The impact of pelagic mussel collectors on plankton

in the western Wadden Sea, the Netherlands

Pascalle Jacobs

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aan mijn ouders
"Gracias a la vida"
Violeta Parra

Abstract

Mussel beds are an important habitat in the Wadden Sea. To better protect these beds, fishing for juvenile mussels from natural beds is replaced with the harvest of mussels from the water column using so-called mussel seed collectors. These pelagic collectors consist of ropes or nets facilitating settlement of mussel larvae (*Mytilus edulis*). The implementation of these collectors will likely increase the number of juvenile mussels in the Wadden Sea. Mussels filter large quantities of water thereby removing suspended matter from the water column. This thesis aims at answering the question whether the harvest of 40 million kilos of juvenile mussels will affect the plankton of the Wadden Sea.

First, settlement and growth of mussel larvae on rope collectors was studied from 2010 to 2013. Both mussel densities on ropes as well as growth rates varied substantially. Mussel density correlated with water temperature after spawning. In years with a relatively high water temperature probably more larvae survived until settlement, resulting in a higher mussel density.

To investigate mussel filtration rate and the type of plankton removed, collector mussels (between 1.5 and 25 mm) were used in incubation experiments. Results showed that larger plankton like nanophytoplankton, heterotrophic nanoflagellates and ciliates was cleared at higher rates than smaller plankton like pico-sized phytoplankton and bacteria. It was also found that mussel clearance rate scales well with squared shell length. The clearance rates reported in this study were among the lowest reported in literature, which can be contributed both to the use of natural sea water in the experiments, compared to the use of algal cultures in older studies and to re-filtration of water at high mussel densities.

A direct short-term effect of mussel filtration on the pelagic food web was an increase in bacterial growth rate and a lower predation mortality rate for phytoplankton. We also reported that heterotrophic nanoflagellates were able to fully recover from mussel filtration within 24 hours. Additional experiments were performed allowing the plankton community to recover from mussel filtration for 9 days, a period comparable to the residence time of water in the western Wadden Sea. These experiments revealed that although larger algae and microzooplankton were able to balance the losses due to mussel filtration by increased growth on some occasions, generally the plankton community did not fully recover to pre-filtration biomasses. For picophytoplankton a relative increase in biomass was hypothesised, but rather a decrease was observed after 9 days.

Finally, a model was set-up to estimate the total filtration pressure of the collector mussels from settlement until harvest in September. At an aimed harvest of 40M kg of mussels, the maximum daily filtration pressure was estimated at 3.2% of the water volume of the western Wadden Sea. Calculations indicated that 8% of all carbon produced was assimilated by these mussels during their presence on the collectors. Including the heterotrophic components in the calculations almost doubled this percentage.

The results described in this thesis make it plausible that mussel filtration and feedback mechanisms will affect the microbial food web in terms of biomasses as well as recycling rates.

Contents

Voorwoord

Het werk is gedaan!
In 2009 kwam ik als afstudeerstudent terecht bij IMARES op Texel, en in 2010 begon ik daar als promovenda. Vier jaar lang werkte ik aan mijn promotieonderzoek en dit boekje is het resultaat. De morele en praktische steun die ik ontving hebben het werk een stuk beter en leuker gemaakt. Hiervoor wil ik graag de onderstaande personen bedanken.

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(Literatuur)onderzoek gaat niet zonder een goede bibliotheek. Die van Wageningen Universiteit is uitstekend, als er toch artikelen niet in de collectie bleken te zitten of boeken nog niet gescand waren bleek één verzoekje voldoende om vaak al de volgende dag een kopie in mijn email-box te vinden. Wat een service.

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Chapter 1

Introduction

Pascalle Jacobs

Introduction

Historically, estuaries and coastal seas have been favourable areas for human settlement and exploration (Lotze *et al.* 2006). At present more than one third of the world's population lives close to the world's coasts, which account for just 4% of the land surface (UNEP 2006). Human pressures resulted in centuries of overexploitation of resources and habitat destruction, leading to biodiversity loss and plant and animal population decline. In developed countries conservation efforts have resulted in a partial recovery of, mainly, higher trophic levels, but have largely failed to restore the former function and structure of these ecosystems (Lotze *et al.* 2006). An example of an estuary with a long record of human settlement in a densely populated area is the Wadden Sea; a shallow sea along the border of Denmark, Germany and the Netherlands covering about 14,700 km². The area consists of a multitude of habitats including tidal channels, sea-grass meadows, mussel beds, sandbars, mudflats, salt marshes, estuaries, beaches and dunes (Unesco c2015, Figure 1.1). The area is home to numerous plant and animal species, including marine mammals such as the harbour seal, grey seal and harbour porpoise, it is considered a site of key importance for migratory birds in the world and the area serves as an important nursery ground for fish from the neighbouring North Sea (Van der Veer *et al.* 2001, Unesco c2015). In 2009, the Wadden Sea was declared a World Heritage site (Wolff *et al.* 2010), and although the Wadden Sea is often regarded as one of the last wilderness areas (Swart *et al.* 2001, Swart & Van der Windt 2005), the area is thus far from pristine.

Wadden Sea

Retreating glaciers and rising sea level 8000-7000 BC created the Wadden Sea and its barrier islands and the first humans started using the wetland, salt marches and estuaries for the harvest of resources as early as 5500 years BC (Knottnerus 2005, Lotze 2005). From that time onwards subsistence usage shifted to commercial exploitation and the modification of the landscape changed from low impact transformation, via construction and destruction of habitats to protection at present. Other historical and ongoing human impacts in the area include pollution, changes in sediment and nutrient load and the introduction of invasive species (Lotze *et al.* 2005). Human interference resulted in an estimated local extinction of 42 species including 4 species of sea mammals, 5 bird and 13 fish species in the last 2000 years (Wolff 2000). In a re-investigation of subtidal macrofauna around the island of Sylt, Germany in the 1970-s, 50 years after the original investigation (1923-1927), large changes in the benthos were revealed (Riesen & Reise 1982). Eelgrass (*Zostera marina*), previously abundant in the area disappeared as a result of wasting disease and never recovered, most likely due to the increased turbidity after the closure of the Zuiderzee in 1932 (Riesen & Reise 1982).The authors also reported the replacement of the reef-building species oysters (*Ostrea edulis*) and *Sabellaria* (polychaete) by mussels

(*Mytilus edulis*). Oysters and tube worm-reefs most likely disappeared as a result of fisheries. Together with the introduction of extensive mussel culture in the western Dutch Wadden Sea in the 1950-s, with annual yields of more than 100 million kg fresh weight (Van der Veer 1989), the mussel became one of the dominant species in terms of biomass in the Wadden Sea. At the beginning of the 1980-s the natural mussel beds covered an area of more than 4000 hectares (Dankers & Fey-Hofstede 2015). To stock cultures, juvenile mussels were fished from littoral mussel beds and subsequently sown on culture lots in the Wadden Sea or exported to other areas like the Oosterschelde in the south-west of the Netherlands. In the period 1980-1990 intensified fisheries and the failure of recruitment resulted in the near disappearance of both cockle (*Cerastoderma edule*) and mussel stocks in the western Wadden Sea (Swart & Van Andel 2008). Public and political concern for impoverishment of the Wadden Sea resulted in protection measure being taken. These measures included closing of some areas to shellfish fishing and the introduction of fishing quotas, but by 1998 only small scale recovery of banks had occurred (Swart & Van Andel 2008). The decline of oystercatchers and mass mortality of eider ducks between 1999 and 2001 (Camphuysen *et al.* 2002) increased public concern even more (Swart & Van Andel 2008). It took until 2003 before more drastic measures were taken, including a ban on large scale mechanical cockle fishing (but still allowing manual harvest), and a joint agreement to replace fishing for juvenile mussels from littoral banks with alternatives (Swart & Van Andel 2008). The main goal of the covenant is twofold; an undisturbed development of juvenile banks as well as a more secure supply of juvenile mussels to the aquaculture sector (Meijer *et al.* 2009).

Mussel collectors

As an alternative for fishing to stock the culture lots, mussel seed collectors or pelagic collectors were developed. Already in the 1950-s people experimented with artificial ways to harvest juvenile mussels (N. Laros, personal comment). Collectors, presently in use in the Netherlands, consist of filamentous nets or ropes, vertical suspended in the water column. The collectors make use of the mussel characteristic to settle on a suitable substrate after having spent several weeks as larvae in the water column (Box 1). Settlement on these pelagic collectors starts around June and mussels are harvested at the end of September, when they have reached a size of approximately 25 mm. In the Netherlands, collectors can be found in de Voordelta, Oosterschelde and the Wadden Sea. When possible, collectors are placed above gullies to assure plenty of food supply for the settled mussels. The policy aim is to upscale the seed collectors to 40M kg harvest by 2020 (Meijer *et al.* 2009). The European bird-and habitat directive, encapsulated in the Dutch nature protection law ('natuurbeschermingswet') requires a first evaluation whether the planned activities are expected to exert significant effects on the protected sites, if so a detailed appropriate assessment is needed to identify the significance of the effect (Defra 2012). To assess the potential impacts of the introduced collectors in the Ooster-

schelde and Wadden Sea an integral research project was set-up commissioned by the former ministry of agriculture, nature and food quality (now ministry of economic affairs) in 2010. In this project several potential impacts of the pelagic collectors on the protected sites were considered including effects on the carrying capacity of the systems via filtration and nutrient regeneration, impacts on the benthic system (biodiversity and sediment characteristics) through deposition of faeces, disturbance or attraction of birds and mammals as well as the formation of plastic litter through wear and tear of the collectors using a combination of experiments, and modelling (Kamermans *et al.* 2014).

Figure 1.1 *The Wadden Sea is an estuarine area, bordering Denmark, Germany and the Netherlands. This World Heritage Site consists of a multitude of habitats including one of the world's largest intertidal areas (Source: CWSS c1998-2013).*

Effects of mussel collectors on plankton

Mussels that settle on pelagic collectors are assumed to have a higher survival chance due to a much lower predation pressure by animals like crabs and starfish. Once settled, pelagic collectors consist of high densities of mussels which have the ability to filter large volumes of water (Box 2). To assess the impact mussels on pelagic collectors can exert on the plankton community, information is needed on their filtration rate as well as the type of particles removed by these juvenile mussels. Most information on this however comes from studies on adult mussels. Based on laboratory studies using algal cultures it was assumed that mussels effectively retain all phytoplankton cells larger than 3 µm, while the retention of smaller cells rapidly decreases (Møhlenberg & Riisgård, 1978).

More recently studies have shown that not only phytoplankton are removed, but that adult mussels also retain other type of particles including microzooplankton (Horsted *et al.* 1988, Kreeger & Newell 1996, Trottet *et al.* 2008a) and mesozooplankton (Horsted *et al.* 1988, Davenport *et al.* 2000, Wong & Levinton 2006, Lehane & Davenport 2006). In addition, results from experiments using natural plankton communities rather than cultured algae, reported variable retention efficiencies

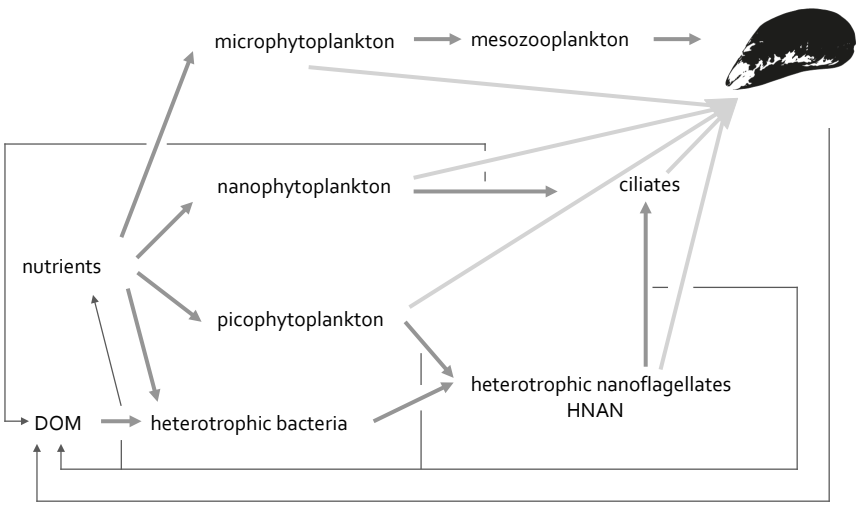

Figure 1.2 *A simplified marine pelagic food web including heterotrophic bacteria (<1 µm) and picophytoplankton (0.2–3 µm), which are considered the main prey for heterotrophic nanoflagellates (HNAN) (2–20 µm) (indicated by the thick dark grey lines). Nanophytoplankton (3-20 µm) is the main prey for ciliates (20–200 µm) together with HNAN. All groups belong to the microbial food web. Microphytoplankton (>20 µm) and mesozooplankton (>200 µm) are part of the classical food web. Ciliates and HNAN, when consumed by mesozooplankton, provide the link between the 2 food webs. The light grey arrows represent the plankton groups hypothesised to be filtered by juvenile blue mussels* Mytilus edulis. *Processes such as filtration produce dissolved organic matter (DOM) and release nutrients. These main remineralisation pathways are indicated by the thin lines. In this thesis the size-definition of both pico-and nanophytoplankton is based on the assumed retention efficiency of mussels and thus is slightly different from the more conventional definition of 0.2-2 µm for picophytoplankton and 2-20 µm for nanophytoplankton.*

(Trottet *et al.* 2008a, Strohmeier *et al.* 2012). It is hypothesised that mussels impact the plankton food web by size-selectively removing plankton (Figure 1.2). Bivalve filter feeders do not effectively remove small plankton but do feed on their predators, the heterotrophic nanoflagellates and ciliates (Dupuy *et al.* 1999). This might result not only in a disruption of the link between small plankton and higher trophic levels (Dupuy *et al.*1999, Wong *et al.* 2003, Greene *et al.* 2011) but also in complex indirect effects. The removal of nano- and micro zooplankton predators by filter feeders might release prey from top-down control, resulting in biomass increases of bacteria and small phytoplankton. In addition mussels can have a bottom-up impact on the plankton community through the excretion of large amounts of particulate organic matter (faeces and pseudofaeces) as well as a dissolved nutrients, stimulating both bacterial and phytoplankton production (Dame & Dankers 1988, Cranford *et al.* 2003, Newell 2004, Richard *et al.* 2006, Van Broekhoven *et al.* 2014). At the same time, by removing suspended matter, mussels, by filtrating, improve the underwater light climate. The pico-sized cells are better competitors for both light and nutrients, so improved growth conditions as a result of mussel filtration will likely favour the smallest cells (Riegman *et al.* 1993), potentially resulting in an increase in small cells at the expense of larger ones (Cranford *et al.* 2009).

Research question & outline

This thesis aims to answer the research question whether a harvest of 40M kg of juvenile mussels will have an effect on the plankton of the Wadden Sea. The different chapters, based on separate journal papers, either published or submitted, deal with the settlement and growth of juvenile mussels on rope collectors, their filtration rate and type of food removed as well as with the recovery of the plankton community after filtration. The data used to answer the research question come from a combination of field data, a field experiment and lab experiments. In the lab experiments, clearance rates of individual mussels were established as well as changes in specific growth and predation mortality rates of bacteria, pico-and nanophytoplankton, HNAN and ciliates resulting from mussel filtration. In outdoor mesocosms, clearance rates of rope mussels were determined by allowing mussels to filter-feed on the plankton community for the duration of a few hours. After this short filtration episode, mussels were removed and the plankton community was allowed to recover for a period of 9 days. This set-up simulated the passage of a water column through a mussel collector and the subsequent recovery of the plankton community for a period equal to the average residence time of water in the Wadden Sea. A simple model was set-up to upscale the results of the experiments to an ecosystem level. The model allowed for a rough estimation of the effect of an annual mussel harvest of 40M kg. For a more elaborate estimation of the effects of juvenile mussels collectors on the Wadden Sea plankton community, the results from this thesis can be also be used in more complex ecosystem models.

Chapter 2 describes the abundance of mussels that settle on the pelagic collector and subsequent growth of these mussels in 2010 and 2011. Differences in growth rate and abundance between the two years will be related to water characteristics like temperature and chlorophyll both for the period when mussels are present as pelagic larvae as well as for the post-settlement period. Hypothesis on the factors that might be responsible for the inter-annual differences will be tested using two years of additional data (2012-2013). These results are described at the end of this thesis in Box 3.

Chapter 3 describes the clearance rates of juvenile mussels varying in shell length between 1.5 and 25 mm, using natural sea water. Rates are determined throughout the growing season of mussels for bacteria (<1 µm), picophytoplankton (0.2-3 µm), nanophytoplankton (3-20 µm) and ciliates (20-200 µm). Measured clearance rates are related to both the shell length and the dry weight of the mussels to derive isometric relations.

Chapter 4 aims at establishing the short-term response of the plankton community to mussel filtration. In this chapter the results of a series of Landry & Hassett (1982) dilution experiments with both mussel-filtered and unfiltered (control) water are presented. These experiments allow for an estimation of changes in both specific growth rates as well as predation mortality rates due to mussel filtration. In addition, clearance rates of juvenile mussels on heterotrophic nanoflagellates (2-20 µm) are reported.

Chapter 5 describes the results of an experiment in which, on several occasions over a period of 2 years, natural plankton communities were exposed to mussel filtration. After removal of the mussels, the plankton community was allowed to recover for several days. The set-up of the experiment allowed for an estimation of the recovery potential of plankton in the western Wadden Sea after a single episode of mussel filtration and subsequent recovery for the duration of the average resident time in the area. Also, simple model calculation based on input of the previous chapters allow for an estimation of the effect of 40M kg of mussels at the time of harvest on the pelagic ecosystem.

Chapter 6 summarises the main results of all previous chapters, the results are discussed, and compared to other research. Findings are placed in the broader context of mussel recruitment in the Wadden Sea. Interesting leads for future research are formulated. The synthesis ends with the main conclusions and some recommendations are given with regard to monitoring the impact of upscaling the number of pelagic collectors.

Box 1 *Mytilus* pelagic life cycle

The life cycle of *Mytilus edulis* (Figure B1.1), like many other bivalves, includes a planktonic larval stage. A temperature cue marks the beginning of spawning (Bayne 1965); the release of eggs and sperm into the water column. Adult mussels of one population or region are thought to synchronise their spawning, increasing the chance of fertilisation (Thorson 1950, Pennington 1985). The number of eggs a single adult can produce is estimated to be up to 8 10^6. Eggs are relatively small (70 µm) and poor in yolk, once fertilised the larvae is fully ciliated with a long flagellum, but without a shell (trochophore; Bayne 1964). Within a few days, depending on water temperature the veliger develops in to a 'straight-hinge' of d-shaped veliger (Pechenik *et al.* 1990); it now has a shell and a velum, which is the swimming and feeding organ in one. The larva now start feeding, retaining particles <9 µm, with a mean size between 2-6 µm, the main food source is assumed to be phytoplankton (Riisgård *et al.* 1980, Sprung 1984b, Olson & Olson 1989). Within weeks the larva will develop in a pediveliger (development of a foot), it has a size of ~270 µm and is ready for settlement and subsequent metamorphosis. When suitable substratum is not available, metamorphosis can be delayed up to 7 weeks, with shell length increasing up to 360 µm (Bayne 1965, Sprung 1984a). During metamorphosis, which can take a few days, the larva loses its velum and develops gills, it is now called a post-settled larva or plantigrade (Bayne 1965).

In laboratory studies both food concentration (as phytoplankton cultures) and temperature were found to positively influence growth and development rates of larvae (e.g. Bayne 1965, Pechenik *et al.* 1990, Phillips 2002). Paulay *et al.* (1985) concluded that maximum growth rates in laboratory studies were generally attained at higher food levels than in situ concentrations, raising the question whether larvae under natural condition are food limited or not. Olson & Olson (1989) argue that larval food has a patchy distribution, making it likely that larvae will be food limited and never attain their maximum growth rate under natural conditions. The authors argue that the real question is whether "larvae are sufficiently food limited that year-to-year fluctuations in their food supply are likely to have a major effect on their recruitment success". There is some recent evidence that variations in food concentrations experienced by larvae determine their condition and the condition of a pool of settling larvae is influencing recruitment success (e.g. Phillips 2002, 2004), with many questions still open for future research.

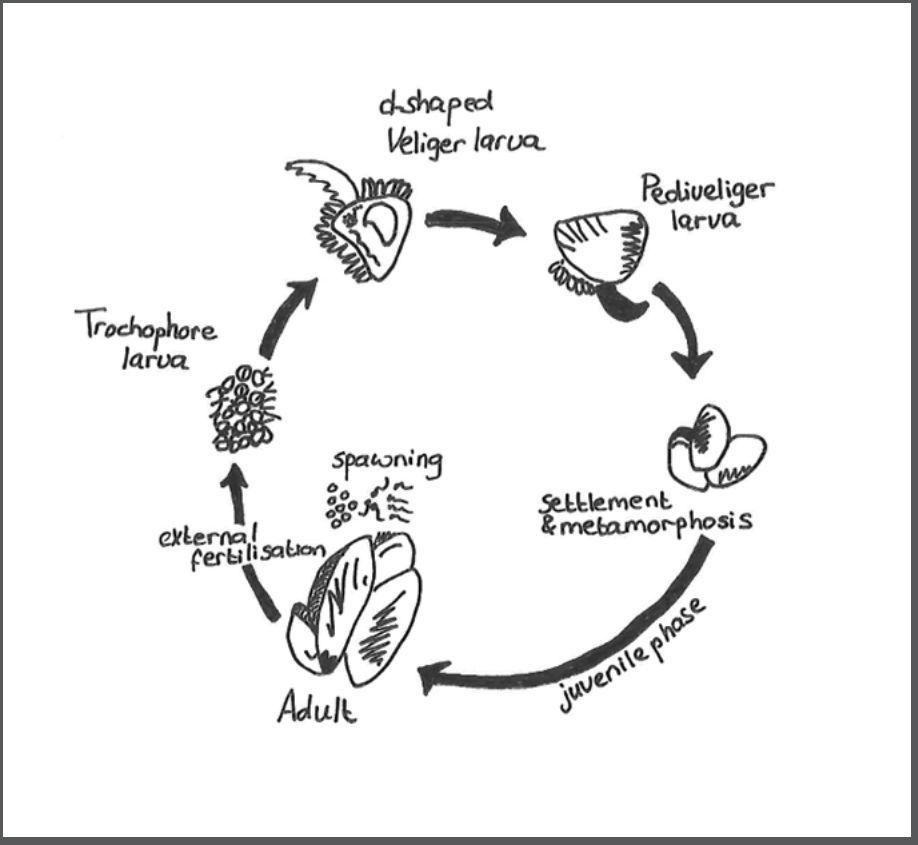

Figure B1.1 *The life cycle of a mussel, picture redrawn after Clark University c2004.*

Box 2 *Mytilus* filter feeding

Filter feeders are organisms that have evolved a sieving mechanism to remove particles from suspension (Wallace & Merritt 1980). Filtration is thus a means to effectively use a very dilute aquatic food source; it is performed by different types of animals ranging from krill and sponges to sharks, baleen whales and even birds like spoonbills and flamingos. For the blue mussel (*Mytilus edulis*) the filter feeding organ is the ctenidium, which also serves as a respiratory organ (gills) (Cranford *et al.* 2011). Filtration is the flow of inhalant water with particles through the mantle and ctenidium, where the lateral cilia create a current, the latero-frontal cilia collect and the frontal cilia transport the captured particles (Winter 1978, Figure B2.1 & B2.2). Exhalant water leaves the mussel and captured particles are either rejected as pseudofaeces, a mucus-bound aggregation of particles regularly expelled, or further transported towards the palps. At the palps, further selection takes places, with non-rejected particles are ingested. Rejection after ingestion is called faeces.

Not all particles are retained equally well, early studies on retention size reported efficient retention of all particles larger than 3 µm for *Mytilus edulis,* while the retention of smaller particles rapidly declined (Møhlenberg & Riisgård 1978). It was assumed that the distance between the filaments largely determined the size of particles retained, with the lamella acting as a sieve. More recent studies describe variable retention of particles, with variability ascribed to particles shape, 'food value' and ambient particle size distribution (Ward & Shumway 2004, Strohmeier *et al.* 2012). The mechanistic explanation for this variable retention remains unclear, suggestions are an ability to adjust the movement and coordination of the latero-frontal cilia (Dral 1967, Figure B2.2) or it is a consequence of a specific interactions between the extracellular matrix of living cells, and the cilia and mucus of the bivalve gills (Ward & Shumway 2004).

The maximum filtration or clearance rate, defined as the volume of water cleared of particles per unit time depends on the gill area (Jones *et al.* 1992), while factors like particle concentration as well as particle quality (fraction organic material) determine the actual clearance rate. Filtration rate appeared to have a temperature optimum in lab experiments, but temperature was not a significant factor influencing filtration rate under natural conditions (Cranford *et al.* 2011). Determining actual filtration rates for mussels under natural conditions is of paramount importance to make a reliable estimation of the impact these organisms have on their surroundings (Cranford *et al.* 2011).

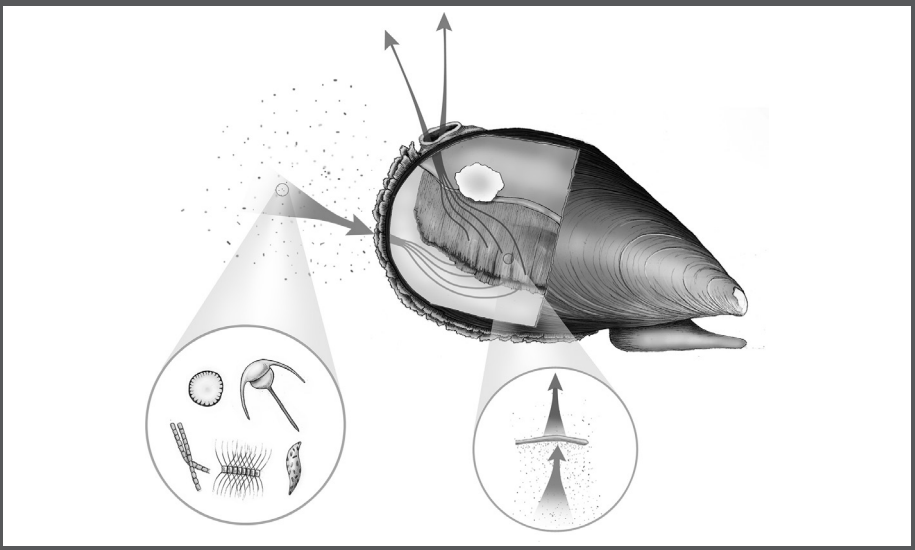

Figure B2.1 *A schematic view of water flow through a mussel with water entering the mussels through the mantle and gills, where particles are retained and non-retained particles and water flow exit the mussel. For a more detailed description see text and figure B2.2. Picture from Kimberly Andrews c2014.*

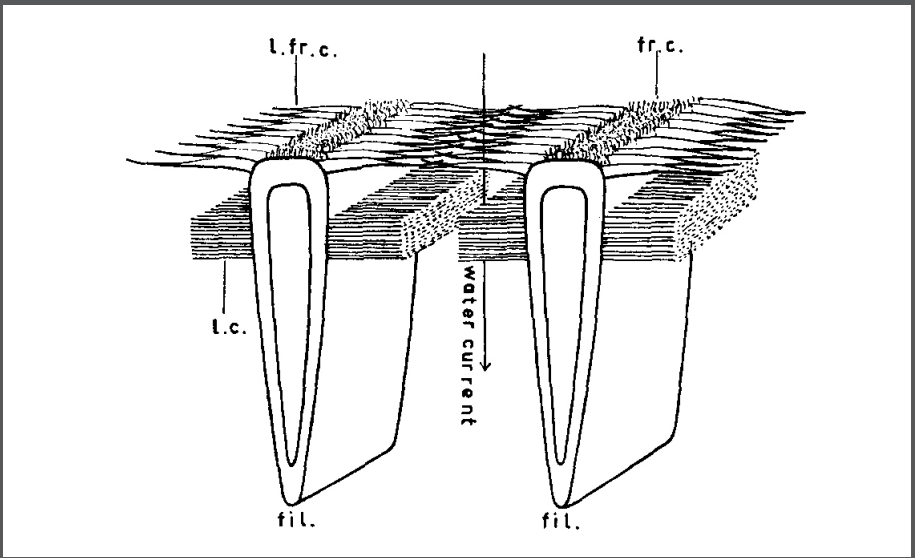

Figure B2.2 *A detailed view of two gill filaments (fil.) of a mussel, showing the latero-frontal cilia (l.fr.c.), the frontal cilia (fr.c.) the latero-cilia (l.c.) and the water current (Dral 1967).*

Chapter 2

Growth of juvenile blue mussels (*Mytilus edulis*) on suspended collectors in the Dutch Wadden Sea

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Abstract

In the Netherlands, fishing for juvenile blue mussels (*Mytilus edulis*) on wild beds is gradually replaced by harvesting of seeds from suspended collectors. Both the relaxation of fishing as well as the up-scaling of the number of seed collectors is expected to result in an increase in the number of mussels in the Wadden Sea. Consequently, an enhanced mussel population will cause an additional filtration impact on the system. The question is raised to what extent collectors can be used without negatively affecting the carrying capacity of an ecosystem. Therefore, a monitoring programme was initiated to study the growth of juvenile mussels on suspended collectors. This growth was related to food availability, measured as chlorophyll-a, and temperature both before and after settlement. Findings will serve as input for mathematical models predicting the carrying capacity for mussel seed collectors in this area.

The results for 2010 and 2011 are presented. In 2011 settled mussels achieved a higher growth rate, while phytoplankton concentrations after settlement were lower. This contradicts the general agreement that higher phytoplankton concentrations result in higher growth rates. We did find a positive relation between chlorophyll-a concentration during the larval period and the growth rate of settled mussels.

The number of settled larvae was higher in 2011. Results from existing studies on settlement and recruitment on tidal flats combined with estimated settlement date in the current study led to the hypothesis that the number of settled mussels on rope collectors is inversely related to the duration of the larval period (determined by water temperature). Our results indicated that in the Wadden Sea, the intra-annual differences in chlorophyll-a and temperature did not have an impact on the juvenile growth rate, while the inter-annual differences did. This is an indication that the larval stage is strongly discriminative in terms of juvenile growth rates.
Modelling growth of juvenile mussels on collectors should thus include conditions before settlement.

Keywords:

Mytilus edulis, larval phase, juvenile growth, settled numbers, Dutch Wadden Sea, environmental conditions

Introduction

In the Netherlands a decrease in the supply of juvenile blue mussels (*Mytilus edulis*) harvested from wild beds together with governmental policy regulations to protect these beds, led to the introduction of artificial mussel collectors. These so called mussel seed collectors are now operational in the Wadden Sea and the Oosterschelde estuary. Decreased fishing effort for juvenile mussels from wild beds as well as the up-scaling of the number of collectors permitted, will increase the amount of mussels in both areas. The consequential increase in filtration pressure might have an effect on the ecosystem where the collectors are placed. Therefore, a monitoring programme was initiated in 2010 to study the growth and filtration capacity of juvenile mussels on suspended collectors in relation to phytoplankton biomass in the Wadden Sea. Findings will serve as input for mathematical models predicting the carrying capacity for mussel seed collectors in this area. The aim of the present study is to present the first results of settlement and growth of juvenile mussels on suspended collectors.

Pelagic larvae of the blue mussel spend 3 weeks up to 3 months (Widdows 1991) in surface waters after which a suitable substratum for settlement is selected. The timing of both peak spawning and settlement seems to be related to temperature. In laboratory experiments higher water temperatures led to a shorter period between hatching and readiness for settlement (Bayne 1965, Pechenik *et al.* 1990, Drent 2002). *Mytilus* larvae attach readily to filamentous structures (Lutz & Kennish 1992), including artificial collectors. At the time of settlement, the larvae (or pediveligers: Bayne 1965) are between 270-360 µm in length (Sprung 1984a, Widdows 1991, De Vooys 1999, Kamermans *et al.* 2009). Once settled, metamorphosis, which includes the development of an adult feeding structure, takes place and the growth rate of juveniles (or plantigrades: Bayne 1965) increases rapidly. On suspended collectors very high growth rates can be reached; a growth to 30-55 mm shell length within 24-48 weeks has been recorded (Walter & Liebezeit 2003, Buck 2007, both southern North Sea). Under controlled conditions, the two most important environmental factors influencing growth rate of juveniles are temperature and phytoplankton biomass (Sprung 1984a , Pechenik *et al.* 1990, Seed & Suchanek 1992). In field populations, variations in phytoplankton alone determine growth rates within the temperature range normally experienced by mussels (Page & Hubbard 1987). Phillips (2002) demonstrated in an experimental study that pre-settlement food conditions are more important for determining growth rate of juvenile mussels than food availability after settlement.

Pelagic seed collectors provide a profitable habitat for mussels, with a constant supply of food particles, compared to wild mussel beds or benthic cultures. At the same time, predation pressure is much lower, because access to the pelagic collectors is complicated for benthic predators. Therefore, mussel collectors provide the

opportunity to harvest large amount of juvenile mussels (Kamermans *et al.* 2009). However, settlement and harvest of bivalves are highly variable and unpredictable in time (e.g. Honkoop *et al.* 1998, De Vooys 1999, Alfaro & Jeffs 2003, Kamermans *et al.* 2009).

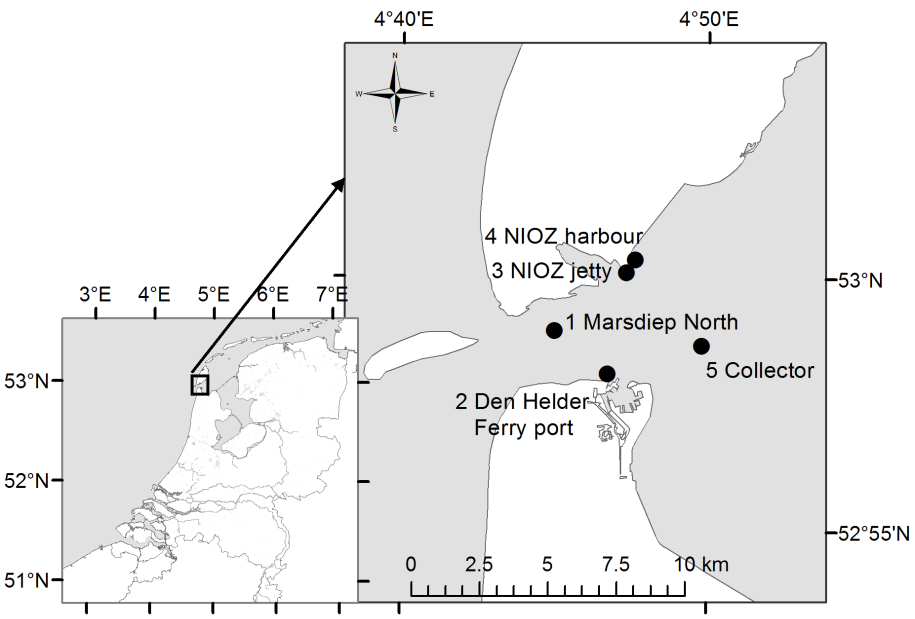

Figure 2.1 *Rijkswaterstaat sampling locations Marsdiep North & Den Helder Ferry port, sampling location NIOZ Jetty, the location where experiments were performed in this study (Chapter 3 & 5) was the NIOZ harbour and the location of the monicube (Collector).*

Material & methods

To explore the settlement and growth of juvenile mussels on collectors, a field study was carried out in 2010 and 2011. In the Netherlands rope collectors are operational in the water from April to October after which period the juvenile mussels are harvested and subsequently distributed among culture lots. In this field study the in situ growth of settled *Mytilus edulis* on an artificial rope collector was studied in the western Wadden Sea. For two consecutive growth seasons, ropes were collected every two weeks to determine the abundance and biomass per unit rope as well as the average length and weight of the mussels. Water samples were collected weekly to provide data on temperature and chlorophyll-a.

Suspended Collector

Ropes set with numerous filaments to facilitate settling of larvae (Xmas tree ropes, Donaghys) of 50 cm length each were tied between metal frames. The metal frames were slid into slots in a so-called monicube (2.5x2x2 m). The monicube contained 36 ropes. There was variation between densities on the ropes within the monicube. Most variation seems to exist between ropes at the top and ropes at the bottom end of the monicube, with the lower placed ropes having a lower density. We think this was due to the fact that at the bottom end the ropes experienced more predation (on the top ropes predation was minimal) and more friction (personal observation). To minimise variation we only collected the top ropes. This floating monicube was placed in the Marsdiep (52˚58'N, 4˚49'E, Figure 2.1). The monicube was in the Marsdiep from the 6th of April to mid-October in 2010 and from the 10th of May to mid-August in 2011 only, after which it sunk and the ropes could not be collected anymore. On August the 9th 2011 no data on mussel density were available. After deployment of the monicube every two weeks ropes in the monicube were visually inspected for settlement of bivalves. As soon as settlement was visible the harvest of ropes started (see below). We calculated the settlement day by assuming a length at settlement of around 300 µm (e.g. De Vooys 1999, Kamermans *et al.* 2009), we then extrapolated to this size using the length increase overtime. In 2010 every other week one or two ropes were collected. Of each rope, subsamples were taken by cutting of a piece of rope (5-20 cm). These subsamples (2-3) were used in the experiments to establish clearance rates. After the experiments all mussels were removed, counted and the length of 50 mussels was measured (±0.1 mm). In 2011 one rope was collected and the number of mussels per rope was counted for 1 subsample only. After each experiment total mussel dry weight was determined after drying at 60°C for 48 hours. Total dry weight per rope divided by the number of mussels yields the average individual weight. Weight included both shell and flesh since the size of the mussels made it difficult to separate the two.

Sampling

Chlorophyll-a and water temperature (±0.5 °C, Hach multimeter) data were obtained from the NIOZ jetty in the Marsdiep (Nioz-jetty, Figure 2.1). At low tide weekly samples were collected from 10th June until 19th Oct in 2010 and from 10th of May until 5th Dec in 2011. Water samples were collected from the surface. Because data on temperature and chlorophyll-a were not collected before the 10th of June in 2010 data from Rijkswaterstaat (www.waterbase.nl) were used for the period 1st of March until the 31st of May. Locations for the Rijkswaterstaat data were 'Den Helder veerhaven' for temperature and 'Marsdiep Noord' for chlorophyll-a (Figure 2.1). We present chlorophyll-a and temperature data from 1 location only. A comparison of sampling data from a sampling station near the monicube (data not shown) revealed that both chlorophyll-a and temperature data patterns were comparable to

those of station 'Jetty'. This supports the assumption that one location can represent differences between years.

Chlorophyll-a analysis

For the determination of total chlorophyll-a, duplicate subsamples (200-300 ml) were filtered (Whatman GF/F) using low vacuum pressure (max -0.4 bar). Filters were stored in the dark at -80°C for no more than 2 months. Chlorophyll-a was extracted by homogenisation of filters in 90% acetone with the addition of glass pearls. Chlorophyll-a was determined fluorometrically (cf. Holm-Hansen *et al.* 1965) using spinach chlorophyll-a (Sigma) as a reference.

Clearance rate

Ropes from the monicube were incubated in 60 litre mesocosms filled with natural sea water for 1-3 hours. Three mesocosms served as replicates and two as control. Clearance rates (R_C) were calculated as the rate of removal of phytoplankton from the water using flow cytometry counts (BD Accuri C6), following the equation (Coughlan 1969):

$$R_C = \frac{V}{wt}\left\{\ln\frac{C_0}{C_t} - \ln\frac{C_{0'}}{C_{t'}}\right\} \qquad \text{(eq. 2.1)}$$

where V is the volume (l) cleared, t is the duration of the measurement (h), w is the dry weight of mussels (g) used in each experiment. C_o is the concentration of phytoplankton at the start of the experiment, C_t is the concentration at the end. $C_{o'}$ and $C_{t'}$ are the concentrations at the start and end of the experiment respectively in the control mesocosms. R_C was expressed as litre per hour per gram mussel dry weight ($l\ h^{-1}\ g^{-1}$ DW). During the experiment, both light transmittance (Wetlab CST) and fluorescence (TriOS MicroFlu) was measured continuously. The experiments were ended when the fluorescence or light transmittance had reached values of 30-50% of the values at the start of the experiment. Values of both parameters never reached levels below 30% of the initial value. The clearance rate of the collector mussels was calculated during June to October; 6 times in 2010 and 5 times in 2011.

Statistical analysis

To see if there were significant differences in the growth rate between years two linear models were made; One model including both length (or weight), year and the interaction between year and length (or weight) and one model without the interaction term. A significant interaction between year and length (or weight) indicates differences in growth rate between years. An ANOVA test was used to investigate the best model. The same procedure was applied to test for differences between the two years regarding the length-weight relation. Regression statistics are known to

be robust with respect to underlying assumptions like normally distributed populations and equal variances (Zar 1996). These assumptions are usually not met with small sample sizes, n=14 and 10 in 2010 and 2011, respectively. The unequal sample size did not influence the outcome of the tests.

The differences in temperature and chlorophyll-a concentration between years and pre and post settlement period were tested using the non-parametric Wilcoxon signed rank. Equal numbers are needed for robustness of this test. In both years temperature and chlorophyll-a measurement were done on different dates. In all analysis only measurements done on the same date (± 2 days) were taken into account. Pre-settlement period was defined as from March to May, post settlement from June to October.

The difference in clearance rate between years was tested using the non-parametric one-tailed Mann-Whitney test. A confidence level of $\alpha<0.05$ was used for all tests. Statistical analysis were performed in R version 2.14.1 © 2011 The R Foundation for Statistical Computing.

Results

Temperature and chlorophyll-a

The temperature during the larval phase of mussels (March-May) was higher in 2011 compared to 2010 ($V_{0.05(2),13}$=0, p=0.002) (Figure 2.2). During the time the juvenile mussels were on the seed collectors (June-October), there was no significant difference in water temperature between 2010 and 2011 ($V_{0.05(2),8}$=17, p=0.3055). Average food concentration (chlorophyll-a) was higher in 2011 compared to 2010 in the larval period (Figure 2.2, 2010: before day 161, 2011: before day 140) ($V_{0.05(2),\ 6}$ = 0, p-value = 0.031), with a higher spring bloom peak in 2011 (22 µg l^{-1}) compared to 2010 (15 µg l^{-1}). The chlorophyll-a concentration after settlement until the harvest of the mussels (June-October, or day 160-297) was significantly lower in 2011 compared to 2010 ($V_{0.05(2),13}$ =31, p=0.019).

Juvenile mussel growth

Calculated settlement of the mussel larvae must have occurred earlier in 2011 (Julian day 140) compared to 2010 (Julian day 161) (Figure 2.3). No additional settlement was observed after the assumed settlement dates (Figure 2.4).

Both length and weight of settled juvenile mussels increased faster in 2011 compared to 2010 (Figure 2.3). For both years, the growth rate, measured as length increase (mm day^{-1}) of the juvenile mussels on seed collectors was constant, within

the course of one growing season. But the growth rate was higher in 2011 (0.21 mm day^{-1}) compared to 2010 (0.12 mm day^{-1}) ($F_{0.05(1)2,24}$=16.16 p=0.0005) (Figure 2.3). For growth rate, measured as weight increase per day, the difference between the years was not significant ($F_{0.05(1)2,4}$=3.716, p=0.066).

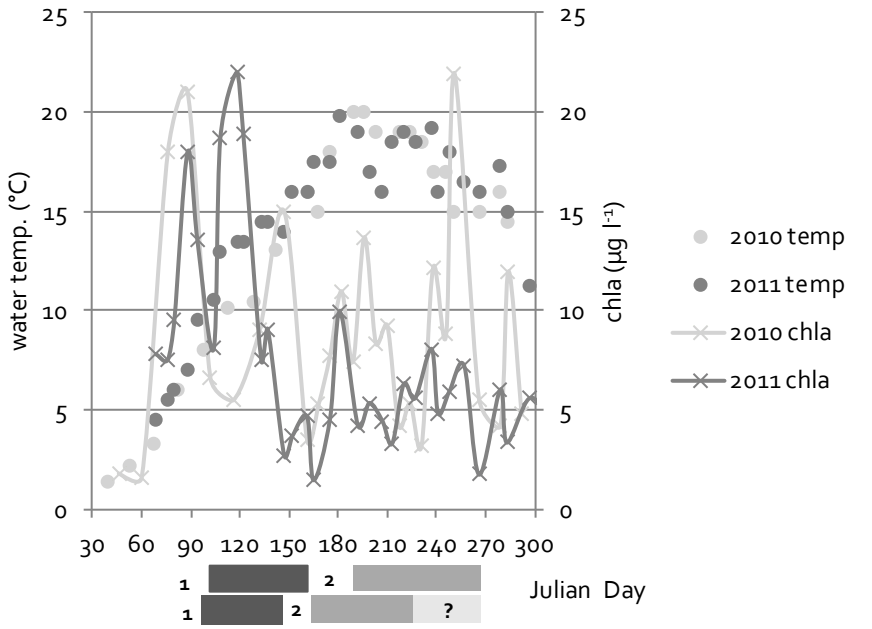

Figure 2.2 *The phytoplankton (chlorophyll-a) dynamics in the Marsdiep area (line with -x- symbols) in 2010 and 2011 and variations in temperature (● symbols) during the sampling period. The bars under the graph indicate the larval phase (dark bars) and the juvenile phase (grey bars) initiated by 1: spawning and 2: settlement. The upper bar represents the situation in 2010, the lower bar 2011.*

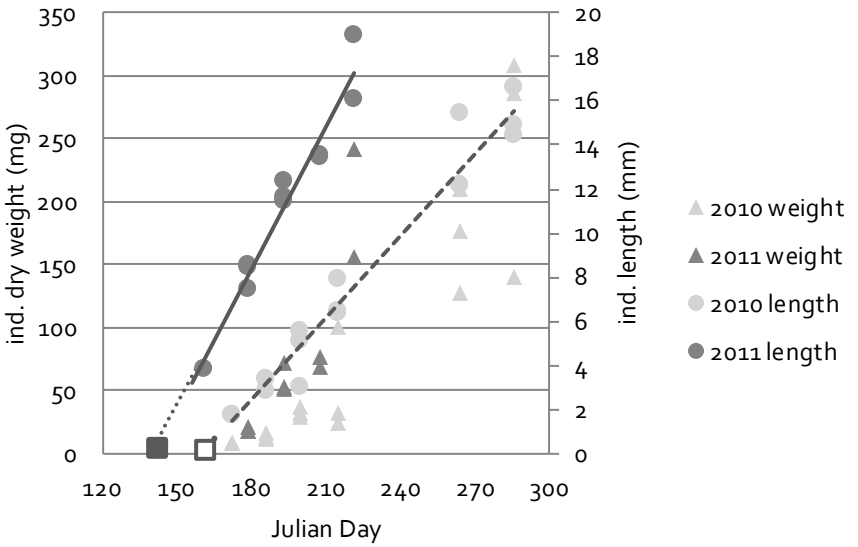

Figure 2.3 *Increase in shell length (circles) and total dry weight (triangles) over time for mussels on collectors for both 2010 and 2011. Each symbol represent the average shell length (mm, n=50) and individual dry weight (mg, n=1) per rope. Different numbers of ropes were subsampled per sampling date (see methods section). For length, regression lines were fitted: with for 2010: 0.12x - 19.3, r^2:0.97 and for 2011: y = 0.21x - 29.2, r^2= 0.95. Dashed lines indicate extrapolation to the day at which the individual length was 300µm, i.e. the size at settlement. Settlement day is indicated with a square symbol.*
For increase of weight over time a power relation was fitted for Julian day minus settlement day. 2010: $y=0.1488x^{1.48}$, R^2= 0.90, 2011: $y=0.0004x^{2.95}$, R^2= 0.96.

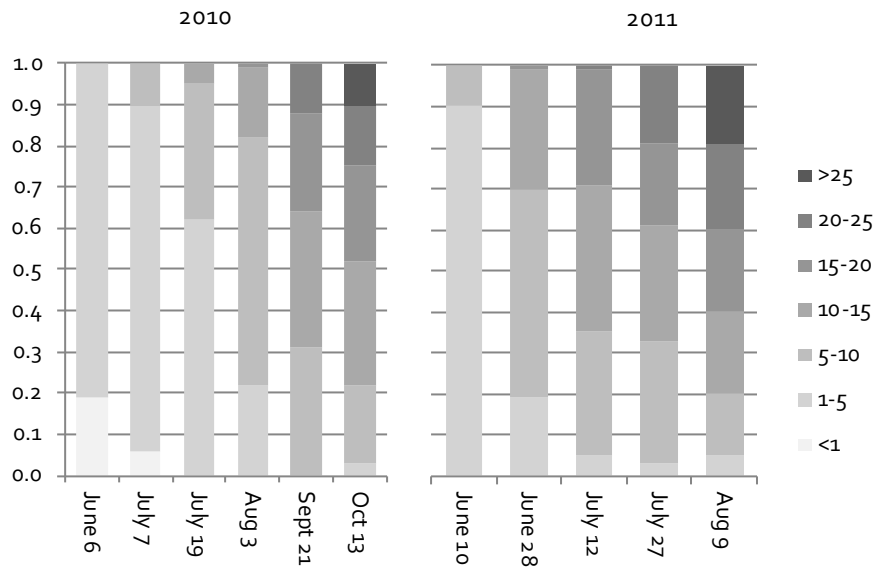

Figure 2.4 *Size-frequency histograms of the juvenile mussels on the collector ropes indicating the proportion of mussels with a certain shell length (mm) per sampling date for both years.*

Mussel condition

There was a difference between years in the length- weight relation (Figure 2.5) between the two years. In 2011 mussel weight increased faster with increasing length compared to 2010 ($F_{0.05(1),2,24}$=10.90, p=0.003). But at any given length the weight was lower in 2011.

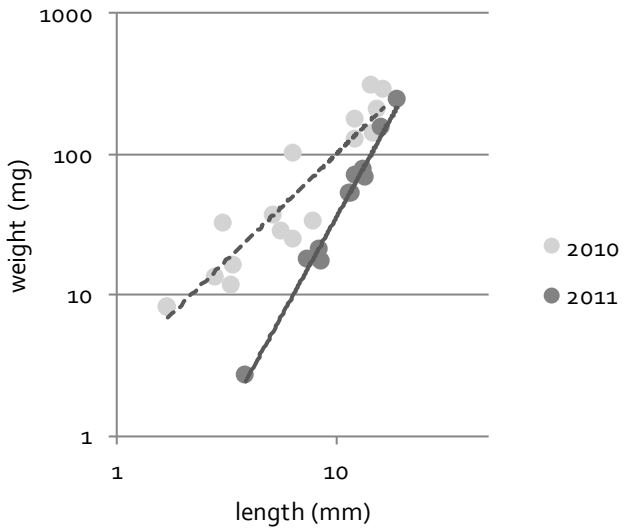

Figure 2.5
Relation between shell length (mm) and total dry weight (mg): $y=a * x^b$, (e.g. Jones et al. *1992) on a log-log scale. 2010 (light symbol): $y=3.12x^{1.51}$, $r^2=0.89$, 2011(dark symbols): $y=0.06x^{2.81}$, $r^2=0.99$. Each symbol represent the average shell length (mm, n=50) and individual dry weight (mg, n=1) per rope. Different numbers of ropes were subsampled per sampling date (see material & methods section 'Suspended Collector').*

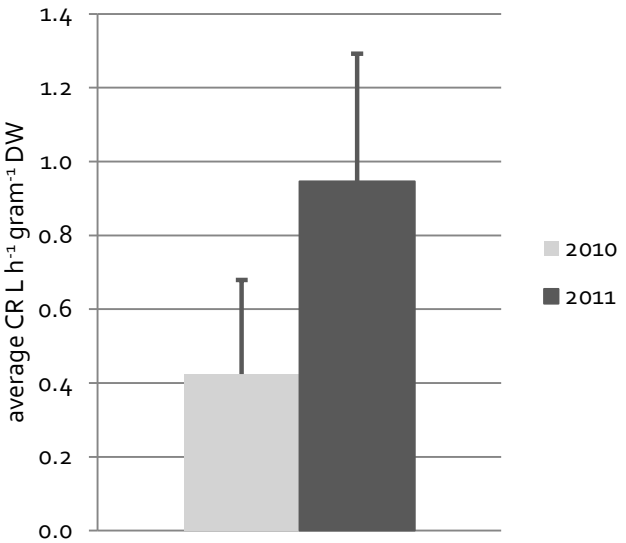

Figure 2.6
The average clearance rate (l h^{-1} g^{-1} dry weight) of juvenile mussels (1.7-20 mm) incl. standard deviation. 2010: 0.42± 0.25 (n=6) and 2011: 0.95± 0.35 (n=5).

Clearance rates

Clearance rates were calculated for collector mussels throughout the growth season in both 2010 and 2011 (Figure 2.6). In 2011 the juvenile mussels removed more phytoplankton out of the water compared to 2010 (U'=11, p= $2.957e^{-05}$) per gram dry weight.

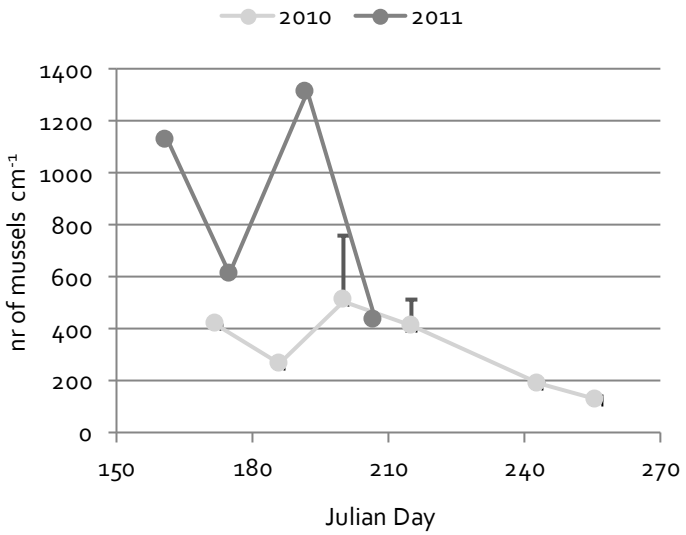

Figure 2.7a
Numbers of mussels per cm rope incl. standard deviation (n=between 1 and 4, see material & method section 'Suspended Collector') in 2010 and 2011. In 2011 the last date with rope density data is day 207 (see methods).

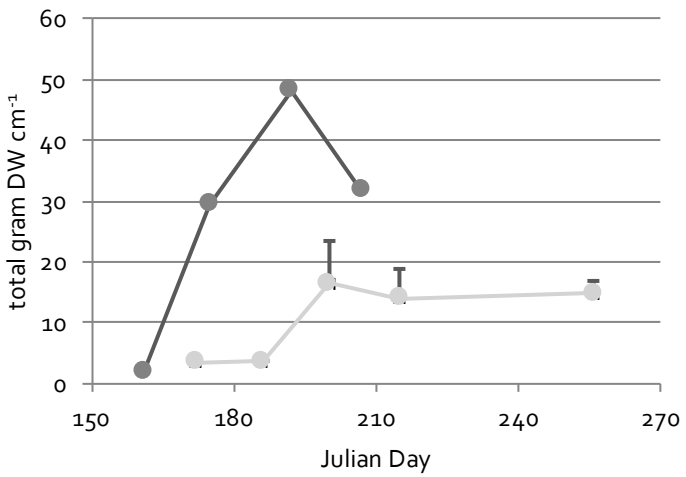

Figure 2.7b
Mussel biomass on a centimetre rope incl. standard deviation (n=between 1 and 4) during the period that the collectors were suspended. Weight is in grams total dry weight (shell and flesh).

Number of settled mussels

Numbers per cm rope fluctuated throughout the season, in 2010 between 71-508 and between 481-1432 in 2011, but the number of mussels per cm rope was higher in 2011 compared to 2010 (Figure 2.7a). The biomass per cm rope in 2011 was higher throughout the first half of the year (Figure 2.7b). Because of the loss of the collector in August 2011 it is unclear whether at the end of the season the biomass remained higher in 2011.

Discussion

Juvenile mussel growth in relation to environmental conditions

In this study, settlement of mussel larvae on rope collectors (Figure 2.3) coincided with a water temperature of 15 degrees Celsius. Water temperature reached 15 degrees at an earlier date in 2011 and throughout the larval phase the water temperature was also higher in 2011 (Figure 2.2). A higher water temperature reduces the time between hatching of the larvae and metamorphosis (Pechenik *et al.* 1990, Honkoop *et al.* 1998, Drent 2002) explaining the earlier estimated settlement day in 2011 in our study. In the year that settlement of larvae occurred earlier, shell length of juveniles was higher throughout the growing season (Figure 2.3). In 2010 the monicube was deployed at 11th of May (JD 130), one month later than in 2011. Spawning in spring occurs after sea water temperature exceeds a temperature of 8-10°C (Bayne 1965). In 2010 could have been taken place from day 100 onwards. There is thus a possibility that in 2010 the first larvae ready to settle were not recorded since the monicube was not in place yet. According to Widdows (1991) a maximum growth rate results in a minimal larval life of 3 weeks. The maximum growth rate is however only reached under ideal growing conditions at a water temperature of 18°C (Widdows 1991). In 2010 the water temperature was low with a temperature of approximately 11°C at the time the monicube was deployed. We therefore assume that there were no larvae readily to settle before the monicube was in place. Additionally, after the monicube was deployed the ropes in it were checked for settlement of bivalves regularly and no settlement was observed until the 21st of June when the first ropes were harvested.

A linear increase in shell length for larvae and juvenile bivalves measured over a short period of time (days to weeks) has been observed (e.g. Bayne 1965, Sprung 1984a). In this study, mussel shell length increased linearly during the course of one growing season after settlement on the seed collectors. The period of linear increase in shell length in this study is thus longer than previously recorded. Linear increase in shell length is based on population measurements, these can be affected by additional spat fall or size dependent mortality, but in this study we did not find additional spat fall (Figure 2.4). Mortality might have occurred by clumps of mussels

falling of the ropes, this can be seen in the number of mussels per cm rope going down (although the variation between ropes is rather high). We did not find many empty shells (dead mussels) when analysis the rope characteristics indicating that size dependent mortality did not play a major role.

The growth rate was higher in 2011 compared to 2010 (Figure 2.3). In 2010 and 2011 the average water temperature experienced by the juvenile mussels was the same, while the food concentration (chlorophyll-a) was lower in the year that the growth rate (in length) of the mussels was higher (2011) (Figure 2.2). Earlier reports of mussel growth rates in lab experiments (Bayne 1965, Sprung 1984a) indicated that mussel growth rates increased with higher temperatures and chlorophyll-a concentrations. These studies were carried out for the period of a few weeks only and under much higher chlorophyll-a concentrations (e.g. 100 µg l^{-1}: Bayne 1965) than occur in the Marsdiep. Page & Hubbard (1987) considered intra-annual variations in growth rates of mussels. They observed higher monthly growth rates at higher chlorophyll-a concentrations (0.5-3.5 µg l^{-1}) and concluded that in field populations phytoplankton is the only factor determining the growth rate of mussels. Results from our study indicated that at chlorophyll-a levels of 10-15 µg l^{-1} no positive correlation between in situ growth rate and chlorophyll-a level existed. We concluded that the higher growth rate of juveniles in 2011 cannot be explained by temperature and phytoplankton biomass during their growth period. It is more likely that juvenile growth rates are related to environmental conditions during the larval phase.

During the larval phase the average chlorophyll-a concentrations were higher in 2011. And, in this year the larval phase coincided with the spring bloom peak, while in 2010 the spring peak occurred after settlement (Figure 2.2). Philips (2002) demonstrated that larvae reared under high food conditions not only obtained a higher growth rate and a longer shell at the time of settlement, but that this higher growth rate was sustained after settlement, independent of food conditions after settlement. The benefits of a longer shell length could be, according to Phillips (2002), the development of a larger surface area for feeding since gill area is proportional to shell length. Phillips (2002) also reported that a longer shell length correlated with higher lipid content at time of metamorphosis. We now argue that, in the present study, a higher energy reserve at time of metamorphosis can contribute to a larger and better developed gill area per unit length. In 2011, the year with higher food availability during the larval phase, we found higher filtration rates as well as higher growth rates, supporting this hypothesis. Clearances rate are expected to scale with shell length of the mussels (Jones *et al.* 1992) and mussels in 2011 were generally longer. However comparing clearance rates of mussels of equal length between the two years reveals the same difference (data not shown, details in Chapter 3). It must be noted that in this study differences in concentration of other food items than phytoplankton, for example ciliates (Trotted *et al.* 2008) were not taken into account. Neither were potential differences in detritus or silt concentration between the years considered (e.g. Kiørboe & Møhlenberg 1981, Bayne *et al.* 1993).

The length-weight relation of juvenile mussels differed between years (Figure 2.5). Weight is expected to scale with length^3 (e.g. Jones *et al.* 1992) as was observed in 2011. For mussels in 2010 weight only increased with a factor 1.5 with length (Figure 2.5). This slower weight increase appeared to be related to the lower individual clearance rate (Figure 2.6). The lower clearance in 2010 might be the result of a smaller or less developed gill area per unit length. A smaller gill area per unit length might be a result of the lower concentration of phytoplankton in this year during the larval period as argued before.

In 2011 the juvenile mussels obtained a maximum average length of 17.5 mm in the beginning of August, after which date the monicube sank. In 2010 the maximum size is 15.3 mm, but this shell length is only reached mid-October. At equal shell length mussel dry weight, which includes the weight of the shell, was lower in 2011 compared to 2010 (Figure 2.5). A possible explanation for the lower weight of mussels at equal shell length in 2011 could be that the mussels put their energy in length growth rather than in weight increase. This could be a successful strategy since clearance rate, one of the factors determining energy intake scales with length. Other possible explanations for the lower observed weight in 2011 might be a thinner shell (lower weight) or a narrower shape.

Number of settled mussels

We observed a difference in the number of settled juvenile mussels on ropes collectors between the two years this research was carried out. In 2011 the number of settled mussels was much higher than in 2010 (Figure 2.7a).

Most studies on wild population do no asses the immediate number of settled bivalves, but rather study recruitment, which is the number of juveniles (0-year class larger than 1 mm) that have survived until 3-5 months after spring spawning (Honkoop *et al.* 1998). There is a large inter-annual variation in the number of bivalve recruits (e.g. Honkoop *et al.* 1998). While the factors influencing this variation are largely unknown, temperature seems to play a major role. Several studies have reported relative successful recruitment after cold winters and failing or less successful recruitment after mild winters (references in Honkoop *et al.* 1998). The lower number of recruits after a mild winter is most likely due to a higher number of predators and their earlier arrival on the tidal flats (Beukema 1992, Honkoop *et al.* 1998, Strasser *et al.* 2001). Higher winter temperatures thus seem to decrease the survival change after settlement.

Before settlement, the planktonic larvae also experience a high mortality (Widdows 1991). This larval mortality is related to the duration of the larval phase; the longer it takes after spawning for larvae to settle, the lower the probability of survival to the settlement stage and beyond (Widdows 1991, Drent 2002). Factors that increase

the development rate and thus reduce the time spent in the pelagic, increase the survival chance to settlement. Several studies have shown a positive relation between water temperatures and development rate in bivalves (Pechenik *et al.* 1990, Drent 2002). For bivalve larvae temperature thus influences the mortality rate, but in the opposite direction as for settled juveniles. On rope collectors earlier settlement, extrapolated from shell size after settlement, resulted in a higher number of settled juveniles (this study). On natural banks or tidal flats recruitment of bivalves is assessed at a more or less fixed date (Honkoop *et al.* 1998), earlier settlement thus increases the time passed until assessment. In years that juvenile bivalves settle early, the exposure time to the dangers on the tidal flats before assessment is longer, possible resulting in a lower number of recruits (Van der Veer *et al.* 1998). A year with a high number of settled larvae might turn out to become an unsuccessful year regarding the number of recruits. This is confirmed by other studies in which a negative relation (De Vooys 1999) or a lack of relation (Honkoop *et al.* 1998, Strasser *et al.* 2001) between the number of settled juveniles and the number of recruits was found.

Here we present the first observations of settlement of juvenile mussels on mussel seed collectors. Based on the observed differences in settled numbers of juvenile bivalves between the two years, we hypothesise that a shorter larval phase will result in a higher density of settled larvae on artificial substrate. This hypothesis is supported by findings presented in a study by De Vooys (1999). Here settlement of mussel larvae was studied on gauze. We re-analysed his data on settlement and related this to the duration of the larval phase, defined in our study as the period in which the water temperature increased from 8-15 degrees Celsius. The new analysis shows an inverse correlation between the duration of the larval period and settled numbers of juvenile mussels (Figure 2.8). This might indicate that larval settlement on artificial substrate is mainly determined by temperature dynamics. The pronounced differences in settlement and growth rates between the two years might have a determinative impact on the population dynamics of the population dynamics on collectors. Differences in the timing of settlement, densities of settled mussels and growth rates are expected to influence the impact these collector rope populations have on the Wadden Sea ecosystem. More insight in the factors influencing settlement and growth on suspended collectors is needed to fine-tune models predicting the carrying capacity for mussel seed collectors in this area.

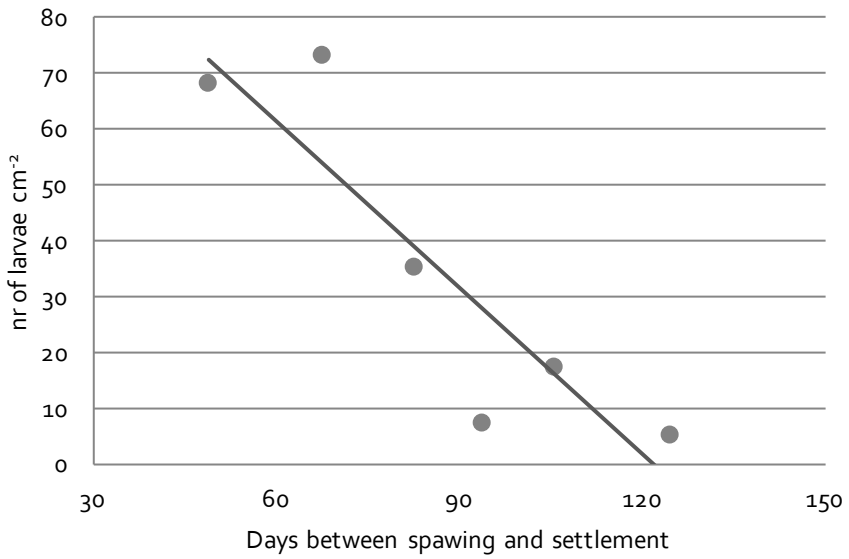

Figure 2.8 *For 6 years (1981-1996) data on the number of settled* Mytilus edulis *larvae per cm² gauze was available in De Vooys (1999). Peak settled numbers per year were plotted as a function of the duration of the larval period. The larval period was defined as the number of days between spawning and settlement, when the water temperature was between 8 and 15 degrees Celsius. Water temperature data came from www.waterbase.nl. The regression equation is given by: y= 121-0.99x, r²=0.80.*

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Chapter 3

Length- and weight-dependent clearance rates of juvenile mussels (*Mytilus edulis*) on various planktonic prey items

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Abstract

Filtration capacity and feeding behaviour has been intensely studied for adult mussels (*Mytilus edulis*), but less information is available for juvenile mussels (1.5-25 mm, <1 year), especially in natural sea water. The recent introduction of mussel seed collectors in the Netherlands prompted the need for more detailed information on juvenile mussel behaviour. To estimate the impact of juvenile populations on ecosystem carrying capacity, information on clearance rate as well as usage of different prey items is essential. Clearance rates were measured in an experimental study, incubating juvenile mussels in natural sea water. Rates were related to isometrics as well as specified for the prey items bacteria, picophytoplankton (<3 µm), nanophytoplankton (3-20 µm) and ciliates.

Results showed that the clearance rate of juvenile mussels depend on shell length^2, while the relation between clearance rate and weight was more variable. Length is thus a better parameter for estimating clearance rate than weight.

Ciliates and nanophytoplankton were cleared at comparable, but variable rates, while picoalgae were cleared from the water at a rate of 11-64% compared to nanophytoplankton. For bacteria the clearance rate was on average 9%. This study showed different retention of particles of similar size (picoalgae and bacteria) as well as variability in particle retention for the different prey items. This variable retention efficiency could not be related to seston concentration or to dominance in cell size. The results from this study can be used to estimate the effect of mussel seed collectors on the carrying capacity of the Dutch Wadden Sea.

Keywords:

juvenile, *Mytilus edulis*, clearance rate, isometrics, planktonic prey, variable retention

Introduction

In estuarine ecosystems suspension-feeding bivalves, like the blue mussel (*Mytilus edulis*), often occur in large numbers, affecting the surrounding ecosystem by filtering vast volumes of water, thereby removing different components of the plankton community (e.g. Verwey 1952, Cadée & Hegeman 1974, Cloern 1982, Dame 1996, Kreeger & Newell 1996, Wong & Levington 2006). The recent introduction of mussel seed collectors in the Netherlands prompted the need for assessing the effect of large numbers of juvenile individuals on the carrying capacity of the surrounding ecosystem. Pelagic seed collectors facilitate the settlement of mussel larvae (300 µm). After settlement in June the juveniles grow in less than 6 months to a maximum size of 25 mm at harvest (Chapter 2). There have been numerous studies performed on the filtration capacity and feeding behaviour of mussels, but these studies were mainly confined to larger (>15 mm) individuals (e.g. Widdows 1978, Bayne & Widdows 1978, Møhlenberg & Riisgård 1979, Riisgård *et al.* 1980, Kiørboe & Møhlenberg 1981, Jones *et al.* 1992, Smaal *et al.* 1997, Riisgård *et al.* 2014), while smaller individuals have been studied far less intensively (but see Riisgård *et al.* 1980). Most studies on filtration rates of mussels were performed under controlled laboratory conditions using algal cultures. These experiments resulted in estimates of the maximum clearance rate, while it can be expected that under natural conditions clearance rates will be lower. The need for information on actual realised clearance rates under natural conditions and the specific usage of natural plankton by these dense collections of juvenile mussels has been recognised (Bunt *et al.* 1992, Cranford *et al.* 2003 & 2011, Trottet *et al.* 2008a).

Mussel larvae are suspension feeders, utilising a ciliated velum to capture food particles (Riisgård *et al.* 1980). After settlement and during metamorphosis the feeding modus changes from a velum to the ctenidium, which also serves as a respiratory organ (gills) (Cranford *et al.* 2011). Lateral cilia on the gill filaments create an inflow; water enters the inhalant chamber and flows through the gills towards the exhalant chamber. Particles in the water flow are captured when the frontal surfaces of the ctenidial filaments encounter and retain them. The size of particles efficiently retained depends on the size and complexity of the latero-frontal cilia of the filaments as well as the current produced by the cirri (Newell & Shumway 1993, Dame 1996, Ward & Shumway 2004). Captured and retained particles are transported to the labial palps. Here particles are either rejected as pseudo faeces or directed further to the mouth (Ward & Shumway 2004). The assumption of isometric relations between length, area and volume (area~length^2 and volume~length^3) this leads to the expectation that theoretically, pumping or filtration rate (R_F) scales with gill surface area, gill surface area is expected to scale with length^2, so R_F= length^2. Since weight scales with volume and volume scale with length^3, gill area will scale with weight$^{2/3}$ and filtration rate will thus also scale with weight$^{2/3}$, so R_F= weight$^{2/3}$ (Jones *et al.* 1992). For veliger and post-metamorphosed larvae, filtration rate was repor-

ted to scale with weight$^{0.8-1}$ (Riisgård *et al.* 1980, Beiras & Camacho 1994). The high scaling factor was attributed to a high non-isometric growth of the gills. In most studies clearance rate (R_C), which is the volume cleared of particles per unit time, is measured rather than the actual pumping or filtration rate. When particles are 100% efficiently retained by the gills, the clearance rate equals the filtration rate. If the filtration efficiency is lower than 100%, the clearance rate is thus lower than the pumping rate. Numerous studies, starting with a study by Møhlenberg & Riisgård (1978), have reported on the particle size range that can be retained by adult mussels (see for overview Strohmeier *et al.* 2012). For a long time is was assumed that mussels do not efficiently retain smaller particles, with studies reporting on 90% retention for 3 µm particles by *Mytilus edulis*, while 1 µm particles are retained with 50% efficiency only (Møhlenberg & Riisgård 1978). Most studies were performed under controlled lab conditions using phytoplankton cultures. Results from experiments using natural plankton communities reported that retention efficiency might be more variable (Trottet *et al.* 2008a, Strohmeier *et al.* 2012). Mussels filter all kinds of particles from the water. Although phytoplankton were traditionally considered the main food source (Nielsen & Maar 2007), several studies have stated the importance of other food particles like dead organic material (Dame & Dankers 1988) and bacteria attached to this (Newell *et al.* 1989), microzooplankton (Horsted *et al.* 1988, Kreeger & Newell 1996, Trottet *et al.* 2008a) and, for larger mussels (>22 mm: Horsted *et al.* 1988), mesozooplankton (Davenport *et al.* 2000, Wong & Levington 2006, Lehane & Davenport 2006).

The aim of this study is to establish realised clearance rate of juvenile mussels (1.5-25 mm) in relation to both shell length and weight. Furthermore, clearance rates will be described for different prey items; bacteria (0.6 µm), picophytoplankton (<3 µm), nanophytoplankton (3-20 µm) and ciliates (20-200 µm). To establish the clearance rates of juvenile mussels, an experimental study was carried out for three years. Juvenile mussels were incubated in sea water originating from the western Wadden Sea. This study is one of the first describing grazing of dense populations of juvenile mussels in natural sea water. The results of this study can be used to estimate the effect of juvenile mussel cultures on the ecosystem of the western Wadden Sea.

Material & methods

In order to measure the clearance rates of juvenile mussels and explore the planktonic prey items removed, an experimental study was carried out between 2010 and 2012. Clearance rates of juvenile mussels in natural sea water were calculated. Before and after the incubation, water samples were analysed for the presence of different prey items.

Study animals

Each year, a small collector was placed in the Marsdiep (52˚58'N, 4˚49'E, Figure 2.1). This collector consisted of filamentous ropes facilitating mussel settlement (Xmas tree ropes, Donaghys). After settlement around June mussels increase in size up to approximately 25 mm when harvested in October. Mussel sizes used in this study were between 1.5 and 25 mm. The day before each incubation experiment, ropes with juvenile mussels were collected, transported in sea water and stored at 4 °C. At the day of the experiment mussels were acclimatised to ambient seawater temperature and pre-incubated.

After each experiment the number of mussels used, average length (± 0.01 mm) and dry weight (dried at 60 °C for 48 h, ± 0.1 mg) was established. Weight included both shell and flesh. In 2012, separate tissue dry weights were determined for an additional series of mussels (7.5-20 mm). The relation between total dry weight and tissue dry weight was used to construct the relation of clearance rate depending on tissue dry weight in 2012, allowing for a comparison with results reported in other studies.

Experimental set-up

Two types of experiments were designed. In 2010 and 2011 pieces of mussel ropes were incubated in mesocosms to calculate the clearance rate of a mussel community. These mussel assemblages on a rope consist of different sized mussels, resulting in a relatively high variation in shell lengths (Table 3.1). In 2012 laboratory experiments were performed, in this set-up the variation in shell length was greatly reduced by removing mussels from a piece of rope, measuring them and sorting them by size. Clearance rates of these equally sized mussels were measured in smaller volumes (Table 3.1).

Mesocosm experiments

To measure the clearance rate of a population of juvenile mussels, pieces of rope were incubated in mesocosms (60-85 litres) in 2010 and 2011. On each experimental date (Table 3.1) 4 or 5 mesocosms were filled with natural seawater by suspension and placed in the NIOZ harbour (Figure 3.1). Both before and after the experiment, complete mixture of the water was checked by comparing the readings of the fluorescence probe (microFlu, TriOS) at different depths. 2 or 3 mesocosms were incubated with mussels, 2 served as control. Mussel ropes were placed in the mesocosm, a rotator enabled gentle mixing of the water to avoid damage of the fragile microzooplankton community. The removal rate of phytoplankton biomass was monitored using a fluorescence probe. Experiments lasted 1-4 hours and were terminated before plankton depletion was expected to have occurred. This assumption was checked at the end of each experiment by verifying the linearity of ln (Fluorescence signal) over time.

Table 3.1 *Overview of most important variables for each experimental date. Temperature is the average water temperature during the experiment, the average number of phytoplankton cells (pico- and nanophytoplankton) as counted with the flow cytometer is given in 10^3 cell per millilitre, N treatment and N control give the number of mesocosms incubated with mussels or kept as control. In 2012 an experiment was sometimes repeated with the same mussels using new sea water, this is than indicated by a 2. On the last experimental date in 2012 the average of 4 separate experiments with 4 individual mussels is given. The number of mussels present per experiment is given as the number of mussels per 100 litres of water (100 l^{-1}). Mean length gives the average shell length in millimetres of the juvenile mussels used per experiment. The last three columns indicate whether clearance rates were measured for each particular prey item on each date. * Mussels originated from a different location than the artificial collector.*

								Clearance rate measured		
Year	Date	Temp (°C)	Phyto (10^3 cells ml^{-1})	Treatment N	Control N	N mussels 100 L^{-1}	Mean length (mm) ± stdev	bacteria	pico and nano	ciliates
Mesocosm experiments										
2010	21-jun	18	7.9 ± 2.1	3	2	0.4	1.71 ± 0.72		v	v
	5-jul	21	36.5 ± 1.9	3	2	2.0	3.18 ± 2.08		v	
	19-jul	20	11.9 ± 2.2	3	2	1.2	4.60 ± 2.58		v	
	3-aug	19	52.5 ± 9.2	3	2	1.1	6.93 ± 2.17		v	v
	21-sep	15	2.3 ± 0.9	3	2	14	13.27 ± 4.42			v
	13-okt	13	24.7 ± 2.8	3	2	11	15.32 ± 6.34		v	
2011	28-jun	19	16.1 ± 0.6	3	2	5.3	8.15 ± 2.90	v	v	v
	12-jul	19	32.4 ± 1.1	3	2	23	11.81 ±4.27	v	v	v
	27-jul	18	33.0 ± 0.6	2	2	13	13.49 ± 5.58	v	v	v
	9-aug	15	42.7 ± 6.5	2	2	31	17.49 ± 7.18	v	v	v
	7-sep*	16	14.1 ± 18.2	2	2	78	20.04 ± 6.00	*v*	*v*	*v*
Laboratory experiments										
2012	5-jun	16	50.5 ± 17.2	2	2	0.3	3.17 ± 0.73	v	v	
	5-jun	13	40.3 ± 13.1	2	2	0.2	1.48 ± 0.49	v	v	
	13-jun	16	7.9 ± 0.7	2	2	1.0	4.60 ± 0.54	v	v	
	13-jun	17	14.8 ± 7.9	2	2	0.7	3.06 ± 0.44	v	v	
	14-jun	17	22.0 ± 0.8	2	2	0.2	2.14 ± 0.42	v	v	
	19-jun	16	19.8 ± 12.6	2	2	1.0	4.96 ± 0.27	v	v	
	20-jun	12	27.7 ± 21.2	2	2	1.3	6.57 ± 0.63	v	v	
	27-jun	14	12.3 ± 0.8	2	2	1.0	4.20 ± 0.20		v	
	27-jun	15	12.6 ± 0.5	1	1	1.3	5.77 ± 0.23		v	
	27-jun	15	11.8 ± 0.9	1	1	1.3	7.16 ± 0.28		v	
	28-jun	15	11.9 ± 1.1	1	1	2.1	8.41 ± 0.24		v	
	11-jul	17	39.8 ± 0.8	1	1	2.5	7.40 ± 0.34	v	v	
	11-jul	16	40.3 ± 2.0	1	1	2.5	10.61 ± 0.35	v	v	
	12-jul	14	32.1 ± 1.7	2	2	3.3	12.03 ± 0.36	v	v	
	7-aug	21	75.6 ± 4.2	1	1	3.6	13.48 ± 0.42	v	v	
	8-aug	21	70.5 ± 3.5	2	2	7.1	15.03 ± 0.30	v	v	
	5-sep	20	56.4 ± 1.4	1	1	10	20.20 ± 0.43		v	
	5-sep	20	49.5 ± 1.0	1	1	10	25.37 ± 0.30		v	
	5-sep	19	49.5 ± 2.1	4	4	10	25.52 ± 0.21		v	

Laboratory experiments

Mussels were gently removed from a piece of rope, measured and sorted by size. 1-100 equally sized mussels (Table 3.1) were placed loosely in petticoat netting (0.5 x 0.5 cm mesh size). For each experiment two glass beakers were filled with natural seawater (0.1-1 litre). To one beaker mussels were added, one beaker served as control. Water was gently stirred, phytoplankton numbers at different depths were compared by means of flow cytometry to check for complete mixture of the water. Phytoplankton cell numbers were monitored throughout the experiment and linearity of the natural logarithm of cell concentration over time was checked afterwards, to verify the absence of depletion. The experiments lasted between 0.75-1.5 hours. On several occasions, mussels were re-used again, repeating the experiment using a new water sample (Table 3.1).

Prey items

Bacteria

Triplicate subsamples (1 ml) for enumerating free-living bacteria were fixed with glutaraldehyde (0.5% final concentration), mixed and then stored at -80 °C until analysis. Analysis was always within one month.

Analyses were performed using a flow cytometer (C6, BD Accuri, excitation with 488 nm laser). Samples were diluted with 10% TE buffer to lower the count rate below 3500 events sec-1, the maximum recording rate of the instrument. SYBR green I (Invitrogen) stain was added (fc 0.1%) and samples were incubated in the dark for 15 minutes, the 530 nm laser (FL1) was used to detect the stained cells.

Pico and nanophytoplankton

Phytoplankton cell counts were performed by means of flow cytometry. Water subsamples (1 ml) in triplicates were processed freshly, immediately after collection. Fluorescence at wavelengths > 670 nm (FL3) was ascribed to chlorophyll-a. Forward scatter (FSC) was used as an indication of cell size (e.g. Li 1995). Based on the relative fluorescence to size, a distinction between phytoplankton and debris was made. Phytoplankton cell counts were further divided in two size classes (<3 µm: pico and 3-20 µm: nano) using 3 µm beads (Polyscience). A minimum cell count of 1000 per size class was applied. Within the picophytoplankton two distinct groups could be identified; those with the pigment phycoerythrin (FL2: 585 nm) ('picocyanobacteria') and those without this pigment ('others'). To calculate an average size per prey item measured with the flow cytometer, additional beads (7 and 10 µm) were used to calibrate forward scatter with size.

Ciliates

For enumeration of ciliates one subsample (0.5-1 litre) was fixed in 4 ml acid Lugol and stored in brown glass bottles at 4 °C until analysis. Samples were concentrated

(10-20 x) and per sample a minimum of 100 individuals was counted or, at very low abundances, all individuals in a maximum of 10% of the concentrated sample. Ciliate cells were counted and divided in 5 size classes (<20 µm, 20-40 µm, 40-60 µm, 60-80 µm and >80 µm) with an inverted microscope using the Utermöhl sedimentation technique (Verweij *et al.* 2010).

Calculation of clearance rates & statistical analysis

Clearance rates (R_C) for each parameter of interest were calculated following the equation (Coughlan 1969):

$$R_C = \frac{V}{nt}\left\{\ln\frac{C_0}{C_t} - \ln\frac{C_{0\prime}}{C_{t\prime}}\right\} \quad \text{(eq. 3.1)}$$

were V is the volume (l) cleared, t is the duration of the measurement (h), n is the number of mussels used in the experiment, C_o is the concentration of a particulate parameter at the start of an experiment, and C_t is the concentration at the end. C_o' and C_t' are the concentrations at the start and end respectively in the control. R_C was expressed as litre per hour per individual mussel. At the end of each experiment linearity of $\ln(C_o/C_t)$ was verified. This 'clearance rate' method is considered reliable when the above condition is met (Riisgård 2001).

To describe clearance rate as a function of either shell length or weight, the removal rate of nanophytoplankton cells was used. For this functional group, with an average size of 6.6 µm, 100 percent efficient retention was assumed. The theoretical relation between clearance rate and shell length or weight can be described by the following equations, for length:

$$R_C = a\ L^b \quad \text{(eq. 3.2)}$$

where R_C is the clearance rate in litres per hour and L the shell length in mm. For weight the equation is given by:

$$R_C = c\ W^d \quad \text{(eq. 3.3)}$$

where R_C is the clearance rate (l h^{-1}) and W is either the total dry weight (shell and tissue, 2010 and 2011) in grams or dry tissue weight (g) (2012).
Under the null hypothesis that clearance rate scales with length to an exponent *b*=2. The exponent for weight *d* is expected to be 2/3 (Jones *et al.* 1992).

To test the potential difference between years for the relation between clearance rate and either length or weight, linear models of $\log^{10}$ transformed data were used (models 1-3). The same kind of models were used to test if the coefficients *b* and *d* differed from their expected values i.e. 2 and respectively (model 4).

model 1: $\log R_{Cij} = \log a + b \log x_{ij} + \varepsilon_{ij}$ (common slope and intercept for all years)

model 2: $\log R_{Cij} = \log a_j + b \log x_{ij} + \varepsilon_{ij}$ (common slope for all years only)

model 3: $\log R_{Cij} = \log a_j + b_j \log x_{ij} + \varepsilon_{ij}$ (slope and intercept differ between years)

model 4: $\log R_{Cij} = \log a + 2\log x_{ij} + \varepsilon_{ij}$ or $\log R_{Ci} = \log a + \log x_{ij} + \varepsilon_{ij}$ (slope equal to 2 or to 2/3, common intercept for all years)

R_C is the clearance rate, a is the intercept, b the slope and ε the error term. The indices i and j refer to observation i in year j. To quantify the clearance rate of picophytoplankton and bacteria relative to the clearance rate of nanophytoplankton linear regression was applied using the individual clearance rates measured. To test whether the clearance rate of juvenile mussels on nanophytoplankton differed from the clearance rate on ciliates the individual rates were compared using a paired t-test.

Statistical analyses were performed in R version 2.14.1 © 2011 The R foundation for statistical computing. A significance level of $\alpha<0.05$ was used for all tests.

Results

Clearance rate of juvenile mussels depending on length and weight

There was no significant interaction of the factor year with the relation between clearance rate and length ($F_{4, 48}=1.42$, p=0.24, model 1 and 3). Neither did the intercepts of this relation differ between the three years ($F_{2, 50}=2.88$, p=0.07 model 1 and 2). The common slope, grouping the measurements of all three years together, did not differ from the theoretically expected value of 2 for b ($F_{1, 52}=2.25$, p=0.14, model 1 and 4). Using this fixed value for b, the intercept was estimated at (0.0004) (Figure 3.1). With no significant differences between the three years ($F_{2, 51}=2.20$, p= 0.12).

The individual clearance rate of juvenile mussels can also be described in relation to the weight of a mussel according to $R_C=c\,W^{d}$. Weight here is defined as the weight of shell and tissue together (Figure 3.2a). The relation of clearance rate with mussel dry weight was not the same for each year ($F_{4, 48}=8.61$, p= $2.547e^{-05}$, model 1 and 3). The intercepts differed between the three years ($F_{2, 50}=14.72$, p= $9.43e^{-06}$, model 1 and 2). Not the slope ($F_{4, 48}=1.94$, p=0.15, model 2 and 3).

Whether the slope differed from the expected value for d=0.67 was tested for each year separately. Only for 2010 the model with a fixed b of 0.67 differed significantly from the estimated d based on the data (2010:$F_{1, 13}=5.18$, p=0.04, 2011:$F_{1, 8}=0.32$,

p=0.59, 2012: $F_{1, 27}$=0.04, p=0.85). The intercepts for 2011 and 2012 are different ($F_{2, 51}$=6.01, p=0.005), so the best fitted lines are given for each year separately (Table 3.2). To compare results on the relation between clearance rates and weight in the current study with results reported in previous studies the relation between clearance rate and tissue dry weight was established (Figure 3.2b). Only for 2012 tissue and shell dry weights were measured separately (material & methods 'study animals'). The relation between tissue dry weight (W, g) and shell length (L, mm) can be described by the relation $W=1.7\ 10^{-5}\ L^{2.7}$ (r^2=0.98). In 2012 the relation between

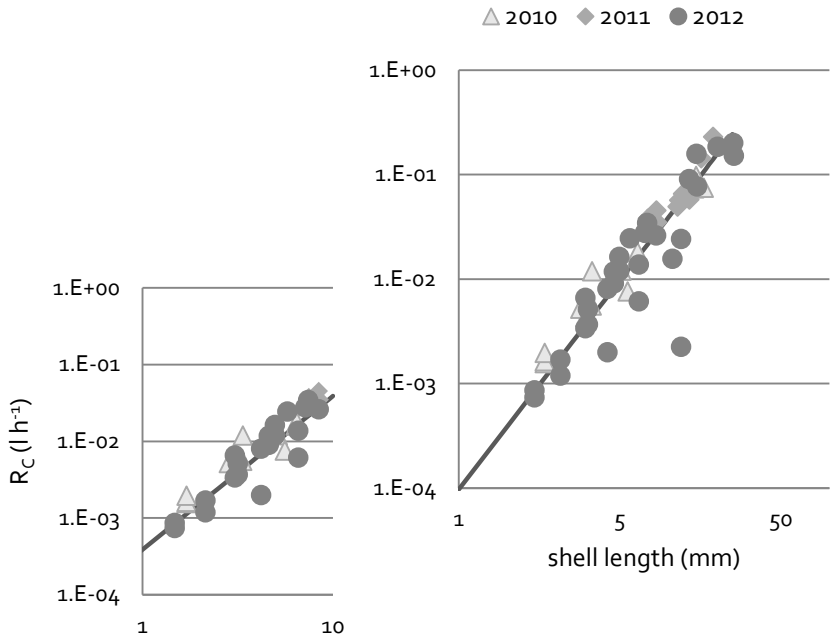

Figure 3.1 *The clearance rate on nanophytoplankton cells, measured for three consecutive seasons for mussels varying in mean size from 1.5-25 mm. The clearance rate is expressed as the litres of water cleared of cells per hour per individual mussel. There were no significant differences in either the slope or the intercept between the three years (model 1-3). The data from the three years were combined and it was further tested whether the regression coefficient different significantly from the expected value of 2 (model 4). The regression coefficient did not differ significantly from the expected value and one regression line was fitted using a slope of two (black line) (log RC= -3.41±0.04 + 2 log length). The small insert on the left shows the clearance rates of the smallest mussels only (<10 mm). Both axes are on log scale.*

Table 3.2 *Estimated value for log c and d (equation 3.3) including the standard error for the relation between clearance rate and the DW (g) of both shell and tissue. The variation explained by this relation is given as r^2. For clarity c is also given.*

year	log *c* ± se	*d* ± se	r^2	c
2010	-3.45 ± 0.22	0.99 ± 0.14	0.80	0.00036
2011	-2.27 ± 0.16	0.62 ± 0.09	0.86	0.0054
2012	-2.62 ± 0.09	0.68 ± 0.07	0.79	0.0024

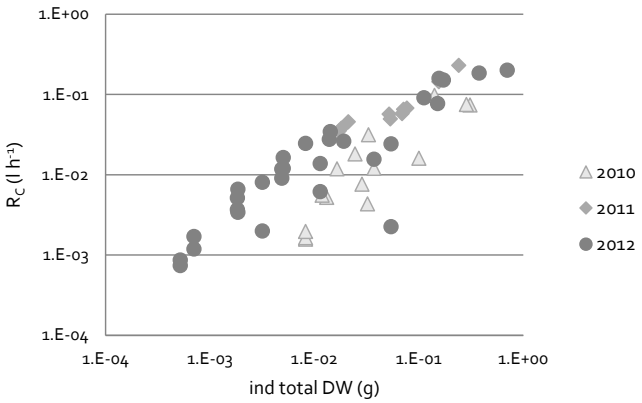

Figure 3.2a (top) *The clearance rate on nanophytoplankton cells, measured for mussels varying in mean size from 1.5-25mm (corresponding to 0.5.-700 mg DW of shell and tissue) for 3 years. The clearance rate is expressed as the litres of water cleared of cells per hour per individual mussel. Both axes are on log scale.*

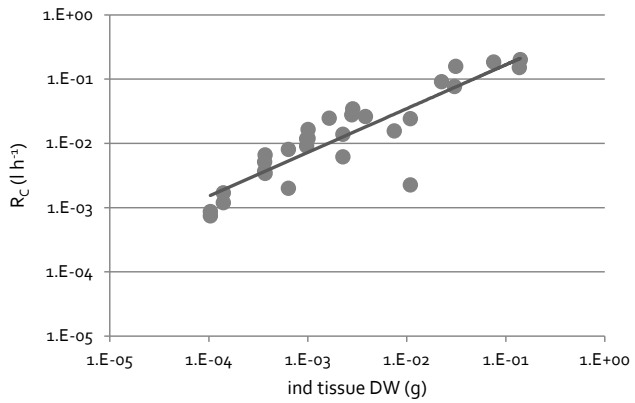

Figure 3.2b (bottom) *The clearance rate on nanophytoplankton cells as a function of the mean individual mussel tissue dry weight. The data were collected in 2012. The regression coefficient did not differ significantly from the expected value of two-thirds. Therefore a regression line was fitted using a slope of two (dark line). The relation between clearance rate (l h^{-1}) and tissue dry weight (g) is best described by the equation: log R_C=-0.13 ± 0.06 + 0.67 log W. Both axes are on log scale.*

clearance rate and tissue dry weight did not differ from the expected value of 0.67 ($F_{1, 27}$=0.02, p=0.90). Clearance rate depends on tissue dry weight according to log R_C= -0.13 ± 0.06 + 0.67 log W.

Clearance rate of juvenile mussels on different prey items

The R_C of juvenile mussels on bacteria is on average 9% of the clearance rate on the better retained nanophytoplankton cells (Figure 3.3a). Picophytoplankton are cleared from the water on average at half the rate of the nanophytoplankton cells (Figure 3.3b). Based on both the auto fluorescence of chlorophyll and phycoerythrin two groups of picophytoplankton could be distinguished; 'others' and 'picocyanobacteria'. The average size of picophytoplankton was 0.7 µm for 'picocyanobacteria' and 1.2 µm for 'others'. There was no difference in the clearance rates of juvenile

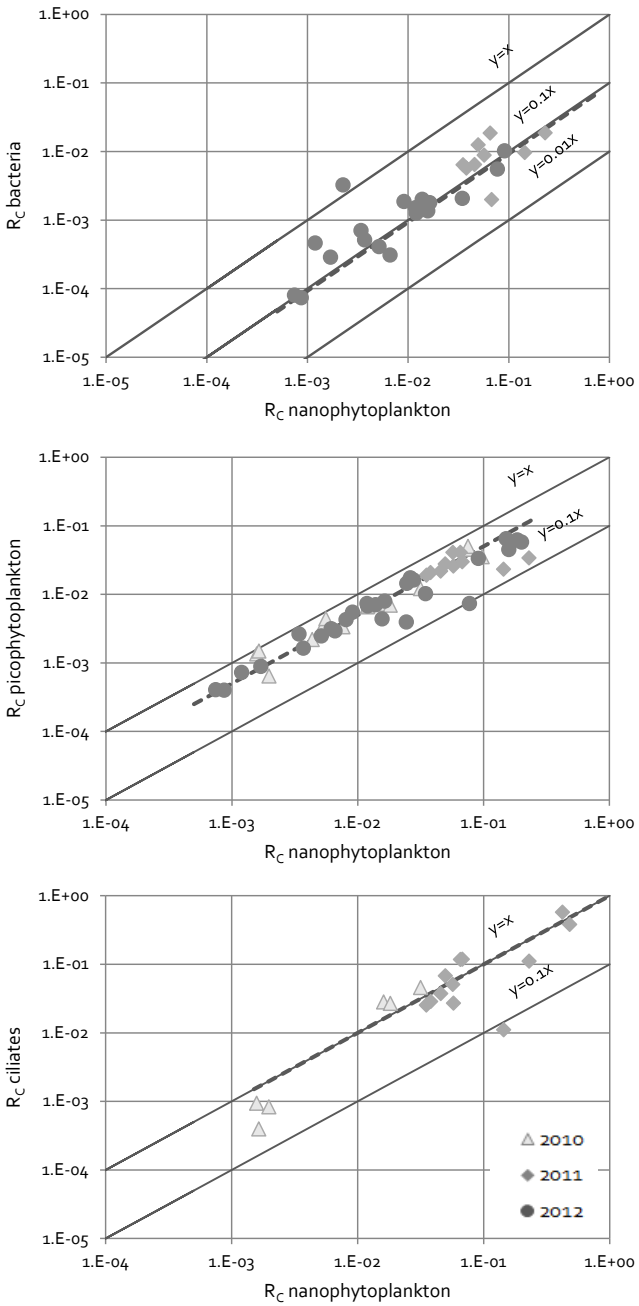

Figure 3.3a-c
The clearance rate (R_C, $l\ h^{-1}$) of juvenile mussels on bacteria (a, top), picophytoplankton (b, middle) and ciliates (c, bottom) relative to the clearance rate on the nanophytoplankton fraction. The clearance rate on bacteria, picophytoplankton and ciliates was assumed to be proportional to the clearance rate on nanophytoplankton (e.g. R_C bact. =a R_C nano). The proportionality-coefficient a was estimated by the antilog of the mean log-ratio of R_C bact, pico and ciliates and R_C nano. The dashed line in figure a & b indicates the estimate for a (all years together) (bacteria: a= 0.09, r^2= 0.75, n=28, picophytoplankton: a= 0.5, r^2=0.95, n=35), for mussels smaller than 10 mm. For ciliates there was no significant difference in clearance rate compared to the clearance rate on nanophytoplankton. (y=x). For reasons of clarity the lines y=x, y=0.1x and y=0.01x are also indicated.

mussels between the two groups of picophytoplankton (data not shown). There was no significant difference between the clearance rate of juvenile mussels on nanophytoplankton and ciliates (t = 0.77, df = 17, p-value = 0.45) (Figure 3.3c).

Table 3.3 *The coefficients a and b in the relation between clearance rate and shell length ($R_c = a\ L^b$) and the coefficients c and d for the relation between clearance rate and tissue dry weight ($R_c = c\ W^d$) as reported in the current and other studies are given. R_c is expressed as litres cleared of particles per hour, shell length in mm and weight in grams. In the current study, with regard to the relation between clearance rate and weight, only data from the year 2012 were used. In this year tissue dry weights were established instead of total (tissue and shell) dry weights. Apart from the current study, most studies referred to in the table have been conducted on Mytilus edulis ranging in size from 10-80 mm using algal cultures thought to be 100% effectively retained. The use of smaller mussels or the use of natural plankton communities instead of cultures is reported under 'comments' in the table. In the current study temperature ranged between 12-21 °C. Temperature ranges in other studies were at a fixed temperature or within a range, but always between 9 and 18 °C except Smaal et al. (0.4-19.5 °C). See original studies for more details.*

a	*b*	reference	comment
0.0004	2.00	This study	1.5-25 mm, natural plankton
0.0002	2.19	Jones *et al.* 1992	mean
0.0004	2.09	Jones *et al.* 1992	max
0.0007	2.14	Kiørboe & Møhlenberg 1981	
0.0035/0.0039	1.72	Filgueira *et al.* 2008	*M. galloprovinciallis,* natural plankton
0.0014	2.08	Riisgard *et al.* 2014	average values
c	*d*		
0.74	0.67	This study (2012)	0.1-140 mg, natural plankton
1.84	0.34	Bayne & Widdows 1978	
2.65	0.38	Widdows 1978	
37.8	1.03	Riisgård *et al.* 1980	post-metamorphosis larvae (0.07-10 mg)
7.45	0.66	Møhlenberg & Riisgård 1979	
7.37	0.72	Riisgård & Møhlenberg 1979	
1.78	0.70	Jones *et al.* 1992	mean
3.16	0.72	Jones *et al.* 1992	max
1.66	0.57	Smaal *et al.* 1997	
5.80/5.02	0.60/0.50	Filgueira *et al.* 2008	*M. galloprovinciallis,* natural plankton
6.90	0.68	Riisgård *et al.* 2014	average values

Discussion

Clearance rate in relation to mussel shell length and weight

There are many studies reporting on clearance rates of mussels. Most of these studies were performed under controlled lab conditions, using cultured algal species, while other, more recent studies established clearance rates under natural conditions. There are large differences in the clearance rates reported and there has been much debate about the causes for these differences. The main arguments to explain the differences between studies are the use of different methodologies (Riisgård 2001, Riisgård *et al.* 2014), differences in mussel condition index (Filgueira *et al.* 2008, Riisgård *et al.* 2014) or food type, with lower clearance rates measured when natural plankton is used (Doering & Oviatt 1986). Nowadays, there seems to be consensus on the concept of considering filtration rates determined in control-

led laboratory experiments using cultured algal species and low mussel densities as maximum rates, while clearance rates established under field conditions can be regarded as realised clearance rates (Cranford *et al.* 2011, Riisgård *et al.* 2014).

In the current study, clearance rates were among the lowest reported (Table 3.3). Although during the experiments complete mixing of the water was aimed for and no gradient of phytoplankton concentration in the experimental units was measured, depletion of algal cells close to an individual mussel cannot be excluded. Especially since in the current study large numbers of closely packed mussels were used in the experiments. Local depletion of food can result in re-filtration of the water. Re-filtration of water might thus provide an additional explanation for the low clearance rates measured in the current study. But it seems that re-filtration was not a constant factor. In 2012, for the smallest mussels, clearance rates were comparable to rates determined in controlled lab experiments on small post-metamorphosed larvae (Riisgård *et al.* 1980). With increasing mussel weight and concentration (Table 3.1), the difference got larger and it seems that the influence of re-filtration on the clearance rate becomes more importance (Figure 3.5). There is thus a difference in the scaling relationship between clearance rate and weight between the current study and the study performed by Riisgård *et al.* (1980). While in the current study clearance rate scaled with an exponent of 2/3 over the entire size range, Riisgård and co-workers found that clearance rate scaled with weight^{1} for small mussels (tissue dry weight < 10 mg), decreasing to 2/3 with increasing weight (Figure 3.5). The difference in scaling exponent between the current study and the study by Riisgård and co-workers is not easy disclosed, but might be due to differences either in morphology or in condition. Clearance rates scaling with weight^{1} could also indicate that gill area does not scale with length^{2}, representing 'high non-isometric growth' of the gills (Riisgård *et al.* 1980). Unfortunately no data is available on the relation between gill area and length, nor on the relation between clearance rate and length. In the current study, weight scaled with length^{3} and clearance rate scaled with length^{2}, making a high isometric scaling of the gills unlikely. However, due to the relative large variation between measurements in our study we cannot rule out that for the maximum clearance rate the relation with weight might be best described by weight^{1}.

Clearance rate of juvenile mussels on different sized prey items

The average diameter of bacteria in the current study was 0.6 µm. Bacteria were cleared from the water with an average efficiency of 9% (Figure 3.3a) of the clearance rate on nanophytoplankton, the most effectively cleared prey item (Figure 3.4). This is somewhat higher than efficiencies reported in other studies. Trottet *et al.* (2008a), using natural sea water, found clearance rates of adult mussels on bacteria to be close to zero. Nielsen & Maar (2007) found no removal of bacteria above a mussel bed. The clearance rate on picophytoplankton was higher than

the average clearance rate on bacteria (Figure 3.4). The clearance rate on the picofraction of phytoplankton occurred on average at half the rate of the clearance on larger nanophytoplankton (Figure 3.3b). The diameter of picophytoplankton was between 0.7 and 1.0 µm and the retention efficiencies found in the current study fall within the range of reported efficiencies for 1 µm (unidentified) particles (e.g. 50%: Møhlenberg & Riisgård 1978, 20%: Riisgård *et al.* 1980, 14-64%: Strohmeier *et al.* 2012).

The difference in diameter between bacteria (0.6 µm) and picophytoplankton (0.7-1.0 µm) is small, while the average retention is much higher for picophytoplankton compared to bacteria. This sharp decline in retention efficiency with decreasing par-

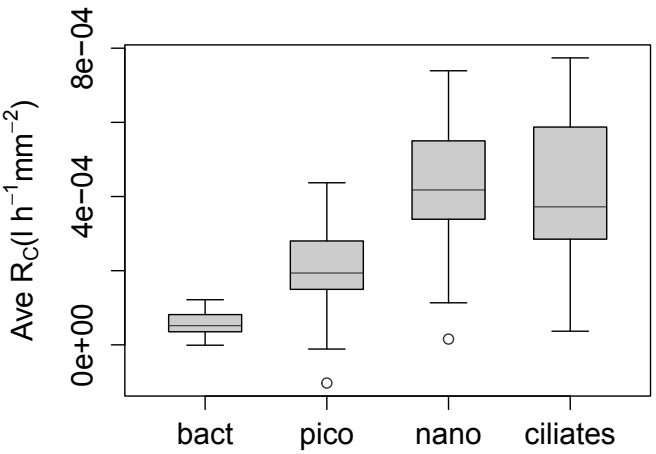

Figure 3.4
Summarizing boxplot indicating clearance rate of juvenile mussels on 4 prey items for all years together. Clearance rate is expressed as litre cleared of items per hour per mm² shell length, to make the R_C independent of shell length.

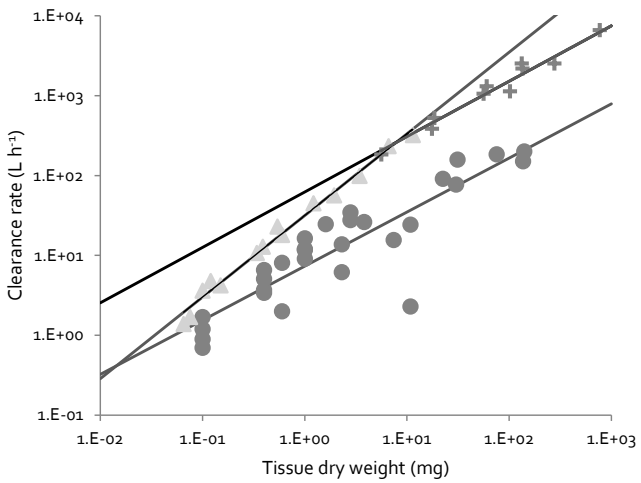

Figure 3.5
The relation between clearance rate (l h^{-1}) and tissue dry weight (mg) of mussels. Both axes are on a log scale. b in the relation log R_C=log a +b log W was reported to be 1 in Riisgård et al. (1980) (Δ), 2/3 in Riisgård & Møhlenberg (1979)/ Møhlenberg & Riisgård (1979) (+) and 2/3 for the current study (2012, o).

ticle size has been reported before (Lucas *et al.* 1987; Matthews *et al.* 1989; Ward & Shumway 2004). Preferential capture of picophytoplankton over bacteria must be based on properties other than cell size alone. Differences in stickiness between species of the same size, affecting capture efficiency by the ctenidium, has been suggested as a possible explanation for the variation in retention of equally sized particles (Ward & Shumway 2004). The average diameter of nanophytoplankton cells was 6.6 µm, while ciliate were much larger, ranging in diameter roughly between 10 and 200 µm, with a weighted average of 28.6 µm (±7.9). Clearance rates on ciliates however were comparable to the clearance rates on nanophytoplankton (Figure 3.3c & 3.4). Optimal retention thus reaches a plateau for particles larger than 6.6 µm in this study.

Variable retention

The retention efficiency for different prey items is not constant (Figure 3.3a-c). For bacteria the retention relative to the retention of nanophytoplankton varied between 1 and 26% and for picophytoplankton retention varied between 11-64%. Mussels can lower the retention efficiency for small particles to some extent by widening the interfilamentary distances of the ctenidium or by shifting the movement of the latero-frontal cilia to the side, so cilia no longer block the passage of smaller particles (Atkins 1937; Dral 1967; Barillé *et al.* 1993; Strohmeier *et al.* 2012). There is a positive relation between the size of a particle and its nutritional value (Ward & Shumway 2004). Assuming that mussels strive to maximize their energy intake, a trade-off is expected with regard to the distance between the filaments; Either a wide interfilamentary distance, creating a low concentration (since abundance is negatively related to size) of large nutritious (Ward & Shumway 2004) particles, or a more narrow distance, resulting in a high concentration of particles, but including a large quantity of low quality particles. A higher inflow of lower quality particles is likely to increase the processing costs (e.g. pumping, handling, selecting and rejection). It can thus be expected that the optimal interfilamentary distance at least balances the costs of processing of different quantity and quality particles with the benefits.

There are studies reporting on higher or lower retention efficiencies in response to variations in natural seston. Strohmeier *et al.* (2012) found that when total cell volume was dominated by small particles, the particle size most efficiently retained decreased (to 6-16 µm). At times when total cell volume was dominated by larger cells, capture efficiency increased to larger particles (20-30 µm). Calculating the carbon per size class for data published in Lucas *et al.* (1987) revealed a similar pattern; retention efficiency for 1.6 µm particles differed between two sites. The highest retention efficiency for these picoparticles corresponded to relative small (8 µm) particles dominating total carbon availability, while at the site with a lower retention the carbon availability was dominated by 12-16 µm particles. Trottet *et al*. (2008a)

investigated clearance rates on different phytoplankton species, heterotrophic flagellates and ciliates. Relative clearance rates between species and taxa varied throughout the year. No consistent relation between cell abundance and clearance rate per species/taxa was found. In the current study seston concentrations varied considerably. During the experiments the suspended matter concentration fluctuated between 16-50 mg l^{-1} with chlorophyll-a concentration between 3-11 µg l^{-1} (data not shown). Variation in retention of the different prey items could however not be related to differences in either suspended matter or chlorophyll-a concentrations. Neither could this variable retention efficiency be attributed to differences in dominant cell size. Whether mussels are able to control particle retention in response to variations in natural seston concentration remains a controversial topic and according to Riisgård *et al.* (2013) the mechanism of modulation of the retention efficiency "lacks a physical explanation".

Conclusion

The current study is one of the first describing realised clearance rates related to length and weight for juvenile mussels. Clearance rates scaled with length^2 in the same way as adult mussels do. Scaling of clearance rate with weight was more variable. Weight is not only expected to fluctuate within a year, but also between years, effecting the relation with clearance rate. In other studies it was already concluded that gill area generally scales well with length and that therefore clearance rate estimates based on length can be considered the actual clearance rates (e.g. Filgueira *et al.* 2008, Riisgård *et al.* 2014).

Clearance rates in the current study were performed on densely populated pieces of ropes or large numbers per water volume. This might have resulted in re-filtration of water, leading to lower clearance rates compared to maximum rates determined in studies performed under controlled lab experiments. Extrapolating maximum rates to estimate the clearance rate exercised by dense populations of juvenile mussels a field situation thus leads to an overestimation. The estimation of realised clearance rates in the current study, including re-filtration of the water, better represent the filtration pressure in a natural situation.

Juvenile mussels exercise comparable clearance rates on nanophytoplankton and ciliates. And, similar to adults, juvenile mussels expressed reduced clearance rates on potential food particles with a diameter less than 3 µm. Size selective removal, as shown by this study might result in relative changes in plankton groups. Information on the potential effect of size-dependent clearance rates of juvenile mussels on the pelagic food web will provide a more realistic estimate of the effect of large populations of filter feeders on the carrying capacity of an ecosystem.

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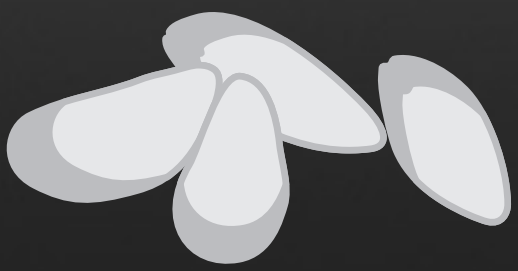

Chapter 4

Impact of the blue mussel *Mytilus edulis* on the microbial food web in the western Wadden Sea, the Netherlands

Pascalle Jacobs, Roel Riegman, Jaap van der Meer

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Abstract

To study the impact of juvenile mussels (*Mytilus edulis*) on the microbial food web in the Dutch Wadden Sea natural sea water was first exposed to mussel filtration. Subsequently, filtered plankton communities were used in a dilution experiment to establish mussel induced changes in bacterial, pico- and nanophytoplankton growth rates as well as heterotrophic nanoflagellates (HNAN) and ciliate induced grazing mortality rates. During the experimental period, from July through to September, mussel filtration had a size-selective impact of the plankton community; on average nanophytoplankton, HNAN and ciliates biomasses were removed at equal rates, while bacterial and picophytoplankton biomasses were affected to a much lower extent. The reduction in HNAN predators by mussels significantly lowered the grazing mortality rates for picophytoplankton. For bacteria grazing mortality did not change, while specific growth rates almost doubled (from 0.65 to 1.16 day^{-1}). There was an increase in HNAN biomass following the enhanced bacterial production. Single exposure to mussel filtration thus led to a stimulation of the bacterial-HNAN pathway. HNAN biomass, although seriously reduced by mussel filtration, recovered to pre-filtration levels within 24 hours. Nanophytoplankton and ciliates did not recover completely within 24 hours. The results from this study reveal potentially important effects of mussel filtration on the pelagic food web not disclosed when considering phytoplankton biomass alone.

Keywords:

microbial food web, dilution technique, *Mytilus edulis,* filtration, growth and mortality rates, carbon flux, Wadden Sea

Introduction

The predators within the microbial plankton community play a central role in marine ecosystems (Azam *et al.* 1983). Bacteria and picophytoplankton (0.2- 3 µm), particles too small to be efficiently used by most other organisms, are the main prey for heterotrophic nanoflagellates (HNAN), while ciliates prey on both HNAN and nanophytoplankton (3-20 µm). Consumption of HNAN and ciliates by larger zooplankton links the microbial food web to the classical food chain (Azam *et al.* 1983, Saiz & Calbet 2011). The specific growth rates of nano-and micro-sized predators (< 200 µm) are high and in the same order as their prey. This allows for a tight control over picoplankton and, to a lesser extent, over nanophytoplankton biomasses (Riegman *et al.* 1993, Kuipers *et al.* 2003). The high growth and grazing rates result in high turn-over rates of organic carbon through the egestion, excretion and 'sloppy feeding' of small sized grazers (Fuhrman 1992, Strom *et al.* 1997 and references herein). The dissolved organic matter (DOM) in turn stimulates bacterial production and, through the regeneration of nutrients, primary production. Predators within the microbial food web can thus be considered the most important remineralisers in the sea (Azam *et al.* 1983) (Figure 4.1).

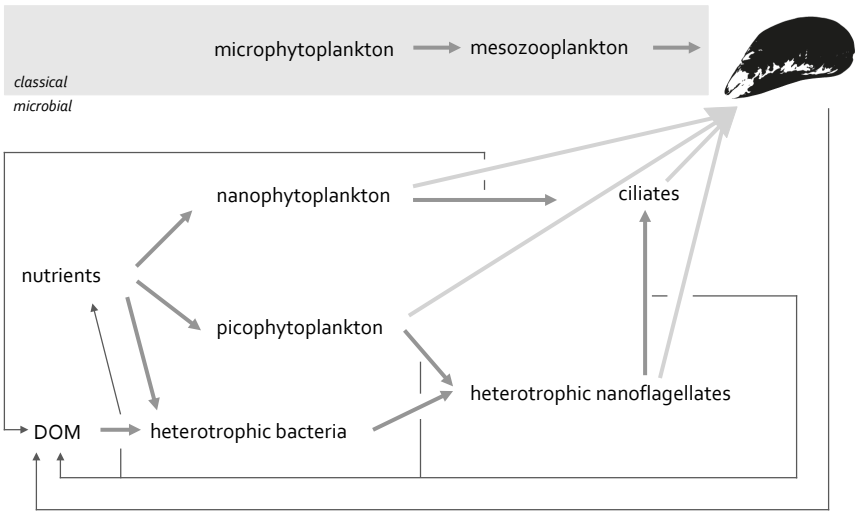

Figure 4.1 *A simplified marine food web indicating both the classical food chain from microphytoplankton (>20 µm) to mesozooplankton (>200 µm) to higher trophic levels as well as the microbial food web. In the microbial food web heterotrophic bacteria (<1 µm) and picophytoplankton (0.2-3 µm) are considered the main prey for heterotrophic nanoflagellates (HNAN) (2-20 µm), while nanophytoplankton and HNAN are considered main prey for ciliates (20-200 µm). Ciliates and HNAN, when consumed by mesozooplankton, provide the link between the two food webs. The light grey arrows represent the functional groups within the microbial food web potentially removed by juvenile mussels (*Mytilus edulis*). Processes like feeding produce dissolved organic matter (DOM) and release nutrients. These main remineralisation pathways are indicated by the thin lines.*

Although the existence of microbial food webs in both oligotrophic as well as eutrophic systems has long been recognised (Riegman *et al.* 1993), it was generally assumed that nano-and microzooplankton grazing dominated in oligotrophic systems, while grazing by larger zooplankton, e.g. the transfer or energy and matter through the 'classical food web', was the most important process in more eutrophic systems (Calbet & Landry 2004). From a meta-analysis of a wide diversity of systems around the globe it was concluded that 59-75% of all primary production was consumed by nano-and microzooplankton (Calbet & Landry 2004), stressing the importance of the microbial food web in all marine pelagic ecosystems. Information on the importance of the microbial food web in shallow benthic (e.g. mussel dominated) systems is lacking (Calbet & Landry 2004). Together with the introduction of bivalve cultures occurring often in pelagic systems, there is a need to gain insight in the impact of bivalves on the microbial food web.

Several authors have suggested an impact of bivalve filter feeders on the microbial food web (Murrell & Hollibaugh 1998, Calbet & Landry 2004, Trottet *et al.* 2008a, Greene *et al.* 2011, Froján *et al.* 2014). Bivalve filter feeders do not effectively remove small plankton, but do feed on their predators, the HNAN and ciliates (Dupuy *et al.* 1999). This might not only result in a disruption of the link between small plankton and higher trophic levels (Dupuy *et al.* 1999, Wong *et al.* 2003, Greene *et al.* 2011), but also in complex indirect effects. The removal of nano-and microzooplankton predators by filter feeders might release prey from top-down control, resulting in bacteria and small phytoplankton biomass increases. Bottom-up, bivalve excretion products result in an increase of dissolved and particulate organic matter as well as a higher availability of nutrients, stimulating both bacterial as well as phytoplankton production (Newell 2004, Van Broekhoven *et al.* 2014). At the same time, by removing suspended matter, mussel filtration improves the underwater light climate. Filtration by bivalves might dramatically change the size distribution of phytoplankton cells; Nanophytoplankton are efficiently removed by mussels, while smaller cells are not. The pico-sized cells are better competitor for both light and nutrients, so improved growth conditions as a result of mussel filtration will likely favour the smallest cells (Riegman *et al.* 1993), potentially resulting in an increase in small cells at the expense of larger ones (Cranford *et al.* 2009). Finally, the removal of HNAN and ciliates, the main remineralisers, by mussels will likely alter the remineralisation process as well.

Surprisingly little research has been performed on this subject (Froján *et al.* 2014). Most studies focussed on the effect of bivalve cultures on phytoplankton biomass only, using total chlorophyll-a as proxy, or combined with the effect on larger mesozooplankton (Lehane & Davenport 2002, Wong *et al.* 2003, Nielsen & Maar 2007, Lonsdale *et al.* 2009). Recently, it was hypothesised that a focus on the classical food web led to an underestimation of the ecosystem impact of bivalve filter feeding (Greene *et al.* 2011). A recent study that included the effect of bivalves on the microbial food web reported a decrease in ciliates biomass following the introduc-

tion of the clam *Corbula amurensis* (Greene *et al.* 2011). Froján *et al.* (2014) reported an in situ decrease in both nano- and microplankton, while picoplankton remained unchanged inside a mussel raft culture (*Mytilus galloprovincialis*). Authors of both studies concluded that bivalves altered the size structure of the microbial community. In both studies the need to include the microbial food web when making an evaluation of bivalve modifications to the ecosystem was stressed.

In 2009 a small number of mussel seed collectors were introduced in the western Wadden Sea, The Netherlands. These collectors facilitate the settlement of large numbers of mussel larvae (*Mytilus edulis*). After settlement these larvae grow up to 25 mm in less than 6 months, after which they are harvested (Chapter 2). To assess the potential impact of an up-scaling of the number of collectors on the microbial food web, a paired set of experiments was designed. First, filtration experiments, incubating juvenile mussels in natural sea water were performed. In these experiments mussels were allowed to remove part of the available plankton biomass. After this mussel filtration experiment, the Landry and Hassett dilution technique (Landry & Hassett 1982) was applied to both mussel-filtered and unfiltered (control) water. This method allowed for an estimation of specific growth and grazing mortality rates of bacteria, pico-and nanophytoplankton as well as changes in these rates due to mussel filtration. Changes in HNAN and ciliate biomasses were also determined.

The aim of this study was to investigate the effect of mussel grazing on different species groups within the microbial food web. Changes in growth and mortality rates within the microbial food web caused by mussels filtration combined with calculation on available carbon per functional groups resulted in estimation of the changes in carbon flow through the food web. Quantifying these pathways within the food web and the mussel induced changes to these pathways give a more realistic depiction of food web processes (Miehls *et al.* 2009). This should allow for a better description of the direct and indirect effects of juvenile mussel filtration on the Wadden Sea food web.

Material & methods

In 2011 and 2013, between July and September, ten paired sets of experiments were executed. Juvenile mussels were incubated in water originating from the western Wadden Sea in a so-called 'filtration experiment'. Subsequently, both mussel-filtered as well as unfiltered (control) water was used in a 'Landry and Hassett dilution experiment' (Landry & Hassett 1982). In 2013 methodological improvements were introduced. The changes in 2013 compared to 2011 consisted of the use of three bottles per dilution level instead of 2 for the Landry and Hassett experiment and additional sampling for HNAN, ciliates and chlorophyll for both sets of experiments.

Additionally a larger experimental volume was used for both sets of experiments. The main reason for using larger volumes in 2013 was to accommodate additional sampling for ciliates and chlorophyll. An unexpected, but positive effect of the use of larger volumes in the dilution experiments in 2013 might have been the reduction in variation between experimental units. Although it was assumed that the experimental volume would not impact the outcome of the results (Hammes *et al.* 2010), it cannot be excluded that in the current study the use of very small volumes in 2011 did have an effect on the outcome.

Mussel filtration experiments

Mussel were collected from a small collector placed in the Marsdiep (52˚58'N, 4˚49'E). This collector consisted of filamentous ropes facilitating mussel settlement (Xmas tree ropes, Donaghys). After settlement around June mussels increase in size up to approximately 25 mm in October. The day before each experiment, ropes with juvenile mussels were collected, transported in sea water and stored at 4 °C. At the day of the experiment mussels were carefully removed from the rope, put in petticoat netting (0.5 x 0.5 cm mesh size) and acclimatised to the ambient seawater temperature. Mussels were placed in natural sea water allowing them to resume feeding normally.

Experiments were conducted in two 3 l glass beakers (2011), filled with natural sea water. In 2013 12 l polycarbonate carboys were used. Mussels were added ('mussel treatment') to one experimental unit, while the other unit served as control. The number of mussels varied between 10 and 50 in 2011 and between 125-450 in 2013, with an average mussel shell length between 7.4 and 23.1 mm. During the experiment the water was gently stirred. At the start of each experiment water samples were taken for bacteria (2013) pico- and nanophytoplankton (2011 and 2013), heterotrophic nanoflagellates and ciliates (2013) and chlorophyll (2013). Samples were taken every 10-15 minutes and phytoplankton cell counts were performed directly with a flow cytometer (see next paragraph) to monitor the decrease. Experiments lasted between 0.5-1.25 hours in 2011 and between 1.5-3 hours in 2013. Grazing pressure (G_p) is the fraction removed by mussels as compared to the fraction available in the control and was calculated per experiment as:

$$G_p = \frac{(N_C - N_M)}{N_C} \qquad \text{(eq. 4.1)}$$

where N is the concentration of prey items (number l^{-1}) (bacteria, pico- or nanophytoplankton, HNAN or ciliates) or chlorophyll (µg l^{-1}) at the end of mussel filtration experiment, C refers to the control treatment and M to the mussel treatment. The number of mussels as well as their length differed between experiments. Although an effort was made to standardise the total biomass removed between experiments by adapting the duration of the filtration experiment, the variation between experiments is large (Table 4.1).

Table 4.1 *Overview of the grazing pressure (G_p) exerted by mussels per experiment for each of the plankton groups. The grazing pressure is calculated as the fraction removed in the mussel treatment compared to the concentration in the control treatment (equation 4.1, material & methods). bact=bacteria, pico=picophytoplankton, nano=nanophytoplankton, hnan= heterotrophic nanoflagellates and cil=ciliates, chlT=total chlorophyll. An empty cell indicates that a parameter was not determined for that experiment.*

exp	date	$G_p$bact	$G_p$pico	$G_p$nano	$G_p$hnan	$G_p$cil	$G_p$chlT
1	28-jul-11		0.72	0.90			
2	10-aug-11		0.67	0.93			
3	9-sep-11		0.77	0.85			
4	26-sep-11		0.61	0.78			
5	2-jul-13		0.25	0.16	0.26		
6	9-jul-13		0.12	0.67	0.55	0.78	
7	24-jul-13	-0.10	0.16	0.75	0.80	0.59	0.57
8	7-aug-13	0.09	0.02	0.49	0.50	0.23	0.55
9	20-aug-13	0.11	0.23	0.44	0.40	0.55	0.29
10	25-sep-13	-0.12	0.29	0.62	0.83	0.67	0.42

Dilution experiments

To study the effect of mussel filtration on the microbial food web, both mussel-filtered water ('mussel treatment') and natural sea water ('control') were serial diluted with filtered (Whatman GF/F filter) sea water. This dilution method (Landry & Hassett 1982) can be used to estimate specific growth rates (growth in the absence of grazers) (μ d^{-1}) and grazing mortality rates (g d^{-1}) of bacteria and phytoplankton (prey). In the current study the specific growth and grazing mortality rates in bottles containing natural sea water were compared to the rates in bottles containing the plankton exposed to mussel filtration. The method is based on measuring the net rate of increase (k t^{-1}) of prey (μ-g) along a gradient of dilutions. The net growth rate in each bottle was calculated according to the equation:

$$k = \frac{1}{t} ln \left(\frac{N_t}{N_0}\right) \qquad \text{(eq. 4.2)}$$

where t is the duration of the experiment (d^{-1}), N_o is the prey concentration at the start of the experiment and N_t is the concentration at the end of the experiment. For both the control treatments and the mussel treatment a separate dilution series was prepared with a ratio of unfiltered: filtered water of 1:0 (100% unfiltered water), 3:1 (75%), 1:1 (50%) and 1:3 (25%). Since mussel grazing resulted in a reduction of larger plankton, further dilutions (below 25%) would result in concentrations of larger phytoplankton, HNAN and ciliates too low to be determined reliably. The use of Whatman GF/F filters (nominal pore size of 0.8 μm) resulted in the passage of bacteria and occasional picophytoplankton cells through these filters. Cell counts were performed for all prepared dilutions. For both pico-and nanophytoplankton the realised ratios of unfiltered to filtered water were close to the ratios aimed for. For bacteria, due to the passage of cells through the filter, the lowest percentage of

unfiltered water was 38% rather than 25% aimed for. For bacteria, pico-and nano-phytoplankton realised dilution fractions were used in the calculations (equation 4.3). Another complication of the passage of bacteria cells through the GF/F filter is the potential increase in the specific growth rate (μ) for these cells in the most diluted concentrations, resulting in a non-linear relation between the net growth (*k*) and the fraction undiluted sea water (Li & Dickie 1985). In the current study no indication for non-linearity was found in the experiments with regard to bacterial growth.

In 2011 each dilution step was performed in duplicate (50 ml Greiner culture flasks), while in 2013 3 replicas were used (500 ml polycarbonate bottles). Bottles and flasks were filled to the rim, to prevent air bubbles, by gently pouring the well mixed water, after which they were attached to a slow rotating plankton wheel for 24 h in a temperature-controlled room set at in situ temperature. Illumination was by daylight fluorescent tubes providing in situ light levels and applying a day-night regime. Changes in the plankton community were established after 24 h. Assumptions for the dilution method are that prey growth is exponential and independent of the dilution level and that the ingestion rate of predators is linearly proportional to concentration of prey (e.g. predators are not food saturated). If these assumptions are met, linear regression of the fraction of unfiltered seawater (f_u) against *k* should yield a slope and an intercept corresponding to the grazing mortality (*g* d^{-1}) and the specific growth rate of prey (μ d^{-1}) respectively (Landry & Hassett 1982):

$$k = \mu - g\, f_u \qquad \text{(eq. 4.3)}$$

To calculate the grazing loss per day, the daily production as well as the net changes in biomass (production-consumption) for bacteria, pico- and nanophytoplankton, the following formulas were used (Landry *et al.* 2000, Calbet & Landry 2004) after first converting cell numbers into carbon:

$$P = \mu\, C_m \qquad \text{(eq. 4.4)}$$

$$G = g\, C_m \qquad \text{(eq. 4.5)}$$

$$C_m = C_0\left[e^{(\mu - g)t} - 1\right]/(\mu - g)t \qquad \text{(eq. 4.6)}$$

where *P* is the production (μg C l^{-1} d^{-1}) for each functional group, μ the specific growth rate (day $^{-1}$) and C_m the integrated concentration during the incubation. *G* is the grazing loss (μg C l^{-1} d^{-1}) experienced by bacteria, pico-or nanophytoplankton, *g* the grazing mortality rate (day^{-1}). C_o is the concentration for each functional group at the start of the incubation and *t* is the duration of the incubation in days.

To calculate carbon specific ingestion rates of predators the following formula was used in which *I* is the units of prey carbon ingested by one unit of predator carbon (d^{-1}):

$$I = {}^{G}/_{C_{m\ predator}} \qquad \text{(eq. 4.7)}$$

Sample analysis

At the start (t_0) and the end (t_{24}) one sample per bottle was analysed for bacteria, pico- and nanophytoplankton for all dilution levels. HNAN were enumerated for each of the 3 undiluted bottles only. Single sub-samples for ciliates and duplicate subsamples for both total and fractionated chlorophyll were taken from the undiluted bottle at t_0 and a mixed sample of the three undiluted bottles at t_{24}. Not all parameters were measured in all experiments (Table 4.1).

Bacteria

Subsamples (1 ml) for enumerating bacteria were fixed with glutaraldehyde (0.5% final concentration), mixed and then stored at -80 °C until analysis. Analysis was always within one month. Analyses were performed using a flow cytometer (BD Accuri C6, excitation with 488 nm laser), samples were diluted with 10% Tris-EDTA buffer. SYBR® Green I (Invitrogen) stain was added (fc 0.1%) and samples were incubate in the dark for 15 minutes. The 530 nm laser (FL1) was used to detect the stained cells. The average diameter of particles was calculated after calibration of forward scatter with size, using beads (3, 7 and 10 µm) (e.g. Li & Dickie, 1985). Assuming a spherical shape, cell counts were converted to carbon biomass using a factor of 4.7 x 10^{-7} µg C μm^{-3} (Verity *et al.* 1992).

Pico and nanophytoplankton

Phytoplankton cell counts were performed by means of flow cytometry. Water subsamples (1 ml) were processed unfixed, immediately after collection. Fluorescence at wavelengths > 670 nm (FL3) was ascribed to chlorophyll. Forward scatter was used as an indication of cell size and based on the relative fluorescence to cell size, a distinction between phytoplankton and debris was made. Phytoplankton cell counts were further divided in two size classes (<3 µm: pico and >3-20 µm: nano) using 3 µm sized beads (spherotech, BD Accuri). The definition of pico- and nanophytoplankton is based on the particle size effectively retained by mussels (Møhlenberg & Riisgård 1978). To convert pico-and nanophytoplankton cell counts into carbon biomass spherical cell shapes were assumed. Conversion factors used were 4.7 x 10^{-7} and 2.2 x 10^{-7} µg C μm^{-3} respectively (Verity *et al.* 1992). It must be noted that flow cytometry counts and subsequent conversion of counts into carbon biomass yielded much lower biomasses of picophytoplankton compared to biomasses based on fractionated chlorophyll. Using a fixed conversion factor of 20 g C/ g chl (Riegman *et al.* 1993), picophytoplankton biomasses were 7 to 20 times higher.

Heterotrophic nanoflagellates (HNAN)

HNAN were counted using flow cytometry applying a slightly modified protocol by Rose *et al.* (2004). Modifications included the use of a smaller volume (4 ml) and a

higher final concentration of Lysotracker® Green (75 nM, Molecular Probes). A flow rate of 100 µl min^{-1} and core size of 40 µm was selected. Count time was 15 minutes. 2.5 µm beads (YG fluorescence, Polysciences) were used for volume and size reference. To convert cell counts to carbon biomass the same procedure was applied as for nanophytoplankton.

Ciliates

For enumeration of ciliates one subsample (0.5-1 litre) was fixed in 2-4 ml acid Lugol and stored in brown glass bottles at 4 °C until analysis. Samples were concentrated (10-20 x) using the Utermöhl sedimentation technique (Verweij *et al.* 2010). Per sample a minimum of 100 individuals was counted or, at very low abundances all individuals in a maximum of 10% of the concentrated sample were counted. Ciliate cells were counted and divided in 5 size classes (<20 µm, 20-40 µm, 40-60 µm, 60-80 µm and >80 µm) based on their length, using an inverted microscope. An oblate spheroid ($4/3\pi\ a^2b$) best represented the average shape of ciliates (Putt & Stoecker 1989). Using the middle of the size class/2 as a and the middle of the size class/4 as b, calculated cell volume was converted into carbon using a factor of 1.65×10^{-7} µg C μm^{-3} (Verity *et al.* 1992).

Chlorophyll

For the determination of total and fractionated chlorophyll, duplicate subsamples (200-300 ml) were filtered over Whatman GF/F and 3.0 µm polycarbonate filters using low vacuum pressure (max -0.4 bar). Filters were stored in the dark at -80 °C for no more than 2 months. Chlorophyll was extracted by homogenisation of filters in 90% acetone with the addition of glass pearls. Chlorophyll was determined fluorometrically (Holm-Hansen *et al.* 1965) using spinach chlorophyll-a (Sigma) as a reference.

Data analysis

Linear regression analysis was performed for each experiment to estimate the specific growth rate (µ) and grazing mortality rate (g) per day for bacteria (when measured), pico- and nanophytoplankton (Table 4.2). On three occasions non-linear responses were detected (Table 4.2), violating the assumption of linearity between predator ingestion rate and prey concentration. The occurrence of non-linear patterns is a common problem for the dilution method (Gallegos 1989, Evans & Paranjape 1992). Previously identified causes of non-linear regressions are the existence of a feeding threshold for grazers, occurring at high dilution levels (Quevedo & Anadon 2001), food saturation of grazers (Gallegos 1989, Evans & Paranjape 1992), a change in the microzooplankton community (Dolan *et al.* 2000), prey selection (Teixeira & Figueiras 2009) or nutrient limitation during the incubation (Landry & Hassett 1982). Teixeira and Figueiras (2009) however reported that specific growth rates and grazing mortality is still robust when calculated over the linear part of the regression. This procedure was applied in the current study.

Table 4.2 *For each experiment a linear regression analysis was performed on the fraction of unfiltered seawater against the change in bacteria, pico-or nanophytoplankton concentration in 24 hours (k). This analysis yielded a slope and an intercept corresponding to the grazing mortality (g d^{-1}) and the specific growth rate of prey (μ d^{-1}) respectively. Analyses were performed for natural sea water (control experiments) and for mussel filtered water. Non-linear responses were detected occasionally (shaded experiments). In these cases, specific growth rates and grazing mortality rates were calculated using the linear part of the response (c.f. Teixeira & Figueiras 2009, see material & methods). These adapted values are given in the table below. · p<0.05, *p<0.01, ** p< 0.001 and ***p<0.0001*

		Control			Mussel		
exp	date	μ (d^{-1}) ± sd	g (d^{-1}) ± sd	R^2	μ (d^{-1}) ± sd	g (d^{-1}) ± sd	R^2
Bacteria							
7	24-jul-13	0.67±0.16**	1.08±0.18***	0.78	1.06±0.17***	0.81±0.21**	0.66
8	7-aug-13	0.09±0.12	1.22±0.16***	0.85	1.50±0.12***	1.59±0.17***	0.90
9	20-aug-13	0.99±0.23**	1.15±0.27**	0.65	1.41±0.12***	0.71±0.15***	0.69
10	25-sep-13	0.86±0.33*	1.14±0.39*	0.47	0.66±0.18**	0.73±0.21**	0.55
Picophytoplankton							
1	28-jul-11	0.22±0.09*	0.78±0.13***	0.87	0.02±0.40	-0.29±0.18	0.39
2	10-aug-11	0.12±0.04*	0.46±0.05***	0.93	0.11±0.04*	-0.01±0.05	0.01
3	9-sep-11	0.13±0.07	-0.06±0.10	0.08	-0.27±0.12.	-0.48±0.17*	0.57
4	26-sep-11	-0.09±0.28	0.13±0.40	0.02	0.25±0.04***	-0.02±0.06	0.02
5	2-jul-13	0.64±0.04***	0.07±0.06	0.16	0.24±0.07*	-0.15±0.11	0.20
6	9-jul-13	0.46±0.05***	0.42±0.07***	0.76	0.94±0.04***	0.22±0.06**	0.60
7	24-jul-13	0.47±0.04***	0.64±0.06***	0.92	0.20±0.08*	-0.21±0.11	0.25
8	7-aug-13	0.26±0.13·	0.91±0.19***	0.71	0.62±0.06***	0.34±0.09**	0.62
9	20-aug-13	0.62±0.11***	0.57±0.15**	0.59	0.54±0.04***	0.20±0.05**	0.59
10	25-sep-13	0.47±0.06***	0.54±0.09***	0.79	0.57±0.03***	0.15±0.05*	0.48
Nanophytoplankton							
1	28-jul-11	0.13±0.08	0.11±0.11	0.13	0.29±0.22	-0.08±0.28	0.02
2	10-aug-11	0.24±0.05**	-0.01±0.06	0.01	0.05±0.14	-0.30±0.22	0.24
3	9-sep-11	0.00±0.15	-0.16±0.26	0.09	-0.23±0.28	-0.12±0.39	0.09
4	26-sep-11	-0.03±0.18	0.06 ±0.25	0.01	0.36X±0.28	0.15±0.16	0.12
5	2-jul-13	0.82±0.13***	0.27±0.19	0.22	0.65±0.07***	0.33±0.12*	0.54
6	9-jul-13	0.71±0.12***	0.80±0.20**	0.62	0.77±0.16***	-0.29±0.23	0.14
7	24-jul-13	0.81±0.07***	0.77±0.12***	0.82	0.79±0.19**	-0.33±0.24	0.16
8	7-aug-13	1.04±0.09***	0.35±0.12*	0.45	1.27±0.06***	0.27±0.08**	0.56
9	20-aug-13	0.95±0.10***	0.31±0.12*	0.40	0.27±0.18	0.42±0.23.	0.26
10	25-sep-13	0.69±0.06***	0.30± 0.09**	0.52	0.42±0.04***	0.16±0.06*	0.44

The estimated specific growth (μ d^{-1}) and grazing mortality rates (*g* d^{-1}) resulting from the regression analyses were used to calculate mean values. In the present study the estimates from all experiments were considered, including those experiments in which grazing mortality rates were low (not significantly different from zero) or even negative (c.f. Latasa 2014, Landry 2014). For the control experiments it can be argued that including negative values in the mean value compensates for

experiments where rates were overestimated (Landry 2014), while leaving out low estimates results in an overestimation of the grazing mortality rates (Latasa 2014). For the mussel treatments however, the occurrence of negative grazing mortality rates for both pico-and nanophytoplankton, indicated by a positive slope, occurred on a regular basis (Table 4.2). The most likely explanation for the occurrence of these positive slopes is the excretion of pseudofaeces by mussels. Pseudofaeces are relatively large particles, consisting of silt and algal cells, loosely bound in mucus. At the start of the dilution experiments pseudofaeces were diluted accordingly, but algal cells bound in pseudofaeces were not enumerated by the flow cytometer due to the large size of the aggregation. During the 24 hour incubations these algal cells were released from the pseudofaeces and were now counted. This resulted in a proportional increase in algal cells with the fraction of unfiltered water, and thus in a positive slope. Since the release of phytoplankton cells from pseudofaeces underestimates the grazing mortality, large positive regression coefficients (>0.20) were set to zero when calculating mean values.

To test for differences in growth and mortality rates in natural sea water and mussel-filtered water, paired t-test were used. A significance level of α=0.05 was applied. Statistical analysis were performed in R version 2.14.1 © 2011 The R Foundation for Statistical Computing.

Results

Mussel filtration experiments

In ten experiments Wadden Sea water was exposed to mussel filtration, resulting in the removal of plankton biomass (Table 4.1). Both the duration as well as the number and size of mussels added, differed between the experiments. This resulted in a different grazing pressure exerted on the plankton community for each experiment. The biomass removed was not equal for all plankton groups. On average, filtration by mussels led to a negligible removal of bacterial biomass and a relatively small but variable (average 20%) amount of picophytoplankton, while the removal of nanophytoplankton, HNAN and ciliates biomasses was substantial with on average 50-60% of available biomasses removed. Total phytoplankton biomass, given as chlorophyll, was removed by half. Besides filtering plankton, mussels also reduced the concentration of other suspended matter like debris and silt, resulting in a higher under water light availability (data not shown). Algae react rapidly to changes in the underwater light climate and more light penetrating the water column might result in a reduction of the light harvesting pigments like chlorophyll (Perry *et al.* 1981). The occurrence of this so-called photo-adaptation was investigated in this study by comparing the net growth for the picophytoplankton fraction of chlorophyll with picophytoplankton cell counts in mussel filtered water after a 24 hour incubation

period. The lower net growth rate measured using chlorophyll was considered proof of photo-adaptation. Therefore, in this study, chlorophyll as a proxy for phytoplankton biomass is considered an unsuitable parameter to study the effect of mussel filtration and results regarding chlorophyll will not be discussed further.

Dilution experiments

After filtration by mussels both mussel filtered ("mussel treatment") water and unfiltered ("control") water were serially diluted and incubated for 24 hours (Figure 4.2 & Table 4.2). The goal of this dilution experiment was to detect changes as a result of mussel filtration in both the specific growth rate (μ) and grazing mortality rate (*g*) for the different plankton functional groups within the microbial food web.

The microbial community in natural sea water

The results from the dilution experiments performed for the control treatments (natural sea water) provide specific growth (μ d^{-1}) and grazing mortality rates (*g* d^{-1}) of the plankton groups of the Dutch Wadden Sea for the study period (July through to September). The specific growth rate varied between 0.09-0.99 per day (average

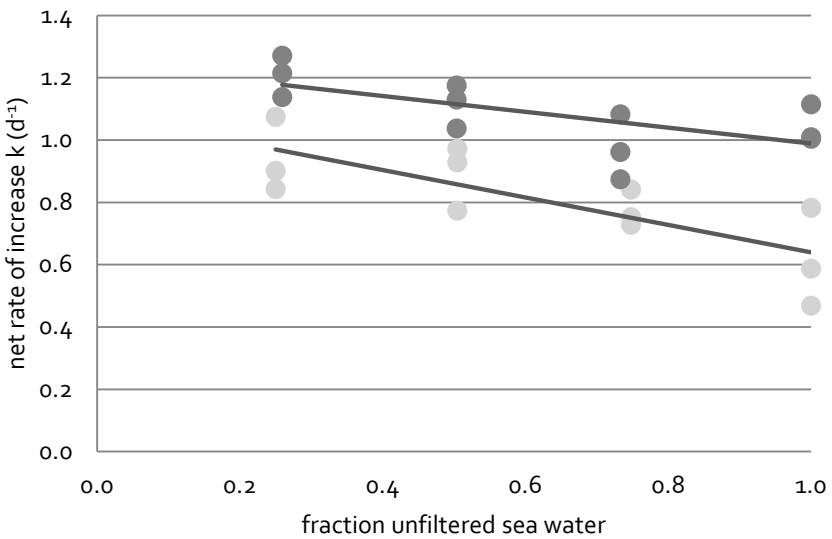

Figure 4.2 *An example of a dilution experiment to establish the specific growth and grazing mortality rate. The dark symbols indicate the change in cell concentration in 24h in the mussel treatment; the lighter symbols indicate the change in the control (natural sea water). On the y-axis the net rate of increase (k: μ-g f_i) is given as the natural logarithm of the change in cell concentration in 24h (1/24 ln (N_t/N_o). The x-axis denotes the fraction unfiltered sea water, 0 indicating 100% filtered sea water and 1.0 indicating 100% undiluted water. Regression of the net increase on the fraction unfiltered water gives an estimate for the specific growth rates (μ d^{-1}, the intercept) and the grazing mortality (g d^{-1}, the regression coefficient).*

0.65±0.37) for bacteria, between -0.09 and 0.64 per day (average 0.33±0.24) for picophytoplankton and between -0.04 and 1.04 per day (0.54±0.41) for nanophytoplankton (Table 4.2). The average μs for bacteria and nanophytoplankton are both higher than the μ for pico-sized phytoplankton.

Grazing mortality rates varied between 1.08 and 1.22 per day for bacteria (average 1.15±0.06), between -0.06 and 0.91 per day for picophytoplankton (average 0.45±0.31) and between -0.16 and 0.80 per day for nanophytoplankton (average 0.28±0.31). During the study period, there was an average net increase per day in predators of 0.65 ±0.28 for HNAN and 0.31±0.61 for ciliates in the control experiments (Figure 4.3a). For bacteria and picoplankton, grazing mortality exceeded the production during the study period, while for nanophytoplankton production exceeded grazing losses (Figure 4.4a). Nanophytoplankton daily specific growth and grazing mortality rates showed a seasonal pattern, with both rates decreasing from July to September.

Effect of mussel filtration

Specific growth & grazing mortality rates

Mussel filtration affected the plankton groups considered in this study differently. For bacteria the average specific growth rate (μ) almost doubled (0.65 to 1.16 day^{-1}) 24 hours after mussel filtration ($t = 3.84$, $df = 3$, $p = 0.031$), while the average μ for both pico-and nanophytoplankton did not show significant differences between the control and mussel treatments (pico.: $t=0.08$, $df=9$, $p=0.94$, nano.: $t=0.46$, $df=9$, $p=0.66$) (Figure 4.3).

Grazing mortality rates (g d^{-1}) showed the opposite pattern as the specific growth rates: for bacteria, after mussel exposure, the grazing loss rate (g d^{-1}) did not differ significantly from the rate measured in the control treatments ($t = -1.19$, $df = 3$, $p = 0.32$), while the remaining pico- and nanophytoplankton after mussel exposure did experience a lower grazing mortality rate (pico.: $t=4.24$, $df=9$, $p=0.0022$, nano.: $t=1.86$, $df=9$, $p=0.096$) (Figure 4.3). For the predators on bacteria and phytoplankton, HNAN and ciliates, only net growth rates (μ-g d^{-1}) were established. In the current study, mussel filtration resulted in an increased net growth rates after 24 hours for HNAN, while for ciliates the differences in net growth rates between the control and mussel treatments were not significant (HNAN: $t=3.75$, $df=4$, $p=0.02$, Ciliates: $t = 0.99$, $df = 4$, $p = 0.38$) (Figure 4.3).

Biomass

HNAN are considered the main predator of bacteria, and although mussel filtration in this study resulted in a substantial reduction of HNAN biomass (Table 4.1), this did not reduce the grazing mortality for bacteria. Instead, within 24 hours after mussel filtration the specific growth rates of bacteria increased, resulting in a substantial

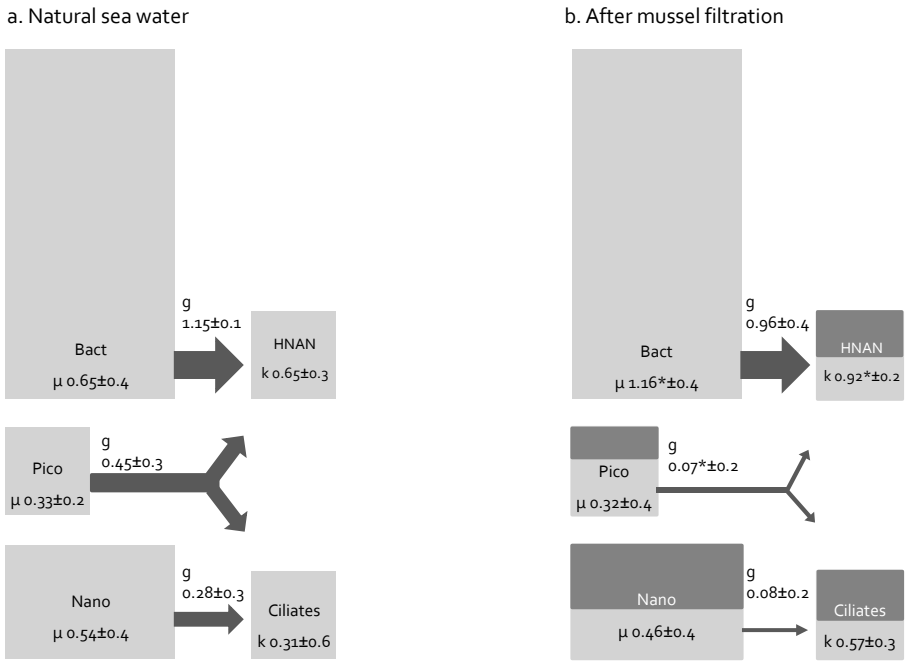

Figure 4.3 *Microbial food web structure in natural sea water (a, left) and after mussel filtration (b, right). Box sizes are indicative for average biomass (μg C l⁻¹) in the Dutch Wadden Sea during the study period for each functional group. For bacteria, pico-and nanophytoplankton in each box the average specific growth rate (μ) per day is given, the arrows indicate grazing mortality rates (g) per day. For HNAN and ciliates the net change in biomass k (μ-g) per day (± stdev) is given. Average biomasses removed by mussels during the experiments are indicated by the dark boxes. Significant changes in rates after mussel filtration are indicated with an asterisk (*).*

increase in bacterial production (Figure 4.4). HNAN increased their net growth rate and within 24 hours their biomass returned to pre-filtration concentrations (Figure 4.4). During the 24 hour incubations HNAN concentrations were lower in the mussel treatment, but carbon specific ingestion rates for HNAN on bacteria were higher; the fast growing HNAN in the mussel treatment had a carbon specific ingestion rate of 37 (μg C μg C^{-1} d^{-1}) compared to a rate of 22 in the control treatments. Picophytoplankton reacted differently to mussel filtration; the removal of a large part of their main predators, the HNAN, by mussels did result in a reduced grazing mortality rate (*g* d^{-1}). This reduced grazing mortality enabled picophytoplankton to recover to pre-filtration concentrations within a day (Figure 4.4). For nanophytoplankton a similar pattern can be seen, but since mussels removed more nanophytoplankton biomass compared to picophytoplankton, after 24 hours the nanophytoplankton biomass did not recover to pre-filtration levels. Ciliates, assumed to prey on nanophytoplankton and HNAN, also did not return to the biomass before mussel filtration (Figure 4.4).

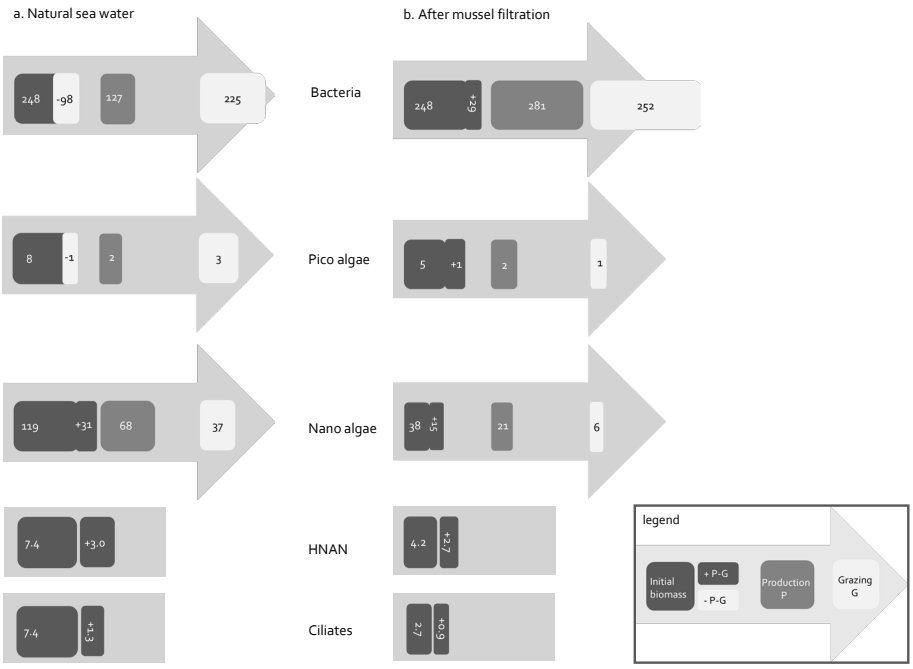

Figure 4.4 *Initial biomass (dark box left) and net change in biomass after 24 hours (+ and dark box right in case of an increase or white box right and – in case of a decrease). For bacteria, pico-and nanophytoplankton the net change is made explicit as the difference between production (grey box) and biomass removed by predators (white box in point of arrow). For HNAN and ciliates only biomass and net biomass change in 24 hours is given. All boxes are in µg C l^{-1}. Figure in the left panel (a) refer to natural sea water, the right panel (b) to the mussel filtration treatment.*

Discussion

The microbial community in natural sea water

Growth and grazing mortality rates vary in both time and space, making it difficult to compare rates between studies. The rates reported in the current study for the control experiments however fall within the range of growth and mortality rates reported before (e.g. Gallegos *et al.* 1996, Calbet & Landry 2004). In natural sea water, net growth rates (µ-g d^{-1}) for bacteria and picophytoplankton were positive on a few occasions, but for most experiments grazing rates exceeded the specific growth rates, resulting in negative net growth (Table 4.2 & Figure 4.3a). In ambient water, bacterial as well as picophytoplankton growth is expected to be balanced by HNAN grazing, resulting in net growth rates oscillating around zero. Although net positive or negative growth rates are commonly reported (e.g. Quevedo & Anadón 2001, Pearce *et al.* 2011, Schmoker *et al.* 2013), it is difficult to determine whether the net growth rates in the current study are part of the expected oscillation or an artefact due to the experimental set-up (Del Giorgio *et al.* 1996, Dolan *et al.* 2000).

The impact of mussel filtration on the microbial community

Mussels removed a negligible amount of bacteria from the water column, but impacted on bacteria indirectly; the increased specific growth rate for bacteria reported in this study are most likely the result of the mussel excretion products. Bacterial production is known to be stimulated by the excretion of particulate and dissolved organic matter (Azam *et al.* 1983). HNAN concentrations, substantially reduced as a result of mussel filtration, responded in the 24 hour incubation period with increased ingestion and growth rates. Both ingestion as well as growth rates are known to increase with increasing food concentration before levelling off at saturating concentration following a Monod response (Heinbokel 1978 and references herein). The simultaneous increase in bacterial specific growth rates and HNAN ingestion and growth rates after mussel filtration thus suggests a tight coupling between HNAN and bacteria as their main prey (Figure 4.3). The lower HNAN concentrations during the 24 hour incubation in the mussel treatments combined with a higher ingestion rate, ultimately resulted in a larger part of bacterial carbon being removed (Figure 4.4). Since only the net growth rate was established (μ-g d^{-1}) it cannot be excluded that also a lower predation pressure experienced by the HNAN, due to the slow recovery of ciliate biomass (Figure 4.3 & 4.4) contributed to the fast recovery of HNAN biomass. A reduced HNAN mortality due to the removal of larger zooplankton (e.g. copepods) cannot be excluded, but is considered less likely since juvenile mussels are inefficient predators on larger zooplankton (Horsted *et al.* 1988).

Next to bacteria, picophytoplankton are also considered prey for HNAN and although HNAN biomass was reduced substantially after mussel filtration, the rapid recovery was expected to enhance grazing mortality rates for picophytoplankton. Instead, grazing mortality rates decreased significantly over a period of 24 hours (Figure 4.3). In natural sea water, bacterial and picophytoplankton biomasses removed by predators seem more or less proportional to their availability (Figure 4.4a). Mussel filtration reduced picophytoplankton concentration with approximately 20 percent in this study (Table 4.1) and increased bacterial production. This caused a 'dilution' of picophytoplankton cells, resulting in a lower encounter rate of predators with picophytoplankton prey and hence lower predation rates. After mussel filtration the remaining picophytoplankton biomass continues to grow with a comparable specific growth rate as before filtration, but since the grazing mortality rate was reduced, there was a consequential increase in picophytoplankton biomass (Figure 4.3 & 4.4). After 24 hours, picophytoplankton biomass recovered to pre-grazing concentrations. For nanophytoplankton a similar pattern can be seen, but since mussels removed a larger part of nanophytoplankton biomass, nanophytoplankton biomass did not recover to the pre-filtration level within one day (Figure 4.4). In the filtration experiments, both the duration of the experiments as well as mussels biomass added differed between experiments, this resulted in a different mussel grazing pressure for each experiment. This means that changes in both specific growth and grazing mortality rates reported attributed to mussel grazing

should be regarded as qualitative rather than qualitative changes since mussel predation pressure was not standardised. At the same time, the differences in mussel grazing pressure in the current study, allow for a first analysis between this mussel grazing pressure (G_p) and a recovery rate of the plankton community. It is hypothesised that there is a relation between the biomass of predators removed by mussels and the net growth rate of prey. In the current study it was found that in those experiments where a larger part of predator biomass was removed, the difference in grazing mortality rate of prey between the mussels and the control treatments was larger. HNAN biomass correlated with the change in picophytoplankton mortality rate (r= -0.52), and ciliates correlated with the change in nanophytoplankton mortality rate (r=-0.84). For bacteria the number of experiments was too small to calculate a relation. Filtration pressure by mussels is thus an important parameter determining the ultimate effect on the microbial food web.

Conclusion

Results from this study show a size-selective removal of plankton by (juvenile) mussels resulting in relative changes in the different functional groups within the microbial food web. In the experiments plankton were exposed to mussel seed filtration for a short period, after which the mussels were removed again. The measured effects, 24 hours after this exposure, are the result of physical removal by filtration as well as chemical changes due to excretion products. The most important effect of the single exposure to mussel seeds was a stimulation of the bacterial-HNAN pathway, most likely due to excretion of DOM by mussels. Furthermore, picophytoplankton recovered faster than nanophytoplankton after mussel exposure due to reduced grazing losses by mussels. Results from the current study revealed the direct as well as indirect effects of mussel exposure on the pathways within the microbial food web over a short period of time. Longer-term effects might include a shift from bacteria to picoalgae production due to complete remineralisation of mussel excretion products by bacteria and a stimulation of primary production due to increased growth conditions (more light and recycled nutrients). Whether HNAN biomass will continue to increase depends on the ability of ciliates to recover and control HNAN biomass. Recovery of ciliate biomass in turn might result in a further reduction of already low concentrations of nanophytoplankton. In the current study the plankton community was exposed to mussel grazing for a single episode only. Continuous exposure to mussel grazing will likely change the outcome since mussels will effectively remove most HNAN and ciliate predators. High or continuous grazing pressure might for example result in a dominance of bacteria or picophytoplankton. Future experiments on the effect of bivalves on the microbial food web lasting longer than 24 hours and with variable grazing exposures might be able to give more insight in the possible effects of filtration on an ecosystem level. Several authors have stressed the need for research on the effect of bivalve filtration on the structure and composition of microbial food web (Murrell & Hollibaugh 1998, Calbet

& Landry 2004, Trottet *et al.* 2008a, Greene *et al.* 2011). To our knowledge, this is the first study describing the short-term effect of mussel filtration on the different components of the microbial food web. The results from this study describe changes in growth and grazing mortality rates within the microbial food web induced by mussel filtration. With these changed rates subsequent modifications in carbon flow though the food web were calculated. The results from this study allow for a better description of the direct and indirect effects of juvenile mussel filtration on the Wadden Sea food web.

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Chapter 5

The impact of introduced juvenile mussel cultures on the pelagic ecosystem of the western Wadden Sea, the Netherlands

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Abstract

To better protect natural mussel beds in the Wadden Sea, the Netherlands, harvest from pelagic collectors will completely replace fishing for juvenile mussels by 2020. In this study the impact of mussel filtration on plankton was assessed. We combined the results of an experiment, in which the passage and subsequent recovery of plankton were mimicked, with model calculations on filtration pressure.

For the experiment, natural plankton were exposed to short-term (hours) mussel filtration after which mussels were removed and the plankton community (bacteria, pico-and nanophytoplankton and ciliates) was allowed to recover for 8 days (the residence time in the area). Two days into the recovery period, net growth rates of bacteria, pico-and nanophytoplankton increased compared to the unfiltered (control) treatment, after 8 days the differences in growth rate had disappeared. At the end of the recovery period bacteria biomasses were not different compared to before filtration, while picophytoplankton biomasses were generally lower. Both nanophytoplankton and ciliates were able to balance losses due to mussel filtration by increased growth in September.

We estimated that the collector mussels exerted a maximum daily filtration pressure of 3.2 percent of the water volume. Collector mussels, in the 4 months between settlement and harvest, consumed 8 percent of all carbon produced, when filtration on heterotrophic organisms was included total carbon consumed doubled. The finding that juvenile mussels remove heterotrophic plankton and the expectation that mussel filtration will impact higher trophic levels through an effect on microzooplankton, justify the inclusion of heterotrophic plankton in future research.

Keywords:
juvenile, mussel, culture, *Mytilus edulis*, autotrophic, heterotrophic, plankton, Wadden Sea

Introduction

In the Wadden Sea, a shallow estuarine system, benthic bivalves are considered key components of the ecosystem (e.g. Verwey 1952, Dankers & Zuidema 1995, Piersma *et al.* 2001), with the blue mussel (*Mytilus edulis*) as one of the most abundant species in terms of biomass (Dekker 1989, Beukema & Cadée 1996, Dekker & Waasdorp 2007). In the Wadden Sea the mussel population consists of four groups, besides natural intertidal beds and subtidal beds, culture lots occur in the subtidal region (Dankers & Zuidema 1995), the recent introduction of pelagic mussel seed collectors added a fourth group. Pelagic collectors are ropes or nets that provide suitable settlement substrate for mussel larvae. After settlement in June juvenile mussels grow to a size of ~25 mm in October when harvest takes place. Both the decrease in the supply of juvenile mussels harvested from natural beds as well as governmental policy regulations to protect these beds, led to the introduction of artificial mussel collectors. The policy aim is to upscale the number of collectors to a yearly harvest of 40M kg of mussels from these collectors by 2020 (Meijer *et al.* 2009). Collectors improve the survival of mussels, raising concern about the carrying capacity of the area. In this study, we describe the potential impact of these collectors on the plankton in the Wadden Sea.

Filtration by mussels results in depletion of plankton at a local scale. Whether depletion will be detectable on larger spatial or temporal scales depends partly on the density of filter feeders and the residence time of an ecosystem as well as on the production capacity of the primary producers or, in other words, whether grazing losses can be balanced by growth (Dame & Prins, 1998). In systems where primary production is limited by nutrient availability, enhanced recycling of nutrients by grazers can result in increased growth rates of primary producers (Newell 2004); these enhanced growth rates might be large enough to compensate for loss of biomass through grazing. In these nutrient limited systems compensation mechanisms might thus result in little or no change in phytoplankton biomass and even an increase in primary production might occur (Caraco *et al.* 1997). In turbid systems, where primary production is expected to be light limited, light attenuation largely depends on non-phytoplankton suspended matter (Caraco *et al.* 1997). Although filter feeders generally remove these particles from the water column, an increase in light availability and thus the potential for phytoplankton to increase their growth rate, depends largely on re-suspension of these particles (Caraco *et al.* 1997). Thus the ultimate response of primary producers to filtration might also depend on whether phytoplankton production is limited by light or nutrient availability (Filgueira *et al.* 2015). Some evidence for this can be found in reported changes in both phytoplankton biomass and primary production as a result of changes in bivalve biomasses. For example, Alpine & Cloern (1992) reported a decrease in both chlorophyll and primary production after the invasion of a clam (*Putamocorbula amurensis*) in San Francisco Bay, USA, while in the same system, 15 years later, a decrease in

total bivalve biomass coincided with an increase in chlorophyll and primary production (Cloern *et al.* 2007). San Francisco Bay is a turbid, nutrient rich system, with primary production limited by light (Alpine & Cloern 1988, Cloern & Dufford 2005). In other systems where primary production was limited by available nutrients such as Grande-Entrée Lagoon, Canada (Trottet *et al.* 2007) or Narragansett Bay, USA (Oviatt *et al.* 2002), an increase in bivalve density was related to an increase in primary production, while chlorophyll concentration did not change (Trottet *et al.* 2008b, Doering *et al.* 1986).

The response of a system to filtration might be complicated by the effect of bivalves on heterotrophic plankton. Heterotrophic plankton can serve as an important food source for bivalves (Kreeger & Newell 1996, Wong *et al.* 2003, Trottet *et al.* 2008a), and filtration can thus result in (local) depletion of heterotrophs like rotifers, heterotrophic nanoflagellates and ciliates. Since these heterotrophs are the main predators on bacteria and small phytoplankton, the removal of these predators by bivalves might alter the competitive outcome between small and larger algae. Since smaller algal cells are assumed to be better competitors for light and nutrients (Riegman *et al.* 1993) an increase in picophytoplankton abundance could be expected to occur under heavy filtration pressure. The few studies that included heterotrophic plankton when examining the effect of bivalves on the plankton community, found a decrease in microzooplankton as a result of bivalve grazing (Lam-Hoai *et al.* 1997, Pace *et al.* 1998, Trottet *et al.* 2008b, Froján *et al.* 2014). A decrease in heterotrophic nanoflagellates predators was suggested as an explanation of the increase in picophytoplankton (Cranford *et al.* 2009), while in other areas a decrease in nano-sized predators did not result in an increase of the picoalgae (Froján *et al.* 2014). The importance of gaining more insight in the effect of bivalve cultures on heterotrophic plankton has recently been recognised (Green *et al.* 2011, Froján *et al.* 2014). The aims of this study are to estimate the impact of filtration by pelagic juvenile mussels on the plankton in the Wadden Sea and to investigate the recovery potential of the different plankton groups after mussel filtration. To meet the aims we followed two approaches, first a mesocosm experiment was set-up. In this experiment natural sea water was exposed to mussel filtration for a few hours during several blocks in 2010-2011, after which the mussels were removed (grazing period). This set-up of the experiment intended to simulate the passage of a volume of water through a mussel collector. Subsequently the plankton community was allowed to recover for 8-9 days (recovery period), duration comparable to the average resident time in the Wadden Sea (Ridderinkhof *et al.* 1990). For bacteria, pico- and nanophytoplankton and, on some occasion, ciliates the response to mussel filtration was determined. In addition to the mesocosm experiment, simulating the small-scale effect of juvenile mussel filtration, a simple model was set-up to estimate the filtration impact when the aimed harvest of 40M kg mussel is realised.

Material and methods

In order to measure the response of the planktonic community to size selective removal by juvenile mussels, an experiment was performed in several blocks during 2010-2011. Natural sea water was incubated with mussels for a few hours after which mussels were removed and the plankton community could recover for a period of 8-9 days. In addition to these recovery experiments, a separate sampling programme in the Marsdiep area was set up between 2011 and 2013 to establish the most important environmental conditions during the mussel growing season. This sampling program included weekly measurements of temperature and light attenuation as well as the analysis of chlorophyll, picophytoplankton (defined here as 0.2-3 µm), nanophytoplankton (3-20 µm), heterotrophic nanoflagellates (HNAN, 2-20 µm, 2013 only) and ciliates (20-200 µm) concentrations.

Recovery experiments

Study animals

In 2010 and 2011, a small collector was placed in the Marsdiep (Figure 2.1, location 5: 'collector'). This collector consisted of filamentous ropes facilitating mussel settlement (Xmas tree ropes, Donaghys). After settlement around June, mussels increase in size up to ~25 mm when harvested in October. Mussel sizes used in this study were between 1.5 and 25 mm. The day before each incubation experiment, ropes with juvenile mussels were collected, transported in sea water and stored at 4 °C. At the day of the experiment mussels were acclimatised to ambient seawater temperature and pre-incubated.

After each experiment the number of mussels used, average length (±0.01 mm) and dry weight (dried at 60 °C for 48 h, ±0.1 mg) was established. Dry weight included both shell and flesh.

Set-up of the experiment

Juvenile mussels, on pieces of rope, were incubated in mesocosms (60-85 litres) in 2010 and 2011. On each date (Table 5.1) 4 or 5 mesocosms were filled with natural seawater by submersion and placed in the NIOZ harbour (Figure 2.1, location 4 'NIOZ Harbour'). Both before and after the incubation, complete mixture of the water was checked by comparing the readings of a fluorescence probe (microFlu, TriOS) at different depths. Two or 3 mesocosms were incubated with mussels, 2 remained without mussels and served as control. Mussel ropes were placed in the mesocosm, a rotator enabled gentle mixing of the water to avoid damage of the fragile microzooplankton community. The removal rate of phytoplankton biomass was monitored using a fluorescence probe. The incubations lasted 1-4 hours and were terminated before plankton depletion was expected to have occurred. This assumption was checked at the end of each experiment by verifying the linearity of ln (Fluo-

Table 5.1 *The mussel incubation experiment was performed in several blocks in the period 2010- 2011. In each block, n=2-3 mesocosms for the mussel treatment and n=2 for the control. The volume of water was 100 litres in 2010 and 60 litres in 2011. The exact water volume per mesocosm was determined at each experimental date and clearance rates were calculated based on the realised volumes. T=average water temperature for all mesocosms at the start of each block, temperature changes during the experiment were always within 0.5 degrees. Tchl= chlorophyll concentration and K_d=attenuation coefficient are start values averaged over all mesocosms including stdev. Exp. time =exposure time to mussels in hours, length=average shell length of mussels used in the mussel treatments and dw = total dry weight of mussels used in the experiments including stdev. Dry weight includes the shell, see methods. For the blocks 2-5 the weight of mussels and exposure time per mesocosm varied substantially and individual exposure time and dry weight of mussels are given separately. Mesocosms within each block are numbered m1-m3 with increasing dry weight applied. Finally the parameters determined per block are indicated as well.*

block	date	day nr	K_d (m^{-1})	Tchl (µg l^{-1})	T (°C)	exp. time (h)	length (mm)	mussels dw (g)
1	21-Jun-10	172	0.86±0.06	5.10±0.97	17	2.6	1.71±0.72	174.0
2	5- Jul-10	186	1.18±0.11	4.53±0.43	21	m1=3.5	2.84±1.88	30.6
						m2=2.5	3.37±1.93	57.5
						m3=1.5	3.34±2.43	92.7
3	19- Jul-10	200	1.28±0.19	4.68±1.05	19	m1=3.6	5.14±2.72	122.1
						m2=2.0	5.59±3.14	256.4
						m3=1.4	3.06±1.88	356.1
4	3-Aug-10	215	0.58±0.15	3.08±0.69	19	m1=0.9	7.96±3.31	70.1
						m2=0.5	7.15±2.21	217.5
						m3=0.3	6.44±2.99	288.2
5	16-Aug-10	228	0.86±0.06	5.02±0.70	19	m1=3.1	15.80±8.40	86.1
						m2=2.4	21.02±5.43	98.1
						m3=1.1	18.72±7.34	248.1
6	21-Sep-10	264	0.23±0.01	1.45±0.08	16	1.5	13.27±4.73	121.5±22.6
7	13-Oct-10	286	0.48±0.06	9.14±1.95	15	1.1	15.32±6.34	178.2±21.5
8	28-Jun-11	179	0.48±0.03	2.93±0.19	19	2.1	8.15±0.58	21.2±0.5
9	12-Jul-11	193	0.85±0.01	4.83±0.08	19	2.5	11.81±0.48	15.1±1.0
10	27-Jul-11	208	0.89±0.03	4.18±0.22	18	3.1	13.49±0.07	32.5±0.4
11	9-Aug-11	221	0.66±0.04	2.36±0.08	15	3.2	17.49±2.05	36.4±1.3
12	7-Sep-11	250	0.80±0.01	3.83±0.63	17	2.8	19.92±0.17	48.2±1.1

rescence signal) over time. At the end of the incubation, mussels were removed and the mesocosms were closed off using a transparent lid in 2010. First results seem to indicate a phytoplankton bloom occurring in both the mussel treatments as well as in the controls, most likely due to the much higher average column irradiance compared to the average irradiance received by algae in the Wadden Sea (see discussion section). In 2011, lids of the mesocosms were covered with shading foil, reducing incoming light with 50%; average column irradiances were now more comparable to column irradiances in the Wadden Sea. In 2010, measurements were performed at the end of the recovery period for temperature, oxygen, salinity (±0.5 °C, Hach multimeter) and light attenuation (Wetlab CST), while samples were taken for total and fractionated chlorophyll, pico-and nanophytoplankton and ciliate cell counts. Mesocosm were then emptied, cleaned and stored for the next experiment. In 2011,

after mussel filtration was terminated and mussels removed, measurements were performed every day, while sampling took place every other day. In 2011, bacteria were sampled in addition to all other parameters.

Sampling programme

In 2011-2013, from April to the end of October weekly water surface samples were taken at location 3 ('NIOZ Jetty', Figure 2.1) to determine total and fractionated (<3 µm) chlorophyll, picophytoplankton (0.2-3 µm) and nanophytoplankton (3-20 µm) cell counts and ciliates. In 2013, at the same location additional samples were taken to perform heterotrophic nanoflagellates (HNAN 2-20 µm) cell counts. Temperature and light attenuation were measured as well. For location 5 ('collector', Figure 2.1) the same sampling program applied, but less frequently; every other week instead of weekly sampling. Average column irradiances (I_C, PAR µE m^{-2} s^{-1}) were calculated according to equation 5.1 (Riley 1957), assuming an average depth (z) for the western Wadden Sea of 4.1 meters. Daily surface irradiances (I_0, J cm^{-2}) were available from the Royal Netherlands Meteorological institute (KNMI) station De Kooy (http://www.knmi.nl/klimatologie) and were converted in PAR µEinstein using a conversion factor of 0.24. Attenuation coefficients (K_d, m^{-1}) were measured at location 1, since these were assumed to best represent the irradiance in the western Wadden Sea:

$$I_C = \frac{I_0(1-e^{-K_d z})}{K_d z} \qquad \text{(eq. 5.1)}$$

Bacteria

Triplicate subsamples (1 ml) for enumerating free-living bacteria were fixed with glutaraldehyde (0.5% final concentration), mixed and then stored at -80 °C until analysis. Analysis was always within one month. Analyses were performed using a flow cytometer (C6, BD Accuri, excitation with 488 nm laser), samples were diluted with 10% TE buffer to lower the count rate below 3500 events sec^{-1}, the maximum recording rate of the instrument. SYBR green I (Invitrogen) stain was added (0.1% final concentration) and samples were incubated in the dark for 15 minutes. The 530 nm laser (FL1) was used to detect the stained cells. The average diameter of particles was calculated after calibration of forward scatter (FSC) with size, using beads (3, 7 and 10 µm) (e.g. Li 1995). Cell diameters were converted in to volumes assuming a spherical cell shape, and volumes were converted into carbon according to: µg C per cell=0.326 volume $(\mu m^3)^{0.891}$ which is the average value for both the indicator and exponent from Verity *et al.* (1992) and Menden-Deuer & Lessar (2000).

Pico and nanophytoplankton

Phytoplankton cell counts were performed by means of flow cytometry. Water subsamples (1 ml) in triplicates were processed unfixed, immediately after collection. Fluorescence at wavelengths > 670 nm (FL3) was ascribed to chlorophyll. FSC was used as an indication of cell size. Based on the relative fluorescence to size, a dis-

tinction between phytoplankton and debris was made. Phytoplankton cell counts were further divided in two size classes (<3 µm: pico and 3-20 µm: nano) using 3 µm beads (spherotech, BD Accuri). The definition of pico- and nanophytoplankton is based on the particle size effectively retained by mussels (Møhlenberg & Riisgård 1978). A minimum cell count of 1000 per size class was applied. To convert pico-and nanophytoplankton cell counts into carbon biomass, the same formulas were used as for bacteria. Picophytoplankton cell counts converted to carbon yielded very low concentrations, suggesting an underestimation of picophytoplankton flow cytometry cell counts. Using the <3 µm fraction of chlorophyll and a fixed conversion factor of 26 g C/ g chl (Greider 1987) yielded 7 to 20 times higher picophytoplankton biomasses. It was decided to use carbon based on chlorophyll rather than based on cell counts.

Heterotrophic nanoflagellates (HNAN)

HNAN were counted using flow cytometry applying a slightly modified protocol from Rose *et al.* (2004). Modifications included the use of a smaller volume (4 ml) and a higher final concentration of Lysotracker® Green (75 nM, Molecular Probes). A flow rate of 100 µl min^{-1} and core size of 40 µm was selected. Counting time was 15 minutes. 3.0 µm beads were used for volume and size reference. To convert cell counts to carbon biomass the same procedure was applied as for bacteria and phytoplankton.

Ciliates

For enumeration of ciliates one subsample (0.5-1 litre) was fixed in 2-4 ml acid Lugol and stored in brown glass bottles at 4 °C until analysis. Samples were concentrated (10-20 x) using the Utermöhl sedimentation technique (Verweij *et al.* 2010). Per sample a minimum of 100 individuals was counted or, at very low abundances all individuals in a maximum of 10% of the concentrated sample were counted. Ciliate cells were counted and divided in 5 length classes (<20 µm, 20-40 µm, 40-60 µm, 60-80 µm and >80 µm), using an inverted microscope. An oblate spheroid ($4/3\pi\, ab^2$) best represented the average shape of ciliates (Putt & Stoecker 1989). Using the middle of the length class/2 as *a* and the middle of the length class/4 as *b*, calculated cell volume was converted into carbon using 0.326 volume $(\mu m^3)^{0.891}$.

Chlorophyll

For the determination of total and fractionated chlorophyll, duplicate subsamples (200-300 ml) were filtered over Whatman GF/F and 3.0 µm polycarbonate filters using low vacuum pressure (max -0.4 bar). Filters were stored in the dark at -80 °C for no more than 2 months. Chlorophyll was extracted by homogenisation of filters in 90% acetone with the addition of glass pearls and the concentration of chlorophyll was determined fluorometrically (Holm-Hansen *et al.* 1965) using spinach chlorophyll-a (Sigma) as a reference.

Light absorption ratio

This ratio, measured as the spectrophotometric absorbance ratio at 480 nm (carotenoid) and 665 nm (chlorophyll) can be used as an indicator of the nutritional status of the phytoplankton populations (Riegman & Rowe 1994). A ratio <1 is an indication that the phytoplankton community is growing light limited, a ratio > 1.5 indicates nutrient limitation while 1<ratio<1.5 indicates dual limitation by both light and nutrients. The absorption was determined in the acetone extraction described in material & method section 'chlorophyll'. The light absorption was corrected for corrected for background absorbance, measured at 750 nm.

Grazing losses & recovery rate

During the grazing period, mussels were incubated in natural sea water. The grazing losses (*Gp*) due to mussel filtration were calculated according to:

$$G_P = \left(\frac{N_{c,0} - N_{m,0}}{N_{c,0}}\right) \qquad \text{(eq. 5.2)}$$

where $Nc_{,o}$ and $Nm_{,o}$ are the concentration of cells at the end of the grazing period in the mussel treatments and control respectively. Concentration in the mesocosms before grazing did not differ significantly for each block.

After the grazing period, mussels were removed from the mesocosm and this marked the beginning of the recovery period. The recovery period lasted 8- 9 days. Based on the changes in concentration of the different plankton groups a net growth rate (k d^{-1}) was calculated after 2 days and at the end of the recovery period according to:

$$k = \left(\frac{1}{t}\right) ln \left(\frac{N_t}{N_0}\right) \qquad \text{(eq. 5.3)}$$

where t is the duration of the recovery period (2 or 8-9 days), N_o and N_t are the concentration of a plankton group directly after the grazing period and at day t during the recovery period respectively.

The recovery rate (R_R, d^{-1}) is defined as:

$$R_R = \left(\frac{1}{t}\right) ln \left(\frac{N_{m,t}/N_{c,t}}{N_{m,0}/N_{c,0}}\right) \qquad \text{(eq. 5.4)}$$

where $N_{m,t}$ and $N_{c,t}$ represent the concentration of cells at day t in the mussel treatments and control treatments respectively.

A model approach

To assess the potential effect of the upscaling of mussel seed collectors to a harvest of 40M kg fresh weight, a simple, zero dimensional, model was set-up to estimate the cumulative filtration pressure of these juvenile mussels.

Input came from field measurements performed from June (settlement) to October (harvest) in 2011 on shell length and weight of settled mussels, the number of juveniles that occupied the ropes and the relation between length and clearance rate (see Chapter 2 &3 for a detailed description of methods).

The shell length of mussels (l_t, mm) on the collectors for each day of the growing season (from settlement day to harvest day) can be described by the equation:

$$l_t = a\,(t - t_0) \qquad \text{(eq. 5.5)}$$

where a= 0.21 (mm d^{-1}), t is the day number and t_o is the settlement day on the collector. The ash free dry weight (afdw) (w_t, mg) on each day during the growing season could be related to length according to:

$$w_t = b\left(\frac{l_t}{l_{ref}}\right)^c \qquad \text{(eq. 5.6)}$$

where b = 0.007 (mg), c = 3.1 (-) and l_{ref} is a reference length which was set equal to 1.

The harvest from the rope collectors is aimed to be 40M kg fresh weight by 2020, this weight is first converted to afdw, using a conversion factor of 0.14 (Chapter 2). This afdw at harvest is divided by the individual afdw at the day of harvest to estimate the number of mussels present. A constant mortality rate (m) per day was calculated according to:

$$m = -\left(\frac{1}{t - t_0}\right) ln\left(\frac{n_h}{n_0}\right) \qquad \text{(eq. 5.7)}$$

where n_h is the number of mussels per unit of rope at harvest, n_o the number of mussels at settlement, t_o the settlement day and t_h the harvest day.
Using this mortality rate, the number of mussels at each day during the growth season was calculated according to:

$$n_t = n_0\, e^{-m\,(t - t_0)} \qquad \text{(eq. 5.8)}$$

where n_t is the number of mussels at day t. The volume of water cleared by an individual mussel (c_t, l d^{-1}) can be estimated assuming clearance rate to be proportional to shell length according to:

$$c_t = d\left(\frac{l_t}{l_{ref}}\right)^e \quad \text{(eq. 5.9)}$$

where d = 0.0004 (l d^{-1}) and e = 2 (-).

To estimate the clearance rate of mussels on each day the individual clearance rate was multiplied by the number of mussels present on each day. The total clearance rate on each day was then divided by the total volume of the western Wadden Sea ($4.66\ 10^{12}$ litres (Philippart *et al.* 2000) to come to the volume filtered daily by collector mussels.

Statistical analysis

All analyses were performed using the R free statistical software environment (R Development Core Team, 2011). A significance level of α=0.05 was used for all tests.

Results

Recovery experiments

In this study, experiments were performed in mesocosms (60-85 litre) filled with natural plankton communities. Some mesocosm were exposed to short-term mussel filtration, others served as controls. After filtration ended, mussels were removed and the plankton community was allowed to recover for 8-9 days. The experiment was performed during several blocks for two years during the period June to October. Throughout this period natural plankton communities changed and mussels increased in size, causing a large variability between the blocks in both mussels induced grazing losses (Table 5.2) and in response to grazing during the recovery period (Figure 5.1). Despite the large variability between blocks some clear patterns arose, with generally a larger part of available nanophytoplankton, ciliates and total chlorophyll removed compared to picophytoplankton and especially bacteria (Table 5.1 & 5.2). After the grazing period had ended the recovery period started. In 2010, samples were taken directly after the mussels were removed from the mesocosms and again only after 8-9 days, at the end of the recovery period. In 2011, samples were taken every other day during the recovery period, showing an alternating pattern of both increases and decreases in plankton concentration. During the recovery period net growth rates were calculated (μ-g) at days 2 (2011) and 8/9 (both years). Bacteria and picophytoplankton concentrations in most mussel treatments showed a peak on day 2, for nanophytoplankton a peak was seen for some of the blocks between day 2 and 4, while ciliate concentrations, determined on two occasions in 2011, showed a peak on day 4 (data not shown). These peaks in concentration are reflected in the net growth rates (μ-g d^{-1}) after 2 days for bacteria, pico-and

nanophytoplankton. Mussel grazing resulted in an increased growth rate of bacteria, pico- and nanophytoplankton after two days, while after 8/9 days this effect of grazing had disappeared (Table 5.3).

During the recovery period, for the control treatments, there was a general decrease in picophytoplankton concentration, an average slight increase in nanophytoplankton and a larger increase in total chlorophyll (Figure 5.1, C). In the mussel treatments, size-selective removal by mussels of the plankton resulted in large increases in both nanophytoplankton as well as total phytoplankton biomass (as chlorophyll) (Figure 5.1, MR). For picophytoplankton there was a decrease in concentration. Comparing the concentration of the different pelagic elements at the end of the recovery period with concentration before mussels grazing it can be seen that with regard to both pico-and nanophytoplankton the concentrations did not recover to the original concentrations before grazing, while total chlorophyll concentrations did (Figure 5.1, MT). When the changes in the control treatment are taken into account it becomes obvious that the mussel treatments lag behind in picophytoplankton ($t=3.12$, $df=34.47$, $p=0.0036$), nanophytoplankton ($t=2.79$, $df=22.38$, $p=0.01$) and chlorophyll concentration ($t=2.12$, $df=27.98$, $p=0.043$), since in the control treatments concentrations decreased less than in the mussel treatments (pico-

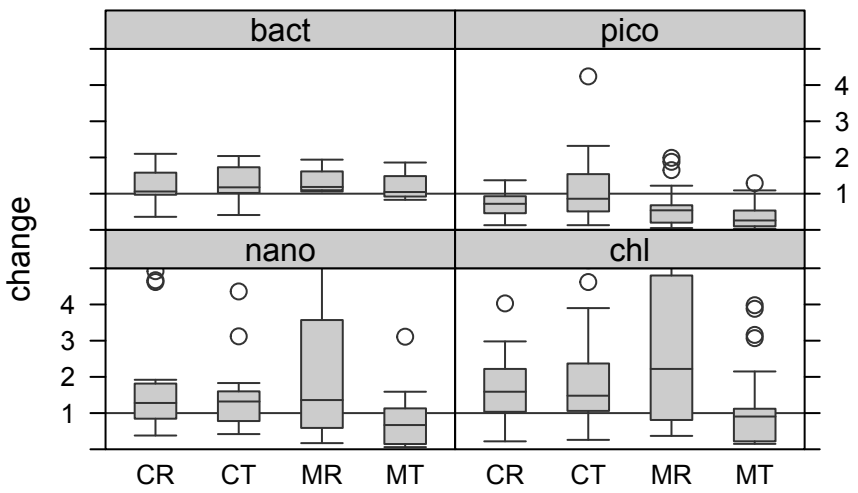

Figure 5.1 *Boxplots indicating the change in concentration during the recovery period plotted as a fraction of the concentration directly after mussel grazing for the control (no grazing) treatments (CR) and the mussel treatments (MR). CT and MT give the change in concentration during the recovery period as a fraction of the concentration before grazing. Note that for the control treatments the difference between before and after grazing is minimal. Changes are given for bacteria, pico-and nanophytoplankton and total chlorophyll. The horizontal line (value=1) indicates no change. Note that the variance between blocks was large especially for nanophytoplankton and total chlorophyll, due to scaling not all outliers are depicted. Data represent the response in both 2010 and 2011 together.*

phytoplankton) or even increased (nanophytoplankton and total chlorophyll). There were differences between the years (not shown) in the response of picophytoplankton during the recovery period; in 2010 in the mussel treatments the increase in concentration was large (up to 200% of concentration directly after grazing), while in 2011 there was a decrease. In 2010 there was a large increase in nanophytoplankton concentration in the control treatments during the recovery period.

Table 5.2 *The mussels grazing losses (eq. 5.2) on the plankton; bact=bacteria, pico=picophytoplankton, nano=nanophytoplankton and Tchl=total chlorophyll. Bacteria removal was measured for the blocks in 2011 only, x denotes that a change was not measured for that block. The shaded cells indicate that grazing losses were determined for these blocks, but concentrations were not determined for the recovery blocks.*

block	date	bact	pico	nano	ciliates	Tchla
1	21-Jun-10	x	0.50	0.65	0.68	0.59
		x	0.61	0.71	0.59	0.70
		x	0.66	0.79	0.69	0.65
2	5-Jul-10	x	0.19	0.37	x	0.20
		x	0.44	0.76	x	0.57
		x	0.45	0.71	x	0.64
3	19-Jul-10	x	0.64	0.76	x	0.72
		x	0.56	0.74	x	0.65
		x	0.53	0.71	x	0.75
4	3-Aug-10	x	0.40	0.65	0.62	0.62
		x	0.43	0.64	0.78	0.68
		x	0.30	0.60	0.69	0.60
5	16-Aug-10	x	0.08	0.18	x	0.62
		x	0.11	0.77	x	0.60
		x	0.24	0.44	x	0.56
6	21-Sep-10	x	0.49	x	0.51	0.55
		x	0.31	0.47	0.67	0.83
		x	-0.12	0.65	0.60	0.57
7	13-Oct-10	x	0.31	0.41	x	0.61
		x	0.35	0.38	x	0.60
		x	0.36	0.60	x	0.63
8	28-Jun-11	0.23	0.53	0.79	0.76	0.78
		0.23	0.55	0.78	0.69	0.75
		0.23	0.57	0.75	0.68	0.74
9	12-Jul-11	0.07	0.28	0.39	0.58	0.55
		0.08	0.31	0.54	0.53	0.50
		0.09	0.39	0.52	0.50	0.65
10	27-Jul-11	0.03	0.47	0.76	0.91	0.79
		0.04	0.45	0.74	0.34	0.75
11	9-Aug-11	0.11	0.23	0.84	0.59	0.79
		0.09	0.24	0.83	0.24	0.78
12	7-Sep-11	0.10	0.40	0.81	0.65	0.75
		0.11	0.41	0.80	0.82	0.79
	Ave ± stdev	0.12 ± 0.07	0.38 ± 0.17	0.62 ± 0.19	0.62 ± 0.15	0.65 ± 0.12

Table 5.3 *The net growth rate (d^{-1}) for bacteria, pico-and nanophytoplankton in both the control and mussel mesocosms, at day 2 and 8 during the recovery period. Day 2 net growth rates were only determined in 2011, for bacteria net growth rates were only determined in 2011. The t-test results from the paired t-test to detect difference between the two treatments are given as well, * denotes a significant difference between treatments.*

	control	mussel			
2 days	µ-g (d^{-1})	µ-g (d^{-1})	t	df	p
Bact.	0.10±0.18	0.28±0.14	3.62	4	0.02*
Pico	0.09±0.19	0.35±0.25	3.68	4	0.02*
Nano	-0.03±0.47	0.32±0.42	3.56	4	0.02*
8 days					
Bact.	0.01±0.08	0.04±0.03	1.01	4	0.37
Pico	-0.07±0.09	-0.05±0.17	-0.08	10	0.94
Nano	0.04±0.09	0.09±0.14	-1.15	10	0.28

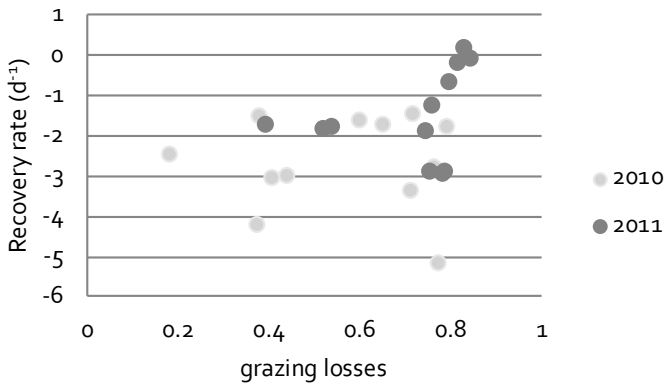

Figure 5.2a (top)
The relationship between losses due to mussel filtration and the subsequent recovery rate (d^{-1}) of nanophytoplankton.

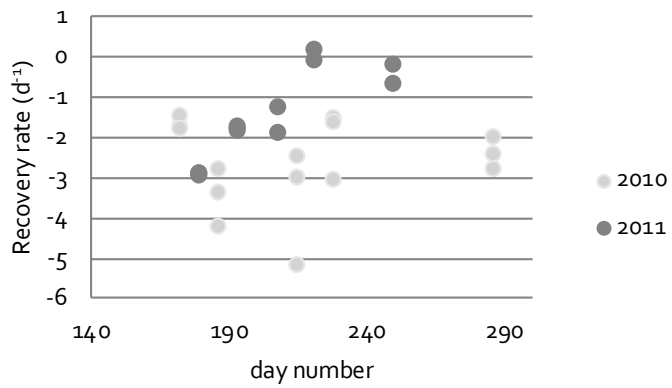

Figure 5.2b (bottom)
The relation between day in the year and the recovery rate (d^{-1}) of nanophytoplankton.

Next to the factor year explaining part of the variation in the response of plankton to mussel induced losses, other factors might attribute to the variation as well. During the grazing period, differences in mussel filtration induced losses of the different plankton groups could also be a source of variation (Table 5.2). For both nanophytoplankton and total chlorophyll the recovery rate was not correlated with the initial fraction of the population that was removed during mussel filtration (Figure 5.2a for nanophytoplankton, for chlorophyll not shown).

For 2011 there the recovery rate (d^{-1}) increased during the season (r=0.86, t=5.28, df=10, p=0.0004), while for 2010 this relation between recovery rate and time was absent. The faster recovery later in the season was not related with water temperature for both years.

Ciliate (as carbon) concentrations were determined before and directly after mussel filtration and at the end of the recovery period for 4 blocks (Figure 5.3, Table 5.2). In two blocks there was a large increase in ciliates carbon during the recovery period, while in the control the changes were small (exp. 6 & 12). This resulted in a higher concentration of ciliates after 8 days compared to the concentration before filtration by mussels. In the other two blocks the ciliates did not or hardly recover (exp. 4 & 9). The two experiments with a large increase in ciliates during the recovery

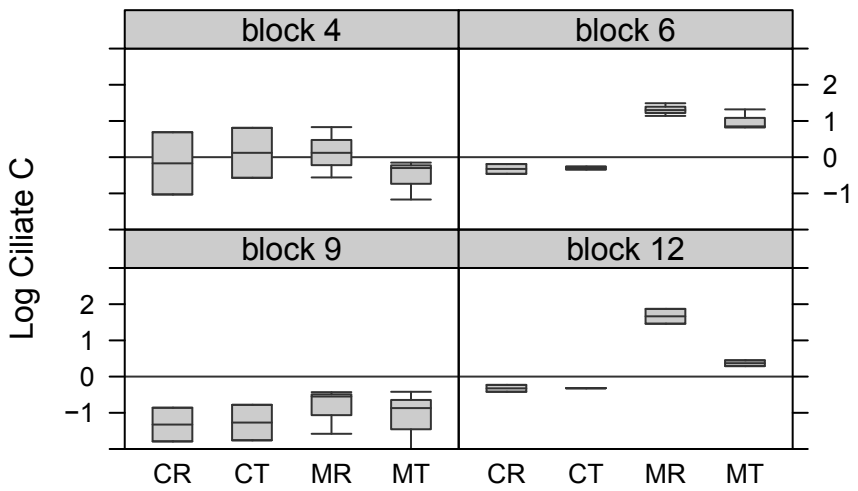

Figure 5.3 *Boxplots indicating the change in ciliate concentration during the recovery period plotted as a fraction of the concentration directly after mussel grazing for the control treatments (CR) and the mussel treatments (MR). CT and MT give change the during the recovery period as a fraction of the concentration before the grazing period. Changes in concentration are ^{10}log transformed for better visualisation of the results since the differences between the blocks were large. The line (value =0) indicates no change. Block 4=August 3rd 2010, 6=September 21st 2010, 9=July 12th 2011 and 12=September 7th 2011. Note that for the control there were only 2 mesocosms, for mussel treatments 2-3 mesocosms.*

period were both in September; the other two blocks took place in the beginning of August and mid-July respectively.

In 2010, the increase in nanophytoplankton concentration during the recovery period was larger than in 2011, this difference might be attributed to the higher column irradiances in the mesocosms compared to the irradiances received by algae in the western Wadden Sea in 2010. If the phytoplankton community receives a non-saturating amount of light, the primary production is assumed to be light limited (Tillmann *et al.* 2000), if these light limited algal cells are transferred from the Wadden Sea to a mesocosm, limitation might be relieved, resulting in a phytoplankton bloom. The average column irradiance in the western Wadden Sea (sampling location 5: 'collector', Figure 2.1) for all experimental dates was estimated (Table 5.4). For 2010 these estimated column irradiances were non-saturating for phytoplankton growth on all but one occasion (21th of June). Transferring the phytoplankton community to the mesocosm exposed them much higher irradiances and in 5 out of 6 experiments light levels now became saturating. In 2011 light reducing foil was applied to the mesocosm lids, reducing incoming light with 50%. In 2011 only one occasion light now became saturating after transferring the phytoplankton community from the Wadden Sea to the mesocosms (28th of June).

Table 5.4 *The average column irradiances (eq. 5.1) in the mesocosms (z=0.8 m) at the start of the recovery experiments as well as the average irradiance during the recovery period. For the experiment on the 21st of June 2010 no attenuation coefficient was determined at the end of the recovery period, for this date no column irradiance was calculated. The average column irradiances in the western Wadden Sea (sampling location 'collector, Figure 2.1) are also given for the day the experiments started. Light is considered saturating for phytoplankton growth when it is above 200 µE m^{-2} s^{-1} (Gieskes & Kraay 1975, Colijn 1982). Non-saturating levels are in italic.*

				average column irradiance (PAR µE m^{-2} s^{-1})				
				control		mussel		Wadden Sea
block	exp start date	day nr	recovery period (days)	start	recovery	start	recovery	start
1	21-Jun-10	172	9	522	-	550	-	295
2	5-Jul-10	186	9	387	415	474	481	*123*
3	19-Jul-10	200	9	390	381	536	424	*118*
4	3-Aug-10	215	8	363	292	422	359	*163*
5	16-Aug-10	228	9	289	272	342	301	*113*
6	9-Sep-10	264	8	*51*	245	*54*	244	*39*
7	13-Oct-10	286	9	*192*	158	*216*	*169*	*96*
8	28-Jun-11	179	8	*153*	253	*172*	265	*154*
9	12-Jul-11	193	8	*148*	*167*	*176*	*185*	*113*
10	27-Jul-11	208	8	*199*	*148*	257	*168*	*143*
11	9-Aug-11	221	8	*182*	*152*	220	*154*	*153*
12	7-Sep-11	250	8	*28*	*98*	*36*	*100*	*19*

The model approach

The mesocosm experiment showed that mussel filtration resulted in a measurable reduction of plankton within a few hours of filtration, with the losses being different for the different plankton groups (Table 5.2). It is generally assumed that mussels effectively retain particles larger than 3 µm and together with the observation that nanophytoplankton were not depleted during the grazing period, we assumed that the grazing losses experienced by nanophytoplankton corresponds to the volume water filtered by mussels. The volume filtered by mussels in the experiment varied between 44 and 84% (Table 5.2). In the following section the filtration pressure exerted by the collector mussels will be estimated.

The policy aim with regard to mussel seed collectors is to upscale their numbers to a harvest of 40M kg. A simple model, using the growth rate and clearance rates experimentally determined in 2011 to calculate the expected filtration pressure (Material & methods 'a model approach'). The result from this model exercise showed that at a maximum 3.2 percent of the water volume of the western Wadden Sea is filtered per day (Figure 5.4). The maximum filtration pressure in 2011 was reached according to the model on the 16th of August and lasted until the 27th of August. With a maximum volume filtered per day of 3.2 percent it would take the collector mussels 31 days to filter the entire volume. The average resident time of 9 days for the water in the western Wadden Sea (Ridderinkhof *et al.* 1990) divided by the time it would take the bivalves to filter the water volume results in a 'clearance efficiency' (Gibbs 2007) of 0.3.

Another way of examining the effect of mussel filtration is to compare the amount of carbon produced by mussels to the carbon produced by primary producers during the same period (Gibbs 2007). 40M kg fresh weight corresponds to 5.6M kg carbon.

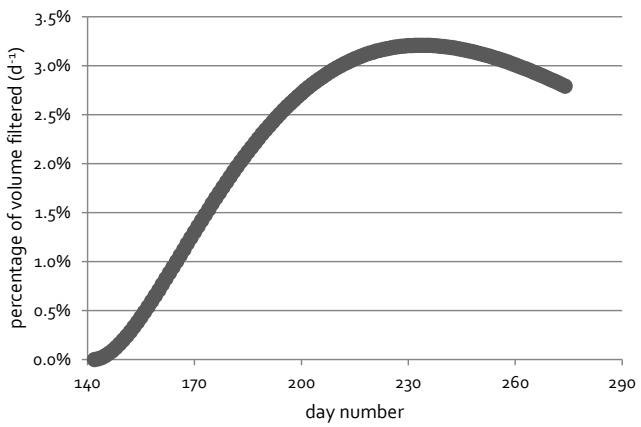

Figure 5.4
Estimated filtration pressure of 40M kilogram mussel (fresh weight) at harvest in percentage of the Wadden Sea volume filtered per day, from settlement day to harvest (day 274).

Assuming an assimilation efficiency of 0.4 (Bayne *et al.* 1993) the total amount of carbon consumed during the period mussels collectors are suspended in the Wadden Sea (days 140-274; end of May -the end of September) was 14M kg carbon. During that period the primary production in the western Wadden Sea was estimated at 183M kg carbon (Kamermans *et al.* 2014). Collector mussels thus removed about 8% of the primary production during the growth season. This rough calculation is based on the assumption that mussels mainly feed on autotrophs. Next to algae, juvenile mussels also remove heterotrophic plankton, ignoring this heterotrophic component underestimates the primary production removed. The degree of under estimation depends on the ratio of autotrophic and heterotrophic plankton removed by mussels during the growth season (Figure 5.5). During the time the collectors are suspended in the water the most abundant carbon source in the pelagic food web (of the groups investigated) shifts from nanophytoplankton towards heterotrophic nanoflagellates. Picophytoplankton are an abundant carbon source for a short period only (day 176-199). Using the clearance rate of mussels at each day and under the assumption that picophytoplankton is cleared by mussels from the water at half the rate of larger plankton (Chapter 3), it was calculated that at the end of the growing season 0.45 part of all carbon consumed came from heterotrophs and 0.55

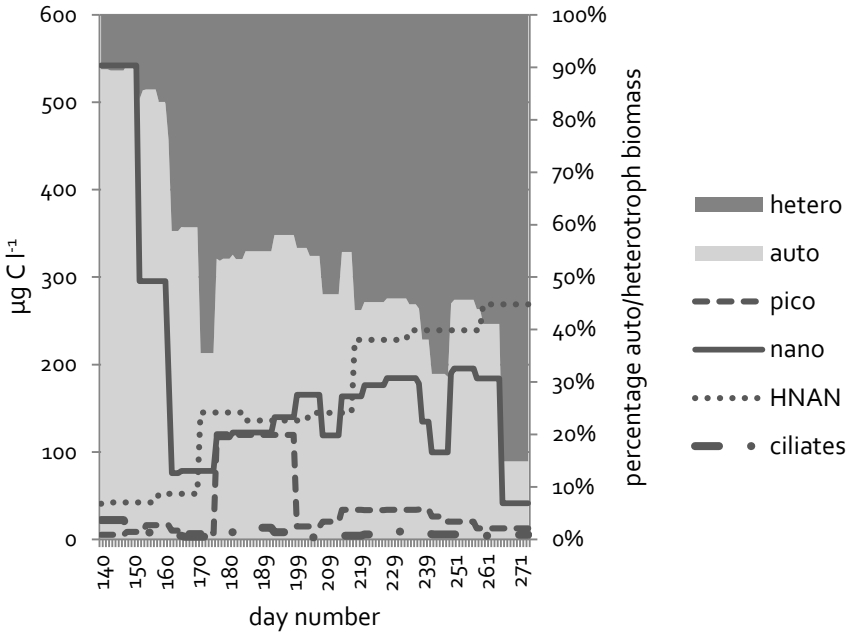

Figure 5.5 *The carbon concentration (µg C l⁻¹) in the western Wadden Sea (location 'NIOZ Jetty', Figure 2.1), measured once a week and extrapolated for the period from mussel settlement on the rope (day 140) and harvest (day 274). In the graph the ratio autotroph versus heterotroph biomass is also shown for this period, Carbon concentration was calculated based on cell counts for nanophytoplankton, HNAN and ciliates and on fractionated chlorophyll for picophytoplankton (see Material & methods section).*

from autotrophs. For each heterotroph 3 algal carbon units are needed (Calbet & Landry 2004). Instead of 8% of the primary production in the period between settlement and harvest, this method estimated that 15% of the carbon produced flowed towards the collector mussels.

Discussion & conclusion

Recovery experiments

Variation in the response of the plankton community to mussel filtration

The mesocosm experiment was performed in several blocks during 2010-2011 and the results show a large variation between the blocks in the response of nanophytoplankton, chlorophyll and ciliates to mussel filtration (Figure 5.1 & 5.3). Part of this variation might be explained by methodological factors. In 2010 the average column irradiances in the mesocosm were higher than the average column irradiances in the Wadden Sea and the higher light environment resulted in a phytoplankton bloom of mainly nanophytoplankton in both the control treatments as the mussel treatments. In 2011 incoming light for the mesocosms was reduced and the average column irradiances were now more comparable to irradiances in the field (Table 5.4) and phytoplankton blooms when occurred in the controls treatments were less intense. Additionally part of the variation between the different blocks of the experiment might be due to different biomasses of mussels incubated in the mesocosms for each block.

For nanophytoplankton it was investigated whether the total losses as a result of mussel filtration could impact the recovery rate during a period of 9 days, but no relation was found (Figure 5.2a). We did however find that the recovery rate was higher later in the season for the period June to September in 2011(Figure 5.2b). This was not correlated to water temperature, but there might be a relation with differences in phytoplankton light limitation during that period. Later in the year, column irradiances in the Marsdiep were lower ($r= -0.45$, $t= -4.22$, $df=70$, $p=0.00007$), implying light limited growth. In the mussel treatments removal of suspended matter by mussels (Table 5.4) resulted in higher column irradiances, stimulating growth of light limited algal cells. This effect of enhanced growth is thus more pronounced later in the season. The absence of a seasonal effect on growth enhancement in 2010, when column irradiances were higher still, might be due to phytoplankton biomass reaching a peak at an earlier day due to excessive light and collapsing by the time sampling took place at day 9. Additionally, nutrient limitation, which is likely to occur during the summer season, might be reduced due to excretion of dissolved nutrients. Since heterotrophic organisms also serve as a food source for mussels (e.g. Kreeger & Newell 1996), not only nutrients stored in algal cells are recycled, but nutrients originating from heterotrophic organisms are also released by mussels,

providing an additional source of nutrients. The contribution to total planktonic carbon in the Wadden Sea from heterotrophs increases from June to September (Figure 5.5), as does the contribution of heterotrophs in water filtered by mussels. Hypothetically, more nutrients might be available for autotrophs later in the season (Van Beusekom & De Jonge 2012).

Effect of mussel filtration on the pelagic food web

For the control treatments, the changes in the plankton community show a general pattern during the recovery period; in all experiments there is a lower ciliate carbon concentration at the end of the 8-9 day period, a lower abundance in picophytoplankton and no change in bacterial abundance (Figure 5.1, CR & CT). There was no shift in ciliate size observed during the recovery period (data not shown), so ciliate mortality is assumed to be the cause of the decline in ciliate carbon. A lower abundance of ciliates might have caused a lower than before grazing pressure experienced by their main prey; the heterotrophic nanoflagellates (HNAN, concentration not determined) and nanophytoplankton. A lower predation pressure on HNAN could have resulted in an increased HNAN abundance, which in turn might have increased the predation rate on their prey, picophytoplankton and bacteria. The lower abundance in picophytoplankton at the end of the recovery period indicates that picophytoplankton specific growth rate was not high enough to compensate for losses, while for bacteria the specific growth rates might be in the same order as their loss rates, resulting in no net change in abundance. In chapter 4 a higher μ (d^{-1}) for bacteria compared to picophytoplankton was recorded. In the control treatments there was, on average, an increase in nanophytoplankton concentration at the end of the recovery period. Nanophytoplankton in some of the experiments showed an increased net growth rate, but whether this was the result of decreased predation by ciliates or an increased growth rate as a result of increased nutrient availability remains unsolved. In two out of four experiments ciliates bloomed at the end of the recovery period after mussel filtration. On these dates there was also a high biomass of nanophytoplankton (MR) suggesting that the increased net growth rate was the result of enhanced nutrient availability not a reduced predation rate by ciliates. In August 2011 (block 9) ciliate biomass decreased during the recovery period, potentially lowering the predation pressure on nanophytoplankton, explaining the observed increase in nanophytoplankton biomass observed for that block.

During the recovery period the net growth rates for bacteria, pico-and nanophytoplankton were initially higher for the mussel treatments compared to the control (Table 5.3). This is an indication that mussel grazing resulted in either an increased specific growth rate or a decreased grazing mortality rate experienced by bacteria, pico- and nanophytoplankton. From experiments performed in 2011-2013 (Chapter 4) it was seen that for bacteria there was an increase in specific growth rate one day after mussel grazing. This increase might be attributed to the excretion of dissolved

organic material by mussels. For pico-and nanophytoplankton a decreased grazing mortality was reported, this possible was the result of a decreased abundance of HNAN and ciliates, their main predators. At the end of the recovery period this difference in net growth rates between control and mussel treatments no longer existed (Table 5.3). In the mussel treatments for two out of four blocks for which ciliate recovery was determined, a large increase in ciliate biomass was seen, while for the control treatments there was always a loss of ciliate biomass after 9 days (Figure 5.3). Based on the mesocosm experiment it might now be hypothesised that the organic carbon excreted by mussels ends up in ciliate biomass, especially later in the season. Since ciliates are the most important food source for copepods (Calbet & Saiz 2005) the introduction of mussels might indirectly enhance the food availability for copepods and thus for trophic levels that depend on copepods as food, like fish larvae.

The model approach

There are several ways to estimate the effect of a bivalve population on the pelagic system. In this study the potential effect of the mussel collectors was estimated using two different methods. Calculations based on total carbon assimilation of collector mussels during the growth season revealed that 8% of the primary production during that period was removed by collector mussels. Including the heterotrophic plankton component in the calculations resulted in an almost doubling of that percentage to 15%. It remains difficult to judge whether a certain percentage is substantial or not. Gibbs (2007) developed the concept of 'clearance efficiency', which is defined as the residence time of a given system divided by the time it would take the filter feeders theoretically to filter the complete volume of that system. In the western Wadden Sea (with an average residence time of 9 days (Philippart *et al.* 2000) and a maximum clearance time of 31 days, the clearance efficiency is 0.30. According to Gibbs (2007) this value suggests that "the present level of culture will be influencing other water-column processes in the area, but not controlling the phytoplankton dynamics." The maximum daily volume filtered refers only to the collector mussels. This filtration is in addition to all other filter feeders in the western Wadden Sea. The maximum filtration pressure of all benthic filter feeders is estimated to be between 0.11 per day (based on survey data 2000-2006 from Scholten *et al.* 2007) and 0.3 (based on model calculations Brinkman 2013). The estimated population of filter feeders thus need between 9 and 3.3 days to filter the entire volume, resulting in an estimation of the clearance efficiency between 1 and 2.7 respectively. According to Gibbs (2007) clearing efficiencies between 1.7 and 5.7 indicate "a control of the total shellfish population on phytoplankton dynamics" (Gibbs 2007).

A controlling role of filter feeders on the phytoplankton community suggests top-down control, but bivalve filter feeding might also include positive feedback mechanisms through nutrient recycling (Newell 2004). The effects of grazing by filter feeders is expected to be different in a system were phytoplankton growth is limited by light or nutrients (Cararo *et al.* 1997, Filgueira *et al.* 2015). It has been suggested that nutrient limitation can only limit primary production when there is no light limitation (Tillmann *et al.* 2000). However, since the phytoplankton community consists of several species, it seems more likely that multiple limitations are present within a community at the same time. To investigate whether at least some species might experience sub-optimal light conditions, it was calculated whether non-saturating conditions existed in the western Wadden Sea during the growth period of collector mussels. It is generally assumed that light is limiting phytoplankton growth when the daily irradiance is below 43 µE m^{-2} s^{-1} and light is assumed to be saturating for phytoplankton growth when daily column irradiance is above 200 µE m^{-2} s^{-1} (Gieskes & Kraay 1975, Colijn 1982). The number of days with saturating light levels in the western Wadden Sea was calculated (Table 5.5). The results for three consecutive years showed that daily column irradiance was at saturating levels only for a limit number of days between June and October (Table 5.5). During the time when collector mussels are present in the western Wadden Sea, phytoplankton production is thus limited by both light and nutrient availability. This is also confirmed by the light absorption ratio (480/665nm) (Riegman & Rowe, 1994). This ratio can be used as tool to investigate the limitation. A ratio below 1 is an indication that phytoplankton are exclusively light limited, while a ratio >1.5 indicates exclusive nutrient limitation (Table 5.5). Simultaneous light and nutrient limitation is reflected by ratios between 1 and 1.5. The ratio thus indicates that during the months when collectors are present in the Wadden Sea, phytoplankton is limited by light and nutrients in 2011 and 2012, while in 2013 light seems the main limiting factor. For 2010 no data on column irradiances and the light absorption ratio is available.

Table 5.5 *For 2011-2013 the average irradiance in the water column (I_c in PAR µE m^{-2} s^{-1}) (eq. 5.1) was calculated for the time of year mussels are present on the mussel collectors (days 140-274). The number of days during the mussel collector growth season that light is below the minimum as well as the number of days light levels were saturating for phytoplankton growth are given. Additionally the light absorption ratio of carotenoid/chlorophyll in algae is given. A ratio < 1 is an indication that phytoplankton are light limited, a ratio > 1.5 indicates nutrient limitation, while 1<ratio<1.5 indicates dual limitation by light and nutrients.*

year	light			ratio 480/665 nm
	nr of days above minimum	nr of days above saturation level	ave I_c (PAR µE m^{-2} s^{-1})	
2011	122	33	138±81	1.27±0.11
2012	128	14	126±59	1.21±0.12
2013	116	2	100±47	1.08±0.09

Conclusions

In the Wadden Sea a harvest of 40M kg of juvenile mussels is aimed for by 2020. In this study we have estimated, using a simple model, the maximum daily filtration pressure of these collector mussels to be 3.2 percent of the water volume. Together with the filter feeder stock already present in the western Wadden Sea, total filtration pressure added up to 14 - 33 percent. In the time between settlement and harvest, the collectors mussels have consumed 15 percent of all carbon produced, including heterotrophic plankton in the calculation. An important question is whether the plankton community is able to recover after filtration by mussels. We therefore designed an experiment in which a natural plankton community was first exposed to mussel filtration for a short time after which the mussels were removed, simulating the passage of a water mass through a mussel collector. Subsequently, the plankton community was allowed to recover for 8-9 days, a period equalling the average resident time in the Wadden Sea. The results from the mesocosm experiment showed a different response for the different plankton groups, while the response also depended on the time scale considered. The short-term (2 days) recovery response demonstrated an increase in the net growth rates of bacteria, pico-and nanophytoplankton. Based on previous reported results (Chapter 4), it seems likely that for bacteria the short-term increase in net growth rate is the result of organic carbon released by mussels, providing substrate for bacteria. The short-term enhancement in net growth rates of pico-and nanophytoplankton were argued to result from lower predation pressure; the predators of phytoplankton were removed to large extent by mussels. After 8-9 days (long-term response) the differences in recovery rate between the control and mussel treatments had disappeared. Comparing the biomasses of the different plankton groups at the end of the recovery period to the situation before mussel filtration it was seen that bacteria biomasses were comparable, leading to the conclusion that mussel filtration likely will not impact bacterial biomass in the Wadden Sea. Picophytoplankton biomass generally decreased in response to mussel filtration, most likely this was an indirect effect of predator recovery, but their main predator, the heterotrophic nanoflagellates, were not assessed in the experiment. For nanophytoplankton and ciliates the response was more variable, with recovery to pre-filtration biomasses occurring mainly at the end of the season. It was hypothesised that later in the season (i.e. September) the phytoplankton community is more severely limited by light and/or nutrients. The (partial) release of these limitations through mussel filtration, either by the removal of suspended matter or by the excretion of dissolved nutrients, is a likely mechanism explaining this seasonal effect.

Feedback mechanisms enhancing phytoplankton growth rates as well as potential changes in the plankton community structure might be able to mask bivalve induced changes for some period of time, but eventually there will be a limit to the stimulating effect on phytoplankton growth rates, simply because the maximum growth rate is achieved. Changes in the heterotrophic plankton community are

likely to occur and it is therefore advisable to monitor the plankton community as well as changes in growth rates of especially phytoplankton. For nanophytoplankton, ciliates and total chlorophyll, the recovery to pre-filtration biomasses was very variable. Considering however the increase in biomasses in the control treatments for both nanophytoplankton and chlorophyll it is obvious that 9 days after mussel filtration removed part of the phytoplankton biomass, algal biomasses lag behind the biomasses in the control treatments. It seems that organic material excreted by mussels end up in ciliate biomass, especially at the end of the season. The finding that juvenile mussels remove heterotrophic plankton and the expected effects of filtration on higher trophic levels through an effect on microzooplankton, warrant the inclusion of these organisms in future research.

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Chapter 6

Synthesis

Pascalle Jacobs

Synthesis

This thesis studied the growth of mussels after settlement on pelagic collectors, their clearance rates on several plankton components and how this filtration activity affected the pelagic food web. The study was part of a research project on the use of pelagic collectors as a measure to protect natural mussel banks in the Dutch part of the Wadden Sea. In this synthesis I will discuss the results presented in the previous chapters, with special focus on mussel population ecology.

Settlement & recruitment

Bivalve population growth and recruitment is limited by both density dependent and density independent factors. Mechanisms that can result in density dependent mortality are for example resource competition (food or habitat), aggregative predation and diseases, while factors like egg and larval drift, water temperature, and winter storms regulate recruitment in a density independent way (Bos *et al.* 2007, Le Pape & Bonhommeau 2013). Total egg production of bivalves generally increases proportionally with adult stock density (Honkoop & Van der Meer 1997), while an obvious stock-recruitment relation is lacking. This suggests a density-dependent mortality in at least one of the pre-recruitment stages; from egg to pelagic larvae to settled larvae to recruit (Figure 6.1, Figure 6.2, Shepherd & Cushing 1980, Bos *et al.* 2007). At the same time, for species with a high fecundity, small eggs and no parental care, the early life stages (eggs, larvae and juveniles) are characterised by a high and variable mortality rate, making the survival of these early stages vulnerable to environmental factors. As a consequence, a small change in mortality rate means a large difference in the number of individuals that will recruit into the adult population (Le Pape & Bonhommeau 2013). The inter-annual variability in mortality of the juvenile stages tends to obscure the stock-recruitment relation (e.g. Van der Meer *et al.* 2001). During the life cycle of the bivalve *Mytilus edulis* several stages can be distinguished (Figure B1.1 & Figure 6.1), where for each stage different factors influencing the survival. Understanding pre-recruitment mortality will improve the understanding of population dynamics of bivalves (Andresen *et al.* 2014). In the next section, I will discuss per life stage whether the chance of survival to the next stage is likely to be determined by density dependent or independent factors.

The survival from egg to pelagic larva is influenced by drift of eggs, water temperature, predation and cannibalism by adults. Predation on eggs and pelagic larvae is high, but is thought to be independent of the size of the adults stock, with different predators occurring in the pelagic and the benthic environment. Mortality due to cannibalism is thought to be density dependent and it is considered a significant mortality factor for pelagic larvae (Lehane & Davenport 2004) (Figure 6.2B). After hatching, larvae start feeding which could result in food competition with other larvae but also between larvae and adults, although the optimal food particle size for

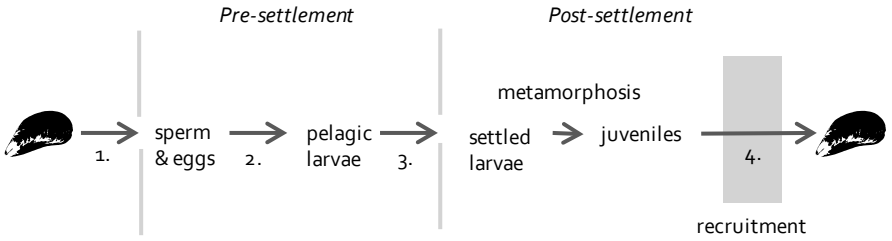

Figure 6.1 *The different stages in the life history of a mussel. During each stage (indicated with a number) there are several factors that influence the survival until the next stage, the most important factors are discussed in the text. 1. Adult spawning results in a large number of eggs and sperm. 2. Eggs hatch into pelagic larvae. 3. After several weeks in the water column, pelagic larvae develop into larvae ready for settlement in the benthic area, after settlement larvae metamorphose changing their feeding mode from a velum to gills (see Box 1), individuals are now called post-settlers, plantigrades or juveniles. 4. The addition of individuals in a population or adult habitat after surviving to a practical moment in time is called recruitment, determined days to months after settlement.*

larvae is smaller than for adults (e.g. Riisgård *et al.* 1980). If food competition occurs, it can result in a higher mortality rate as well as in a lower development rate, exposing the larvae to pelagic predators for a longer period of time. Water temperature, also influences the development rate of larvae (e.g. Bayne 1965, Drent 2002, Honkoop *et al.* 1998, Pechenik *et al.* 1990) and might have the same effect as food competition in that it exposes larvae to predation for a longer period of time. Predation mortality can thus be high and variable depending on the time spent in the water column, which in turn is determined by both the density independent factor water temperature as well as the density dependent factor food competition (Figure 6.2C). Pelagic larvae eventually become ready for settlement in the benthic area, where suitable settling habitat includes existing mussel beds (e.g. Verwey *et al.* 1952). Le Pape & Bonhommeau (2013) assumed for fish larvae that the relation between the number of pelagic larvae and the number of benthic ('settled' in bivalves) larvae was density independent, while survival from settlement to recruit was density dependent (Figure 6.2B, C & D). Doherty *et al.* (2004) reported for a tropical coral reef fish species, that after settling from a pelagic habitat into a benthic habitat, early post settlement mortality was density dependent and the authors concluded that predation pressure at this life stage was structuring recruitment. Survival from settled larvae to recruit (Figure 6.2D) is often hypothesised to be density dependent due to competition for food and space with adults, removal of larvae by adult filtration and predation (Bos *et al.* 2007 and references therein, Fraschetti *et al.* 2013), but there is a large gap in knowledge on the actual processes operating in this life stage, since in most studies only recruitment is measured (Figure 6.1 & caption) such that settlement success and early post-mortality cannot be estimated separately (Van der Meer *et al.* 2001).

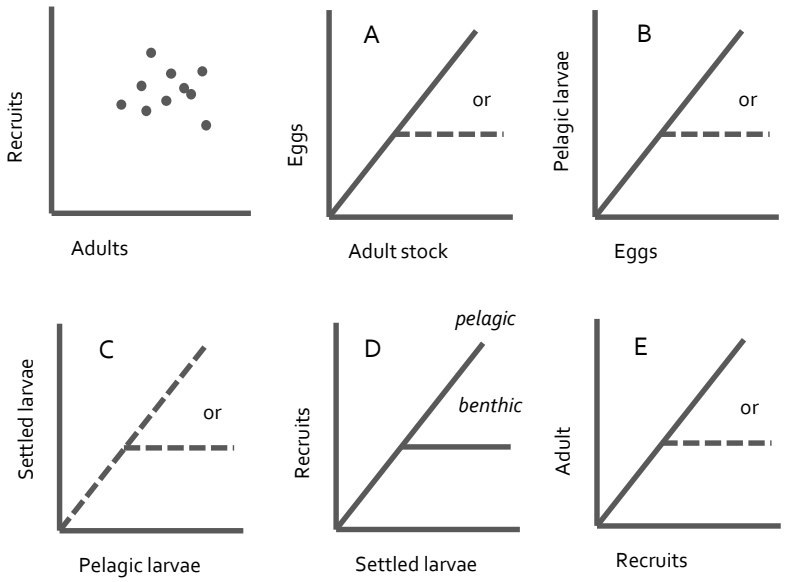

Figure 6.2 *The hypothetical relations between different stages in the life cycle of a mussel (see also Figure 6.1). For sake of simplicity two alternative relations for each two combinations of stages are indicated; the solid line is the most likely relation based on studies reported in literature, the dashed line indicated the alternative. See text for further explanation.*

In our study we found that for collector mussels the number of recruits was proportional to the number of settled larvae (Chapter 2 & Box 3). The absence of predators in the water column that feed on settled larvae is the most likely explanation, shaping the relation between post-settled larvae and recruit density from a likely density dependent to an independent relation (Figure 6.2D). Additionally, the placement of the collectors in a high food environment might have released the larvae from density dependent food competition playing a role in adult habitat. Towards the end of the growing season, clumps of mussels started to fall off the ropes, reducing the number of mussels per unit rope. This indicates that eventually competition for food or space is regulating the number of mussels a rope. At the end of this synthesis I will further discuss factors that have a dominant role in determining successful recruitment of bivalves in the Wadden Sea and how changes in the last decades have changed the relative importance of these factors.

Growth

Growth rates of mussels on collectors were determined and related to both pre- and post-settlement conditions (Chapter 2). After comparing two years of data, the hypothesis was formulated that inter-annual variations in food availability (as chlorophyll) during the time between hatching and settlement (larval or pre-settlement period) can explain differences in growth rates after settlement. Laboratory studies

(e.g. Philips 2002) showed the importance of environmental conditions during the larval phase in determining the post-settlement growth rates. The rationale behind the hypothesis is that when phytoplankton conditions available to larvae are prosperous, this will result in a high energy reserve at the time of settlement and subsequent metamorphosis. Since gill development takes place during metamorphosis, a higher energy reserve likely results in the development of larger gills or a better gill structure. Since gill area is expected to scale with filtration rate (Jones *et al.* 1992) a large gill surface area will thus result in higher filtration rates and more food filtered from the water per time unit compared to equal sized mussels with less developed gills. More food filtered per unit of time could result in a higher growth rate, leading to larger animals. With only two additional years of data (Box 3), the hypothesis could not be verified. However, this field of study warrants more research. Future experiments or field studies for example should include the condition of early-post settled larvae as well as other food sources besides phytoplankton both in terms of quantity and quality since in laboratory experiment these factors have proven to be of influence as well (Laing 1995, Wacker & Von Elert 2002, Phillips 2004).

In our study, water temperature and food concentrations after settlement could not explain the differences in inter-annual growth rates. Most studies on the influence of environmental conditions like water temperature and food concentration on the growth of mussels have been conducted on adult mussels (but see e.g. Bayne 1965, Sprung 1984). Laboratory experiments often indicate a positive relation between food concentration (phytoplankton) and growth rate and between water temperature and growth rate, while the results from studies performed in natural systems are more variable. For example Page & Hubbard (1987) reported a positive relation between growth rate and chlorophyll, but not with water temperature, while Kirby-Smith & Barber (1974) found a positive relation between growth rate and water temperature, but not with chlorophyll. Babarro *et al.* (2000) found both temperature and chlorophyll explaining a large part of the variation in growth rate and Fuentes *et al.* (1992) found the differences within rafts to be larger than between rafts, indicating an effect of food depletion within the raft. For the first three years (2010-2012) we did not find an indication of density-dependent growth for the mussels on the pelagic collectors (Chapter 2 & Box 3). For many species, including filter-feeders, there is a negative relation between density and growth rate, but mussels are able to re-orient their position to minimise food competition with their neighbours (Fuentes *et al.* 2000). In 2013, when the occupation of mussels on the ropes was extremely low, the highest growth rates were observed (Box 3) indicating inversely density-dependent growth at low densities and growth rate becoming density independent at higher densities. Additionally, pelagic collectors are placed in areas with relatively high current speed, so another explanation for the lack of relation between phytoplankton and growth rate is that there simple is no food limitation, while for benthic mussels, situated in locations with lower rates of water circulation and thus water replacement, food limitation might be a realistic scenario.

Box 3 Chapter 2 revisited

In chapter 2, the growth rates and the density of juvenile mussels on pelagic rope collectors was described for 2 years (2010 and 2011) and differences were related to environmental conditions pre- and post-settlement. In 2012 and 2013 additional data was collected. In this box the results from all 4 years will be presented and the conclusions and hypothesis posed at the end of chapter 2 will be revisited.

Pre-settlement: the importance of the larval stage

In chapter 2 it was shown that for 2011 the growth rate of juvenile mussels, for the period between settlement on the ropes and harvest 6 months later, was higher compared to 2010. The higher growth rate in 2011 was hypothesised to be the result of the higher food concentrations (as chlorophyll) experienced by larvae (pre-settlement) in 2011 compared to 2010. In 2012 and 2013 additional data was collected (Table B3.1), for 2012 the growth rate, in this thesis expressed as the daily increase in millimetre shell length, was 0.18. This rate is intermediate between the growth rates of 2010 (0.12) and 2011 (0.21). The average food concentration (as chlorophyll) during the larval period[1] was also in the midst of the concentrations found in the two previous years (Table B3.1, Figure B3.1), with a chlorophyll peak that was comparable to 2011. For the last year, 2013, the observed growth rate was the highest, with co-occurring high chlorophyll concentrations and the highest chlorophyll peak of all 4 years. However, after statistically analysing the data there was no significant correlation between the peak or the average chlorophyll concentration during the larval period and the growth rate (length or dry weight).

In 2011 the density of settled mussels per unit rope was higher compared to 2010. In chapter 2 it was hypothesised that the duration of the larval period, in other words how long it takes before larvae are ready to settle, is largely determined by water temperature. Since pelagic larval mortality is high, the longer the time spent drifting in the water, the lower the number of larvae that will survive until settlement. In 2013 water temperatures remained low for a long time in spring, resulting in mussels spawning only late in the season. With water temperatures remaining low, it lasted a long time before the first mussels settled. The numbers that eventually settled was the lowest for the 4 years investigated (Table B3.1). The numbers

1 In this study it was assumed that mussel spawning begins when water temperatures reach a minimum temperature of 10°C (Bayne 1965). The larval or pre-settlement period in this study is defined as the number of days between spawning and settlement on the rope collector. Settlement day was back-calculated using daily length growth assuming a length at settlement of ~300 µm (e.g. Sprung 1984a).

of settled larvae on the collector ropes might thus be positively correlated to pre-settlement water temperature. The duration of the larval period (in days) was found to negatively correlate with the number of settled mussels on the ropes (r=-0.98, t=-6.25, df=2, p=0.025). Note however that this correlation is based on four years only (Table B3.1).

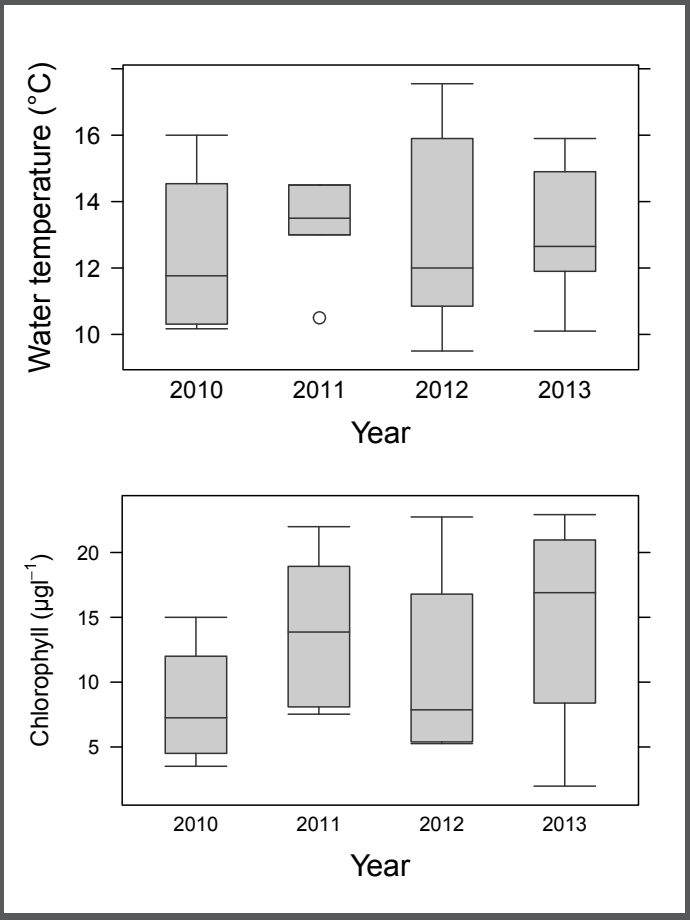

Figure B3.1
Box plots for water temperature (top) and chlorophyll concentration (bottom) for each year for the period between spawning (~10 °C) and settlement. The peak in chlorophyll is indicated by the upper whisker.

Post-settlement conditions

In chapter 2, the post-settlement conditions for 2010 and 2011 indicated a higher chlorophyll concentration in 2010 and no differences in average water temperature between the two years. It was therefore cautiously concluded that intra-annual differences in temperature and chlorophyll post-settlement could not explain the higher growth rate of the juvenile mussels in 2011. The additional data reinforce this conclusion (Table B3.1 & Figure B3.2). These data collected also indicated that 2013 was a completely different year compared to the previous three years; initially a very low number of mussels settled on the ropes and first settlement occurred only very late in spring. The growth rate of these initial settlers was exceptionally high (Table B3.1). After approximately 6 weeks new settlement occurred and throughout

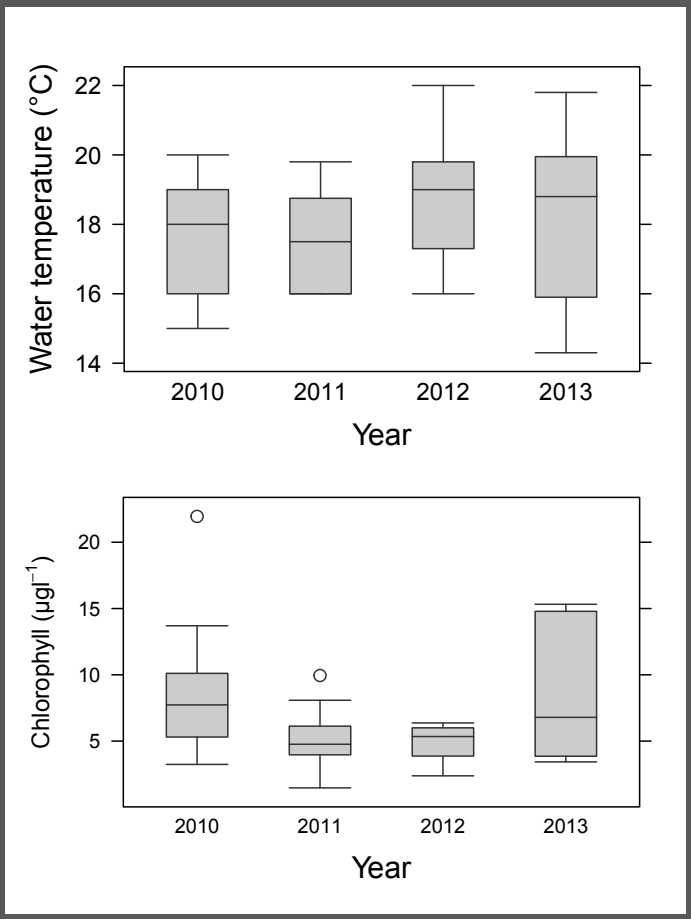

Figure B3.2
Box plots for water temperature (top) and chlorophyll concentration (bottom) for the period between settlement and harvest for the four years investigated. For the chlorophyll graph the upper whisker indicated the peak in chlorophyll concentration.

the season additional settlement was observed. Since the growth rate was calculated as the increase in average shell length, the growth rate per day approached zero in 2013. For the growth rate calculated in dry weight per cm rope this decrease was less pronounced since the weight of the new settlers was very low compared to the mussels already present.

The conclusions after 4 years of research is thus that pre-settlement condition are important for performance (e.g. growth rate) after settlement as well as for the number of larvae that survive until settlement. The influence of post-settlement environmental conditions on mussel growth rate is less clear. The conclusion from chapter 2 that growth rates were not density dependent was not supported with the additional data. In 2013 the very low number of settlers showed an initial higher growth, indicating some density dependence at least at low densities.

Table B3.1 *Main characteristics related to mussel growth are given for the years 2010-2013, differentiating between the pre-settlement (spawning to settlement) and the post-settlement period (settlement to harvest). The growth rate of mussels is given for shell length as mm day^{-1}, while for weight the growth rates relates to mg dry weight day^{-1}. In 2013 the initial number of mussels that settled as well as the initial growth rates is indicated with an asterisk (*). Additional settlement later in the season resulted in a higher average density on the ropes and lower average growth rate (length). For more detailed information see the Material and methods section of chapter 2.*

		2010	2011	2012	2013
Pre-settlement	First day of spawning	112	99	109	120
	First day of settlement	162	144	157	176
	Larval period (days)	50	45	48	56
	Ave temp (°C)	12.4 ± 2.7	13.3 ± 1.5	13.1 ± 3.0	13.1 ± 2.0
	Ave chl (µg l^{-1})	8.3 ± 5.0	14.1 ± 6.5	11.0 ± 7.2	14.7 ± 9.1
	Peak chl (µg l^{-1})	15.0	22.0	22.7	22.9
Post-settlement	Mussels (cm^{-1})	416	613	585	69*/478
	Growth rate (day^{-1}): length	0.12	0.21	0.18	0.50*/~0.0
	Growth rate (day^{-1}): weight	0.03	0.07	0.08	0.09*/0.03
	Ave temp (°C)	17.6 ± 1.9	17.5 ± 1.4	18.8 ± 1.7	18.1 ± 2.4
	Ave chl (µg l^{-1})	8.5 ± 4.9	5.1 ± 2.2	4.9 ± 1.5	8.5 ± 5.5
	Peak chl (µg l^{-1})	21.9	9.9	6.4	15.3

Clearance rates in natural sea water

When estimating the impact of mussels on the pelagic ecosystem it is important to have insight in the type of particles mussels remove from the water and at what rate. Most research on this subject has been conducted using adult mussels (but see e.g. Riisgård *et al.* 1980) under controlled conditions (but see e.g. Trottet 2008a). In chapter 3 clearance rates (R_C, l d^{-1}) of juvenile mussels were determined using natural sea water. In this chapter it was shown that for juvenile mussels the clearance rate scaled with squared shell length, as it does for adult mussels (Jones *et al.* 1992), but that the indices, a in the relation $R_C = a\ \text{length}^b$ was very low, resulting in clearance rates that were among the lowest reported in literature. These lower rates are partly explained by the use of natural plankton (Doering & Oviatt 1986). Nowadays, there seems to be consensus to consider filtration rates determined in controlled laboratory experiments using cultured algal species and low mussel densities as maximum rates, while clearance rates established under field conditions can be regarded as realised clearance rates (Cranford *et al.* 2011, Riisgård *et al.* 2014). Additionally, the lower clearance rates reported in chapter 3 could be due to the occurrence of depletion of algal cells at the scale of an individual mussel. Local depletion of food can result in re-filtration of the water and thus lower clearance rates. This study showed that the effect of re-filtration increased with mussel size (shell length). These variable 'community' clearance rates provide valuable input for models, calculating the

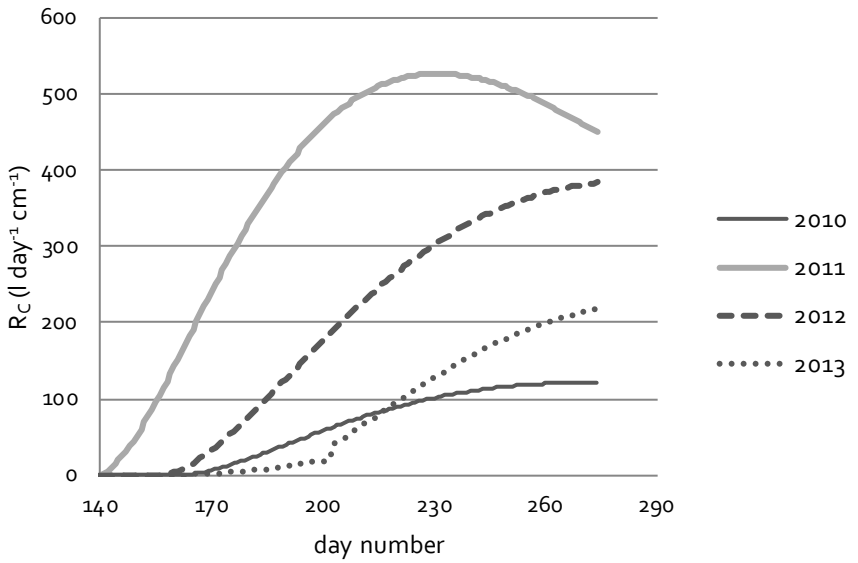

Figure 6.3 *The clearance rate (R_C) per centimetre collector rope (l day^{-1} cm^{-1}) determined as the volume of water cleared of nanophytoplankton per day, estimated based on mussel shell length per day, number of mussels per cm collector rope and the clearance rate at length relation experimentally determined. The clearance rates are estimated for the four years, from the day of settlement to the day of harvest. The model is described in detail in chapter 5.*

impact of mussel seed collectors form June to October when mussels grow from 0.3 to 25 mm shell length (Chapter 5). Combining the results on growth and density on collector ropes (Chapter 2 & Box 3) with community clearance rates (Chapter 3) an average clearance rate per unit rope was calculated for each day during the period the collectors were suspended in the Wadden Sea (Figure 6.3). Differences in both the density of mussels per unit rope and growth rates during the growth season resulted in different clearance rates between years (Figure 6.3). Variations between years in the clearance rate per rope were large and will influence the estimation of the total filtration pressure exerted by mussels on rope collectors (Chapter 5).

Using a simple model, we calculated for the year 2011 the daily percentage of the western Wadden Sea volume filtered by the mussels on collectors from the moment of settlement until harvest, using the 40M kg fresh weight as target. The estimated maximum daily percentage of the western Wadden Sea volume filtered was 3.2 percent. Together with the resident filter feeding population, the total volume filtered daily was estimated to be between 14 - 33 percent. For the collector mussels, in the time between settlement and harvest, it was calculated that they consumed 8 percent of all carbon produced. When the filtration of mussels on heterotrophic organisms was included in the calculations, the estimation of the total amount of carbon consumed almost doubled. In all other years for which data was available maximum daily volume cleared by the collectors was lower (Figure 6.3).

Impact on the plankton community

Biomass

Heterotrophic plankton like heterotrophic nanoflagellates (HNAN) and ciliates are important predators as well as recyclers within the marine microbial food web (Azam *et al.* 1983). Since juvenile mussels remove the planktonic heterotrophs at comparable rates as nanophytoplankton (Chapter 3 & 4), and since these heterotrophic organisms constitute a substantial part of the pelagic plankton in terms of carbon available to juvenile mussels (Chapter 5), the question was raised to what extent mussel filtration impacts the microbial food web. Results from dilution experiments, using mussel filtered as well as unfiltered water (control), showed that one day after mussel filtration had ended, the removal of both HNAN and ciliates by mussels resulted in a reduced mortality rate of their prey, especially for picophytoplankton. The results from the experiments also demonstrated a very high recovery rate of HNAN, most likely due to the increased specific growth rate of bacteria, an important prey for HNAN. The most plausible explanation for this boost in bacterial specific growth rate is the increased availability of substrate, dissolved organic matter, excreted by the mussels. Two days after mussel filtration the net growth rates of bacteria as well as pico-and nanophytoplankton were still higher compared to the unfiltered, control experiments, while after 8-9 days, at the end of the recovery period, these differences had disappeared (Chapter 5).

The expected effects of mussel filtration on the plankton community included increased growth rates of especially smaller phytoplankton as a result of nutrient release by mussels. If phytoplankton growth is limited by the availability of nutrients, the release of nutrients by mussels could enhance the specific growth rate. The fact that this effect was not found in the short-term (i.e. one day) experiments (Chapter 4) can be explained by the mechanism of a delay in carbon uptake after the limiting nutrient has become available, in favour of an increased nutrient uptake rate (Lean & Pick 1981). In the experiments where the plankton community was allowed to recover 8-9 days after mussel filtration, the picophytoplankton concentration generally decreased, indicating that if nutrient release caused an increase in specific growth rates, this was not high enough to compensate for losses through HNAN consumption (Chapter 5). The results from the recovery experiments (Chapter 5) indicated that within the timeframe of the average residence time of the western Wadden Sea, 9 days, both nanophytoplankton and heterotrophs were able to balance losses due to mussel filtration by increased growth on at least some occasions. The recovery rate was not related to the initial filtration pressure, but there were indications that the recovery rates were influenced by growth limitation of the phytoplankton community and the feedback mechanisms of mussel filtration partially relieving these limitations. The discussion presented in chapter 4 gave rise to the question whether mussel filtration not only changes planktonic biomasses, but also effects the recycling of matter through the microbial food web.

Recycling

Heterotrophic nanoflagellates (HNAN) and ciliates are the most important recyclers in a marine ecosystem (Azam *et al.* 1983). These organisms directly impact the flux of both inorganic nutrients and dissolved organic carbon by excreting these components during consumption (Taylor *et al.* 1985). Mussels, by removing part of the heterotrophic nanoflagellates (HNAN) and ciliate biomasses and storing carbon in their tissue, potentially reduce the pelagic recycling rates. At the same time, mussels also produce losses (pseudofaeces and faeces) and excrete nutrients (Newell 2004). The introduction of mussel collectors could thus potentially influence the cycling of pelagic carbon. Whether recycling rates will increase or decrease is not a question that can be answered at present, but to illustrate the potential change in recycling rates within the microbial food web some 'back on the envelope' calculation are provided (Box 4). In this example first the daily recycling by heterotrophic nanoflagellates and ciliates is estimated for a hypothetical litre of water. Then a mussel is added to this litre of water of such a size that this volume is completely filtered within one day. Since both HNAN and ciliates are retained with great efficiency (Chapter 3 & 4), their population is expected to be largely reduced and recycling will be solely performed by the mussel now. In this example total carbon recycling after the addition of a mussel to the litre of water is less than half compared to the recycling by HNAN and ciliates (Figure B4.1). It can now be hypothesised that in a situation in which filter feeders and the microbial food web co-exist, total recycling can even be

enhanced. The excreted organic material by mussels originating from both autotrophic as well as heterotrophic plankton provides a substrate for bacteria. As was seen in chapter 4, HNAN and to a lower extent, ciliates can responds rapidly by increasing their intake rate and therefore their growth rate. This 'recovery' mechanism allows them to compensate for mussel induced filtration losses and will enhance the recycling, since this is coupled to intake and growth rates (Dolan 1997).

Box 4 Recycling

Definitions

Total recycling is the sum of loss and excretion. In this example losses are defined as the total amount of organic carbon that is produced during sloppy feeding, incomplete ingestion and faeces from consumption. We assume the production of dissolved organic carbon by phytoplankton to be negligible (Strom *et al.* 1997). Excretion generally refers to the excretion of inorganic nutrients, but in this example excretion is defined as the amount of carbon excreted. Literature rates of nutrient excretion were converted into carbon units using the Redfield ratio.

Example

In 1 litre of Wadden Sea water, the heterotrophic carbon consists of heterotrophic nanoflagellates (HNAN) (7.4 µg) and ciliates (7.4 µg) (Chapter 4). Using an allometric relation between average cell size (Chapter 4) and maximum intake rate (Moloney & Field 1989) the daily intake rates for HNAN and ciliates are 22.8 and 9.4 g C /g C respectively. The total amount of carbon taken in by the heterotrophic community in 1 litre of Wadden Sea water is the sum of HNAN intake (169 µg) and ciliate intake (70 µg) (Figure B4.1). The losses are considered to be a fraction of intake, which are estimated at 0.52 and 0.36 for HNAN and ciliates respectively (Dolan 1997). The remaining fraction (0.48 and 0.64) gets ingested. Of the ingested material 15 (HNAN) and 30 (ciliates) percent gets excreted. The total daily recycling of HNAN and ciliates in a litre of sea water, the sum of losses and excretion, in this example adds up to 140 µg C (Figure B4.1).

Now imagine adding 1 mussel with a shell length of 10 mm to the litre of water. This size mussel has a clearance rate of 0.96 l d^{-1} (Chapter 3) and a carbon content of 8.7 mg (Chapter 4). The clearance rate * concentration of prey gives the total amount of carbon cleared from the water by this mussel. Prey items include nanophytoplankton, HNAN and ciliates and total

prey biomass was estimated at an average of 140 µg C l^{-1} (Chapter 3 & 4). The total amount of carbon taken in by a mussel is thus 134 µg (Figure B4.1). It is assumed that a fraction of 0.3 of filtered carbon is removed before ingestion as pseudofaeces (Bayne *et al.* 1993) and 0.7 is ingested (94 µg C l^{-1} d^{-1}). Of all carbon taken in, a fraction of 0.14 (13 µg C l^{-1} d^{-1}) is considered as loss (faeces) and the same fraction is excretion (Bayne *et al.* 1993). The total amount of carbon recycled daily in a litre of water is 66 µg, less than half of the carbon recycled by the microbial food web.

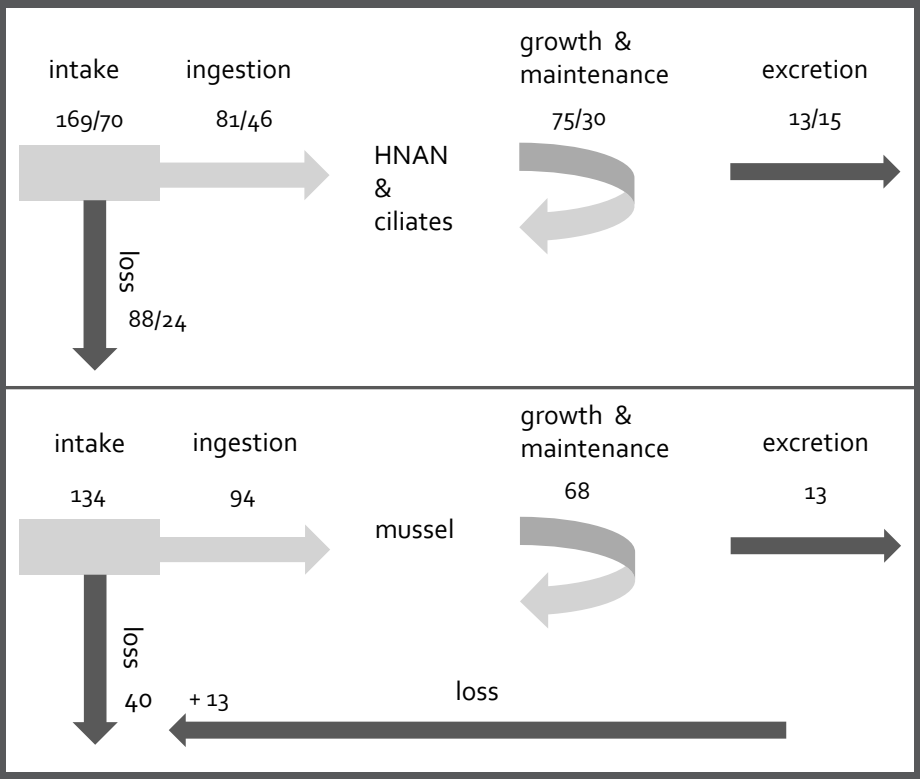

Figure B4.1 *A numerical example of the difference in recycling rate between a hypothetical situation with recycling solely occurring by the microbial food web (top) and a situation with recycling occurring solely by a mussel (below). For explanation of the rates see text. In the microbial food web, the first number refers to the rate of HNAN and the second to ciliates. Rates are in µg l^{-1} d^{-1}.*

Recovery of mussel beds

Population growth in most species is regulated by density dependent factors like predation, parasites or competition, while density independent factors, biotic and abiotic environmental factors are a source of large inter-annual variation especially for broadcast spawners like the bivalve *Mytilus edulis*. This density dependent regulation for bivalves as well as many marine fish species is thought to occur mainly in the juvenile stages (Shepherd & Cushing 1980, Bos *et al.* 2007). As was argued in a previous section of this synthesis ('Settlement & recruitment') for the natural *Mytilus* population in the Wadden Sea, density dependent regulation of the population seems to be most prominent in the transition from the settlement stage to recruitment (Figure 6.1), with predation as the main regulating factor. With the introduction of the pelagic collectors, settled mussels experienced much lower predation rates compared to their benthic counterparts, while density dependent food competition is prevented, either by the mussel farmers by thinning or by a self-thinning or dislodgement (Fuentes *et al.* 2000). For mussels in aquaculture, density dependent mortality is thus expected to occur at the stage from recruit to adult i.e. in the period between sowing of the juveniles on the culture lots until harvest as adults. The introduction of juvenile mussel collectors might thus change the life stage when density dependence will have an effect on the adult population from the settling and subsequent recruitment phase to the adult stage.

Historical developments in the Wadden Sea (Chapter 1) pointed at the replacement of oyster and possible *Sabellaria* reefs by mussels as well as the large scale introduction of mussel culture in the area. This resulted in an increased mussel density in the Wadden Sea by the 1970-s (Riesen & Reise 1982, Van der Veer 1989), with an estimate of 4000 hectares coverage of natural banks (Dankers & Fey-Hofstede 2015). It is likely that the population at that time was regulated by resource competition either for food or hard substrate or a combination of both. Harvest of a part of the juvenile mussel stock by both animal as well as human predators might have been compensated for by an increased growth and survival of the remaining mussels (compensatory regulation; Hunt & McKinnell 2006). Increased fishing activities for juvenile mussels since 1985 (Wolff *et al.* 2010) resulted in a higher loss rate, with predation now becoming the main determinant of the size of the mussel population. According to Hunt & McKinnell (2006) further removal of individuals by fishery can result in intensified per capita losses to predation with fished stocks being further depleted. In the 1990-s overfishing of the mussel stock resulted in the almost complete loss of this species in the area. In addition, repeated fishing of the same mussel beds likely damaged mussel habitat, reducing the system wide availability of suitable settling habitat (McGrorty *et al.* 1993, Dare *et al.* 2004, Van der Heide *et al.* 2014). The target for future coverage of mussel beds in the Wadden Sea is 4000 ha (Dankers & Fey-Hofstede 2015) and studies have indicated that this is a realistic target both based on historic coverage as well as on the ecologic space (carrying capacity) at present (Dankers *et al.* 2003, Brinkman & Jansen 2007). From the above

I would now like to hypothesise that in the past, density dependent competition for food most likely interfered with population dynamics in the adult stage, while in the present time suitable settling habitat might be the bottleneck for the recovery to the target coverage of stable mussel beds.

Conclusions & recommendations

- After 4 years of monitoring the abundances and growth rates of mussels on ropes collectors, large inter-annual differences were reported. As a result of this large inter-annual variation, placing a fixed number of collectors in the Wadden Sea might not always result in a targeted annual harvest of 40M kg of mussels. The large differences between years also has consequences for model estimates of the total filtration pressure of pelagic mussels in the western Wadden Sea.

- Clearance rates in this study were lower than commonly reported in literature. This was most likely due to the use of natural plankton rather than phytoplankton cultures in experiments, as well as re-filtration of water. These more realistic clearance rates provide valuable input for ecosystem models to make reliable estimates of the total clearance capacity of collector mussels.

- Results from this study confirmed that mussels remove plankton size-selectively from the water. Generally, smaller plankton were removed at a lower rate compared to larger plankton. We also reported a large variation in the retention of equally sized particles, suggesting that other characteristics than size alone play a role in the retention of particles by mussels.

- Heterotrophic nanoflagellates (HNAN) and ciliates are important predators on both pico-and nanophytoplankton. We reported that mussels remove heterotrophic plankton at comparable rates as nanophytoplankton, while picophytoplankton were removed at lower rates. Initially, we also assumed small organisms to be able to respond faster to improved growth conditions than larger organisms. We therefore hypothesised that at a high mussel filtration pressure, picophytoplankton would increase in relative abundances. In this thesis we did not observe an increase in picophytoplankton. On the contrary, we found that nanophytoplankton were better in balancing losses with growth than picophytoplankton. The nano-sized cells had slightly higher growth rates and presumably experienced less predation due to the lower growth rate of their main predators; the ciliates. Build-up of picophytoplankton biomass was most likely prevented due to grazing control of the HNAN. HNAN were able to profit from increased bacterial growth rates, increasing their intake and growth rate, allowing them to keep the small algae under numerical control.

- In this thesis it was estimated that an annual harvest of 40M kg of collector mussels per year results in maximum daily filtration pressure of 3.2 percent of the volume of the western Wadden Sea. Under the assumption that mussels mainly remove phytoplankton, it was calculated that 8 percent of carbon was assimilated by these mussels during the time that they were present on the collectors. Results presented in this thesis showed however that heterotrophic plankton were removed by mussels at comparable rates as nanophytoplankton. Both groups also contribute about the same amount of carbon, as monitoring data indicated. Carbon assimilated by mussels, including the heterotrophic component is estimated at 15 percent of carbon produced.

- Ongoing nutrient reductions in surface water, in the context of the water framework directive and the marine strategy framework directive, are expected to result in a more dominant role of the microbial food web in total carbon flow. Both the size-distribution of phytoplankton as well as recycling rates within this food web are expected to be affected by a high filtration pressure as was argued in the synthesis. In addition, a more dominant role of the microbial food web might also impact the flux of food from the pelagic towards the sediment and thus the food availability for benthic organisms, including mussels on natural beds.

- Mussels excrete both inorganic nutrients as well as organic material. This results in feedback mechanisms potentially increasing growth rates of both phytoplankton and heterotrophic plankton. A small increase in mussel filtration pressure might be compensated by increased plankton growth. There are however limits to this compensatory regulation. It is thus recommended for future management purposes, to monitor growth rates of plankton organisms like nanophytoplankton, heterotrophic nanoflagellates (HNAN) and ciliates.

Addendum

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Summary

Coastal areas have a long history of human resource use. Increasing pressure has resulted in overexploitation and habitat destruction, ultimately leading to declining plant and animal populations. The bivalves of the Wadden Sea, a shallow sea bordering Denmark, Germany and the Netherlands, are an example of an exploited resource. They are key biological components of the ecosystem. Since the 1950-s bottom cultures of the blue mussel (*Mytilus edulis*) began to expand and to stock these cultures, juvenile mussels were harvested from natural littoral banks. Intensified fisheries as well as years of failing recruitment resulted in the near disappearance of mussels in the Wadden Sea in the period 1980-1990. To better protect the natural mussel banks and to supply the shellfish culture with a steady supply of mussels, an agreement was negotiated between several parties including fishery organisations, nature-and bird protection organisations as well as the government to gradually replace mussel fishing from natural beds by the harvest from pelagic collectors. Pelagic collectors consist of filamentous ropes or nets that are suspended in the water column and they make use of the settlement urge of mussel larvae a few weeks after spawning. High densities of mussels can settle on these pelagic collectors, filtering large volumes of water, thereby removing suspended particles from the water column.

This thesis aimed to answer the research question whether an annual harvest of 40M kg juvenile mussels from collectors will have an effect on the plankton of the Wadden Sea. To answer this question the settlement and growth of juvenile mussels on rope collectors was studied first. Clearance rates of mussels on bacteria, pico-and nanophytoplankton, heterotrophic nanoflagellates and ciliates were determined. Changes in specific growth rate and mortality rates of these different planktonic organisms due to mussel filtration were studied. Mesocosms experiments were executed, simulating the passage of a water mass through a pelagic collector and the subsequent recovery of the plankton community. The duration of the recovery period equalled the residence time of water in the Wadden Sea. Finally, to upscale the results of the experiments to the ecosystem level, allowing for an estimation of the effect of a 40M kg harvest of juvenile mussels, a simple model was set-up.

The growth of mussels after settlement on the rope collectors was described for the years 2010 and 2011 in **chapter 2**. Mussels settle at a size of 300 µm and at the time of harvest (September) the average size was up to 25 mm. For 2011 a much higher growth rate was reported (0.21 mm day^{-1}) compared to 2010 (0.12 mm day^{-1}) as well as higher abundances per unit rope in 2011. These differences were related to food concentration, indicated by chlorophyll-a, and water temperature, two environmental variables generally assumed to explain growth rates in bivalves in laboratory experiments. We distinguished between two periods; the larval stage (before settlement) and the post settlement stage (the time after settlement until harvest).

The average chlorophyll concentration during the larval stage was higher in 2011 compared to 2010, generating the hypothesis that food concentration as experienced by larvae influences the growth rate after settlement. Analysis of two additional years of data (Box 3) this hypothesis could not be confirmed. We also reported a higher density of mussels on ropes in 2011. In this year the water temperature also rose more rapidly than in the previous year. Since larvae develop faster in warmer water, they can settle earlier which results in a shorter period of time exposed to pelagic predators. Based on two years of data it was hypothesised that the duration of the larval period was negatively related to the numbers surviving until settlement. A re-analysis of data on water temperature and the number of settled larvae from a previous study (De Vooys 1999) as well as two years of additional data (Box 3) supported this second hypothesis. No relation between mussel growth rates in the different years and the food concentration (as chlorophyll) nor water temperature after settlement could be detected. The near absence of predation on rope mussels compared to the large predation mortality experienced by benthic mussels led to the hypothesis that density dependent predation on settling mussels, shapes the natural mussel population. For cultured mussels receiving input from rope collectors, density dependent mortality will play a role later in life, from the moment of sowing on the culture lots to the moment of harvest. Due to the large inter-annual differences in abundances and growth rates of mussels on the collectors, it is possible that the targeted harvest of 40M kg of juvenile mussels will not be reached every year. The large differences between years also have consequences for model estimates of the total filtration pressure exerted by pelagic mussels.

To investigate the potential effect of the collector mussels on the pelagic food web, an estimate was needed on individual clearance rate, as well as insight in the type of particles cleared from the water column. In **chapter 3**, natural sea water was exposed to juvenile mussels (1.5-25 mm) from rope collectors. Clearance rates were related to shell length and dry weight of mussels and specified for the prey items bacteria (<1 µm), picophytoplankton (<3 µm), nanophytoplankton (3-20 µm) and ciliates (20-200 µm). Results showed that the clearance rate of juvenile mussels scaled well with squared shell length, while the relation between clearance rate and weight was less constant. Ciliates and nanophytoplankton were cleared at comparable, but variable rates, while picoalgae were cleared from the water at a rate only 11-64% of the clearance rate on nanophytoplankton. For bacteria the efficiency was even lower, on average 9%. This study confirmed that retention efficiency is generally higher for particles larger than 3 µm, but that retention does not depend on particles size alone. Clearance rates were lower than commonly reported, most likely due to re-filtration of water. These more realistic estimates are an important input for ecosystem models estimating the total clearance capacity of collector mussels in the field.

In **chapter 4** mussel filtered plankton communities were used in a series of dilution experiments to establish mussel induced changes in net growth rates of bacteria, pico- and nanophytoplankton, heterotrophic nanoflagellates (HNAN) and ciliates. Using the Landry & Hassett (1982) dilution method it was possible to specify whether mussel filtration resulted in increased specific growth rates or reduced predation rates within the microbial food web. Also in this chapter, due to the availability of a relatively simple counting method using flow cytometry, clearance rates of mussels on heterotrophic nanoflagellates (HNAN) were reported for the first time (2013). The results from this chapter were in line with the results from the previous chapter, indicating that mussel filtration had a size-selective impact on the plankton community. On average, nanophytoplankton, HNAN and ciliates biomasses were removed at equal rates, while bacterial and picophytoplankton biomasses were affected to a much lower extent. The reduction in HNAN predators by mussels significantly lowered the predation mortality rates for picophytoplankton. For bacteria, predation mortality did not change, while specific growth rates almost doubled (from 0.65 to 1.16 day^{-1}). There was an increase in HNAN biomass following the enhanced bacterial production. Single exposure to mussel filtration resulted in a stimulation of the bacterial-HNAN pathway. HNAN biomass, although seriously reduced by mussel filtration, recovered to pre-filtration levels within 24 hours, while nanophytoplankton and ciliates did not recover completely. The results from this chapter revealed potentially important effects of mussel filtration on the pelagic food web. Such effects are not disclosed in studies when only the effect on the phytoplankton biomass is considered. In the next chapter, the recovery of plankton to mussel filtration was investigated for a longer time period.

In **chapter 5** a mesocosm experiment (2010-2011) was designed to study the net-effect of mussel filtration and subsequent recovery. Natural plankton were first exposed to mussel filtration for a few hours after which mussels were removed. This set-up mimicked the single passage of a water mass through a mussel collector. The plankton community (bacteria, pico-and nanophytoplankton and ciliates) was then allowed to recover for 9 days, which equals the average residence time of the water in the Wadden Sea. The results showed that two days into the recovery period, net growth rates of bacteria, pico-and nanophytoplankton increased compared to the unfiltered (control) treatment. This result was in line with the increase in net growth rate for bacteria and reduced predation mortality rate of both pico-and nanophytoplankton after 24 hours presented in chapter 4. At the end of the 9 days recovery period, the differences in growth rates for bacteria and phytoplankton between the mussel treatment and the unfiltered control had disappeared. At the end of the recovery period, bacteria biomasses were not different compared to before filtration, while picophytoplankton biomasses were generally lower. Both nanophytoplankton and ciliates were, at least at the end of the season, able to balance losses due to mussel filtration by increased growth.

At the start of this study it was hypothesised that a high mussel filtration pressure would result in the relative increase in picophytoplankton numbers. This expectation was based on the assumptions that the removal of heterotrophic nanoflagellates (HNAN) by mussels would lower the predation mortality for picophytoplankton and that mussel filtration, by the excretion of inorganic nutrients as well as the removal of suspended matter (more light) would improve the growth conditions for autotrophs. Small sized organisms were expected to be able to respond more rapidly to these enhanced growth conditions due to their high surface to volume ratio. In this thesis we did not observe an increase in picophytoplankton. On the contrary, we found that nanophytoplankton were better in balancing losses with growth than picophytoplankton. These nano-sized cells had slightly higher growth rates and presumably experienced less predation due to the lower growth rates of their main predators; the ciliates. Build-up of picophytoplankton biomass was most likely prevented due to predation control by the rapidly recovering HNAN. Removal of biomass by a filter feeder might thus be compensated by increased growth. There are however limits to this compensatory regulation.
At the end of chapter 5, the filtration pressure of the collector mussels was estimated from the moment of first settlement of mussels on the collectors until harvest in September. Aiming for a harvest of 40M kg the maximum daily filtration pressure was estimated at 3.2 percent of the water volume of the western Wadden Sea. Calculations indicated that 8 percent of all carbon produced, was assimilated by these mussels during the time they were present on the collectors. Including the heterotrophic components in the calculations almost doubled this percentage.

The results described in this thesis made it plausible that both mussel filtration and their feedback mechanism will affect the microbial food web in terms of biomasses as well as recycling rates. The dominant role of heterotrophic nanoflagellates and ciliates in this microbial food web warrants their inclusion in research and monitoring programs regarding the future of the Wadden Sea.

Samenvatting

Overal ter wereld worden kustgebieden al lange tijd door mensen beïnvloed. De toegenomen druk op deze gebieden in de afgelopen decennia heeft in veel gevallen geleid tot overexploitatie en vernietiging van habitat, uiteindelijk uitmondend in afnemende soortenrijkdom. Een voorbeeld van een hulpbron in kustgebieden die al lange tijd door mensen wordt beïnvloed zijn de schelpdierpopulaties in de Waddenzee. Schelpdieren kunnen worden gezien als sleutelsoorten; soorten waarvan de invloed op de omgeving veel groter is dan gedacht zou kunnen worden op basis van hun voorkomen of biomassa. In de jaren 50 van de vorige eeuw nam de antropogene druk op deze populaties toe; het areaal aan mosselbodemcultuur groeide. Om de percelen van mosselen te voorzien werden jonge mosselen (*Mytilus edulis*) geoogst van de natuurlijke, droogvallende en van de permanent onderwater liggende banken in de Waddenzee. De almaar toenemende visserijdruk en het uitblijven van nieuwe aanwas leidden tot het verdwijnen van mosselbanken in de periode 1980-1990. In de jaren daarna herstelden de banken maar langzaam, en het besef groeide dat natuurlijke mosselbanken beter beschermd moesten worden. In 2008 werd daartoe een convenant gesloten tussen vertegenwoordigers uit de visserijsector, natuur-en milieuorganisaties en de Nederlandse overheid. De gemaakte afspraak gaat uit van een geleidelijke omschakeling van visserij van jonge mosselen die op de natuurlijke banken op de zeebodem liggen naar mosseloogst van mossellarven vanuit de waterkolom met hulp van zogenaamde mosselzaadinvanginstallaties (MZI's). Een MZI bestaat uit een groot aantal touwen of netten die in het water worden opgehangen. Aan deze touwen of netten kunnen zich hoge dichtheden aan mossellarven vestigen. Wanneer er van wordt uitgegaan dat het wegvangen van larven geen tot weinig effect heeft op het ontstaan van natuurlijke mosselbanken, dan zorgt het gebruik van MZI's voor een toename van het aantal jonge mosselen in de Waddenzee. Mosselen filtreren grote hoeveelheden water en verwijderen daarbij zwevend materiaal uit de waterkolom. Een toename in de hoeveelheid jonge mosselen kan hiermee invloed hebben op het aanwezige plankton in de Waddenzee.

Dit proefschrift beoogt de onderzoeksvraag te beantwoorden of het jaarlijks opkweken van 40 miljoen kilo aan jonge mosselen van MZI's een effect heeft op het plankton in de Waddenzee. Om deze vraag te beantwoorden is allereerst begonnen met het bestuderen van de vestiging en groei van mossellarven op de MZI-touwen. In **hoofdstuk 2** is de groei van mosselen na vestiging op MZI-touwen beschreven voor de jaren 2010 en 2011. Mossellarven vestigen zich wanneer ze een lengte van 300 µm bereiken. In september, wanneer de oogst plaatsvindt, is de schelplengte toegenomen tot maximaal 25 mm. De groeisnelheid van de jonge mosselen was veel hoger in 2011 (0.21 mm per dag) dan in 2010 (0.12 mm per dag). De groeisnelheid bij schelpdieren wordt onder gecontroleerde omstandigheden, zoals in laboratoria,

beïnvloed door voedselconcentraties en watertemperatuur. Om te onderzoeken of dit ook het verschil in groeisnelheid tussen 2010 en 2011 kon verklaren werden deze variabelen bekeken in twee perioden; de larvale en de postvestiging periode. Een opvallend verschil tussen de twee jaren was dat de gemiddelde voedselconcentratie (als chlorofyl) in het larvale stadium hoger was in 2011 dan in 2010. Na vestiging was dit juist andersom. De gevonden resultaten leidden tot de hypothese dat de voedselconcentratie waar pelagische larven mee worden geconfronteerd wellicht de groeisnelheid na vestiging bepaald. Deze hypothese kon echter met de gegevens van de volgende twee jaren niet worden bevestigd (Box 3). Er werd geen bewijs gevonden voor een relatie tussen de groeisnelheid van jonge mosselen en de watertemperatuur zowel in de periode voor als de periode na vestiging.

In **hoofdstuk 2** beschrijven we ook een hogere dichtheid aan mosselen op de touwen in 2011. In dit jaar warmde het water sneller op dan in 2010 en omdat larven zich over het algemeen sneller kunnen ontwikkelen in warmer water, duurde in dit jaar de larvale periode korter. Zwevend in het water lopen larven een groot risico te worden opgegeten, na vestiging lijkt dat risico kleiner. Op basis van gegevens die we in deze twee jaren hebben verzameld formuleerden we de hypothese dat de duur van de larvale periode negatief correleert met het aantal mosselen dat overleeft tot het moment van vestiging. Gegevens uit de literatuur en de twee volgende jaren onderzoek lijken deze hypothese te bevestigen.

Als gevolg van de in dit proefschrift gevonden verschillen tussen mosseldichtheid op de MZI-touwen en de groeisnelheid van deze mosselen in de bestudeerde jaren is het mogelijk dat de beoogde oogst van 40 miljoen kilo niet altijd zal worden gehaald. De grote verschillen tussen jaren hebben ook gevolgen voor de inschatting van de totale filtratiedruk die MZI- mosselen uitoefenen.

Om het effect van MZI-mosselen op het pelagische voedselweb te bestuderen is een inschatting nodig van het type plankton dat wordt verwijderd en de snelheid waarmee dit gebeurt. Om dit te onderzoeken werden voor **hoofdstuk 3** jonge mosselen (1.5-25 mm) in zeewater geplaatst om te filtreren. Verwijdersnelheden werden gerelateerd aan de schelplengte en het drooggewicht van de mosselen om zo tot een algemeen geldende relatie te komen. De snelheden werden bepaald voor bacteriën (<1 µm), picofytoplankton (<3 µm), nanofytoplankton (3-20 µm) en ciliaten (20-200 µm). Uit de resultaten blijkt dat de verwijdersnelheid door een mossel goed te beschrijven is met de gekwadrateerde schelplengte, terwijl de relatie tussen verwijdersnelheid en gewicht veel meer variatie vertoonde. Ciliaten en nanofytoplankton werden met een vergelijkbare, maar variabele snelheid uit het water gehaald door mosselen, terwijl kleinere algen veel minder snel werden verwijderd. Voor bacteriën was de snelheid nog lager, gemiddeld maar 9% van de snelheid waarmee nanofytoplankton uit het water werd verwijderd. Deze

studie bevestigt dat de retentie van plankton over het algemeen beter is voor deeltjes groter dan 3 µm, maar dat grootte niet alleen bepaalt of een deeltje wordt vastgehouden door een mossel.

De verwijdersnelheden gerapporteerd in dit proefschrift zijn lager dan de waarden die in de meeste studies worden genoemd. Onze meer realistische waarden vormen een belangrijke input voor modellen die het effect van filtratie op het ecosysteem berekenen.

In **hoofdstuk 4** wordt beschreven wat er gebeurt met de planktongemeenschap na mosselbegrazing. Om vast te stellen wat de veranderingen zijn in groei- en sterftesnelheid van het plankton is gebruik gemaakt van een serie verdunningsexperimenten. Verder beschrijven we in dit hoofdstuk de verwijdersnelheid van heterotrofe nanoflagellaten (HNAN) door MZI-mosselen. De resultaten lieten zien dat, net als in het vorige hoofdstuk, mosselen plankton grootte-selectief uit het water verwijderden. HNAN en ciliaten zijn belangrijke predatoren op bacteriën en algen en het feit dat deze organismen sterk in aantal werd gereduceerd door mosselen resulteerde in een verlaagde sterftesnelheid van de kleine algen. Bacteriën ondervonden geen lagere sterftesnelheid, maar vertoonden een veel hogere specifieke groeisnelheid na mosselbegrazing vergeleken met de controle situatie zonder mosselen (respectievelijk 0.65 en 1.16 per dag). De verhoogde bacteriële productie leidde tot een toename in aantallen heterotrofe nanoflagellaten. Hoewel mosselen in eerste instantie de aantallen HNAN dus flink reduceerden, herstelden de HNAN binnen 24 uur na begrazing. Nanofytoplankton en ciliaten herstelden niet volledig van de begrazing door mosselen.

In **hoofdstuk 5** worden de resultaten beschreven van een mesocosm studie (2010-2011). De studie had als doel het herstel van de planktongemeenschap te bestuderen voor een periode langer dan 1 dag. De resultaten geven het netto effect (groei minus sterfte) van mosselfiltratie op de planktonbiomassa door het jaar heen. Waddenzee-plankton werd eerst blootgesteld aan mosselfiltratie voor een aantal uren waarna de mosselen werden verwijderd. Deze opzet simuleerde een eenmalige passage van een watermassa door een MZI-installatie. De planktongemeenschap (bacteriën, pico-en nanofytoplankton en ciliaten) werd daarna gemonitord om het al dan niet optredende herstel na mosselbegrazing te kunnen kwantificeren. De duur van de herstelperiode kwam overeen met de gemiddelde verblijftijd van water in de Waddenzee.

Na twee hersteldagen bleek dat de netto groeisnelheden van bacteriën, pico-en nanofytoplankton hoger waren in de mesocosm waar mossel hadden gefiltreerd dan in de controle mesocosms. De resultaten kwamen overeen met de gevonden resultaten na 24 uur herstel zoals gepresenteerd in hoofdstuk 4. Aan het einde van de herstelperiode, na 9 dagen, was het verschil in netto groeisnelheid tussen de mossel-

gefiltreerde en de controle mesocosms verdwenen. Wat betreft bacteriebiomassa bleek er aan het einde van de 9 dagen geen meetbaar verschil tussen mossel en controle mesocosm te zijn, terwijl de biomassa aan picoalgen over het algemeen lager was in de mossel mesocosm. Zowel het nanofytoplankton als de ciliaten leken in sommige experimenten in staat het verlies in biomassa door mosselbegrazing te compenseren door een toename in netto groei. Een belangrijke conclusie van dit hoofdstuk is dat verwijdering van biomassa door mosselen gecompenseerd kan worden door toegenomen groei voor sommige organismen. Er zit echter een grens aan dit compensatie mechanism.

Aan het einde van **hoofdstuk 5** is een inschatting gemaakt van de filtratiedruk die de mosselen op MZI-installaties gedurende het groeiseizoen zullen uitoefenen. Uitgaande van de beoogde oogst van 40 miljoen kilogram mosselen werd de maximale dagelijkse druk geschat op 3.2 procent van het totale volume van de westelijke Waddenzee. Berekeningen lieten ook zien dat de MZI-mosselen gedurende hun tijd op de installatie 8 procent van al het geproduceerd koolstof omzetten. Wanneer echter ook koolstof uit heterotroof plankton in de berekening werd meegenomen, mosselen filtreren ten slotte ook HNAN en ciliaten zoals blijkt uit hoofdstuk 3 en 4 van dit proefschrift, dan verdubbelt de schatting aan gebruikt koolstof bijna.

De resultaten beschreven in dit proefschrift maken het aannemelijk dat mosselfiltratie en de terugkoppelingsmechanismen het microbieel voedselweb zullen beïnvloeden, zowel wat betreft veranderingen in biomassa's van de verschillende componenten als wat betreft recyclingsnelheden. De dominante rol van heterotrofe nanoflagellaten en ciliaten in het microbieel voedselweb rechtvaardigt het toevoegen van deze groepen van organismen in onderzoeks-en monitoringsprogramma's met betrekking tot de ontwikkeling van het Waddenzee-ecosysteem.

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- Microbial ecology (2015)

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- *Being aPhD at IMARES.* IMARES PhD Day, 25 November 2011, Den Hoorn, Texel
- *Growth of juvenile mussels on suspended collectors in the western Wadden Sea*. IMARES PhD Day, 29 November 2013, Den Hoorn, Texel
- *Impact of the blue mussel (Mytilus edulis) on the microbial food web in the western Wadden Sea*, The Netherlands. European Marine Biology Symposium, 8-12 September Saint Petersburg, Russia
- *Invloed van mosselen (Mytilus edulis) op het microbiële voedselweb in de westelijke Waddenzee.* Mosselworkshop - Institute for Marine Resources & Ecosystem Studies (IMARES) and Royal Netherlands Institute for Sea Research (NIOZ), 30 September - 1 October 2014, Den Hoorn, Texel

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