Larviphagy in native bivalves and an introduced oyster

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ABSTRACT

Introduced Pacific oysters *Crassostrea gigas* have expanded rapidly in the Dutch Oosterschelde estuary, while stocks of native bivalves declined slightly. As a consequence, total filtration pressure increased significantly, which may affect the mortality of bivalve larvae. Better escape abilities in Pacific oyster larvae might be a contributing factor to their rapid geographic expansion. To study whether *C. gigas* larvae are filtered less than larvae of native bivalves, we investigated filtration and ingestion of the larvae of the native *Mytilus edulis* and introduced *C. gigas* by the adults of *C. gigas* and *M. edulis* as well as the native *Cerastoderma edule*.
We measured filtration rates of *C. gigas* and *M. edulis* larvae by the adult bivalves (*C. gigas*, *M. edulis* and *C. edule*), and compared these to filtration rates of algae. Additionally, we studied the fate of filtered larvae. All three adult species filtered both *C. gigas* and *M. edulis* larvae. *M. edulis* larvae were filtered by all three bivalve species with the same filtration rates as algae, whereas filtration rates of *C. gigas* larvae were roughly 50% lower than filtration rates of algae. This suggests that *C. gigas* larvae can somehow reduce their filtration risk, whereas larvae of *M. edulis* cannot. The majority of filtered *C. gigas* and *M. edulis* larvae were ingested.

1. Introduction

Nowadays, one of the most abundant bivalve filter feeders in the Dutch Oosterschelde estuary is the non-native Pacific oyster *Crassostrea gigas* (Thunberg) (Drinkwaard, 1999; Geurts van Kessel et al., 2003). After their deliberate introduction in 1964 (Drinkwaard, 1999), these oysters expanded rapidly throughout Dutch estuaries, forming large and dense oyster reefs in the intertidal and subtidal (Drinkwaard, 1999; Wolff and Reise, 2002; Dankers et al., 2006). In the Oosterschelde estuary the intertidal area occupied by oyster beds is estimated to have increased from 0.15 km² in 1980 to 6.4 km² in 2002 (Geurts van Kessel et al., 2003). Within this period, during the 1990s, stocks of the native blue mussel *Mytilus edulis* L. and common cockle *Cerastoderma edule* (L.) showed a slight decrease in this estuary (Geurts van Kessel et al., 2003; Dankers et al., 2006). In the Wadden Sea in Germany and the Netherlands, Pacific oysters are also increasing in numbers and they are reported to invade mussel beds (Reise, 1998; Smaal et al., 2005; Dankers et al., 2006).

While the Pacific oyster stock in the Oosterschelde estuary increased, so did the total filtration pressure. The total filtration pressure was estimated to have increased from 289 million m³ day⁻¹ in 1990 to 398 million m³ day⁻¹ in 2000 (Geurts van Kessel et al., 2003; Kater, 2003). This will lower available food levels, thereby increasing food competition. The increased filtration pressure may

also cause a declining recruitment of bivalve filter feeders through larviphagy.

Bivalve filter feeders such as *C. gigas* and *M. edulis* have a pelagic larval stage (e.g. Bayne, 1964; Widdows, 1991; Wildish and Kristmanson, 1997). The pelagic larvae experience very high mortality rates due to various factors (e.g. Thorson, 1950; Rumrill, 1990; Gosselin and Qian, 1997). Mortality estimates of 0.13 day⁻¹ (Jørgensen, 1981) up to 0.8 day⁻¹ (Ayers, 1956) have been made for respectively *M. edulis* and *Mya arenaria*. Apart from pelagic predators (Johnson and Shanks, 2003), bivalve veligers encounter various benthic predatory species (Thorson, 1950), such as adult bivalve filter feeders. It seems plausible that most bivalve filter feeders filter pelagic bivalve larvae, as has already been demonstrated for *M. edulis* (Thorson, 1946; Maar et al., 2007; Lehane and Davenport, 2002, 2004), *Mytilus galloprovincialis* (Jasprica et al., 1997), *C. edule* (André and Rosenberg, 1991), *Crassostrea virginica* (Tamburri and Zimmer-Faust, 1996) and *C. gigas* (Tamburri et al., 2007). Once filtered, bivalve larvae are either ingested or rejected in pseudofaeces. If ingested, almost all larvae die in the digestion process or in the faeces (Mileikovsky, 1974; Lehane and Davenport, 2004; Tamburri et al., 2007). Rejection in pseudofaeces generally also leads to death (Mileikovsky, 1974; Tamburri and Zimmer-Faust, 1996; Lehane and Davenport, 2004; Tamburri et al., 2007).

Timko (1979) introduced the term 'larviphagy' for the feeding on larvae by adults of the same species, but here we extend the definition to the feeding on bivalve larvae by adult bivalve filter feeders in general. Because of the bivalve feeding mode, bivalve filter feeders are not likely to selectively filter larvae of specific bivalves. Their gills retain anything above their specific size limit for complete retention of

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particles, which is in the order of a few micrometers (2–7 μm, Møhlenberg and Riisgård, 1978). Bivalve larvae are generally larger than 70 μm (e.g. Hendriks et al., 2005) and are thus retained on most bivalve gills. An increased filtration pressure may result in a reduction in the numbers of bivalve larvae. Eventually, reduced larval numbers may result in a reduced recruitment success.

The strong increase of *C. gigas*, the increase of total bivalve filter feeder biomass, and the slight decrease of biomass of native filter feeders may have been brought about or at least stimulated by different responses of the larvae of the various bivalve species to larviphagy. Larvae of bivalve species are reported to escape from adverse conditions by increasing their upward swimming speed (Cragg, 1980; Prael et al., 2001), or by retracting the velum and sinking rapidly (LaBarbera, 1974; Cragg, 1980). However, Troost et al. (2008) found no escape responses in larvae of *C. gigas* and *M. edulis* in an artificial suction flow field mimicking a bivalve's inhalant current.

The increased abundance and distribution of the Pacific oyster and its large filtering capacity have the potential to influence bivalve larval mortality. In this context, we investigated whether *C. gigas* larvae are filtered less by adult bivalves than the larvae of the native *M. edulis*.

We tested the null hypothesis that *C. gigas* larvae are filtered at the same rate as *M. edulis* larvae by adult *C. gigas*, *M. edulis* and *C. edule*. We used a clearance rate approach to determine filtration rates of *C. gigas* and *M. edulis* larvae in a laboratory set-up. To enable a comparison between the larvae of both species, tested in different months, these filtration rates were compared to filtration rates of algae by the adult suspension feeders in the same experimental set-up. Our aim was not to obtain actual clearance rates, but to study whether oyster and mussel larvae are equally prone to predation by bivalves. Additionally, we studied the fate of filtered *C. gigas* and *M. edulis* larvae. We examined stomach contents and pseudofaeces of adult bivalves that had been fed with bivalve larvae to assess the proportions of larvae that were either ingested or rejected.

2. Materials and methods

2.1. Experimental animals

Adult animals were collected from the field. *C. gigas* were collected by hand from an intertidal oyster bed in the Oosterschelde estuary (SW Netherlands). They ranged from 0.53 to 1.23 g ash-free dry flesh weight (afdw). *M. edulis* were dredged from a subtidal bottom-culture plot in the Oosterschelde estuary and ranged from 0.77 to 1.11 g. *C. edule* were collected by hand from an intertidal mudflat in the Dutch Wadden Sea and ranged from 0.18 to 0.33 g. All specimens were transported dry and on ice to the laboratory at Yerseke as soon as possible but within 24 h. They were left to acclimatize in aerated natural seawater (30 psu and 18/21 °C, depending on the experiment) for at least a week. They were fed with the Instant Algae® Shellfish Diet ® (Reed Mariculture Inc., Campbell, CA, USA), containing *Isochrysis* sp., *Tetraselmis* sp., *Pavlova* sp. and *Thalassioseira weissflogii*. We consulted Helm et al. (2004) and Reed Mariculture (www.reedmariculture.com) to calculate food rations suitable for growth.

C. gigas veliger larvae were purchased from a commercial hatchery (Seasalter Shellfish (Whitstable) Ltd., UK), and shipped to our laboratory at Yerseke, the Netherlands. We performed three types of experiments with these larvae. In experiments on filtration rates (experiments A and B) we used two different batches with average lengths of 151.5±14.3 and 241.2±19.1 μm, respectively (Table 1). Both groups were in the veliconcha (umbo) stage. Larvae of the second batch were also used to study the fate of filtered *C. gigas* larvae. The larvae were reared at 27 °C and 30 psu. During transport, the larvae were kept on ice in moist filtration paper. Transport took no more than 24 h. Upon arrival, the larvae were submerged in 2–3 l of natural filtered (0.2 μm) seawater of 4–5 °C and 30 psu. They were then placed in a climate chamber to acclimatize to 21 °C over a period of at least 4 h (protocol after Helm et al., 2004). This temperature corresponds with seawater temperatures during the reproductive season (in the Netherlands July–September; unpublished data IMARES and the National Institute for Coastal and Marine Management RIKZ) . While acclimatizing they were fed the same algal mix they had been reared on (*Pavlova* sp., *Isochrysis* sp., *Chaetoceros muelleri* and *Tetraselmis* sp.). We followed Helm et al. (2004) in calculating a food ration suitable for growth. *M. edulis* larvae were produced in the experimental mussel hatchery of Wageningen IMARES at Yerseke and were transported over a short distance in the same containers they were reared in, at a constant temperature of 18 °C. We performed the same experiments with these larvae as we did with *C. gigas* larvae. In experiments on filtration rates (experiments C and D) we used two different batches with average lengths of 172.7±18.1 and 112.4±4.9 μm, respectively (Table 1). Both groups were in the veliconcha (straight-hinge) stage. Larvae of the first batch were also used to study the fate of filtered larvae. The *M. edulis* larvae were reared at a water temperature of 18 °C and this temperature was maintained throughout all experiments with these larvae. This temperature is at the higher end of the range in seawater temperatures occurring during the reproductive season (in the Netherlands May–June; unpublished data IMARES and RIKZ). *C. edule* larvae were not included because they could not be obtained in sufficient numbers.

2.2. Filtration experiments

Adult bivalves were placed in individual grazing chambers filled with natural filtered (60 μm) seawater of 30 psu. As grazing chambers we used buckets with different volumes for different species, roughly corresponding to their relative filtration capacities. We put oysters in 9 l of water, mussels in 5 l and cockles in 2.5 l of water. Per species we used an additional pair of chambers without adult bivalves as control chambers. The water temperature in the grazing chambers was kept constant by placing the chambers in water baths that were heated with heater thermostats. In experiments with *C. gigas* larvae the temperature was kept constant at 21 °C and in experiments with *M. edulis* larvae at 18 °C. For the filtration experiments, the adult animals were left to acclimatize to the grazing chambers for at least 1 h, while they were fed with a mixture of *Isochrysis galbana* and *Pavlova lutherii* that were cultivated at the Wageningen IMARES experimental mussel hatchery. Experiments were carried out in sets over a period of 2 days. On the first day of a set, filtration rates of algae were determined. On the second day

Table 1
Initial concentrations and larval shell lengths as used in the experiments on larviphagy

Larval species	Set of experiments	Experiment	Date	Initial larval concentration (l⁻¹)	Age larvae (days from fertilization)	Length larvae±s.d. (μm)
Crassostrea gigas	A	CR algae	July 6 2005			
	A	CR larvae	July 7 2005	451	6	151.5±14.3
	B	CR algae	July 19 2005			
	B	CR larvae	July 20 2005	240	10	241.2±19.1*
Mytilus edulis	C	CR algae	March 30 2005			
	C	CR larvae	March 31 2005	253	9	172.7±18.1*
	D	CR algae	April 5 2006			
	D	CR larvae	April 6 2006	629	3	112.4±4.9

Larval batches that were also used to study the fate of filtered larvae are indicated by an asterisk.

of a set, filtration rates of either *C. gigas* larvae or *M. edulis* larvae were determined. We used the 'indirect' clearance method to determine filtration rates (see Riisgård, 2001; Petersen et al., 2004). Overnight, in between the experiments of one set, the adult bivalves were placed back in a tank with aerated natural seawater of the same temperature and salinity as used in the experiments.

We carried out two sets of experiments per larval species (Table 1). At the end of each set, the adult animals were dried at 70 °C for 3 days and incinerated at 550 °C for 4 h to determine their ash-free dry weights.

On the day after the filtration experiments, we studied the fate of filtered larvae by examining stomach contents and pseudofaeces of adults. These experiments were performed only once for each species of larvae.

2.3. Filtration rates of algae

Because the experiments with *C. gigas* and *M. edulis* larvae were carried out in different months and with different individuals of adult suspension feeders, we related filtration rates of larvae to filtration rates of algae. Filtration rates of algae were determined by measuring clearance rates of micro-algae that are retained with 100% efficiency by the bivalve gill (Møhlenberg and Riisgård, 1978; Riisgård, 2001): *I. galbana* and *P. lutherii*. We used the set-up as described above with eight adults in individual grazing chambers. The water temperature was 18 or 21 °C, depending on whether we would use *M. edulis* or *C. gigas* larvae, respectively, the next day. After the bivalves' acclimatization period, we replenished the algal mixture (*I. galbana* and *P. lutherii*) in the chambers to reach 3×10^4 to 4×10^4 cells ml^{-1} and stirred gently. We first waited 20 min for larger particles (e.g. pseudofaeces) to settle down again to prevent an over-estimation of clearance rates. Then, every 20 min we took water samples of 12 ml with a pipette to determine the algal concentration. We moved the pipette gently around through the grazing chamber while sucking. All particles in the size range 4–12 μm were counted with a Z2™ Coulter Counter®. Per count, only 1 ml was removed for counting and we returned the remaining 11 ml (with an unchanged algal concentration) to the grazing chambers to maintain the same volume throughout the experiment. We counted algal numbers 4 to 7 times per experiment. The experiment lasted for 1.5 to 2.5 h. From the reduction in algal concentration, we calculated the clearance rate according to equation 3 in Riisgård (2001):

$$Cl = (V/t) \times \ln(C_0/C_t) \tag{1}$$

where Cl is the clearance rate (the volume of water that is cleared of all particles per unit of time per individual, in l h^{-1} ind^{-1}), V is the volume (l) of the grazing chamber, t is the time (h) spent filtering, and C_0 and C_t are particle concentrations (particles l^{-1}) at times 0 and t, respectively. Because filtration rates of bivalves are related to their body weight (Møhlenberg and Riisgård, 1979), we standardized the clearance rates for body weight by dividing clearance rates by the ash-free dry tissue weight (in g) of the individual bivalves, resulting in clearance rates expressed in l h^{-1} g^{-1}. A prerequisite for the use of Eq. (1) to calculate clearance rates is that the water should be well mixed, to ensure that the decline in particle concentration is exponential (Riisgård, 2001). Because the only mixing in our chambers was created by the pumping activity of the adult bivalves, this prerequisite was possibly not fully met. We investigated whether the decrease in algal concentration in our experimental chambers was exponential by fitting a linear regression through log-transformed algal concentrations plotted against time.

2.4. Larviphagy

In the experiments with larvae, we used the same eight adults per species as in the experiment with algae the previous day. The animals were left to acclimatize for 1 h, while feeding on the same algal mix as in the experiment with algae the previous day. After the acclimatization period, as soon as all animals were observed to be filtering actively, we first added algae until the same algal concentrations were reached as we used in the experiment the day before (*I. galbana* and *P. lutherii*, 3×10^4 to 4×10^4 cells ml^{-1}). After that we added either *M. edulis* or *C. gigas* larvae in known numbers to the grazing chambers ($t=0$) (Table 1). We stirred very gently immediately afterwards to distribute the larvae as evenly as possible. We let the adults feed for 1 h and then we removed them from the chambers. When removing the animals, they were rinsed with filtered seawater, to make sure that no larvae were removed along with the adult animals. The water from the grazing chambers was then sieved through a 60 μm mesh, and the walls and bottom of the chambers were flushed and sieved as well, to collect all remaining larvae. The larvae were submerged in a little seawater and fixed with 4% formaldehyde buffered with borax. Afterwards, larval numbers were counted using an inverted microscope. Filtration rates were calculated as clearance rates with Eq. (1).

2.5. Fate of filtered larvae

Of adult *C. gigas*, *M. edulis* and *C. edule*, ten individuals were placed in individual chambers filled with 2 l natural seawater. Five of them would be used for stomach content analysis and the other five for analysis of pseudofaeces. An algal mixture (*I. galbana* and *P. lutherii*) was added to stimulate feeding. Above a certain particulate matter level, the pseudofaeces threshold level, bivalves produce pseudofaeces by rejecting unpalatable particles in a mucus cover. To be able to study the choice of the adult bivalves for either rejection or ingestion, we stimulated pseudofaeces production by adding silt (incinerated and sieved over a 40 μm screen). A silt concentration of 15 mg l^{-1}, which is above the pseudofaeces threshold level (Hawkins et al., 1998), was maintained throughout the experiment. The adults were left to acclimatize for at least 1 h. Then, when they were observed to be actively feeding (valves opened and mantle and/or siphons extended) we added either *M. edulis* or *C. gigas* larvae to their inhalant feeding current ($t=0$). We pipetted larvae as close as possible to the inhalant feeding aperture. In the experiment with *C. gigas* larvae we used 400 larvae and in the experiment with *M. edulis* larvae 600 larvae. The *C. gigas* larvae were 241.2 ± 19.1 μm in length, and the *M. edulis* larvae 172.7 ± 18.1 μm. Before starting the experiment, we had determined the time it took for black carbon particles to reach the mouth or to be expelled in pseudofaeces, in adult oysters, mussels and cockles. These animals were placed in seawater with the same diet as used in the experiment. One shell valve was removed, and the carbon particles were pipetted onto the gills as far away from the mouth as possible. Transport of the particles to the mouth, and expulsion in pseudofaeces never took more than 5 min. Following these observations, and the methodology of Lehane and Davenport (2004), 5 min after introducing the larvae to the inhalant apertures of the adult bivalves, we removed five adults per species from their chambers and extracted the stomach contents within 5 min more. We extracted the stomach contents of oysters using a glass pipette that was inserted through the mouth into the stomach. Stomach contents of mussels and cockles were extracted by inserting a glass pipette through a small incision in the stomach wall. The stomachs of all adults were flushed several times with filtered (0.2 μm) seawater to remove as many ingested larvae as possible. Removed stomach contents were fixed with 4% formaldehyde buffered with borax. The five remaining adults per species were left to feed for 15 min after $t=0$. At $t=15$ their pseudofaeces were collected with a pipette. To collect all larvae that were potentially left in the surrounding water, we sieved the water from each chamber through a 60 μm mesh, while flushing the walls and bottom with filtered seawater. The residue was fixed in seawater with buffered formaldehyde, to be able to count how many larvae were spilled into the surrounding water while adding them to the inhalant feeding current. Afterwards, larval numbers in all collected samples were
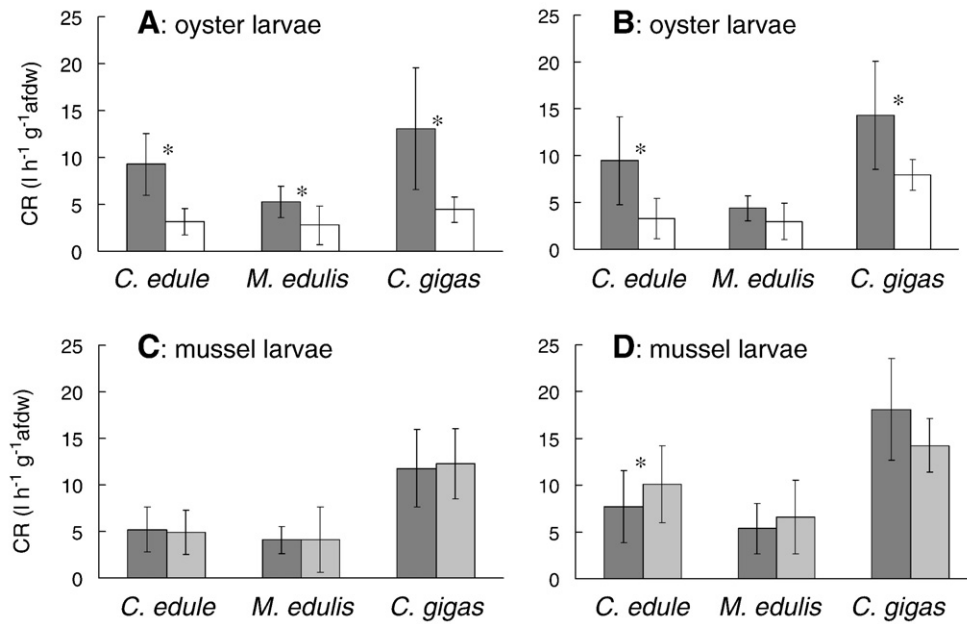
Fig. 1. Clearance rates (CR) by adult bivalves of algae (A–D, dark grey bars), *C. gigas* larvae (A–B, white bars), and *M. edulis* larvae (C–D, light grey bars). Average clearance rates with standard deviations are given in litres per hour per individual of a standardized 1 g ash-free dry weight ($n = 8$). Statistically significant differences are indicated by an asterisk (paired *t*-test, $P < 0.05$). A, B, C, and D are separate series of experiments.

counted using an inverted microscope. We subtracted the number of spilled larvae from the number of pipetted larvae to calculate the actual number of larvae that were added to the animal. We calculated percentages of retrieved larvae in stomach and pseudofaeces samples by relating the numbers of retrieved larvae to the actual number of larvae that were added to the animal.

2.6. Statistical analysis

All statistical tests were performed with SPSS® 12.0.1. Data were visually checked for normality using a Q–Q plot, and for equality of variances by plotting studentized residuals against predicted values. If the prerequisites were not met, the data were ln-transformed before testing. A significance level of $\alpha = 0.05$ was maintained.

3. Results

3.1. Larviphagy

The larvae of both species appeared to be healthy and behaving normally. All three bivalve species filtered *C. gigas* larvae with filtration rates that were on average roughly half of the filtration rates of algae (Fig. 1A–B). In experiment A (Fig. 1A) we found the filtration rates of *C. gigas* larvae by all three species to be significantly lower than their filtration rates of algae (paired *t*-test, $P < 0.05$). In experiment B we also observed the filtration rates by all three species of *C. gigas* larvae to be lower than their filtration rates of algae (Fig. 1B), although the observed difference was not significant in the case of adult mussels ($P > 0.05$). *C. gigas* larvae were filtered with filtration rates that were on average 50.5% of the filtration rates of algae. This ratio did not differ significantly between adult species (ANOVA, $P > 0.05$).

All three bivalve species filtered *M. edulis* larvae with the same filtration rates as they had filtered algae with, the previous day (Fig. 1C–D). We found this result for both experiments performed with *M. edulis* larvae. In experiment C (Fig. 1C) we found no significant differences between the filtration rate of algae and the filtration rate of larvae (paired *t*-test, $P > 0.05$). In experiment D (Fig. 1D) we also found no significant

differences, except for cockles that had filtered *M. edulis* larvae with a significantly higher filtration rate than algae ($P < 0.05$).

Algal concentrations showed an exponential reduction in time in all chambers and for all actively filtering adult bivalves (linear regression on log-transformed algal concentrations plotted against time: $R^2 > 0.85$; $P < 0.05$). In the control chambers ($n = 2$ per species), no reduction in algal concentrations or larval numbers was observed (deviation of the difference between C_0 and C_t from 0, tested with a
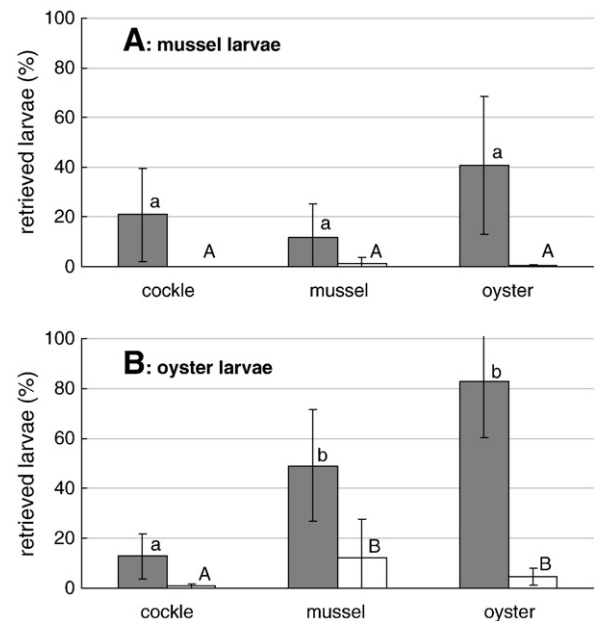

Fig. 2. Mean percentages of actual number of larvae added, retrieved from stomach (dark bars) and pseudofaeces (white bars) samples, with standard deviations. Different letters indicate significant differences (ANOVA+Bonferroni, $P < 0.05$ after ln-transformation) between adult species, for stomach (lower case letters) and pseudofaeces (upper case letters) samples.

one-sample t-test ($n=8$ per adult species for algae, $n=4$ per adult species for oyster and mussel larvae): $P>0.05$).

3.2. Fate of filtered larvae

All three bivalve species ingested *M. edulis* and *C. gigas* larvae (Fig. 2). Only 0.1 to 1.3% of all *M. edulis* larvae that were added were found back in the pseudofaeces of the adult filter feeders. Of all added *C. gigas* larvae, 0.9 to 12% were retrieved from the pseudofaeces. Percentages of *M. edulis* larvae retrieved from the stomach and pseudofaeces did not differ between species of adult filter feeders (ANOVA, $P>0.05$). *C. gigas* larvae were retrieved in significantly lower percentages from *C. edule* stomachs and pseudofaeces, compared to *M. edulis* and *C. gigas* (ANOVA, $P<0.05$). Because of the large size difference between *C. gigas* and *M. edulis* larvae, and because we only used one batch per species, we did not address and test differences in proportions rejected and ingested between the two species. The larvae appeared healthy and behaving normally before adding them to the adults.

4. Discussion

4.1. Larval stage

We compared umbo-stage *C. gigas* larvae to straight-hinge *M. edulis* larvae. These are the stages that make up the largest part of the pelagic life of these species. All *C. gigas* larvae were observed to be in the veliconcha umbo stage. None of these larvae had already developed a foot, and the velum appeared not to be resorbed yet. In European *C. gigas*, straight-hinge veliger larvae develop at 80 μm, the umbo is developed at a length of about 120 μm, a foot is developed and the velum resorbed at about 300 μm, when the larvae become pediveligers. The umbo veliconcha stage is by far the lengthiest stage (e.g. Hendriks, 2004). All *M. edulis* larvae were observed to be in the veliconcha straight-hinge stage. They had not yet developed an umbo and a foot. In European *M. edulis*, straight-hinge veliger larvae develop at 94 μm and the umbo and foot are developed at a length of about 185–200 μm, making the straight-hinge stage the lengthiest stage (e.g. Hendriks, 2004).

4.2. Larviphagy

All three species filtered *M. edulis* and *C. gigas* larvae. *C. gigas* larvae were filtered with a 50% lower rate than algae. Since *M. edulis* larvae were filtered with the same rate as algae, we can conclude that in our experiment *C. gigas* larvae were filtered with 50% lower rates than *M. edulis* larvae.

C. gigas larvae were filtered 50% less than expected for 'inert' particles such as micro-algae. We assumed that the flagellated micro-algae moved randomly, and with low displacement rates. In advance we had observed the micro-algae to remain distributed homogeneously in a 9 l chamber during 1.5 h. The lower filtration rates of *C. gigas* larvae in all three adult species suggest that the oyster larvae somehow avoided being filtered. There are few possibilities for zooplankton to detect filter feeders or their inhalant feeding current. Zooplankton may detect hydromechanical stimuli in the inhalant flow field (e.g. Singarajah, 1975; Jakobsen, 2001; Kingsford et al., 2002), or they may detect filter feeders chemically (see waterborne chemical attraction in settling larvae, Fitt and Coon, 1992; Tamburri et al., 1996). Troost et al. (2008) showed that *C. gigas* and *M. edulis* did not respond to hydromechanical stimuli in a suction current, leaving the option that the larvae detected the adult filter feeder chemically, and responded by moving away from its direct vicinity. Further investigations should address this possibility.

Observed differences in filtration rates were not likely caused by gradients in oxygen concentration. We stirred the water completely but gently after adding the larvae to the chambers, thereby homogenizing possible oxygen gradients. Similar rates of oxygen consumption in larvae of *C. gigas* and *M. edulis* (Riisgård et al., 1981; Gerdes, 1983; Sprung, 1984) cannot explain observed differences in filtration rates. Moreover, Mann and Rainer (1990) found that larvae of *C. virginica* did not alter their vertical swimming speed when exposed to hypoxia. They argued that aerobic metabolism can be maintained at low oxygen saturation due to the large surface to volume ratio in oyster larvae. Finally, eventual oxygen gradients were probably too weak to cause an effect, since an adult oyster consumes roughly 1.25 μl O_2 l^{-1} in 2 h (Gerdes, 1983), which is only 0.02% of full O_2 saturation (Mann and Rainer, 1990).

In the second filtration experiment with mussel larvae (experiment B, Table 1; Fig. 1B), we found that adult cockles filtered larvae with a significantly higher filtration rate than algae. Since it is highly unlikely that mussel larvae swam directionally towards the inhalant siphon of the cockles, we assume that this is a chance result.

4.3. Fate of filtered larvae

Most larvae of both species were ingested by the adult bivalves, and only 0.1 to 12.0% of the larvae were rejected in pseudofaeces. Different authors observed bivalve larvae to be unable to free themselves from pseudofaeces, or even to have died in the rejection process (Mileikovsky, 1974; Tamburri and Zimmer-Faust, 1996; Lehane and Davenport, 2004; Tamburri et al., 2007). Thus, survival of filtration through rejection in pseudofaeces does not seem a successful survival strategy for *C. gigas* larvae. Although the added silt stimulated the rejection of less digestible particles in pseudofaeces, only few larvae were rejected, suggesting that veliger larvae are preferentially ingested by the bivalve filter feeders.

All ingested larvae likely died, either in the digestion process or in the faeces where they would be covered in mucus and faecal material. Different authors found a few intact bivalve larvae in bivalve faeces, but these larvae were generally unable to extricate themselves and in most cases died (Mileikovsky, 1974; Lehane and Davenport, 2004; Tamburri et al., 2007).

C. edule rejected significantly fewer *C. gigas* larvae than *M. edule* and *C. gigas*, which corresponds to the findings of Hawkins et al. (1998) that *C. edule* reject smaller proportions of all filtered material than *M. edulis* and *C. gigas*. We also found significantly fewer *C. gigas* larvae in cockle stomachs than in mussel and oyster stomachs. *C. gigas* larvae are possibly processed more quickly in cockle stomachs, and pass faster from the stomach into the guts or into the digestive diverticula. After having extracted all stomach contents within 5 to 10 min after having added the larvae, we only retrieved 22 to 89% of the total number of added larvae in stomach and pseudofaeces samples of all adults. This is in accordance with the findings of Lehane and Davenport (2004), who also retrieved only a small proportion (<25%) of added larvae from stomachs of *M. edulis* after 5 min. The missing larvae must have been located either somewhere between the gills and the stomach, or somewhere between the stomach and the anus, or they were already located in the digestive diverticula. In the first case, the larvae might still have been present in the pallial cavity, where handling times were found to range up to 10 min for *C. virginica* and *M. edulis*, even up to 23 min in *C. virginica* fed on a low-quality diet (Milke and Ward, 2003). However, we observed a handling time for carbon particles of less than 5 min for all three species fed on the experimental diet. Therefore, the larvae were more likely transported beyond the stomach already, due to post-ingestive selection processes (Brillant and MacDonald, 2002). If the missing larvae were located in the digestive diverticula or beyond the stomach, they were ingested and most likely dead. Retrieval of low numbers of larvae from the pseudofaeces after 15 min confirms that most larvae were ingested. This is in accordance with the findings of Tamburri et al. (2007), who observed that of *C. gigas* larvae introduced to the pallial cavity of adult *C. gigas*, 73.9% were ingested and 17.4% were expelled in pseudofaeces. In stead of counting larval numbers from

stomach contents, these authors calculated the percentage of ingested larvae by subtracting the number of larvae in pseudofaeces from the number of larvae introduced into the pallial cavity. If we were to do the same, we would find that 98.7 to 99.9% of the offered *M. edulis* larvae were ingested, and 88.0 to 99.1% of all *C. gigas* larvae. Ingested larvae are likely to be fully digested, including the shell, as was observed for larvae of *C. gigas* (Tamburri et al., 2007), *C. virginica* and *Mercenaria mercenaria* (Tamburri and Zimmer-Faust, 1996), and *Tapes philippinarum* (Lehane and Davenport, 2004).

4.4. Ecological implications

Because we cannot translate our still-water results directly to the field, we can only speculate about the ecological implications of our results. We found larvae of *C. gigas* to be filtered 50% less than larvae of *M. edulis*. In combination with the higher reproductive output of *C. gigas* per female (50 to 200 million eggs; Utting and Spencer, 1992; Kang et al., 2003; Helm et al., 2004) in comparison to *M. edulis* (5 to 12 million eggs; Bayne et al., 1978; Helm et al., 2004), this could potentially (and partially) explain the fast expansion of Pacific oysters in Dutch waters, and possibly also the slight decline in stocks of native bivalve filter feeders in the enclosed Oosterschelde estuary.

Tamburri et al. (2007) studied filtration of *C. gigas* larvae by conspecific adults in a flume tank. *C. gigas* pediveliger larvae that were competent to settle were rarely entrained by inhalant feeding currents of adult *C. gigas*. The authors attribute this to the small relative gape surface area and the observed weak inhalant feeding currents (~1.65 mm s^{-1}) of the adults. The implication of this study is that avoidance of filtration by adult *C. gigas* is not necessary in bivalve veliger larvae, because the risk of being filtered is very low. However, this is not in agreement with our results and results by Troost et al. (submitted), who found a much higher inhalant current velocity than Tamburri et al. (2007) did, in comparably sized *C. gigas*.

Troost et al. (submitted) also found an increase of inhalant current velocity with body weight, ranging up to more than 15 mm s^{-1} in the largest oyster studied. In our still-water experimental chambers veliger larvae of *C. gigas* and *M. edulis* were entrained in high numbers by the adult *C. gigas*. Furthermore, in stomach contents of adult *C. gigas* collected from the field we found numerous (parts of) zooplankters, even copepod nauplii and parts of copepods (unpublished observations KT), indicating that *C. gigas* are very well capable of entraining zooplankton, even zooplankton with advanced escape capabilities (for nauplii see Titelman and Kiørboe, 2003). Finally, although local effects may be small, significant effects on a larger scale may occur in areas with a high cover of suspension feeders (Peterson and Black, 1987; André et al., 1993), and when larvae become trapped in the benthic boundary layer at moderate current velocities (Jonsson et al., 1991).

4.5. Methodological considerations

The method we used to determine filtration rates, the 'indirect' clearance method, is considered to be a reliable method (Riisgård, 2001; Petersen et al., 2004). However, the reduction in particle concentration over time may be a disadvantage since this may cause the bivalves to adjust their clearance rates during the experiment (Riisgård, 2001). Furthermore, we did not stir the water because we wanted to exclude background turbulence from our experiments, to study the effects of larval behaviour alone. Therefore, recirculation of cleared water may have occurred, leading to an underestimation of filtration rates. The use of formula 1 to calculate filtration rates was justified since algal concentrations declined exponentially with time (Riisgård, 2001). The 'indirect' clearance method was very suitable for our purpose, but the obtained filtration rates may not apply directly to a field situation where flow and turbulence play significant roles.

Furthermore, we do not expect differences in larval concentrations (Table 1) to have affected our results. In Pacific oyster hatcheries,

concentrations of 5 up to 57 ml^{-1} are generally used without significant negative effects on larval health and development (Helm et al., 2004). Therefore, significant effects on larval behaviour were also not expected.

4.6. Conclusions

Our study shows that adult edible cockles, blue mussels and Pacific oysters filter and for a large part ingest *M. edulis* and *C. gigas* larvae. Smaller numbers of larvae were rejected and ended up in pseudofaeces. *C. gigas* larvae appear to avoid filtration in some way, but this study does not elucidate the mechanisms. The larvae do not respond to hydromechanical stimuli (Troost et al., 2008), leaving other mechanisms, such as chemical detection, for further research.

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