MASTER'S THESIS

The impact of suspended sediments and phosphorous scarcity on zebra mussel and quagga mussel growth

Name: Jeroen Mandemakers
Student number: 3346110
E-mail address: j.j.mandemakers@students.uu.nl
Postal address: 2964CS-33

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Supervisor (UU): Dr. Maarten Eppinga

Supervisor (NIOO-KNAW): Dirk Sarpe MSc

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Abstract

Dreissenids are a key stone species for the IJsselmeer area in the Netherlands. However, the zebra mussel (*Dreissena polymorpha)* populations in the IJsselmeer and Markermeer are in poor conditions relative to 20 years ago. Nowadays, quagga mussels (*Dreissena rostriformis bugensis*) are dominant in both lakes after their recent invasion. It was tested whether the high suspended sediment concentrations in the Markermeer and/or the relatively low phosphorous content of the phytoplankton in both lakes might underlie the observed population developments. Two full factorial microcosm experiments were conducted with zebra and quagga mussels. In the first experiment, the mussels were reared under four different suspended sediment concentrations (0, 32, 80 and 200 mg l^{-1}). In the second experiment, zebra and quagga mussels were fed with *Scenedesmus obliquus* with C:P ratios of 520 and 287.

Growth rates were very constant over the suspended sediment levels and did not differ between both species but at the highest sediment level, where zebra mussel growth rate peaked. Mussel tissue dry weight was significantly lower in the high C:P level than in the low C:P level. However, the mussel tissue C:P ratio was not significantly different between the two C:P ratio treatment levels. These results suggest an upper tissue C:P limit of 110 for zebra mussels and 150 for quagga mussels. Furthermore, quagga mussels appeared to have lower phosphorous requirements than zebra mussels. The current suspended sediment levels in the Markermeer are unlikely to hinder zebra mussel growth or to benefit quagga mussel growth over zebra mussel growth. In contrast, the relative low phosphorous content of the phytoplankton in both lakes might be detrimental to zebra mussel growth, while quagga mussel growth will be less affected.

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1. Introduction

1.1 Motivation

The wetland ecosystems in IJsselmeer area (figure 1.1) are assigned as Ramsar sites and Natura 2000 areas because of their international importance for about one million water birds per year. However, the population size of some bird species which feed on mussels and fish has declined in the IJsselmeer and Markermeer over the last decades (Noordhuis et al., 2010). Simultaneously, the number of certain mussel and fish species showed a declining trend. Hence, ANT-studies (*Autonome Neerwaardse Trend*) focus on opportunities to halt the declining trends and to improve the ecological quality of the IJsselmeer area. In order to come up with feasible measures, the mechanisms which might be responsible for the observed declining trends should be clarified.

In the IJsselmeer and Markermeer, densities of the bivalve filter-feeder *Dreissena polymorpha* (Pallas, 1771), the zebra mussel, showed a 75 per cent decline between 1981 and 2006/07 (Noordhuis, 2009). This might have attributed to the general declining trend in bird numbers in this area because zebra mussels are a major food source for diving ducks in these lakes (de Leeuw, 1997). Moreover, zebra mussels can substantially decrease the turbidity of the water by filtration (e.g. Reeders et al., 1989) which in turn influences the conditions for fish-eating bird species (Noordhuis et al., 2010). Hence, zebra mussels might be considered as a key stone species for the IJsselmeer and Markermeer ecosystem.

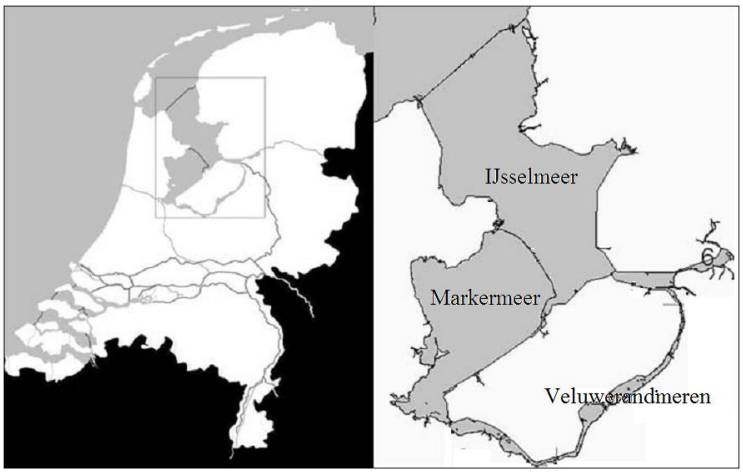

Figure 1.1 Map of the IJsselmeer area in the Netherlands. Adapted from Lammens et al., 2008.

In line with the ANT-study on filter feeders, this research focused on the influence of bottom up factors (i.e. resource availability) instead of on top down factors (i.e. predation) on dreissenids. More precisely, the scope of this research was the impact of phytoplankton quality and suspended sediment levels on dreissenids. In turn, the quality of the phytoplankton and the suspended sediment concentrations are influenced by anthropogenic processes like eutrophication and oligotrophication, climate change and damming. Although this study focused mostly on the relation between the environment and individual species, interrelations between ‘humans’, ‘the environment’ and ‘biodiversity’ are obviously present. Therefore, this study fits into the research of the Environmental Sciences group of the Copernicus Institute.

1.2 Problem definition

1.2.1 Dreissenid trends in the IJsselmeer and Markermeer

In the Netherlands, the zebra mussel have been present for almost 200 years. Moreover, zebra mussels were among the first colonizers of the IJsselmeer after the enclosure of this lake from the sea in 1932 (Noordhuis et al., 2010). The zebra mussel densities were relatively constant between 1981 and 1999, after which the mussel concentrations declined by almost 90 per cent (figure 1.2). In the Markermeer, which was segregated from the IJsselmeer by a dam (the

Houtribdijk) in 1976 (see figure 1.1), the population shifted from a stable state with high densities into another stable state with low densities in the early nineties of the previous century (figure 1.2; Noordhuis & Houwing, 2003). However, the exact causes of the crash of the zebra mussel populations in the IJssel- and Markermeer are unclear (Noordhuis, 2009).

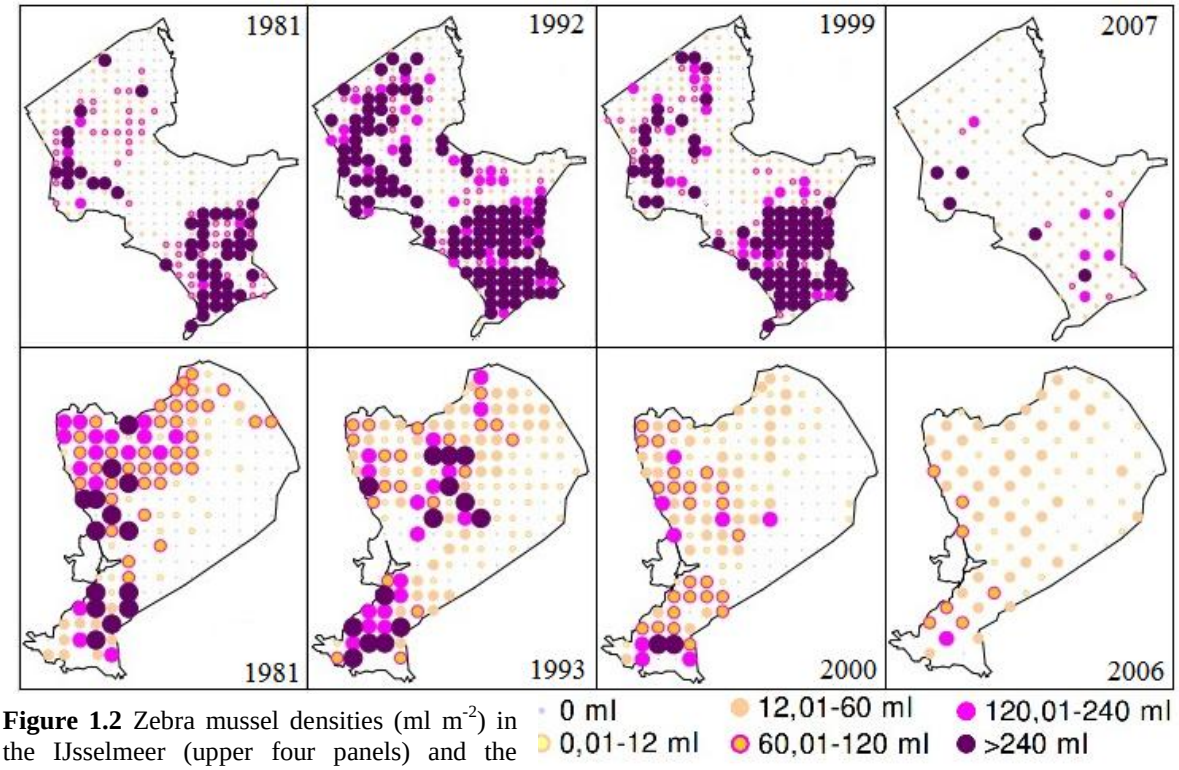

Figure 1.2 Zebra mussel densities (ml m^{-2}) in the IJsselmeer (upper four panels) and the Markermeer (lower four panels) for four samplings. Adapted from Noordhuis, 2009.

1.2.2 Dreissenid trends in the Veluwerandmeren

Compared with the developments of the zebra mussel population in the IJssel- and Markermeer, the populations in the Veluwerandmeren (see figure 1.1) showed a converse trend. Zebra mussels were abundant in these lakes in the 1960s, but the mussel populations strongly declined in the 1970s (Noordhuis et al., 2010). A recovery of the populations took place in the late 1990s and for most lakes relatively high mussel densities are observed today (Noordhuis et al., 2010). These trends coincide with the eutrophication and subsequent re-oligotrophication in the Veluwerandmeren during this period. High nutrient loadings in the 1970s pushed the ecosystem to a turbid state with severe algal blooms (Ibelings et al., 2007). During this phase, the chemical and biological oxygen demand (figure 1.3), the pH and the ammonia concentrations reached very high values.

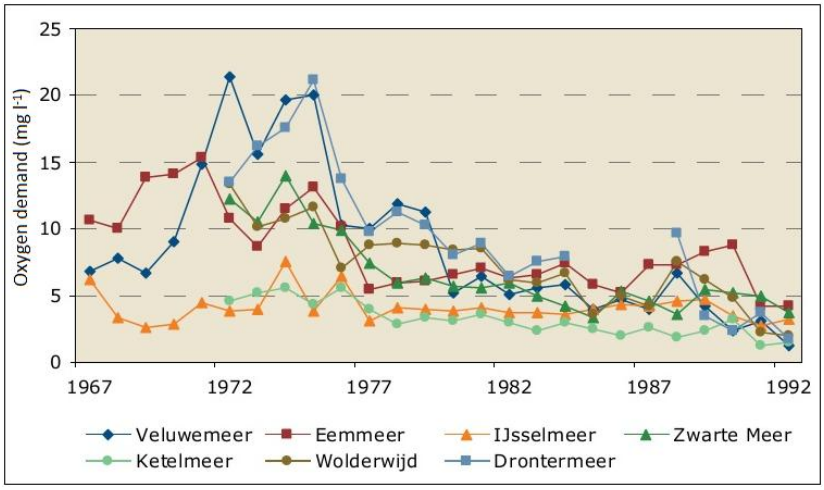

Figure 1.3 Biological plus chemical oxygen demand (mg l^{-1}) for several water bodies in the IJsselmeer area. The oxygen demand in the IJsselmeer was relatively constant over time, while the oxygen demand in most other lakes peaked in the 1970s. Figure from Noordhuis et al., 2010.

Reduced nutrient loadings (figure 1.4), biomanipulation and flushing resulted in a shift to a clear state in some of the Veluwerandmeren in the 1990s (Ibelings et al., 2007). Chlorophyll levels declined very rapidly (figure 1.5) and water transparency increased substantially (figure 1.6A). However, as the phosphorus concentrations did not decline so strongly, the ratio of chlorophyll to total phosphorus dropped. Subsequently to the improvements of the environmental conditions of the ecosystem, the dreissenid populations in the Veluwerandmeren recovered to their previous level. Additionally, zebra mussels itself might have contributed to the environmental improvements, e.g. by grazing on cyanobacteria (Gulati et al., 2008).

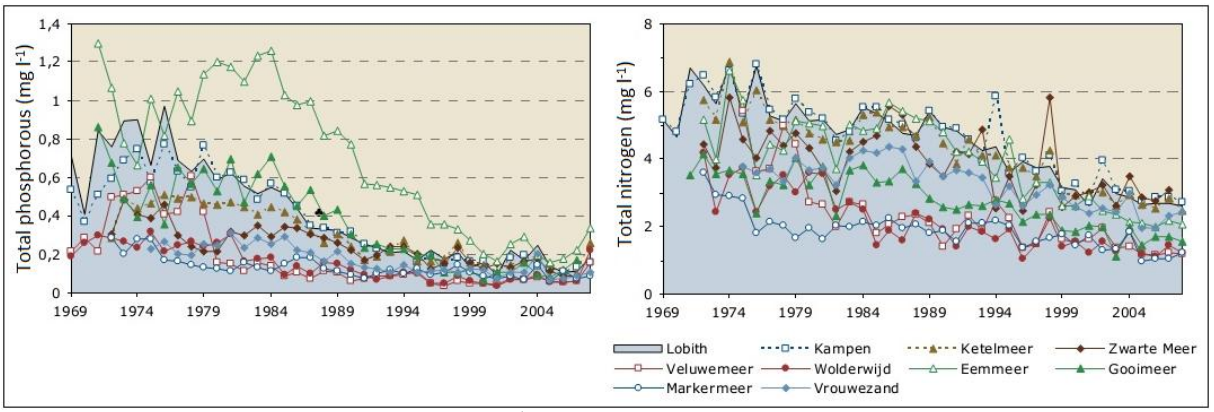

Figure 1.4 Mean summer concentrations (mg l^{-1}) of total phosphorus (left) and total nitrogen (right) for several water bodies in the IJsselmeer area and for the supplying river Rhine (near Lobith, blue shaded). Figure from Noordhuis et al., 2010.

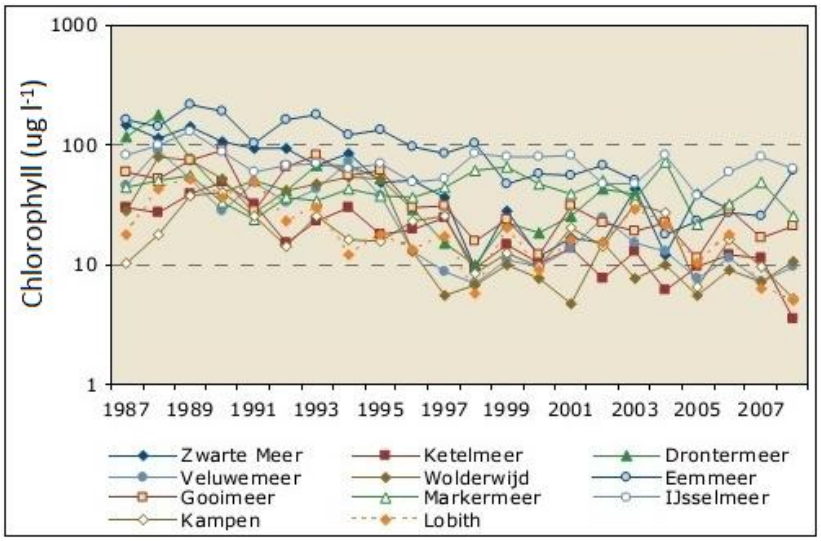

Figure 1.5 Summer mean chlorophyll concentration (µg l^{-1}) for several water bodies in the IJsselmeer area. Figure from Noordhuis et al., 2010.

1.2.3 Steering factors in the IJsselmeer and Markermeer

The zebra mussel population trends in the Veluwerandmeren might easily be linked to the prevailing environmental conditions. However, this is not the case for the IJssel- and Markermeer. The deterioration of the zebra mussels in IJsselmeer and Markermeer occurred under declining nutrient (figure 1.4) and chlorophyll concentrations (figure 1.6A). Also, the concentrations of heavy metals (zinc, copper, lead, cadmium and mercury) are considerably decreased over the past four decades (Noordhuis et al., 2010). Furthermore, concentrations of organic pollutants like pesticides were mostly far below safety norms (Noordhuis et al., 2010). However, the Veluwerandmeren and the Marker- and IJsselmeer differ substantially regarding two environmental factors which might underlie the dreissenid population developments in the Marker- and IJsselmeer.

The Markermeer is characterized by high suspended sediment concentrations and a very low water transparency (figure 1.6C). In contrast to the sandy IJsselmeer, the bed of the Markermeer consist mainly of clay and loam. Silt is deposited on the clay and loam layers and on top of the silt a thin oxidized 'fluffy' mud layer is found (Vijverberg et al., 2011). The total suspended matter concentration in the Markermeer is around 40 mg l^{-1} in summer and 70 mg l^{-1} in winter (Penning et al., 2012). There appears to be a slightly increasing trend over the last four decades, but the actual concentration shows large wind-driven fluctuations (Vijverberg et al., 2011).

A modeling study by Vijverberg et al. (2011) showed that the fluffy layer will be mixed over the whole water column at wind speeds higher than 4 m s^{-1} (3 Bft), causing a suspended sediment concentration of 80 mg l^{-1}. The coarser silt layer will resuspend at wind speeds higher than 10 m s^{-1} (5 Bft), resulting in total sediment concentrations of 200 to 300 mg l^{-1}. The suspended sediment concentrations near the bed might be up to 1000 mg l^{-1} directly after the peak of a storm due to settling of the silt particles (Vijverberg et al., 2011).

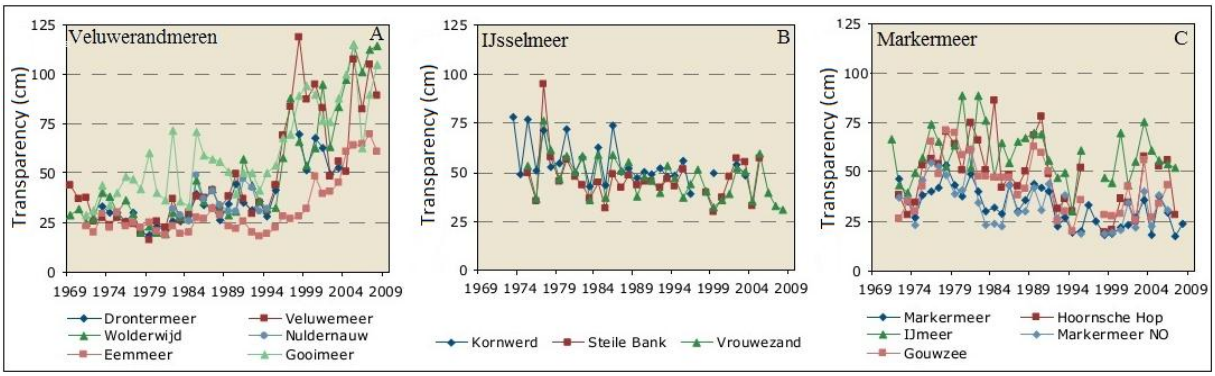

Figure 1.6 Summer mean **w**ater transparency (cm) over the period 1969-2009 for several of the Veluwerandmeren (A) and several locations in the IJsselmeer (B) and the Markermeer (C). Figures from Noordhuis et al., 2010.

In addition to the high suspended sediment concentrations in the Markermeer, phosphorous limited growth of the phytoplankton might have led to a poor food quantity and quality for dreissenids (Noordhuis & Houwing, 2003). The total phosphorous (TP) concentration decreased from 0.3 mg l^{-1} in 1970 to 0.1 mg l^{-1} in 1990. The chlorophyll concentration shows no decreasing or increasing trend over this period, but was relatively low in the early 1990s. So, the concurrence of high suspended sediment levels and low phytoplankton concentrations might have caused the observed mussel crash in the Markermeer (Noordhuis & Houwing, 2003).

Moreover, a relatively low phosphorous content of the phytoplankton might also explain the deterioration of the zebra mussel population in both the Markermeer and the IJsselmeer. As in the Markermeer, the TP concentration in the IJsselmeer declined from around 0.25 to 0.1 mg l^{-1} between 1980 and 2010, while the chlorophyll concentration declined much less and still exceeds 50 µg l^{-1} (figure 1.5). As a result, the ratio of carbon to TP in the IJsselmeer has increased from roughly 150 to 250 over the last three decades (Sarpe et al., in preparation). Moreover, the mean C:P ratio of the seston—i.e. what the mussels are actual feeding on—was 294 in the IJsselmeer (combined from three locations; N = 83) and 347 in the Markermeer (combined from three locations; N = 93) over the period 2010 to 2012 (*pers. com.* D. Sarpe, 2013).

1.2.4 Recent dreissenid developments in the IJsselmeer and Markermeer
In the years following the crash in the Markermeer, observations indicate very successful spawning and the yearly offspring (mussels < 13 mm) accounted for over 80% of the total population (Noordhuis & Houwing, 2003). Nevertheless, there were no signs of recovery of the dreissenid population through 2006 (Noordhuis, 2009). Suddenly, the biovolume of dreissenids increased almost five-fold between 2006 and 2011 and this was completely due to the invasion of the quagga mussel, *Dreissena rostriformis bugensis* (Andrusov, 1897), which made up 80% of the total dreissenid population in 2011 (Sarpe et al., 2012). Also in the Southern part of the IJsselmeer, sampling in 2008 indicated a significant recovery of the dreissenid population with more than 50% quagga mussels (Noordhuis, 2009; bij de Vaate & Jansen, 2010).

1.2.5 Problem summary
The dreissenid populations in the Marker- and IJsselmeer crashed and did not recover until the massive invasion of the quagga mussel. In contrast to the Veluwerandmeren, the population developments cannot easily be linked to the prevailing environmental conditions and the exact causes for the mussel crash are unknown. However, there are two major differences between the Veluwerandmeren and the Marker- and IJsselmeer which might explain the observed population developments. On the one hand, the concentration of suspended sediments in the Markermeer is relatively high, which might be detrimental to dreissenids. On the other hand, dreissenid growth might be hampered by the relatively high ratio of chlorophyll to phosphorous in the Marker- and IJsselmeer. Moreover, the invasion of quagga mussels suggest that they are less affected by these conditions than zebra mussels.

However, the exact causes for the crash of the zebra mussel populations and the different response of zebra and quagga mussels to the prevailing environmental conditions in the Marker- and IJsselmeer are unclear. First, a literature review was performed in order to elucidate the possible impacts of suspended sediments and phosphorous availability on both zebra and quagga mussels (see section below).

1.3 Scientific state of the art

1.3.1 Distribution of zebra and quagga mussels
The native range of the zebra mussel *Dreissena polymorpha* (Pallas, 1771) and the quagga mussel *Dreissena rostriformis bugensis* (Andrusov, 1897) is located within the Ponto-Caspian region (i.e. the Black, Caspian and Azov Sea) (Son, 2007). Dreissenids have very successfully invaded numerous freshwater bodies in Europe and North America over the past decades, causing serious impacts on the food web of these systems (Miehls et al., 2009; Strayer et al., 1999). Although the zebra and quagga mussel are closely related species, the time between introduction and maximum population density differs significantly between zebra and quagga mussels (2.5 and 12.2 years on average, respectively) (Karatayev et al., 2011a). They can co-occur, but zebra mussels tend to be replaced by quagga mussels in many water bodies (Karatayev et al., 2011b). Several mechanisms are suggested to explain the displacement of zebra mussels by quagga mussels (see citations in Karatayev et al., 2011b), but a conclusive framework is still lacking.

1.3.2 Food quantity and energy allocation
Dreissenids are filter feeders and graze on most seston particles which can pass their inhalant siphon (Horgan & Mills, 1997). Like other bivalves, zebra mussels can either digest the

filtered particles or expel them as pseudofeces (Kiørboe & Møhlenberg, 1981; Berg et al., 1996). Dreissenids have a Type 1 functional response of clearance rate (V mussel $^{-1}$ T^{-1}) and ingestion rate (M mussel^{-1} T^{-1}) in dependence on the phytoplankton concentration (M V^{-1}) (Walz, 1978). This implies that clearance rates decrease at phytoplankton concentrations above the incipient limiting concentration (ILC) (figure 1.7A) and that food ingestion is maximal at the ILC (figure 1.7B). Based on laboratory experiments, Walz (1978) reported an ILC for zebra mussels of 2 mg C l^{-1}. However, the ILC might differ with mussel size and phytoplankton species (Berg et al., 1996).

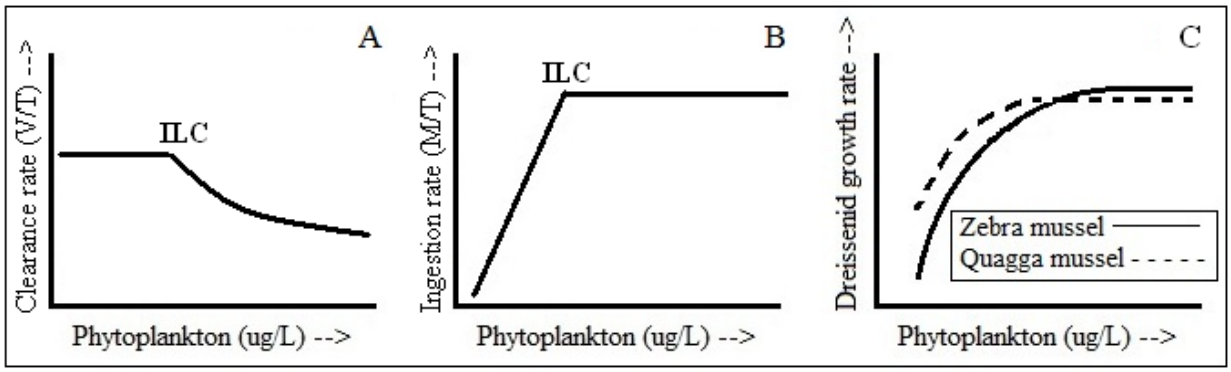

Figure 1.7 Sketched dependence of clearance rate(A) and food ingestion (B) of dreissenids and dreissenid growth rate (C) on phytoplankton levels. Walz (1978) reported an incipient limiting concentration (ILC) for zebra mussels of 2.0 mg C l^{-1}. In figure C, the relative position of the lines to each other is based on the results of the growth experiments by Baldwin et al., 2002.

The energy available for organisms to grow or to reproduce equals the energy ingested via their food minus metabolic demands. Stoeckmann & Garton (1997) measured the energy budget of zebra mussels from May through October and found an average energy expenditure of 95% on metabolism and only 5% on growth and reproduction. Furthermore, the energy allocation between growth and reproduction of zebra mussels appears to be flexible. Mussels larger than 15 mm invested most in reproduction and showed degrowth over the study period, while smaller individuals invested more in somatic growth (Stoeckmann & Garton, 1997). Moreover, zebra mussels appear to allocate energy from growth to reproduction under stressful conditions, namely low food quantity and high temperatures, but above all low food quality (i.e. absence of PUFAs) (Stoeckmann & Garton, 2001).

Baldwin et al. (2002) did several comparative growth and grazing experiments with zebra and quagga mussels. They reared juvenile mussels (6-9 mm) at 23^{o}C under mean food concentrations ranging from 0.05 to 8.68 µg chlorophyll a l^{-1}. Quagga mussels had significant higher growth rates (in terms of wet mass) than zebra mussels over all food concentrations but at the highest food level (Baldwin et al., 2002). Based on these results, Baldwin et al. (2002) hypothesized that zebra mussel growth is more likely to be food limited than quagga mussel growth.

Furthermore, quagga mussels had lower clearance rates per unit of mussel biomass than zebra mussels and this suggest that zebra mussels have a lower assimilation efficiency and/or that they have higher metabolic costs compared to quagga mussels (Baldwin et al., 2002). Indeed, Stoeckmann (2003) found that quagga mussels had lower metabolic costs and this energetic benefit was predominantly invested in shell growth and tissue mass. On the other hand, zebra mussels had higher metabolic costs and invested relatively more energy in reproduction (Stoeckmann, 2003).

Zebra mussels appear as typical *r* strategists focusing on reproduction, which will hamper their growth and survival under stressful environmental conditions. Relative to zebra mussels, quagga mussels behave more like K strategists investing in growth instead of in reproduction. These results might support the earlier mentioned observations that zebra mussel invasions are often followed by a quagga mussel dominance.

1.3.3 Suspended sediments and O:I ratio

Several studies indicate a negative relationship between the amount of suspended (inorganic) matter and zebra mussel growth rate (Karatayev et al., 2006). An increase in inorganic suspended sediment will lower the fraction of edible organic particles in the seston. Hence, with a decreasing O:I ratio, the food quality of the seston is lowered (Schneider et al., 1998).

Pseudofeces production by zebra mussels tends to increase with inorganic sediment concentrations (Baldwin et al., 2002; Schneider et al., 1998). However, several studies found no difference between the O:I ratio of the seston and the excreted pseudofeces at low seston food quality (at 50 and 100 mg clay l^{-1}, Baldwin et al., 2002; and at an O:I ratio below 0.33, Schneider et al., 1998). This implies that selective feeding by zebra mussels ceases at high suspended sediment concentrations and hence, pseudofeces production will be no appropriate strategy for dreissenids to cope with very turbid conditions.

Instead, results of Madon et al. (1998) suggest that the food intake of zebra mussels under increasing suspended sediment levels up to 100 mg l^{-1} is mainly controlled via a reduction in their clearance rate. This is supported by field experiments of Reeders et al. (1989) in the IJsselmeer area, where the clearance rate of zebra mussels was inverse exponential related to the concentration of suspended material (ranging from 5 to 80 mg l^{-1}). This strategy saves energy related to filtration activity at high suspended sediment concentrations, but also involves reduced ingestion of particulate organic matter which leads to decreased assimilation efficiency, increased maintenance costs and reduced scope for growth (Madon et al., 1998).

Baldwin et al. (2002) also found decreasing clearance rates and decreasing assimilation efficiency in response to increasing clay concentrations (from 0 to 100 mg l^{-1}). Moreover, zebra and quagga mussels responded very similar to high sediment levels in their study (Baldwin et al., 2002). Summers et al. (1996) compared the oxygen consumption of zebra and quagga mussels at zero and high turbidity (500 mg sediment l^{-1}) and they also found no discrepancy between both species in their response to the high sediment concentration.

Penning et al. (2012) did a grazing experiment in which they discriminated sediment particles from algae particles (*Scenedesmus obliquus*). They found decreasing clearance of suspended sediments with increasing sediment levels (from 250 to 1000 mg l^{-1}) for mussels smaller than 15 mm. However, the clearance of algae increased with increasing sediment levels (0 to 500 mg l^{-1}) and declined at higher sediment concentrations (750 to 1000 mg l^{-1}). These findings suggest that zebra mussels are able to select algae particles from the seston even at relatively high sediment concentrations (Penning et al., 2012). However, this is contrasting with the earlier mentioned studies which argue that dreissenids cannot selectively feed on algae at high sediment levels (Baldwin et al., 2002; Schneider et al., 1998) and that organic matter ingestion declines at high sediment concentrations (Madon et al., 1998).

1.3.4 Food quality

The quality of phytoplankton as a food source for dreissenids is to a large extent determined by the phosphorous content. First, dreissenid performance might be strongly affected by the

polyunsaturated fatty acids (PUFAs) content of phytoplankton (Stoeckmann & Garton, 2001; Vanderploeg et al., 1996). As the content of PUFAs will decrease with increasing P-limitation, algae with low phosphorus content are low quality food for grazers (Saikia & Nandi, 2010). Moreover, the growth rate of organisms has been hypothesized to be closely related to their phosphorus content, the so called 'growth rate hypothesis' (GRH) (Elser et al., 2003). Hence, the relative phosphorous content in consumers and their food is an important characteristic to describe food quality. A useful approach in this context is ecological stoichiometry, the study of the balance of chemical elements and energy between ecosystem interactions (Sterner & Elser, 2002).

The tissue C:N:P stoichiometry (i.e. molar ratio of C to N to P) of autotrophs like phytoplankton varies over a wide range, largely reflecting the nutrient availability in their environment (van de Waal et al., 2009). In contrast to autotrophs, most heterotrophic organisms are strictly homeostatic (as depicted in figure 1.8), i.e. they have a fixed tissue stoichiometry (Sterner & Elser, 2002). Optimal somatic growth will be achieved when the chemical composition of the food is balanced with the requirements of the consumer for metabolism, somatic growth and reproduction (Boersma & Elser, 2006). Hence, organisms which can adapt their tissue stoichiometry in response to food stoichiometry might obtain high growth and reproduction rates because they are able to overcome elemental imbalances (Gonzáles et al., 2010).

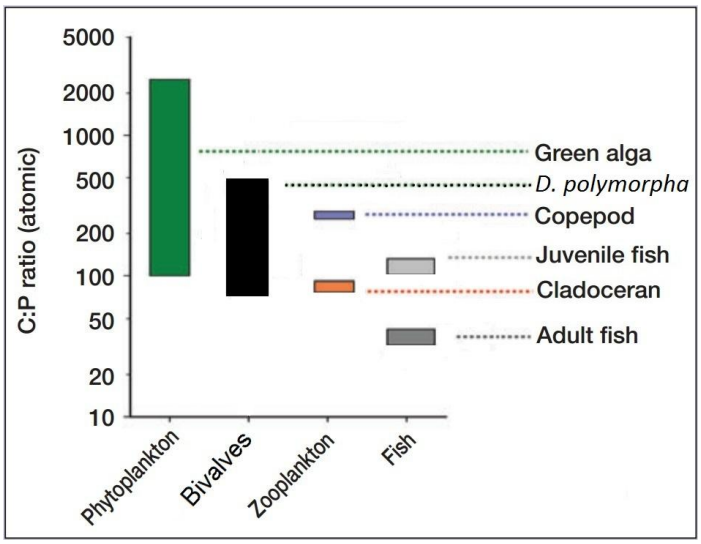

Figure 1.8 Observed tissue stoichiometric ranges (molar C:P) of several aquatic organisms. Adapted from van de Waal et al. (2009) with additional data for bivalves (*D. polymorpha)* from Naddafi et al. (2012).

Little research has been done so far regarding the tissue stoichiometry of dreissenids. Naddafi et al. (2009) studied the relation between zebra mussel condition and tissue P content in two Swedish lakes. Next, they studied the variability of mussel tissue stoichiometry at different locations in four lakes (Naddafi et al., 2012). In the latter study, they found a large variability of tissue C:P ratio within and between lakes (ranging from 69 to 480, figure 1.8). In general, mussels from more productive lakes—with higher TP concentrations—had lower C:P ratios and moreover, tissue C:P ratio was found to be positively correlated to the seston C:P ratio (Naddafi et al., 2012). Also, Naddafi et al. (2009) found that mean tissue C:P ratio was not significantly different from the mean seston C:P ratio in the studied lakes.

However, mussel condition (expressed as the ratio of dry tissue weight to dry shell weight) was not clearly related to the P content of their tissue. Naddafi et al. (2009) found no significant relation between mussel tissue C:P ratio and mussel condition. Instead, they found that tissue N:P ratio was weakly negatively related with mussel condition (Naddafi et al., 2009). This partly supports the GRH, although a direct link between tissue P content and mussel growth was absent (Naddafi et al., 2009; 2012).

In contrast to many other aquatic organisms, the C:P ratio of zebra mussels varies over a broad range (figure 3, see also Liess & Hillebrand, 2005). Therefore, they are called rheostatic

(i.e. their nutrient stoichiometry can vary over a wide range depending on the external surroundings; definition from Villar-Argaiz, 2002). According to the GRH, this would suggest that they have relatively low growth rates in P deficient environments. On the other hand, rheostatic organisms might be able to adjust their tissue stoichiometry in response to their food and hence, prevent elemental imbalances. This strategy might enable zebra mussels to meet their nutrient requirements even under low phosphorus levels, which enables them to invade low-nutrient environments (Gonzáles et al., 2010). Moreover, the P content of zebra mussel tissue might increase in high-nutrient environments, which will (based on the GRH) result in higher growth rates (Gonzáles et al., 2010). Altogether, this suggests that zebra mussels can perform well over a wide range of nutrient levels which might have contributed to their successful invasion of numerous freshwater systems.

Naddafi et al. (2009) argued for experiments which elucidate the link between phytoplankton C:P ratios, mussel tissue C:P ratios and growth rates. Moreover, no data at all was available up to now regarding the tissue stoichiometry of quagga mussels. Morehouse et al. (2013) performed two microcosm experiments in which zebra mussel growth was examined at two very low C:P food ratios (20 and 45) and at a much higher ratio of 380. The found a negative effect of P excess on mussel growth. Moreover, the mean tissue stoichiometry differed per treatment level, indicating that zebra mussels are able to adjust their stoichiometry in response to their food (Morehouse et al., 2013). However, the study by Morehouse et al. (2013) does not clarify the linkage between food and tissue stoichiometry and growth rates due to the extreme low C:P food ratios used.

1.3.5 Conclusion

The crash of the zebra mussel population in the Markermeer in the early 1990s might be explained by high suspended sediment concentrations. Most studies predict negative effects of high suspended sediment concentrations on zebra mussel growth as organic matter ingestion and assimilation efficiency declined with increasing suspended sediment concentrations (Baldwin et al., 2002; Schneider et al., 1998; Madon et al, 1998; Summers et al., 1996). However, Penning et al. (2012) found increased clearance of algae cells by zebra mussels up to sediment concentrations of 500 mg l^{-1} which suggest that growth rates will not be affected by the suspended sediment concentrations in the Markermeer. So, the actual impact of suspended sediments on dreissenids is not undisputed and additional research is required to elucidate whether the observed population developments in the Markermeer might have been caused the suspended sediment concentrations.

Moreover, the possible different responses of zebra and quagga mussels to suspended sediments should be explicitly researched. Two comparative studies found no discrepancy in the response of both species to high suspended sediment concentrations (Baldwin et al., 2002; Summers et al., 1995). However, the current quagga mussel dominance in the Markermeer suggests that quagga mussels are less affected by suspended sediments. Moreover, quagga mussels have relatively low metabolic costs and had higher growth rates than zebra mussels when food was not abundant (Stoeckmann, 2003; Baldwin et al., 2002). This implies that quagga mussels might be better able to cope with a low O:I ratio caused by high suspended sediment concentrations.

The tissue C:P ratio of zebra mussels can vary over a wide range which suggests that they are able to adjust their tissue stoichiometry in order to prevent elemental imbalances (Nadaffi et al., 2012). However, it is unknown to what extent dreissenids can adapt their tissue stoichiometry in response to the phytoplankton stoichiometry. Moreover, the linkage between

tissue C:P ratio and growth rate is not evident (Nadaffi et al., 2009; 2012). Hence, it is impossible to conclude whether the current phytoplankton stoichiometry induces phosphorous limitation for zebra mussels in the Marker- and IJsselmeer. Moreover, it is unknown whether quagga mussels differ from zebra mussels concerning their tissue stoichiometry and elemental requirements. Hence, in order to conclude whether the observed population developments in the Marker- and IJsselmeer might be caused by the phosphorous content of the phytoplankton, additional research is required which quantifies the linkage between phytoplankton stoichiometry, mussel stoichiometry and growth rates.

The current scientific knowledge is inadequate to elucidate the impact of suspended sediments and phosphorous availability on dreissenid performance. The proved and hypothesized relations between the environmental conditions and mussel growth are synthesized in a conceptual model (figure 1.9). In the Markermeer, the suspended sediment concentrations are high relative to the chlorophyll concentration. This results in a low seston O:I ratio, which is expected to lower the organic matter ingestion of mussels. This in turn will hamper dreissenid growth. In the Markermeer and IJsselmeer, the chlorophyll concentration is high relative to TP, resulting in a high seston C:P ratio. Possibly, this increases the dreissenid tissue C:P ratio, leading to lower growth rates. As the literature provides no indications of a discrepancy between zebra and quagga mussels with respect to suspended sediments or tissue stoichiometry, the conceptual model applies to both species.

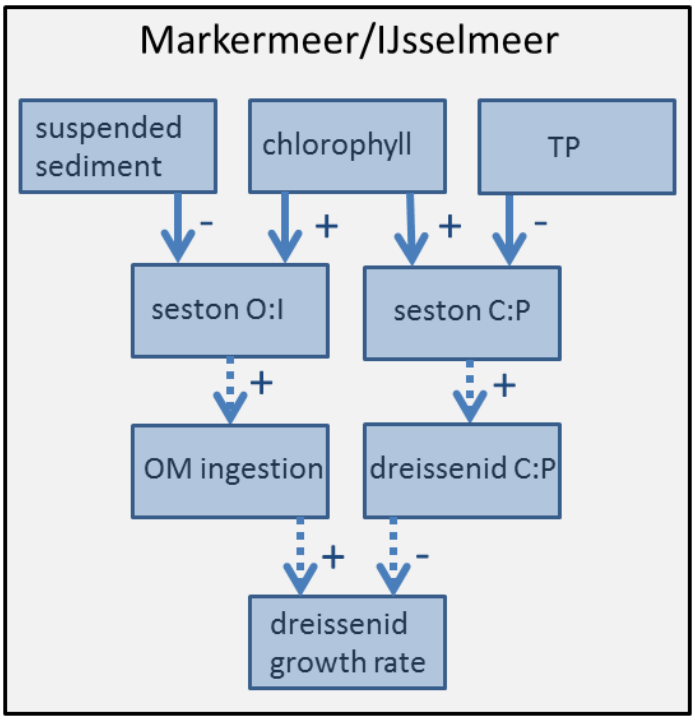

Figure 1.9 Conceptual model of the Markermeer and IJsselmeer ecosystem with respect to dreissenid performance. Solid arrows represent proved correlations, hypothesized relations are showed by dotted lines. (Expected) positive (+) and negative (-) correlations are indicated. TP = total phosphorus concentration; C:P = molar carbon to phosphorous ratio; O:I = organic to inorganic mass ratio; OM = organic matter (mass).

1.4 Research aims and hypotheses

The scientific literature cannot unambiguously explain the observed dreissenid developments in the Marker- and IJsselmeer. Hence, the main aim of this research was to stop the gap of knowledge regarding the causes of the deterioration of the zebra mussel and the recent quagga mussel invasion in the Marker- and IJsselmeer. Therefore, the growth of zebra and quagga mussels in dependence of suspended sediment concentrations and of phytoplankton phosphorous content was researched in two experiments.

The aims of the experiments were 1) to quantify the effect of suspended sediment on mussel growth rates, 2) to quantify to what extent dreissenid tissue stoichiometry is coupled to phytoplankton stoichiometry and how tissue stoichiometry influences growth rates and 3) to compare zebra and quagga mussel growth and tissue stoichiometry to varying food stoichiometry and suspended sediment levels.

Based on the observations in the Markermeer and the current scientific understanding of the influence of suspended sediments on dreissenid performance, I hypothesize that (*hyp* 1):

- *Dreissenid growth rates decrease with increasing suspended sediment levels, because of increased maintenance costs, decreased organic matter ingestion and decreased assimilation efficiency.*

With respect to the second research aim, I hypothesize that (*hyp* 2a):

- *Dreissenid growth rate will decrease with increasing C:P ratio of the phytoplankton, because less phosphorous will be available to the dreissenids.*

Furthermore, because the tissue C:P ratio of zebra mussels varies over a wide range (figure 1.8), I hypothesize that (*hyp* 2b):

- *Dreissenid tissue P content decreases with increasing phytoplankton C:P ratio, because zebra mussels have a flexible tissue C:P ratio.*

The scientific literature provides no clear distinction between zebra and quagga mussels regarding suspended sediment concentrations or tissue stoichiometry. However, quagga mussels outperformed zebra mussels under turbid conditions in Markermeer. Moreover, quagga mussels appeared to have lower metabolic costs than zebra mussels (Stoeckmann, 2003). Hence, I hypothesize that (*hyp* 3a):

- *Under high suspended sediment concentrations, quagga mussels have higher growth rates than zebra mussels, because quagga mussels spend a larger part of ingested food to tissue growth.*

Furthermore, quagga mussels outperformed zebra mussels in the IJsselmeer and Markermeer under decreasing P abundance. From this, it might be concluded that quagga mussels maintain higher growth rates when P is scarce. This suggest that quagga mussels are more capable to prevent elemental imbalances between their food and their needs and hence, that they are even more rheostatic than zebra mussels. Hence, I hypothesize that (*hyp* 3b):

- *Under low P content of the phytoplankton, quagga mussels maintain higher growth rates than zebra mussels because they are more capable to adjust their tissue stoichiometry to prevent elemental imbalances.*

To test the hypotheses under controlled circumstances two indoor microcosm experiments were performed at the NIOO-KNAW in Wageningen from April to June 2013. Zebra and quagga mussels from the Markermeer were reared under four different suspended sediment concentrations to test the effect on the growth rate of the two species. In the second experiment zebra and quagga mussels from the IJsselmeer were reared under two different phytoplankton C:P ratios to test the effect of phosphorous availability on mussel growth and tissue stoichiometry. A detailed description of the methodology of both experiments is given in chapter 2. Next, chapter 3 gives the main results of both experiments and the outcomes are discussed in chapter 4. Finally, the major implications of this research are given in the conclusion (chapter 5).

2. Methodology

2.1 Sediment experiment

2.1.1 Mussel and sediment collection

Zebra and quagga mussels were dredged from the Northern part of the Markermeer in April 2013 (N 52 38.093, E 5 12.073). After collection, individuals of both species between 8 and 14 mm shell length (SL) were sorted and acclimatized in tanks with aerated groundwater for 2 weeks. The water temperature of these tanks was gradually increased from 10^{o}C to 15^{o}C. Because this research aimed to predict the effect of suspended sediments on dreissenids, natural sediment was collected in the Markermeer. The upper 15 cm of the silt layer and the fluffy layer on top (according to Vijverberg et al., 2011) was collected in the North Eastern part of the lake (N 52 34.431, E 5 24.893) in April 2013. Twelve sediment cores were collected with a plastic tube of 1 meter height and a diameter of 10 cm. The tubes were transported to the NIOO and left undisturbed for a few days for most particles to settle. Next, most of the water layer was removed, the fluffy layer carefully collected and mixed together in a large beaker and subsequently distributed to five stocks.

The dry sediment concentration (mg l^{-1}) of each stock was determined by filtration of stock samples over pre-dried (for at least 12 hours at 60^{o}C) and pre-weighted Whatman GF/F filters (N = 6 per stock). After filtration, the filters were dried for at least 12 hours at 60^{o}C and subsequently weighted. The dry mass of the sample volume was calculated by subtracting the initial filter weight from the final filter weight. Next, the percentage of organic matter (%OM) of the sediment was calculated by burning the organic material on the filters at 550^{o}C for at least 2 hours ('Loss On Ignition'). The mean sediment concentration of the stocks was 44 mg ml^{-1} with on average 6.6% organic matter.

2.1.2 Experimental setup

The experiment had a full factorial design with two species (zebra and quagga mussels) and four treatment levels (zero, low, intermediate and high suspended sediment concentrations of 0, 32, 80 and 200 mg sediment l^{-1}, respectively), resulting in eight experimental groups. There were four vessels per group, with four mussels per vessel (i.e. 8 experimental groups (4 treatment levels * 2 species); 4 vessels per group; 4 individual mussels per vessel). Individuals of both species were randomly selected from the acclimatization tanks at the start of the rearing period. The shell dimensions of each mussel were measured with a caliper to 0.1 mm and their wet weight (WW) was determined to 1 mg. Next, each mussel was uniquely marked with a bee tag and used as an experimental or as a baseline mussel. The latter were frozen at minus 80^{o}C for eventual later analysis.

The experimental vessels were randomly divided over two 'handling groups'. The second group was started one day after the first group to limit the daily maintenance work. Nevertheless, the conditions and handling were exactly the same for both groups. Also the description of the experimental setup and conditions holds for both handling groups.

The experimental mussels were placed into cylindrical vessels which were filled with two liter aerated groundwater enriched with chalk. The vessels were placed in water baths with a controlled temperature of 15^{o}C. A 15:9 light:dark cycle was used with a rather low light intensity ranging from 0.1 to 0.4 µmol s^{-1} m^{-2}. A magnetic stirrer was placed at the bottom of each vessel to keep the medium in suspension. After the mussels were placed in the vessels, the vessels were left undisturbed overnight to give the mussels the opportunity to attach to the walls. The next day, sediment from the stocks was added to get the desired treatment levels of 0, 32, 80 and 200 mg dry sediment per liter. Furthermore, an instant shellfish and rotifer diet

(Reed Mariculture Inc, USA) at a concentration of 4 mg C l^{-1} was provided as food to all experimental vessels. The average carbon concentration ('total organic carbon' minus 'dissolved organic carbon') in the Markermeer was around 2.6 mg C l^{-1} over the last two years (live.waterbase.nl, 2013). Because this is above the incipient limiting concentration (Walz, 1978), it is assumed that food *per se* was not limiting in the Markermeer. Hence, food was added at a rather high concentration as it should not become a limiting factor during this experiment.

The vessel medium was exchanged every two days. After removal of the old medium, the vessels were carefully cleaned. Next, the vessels were again filled with aerated groundwater enriched with chalk, sediment (at the required treatment concentration) and food. Note that the mussels remained attached to the bottom or the walls of the vessels when the medium was exchanged. The experiment lasted 42 days.

2.1.3 Experimental conditions

A set of measurements was performed to quantify the experimental conditions in terms of food availability and suspended sediment concentrations during the time between refreshing the vessel medium (48 hours). 60 ml of vessel medium was sampled from 16 experimental vessels at 0, 8, 23 and 47 hours after exchanging the vessel medium. To discriminate between algae and sediment particles, two additional samples per treatment level were taken after the sediment was added at t0, but just before the algae food was added. The size distribution of particles between 2 and 60 µm for each sample was measured with a Beckman Coulter Counter (Multisizer 3). Next, the total dry matter concentration (mg l^{-1}) was determined as described in section 2.1.1 for the sediment stocks. The total carbon concentration (sum of organic + inorganic carbon) was calculated by measuring the carbon mass on a small piece of the filters with a FlashEA 1112 NC analyzer (Thermo Electron Corporation). Furthermore, the %OM of a subset of the filters was determined by LOI at 550°C.

Figure 2.1 shows that the sediment particle concentration increased in accordance with the consecutive treatment levels. The same amount of food was added to the vessels, so the concentration of algae particles was similar among the treatment levels. The ratio of algae to sediment particles was 21, 13 and 6 for the low, intermediate and high sediment level, respectively (the zero sediment level consisted for 100% of algae).

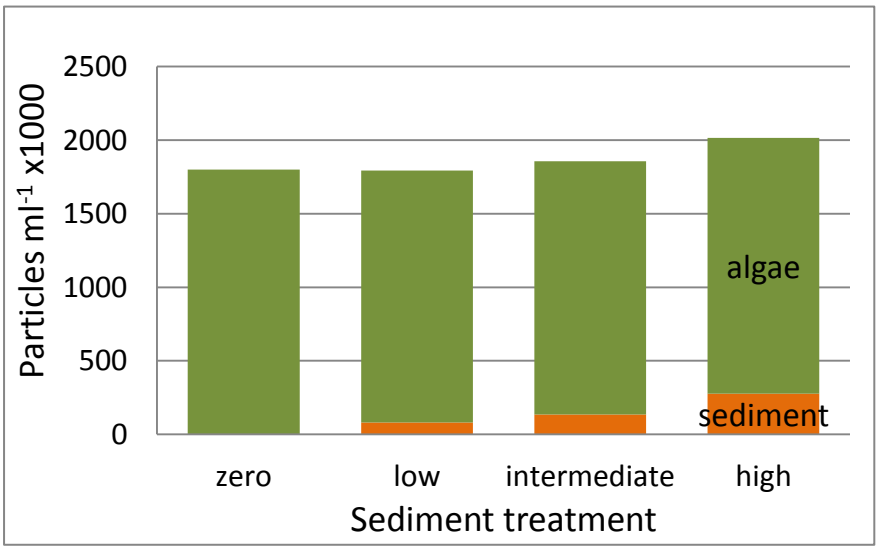

Figure 2.1 Particle concentrations for the four sediment treatment levels at t0 (N = 4 per treatment). Algae concentrations were approximated by subtracting the number of sediment particles from the total number of particles measured at t0.

The concentration of algae and sediment particles in the experimental vessels declined by around 75% over the 48 hours between refreshing of the medium (figure 2.2). For quagga mussels, the particle concentration declined relatively gradually. For zebra mussels, there was a stronger drop between t8 and t23 than between t0 and t8 and between t23 and t47. However, the particle concentration in the zebra and quagga mussel vessels differed siginificantly only in the intermediate sediment level at t23 and t47 (*species* ANOVA with Tukey multiple comparisons, p = 0.09 and p = 0.001 for t23 and t47, respectively). Note that it was only possible to distinguish sediment particles from algae particles at t0.

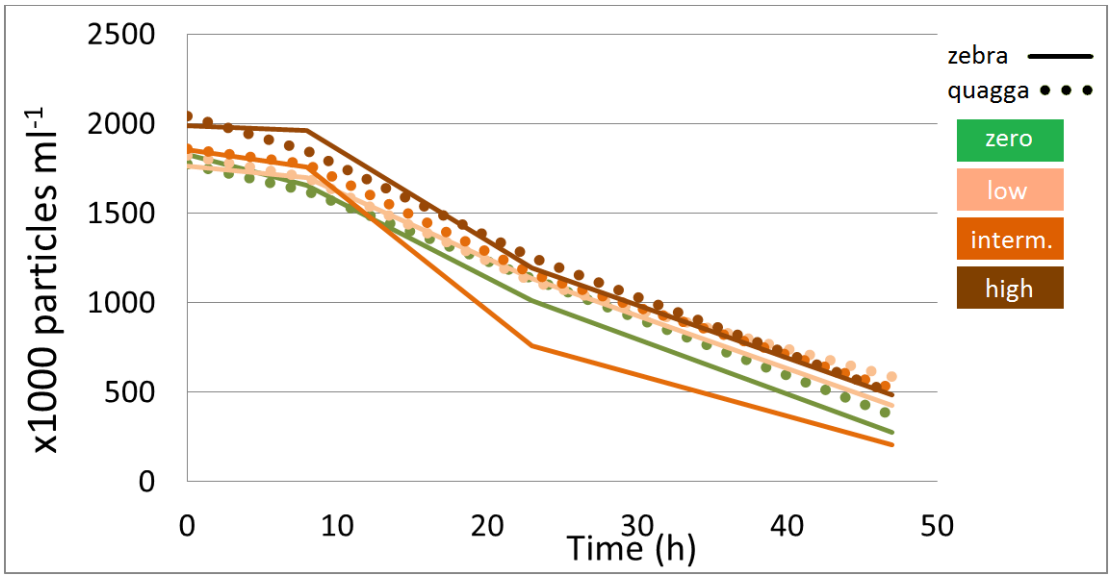

Figure 2.2 Particle concentrations in the experimental vessels (zebra and quagga mussels; zero, low, intermediate and high suspended sediment levels) over the 48 hours between refreshing of the medium (N = 2 per species per treatment level at t0, t8, t23 and t47).

The average carbon concentration in the zero sediment treatment dropped from 4.4 to 2.5 mg C l^{-1} over 48 hours. The carbon concentration was higher in the consecutive sediment treatment levels, which could be attributed to the organic and inorganic carbon fraction of the added sediment. The carbon concentration in each treatment level dropped by about 30 percent over the 48 hours between refreshing. However, the amount of food over time in terms of organic carbon could not be quantified because it was impossible to distinguish the inorganic and organic carbon fractions.

The total dry matter concentration dropped with approximately 70% in the highest sediment level, with 60% in the intermediate level and with 50% in the low sediment level during the 48 hours between refreshing (figure 2.3). Note that the concentrations in figure 2.3 represent the sum of the inorganic and the organic part of the sediment and the food. Next, the mean organic matter concentration (mg OM l^{-1}) for each treatment level over time was calculated by multiplying the %OM by the total dry matter concentration (N = 2 per treatment level per time, table 2.1).

The %OM was lower in the higher sediment levels and was relatively constant over time, except for the zero treatment level. In contrast to the %OM, the OM concentration was higher in the higher sediment levels and decreased substantially over time. The average organic to inorganic mass ratio (O:I ratio) was calculated by dividing the mean OM concentration by the mean inorganic material concentration (total dry matter concentration minus OM

concentration). The O:I ratios were lower for the higher sediment levels at t = 0. However, the O:I ratios of the treatment levels converged after t0.

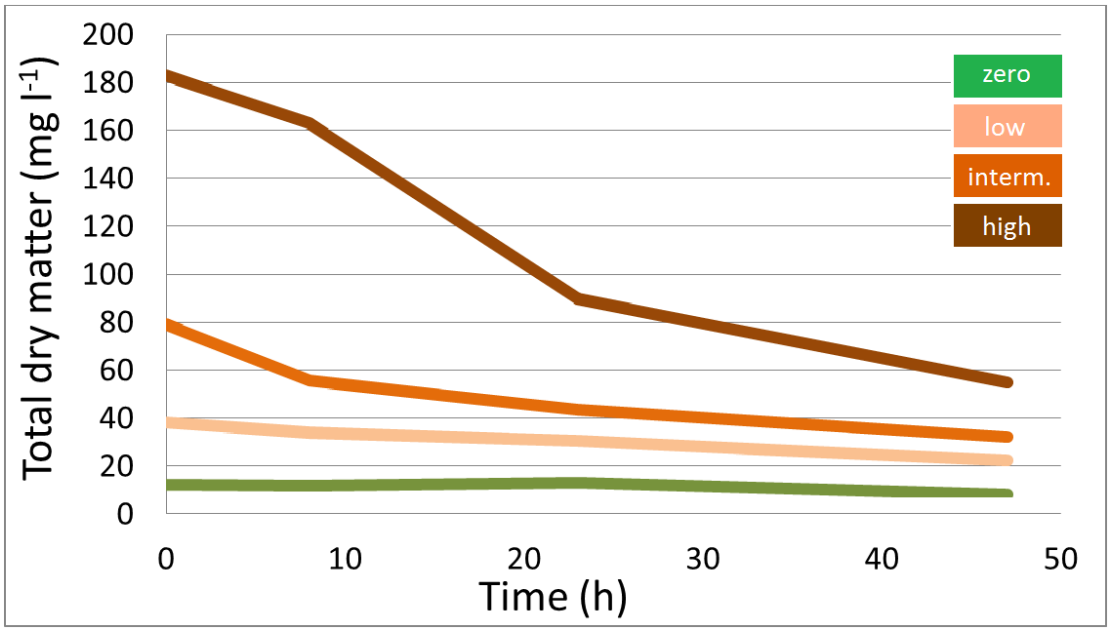

Figure 2.3 Total dry inorganic plus organic matter concentration in the experimental vessels (zero, low, intermediate and high suspended sediment levels) over the 48 hours between refreshing of the medium (N = 4 per treatment level at t0, t8, t23 and t47).

Table 2.1. Average percentage of organic matter (%OM), concentration of organic matter (mg OM l^{-1}) and the mass ratio of organic to inorganic material in the vessel medium of the four different treatments during one time cycle of 48 hours. The values between brackets are the rounded deviation from the actual measurements to the mean (N = 2).

Treatment	%OM			mg OM l^{-1}			mass O:I ratio		
t (h)	0	23	47	0	23	47	0	23	47
Zero	**83** (2)	**31** (2)	**23** (0)	**10** (0)	**4** (0)	**2** (0)	**5.59**	**0.37**	**0.29**
Low	**39** (8)	**26** (1)	**24** (0)	**13** (1)	**7** (1)	**5** (1)	**0.50**	**0.31**	**0.28**
Intermediate	**20** (1)	**25** (1)	**20** (3)	**16** (1)	**11** (2)	**7** (0)	**0.26**	**0.34**	**0.28**
High	**12** (1)	**19** (0)	**19** (2)	**24** (0)	**19** (0)	**11** (1)	**0.15**	**0.27**	**0.26**

2.1.4 Reflection on experimental conditions

The sediment levels were not constant over the 48 hours between refreshing of the vessel medium (figure 2.3) and therefore, they were not considered as continuous variables but as categorical variables (i.e. zero, low, intermediate and high sediment levels). Possibly, the drop in the total dry matter concentration was caused by settling of the suspended sediment and by filtering activity of the mussels. Still, the actual total dry matter concentration was higher in the consecutive sediment treatment levels at each moment of time. Furthermore, the organic matter concentration was also higher in the higher sediment levels (table 2.1). Given the high organic matter concentration, food availability was probably above the incipient limiting concentration in all treatment levels during the whole experiment, as intended.

There was an important discrepancy between the O:I mass ratio and the algae:sediment particle ratio. The former was below one while the latter was much higher than one—at least at t0 (figure 2.1). So, the experimental mussels were exposed to relatively few sediment particles (even in the highest treatment level) which made up the largest fraction of the total

dry material. Moreover, the experimental conditions were hardly distinctive in terms of mass ratio among the four treatment levels from t23 onward. In contrast, the divergent total dry matter concentrations might suggest that the particle ratios remained more distinctive among the treatment levels over time. Altogether, the outcomes of the experiment were carefully interpret as the experimental conditions were not beyond question.

2.1.5 Response variables

The major response variables of this experiment were mussel growth and survival. Survival of the experimental mussels was recorded every second day. Some mussels died due to handling errors (e.g. an erroneous setting of the magnetic stirrer bar). Hence, a distinction was made between those mussels and mussels which died for 'natural' reasons. To compare growth among treatment levels and species, shell length (SL), height (SH) and width (SW) and wet weight (WW) were measured again at the end of the rearing period. Also, the volume (V) of each mussel was approximated by an ellipsoid shape:

$$V = pi * \frac{4}{3} * \frac{SL}{2} * \frac{SH}{2} * \frac{SW}{2}$$

Shell length, height and width were differently correlated for zebra mussels and quagga mussels. This coincide with the particular shape of the species; quagga mussels being higher and less wide than zebra mussels of the same length. Nevertheless, the volume and WW of the mussels from both species was strongly correlated to their shell length (R^2 = 0.9). Moreover WW was strongly correlated to mussel volume for both zebra and quagga mussels (for both species: R^2 = 0.99, N = 64).

Growth of the experimental mussels was computed by comparing the difference in dimensions between the start and end of the experiment. SL growth was reported for comparison with other studies. However, only WW growth was used and reported for analyses of the different experimental groups. Using WW as growth variable enables the comparison with the extensive growth experiments of Baldwin et al. (2002). Also, the WW of all experimental mussels was measured, while SL (and volume) could not be recorded for all experimental mussels because the shell of some mussel was broken during the final measurements.

Growth was expressed as absolute SL change per day (mm day^{-1}):

$$\Delta SL = \frac{(SL2 - SL1)}{T}$$

As percentage mass change over the whole rearing period (%):

$$\%\Delta WW = 100 * \frac{WW2 - WW1}{WW1}$$

And as instantaneous rate of mass change (day^{-1}), which could be interpret as percentage mass change per day, i.e. the specific growth rate (SGR):

$$SGR = 100 * \frac{lnWW2 - lnWW1}{T}$$

The experimental design induced spatial pseudoreplication because four mussels were placed together in one experimental vessel. Hence, these mussels are no independent replicates and instead, the vessels were considered as replicates. Growth per vessel was calculated by averaging the growth of the individual mussels in that particular vessel. However, because not all mussels survived, the number of individuals per vessel ranged between one and four at the end of the experiment. Therefore, the number of survivors per vessel was included in the statistical model as a covariate to check whether it had an effect on growth.

To test the first hypothesis that dreissenid growth rates decrease with sediment level, the effect of sediment treatment level (independent variable) on the growth rate (dependent variable) was analyzed separately for zebra and quagga mussels. When *treatment* had a significant effect, a Tukey post hoc test was performed to see which groups within the concerning species differed. To test whether quagga mussels had higher growth rates than zebra mussels at the highest sediment level (*hyp* 3a), a *species*treatment* two-way ANOVA was performed. This enabled the comparison of species in each treatment level in one single test, which was preferred over testing the species effect separately for each treatment level. Significant interaction effects were unraveled by looking at the simple main effects. To this, the effect of species was analyzed for each treatment level separately (applying Bonferroni adjustment for multiple comparisons, only corrected p-values were reported). All statistical analyses were performed in IBM SPSS Statistics 21.

2.2 Stoichiometry experiment

2.2.1 Mussel collection

Zebra and quagga mussels were dredged from the IJsselmeer near Enkhuizen in April 2013 (N 52 41.929, E 5 18.744). After collection, individuals of both species between 10 and 15 mm shell length were sorted and kept in acclimatization tanks for 4 weeks under the same conditions as the mussels from the Markermeer (section 2.1.1).

2.2.2 Algae culturing

Scenedesmus obliquus were cultured in two chemostats (2 liter) to get algae with two distinct molar carbon to phosphorous ratios. *S. obliquus* were reared on COMBO medium (Kilham et al., 1998) containing 17.5 and 50.0 µmol phosphorous per liter (LP and HP chemostat, respectively). The dilution rate of the LP and HP chemostat was 0.3 day^{-1} and 0.2 day^{-1}, respectively. The average irradiance for both chemostats was 40 µmol m^{-2} s^{-1} and the temperature was kept just below 20^{o}C. The incoming air was enriched with circa 0.4 liter CO_2 per hour for each chemostat.

The carbon concentration in both chemostats was strongly correlated to the optical density at a wavelength of 750 nm (N = 8, R^2 = 0.96). During the experiment, the average carbon concentration in the LP and HP chemostat was 106 and 150 mg C l^{-1}, respectively. The C:P ratio of *S. obliquus* ranged between 450 and 600 in the LP chemostat and was just below 300 in the HP chemostat (figure 2.4).

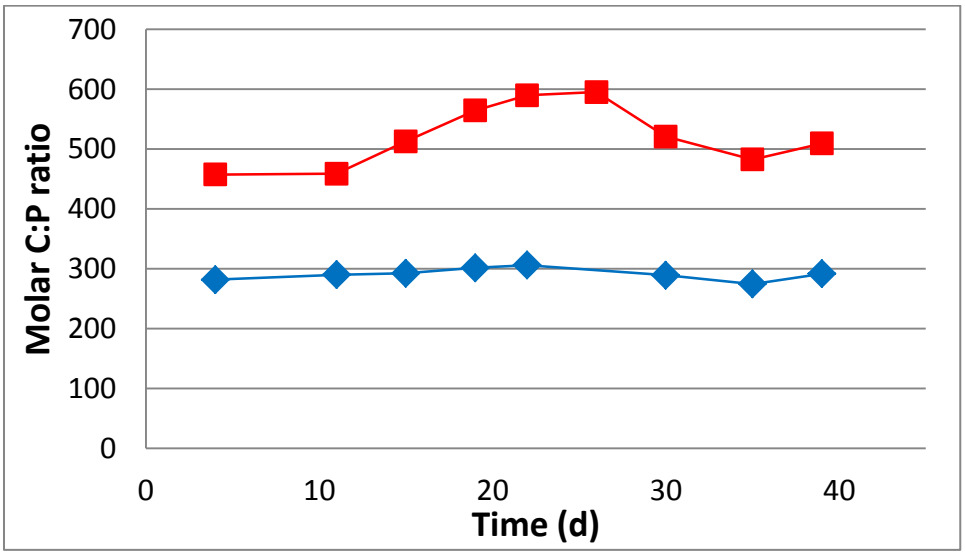

Figure 2.4 The molar C:P ratio of the LP (*red*) and HP chemostat (*blue*) over time during the experiment. The *squares* and *diamond shapes* represents actual measurements (N = 2).

2.2.3 Experimental setup

The experiment had a full factorial design with two species (zebra and quagga mussels) and two treatment levels (low and high molar C:P ratio, i.e. the HP and LP treatment levels, respectively), with 15 replicates per experimental group. The mussels were reared for 42 days.

At the start of the experiment 60 individuals of both mussel species were randomly selected from the acclimatization tanks. The dimensions of these mussels were measured to 0.1 mm and the wet weight (WW) was determined to 1 mg. Next, 30 individuals from both species were used as experimental mussels while the other 30 individuals were frozen at minus 80^{o}C

and were used as a baseline. The experimental mussels were randomly assigned to the LP and HP treatment levels.

Each individual mussel was placed in a beaker which contained 250 ml food medium which was renewed daily. The medium was a mixture of a P-free artificial freshwater (ADaM, Klüttgen et al., 1994) and *S. obliquus* from the LP or HP chemostat. Food quantity in terms of carbon was kept at an equal amount for both treatment levels. As food should not be a limiting factor during this experiment, the food medium had a rather high concentration of 4 mg C l^{-1} (well above the incipient limiting concentration of 2 mg C l^{-1} (Walz, 1978)). The optical density of both chemostats was measured daily to estimate the actual carbon concentration in the chemostats. Next, the required chemostat volume was sampled and mixed with ADaM to get a food mix with a carbon concentration of 4 mg C l^{-1}.

The beakers were kept in a water bath with a constant temperature of 15^{o}C. The light intensity in the experimental room ranged from 0.5 to 0.9 µmol s^{-1} m^{-2} with a 15:9 light to dark cycle. Air bubbling in the beakers was used to prevent that the algae would settle at the bottom.

2.2.4 Experimental conditions

The average C:P ratio of the *S. obliquus* was 287 and 520 for the HP and LP chemostat, respectively (see also figure 2.4). Additionally, the actual carbon concentration, the *S. obliquus* cell density and the C:P ratio in the experimental beakers was monitored over 24 hours (i.e. the time between refreshing the food medium) at day 30 of the experiment. For this test, eighteen experimental beakers (2 treatment levels * (2 species + 1 control (i.e. no mussel)) * 3 replicates) were sampled at 0, 8 and 20 hours after the food medium was refreshed.

The particle concentration of the samples was measured with a Coulter Counter (see section 2.1.3 for a more detailed description of the used technics). The cell concentration in the experimental beakers was approximated by the measured number of particles minus the number of particles in ADaM to which no algae was added. Next, each sample was filtered over a Whatman GF/F filter and further prepared for CN and P analysis. A small piece of each filter was used for carbon and nitrogen measurements with a NC analyzer. The remaining part of each filter was used for the measurement of particulate phosphorous according the persulfate digestion method. For this, the filter samples were ashed at 550^{o}C for at least one hour. Next, 2.5% persulfate was added and subsequently the samples were autoclaved for 30 minutes. Finally, phosphorous concentrations were measured with an auto analyzer.

The number of *S. obliquus* cells was analysed over time for species and treatment level separately. For zebra mussels, the number of algae cells dropped significantly between t0 and t8 in both the LP and HP treatment level (figure 2.5). For quagga mussels, the number of algae cells dropped significantly between t = 0 and t = 8 in the HP treatment level only. The mean cell densities of the control beakers were not significantly different over time in LP treatment level. However, in the HP treatment level, the number of algae cells dropped significantly between t0 and t8. Next, the effect of species on the mean cell concentration was analysed for each time, separately for both treatment levels (*species*time* ANOVA). In both the LP and HP treatment level the mean cell concentration did not differ significantly among zebra mussels, quagga mussels and the control at any moment of time (LP: $F_{(2,18)} = 2.136$, $p = 0.147$; HP: $F_{(2,18)} = 0.092$, $p = 0.092$).

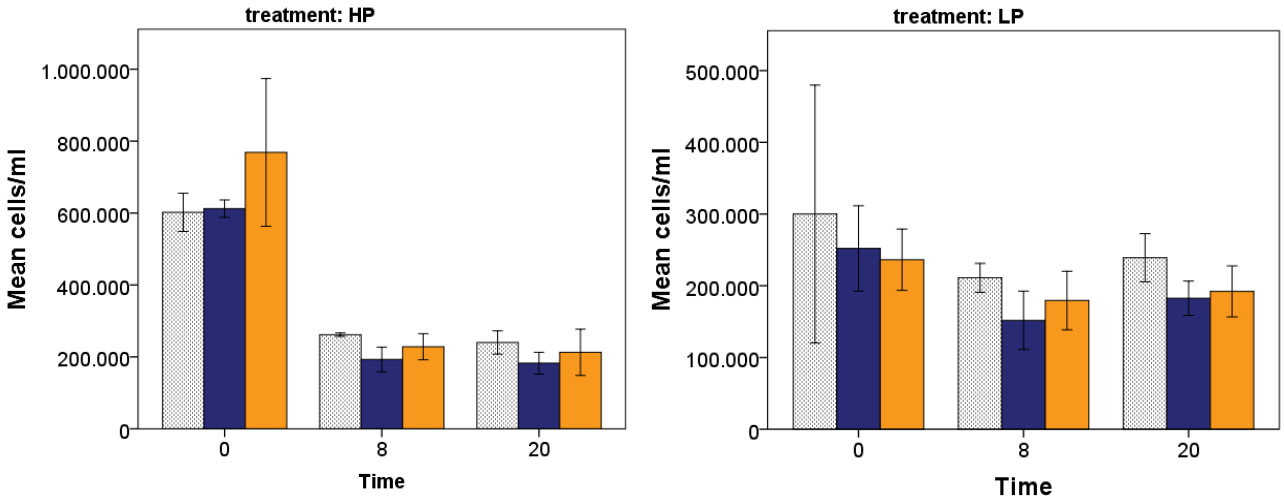

Figure 2.5 Mean *Scenedesmus* concentration (cells ml^{-1}) over time after refreshing of the food medium at t = 0. For control (i.e. no mussel; *grey raster*), zebra (*blue*) and quagga (*orange*) beakers (N = 3 for each group at each time). Error bars show +/- 2 SE.

The LP chemostat (106 mg C l^{-1}) had a *S. obliquus* concentration of 5.15E6 cells ml^{-1}, against 10.42E6 cells ml^{-1} in the HP chemostat (145 mg C l^{-1}). This implies that *S. obliquus* in the LP chemostat contains more carbon per cell than in the HP chemostat (20.6 and 13.9 pg C $cell^{-1}$, respectively). This explains the much higher cell concentration in the HP treatment level than in the LP level at t0, for the carbon concentration had to be similar in both treatment levels. Indeed, carbon concentrations where very similar for the LP and HP food mix at t = 0 (figure 2.6). The carbon concentration dropped over time, and in general more strongly for beakers with mussels than for the control beakers. However, the carbon concentration did not drop below the incipient limiting concentration of 2 mg C l^{-1} (Walz, 1978). The mean C:P ratio of the food mix dropped between t = 0 and t = 20 from 435 to 386 in the LP (N = 9) and from 267 to 226 in the HP (N = 9). Note that the C:P ratio of the food mix at t = 0 was lower than of the chemostats, which had a C:P ratio of 521 and 289, LP and HP respectively.

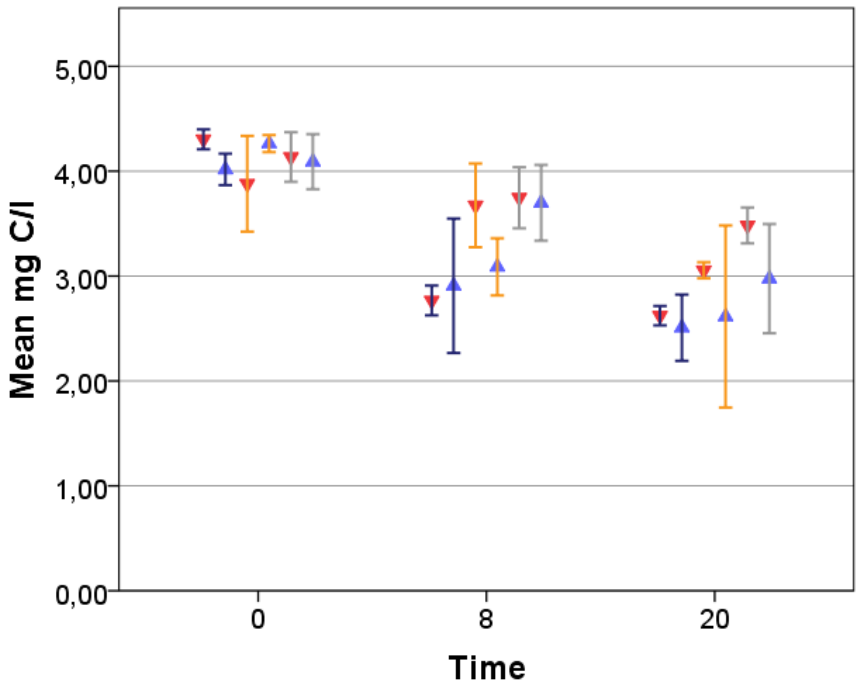

Figure 2.6 Mean carbon concentration (mg l^{-1}) over time for zebra mussels (*blue*), quagga mussels (*orange*) and control (grey). Separately for LP (*red triangles*) and HP (*blue triangles*). Error bars indicate +/- 2 SE.

2.2.5 Reflection on experimental conditions

Both the algae particle concentration (figure 2.5) and the carbon concentration (figure 2.6) suggest that food availability was not differently for both species. Moreover, food availability was no limiting factor to mussel growth as the carbon concentrations were above the incipient limiting concentration during the 24 hours between refreshing the food mix of the beakers. The actual C:P ratio in the experimental beakers was slightly lower than in the chemostats. Nevertheless, the LP and HP treatment levels were still clearly distinctive and hence, adequate to test the hypotheses.

2.2.6 Response variables

Survival was recorded daily when the food medium was refreshed. The numbers of dead mussels per species and treatment were compared with a Fisher's Exact test. Dead mussels were not incorporated in any other analyses.

Wet weight (WW) and shell dimensions were measured at the end of the experiment (t = 42 days). A Paired Samples T Test for each experimental group was performed to test whether the final WW was significantly different from the initial WW (i.e. to test whether there was significant growth). Next, the specific growth rate (SGR) in terms of WW was calculated, as well as absolute SL growth (see section 2.1.5 for detailed calculations).

To test whether growth rates decreased with increasing C:P ratio of the phytoplankton (*hyp*. 2a), the growth rates between the treatment levels LP and HP were compared for each species separately with an Independent Samples Test. Next, a *species*treatment* two-way ANOVA was performed to test if there were significant differences in growth between the four experimental groups. The latter analysis was used to test the hypothesis that quagga mussels maintain higher growth rates than zebra mussels at high C:P ratios of the phytoplankton (*hyp* 3b).

Dry tissue weight

Next to WW and volume, dry tissue weight (DW) was used to test the hypotheses regarding the linkage between phytoplankton stoichiometry and mussel growth rates. The DW was determined for the baseline mussels at the start of the experiment and for the experimental mussels at the end of the experiment. For this, the entire mussel tissue—except the anterior adductor muscle—was retrieved from the shell and beat beaded with a small iron ball in a tube. Subsequently, the mussel tissue was dried in a freeze drier for three days before the DW was measured to 1 µg.

As the baseline mussels did not differ significantly from the experimental mussels in terms of initial WW and volume, it was assumed that the mean initial DW was not different for experimental and baseline mussels. Nevertheless, DW growth was not calculated, because the initial DW of the experimental mussels could not be estimated very precisely (the correlation for the baseline mussels between DW and volume was significant, but not very strong; R^2=0.59 for zebra mussels, R^2=0.61 for quagga mussels). Hence, the DW per treatment level (LP, HP and baseline) was analyzed for zebra and quagga mussels separately to test whether DW was significant lower in the LP treatment level—to approximate hypothesis 2a (*treatment* one-way ANOVA). Also, the DW of the four experimental groups and the two baseline groups was compared amongst each other to analyze species differences, as approximation of *hyp*. 3b (*species*treatment* two-way ANOVA; simple main effects of species per treatment level were analyzed with Bonferroni adjustment for multiple comparisons, only corrected p-values were reported). All statistical analyses were performed in IBM SPSS Statistics 21.

Subsequently, the DW per unit of WW (µg mg^{-1}) was calculated and used as an indicator for mussel condition. Mussel condition is often similarly indicated by tissue DW to dry shell weight (Nadaffi et al., 2012) or by tissue DW to volume (Noordhuis, 2009). DW by WW is no growth variable, but was used to compare the condition of the mussels in the experimental and baseline groups. Hypotheses 2a and 3b were tested similarly as for DW.

Stoichiometry

The dry tissue of each experimental and baseline mussel was analyzed on carbon, nitrogen and phosphorous content. A small part of each tissue was used for carbon and nitrogen measurements with the NC analyzer. The largest part of each tissue was used for phosphorous measurements according to the sulfate digestion method (see section 2.2.4).

Hypothesis 2b states that dreissenid tissue P content (%P) decreases with increasing food C:P ratio because dreissenids would have a flexible tissue stoichiometry. First, it was tested whether tissue C:P ratios differed with treatment level (including the baseline as a treatment level) (*treatment* one-way ANOVA for zebra and quagga mussels separately). Next, it was tested for each species whether the %P was lower in the LP than in the HP treatment level, and also compared with the baseline (*hyp* 2b; *treatment* one-way ANOVA). Also, a *species*treatment* two-way ANOVA was performed to compare zebra and quagga mussels C:P ratio and %P.

3. Results

3.1 Sediment experiment

3.1.1 Survival

Thirteen of the 128 experimental mussels died during the rearing period. Six mussels died due to handling errors and seven mussels died for other reasons. In total, 90% of the experimental mussels survived during the rearing period and only 5% died for reasons other than handling. The latter involved three zebra mussels, one in the zero and two in the intermediate sediment level and four quagga mussels, one in each sediment level. Because of the low number of deceased mussels no further survival analysis has been performed.

In one experimental vessel (quagga mussels, intermediate sediment level) three mussels were literally crushed by an erroneous stirrer bar. The only surviving mussel showed hardly any growth at the end of the rearing period and therefore this vessel was an outlier with respect to the other vessels. Hence, the concerning vessel was not included in growth analyses.

3.1.2 Growth

The initial dimensions of zebra and quagga mussels are summarized in table 3.1. The average dimensions of the experimental vessel did not differ significantly by species or treatment level at the start of the experiment (*species*treatment* ANOVA).

	Zebra	**Quagga**
SL (mm)	10.0 (1.4)	10.2 (1.4)
Volume (mm^3)	118.4 (52.5)	107.7 (47.3)
WW (mg)	120.8 (51.1)	102.8 (42.7)

Table 3.1 Mean (Std. Deviation) shell length (mm), volume (mm^3) and wet weight (mg) per species at the start of the experiment (N = 64 for both species).

Almost all mussels had increased in SL, volume and WW at the end of the rearing period (t = 42 days). Moreover, the mean WW growth was positive for all experimental vessels. Figure 3.1 shows the mean SGR (of WW) of the eight experimental groups (with four vessels per group). The absolute SL growth and the relative WW growth showed the same pattern as the SGR of WW (see table 3.2).

The number of survivors per experimental vessel—as covariate—had no significant effect on the SGR for zebra mussels (ANCOVA; $F_{(1,11)} = 0.029$, $p = 0.867$) or quagga mussels (ANCOVA; $F_{(1,10)} = 1.314$, $p = 0.278$). Hence, the number of survivors was excluded from the statistical model. Next, treatment level had a significant effect on the SGR of zebra mussels (ANOVA; $F_{(3,12)} = 4.915$, $p = 0.019$) but not on the SGR of quagga mussels (ANOVA; $F_{(3,11)} = 3.112$, $p = 0.071$). The SGR of zebra mussels in the high sediment level was significantly higher than in the zero and low sediment level (Tukey Multiple Comparisons, $p = 0.021$ and 0.048, respectively).

When comparing the SGR of zebra and quagga mussels over the four sediment levels (figure 3.1), there was a significant *species*treatment* interaction effect (two-way ANOVA; $F_{(3,23)} = 3.636$, $p = 0.028$). In the high sediment level, the SGR of zebra mussels was significantly higher than of quagga mussels (simple main effect; $F_{(1,23)} = 11.145$, $p = 0.003$). In all other treatment levels, species had no effect on the mean SGR (i.e. zebra mussel growth rates equaled quagga mussel growth rates).

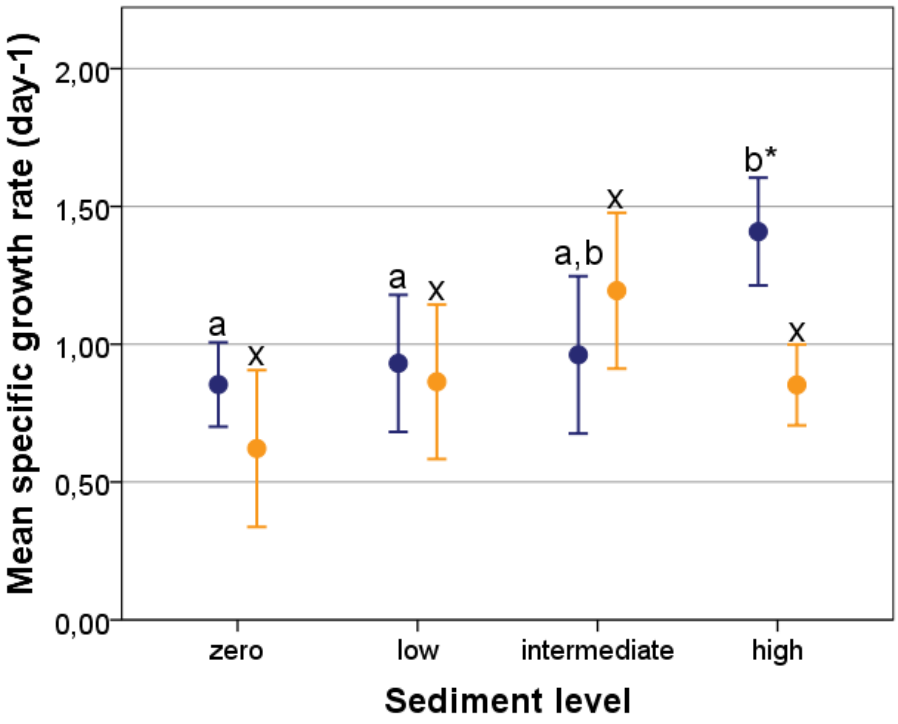

Figure 3.1 Mean specific growth rate (day^{-1}) in terms of wet weight for zebra mussels (*blue*) and quagga mussels (*orange*). N = 4 for all groups, except for quagga mussels at the intermediate sediment level where N = 3. Error bars shows +/- 2 SE. Significant higher growth than the other species in same treatment is given by an asterisk (α = 0.05) (two-way ANOVA). Super script letters (a,b,c for zebra mussels; x,y,z for quagga mussels) classify groups within a species with no significantly different mean growth rates (Tukey Multiple Comparisons for zebra and quagga mussels separately, α = 0.05).

Table 3.2 Mean shell length growth (mm day^{-1}), relative wet weight growth (%) and specific growth rate (day^{-1}) with the standard error given between brackets. N = 4 for all groups, except for quagga mussels in the intermediate treatment, where N = 3. Significant higher growth than the other species in same treatment is given by an asterisk (α = 0.05) (two-way ANOVA). Super script letters within a species (a,b,c for zebra mussels; x,y,z for quagga mussels) classify groups within a species with no significantly different mean growth rate (Tukey Multiple Comparisons for zebra and quagga mussels separately, α = 0.05).

	Shell length growth (mm day^{-1})		*Wet weight growth (%)*		*Wet weight specific growth rate (day^{-1})*	
	Zebra	**Quagga**	**Zebra**	**Quagga**	**Zebra**	**Quagga**
Zero	0.029 (0.003)^{a,*}	0.012 (0.004)^{x}	45.0 (5.0)^{a}	31.0 (7.8)^{x}	0.854 (0.077)^{a}	0.622 (0.142)^{x}
Low	0.032 (0.003)^{a}	0.020 (0.005)^{x}	49.4 (7.9)^{a}	44.9 (8.4)^{x}	0.931 (0.124)^{a}	0.864 (0.140)^{x}
Interm.	0.033 (0.005)^{a,b}	0.029 (0.008)^{x}	52.1 (8.8)^{a,b}	66.9 (9.7)^{x}	0.961 (0.143)^{a,b}	1.194 (0.141)^{x}
High	0.047 (0.003)^{b,*}	0.016 (0.001)^{x}	82.5 (7.5)^{b,*}	44.3 (4.7)^{x}	1.409 (0.098)^{b,*}	0.853 (0.073)^{x}

3.2 Stoichiometry experiment

3.2.1 Survival

One quagga mussel died during the experiment due to a handling error and was therefore not included in the survival analysis. Five zebra mussels diet for other reasons than handling, three in the LP treatment level and two in the HP level. So, seventeen percent of the zebra mussels died. However, survival was not significantly different between species or between the LP and HP zebra mussel groups (Fisher's Exact test, between species $p = 0.052$; between LP and HP zebra mussels $p = 1.000$).

3.2.2 Wet weight and volume growth

The initial dimensions in terms of shell length (SL), volume and wet weight (WW) were not significant differently among the experimental groups (*species*treatment* ANOVA; see table 3.3 for species means). WW and volume were strongly correlated ($R^2 = 0.98$ for both species) and this relation was hardly changed at the end of the experiment.

Table 3.3 Mean (Stand. Dev.) initial dimensions (SL (mm), volume (mm^3) and WW (mg)) of zebra and quagga mussels (N = 30 for each species).

	Zebra	**Quagga**
SL (mm)	13.0 (1.2)	13.1 (1.4)
Volume (mm^3)	272 (84)	250 (82)
WW (mg)	254 (78)	231 (72)

A small degrowth was observed for the majority of the zebra and quagga mussels during the experiment (table 3.4). Indeed, the mean WW of all experimental groups was significantly lower at the end of the experiment than at the start of the experiment (Paired Samples T Test, $p < 0.05$ for all experimental groups). The SGR of each species was not significantly different between the LP and HP level (Independent Samples Tests for zebra mussels and quagga mussels; $p = 0.140$ and $p = 0.941$, respectively). Furthermore, the mean SGR did not differ significantly among the four experimental groups (*species*treatment* ANOVA).

Table 3.4 Mean values (Std. Error) of total SL growth (mm) and SGR of WW (d^{-1}).

	Zebra mussels		**Quagga mussels**	
	LP (N = 12)	**HP** (N = 13)	**LP** (N = 14)	**HP** (N = 15)
SL growth (mm)	-0.067 (0.036)	0.008 (0.014)	-0.057 (0.020)	-0.087 (0.022)
WW SGR (d^{-1})	-0.047 (0.009)	-0.028 (0.009)	-0.045 (0.015)	-0.047 (0.026)

3.2.3 Dry tissue weight

SL and WW growth was very small and mostly not significantly different among the species and treatment levels (table 3.4). In contrast, the mean dry tissue weight (DW) of the mussels showed substantial differences among the experimental groups. The mean DW of mussels from the LP treatment level was almost forty percent lower than in the baseline, while the mean DW in the HP level was very similar to the baseline (left panel in figure 3.2; baseline is treated as another treatment level here).

For zebra mussels, the mean DW in the LP level was significantly lower than in the baseline (Tukey multiple comparisons, $p = 0.004$). For quagga mussels, the mean DW of the LP level was significantly lower than both the baseline (Tukey multiple comparisons, $p = 0.0003$) and the HP level (Tukey multiple comparisons, $p = 0.001$). Moreover, while the effect of

treatment on the DW was significant, the effect of *species* was not (*species*treatment* ANOVA, *treatment* $F_{(2,105)}$ = 14.200; p < 0.0001). So, the mean DW of zebra and quagga mussels did not differ significantly within any of the treatment levels (left panel figure 3.2).

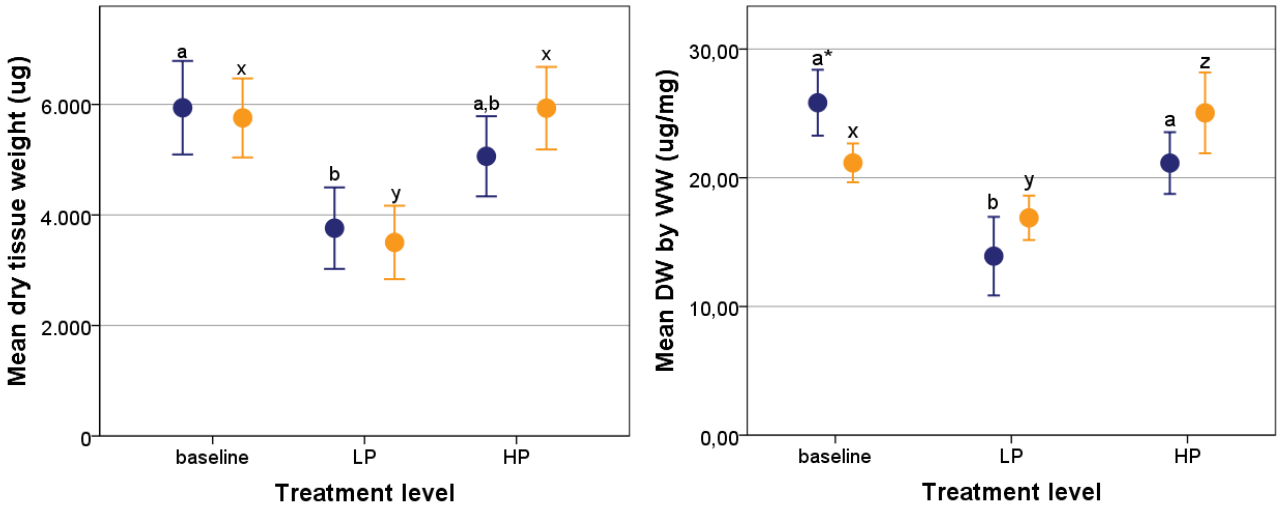

Figure 2.2 Mean dry tissue weight (DW in µg) (left) and mean condition (DW by WW in µg mg^{-1}) of zebra mussels (*blue*) and quagga mussels (*orange*) from the baseline (N = 29; N = 29, respectively) and experimental mussels from the LP (N = 12; N = 14, resp.) and HP (N = 13; N = 14, resp.) treatment level. The error bars indicate +/- 2 SE. Super script letters classify groups within a species which did not differ significantly from each other (separately for zebra mussels (a,b,c) and quagga mussels (x, y, z), *treatment* one-way ANOVA with Tukey multiple comparisons, α = 0.05). An asterisk denotes significant higher growth than other species within the same treatment level (*species*treatment* two-way ANOVA, simple main effects with α = 0.05).

The mean condition (DW by WW) of zebra mussels in the LP treatment level was significantly lower than in the baseline (Tukey multiple comparisons, p < 0.0001) and then in the HP level (Tukey multiple comparisons, p =0.012). The condition of quagga mussels was significantly different among all treatment levels, as depicted in the right panel of figure 3.2. The mean condition revealed a significant interaction effect of *species* and *treatment* (*species*treatment* ANOVA, $F_{(2,105)}$ = 8.280, p = 0.0005). In the baseline, zebra mussels had a significantly better condition than quagga mussels ($F_{(1,105)}$ = 11.437, p = 0.001). However, the condition of zebra and quagga mussels was not significantly different in both the LP and HP treatment level.

3.2.4 Stoichiometry

In general, the C:P ratio of the food was considerably higher than the C:P ratio of mussel tissue (table 3.5). The molar C:P ratio of the baseline and experimental mussels ranged from 79 to 143 for zebra mussels and from 97 to 205 for quagga mussels. There were no significant differences in tissue C:P ratio among the treatment levels for zebra mussels (*treatment* one-way ANOVA $F_{(2,51)}$=0.551, p = 0.580). In contrast, for quagga mussels, *treatment* had a significant effect on mean C:P ratio (one-way ANOVA $F_{(2,54)}$= 5.331, p = 0.008) with a higher C:P ratio in the HP treatment level than in the baseline (Tukey multiple comparisons, p = 0.013).

The mean C:P ratio was higher for quagga mussels than for zebra mussels in all treatment levels (*species*treatment* two-way ANOVA with significant interaction effects, $F_{(2,105)}$ = 4.313, p = 0.016; simple main effects of species, p < 0.0001 for each treatment level, see also figure 3.3).

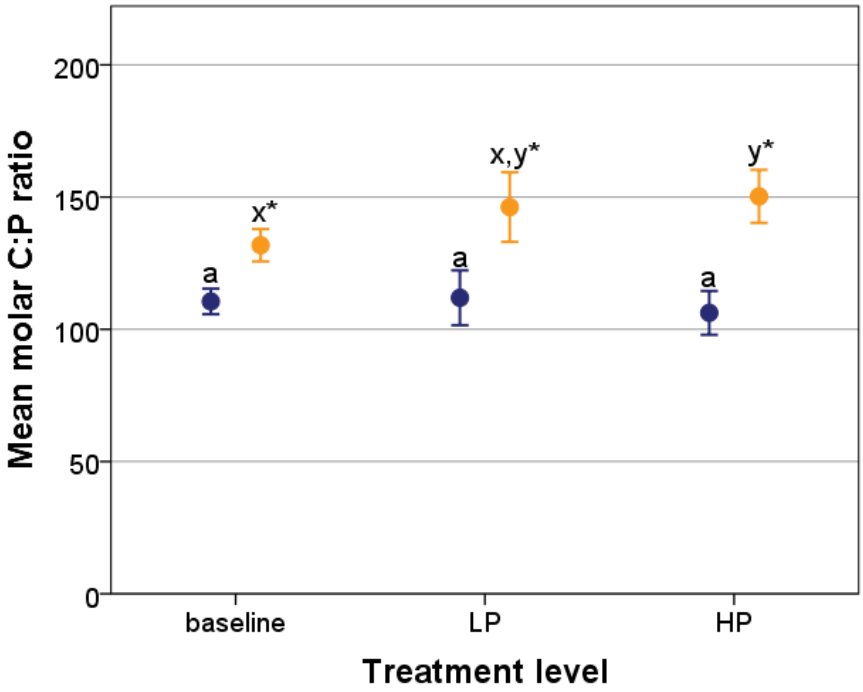

Figure 3.3 The mean molar C:P ratio for zebra mussels (*blue*) and quagga mussels (*orange*) +/- 2 SE. Super script letters classify groups within a species which did not differ significantly from each other (separately for zebra mussels (a,b,c) and quagga mussels (x, y, z), *treatment* one-way ANOVA with Tukey multiple comparisons, $\alpha = 0.05$). An asterisk denotes significant higher growth than other species within the same treatment level (*species*treatment* two-way ANOVA). For N, see caption table 3.5.

The %P of zebra mussels was not different among the treatment levels. In contrast, for quagga mussels, *treatment* had a significant effect on %P (one-way ANOVA $F_{(2,54)}= 5.994$, $p = 0.004$). The mean %P in the baseline was higher than in the HP treatment level (Tukey multiple comparisons, $p = 0.006$). When comparing the %P over the six groups, there was a significant interaction effect of *species*treatment* (two-way ANOVA $F_{(2,105)} = 4.327$, $p = 0.016$, see also table 3.5). In all three treatment levels, zebra mussels had significant higher %P than quagga mussels (simple main effects, $p < 0.0001$ in all treatment levels).

Table 3.5 Mean %P, C:P, C:N and N:P ratios (Std. Error) for food, zebra and quagga mussels. 'Food' encompass an instant diet for baseline mussels (applied during acclimatizing, see section 2.2) and S. obliquus from the chemostats for the LP and HP treatment levels. Asterisks denote species with significant higher mean than the other species for the corresponding variable (* $p < 0.01$; ** $p \leq 0.001$). Baseline food, N = 4; LP food, N = 20; HP food, N = 18. Baseline zebra mussels (ZM), N = 29; baseline quagga mussels (QM), N = 29; LP ZM, N = 12; LP QM, N = 14; HP ZM, N = 13; HP QM, N = 14.

		Baseline	LP	HP
%P	Zebra	1.13 (0.03)**	1.13 (0.06)**	1.19 (0.04)**
	Quagga	0.96 (0.02)	0.87 (0.04)	0.84 (0.03)
C:P	Food	235	520	287
	Zebra	111 (2.5)	112 (5.2)	106 (4.1)
	Quagga	132 (3.1)**	146 (6.6)**	150 (5.0)**
C:N	Food	11.9	10.8	15.5
	Zebra	4.6 (0.1)	4.5 (0.1)	4.6 (0.1)
	Quagga	4.4 (0.0)	4.7 (0.1)	5.1 (0.1)**
N:P	Food	20.7	48.4	18.6
	Zebra	24.1 (0.5)	24.8 (1.0)	23.4 (1.0)
	Quagga	29.7 (0.7)**	31.1 (1.6)**	29.9 (1.1)**

For quagga mussels, the mean C:N ratio in the HP treatment level was significant higher than in the baseline and the LP treatment level (*treatment* one-way ANOVA with Tukey multiple comparisons $p < 0.0001$ and $p = 0.037$, respectively). For zebra mussels, the mussel tissue C:N ratio did not differ among the treatment levels. The C:N ratio was not different for zebra

mussels and quagga mussels, but in the HP treatment level, where quagga mussels had a significant higher C:N ratio than zebra mussels (*species*treatment* two-way ANOVA simple main effect $p = 0.0002$).

The tissue N:P ratio was not different per treatment level within both species. Between species, the N:P ratio was higher for quagga mussels than zebra mussels in all treatment levels (*species*treatment* two-way ANOVA with significant *species* effect; simple effect analyses, $p < 0.0001$).

4. Discussion

4.1 Suspended sediment experiment

4.1.1 Growth response to suspended sediments

The experimental results do neither support nor falsify the hypothesis which predicted lower growth rates at higher sediment levels (*hyp.* 1). For zebra mussels, growth rates in the low and intermediate sediment levels were similar to the growth in the control vessels without sediment. Moreover, growth rates in the highest sediment level were even higher than in the zero and low sediment level. For quagga mussel growth, there were no significant differences between any of the sediment levels. Furthermore, the growth rate of zebra and quagga mussels was similar at each sediment level, except at the highest suspended sediment concentration, where zebra mussel growth was significant higher than quagga mussel growth. This falsified the hypothesis that quagga mussels have higher growth rates than zebra mussels at the highest sediment level (*hyp. 3a*).

The SGR of quagga mussels in terms of WW was comparable to values reported by Baldwin et al. (2002). In contrast, they reported much lower SGRs for zebra mussels than found in this study, but at the highest rations, where they found higher growth rates (Baldwin et al., 2002). Note that even the highest food concentration used by Baldwin et al. (2002) was much lower than supplied in this study. To mimic the conditions in the Markermeer, 4 mg C l^{-1} was added to the experimental vessels to prevent food limitation. Also, an extra amount of food was available in the form of organic matter in the sediment which was used. Hence, the food (i.e. organic matter) concentration was higher with increasing sediment level (see table 2.1). Moreover, the O:I ratio was very similar for all treatment levels 23 hours after refreshing the vessel medium. The combined effect of abundance of food and very similar O:I ratios might underlie the absence of different growth rates among the sediment levels.

The actual O:I ratio suggest very adverse conditions for mussels in all suspended sediment levels. Schneider et al. (1998) identified an O:I threshold of 0.5, below which scope for growth would be negative according to their results. Nevertheless, growth was positive in this study despite O:I ratios below 0.5 in all sediment levels during almost the whole experiment. Several reasons might underlie this discrepancy between the observed and expected growth.

First of all, most studies which researched the response of mussels to suspended sediments did grazing experiments in which clearance and respiration rates, pseudofeces production etc. were measured (e.g. Penning et al., 2012; Baldwin et al., 2002; Schneider et al., 1998; Madon et al., 1998). These experiments lasted between one and three hours and hence, the results might be considered as a snapshot of the food clearance and processing by dreissenids in response to suspended sediments. Moreover, the mussels were usually acclimatized to the experimental conditions only a few hours before the start of the experiment. However, Summers et al. (1996) found that zebra and quagga mussels were less sensitive (in terms of respiration) to high turbidity when the mussels were acclimatized for four weeks to turbid conditions. So, the results of grazing experiments without a prolonged acclimatization time might be biased because the mussels were not (yet) habituated to the experimental conditions.

Several studies suggest that dreissenids might not be able to withstand turbid conditions, as the resulting clearance rate reduction was accompanied by decreased organic matter ingestion (Madon et al., 1998) and because particle selection appeared to cease at high sediment concentrations (Baldwin et al., 2002; Schneider et al., 1998). In contrast, Penning et al. (2012) found increased clearance of algae particles by zebra mussels up to sediment concentrations of 500 mg l^{-1}, which suggest that dreissenids still select food particles at very high sediment

concentrations. So, their results are not conflicting with the found positive growth rates in this experiment, despite the low O:I ratios. Hence, the study by Penning et al. (2012) supports the major outcome of this growth experiment.

Furthermore, the results from Penning et al. (2012) suggest a more efficient particle selection at higher sediment concentrations, which might explain the significant higher growth rate of zebra mussels in the highest sediment level. Moreover, Penning et al. (2012) used sediment from the most mobile, fluffy layer in the Markermeer, like was used in this experiment. In contrast, clay or ashed sediments were used in most other grazing experiments (e.g. Baldwin et al., 2002; Madon et al., 1998). This might be another reason for the discrepancy between the observed growth in this experiment and the expected growth based on the majority of grazing experiments.

Finally, in contrast to the relatively low mass O:I ratio, the ratio of algae particles to sediment particles was much higher than 1 in all treatment levels (see figure 2.1). In the highest sediment level, there were still six times more algae particles than sediment particles, while the mass O:I ratio was only 0.15 at t0. The particle ratio over time was not calculated, but it is not unlikely that the amount of algae particles remained higher than the number of sediment particles during the whole experiment. This might imply that the conditions (in terms of food to sediment ratios) were not so adverse as suggested by the low ratio of organic mass to inorganic mass. And hence, if sediment particles make up only a minor part of the 'seston' and food was not limiting in any of the treatment levels, this might have caused the equal, positive growth rates among most sediment levels.

4.1.2 The conceptual model—suspended sediments

The conceptual model presented in section 1.3.5 (figure 1.9) summarizes the current scientific knowledge regarding the impact of suspended sediments on dreissenid growth rates. It was predicted that increased suspended sediment concentrations would lower the seston O:I ratio, which in turn leads to a declined organic matter ingestion resulting in lower growth rates. However, the experimental results did not show a relation between suspended sediment concentrations and growth rates. On the one hand, this might be an artifact of the indistinctive O:I mass ratios among the treatment levels or of the high algae to sediment particle ratio in all treatment levels. On the other hand, the experimental results might imply that the organic matter ingestion by dreissenids was not hampered by the suspended sediment concentrations used in this study (which is supported by Penning et al. (2012)). The latter concept suggest that the organic matter ingestion by dreissenids is independent of the seston O:I ratio. In conclusion, the effect of suspended sediments on dreissenid growth is not irrefutably quantified as the adequacy of actual experimental conditions was questionable.

The experimental results suggest that that quagga mussels are not less sensitive to high suspended sediment concentrations than zebra mussels. This corresponds to the studies of Baldwin et al. (2002) and Summers et al. (1996), who found no difference in the response to suspended sediments (in terms of clearance rate, particle selection, pseudofeces production and respiration) between both species.

4.1.3 Implications for the Markermeer

In this experiment, Markermeer mussels were exposed to very similar conditions as in the lake itself. Sediment from the mobile sediment layers from the Markermeer was used, albeit the fraction of organic matter (6.6%) was relatively low compared to the measured organic fraction of suspended sediments in the water column (18 to 76% according to Vijverberg et

al., 2011). The sediment concentrations in the intermediate and high treatment level corresponded to the actual average sediment concentrations in the Markermeer (30 to 80 and 60 to 200 mg total dry suspended matter l^{-1}, respectively). Moreover, the sediment was refreshed every other day to ensure that mussels could use the organic part of the sediment as food.

The general result from this growth experiment was that turbid conditions are not per se deteriorative for dreissenids, as growth rates did not decrease with increasing suspended sediment levels. This finding questions whether high suspended sediment concentrations are the actual reason for the poor condition of the zebra mussel population in the Markermeer.

The zebra mussel population in the Markermeer was already exposed to turbid conditions for decades before the population crashed. However, during the crash of the population in the early 1990s, the sediment concentrations appeared to be much higher while the chlorophyll levels were relatively low (Noordhuis & Houwing, 2003). It was not tested in this experiment whether these conditions indeed have caused the mussel crash, because sediment concentrations in this experiment were much lower and food concentrations much higher than during the crash. Instead, the experimental conditions reflected the current sediment and food concentration of the Markermeer. However, the experimental results suggest that zebra mussels are not negatively affected by the prevailing conditions, which implies that zebra mussels in the Markermeer should be able to recover.

Moreover, the current dominance of quagga mussels in the Markermeer cannot be explained by species differences in their response to suspended sediments. The higher SGR of zebra mussels than of quagga mussels at the highest sediment level even suggests that zebra mussels are less sensitive to high sediment concentrations, at least at relatively high food concentrations. Altogether, it is not likely that the poor condition of the zebra mussel population is caused by the current suspended sediment concentration, nor that the suspended sediment levels have contributed to the invasion success of quagga mussels.

4.1.4 Future developments in the Markermeer

The whole Markermeer ecosystem will probably undergo drastic changes in the near future. Currently, the Dutch nature conservation association *Natuurmonumenten* is planning and initiating the construction of an archipelago called the *Marker Wadden*. The archipelago should finally make up 15% of the total lake surface area and might be a boost for the ecosystem of the whole IJsselmeer area (Natuurmonumenten, 2012). For the construction of the islands and mud flats, mobile sediment (mostly silt and fluffy material) will be intercepted in channels in the lake bed and transported by gravity to mud depots.

The construction of the *Marker Wadden* might substantially reduce the average concentration of suspended sediments in the Markermeer. However, based on the results of this experiment, is it unlikely that reduced sediment concentrations will improve mussel growth. Nevertheless, decreased sediment concentrations might have indirect effects on the dreissenid population. Currently, suitable substratum for dreissenids to attach to is scarce because most solid substratum is covered by the mobile sediment (Noordhuis & Houwing, 2003). Hence, lower suspended sediment concentrations might enhance the possibilities for dreissenids to settle as more suitable substratum will be available.

4.2 Stoichiometry experiment

4.2.1 Mussel growth and condition in relation to food stoichiometry

Very small negative mean growth rates were calculated for both zebra and quagga mussels in the LP and the HP treatment levels. In terms of WW, this degrowth was significant for all experimental groups—albeit the negative growth was less than 2% over the whole rearing period. The mean SGR in terms of WW was not significantly different among the four experimental groups. Hence, the hypothesis that dreissenid growth rates decrease with increasing C:P ratios was not supported by these results (*hyp*. 2a). Also the hypothesis that quagga mussels have higher growth rates than zebra mussels under low phosphorous conditions was not supported (*hyp*. 3b).

Calcium is a very important element for dreissenid shell growth and might be a limiting factor for normal mussel development and long-term survival (Jones & Ricciardi, 2005). However, calcium was likely not limiting in this experiment, as the calcium concentration in the artificial fresh water (ADaM, 1.84 mmol Ca^{2+} l^{-1}) was very similar to concentrations in the IJsselmeer (live.waterbase.nl, 2013) and much higher than the reported threshold concentrations for dreissenid development (Jones & Ricciardi, 2005). Also other physic-chemical properties like pH, salinity, oxygen saturation, light intensity and temperature were unlikely to limit mussel growth in this experiment. However, the experimental mussels were supplied with the green algae *Scenedesmus obliquus*, which is deficient in long-chained PUFAs (Wacker & Elert, 2004). Wacker & Elert (2004) found that zebra mussels which were fed with *S. obliquus* produced relatively low egg masses. Hence, the exposure of the mussels to a diet of only *S. obliquus* might explain the absence of positive growth rates in this experiment. On the other hand, zebra mussels grown with 0.015 mm day^{-1} on *S. obliquus* with a C:P ratio of 380 in an experiment by Morehouse et al. (2013).

In contrast to shell and WW growth, the DW and condition (DW by WW) of the experimental mussels differed substantially per treatment level (see figure 3.2). Both species had a significantly better condition in the HP than in the LP treatment level. Furthermore, their DW and condition in the LP treatment level was significantly lower than in the baseline. This implies that the experimental mussels in the LP level had a negative DW growth and that their condition was deteriorated. Hence, this might support hypothesis 2a, as DW (for qugga mussels) and condition (for both species) was lower at the high C:P ratio than at the low C:P ratio of the food.

Note that the main indicators of growth (WW *vs*. DW and condition) leads to divergent conclusions regarding the hypothesis that growth changes with phosphorous availability. However, neither the WW nor the DW indicated a significant positive growth. The major discrepancy between both indicators was found in the LP treatment level, where the DW was substantially lowered (relative to the baseline) while the WW had hardly declined. As WW is largely determined by shell volume, WW will not substantial decline under adverse conditions (i.e. phosphorous scarcity). In contrast, mussel tissue is likely to be much more sensitive to adverse conditions. Hence, this might explain the different growth in terms of WW and DW in the LP treatment level.

There were no differences in DW and condition between zebra and quagga mussels in the LP or HP treatment level. However, the condition of zebra mussels declined much more in the LP level compared to the baseline (-46%) than for quagga mussels (-20%). Furthermore, the condition of quagga mussels in the HP treatment level was significantly better than in the

baseline, while this was not the case for zebra mussels. These findings suggest on the one hand that quagga mussels are less affected by low phosphorous availability than zebra mussels (because of the relatively small drop in quagga mussels condition in the LP treatment compared to the baseline) and on the other hand that they profited more than zebra mussels from the increased P availability in the HP treatment level (because quagga mussels, in contrast to zebra mussels, had a significant higher DW in the HP than in the LP level and a better condition than in the baseline). Nevertheless, hypothesis 3b is not supported by these results, as the DW and condition of quagga mussels was not better than of zebra mussels in the LP treatment level—nor in the HP treatment level.

4.2.2 Mussel stoichiometry and relation to growth

The molar C:P ratio of zebra mussels fell within the lower half of the range as found by Naddafi et al. (2012). The C:P ratio for quagga mussels fell in this range as well, but the average C:P ratio was significantly higher than for zebra mussels in each treatment level (including the baseline). Within both species, the tissue C:P ratio as well as the tissue P content was very similar among the treatment levels. Hence, these results do not support the hypothesis that tissue P content decreases with increasing phytoplankton C:P ratio (*hyp.* 2b). This is conflicting with the results of Naddafi et al. (2009, 2012), as their results suggest a positive relation between seston C:P ratio and mussel tissue C:P ratio.

So, tissue stoichiometry appeared to be inflexible because it did not change with food stoichiometry. However, on the one hand, the rearing period was possibly too short for the experimental mussels to adapt their tissue stoichiometry. Note that Naddafi et al. (2009, 2012) sampled mussels from their original location, where the mussels can gradually adapt to the prevailing seston C:P ratio. On the other hand, the absence of an adaptation to food stoichiometry might be caused by a stoichiometric limit. The DW and condition of zebra and quagga mussels in the LP treatment level showed deterioration and this implies an adverse elemental ratio between the supplied food and the needs of the mussels. If the mussels were capable to adjust (i.e. *increase*) their tissue C:P ratio in LP treatment level, that would have diminished elemental imbalances and hence, that would have benefited growth and condition. However, the tissue C:P ratios in the LP treatment level were *not increased,* which suggest that the zebra and quagga mussels were not capable to increase their tissue C:P ratios above 110 and 150, respectively.

Furthermore, the actual phytoplankton C:P ratio in the HP treatment level (just below 300) was actually not so low in comparison with seston C:P ratios usually found in freshwater lakes (see below). Possibly, dreissenid growth was still P limited in the HP treatment level, which might explain the absence of positive growth. This might at least be true for zebra mussels, which DW did not significantly differ between the LP and HP treatment level. In contrast, the DW of quagga mussels was significantly higher in the HP than in the LP level, which suggest that phosphorous was no longer—or less—limiting quagga mussel growth in the HP level. Moreover, this corresponds with the higher mean tissue C:P ratio of quagga mussels which was closer to the food stoichiometry, reducing the elemental imbalance between their requirements and supplied food. Altogether, this suggest that quagga mussels might have lower phosphorous requirements than zebra mussels and this is in line with the reasoning of hypothesis 3b that quagga mussels perform better than zebra mussels under relatively low phosphorous availability.

4.2.3 The conceptual model—stoichiometry

Based on the studies by Naddafi et al. (2009; 2012), the conceptual model (figure 1.9) predicts a positive correlation between seston and mussel tissue C:P ratios. However, this was not confirmed by the experimental results. Probably, the positive correlation only exists within a certain C:P range in which mussels can change their tissue stoichiometry. The found C:P ratios in this experiment suggest an upper tissue C:P limit of 110 and 150 for zebra and quagga mussels, respectively. However, as Naddafi et al. (2009; 2012) found much higher C:P ratios for zebra mussels, the (short term) stoichiometric flexibility might differ per population.

The absence of divergent tissue C:P ratios within the species makes it impossible to test the predicted correlation in the conceptual model between tissue C:P ratio and mussel growth rates. Nevertheless, growth as indicated by DW and condition (see figure 3.2) was lower in the high C:P treatment level. This implies a direct negative correlation between seston C:P and dreissenid growth rates when phosphorous is limiting and mussel tissue is at its maximum C:P ratio.

4.2.4 Stoichiometric patterns in the field

The phosphorous availability was relatively low in both treatment levels. Sterner et al. (2008) found that only 17% of 130 sampled lakes and ponds in the USA had seston C:P ratios larger than 300. Nadaffi et al. (2009, 2012) reported seston C:P ratios between 100 and 250 in Lake Erken, Sweden. The mean seston C:P ratio was just below 300 in the IJsselmeer and around 350 in the Markermeer in 2010-2012 (*pers. com.* D. Sarpe, 2013). So, the phytoplankton C:P ratio in the HP treatment level was very similar to the seston stoichiometry in the IJsselmeer, but relatively high compared to many other freshwater bodies.

The results suggest that phosphorous might limit zebra mussel growth, but not quagga mussel growth in the Markermeer and IJsselmeer. Furthermore, based on the criteria of González et al. (2010), quagga mussels will be better invaders than zebra mussels. Quagga mussels might perform better in low-nutrient environments (because they have a higher maximum C:P tissue ratio and hence will be less susceptible to phosphorous scarcity) and in high-nutrient environments (because they appear to profit more from the increased phosphorous availability in this experiment).

Note that the phytoplankton C:P ratios in this experiment were relatively high. When the phytoplankton C:P ratios are lowered below values used in this study, initially quagga mussels might benefit more than zebra mussels. However, it is not unlikely that this will switch at a certain phytoplankton C:P threshold ratio, which will be reached when zebra mussels become in elemental balance with the phytoplankton. From that point, decreasing C:P ratios will favor zebra mussel growth over quagga mussel growth (assuming that zebra mussels still have a lower tissue C:P ratio than quagga mussels, the GRH predicts that their growth rate will be higher (Elser et al., 2003)). Moreover, this postulated switch at a certain C:P threshold ratio might be linked to the observation that zebra mussels only outgrow quagga mussels under high food conditions (Baldwin et al., 2002), because high food quantities are often accompanied by low seston C:P ratios (Elser et al., 2008).

The different phosphorous requirements of zebra and quagga mussels match with the deterioration of zebra mussels and the rapid spread of quagga mussels in the Marker- and IJsselmeer. So, the relatively low phosphorous availability might explain the population developments in these lakes. However, a rapid expansion of the quagga mussel and possibly

even displacement of zebra mussels is observed in many water bodies in the Netherlands (Matthews et al., 2013), Europe and North America (references in: Karatayev et al., 2011b). It is questionable whether phosphorous scarcity in itself can explain this widespread trend.

Based on multiple experiments, Vanderploeg et al. (2009) hypothesized that zebra mussels can induce a regime shift in the phytoplankton composition which fed back in a deteriorated condition of the mussel population because the 'new' composition is of lower quality to the zebra mussels. Interestingly, if quagga mussel are better capable to utilize the newly created phytoplankton composition, than this will give them a competitive advantage over zebra mussels. This mechanism might explain the widespread zebra mussel deterioration and subsequent quagga mussel expansion. Moreover, based on the results of the stoichiometry experiment, it might be predicted that for zebra mussels, the 'good' phytoplankton composition consist of algae with relatively low C:P ratios and the 'worse' phytoplankton composition of algae with higher C:P ratios. As quagga mussels might be better able to withstand a relatively low phosphorous availability, they might displace the zebra mussel under the low phosphorous phytoplankton composition.

5. Conclusion

The zebra mussel populations in the Markermeer and the IJsselmeer are hardly recovered after they crashed 20 and 10 years ago, respectively. Suggested causes for their crash and the lack of recovery are the high suspended sediment concentrations in the Markermeer and the relatively high seston C:P ratio in both lakes. However, both lakes are recently invaded by the quagga mussel which is currently dominating the dreissenid populations. This suggests that quagga mussels are less affected by the prevailing environmental conditions.

However, the results from the suspended sediment experiment do not suggest that quagga mussels are less affected by suspended sediment, as growth was very similar between both species. Furthermore, there was a substantial positive growth of both species in all sediment levels and neither zebra nor quagga mussel growth rates decreased with increasing sediment levels. Therefore, it is unlikely that suspended sediments halt the recovery of the zebra mussel population in the Markermeer.

No causal relation was found between suspended sediment concentrations and mussel growth rates at sediment concentrations ranging from zero to 200 mg l^{-1}. Still, dreissenids might be negatively affected by the very high suspended sediment concentrations which do occur during storm events on the Markermeer. On the other hand, the experimental results suggest that structurally lower average suspended sediment concentrations will not directly lead to an improvement of zebra or quagga mussel growth. Nevertheless, lower sediment concentrations in the Markermeer might indirectly benefit the dreissenid populations, although the exact impacts should be clarified by further research. Interestingly, the construction of the *Marker Wadden* will offer a great opportunity to study the direct and indirect effects of declining suspended sediment concentrations and turbidity on both the zebra and quagga mussel populations.

The results from the stoichiometry experiment partly clarify the linkage between food C:P ratios, mussel tissue C:P ratios and growth rates. Mussels were in better condition and had a higher dry tissue weight in the highest phosphorous treatment level. However, the tissue C:P ratio of the mussels did not change with phytoplankton C:P ratios. Possibly, the mussels were phosphorous limited and incapable to increase their tissue C:P ratio in order to reduce the elemental imbalance between their requirements and their food. This implies that zebra and quagga mussels from the IJsselmeer population have a maximum mean C:P ratio of around 110 and 150, respectively.

Quagga mussels benefited more from high phosphorous availability in the low C:P treatment level than zebra mussels, and were less affected by the low phosphorous availability in the other treatment level. Hence, it might be concluded that quagga mussels have lower phosphorous requirements, which is in accordance with their higher tissue C:P ratio. Therefore, they might outreach zebra mussel growth up to a certain phytoplankton C:P threshold ratio, below which zebra mussels will have higher growth rates. Additional experiments should focus on lower C:P ratios to determine the actual threshold ratio were zebra mussel growth will surpass quagga mussel growth.

The current C:P ratio of the seston in the Marker- and IJsselmeer is relatively high compared to many other lakes. The results from the stoichiometry experiment suggest that phosphorous might be limiting to zebra mussel growth in the Marker- and IJsselmeer. In contrast, the rapid quagga mussel expansion in both lakes might be explained by their lower phosphorous requirements. So, the declining phosphorous content of the phytoplankton might have caused

the observed dreissenid population developments in the Markermeer and in the IJsselmeer of the last three decades.

However, zebra mussels are recently displaced by quagga mussels in many water bodies in Europe and North America and hence, it is questionable whether phosphorous availability in itself can explain this trend. This experiment clarified that zebra and quagga mussels from the same water body can have different tissue C:P ratios, which might be an important discrepancy between the species. However, it should be further researched whether this discrepancy underlies the widespread trend of zebra mussel displacement by quagga mussels.

Finally, it is largely unclear whether quagga mussels have different impacts on ecosystems than zebra mussels (Matthews et al., 2013). Possibly, quagga mussels have taken over the key stone function of zebra mussels in the Marker- and IJsselmeer. This should be elucidated by additional research because dreissenids can have a significant impact on the ecological status of the whole IJsselmeer area.

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