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ORIGINAL ARTICLE

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

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Abstract Filtration capacity and feeding behaviour has been intensely studied for adult mussels (*Mytilus edulis*), but less information is available for juvenile mussels (1.5–25 mm, <1 year), especially in natural sea water. The recent introduction of mussel seed collectors in the Netherlands prompted the need for more detailed information on juvenile mussel behaviour. To estimate the impact of juvenile populations on ecosystem carrying capacity, information on clearance rate as well as usage of different prey items is essential. Clearance rates were measured in an experimental study, incubating juvenile mussels in natural sea water. Rates were related to isometrics as well as specified for the prey items bacteria, picophytoplankton (<3 µm), nanophytoplankton (3–20 µm), and ciliates. Results showed that the clearance rate of juvenile mussels depends on shell length^2, while the relationship between clearance rate and weight was more variable. Length is thus a better parameter for estimating clearance rate than weight. Ciliates and nanophytoplankton were cleared at comparable, but variable rates, while picoalgae were cleared from the water at the rate of 11–64 % compared to nanophytoplankton. For bacteria, the clearance rate was on average 9 %. This study showed different retention of particles of similar size (picoalgae and bacteria) as well as variability in particle retention for the different prey items. This variable retention efficiency could not be related to seston concentration nor to dominance in cell size. The results from this study can be used to estimate the effect of mussel seed collectors on the carrying capacity of the Dutch Wadden Sea.

Keywords Juvenile · *Mytilus edulis* · Clearance rate · Isometrics · Planktonic prey · Variable retention

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Introduction

In estuarine ecosystems, suspension-feeding bivalves, like the blue mussel (*Mytilus edulis*), often occur in large numbers, affecting the surrounding ecosystem by filtering vast volumes of water, thereby removing different components of the plankton community (e.g. Verwey 1952; Cadée and Hegeman 1974; Cloern 1982; Dame 1996; Kreeger and Newell 1996; Wong and Levinton 2006). The recent introduction of mussel seed collectors in the Netherlands prompted the need for assessing the effect of large numbers of juvenile individuals on the carrying capacity of the surrounding ecosystem. Pelagic seed collectors

facilitate the settlement of mussel larvae (300 µm). After settlement in June, the juveniles grow in <6 months to a maximum size of 25 mm at harvest (Jacobs et al. 2014). There have been numerous studies performed on the filtration capacity and feeding behaviour of mussels, but these studies were mainly confined to larger (>15 mm) individuals (e.g. Widdows 1978; Bayne and Widdows 1978; Møhlenberg and Riisgård 1979; Riisgård et al. 1980, 2014; Kiørboe and Møhlenberg 1981; Jones et al. 1992; Smaal et al. 1997), while smaller individuals have been studied far less intensively (but see Riisgård et al. 1980). Most studies on filtration rates of mussels were performed under controlled laboratory conditions using algal cultures. These experiments resulted in estimates of the maximum clearance rate, while it can be expected that under natural conditions, clearance rates will be lower. The need for information on actual realised clearance rates under natural conditions and the specific usage of natural plankton by these dense collections of juvenile mussels has been recognised (Bunt et al. 1992; Cranford et al. 2003, 2011; Trottet et al. 2008).

Mussel larvae are suspension feeders, utilising a ciliated velum to capture food particles (Riisgård et al. 1980). After settlement and during metamorphosis, the feeding modus changes from a velum to the ctenidium, which also serves as a respiratory organ (gills) (Cranford et al. 2011). Lateral cilia on the gill filaments create an inflow; water enters the inhalant chamber and flows through the gills towards the exhalant chamber. Particles in the water flow are captured when the frontal surfaces of the ctenidial filaments encounter and retain them. The size of particles efficiently retained depends on the size and complexity of the latero-frontal cilia of the filaments as well as the current produced by the cirri (Newell and Shumway 1993; Dame 1996; Ward and Shumway 2004).

Captured and retained particles are transported to the labial palps. Here, particles are either rejected as pseudofaeces or directed further to the mouth (Ward and Shumway 2004).

The assumption of isometric relationships between length, area, and volume (area $\sim$ length^2 and volume $\sim$ length^3) was more variable; this leads to the expectation that theoretically pumping or filtration rate (R_F) scales with gill surface area, and gill surface area is expected to scale with length^2, so R_F = length^2. Since weight scales with volume and volume scales with length^3, gill area will scale with weight$^{2/3}$ and filtration rate will thus also scale with weight$^{2/3}$, so R_F = weight$^{2/3}$ (Jones et al 1992). For veliger and post-metamorphosed larvae, filtration rate was reported to scale with weight$^{0.8–1}$ (Riisgård et al. 1980; Beiras and Camacho 1994). The high scaling factor was attributed to a high non-isometric growth of the gills.

In most studies, clearance rate (R_C), which is the volume cleared of particles per unit time, is measured rather than the actual pumping or filtration rate. When particles are 100 % efficiently retained by the gills, the clearance rate equals the filtration rate. If the filtration efficiency is lower than 100 %, the clearance rate is thus lower than the pumping rate.

Numerous studies, starting with a study by Møhlenberg and Riisgård (1978), have reported on the particle size range that can be retained by adult mussels (see for overview Strohmeier et al. 2012). For a long time, it is was assumed that mussels do not efficiently retain smaller particles, with studies reporting on 90 % retention for 3-µm particles by *Mytilus edulis*, while 1-µm particles are retained with 50 % efficiency only (Møhlenberg and Riisgård 1978). Most studies were performed under controlled laboratory conditions using phytoplankton cultures. Results from experiments using natural plankton communities reported that retention efficiency might be more variable (Trottet et al. 2008; Strohmeier et al. 2012).

Mussels filter all kinds of particles from the water. Although phytoplankton was traditionally considered the main food source (Nielsen and Maar 2007), several studies have stated the importance of other food particles like dead organic material (Dame and Dankers 1988) and bacteria attached to this (Newell et al. 1989), microzooplankton (Horsted et al. 1988; Kreeger and Newell 1996; Trottet et al. 2008) and, for larger mussels (>22 mm; Horsted et al. 1988), mesozooplankton (Davenport et al. 2000; Wong and Levinton 2006; Lehane and Davenport 2006).

The aim of this study was to establish realised clearance rate of juvenile mussels (1.5–25 mm) in relation to both shell length and weight. Furthermore, clearance rates will be described for different prey items: bacteria (0.6 µm), picophytoplankton (<3 µm), nanophytoplankton (3–20 µm), and ciliates (10–200 µm). To establish the clearance rates of juvenile mussels, an experimental study was carried out for 3 years. Juvenile mussels were incubated in sea water originating from the western Wadden Sea. This study is one of the first describing grazing of dense populations of juvenile mussels in natural sea water. The results of this study can be used to estimate the effect of juvenile mussel cultures on the ecosystem of the western Wadden Sea.

Materials and methods

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

Study animals

Each year, a small collector was placed in the Marsdiep (52°58′N, 4°49′E, Fig. 1). This collector consisted of filamentous ropes facilitating mussel settlement (Xmas tree ropes, Donaghys). After settlement around June, mussels increase in size up to approximately 25 mm when harvested in October. Mussel sizes used in this study were between 1.5 and 25 mm. The day before each incubation experiment, ropes with juvenile mussels were collected, transported in sea water, and stored at 4 °C. At the day of the experiment, mussels were acclimatised to ambient sea water temperature and pre-incubated.

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

Experimental set-up

Two types of experiments were designed. In 2010 and 2011, pieces of mussel ropes were incubated in mesocosms to calculate the clearance rate of a mussel community. These mussel assemblages on a rope consist of different-sized mussels, resulting in a relatively high variation in shell lengths (Table 1). In 2012, laboratory experiments were performed; in this set-up, the variation in shell length was greatly reduced by removing mussels from a piece of rope, measuring them, and sorting them by size. Clearance rates of these equally sized mussels were measured in smaller volumes (Table 1).

Mesocosm experiments

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

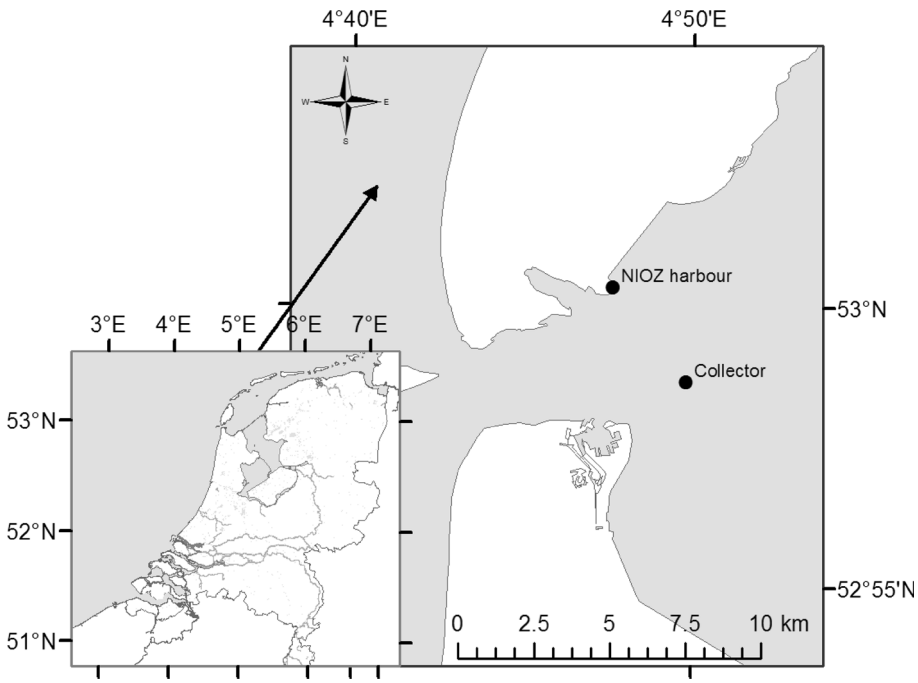

Fig. 1 Locations for the collector and experimental site (NIOZ harbour) in the Dutch Wadden Sea

Table 1 Overview of most important variables for each experimental date

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

Temp is the average water temperature during the experiment, Phyto is the average number of phytoplankton cells (pico- and nanophytoplankton) as counted with the flow cytometer in 10^3 cell per millilitre, *N* treatment and *N* control give the number of mesocosms incubated with mussels or kept as control respectively. In 2012, an experiment was sometimes repeated with the same mussels using new sea water; this is than indicated by a two. On the last experimental date in 2012, the average of four separate experiments with four individual mussels is given. The number of mussels present per experiment is given as the number of mussels per 100 L of water (100 L^{-1}). Mean length gives the average shell length in millimetres of the juvenile mussels used per experiment. The last three columns indicate whether clearance rates were measured for each particular prey item on each date

[a] Mussels originated from a different location than the artificial collector

Laboratory experiments

Mussels were gently removed from a piece of rope, measured, and sorted by size. 1–100 equally sized mussels (Table 1) were placed loosely in petticoat netting (0.5 × 0.5 cm mesh size). For each experiment, two glass beakers were filled with natural sea water (0.1–1 L). To one beaker, mussels were added, one beaker served as control. Water was gently stirred, and phytoplankton numbers at different depths were compared by means of

flow cytometry to check for complete mixture of the water. Phytoplankton cell numbers were monitored throughout the experiment, and linearity of the natural logarithm of cell concentration over time was checked afterwards, to verify the absence of depletion. The experiments lasted between 0.75–1.5 h. On several occasions, mussels were reused again, repeating the experiment using a new water sample (Table 1).

Prey items

Bacteria

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

Analyses were performed using a flow cytometer (C6, BD Accuri, excitation with 488 nm laser), and samples were diluted with 10 % TE buffer to lower the count rate below 3,500 events s^{-1}, the maximum recording rate of the instrument. SYBR green I (Invitrogen) stain was added (fc 0.1 %), and samples were incubated in the dark for 15 min. The 530 nm laser (FL1) was used to detect the stained cells.

Pico- and nanophytoplankton

Phytoplankton cell counts were obtained by means of flow cytometry. Water subsamples (1 mL) in triplicates were processed freshly, immediately after collection. Fluorescence at wavelengths >670 nm (FL3) was ascribed to chlorophyll *a*. Forward scatter was used as an indication of cell size (e.g. Li 1995). Based on the relative fluorescence to size, a distinction between phytoplankton and debris was made. Phytoplankton cell counts were further divided into two size classes (<3 µm: pico and 3–20 µm: nano) using 3-µm beads (Polyscience). A minimum cell count of 1,000 per size class was applied. Within the picophytoplankton, two distinct groups could be identified: those with the pigment phycoerythrin (FL2: 585 nm) ('picocyanobacteria') and those without this pigment ('others').

To calculate an average size per prey item measured with the flow cytometer, additional beads (7–10 µm) were used to calibrate forward scatter with size.

Ciliates

For enumeration of ciliates, one subsample (0.5–1 L) was fixed in 4 mL acid Lugol and stored in brown glass bottles at 4 °C until analysis. Samples were concentrated (10–20×), and per sample, a minimum of 100 individuals were counted or, at very low abundances, all individuals in a maximum of 10 % of the concentrated sample. Ciliate cells were counted and divided into five size classes (<20, 20–40, 40–60, 60–80, and >80 µm) with an inverted microscope using the Utermöhl sedimentation technique (Verweij et al. 2010).

Calculation of clearance rates

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

$$R_C = \frac{V}{nt}\left\{\ln\frac{C_0}{C_t} - \ln\frac{C_{0'}}{C_{t'}}\right\} \quad (1)$$

where V is the volume (L) cleared, t is the duration of the measurement (h), n is the number of mussels used in the experiment, C_0 is the concentration of a particulate parameter at the start of an experiment, and C_t is the concentration at the end. C_0' and C_t' are the concentrations at the start and end, respectively, in the control. R_C was expressed as litre per hour per individual mussel. At the end of each experiment, linearity of $\ln(C_0/C_t)$ was verified. This 'clearance rate' method is considered reliable when the above condition is met (Riisgård 2001).

Statistical analysis

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

$$R_C = aL^b \quad (2)$$

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

$$R_C = cW^d \quad (3)$$

in which R_C is the clearance rate (L h^{-1}) and W is either the total dry weight (shell and tissue, 2010 and 2011) in grams or dry tissue weight (g) (2012).

Under the null hypothesis, that clearance rate scales with length to an exponent $b = 2$. The exponent for weight d is expected to be 2/3 (Jones et al. 1992).

To test the potential difference between years for the relationship between clearance rate and either length or weight, linear models of $\log^{10}$-transformed data were used (models 1–3). The same kind of models was used to test whether the coefficients b and d differed from their expected values, i.e. 2 and 2/3, respectively (model 4). model 1: $\log R_{Cij} = \log a + b \log x_{ij} + \varepsilon_{ij}$ (common slope and intercept for all years) model 2: $\log R_{Cij} = \log a_j +$

$b \log x_{ij} + \varepsilon_{ij}$ (common slope for all years only) model 3: $\log R_{Cij} = \log a_j + b_j \log x_{ij} + \varepsilon_{ij}$ (slope and intercept differ between years) model 4: $\log R_{Cij} = \log a + 2\log x_{ij} + \varepsilon_{ij}$ *or* $\log R_{Ci} = \log a + \log x_{ij} + \varepsilon_{ij}$ (slope equal to 2 or to 2/3, common intercept for all years) R_C is the clearance rate, a is the intercept, b the slope, and ε the error term. The indices i and j refer to observation i in year j.

To quantify the clearance rate of picophytoplankton and bacteria relative to the clearance rate of nanophytoplankton, linear regression was applied using the individual clearance rates measured.

To test whether the clearance rate of juvenile mussels on nanophytoplankton differed from the clearance rate on ciliates, the individual rates were compared using a paired t test.

All data were analysed using R version 2.14.1 [(C) 2011, The R Foundation for Statistical Computing].

A significance level of $\alpha < 0.05$ was used for all tests.

Results

Clearance rate of juvenile mussels depending on length and weight

The clearance rate of mussels depending on mussel shell length

There was no significant interaction of the factor year with the relationship between clearance rate and length ($F_{4,48} = 1.42$, $p = 0.24$, models 1 and 3). Neither did the intercepts of this relation differ between the 3 years ($F_{2,50} = 2.88$, $p = 0.07$ models 1 and 2).

The common slope, grouping the measurements of all 3 years together, did not differ from the theoretically expected value of 2 for b ($F_{1,52} = 2.25$, $p = 0.14$, models 1 and 4). Using this fixed value for b, the intercept was estimated at (0.0004) (Fig. 2) with no significant differences between the 3 years ($F_{2,51} = 2.20$, $p = 0.12$).

The clearance rate of mussels depending on mussel weight

The individual clearance rate of juvenile mussels can also be described in relation to the weight of a mussel according to $R_C = cW^d$. Weight here is defined as the weight of shell and tissue together (Fig. 3a).

The relation of clearance rate with mussel dry weight was not the same for each year ($F_{4,48} = 8.61$, $p = 2.547e{-}05$, models 1 and 3). The intercepts differed between the 3 years ($F_{2,50} = 14.72$, $p = 9.43e{-}06$, models 1 and 2), not the slope ($F_{4,48} = 1.94$, $p = 0.15$, models 2 and 3).

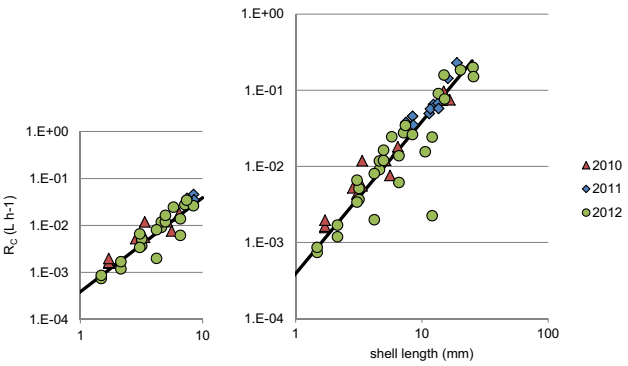

Fig. 2 Clearance rate on nanophytoplankton cells, measured for three consecutive seasons for mussels varying in mean size from 1.5 to 25 mm. The clearance rate is expressed as the litres of water cleared of cells per hour per individual mussel. There were no significant differences in either the slope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

Whether the slope differed from the expected value for $d = 0.67$ was tested for each year separately. Only for 2010, the model with a fixed b of 0.67 differed significantly from the estimated d based on the data (2010: $F_{1,13} = 5.18$, $p = 0.04$, 2011: $F_{1,8} = 0.32$, $p = 0.59$, 2012: $F_{1,27} = 0.04$, $p = 0.85$). The intercepts for 2011 and 2012 are different ($F_{2,51} = 6.01$, $p = 0.005$), so the best fitted lines are given for each year separately (Table 2).

To compare the results on the relationship between clearance rates and weight in the current study with results reported in the previous studies, the relationship between clearance rate and tissue dry weight was established (Fig. 3b). Only for 2012, tissue and shell dry weights were measured separately (methods 2.1). The relationship between tissue dry weight (W, g) and shell length (L, mm) can be described by the relation $W = 1.7 \times 10^{-5} L^{2.7}$ ($r^2 = 0.98$).

In 2012, the relationship between clearance rate and tissue dry weight did not differ from the expected value of 0.67 ($F_{1,27} = 0.02$, $p = 0.90$). Clearance rate depends on tissue dry weight according to $\log R_C = -0.13 \pm 0.06 + 0.67 \log W$.

Clearance rate of juvenile mussels on different prey items

The R_C of juvenile mussels on bacteria is on average 9 % of the clearance rate on the better retained nanophytoplankton cells (Fig. 4a).

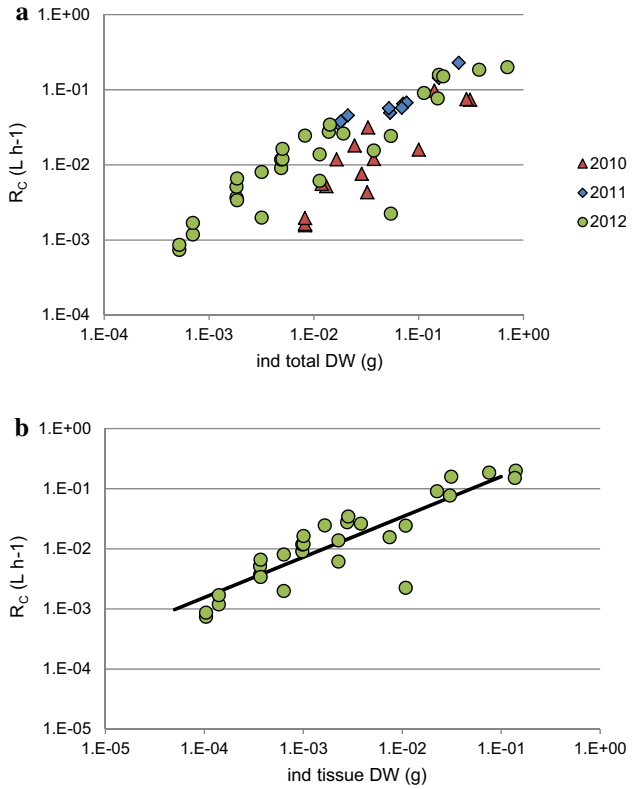

Fig. 3 **a** Clearance rate on nanophytoplankton cells, measured for mussels varying in mean size from 1.5 to 25 mm (corresponding to 0.5–700 mg DW of shell and tissue) for 3 years. The clearance rate is expressed as the litres of water cleared of cells per hour per individual mussel. Both axes are on log scale. **b** The clearance rate on nanophytoplankton cells as a function of the mean individual mussel tissue dry weight. The data were collected in 2012. The regression coefficient did not differ significantly from the expected value of two-thirds. Therefore, a regression line was fitted using a slope of two (*black line*). The relationship between clearance rate (L h^{-1}) and tissue dry weight (g) is best described by the equation $\log R_C = \log(-0.13 \pm 0.06) + 0.67 \log W$. Both axes are on log scale

Table 2 Estimated value for log *c* and *d* (Eq. 3) including the standard error for the relationship between clearance rate and the DW (g) of both shell and tissue. The variation explained by this relation is given as r^2. For clarity, *c* is also given

Year	Log *c* ± SE	*d* ± SE	r^2	*c*
2010	−3.45 ± 0.22	0.99 ± 0.14	0.80	0.00036
2011	−2.27 ± 0.16	0.62 ± 0.09	0.86	0.0054
2012	−2.62 ± 0.09	0.68 ± 0.07	0.79	0.0024

Picophytoplankton is cleared from the water on average at half the rate of the nanophytoplankton cells (Fig. 4b). Based on both the auto fluorescence of chlorophyll and phycoerythrin, two groups of picophytoplankton could be distinguished: 'others' and 'picocyanobacteria'. The average size of picophytoplankton was 0.7 µm for 'picocyanobacteria' and 1.2 µm for 'others'. There was no difference in the clearance rates of juvenile mussels between the two groups of picophytoplankton (data not shown).

There was no significant difference between the clearance rate of juvenile mussels on nanophytoplankton and ciliates ($t = 0.77$, $df = 17$, p value $= 0.45$) (Fig. 4c).

Discussion

Clearance rate in relation to mussel shell length and weight

There are many studies reporting on clearance rates of mussels. Most of these studies were performed under controlled laboratory conditions, using cultured algal species, while other, more recent studies established clearance rates under natural conditions. There are large differences in the clearance rates reported, and there has been much debate about the causes for these differences. The main arguments to explain the differences between studies are the use of different methodologies (Riisgård 2001; Riisgård et al. 2014), differences in mussel condition index (Filgueira et al. 2008; Riisgård et al. 2014) or food type, with lower clearance rates measured when natural plankton is used (Doering and Oviatt 1986). Nowadays, there seems to be consensus on the concept of considering filtration rates determined in controlled laboratory experiments using cultured algal species and low mussel densities as maximum rates, while clearance rates established under field conditions can be regarded as realised clearance rates (Cranford et al. 2011; Riisgård et al. 2014).

In the current study, clearance rates were among the lowest reported (Table 3). Although during the experiments complete mixing of the water was aimed for and no gradient of phytoplankton concentration in the experimental units was measured, depletion of algal cells close to an individual mussel cannot be excluded; especially, since in the current study, large numbers of closely packed mussels were used in the experiments. Local depletion of food can result in re-filtration of the water. Re-filtration of water might thus provide an additional explanation for the low clearance rates measured in the current study. But it seems that re-filtration was not a constant factor. In 2012, for the smallest mussels, clearance rates were comparable to rates determined in controlled laboratory experiments on small post-metamorphosed larvae (Riisgård et al. 1980). With increasing mussel weight and concentration (Table 1), the difference got larger and it seems that the influence of re-filtration on the clearance rate becomes more importance (Fig. 5).

There is thus a difference in the scaling relationship between clearance rate and weight between the current

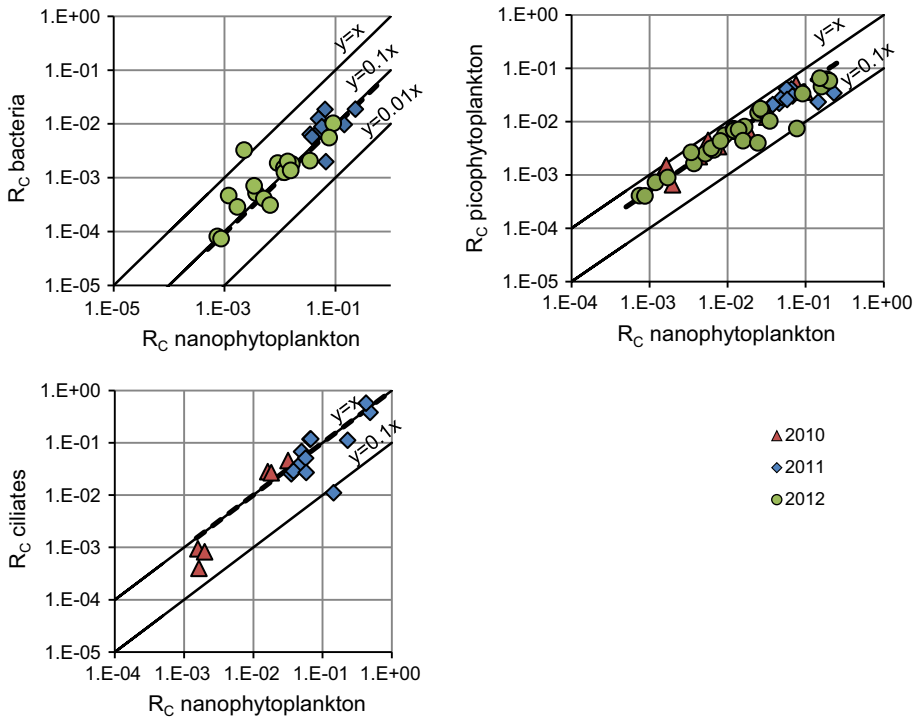

Fig. 4 Clearance rate (R_C, L h^{-1}) of juvenile mussels on bacteria (**a**, *top left*), picophytoplankton (**b**, *top right*), and ciliates (**c**, *bottom left*) relative to the clearance rate on the nanophytoplankton fraction. The clearance rate on bacteria, picophytoplankton, and ciliates was assumed to be proportional to the clearance rate on nanophytoplankton (e.g. R_C bact. $= a\, R_C$ nano). The proportionality coefficient a was estimated by the antilog of the mean log ratio of R_C bact, pico and ciliates and R_C nano. The *black dashed line* (**a**, **b**) indicates the estimate for a (all years together) (bacteria: $a = 0.09$, $R = 0.75$, $n = 28$, picophytoplankton: $a = 0.5$, $R = 0.95$, $n = 35$, for mussels smaller than 10 mm. For ciliates, there was no significant difference in clearance rate compared to the clearance rate on nanophytoplankton. ($y = x$). For reasons of clarity, the lines $y = x$, $y = 0.1x$, and $y = 0.01x$ are also indicated

study and the study performed by Riisgård et al. (1980). While in the current study clearance rate scaled with an exponent of two-thirds over the entire size range. Riisgård et al. found that clearance rate scaled with weight[1] for small mussels (tissue dry weight <10 mg), decreasing to two-thirds with increasing weight (Fig. 5).

The difference in scaling exponent between the current study and the study by Riisgård et al. is not easy disclosed, but might be due to differences either in morphology or in condition. Clearance rates scaling with weight[1] could also indicate that gill area does not scale with length[2], representing 'high non-isometric growth' of the gills (Riisgård et al. 1980). Unfortunately, no data are available on the relationship between gill area and length, nor on the relationship between clearance rate and length. In the current study, weight scaled with length[3] and clearance rate scaled with length[2], making a high isometric scaling of the gills unlikely. However, due to the relative large variation between measurements in our study, we cannot rule out that for the maximum clearance rate and the relation with weight might be best described by weight[1].

Clearance rate of juvenile mussels on different-sized prey items

The average diameter of bacteria in the current study was 0.6 µm. Bacteria were cleared from the water with an average efficiency of 9 % (Fig. 4a) of the clearance rate on nanophytoplankton, the most effectively cleared prey item (Fig. 6). This is somewhat higher than efficiencies reported in other studies. Trottet et al. (2008), using natural sea water, found clearance rates of adult mussels on bacteria to be close to zero. Nielsen and Maar (2007) found no removal of bacteria above a mussel bed. (Fig. 5).

The clearance rate on picophytoplankton was higher than the average clearance rate on bacteria (Fig. 6). The clearance rate on the picofraction of phytoplankton occurred on average at half the rate of the clearance on larger nanophytoplankton (Fig. 4b). The diameter of picophytoplankton was between 0.7 and 1.0 µm, and the retention efficiencies found in the current study fall within the range of reported efficiencies for 1 µm (unidentified) particles (e.g. 50 %: Møhlenberg and Riisgård 1978; 20 %: Riisgård et al. 1980; 14–64 %: Strohmeier et al. 2012).

Table 3 Coefficients a and b in the relationship between clearance rate and shell length ($R_C = aL^b$), and the coefficients c and d for the relationship between clearance rate and tissue dry weight ($R_C = cW^d$) as reported in the current and other studies are given

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

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

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

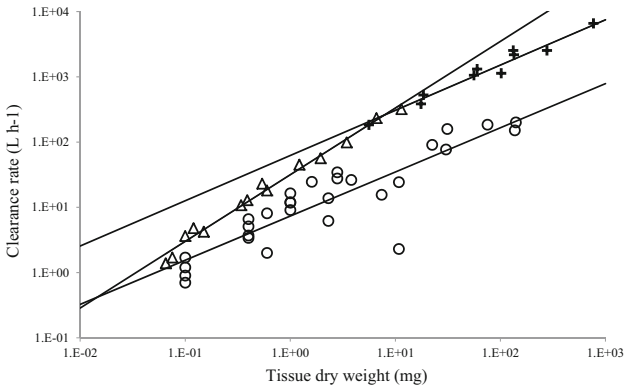

Fig. 5 Relationship between clearance rate (L h^{-1}) and tissue dry weight (mg) of mussels. Both axes are on a log scale. b in the relation to log R_C = log a + b log W was reported to be 1 in Riisgård et al. (1980) (Δ), 2/3 in Riisgård and Møhlenberg (1979)/ Møhlenberg and Riisgård (1979) (+), and 2/3 for the current study (2012, o)

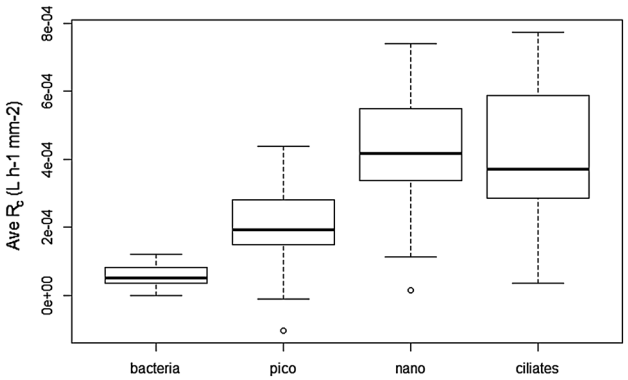

Fig. 6 Summarising *boxplot* indicating the clearance rate of juvenile mussels on four prey items for all years together. Clearance rate is expressed as litre cleared of items per hour per mm^2 shell length, to make the R_C independent of shell length

efficiency with decreasing particle size has been reported before (Lucas et al. 1987; Matthews et al. 1989; Ward and Shumway 2004). Preferential capture of picophytoplankton over bacteria must be based on properties other than cell size alone. Differences in stickiness between species of the same size, affecting capture efficiency by the ctenidium, has been suggested as a possible explanation for the variation in retention of equally sized particles (Ward and Shumway 2004).

The average diameter of nanophytoplankton cells was 6.6 µm, while ciliate were much larger, ranging in diameter roughly between 10 and 200 µm, with a weighted average of 28.6 µm (±7.9). Clearance rates on ciliates however were comparable to the clearance rates on nanophytoplankton (Figs. 4c, 6). Optimal retention thus reaches a plateau for particles larger than 6.6 µm in this study.

Variable retention

The retention efficiency for different prey items is not constant (Fig. 4a–c). For bacteria, the retention relative to the retention of nanophytoplankton varied between 1 and 26 % and for picophytoplankton, retention varied between 11 and 64 %.

Mussels can lower the retention efficiency for small particles to some extent by widening the interfilamentary distances of the ctenidium or by shifting the movement of the latero-frontal cilia to the side, so cilia no longer block the passage of smaller particles (Atkins 1937; Dral 1967; Barillé et al. 1993; Strohmeier et al. 2012).

There is a positive relationship between the size of a particle and its nutritional value (Ward and Shumway 2004). Assuming that mussels strive to maximize their energy intake, a trade-off is expected with regard to the distance between the filaments, either a wide interfilamentary distance, creating a low concentration (since abundance is negatively related to size) of large nutritious (Ward and Shumway 2004) particles, or a more narrow distance, resulting in a high concentration of particles, but including a large quantity of low-quality particles. A higher inflow of lower-quality particles is likely to increase the processing costs (e.g. pumping, handling, selecting, and rejection). It can thus be expected that the optimal interfilamentary distance at least balances the costs of processing of different quantity and quality particles with the benefits.

There are studies reporting on higher or lower retention efficiencies in response to variations in natural seston. Strohmeier et al. (2012) found that when total cell volume was dominated by small particles, the particle size most efficiently retained decreased (to 6–16 µm). At times when total cell volume was dominated by larger cells, capture efficiency increased to larger particles (20–30 µm). Calculating the carbon per size class for data published in Lucas et al. (1987) revealed a similar pattern; retention efficiency for 1.6-µm particles differed between two sites. The highest retention efficiency for these picoparticles corresponded to relative small (8 µm) particles dominating total carbon availability, while at the site with a lower retention the carbon availability was dominated by 12- to 16-µm particles. Trottet et al (2008) investigated the clearance rates on different phytoplankton species, heterotrophic flagellates, and ciliates. Relative clearance rates between species and taxa varied throughout the year. No consistent relationship between cell abundance and clearance rate per species/taxa was found. In the current study, seston concentrations varied considerably. During the experiments, the suspended matter concentration fluctuated between 16 and 50 mg L^{-1} with chlorophyll *a* concentration between 3 and 11 µg L^{-1} (data not shown). Variation in retention of the different prey items could however not be related to differences in either suspended matter or chlorophyll *a* concentrations. Neither could this variable retention efficiency be attributed to differences in dominant cell size. Whether mussels are able to control particle retention in response to variations in natural seston concentration remains a controversial topic, and according to Riisgård et al. (2013), the mechanism of modulation of the retention efficiency 'lacks a physical explanation'.

Conclusion

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

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

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

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