic nutrient pools are presented as absolute values, and the percentages are relative to initial uptake. The dissolved inorganic nutrients pool represents the net balance of the budget, i.e. negative numbers indicate net uptake by SMCs from the pool. Data sources: Chapter 3 – mussel density; Chapter 4 – individual mussel biomass, AFFOM biomass, SMC intact section N-P-Si excretion rates; Chapter 5 – feces & pseudofeces N-P-Si regeneration rates; unpublished data (Box 1) – mussel N-P content, AFFOM N-P content, feces & pseudofeces production rate, feces & pseudofeces N, P, Bsi content; national monitoring (MWTL; waterinfo.rws.nl) – PON-POP concentrations.

Box 1. Calculations and unpublished data used to construct the integrated nutrient budget

Budget calculations

The nutrient budget over the full SMC growing season was obtained by summing daily interpolated values between pelagic chamber measurement dates (Chapter 4). Excretion measured in pelagic chambers (Chapter 4) was interpolated linearly between dates. Daily feces and pseudofeces remineralisation was calculated by tracking the actively remineralising pool and applying daily remineralisation fractions (Chapter 5). Nitrogen and phosphorus remineralisation were continued for 18 days, and Si remineralisation was continued until depletion. The remaining fraction was considered as not (quickly) contributing to remineralisation. Loss of mussels was calculated from declining mussel densities, multiplied by the mean individual biomass at the time of loss (Chapter 4). Total uptake was calculated as the sum of mussel growth, associated fauna flora and organic matter (AFFOM growth), excretion, feces and pseudofeces production.

Unpublished data

Feces & pseudofeces production

Feces and pseudofeces production rates were measured using the mussels that produced biodeposits for incubations, as described in Chapter 5. Holding conditions including food availability are described there. Production rates were quantified by means of vertical flow-through chambers in which mussels were held on a mesh. The water was allowed to overflow at the top, at a rate visually observed to be just below the rate at which biodeposits started to be carried upwards. Dry and ash-free dry masses were determined by gravimetry (see Chapter 5; also for nitrogen, phosphorus, and silicon content of the biodeposits). Rates were determined for a range of mussel sizes using a controlled and repeated setup. Production rates were then calculated for the measurement dates (Chapter 4) using the full observed distribution of mussel sizes per measurement date (Chapter 4). These were subsequently scaled to PON and POP concentrations taken from national monitoring data (MWTL; waterinfo.rws.nl) and interpolated linearly between sampling dates, relative to concentrations in the mussels' (natural) water supply during measurements. Finally, daily production rates were derived using an exponential relation of individual production rate over time, combined with daily mussel density on SMC ropes (Chapter 2).

Mussel nitrogen, phosphorus content

Nitrogen and phosphorus content were determined for 0.2–20.0 mm shell length mussels taken from across the SMC growing season by means of segmented flow analysis after selenium, salicylic acid and sulfuric acid digestion following homogenisation, according to Temminghof & Houba (2004). For mussels > 12.0 mm shell length, tissue and shell were analysed separately. The mean proportion of the total nitrogen which was contained in the shell was 31.6%, and the corresponding value for phosphorus was 23.3%.

AFFOM nitrogen, phosphorus content

Nitrogen and phosphorus content were determined for AFFOM at each measurement date (see Chapter 4). For the fraction > 1000 µm, the procedure was the same as described for mussels above. Smaller AFFOM was captured on GF/C filters and its nitrogen content was determined after powderisation by means of organic element analyser according to Nieuwenhuize et al. (1994). Phosphorus content was determined by means of inductively coupled plasma optical emission spectroscopy according to Poussel et al. (1993).

By the end of the SMC growing season, 31% of nitrogen and 11% of phosphorus uptake by the SMC assemblage had been sequestered in mussel spat biomass. Approximately three quarters of this was harvested at the end of the season, while the remaining one quarter was lost with mussels detaching from the SMC over the course of the growing season. Through harvest of both mussels and AFFOM, 31% of the nitrogen uptake and 14% of the phosphorus uptake were collected. Although a small part of the AFFOM may directly or indirectly arise from mussel biodeposition and would thus be counted twice in the overall nutrient uptake estimate, the majority of the AFFOM consisted of independently feeding organisms such as hydroid polyps and green filamentous algae (Chapter 4). The AFFOM was therefore counted as a separate component. The relative magnitude of sequestration of nitrogen by the AFFOM was roughly a quarter of the amount incorporated by the mussels. For phosphorus this was half the amount, demonstrating the importance of assessing the SMC community as a whole. It shows that measurements on whole communities (as in Chapter 4) are more representative of actual rates in the field than extrapolations of individual measurements that do not take the AFFOM into account (Chapter 4; Richard et al. 2006, 2007a, Jansen 2012). Chapter 4 also underlined the importance of accounting for weight-specific activity when assessing impacts of SMCs on the ecosystem. A comparison of weight-specific activity of SMC mussels to the literature showed much higher values in our study (Chapter 4), as can be expected based on allometric scaling (Bayne et al. 1976a b).

Total nutrient uptake, calculated as the sum of mussel growth, associated fauna flora and organic matter (AFFOM) growth, excretion, and feces and pseudofeces production, was, however, not fully matched by filtration rates empirically measured in the pelagic chambers (Chapter 4). Measured clearance rates could account for 35–98% of calculated total uptake rates, with the ratio varying per nutrient and over time. Compared to the present study, Jansen (2012) found a closer match between community filtration rates and calculated uptake (102–127%) for *M. edulis* in oligotrophic Norwegian fjords. It is possible that adaptation to oligotrophic conditions in these mussels resulted in a more persistent high activity level (Strohmeier et al. 2009) than in mussels accustomed to eutrophic conditions, as in the present study. Jacobs et al. (2015) also measured apparently low filtration rates, using grazing chambers in the Western Wadden Sea. These authors aimed for complete mixing of the grazing chambers similar to the pelagic chamber measurements in this thesis (Chapter 4), but hypothesised that refiltration still occurred and might explain the low rates. Mixing appeared to be more vigorous in the grazing chambers applied in the current study (personal observation), but refiltration as a possible explanation cannot be ruled out entirely. It seems unlikely that the difference was caused by release of nutrients trapped in the organic matrix due to handling of the ropes. This would be measured as part of excretion, which for Si was minimal while the discrepancy between measured filtration and calculated uptake was in a similar range as for nitrogen and phosphorus. What may have contributed to the difference between

calculated uptake rates and measured filtration rates is a difference in timescale and timing of the experiments. Filtration rates were determined in short incubations of around one hour during daytime, whereas certain uptake rates (e.g. growth) were based on measurements over much longer timescales ranging from days to weeks. In mussels, the presence of light has been shown to affect filtration rates (Hills et al. 2020), relocation behaviour (Kobak & Nowacki 2007), and growth and survival of newly settled specimens (Mero et al. 2019). *Mytilus edulis* has been found to display greater levels of valve gape opening, feeding activity, and growth at night during darkness than during daylight hours (Strömgren 1976, Nielsen & Strömgren 1985, Gnyubkin 2010, Robson et al. 2010). All of the above may have contributed to the observed difference, since the pelagic chamber measurements (Chapter 4) were carried out during daylight hours. Future studies, at least in eutrophic environments, should validate filtration measurements to account for any circadian variability.

The fraction of regenerated nutrients relative to their uptake varied strongly between the elements. Approximately one third of the nitrogen, half of the phosphorus, and all of the silicate was regenerated and thus made available again for primary producers. The pathways contributing to regeneration also varied between the elements. Excretion by the mussel spat community (Chapter 4) was the dominant regeneration pathway for nitrogen (accounting for 86% of total nitrogen regenerated) and phosphorus (92%), but silicon regeneration occurred primarily through biodeposit decomposition (98%, Chapter 5). This study demonstrates the importance of characterizing biodeposit remineralization when assessing the impact of bivalve cultures on nutrient cycling (Chapter 5). The relative importance of excretion, biodeposit production, and incorporation into mussel biomass (Figure 7.3) followed similar patterns as reported by Jansen et al. (2011) for suspended rope culture in an oligotrophic Norwegian Fjord (see also Chapter 3). In both studies, nitrogen was divided more equally over the three pathways than phosphorus. For phosphorus the excretion pathway was greater than the fraction incorporated in tissue. Given the difference in food availability, it was remarkable that the proportion of nutrients allocated to biodeposition was similar between the two studies as mussels generally do not produce pseudofeces under oligotrophic conditions. Despite rejecting 16% of the filtered nitrogen in pseudofeces, the mussel seed in our study seemed to make more efficient use of their food as they converted more of the filtered nitrogen into growth (33% in our study compared to 24% in the Norwegian case). It is hypothesized that these differences can be explained by lower metabolic requirements (lower excretion) or by higher absorption efficiencies (lower egestion in feces).

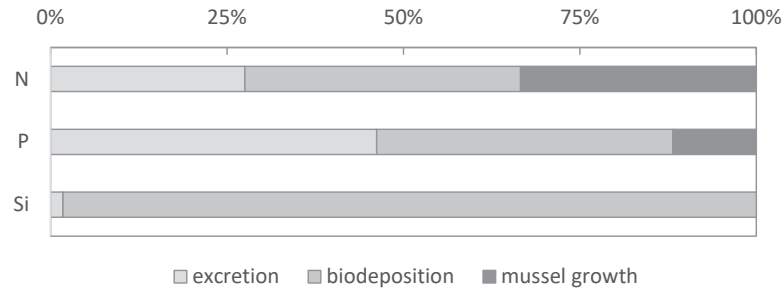

Figure 7.3. Relative importance of physiological processes based on the allocation of nutrients, summed over the full 2012 SMC growing season (until harvest).

7.2.2. Temporal dynamics over the SMC growing season

Three apparent phases in nutrient pathways over the course of the growing season can be conceptually distinguished (Figure 7.4, left panels): i) initial uptake increase; ii) levelling phase; iii) final uptake increase. The first phase (start until measurement 3, July 11) was characterised by a buildup of total uptake, with the majority of captured nitrogen and phosphorus being directly excreted. Biodeposit production gained in importance after some time. In the second phase (measurement 3, July 11 to measurement 4, August 9), total uptake levelled off (nitrogen, phosphorus) or decreased (silicon). Biodeposit production gained a share of captured nutrients for both nitrogen and phosphorus. And while excretion of nitrogen did not increase further, excretion of phosphorus continued to increase. Possibly, these patterns are linked to the increase of mussel biomass on the ropes during this interval due to increasing individual size (Chapter 2), and differences in processing of nitrogen and phosphorus during growth. In the third phase (measurement 4, August 9 onwards), uptake increased again due to an accelerated mussel growth rate. In this phase, a large share of captured nitrogen and phosphorus was incorporated into mussel biomass. The ratio between nutrients (nitrogen and phosphorus) incorporated into mussel biomass and excreted nutrients increased strongly. This suggests that mussels were growing more efficiently, at least in terms of nitrogen and phosphorus incorporation. The transition to the third phase coincided with the levelling off of previously high rates of mussel loss from the ropes (Chapter 2). It is possible that the increased growth efficiency in the third phase reflects the apparently conducive individual circumstances of the remaining mussels. The high loss rates of mussels prior to this point may be linked to inadequate individual circumstances, likely including orientation with respect to the ability to capture incoming food particles. For nitrogen and phosphorus, nutrient regeneration was dominated by excretion throughout the SMC growing season, while for silicon, biodeposit regeneration was the dominant pathway (Figure 7.4, right panels). Post-harvest, comparatively little nitrogen and phosphorus continued to be regenerated, whereas silicon regeneration continued at a considerable rate.

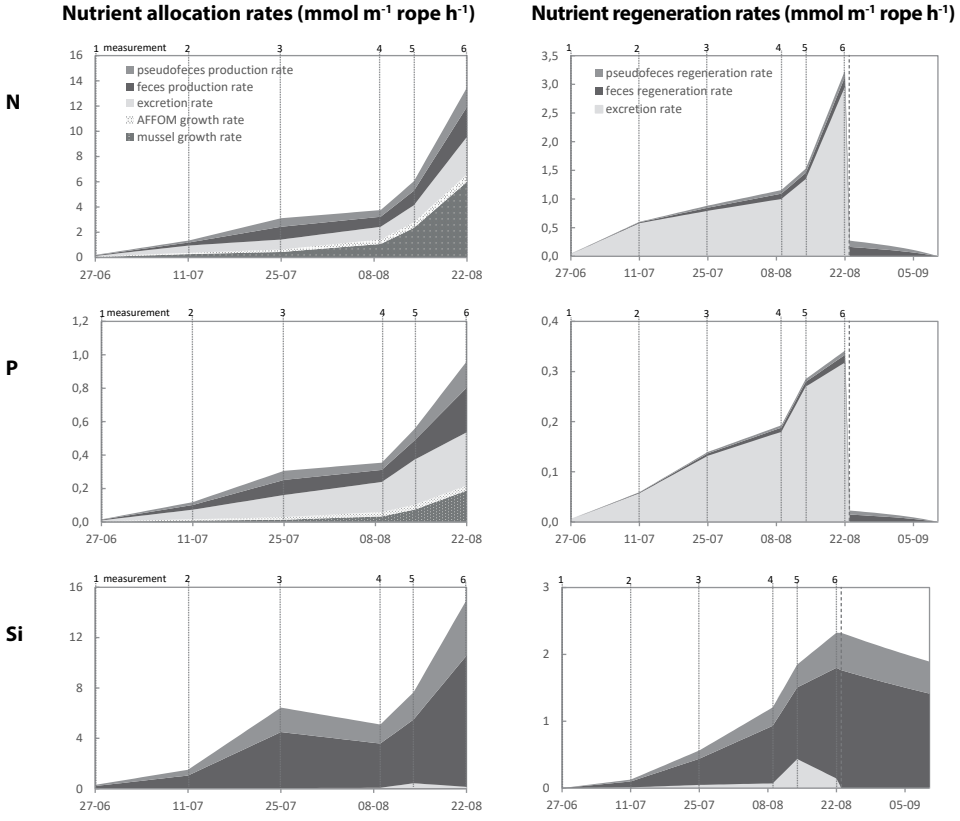

Figure 7.4. nutrient pathways that together represent total uptake (left panels), and those that together represent total regeneration (right panels). Right panels continue beyond harvest date (vertical large dash). Remineralisation of Si continues beyond the range shown, until depletion of the accumulated biodeposit pools. Vertical small dashed lines indicate the measurement days.

7.3. Ecosystem interactions of SMCs in the Oosterschelde bay

To understand how the regeneration of nutrients may contribute to the functioning of the ecosystem, I evaluate SMC-ecosystem interactions for the Oosterschelde bay in this section. Specifically, the extent to which negative and positive feedbacks take place, including the ability of SMCs to fertilise their phytoplankton food source during the course of the growing season. I used a set of indices (Box 2) to inform this analysis, where filtration and nutrient regeneration rates are combined with either: SMC biomass over the growing season, phytoplankton nutrient requirements, nutrient limitation status, and Oosterschelde bay compartment water masses. In addition to the basin as a whole, two contrasting compartments are shown: the most exposed mouth section on one hand, and the most sheltered Slaak gully on the other hand (Figure 7.5, characteristics in Table 7.1).

The largest influence of SMCs was expected in the enclosed Slaak gully (for this reason the location was investigated as part of the farm scale study, Chapter 6).

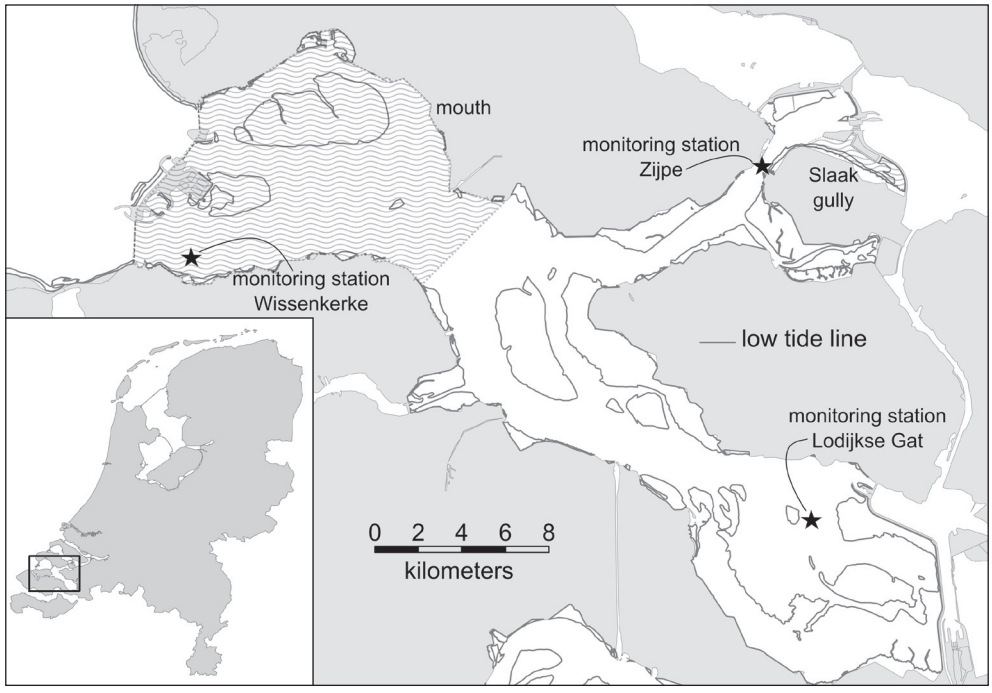

Figure 7.5. Map of the Oosterschelde bay indicating mouth and Slaak gully compartments (hatched areas), and the three Oosterschelde bay monitoring stations of the national water quality monitoring programme (www.waterbase.nl).

Table 7.1. Physical, environmental and biological characteristics of the Oosterschelde bay at the level of the basin and for the mouth and Slaak gully compartments.

Parameter	Basin	Mouth	Slaak gully	Source
surface area at mean water level NAP (Amsterdam gauge)	304 km^2	106 km^2	2.5 km^2	1,2
water volume at mean water level NAP	$274*10^7$ m^3	$115*10^7$ m^3	$1*10^7$ m^3	1,3
(mean) water residence time	0–150 (40) d	52 d	150 d	4
SMC *M. edulis* harvest, 2012	$3.2*10^6$ kg	$2.2*10^6$ kg	$0.2*10^6$ kg	5
Wild and culture plots *M. edulis* biomass, 2012	$44.7*10^6$ kg	$17.0*10^6$ kg	$0.4*10^6$ kg	5
Other bivalve filter feeder biomass, 2012	$66.6*10^6$ kg	$15.4*10^6$ kg	$1.1*10^6$ kg	5
[DIN-N] range Jun-26 – Aug-21 2012	1.6–8.2 (µmol L^{-1})	1.4–8.7 (µmol L^{-1})	1.1–7.1 (µmol L^{-1})	6
[phosphate-P] range Jun-26 – Aug-21 2012	0.6–1.1 (µmol L^{-1})	0.6–1.0 (µmol L^{-1})	0.5–1.5 (µmol L^{-1})	6
[silicate-Si] range Jun-26 – Aug-21 2012	2.1–7.7(µmol L^{-1})	0.7–7.4 (µmol L^{-1})	1.5–8.3 (µmol L^{-1})	6

Sources: 1: (Smaal & Boeije 1991a); 2: calculated from map; 3: estimated from depth chart; 4: Jiang et al. (2019); 5: Jansen et al. (2019); 6: national biweekly routine monitoring programme, www.waterbase.nl (mouth: Wissenkerke monitoring station; Slaak gully: Zijpe station; Basin: mean of the three stations). DIN = Dissolved Inorganic Nitrogen (sum of ammonia, nitrate, and nitrite).

7.3.1. Nutrient limitation status and local primary production

To assess whether nutrients regenerated by SMCs in the Oosterschelde bay during the summer growing season may contribute to primary production, it is first of all important to identify which nutrients are limiting primary production. I evaluated this nutrient limitation status by two methods: i) comparing nutrients in the bay with Redfield (1934) and Brzezinski (1985) ratios for phytoplankton; ii) Monod-scaling of nutrient concentrations (Figure 7.6).

In the first method, the Redfield ratio applies to all phytoplankton, and the Brzezinski ratio is relevant for diatoms since these require silicon to grow their frustules. In all compartments, DIN:DIP ratios (dissolved inorganic nitrogen / phosphorus) were below the Redfield ratio of 16:1 and decreased during the SMC growing season (Figure 7.6, top panels), indicating a relative shortage of nitrogen. DIN:DSi (dissolved inorganic nitrogen / silicon) ratios were below the Brzezinski ratio of 16:15 in the second half of the season in all compartments. In Slaak gully they were lower from the start (Figure 7.6, bottom panels). This indicates a shift from a relative silicon to nitrogen shortage for diatoms.

Box 2. Ecosystem interaction indices

This box details the calculation of indices used here to evaluate ecosystem interactions resulting from nutrient cycling by SMCs.

List of abbreviations used in the formulae:

E	= Excretion rate	RT	= Residence Time
FR	= Feces Regeneration rate	PPT	= Primary Production Time
PFR	= Pseudofeces Regeneration rate	AN	= Ambient Nutrient concentration
AM	= Assimilation rate into mussels	V	= water Volume
AA	= Assimilation rate into AFFOM	BM	= Biomass of Mussels
FP	= Feces Production rate	Up	= nutrient Uptake rate by phytoplankton
PFP	= PseudoFeces Production rate	SA	= Surface Area
CT	= Clearance Time		

Source and sink fractions of nutrients

The source fraction is given by the sum of nutrient regeneration pathways as a fraction of the sum of nutrient destinations after uptake, on a particular day (Equation 1). Or more simply put: the nutrients returned to the system, as a proportion of nutrients taken in on the same day. The sink fraction is the inverse, given by: 1 – (source fraction), see Chapter 3.

Equation 1. Source fraction

$$Source\ fraction = \frac{E + FR + PFR}{AM + AA + E + FP + PFP}$$

Clearance Ratio (CR) and Grazing Ratio (GR)

The Clearance ratio is given by the clearance time over the residence time, and the Grazing ratio is given by the clearance time over the primary production time (Dame and Prins, 1998). The CR and GR were taken from Jiang et al. (2019).

Equation 2. Clearance Ratio (CR)

$$CR = \frac{CT}{RT}$$

Equation 3. Grazing Ratio (GR)

$$GR = \frac{CT}{PPT}$$

Nutrient Turnover Time (NTT)

NTT represents the quantity of dissolved nutrients present in the system, over the daily regeneration of nutrients in that system. More simply put: the number of days it would take to completely replace the dissolved nutrients in the system (see Chapter 3). A SMC mussel biomass estimate per compartment for each of the measurement dates (Chapter 4) was derived by scaling the SMC mussel biomass harvest (Table 11) using the growth curve at the Galgeplaat SMC (chapter 4).

Equation 4. Nutrient Turnover Time

$$NTT = \frac{AN * V}{BM(E + FR + PFR)}$$

Box 2. Continued

Potential Extent of Fertilisation (PEF)

PEF represents the daily regeneration of nutrients in the system, over the daily nutrient uptake by phytoplankton in that system. More simply put: the proportion of daily phytoplankton uptake potentially provided by SMCs. Monthly nutrient uptake rates by phytoplankton were based on 1991 – 2010 monthly primary production estimates by Malkin, Kromkamp and Herman (2010). Mean monthly C uptake rates for 2012 were extrapolated using negative linear functions based on 1991 – 2012 annual gross primary production values (Kromkamp 2017, pers.comm.) to account for the negative trend in recent decades (Kromkamp and Ihnken, 2012; Kromkamp *et al.*, 2013). The C uptake rates were converted to N, P, and Si using Redfield's (1934) and Brzezinski's (1985) ratios.

Equation 5. Potential Extent of Fertilisation

$$PEF = \frac{BM(E + FR + PFR)}{Up * SA}$$

In the second method, the Monod equation relates growth to the concentration of a limiting nutrient, and can be used to evaluate which nutrient is most limiting (following Philippart et al. 2007). For non-diatom phytoplankton, DIN turned out to be most limiting. In the Slaak gully early in the season and in the mouth and the basin as a whole, Monod-scaled DIN concentration was clearly lower than Monod-scaled DIP concentration from about midway during the SMC growing season. For diatoms, DSi was most limiting in the mouth and basin as a whole during the first half of the SMC growing season, after which DIN took over. In Slaak gully, DIN and DSi were relatively close during the first half of the SMC growing season, before DIN clearly became most limiting. Evaluated by monthly means for July, August, and September, the pattern of either DIN or DSi as the most limiting nutrient generally applied for all Oosterschelde bay compartments over the entire period from 2010 when spat production using SMCs started, (see Chapter 3) to 2021 (most recent data availability, waterinfo.rws.nl; results not shown).

Subsequently it is relevant to identify whether filter feeders depend on locally produced phytoplankton or if most feed is imported (external primary production). I evaluated this by means of the Clearance Ratio (CR) and Grazing Ratio (GR) indices (Box 2). A CR>1 is considered indicative of a relatively open exchange with external waters. A CR<1 then indicates a relatively closed system, where carrying capacity depends more on internal primary production. In that case, the Grazing Ratio is considered a relevant measure, expressing filtration pressure on the local primary production. Values <1 would indicate unsustainable overgrazing. The Aquaculture Stewardship Council's bivalve standard stipulates that if CR<1, GR should be >3 (Threshold for Potential Concern, Aquaculture Stewardship Council 2019).

In the Oosterschelde bay, Jiang et al. (2019) reported CR<1 and GR>5 for the period 1992 – 2010 (no primary production data were available after 2010). This implies a dependence of filter feeders in the system on local primary production, and on average no excessive grazing pressure. However, Comeau et al. (2023) caution that structural shifts in phytoplankton composition can still occur at higher GR values: from approximately 15 in the case of Prince Edward Island (Canada) embayments. In a recent update investigation on the Oosterschelde bay, no indications were found for changes to the general picture for 1992–2010 (Craeymeersch et al. in prep). Although seston imported from the North Sea is argued by these authors to be able to meet the basic metabolic demands of bivalve shellfish stocks to a large extent, this situation is likely different under nutrient limitation during the SMC growing season. Note also that the basic metabolic demand assessed in the study may underestimate actual metabolic requirements during the SMC growing season since the model that the authors used did not include growth, and did not account for the effect of high water temperatures on metabolic rates in this season.

In any case, a high phytoplankton demand for nutrients combined with water residence times in the order of weeks to months (Smaal & Boeije 1991b, Jiang et al. 2019), will lead to recirculation of regenerated nutrients via primary producers back to the filter feeding populations during the SMC growing season. Although it is indirectly observed via mussel growth rates, directly linking nutrient regeneration via primary production to bivalve filter feeder growth rates was beyond the scope of this thesis. This presents an interesting avenue for further research.

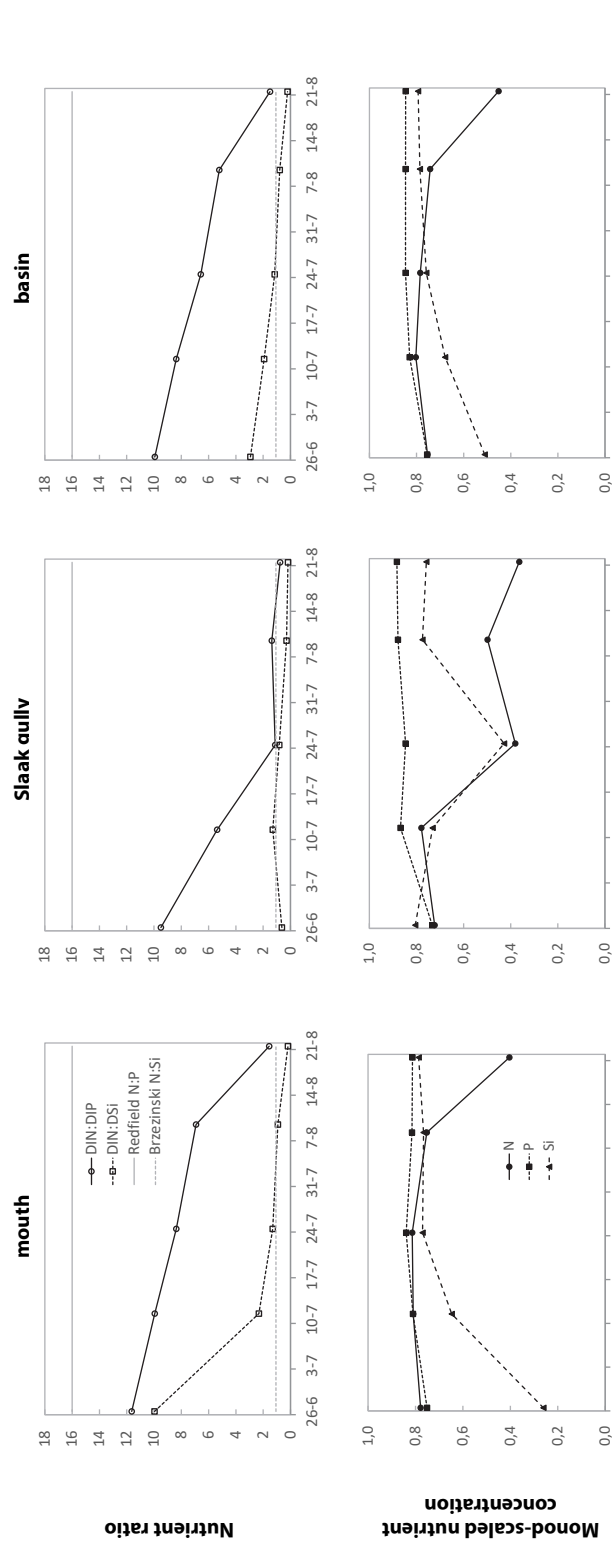

Figure 7.6. Top panels: nutrient ratios in the Oosterschelde bay, with Redfield N:P and Brzezinski N:Si ratios for reference. Bottom panels: Monod-scaled nutrient concentrations per compartment (panels) and nutrient (line and symbol types), calculated according to Philippart et al. (2007) as $f_x = x / (x + x_k)$, where x is the nutrient concentration (mmol m^{-3}) and x_k is the half-saturation coefficient: 2 mmol m^{-3} for DIN and DSi, and 0.2 mmol m^{-3} for DIP. The most limiting nutrient is the one with the lowest f_x.

7.3.2. Nutrient cycling and feedback

Having established the occurrence of nutrient limitation and the relevance of local primary production for filter feeders during the SMC growing season, I assessed the role of SMCs as nutrient sink and source, the influence on stoichiometric ratios in the environment, and evaluated the potential extent of positive feedback by means of the Nutrient Turnover Time (NTT, Box 2) index and the newly developed Potential Extent of Fertilisation (PEF, Box 2) index.

Nutrient sink and source

The SMCs effectively act as a sink and a source of nutrients, both removing nutrients by filtration and returning nutrients by regeneration (Box 2). In this study the SMC represented a sink for 70% of all nitrogen taken in, and a source for the 30% that was regenerated. These values were close to suspended culture rope systems in an oligotrophic Norwegian fjord system and mussel beds in the German Wadden Sea, but differed from measurements on cultured mussel beds in the Oosterschelde bay which acted as a source of 85–100% of nitrogen taken in (Chapter 3). For phosphorus, the SMC acted as a sink for 52%, and a source for 48%. This was relatively similar to suspended culture in an oligotrophic Norwegian fjord system (56% source), but differed from cultured mussel beds in the Oosterschelde bay (33–100% source depending on time of measurement). Given the full remineralisation of silicon from biodeposits assumed in the current study based on Chapter 5, the SMC acted as a source for 100% of silicon taken in. Mussel beds in the Oosterschelde bay were previously found to also act as a source for 100% of silicon, but 48% has also been reported (Chapter 3). In contrast to suspended cultures such as SMCs, mussel beds are able to progressively accrete organic material over time, which can explain functioning as a sink in certain conditions despite the mussels themselves not incorporating any silicon into tissue or shell. Expressed on a daily basis (Box 2), nitrogen and phosphorus regeneration took place right from the start of the culture cycle, whereas silicon regeneration was relatively delayed (Figure 7.7). The maximum source values achieved by the SMC reached 45% for nitrogen earlier on in the SMC growing season, and 54% for phosphorus and 28% for silicon at a later stage. The absence of a substantial excretion component for silicon, together with the absence of incorporation of silicon into mussels or AFFOM, led to lower source values early on in the growing season. The reduction of source proportions near the end of the season was caused by acceleration of the rate of biodeposit accumulation due to accelerated mussel biomass growth.

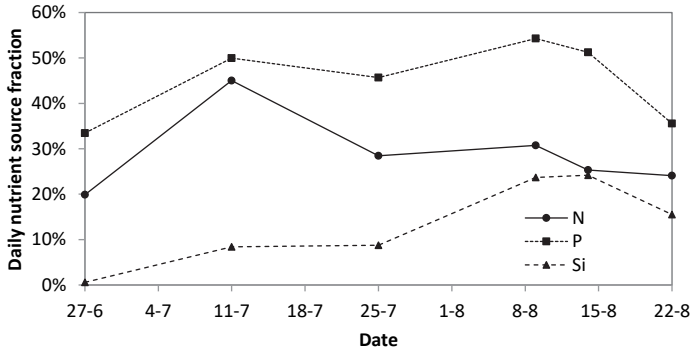

Figure 7.7. Source fraction for N, P, and Si, representing the nutrients returned to the system as a proportion of nutrients taken in on the same day.

It should be noted that nutrient release continues post-harvest. A fraction of biodeposits is mineralised on a relatively short timescale of days to weeks (Chapter 5 and paragraph 7.2.2). However, a proportion of the less labile fractions of biodeposits will likely eventually be remineralised. Furthermore, the measurements on remineralisation of biodeposits (Chapter 5) were performed in the absence of macrofauna, whereas biodeposits can be a food source for such organisms (e.g. a deposit feeding polychaete, Bergström et al. 2019). Biodeposit remineralisation may thus occur faster or more completely under natural circumstances. Furthermore, in case the mussel seed is relayed after harvest in the same ecosystem, mortality of mussel seed represents another significant return flow of nutrients. Given the high mortality of SMC mussels between relaying on bottom culture plots and final harvest for consumption (92%, Capelle et al. 2016), the quantity of nutrients locked away temporarily represents over an order of magnitude more than the quantity that is passed on to adult mussels and ultimately harvested for human consumption. These nutrients are not available for primary production and thereby for other filter feeders during the SMC growing season. SMCs contrast in this regard with the adult mussel culture in the system, which locks away a comparatively small quantity of nutrients per unit harvested biomass during the growing period. Approximately 42% of mussel seed is lost directly upon seeding (Capelle et al. 2016), so that this fraction can be expected to remineralise on a timescale of days to months from harvest. The remainder of mussel loss occurs more gradually between seeding and harvest, so that these nutrients will be returned to the environment over a period of several years.

Stoichiometry

Apart from assessing nitrogen, phosphorus, and silicon dynamics, it is relevant to look at nutrient stoichiometry while assessing (potential) aquaculture-ecosystem interactions (Prins et al. 1998). In principle, phytoplankton composition can be altered when nutrients are regenerated in proportions that differ from ambient ratios in the system (Prins & Smaal 1994, Turner et al. 1998, Philippart et al. 2000, Cloern 2001, Richard et al. 2006, Jansen et al. 2011). Differences in regeneration of the elements resulted in nutrient ratios that differ both from Redfield's and Brzezinski's mean ratios, and from ambient ratios of the inorganic nutrients. This implies a potential effect on phytoplankton community dynamics. DIN:DIP and DSi:DIP ratios in the Oosterschelde bay in reference year 2012 were considerably lower than Redfield's (1934) and Brzezinski's (1985) ratios, while the DIN:DSi ratio was slightly lower (Table 7.2). This was the case during the SMC growing season, and also over the full year. There were no obvious trends over the period 2010–2021. These ratios indicate relative shortages of nitrogen and silicon compared to phosphorus, with nitrogen in shortest supply. This is in line with the nitrogen and silicon limitation status of the system described in paragraph 7.3.1.

Compared to the nutrients present in the water during the SMC growing season, the SMCs provided some more nitrogen and silicon relative to phosphorus (Table 7.2). However, compared to Redfield's and Brzezinski's ratios the mussels still returned a relative shortage of nitrogen. During the SMC growing season the difference between regenerated nutrient ratios and ambient nutrient ratios in the water column were <20%. But after harvest of the SMCs, a further fraction of nutrients was regenerated from biodeposits (Table 7.2, and see paragraph 7.2.1). This was particularly the case for silicon. Over this extended period the SMCs still returned a relative shortage of nitrogen and a relative surplus of phosphorus, but also a relative surplus of silicon.

Finally, as an extractive aquaculture technique, bivalve culture constitutes a net downward pressure on nitrogen and phosphorus present in the system on longer timescales. After harvest of the SMCs, the young mussels are relayed onto bottom culture plots for growing to commercial size. If the mussel seed is relayed in the same system, if the AFFOM associated with SMCs is assumed to remain in the system, and if nutrients contained in the less labile fractions of biodeposits are also counted in the remaining pool of nutrients, then the net removal from the system is formed only by the SMC mussels that survive until their final harvest as full-grown mussels. The molar N:P ratio in SMC mussels established in this study was 31.9 (similar to adult *M. edulis* tissue in cultivation areas around the world, see chapter 3), and the mussels contained no Si (see Box 1). . This means that the pressure on nitrogen in the system caused by harvest for consumption was greater than on phosphorus, since this N:P ratio was greater than ambient concentrations (Table 7.2).

Table 7.2. Comparison of nutrient stoichiometry as regenerated by SMCs, and as present in the Oosterschelde bay in reference year 2012, with reference values for phytoplankton as described by Redfield (1934) and Brzezinski (1985). Nutrient concentrations in the Oosterschelde bay from the Dutch national monitoring programme "MWTL" (www.waterbase.nl).

		N:P	Si:P	N:Si
Regenerated nutrients by SMC during SMC growing season		7.0	6.4	1.1
Regenerated nutrients by SMCs including post-harvest phase		7.2	30.5	0.2
Dissolved Inorganic nutrients*	during 2012 SMC growing season	5.6	5.7	1.0
	during the year	9.1	9.8	0.9
Redfield's & Brezezinski's ratios		16	15	1.1

*Ambient values in the Oosterschelde bay 2012

Nutrient turnover & potential fertilization effect

The Nutrient Turnover Time (NTT; Box 2), or the time it would take for the SMCs to fully replace the dissolved inorganic nutrients present in the system compartment, can be compared to the water Residence Time (RT). If NTT<RT, the SMCs are considered to meaningfully mediate nutrient cycling locally (Chapter 3) during the SMC growing season, although this should not be regarded as a hard boundary and some degree of influence occurs before this point. Figure 7.8 (left panels) shows how the influence of SMCs differs between nutrients and system compartments. In the Slaak gully, SMCs mediated nutrient cycling within the local compartment for almost the entire duration of the growing season: NTT became lower than RT for all nutrients early on in the SMC growing season. For the mouth and the basin as a whole, this occurred only for nitrogen, at the end of the growing season (Figure 7.8, left panels). In Chapter 3, the Nutrient Turnover Time for nitrogen was compared with Residence Time in the same way for other mussel aquaculture areas in the world. The only other location where NTT<RT occurred was Great Entry Lagoon. This would suggest that the nitrogen interaction exhibited by SMCs in the Oosterschelde is among the strongest mussel-ecosystem nitrogen interactions in the world. However, the comparison in Chapter 3 is based on averaged values, whereas in the present study the maximum is evaluated.

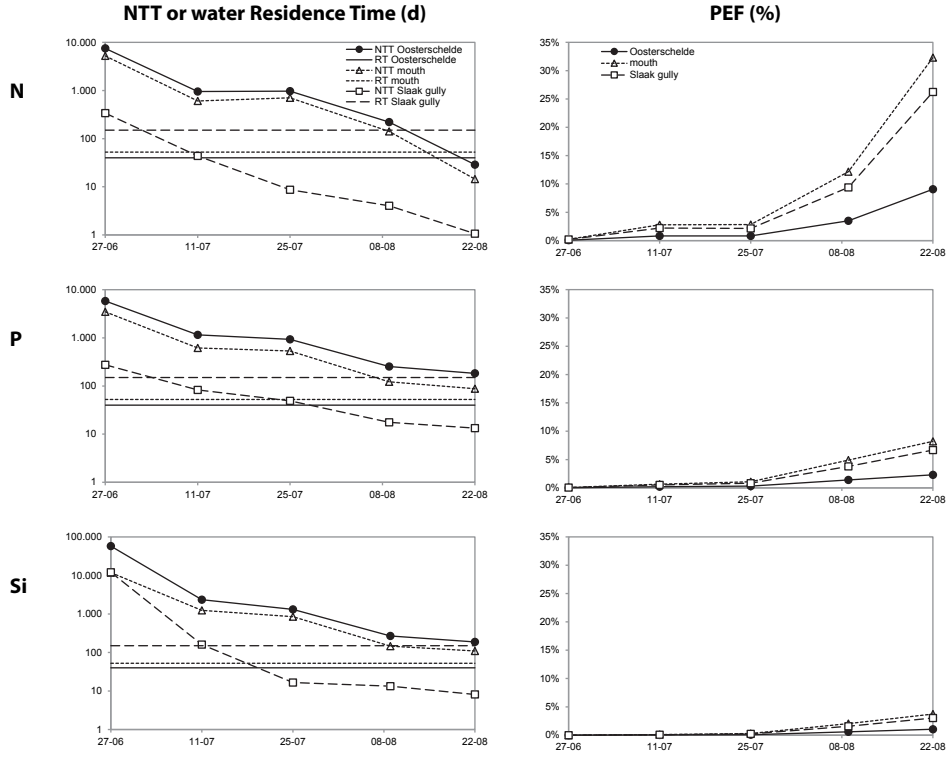

Figure 7.8. Left panels: Nutrient Turnover Time (NTT) calculated per compartment (symbol types) and per nutrient (panels), representing the time required for SMCs to fully replace dissolved inorganic nutrients in the compartment. Water Residence Times (RT) are shown for comparison. Right panels: Potential Extent of Fertilisation (PEF) calculated per compartment (symbol types) and per nutrient (panels), representing the proportion of nutrient uptake by phytoplankton provided by SMCs.

The Potential Extent of Fertilisation (PEF, Box 2) reflects the proportion of nutrient uptake by phytoplankton provided by SMCs. As for the Nutrient Turnover Time (NTT), the fertilisation potential (PEF) was largest in terms of nitrogen, reaching approximately order of magnitude higher levels than for phosphorus and silicon (Figure 7.8, right panels). For the basin as a whole, the Potential Extent of Fertilisation indicated a low contribution of 0.1% to silicon requirement of diatoms when silicon was most limiting (on Julian day 206), but there was a substantial positive feedback in terms of nitrogen of 9% of the phytoplankton primary production requirement at the time of harvest when nitrogen was most limiting (Figure 7.8, right panels). In the mouth of the Oosterschelde bay, PEF values were highest. For diatoms, silicon was most limiting during the first period, but the subsidy of silicon from SMCs to the system was low around this time (0.3% on Julian day 206). Nitrogen was most limiting at the end of the SMC growing season, and at the time of harvest up to 32% of the phytoplankton primary production requirement was

contributed by SMCs. In the enclosed Slaak gully, PEF values were almost as high as in the mouth, supplying at the time of SMC harvest up to 26% of the phytoplankton primary production nitrogen requirement. These findings indicate that especially toward the end of the SMC growing season when the mussels have grown and their total activity is highest, a substantial positive feedback of regenerated nutrients via primary producers to the SMC mussels, and to other filter feeders in the system, occurs. This conclusion has relevance not only for the evaluation of changing aquaculture practices in existing situations, but also for understanding the consequences of invading filter feeding species into new systems.

Finally, it is interesting to make a comparison of SMC and adult mussel activity in the Oosterschelde bay, which mediate nutrient cycling in the same system. The Oosterschelde bay contains a sizeable adult mussel population mostly on mussel culture lease plots fluctuating around $40*10^6$ kg since reference year 2012 (Craeymeersch et al. in prep; data until 2021), with SMCs amounting to an order of magnitude lower biomass of approximately $3*10^6$ kg (Chapter 2). The greater weight-specific SMC filtration and nutrient regeneration rates of the smaller specimens (chapter 4; Cranford et al. 2011) brought SMC activity per unit biomass at or above the upper end of the range reported for adult mussel beds in the Oosterschelde bay (Chapter 3). Taking biomass into account, at the peak of the SMC growing season SMCs captured and released significant amounts of nutrients compared to the adult mussel stock. Summarising over the mouth, Slaak gully, and basin as a whole, SMC clearance rates represented 0.4–22.0 times those of the adult mussel stock, DIN excretion 0.1–9.6 times, phosphate excretion 0.1–22.0 times, and silicate excretion 0.7–5.2 times. This comparison shows that SMC mussels are able to match, and exceed by an order of magnitude, the activity of the adult mussels in the Oosterschelde bay during the growing season.

7.4. Concluding remarks

In this chapter I combined data from Chapters 2, 4, and 5 and showed substantial nutrient regeneration potential of SMCs during the growing season in the Oosterschelde bay, in combination with nutrient limitation of primary production. Substantial nutrient regeneration was confirmed by a field study that evaluated filtration and nutrient regeneration from commercial SMCs directly at the scale of an entire SMC (Chapter 6).

It is noteworthy that assessments of aquaculture-ecosystem interactions are frequently made on an annual scale. A recommendation following from the findings in this study is to assess effects with appropriate spatial and temporal resolution. This chapter showed a pronounced difference between spatial scales with regard to the magnitude of potential effects, with Slaak gully and the mouth of the bay experiencing much stronger effects

than the Oosterschelde bay as a whole. Temporal scale is equally relevant: SMCs were shown to be able to provide a positive feedback during the summer growing season, but the occurrence of nutrient limitation of phytoplankton during the summer SMC growing season would not be detected when averaged on an annual scale. The spatial and temporal resolution of assessments should reflect both the physical and temporal distribution of the aquaculture population under study in conjunction with functional characteristics of ecosystem compartments. Important factors in this regard are water mass residence time, and the occurrence of any nutrient limitation for phytoplankton, the food source for filter feeding species.

In conclusion, this thesis provided evidence for strong ecosystem interactions in terms of top-down and bottom-up control, at local and potentially at bay-wide scale. SMCs affect phytoplankton populations through grazing activity, but in return may regenerate a substantial proportion of the assimilated nutrients and thereby potentially stimulate primary production. This results in a lower overall pressure on phytoplankton populations than may be expected based on grazing pressure alone. SMCs in the Oosterschelde bay provide a substantial positive feedback to primary producers, particularly in terms of nitrogen. Disregarding this effect when assessing environmental impacts of SMCs may substantially overestimate the impact on carrying capacity for filter feeding populations in the system.

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Dorine van Broekhoven

Summary

Summary

Bivalve molluscs have been consumed by humans for over 100,000 years. In the present day, the vast majority of production is aquaculture based. Globally, farming of bivalves is still heavily reliant on supply of seed from wild fisheries. These fisheries are increasingly under pressure because of a growing concern about potential negative effects on the natural environment and conservation goals. Seed collector systems consisting of artificial substrate are successfully used in mussel aquaculture sectors such as the bouchot, raft and longline cultures around the globe. In the blue mussel (*Mytilus edulis*) aquaculture industry in the Netherlands, suspended seed collectors, known as Seed Mussel Collector (SMC) systems, are gradually replacing wild seed fishery. This results in an elevated biomass of mussels in the ecosystem during the summer growing season, spanning from spatfall in spring to harvest and relaying to bottom culture plots in autumn. Since mussel seed exhibit a proportionally high metabolic activity, the introduction of SMCs has the potential to increase pressures on the ecosystem from the mussels' filtration and metabolic activity. Bivalve suspension feeders exercise top-down (filtration) and bottom-up (nutrient regeneration) control over phytoplankton populations. When phytoplankton becomes depleted, top-down filtration control can constitute a negative feedback onto growth rates of the bivalves, thus reducing the carrying capacity of the system for suspension feeding populations. On the other hand, bottom-up nutrient regeneration processes can constitute a positive feedback when phytoplankton productivity is stimulated and promotes growth rates of the bivalves. As the SMC culture cycle takes place during summer, it coincides with the period when phytoplankton is nutrient limited. Regenerated nutrients may therefore directly benefit primary production rates in the system, potentially counteracting part of the top-down control on phytoplankton.

A quantitative understanding of nutrient cycling by mussel seed assemblages has been lacking. Previous studies in relation to nutrient cycling by bivalve suspension feeders investigated (adult) individual specimens in isolation, investigated a single direction of control i.e. top-down or bottom-up, or investigated a single macronutrient. A few studies have pointed to the significant role played by associated fauna, flora and organic matter (AFFOM) on suspended bivalve culture assemblages in nutrient cycling. Appraising the importance of community level interactions, the aim of this thesis was to investigate the contribution of suspended mussel seed collector communities to nutrient cycling. This was addressed via four objectives:

i) Quantify filtration and nutrient release by SMC communities;
ii) Develop a multi-element (N,P,Si) nutrient budget for an SMC community;
iii) Measure farm scale depletion of seston and accumulation of nutrients under macrotidal conditions;

iv) Explore the top-down and bottom-up regulation of phytoplankton by mussel seed, and evaluate its ecological relevance in the transition of wild seed fishery to SMCs.

The thesis starts with a general introduction to the subject matter and the research aims (**Chapter 1**). **Chapter 2** then describes the transition to SMCs in the Dutch mussel sector, and presents detailed harvest data from 2010 until 2022. These data were analysed to investigate the efficiency of different systems, identify differences between years and areas, and assess how production can be optimized. Additionally, numerical density, biomass, and shell lengths of mussels from 0.375 mm shell length were recorded on SMC ropes at one SMC location during a full growth season to evaluate biomass-density relations and assess the process of self-thinning on the ropes. Total harvest of SMC mussel seed increased over the period 2010-2022, from 8.0×10^6 kg to 21.0×10^6 kg fresh weight. Harvest per unit substrate was remarkably stable over the years across sites, with a lower mean in the Oosterschelde Bay (~2.56 kg m^{-1}) than in the Wadden Sea (~3.28 kg m^{-1}). Ropes were found to provide a greater yield per unit area than nets, but nets are less labor intensive to use. Occurrence of density-dependent growth on the ropes was indicated by the allometric relation between mussel biomass and mussel density. A positive relation between density and growth rate suggested that competition increased with growth rate. In the growth data covering a full SMC season, we first observed a rapid numerical increase as newly settled mussels continued to grow into the measured size range. This was followed by a period of rapid numerical reduction and increasing biomass, indicating self-thinning. The analysis shows that SMC seed is a robust and annually more reliable alternative to wild capture fishery as a seed provisioning resource for mussel culture. Production per unit substrate does not appear to be easily amenable to further improvement. Production per unit area showed no indication of overstocking on the scale of the SMC plots, suggesting that production gains could be made by increasing substrate density.

The general introduction to the subject (**Chapter 1**) is complemented by an extensive literature review exploring nutrient cycling mediation by cultured mussels (**Chapter 3**). The review evaluates how cultured mussel stocks regulate nutrient dynamics for ecosystems that vary in trophic state. It examines (i) the eco-physiological response of mussels, and (ii) the positive and negative feedback mechanisms between mussel stocks and the surrounding ecosystem. It was found that despite differences in eco-physiological rates, the proportion of nutrients regenerated was similar between (deep) nutrient-poor and (shallow) nutrient-rich areas. Of the filtered nutrients, 40% to 50% is regenerated and thus made available again for phytoplankton growth, and 10% to 50% of the filtered nutrients is stored in tissue and could be removed from the system by harvest. The review shows that due to the physical characteristics (volume, water residence time), estimated effects

were stronger in shallow nutrient-rich areas with more intensive aquaculture activities, especially in terms of the negative feedback mechanisms (filtration intensity), while the effects were lower in nutrient-poor systems with lower mussel densities. The potential for a positive feedback via nutrient regeneration is considered particularly relevant for SMCs in the Netherlands because of their specificity to the summer, when phytoplankton can be nutrient limited.

Chapter 4 provides new data on nutrient uptake and release dynamics, and potential implications for availability and stoichiometry of nutrients, for SMCs in the Netherlands. Uptake and release rates were measured *in situ* on intact seed collector ropes and related to development of ropes in terms of mussel biomass and AFFOM. There was a good fit between uptake/release rates and mussel weight based on allometric scaling functions, despite the occurrence of a substantial biomass of AFFOM on ropes. Per unit biomass, nutrient release rates were much higher than reported in other studies, and a link is made to the greater activity of juvenile mussels. It is demonstrated that seed collectors can affect relative availabilities of nitrogen, phosphorus and silicon.

Chapter 5 addresses the lack of quantitative information on mussel feces and pseudofeces quality, and associated nutrient regeneration rates. In this chapter nutrient regeneration rates were determined during decomposition of feces and pseudofeces produced by mussel seed. Apart from a trial in the nineties, this study is the first to present nutrient regeneration dynamics of feces and pseudofeces separately. Dissolved inorganic nitrogen and phosphate regeneration continued at stable rates for approximately three weeks, after which 13.1% and 12.4% of the available nitrogen and 8.7% and 7.9% of the available phosphorus had been regenerated from feces and pseudofeces, respectively. Rates of silicate regeneration declined continuously, which is attributed to its accumulation in the experimental setup. Overall dissolved inorganic nitrogen regeneration rates were similar between feces and pseudofeces, but depletion of ammonia was initially more rapid for pseudofeces due to stronger nitrification. Phosphate and silicate regeneration rates were 1.1 and 1.4 times greater from feces than pseudofeces, respectively.

Chapters 4 and 5 quantified how suspension-feeding bivalves, specifically mussel seed, can filter large quantities of particulate matter from the water column, while at the same time returning inorganic nutrients to the environment. However, *in situ* observations of these effects are scarce. **Chapter 6** presents a field study aiming to identify whether reduction of seston and enhanced nutrient concentrations in the water column could be demonstrated for suspended mussel farms in a highly dynamic bay. Two complementary field campaigns were carried out at three SMCs and one suspended grow-out mussel farm in the Oosterschelde bay: (i) a discrete sampling approach limited in spatio-temporal resolution investigating a range of parameters (TPM, Chl *a*, particle concentration, POC,

PON, DIN, DIP), (ii) a towed glider approach investigating TPM and Chl *a* concentrations at high resolution. Reduction of seston concentrations (up to 65%, POC) and increase of overall macronutrient concentrations (up to 208%, DIN) were observed. Large variability between farms and parameters reflected potential hydrodynamic effects such as water intrusion due to body drag of the farms, and patchy water mass contents as indicated by the towed glider measurements. This led to a few cases where reversed trends were apparent. This study is one of the first to simultaneously show both top-down control and bottom-up feedback processes *in situ* for suspended bivalve cultures.

In the final chapter (**Chapter 7**) the findings from the previous chapters are synthesised to construct a (cumulative) nutrient budget over the summer growing season. This allows an assessment of the magnitude of impact of suspended mussel seed collectors on nutrient cycling in the host ecosystem. By the end of the SMC growing season, 31% of nitrogen and 11% of phosphorus uptake by the SMC assemblage had been sequestered in mussel seed biomass. Approximately three quarters of this was harvested at the end of the season, while the remaining quarter was lost with mussels detaching from the SMC over the course of the growing season. The remaining mussels were harvested along with the AFFOM, together representing 31% of the total nitrogen and 14% of the total phosphorus uptake. The fraction of regenerated nutrients relative to their uptake varied strongly between the elements. Approximately one third of the nitrogen, half of the phosphorus, and all of the silicate was regenerated and thus made available again for primary producers. The pathways contributing to regeneration also varied between the elements. Excretion by the mussel seed community (**Chapter 4**) was the dominant regeneration pathway for nitrogen (accounting for 86% of total nitrogen regenerated) and phosphorus (92%), but silicon regeneration occurred primarily through biodeposit decomposition (98%; **Chapter 5**).

Subsequently, building on new and existing indicators, the (potential) level of ecological influence exerted by SMCs is evaluated by a set of approaches: (i) sink and sources evaluation, (ii) nutrient stoichiometry, (iii) nutrient turnover times, and (iv) fertilisation potential. This was done at the level of the Oosterschelde bay as a whole as well as separate compartments within the bay. The Slaak gully is thereby specifically reported on given that most pronounced effects are expected due to local hydrodynamic conditions. The evaluation is followed by a comparison with adult mussels in the system. But first, evidence for the occurrence of nutrient limitation, and relevance of local phytoplankton production for filter feeders, during the SMC growing season were established. A comparison of ambient nutrient ratios with Redfield's and Brzezinski's ratios, and an evaluation of Monod-scaled nutrient concentrations, both pointed to a nitrogen limitation of phytoplankton. In addition, in the mouth and the bay as a whole, there was evidence of silicon limitation – relevant for diatoms – during the first half of the SMC growing season.

Clearance Ratio and Grazing Ratios reported previously implied a dependence of filter feeders on local primary production. Furthermore, a high phytoplankton demand for nutrients combined with water residence times in the order of weeks to months implied a recirculation of regenerated nutrients via primary producers back to the filter feeding populations during the SMC growing season.

Having established the relevance of nutrient regeneration by SMCs for local primary production and a probable feedback to filter feeders in the system, the role of SMCs as sink versus source of nutrients is analysed. Over the SMC growing season and including remineralisation of biodeposits after harvest, the SMC represented a sink for ~70% of all nitrogen taken in, and a source for the other ~30%. For phosphorus, the SMC acted as a sink for ~50%, and a source for ~50%. The SMCs were considered to acted as a source for 100% of silicon. This is followed by an investigation of the influence of SMCs on relative nutrient availability (stoichiometry). The ambient N:P ratio of 5.6 was lower than Redfield's ratio of 16, and SMCs did not change this situation since the N:P ratio of regenerated nutrients was also lower at 7.0. The ambient N:Si ratio was 1.0, close to the 1.1 ratio of nutrients regenerated during the SMC growing season. However, when considering post-harvest release of silicon from accumulated biodeposits, a relative surplus of silicon was returned (N:Si ratio 0.2). Therefore on the whole, considering the nutrient requirements of phytoplankton, the nutrients returned by the SMCs were lacking in nitrogen. Subsequently, a comparison of nutrient turnover time and water residence time for the Slaak gully and mouth compartments as well as the entire Oosterschelde bay, shows how the influence of SMCs differs between nutrients and system compartments. In the enclosed Slaak gully, SMCs mediated nutrient cycling within the local compartment for almost the entire duration of the growing season. For the mouth and the basin as a whole, this was only the case for nitrogen, at the end of the growing season. Finally, the proportion of nutrient uptake by phytoplankton that is provided by SMCs is quantified. The newly established indicator on the fertilisation potential was most significant for nitrogen, reaching approximately an order of magnitude higher levels than for phosphorus and silicon. Values were highest in the mouth of the Oosterschelde bay. Here, nitrogen was most limiting at the end of the SMC growing season, and at the time of harvest >30% of the phytoplankton primary production requirement was contributed by SMCs. In the Slaak gully, this was ~25%, and in the bay as a whole ~10% was reached. For context, a comparison to adult mussels shows that SMC mussels are able to match, and exceed by an order of magnitude, the activity of the adult mussels in the Oosterschelde bay during the SMC growing season.

This thesis sheds a light on the implications for nutrient cycling of the juvenile mussel community that forms on suspended substrates every summer as a result of the transition from wild capture seed fishery to SMCs. This rapidly growing mussel cohort together with

its AFFOM was not present traditionally in the Oosterschelde bay when wild seed was imported from the Wadden Sea. In this thesis, filtration and nutrient release rates by SMC communities were quantified and combined into a nutrient budget, and the effects were observed *in situ* at the farm scale. These data were used to evaluate the ecological relevance of the nutrient cycling pathways via which the SMCs interact with their environment. It is concluded that SMCs are able to exercise significant top-down and bottom-up control on phytoplankton at local, and potentially bay wide scale. SMCs in the Oosterschelde Bay provide a substantial positive feedback to primary producers, which is particularly relevant for nitrogen given the shortage of this nutrient during the SMC growing season. Disregarding this effect when assessing environmental impacts of SMCs may substantially overestimate the impact on carrying capacity for filter feeding populations in the system.

Margot van Broekhoven

Acknowledgements

Acknowledgements

This study for me began with a trip to Norway together with Aad, to join a measuring campaign of his PhD student Henrice. Between anecdotes and plus size king crab legs with which the research vessel's freezer turned out to be stuffed full, it began to dawn on me during incubations and clearance rate measurements what this investigation was going to involve. Now Zeeland has its beauty but it's no match for a Norwegian fjord as a backdrop for doing fieldwork. Nevertheless, the role of mussels in nutrient cycling is a fascinating study topic, and the rich Zeeland waters with their concentrated mussel industry have a unique dynamic to them. I also recommend to all to delve a little into the workings of the mussel itself. The foot is surprisingly nimble, the mussel is equipped with a rotating grinding stone of sorts to digest its food, and the filtration and disposal system for suspended matter is as sharply tuned as a precision watch. Aad, we have on occasion approached things differently, but I have learned a lot from you, professionally but also about having fun in your work and about the importance of putting things into perspective. Thank you for your confidence and the wonderful insights into the mussel itself and the industry surrounding it!

Henrice, I also learned a great deal from you. My subject and yours were closely related and this meant that I could bounce ideas and the inevitable practical problems associated with practical research off you very well. I also appreciate your unwavering focus on the main storyline and your way of structuring an experiment or a paper. This has helped me more than once to make sense of a jumble of data with all kinds of ifs and buts. I should also note here that you were the primary instigator for starting the final push to compile my work into this thesis. Over time shellfish had become increasingly relevant to my work again, and you had taken up a position more directly related to the university. It was meant to be. Thank you for all you help and wisdom, it has been a pleasure to work together!

Karin, before we embarked on this adventure I had already had the opportunity to do an internship in Yerseke as part of your PhD studies: simply put, the identification of critters on oyster banks. I learned a lot from you about working systematically, and thanks to you I also know that a Mercedes van can always be backed up into salt water a bit more if that helps to launch your boat in a gentle manner. Your calmness and your admiration for nature contributed to my continuing interest in shellfish, what these animals are up to, and what they can bring to us humans. Thank you for your help, your support, and your sober look on matters!

Geert, I greatly valued our collaboration. In an inimitable way you are able to work to-the-point and efficiently but at the same time remain a very sympathetic human being.

This characteristic contributed to my conviction that finishing my thesis in spare time was going to be feasible and I would not be on my own. Thank you for agreeing to be my promotor!

Pauline, my research took place within the context of your research project on mussel seed collectors. If possible, you are even calmer than the others, and it was very pleasant to be able to work that way. I have great memories of boating around with the Acrobat glider in tow, and also of the field measurements a few years later. These days we again meet regularly in our professional lives, this time around oysters instead of mussels. I look forward to continuing to work together!

De mosselen voor mijn experimenten waren vooral afkomstig van de MZI van Wout van den Berg. Wout bedankt dat ik mosseltouwen bij je vlottensysteem mocht hangen en hier observaties en metingen mocht verrichten!

Many fine colleagues and relations have kindly shared their knowledge and experience and thus contributed to this thesis. Peter, your visit with the Acrobat towed glider at the start of my PhD and the stories you told of other ecosystems with quite different shellfish aquaculture industries were highly inspiring. Thank you for kindly providing your data and figures of our campaign, and your willingness to take another look at this work all these years later. Sven, your unique perspective on science and on anything really were inspiring, and I very much enjoyed our collaboration on the field campaign and a bunch of related measurements. Eric Struyf, we got in touch about silicon cycling in ecosystems and our collaboration, although short, has brought me much insight. Marc Verdegem, thank you for your ever efficient and spot on advice that I was able to put to good use. Coming from a practical research setting, it was great to be able to seek advice from someone with such academic knowledge and experience. At the same time, Jeroen Wijsman, one of the benefits of working in a practical research institute is that people such as yourself work there too. Thank you for a short brainstorm now and then about the functioning of mussels in the Oosterschelde bay, and thank you for sharing your knowledge and experience. Pascalle, we never really found the time to run parallel experiments in the Oosterschelde bay and Wadden Sea. Still it was nice to both be working on SMCs and be able to share some ups and downs around this, thank you for these exchanges over time. Matt, I am sorry that you have had to suffer recurring thoughts about feces and pseudofeces ever since I asked you to review my manuscript for chapter 4. But I am happy that we are still in touch despite all that, and I hope to repay you a visit sometime soon, in a far or near corner of the world.

Mijn onderzoek had niet uitgevoerd kunnen worden zonder de praktische hulp van een veelheid aan collega's en relaties. Sander Ruizeveld de Winter en Adrie Albregtse,

jullie praktische insteek was onmisbaar tijdens de productie van grote hoeveelheden mosseluitwerpselen voor mijn incubatie-experimenten. Bedankt ook voor de gastvrijheid op de faciliteit in Colijnsplaat! Sairah Malkin, Dirk Burggraaf, thank you for kindly helping out with some niche technical measurements. Ronald Booms, je creatieve geest was van grote waarde bij het verwerken van allerhande moeilijke monsters in Wageningen. Ik heb genoten van onze samenwerking! Noortje, ook jij was een grote hulp in het lab. Angelo, zonder jouw hulp en advies had ik die vervloekte autoanalyser niet aan de praat gekregen en zeker niet gehouden!

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Among my fellow PhD students at Imares were Jurgen and Jacob, my paranymphs. Jurgen, we have known each other since our student days in Groningen and I hold many fond memories of shared experiences over the years. You have played a major role in terms of introductions leading to an eventful job position, among other important things in life. Hopefully finishing this thesis finally gives me some free time to catch up on some overdue Belgian beer tasting. Thank you for everything. Jacob, you were my roommate from day one at Imares in Yerseke. I am grateful to have had your down-to-earth and laidback attitude, your improvised aquarium, and your insight into all things Zeeland and mussel related on my side! Thank you also for our collaboration on the latest publication to have become a part of this thesis as chapter 2. Working on that provided the flywheel effect to finish the rest of the work and get to this point. There were many other fellow PhD students with whom I have shared a laugh or a frustration. Among the most notable, Antonios: it's always a pleasure to meet up or joke around. Antonio, for being my other roommate in Yerseke, for some thoughtful discussions and for briefly sharing a house. Dorina, your support helped me get back on track on some difficult days, this was highly appreciated! And Brenda, for providing a place to stay during the first days in Yerseke and for bringing me along on the coldest fieldwork during my entire PhD study (leaky rubber boots did not help!).

At various stages during my research I was lucky to have the help of my enthusiastic students: Eliyah Nurul Khasanah, Marloes Groeneveld, Jesse Barnaart, Marion Roger,

Joffrey Lambert, Angeline Grente, and Emilie de Medts. Thank you all for your invaluable help along the way!

Hoewel het er met de tijd vanwege iedereens drukke banen en alle kinderen zeker niet eenvoudiger te organiseren op is geworden om de bloemetjes buiten te zetten gaat mijn dank ook uit naar fijne vrienden; Stefan, Sarah, Laura, Steijn, Esther, Luuk, Huygen, Sabina, Ivo, Martijn, Maarten J, Maarten V, Paul, Dirk Jan, Mats, Inge, en bij voorbaat mijn excuses voor wie ik hier niet heb genoemd. Over de hele periode van mijn proefschrift heen heb ik veel energie geput uit de feestjes die we hebben gevierd, de gesprekken die we hebben gevoerd, en de eenvoudige wetenschap dat jullie er zijn.

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Marieke, uiteraard wordt een proefschrift niet geschreven zonder dat de partner daar een belangrijke rol in heeft. Er zijn heel veel avonden in gaan zitten en het is soms best een uitdaging geweest om alles te kunnen bolwerken. Bedankt dat je me de ruimte hebt gegeven om dit werk te voltooien. Voor de steun die ik van je krijg en voor heel veel mooie dingen die we samen hebben meegemaakt. Ik ben nu zelf eigenlijk ook wel klaar met dit onderzoek. Dankjewel ook voor de mooie illustratie op de omslag, zo komen jouw inspanningen ook zichtbaar tot uiting in het eindresultaat.

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Liefs, Wouter

About the author

Wouter was born on the 23rd of May 1980 in Haarlem, near the Dutch coastline. His fascination for aquatic environments in general and marine biology specifically arose from sailing with the seascouts and from holidays spent on the Wadden Islands and places like Brittany in France. After graduating his pre-university education at the Stedelijk Gymnasium in Haarlem in 1998, he backpacked through Australia where he obtained his first scuba diving certification on the Ningaloo Reef. During his university education at the University of Groningen leading to a MSc degree in Marine Biology, he undertook practical research projects on mutual mate choice in the Sphinx blenny on Corsica, the effects of ultraviolet radiation on marine bacterial communities from Antarctica in the lab, and his first shellfish-centred topic: faunal diversity on beds of Pacific oyster in the Oosterschelde bay in the Netherlands. Following his studies he participated in the Japan Prizewinners Programme. He spent one year immersed in Japanese culture and language and participated in research at the Japanese National Institute for Environmental Studies in Tsukuba, investigating the local extirpation of hamaguri, the Asian hard clam, from Tokyo Bay.

On return to the Netherlands Wouter worked as a policy officer, taking part in the government fast stream programme (Rijkstraineeprogramma). Topics included marine environmental policy, and implementation of the then new European Marine Strategy Framework Directive. He also fulfilled a secondment with the Science and Technology attaché at the Netherlands Embassy in London. He then undertook a second MSc degree (graduated with Distinction) on Conservation Science, obtained from Imperial College London. His practical research took place in the Philippines and focused on perceptions of rural coastal communities on nearby small scale marine protected areas, aiming to understand obstacles to upscaling protective measures for the marine environment. On return again to the Netherlands he commenced his PhD research on another shellfish species, the blue mussel, at Imares (now Wageningen Marine Research) in Yerseke. The last part of his PhD thesis – completing two chapters and producing a synthesis – was completed recently and in spare time. Directly following the practical work in Yerseke Wouter took up a position at VisNed, the Dutch fishermen's representative organisation, working first as science and policy advisor and subsequently as chief scientist. Here he initiated and participated in various research projects around improving the environmental performance of the demersal fishing sector, as well as coordinated the Dutch part of an international Marine Stewardship Council certification assessment, a combined application covering nearly 1000 vessels. He then worked for Witteveen+Bos, an engineering consultancy firm, as a marine biologist. He was involved in a range of projects, most notably on nature inclusive designs for offshore wind farms. Wouter presently works for Van Oord, a marine contractor, in the environmental engineering department. His work involves developing, implementing, and scaling up nature restoration solutions. Bivalve shellfish species such as the European native oyster, *Ostrea edulis*, again feature in this work.

WIAS Training and Supervision Plan (TSP)

WIAS Training and Supervision Plan (TSP)

GENERAL INFORMATION	
Name PhD candidate	Wouter van Broekhoven
Project title	Nutrient cycling by mussel seed collectors
Group	Aquaculture and Fisheries
Promotor	Prof. Dr GF (Geert) Wiegertjes
(Co)promotor	Dr HM (Henrice) Jansen
(Co)promotor	Dr K (Karin) Troost
Project term	From 15-08-2010 Until 31-12-2023

EDUCATION AND TRAINING (minimum 30 credits)

A. The Basic Package		year	credits *
WIAS Introduction Day (mandatory)		waived	0,0
WGS Scientific Integrity course (mandatory)		2024	0,6
WGS Ethics in Animal Sciences course (mandatory)		2012	1,5
Subtotal Basic Package			**2**
B. Disciplinary Competences *(minimum 2 courses)*		year	credits
Writing research proposal		2015	6,0
Aquatic and Microbial Ecology(University of Southern Denmark), 1-19 August		2011	10,0
Analysing biological and environmental data		2013	1,4
Design of experiments course		2010	1,0
Subtotal Disciplinary Competences			**18**
C. Professional Competences *(minimum 2 courses)*		year	credits
Techniques for writing and presenting scientific papers (WUR)		2012	1,2
Course on Organisation and Leadership for PhD students		2013	4,0
Voice Matters course		2013	0,4
Organising first annual IMARES PhD day (chair of organizing committee)		2011	3,0
Subtotal Professional Competences			**9**
D. Presentation Skills *(max 4 credits)*		year	credits
Impact of seed mussel collectors on carrying capacity, European Aquaculture symposium, Rhodes, Greece	Oral	2011	1,0
Filtration and bio-deposition impacts of mussel seed on ecological carrying capacity, mini-symposium Wageningen, The Netherlands	Oral	2012	1,0
Nutrient regeneration by mussel Mytilus edulis spat collectors in a productive macrotidal system, European Marine Biology Symposium, Galway, Ireland	Oral	2012	1,0
Nutrient regeneration by mussel Mytilus edulis spat collectors in a productive macrotidal system, ASLO, Honolulu, USA	Oral	2014	1,0
Subtotal presentations			**4**
E. Teaching competences *(max 6 credits)*		year	credits
Supervising 3 BSc thesis students		2011-2013	3,0
Supervising 1 MSc thesis student		2011/2012	2,0
Subtotal Teaching competences			**5**
Education and Training Total (minimum 30 credits)*			**38**

**One ECTS credit equals a studyload of approximately 28 hours*

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