Pacific Oysters in Dutch Estuaries

Causes of Success and Consequences for Native Bivalves

This project was funded by the Netherlands Organization for Scientific Research – Earth and Life Sciences (NWO-ALW; project number 812.03.003).

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ISBN: 978-90-367-4034-0

RIJKSUNIVERSITEIT GRONINGEN

Pacific Oysters in Dutch Estuaries
Causes of Success and Consequences for Native Bivalves

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
vrijdag 9 oktober 2009
om 16.15 uur

door

Karin Troost
geboren op 6 oktober 1977
te Heerenveen

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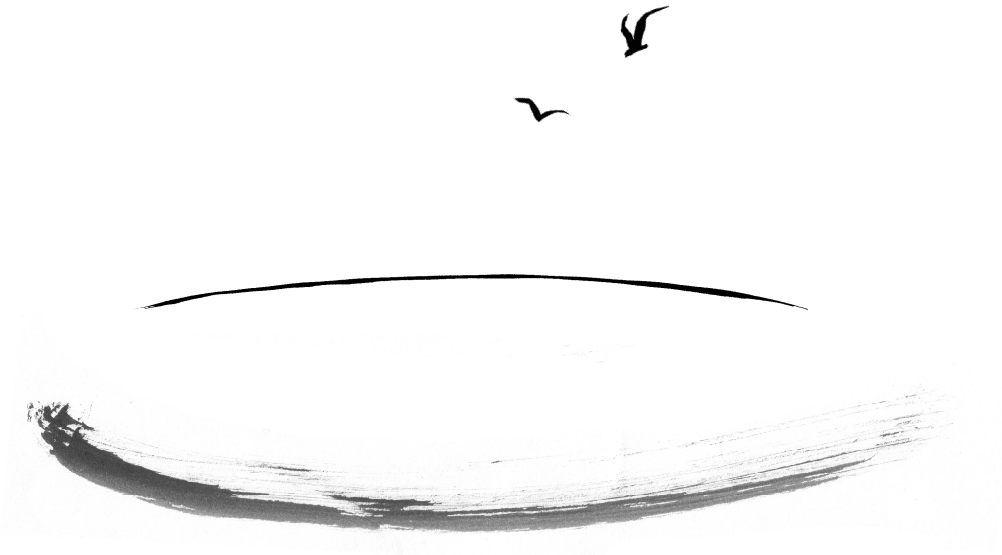
Chapter 1



Causes of the Pacific oysters' success and consequences for native bivalves:

General introduction & outline of the thesis

Karin Troost

1.1. Introduction

Oyster farmers first introduced the Pacific cupped oyster *Crassostrea gigas* (Thunberg, 1793) in the Dutch Oosterschelde estuary in 1964 (Drinkwaard 1999b). Although at that time this species was deemed unable to reproduce in the 'cold' Dutch waters, a decennium and repeated introductions later a first extensive spatfall was recorded. The Pacific oyster had established itself successfully (Drinkwaard 1999b). It spread rapidly throughout the Oosterschelde estuary and to other Dutch estuaries, forming large and dense oyster reefs in the intertidal and subtidal (Dankers et al. 2006). When the current investigation was started in 2002, it had become clear that Pacific oysters had a major impact on Dutch estuarine ecosystems (Figure 1.1). In a worst-case scenario the expansion of *C. gigas* was supposed to eventually lead to a complete replacement of native bivalves. Therefore this thesis investigates the causes of its remarkably successful establishment in the Netherlands as well as the consequences for native bivalves. Results presented in this thesis will contribute to elucidate general mechanisms in marine invasion ecology.

1.2. Biological invasions

Globalisation of human activities increasingly promotes homogenisation of the earth's biota by introducing species outside their natural ranges (Lodge 1993; Gray 1997; Vitousek et al. 1997; Bax et al. 2003; Olden and LeRoy Poff 2003; Galil 2007). Biological invasions are considered as an important element of global change (together with e.g. increasing CO_2 concentrations, climate change, changes in land use, and

overexploitation of natural resources; Vitousek et al. 1997) and occur everywhere: in terrestrial, aquatic and marine ecosystems, on islands, on continents, in coastal areas and oceans. Biological invasions pose a serious threat to global biodiversity (Occhipinti-Ambrogi and Savini 2003; Occhipinti-Ambrogi 2007), and can change the functioning of ecosystems in extreme cases in such a way that they change the rules of existence for all species (Vitousek et al. 1997). In this thesis, the term 'biological invasions' refers to species moved outside their natural range by human activities (deliberate and accidental introductions) and natural range expansions (after Carlton

Figure 1.1. An oyster bed covering a large area in the intertidal of the Oosterschelde estuary (picture taken at 'Zandkreek').

1989). The term 'introduced species' is used specifically for species introduced by human activities, while 'invasive species' refers to introduced species that manage to establish successfully and have a certain impact on the receiving ecosystem.

Examples of individual invaders that caused major changes in the receiving ecosystems are the brown tree snake *Boiga irregularis*, the zebra mussel *Dreissena polymorpha*, the Nile perch *Lates niloticus*, and the 'killer weed' *Caulerpa taxifolia*. The

accidentally introduced brown tree snake largely depleted the island of Guam of native birds, bats and reptiles (Fritts and Rodda 1998). The snake also causes electric power outages on a large scale by climbing energy lines and creating short-circuits (Fritts 2002). The zebra mussel invaded the Great Lakes in North America where it smothers indigenous unionid bivalves, affects the food web by increasing water clarity and controls phytoplankton and zooplankton community structure by its high grazing rate. It is now the main biofouling organism of water intakes and other man-made littoral structures in the Great Lakes (Hebert et al. 1991; Mills et al. 1993). The Nile perch, introduced in Lake Victoria in Africa, drove approximately 200 fish species extinct in less than a decade and caused far reaching changes in the food web (Ogutu-Ohwayo 1990; Goldschmidt et al. 1993). In the Mediterranean Sea the green alga *Caulerpa taxifolia* is associated with a reduction in species richness of native hard substrate algae by 25 – 55%. It threatens ecologically important species-rich seagrass meadows with replacement by species-poorer dense *Caulerpa taxifolia* meadows (Boudouresque et al. 1995; Galil 2007).

Biological invasions occur at a global scale, and in large numbers. Most countries count 10^2 – 10^4 documented introductions (Lodge 1993). Apart from causing dramatic ecological effects, non-indigenous species can also cause high economical damage. In the United States only, damage caused by the approximately 50,000 foreign species is estimated to amount to almost $120,000,000,000 per year (Pimentel et al. 2005).

1.2.1. Pathways of marine invasions

Globalisation of human activities increases the rate of biological invasions by creating and expanding invasion pathways. In the marine environment, five principal pathways of marine invasions can be discerned, that are created or caused by humans: intentional release of species (e.g. for mariculture), unintentional escape from captivity (e.g. from culture, aquariums), contaminants of species kept and transferred by humans (e.g. parasites, commensals), stowaways in human transport vessels (e.g. on hulls or in ballast water of ships), and via man-made corridors (e.g. canal systems). All pathways listed above can be followed by natural dispersal of the exotic species in recipient regions (Reise et al. 1999; Wolff 2005; Hulme et al. 2008).

In the marine environment, the bulk of exotic species is imported by accident as stowaways on ships' hulls and in ballast water (Carlton 1987; Carlton and Geller 1993; Gollasch 2002; Wonham and Carlton 2005; Drake and Lodge 2007; Barry et al. 2008). The wood-boring shipworm *Teredo navalis* was probably introduced into the Netherlands as a stowaway in ships' hulls, and caused massive damage to wooden

constructions in seawalls in the 18th and 19th centuries (Wolff 2005). Another infamous stowaway is the green crab *Carcinus maenas*, that was introduced world-wide in either ballast water, solid ballast, in ships' hulls in holes bored by *T. navalis*, or in seaweeds used for packing marine products (Carlton and Cohen 2003). This voracious predator has a major ecological impact at Atlantic and Pacific coasts of North America (Grosholz et al. 2000; Behrens Yamada et al. 2005; Taylor 2005).

Another major invasion pathway is found in shellfish imports for mariculture (Wonham and Carlton 2005), especially in oyster transfers (Wolff and Reise 2002; Wolff 2005). These transfers not only present an invasion pathway for the target shellfish species themselves, but also for non-target species that are transferred along with the shellfish. An overview of the long history of oyster transfers in northwestern Europe is given by Wolff (2005). Main species of interest for shellfish culture in northwestern Europe, historically and at present, include the oysters *C. gigas*, *Crassostrea angulata*, *Crassostrea virginica*, *Ostrea edulis* and the mussel *Mytilus edulis* (Wehrmann et al. 2000; Wolff 2005; Wijsman and Smaal 2006). An overview of global introductions of *C. gigas* is given in Box 1.1. Many species have been introduced globally with oyster imports (as contaminants), such as the protist parasite *Bonamia ostreae*, the snail *Crepidula fornicata* and the seaweed *Sargassum muticum* in Europe (Blanchard 1997; Eno et al. 1997; Wolff 2005). *B. ostreae* was introduced in the Netherlands with oysters (*O. edulis*) from Brittany in 1980. It caused the nearly complete disappearance of Dutch culture of *O. edulis* and is still infecting oysters in the Oosterschelde estuary and Lake Grevelingenmeer (Van Banning 1991; Wolff 2005).

Man-made corridors also caused high rates of introductions into new areas. Well-known examples are the canal systems that connect the Ponto-Caspian region to the Baltic and North Sea coasts (Bij de Vaate et al. 2002; Ketelaars 2004), the Suez canal connecting the Red Sea with the Mediterranean Sea (Galil 2007) and the Welland canal and St-Lawrence Seaway connecting the Great Lakes to the Atlantic Ocean (Mills et al. 1993).

An example of an 'escapee' is the macro-alga *Caulerpa taxifolia*, that probably escaped from aquariums of Monaco's Oceanographical Museum into the Mediterranean Sea where it causes large-scale ecological effects (Boudouresque et al. 1995).

Finally, the Chinese mitten crab *Eriocheir sinensis* is a good example of an invader with a fast natural range expansion. It was introduced into northern Germany and southern France, and showed maximum rates of natural range expansion of 562 km per year in northern Europe (1928 – 1938) and 380 km per year in southern France (1954 – 1960). It is now widely distributed in Europe (Herborg et al. 2003).

BOX 1.1.
Global introductions of *Crassostrea gigas*

Pacific oysters originate from the Sea of Japan and the Pacific coasts of the Japanese islands. Their native range lies between the Russian island of Sakhalin and Primorskiy Kray on the continent in the north (latitude ~48° north), and the Japanese island of Kyushu and the east coast of southeast Asia in the south (latitude ~30° north; Arakawa 1990a). Pacific oysters *C. gigas* have long been cultured in Hokkaido, Miyagi, Hiroshima and Kumamoto prefectures in Japan. In 1902 and 1903 first export shipments were made to Washington State in the USA, and British Columbia in Canada (Arakawa 1990a; Shatkin et al. 1997). Pacific oyster culture became widespread along the Pacific coast (hence the English name of the species) from British Columbia in Canada to California in the United States (Arakawa 1990a). British Columbia became a major source of new introductions. From Japan, *C. gigas* was first introduced in Australia in 1947 and in France in 1966. From British Columbia, *C. gigas* was first introduced in the Netherlands in 1964 and in the UK in 1965. The Pacific oyster was subsequently imported from Wales into Ireland in 1969, from the UK into Germany in 1971 and from Germany into Denmark in 1979. In New Zealand, accidentally introduced *C. gigas* were discovered in 1970 (Shatkin et al. 1997; Wolff 2005).

At present, *C. gigas* is the species with the greatest global production volume in aquaculture, with about 4.4 million tonnes (FAO 2004). Producer countries are Canada, the USA, Mexico, Peru, Argentina, Chile, Norway, Ireland, UK, Germany, the Netherlands, France, Portugal, Spain, Morocco, Algeria, Tunisia, Senegal, Namibia, South Africa, China, Japan, Korea, Taiwan, Australia, New Zealand, and New Caledonia. Introductions of *C. gigas* have also been recorded in Belgium, Denmark, Sweden, Ecuador, Belize, Costa Rica, Puerto Rico, the United States Virgin Islands, Brazil, Israel, Philippines, Malaysia, Romania, the Ukraine, the Seychelles, Fiji, French Polynesia, Guam, Palau, Samoa, and Vanuatu (FAO 2004).

The Portuguese oyster *Crassostrea angulata*, originally a strain of *C. gigas* from Taiwan (Ó Foighil et al. 1995; Boudry et al. 1998), was introduced in Portugal already somewhere between 1500 and 1800. From there, *C. angulata* has been introduced elsewhere. In the Netherlands it was introduced in the 19th century and in the 20th century until a few years after the Second World War. The species never established itself in Dutch estuaries (Wolff and Reise 2002).

1.2.2. Number of introductions underestimated

The number of recorded introductions probably underestimates the total number of introductions dramatically (Lodge 1993). Many introductions are not noticed and the distinction between indigenous and non-indigenous species can be very difficult, if not

impossible. For instance, some non-indigenous species were introduced long before biological research started. This was the case with *Mya arenaria* that is thought to have been introduced from North America into NW Europe by the Vikings before 1245 - 1295 (Petersen et al. 1992). Some species cannot be reliably demonstrated to be either native or exotic. These are termed 'cryptogenic' species (Carlton 1996). Invaders may also be considered as native species if they closely resemble native species. Often such cryptic species cannot be distinguished from native species based on morphological differences alone. Molecular genetic techniques are often required. These have proven a powerful tool in differentiating cryptogenic taxa, and in taxonomic verification and identification of source populations and vectors (Geller et al. 1997; Holland 2000; Wares et al. 2002). Furthermore, what seems to be a human-induced introduction may in fact be a natural range expansion. During the last glaciation (appr. 18,000 years ago), the sea level dropped at least 110 m, exposing the North Sea floor as arctic dry land. After the ice age, marine organisms recolonised the North Sea from refuges. This may still be going on today (Reise et al. 1999, and references therein).

1.2.3. Characteristics of successful invaders and invasible ecosystems

Since numbers of invasions are still increasing, it becomes more and more important to be able to understand and predict invasion patterns and mechanisms. Without proper knowledge about why and how exotic species are able to invade and establish themselves in other ecosystems and in what ways these invasions are facilitated by man, trying to manage or even prevent unwanted introductions will be very difficult if not impossible. In many cases however, an invader is not discovered until after the invasion event, at a point where the invader already is a part of the ecosystem (Williamson 1996). Consequently, ecological responses to the invasion may go unnoticed for a long time, and the mechanism of invasion and the causes of its success may never be elucidated.

A large body of scientific work is devoted to finding general rules in invasion ecology. What characteristics determine whether species are invasive? And what characteristics determine whether a community is invasible? Although the relatively high amount of attention given to pests may give the impression that most introduced species cause trouble, proportionally only very few introduced non-native species actually become pests (Lodge 1993; Williamson and Fitter 1996). Species that are introduced into new habitats encounter many abiotic and biotic barriers (Colautti et al. 2006). They have to be able to live in or adapt to the new habitats. Generally three determining stages are identified in invasion ecology: 1) colonization of the receiving

habitat, 2) establishment in the receiving habitat, 3) natural range expansion after establishment (Sakai et al. 2001). Common causes of failure to establish are: an unsuitable climate, disturbance, predation, competition and disease. Species that do manage to establish themselves face many different interactions with native species in the new community (Lodge 1993; Sakai et al. 2001). Many attempts have been made to identify characteristics of species that allow predictions about their invasiveness (Lodge 1993; Williamson and Fitter 1996; Morton 1997; Kolar and Lodge 2001; Sakai et al. 2001). For successful establishment in the receiving ecosystem, other traits may be required than for successful colonization and natural range expansion (Sakai et al. 2001). Successful colonists are generally species with fast reproductive rates. They are characterized by fast growth rates, rapid sexual maturation and a high fecundity (Lodge 1993; Williamson and Fitter 1996; Morton 1997; Sakai et al. 2001) (Table 1.1).

Table 1.1. A selection of characteristics generally attributed to successful invaders, especially relevant for bivalve invaders and for the three principal stages from first colonization to natural range expansion (from Lodge 1993; Williamson and Fitter 1996; Morton 1997; Sakai et al. 2001; Marvier et al. 2004; Wallentinus and Nyberg 2007; and references therein).

Stage	**Trait**
Colonization	rapid growth
	rapid sexual maturation
	high fecundity
	ability to colonize wide range habitat types
	broad diet
	tolerance to wide range environmental conditions
	gregarious behaviour
	genetic variability & phenotypic plasticity
	ability to recolonize after population crash
Establishment	competitiveness
	lack of predators, parasites and diseases
	association with humans
	repeated introductions
	ecosystem engineering
	genetic variability & phenotypic plasticity
Natural range expansion	dispersability
	traits of successful colonists (see above)

These are traits of an *r*-selected life history strategy (*r*-selected species are opportunistic, adapted to unstable environments by investing in high numbers of

offspring and fast growth rates, whereas *K*-selected species are more adapted to stable environments by investing in the quality rather than the quantity of offspring, and a long life rather than high growth rates; Pianka 1970). Habitat generalists may also be more successful in colonizing (Marvier et al. 2004). Characteristics of habitat generalists include: broad tolerances for wide ranges of environmental conditions, the ability to occupy a wide range of habitat types, and a broad diet (Lodge 1993; Morton 1997; Sakai et al. 2001; Marvier et al. 2004). For successful establishment, other traits seem more important. Highly competitive species seem more successful in establishing, as well as species that are defended against predators or that lack predators in the receiving ecosystem (Lodge 1993; Sakai et al. 2001). An association with humans may result in repeated introductions, which may increase establishment opportunities (Lodge 1993; Sakai et al. 2001). Also a wide genetic variation and phenotypic plasticity may result in higher chances of establishing successfully, since this provides the invading species with a wider range of tools to adapt to a new environment (Sakai et al. 2001). Ecosystem engineers also seem successful invaders (Jones et al. 1994; Wallentinus and Nyberg 2007). They modify the habitat to their own requirements (Gutiérrez et al. 2003).

Life history theory predicts a trade-off between fast reproductive rates, that facilitate colonization, and competitive ability, that facilitates establishment (Pianka 1970). However, in some invaders both strategies are represented (Keddy et al. 1994). Blossey and Notzold (1995) suggested that invasive species that have been released from the pressure of diseases or predators in their native habitat, reallocate energy used for defence into reproduction and growth.

Once an invader is established, subsequent spread is related to the dispersability of the invader. For successful natural dispersion, again colonization capabilities and broad tolerances are considered useful characteristics (Sakai et al. 2001).

Many attempts have also been made to identify characteristics that determine the invasiveness of receiving communities. Despite conflicting evidence, disturbance is often considered such a characteristic (Lodge 1993; Occhipinti-Ambrogi and Savini 2003; Marvier et al. 2004). Evidence from palaeobiological reconstructions, from impacts of invaders on islands and in brackish waters, and from modelling exercises suggests that species-poor communities are more susceptible to invasions than species-rich and more saturated communities (Wolff 1973; Lodge 1993; Wolff 1999). In the marine environment, estuaries seem to be common sites of invasions worldwide (e.g. Filice 1958; Wolff 1973; Cohen and Carlton 1998; Smith et al. 1999; Wolff 1999; Wonham and Carlton 2005; Nehring 2006). In the North Sea, numbers of non-indigenous species increase from open sea towards the coast, reaching a

maximum in the brackish reaches of estuaries (5 – 20 psu; 20% of all species non-indigenous) and decreasing again land inward (Reise et al. 1999; Wolff 1999; Nehring 2006). Possible explanations for this phenomenon were investigated in Dutch estuaries by Wolff (1973, 1999) and in German estuaries by Nehring (2006). They concluded that the following explanations may have played a part in enhancing the number of exotic brackish-water species in NW European estuaries: 1) most ports are situated in brackish regions, giving brackish-water species a better chance of being transported; 2) brackish-water species have a better chance of surviving transport in ballast water because they are more tolerant to conditions in ballast water tanks; 3) because the species number in brackish water is relatively low, it is easier for an introduced species to establish itself; 4) salt-tolerant limnetic alien species introduced into inland water reach the coast at first in the estuaries. Of the 80 non-indigenous species that were assumed by Reise et al. in 1999 to have been established in the North Sea, 22 occur preferentially in brackish waters. This is likely an underestimation of the actual number of introductions (Reise et al. 1999), as mentioned in the previous section. Furthermore, a lack of predators in the receiving community is often suggested as a reason for fast proliferation of introduced non-indigenous species ('enemy release' hypothesis: Williamson and Fitter 1996; Keane and Crawley 2002; Liu and Stiling 2006).

Despite all effort and some successes in finding general characteristics, all generalizations are characterized by a large variance and many exceptions (Lodge 1993). Different characteristics will be important in different habitats (Williamson 1996). It is therefore difficult (and according to some: impossible) to predict the outcome of an introduction (Lodge 1993). It is easier to describe invasions and to find explanations for specific invasions, than to predict future invasions (Williamson 2006).

1.2.4. *Crassostrea gigas* as an invader

By 2005, the Netherlands counted at least 112 non-indigenous marine and estuarine species (Wolff 2005). At present the Pacific oyster *C. gigas* (Box 1.2) is one of the best known non-indigenous animals in the Netherlands. While some consider it an enrichment of Dutch estuarine biodiversity (Cadée 2007), it is considered a nuisance by many. Its dense beds change the appearance and structure of tidal flats as people knew them, the razor sharp edges of oysters growing in an upright position in intertidal oyster beds sometimes cause severe injuries among wind surfers and scuba divers (Anonymous 2002; Smaal et al. 2006; pers. comm. Marnix Poelman), and commercial mussel stocks and culture plots are fouled by spat of *C. gigas* (Smaal and

BOX 1.2.
Biology of *Crassostrea gigas*

Pacific oysters are lamellibranch suspension-feeding bivalves of the class Pelecypoda. They live attached to hard substrates along exposed shores and form reef structures on tidal flats (Arakawa, 1990; Reise, 1998; Dupuy et al., 1999).

In NW European estuaries, colonization of tidal flats generally starts with few individuals colonizing pieces of hard substrate such as shell fragments or stones, or with colonization of mussel beds (Reise 1998; Diederich 2005a; Nehls et al. 2006). As the density of the oysters increases, oysters settle onto each other, forming aggregations, until eventually larger patches and entire reefs of upright oriented oysters are formed (Figure B1.2.1.; Diederich et al. 2005). Pacific oysters are oviparous; in the northern hemisphere they release their gametes into the water mainly in July and August, when water temperatures are highest. After a pelagic phase of about 3 weeks (Box 1.5), the veliger larvae settle onto hard substrate: rock, stones, or pieces of other hard substrate. After settlement, their lower (left) cupped valve becomes partially or almost completely cemented to the substrate. By doing so, generally the shell assumes the form of the substrate to which it attaches (Arakawa 1990a; Reise 1998; Gosling 2003). With a maximum shell length of about 30 cm, the Pacific oyster is the largest bivalve in Dutch waters since the near-disappearance of *O. edulis*. Pacific oysters feed by filtering planktonic organisms and detritus from the surrounding water. Relative to other bivalve species in Dutch estuaries they process large volumes of water per time unit (Box 1.3).

Figure B1.2.1. Left: Oyster bed in the Oosterschelde estuary (Neeltje Jans); Right: aggregation of oysters settled onto each other.

Lucas 2000). Moreover, regarding dramatic negative impacts by introduced species worldwide, considering that native bivalve filter-feeders largely utilize the same food source and habitat as *C. gigas*, and considering the large filtration capacity of *C. gigas* (Box 1.3), Pacific oysters may have the potential to out-compete and replace native bivalves.

1.3. Introduction of *C. gigas* in the Netherlands and neighbouring countries

1.3.1. Short description of Dutch estuaries

The Netherlands count two estuarine areas: the Dutch part of the Wadden Sea in the north, fed by the rivers Ems and Rhine (the latter through the rivers IJssel and Utrechtse Vecht, through Lake IJsselmeer) and the Delta area in the south-west where the rivers Scheldt, Rhine and Meuse reach the North Sea (Figure 1.2). From south to north, the Delta area consists of the Westerschelde estuary, Lake Veerse Meer, the Oosterschelde estuary, Lake Grevelingenmeer and Lake Haringvliet. The Westerschelde estuary is the mouth of the river Scheldt, still in open connection with the North Sea. The other water bodies used to drain the rivers Rhine, Meuse and also the Scheldt, but were partially closed off from river inputs and the North Sea by a coastal engineering project, the 'Delta' project. The Haringvliet, nowadays containing freshwater, was closed off from the North Sea by a dam and sluices, but still discharges water from the rivers Rhine and Meuse. The Grevelingen estuary was dammed off from the North Sea and from river inputs, resulting in a brackish Lake Grevelingenmeer. Lake Veerse Meer is also a brackish lake since it was dammed off from the Oosterschelde estuary in the east and the North Sea in the west. Since 2004 a sluice in the Zandkreek dam, separating the lake from the Oosterschelde estuary, allows a tidal exchange and a moderate tidal amplitude (Escaravage et al. 2006). The Oosterschelde estuary was largely closed off from river inputs by compartmentalization dams and locks. A storm surge barrier (Box 1.4), which can be closed in times of dangerously high water levels during storm surges, was built in the mouth of the estuary. This reduced the tidal amplitude and current velocities in the estuary.

The Wadden Sea and the Oosterschelde and Westerschelde estuaries are characterized by a relatively large tidal amplitude of about 1.5 – 3.0 m in the Wadden Sea and about 3.0 – 4.5 m in the Oosterschelde and Westerschelde estuaries. These tidally driven

BOX 1.3.
Filter-feeding in bivalves

Bivalve filter-feeders (also called 'suspension-feeders') collect their food by filtering and sorting particles from the water column. Their ciliated gills create a water current through the mantle cavity and over the gills. Particles above a certain threshold size, generally 2 – 7 µm (Møhlenberg and Riisgård 1978), are retained efficiently. Different types of particles can serve as food for bivalves: phytoplankton, zooplankton, protists and dead particulate organic matter (Fréchette et al. 1989; Smaal 1997; Dupuy et al. 1999; Riera et al. 2002; Wong and Levinton 2006). Selection of particles for ingestion takes place on the gills and labial palps (Figure B1.3.1.; Shumway et al. 1985; Ward et al. 1998). Particles retained on the gills are transported towards the labial palps through ciliary movement. At the labial palps, rejected particles are covered in mucus and excreted as pseudofaeces. Particles selected for ingestion move into the stomach through the mouth (Gosling 2003). Post-ingestive selection of particles for absorption occurs in the stomach and guts (Brillant and MacDonald 2002).

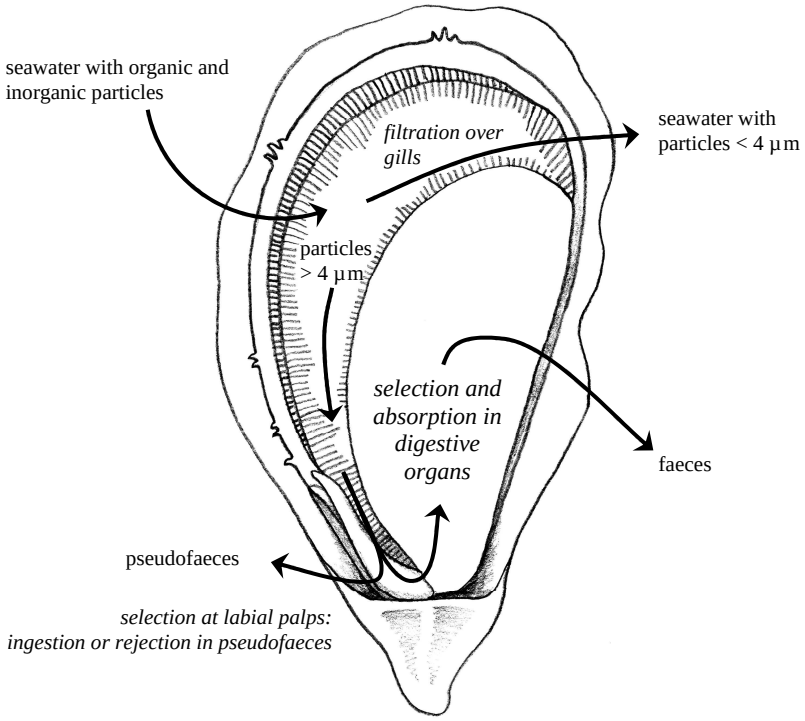


Figure B1.3.1. Schematic drawing of filtration, selection, ingestion and digestion of food particles in bivalves (example: *C. gigas*).

Filtration rates are generally measured as clearance rates. By filtering water over their gills, bivalve filter-feeders clear the water from particles. Whereas the filtration rate is defined as the volume of water pumped, clearance rate is defined as the volume of water cleared of all particles per time unit (Riisgård 2001). Hence, the clearance rate is only equal to the filtration rate if the particles offered are large enough to be retained with 100% efficiency (Riisgård 2001). For example, if a bivalve filters 4 litres of water in one hour, and thereby retains 50% of a certain type of particles, the filtration rate is 4 l h^{-1} but the clearance rate of that type of particles is 2 l h^{-1}. In most cases, when clearance rates are given in literature, clearance of neutrally buoyant particles retained with 100% efficiency, and thus filtration rates, are meant.

Bivalve filter-feeders, and particularly *C. gigas*, can process large volumes of water (Table B1.3.1). Clearance rates in *C. gigas* can be as high as 12.5 l h^{-1} per individual (Walne 1972). Clearance rates vary with many environmental parameters such as temperature (Bougrier et al. 1995), seston (particulate matter) concentration (Rueda and Smaal 2002; Riisgård et al. 2003) and composition (Pouvreau et al. 2000; Hawkins et al. 2001; Rueda and Smaal 2002), and current velocity (Newell et al. 2001). Pacific oysters may even adjust their retention threshold from about 4 to 12 μm in response to a high seston load (Barillé et al. 1993). At high seston loads *C. gigas* will therefore not be able to feed on all phytoplankton cells smaller than 12 μm, but it also prevents clogging of the gills (Barillé et al. 1993).

estuaries are turbulent and well-mixed. On the extensive muddy to sandy tidal flats benthic biomass is dominated by bivalve molluscs. These bivalves serve as an important food source for estuarine birds, and some are also exploited economically. The dominant species are the blue mussel *M. edulis*, the common cockle *Cerastoderma edule*, the Baltic tellin *Macoma balthica*, the soft-shelled clam *M. arenaria*, the American razor clam *Ensis directus* (introduced from North America around 1980, Wolff 2005) and recently also the Pacific oyster *C. gigas*. Before the introduction of *C. gigas*, *M. edulis* and the native European flat oyster *O. edulis* were the only epifaunal bivalves. The latter disappeared almost completely during the 20th century (Drinkwaard 1999a).

Bivalve shellfish in Dutch estuaries are heavily predated by estuarine birds. These feed preferentially, and in some cases obligatory, on especially *M. edulis*, *M. balthica* and *C. edule* (Meire 1993; Zwarts and Wanink 1993; Van de Kam et al. 2004). The birds share this resource with mussel farmers and cockle dredgers (Dankers and Zuidema 1995; Kamermans and Smaal 2002). In the autumn and following spring, mussel farmers dredge young-of-the-year mussels ('seed mussels') in the Wadden Sea, and sow them onto culture plots in the Wadden Sea and Oosterschelde estuary. Cockles are dredged in the Oosterschelde and Westerschelde estuaries, and hand-raked in the

Wadden Sea, and are immediately sold for consumption (Dijkema 1997; Smaal and Lucas 2000).

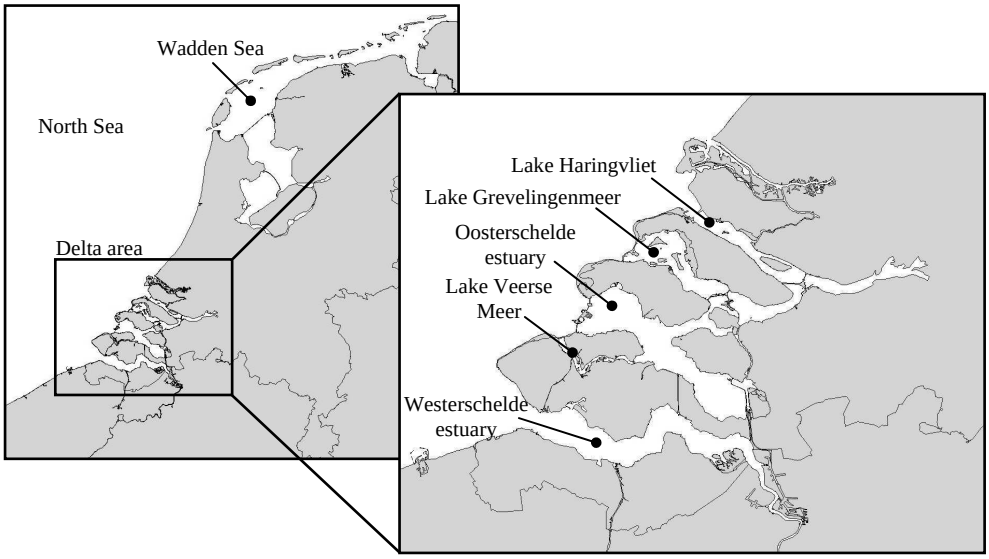


Figure 1.2. Situation of Dutch estuaries: the Wadden Sea in the north and the Delta area in the south, including the Oosterschelde estuary.

1.3.2. History of oyster culture in the Netherlands

Nowadays, *C. gigas* is the main oyster species cultured in the Netherlands. Traditionally however, from the 1870s until the 1970s, Dutch oyster culture concentrated on European flat oysters *O. edulis*. A combination of severe winters, mixing of native brood stocks with foreign strains and the accidental introduction of a parasite led to the downfall of the European oyster in Dutch waters (Drinkwaard 1999b). In the extremely severe winter of 1962 - 1963 the stock size of cultured European oysters in the Oosterschelde estuary decreased sharply from 120 million to about 4 million. In order to replenish *O. edulis* stocks, considerable amounts of spat and seed oysters were imported from France, Italy, Greece, England, Ireland and Norway until 1977. Then, a spatfall of a completely mixed brood stock suffered high mortality rates, probably due to the lower winter hardiness compared to the original stock (Drinkwaard 1999b). In 1980 the Bonamia parasite was accidentally introduced into the Oosterschelde

BOX 1.4.
The Oosterschelde storm surge barrier

In 1953 in the southern North Sea a northwesterly storm induced tides up to 3 meters above normal tidal levels. In the Delta area (Figure 1.2) 1,600 km² of polder land flooded and 1,836 people were killed. This was the incentive for a large-scale engineering project: the Delta project ('Deltawerken'). All main tidal estuaries except the Westerschelde estuary were closed from the North Sea and river inputs. According to original plans the Oosterschelde estuary should have been dammed off by 1978, which would have resulted in a stagnant lake. However, many people including shellfish farmers and scientists opposed to this plan. They wanted to retain the unique habitat and shellfish culture. As a consequence plans changed and a storm surge barrier (Figure B1.4.1) that allowed the tides to enter freely was constructed between 1976 and 1986. Compartmentalization dams had already been built to reduce the tidal volume and to close the estuary off from river inputs. Although the new design was less drastic, it still induced many changes that are still going on at present. Gullies are slowly filling up with sediments from tidal flats, causing erosion of tidal flats and a reduction of emersion time ('sand hunger'; Van Zanten and Adriaanse 2008). Hence, the habitat for intertidal bottom fauna is slowly disappearing, and with it food sources for estuarine birds. Erosion of tidal flats locally exposes deeper peat layers, potentially resulting in a reduced water clarity and primary production (Nienhuis and Smaal 1994a; Geurts van Kessel et al. 2003). The slow disappearance of tidal flats and even salt marshes will also result in an increased risk of dike bursts and flooding during storm surges, because the dikes become more exposed to wave action (Van Zanten and Adriaanse 2008).

Figure B1.4.1. The Oosterschelde storm surge barrier, viewed from the North Sea.

estuary with oyster imports from France (Drinkwaard 1999b; Haenen 2001). *Bonamia ostreae* is a protozoan that infects the granular blood cells of the flat oyster. With the introduction of *B. ostreae*, which caused high mortality rates, culture of European flat oysters in the Oosterschelde came to an end (Drinkwaard 1999b). Earlier, around 1940, flat oysters had also disappeared from the Dutch Wadden Sea due to habitat change and overfishing (Drinkwaard 1999a). Although Lake Grevelingenmeer is infested with *B. ostreae* since 1988 (Haenen 2001), still some flat oysters are being cultured here at present.

1.3.3. Culture of other oyster species after the decline of *Ostrea edulis*

Because of high mortalities among native oysters, farmers were searching for alternatives. In fact, before culture of *O. edulis* started in 1870, fishery of *O. edulis* already faced declining stocks due to over-exploitation of the wild beds. Hence, oysters from abroad had already been imported on occasion (Dijkema 1997). From the late 19th century until 1963, experiments were conducted with Portuguese oysters *C. angulata* (Korringa 1965). Although *C. angulata* built up gonads, temperatures in Dutch waters appeared too low for this species to release its gametes, resulting in a low quality of the oyster meat and unappreciated flavour (Korringa 1965). Some experiments were also conducted with the American Atlantic oyster, *C. virginica*, but this species never established itself in Dutch estuaries (Wolff and Reise 2002). In 1964, Dr. P. Korringa and J. Bol of the Netherlands Institute for Fisheries Research (RIVO, presently part of Wageningen IMARES), together with an oyster grower, imported spat of the Pacific oyster from British Columbia into the Oosterschelde estuary (Shatkin et al. 1997; Drinkwaard 1999b). The spat, settled onto empty shells, was placed in the water supply channel of a lobster storage park at Yerseke. The spat grew fast and were relocated after the first winter to an oyster culture plot of the RIVO in the eastern part of the Oosterschelde estuary, at the Yerseke Bank. More introductions followed. In 1966, oyster farmers were told that the introduction of the Pacific oyster was acceptable since water temperatures in the Netherlands were too low for this species to be able to reproduce, as had been the case with *C. angulata* (Dijkema 1997; Drinkwaard 1999b). Additionally, plans for closing off the Oosterschelde estuary from the North Sea had already been made. According to plan, this would have resulted in a fresh or brackish tide-free lake, unsuitable for oyster growth and reproduction. But plans were changed and the Oosterschelde estuary remained a marine tidal system (Smies and Huiskes 1981). A few years after oyster farmers had been told that Pacific oysters were not able to reproduce in the

Netherlands, natural spatfall was observed (see Box 1.5. for the bivalve life cycle). In 1971, young *C. gigas* of approximately one year old were collected from the harbour of Zierikzee by F. Kerckhof (in prep.). In 1975, Pacific oyster spat were observed to have settled onto mussel shells that were laid out as collectors for *O. edulis* spat, and also onto some intertidal mussel beds. In 1976 and 1982 extensive spatfalls were observed, which were attributed to prolonged periods of high water temperatures. From then on, most oyster farmers started to culture *C. gigas* (Drinkwaard 1999b).

1.3.4. Feral Pacific oysters in Dutch estuaries

Natural spatfall of *C. gigas* (Box 1.5) throughout the Oosterschelde estuary resulted in the formation of large and dense feral oyster reefs in the intertidal and subtidal areas. By means of stock assessments and reconstructions, the RIVO estimated that on the 118 km² of intertidal flats in the Oosterschelde estuary the cover by oyster beds increased from 0.25 km² in 1980 to 8.1 km² in 2003 (Kater and Baars 2004; Dankers et al. 2006). Oyster cover on hard substrates (160 km of dikes and sea walls, 2 - 4% of the total bottom surface area; Leewis et al. 1994) generally increased from 0 – 10% in 1985 to 50 - 60% in 2002, and even to 90% on some locations (AquaSense 2003). Within this period, during the 1990s, stocks of the native blue mussel *M. edulis* and common cockle *C. edule* showed a slight decrease (Geurts van Kessel et al. 2003; Dankers et al. 2006).

The introduction of *C. gigas* into the Wadden Sea goes back to the late 1970s. Bruins (1983) described finding *C. gigas* individuals attached to the dike near the outlet of the cooling water basin of a power and desalinization plant at Oudeschild, at the island of Texel, in 1983. In the cooling water basin itself he found larger specimens and estimated they were 6 – 7 years old. It is not clear whether these oysters originated from spat that was released there by someone in 1976 (Tydeman 2008), or from juveniles that were released there by the RIVO in 1978 (Smaal et al. in press). In 1978, the facilities of the RIVO at Texel were used for the culture of *O. edulis* spat from a French hatchery. The *O. edulis* spat was mixed with juvenile *C. gigas*, of which larger specimens were placed in the warmer cooling water basin (Smaal et al. in press). About every five years the cooling water basin was dredged, and apparently the mud was largely dumped directly in the Wadden Sea. If this mud contained oysters, the dredging may have contributed to the dispersal of *C. gigas* from the basin into the Wadden Sea (Tydeman 2008). Today, the oysters are still steadily spreading throughout the Dutch Wadden Sea where they locally cover dikes and tidal flats in high densities (Cadée 2001; Tydeman et al. 2002; Wolff 2005; Dankers et al. 2006). In

Lake Grevelingenmeer a first natural spatfall of *C. gigas* was observed in 1987 (Drinkwaard, 1999) and the Pacific oyster is now one of the dominant species (Sistermans et al. 2005). In the Westerschelde estuary, Pacific oysters were only found sporadically during the 1980s. During the early 1990s numbers increased slightly (Drinkwaard, 1999). These oysters may originate through natural dispersal from the brood stock in the Oosterschelde estuary, or from an experimental nursery facility at Ostend, Belgium (Drinkwaard 1999b). At the beginning of the 21st century, still no Pacific oysters are observed on the tidal flats of the Westerschelde estuary although they are now rather common on dikes and jetties where they seem to remain quite small (pers. obs.; pers. comm. J.Kesteloo-Hendrikse).

1.3.5. Introduction and spread of Pacific oysters in Germany

Pacific oysters were introduced in many countries worldwide (Box 1.1), and are also spreading throughout German (Reise 1998; Diederich et al. 2005; Nehls et al. 2006) and Danish parts (Diederich et al. 2005) of the Wadden Sea (Figure 1.3). The western part of the German Wadden Sea, the East Frisian Wadden Sea, has been systematically searched for wild *C. gigas* on mussel beds since 1996 (Wehrmann et al. 2000). Here, the first naturally dispersed oysters were detected in 1998 (Figure 1.3). Although an experimental culture plot for *C. gigas* had existed for one farming season in 1987 near the island of Norderney, this was considered an unlikely source for the observed wild *C. gigas*. Instead, the East Frisian Wadden Sea was probably invaded from the Dutch Wadden Sea (Wehrmann et al. 2000). The northern part of the German Wadden Sea, the Wadden Sea of Schleswig-Holstein, was colonized from an oyster culture site at the northernmost German island of Sylt (Reise 1998; Wehrmann et al. 2000). Here, *C. gigas* spat from a Scottish hatchery were imported for the first time in 1971 and again in 1972 for a raft culture experiment (Drinkwaard 1999b). At the end of the 1970s, outdoor experiments on growth and fattening and indoor experiments on rearing of spat from larvae were continued (Drinkwaard 1999b). At Sylt, regular oyster culture on trestles started in 1986 (Reise 1998; Drinkwaard 1999b). The first *C. gigas* individual outside the culture plot at the island of Sylt was observed in 1991 in the Königshafen Bay (Reise 1998; Drinkwaard 1999b). Since 1995, *C. gigas* is also found on mussel beds near the island of Amrum, south of Sylt, which became the second centre of oyster distribution in the area (Nehls et al. 2006). Since 2000, the abundance of *C. gigas* in the Wadden Sea of Schleswig-Holstein increased markedly. In Schleswig-Holstein, as in

BOX 1.5.
Bivalve life cycle

The life cycle of most intertidal bivalves includes a planktonic larval stage and benthic juvenile and adult stages (Thorson 1950) (Figure B1.5.1). Triggered by an environmental cue, bivalve filter-feeders release eggs and sperm into the water column where fertilisation takes place. The gametes have a limited life span and diffuse easily in dynamic systems (Bayne 1976). Therefore, to ensure fertilisation success many bivalve species and other benthic invertebrates spawn synchronously and live in dense aggregations (Levitan 1995; Claereboudt 1999; Luttikhuizen et al. 2004; Levitan 2006).

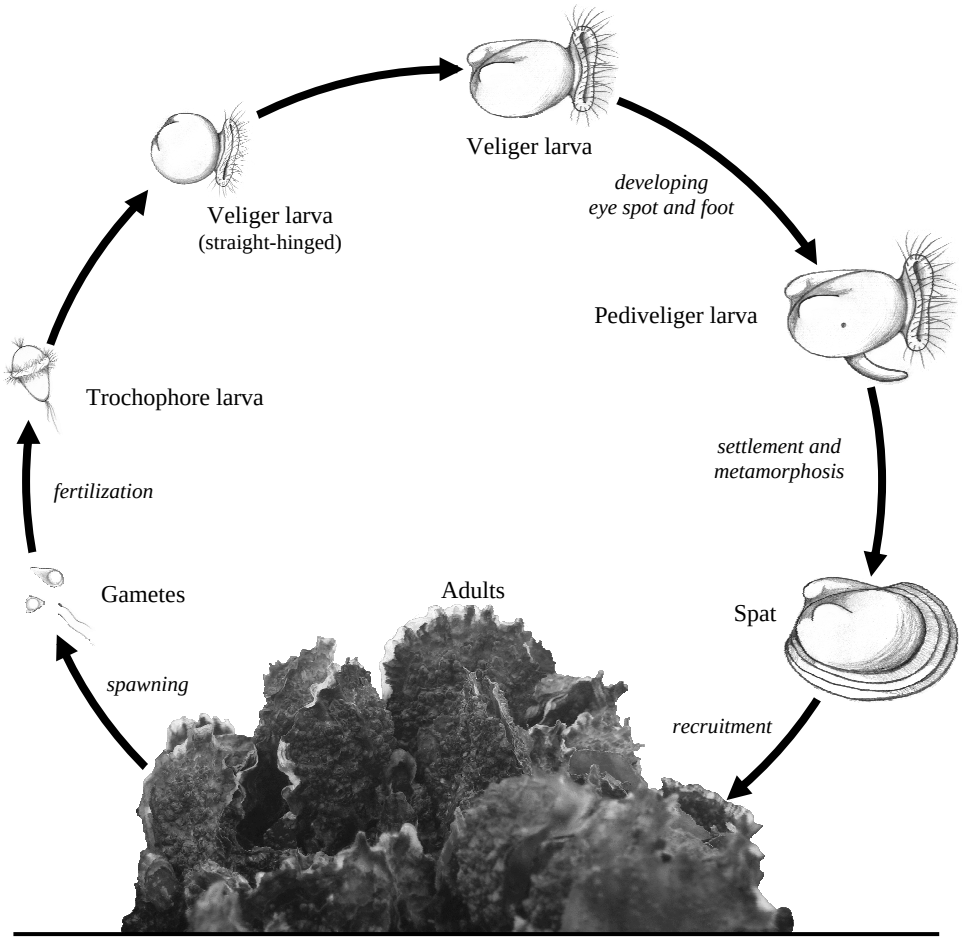


Figure B1.5.1. Bivalve life cycle (*C. gigas* as example; not drawn to scale; references in text).

Spawning is often triggered by environmental cycles that occur with regularity, such as lunar and tidal rhythms (Morgan 1995; Gosling 2003). Spawning may also be triggered by temperature or chemical cues (Gosling 2003; Helm et al. 2004). Fertilised eggs usually develop via the trochophore stage into veliger larvae within approximately 2 days (Gosling 2003). The veliger larvae, about 70 to 170 - 300 µm in length, swim and forage with their velum. The velum is a lobed structure carrying cilia on the outer margin (Strathmann and Leise 1979; Strathmann and Grünbaum 2006). The first veliger stage is called the D-veliger or straight-hinged veliger. In this stage the larvae shift from obtaining their nutrition from the egg yolk energy reserves to active foraging and feeding. The planktonic stage allows bivalves to disperse over large distances, but the larvae are subject to high rates of mortality (Pechenik 1999; Allen 2008). Most bivalve filter-feeders release large amounts of gametes resulting in high numbers of larvae (Thorson 1950). *O. edulis* breeds the fertilised eggs internally, in the mantle cavity (Gosling 2003; Helm et al. 2004), resulting in lower numbers of larvae but with a shorter pelagic stage. Veligers continue to develop through the veliconcha stage into the pediveliger stage, in which the larvae have developed a foot and eye spot (Gosling 2003). At this stage the velum begins to degenerate, resulting in reduced swimming abilities. The larvae are now competent to settle on a suitable substrate and to metamorphose into the benthic juvenile stage, approximately 3 weeks after fertilisation. If a suitable substrate is not found, larvae are able to postpone settlement and metamorphosis (Butman 1987; Gosling 2003). Larvae of many species may even re-enter the water column as post-larvae after metamorphosis, to migrate to a more suitable location (Bayne 1964a; Seed 1976). This strategy is commonly found in bivalve species that use specific nursery areas (Hiddink 2002, and references therein). In species such as *C. gigas* that attach themselves permanently to the substrate during metamorphosis (Seed 1976; Arakawa 1990b), secondary migration does not occur. Metamorphosis is a critical phase because a massive re-organisation of body parts takes place to adapt to a sessile existence (Gosling 2003). The benthic juveniles grow and recruit into sexually mature adults. During all benthic stages the bivalves are vulnerable to benthic predators; in early stages e.g. small crabs and shrimps (Jensen and Jensen 1985; Van der Veer et al. 1998; Hiddink et al. 2002) and in later stages e.g. crabs, starfish and birds (Meire 1993; Leonard et al. 1999; Diederich 2005b).

the East Frisian Wadden Sea and the Dutch Wadden Sea, *C. gigas* settles preferentially on mussel beds (Reise 1998), and by 2004 almost all mussel beds in the List tidal basin near Sylt had been colonized (Nehls et al. 2006). Densities of *C. gigas* on mussel beds in Schleswig-Holstein were on average 290 m^{-2}, up to a maximum of 600 m^{-2} (Nehls et al. 2006). Highest densities were found in the List tidal basin near Sylt (Diederich et al.

2005). From Sylt, the oysters also spread to the Danish Wadden Sea. The first wild oysters on the offshore German island of Helgoland were found in 2003 (Diederich et al. 2005).

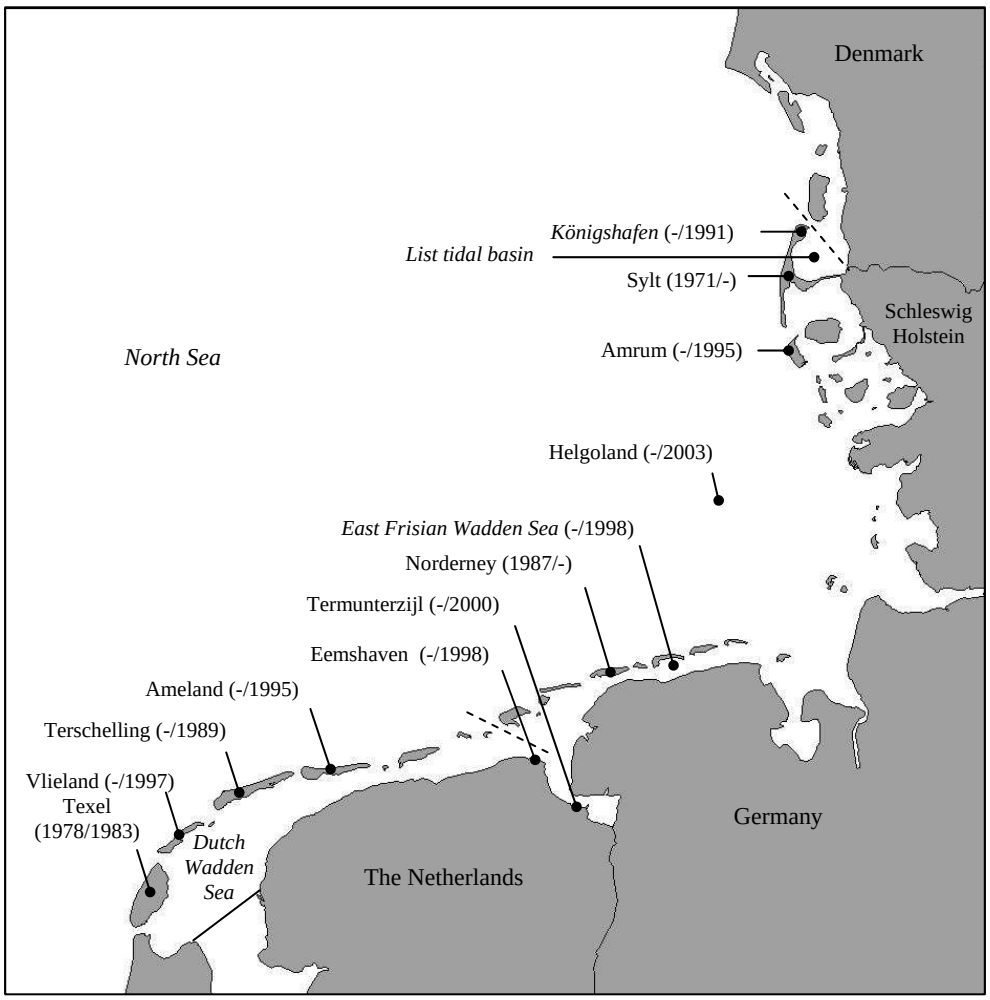


Figure 1.3. Locations where *C. gigas* was first introduced/encountered (years between brackets) in the Wadden Sea area (Bruins 1983; Reise 1998; Drinkwaard 1999b; Tydeman 1999; Wehrmann et al. 2000; Tydeman et al. 2002; Diederich et al. 2005; Wolff 2005; Nehls et al. 2006). Locations of islands and water bodies are given, not exact locations of introductions/encounters.

1.3.6. Introduction and spread of Pacific oysters in Belgium

In Belgium, oysters of different species and from different regions have been imported in the Sluice Dock of Ostend (Kerckhof et al. 2007). The Sluice Dock was used for cultivating and/or relaying oysters from the 1930s until World War II and again from 1957 to 1974. European flat oysters *O. edulis* were cultured here, but also *C. virginica* imported from the east coast of the United States and *C. angulata* imported from southern Europe (Kerckhof et al. 2007). Neither *C. virginica* nor *C. angulata* became established in Belgian coastal waters. In 1969 and the early 1970s, Pacific oysters *C. gigas* were imported. The first oysters originated from the Netherlands, but later imports were also made from Japan, Canada, France and the Mediterranean (Kerckhof et al. 2007; F. Kerckhof, pers. comm.). In 1974 oyster culture in the Sluice Dock stopped because of poor water quality. Although all imports and culture activities were stopped, *C. gigas* remained a resident of the Sluice Dock. Apparently this species was able to reproduce in Belgian waters (Kerckhof et al. 2007). Since the 1970s the Pacific oyster has colonized the Belgian coast, and now forms extensive reefs in the harbours of Ostend, Nieuwpoort, Zeebrugge and Blankenberge (Kerckhof et al. 2007). The species is regularly found living on piers and jetties and washed ashore (Kerckhof 1997; Jonckheere 2006). Especially the 1990s saw a rapid proliferation of the species in Belgian waters (F. Kerckhof, pers. comm.). Since 1996, oyster culture in the Sluice Dock has resumed. Next to the limited use of local brood stock, again *C. gigas* are imported from European countries and Canada (Kerckhof et al. 2007).

1.4. Causes of the Pacific oysters' success and consequences for native bivalves

This thesis focuses on causes of establishment and expansion of *C. gigas* in Dutch estuaries, and on potential consequences for native bivalve filter-feeders. Special attention is given to effects on and interactions with *M. edulis* that was the only creator of large biogenic structures on soft sediments until the invasion of *C. gigas*. Causes for invasion success are sought in characteristics generally attributed to successful invaders (Table 1.1). Some of these characteristics, particularly competitiveness, may lead to negative effects on reproduction, recruitment and growth of native bivalve filter-feeders.

Pathways according to which characteristics of *C. gigas* may affect native bivalves are shown in Figure 1.4. The figure is a schematic representation of the life cycle of bivalve filter-feeders in general, with the main factors affecting survival, growth and

mortality. As a starting point, the cycle represents the life cycle of *C. gigas*. Through responses to and effects on driving parameters (e.g. shelter, predation and food availability), *C. gigas* may affect different stages in its own life cycle and in the life cycles of native bivalve species. Summarized, the figure shows effects of different life stages of *C. gigas* on different life stages of any bivalve in Dutch estuaries.

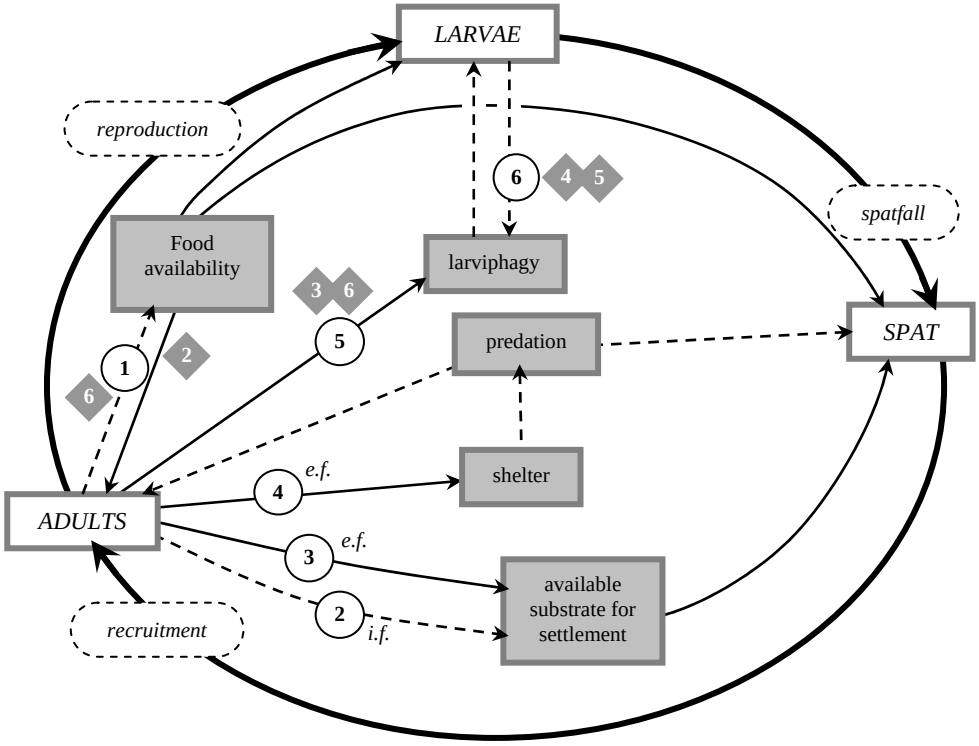


Figure 1.4. Schematic representation of the life cycle of bivalve filter-feeders in general, with the main factors affecting survival, growth and mortality. Effects of different life stages of *C. gigas* on different life stages of any bivalve in Dutch estuaries are indicated with solid arrows representing a positive effect (increase) and dashed arrows representing a negative effect (decrease). Numbers in circles refer to the explanatory text in section 1.5. Numbers in grey diamonds refer to the different chapters in this thesis. All factors and effects will be reviewed in Chapter 7. Where necessary a distinction is made in effects relevant to 'epifaunal bivalves' (*e.f.*) and 'infaunal bivalves' (*i.f.*).

Because of their relatively large filtration rate (Box 1.3), and an estimated overall increase in filtration pressure in the Oosterschelde estuary due to the increase in oyster stock (Geurts van Kessel et al. 2003), phytoplankton biomass and production and therefore food availability for bivalve filter-feeders and their larvae and spat may have

been reduced (1 in Figure 1.4). This may result in reduced reproductive output, reduced growth and survival of larvae, spat and adult bivalves, and eventually in a reduced stock size of bivalve species that are most vulnerable to low food levels. Possibly *C. gigas* is better equipped to deal with low food levels, and is therefore less affected by the reduced food levels that were caused by its own expansion.

Pacific oysters are ecosystem engineers. They build three-dimensional structures where previously only bare soft sediments existed. On the one hand expansion of these beds may reduce the area suitable for colonization by infaunal species, and may therefore reduce the spatfall and recruitment of these infaunal species (2 in Figure 1.4). On the other hand, expansion of these beds will increase the area suitable for epifaunal species of hard substrates such as the oyster itself, and thereby increase spatfall and recruitment success of these epifaunal species (3 in Figure 1.4). In oyster beds, the epifaunal bivalves *C. gigas* and *M. edulis* may find shelter from predators, thereby reducing mortality rates of spat and adults due to predation (4 in Figure 1.4).

Bivalve suspension-feeders may reduce bivalve larval abundance (including their own larvae) because of their large filtration capacity. Bivalve larvae may be filtered and ingested by adult bivalves ('larviphagy'), possibly resulting in a reduced spatfall and recruitment success (5 in Figure 1.4). The extent of this potential mortality factor may be influenced by capabilities of the larvae themselves to avoid or escape filtration (6 in Figure 1.4).

Additionally, some characteristics of successful invaders as listed in Table 1.1 may offer an indirect competitive advantage. For instance, a lack of natural enemies may result in a faster population increase relative to native species.

1.5. Research questions & Outline of this thesis

This thesis focuses on two main research questions:

1. *Causes of success*: What characteristics of *C. gigas* contributed to its fast colonization, successful establishment and rapid natural range expansion in Dutch estuaries?
2. *Consequences for native bivalves*: What characteristics of *C. gigas* affect native bivalves negatively, potentially leading to their decline?

In Chapter 2, possible advantages of *C. gigas* in food intake in comparison to the native *M. edulis* and *C. edule* are explored. Potential differences in individual feeding current characteristics of the three species, which may ultimately result in a differential food intake, were studied. Similar feeding current characteristics would imply similar

food intake rates at similar near-bed food concentrations, and a similar filtration rate of zooplankton species. In addition, similar exhalant jet speeds would imply a similar influence on near-bed turbulence levels, and therefore a similar influence on food flux towards the bivalves based on filtration activity alone and disregarding effects of bed roughness.

An increase in total filtration pressure as a consequence of an increasing oyster stock in the Oosterschelde estuary may result in a reduction of bivalve larval abundance through larviphagy (after Timko 1979). Chapter 3 investigates whether larvae of *C. gigas* and *M. edulis* are filtered by adult bivalves, and whether larvae of *C. gigas* are less susceptible to larviphagy than larvae of the native *M. edulis*. This would imply a reduced effect of an increased filtration pressure on the oyster stock compared to native stocks, which may have contributed to the fast natural range expansion of *C. gigas* while stocks of native bivalves remained more or less the same. A clearance rate approach was used to determine filtration rates of *C. gigas* and *M. edulis* larvae by adult bivalves in a laboratory set-up. Additionally, the fate of filtered *C. gigas* and *M. edulis* larvae was investigated. Adult bivalves were fed larvae of *C. gigas* and *M. edulis* and their stomach contents and pseudofaeces were subsequently analyzed to investigate whether filtered larvae may be used as a food source.

Because Chapter 3 shows that larvae of *C. gigas* are filtered less by adult bivalves than larvae of *M. edulis*, Chapter 4 investigates whether *C. gigas* larvae are better able to detect and/or escape inhalant feeding currents of adult bivalves, than *M. edulis* larvae. Escape responses of *C. gigas* and *M. edulis* larvae were studied in an artificial flow field simulating a bivalve feeding current. Differences in swimming speeds were studied as well. If larvae of both species are able to react to fluid disturbances, higher swimming speeds would enable oyster larvae to escape faster and therefore more successfully. Additionally, regardless of an ability to react to fluid disturbances, higher swimming speeds and faster vertical displacement may increase survival chances in general.

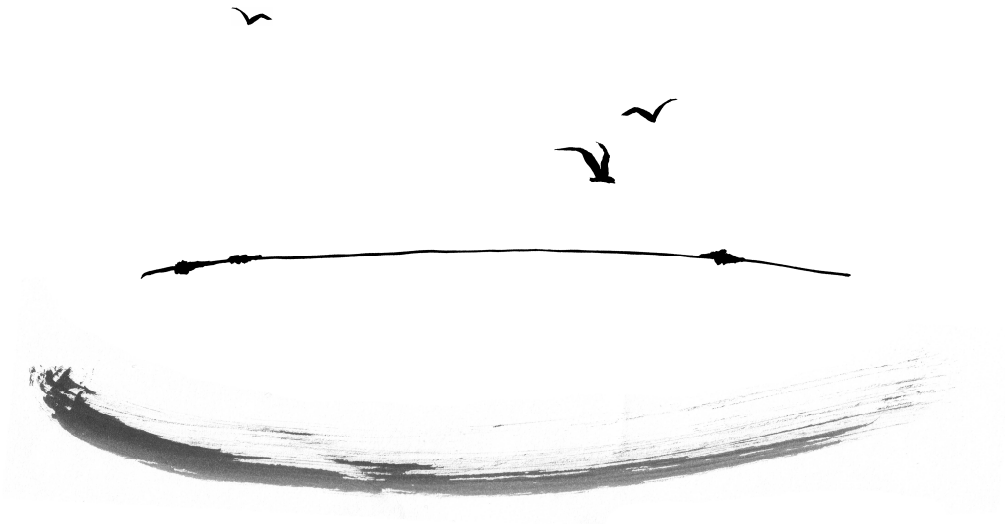
Chapter 5 investigates whether Pacific oyster larvae are filtered less than larvae of *M. edulis* by migrating upwards in the water column in response to the presence of adult bivalves. If such a response is present in *C. gigas* larvae, but not in *M. edulis* larvae, this may explain why Pacific oyster larvae were less susceptible to larviphagy in Chapter 3.

The aim in Chapter 6 was to find field-evidence for effects of larviphagy on the abundance of bivalve larvae in the Oosterschelde estuary. The potential impact of

larviphagy was studied on three scales. First, literature evidence on larviphagy in individual *C. gigas*, *M. edulis* and *C. edule* was confirmed for the Oosterschelde estuary by field sampling. Second, local effects of a dense bed of filter-feeding bivalves (*C. gigas*) on bivalve larval abundance in the overlying water column were studied. Third, existing time-series of larval abundance of *C. gigas* and *M. edulis* in the Oosterschelde estuary were related to the increase in total filter-feeder stock and filtration pressure caused by the increasing stock of *C. gigas*. The magnitude of mortality among bivalve larvae due to larviphagy in the estuary was estimated using a mathematical model. The assumption that larvae are distributed homogeneously over the water column was tested using existing results of mussel larvae monitoring in near-surface and near-bottom water samples.

Chapter 7 is a review of the causes of the Pacific oyster's success in Dutch estuaries and of the potential consequences for native bivalves. First, characteristics of *C. gigas* are discussed that may have contributed to its successful establishment and natural range expansion throughout the Oosterschelde estuary and to other estuaries, after its initial introduction for culture. Second, comparisons between *C. gigas* and native bivalves are made. Competition for food and space as well as effects of larviphagy (chapters 2 to 6) are discussed. Potential effects of *C. gigas* on native bivalve filter-feeders, and other species, are evaluated. Finally, some management aspects are discussed.

Chapter 2



Abstract
Introduced Pacific oysters (*Crassostrea gigas*) have shown rapid expansion in the Oosterschelde estuary, while stocks of native bivalves declined slightly or remained stable. This indicates that they might have an advantage over native bivalve filter-feeders. Hence, at the scale of individual bivalves, we studied whether this advantage occurs in optimizing food intake over native bivalves. We investigated feeding current characteristics, in which potential differences may ultimately lead to a differential food intake. We compared feeding currents of the invasive epibenthic non-siphonate Pacific oyster to those of two native bivalve suspension feeders: the epibenthic siphonate blue mussel *Mytilus edulis* and the endobenthic siphonate common cockle *Cerastoderma edule*. Inhalant flow fields were studied empirically using digital particle image velocimetry and particle tracking velocimetry. Exhalant jet speeds were modelled for a range of exhalant aperture cross-sectional areas as determined in the laboratory and a range of filtration rates derived from literature. Significant differences were found in inhalant and exhalant current velocities and properties of the inhalant flow field (acceleration and distance of influence). At comparable body weight, inhalant current velocities were lower in *C. gigas* than in the other species. Modelled exhalant jets were higher in *C. gigas*, but oriented horizontally instead of vertically as in the other species. Despite these significant differences and apparent morphological differences between the three species, absolute differences in feeding current characteristics were small and are not expected to lead to significant differences in feeding efficiency.

Feeding current characteristics of three morphologically different bivalve suspension feeders, *Crassostrea gigas*, *Mytilus edulis* and *Cerastoderma edule*, in relation to food competition

Karin Troost, Luca A. van Duren, Eize J. Stamhuis, Wim J. Wolff

2.1. Introduction

2.1.1. Introduced oysters

Since their initial introduction in the Oosterschelde estuary (SW Netherlands) in 1964 (Drinkwaard 1999b), Pacific oysters *Crassostrea gigas* (Thunberg) have been spreading rapidly, forming large and dense oyster reefs in the intertidal and subtidal (Drinkwaard 1999a; Wolff and Reise 2002; Dankers et al. 2006). While the Pacific oyster stock in the Oosterschelde estuary was expanding, stocks of the most common native bivalves, the blue mussel *Mytilus edulis* L. and the edible cockle *Cerastoderma edule* (L.) were slightly declining or stable (Geurts van Kessel et al. 2003; Dankers et al. 2006; Chapter 6). This suggests an advantage of *C. gigas* over native bivalve filter-feeders. One possible advantage may be found in differences in food intake, caused by a combination of differences in filtration rate and different feeding current characteristics due to differences in morphology.

2.1.2. Morphology and living habits

In our study area the blue mussel, *M. edulis*, is an epifaunal species living in large beds on hard and soft bottoms both intertidally and subtidally. *M. edulis* circulates water for filtration and respiration through its mantle cavity via in- and exhalant siphons. These siphons are extendible up to a few millimetres. The inhalant siphon is continuous along the entire length of the ventral to posterior edge of the shell and the exhalant siphon is small and conical (Bayne 1976; Gosling 2003). Pacific oysters are epifaunal, and live in beds on hard and soft bottoms both in the intertidal and subtidal. They

inhale water through the gape between both mantle folds and the exhalant opening is small relative to the inhalant opening (Gosling 2003). The cockle *C. edule* is an infaunal species living buried in soft sediments both in the intertidal and subtidal. It inhales and exhales water through clearly separated posterior siphons of comparable size that extend several millimetres beyond the margin of the shell (Gosling 2003). When buried the tips of the siphons are usually flush with the sediment, so in the field this species causes very little additional topographic roughness to the sediment surface.

2.1.3. Feeding currents and food intake

Food intake is for a large part determined by filtration rate, but not entirely. Food intake may for instance be reduced by refiltration of already filtered water. Filtration rates have been extensively studied in many bivalves, including *C. gigas* (Walne 1972; Gerdes 1983b; Bougrier et al. 1995; Dupuy et al. 2000), *M. edulis* (Walne 1972; Winter 1973; Foster-Smith 1975; Riisgård 1977; Møhlenberg and Riisgård 1979; Famme et al. 1986; Prins et al. 1996; Smaal and Twisk 1997; Petersen et al. 2004), and *C. edule* (Vahl 1972; Foster-Smith 1975; Møhlenberg and Riisgård 1979; Fernandes et al. 2007; Widdows and Navarro 2007). Filtration rates are in most cases determined by measuring clearance rates of particles that are retained 100% efficiently. Clearance rate is defined as the rate at which a bivalve clears a certain water volume of all suspended particles (Riisgård and Larsen 2000). Clearance rate measurements by different authors have yielded large differences that are related to differences in e.g. experimental set-up, environmental factors, food quantity and quality, and origin and history of the animals (Riisgård 2001). It is also important to distinguish between results obtained in experiments on actively filtering individuals and experiments on assemblages or even entire shellfish beds. Average clearance rates in a bed will generally be lower than individual clearance rates due to the facts that not all individuals may be active and that within a bed refiltration of previously filtered water can occur. Comparisons between species should therefore ideally be made in the same study. Møhlenberg and Riisgård (1979) compared 13 different species of bivalves, and showed that *C. edule* had higher clearance rates than *M. edulis* at comparable body weight. Walne (1972) compared five species of bivalves and showed that clearance rates of *C. gigas* were more than twice the clearance rates of *M. edulis* at comparable body weight. Based on clearance rates alone, food intake would thus be expected to be higher for *C. gigas* and *C. edule* than for *M. edulis*, at comparable body weight. Since *C. edule* generally has a lower body weight than *M. edulis* and *C. gigas*, and filtration rate is positively related to

body weight (Møhlenberg and Riisgård 1979), clearance rates per individual should generally be higher in *C. gigas* than in both *M. edulis* and *C. edule*.

Differences in inhalant feeding current characteristics may also result in differences in food intake. Higher inhalant current velocities will deflect passing larger particles (such as bivalve larvae, see Tamburri et al. 2007) more strongly towards the inhalant aperture, thereby increasing the intake rate of larger food particles. Larger food particles can be larger phytoplankton cells but also zooplankton individuals (Lehane and Davenport 2002; Wong and Levinton 2006; Maar et al. 2007). An ability of adult bivalves to utilize zooplankton as an additional food source may give them an advantage in food competition with species less able to feed on zooplankton (see Wong and Levinton 2004). Zooplankton species vary widely in swimming and escape capabilities (Singarajah 1969, 1975; Kiørboe and Visser 1999; Visser 2001). Higher and more strongly accelerating inhalant current velocities are likely to entrain more slow-swimming zooplankton species (Singarajah 1969), although bivalve predation on zooplankton species is also dependent on the sensitivity of the zooplankters to flow-field disturbances and their behavioural reaction to these hydromechanical stimuli (Kiørboe et al. 1999; Titelman and Kiørboe 2003). Inhalant flow fields that extend further into the water column allow for foraging in higher water levels, thereby increasing plankton intake rate (Fréchette et al. 1989).

The supply of phytoplankton and zooplankton to the bivalves is mediated by turbulent mixing of the water column. Turbulent mixing is caused by physical forcing of the system (e.g. tidal forcing). Near-bed turbulence is enhanced by roughness created by biogenic structures (Wright et al. 1997) such as beds of epifaunal bivalves (Butman et al. 1994; Nikora et al. 2002). Turbulence levels are also enhanced by biomixing through the feeding activity of the bivalves. The momentum of exhalant jets increases mixing inside and near the bed, thereby increasing the flux of phytoplankton towards the bivalves (Ertman and Jumars 1988; O'Riordan et al. 1995; Lassen et al. 2006; Van Duren et al. 2006; Fernandes et al. 2007). Turbulence levels near the bivalve bed may also affect the escape success of zooplankton (Maar et al. 2007). The 'background noise' caused by turbulence may interfere with the perception of the predator (bivalve) signal and thereby enhance the predation risk (Kiørboe et al. 1999). Zooplankton may respond to hydromechanical signals that are present in the inhalant and exhalant flow fields. Exhalant current velocities are generally higher and may present stronger stimuli for escape reactions, but for survival the response to inhalant current should be of more immediate concern.

2.1.4. Suspension-feeder – flow interactions

Ultimately the effect of the inhalant and exhalant currents on food intake of bivalves is a result of the interaction between the feeding currents and the overlying flow. In turn, the total effect of the presence of bivalves on transport of food from the water column towards the bed is a combination of the interactive effect of their feeding currents with the ambient flow and the interaction of biogenic roughness and ambient flow. For infaunal species such as cockles the latter effect is fairly minor and the filtration activity is important for increasing near-bed mixing and reduction of near-bed depletion (Fernandes et al. 2007). For epibenthic species, such as mussels and Pacific oysters, generally the mixing effect caused by the roughness of the shell aggregations has a more profound effect than the exhalent jets (Wiles et al. 2006), although in some situations, e.g. at low ambient flow conditions, the jets may still have a significant influence (Lassen et al. 2006; Van Duren et al. 2006).

Suspension-feeder – flow interactions in relation to food intake can be studied on different scales: on the scale of the individual, on patch or bed scale, and on estuary scale (see Nikora et al. 2002). At these different scales, different processes are relevant. For a complete understanding of how bivalve suspension feeders affect biotic and abiotic parameters and processes and how bivalves are in turn affected by these parameters (see Butman et al. 1994; Dame 1996; Wildish and Kristmanson 1997; Nikora et al. 2002; Porter et al. 2004; Van Duren et al. 2006), all scales should ideally be combined. In the present study, we considered one piece of the puzzle: the scale of individual bivalves.

2.1.5. Aim

Our aim was to study potential differences between feeding current characteristics of individually studied bivalves of three morphologically different species, invasive Pacific oysters *C. gigas* and native mussels *M. edulis* and cockles *C. edule*. These differences may ultimately result in a differential food intake between these species. The study consisted of two parts. First, we empirically studied characteristics of the inhalant flow field. Our null hypothesis was that inhalant feeding current velocities and the acceleration and distance of influence of the inhalant flow field in *M. edulis*, *C. edule* and *C. gigas* are the same (at comparable body weight or shell length). To test this, we analysed inhalant flow fields in still water using digital particle image velocimetry (DPIV) and particle tracking velocimetry (PTV). Velocity gradients and distances up

to which the flow fields influence the surrounding water were determined from the velocity profiles.

Second, we studied whether the three different species of bivalves affect the overlying water column differently with their exhalant jets. With these jets bivalves transfer momentum to the overlying water that may be converted into turbulent kinetic energy. Kinetic energy transfer is a product of the exhalant jet speed and the cross-sectional area of the exhalant aperture (Tritton 1988). Our aim was to explore the order of magnitude of differences in jet speeds between the three bivalve species. Experimental flow quantifying methods such as DPIV and PTV could not be used to study exhalant jet speeds since the bivalves cleared all particles from the water, resulting in an empty exhalant jet. We therefore chose a modelling approach to explore differences in exhalant jet speeds between the three species for a range of exhalant siphon cross-sectional areas and filtration rates. We used dimensions of the exhalant apertures measured by ourselves and filtration rates from literature as input. Implications of differences in exhalant jet speed and exhalant aperture cross-sectional area for kinetic energy transfer to overlying water layers are discussed.

2.2. Materials and methods

2.2.1. Experimental animals

Experimental animals were collected from the field. Per species, we collected individuals of different sizes. *C. gigas* were collected by hand from an intertidal oyster bed in the Oosterschelde estuary. Shell lengths ranged from 29 to 174 mm (0.04 – 1.10 g ash-free dry tissue weight). *M. edulis* were dredged from a subtidal bottom culture plot in the Oosterschelde estuary and ranged in shell length from 11 to 80 mm (0.02 – 1.21 g). *C. edule* were collected by hand from an intertidal mudflat in the Dutch Wadden Sea. They ranged in shell length from 20 to 32 mm (0.07 – 0.15 g). All collected animals were transported dry and cooled with ice-packs to the laboratory at Haren as soon as possible, within 24 hours. They were left to acclimate for 3 days in an aerated glass aquarium with running seawater of 18 °C and 30 psu. The animals were fed with the Instant Algae® Shellfish Diet® (Reed Mariculture Inc., Campbell, CA, USA), containing *Isochrysis* sp., *Tetraselmis* sp., *Pavlova* sp. and *Thalassiosira weissflogii*. We consulted Helm et al. (2004) and Reed Mariculture (www.reed-mariculture.com) to calculate food rations suitable for growth (2 g dry weight of Shellfish Diet® for every 100 g wet meat weight of bivalves per day).

2.2.2. Mapping flow fields

Inhalant flow fields of adult cockles, mussels and oysters were mapped using digital particle image velocimetry (DPIV; e.g. Stamhuis 2006). Per experiment, one animal that was seen to be filtering actively was transferred from the aquarium to a still-water tank (dimensions 40 x 40 x 50 cm), containing filtered seawater that had been well aerated for more than 1 hour in advance. All experimental animals were given the same amount of algae (Instant Algae® Shellfish Diet®) upon transfer to the experimental tank, to stimulate feeding. To visualise water movement generated by the bivalve, the water was seeded with neutrally buoyant synthetic white particles (Pliolyte, BASF, diam. 25-50 μm). By transmitting laser light through an optical fibre to a sheet probe, a vertical two-dimensional laser sheet (thickness 0.5 ± 0.2 mm) was projected in the still-water tank. Only particles in this 2D plane were illuminated. We used a CW Krypton laser (Coherent Innova K, Coherent Lasers Inc., Santa Clara, CA, USA; λ = 647 nm, P_{max} = 1 W) for illumination. A high resolution digital camera (Kodak MEGAPLUS ES 1.0, 30 fps at 1018 x 1008 px resolution) was mounted perpendicular to the illuminated plane. The camera was linked to a digital acquisition system. For calibration, a piece of plastic centimetre scale was placed in the focal plane next to the experimental animal. After recording a few frames it was removed before the actual experiment started. Analysis of the recorded images was performed with the DPIV software Swift 4.0 (developed at the University of Groningen). Successive filmed frames were analysed following Stamhuis (2006). We used interrogation areas (sub-images) of 65 x 65 pixels that overlapped by 50%, after image enhancement to remove unevenly lit backgrounds and with increased contrast. Displacement of the particle pattern in the interrogation areas was determined using 'convolution filtering', and the displacement peak was located using the 'centre of gravity weighed to grey value' (see Stamhuis 2006 for explanations, details and references). When light-coloured body parts of an animal caused diffusion and reflection of light, thereby possibly disturbing the DPIV analysis close to the animal (see Frank et al. 2008), the images to be analysed were treated in advance by masking the animal itself in Adobe® Photoshop®. Results of the DPIV analyses were exported to Microsoft® Excel© for further analysis.

Because suspension feeders have been reported to reduce their filtration rate in response to high particle loading (e.g. Foster-Smith 1975; Riisgård and Randløv 1981), we kept the seeding density as low as possible, without losing too much resolution in the DPIV analysis. In general, a DPIV interrogation area should contain 8 to 15 particles (Hinsch 1993). During the experiment, seeding particles had to be replenished regularly because the particles were filtered out by the bivalves.

Concentrations of seeding particles ranged approximately between 5 and 15 x 10^3 per ml.

2.2.3. Localizing inhalant apertures in oysters

To find the locations of strongest inhalant flow in *C. gigas*, the entire flow field of one oyster (78 mm shell length) was mapped using DPIV. Because shell edges of a Pacific oyster are highly irregular and undulating, it was not possible to map the entire inhalant flow field in one 2D plane. Therefore, multiple 2D maps were recorded, with the laser sheet projected at different locations parallel to the sagittal plane (Figure 2.1A). After analysing image pairs at these different locations, maximum velocity vectors were combined and the area of strongest inhalant current velocities determined. Further recording of inhalant currents of *C. gigas* focused on this area.

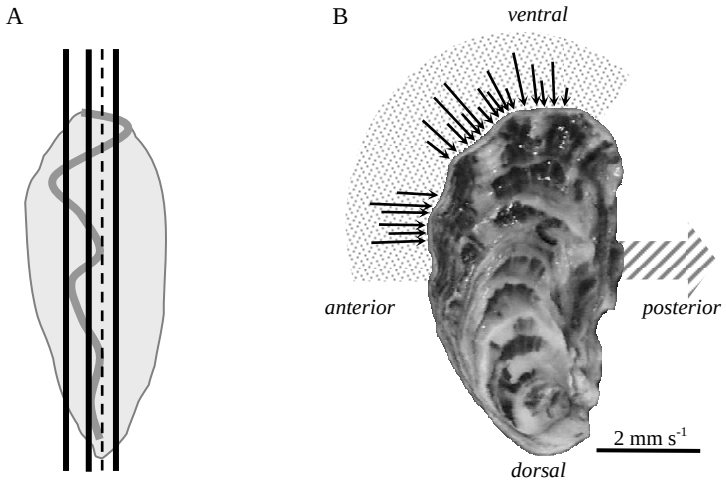


Figure 2.1. Indication of the different laser sheet projections (A) used to determine the area of in- and outflow in *C. gigas* (B). A: The frontal view of an oyster, showing both valves and undulating shell edges. The dashed line indicates the sagittal plane. Different projections of the laser-sheet, parallel to the sagittal plane, are indicated as black lines. B: Areas of in- and outflow in a Pacific oyster. The dotted area is the inflow area, and the hatched arrow indicates the location and direction of the exhalant flow. Arrows in the inflow area indicate inhalant current velocities determined in the DPIV analysis. The length of an arrow indicates its magnitude, according to the scale bar below.

2.2.4. Inhalant feeding currents

Recording of inhalant feeding currents started one hour after transferring an animal from its tank to the experimental still-water tank, provided with food (Instant Algae® Shellfish Diet®, approximately 2 x 10^4 - 4 x 10^4 cells ml^{-1}). White synthetic particles were added as soon as the animal was observed to be feeding in its new environment.

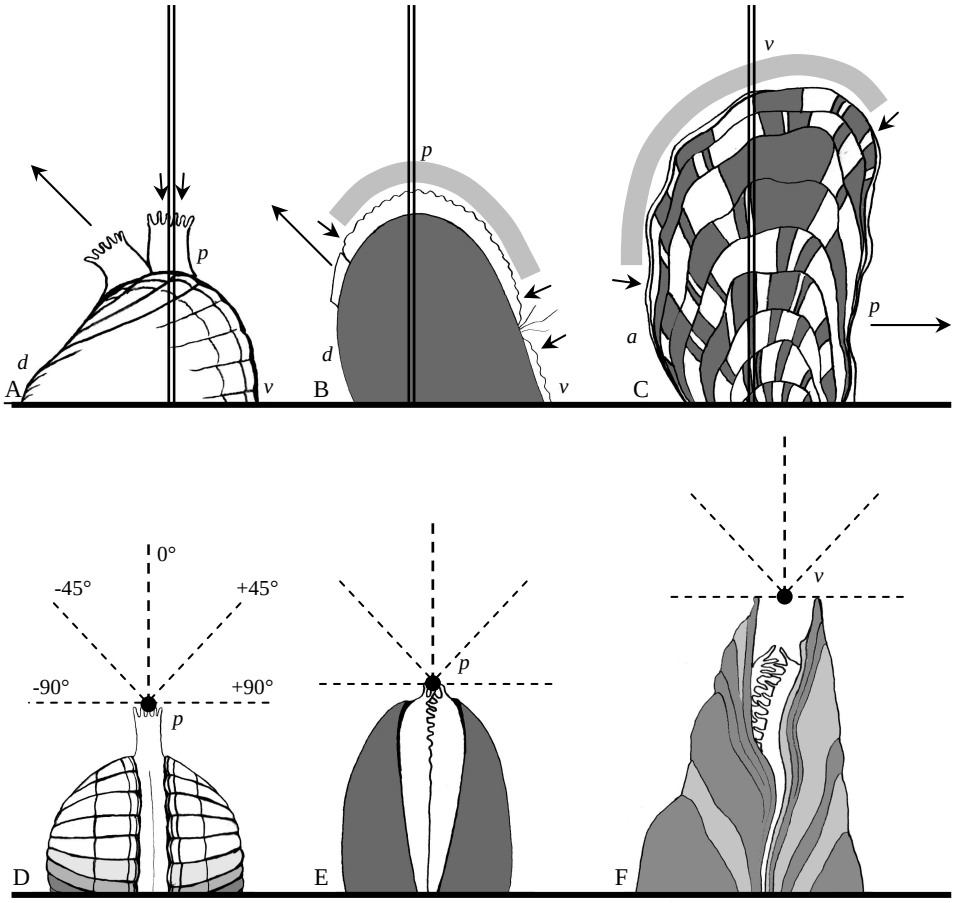


Figure 2.2. The orientation of the three species in the DPIV set-up, indicating the cross-sectional plane of the laser sheet: (A) *C. edule*, B) *M. edulis*, C) *C. gigas*) and the orientation of transects along which velocity gradients were studied: (D) *C. edule*, E) *M. edulis*, F) *C. gigas*). In Figures A-C, the inhalant areas are indicated with short arrows. A larger inflow area (B-C) is indicated as a grey area between these arrows. The exhalant jet is depicted as a long arrow. In Figures D-F, the point of inflow (distance = 0) is marked with a black dot. *d* = dorsal, *v* = ventral, *a* = anterior and *p* = posterior.

In order to determine maximum velocities at the inhalant apertures of the bivalves, the laser sheet was projected to cross-sect the plane between valves (the sagittal plane) at a location along the shell edge of interest for *C. gigas* and *M. edulis* and cross-secting the inhalant siphon in *C. edule* (Figure 2.2A-C). Cockles were placed upright in black grit, buried halfway. In a natural situation they would also be oriented upright, but buried completely. Mussels were placed upright, with the anterior end of the shell stuck loosely in a rubber ring that was buried in the grit (Figure 2.2B). Valve movement was unobstructed by this ring. The experimental position of the mussels roughly corresponded to the orientation of mussels in natural mussel beds with high densities. Mussels generally attach with their ventral surface to the substrate, or to each other in more crowded circumstances when they show a preference for an upright position with the anterior end pointed downward (Maas Geesteranus 1942). Small oysters (78 – 82 mm) were placed upright in the black grit (Figure 2.2C). This is a natural orientation for oysters in dense oyster beds. Larger oysters were placed horizontally, lying on their cupped valve. The dorsal end of the shell was resting on a mound of grit, thereby creating sufficient distance between the gape at the ventral end of the shell and the bottom of the tank to ensure an unobstructed flow field.
To facilitate a comparison between the three species, we determined inhalant current velocities at the entrance of the furthest protruding structure: in *C. gigas* at the shell entrance (the mantle entrance was not always visible and never protruded beyond the shell entrance), in *M. edulis* at the mantle entrance, and in *C. edule* at the siphon entrance (point of inflow, Figure 2.2D-F). The experimental animals were kept no longer than 4 hours in the set-up, including acclimation time, and were then returned to their tank with clean seawater (without synthetic particles) and algae.

Flow fields and inhalant current velocities were studied in 7 individual specimens for *C. gigas*, 7 for *M. edulis* and 8 for *C. edule*. Per individual, 5 to 10 sequences were recorded during periods of active pumping (when seeding particles were observed to be sucked in with relatively high speeds). In the DPIV analysis, one pair of filmed frames was analysed per sequence (selected visually). Overview flow fields were exported to a spreadsheet to analyse velocity profiles.

Because DPIV cannot resolve velocities closest to the animal (see Frank et al. 2008), inhalant feeding current velocities at the inhalant aperture were analysed in more detail using PTV. Single particles were tracked (using Didge© 2.3b1 by A.J. Cullum, Creighton University, Omaha, NE, USA) by manually pointing out corresponding particles in the same digital images series as used for the DPIV analysis. Changes in displacement in x and y direction were calculated, and from these, particle velocities that represent water current velocity. Per individual bivalve, five sequences

of ≥ 20 frames were analysed in Didge. Per sequence, at least five particles were tracked.

Upon completion of the experiments, the experimental animals were dried and incinerated to determine the ash-free dry weight of their flesh. The flesh was dried for three days at 70 °C and incinerated at 550 °C for four hours. Mean current velocities at the inhalant aperture were related to ash-free dry body weight per individual.

2.2.5. Velocity profiles

The DPIV overview flow fields were processed to velocity profiles. Inhalant current velocities were related to the distance from the point of inflow along transects in the laser plane that cross-sectioned the sagittal plane perpendicularly (Figure 2.2D-F). We compared velocity profiles from different directions towards the inhalant aperture, at angles of 0°, 45° and 90° relative to the sagittal plane of the bivalves (Figure 2.2D-F). In *C. edule*, the point of inflow (distance = 0 mm) was localised at the centre of the tip of the inhalant siphon, in *M. edulis* at the centre of the inhalant aperture, at the same height as up to where the mantle protruded, and in *C. gigas* at the centre of the inhalant aperture, at the same height as up to where the shell valves protruded (Figure 2.2D-F). All velocity profiles for each individual and each transect were processed to scatter graphs (Sigmaplot® 2001) and curves representing exponential decay with distance were fitted corresponding to the formula

$$v(r) = v_0 + ae^{-br} \qquad (2.1)$$

where v (in mm s^{-1}) is the incurrent velocity at distance r (in mm) from the point of inflow in the inhalant aperture, v_0 is the background velocity (that should be 0 mm s^{-1} in still water), and 'a' and 'b' are constants. Constant 'a' describes the maximum inflow velocity at r = 0 mm due to the pumping activity of the animal. Constant 'b' is the acceleration coefficient: it describes the slope of the curve, and thereby the acceleration of the inhalant feeding current towards the point of inflow. Acceleration coefficients were compared between species.

Feeding currents can be considered to influence the surrounding water up to the distance where v becomes v_0. Because of the asymptotical nature of Equation 2.1, v can never become v_0, but only approaches this value. According to Equation 2.1, if v approaches v_0, ae^{-br} approaches 0. We chose to calculate the distance of influence d_{infl} from ae^{-br} = 0.01 mm s^{-1}, and thus from the following formula (modified from Equation 2.1).

$$d_{infl} = - \ln(0.01/a) / b \qquad (2.2)$$

The distance of influence d_{infl} along the 0° transect parallel to the sagittal plane was calculated per individual, using the average 'a' and 'b' values of all analysed sequences, and compared between species.

The velocity profile of the half-buried *C. edule* in our set-up will be different from the field where cockles are generally buried completely in the sediment. We applied a mathematical correction to compensate for this difference. In half-buried *C. edule*, iso-velocity surfaces will be approximately sphere-shaped (with some interference from the animal itself), but for a completely buried cockle the iso-velocity surfaces would be in the shape of a hemisphere (see André et al. 1993). When comparing a sphere and a hemisphere with the same (filtered) volume, the radius of the hemisphere is 1.26 times larger than the radius of the full sphere (according to the formula to calculate the volume of a sphere: $^4/_3 \cdot \pi \cdot \text{radius}^3$). Since the inflow velocity remains the same, the distance of influence will be 1.26 times larger and the acceleration coefficient will be 1.26 times smaller if the cockles are buried. Hence, we multiplied both parameters with correction factors 1.26 and 1/1.26, respectively.

2.2.6. Exhalant jets

The location and direction of the exhalant jets were determined visually. This was facilitated by the efficient retention of the Pliolyte particles on the bivalve gills, resulting in an excurrent jet of particle-depleted water that was clearly visible in the particle seeded field. However, the empty exhalant jet did not allow for direct current-velocity analyses. High-velocity particles are visible just adjacent to the jet, but these are entrained particles, accelerated by the shear of the jet. Although exhalant jet speeds may be reconstructed using spline interpolation (Spedding and Rignot 1993; Stamhuis et al. 2002), this will introduce an additional error (see Frank et al. 2008 and note the in this context complicating bifurcated exhalant jet). Rather, we chose to estimate average exhalant jet speeds using a mathematical model. Average jet speeds (in cm s^{-1}) were calculated as Q/A_{exh} with Q being the volume flux (pumped volume of water; in cm^3 s^{-1}) and A_{exh} being the cross-sectional area of the exhalant aperture (in cm^2). For Q we used a range of filtration rates from literature. A_{exh} was determined from the frames that were recorded for the DPIV and PTV analyses by measuring the exhalant siphon diameters from dorsal and lateral recordings. During recording, additional images have been recorded that allowed for measurements of exhalant apertures and

shell gapes. In *C. edule*, A_{exh} was calculated as the surface area of a circle: as $\pi \cdot r^2$ with r being the radius of the opening of the extended exhalant siphon. In *M. edulis* and *C. gigas*, A_{exh} was calculated as an oval: as $\pi \cdot \frac{1}{2} d_1 \cdot \frac{1}{2} d_2$ with d_1 being the largest diameter (along the sagittal plane) and d_2 the smallest diameter (perpendicular to the sagittal plane). In *M. edulis*, these diameters were measured from dorsal recordings (camera flush with the sagittal plane) of upright mussels. In *C. gigas* the dimensions could not be measured directly since the exhalant aperture is located inside the shell. We assumed that the smallest diameter d_2 of the exhalant siphon was equal to the shell gape at the location of the exhalant aperture. The shell gape was determined from dorsal recordings (camera oriented flush with the sagittal plane). From lateral recordings with clearly distinguishable exhalant jets, the largest diameter d_1 of the exhalant aperture was estimated by measuring the width of the exhalant jet. Recorded sequences of three individual oysters were clear enough to allow for a reliable estimate of d_1. For these individuals, the ratio of d_1 to d_2 ranged from 1 to 2. Therefore, d_1 was assumed to range from d_2 to $2d_2$, and A_{exh} was thus assumed to lie between $\pi \cdot \frac{1}{2} d_2 \cdot \frac{1}{2} d_2$ and $\pi \cdot \frac{1}{2} d_2 \cdot d_2$. Jet speeds were modelled with A_{exh} values calculated with both formulas, as upper and lower limits. Jet speeds were modelled for a range of filtration rates and a range of siphon cross-sectional areas, since both are variable with body weight, trophic conditions and other parameters (Newell et al. 2001, and references therein).

2.2.7. Statistical analysis

Curve fitting and non-linear regression analysis were performed in Sigmaplot® 2001. All other statistical tests were performed in 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. Additionally, Levene's test for homogeneity of error variances was used. If the prerequisites were not met, the data were ln-transformed before testing. A significance level of $\alpha = 0.05$ was maintained. In testing differences between species with GLM in SPSS® 12.0.1. (aided by Norušis 2008), 'species' was always included as fixed factor, along with either 'shell length' or 'body weight' as covariate. Homogeneity of slopes was tested first, by including the effects of the fixed factor, the covariate, and the interaction term *fixed_factor*covariate* in the model. If slopes were equal (*fixed_factor*covariate*: $p > 0.05$), the full factorial model was tested to find differences between species (in intercepts). If significant differences in intercepts were found (*fixed_factor*: $p < 0.05$), multiple pair-wise comparisons were performed (the same GLM analysis, three combinations of 2 species tested separately).

2.3. Results

All experimental animals were observed to be filtering actively, cockles with open and extended siphons, mussels with open and extended mantles, and oysters with open and extended mantles inside the open shell. The animals appeared to be healthy and their behaviour normal. The seeding particles appeared not to hamper the filtration activity.

2.3.1. Localizing inhalant apertures

Inhalant feeding-current velocities of *C. gigas* showed high small-scale fluctuations along the gape. This is probably due to the undulating form of the shell edges, causing different gape widths along the shell edge. Inflow occurred along a large part (appr. 30%) of the anterior to ventral gape (Figure 2.1B). On a larger scale incurrent velocities did not differ considerably between different parts of the shell (e.g. the anterior and ventral parts).

Although for *M. edulis* incurrent velocities were highest in the area at the posterior end of the shell, inflow was observed along the entire ventral to posterior gape between somewhat below the byssal opening and the exhalant siphon. *C. edule* showed inhalant flow through the inhalant siphon. In some cases, however, the halfway buried cockles were occasionally observed to inhale water through the opening between both mantle folds that is normally used for extension of the foot, while still inhaling water through the inhalant siphon. Because the opening of this third aperture reduced the incurrent velocity in the inhalant siphon, and will not occur in fully buried cockles, such observations were excluded from further analysis.

2.3.2. Inhalant feeding current velocities

In all three species, larger individuals generally showed higher inhalant current velocities than smaller individuals (Figure 2.3). A relationship with shell length was significant for *C. gigas* (p = 0.04; Table 2.1) but not for *M. edulis* and *C. edule* (respectively p = 0.14 and 0.08; Table 2.1). Relationships with body weight (g AFDW) were not significant (Table 2.1; p = 0.05 (*C. gigas*), 0.34 (*M. edulis*), 0.08 (*C. edule*)). Although a significant effect of shell length and body weight was lacking in all cases but one, p-values in *C. gigas* and *C. edule* approached the significance level of 0.05. Therefore an effect of both variables could not convincingly be rejected, and we

included shell length and body weight as covariates in GLM analyses to test differences between species.

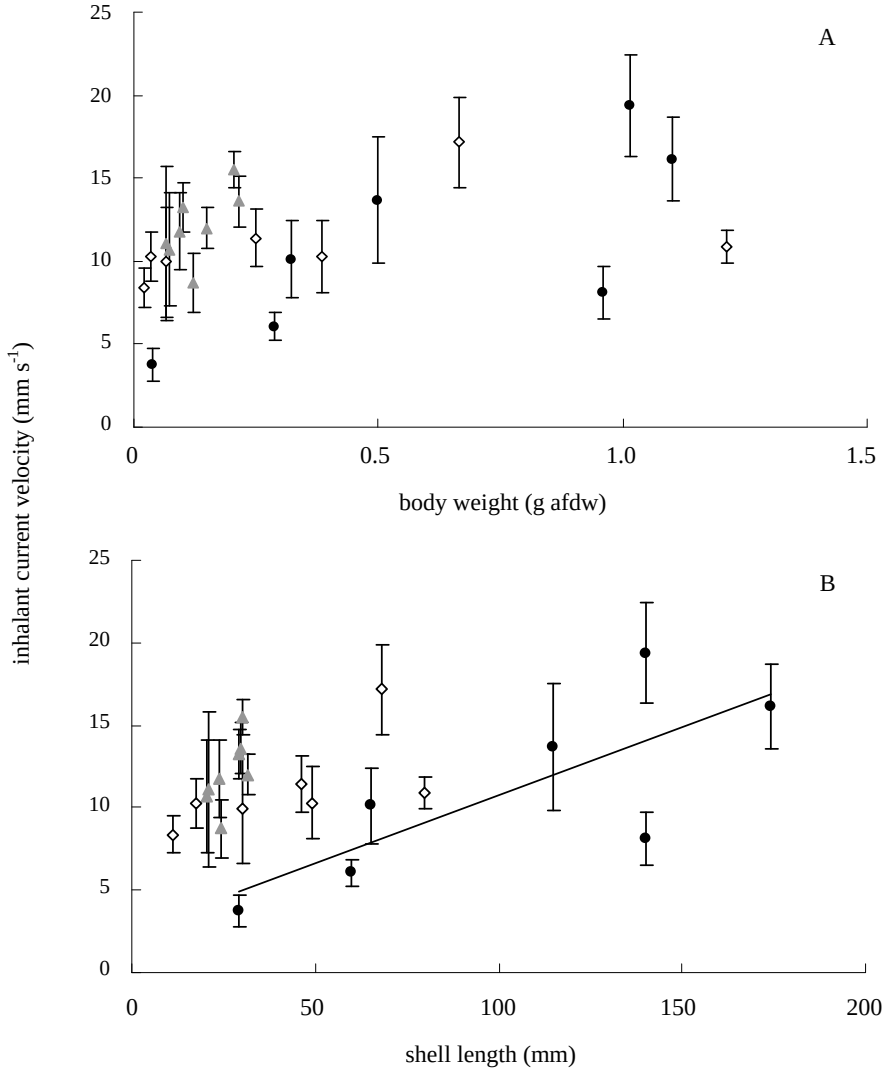


Figure 2.3. Mean inhalant feeding current velocities at the inhalant aperture per individual in mm s^{-1} (with standard deviations), plotted against body weight (A) and shell length (B) for *C. gigas* (black filled circles), *M. edulis* (open diamonds) and *C. edule* (grey filled triangles). A regression line is drawn through inhalant feeding current velocities of *C. gigas*, plotted against shell length (C; linear regression: $R^2 = 0.60$, $p < 0.05$).

Table 2.1. Results of linear regression analysis; for each bivalve species relationships of inhalant feeding current velocity, acceleration coefficient 'b' and distance of influence d_{infl} with shell length (mm) and body weight (g AFDW, ln-transformed) were tested. Sample sizes (*n*), degrees of freedom (*df*), R^2, *F*- and *p*-values are given. Significant relationships ($p < 0.05$) are underlined.

				independent: shell length			independent: body weight		
dependent	species	*n*	*df*	R^2	*F*	*p*	R^2	*F*	*p*
inhalant velocity	*C. edule*	8	7	0.42	4.36	0.08	0.42	4.38	0.08
	M. edulis	7	6	0.38	3.01	0.14	0.18	1.1	0.34
	C. gigas	7	6	0.6	7.57	0.04	0.56	6.42	0.05
'b'	*C. edule*	8	7	0.6	8.98	0.02	0.47	5.37	0.06
	M. edulis	7	6	0.8	19.33	0.01	0.84	26.00	0.00
	C. gigas	6	5	0.21	1.07	0.36	0.56	5.07	0.09
d_{infl}	*C. edule*	8	7	0.7	13.66	0.01	0.42	4.29	0.08
	M. edulis	7	6	0.6	7.34	0.04	0.64	8.72	0.03
	C. gigas	6	5	0.19	0.92	0.39	0.58	5.45	0.08

Table 2.2. Differences in inhalant feeding-current velocity and the velocity-profile parameters 'b' (acceleration coefficient) and d_{infl} (distance of influence) between three species of bivalves; statistical results of GLM with 'species' as fixed factor and either 'shell length' (mm) or (ln-transformed) body weight (g AFDW) as covariate. Degrees of freedom (*df*), *F*-values and *p*-values are given. Where significant differences in slopes or intercepts between the regression lines for the three species were found, *p*-values are underlined. Sample sizes (*n*) per species are given in Table 2.3.

	GLM *covariate = shell length*			GLM *covariate = body weight*		
	df	*F*	*p*	*df*	*F*	*p*
inhalant velocity						
slopes (effect of *species*covariate*)	2	0.50	0.62	2	2.07	0.16
intercepts (effect of *species*)	2	6.71	0.01	2	2.4	0.12
'b'						
slopes (effect of *species*covariate*)	2	7.76	0.01	2	1.08	0.36
intercepts (effect of *species*)	2	*n.t.*	*n.t.*	2	14.76	0.00
d_{infl}						
slopes (effect of *species*covariate*)	2	8.24	0.00	2	2.67	0.10
intercepts (effect of *species*)	2	*n.t.*	*n.t.*	2	6.73	0.01

n.t. = not tested since the prerequisite homogeneity of slopes was not met.

Table 2.3. Differences in inhalant feeding-current velocity and the velocity-profile parameters 'b' (acceleration coefficient) and d_{infl} (distance of influence) between three species of bivalves; results of GLM (descriptives and parameter estimates) tested with 'species' as fixed factor and either 'shell length' (mm) or (ln-transformed) body weight (g AFDW) as covariate (see *F*- and *p*-values in Table 2.2). Mean values and standard errors are given. For *C. edule*, mean values of 'b' and d_{infl} are corrected for not being buried completely in the experiments (uncorrected means between brackets). The estimates of intercepts are relative. Intercepts for *C. gigas* are set at zero, and intercepts for the other species are given relative to *C. gigas*' intercepts. Significant differences between intercept, tested in multiple pair-wise comparisons, are given with uppercase letters in superscript.

	C. edule	*M. edulis*	*C. gigas*
inhalant velocity			
Mean	12.08	11.18	11.03
standard error	0.73	1.06	2.12
n	8	7	7
GLM: intercepts (*shell length*)	7.31^{A}	5.05^{A}	0.00^{B}
GLM: intercepts (*body weight*)	4.10	1.62	0.00
'b'			
Mean	0.34	0.37	0.53
(*C. edule* uncorrected)	(0.43)		
standard error	0.05	0.09	0.06
n	8	7	6
GLM: intercepts (*shell length*)	*n.a.*	*n.a.*	*n.a.*
GLM: intercepts (*body weight*)	-0.33^{A}	-0.27^{A}	0.00^{B}
d_{infl}			
mean	21.04	25.68	13.24
(*C. edule* uncorrected)	(16.70)		
standard error	2.27	7.58	1.24
n	8	7	6
GLM: intercepts (*shell length*)	*n.a.*	*n.a.*	*n.a.*
GLM: intercepts (*body weight*)	16.05^{A}	19.00^{A}	0^{B}

n.a. = not available. The difference in intercepts was not tested since the prerequisite homogeneity of slopes was not met (see Table 2.2).

At comparable shell lengths, inhalant feeding-current velocities in *C. gigas* were significantly lower than inhalant feeding-current velocities in both *M. edulis* and *C. edule* (GLM tested with 'shell length' as covariate: equal slopes, but different intercepts; Table 2.2). Inhalant velocities in *M. edulis* were 5.05 mm s^{-1} higher than in *C. gigas*, and in *C. edule* 7.31 mm s^{-1} higher than in *C. gigas* (Table 2.3). No significant differences

between species were found when tested with body weight as covariate (Tables 2.2 and 2.3). Disregarding the (potential) effect of shell length and body weight, mean values of the acceleration coefficient 'b' over the entire ranges of shell lengths and body weights did not differ between species (one-way ANOVA: $F = 0.17$, $p = 0.84$; for n see Table 2.1).

2.3.3. Velocity profiles

Acceleration coefficients of velocity profiles from different directions towards the point of inflow (Figure 2.2), at angles of 0°, 45° and 90° relative to the sagittal plane of

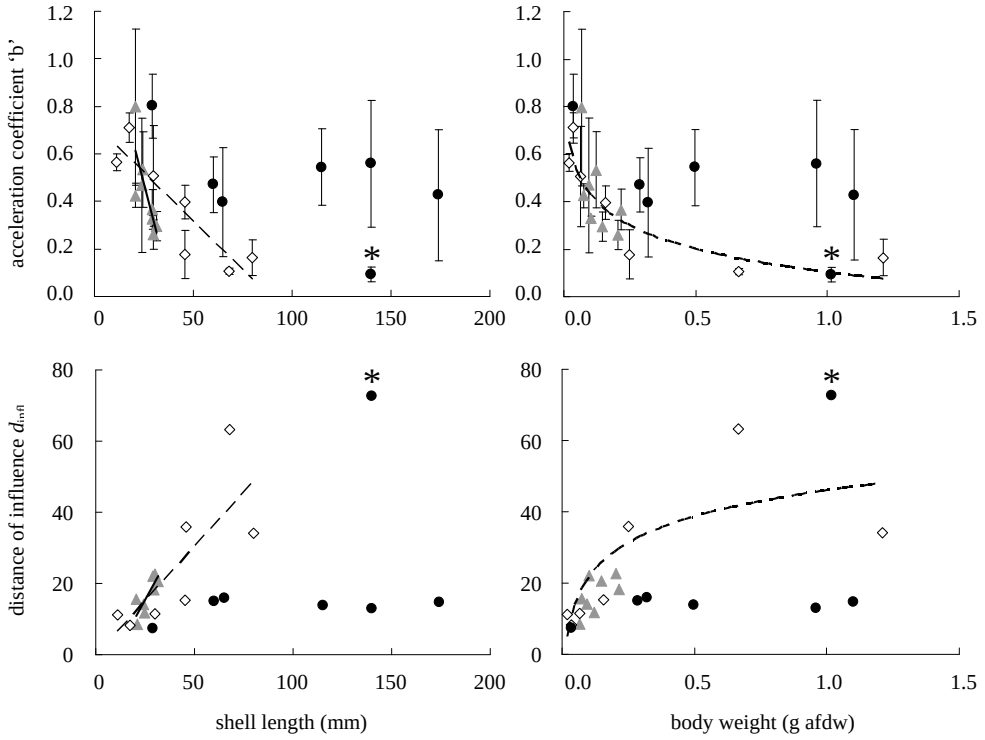


Figure 2.4. Acceleration coefficient and distance of influence plotted against shell length and body weight for *C. edule* (grey triangles), *M. edulis* (open diamonds), and *C. gigas* (black circles). Error bars represent standard deviations. Significant trendlines ((non)linear regression: $p < 0.05$) are drawn for *C. edule* (solid line; shell length: 'b': $R^2 = 0.60$; d_{infl}: $R^2 = 0.69$) and *M. edulis* (dashed line; shell length: 'b' linear: $R^2 = 0.79$, d_{infl} linear: $R^2 = 0.59$; body weight: 'b' logarithmic: $R^2 = 0.84$, d_{infl} logarithmic: $R^2 = 0.64$). Extreme values for *C. gigas* that were excluded from statistical analysis (GLM) are indicated with an asterisk.

the bivalves, were not significantly different (Friedman test, Table 2.4). Therefore we continued our analysis of velocity profiles along transects at an angle of 0° only.For *C. gigas*, one individual that yielded extreme values for 'b' (0.075) and d_{infl} (75.8 mm) (see Figure 2.4), as revealed by a simple boxplot, was removed from further analysis. This reduced the sample size to 6 for oysters (see Table 2.3).

In *C. gigas*, the velocity profile parameters 'b' and d_{infl} showed no relationship with either shell length or body weight (Table 2.1; Figure 2.4). For the other two species we did find significant relationships. The acceleration coefficient 'b' decreased linearly with shell length in *M. edulis* and *C. edule*, and logarithmically with body weight (ln-transformed in linear regression analysis) in *M. edulis* (Table 2.1; Figure 2.4). The distance of influence d_{infl} increased linearly with shell length in *M. edulis* and *C. edule*, and logarithmically with body weight (ln-transformed in linear regression analysis) in *M. edulis* (Table 2.1; Figure 2.4). Because both shell length and body weight affect velocity profile parameters in at least two of the three species (note also the low *p*-values in non-significant regressions), we included these variables as covariates in GLM analyses to test differences in 'b' and d_{infl} between species.

After correction of results for *C. edule* for not being buried completely (see Table 2.3), the acceleration coefficient 'b' was significantly higher for *C. gigas* than for both *M. edulis* and *C. edule* at comparable body weight (Tables 2.2 and 2.3). Acceleration coefficients for *M. edulis* and *C. edule* were, respectively, 0.27 and 0.33 lower than for *C. gigas* (Table 2.3). Testing differences between species with shell length as covariate yielded no results since the slopes of the regression lines were significantly different (Table 2.2). Disregarding the (potential) effect of shell length and body weight, mean values of 'b' over the entire ranges of shell lengths and body weights did not differ between species (one-way ANOVA: $F = 2.15$, $p = 0.15$; for *n* see Table 2.1).

After correction of *C. edule* results for not being buried completely, d_{infl} (the distance where $v = v_0 + 0.01$) was significantly smaller for *C. gigas* than for both *M. edulis* and *C. edule* at comparable body weight (Tables 2.2 and 2.3). Distances of influence d_{infl} for *M. edulis* and *C. edule* were, respectively, 19.00 and 16.05 mm larger than for *C. gigas* (Table 2.3). Testing differences between species with shell length as covariate yielded no results since the slopes of the regression lines were significantly different (Table 2.2). Disregarding the (potential) effect of shell length and body weight, mean values of d_{infl} over the entire ranges of shell lengths and body weights did not differ between species (one-way ANOVA: $F = 1.66$, $p = 0.22$; for *n* see Table 2.1).

Velocity profiles of all three species, modelled with Equation 2.1 and 'b' and inhalant feeding-current velocities at the aperture (mean values for all individuals per species), are shown in Figure 2.5. The velocity profile of *C. gigas* is steeper and has a smaller distance of influence compared to *M. edulis* and *C. edule*.

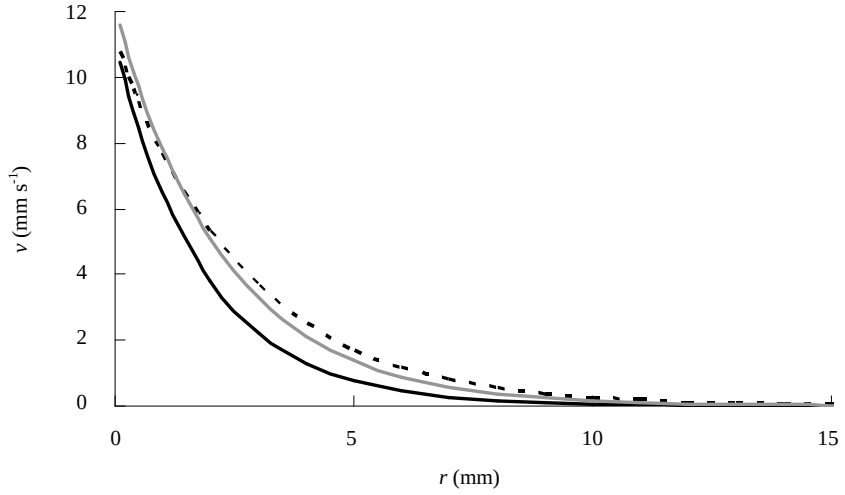


Figure 2.5. The average velocity profiles in the inhalant flow fields of *C. gigas* (solid black line), *M. edulis* (dashed black line) and *C. edule* (solid grey line), representing an exponential decay of the inhalant current velocity v with distance r from the inhalant aperture. The curves are based on Equation 2.1, but without background current y_0: $v(r) = ae^{-br}$, with the mean 'a' and 'b' parameters per species used as input.

2.3.4. Exhalant jets

Exhalant jets were distinctly visible as particle-depleted plumes in a particle-seeded field. In *C. gigas*, exhalant jets originated from the posterior region of the oyster (near the anus) in horizontal direction (Figure 2.6A). In upright mussels in our set-up (oriented as in Figure 2.2B) the exhalant jet was directed away from the bottom (Figure 2.6B), roughly at an angle of 50° - 70° relative to the bottom. The mussels appeared to be capable of modifying the direction of the exhalant jet somewhat relative to the shell. In *C. edule*, the exhalant jet was directed vertically and away from the bottom (Figure 2.6C).

The cross-sectional area of the exhalant aperture A_{exh} ranged from 0.03 to 0.08 cm² in *C. edule*, from 0.003 to 0.16 cm² in *M. edulis* and, as estimated, from 0.001 to

0.28 cm² in *C. gigas* (Table 2.5). A_{exh} increased with shell length and body weight in *M. edulis* and *C. gigas*, but showed no relationship with both variables in *C. edule* (non-linear regression; Table 2.6).

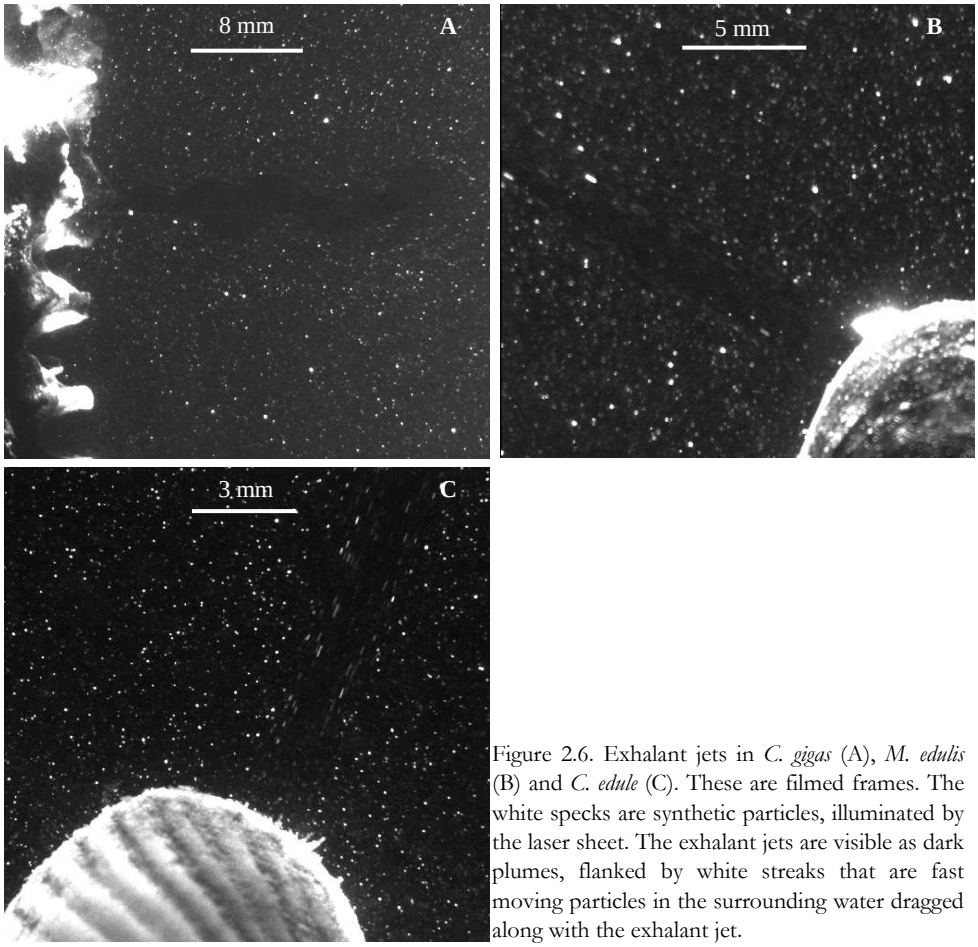


Figure 2.6. Exhalant jets in *C. gigas* (A), *M. edulis* (B) and *C. edule* (C). These are filmed frames. The white specks are synthetic particles, illuminated by the laser sheet. The exhalant jets are visible as dark plumes, flanked by white streaks that are fast moving particles in the surrounding water dragged along with the exhalant jet.

A range of filtration rates (Q) was derived from literature; these ranged from approximately 1.0 to 5.0 l h^{-1} in *C. edule* (Vahl 1972; Foster-Smith 1975; Møhlenberg and Riisgård 1979), from 1.5 to 6.0 l h^{-1} in *M. edulis* (Walne 1972; Riisgård 1977; Møhlenberg and Riisgård 1979) and from 3.8 to 12.5 l h^{-1} in *C. gigas* (Walne 1972; Gerdes 1983b; Bougrier et al. 1995) (Table 2.5) in animals of different sizes and measured under different experimental conditions (see cited papers).

Table 2.4. Differences between acceleration coefficients 'b' of velocity profiles in three directions (0°, 45° and 90°); results of the Friedman test for multiple related samples, tested for each species separately. No significant differences were found.

	C. edule	*M. edulis*	*C. gigas*
Chi-square	1.33	1.6	4.67
n	6	5	9
df	2	2	2
p	0.51	0.45	0.10

Table 2.5. Values for A_{exh} (measured and estimated cross-sectional areas of the exhalant aperture, in cm^2) and *FR* (filtration rates derived from literature, in $l\ h^{-1}$ $individual^{-1}$) that were used to calculate exhalant jet speeds for all three species. Mean values are given, with ranges in brackets. Expressed in $cm^3\ s^{-1}$, *FR* was used as Q (volume flux) to calculate exhalant jet speeds (in $cm\ s^{-1}$) according to the formula: jet speed = Q/A.

Species	A_{exh} (cm^2)	*FR* ($l\ h^{-1}$)	Jet speed ($cm\ s^{-1}$)
C. edule	0.04 (0.03 – 0.08)	3.0 (1.0 – 5.0)	20.8
M. edulis	0.06 (0.003 – 0.16)	4.0 (1.5 – 6.0)	18.5
C. gigas	0.04 – 0.08 (0.001 – 0.28)	7.0 (3.8 – 12.5)	24.3 - 48.6

Table 2.6. Results of non-linear regression analysis of relationships between exhalant siphon area A_{exh} and either shell length (mm) or body weight (g AFDW) as independent factors according to the equations $A_{exh} = a \cdot (independent)^b$ (a and b are constants). Significant relationships ($p < 0.05$) are underlined.

independent	species	*n*	R^2	*F*	b	*p*
shell length						
	C. edule	8	0.32	2.80		0.15
	M. edulis	10	0.83	38.21	1.56	<u>0.00</u>
	C. gigas	9	0.67	14.32	3.35	<u>0.01</u>
body weight						
	C. edule	8	0.23	1.81		0.23
	M. edulis	10	0.83	39.42	0.65	<u>0.00</u>
	C. gigas	9	0.69	15.29	0.86	<u>0.01</u>

Jet speeds were calculated using average values of A_{exh} and Q (see Table 2.5). Average jet speeds were thus calculated to be 20.8 $cm\ s^{-1}$ in *C. edule*, 18.5 $cm\ s^{-1}$ in *M. edulis* and 24.3 - 48.6 $cm\ s^{-1}$ (calculated with upper and lower estimates for A, see

methods) in *C. gigas*. Since A_{exh} and Q are both variable parameters, jet speeds were also modelled for a range of both parameters. When modelling a range of A_{exh}, Q was kept constant at average values, as derived from literature, of 3.0 l h^{-1} for *C. edule*, 4.0 l h^{-1} for *M. edulis* and 7.0 l h^{-1} for *C. gigas*. When modelling a range of Q, A_{exh} was kept constant at average values, as measured and estimated in this study over a range of body weight per species, of 0.04 for *C. edule*, 0.06 for *M. edulis* and for *C. gigas* 0.04 (upper limit) and 0.08 (lower limit). Figure 2.7 illustrates how the jet speed in all three species varies with A_{exh} and Q. With increasing A_{exh} but constant Q, jet speeds decreased. Because of the higher filtration rate in *C. gigas*, its modelled jet speeds are higher at similar cross-sectional areas of exhalant apertures. With increasing Q but constant A_{exh}, modelled jet speeds increased. Jet speeds of *M. edulis* were within the range of jet speeds estimated for *C. gigas*. Jets speeds in *C. edule* were equal to the upper estimated limit of *C. gigas* at similar filtration rates, but because *C. gigas* display wider ranges in filtration rate (due to a larger natural range in body weight and its relationship with filtration rate, for the latter see Møhlenberg and Riisgård 1979), over the entire range *C. gigas* may show higher jet speeds.

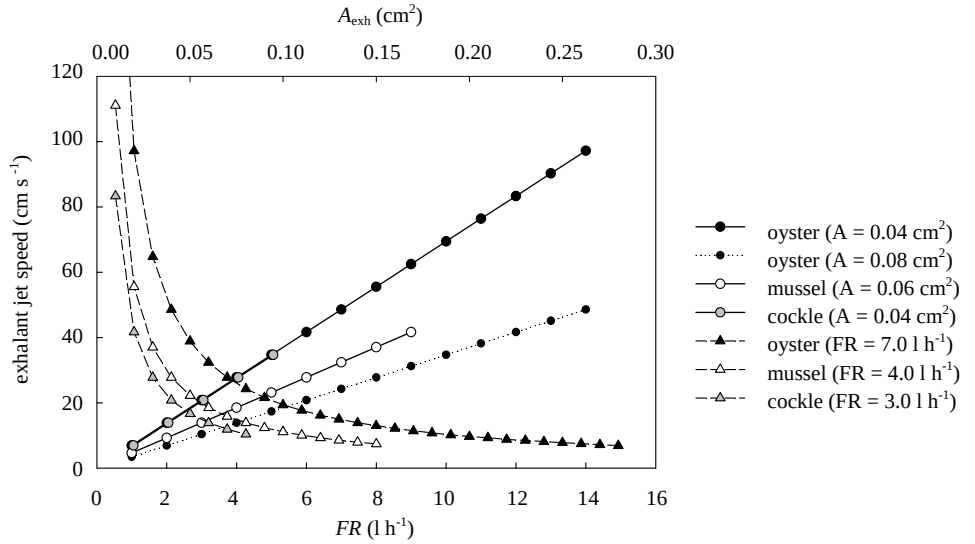


Figure 2.7. Model calculations on exhalant jet speeds (in cm s^{-1}) in *C. gigas*, *M. edulis* and *C. edule*, with varying exhalant aperture cross-sectional area (A_{exh}, in cm^2) and filtration rate (FR, in l h^{-1}).

2.4. Discussion

2.4.1. Methodological considerations

Performing the experiments in still water did not exclude background water currents completely. The strong exhalant jets caused disturbance of water movement in inhalant flow fields when located nearby, especially in cockles, and when reflected by the nearby tank wall. The exhalant jets in general caused some slight background circulation that was in most cases well below 1 mm s^{-1} in different directions (measured with DPIV). Recordings with higher background currents were excluded from the analysis.

Variations in inhalant feeding-current velocities between and within species were high. This may be caused by small variations in siphon diameter, valve gape and mantle gape, and by local variations along the irregularly shaped shell and mantle edges of *C. gigas* and mantle of *M. edulis*. Additionally, the animals may have adjusted their clearance rates to short-term fluctuations in particle and algal concentrations caused by the continuous depletion by the bivalves' filtration activity and periodical replenishments (see Hawkins et al. 2001; Riisgård et al. 2003).

Exhalant jet speeds could not be measured directly because the particles used to visualize water movement were retained efficiently by the bivalve gills. Frank et al. (2008) solved this problem by using particles that were too small (appr. 2 μm) to be retained efficiently by the gills in measuring exhalant jet speeds of several bivalve species using DPIV. With the magnification used in our set-up, such small particles would not have been visible, particularly at the highest water-current velocities measured.

2.4.2. Inhalant flow field

In *M. edulis*, mean inhalant feeding-current velocities at the inhalant aperture ranged up to 17.2 mm s^{-1} (68 mm shell length). Green et al. (2003) measured inhalant feeding-current velocities in *M. edulis* (22.6 – 23.7 mm shell length) of up to 6 mm s^{-1} at a slight distance from the gape (< 1 mm), at 17 °C by tracking particles. We found higher inhalant current velocities at the inhalant aperture itself, but the modelled velocity profiles (Figure 2.5) show a velocity of 7.7 mm s^{-1} at a distance of 1 mm from the aperture, corresponding to the results by Green et al. (2003).

In *C. gigas* of 9.0 to 11.0 cm shell length, at a seawater temperature of 12 – 14 °C, Tamburri et al. (2007) found a mean inhalant current velocity of 1.65 ± 0.10 (s.e.) mm

s^{-1} at a distance of ~1.5 mm from the gape. We found much higher inhalant feeding current velocities for a *C. gigas* individual of similar size: for an oyster of 11.5 cm shell length, we found an inhalant feeding current velocity of 13.7 mm s^{-1} at the inhalant aperture, that had decreased according to the velocity profile for this individual to 8.6 mm s^{-1} at a distance of 1.5 mm from the inhalant aperture. We also found a larger distance of influence in *C. gigas* than Tamburri et al. (2007) did. For *C. gigas* kept in still water, Tamburri et al. (2007) observed an influence of inhalant feeding currents at distances of 1 – 2 mm from the gape, but not at distances of 4 – 20 mm. For the same species, we found distances of up to 13.2 mm on average where the inhalant velocity had decreased to 0.01 mm s^{-1}. Differences in inhalant current velocity and distance of influence may be partially due to a difference in methodology. Tamburri et al. (2007) injected a 1 µl bolus of a neutrally buoyant dye at different distances from the gape and analysed the velocity in the initial 5 second interval after injection. A speed as minimal as 0.01 mm s^{-1}, more or less arbitrarily chosen in our study to facilitate a comparison of d_{infl} between species, may be very difficult to detect with a method such as Tamburri et al. (2007) used. Differences may also have been induced by a difference in experimental temperature. *C. gigas* increase their filtration rate with increasing temperature up to a maximum at about 19 °C (Bougrier et al. 1995). The higher temperature used in our study (18 °C) than in the study of Tamburri et al. (2007; 12 – 14 °C) may have induced a larger filtration rate (Walne 1972) and subsequently higher inhalant current velocities and a larger d_{infl} (see Equation 2.1). Additionally, it is also possible that differences in condition, origin of the animals, food concentrations and the food type used in the experiments caused differences in filtration activity.

André et al. (1993) measured inhalant current velocities in *C. edule* with 15 – 43 mm shell length of up to 12 mm s^{-1} at 17 °C, for one individual even up to 22 mm s^{-1}, by tracking particles. This corresponds well with our results. We found inhalant feeding current velocities ranging up to 15.5 mm s^{-1} at the inhalant aperture in *C. edule* (of 30 mm shell length). The mean distance of influence of 21.0 mm (corrected for not being buried completely) of the inhalant flow field of *C. edule* corresponds to results for another cockle species. Ertman and Jumars (1988) observed influence of the inhalant siphon of *Clinocardium nuttallii* (Conrad) up to a distance of 10 - 20 mm vertically. These observations were made in a flume tank at a current velocity of 2.8 cm s^{-1} (free-stream velocity), and the observed distance of influence of 10 – 20 mm was therefore likely smaller than it would have been in still water (Ertman and Jumars 1988; André et al. 1993).

No significant relationships between inhalant current velocities and body weight were found, and a significant relationship with shell length only in *C. gigas*. The *p*-values for the linear regression of inhalant feeding-current velocities with body weight and shell length in *C. edule* and *C. gigas* were close to 0.05 (Table 2.1). We could therefore not dismiss a potential relationship of inhalant feeding current velocity with body size in testing differences between species. Our results suggest that the filtration rate (*FR* or *Q*) increased faster with body size than the inhalant aperture area A_{inh}, resulting in an increase in inhalant feeding-current velocity. However, both gill area and pumping rate approximately scale with length^2 and with weight$^{0.67}$ in many bivalve species (*M. edulis*, *C. edule* and 11 other species of suspension feeding bivalves, Møhlenberg and Riisgård 1979; Jones et al. 1992). Inhalant aperture area, being a 2-dimensional variable, is also expected to scale with length^2 and with weigth$^{0.67}$, theoretically resulting in an equal rate of increase of A_{inh} and Q with body size, and therefore in a constant inhalant feeding-current velocity. In contrast with this theory, we found a significant relationship with shell length in *C. gigas* (Table 2.1; Figure 2.3). Considering the large variation in our results, sample sizes may not have been large enough to detect positive trends with shell length or body weight in *C. edule* and *M. edulis*. A detailed study of allometric relationships would require close monitoring of valve gape, inhalant and exhalant aperture areas, and filtration rates. This was, however, beyond the scope of our study.

The increase in the distance of influence with body size (Figure 2.4) is probably a direct result of the decrease in acceleration coefficient (potentially in combination with an increase in inhalant feeding current velocity) in *M. edulis* and *C. edule*. The decrease of the acceleration coefficient with body size is more difficult to explain. It may be related to changes in the shape of the animal surrounding the inhalant aperture (Anayiotos et al. 1995), or to changes in aspect-ratio (length/width) of the inhalant aperture (Anayiotos et al. 1997). For a liquid with the same viscosity as blood, Anayiotos et al. (1997) showed that a higher aspect-ratio in an oval shaped orifice resulted in a steeper velocity profile. The same rule may apply to our results. The length of the inhalant aperture along the edge of the shell may be larger in *C. gigas* than in *M. edulis*, relative to the width of the inhalant aperture. Obviously, the ratio of length to width of the inhalant aperture of *C. gigas* is higher than the aspect-ratio of the circular inhalant aperture of *C. edule* (≈ 1). This theoretically agrees with the significantly lower acceleration coefficient of *C. edule* and *M. edulis* compared to *C. gigas*.

The influence of inhalant feeding currents of *C. edule* and *M. edulis* extends significantly further into the water column than the influence of inhalant feeding

currents of *C. gigas* (Table 2.3; Figure 2.4). In still water, a larger distance of influence may allow these species to forage in higher water layers than *C. gigas*, possibly resulting in a larger phytoplankton intake rate (see Fréchette et al. 1989) at comparable near-bed phytoplankton concentrations and comparable filtration rates. However, still water is a rare occurrence in the vicinity of bivalve beds. Absolute differences between species were small, and potentially different effects on food flux towards the bivalves may be overwhelmed by turbulent boundary layer mixing in the field.

The significantly higher steepness of the inhalant velocity profile in *C. gigas* may have consequences for the entrainment of zooplankton species that can detect and escape from hydromechanical stimuli such as critical deformation rates and acceleration (see Kiørboe and Visser 1999; Titelman and Kiørboe 2003). However, absolute differences in steepness were small between species and are not expected to lead to differences in the capture rate of zooplankton against background levels of turbulence caused by exhalant jets and shell roughness. The increased roughness of mussel beds and particularly oyster beds strongly increases near-bed turbulence and therefore ambient fine-scale deformation rates. Higher deformation rates in the inhalant feeding currents of *C. gigas* may therefore not have major negative impact of the capture rate of zooplankton. We did not, however, study hydromechanical stimuli (e.g. deformation rate) in the flow fields of the three species. Whether hydromechanical stimuli in the inhalant currents differ significantly from the ambient flow (and hence can be detected by potential prey items) can only be evaluated in flowing water.

In conclusion, the lower inhalant current velocity and smaller distance of influence and steeper velocity profile of the flow field of *C. gigas* may result in a reduced capacity to deflect larger particles, entrain slow-swimming zooplankton and forage from higher water layers. At comparable filtration rates this would imply a reduced ability to capture motile prey compared to *M. edulis* and *C. edule*. However, differences were found at comparable shell length and/or body weight. The natural size range of *C. gigas* is much larger than the natural size ranges of *C. edule* and *M. edulis*, and *C. gigas* can reach larger sizes and higher body weights. Therefore, when considering natural size ranges of the different species in the field, differences found are expected to be reduced (as an indication: one-way ANOVA gave no significant differences between mean inhalant feeding current velocities, 'b', and d_{infl} of the three species over the entire range of body sizes used). Furthermore, *C. gigas* individuals generally have higher filtration rates than *M. edulis* (Walne 1972) and *C. edule* (see introduction; own unpublished results). Considering the relatively small differences found in inhalant feeding-current characteristics, differences in filtration rates are

expected to be more determining for differences in food intake. Finally, potential differences in feeding efficiency as a result of small differences in inhalant feeding-current characteristics are expected to be overwhelmed by differences in food flux towards the bed due to differences in near-bed turbulence levels.

2.4.3. Exhalant jets

Modelled exhalant jet speeds were almost always higher in *C. gigas*, due to its higher filtration rate but similar cross-sectional area of the exhalant aperture. Although A_{exh} was estimated and not measured directly for *C. gigas*, the order of magnitude should be reliable. The smallest diameter ($d2$) of the exhalant aperture cannot have been wider than the shell gape. The largest diameter ($d1$) of the exhalant aperture ranged from one to two times $d2$, indicating a round to oval shaped exhalant aperture, as in *M. edulis* (Newell et al. 2001). Frank et al. (2008) measured a cross-sectional area of the exhalant aperture of 0.03 – 0.16 cm^2 in *M. edulis* (51 – 62 mm shell length), which corresponds to our results. Highest exhalant jet speeds were determined at 4.06 cm s^{-1} for *Crassostrea virginica*, 4.80 cm s^{-1} for *Mercenaria mercenaria*, 12.31 cm s^{-1} for *M. edulis* and 15.20 for *Argopecten irradians* (Frank et al. 2008). Their *M. edulis* were estimated to have filtered with a rate of 0.2 to 2.0 l h^{-1}. These values correspond well to our model results (Figure 2.7). Newell et al. (2001) measured exhalant-aperture cross-sectional areas of 0.14 to 0.65 cm^2 in large *M. edulis* individuals of 81.2 mm mean shell length. This is larger than what we measured, but so were the animals.

Although generally higher exhalant jet speeds were calculated for *C. gigas*, this does not necessarily mean a higher kinetic energy input in the benthic boundary layer. Kinetic energy input is determined by a balance between exhalant jet speeds and exhalant aperture cross-sectional areas. The rate of transport of kinetic energy in a jet (E, in Watt) can be calculated as

$$E = \tfrac{1}{2}\rho \cdot u^3 \cdot A \qquad \text{(Tritton 1988)} \qquad (2.3)$$

where ρ (kg m^{-3}) is the density of the medium, u (m s^{-1}) is the average jet speed and A (m^2) is the exhalant aperture cross-sectional area (Tritton 1988). Using the average values measured and estimated for A, and average modelled jet speeds (both in Table 2.3), rates of kinetic energy transport in exhalant jets (E) of *C. edule* and *M. edulis* can be calculated to be approximately 2 x 10^{-5} W. Rates of kinetic energy transport in exhalant jets of *C. gigas* appear, thus calculated, an order of magnitude higher: ranging from 6 x 10^{-5} to 2 x 10^{-4} W (for upper and lower limits of A estimates).

Increased turbulent mixing inside and just above the bed enhances turbulent transport of phytoplankton towards the bivalves and thereby increases the food availability (Fréchette et al. 1989; Larsen and Riisgård 1997). At the same time, vertical exhalant jets reduce refiltration of already filtered water inside the bed (Jonsson et al. 2005; Widdows and Navarro 2007), and may blend near-bottom water, thereby increasing the thickness of the water layer available to suspension feeders (suggested by Larsen and Riisgård 1997). Bivalve suspension feeders can seriously deplete overlying phytoplankton concentrations (Dolmer 2000; Jonsson et al. 2005), which may lead to food-limited growth in dense beds (Kamermans 1993). Therefore, enhancing turbulent mixing probably contributes directly to enhanced growth. Regarding the higher modelled exhalant current velocities and the roughly estimated individual kinetic energy transfer only, *C. gigas* may affect near-bed turbulence levels through biomixing more strongly than *M. edulis* and *C. edule*. However, this may be counteracted by the different orientation of exhalant jets in *C. gigas* compared to the native species. Exhalant jets of *C. edule* are directed vertically and away from the bottom. In the field, exhalant jets in *M. edulis* are mostly directed away from the bed at angles varying roughly from 40 – 90° relative to the bottom (Maas Geesteranus 1942). Exhalant jets of *C. gigas* are not directed away from the bed but horizontally, parallel to the bottom (for oysters growing upright in beds on soft sediments as well as oysters living attached to hard substrates with their cupped valve; pers. obs.). This suggests a certain level of refiltration inside the oyster bed, which may reduce the food intake rate but may also be counteracted by the relatively large filtration rate of *C. gigas* individuals.

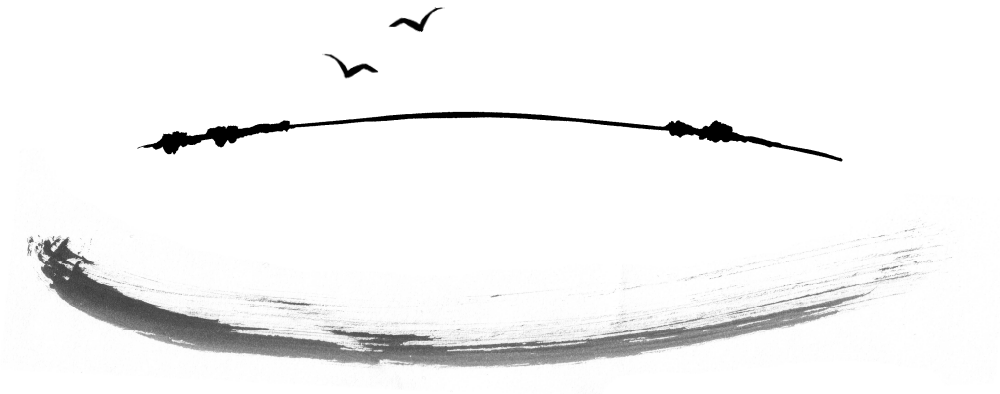
Besides through biomixing, epibenthic bivalves also affect turbulence levels by their physical presence on the sediment (Fréchette et al. 1989). Mussel beds and oyster reefs represent large biogenic roughness structures that may enhance near-bed turbulence levels significantly (Nikora et al. 2002). The effect of topographic roughness on turbulence is often assumed to scale with the length of the roughness structures (Butman et al. 1994 and references therein; Van Duren et al. 2006). In a closely packed experimental mussel patch (1,800 mussels m^{-2}), the average roughness height was estimated at 25 – 30 mm, as the difference between the lowest and the highest point of the mussel bed (Van Duren et al. 2006). Roughness height in an oyster bed is roughly in the order of 10 to 20 cm (own observations from the Oosterschelde estuary, SW Netherlands), which is an order of magnitude larger than in mussel beds. Because cockles cause bioturbation of sediments, they do affect bottom topography, but to a much smaller extent than mussels and oysters (Ciutat et al. 2007). Increased topographic roughness in a cockle bed, compared to similar

sediment without cockles is in the order of a few mm at most (Fernandes et al. 2007). The difference in roughness height therefore seems more determining for potential differences in near-bed turbulence levels, food flux towards the bed, and entrainment of zooplankton prey. Because cockles hardly increase mixing by physical roughness, increased mixing due to exhalent jets (Ertman and Jumars 1988) may be more relevant to them, as may be optimizing the distance of influence of the inhalant flow field.

2.4.4. Conclusions – implications for food intake

Our study shows that differences in inhalant feeding currents on the scale of equal-sized individual bivalves are small despite apparent differences in morphology between the species. Differences in inhalant feeding currents may even diminish when considering the natural size ranges of the species studied. Modelled exhalant jets of *C. gigas* were generally stronger than jets of native bivalves. This seems to result in a higher kinetic energy input in the boundary layer by individual oysters. However, implications of the horizontal orientation of the exhalant jets of *C. gigas* for food intake are unknown. We furthermore expect that the obviously large difference in roughness scale between beds of the invasive *C. gigas* and the native *M. edulis* and *C. edule* may be more relevant for potential differences in phytoplankton flux and zooplankton predation. Possible differences in food intake between the species should further be studied on the scale of a patch in the full range of biogenic interactions in a boundary flow.

Chapter 3



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.

Larviphagy in native bivalves and an introduced oyster

Karin Troost, Pauline Kamermans, Wim J. Wolff

3.1. 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 1999a; Geurts van Kessel et al. 2003). After their deliberate introduction in 1964 (Drinkwaard 1999a), these oysters expanded rapidly throughout Dutch estuaries, forming large and dense oyster reefs in the intertidal and subtidal (Drinkwaard 1999a; 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 0.25 km^2 in 1980 to 8.1 km^2 in 2003 (Kater and Baars 2004; Dankers et al. 2006). 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 m^3 day^{-1} in 1990 to 398 million m^3 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 1976; 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; Lehane and Davenport 2002, 2004; Maar et al. 2007), *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 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. (2008b, Chapter 4) 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.

3.2. Materials and methods

3.2.1. 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 hours. 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.reed-mariculture.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 3.1). 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 hours. Upon arrival, the larvae were submerged in 2-3 litres 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 hours (protocol after Helm et al. 2004). This temperature corresponds with seawater temperatures during the reproductive season (in the Netherlands July – September; unpublished data Wageningen 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 3.1). 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 Wageningen IMARES and RIKZ). *C. edule* larvae were not included because they could not be obtained in sufficient numbers.

3.2.2. 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 litres of water, mussels in 5 litres and cockles in 2.5 litres 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 one hour, 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 two days. On the first day of a set, filtration rates of algae were determined. On the second day 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 3.1). At the end of each set, the adult animals were dried at 70 °C for three days and incinerated at 550 °C for four hours 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.

Table 3.1. Initial concentrations and larval shell lengths as used in the experiments on larviphagy. Larval batches that were also used to study the fate of filtered larvae are indicated with an asterisk.

Larval species	Set of experiments	Experiment	Date	Initial larval concentration (l^{-1})	Age larvae *(days from fertilization)*	length larvae ± s.d. *(µm)*
C. 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 *
M. 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

3.2.3. 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 x 10^4 to 4 x 10^4 cells ml^{-1} and stirred gently. We first waited 20 minutes for larger particles (e.g., pseudofaeces) to settle down again to prevent an over-estimation of clearance rates. Then, every 20 minutes 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 hours. From the reduction in algal concentration, we calculated the clearance rate according to Riisgård (2001, equation 3):

$$Cl = (V/t) \times \ln(C_0 / C_t) \qquad (3.1)$$

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 Equation 3.1 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.

3.2.4. 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 one hour, 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 x 10^4 to 4 x 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 3.1). We stirred very gently immediately afterwards to distribute the larvae as evenly as possible. We let the adults feed for 1

hour 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 Equation 3.1.

3.2.5. 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 one hour. 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 minutes. Following these observations, and the methodology of Lehane and Davenport (2004), five minutes 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 minutes 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 minutes 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 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.

3.2.6. 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.

3.3. Results

3.3.1. 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 (Figure 3.1A - B). In experiment A (Figure 3.1A) 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 (Figure 3.1B), 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, $p > 0.05$).

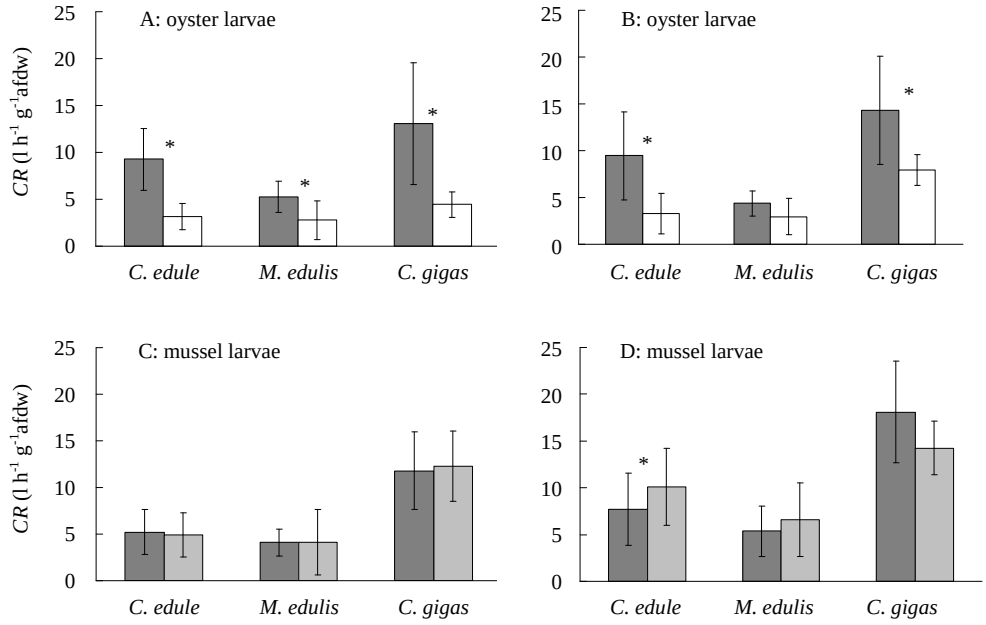


Figure 3.1. Clearance rates (*CR*) by adult bivalves of algae (A - D, dark grey bars), *C. gigas* larvae (A - B, white bars), and *M. edulis* larvae (C - D, light grey bars). Average clearance rates with standard deviations are given in litres per hour per individual of a standardized 1 gram ash-free 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.

All three bivalve species filtered *M. edulis* larvae with the same filtration rates as they had filtered algae with, the previous day (Figure 3.1C - D). We found this result for both experiments performed with *M. edulis* larvae. In experiment C (Figure 3.1C) 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 (Figure 3.1D) 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 one-sample t-test (n = 8 per adult species for algae, n = 4 per adult species for oyster and mussel larvae): $p > 0.05$).

3.3.2. Fate of filtered larvae

All three bivalve species ingested *M. edulis* and *C. gigas* larvae (Figure 3.2). Only 0.1 to 1.3% of all *M. edulis* larvae that were added were found back in the pseudofaeces of

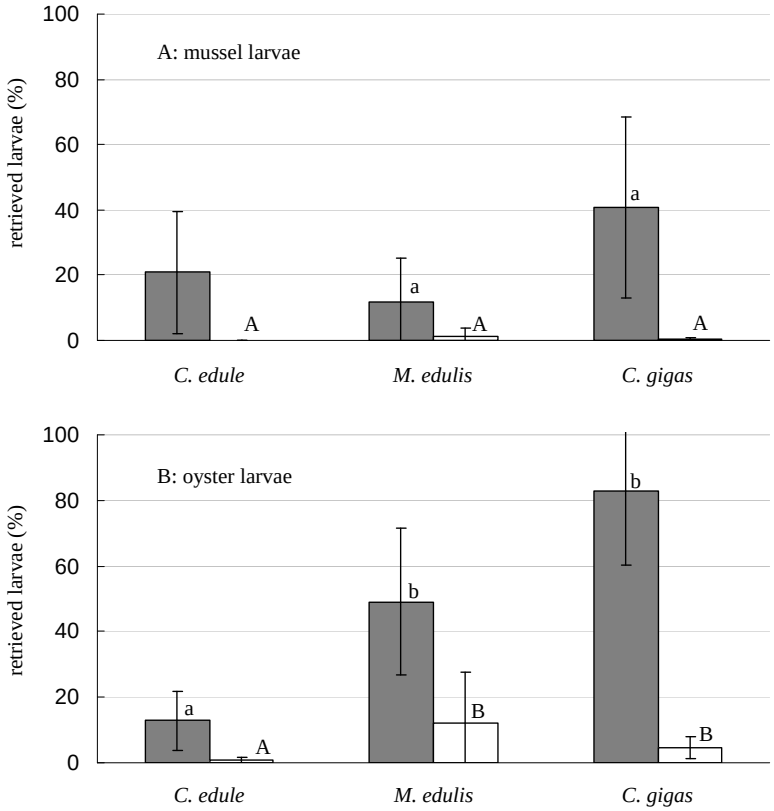


Figure 3.2. Mean percentages of actual number of larvae added, retrieved from stomach (dark bars) and pseudofaeces (white bars) samples, with standard deviations. Different letters indicate significant differences (ANOVA + Bonferroni, $p < 0.05$ after ln-transformation) between adult species, for stomach (lower case letters) and pseudofaeces (upper case letters) samples.

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.

3.4. Discussion

3.4.1. 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).

3.4.2. 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 litre chamber during 1.5 hours. 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 (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. (2008b, Chapter 4) 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 1983a; 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 hours (Gerdes 1983a), 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 3.1; Figure 3.1B), 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.

3.4.3. 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 minutes 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 minutes. 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 minutes for *C. virginica* and *M. edulis*, even up to 23 minutes 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 minutes 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 minutes 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).

3.4.4. 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; 5 to 12 million eggs; 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. (2009b, Chapter 2), who found a much higher inhalant current velocity than Tamburri et al. (2007) did, in comparably sized *C. gigas*.

Troost et al. (2009b, Chapter 2) 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).

3.4.5. 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 3.1) 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.

3.4.6. 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. 2008b, Chapter 4), leaving other mechanisms, such as chemical detection, for further research.

Chapter 4

Abstract
While the stock of introduced Pacific oysters (*Crassostrea gigas*) increased in the Oosterschelde estuary (SW Netherlands), so did the filtration pressure of all bivalve species together. In the same period, stocks of native bivalves declined slightly. The expansion of Pacific oysters in Dutch estuaries might be partially due to better abilities of their larvae to avoid or escape filtration, compared to larvae of native bivalves. In this context, escape and swimming abilities of Pacific oyster larvae and the larvae of the native blue mussel (*Mytilus edulis*) were compared.
Swimming behaviour of *C. gigas* larvae and larvae *of M. edulis* was recorded in still water and in a suction current mimicking a bivalve feeding current, in a horizontal and in a vertical plane. Larval swimming behaviour in a suction flow field was reconstructed by subtracting local water movement vectors from the total movement of larvae, yielding movement paths due to larval swimming alone.
Swimming speeds and the rate of displacement in vertical direction of *C. gigas* and *M. edulis* larvae were related to larval shell length, and to the pitch of up- or downward swimming.
Larvae of both species did not show escape reactions in a suction flow field. With increasing shell length, larval swimming speeds of both species increased significantly. Swimming speeds of *C. gigas* larvae were significantly higher than swimming speeds of *M. edulis* larvae, resulting in a faster vertical displacement. The ability to migrate to more favourable water layers faster may offer *C. gigas* an advantage over native bivalves with slower swimming larvae.

Can bivalve veligers escape feeding currents of adult bivalves?

Karin Troost, Ronald Veldhuizen, Eize J. Stamhuis, Wim J. Wolff

4.1. Introduction

4.1.1. Introduced oysters

Pacific oysters (*Crassostrea gigas* (Thunberg)) were introduced in the Netherlands in the Oosterschelde estuary in 1964 (Drinkwaard 1999b, 1999a). They spread rapidly throughout all Dutch estuaries (Bruins 1983; Drinkwaard 1999b; Wolff and Reise 2002; Smaal et al. 2005; Dankers et al. 2006) and are now a potential threat to native bivalve filter-feeders. While Pacific oyster stock increased, stocks of native bivalves slightly declined. As a consequence, the total filtration pressure in the Oosterschelde estuary was estimated to have increased from 289 million m^3 water day^{-1} in 1990 to 398 million m^3 day^{-1} in 2000. All filter-feeding bivalves together are estimated to filter a volume equal to that of the estuary in one week. Of this total filtration capacity, roughly 2/3 can be ascribed to the Pacific oysters while they contribute 'only' 50% to the total filter-feeder biomass (Geurts van Kessel et al. 2003; Kater 2003). 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, i.e. the filtering of bivalve larvae by adults of their own and other species.

4.1.2. Larviphagy

During the first one to four weeks of their lives, many bivalve species are part of the zooplankton. This is their pelagic larval stage, after which they search for a suitable substrate to settle (Wildish and Kristmanson 1997). During this pelagic stage, bivalve larvae experience very high mortality rates due to various factors (Thorson 1950; Rumrill 1990; Gosselin and Qian 1997). It has been demonstrated that bivalve larvae are filtered by adult bivalves (André and Rosenberg 1991; Tamburri and Zimmer-

Faust 1996; Jasprica et al. 1997; Lehane and Davenport 2002, 2004; Maar et al. 2007). Since adult bivalves filter all particles above a certain threshold size non-selectively (Møhlenberg and Riisgård 1978), they may also filter their own larvae. This has indeed been demonstrated for several species: the blue mussel *Mytilus edulis* (Cowden et al. 1984; Maar et al. 2007), the edible cockle *Cerastoderma edule* (André and Rosenberg 1991), the American oyster *Crassostrea virginica* (Tamburri and Zimmer-Faust 1996) and the zebra mussel *Dreissena polymorpha* (MacIsaac et al. 1991). Once filtered, bivalve larvae are either ingested or rejected in pseudofaeces. If ingested they most likely die in the digestion process or in the faeces (Mileikovsky 1974; Lehane and Davenport 2004; Troost et al. 2008a, Chapter 3). Larvae that are rejected in pseudofaeces are also likely to die (Mileikovsky 1974; Tamburri and Zimmer-Faust 1996; Lehane and Davenport 2004). Bivalve larvae are not likely to reach a size refuge from bivalve filtration before settlement, since *C. gigas* larvae with a shell length of 241 μm were readily filtered and ingested by adult *C. gigas*, *M. edulis* and *C. edule* (Troost et al. 2008a, Chapter 3). Furthermore, (parts of) zooplankton species with widths up to 300 μm and lengths of up to even 1000 μm were commonly found in stomachs of *C. gigas* and *M. edulis* (unpublished field observations, KT). Many bivalve larvae settle at sizes of 200-350 μm (see Hendriks et al. 2005).

Larvae of some species might be better in avoiding or escaping bivalve filtration than larvae of other species. This may lead to different mortality rates and, potentially, to differences in recruitment success. Kimmerer et al. (1994) already suggested that selectivity caused by differences in escape responses could make bivalve predation an important factor influencing biomass and species composition of inshore zooplankton. In extension, bivalve predation of larvae could influence stocks of macrobenthic species with pelagic larvae (such as bivalves).

4.1.3. Escape abilities

Since aggregations of filter-feeding bivalves can be a serious threat to conspecific larvae, one would expect filter-feeding bivalve species to have evolved some kind of survival strategy. One strategy can be the production of an excess of larvae, allowing them to cope with high losses due to predation. Most bivalves produce large amounts of larvae (Helm et al. 2004). Another strategy can be to provide their larvae with means to avoid or escape predation. Although bivalve larvae do have sensory abilities (LaBarbera 1974; Hidu and Haskin 1978; Cragg 1980; Prael et al. 2001; Kingsford et al. 2002), it is still unknown if they are able to detect and act on hydromechanical signals created by, for instance, a filtering bivalve. Behavioural reactions to

hydromechanical signals (rheotaxis) have been observed in several zooplanktonic species (e.g. Singarajah 1975; Jakobsen 2001; Kingsford et al. 2002) and studied extensively in copepods and their nauplii (e.g. Fields and Yen 1997; Kiørboe et al. 1999; Titelman 2001; Green et al. 2003; Titelman and Kiørboe 2003), but not in bivalve larvae. In the copepod studies, shear rate (or shear deformation, see Kiørboe et al. 1999) turned out to be the strongest cue for escape jumps. Bivalve larvae may respond to the same cue, or they may be triggered to escape by other properties of a suction current such as acceleration or strain rate (or longitudinal deformation rate, see Kiørboe et al. 1999). The direction of potential escape reactions in bivalve larvae is most likely to be orientated vertically, because of their swimming mode and the spatial position of bivalve filter-feeders. Most bivalve veliger larvae alternately swim upward and sink. When swimming upward, they typically do so in a helical pattern (Figure 4.1) (Cragg 1980). Upon encountering a disturbance they may either increase their swimming speed in vertical direction (Hidu and Haskin 1978; Cragg 1980; Prael et al. 2001), e.g. by increasing their absolute swimming speed along the helix or by increasing the pitch of upward or downward swimming, or they may close their shell valves and sink rapidly (LaBarbera 1974; Hidu and Haskin 1978; Cragg 1980). It is also possible that bivalve larvae are able to detect hydromechanical signals, but unable to escape inhalant feeding currents, or they may not be able to do either.

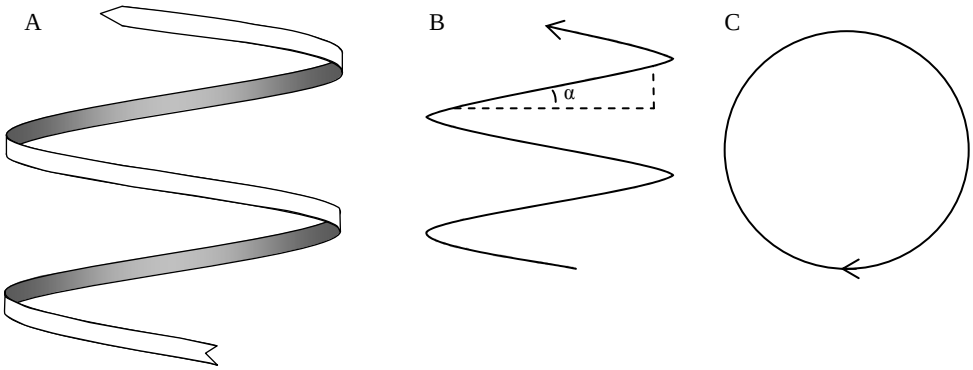


Figure 4.1. Upward helical swimming pattern of a bivalve veliger larva. A: overview, B: side-view (α = pitch of upward swimming), C: view from above.

4.1.4. Hypothesis

Regarding the expansion success of *C. gigas* in Dutch estuaries, and the parallel decline of native bivalves, we expect *C. gigas* to have a competitive advantage over native bivalves. In this context, we investigated whether *C. gigas* larvae are better able to escape or avoid filtration by adult bivalves than the larvae of the native *M. edulis*.

We hypothesized that both *C. gigas* and *M. edulis* larvae are able to detect adult bivalve feeding currents, and we studied escape responses of *C. gigas* and *M. edulis* larvae in an artificial flow field simulating a bivalve feeding current to test this hypothesis. In addition, we hypothesized that *C. gigas* larvae have higher swimming speeds than *M. edulis* larvae. If both are able to detect fluid disturbances, this may enable oyster larvae to escape faster and therefore more successfully. Additionally, higher swimming speeds and faster vertical displacement may increase survival chances in general. So firstly, we determined mean and maximum absolute swimming speeds for *C. gigas* and *M. edulis* larvae of different sizes. Secondly we compared absolute swimming speeds and vertical rate of displacement in relation to the pitch of upward and downward swimming between larvae of *C. gigas* and *M. edulis* of the same size.

4.2. Materials and methods

4.2.1. Experimental larvae

C. gigas larvae were purchased from a commercial hatchery (Seasalter Shellfish (Whitstable) Ltd., U.K.), and shipped to the laboratory at Haren, the Netherlands. They had been reared at 27 °C and 30 psu salinity. During transport they were kept in moist filtration paper in a plastic Petri-dish, cooled at 4 – 5 °C with ice packs. Transport took no more than 24 hours. *M. edulis* larvae were transported in a similar manner from the experimental *M. edulis* hatchery of Wageningen IMARES at Yerseke, the Netherlands. These larvae had been reared at 18 °C and 30 psu salinity. Different age groups were used (Tables 1-2), which were ordered and shipped separately. Upon arrival the larvae were suspended in seawater with a temperature of 4 – 5 °C and salinity of 30 psu. They were then placed in a climate chamber, at a concentration between 10 and 50 ml^{-1}, to reach a temperature of 17 °C over a period of at least three hours (see Helm et al. 2004 for protocols for transporting and acclimatizing larvae). After reaching 17 °C, the larvae were left for another hour before using them in the experiments. During the experiments, the larvae were fed with the same algae as

they had been reared on (*M. edulis*: *Isochrysis galbana* and *Pavlova lutheri*; *C. gigas*: *Pavlova* sp., *Isochrysis* sp., *Chaetoceros muelleri* and *Tetraselmis sp.*). All experiments were carried out in natural seawater with a salinity of 30 psu and completed within 4 hours. Across all size groups, the size of *C. gigas* larvae ranged from 68 to 279 μm shell length (measured as the longest distance from anterior to posterior, parallel to the hinge), and the size of *M. edulis* larvae ranged from 73 to 166 μm shell length. All larvae in one size group were of the same age.

4.2.2. Suction current

A constant suction flow field was created with an automatic pipette (Eppendorf Multipette® pro). A tube with an inner diameter of 7 mm led from the tip of the pipette horizontally into the experimental chamber (Figure 4.2). We used the lowest possible suction speed of 2199.41 $mm^3\ s^{-1}$. In the resulting flow field, flow velocities similar to those that occur in a natural bivalve feeding flow field (Troost et al. 2009b, Chapter 2) were present at a short distance from the tube inflow.

The experimental chamber was a Plexiglas flask (150 x 110 x 36 mm). The chamber was submerged in a glass aquarium filled with seawater: water removed from the flask by suction was immediately replaced through the flask opening, and water temperature changes and advection or convection currents were practically absent. By using a small experimental volume, we reduced the observation area and the amount of larvae necessary to create a sufficiently high concentration during swimming speed measurement experiments.

To characterize the suction flow field, we used neutrally buoyant synthetic white particles (Pliolyte, BASF, diam. 25 – 50 μm) to visualize the water movement. In the darkened room we then projected a laser sheet with 0.5 ± 0.2 mm thickness through the experimental vessel towards the centre of the suction tube. We used a CW Krypton laser (Coherent Innova K, Coherent Lasers Inc., USA; λ = 647 nm, P_{max} = 1 W), projected through an optical lens. Only the particles in the laser sheet were illuminated, and their movement was recorded using a high resolution digital camera (Kodak MEGAPLUS ES 1.0, 30 fps at 1018 x 1008 px resolution) that was mounted perpendicular to the laser sheet. The camera was linked to a digital acquisition system, and all filmed frames were saved in uncompressed tiff format. We recorded particle movement in a vertical as well as a horizontal plane. Digital Particle Image Velocimetry (Stamhuis and Videler 1995; Stamhuis et al. 2002) was used to obtain an overview of the entire velocity field. Image pairs were analyzed with the DPIV analysis software Swift 4.0 (developed at the University of Groningen) using convolution

filtering with interrogation areas of 65 x 65 pixels, after image enhancement to remove unevenly illuminated backgrounds and increase contrast. To locate convolution peaks, the COGW (centre of gravity, weighed to grey value, Stamhuis et al. 2002) was used. To obtain more detailed velocity vectors, movement of single particles was traced using the image digitizing software program Didge© 2.3b1 (A.J. Cullum, Creighton University, Omaha, NE, USA). Changes in direction in x (dx) and y (dy) direction were calculated, and from these, particle velocities that represent water current velocity.

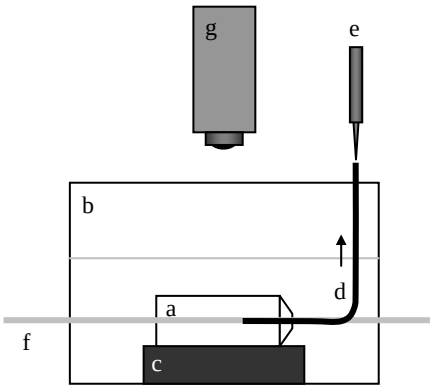


Figure 4.2. Schematic drawing of the experimental horizontal set-up for mapping the suction current velocity profile using digital particle image velocimetry and recording larval behaviour in the flow field. The experimental chamber (a) was placed in an aquarium (b), elevated on a black block (c) that also provided a black background for high contrast. The suction tube (d) was connected to an automatic pipette (e). A 2D laser sheet (f) was projected horizontally through the centre of the suction tube for observations in a horizontal plane (this figure), and vertically through the centre of the suction tube for observations in a vertical plane. After placing larvae in the experimental chamber, their movements were filmed with a high-resolution digital camera (g) that was mounted perpendicular to the laser sheet.

4.2.3. Larval movements in suction flow

To study escape reactions of larvae, the experimental chamber and aquarium were filled with filtered seawater of 17 °C. In stead of synthetic particles, bivalve larvae were added. We performed two separate experiments.

Experiment 1

In the first experiment, we used *C. gigas* larvae of 123 ± 11 μm and *M. edulis* larvae of 120 ± 18 μm in high concentrations (resp. 115 and 50 ml^{-1}) in separate experiments (Table 4.1). We used an experimental chamber with dimensions 100 x 77 x 36 mm. After introducing the larvae, they were left for one hour before a suction current was applied. For both species, movements of illuminated larvae in the suction current were recorded in a horizontal laser sheet. After this first experiment, we visualized the movement tracks of the larvae, by super-imposing a succession of 44 -100 filmed frames (after thresholding to monochrome black/white values) in Adobe Photoshop. We examined these movement paths in search for movements that could indicate escape behaviour.

Table 4.1. Larvae used in the experiments on escape reactions (Exp. 1 and 2). The age and average length (with standard deviation) are given, as well as larval concentrations, numbers of larvae digitized, and numbers of filmed frames analysed.

Exp.	Species	Date	Age	Length	Concentration	Plane	Suction	Larvae	Frames
			(days)	*(μm)*	*(n ml^{-1})*			*n*	*n*
1	*C. gigas*	6-13-'05	4	123 ± 11	115			*	
	M. edulis	10-17-'05	21	120 ± 18	50			*	
2	*C. gigas*	3-16-'07	8	173 ± 25	30	vertical	yes	42	100
	"	"	"	"	"	vertical	no	35**	100
	"	"	"	"	"	horizontal	yes	23	100
	"	"	"	"	"	horizontal	no	20	100
	M. edulis	3-14-'07	14	166 ± 12	10	vertical	yes	47	100
	"	"	"	"	"	vertical	no	23**	100
	"	"	"	"	"	horizontal	no	27	100

* these larvae were also used to study swimming speeds in a horizontal plane (§ 4.2.4. '*Horizontal*')
** these larvae were also used to study swimming speeds and pitch in a vertical plane (§ 4.2.4. '*Vertical*')

Experiment 2

In the second experiment, we used *C. gigas* larvae and *M. edulis* larvae from one size group. In separate experiments, *C. gigas* larvae of 173 ± 25 μm and *M. edulis* larvae of 166 ± 12 μm shell length were used in respective concentrations of 30 and 10 ml^{-1} (Table 4.1). After introducing the larvae we waited for one hour to start the experiment. For both larval species, movements of illuminated larvae in a vertical and

a horizontal laser sheet were recorded in separate experiments. Larval movements were first recorded in still water for approximately 10 minutes. Then, recording continued as a suction current was applied. We traced the positions of 20 - 47 *C. gigas* and *M. edulis* larvae using Didge©, in both still water and in a suction flow field throughout 50-100 filmed frames. Movement paths of larvae in a suction flow, and swimming paths of larvae in still water, were visualized by plotting the digitized *x* and *y* coordinates of the paths in a 2D map. To study swimming behaviour of larvae in a suction flow field, velocity vectors (d*x* and d*y*) of synthetic particles, representing water current vectors, were subtracted from larval movement vectors to calculate velocity vectors caused by the swimming activity of the larvae alone. In order to subtract water current vectors, the image area of 1018x1008 px was subdivided in cells of 20x20 px. Per cell, average d*x* and d*y* of single synthetic particles (traced using Didge©) were calculated from frame to frame. Per cell, these values were subtracted from all d*x* and d*y* from frame to frame of digitized movement paths of *C. gigas* and *M. edulis* larvae in the suction flow field. This yielded d*x* and d*y* net swimming vectors of the larvae. Using these swimming vectors, swimming paths of larvae in a suction flow field were reconstructed and plotted in a 2D map. In vertical planes, displacement in vertical direction was calculated from net d*y*. Rates of vertical displacement in a suction flow field were related to the distance from the suction tube aperture.

4.2.4. Swimming speeds

Horizontal

Swimming speeds of 60 *C. gigas* larvae and 30 *M. edulis* larvae were analysed, for both species in 7 size groups (Table 4.2). Larvae were filmed in a Petri-dish in seawater of 17 ± 1 °C against a black background, lit by cold light from the side. The larvae were filmed from above with the digital camera described above. A recording was considered successful when during playback a significant displacement was observed. The water column in the Petri-dish was approximately 1 cm high, and the depth of sharpness of the camera was narrower than that, resulting in recordings of swimming in a horizontal plane only. The filmed images were saved frame by frame in uncompressed tiff format to prevent digital compression artefacts that might affect swimming speed measurements. Swimming speeds were analysed frame by frame. To filter out noise, running means were calculated by averaging each swimming speed per frame with the previous and next swimming speed in the swimming trajectory of each

larva. For both species, mean swimming speeds of measured larvae were related to the mean shell length per size group.

Table 4.2. Larvae used in the horizontal swimming speed experiment. The age and average length (with standard deviation) are given, as well as numbers of larvae used in the experiment, and numbers of filmed frames that were analysed.

Species	Date (2005)	Age (days)	Average length (µm)	*n* larvae filmed	*n* frames analysed
C. gigas	April 6	8	166 ± 23	3	18-41
	April 7	2	68 ± 17	1	42
	April 12	14	279 ± 18	2	13-31
	July 1	7	183 ± 25	12	15-85
	July 13	4	123 ± 11 *	11	19-87
	July 20	10	214 ± 19	17	13-55
	July 22	12	246 ± 17	14	22-37
M. edulis	March 30	6	87 ± 7	3	18-40
	April 5	14	153 ± 18	3	42-79
	April 5	12	157 ± 14	1	19
	June 2	2	101 ± 5	2	50-62
	June 6	2	103 ± 5	4	21-61
	June 14	14	73 ± 5	9	29-204
	October 17	21	120 ± 18 *	8	21-117

* these groups were used to visualize swimming tracks (by super-imposing filmed frames) in Experiment 1 (§4.2.3.)

Vertical

To determine and compare rates of vertical displacement of *C. gigas* and *M. edulis* larvae, and relate these to the pitch of upward or downward swimming (Figure 4.1), we used the digitized swimming trajectories of larvae of both species in vertical planes without suction (section 4.2.3. *'Experiment 2'*). Per swimming trajectory per larva, we identified and isolated sections with a constant pitch. Resulting trajectory sections with constant pitch were plotted as y against x coordinates of the separate digitized locations of the larvae per filmed frame. The pitch of upward or downward swimming was determined from the slope of a fitted linear regression line. The slope was converted to a pitch in degrees by taking the inverted tangent function (pitch (°) = $\tan^{-1}$(slope)). Rates of vertical displacement encountered along these trajectories with

constant pitch were calculated from d*y*, and running means were calculated as described in the previous section. Rates of vertical displacement were related to the pitch of upward and downward swimming (ranging from -90° to 90°), and a comparison was made between *C. gigas* and *M. edulis* larvae. Sinking velocities were removed from the analyses by excluding all vertical displacement rates at negative angles larger than -85°. This was done because some sinking velocities were exceptionally large, in comparison to other swimming speeds, likely caused by a complete closure of the shell valves (Cragg, 1980).

4.2.5. Statistical analysis

Curve-fitting and non-linear regressions were performed with Sigmaplot® 2001. All other 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.

4.3. Results

4.3.1. Suction current

The suction flow field was radially symmetrical, and the velocity profile (Figure 4.3) closely fitted an exponential decay function

$$U_W(r) = u_{max} \cdot e^{-br} \qquad (4.1)$$

that relates the water current velocity U_w (mm s^{-1}) to the distance from the tip of the suction tube r (mm), with a maximum current velocity at the tip of the suction tube u_{max} of 75.85 mm s^{-1} and constant 'b' of 0.26 (non-linear regression: $R^2 = 0.95$, $p < 0.05$).

4.3.2. Escape reactions

The movement paths of larvae in a suction field, as visualized by the super-imposed filmed frames in experiment 1, did not indicate any escape reactions (Figure 4.4). Movement paths were clearly circular at the outer margins, at longer distances from the suction tube. This reflects the helical swimming behaviour of bivalve veliger larvae (Figure 4.1). Coming closer to the suction tube, movement paths became more

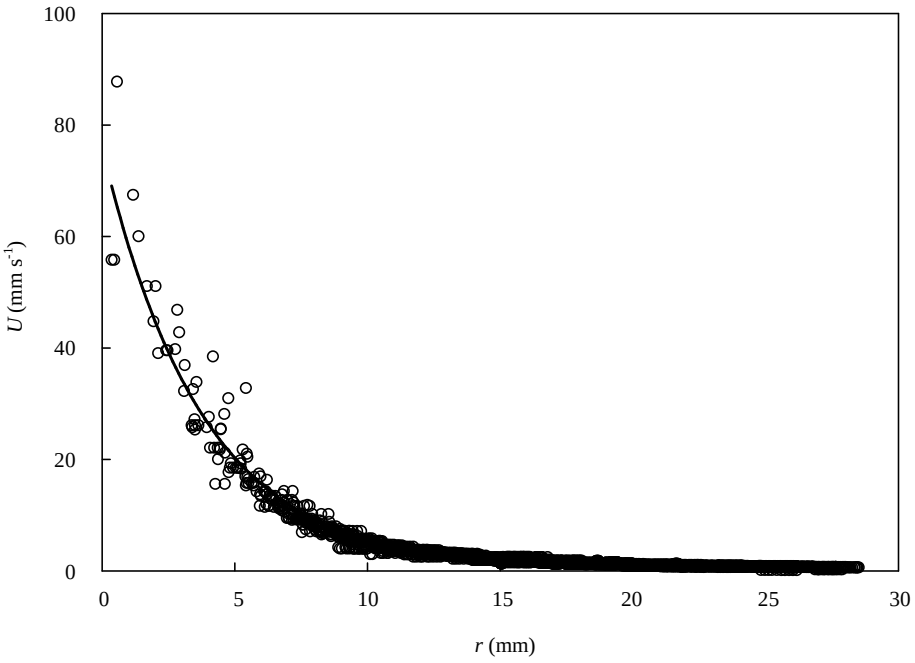


Figure 4.3. Velocity profile of the suction current, resulting from the tracing of single synthetic particles in Didge©. The suction current velocity *u* follows an exponential decay function of the distance *r* from the suction tube aperture (at $r = 0$ mm) (non-linear regression, $R^2 = 0.95$, $p < 0.05$).

elongated as their motion was distorted by the increasing super-imposed water current. No escape jumps were observed, neither were the larvae observed to turn and swim against the current. Also in experiment 2, where we included observations on movement in a vertical plane, no escape reactions were observed in the larvae of both species. This can be seen in the digitized movement paths and the reconstructed swimming paths of larvae in a suction current (water movement subtracted), in comparison to swimming paths of larvae in still water in both vertical (Figure 4.5) and horizontal (Figure 4.6) planes. The larvae of both species clearly did not show escape jumps, nor were they observed to turn to swim against the current. They continued their helical swimming behaviour. As the larvae were being sucked towards the suction tube, their swimming behaviour appeared to remain the same. In a suction flow field only 2 *M. edulis* and 4 *C. gigas* larvae were observed to sink (Figure 4.5).

These were not regarded as escape reactions since in still water similar low numbers of sinking larvae were observed (3 larvae per species; Figure 4.5). Results for sucked *M. edulis* larvae in a horizontal plane are lacking because the filmed frames were lost in a computer hard-disk failure.

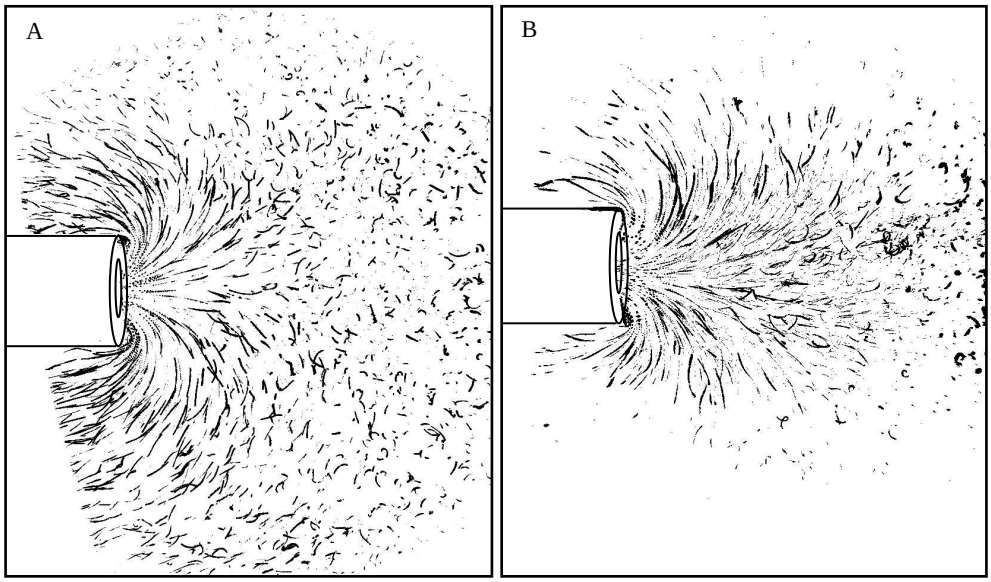


Figure 4.4. Movement paths of individual *C. gigas* larvae (A) and *M. edulis* larvae (B) in the suction flow field. A is a succession of 44 filmed frames in 1.47 seconds and B of 100 frames in 3.33 seconds. The suction tube (indicated in the left) is 12.8 mm in outer diameter.

Larvae recorded in a vertical laser sheet did not show escape responses in the form of suddenly increased rates of vertical displacement (either upward or downward due to sinking) at a certain distance from the suction tube (Figure 4.7). Rates of vertical displacement showed no relationship with distance from the suction aperture (linear regression $p > 0.05$). Coming closer to the suction aperture, the variance in vertical displacement rate increased, especially in *M. edulis* larvae. This is likely due do methodological artefacts, and not to larval behaviour. This will be explained in section 4.4.3.

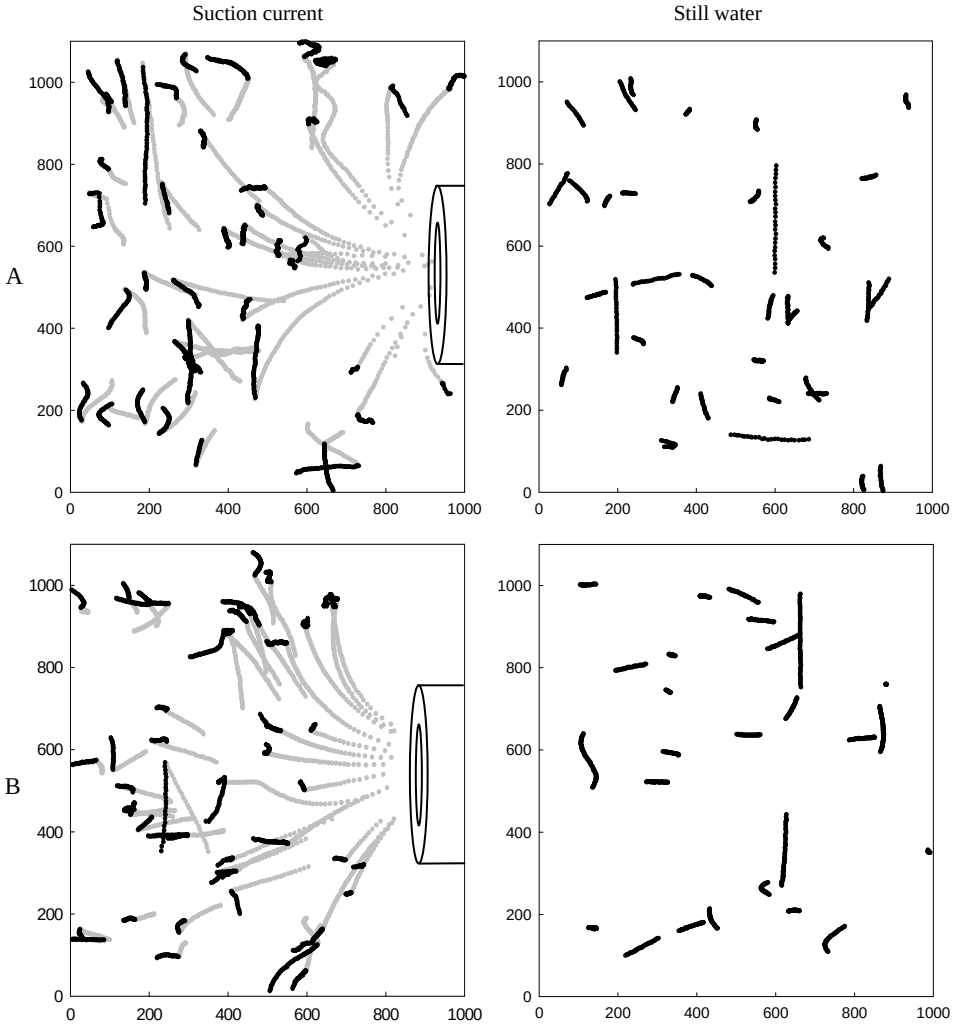


Figure 4.5. Total movement paths (grey) and (reconstructed) swimming paths (black) in a vertical plane, of individual larvae in a suction current and in still water: A) *C. gigas* larvae in a vertical plane (1000 px = 30 mm); B) *M. edulis* larvae in a vertical plane (1000 px = 30 mm). Values on the axes are in pixels (px). The suction tube (indicated in the right) is 12.8 mm in outer diameter.

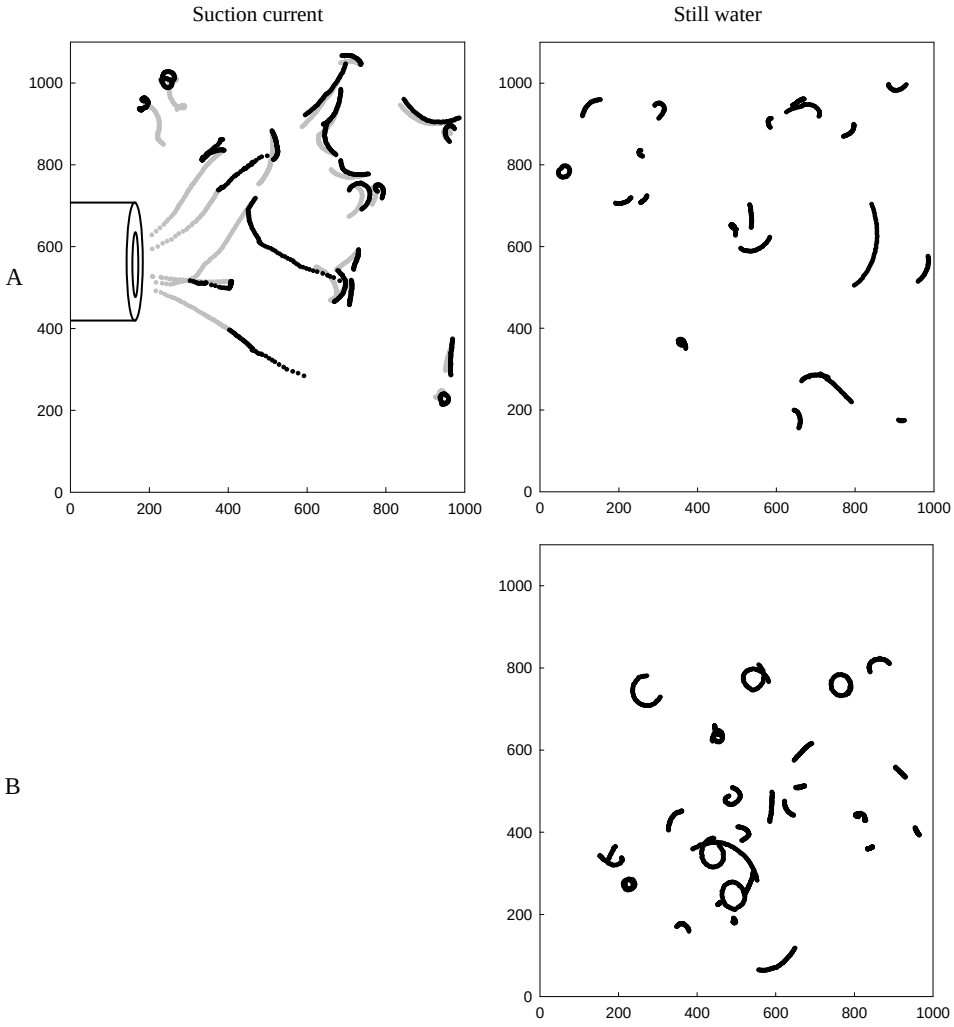


Figure 4.6. Total movement paths (grey) and (reconstructed) swimming paths (black) in a horizontal plane, of individual larvae in a suction current and in still water: A) *C. gigas* larvae in a horizontal plane (1000 px = 45 mm); B) *M. edulis* larvae in a horizontal plane (1000 px = 42 mm; no suction current observations). Values on the axes are in pixels (px). The suction tube (indicated in the left) is 12.8 mm in outer diameter.

4.3.3. Swimming speeds

Horizontal

In both species, recorded in a horizontal plane, mean swimming speeds per larva showed a linear relationship with shell length (Figure 4.8; linear regression, *C. gigas* $R^2 = 0.65$, $p < 0.05$, *M. edulis* $R^2 = 0.56$, $p < 0.05$). Swimming speeds of *C. gigas* larvae

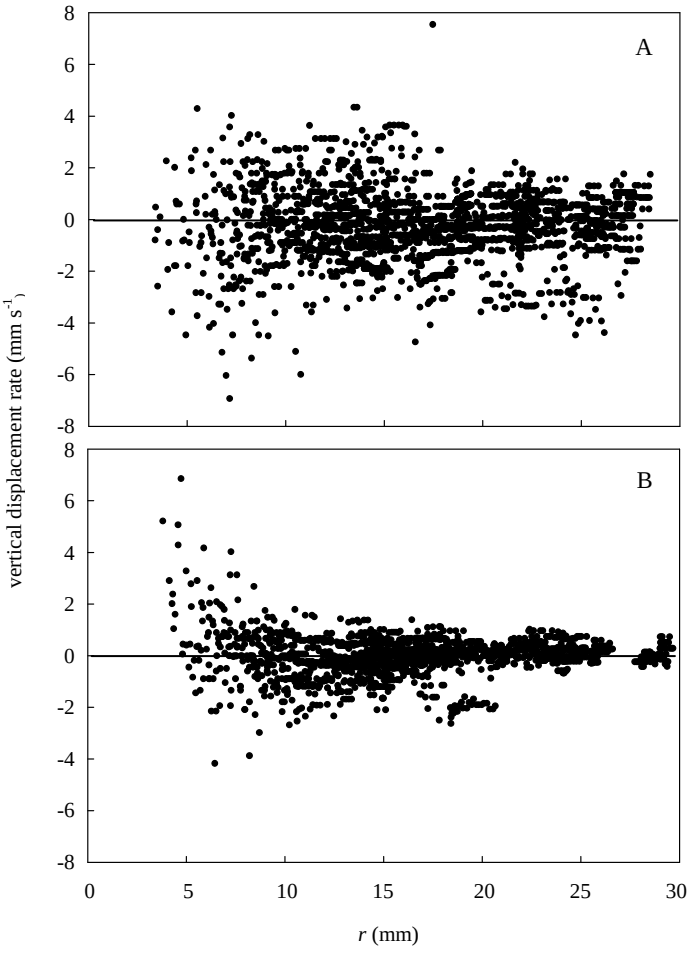


Figure 4.7. Rate of vertical displacement (in mm s^{-1}) plotted against the distance from the suction tube (*r* in mm), for: A) *C. gigas* larvae and B) *M. edulis* larvae. Complete sets of running averages of each individual larva are plotted. For both species, there was no relationship between vertical displacement rate and distance from the suction tube (linear regression $p > 0.05$).

were significantly higher than swimming speeds of *M. edulis* larvae across all size groups (Figure 4.8; GLM after ln-transformation: slopes $p > 0.05$, intercepts $p < 0.05$, difference between intercepts: 2.4 mm s^{-1} (recalculated from ln-transformed value)). Swimming speeds found in *C. gigas* larvae ranged from 0.7 to 6.5 mm s^{-1} and swimming speeds in *M. edulis* from 0.6 to 2.2 mm s^{-1} (Table 4.3).

Vertical

Speeds of vertical displacement and absolute swimming speeds were plotted for each trajectory section with a constant pitch (Figure 4.9). With an increased pitch of upward and downward swimming, vertical displacement rates of both *C. gigas* and *M. edulis* larvae increased significantly (Figure 4.9A; linear regression: *C. gigas* $R^2 = 0.86$, $p < 0.05$; *M. edulis* $R^2 = 0.91$, $p < 0.05$). With an increasing pitch, from -90° to +90°, rates of vertical displacement in *C. gigas* larvae showed a significantly stronger increase

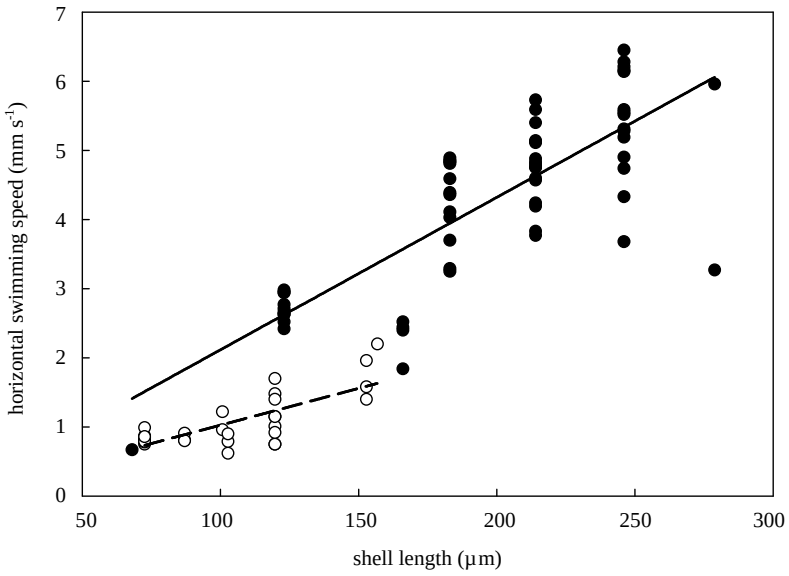


Figure 4.8. Mean swimming speeds per larva (● = *C. gigas*, ○ = *M. edulis*) in mm s^{-1}, plotted against the mean shell length in µm. Both data sets fit a linear regression (*C. gigas*, solid line, $R^2 = 0.65$, $p < 0.05$; *M. edulis*, dashed line, $R^2 = 0.56$, $p < 0.05$). After ln-transformation (not shown in this figure), intercepts were significantly different (GLM, $p < 0.05$) but slopes were not (GLM, $p > 0.05$).

than in *M. edulis* larvae (GLM slopes $p < 0.05$, intercepts $p > 0.05$). On average, larvae of *C. gigas* and *M. edulis* showed a slight downward displacement in experiments

without suction. The average rate of vertical displacement was -0.18 ± 1.26 mm s^{-1} for *C. gigas* and -0.20 ± 0.55 mm s^{-1} for *M. edulis*. This deviated significantly from 0.0 (one-sample t-test $p < 0.05$).In both species, absolute swimming speeds were not related to the pitch (Figure 4.9B; linear regression, *C. gigas* $R^2 = 0.02$, $p > 0.05$; *M. edulis* $R^2 = 0.10$, $p > 0.05$). Again, sinking velocities were excluded from the analysis.

4.4. Discussion

Both *C. gigas* and *M. edulis* larvae did not show escape responses to the simulated inhalant current. Either they can detect hydromechanical stimuli but cannot react to them, or they are unable to detect the hydromechanical signals created by a filter-feeding bivalve. Larvae of *C. gigas* swam faster than larvae of *M. edulis*, resulting in a faster displacement in vertical direction.

4.4.1. Absence of escape responses

The absence of any escape response suggests that bivalve larvae are not able to detect inhalant current velocities of adult bivalves, or at least that they are not able to induce an escape response after having detected an inhalant current. If larvae were not sensitive enough to detect our simulated feeding current, they are not likely to escape bivalve feeding currents either. In the simulated flow field, higher current speeds were present than in the inhalant flow field of a live filter-feeding bivalve. This provided a wide range in values for different possible triggers (e.g. acceleration, shear and strain rate) to respond to. If larvae are able to detect and escape from bivalve inhalant feeding currents, we should at least have seen some escape attempts. Possibly, larvae are physically unable to perform escape swimming, which should at least involve higher swimming speeds than the normal cruising speeds. From the next paragraph it follows that the larvae may already have been swimming at their maximum speeds. The larvae appear not to be equipped with sophisticated sensory organs such as the antennae of copepods to detect hydromechanical signals (e.g. Visser 2001), or swimming legs to perform quick escape jumps (e.g. Van Duren and Videler 2003).

4.4.2. Swimming speeds

In a horizontal plane, *C. gigas* larvae swam significantly faster than *M. edulis* larvae across all size groups. We also found that *C. gigas* larvae increase their rate of vertical displacement faster with increasing pitch (either upward or downward). Since absolute

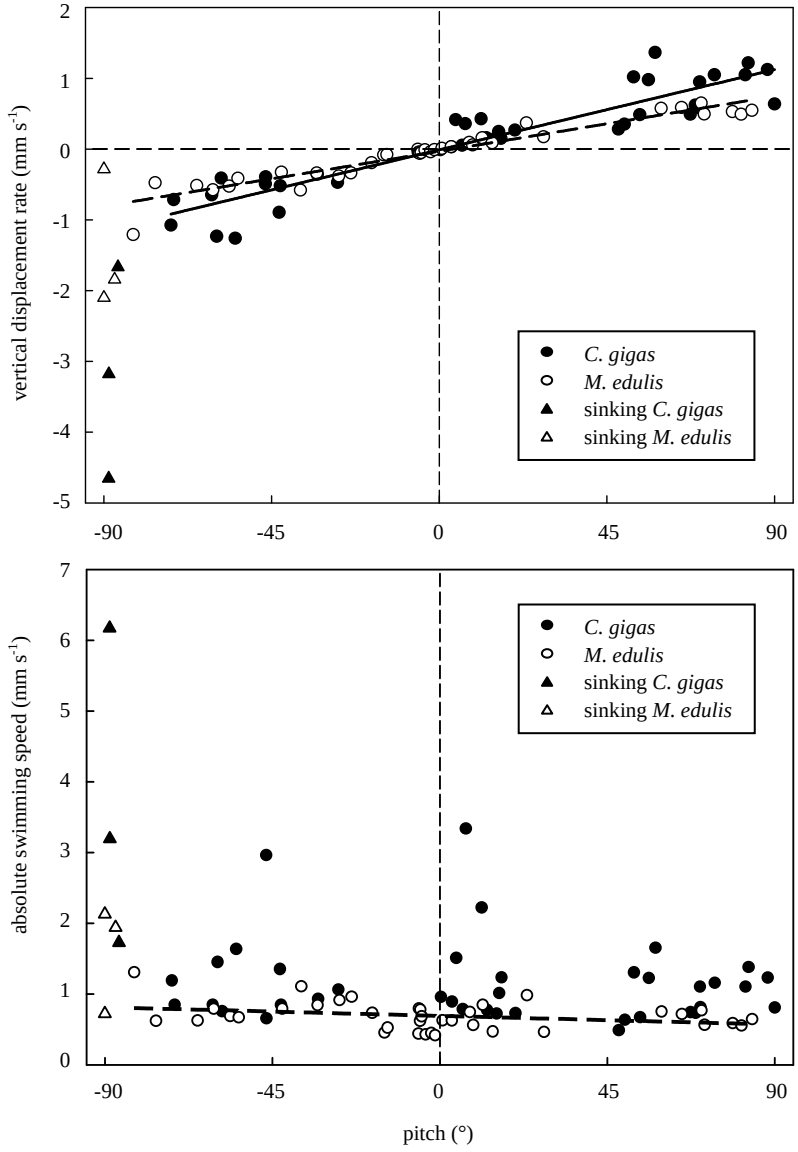


Figure 4.9. Mean vertical displacement rate and mean absolute swimming speed per larva, in *C. gigas* and *M. edulis* larvae, plotted against the pitch. Vertical displacement speeds of *C. gigas* and *M. edulis* both fit a linear regression (*C. gigas*: $R^2 = 0.82$, $p < 0.05$, solid line; *M. edulis*: $R^2 = 0.81$, $p < 0.05$, dashed line). Slopes differ significantly (GLM $p < 0.05$). Absolute swimming speeds of *C. gigas* do not fit a linear regression ($R^2 = 0.00$, $p > 0.05$), absolute swimming speeds of *M. edulis* do ($R^2 = 0.05$, $p < 0.05$, slope = -1.3×10^{-3}, dashed line). Sinking speeds (filled and open triangles) were not included in the regression analyses on vertical and absolute swimming speeds.

swimming speeds of both species were not related to the pitch, we can say that the positive relationship between vertical rate of displacement and pitch is due to an increase in the pitch itself and not due to an increase in absolute swimming speeds. The observed pitch, however, will be lower than the actual pitch of swimming because of the gravitational force on the larva (Jonsson et al. 1991). Thus, the higher increase in vertical displacement with increasing pitch of *C. gigas* larvae is a direct result of their higher swimming speeds. This means that the difference between absolute swimming speeds of *C. gigas* and *M. edulis* larvae that we measured for different size groups in a horizontal plane, is directly reflected in differences in vertical displacement rates. Although we did not compare rates of vertical displacement for a whole size range of *C. gigas* and *M. edulis* larvae, we can conclude from our results that *C. gigas* larvae move faster in vertical directions than *M. edulis* larvae. Since larvae swim with pitches of up to 90°, we can estimate that the maximum rate of vertical displacement is likely about 2 mm s^{-1} for *M. edulis* larvae and about 6 mm s^{-1} for *C. gigas* larvae, based on the results of swimming speeds in a horizontal plane and the size range of the larvae.

The double function of the velum in bivalve veliger larvae, propulsion and feeding (Widdows 1991), may explain why these larvae regulate their vertical displacement rate by changing the pitch: It allows them to continue swimming at maximum speed, thereby maximizing food intake (Jonsson et al. 1991).

Considering the above, we can say that our horizontal swimming speeds are actually swimming speeds in 'any direction', that are classified as horizontal swimming speeds because the water column was merely 1 cm high, forcing the larvae to swim in a horizontal plane and not allowing them to swim upward.

Observed swimming speeds of *M. edulis* larvae were in the same range as swimming speeds of bivalve larvae found in earlier studies (Table 4.3). *C. gigas* larvae swam significantly faster than all previously studied bivalve larvae except for one species: observed swimming speeds of *C. gigas* larvae were comparable to swimming speeds of the larvae of *Crassostrea virginica*. The values for upward and downward swimming, as well as sinking, were comparable to the values found by Hidu and Haskin (1978) and Mann (1988). Mann and Rainer (1990) recorded an upward maximum vertical displacement of 5.0 mm s^{-1} in *C. virginica* larvae of 290 μm, which is much higher than our 0.9 mm s^{-1} for *C. gigas*, but agrees well with the maximum swimming speed of around 6 mm s^{-1} we estimated above. Wood and Hargis (1971) reported an observed absolute swimming speed of 10 mm s^{-1} for larvae of *C. virginica*, but this is an anecdotal remark and the authors do not elaborate on methodology and results.

Table 4.3. Mean swimming speeds of bivalve larvae. Where maximum values are given, these are expressed in *italics*. Where a range of all recorded speeds is given, in stead of a range in mean or maximum values, this is indicated with an *'r'*. All swimming speeds were determined at salinities between 22 and 33 psu, at 1 bar pressure and at temperatures between 12 and 25 °C (see references). In the cases of swimming in 'any direction', the exact direction was unspecified for *A. islandica* and *M. edulis*.

Species	Size	Mean/*max.* swimming speeds					Source
		horizontal	up	down	sinking	any direction	
	(µm)	*(mm s^{-1})*	*(mm s^{-1})*	*(mm s^{-1})*	*(mm s^{-1})*	*(mm s^{-1})*	
Arctica islandica	170 - 202	-	0.3 – 0.4	-	-	0.5 – 0.8	1
Cerastoderma edule	280	-	0.9	1.3	1.7	-	2
Crassostrea gigas	68 - 279	0.7 – 6.5 *r*	-	-	-	-	TS
" "	173	-	0.9	1.4	3.2	-	TS
Crassostrea virginica	75 - 300	*0.3 – 0.8*	*0.8 – 2.3*	-	*1.7 – 8.3*	-	3
" "	65 - 160	-	0.4 – 1.0	-	-	-	4
" "	120 - 300	-	-	-	1 - 4	-	4
" "	77 - 290	-	*1.4 - 5.0*	-	-	-	13
Ostrea edulis	-	-	1.2	-	-	-	6 (in 5)
Mercenaria mercenaria	-	-	*1.3*	-	-	-	9 (in 8)
" "	-	-	1.2 – 1.3	-	-	-	7 (in 5)
Mytilus edulis	255	-	-	-	-	1.1	10
" "	226 - 261	1.3 – 3.3	-	-	-	-	11
" "	73 - 157	0.6 – 2.2 *r*	-	-	-	-	TS
" "	166	-	0.7	0.4	1.4	-	TS
Pecten maximus	(3-41 days)	-	0.2 – 0.5	-	-	*2.2*	5
Spisula solidissima	96 - 196	-	0.2 – 0.5	0.2 – 0.4	0.6 – 2.2	-	12

1) Mann and Wolf 1983; 2) Jonsson et al. 1991; 3) Hidu and Haskin 1978; 4) Mann 1988; 5) Cragg 1980; 6) Cragg and Gruffydd 1975; 7) Turner and George 1955; 8) Chia et al. 1984; 9) Carriker 1961; 10) Konstantinova 1966; 11) Sprung 1984; 12) Mann et al. 1991; 13) Mann and Rainer 1990; TS) this study.

Although linear functions described the relationship between larval length and swimming speed most accurately (Figure 4.8), the swimming speeds likely reach a plateau or an optimum at a certain shell length, such as found by Cragg (1980) for larvae of *Pecten maximus* and by Hidu and Haskin (1978) for larvae of *C. virginica*. The largest size group of *C. gigas* larvae indeed show decreased swimming speeds, although only in two observations, indicating a cessation in the increase in swimming speeds or even a decrease. *M. edulis* larvae do not show a plateau or an optimum at all. An optimum was likely not yet reached in *M. edulis* larvae because we did not include the largest larval stages (up to pediveliger stage).

The difference in swimming speed between *M. edulis* and *C. gigas* is not only caused by a difference in size or size range used. Also at comparable shell lengths, *C. gigas* larvae swam faster than *M. edulis* larvae. Regarding swimming speeds in a horizontal plane (Petri-dish), *C. gigas* larvae from all size groups swam on average with a speed of 18.0 body lengths (bl = shell lengths) s^{-1} and *M. edulis* larvae with a speed of 10.5 bl s^{-1}, both independent of shell length or age (linear regression, $p > 0.05$).

4.4.3. Methodological considerations

Due to the limited imaging frequency of our camera (30 fps) and the linear approximation of the particle and larval velocities, we might have underestimated particle displacement and larval swimming velocities close to the suction tube aperture. This is, however, expected to have had minor consequences for our results since the underestimation can be assumed to be the same for both particles and larvae at the same location in the flow field.

Observations and measurements on particles and on larvae were made in separate experiments. Minor differences in e.g. the position of the suction tube in the transparent measurement chamber may have decreased the fit of water movement (synthetic particles) and larval movement. This may have increased the variances in the resulting data, especially closer to the suction tube opening, as e.g. shown in the vertical displacement rates towards the suction tube (Figure 4.7). The conclusions based on the experimental data do, however, hardly suffer from this increase in variance.

Furthermore, we do not expect differences in larval concentrations (Table 4.1) to have affected swimming speeds or inhibited escape reactions. In Pacific oyster hatcheries, concentrations of 5 up to 57 ml^{-1} are generally used without significant negative effects on larval health (Helm et al. 2004). Therefore, significant effects on larval behaviour were also not expected. During the experiments, collisions between larvae were observed only occasionally.

From hatchery to experiments *C. gigas* larvae experienced a change from 27 to 5 to 17 °C. *M. edulis* larvae experienced a change from 18 to 5 to 17 °C. This falls within the limits of, a protocol for transport and acclimatization that is generally used in hatchery practice (Helm et al. 2004). On visual inspection the larvae appeared to be healthy and behaving normally. Therefore, we do not expect serious effects on swimming performance.

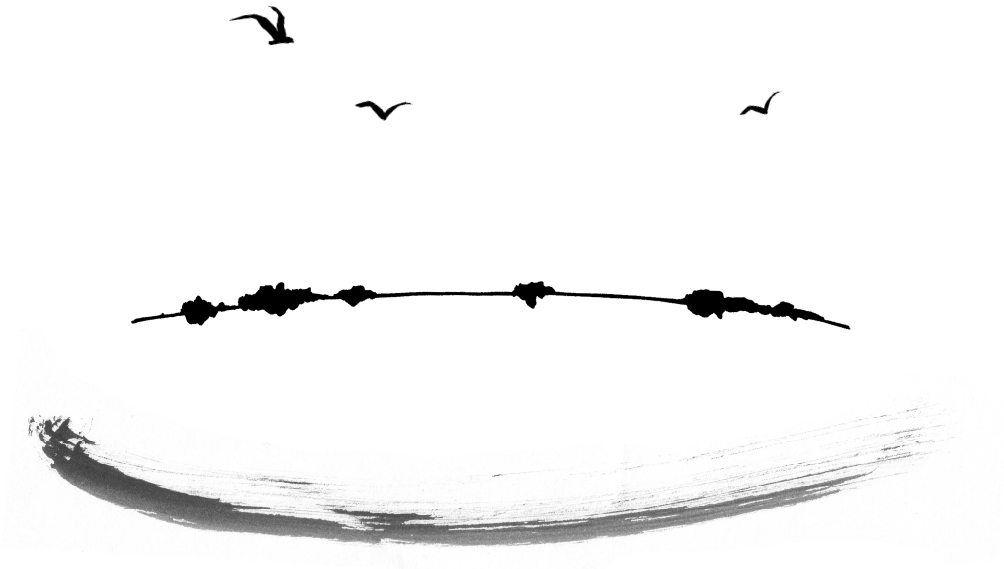
The larvae showed no reaction to the laser light; no attraction, nor avoidance.

4.4.4. Ecological implications

Since larvae of both species showed no escape responses, the expansion of *C. gigas* in Dutch waters, seemingly at the cost of native bivalves, cannot be explained by better escape abilities of their larvae in comparison to the larvae of native bivalves. The absence of escape responses in bivalve larvae theoretically makes them an easy prey for bivalve filter-feeders. Besides various other sources of high natural mortality (e.g. Thorson, 1950; Rumrill, 1990; Gosselin and Qian, 1997), bivalve larvae might suffer substantial mortality due to bivalve filtration. High mortality rates are, however, a natural phenomenon for planktonic larvae that are produced in very high numbers (see Helm et al., 2004).

Additionally, larvae may not have shown direct escape responses to a suction current, but they might avoid filtration by benthic suspension feeders in a more indirect manner through regulation of their vertical position in the water column. Bivalve larvae have been shown to migrate in vertical directions and to respond to directional indicators such as pressure, gravity and light (Bayne 1963, 1964b; Mann and Wolf 1983). For instance, young *M. edulis* larvae are reported to occupy higher water levels through phototaxis and negative geotaxis, thereby possibly avoiding benthic filter-feeders (Thorson 1950; Bayne 1964b). In this respect, the significantly higher swimming speeds of Pacific oyster larvae can be speculated to offer them competitive advantages over larvae of native bivalves. Theoretically, *C. gigas* larvae can move faster vertically to other water layers than *M. edulis* larvae, enabling them to either avoid benthic predators, find layers with more food (Raby et al. 1994) or to transport themselves with the tides in favourable directions (Shanks and Brink 2005). Whether they actually do so in the field remains open for further research.

Chapter 5



Abstract
Predation by adult bivalves on bivalve larvae has been suggested to significantly reduce numbers of bivalve larvae and their settlement success in areas with high filter-feeder biomass. Larvae of *Crassostrea gigas* have been found to avoid predation in laboratory experiments and must therefore be able to detect adult bivalve filter-feeders. *C. gigas* larvae do not respond to inhalant feeding currents, leaving the possibility that they avoid filtration by detecting and responding to chemical substances associated with adult bivalve filter-feeders. A simple experiment was conducted to establish whether bivalve larvae (*C. gigas* and *Mytilus edulis*) avoid the vicinity of an adult bivalve filter-feeder (*C. gigas*) by migrating to higher water layers. The adult bivalve was tied shut, preventing it from circulating water and predating larvae, after it had been feeding for 2 hours to allow a build-up of metabolite concentrations. The results showed that *C. gigas* larvae responded to the presence of adult bivalves by migrating upward and by keeping away from the bottom. Larvae of *M. edulis* did not redistribute to higher water layers, but did show a response in that more larvae stayed clear from the bottom. This study indicates an ability of bivalve larvae to chemically detect an adult bivalve, to avoid its vicinity and thereby avoid predation.

Vertical migration of bivalve veliger larvae in response to adult filter-feeder presence

Karin Troost

5.1. Introduction

Predation by adult bivalves on bivalve larvae has been suggested to significantly reduce numbers of bivalve larvae and their settlement success in waters with high bivalve filter-feeder biomass (André and Rosenberg 1991; Lehane and Davenport 2004; Troost et al. 2009a, Chapter 6). Larvae of many bivalve species settle in dense aggregations (Bayne 1969; Hidu et al. 1978; Burke 1986; Cáceres-Martínez et al. 1994; Pascual and Zampatti 1995; Tamburri et al. 2007). Competent larvae move towards the bottom to find a suitable substrate to settle onto. For these larvae, advantages of settling into a bed of conspecific adults (see Jonsson et al. 1991; André et al. 1993; Rodríguez et al. 1993; Pechenik 1999) may outweigh the risk of being filtered. For younger larvae that are not yet competent to settle it would seem more advantageous to avoid benthic filter-feeders.

The ability of bivalve larvae to avoid or escape filtration has seldom been studied. Early-stage veliger larvae of *Mytilus edulis* L. have been demonstrated to occupy higher water layers as a result of phototaxis and negative geotaxis (Bayne 1964b), thereby avoiding benthic filter-feeders. However, veliger larvae of the same species have also been shown to be distributed homogeneously throughout the water column in tidally driven turbulent waters such as the Irish Sea (Knights et al. 2006) and the Dutch Oosterschelde estuary (Troost et al. 2009a, Chapter 6). Results of a laboratory study by Troost et al. (2008a, Chapter 3) indicated an ability of *Crassostrea gigas* (Thunberg) larvae to avoid predation by bivalve filter-feeders. Larvae of *C. gigas* were filtered 50% less than either *M. edulis* larvae or micro-algae by three species of adult bivalves. In another study, Troost et al. (2008b, Chapter 4) showed that larvae of *C. gigas* and *M. edulis* showed no avoidance nor escape responses to an artificial inhalant feeding current and suggested that they are unable to detect bivalve feeding currents. Chemical detection of adult bivalve filter-feeders may offer an alternative explanation for the

observed lower filtration rate of *C. gigas* larvae, in comparison to *M. edulis* larvae, by adult bivalves (Troost et al. 2008a, Chapter 3). Larvae of bivalve species are reported to respond to contaminants, increased hydrostatic pressure and mechanical disturbances by increasing their upward swimming speed (Cragg 1980, Prael et al. 2001), or by retracting the velum and sinking rapidly (LaBarbera 1974, Cragg 1980). The null-hypothesis to be tested was that the larvae of both species do not respond to the presence of an adult bivalve by migrating upward or by sinking downward.

5.2. Materials and methods

5.2.1. Experimental animals - adults

In November 2006, adult oysters (*C. gigas*) were collected by hand from an intertidal oyster bed in the Oosterschelde estuary. They ranged from 0.53 to 1.23 grams ash-free dry flesh weight (afdw) and measured 9 ± 1 cm. All specimens were transported dry and on ice to the laboratory at Yerseke as soon as possible but within 24 hours. They were left to acclimatize in aerated natural seawater (30 psu and 18 / 21 °C, depending on the experiment) for at least two weeks. 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*. Helm et al. (2004) and Reed Mariculture (www.reed-mariculture.com) were consulted to calculate food rations suitable for growth (approximately 2 g dry weight of Shellfish Diet® per 100 g wet meat weight of bivalves).

5.2.2. Experimental animals - larvae

C. gigas veliger larvae were purchased from a commercial hatchery (Seasalter Shellfish (Whitstable) Ltd., UK) in December 2006, and shipped to the laboratory at Yerseke, the Netherlands. These larvae were in the veliconcha (umbo) stage, and their average shell length was 88.1 ± 1.4 (s.e., n = 30) µm. The larvae were reared at 27 ºC and 30 psu. During transport, the larvae were kept on ice in moist filtration paper. Transport took no more than 24 hours. Upon arrival, the larvae were submerged in 2-3 litres 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 hours (protocol after Helm et al. 2004). This temperature corresponds with seawater temperatures during the reproductive season (in the Netherlands July – September; unpublished data Wageningen 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.*). Helm et al. (2004) and Reed Mariculture (www.reed-mariculture.com) were followed in calculating a food ration suitable for growth. *M. edulis* larvae were produced in January 2007 in the experimental mussel hatchery of Wageningen IMARES at the laboratory at Yerseke. The larvae were in the veliconcha (straight-hinge) stage, and their average shell length was 129.3 ± 1.2 μm. The *M. edulis* larvae were reared at a water temperature of 18 °C and this temperature was maintained during the experiment. This temperature is at the higher end of the range of seawater temperatures occurring during the reproductive season (in the Netherlands May – June; unpublished data Wageningen IMARES and RIKZ).

5.2.3. Vertical distribution

Responses of *C. gigas* and *M. edulis* larvae were studied in two separate experiments. Per experiment eight grazing chambers with 8 litres of filtered (0.2 μm) seawater were used. At the bottoms of four of these chambers one adult oyster (*C. gigas*) of 9 ± 1 cm shell length was placed. Algae (*I. galbana* and *Chaetoceros calcitrans*) were added to these 4 chambers and the animals were left to feed for 2 hours, to allow metabolites to accumulate in the water column. The valves of the oysters were then tied together with elastic bands to exclude the effect of filtration of larvae on the vertical distribution of the larvae. Algae were added to the other 4 chambers without adult oysters (3×10^4 to 4×10^4 cells ml^{-1}) and replenished in the chambers with oysters to the same concentration. After adding *C. gigas* larvae to a concentration of 10.4 ± 0.5 (s.e., $n = 8$) larvae ml^{-1} in each chamber in the first experiment at t = 0 and *M. edulis* larvae to a concentration of 5.0 ± 0.4 (s.e., $n = 8$) larvae ml^{-1} in the second experiment, the water was stirred gently for a few seconds. After 50 minutes, water samples of 12 ml were taken from all chambers, at three different levels above the bottom: 2, 11 and 19.5 cm. After 90 minutes, a second set of water samples was taken. Samples were fixated with Lugol's solution and the larvae were counted the next day. Larval concentrations at all three levels were determined and the proportion of added larvae present in the water column was calculated by multiplying the average concentration per chamber (average of all three levels, in number ml^{-1}) with the water volume (in ml).

5.3. Results

5.3.1. Oyster larvae

At t = 50 (min.), *C. gigas* larvae were distributed homogeneously throughout the water column in chambers both with and without oysters; there were no significant differences between levels and treatments (Figure 5.1; ANOVA, $p > 0.05$). At t = 90 the larvae in chambers without adult oysters were still distributed homogeneously over

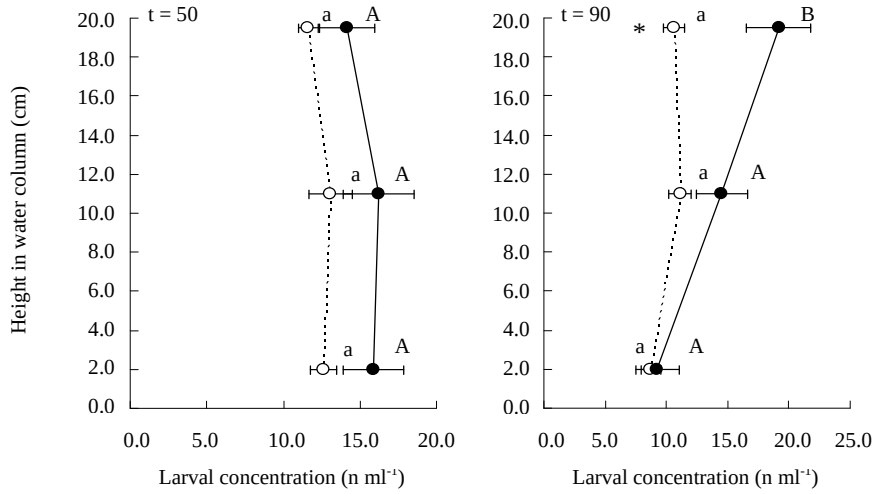


Figure 5.1. Average *Crassostrea gigas* larvae concentrations ($n = 4$) at three levels in chambers with (filled circles) and without (open circles) an adult oyster at t = 50 and t = 90, with standard errors. An asterisk denotes a significant difference between concentrations in presence and absence of an oyster at a specific level (ANOVA, $p > 0.05$). Different letters indicate significant differences (ANOVA + Bonferroni, $p < 0.05$) between concentrations at different levels for chambers with (upper case) and without (lower case) oysters separately.

the water column (ANOVA, $p > 0.05$), whereas larvae in chambers with oysters had redistributed to higher water layers (Figure 5.1). They were found in significantly higher concentrations near the surface than near the bottom (ANOVA + Bonferroni, $p < 0.05$). At the near-surface level, larval concentrations were higher in chambers with oysters than in chambers without oyster (ANOVA, $p < 0.05$). Of the larvae added, 93% was still in suspension in chambers with oysters at t = 50, and 75% in chambers without oysters (Table 5.1). At t = 90, 85% was still in suspension in

chambers with oysters and 61% in chambers without oysters. These differences between treatments were not significant (ANOVA, $p > 0.05$). The proportion of larvae in suspension declined significantly from t = 50 to t = 90 in chambers without oysters (ANOVA, $p < 0.05$) but not in chambers with oysters (ANOVA, $p > 0.05$).

Table 5.1. Mean proportions of added larvae still in suspension at t = 50 and t = 90 with standard errors. Significant differences per experiment (larval species) between chambers with and without an adult oyster are shown with asterisks (ANOVA, $p < 0.05$). Significant differences per experiment between t = 50 and t = 90 are given in a separate column (ANOVA, $p < 0.05$).

Larval species	Adult oyster	Replicas	Mean proportion in suspension ± s.e.		Significant difference
		(n)	*t = 50 (%)*	*t = 90 (%)*	*(p < 0.05)*
Crassostrea gigas	*absent*	4	74.7 ± 4.3	60.7 ± 3.0	*
	present	4	92.5 ± 4.0	85.0 ± 5.6	n.s.
Mytilus edulis	*absent*	4	23.0 ± 4.7 *	25.3 ± 6.3 *	n.s.
	present	4	68.9 ± 7.7 *	49.2 ± 7.3 *	n.s.

5.3.2. Mussel larvae

The vertical distribution pattern of *M. edulis* larvae showed no difference between treatments. In both treatments, at t = 50 and t = 90, larvae were distributed homogeneously (Figure 5.2). Larval concentrations were consistently lower in chambers without oysters, significantly so at levels of 2 and 11 cm above the bottom at t = 50 (ANOVA, $p < 0.05$). At t = 90 no differences were found between levels per treatment and between treatments (ANOVA, $p > 0.05$). A higher proportion of larvae remained in suspension in chambers with oysters than in chambers without oysters, at t = 50 (respectively 69 and 23%; Table 5.1) and at t = 90 (49 and 25%; Table 5.1) (ANOVA, $p < 0.05$). The proportion of larvae in suspension did not change significantly between t = 50 and t = 90 in both treatments (ANOVA, $p > 0.05$).

5.4. Discussion

The presence of an adult oyster stimulated *C. gigas* larvae to keep away from the bottom and to migrate to higher water layers. This follows not only from the difference in vertical distribution pattern between treatments, but also from the

observation that proportions of larvae still in suspension decreased in chambers without oysters but not in chambers with oysters. The latter decrease was likely caused by larvae accumulating at the bottom, which was indeed observed visually. In chambers without oysters a clearly higher accumulation of *C. gigas* larvae was observed at the bottom (visible as a brownish hue) than in chambers with oysters.

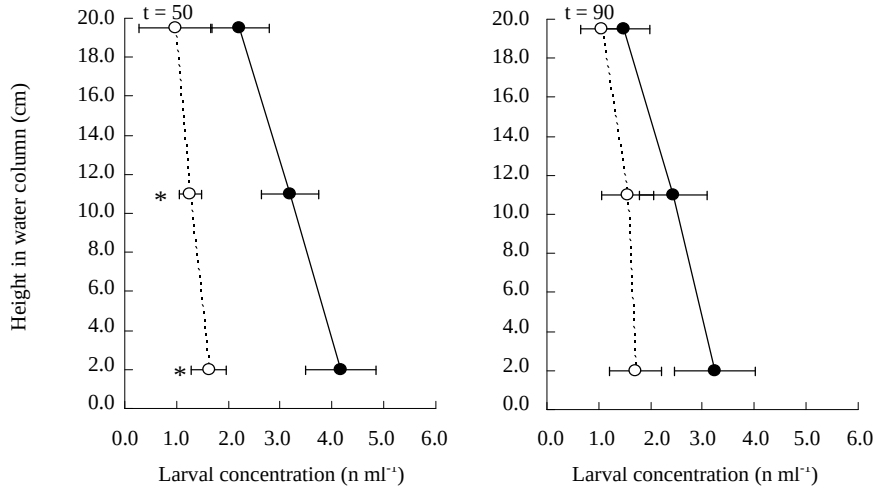


Figure 5.2. *Mytilus edulis* larvae concentrations at three levels in chambers with (filled circles) and without (open circles) an adult oyster at t = 50 and t = 90, with standard errors. An asterisk denotes a significant difference between concentrations in presence and absence of an oyster at a specific level (ANOVA, $p < 0.05$). No significant differences in larval concentrations were found between different levels.

Although *M. edulis* larvae did not change their vertical distribution pattern in response to the presence of an oyster, they did remain in suspension in higher proportions in chambers where oysters were present. The presence of an adult oyster must have stimulated the *M. edulis* larvae to stay in the water column, although it did not stimulate them to move to higher water levels.

Differences between species in the number of larvae added are not expected to have affected the comparison between species. Concentrations of *C. gigas* larvae were higher but always below 30 ml^{-1}. In Pacific oyster hatcheries, concentrations of 5 up to 57 ml^{-1} are generally used without significant negative effects on larval production (Helm et al. 2004). At the higher end of this range, growth and survival are decreased slightly, but mainly because the food supply becomes more critical. The experiments with oyster and mussel larvae only lasted for a few hours.

Larvae of *M. edulis* accumulated in much higher proportions at the bottom than larvae of *C. gigas*. This does not necessarily imply that *M. edulis* larvae were stressed more than *C. gigas* larvae. *C. gigas* larvae received a more stressful treatment (transport of at least 18 hours and subsequent acclimatization) than *M. edulis* larvae that were hatched in the same laboratory as where the experiment was carried out. The *C. gigas* larvae performed well and did not appear stressed. Larvae of both species from the same batches were observed for a few days after the experiments. They appeared healthy and did not show abnormal mortalities. Moreover, in bivalve hatcheries larvae are commonly observed to accumulate near the bottom when the water is not mixed vigorously, without showing signs of stress, disease or mortality (pers. comm. Antoine Pennec). These larvae are generally observed to be moving lively in a water layer of < 1 cm above the bottom (pers. comm. Ainhoa Blanco and Pauline Kamermans). I am therefore confident that the larvae were healthy and that the observed differences between the treatments were caused by the treatments alone. Furthermore, larvae accumulating near the bottom only reduced the concentrations but did not affect the comparison between treatments of vertical distributions in the water column. Why the larvae accumulated at the bottom is not clear.

Concluding, larvae of both species must have detected the presence of the adult oyster in some way. For both species significant differences were found between treatments that can only have been caused by the absence or presence of an oyster. Since the oysters were tied there were no hydromechanical differences between the treatments. The only differences between treatments were the physical presence or absence of an oyster and the presence or absence of its metabolites. There is also the hypothetical possibility that the rubber bands used to tie the oysters had an effect. This is unlikely, however, because the results of this study match so well with the results found by Troost et al. (2008a, Chapter 3) in whose experiments no rubber bands were used. Since in those experiments *C. gigas* larvae avoided filtration by three different bivalve species, it seems likely that the larvae detected and responded to a chemical substance that is produced commonly by these adult bivalves. In our experiment, responses of *C. gigas* and *M. edulis* larvae to the presence of an adult oyster may have been induced by the oyster's metabolites or substances originating from, or bound to, its shell. *C. gigas* larvae may for instance use the same waterborne triggers (e.g. metabolites, see Turner et al. 1994 and references therein) or substrate-bound triggers (e.g. biofilms, see Turner et al. 1994 and references therein) that induce settlement in later life stages to avoid adult filter-feeders in earlier life stages. This study was designed to investigate whether bivalve larvae respond to the presence of an adult filter-feeder, but not to identify the chemical cue. Reduced levels of oxygen due

to the metabolic activity of adult oysters are not a likely cause for the observed differences, since an adult oyster consumes roughly 1.25 µl O_2 l^{-1} in 2 hours (Gerdes 1983) which in my experiments is only 0.02% of full O_2 saturation (Mann & Rainer 1990).

The results indicate an ability of bivalve veliger larvae to detect and avoid the presence of adult bivalve filter-feeders. The upward migration of *C. gigas* in response to the presence of adult *C. gigas* may at least partly explain why Troost et al. (2008a, Chapter 3) found *C. gigas* larvae to be filtered less than *M. edulis* larvae.

Settlement of oyster larvae in response to waterborne adult chemical cues is enhanced in still water as well as flowing water resembling hydrodynamic conditions in the field (Turner et al. 1994; Tamburri et al. 2007). Whether the observed avoidance of adult filter-feeders by *C. gigas* larvae, which also appears to be chemically-induced, effectively reduces the filtration risk in turbulent conditions in the field remains open for further research.

Chapter 6

Abstract
Predation by adult bivalves on bivalve larvae has been suggested to reduce larval abundance in areas with high bivalve filter-feeder biomass. Although the occurrence of larviphagy is well-studied in the laboratory, its effects in the field have scarcely been studied. We studied larviphagy at different spatial scales in the Oosterschelde estuary. On the scale of individuals, we confirmed that larviphagy occurs in *Crassostrea gigas* and *Mytilus edulis* in the Oosterschelde estuary, by examining stomach contents of adult bivalves. On a local scale, we studied effects of larviphagy by a Pacific oyster (*C. gigas*) bed on presence of larvae in the overlying water column by sampling larvae with fixed plankton nets. Abundance of blue mussel (*M. edulis*) larvae was significantly reduced by the oyster. Abundance of *C. gigas* larvae did not seem to be reduced by the oyster bed, but spawning by the adult oysters during the sampling period may have affected the results. On estuary-scale, the effect of larviphagy on larval abundance of *C. gigas* and *M. edulis* was studied using existing monitoring data over 6 years for *M. edulis* and 13 years for *C. gigas*. Numbers of *M. edulis* larvae showed no significant trend over the 6 years studied. Abundance of *C. gigas* larvae declined with an increasing filter-feeder stock (that was mainly caused by an increase in *C. gigas* stock). This decline may be due to direct effects of larviphagy or indirect effects such as lowered food levels, and was not compensated by an increased larval production. All results combined, complemented with a theoretical estimate of the effect of larviphagy on estuary-scale, strongly suggest that larviphagy is major source of mortality for bivalve larvae in the Oosterschelde estuary.

Effects of an increasing filter-feeder stock on larval abundance in the Oosterschelde estuary (SW Netherlands)

Karin Troost, Edzard Gelderman, Pauline Kamermans, Aad C. Smaal, Wim J. Wolff

6.1. Introduction

Predation by adult bivalves on bivalve larvae seems a wide-spread phenomenon. It is suggested to reduce numbers of bivalve larvae in waters with a high adult bivalve filter-feeder biomass (Lehane and Davenport 2004) and it has been demonstrated to reduce settlement success of conspecific larvae in *Cerastoderma edule* (L.) (André and Rosenberg 1991). Timko (1979) defined the term 'larviphagy' as the feeding by adults on their own larvae. Some species have been shown to predate their own larvae: *Mytilus edulis* L. (Lehane and Davenport 2004), *C. edule* (Kristensen 1957; André et al. 1993), *Crassostrea virginica* (Gmelin) (Tamburri and Zimmer-Faust 1996) and *Dreissena polymorpha* (Pallas) (MacIsaac et al. 1991). Lehane & Davenport (2004) already suggested that bivalves routinely filter larvae from the surrounding water. Because bivalve filter-feeders filter all particles above a certain threshold size (Møhlenberg and Riisgård 1978), they do not seem able to select certain particles above this threshold size. Selection only seems to occur afterwards by the gills, labial palps, stomach and guts (Shumway et al. 1985; Ward et al. 1998; Brillant and MacDonald 2002). Hence, we broadened the definition of larviphagy following Troost et al. (2008a, Chapter 3) to: filtration and ingestion of bivalve larvae by adult bivalves in general. Overall, larviphagy may pose a significant threat to larvae of all bivalve filter-feeders as well as other meroplankton with weak swimming and escape abilities (see Singarajah 1969, 1975; Kiørboe and Visser 1999; Troost et al. 2008b, Chapter 4).

Larviphagy has been demonstrated in laboratory experiments for *M. edulis* (Lehane and Davenport 2004; Troost et al. 2008a, Chapter 3), *C. edule* (Kristensen 1957; André et al. 1993; Troost et al. 2008a, Chapter 3), *C. virginica* (Tamburri and Zimmer-Faust 1996), *Crassostrea gigas* (Thunberg) (Tamburri et al. 2007; Troost et al. 2008a, Chapter 3), and *D. polymorpha* (MacIsaac et al. 1991). Studying larviphagy in the laboratory

generally focused on clearance experiments in confined volumes of water (MacIsaac et al. 1991; Troost et al. 2008a, Chapter 3), analysis of stomach contents and excreta (MacIsaac et al. 1991; Tamburri et al. 1996; Lehane and Davenport 2004; Tamburri et al. 2007; Troost et al. 2008a, Chapter 3) and observations on larvae being sucked in by individual bivalves (André et al. 1993; Tamburri et al. 2007). In field experiments larviphagy has been shown to occur in *M. edulis* (Thorson 1946; Lehane and Davenport 2002, 2004; Maar et al. 2007) and *Mytilus galloprovincialis* Lamarck (Jasprica et al. 1997). In these experiments, stomach contents and excreta were analyzed (Jasprica et al. 1997; Lehane and Davenport 2002, 2004). Although previous laboratory and field studies convincingly demonstrated the occurrence of larviphagy in individual bivalves, effects of larviphagy on a larger scale in the field are still scarcely studied (André and Rosenberg 1991; Maar et al. 2007).

In the Oosterschelde estuary (SW Netherlands), larval mortality due to larviphagy is expected to have increased over the last three decades due to rapid expansion of the introduced Pacific oyster *C. gigas*. After being first introduced in 1964 (Drinkwaard 1999b) these oysters started to expand rapidly throughout Dutch estuaries in 1975. They developed large and dense oyster reefs in the intertidal and subtidal (Drinkwaard 1999b; Wolff and Reise 2002; Dankers et al. 2006), and are now potentially in competition with native bivalve filter-feeders. In the Oosterschelde estuary the share of the soft-bottom intertidal area (118 km^2) occupied by oyster beds is estimated to have increased from 0.25 km^2 in 1980 to 8.09 km^2 in 2003 and a similar cover and absolute increase was estimated for subtidal soft bottoms (Geurts van Kessel et al. 2003; Kater 2003; Kater and Baars 2004; unpublished data Wageningen IMARES). Oyster cover on hard substrates (mainly consisting of 160 km of dikes and sea walls, area estimated at 2 - 4% of the total Oosterschelde area, Leewis et al. 1994) generally increased from 0 – 10% in 1985 to 50 - 60% in 2002, and even to 90% on some locations (AquaSense 2003). Within this period, stocks of the native blue mussel *M. edulis* and common cockle *C. edule* showed a slight decrease (Geurts van Kessel et al. 2003; Dankers et al. 2006). The total stock of *C. gigas*, *M. edulis* and *C. edule* combined was estimated to have increased from 150 million kg fresh weight (including shells) in the early 1990s to 255 million kg around 2000. As a consequence, the filtration pressure in the Oosterschelde estuary was roughly estimated to have increased from 289 million m^3 water day^{-1} in 1990 to 398 million m^3 day^{-1} in 2000 (Geurts van Kessel et al. 2003; Kater 2003). This may have resulted in a considerable increase in larviphagy and hence a reduction in larval numbers on estuary scale. Moreover, the increased filtration pressure may not only have affected bivalve larval

numbers, it may have affected populations of benthic species with pelagic larval stages in general.

The aim of this study was to find field-evidence for effects of larviphagy on numbers of bivalve larvae. We considered the potential impact of larviphagy on three scales. First, we tested the hypothesis that individual bivalve filter-feeders in the Oosterschelde estuary ingest bivalve larvae by analyzing stomach contents of bivalves from the field. Additionally, we sought evidence for larviphagy in *C. gigas*, *M. edulis* and *C. edule* in literature. Second, we studied local effects of a dense bed of filter-feeding bivalves (*C. gigas*) on bivalve larval abundance in the overlying water column. We tested the null-hypothesis that larval abundance was unaffected by the presence of adult filter-feeding bivalves. We expected to find reduced numbers of bivalve larvae above the oyster bed, in comparison to a nearby bare reference site. Third, we related existing time-series of larval abundances of *C. gigas* and *M. edulis* in the Oosterschelde estuary to the increase in total filter-feeder stock (≈ filtration pressure). Our null-hypothesis was that larval abundance remained the same throughout the years and showed no relationship with an increased filter-feeder stock. Since the increase in total bivalve filter-feeder stock was mainly due to an increase in *C. gigas* stock, our expectations are different for larvae of *C. gigas* and *M. edulis*. Numbers of *M. edulis* larvae were expected to decline with an increase in total bivalve filter-feeder stock. Production of *C. gigas* larvae and the total volume of water filtered by the oyster population were both expected to increase proportionally to the increase in oyster biomass. Since part of the filtered water will be re-filtered inside oyster beds (see Jonsson et al. 2005), and since filtration is a dilution process (Riisgård 2001; Riisgård et al. 2004), a potential increase in larval production of *C. gigas* was expected to be higher than, or at least compensate for, a potential decrease in larval numbers due to larviphagy. Our expectations about larval numbers in the estuary are based on the assumption that larvae are distributed homogeneously over the water mass. If, however, in the Oosterschelde estuary early stage larvae occupy higher water layers, as described for *M. edulis* by Bayne (1964b), this behaviour may offer the larvae a refuge from predation by benthic filter-feeders. We therefore used existing monitoring data to test the null-hypothesis that young *M. edulis* larvae are distributed homogeneously over the water column. We expected to find a homogeneous distribution since the Oosterschelde estuary is vertically well mixed (see Hendriks et al. 2006).

6.2. Methods

6.2.1. Study area

The Oosterschelde estuary (SW Netherlands; Figure 6.1) is a macrotidal system where tidal currents force an extensive vertical mixing. The estuary has a mean tidal volume of 880 million m^3, a total volume at half tide of 2,750 million m^3, and a surface area of 351 km^2 of which 118 km^2 tidal flats. Salinity is high, generally > 30 psu, throughout the estuary. Freshwater discharge into the estuary is very limited (1 million m^3 per tide) and does not cause salinity stratification. Water residence time is 10 – 150 days. The mean tidal amplitude ranges from 2.47 m near the mouth to 2.98 m in the most northern part (near Krammer locks) and 3.39 m at the southeast end. The maximum current velocity is about 1.0 m s^{-1}. The water temperature varies over the season from 0 - 5 to 18 - 22 °C (Nienhuis and Smaal 1994a). The average annual chlorophyll-a

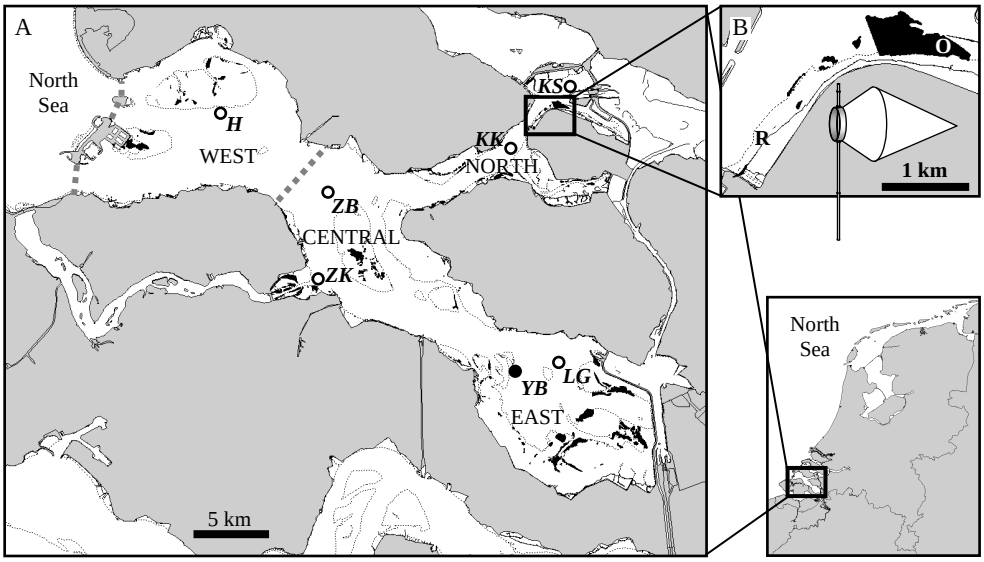


Figure 6.1. Larvae sampling locations in the larvae monitoring programme (A) and the local-scale experiment with plankton nets (B). The western and central compartments of the Oosterschelde estuary are separated by a bridge (grey dashed line). The estuary is separated from the North Sea by a storm surge barrier (grey dashed line). Black dotted lines follow the mean low tide level. Intertidal areas covered with oysters are indicated in black. The sampling location of *C. gigas* larvae at Yerseke Bank (YB) is indicated with a black dot. Sampling locations of *M. edulis* larvae (H, ZB, ZK, KK, KS, LG, see Table 6.1) are indicated with white dots. The local-scale sampling location in the oyster bed is indicated with the letter 'O', at the reference site with the letter 'R'.

concentration is about 5 μg l^{-1}, reaching maximum values of about 40 - 50 μg l^{-1} in May - June (Wetsteyn and Kromkamp 1994).

The Oosterschelde estuary is extensively used for culture of *C. gigas* and *M. edulis* on subtidal bottom culture plots (Dijkema 1997; Smaal and Lucas 2000). The total *M. edulis* stock varies roughly between 20 and 80 million kg fresh weight (Kater and Kesteloo 2003) and is for > 95% controlled through import and removal by mussel farmers. Only 5% of the total stock originates from the Oosterschelde estuary and is mainly found on intertidal hard substrates. The rest is imported, mainly from the Dutch Wadden Sea (Van Stralen and Dijkema 1994). The majority of the total *C. gigas* stock in the Oosterschelde consists of feral oysters (189 million kg fresh weight in 2002; Kater 2003). The annual total cultured *C. gigas* stock is about 0.7 million kg fresh tissue weight (7 million kg total fresh weight) (Perdon and Smaal 2000; Smaal and Lucas 2000). Spat of *C. gigas* is collected within the Oosterschelde estuary by spreading and retrieving spat collectors (usually broken mussel shells), and seeded on subtidal bottom plots in the eastern compartment where it is left to grow to consumption size (Dijkema 1997). Another bivalve occurring in relatively high numbers is the edible cockle *C. edule*. This species is fished for consumption but not cultured (Dijkema 1997). The stock size fluctuates between roughly 2 and 10 million kg fresh tissue weight (about 15 - 70 million kg fresh weight) (Kesteloo et al. 2007).

6.2.2 Larviphagy in individual bivalves

Since many authors already described the occurrence of larviphagy in bivalves, and some authors already demonstrated it specifically for the bivalve species that are considered in this paper (discussed in section 6.4.1.), we conducted only a simple experiment in 2003 to check whether larviphagy also occurs among bivalves in the Oosterschelde estuary. Oysters and mussels, 32 per species, were suspended at Yerseke in the Oosterschelde estuary in cages (mesh size 15 mm) at a tidal height of about 0.5 m above the mean low water level. The average shell length was 110 mm for oysters and 50 mm for mussels. On six dates in May and June, the first date one week after suspending the animals, we took water surface samples of 200 l with a bucket and removed 4 – 6 individuals per species half an hour before high tide. The stomach contents were immediately removed and analyzed for bivalve larvae as described by Troost et al. (2008a, Chapter 3). From the water samples, filtered over a mesh of 60 μm, bivalve larval concentrations were determined the same day. Bivalve larvae from stomach contents and water samples were counted and classified as '*C. gigas*', '*M. edulis*' and 'other' (methods described in section 6.2.5.).

6.2.3. Larviphagy by an oyster bed

Study site

We studied local effects of a filter-feeder bed on bivalve larval abundance in the overlying water column in 2003, at an extensive oyster bed in the northern part of the estuary (Figure 6.1B). This bed covers an area of 280,000 m², at a vertical range of 1.2 – 2.0 m below mean tidal level (MTL; Dutch: NAP). We conducted the experiment at 1.4 m below MTL. A reference location, also at 1.4 m below MTL, was chosen at a distance of approximately 2 km. Here, there were no beds of bivalve filter-feeders present in a radius of at least 1 km.

Filter-feeder biomass

At the two locations, density and biomass of filter-feeding bivalve species were determined. At the reference location, in an area with a radius of 50 m, 20 cores of 78.5 cm² and 30 cm deep were taken randomly and sieved over a 1 mm mesh. All collected bivalves were taken to the laboratory for further analysis. At the oyster bed, the average cover by bivalves was determined with a 1 x 1 m square divided in 100 sections, at twelve random locations within an area with a radius of 50 m. At three random locations, all bivalves in a square of 0.25 x 0.25 m with 100% bivalve cover were removed and taken to the laboratory for analysis. At the laboratory, all bivalves collected at both locations were identified to species level and their flesh was dried (3 days at 70 °C), incinerated (4 h at 550 °C) and weighed. From the average biomass in the 0.25 x 0.25 squares with 100% cover, and the average percentage of bivalve cover, the mean biomass per m² at the oyster bed was calculated for all bivalve species present. For the reference site, biomass per m² was calculated directly from the bivalve biomass per sampled surface area.

To estimate area-specific filtration rates, we used clearance rates as determined for *C. gigas* by Bougrier et al. (1995) and as determined for *M. edulis* and *C. edule* by Smaal et al. (1997) in the Oosterschelde estuary. We used a temperature of around 8 °C for April and around 18 °C for the summer months (June – August). Based on average seawater temperatures of approximately 8 °C in April and 18 °C in June - August (2003 – 2006, measured by Wageningen IMARES and the Netherlands Institute of Ecology (NIOO-CEME)), we used clearance rates of 6.8 – 8.7 l h^{-1} g^{-1} (DTW) for *C. gigas*, 1.5 – 1.8 l h^{-1} g^{-1} for *M. edulis* and 1.6 – 1.9 l h^{-1} g^{-1} for *C. edule*.

Larval abundance

We used plankton nets (Figure 6.1B; opening diameter 10.0 cm; mesh size 60 μm) to collect bivalve larvae during a complete tidal cycle. These nets could rotate freely around a fixed bamboo pole, and openings of the nets were thus always oriented towards the current (see Armonies 1994). The advantage of collecting plankton with fixed plankton nets is that bivalve larvae are collected during a complete tidal cycle. This yields larval numbers integrated over a tidal cycle. The nets were placed at mean low water level (MLW = 1.40 m below MTL), with the centre of the opening 30 cm above the substrate. On each location (Figure 6.1B), 4 nets were deployed during low tide and collected during the next low tide. For logistic reasons we deployed the nets at low tide in the early evening and collected them early the next morning. The still moist nets were transported in plastic bags to the laboratory, where their content was flushed into plastic containers and fixed with buffered formaldehyde for further analysis. The nets were deployed on 5 dates in 2003 (April 28, June 12, July 10, August 12 and 25), during the main spawning seasons of *M. edulis* (April – June) and *C. gigas* (July – September).

Current velocity

To be able to correct for differences in larval numbers due to possible differences in current velocity between both locations we measured current velocities with a StreamPro Acoustic Doppler Current Profiler (ADCP; RD Instruments, CA, USA). The ADCP consisted of a floating body with a transducer pointing downward. Current velocities were measured at both locations, at three positions that were 5 – 6 m apart. The ADCP was operated from a dinghy that was anchored 10 m downstream. Communication between the ADCP and the dinghy was established by a Bluetooth connection with a hand-held pocket PC. Measurements were made at water levels of 100, 150, and 200 cm above the bottom, while the tide was coming in. Current velocities were measured in 20 bins (depth cells), ranging from the bottom to the surface. The transducer frequency was 2.0 MHz. At the laboratory, the measured data were read into the software package WinRiver (RD Instruments), and exported to an Excel spreadsheet for further analysis. Per measuring position, 23 to 56 measurements were averaged per bin.

Measurements were made on four successive days in August. On the first and third day (August 1st and 3rd), measurements were made at the oyster bed site. On the

second and fourth day (August 2nd and 4th), measurements were made at the reference site.

6.2.4. Larviphagy on estuary-scale

Bivalve filter-feeder stock

The total filter-feeder stock in the Oosterschelde estuary, comprising the dominant species *M. edulis*, *C. edule* and *C. gigas*, was estimated from 1990 onward. Stock sizes of these commercial shellfish species are assessed on a regular basis by Wageningen IMARES. These species are also the most dominant bivalves in the estuary. Filter-feeding razor clams *Ensis* spp. and slipper limpets *Crepidula fornicata* also occur in relatively high biomass (Sistermans et al. 2005), but stock sizes have not been assessed for these species. They were therefore not included in the estimated total filter-feeder stock. Filter-feeders on hard substrates (bivalves, *C. fornicata* and tunicates) were also not included since no stock assessments were available, and the total surface area of hard substrates in the Oosterschelde is only 2 – 4% of the total surface area of the Oosterschelde estuary (Leewis et al. 1994). Time series of stocks of *C. gigas*, *M. edulis* and *C. edule* were available for the period 1992 – 2007 for *M. edulis* (methods described by Kater and Kesteloo 2003) and for the period 1990 – 2007 for *C. edule* (methods described by Kesteloo et al. 2007). The *M. edulis* stock, assessed annually in June – July, consisted mainly of mussels on subtidal culture plots and only few mussels were found on intertidal culture plots and as wild stocks (Kater and Kesteloo 2003). The *C. edule* stock, assessed annually in May, consisted entirely of wild animals (Kesteloo et al. 2007). The intertidal cover of *C. gigas* beds was determined in the period 2000 – 2003 and a reconstruction was made for the years 1980 and 1990 using aerial photographs (Kater and Baars 2004; Dankers et al. 2006). Oyster cover in the subtidal was surveyed with side-scan sonar in March 2002, yielding a rough estimate of the subtidal oyster cover (Kater 2003). From biomass samples taken in January – February in the period 2002 – 2003 the intertidal stock size was determined. Stock sizes in the subtidal in 2003 and in the intertidal and subtidal in 1990 were roughly estimated based on two assumptions: 1) the biomass to surface area ratio was the same in the subtidal area as in the intertidal; 2) the ratio of subtidal to intertidal oyster cover was the same in 1990 as in 2002-2003 (Kater 2003). The oyster stock was not assessed between 1990 and 2000, but was observed to increase rapidly (Kater and Baars 2004; pers. obs. K. Baaij). After 2003, additional surveys that did not cover the entire estuary indicated a continued increase at roughly the same rate (Dankers et al. 2006; Smaal et al. in press;

unpublished data Wageningen IMARES). We therefore interpolated the *C. gigas* stock in the intermediary period assuming a linear increase in stock size from 1990 to 2003. We extrapolated the oyster stock for the years 2004 – 2007 by assuming the same linear increase for the period after 2003. In 1975 the first extensive spatfall of *C. gigas* in the Oosterschelde was recorded (Drinkwaard 1999b), so the cover by wild oysters was assumed to be zero in this year. For the period 1992 – 2007 the annual total filter-feeder stock was estimated by summing the stock sizes of *M. edulis* and *C. edule* and the inter- and extrapolated stock of *C. gigas*.

Table 6.1. Sampling locations in the larvae monitoring programme. Water depth at the sampling locations is given in meters below MTL, and maximum current velocities near the surface during mean spring tide are given in cm s^{-1}.

Sampling location	Code	Compartment	Depth *(m)*	Maximum current velocity *(cm s^{-1})*	Target species	Week numbers sampled	Years sampled
Hammen	H	West	12	82	*M. edulis*	6 - 40	1998 - 2003
Zeelandbrug	ZB	Central	17	67	*M. edulis*	6 - 40	1998 - 2000
Zandkreek	ZK	Central	20	51	*M. edulis*	6 - 40	2001 - 2002
Lodijkse Gat	LG	East	13	51	*M. edulis*	6 - 40	1998 - 2002
Keeten - Krabbenkreek	KK	North	14	31	*M. edulis*	6 - 40	1998 - 2002
Krammersluizen	KS	North	10	15	*M. edulis*	6 - 40	2003
Yerseke Bank	YB	East	5	46	*C. gigas*	26 - 36	1994 – 2006

Larval abundance

To optimize the timing of spat and seed collection by mussel and oyster farmers, Wageningen IMARES has monitored the abundance of *M. edulis* and *C. gigas* larvae during their respective spawning seasons. We used the resulting time series to study a potential trend in larval abundances of the two species. The abundance of *C. gigas* larvae was monitored at the Yerseke Bank (Figure 6.1A) in the period 1994 – 2006, throughout weeks 26 to 36 (Table 6.1). The abundance of *M. edulis* larvae was monitored at several locations in the Oosterschelde estuary (Figure 6.1A) in the period 1998 – 2003, throughout weeks 6 to 40 (Table 6.1). All sampling locations were located in gullies, at water depths of 10 to 20 m below mean tidal level (except Yerseke Bank: 5 m; Table 6.1). Maximum current velocities (at mean spring tide) at

the sampling locations range from 82 cm s^{-1} in the western compartment (Hammen) to 15 cm s^{-1} in the northern compartment (Krammersluizen) (Anonymous 1992). Weekly samples of 100 litres surface water per station were taken with a bucket, and filtered over a 55 μm mesh for *M. edulis* larvae and a 100 μm mesh for *C. gigas* larvae. The residue was flushed into a 1 l bottle and brought to the lab within the same day, where the sample was fixed for further analysis within 48 h. Until 2000 the samples were fixed and preserved with 70% ethanol. From 2000 onwards formaldehyde buffered with borax (pH 8.0 – 8.2) was used. Larval numbers were compared to trends in adult stocks of the same species and to the trend in estimated total bivalve filter-feeder stock.

Chlorophyll-a concentrations during the larval monitoring period were obtained from monitoring data by the National Institute for Coastal and Marine Management (RWS-RIKZ; www.waterbase.nl) at the location Lodijkse Gat for a comparison with numbers of oyster larvae and at the locations Hammen, Keeten-Krabbenkreek and Lodijkse Gat for a comparison with numbers of mussel larvae. Chlorophyll-a concentrations were determined twice per months at these locations. We related larval numbers to maximum chlorophyll-a concentrations occurring during the monitoring period (week 6 – 40) and also to average chlorophyll-a concentrations over the monitoring period. In the comparison with mussel larvae maximum values per location occurring in the monitoring period were averaged to one maximum value for the entire estuary. Monthly surface water temperatures, obtained by Wageningen IMARES and NIOO-CEME, were available for the different compartments.

Vertical distribution of bivalve larvae

In 2003 samples of *M. edulis* larvae were not only taken from the surface, but also from near the bottom (0.5 m height) at the sampling locations Hammen in the western compartment and Krammersluizen in the northern compartment (Table 6.1). At location Hammen the water depth during sampling was between 10 and 14 m depending on the tidal phase, at location Krammersluizen between 8 and 12 m. Both surface and near-bottom samples were taken with a submersible water pump. Further treatment of the samples was the same as in the monitoring programme (see previous paragraph). We compared numbers and lengths of the larvae between the surface and bottom water layers in weeks 14 - 30, in which highest densities were observed.

Mortality rate on estuary-scale

We estimated the order of magnitude of the effect of larviphagy on larval mortality in the Oosterschelde estuary by using a general formula for calculation of clearance rates. We modified the formula from Riisgård (2001, his equation 3) to calculate the fraction f of all bivalve larvae in the Oosterschelde estuary that are filtered in time t (in days):

$$f = 1 - e^{-CR \cdot t/V} \qquad (6.1)$$

CR is the potential clearance rate of the main bivalve filter-feeders in the estuary (in m^3 day^{-1}, by *C. gigas*, *M. edulis*, *C. edule*) and V is the mean volume of water in the Oosterschelde estuary (2,750 million m^3). The model assumes a homogeneous distribution of larvae throughout the Oosterschelde estuary, a homogeneous distribution of adult bivalves on the bottom of the estuary, a continuous complete mixing of the estuary and no exchange of larvae with the North Sea with tidal exchange. A clearance rate CR of 398 million m^3 day^{-1} was used, that is representative for the year 2000 as estimated by Kater (2003).

6.2.5. Analysis and identification

Samples for larval counts were stored cool and dark until analysis. Bivalve larvae were counted using a universal camera microscope (Reichert Me-F2, 52.6x) and an inverted microscope (Olympus IMT-2, 60x). Larvae of *M. edulis* and *C. gigas* were identified according to Loosanoff et al. (1966) and Hendriks et al. (2005). Lengths of the larvae were measured as the longest distance from anterior to posterior, roughly parallel to the hinge (as in Loosanoff et al. 1966). From the Wageningen IMARES monitoring samples, all counted larvae were measured up to a maximum of 100 larvae. Larval counts were integrated over time by calculating the surface under the curve of larval density (numbers per 100 l^{-1}) versus time (in days) in SigmaPlot® 2001. Assuming that larval density was zero outside the sampling interval, the resulting number expresses how many larval days (per 100 l) were spent in the estuary in each year. From the plankton-net samples, all counted larvae were measured up to a maximum of 25, amounting to a maximum of 100 measured larvae per location per sampling date.

6.2.6. 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, resulting in a normal distribution and equal variances. Differences in means with unbalanced sample sizes were tested with a non-parametric test. A significance level of $\alpha = 0.05$ was maintained.

6.3. Results

6.3.1. Larviphagy in individual bivalves

Stomach analyses of suspended adult *C. gigas* and *M. edulis* confirmed that these bivalves ingest bivalve larvae in the Oosterschelde estuary. The stomachs contained larvae of *C. gigas*, *M. edulis* and unidentified molluscs. In *C. gigas* we found on average 8.2 mussel larvae and 1.5 oyster larvae per individual (Table 6.2), with maximum numbers of 25 mussel larvae and 9 oyster larvae. In *M. edulis* we found on average 2.2 mussel larvae and 0.3 oyster larva per individual (Table 6.2) with maximum numbers of 4 mussel larvae and 1 oyster larva. We found 2 unidentified larvae in oyster stomachs. Average larval concentrations in the surrounding water over the entire sampling period (May – June) in the surrounding water were as high as 220 (12 – 493) mussel larvae and 2 (0 – 12) oyster larvae per 100 l.

Table 6.2. Numbers of mussel and oyster larvae found in stomach contents of adult *M. edulis* and *C. gigas*. The examination of 32 individuals per species was spread over 6 dates in May and June. Mean numbers for all 32 individuals are given, with maximum numbers found per stomach between brackets.

Species	*n* examined	Mean (and maximum) number of larvae in stomach contents			
		C. gigas larvae	s.e. (n = 32)	*M. edulis* larvae	s.e. (n = 32)
C. gigas	32	1.5 (9)	0.5	8.2 (25)	1.6
M. edulis	32	0.3 (1)	0.1	2.2 (4)	0.2

6.3.2. Larviphagy by an oyster bed

Filter-feeder biomass

Filter-feeder biomass was much higher at the oyster bed site than at the reference site, resulting in much higher area-specific filtration rates (Table 6.3). Mean biomass of filter-feeders at the experimental site within the oyster bed was 490.0 g ash-free dry weight (afdw), consisting of *C. gigas* (471.2 g) and *M. edulis* (18.8 g). The mean biomass of filter-feeders at the reference site was 4.4 g afdw m^{-2}, consisting of *C. edule* (3.9 g) and *M. edulis* (0.5 g). Area-specific filtration rate was roughly estimated to be 70 times higher in the oyster bed than at the reference site. In April, at a water temperature of about 8 °C, filtration rates were estimated to be 1,200 l h^{-1} m^2 in the oyster bed and 16 l h^{-1} m^2 at the reference site. In June – August, at seawater temperatures of around 18 °C, filtration rates were estimated to be 1,600 l h^{-1} m^2 in the oyster bed and 26 l h^{-1} m^2 at the reference site.

Current velocity

Water current profiles above the oyster bed and the reference location were fairly similar. Current velocities ranged roughly between 15 and 40 cm s^{-1}, and did not differ much over the entire depth range near high tide. However, around 30 cm above the bottom, the level at which the plankton nets were positioned, we found significant differences between the different days at which current velocities were measured (Table 6.4; ANOVA). All significant differences except one showed higher current velocities at the oyster bed. There are no results for day 1 at a water level of 100 cm because measurements started at a water level of 150 cm. The maximum difference found between current velocities measured at both locations was a factor 2.1 at a water level of 150 cm (Table 6.4). Weather conditions were similar: dry and sunny with air temperatures between 20 and 25 °C. Wind speed was low on days 1, 2 and 4 (3.3 - 4.9 m s^{-1}) and moderate on day 3 (5.5 m s^{-1}) (Zeeland Centre for Hydrology and Meteorology, www.hmcz.nl).

Larval abundance and size

Numbers of *M. edulis* larvae were reduced at the oyster bed site, but numbers of *C. gigas* larvae were not. In April and June we mainly found *M. edulis* larvae in our samples (> 90%), and no *C. gigas*. On both dates, larval numbers at the reference site were

significantly higher than those in the water overlying the oyster bed (ANOVA: $p < 0.05$; Figure 6.2A), by an average factor of 3.3. Higher current velocities at the oyster bed cannot account for this difference. Correcting for the maximum difference in current velocities found (factor 2.1) doubled the difference in larval numbers between both sites to a factor 6.9 (Figure 6.2B). In July and August we mainly found *C. gigas* in

Table 6.3. Average area-specific filter-feeder biomass (in g afdw m^{-2}) and filtration rate (*FR* in l h^{-1} m^{-2}) at the oyster bed (O) and reference site (R). Area-specific filtration rates were estimated for April and the summer months (June – August) according to literature values of clearance rates (*CR*) depending on body weight (g DTW = dry tissue weight) and temperature (assumptions: April 8 °C, summer 18 °C) (*C. gigas*: Bougrier et al. 1995; *M. edulis* and *C. edule*: Smaal et al. 1997).

	Oyster bed (O)				Reference site (R)			
	Biomass (*g afdw m^{-2}*)	Body weight (*g DTW*)	*CR* (*l h^{-1} g DTW*)	*FR* (*l h^{-1} m^{-2}*)	Biomass (*g afdw m^{-2}*)	Body weight (*g DTW*)	*CR* (*l h^{-1} g DTW*)	*FR* (*l h^{-1} m^{-2}*)
M. edulis	18.8	0.20			0.5	0.06		
April			1.5				1.5	
Summer			1.8				1.8	
C. gigas	471.2	3.80			0	-		
April			6.8					
Summer			8.7					
C. edule	0	-			3.9	0.49		
April							1.6	
Summer							1.9	
All bivalves	490.0				4.4			
April				1,200				16
Summer				1,600				26

our samples (> 90%), and few *M. edulis* individuals (> 5%). On August 12th we found significantly more larvae in the water overlying the oyster bed than at the reference site (factor 8.2; ANOVA: $p < 0.05$; Figure 6.2A). On the other two dates we found no significant differences (ANOVA: $p > 0.05$). Correcting for the maximum difference in current velocities found halved the difference in larval numbers between both sites, but did not change the statistical results.

Table 6.4. Current velocities measured at the oyster bed (O) and reference location (R), at three tidal water levels (in cm above the bottom), measured at heights around 30 cm above the bottom. Mean values (n = 3) and standard errors are given. If present, significant differences are indicated with lowercase letters between brackets (ANOVA: $p < 0.05$). The maximum factors of difference between the highest and lowest current velocities measured at the oyster bed and reference site are given in columns 'O/R' and 'R/O' (e.g. O/R: 3/4 = highest current velocity at oyster bed (O) on day 3 divided by lowest velocity at reference site (R) on day 4). Significance of the differences is indicated with an asterisk.

Tidal level	Height above bottom	Current velocity *(cm s^{-1} ±s.e.)*				Max. factor	
		oyster bed (O)		reference site (R)			
(cm)	*(cm)*	*day 1*	*day 3*	*day 2*	*day 4*	*O/R*	*R/O*
100	26.5 - 31.5	-	43.7 ± 3.8 (*b*)	30.2 ± 0.4 (*a*)	29.1 ± 2.1 (*a*)	3/4 = 1.5 *	2/3 = 0.7 *
	31.5 - 36.5	-	31.3 ± 2.1	28.8 ± 1.0	24.7 ± 1.3	3/4 = 1.3	2/3 = 0.9
150	23.0 - 31.3	19.2 ± 1.4 (*a*)	40.2 ± 0.4 (*b*)	19.4 ± 1.1 (*a*)	19.0 ± 0.7 (*a*)	3/4 = 2.1 *	2/1 = 1.0
	31.3 - 38.8	20.8 ± 3.2 (*a*)	41.7 ± 6.3 (*b*)	23.7 ± 2.3 (*a*)	20.0 ± 0.7 (*a*)	3/4 = 2.1 *	2/1 = 1.1
200	19.0 - 29.0	16.7 ± 3.8	20.4 ± 1.7	21.5 ± 1.8	16.6 ± 0.2	3/4 = 1.2	2/1 = 1.3
	29.0 - 39.0	25.0 ± 2 (*a*)	21.9 ± 2.2 (*ab*)	22.5 ± 1.9 (*ab*)	17.8 ± 0.6 (*b*)	1/4 = 1.4 *	2/3 = 1.0

In April and June (mainly *M. edulis*) the mean larval length did not differ between the two locations (Figure 6.2C; ANOVA: $p > 0.05$). In July and August (mainly *C. gigas*) the mean larval length was smaller above the oyster bed than at the reference site. This difference was significant on July 10th (ANOVA: $p < 0.05$), and was caused by a relatively higher abundance of small-sized larvae (80 - 90 μm) at the oyster bed location.

6.3.3. Larviphagy on estuary-scale

Vertical distribution of mussel larvae

The abundance of *M. edulis* larvae was not predominantly higher in near-bottom or surface samples at both sampling locations (Figure 6.3; Wilcoxon Signed Rank test: $p > 0.05$).

Although significant differences were found in larval shell length between near-bottom and surface samples, mean differences were small (5.1 μm in the western compartment and 17 μm in the northern compartment). The two locations showed contrasting results. Whereas in the western compartment the largest larvae were

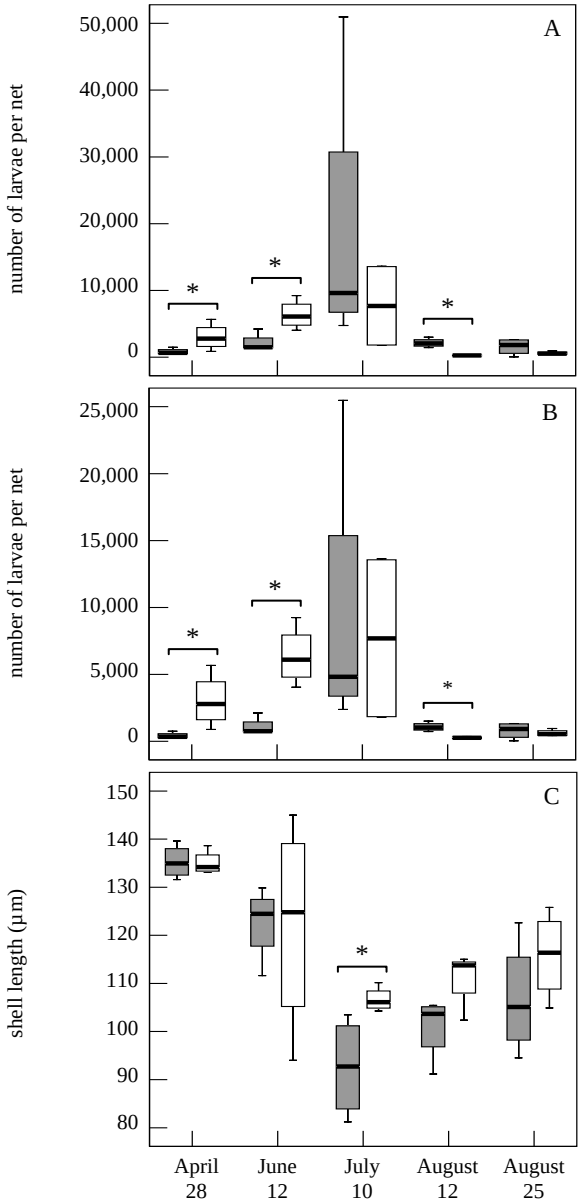


Figure 6.2. Numbers of bivalve larvae collected with plankton nets at the oyster bed (grey boxes, n = 4) and reference site (white boxes, n = 4) (A), and the same numbers corrected for a maximum difference in current velocity between locations with factor 2.1 (B). The box-plots represent the median and quartiles. An asterisk denotes a significant difference (ANOVA: $p < 0.05$). In April and June numbers consisted mainly of *M. edulis* larvae, in July and August mainly of *C. gigas* larvae. Figure C shows shell lengths of bivalve larvae collected with plankton nets at the oyster bed (grey boxes, n = 64 - 100) and reference site (white boxes, n = 58 - 100).

generally found in near-bottom samples (Figure 6.3), in the northern compartment larvae were generally larger in surface samples. The two sampling locations differ considerably in nature. Location Hammen (H) in the western compartment is a tidal channel with high current speeds (max. 82 cm s^{-1} at mean spring tide) and turbulence; location Krammersluizen (KS) in the northern compartment has lower current speeds (max. 15 cm s^{-1} at mean spring tide) and turbulence levels.

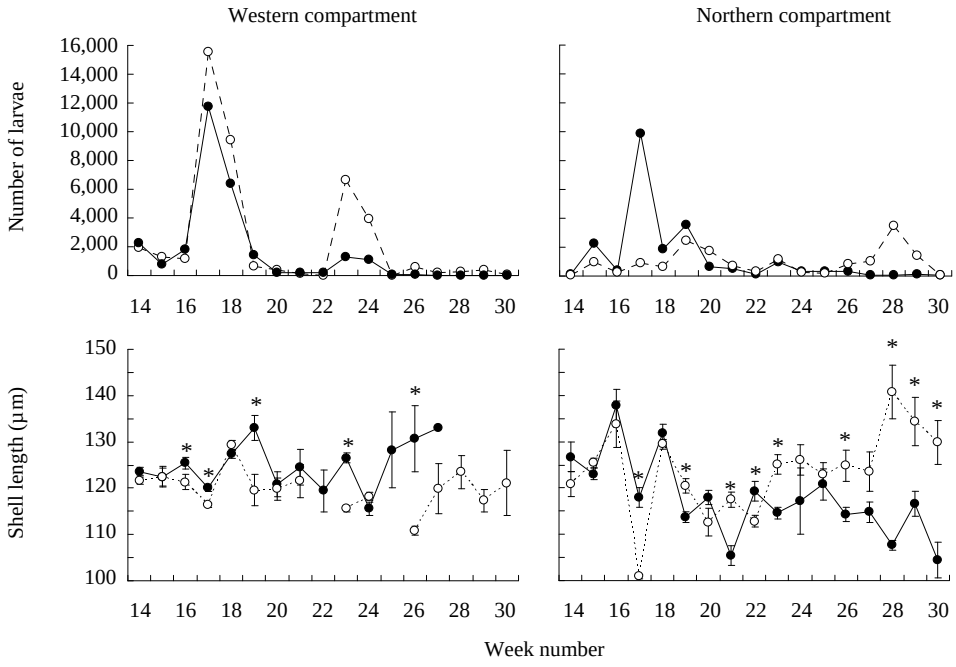


Figure 6.3. Weekly numbers (per 100 l) and lengths of *M. edulis* larvae counted in bottom (filled circles) and surface (open circles) samples in 2003 in the western ('Hammen') and northern ('Krammer') compartments. Bars indicate standard errors (n = 3 – 440). Asterisks denote significant differences between bottom and surface samples (Mann-Whitney U test: $p < 0.05$). Where data points are missing, no larvae were present in the samples.

In the northern compartment in near-bottom samples, larval lengths decreased in time (linear regression: $R^2 = 0.43$, $p < 0.05$). None of the larvae measured was larger than 181 µm and therefore none of them was competent to settle yet (Hendriks et al. 2005).

Filter-feeder stock

The *C. edule* stock fluctuated between 8 and 45 million kg fresh weight, and did not show an increase or decline in the period 1990 – 2007 (Figure 6.4; linear regression: $p > 0.05$). The *M. edulis* stock showed a decline in the period 1996 – 2007 (linear regression: $R^2 = 0.47$, $p < 0.05$), from about 80 to 30 million kg fresh weight. The total *C. gigas* stock was estimated at 66 million kg in 1990 and 217 million kg in 2003 (assessed in 2000 - 2003; Kater 2003; Kater and Baars 2004; Dankers et al. 2006). Accumulating stocks of all three bivalves per year resulted in an increase in estimated total bivalve filter-feeder stock from 160 million kg in 1992 to 320 million kg in 2007.

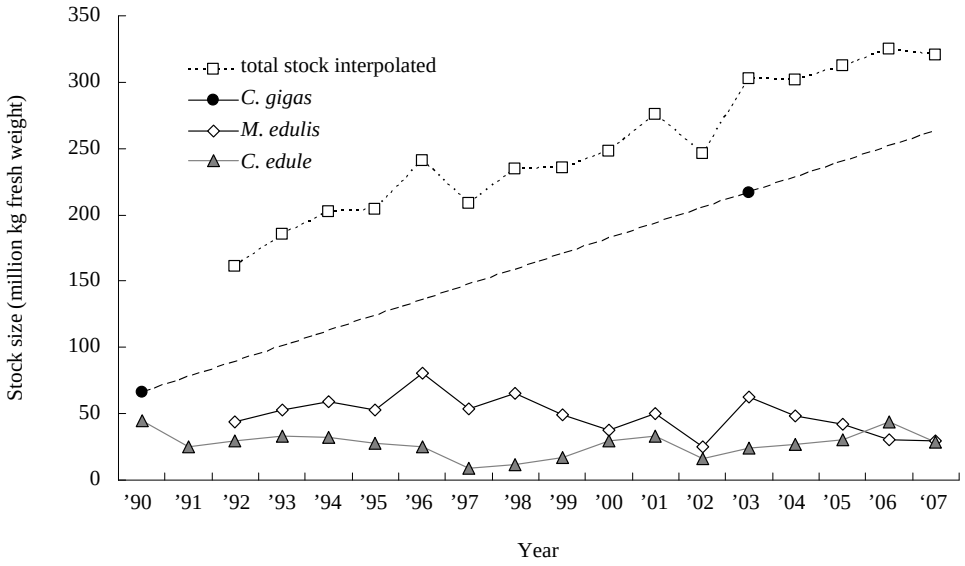


Figure 6.4. Stocks of the most dominant bivalve filter-feeders in the Oosterschelde estuary in million kg fresh-weight (including shells). The total filter-feeder stock is calculated by accumulating stocks of *M. edulis* (Kater and Kesteloo 2003; unpublished data Wageningen IMARES), *C. edule* (Kesteloo et al. 2007), and the inter- and extrapolated stock of *C. gigas* (Kater and Baars 2004; Dankers et al. 2006; unpublished data Wageningen IMARES) (using the long-dashed trendline for *C. gigas* for the period 1990-2003).

Larval abundance

Abundance of *M. edulis* larvae (expressed in larval days per 100 l) showed no trend over the period 1998-2003 (Figure 6.5A), and no relationship with the adult *M. edulis*

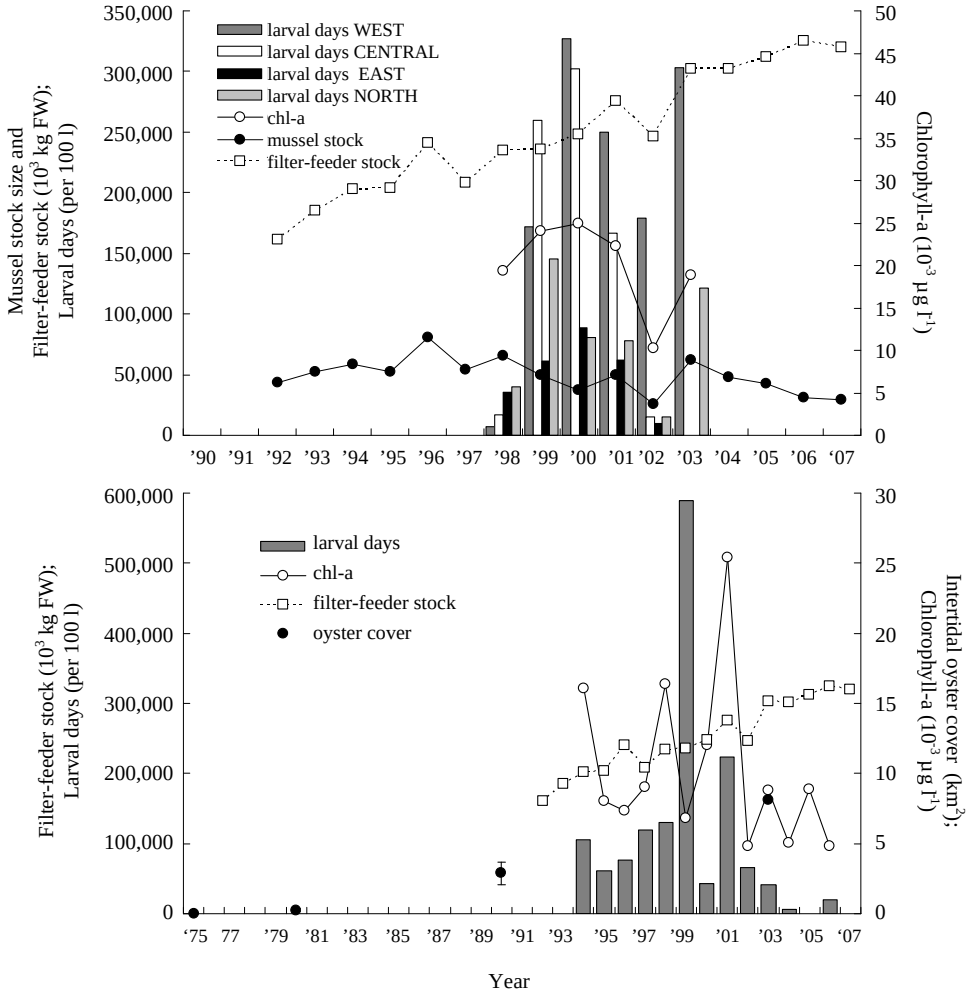


Figure 6.5. Larval abundance of *M. edulis* (A) and *C. gigas* (B) and adult filter-feeder stocks in the Oosterschelde estuary. Larval abundance is expressed in larval days (larval numbers per 100 l, integrated over time), for *M. edulis* per compartment (west, central, east and north). Adult *M. edulis* stock and total filter-feeder stock are given in million kg fresh weight, estimated total oyster cover in the intertidal is given in km². Highest chlorophyll levels (in µg l^{-1}; x1000) measured during the sampling period (February/July – September) are also given.

stock (linear regression: $p > 0.05$) nor with the bivalve filter-feeder stock (linear regression: $p > 0.05$). Larval abundance seemed to roughly follow the maximum annual chlorophyll-a concentration, although a significant correlation was only found

for the eastern compartment (linear regression: $R^2 = 0.68$, $p < 0.05$). Abundance of *M. edulis* larvae showed no relationship with mean chlorophyll-a concentrations during the sampling period (week 6 – 40) either, nor with mean annual temperatures in the different compartments (calculated from monthly temperatures; data not shown). Highest larval abundance was found in the western and central compartments.

Abundance of *C. gigas* larvae (expressed in larval days per 100 l) showed an overall decline over the entire monitoring period of 1994 – 2006 (Figure 6.5B), while *C. gigas* and bivalve filter-feeder stocks were increasing. After log-transformation (^{10}log) a negative linear trend in time was significant (linear regression: $R^2 = 0.41$, $p < 0.05$). Log-transformed larval abundance of *C. gigas* also declined significantly with an increasing estimated stock of *C. gigas* (linear regression: $R^2 = 0.42$, $p < 0.05$) and with an increasing estimated total stock of bivalve filter-feeders (Figure 6.6; linear regression: $R^2 = 0.45$, $p < 0.05$). Abundance of *C. gigas* larvae seemed to follow the maximum annual chlorophyll-a concentration, but no significant correlation was found (linear regression: $p > 0.05$). Larval abundance of *C. gigas* was not correlated with average chlorophyll-a levels in the sampling period either ($p > 0.05$), nor with mean annual sea surface temperatures at Yerseke Bank ($p > 0.05$; data not shown).

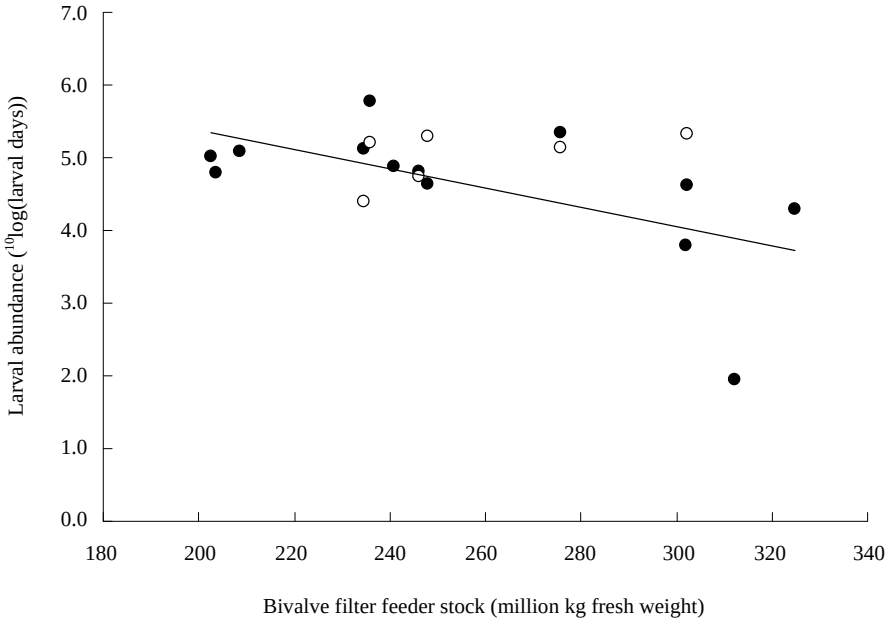


Figure 6.6. Log-transformed larval abundance (in larval days per 100 l) of *C. gigas* (filled circles) and *M. edulis* (open circles), plotted against the total bivalve filter-feeder stock (in million kg fresh weight). Linear regression was significant for *C. gigas* (linear regression: $R^2 = 0.37$, $p < 0.05$) but not for *M. edulis*.

Mortality rate on estuary-scale

During an average pelagic stage of 20 days (t = 20 in Equation 6.1), with a total bivalve clearance rate of 398 million m^3 day^{-1} (situation in 2000; by *C. gigas*, *M. edulis* and *C. edule*), 95% of the bivalve larvae in the Oosterschelde estuary (f = 0.95) are estimated to be filtered by adult bivalve filter-feeders (*C. gigas*, *M. edulis*, and *C. edule*). With an increasing clearance rate the fraction f of filtered larvae (the mortality rate due to larviphagy) will approach the asymptotical value of 1.00 (100% mortality) further. The fraction f is also dependent on the actual time spent by the larvae in the pelagic phase. With a constant CR of 398 million m^3 day^{-1}, 95% of the larvae are filtered during a period of 20 days, and over a period of over 32 days more than 99% of the larvae will be filtered.

6.4. Discussion

6.4.1. Larviphagy in individual bivalves

Our results confirm that larviphagy occurs among *M. edulis* and *C. gigas* in the Oosterschelde estuary. This supports the idea that larviphagy is a general mechanism in bivalve filter-feeders, as a consequence of their feeding mode (Lehane and Davenport 2004; Troost et al. 2008a, Chapter 3). Previous studies by different authors have also demonstrated the occurrence of larviphagy in *M. edulis* and *C. gigas*. It has been conclusively shown that *M. edulis* filters and ingests bivalve larvae, including its own, in the field. Stomach content analyses of farmed mussels *M. edulis* in Bantry Bay (SW Ireland) showed that bivalve larvae were ingested year-round, with peak numbers occurring during spawning periods of *M. edulis* (Lehane and Davenport 2004). C. *gigas* was found to predate its own larvae and larvae of *M. edulis* in laboratory experiments (Tamburri et al. 2007; Troost et al. 2008a, Chapter 3). *C. edule* also predates bivalve larvae, as was shown in laboratory experiments (Kristensen 1957; André et al. 1993; Troost et al. 2008a, Chapter 3) and field experiments in the Gullmarsvik, Sweden (André and Rosenberg 1991), and the Dutch Wadden Sea (Kristensen 1957). We conclude that larviphagy at the scale of the individual occurs in *C. gigas*, *M. edulis* and *C. edule* in the Oosterschelde estuary.

6.4.2. Larviphagy by an oyster bed

Presence of an oyster bed reduced the abundance of *M. edulis* larvae (collected in April – June) but not of *C. gigas* larvae (collected in July – August). This may be a result of differences in escape or avoidance success between the larvae of both species. Troost et al. (2008a, Chapter 3) found that both *C. gigas* and *M. edulis* larvae were filtered by adult bivalve filter-feeders in a laboratory set-up, but that *C. gigas* larvae were filtered at a 50% lower rate than *M. edulis* larvae. However, we need to be careful with such an interpretation. While we had no reasons to assume that the *M. edulis* larvae found at both locations originated from different larval pools, because mean larval lengths were the same and because adult brood stocks are located all around in the area, such an assumption may not be valid for the *C. gigas* larvae. We propose the possibility that spawning by adult oysters in the oyster bed hampered our study of effects of larviphagy on *C. gigas* abundance at local scale. This hypothesis is supported by the following observations. *C. gigas* larvae were collected in higher numbers at the oyster bed site and mean larval lengths at this site were smaller. Smaller lengths were caused by a relatively larger amount of small veliger larvae of 80 – 90 μm shell length found at the oyster bed location. These larvae had likely hatched recently. Laboratory hatched *C. gigas* larvae reach a length of 80 – 90 μm in only 2 – 3 days after fertilization of the eggs (Helm et al. 2004; Hendriks et al. 2005). Part of the *C. gigas* larvae may therefore have been produced recently by the adult brood stock in the oyster bed. This oyster bed is by far the largest bed in the northern compartment. Other oysters in the northern area are located in small patches of several squared meters scattered throughout the area. If the larvae were produced while the tide was coming in, the tide would transport the larvae further land inward. With the next ebb tide the larvae would be transported back towards the bed without passing the reference site. Over the following tides the newly produced larvae would become more and more dispersed (see Korringa 1941). Together with natural mortality leading to lower concentrations locally, this would result in an increased similarity between both locations.

The reduction in larval abundance at the oyster bed in April – June was not caused by a difference in local current velocity, since current velocities were either similar or higher above the oyster bed. The higher number of oyster larvae found at the oyster bed at August 12th was not caused by a higher current velocity at the oyster bed since the difference remained significant after correction for differences in current velocity. Although the current velocity measurements may have been influenced by the rather unsteady position of the transducer in a floating body on the water surface, they are

suitable for obtaining an indication of the difference in current velocities between both sites. The higher velocities measured at day 3 at the oyster bed did not occur at the same location on day 1, and not at all water levels studied on day 3 either. Therefore we assumed it to be safe to take the maximum difference of 2.1 found as the maximum difference ever occurring between both locations at similar weather conditions as encountered during our study, integrated over a complete tidal cycle. The higher current velocities on day 3 may have been caused by a stronger wind that day (5.5 m s^{-1}). A comparable wind speed also occurred during sampling with the plankton nets at April 28th (5.7 m s^{-1}) and August 25th (5.8 m s^{-1}).

Effects of larviphagy on the abundance of bivalve larvae in the field have scarcely been studied. Nielsen and Maar (2007) compared the vertical distribution of different zooplankton species groups above a mussel bed and a bare sandy site in the Limfjord, Denmark. They demonstrated significant grazing by the mussels on all major components of the pelagic food web, including bivalve larvae. They stated that protozooplankton and bivalve veliger larvae seemed more vulnerable to predation than either polychaete trochophores or copepods. André & Rosenberg (1991) demonstrated that settlement success of bivalve larvae in a shallow bay was reduced by 40% by dense beds of *C. edule* and by 20% by dense beds of *Mya arenaria*. Based on observations by André et al. (1993) they concluded that larviphagy was mainly responsible. This was in agreement with an earlier study by Kristensen (1957), who also found a reduced settlement of *C. edule* in areas with dense aggregations of adult conspecifics in the Dutch Wadden Sea. Our study likewise shows that larviphagy by adult *C. gigas* at bed scale significantly affects the abundance of bivalve (*M. edulis*) larvae in the overlying water column. Dense oyster beds therefore have the potential to reduce bivalve larval numbers significantly, potentially leading to a reduced recruitment locally. Dense filter-feeder beds may have the same effect on benthic species with (slow-swimming) pelagic larvae in general.

6.4.3. Larviphagy on estuary-scale

Vertical migration

Numbers and lengths of *M. edulis* larvae collected in surface and near-bottom samples confirmed our expectation that, due to extensive vertical mixing, young mussel larvae find no refuge from predation in higher water layers. *M. edulis* larvae did not collectively and consistently occupy higher water levels. There were, however, striking differences between the two locations sampled. The results from the western

compartment correspond to results by Bayne (1964b), who found that young *M. edulis* larvae occupy higher water levels and competent larvae accumulate near the bottom, in that mussel larvae found in our near-bottom samples were slightly larger. Our results differed from Bayne's (1964b) results in that none of the larvae in our samples were competent yet. In the northern compartment where current velocities are lower, we found the opposite pattern. Here, larval lengths seemed to increase over time in the surface layer and they decreased over time in the near-bottom layer, suggesting that larvae move to higher water layers as they get older. This study offers no explanation for the observed difference.

The collection of high numbers of young larvae in the plankton nets fixed at 30 cm above the bottom confirms that *M. edulis* larvae, that are not yet competent to settle, come into contact with benthic filter-feeders in high numbers in the turbulent Oosterschelde estuary. These results agree with the results of Knights et al. (2006) who found that larval size has no effect on vertical distribution patterns in the tidally driven Irish Sea. We cannot conclude from our results whether young *C. gigas* larvae will also be distributed homogeneously over the water column, although it seems likely regarding the extensive vertical mixing. Although we found no studies on vertical distribution patterns of *C. gigas* larvae, smaller veliger larvae of the well-studied relative *C. virginica* are distributed homogeneously over the water column in estuaries with different tidal ranges and mixing regimes (Carriker 1951), as well as in a flume tank (Finelli and Wethey 2003). *C. virginica* larvae are comparable to *C. gigas* larvae in appearance, size (compare Hidu and Haskin 1978; Hendriks et al. 2005) and swimming speed (see Troost et al. 2008b, Chapter 4).

Filter-feeder stock and larval abundance

The decline in larval abundance of *C. gigas* was contrary initial expectations. Declining numbers of larvae, possibly due to larviphagy, were apparently not compensated by an increased larval production by the increasing adult stock. The observed decline may be explained by a reduced production of *C. gigas* larvae or an increased mortality of the larvae. An increased mortality may be explained by increased larviphagy due to an increase in total filter-feeder stock. Our estimated mortality rate due to larviphagy *f* of 0.95 is in the same order of magnitude as total natural mortality rates generally estimated or determined for bivalve larvae and larvae of other benthic invertebrates (Thorson 1950; Rumrill 1990). Therefore, larviphagy appears to contribute significantly to mortality of bivalve larvae in the Oosterschelde estuary, and may consequently affect recruitment as well. Based on these calculations, and earlier

findings that *C. gigas* larvae are filtered less by adult bivalves than *M. edulis* larvae (Troost et al. 2008a, Chapter 3), the mortality rate due to larviphagy *f* (being dependent on *CR*) would be expected to affect *M. edulis* larval abundance more than *C. gigas* larval abundance. For example, a reduction of the clearance rate of *C. gigas* larvae with 50% (as found by Troost et al. 2008a in the laboratory, Chapter 3) would result in a reduction of *f* with 0.17 (17%). Although this mathematical exercise offers a rough estimate only, it does give an indication of the order of magnitude of the effects of larviphagy on estuary scale. Some assumptions had to be made, that are not that representative of the actual situation in the field. Although larval abundance does seem to be distributed homogeneously vertically in the water column, larvae are not distributed homogeneously throughout all compartments of the estuary. More larvae are found in areas with higher concentrations of conspecific adults. How this would influence the estimate of *f* is difficult to grasp since the adult stock of bivalve filter-feeders is not distributed homogeneously over the entire estuary either. Furthermore, no exchange of bivalve larvae with the North Sea was assumed. If we would include this as a dilution factor, even less larvae would survive until settlement. Finally, errors in estimates of clearance rates may have been large (Kater 2003). Clearance rates estimated by Kater (2003) were based on literature values on individual clearance rates and filtration time activity, and empirical values on stock sizes and submersion times. However, total clearance rates of all filter-feeding molluscs in the estuary combined were more likely underestimated than overestimated since other shellfish species than *C. gigas*, *M. edulis* and *C. edule* (such as *Crepidula fornicata* and *Ensis directus*) were not included in the calculations.

Within the relatively short time series of *M. edulis* larval abundance no apparent trend was detected. We can therefore not draw any conclusions on whether mussel larvae are declining as a result of an increased filtration pressure.

The degree of variation must have been high in the estimated increase in oyster stock (Kater 2003), and therefore in the estimated increase of total bivalve filter-feeder stock. The linear inter- and extrapolation between 1990 and 2003 and after 2003 will also have introduced errors. Nevertheless, it is clear that the oyster stock did increase considerably in the period studied, and we expect that the order of magnitude of the estimated increase in bivalve filter-feeder stock is reliable. An alternative explanation for an increased mortality of larvae and a reduced production of larvae by adults may be another aspect of an increased filtration pressure: reduced food levels. Primary production, chlorophyll-a concentration and phytoplankton composition all changed in the Oosterschelde estuary in the period 1990 – 2000. Phytoplankton composition shifted from larger (>20 μm) to smaller (< 20 μm) species around 1995, primary

production declined and chlorophyll-a also seemed to decline in the second half of the 1990s (Wetsteyn et al. 2003). These shifts coincide with an increase in total filter-feeder stock between 1990 and 2000, but also with on-going changes as a result of the construction of a storm surge barrier before 1986 (Nienhuis and Smaal 1994a) and may have continued after 2000. A shift towards smaller and faster growing phytoplankton species is a common result of increased non-size-selective filtration pressure (e.g. Noren et al. 1999). This seems to confirm that the total filtration pressure in the Oosterschelde indeed increased as was estimated by Kater (2003). The decrease in primary production however appears to be related to a decrease in transparency due to the engineering project (Wetsteyn et al. 2003). Possibly food availability and/or quality dropped below a threshold after 1999, causing a decline in *C. gigas* larval survival and/or larval production by adults due to deteriorated trophic conditions. However, we have no explanation for the peaks in larval numbers in 1999 and 2001. These years did not seem to deviate from other years in terms of chlorophyll-a levels and mean monthly sea surface temperatures in the different compartments. Also periods of prolonged high temperatures in 1999 and 2001 did not seem different from other years. Additionally, in terms of food availability, decreasing phytoplankton abundance may not necessarily imply reduced food levels for bivalve larvae if it is compensated by a shift to smaller phytoplankton species that may be more suitable as a food source for the larvae.

Identification of *M. edulis* larvae was probably not always 100% accurate as discrimination from larvae of other bivalve species is difficult for certain size categories (see Hendriks et al. 2005). *C. gigas* larvae could be easily distinguished from larvae of other species in the Oosterschelde estuary, although not always with 100% certainty for the smallest larvae that had not developed an umbo yet (< 105 μm, see Hendriks et al. 2005). This is not expected to have affected comparisons between locations and years. Potential uncertainties in identification of small *C. gigas* larvae did not play a role in the monitoring programme since samples were sieved over a 100 μm mesh and the smallest larvae (70 – 100 μm) were not collected. Larval abundance of *C. gigas* was therefore underestimated in the monitoring programme, but this will not have affected our comparison between years.

We conclude that larviphagy occurs among *C. gigas*, *M. edulis* and *C. edule* individuals in the Oosterschelde estuary. We found evidence for effects of larviphagy by an oyster bed on larval numbers of *M. edulis*, but no evidence for effects of larviphagy on numbers of *M. edulis* larvae on estuary-scale. Six years may have been too short to detect a trend in larval abundance of *M. edulis*. The decline in numbers of *C. gigas* larvae may point to effects of increased larviphagy, but effects of an increased

filtration pressure on food availability for bivalves and their larvae may also have played a significant role. Furthermore, the mathematical exercise suggests a large effect of larviphagy on estuary scale. All results combined strongly suggest that bivalve filter-feeders have a large impact on larval abundance of bivalve filter-feeders. Whether larviphagy also affects stocks of adult bivalves and other benthic species seems likely, but remains open for further research.

Chapter 7

Introduction

In this Chapter the causes of the Pacific oyster's success in Dutch estuaries and the potential consequences for native bivalves are reviewed. In section 7.1 characteristics of *Crassostrea gigas* that may have contributed to its successful establishment and natural range expansion throughout the Oosterschelde estuary and other Dutch estuaries will be discussed, as well as the invasibility of Dutch estuaries. The review in this section is mainly based on a literature study. In section 7.2 competition for food and space between *C. gigas* and native bivalves as well as effects of larviphagy are discussed. This is mainly based on the research results reported in the previous Chapters. In section 7.3 all findings are synthesized and conclusions are presented on the causes of success of the Pacific oyster and the consequences for native bivalves. Consequences for other species are discussed, as well as some management aspects.

Causes of the Pacific oysters' success and consequences for native bivalves:

General discussion

Karin Troost

7.1. Causes of success: characteristics of *Crassostrea gigas* enabling its successful invasion

Of characteristics generally attributed to successful invaders, many are applicable to *C. gigas* (Table 7.1). First colonization of Dutch estuaries by *C. gigas* occurred through introductions by oyster farmers, who sowed oyster spat and juveniles on culture plots in the Oosterschelde estuary, and was therefore entirely facilitated by humans. Characteristics of the Pacific oyster itself facilitated successful establishment and a rapid natural range expansion in the Oosterschelde estuary and colonization of other Dutch estuaries. These characteristics are discussed below.

7.1.1. Establishment of *C. gigas* in Dutch estuaries

For successful establishment, competitive ability seems of key importance (Sakai et al. 2001). Competitive interactions between *C. gigas* and the most common native bivalve filter-feeders are discussed in section 7.2. Furthermore, lack of natural enemies is also expected to contribute significantly to establishment success (Sakai et al. 2001), and close association to humans, ecosystem engineering capabilities, repeated introductions, genetic variation and phenotypic plasticity are assumed to be contributing traits (Table 7.1; see Chapter 1).

Lack of natural enemies

A lack of natural enemies in the receiving community is often suggested as a reason for fast proliferation of introduced non-indigenous species (Williamson and Fitter

1996; Keane and Crawley 2002; Liu and Stiling 2006). To find out whether this 'enemy release hypothesis' is applicable to the successful establishment and rapid expansion of *C. gigas* in Dutch waters, an overview of predators, parasites and diseases affecting *C. gigas* in the North-West Pacific and in the Netherlands is given here.

Table 7.1. A selection of characteristics generally attributed to successful invaders, especially relevant for bivalve invaders and for the three principal stages from first introduction to natural spread to other locations (from Lodge 1993; Williamson and Fitter 1996; Morton 1997; Sakai et al. 2001; Marvier et al. 2004; Wallentinus and Nyberg 2007; and references therein). This table was copied from Chapter 1 (Table 1.1).

Stage	**Trait**
Colonization	rapid growth
	rapid sexual maturation
	high fecundity
	ability to colonize wide range habitat types
	broad diet
	tolerance to wide range environmental conditions
	gregarious behaviour
	genetic variability & phenotypic plasticity
	ability to recolonize after population crash
Establishment	competitiveness
	lack of predators, parasites and diseases
	association with humans
	repeated introductions
	ecosystem engineering
	genetic variability & phenotypic plasticity
Natural range expansion	dispersability
	traits of successful colonists (see above)

In the Netherlands native bivalves are generally heavily preyed upon by various bird species (Reise 1978; Nehls et al. 1997), but *C. gigas* is affected much less by bird predation. Herring gulls (*Larus argentatus*) and oystercatchers (*Haematopus ostralegus*) are the only bird species reported to feed on *C. gigas*. Herring gulls prey upon *C. gigas* locally. They take a loose individual up in the air, and break the shell by dropping the oyster several times on a hard surface, usually a stone-covered dike (Cadée 2001 at Texel, Wadden Sea; own unpublished observations in the Oosterschelde estuary). Recently, also oystercatchers *H. ostralegus* learnt to feed on *C. gigas* in the Wadden Sea

(Cadée 2008). However, they appear unable to open the shells of healthy individuals. They were solely observed to eat flesh of individual oysters that were washed ashore, likely of individuals that were desiccated and gaping (Cadée 2008). Predation by herring gulls and oystercatchers occurs only locally at low rates and is not expected to cause significant losses (Cadée 2008).

Little information on the role of bird predation in the North-West Pacific could be found. The local species of oystercatcher (*H. ostralegus osculans*) is said to be an uncommon species (Del Hoyo et al. 1996). The black-tailed gull *Larus crassirostris* is a common omnivore in Japan, and reported to feed on molluscs (Del Hoyo et al. 1996). However, no information was found on whether Pacific oysters are also included in its diet. No indications were found that bird predation pressure in the Pacific differs very much from that in the Netherlands.

Fish species in the Netherlands reported to feed on bivalve spat are the gobies *Pomatoschistus microps* and *P. minutus*, and juvenile flatfish of the species *Pleuronectes platessa*, *Platichthys flesus* and *Solea solea* (Hiddink et al. 2002 and references therein). Whether these fish also predate on spat of *C. gigas* is not known. In Japan, Pacific oyster spat is reportedly predated by the black sea bream *Acanthopagrus schlegelii* and the fine-patterned puffer *Takifugu poecilonotus* in Hiroshima Bay (Saito et al. 2008).

In the Netherlands especially juvenile stages of bivalves are preyed upon by a variety of epibenthic invertebrate predators (Beukema 1991; Beukema et al. 1998; Van der Veer et al. 1998; Hiddink et al. 2002). The most common invertebrate shellfish predators in the Wadden Sea and Dutch estuaries are the brown shrimp *Crangon crangon*, the shore crab *Carcinus maenas* and the common starfish *Asterias rubens*. Of these the shore crab and the starfish have been shown in laboratory experiments to predate on *C. gigas* as well (Diederich 2005b: oysters with shell lengths of up to 40 mm offered to and predated by *C. maenas*, and oysters of up to 60 mm offered to and predated by *A. rubens*). However, Diederich (2005b) found no effects of predation on post-settlement survival of *C. gigas* juveniles in the German Wadden Sea at Sylt, and she also showed a strong preference of *C. maenas* and *A. rubens* for *Mytilus edulis* over *C. gigas* of similar shell length in laboratory feeding experiments. *C. crangon* causes high mortality rates among native bivalve spat (Van der Veer et al. 1998), but no reports on whether it also feeds on *C. gigas* spat were found.

In its native range, the Pacific oyster is predated by crabs (Fukui 1988). It is also attacked by a variety of other epibenthic predators not occurring in Dutch waters. In Japan oysters are predated by several species of predatory flatworms (Turbellaria, Polycladida; Kato 1944; Galleni et al. 1980) among which *Pseudostylochus ostreophagus*, especially dangerous to oyster spat, and *Stylochus ijimai* that predates on adult oysters

(Korringa 1976b). Gruet et al. (1976) describe how Pacific oysters imported from Japan into France were immersed in freshwater to kill these flatworms; apparently this treatment was successful. Fujiya (1970) describes the following natural enemies of *C. gigas* in hanging-culture in Japan, all oyster drills: *Thais tumulosa clavigera*, *Thais bronni*, *Tritonalia japonica*, *Rapana thomasiana* (presently referred to as *R. venosa*), and *Ceratostoma burnetti*. Among these species, *T. tumulosa clavigera* is the most serious enemy of oysters (Fujiya 1970). Some of these predators were introduced with early oyster shipments to North America, before Canadian and American authorities developed and implemented control measures in cooperation with Japanese authorities and seed growers (Quayle 1988). Lavoie (2005) mentions the flatworm *P. ostreophagus* and the Japanese oyster drill *Ocenebra japonica* (Dunker, 1860) (also called *Tritonalia japonica* (Dunker, 1850), *Ceratostoma inornatum* (Recluz 1851), and presently referred to as *Ocinebrellus inornatus* (Recluz 1851)). This oyster drill originates from the same geographical range as *C. gigas*, and was introduced in North America in 1924 and, from there, to France in 1995 (Martel et al. 2004). The veined whelk *Rapana venosa* was introduced to several areas over the world (Black Sea, Aegean Sea, Adriatic Sea, Uruguay, the eastern USA, north-western France) and was found in the southern North Sea in 2005 (Kerckhof et al. 2006). So far no veined whelks or flatworms predating on *C. gigas* have been reported from Dutch estuaries. The oyster drills *Ocinebrellus inornatus* and *Urosalpinx cinerea* (native to the US east coast) have been found in the Oosterschelde estuary in 2007 and 2008 (Faasse and Ligthart 2007; Goud et al. 2008). They were likely introduced only recently and still occur very locally and in low numbers. It may therefore be concluded that the predation pressure from epibenthic invertebrate predators in previous years was likely to be much higher in the North-West Pacific than in the Netherlands, thus giving support to the 'enemy release hypothesis' as an explanation for the proliferation of *C. gigas* in Dutch estuaries.

From Japan and British Columbia, live adult Pacific oysters were introduced directly into the Oosterschelde estuary. Therefore, most parasites and diseases that are present in the areas of origin and that have been able to survive and establish in the receiving ecosystem, are likely to be present in the Oosterschelde estuary. The copepod parasites of the Pacific oyster *Mytilicola orientalis* and *Myicola ostreae* were thus introduced with Pacific oyster imports from Japan or British Columbia (Stock 1993; Wolff 2005). It is not known whether the latter species is presently established in Dutch waters (Wolff 2005). *M. orientalis* causes loss of gonadal mass in *M. edulis* (Mann 1956 and Williams 1969 in Steele and Mulcahy 2001), but it hardly affects *C. gigas* (Steele and Mulcahy 2001). The bacterium *Nocardia crassostreae* also seems to have been introduced from Japan and/or the west coast of North America. In these regions,

occurrence of this bacterium in Pacific oysters is associated with summer mortalities (references in Engelsma et al. 2008). *N. crassostreae* was recently found, together with the bacterium *Vibrio aestuarianus*, in *C. gigas* from Lake Grevelingenmeer (The Netherlands; Engelsma et al. 2008). The infected oysters were collected after an extensive mortality in the summer of 2006. However, it was concluded that the oysters had mainly died because of physiological stress due to adverse environmental conditions in Lake Grevelingenmeer. The bacteria may only have contributed as a secondary cause to the observed oyster mortality (Engelsma et al. 2008). Parasites of *C. gigas* that were already described from Dutch estuaries before the first introduction of *C. gigas* are the fungus *Ostracoblabe implexa* and spionid polychaetes of the genus *Polydora* (Korringa 1952). The fungus *O. implexa* affects and weakens *C. gigas*' shell, but this does not seem to lead to significant mortalities (Engelsma and Haenen 2004). *Polydora* spp. weaken the shell of *C. gigas* and the native oyster *Ostrea edulis* by burrowing into it (Korringa 1951; Almeida et al. 1996), but this does not appear to cause mortalities among the two species (Engelsma and Haenen 2004). In Japan, *C. gigas* is also infected by polychaetes of the genus *Polydora*, but as in the Netherlands these do not cause serious damage (Fujiya 1970). The Pacific oyster is furthermore not affected by bonamiosis (Renault 1996). After its introduction with oyster imports from Brittany in 1980, bonamiosis has caused very high mortality rates among *O. edulis* (Drinkwaard 1999a). The protist *Bonamia ostreae* that causes bonamiosis possibly originates from the north-east Pacific (Wolff 2005 and references therein). Tentatively, it is concluded that infestation of *C. gigas* by parasites and diseases in the Netherlands is at a similar level as in the native area of the Pacific oyster.

In conclusion, bird predation rates on *C. gigas* are very low in the Netherlands, as seems to be the case in the north-west Pacific. However, invertebrate predation on *C. gigas* seems to be considerably higher in its native range compared to the Netherlands. *C. gigas* is not free from parasite pressure in Dutch waters. It took at least some of its parasites with it from the north-west Pacific, and is also infected by European parasites. However, parasites and diseases do not appear to cause significant mortality among *C. gigas* in the Netherlands. Infestation of *C. gigas* by parasites and diseases in the Netherlands may be at a similar level as in its native area. The apparent lack of natural predators and the low vulnerability to parasites and diseases in the Netherlands likely contributed to the oyster's invasion success.

Ecosystem engineering by *C. gigas*

Pacific oysters are ecosystem engineers (Jones et al. 1994; Gutiérrez et al. 2003). Ecosystem engineers are organisms that affect access of other species to resources by modifying the habitat (Jones et al. 1994). By creating large shell volumes and adding structural complexity to the ecosystem, bivalve molluscs affect opportunities for settlement and refuge from predation and environmental stress (Gutiérrez et al. 2003). They may also affect particle and solute transports by altering near-bed flow (Gutiérrez et al. 2003). Habitat modification by invasive ecosystem engineers may facilitate establishment and subsequent colonization of the new area by adapting the encountered habitat, that may have been suboptimal, to the demands of the invader (Cuddington and Hastings 2004). In addition, engineering invaders seem to impact receiving ecosystems more severely than non-engineering invaders (Vitousek 1990; Wallentinus and Nyberg 2007).

Pacific oysters build dense oyster reefs with a high three-dimensional structure. The oysters change the habitat locally to their demand. The roughness of oyster reefs enhances near-bed turbulence levels, thereby increasing the food flux towards the bivalves and reducing refiltration of already filtered seawater (Jonsson et al. 2005; Widdows and Navarro 2007; Chapter 2). The three-dimensional structure provides shelter against predation from e.g. birds that have difficulty reaching into the oyster bed, but also from benthic predators such as crabs. The high degree of structural complexity in an oyster reef reduces the predator-prey encounter rate (Bartholomew et al. 2000; Grabowski 2004) resulting in higher growth and survival of oyster spat (Nestlerode et al. 2007). Oyster reefs also offer the spat shelter from extreme environmental conditions such as heat and desiccation (Bartol et al. 1999; Gutiérrez et al. 2003) and enhance larval settlement by baffling water movements (Commito and Rusignuolo 2000). In conclusion, ecosystem engineering by the Pacific oyster likely contributed to its fast establishment in Dutch waters.

Other traits contributing to successful establishment of *C. gigas*

Pacific oysters are obviously associated closely to humans since they are cultured world-wide for consumption. In the Netherlands, Pacific oysters have been introduced repeatedly from 1964 to around 1980 (Drinkwaard 1999b), because of desirable traits for mariculture (Korringa 1976b; Shatkin et al. 1997) and promising initial results of culturing trials in 1964 (see Chapter 1). Propagule pressure was therefore high, increasing the genetic variation of the introduced stock, widening the

genetic bottleneck and increasing chances of establishment and adaptation to the new environment. Japanese populations show a high genetic variation, and most of this high variation appears to have been retained by populations of *C. gigas* introduced for mariculture world-wide (Hedgecock et al. 1996; English et al. 2000; references therein). In addition to the relatively large gene pool in the new area, the species also appears highly adaptable phenotypically, as are many species of sessile bivalves (Bayne 2004). In terms of survival, growth and reproductive effort, *C. gigas* responds plastically to spatial variability in food abundance (Ernande et al. 2003). Pacific oysters are also flexible in the morphology of their feeding organs (relative sizes of gills and labial palps; Honkoop et al. 2003), and in their limits of thermal tolerance (Hamdoun et al. 2003). As in many other bivalves, also the larvae and spat of *C. gigas* exhibit high phenotypic variation, particularly in terms of growth and survival (Taris et al. 2006). High phenotypic flexibility enables an invader to adapt to a wide range of conditions, and increases chances of becoming established and of competing successfully with native species.

Repeated or multiple introductions are often correlated with the eventual success of non-native species' establishment and invasiveness (Sakai et al. 2001). Multiple invasions are thought to provide the invader with the genetic variation necessary for adaptive evolution. The time needed to gather the genetic variation necessary to adapt evolutionary to the new environment in many cases results in a time-lag between first colonization and establishment. A time-lag of several years between first introduction and establishment has been described for the Dutch Oosterschelde estuary (Drinkwaard 1999b) and the German Wadden Sea (Reise 1998; Schmidt et al. 2008). However, regarding the high genetic variation and phenotypic flexibility among populations of *C. gigas*, this may rather be a time-lag between first introduction and first human observation of feral oysters than an actual time-lag between first introduction and establishment of the species. In the first years after establishment, naturally produced spat would have been low in abundance and therefore not very conspicuous. Indeed, although Drinkwaard (1999b) described a first spatfall in the Oosterschelde estuary in 1975, Kerckhof (in prep.) recently reported on finding *C. gigas* of at least one year old in the Oosterschelde estuary in 1971. This supports the hypothesis that there was no time-lag between first introduction and establishment. This hypothesis is further supported by the comparable sea surface temperatures in the area of origin of the Miyagi and Kumamoto strains (that were mainly imported, Box 7.1) and in Dutch estuaries (Oceanographic atlas of the Bering Sea, Okhotsk Sea and Japan/East Sea, www.pacificinfo.ru, January 2009). If environmental conditions

were similar, perhaps the Pacific oyster did not need to adapt to Dutch estuaries that much.

In conclusion, *C. gigas*' close association to humans, its high genetic variation, and its phenotypic flexibility are all likely to have contributed to a rapid establishment in the Oosterschelde estuary.

7.1.2. Natural range expansion of *C. gigas* in NW Europe

The Pacific oyster shows a large capability of spreading rapidly after first introduction and establishment. From the moment the first spatfall was observed the rate of spread to new areas has been very fast in France, The Netherlands and Germany (Grizel and Héral 1991; Kater and Baars 2004; Diederich 2005b; Cognie et al. 2006; Dankers et al. 2006; Nehls et al. 2006). In the Oosterschelde estuary, the intertidal area covered by

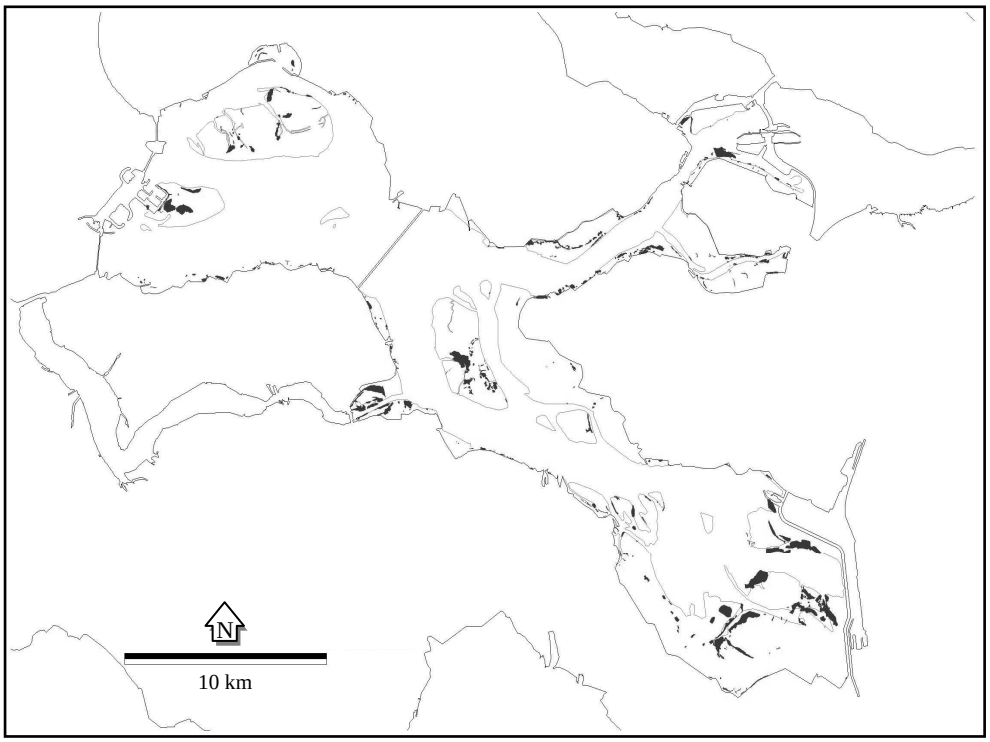


Figure 7.1. Oyster cover (dark areas; a total of 8.1 km²) in the intertidal of the Oosterschelde estuary in 2003 (Wageningen IMARES, Yerseke). Mean low water level is indicated with a thin line.

oyster beds increased from 0.25 km² in 1980 to 8.1 km² in 2003 (Figure 7.1; Kater and Baars 2004; Dankers et al. 2006). This illustrates the high potential for range expansion of *C. gigas,* which is probably a result of a high dispersion rate and of characteristics generally attributed to colonization success (Table 7.1). Characteristics attributed to colonization success are also believed to contribute to a fast natural range expansion (see also Chapter 1).

Dispersability

Pacific oysters are broadcast spawners and are highly fecund. An oyster female may produce more than 50 million eggs per spawning, which is high compared to native bivalves (Helm et al. 2004). A *M. edulis* female may produce 5 – 12 million eggs per spawning (Helm et al. 2004), a *C. edule* female 0.2 – 0.7 million eggs (Honkoop and Van der Meer 1998) and a *Macoma balthica* female 0.02 – 0.07 million eggs (Honkoop and Van der Meer 1998). Per square meter bed, however, total egg production of *C. gigas* and *M. edulis* is comparable because the latter generally occurs in higher densities (see Nehls et al. 2006). In the Netherlands, Pacific oysters produce more and relatively smaller eggs than their more southern kin in France (Cardoso et al. 2007). These smaller eggs have a lower energy content and therefore result in a longer duration of the pelagic larval phase (Van der Veer et al. 2006; Cardoso et al. 2007). This enables a wider dispersion range, although it may also result in a lower fertilization rate (Luttikhuizen et al. 2004). Although the bulk of the larvae travel up to 5 to 15 km, a smaller part will be carried further with residual currents (Wehrmann et al. 2000; Brandt et al. 2008). In conclusion, the high dispersability of *C. gigas*, caused by its production of relatively large amounts of eggs and larvae and the lengthy pelagic stage of its larvae, will have contributed to the species' fast natural range expansion after establishment.

r-selected traits of *C. gigas*

The Pacific oyster exhibits many features of an *r*-selected life-history strategy (Pianka 1970) that is generally believed to contribute greatly to colonization success (Sakai et al. 2001) and therefore also to a fast natural range expansion (Table 7.1). Growth of Pacific oysters is rapid. Two-year old Pacific oysters have been reported to reach lengths of up to 80 mm at Sylt, Germany (Diederich 2006), and lengths of 30 mm on average at Texel and Yerseke, the Netherlands (Cardoso et al. 2007). The oysters, that can live up to about 10 years (Cardoso et al. 2007; Global Invasive Species Database

BOX 7.1.
Origin of *Crassostrea gigas*

Pacific oysters occur from the Russian island of Sakhalin and Primorskiy Kray on the continent in the north (latitude ~48° N) to the Japanese island of Kyushu and the east coast of China in the south (latitude ~30° N; Figure B7.1.1; Arakawa 1990a). In Japan four regional strains of *C. gigas* were discerned by Imai and Sakai (1961), that originate from different geographical areas: Miyagi, Hiroshima, Kumamoto and Hokkaido. However, the Kumamoto oyster was later shown to be a different species, *Crassostrea sikamea*, by Buroker et al. (1979) for Japanese populations and by Banks et al. (1994) for cultured oysters from the United States. The Miyagi and Hokkaido oysters come from a relatively cool climate with temperate conditions. They are relatively larger and grow faster than Hiroshima oysters that originate from a warmer region (Imai and Sakai 1961). Oysters imported in British Columbia and the Netherlands were mainly (but not exclusively) of the Miyagi and Kumamoto strains, but experiments with Hiroshima oysters have also been conducted in the Netherlands (Shatkin et al. 1997; Drinkwaard 1999b). It is not clear what happened with the Kumamoto *C. sikamea* oysters in the Netherlands. They may have hybridized with *C. gigas*, or they may have disappeared (see English et al. 2000). *C. sikamea* also seems to have disappeared from its native range in Japan and may now only be found in culture in North America (Banks et al. 1994). Mann et al. (1991) described *C. gigas* oysters in North American and European cultures as Miyagi-like and pointed out that there has been much intentional inter-breeding of introduced stocks, but that precise pedigrees are lacking.

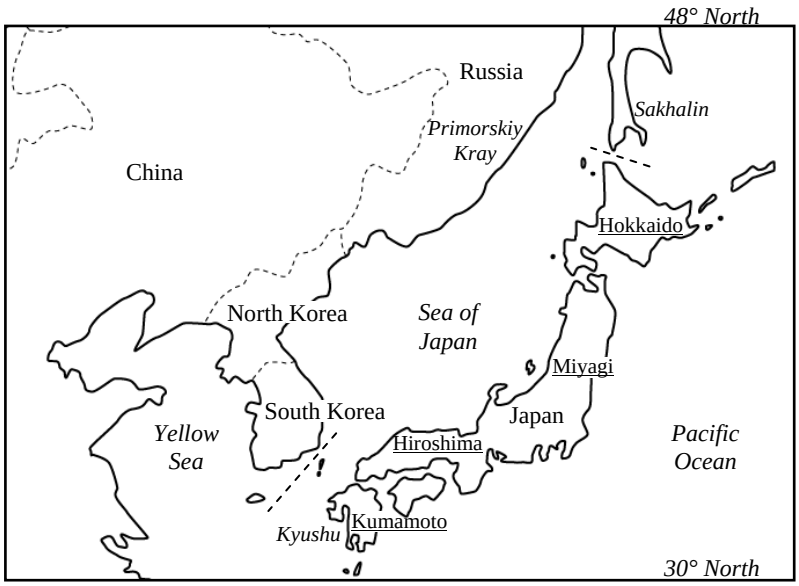


Figure B7.1.1. Native range of the Pacific oyster *C. gigas*.

www.issg.org 5-21-2008), reach maximum lengths of up to 300 mm (Reise 1998; Cardoso et al. 2007; own unpublished observations). In comparison, exceptionally large shell lengths reported for NW European native bivalves are: 30 mm for *M. balthica*, 60 mm for *Cerastoderma edule*, 150 mm for *M. edulis*, and 150 mm for the historically introduced *Mya arenaria* (Van der Veer et al. 2006, and references therein). Fast growth may enable the oysters to reach a size refuge from invertebrate and fish predation quickly. In addition, Diederich (2006) observed very high survival rates of *C. gigas* juveniles in comparison to native species. The combination of fast growth and high survival may account for a fast population increase, and may compensate for recruitment failures near the distribution limits (Diederich 2006). Pacific oysters, as well as the native *M. edulis* and *C. edule*, may already spawn in the season following their settlement.

Pacific oysters are generalists

Pacific oysters are able to colonize a wide range of habitat types (Quayle 1988). Although in first instance they settle onto hard substrates in the subtidal and intertidal, they also develop beds on soft bottoms by first settling onto small pieces of shell and stones (Quayle 1988; Mann et al. 1991; Leewis et al. 1994; Wolff 2005; Dankers et al. 2006). This is facilitated by their gregarious settling behaviour. Oyster larvae preferentially settle onto oyster shells (Diederich 2005a) and settlement is triggered by presence of adult oysters (Tamburri et al. 2007) and likely also by previously settled spat (Box 7.2). The native geographical range of *C. gigas* is very wide, comprising a large range of abiotic conditions (Box 7.1). The regions where *C. gigas* was successfully introduced also cover a wide geographical range (see Chapter 1, Box 1.1). Hence, the Pacific oyster was already adapted to a wide range of environmental conditions, and appears able to quickly adapt to new habitats. This is confirmed by its ability to sustain a wide range of environmental conditions. The oysters can survive water temperatures up to 40 °C (Shamseldin et al. 1997) and at low tide air temperatures as low as -5 °C (Korringa 1952) and even lower, depending on the salinity of the water enclosed in their shells (>75% survival at 30 psu, at -12 °C air temperature; exposure during 7 days, 6 hours per day, mimicking tidal emersion; Wa Kang'eri 2005). Growth occurs between 10 and 40 °C and 10 – 30 psu, and spawning between 16 and 30 °C and 10 - 30 psu. Larvae can sustain temperatures between 18 and 35 °C and salinities between 19 and 35 psu (Mann et al. 1991 and references therein; Rico-Villa et al. 2009). In conclusion, Pacific oysters indeed appear to be generalists, which will have contributed to their rapid natural range expansion.

Ability of *C. gigas* to repopulate previously colonized habitats

Dutch estuaries consist largely of soft sediments, with occasional patches of hard substrate created by man (e.g. dikes) and epifaunal bivalves. Mussel beds consist of many mussels clumped to small pieces of hard substrate and to each other with byssus threads. Such byssal attachments are temporary and allow the mussels to detach, move to another location and re-attach within the bed (see Van de Koppel et al. 2008). Mussel beds may disappear locally following mass mortality (Brinkman et al. 2002). When the mussels die, most of the empty shells will be washed away with the tides. Mussel beds may also disappear due to storms or ice scouring (Nehls and Thiel 1993; Strasser et al. 2001). Oyster reefs are more persistent. Oyster reefs consist of many oysters that are permanently attached together into large clumps or patches. Following mass mortality, the reef itself, now consisting mainly of empty shells cemented together, will remain in place. Pacific oysters therefore increase the area suitable for settlement by building reefs, and in this way ensure settlement possibilities on a longer time scale. Following mass mortality, an extensive spatfall of larvae from another brood stock will be sufficient to recolonize the entire reef. This process will be enhanced by the oysters' gregarious settling behaviour (Box 7.2) and high dispersability.

7.1.3. Invasiveness of the Dutch estuaries

The highest rate of population increase of *C. gigas* was observed in the Oosterschelde estuary, where the oyster was initially introduced. From there, other estuaries in the Netherlands were colonized (although oysters in the Westerschelde estuary may also originate from Ostend, Belgium). How invasible were these Dutch estuaries, in particular the Oosterschelde estuary, in terms of: the level of disturbance, species-richness and predator abundance?

Disturbance of the Dutch estuarine ecosystems

Although disturbance is a factor which is hard to quantify, it may be suggested that it has played a part in the establishment of *C. gigas* in Dutch estuaries. The Oosterschelde estuary may be considered a disturbed ecosystem, because of the Delta Project which is still causing on-going changes (Chapter 1, Box 1.4). In 1975, when the first large natural spatfall of *C. gigas* was recorded, the Oosterschelde estuary was still in open connection with the North Sea. However, the estuary had already been

hydromorphologically modified through its separation of the present Lake Veerse Meer in 1960, the Grevelingen estuary (presently Lake Grevelingenmeer) in 1964 and the Haringvliet estuary (presently Lake Haringvliet) and riverine input from the rivers Rhine and Meuse in 1969. Other sources of disturbance in the Oosterschelde estuary are fishery activities, shellfish culture and shipping. The Delta project also had a large impact on Lake Grevelingenmeer that was turned from a tidal estuary in to a stagnant and brackish lake. The Westerschelde estuary, although not much affected by the Delta project since it remained an estuary with open connection to the North Sea, was heavily polluted in the 1970s and 1980s (Van Eck et al. 1998), and large quantities of sand and mud are dredged and dumped continuously (Ysebaert et al. 2000). It is intensively used as a shipping channel to the harbours of Antwerp, Ghent, Terneuzen, and Flushing (Vlissingen).

The Dutch Wadden Sea received pollutants from the river Rhine that was severely polluted in the 1960s, 1970s and 1980s (De Jonge et al. 1993). Furthermore, it has been modified by the construction of the Afsluitdijk, a barrier dam that separates Lake IJsselmeer (the former Zuiderzee estuary) from the Wadden Sea since 1932. This has induced still on-going changes in geo- and hydromorphology and salinity gradients (De Jonge et al. 1993; De Jonge and De Jong 2002). Shores along all Dutch estuaries have been modified drastically by building dikes, to protect the land from flooding. In conclusion, all Dutch estuaries experience(d) some level of human-induced disturbance which may have facilitated establishment and spread of *C. gigas* to some extent.

Low species richness in estuaries

In general, species-poor estuarine communities seem more susceptible to invasions than species-rich, more saturated communities (Wolff 1973; Lodge 1993; Wolff 1999). This is thought to be a contributing factor to the relatively large number of non-indigenous species recorded in brackish (5 – 20 psu) estuaries (Wolff 1973; Wolff 1999; Nehring 2006). In the case of *C. gigas* it may have contributed to the rapid colonization of other estuaries than the Oosterschelde estuary, although highest densities of *C. gigas* are found at salinities higher than 20 psu. The Oosterschelde estuary itself is not at all poor in native species (Wolff 1973; Hostens and Hamerlynck 1994; Sistermans et al. 2005; Wolff 2005), and moreover harbours many non-indigenous species that were imported through shellfish culture activities (Wolff 2005). Species richness therefore did not play a part in the establishment of *C. gigas*.

BOX 7.2.
Preferential settlement of *Crassostrea.gigas* larvae near conspecific spat

Anne Vos and Karin Troost

Introduction. Pacific oysters *C. gigas* settle gregariously. They prefer to settle on oyster shell over mussel shell (Diederich 2005a) and settlement is induced by an adult cue (Tamburri et al. 2007). We studied whether *C. gigas* larvae also settle preferentially near previously settled conspecific spat, as was demonstrated earlier for *C. virginica* (Hidu 1969; Hidu et al. 1978).

Methods. To collect oyster spat, we used 6 cages each containing 6 spat collectors (Figure B7.2.1). These were roughened perspex panels of 15 x 15 cm, all covered with the same thin layer of a mix of cement, plaster and silver sand (4:2:1). Historically in the Netherlands, such a plaster mix was used to cover roof tiles as spat collectors for *Ostrea edulis* (pers. comm. Jaap Poelman; Korringa 1976a). The roughness provided by the plaster mix is supposed to aid in the settlement of oyster larvae. The experiment lasted for 2 weeks in August 2004. During the first week, 3 cages were placed in the intertidal of the Oosterschelde estuary at 1.4 m below MTL to collect oyster larvae. The other 3 cages were placed in an outdoor basin with running seawater that was pumped directly from the Oosterschelde estuary. These cages were covered in mesh bags (200 μm mesh) to prevent settlement of *C. gigas* larvae, still allowing for the formation of a biofilm similar in thickness to the biofilm formed on the collectors in the field (see Keough 1998, for effects of biofilms on settlement). After the first week, all cages were retrieved and numbers of oyster spat counted on the collectors from the field. The collectors from the basin were checked for absence of spat. On the same day, all cages were placed at the field location to collect spat for one more week. After the second week, oyster spat was counted on all collector panels.

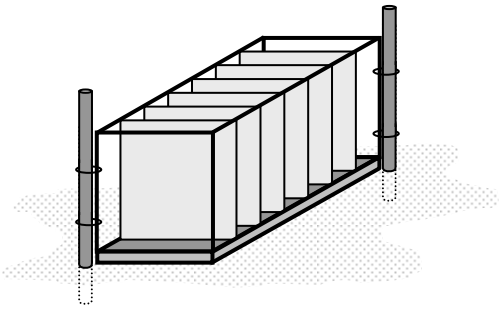


Figure B7.2.1. Schematic representation of a cage with six collector panels, as used in the field experiment. The cage was made of stainless steel, with a solid bottom and mesh at the sides and top (the mesh itself is not shown in the figure). It was fixed to the sediment with two bamboo poles and tie-wraps.

Results. Spat collectors that had already collected spat during the first week, collected significantly more spat during the second week (Figure B7.2.2; ANOVA: $p = 0.00$, $F = 360.1$).

Discussion. The presence of previously settled spat stimulated settlement of more spat. The new settlers may be attracted chemically to the previous settlers (see Hidu et al. 1978), or an increased surface roughness due to the presence of previously settled spat may be responsible for the observed enhanced spatfall on prespatted collectors.

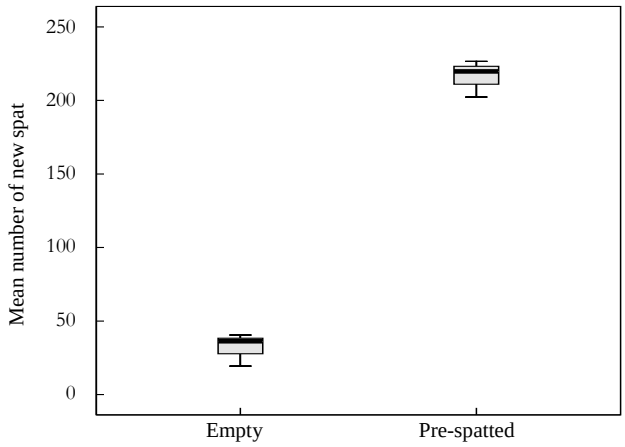


Figure B7.2.2. The mean number of new spat on pre-spatted and empty collectors. Per treatment, the mean number of new spat per cage is given ($n = 3$ cages). Six collector panels were averaged per cage. The box-plot represents the median and quartiles.

Predator abundance in estuaries

Dutch estuaries harbour plenty predators of bivalve shellfish, such as brown shrimp *Crangon crangon,* shore crabs *Carcinus maenas*, starfish *Asterias rubens*, oystercatchers *Haematopus ostralegus*, eider ducks *Somateria mollissima*, knot *Calidris canutus*, herring gulls *Larus argentatus*, and siphon-cropping flat-fish such as flounder *Platichthys flesus* and plaice *Pleuronectes platessa*. However, as already discussed (section 7.1.1 *'Lack of natural enemies'*), for as far as anything is known about predation on *C. gigas* by these predators, predation rates appear very low. Natural predators of *C. gigas* originating from the NW Pacific are not (yet) present in the Netherlands, or were introduced only recently and

still occur in low numbers. This lack of predators may be a contributing factor to the oysters' success in establishing in Dutch estuaries.

7.2. Consequences of *C. gigas*' successful invasion for native bivalves

7.2.1. Competition with native bivalve filter-feeders

Food competition

Bivalve filter-feeders feed on phytoplankton, but also on other particles in the water column that are large enough to be retained by the gills and that are not too large or evasive, such as dead particulate organic material or certain species of zooplankton (Fréchette et al. 1989; Navarro et al. 1992; Smaal 1997; Dupuy et al. 1999; Davenport et al. 2000; Dupuy et al. 2000; Karlsson et al. 2003; Lehane and Davenport 2004; Chapter 3). All particles above a species-specific retention threshold (2 – 12 μm, Møhlenberg and Riisgård 1978; Barillé et al. 1993) are retained by the gills. The species may differ in selection and absorption efficiencies, but also in how they optimize a food flux towards the bed, how they minimize refiltration inside the bed, and how efficiently they entrain zooplankton. Food-limited growth is a common phenomenon among bivalves, demonstrated to occur on a local scale in the Wadden Sea (Kamermans 1993; Beukema and Cadée 1997) and on a larger scale in the Oosterschelde estuary (Hoek 1902; Van Stralen and Dijkema 1994; Smaal et al. 2001).

Food intake of bivalves in relation to hydrodynamics

Chapter 2 showed that differences in inhalant feeding currents of individual *C. gigas, M. edulis* and *C. edule* are small despite apparent differences in morphology. Differences in inhalant feeding currents are therefore not expected to result in differences in food intake between the introduced oyster and native bivalves. However, processes on a larger scale (a patch or bed of bivalves) may be more determining. Bivalve filter-feeders can optimize food flux towards the bed by enhancing near-bed turbulence levels with their filtration activity and/or physical roughness of the bed. Modelling exhalant jet speeds revealed that also these are not very different between the three species (Chapter 2). Although exhalant jet speeds of *C. gigas* were generally higher than in the two other species, differences were modest. Moreover, exhalant jets in *C. gigas* were oriented horizontally instead of vertically as in *M. edulis* and *C. edule*. How this affects differences between the species in their effect

on the benthic boundary layer is not known yet. In conclusion, no important differences in food intake are expected as a result of differences in exhalant jet speeds of individual animals.

The much larger differences in roughness height of oyster, mussel and cockle beds are expected to contribute more strongly to differences in food intake. Oysters create larger roughness structures than mussels and cockles. Cockles do not produce any protruding roughness structures at all and seem entirely dependent on roughness created by their filtration activity. By creating larger roughness structures, Pacific oysters probably affect near-bed turbulence levels more strongly than native bivalves (that are all infaunal except for *M. edulis* and the now rare *O. edulis*). In that way they may enhance food flux and intake rate that is also facilitated by their large filtration capacity (Chapter 1, Box 1.3). Additionally, since many zooplankton species use hydromechanical signals to detect and escape from predators, higher levels of turbulent mixing cause more 'background noise' to these zooplankters, reducing (the effectivity of) escape reactions, potentially resulting in a higher zooplankton intake rate by oysters than by mussels and cockles (Chapter 2).

Diet of filter-feeding bivalves

Some bivalves may utilize a broader range of particles than others. Bivalve filter-feeders filter all particles above their retention threshold size (Møhlenberg and Riisgård 1978), as long as the particles do not escape (see Green et al. 2003; Maar et al. 2007; and Chapters 3, 4, 6). Pacific oysters may be more efficient in entrapping zooplankton because they probably create more background turbulence than native bivalves (although this yet needs to be studied, see previous section). Hence, they may utilize a broader diet. Zooplankton has been shown to be a useful additional food source. Bivalves grown on a mix of phytoplankton and zooplankton showed faster growth than bivalves fed with phytoplankton only (Wong and Levinton 2004).

Ostreids have reduced eulatero-frontal cirri and have a slightly higher retention threshold than species with long latero-frontal cirri (such as *C. edule*, *M. edulis* and *M. arenaria*). In the latter, all particles larger than 4 μm are 100% efficiently retained (Møhlenberg and Riisgård 1978). Ostreids have a retention threshold of 4 – 6 μm (Møhlenberg and Riisgård 1978; Riisgård 1988; Barillé et al. 1993). In addition, at high seston load the retention threshold in *C.*

BOX 7.3.
Size ranges of particles ingested by *Crassostrea.gigas* and *Mytilus edulis*

Edzard Gelderman and Karin Troost

Introduction. An upper size limit to what bivalves can still filter and ingest has hardly been studied. Davenport et al. (2000) found that mussels *M. edulis* routinely ingest particles between 100 and 1000 µm, and occasionally 3 – 6 mm. Since the inhalant feeding aperture of *C. gigas* is much larger than the inhalant aperture of *M. edulis*, *C. gigas* may filter and ingest larger particles, and thereby utilize a wider size range of food particles. We tested the hypothesis that *C. gigas* ingests larger particles than *M. edulis* by measuring lengths of all particles other than micro-phytoplankton encountered in stomach contents of both species.

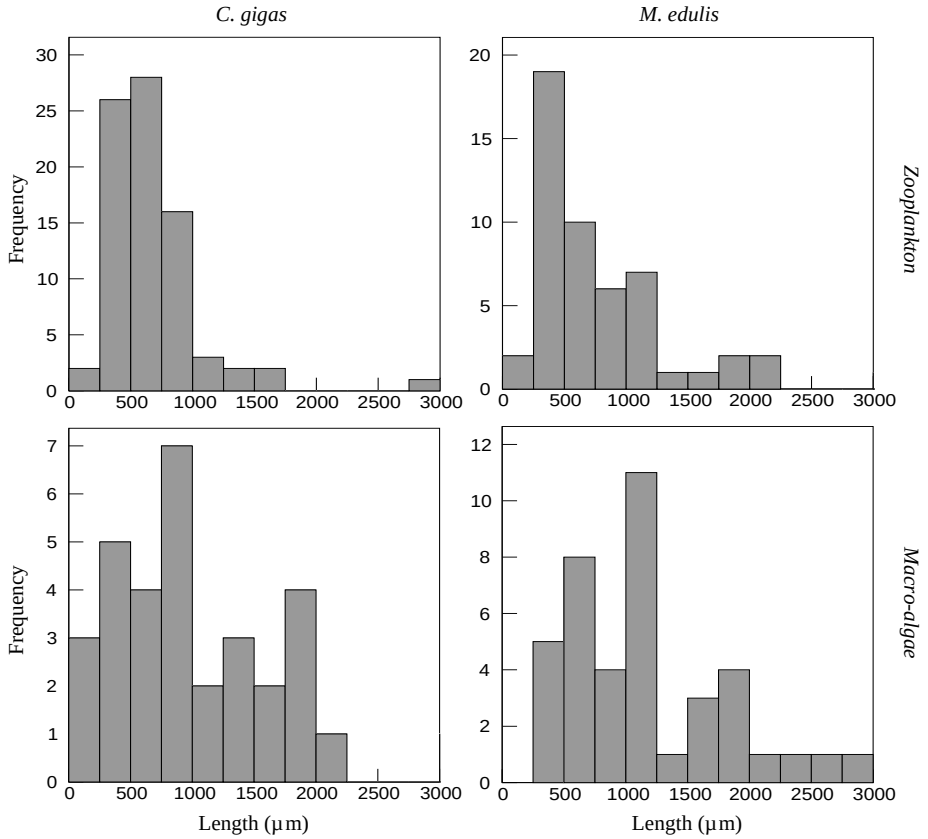


Figure B7.3.1. Length frequency distributions of particles of animal (zooplankton) and macro-algal origin, found in stomach contents of *C. gigas* and *M. edulis* (n = 32 stomachs per species).

Methods. Adult oysters *C. gigas* and mussels *M. edulis*, 32 per species, were suspended in cages in the Oosterschelde estuary during 6 weeks in May and June 2003. They were suspended at a tidal level of approximately 40 cm above MLW and were therefore emerged during low tide. Once a week, 4-6 individuals per species were removed 30 minutes before high tide. Their stomach contents were removed as quickly as possible and fixated with buffered formaldehyde, as described in Chapter 3. All zooplankton and macro-algal particles were measured to the nearest 10 μm.

Results. There were no apparent differences in the size range of prey items found in stomachs of *M. edulis* and *C. gigas* (Figure B7.3.1). We found no differences between species in average zooplankton prey size (Mann-Whitney U test: $p = 0.98$) and average prey size of macro-algal particles (Mann-Whitney U test: $p = 0.52$). In both species, sizes of prey found in stomach contents ranged up to 3 mm.

Discussion. We were unable to determine an upper size limit of potential prey items from our results since the length frequency distribution of prey items found in the stomachs may reflect length frequency distributions of zooplankton and algal material available in the water column. This would require a detailed comparison of prey sizes found in stomach contents to prey sizes available in the surrounding water. No differences in prey selection were found between the two species. The results do show that both species filter a wide range of prey sizes, up to a few mm in length. This corresponds to the result by Davenport et al. (2000).

gigas changes from about 4 μm to 12 μm (Barillé et al. 1993). This is thought to ensure a good functioning of the ciliary systems at the gills and labial palps, but it also renders *C. gigas* unable to utilize plankton < 12 μm at high seston load (Barillé et al. 1993). In addition to this mechanism and in comparison to Dutch native species of bivalve filter-feeders, larger components of the seston, such as ciliates, larger phytoplankton cells, debris of macro-algae and zooplankton, may play a more important role in the diet of *C. gigas* (see Dupuy et al. 1999). Although lower thresholds have been determined experimentally, it is more difficult to determine the upper size limit of what bivalves can still filter. Davenport et al. (2000) found 260 planktonic animals in stomach contents of 100 mussels *M. edulis* from the field. The mussels appeared to routinely ingest particles of 100 – 1000 μm, and occasionally particles of 3 – 6 mm. This corresponds to what we found for *M. edulis* and *C. gigas* from the Oosterschelde estuary (Box 7.3).

That Pacific oysters and native bivalves may not utilize the exact same diet was shown in various studies. Bougrier et al. (1997) showed in the laboratory that from various algal species simultaneously available in the surrounding water, *C. gigas* and *M.*

edulis selected different species for ingestion. Based on different stable isotope signatures of $\delta^{13}C$ (ratio of ^{13}C to ^{12}C) and $\delta^{15}N$ (ratio of ^{15}N to ^{14}N) of the bivalve tissue, *C. gigas* was found to utilize a diet different from that of *M. edulis* and the filter-feeding snail *Crepidula fornicata* in the French Bays of Veys (Dubois et al. 2007) and Mont Saint Michel (Riera 2006) and the Dutch Oosterschelde estuary (Riera et al. 2002). Differences in these signatures can indicate different food sources, but also a utilization of the same food sources but in different proportions is possible. Riera et al. (2002) and Riera (2006) hypothesized that the differences found between *C. gigas* and *C. fornicata* in the Oosterschelde estuary and Bay of Mont Saint Michel may be due to utilization of different size classes of consumed particulate organic matter. They therefore concluded that both species may not necessarily be competitors for the same food sources. Results from the Bay of Veys indicated that *C. gigas* is capable of a greater trophic plasticity than *M. edulis*. Pacific oysters showed a larger spatial variation in isotope signatures, indicating that they are better able to adapt to the local environmental availability of food items (Dubois et al. 2007).

Although Pacific oysters and native bivalves appear not to utilize the exact same diet, there does seem to be a large similarity in which particles they filter from the surrounding water (barring zooplankton species that may have different escape successes for different species of bivalves). This includes particles that they do not ingest, but instead reject in their pseudofaeces. Therefore, even if they do not compete directly for the same food sources, they do interfere with each other by reducing food levels available to other species (Green 1971; Case and Gilpin 1974).

Feeding physiology of filter-feeding bivalves

Bivalve filter-feeders adjust their feeding rate and feeding and absorption efficiencies to changes in total particulate matter (TPM) and organic content of the TPM (e.g. Navarro et al. 1992; Hawkins et al. 1998; Bayne 2002). Species with a relatively high food intake, efficient particle selection and absorption, and low metabolic loss will have a relatively high net energy gain and will be stronger competitors for food (Hawkins et al. 1998; Bayne 2002). In the Bay of Marennes-Oléron, at the French Atlantic coast, Hawkins et al. (1998) found differences in feeding physiology parameters between *C. gigas*, *M. edulis* and *C. edule* (after standardizing for 0.5 g dry soft tissue weight). The infaunal *C. edule*, feeding on natural seston, was found to have a lower capacity to selectively ingest organic matter at higher TPM. This was hypothesized to be compensated by a longer gut passage time for the extraction of available nutrients (Hawkins et al. 1998). Differences between *C. edule* and the other

two species were hypothesized to be due to the cockle living in environments that are less turbid, in sandy sediments (Hawkins et al. 1998). In comparison with *M. edulis*, *C. gigas* appeared less efficient in net selection of organic matter and digestion and/or assimilation of ingested organics. *C. gigas* rejected a smaller proportion of filtered material, resulting in a faster ingestion rate compared to *M. edulis*. However, *M. edulis* compensated the difference with a more efficient selection of organic matter before ingestion, resulting in similar net organic ingestion rates. Because the absorption rate of ingested organic matter in the stomach and gut by *M. edulis* was twice as fast compared to *C. gigas*, ultimately *C. gigas* gained less energy from filtered matter than *M. edulis* (Hawkins et al. 1998). Additionally, *C. gigas* may be metabolically more efficient than native bivalves. *C. gigas* has a competitive advantage over the Sydney rock oyster *Saccostrea glomerata* in Australia, due to faster rates of feeding and greater metabolic efficiencies of both feeding and growth (Bayne 2002).

Growth of bivalve filter-feeders

Using the Dynamic Energy Budget theory (Kooijman 1986, 2000), Cardoso et al. (2006) reconstructed food conditions for different bivalve species in Dutch estuaries, based on growth curves determined from the field. The results suggested that growth of *M. edulis* and *C. gigas* is suboptimal in Dutch coastal waters, in contrast to growth of *M. balthica* and *C. edule*. This was hypothesized to be due to food limitation during some months (Cardoso et al. 2006). Despite this, growth in *C. gigas* is still rapid in the Wadden Sea (Diederich 2006; Cardoso et al. 2007). Growth of *C. gigas* in the Oosterschelde estuary around 2002-2003 was similar to growth in the Dutch Wadden Sea (Cardoso et al. 2007), but over the past years growth of mussels and oysters has declined. This is probably due to a reduced carrying capacity and will be discussed further below (section '*Carrying capacity of the Oosterschelde estuary for filter-feeding bivalves*').
In the German Wadden Sea, growth of juvenile *C. gigas* settled on ceramic tiles was not affected by substrate type of the bed (i.e. sand, mussels, oysters), barnacle epibionts and tidal level, whereas growth of juvenile *M. edulis* was lower in mussel and oyster beds than on a sand flat and higher in the subtidal than in the intertidal (Diederich 2006). This suggests that oysters are more generalistic in where they settle, possibly with a broader tolerance for extreme values of environmental factors. It also suggests that in the German Wadden Sea growth in *M. edulis* was density-dependent whereas growth in *C. gigas* was not. This corresponds to the results of an MSc study in the Oosterschelde estuary (Box 7.4). Here, growth of caged mussels in an intertidal bed of wild Pacific oysters was negatively related to local oyster biomass, whereas

BOX 7.4.
Oyster-density-dependent growth of caged mussels and oysters

Ingmar Hans and Karin Troost

Introduction. In the Oosterschelde estuary, Pacific oysters *C. gigas* and blue mussels *M. edulis* appear to be competing for food. The species least affected in growth by reduced food levels is expected to have a competitive advantage over the other species. Both species co-occur in Pacific oyster beds. To determine which species is affected most by reduced food levels, we studied growth of caged *M. edulis* and *C. gigas* in an oyster bed, in relation to local biomass of the wild oysters.

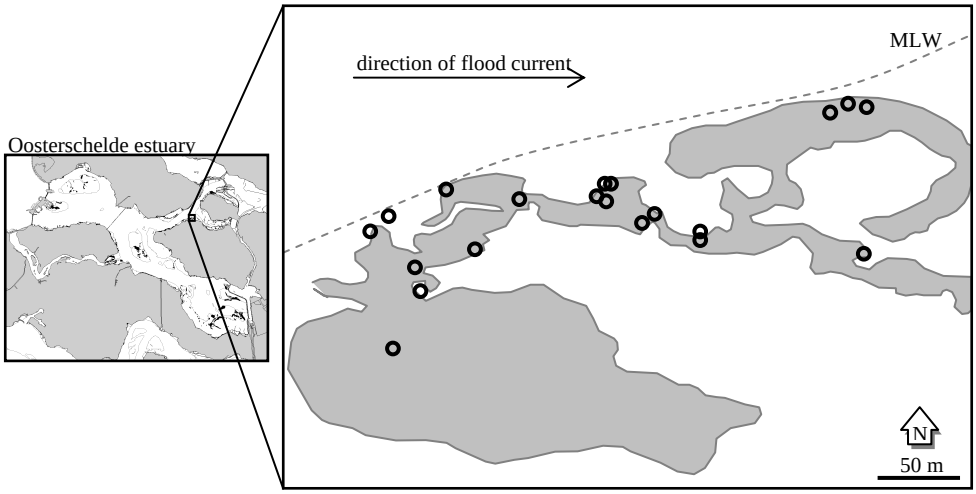


Figure B7.4.1. Experimental locations (circles) within the intertidal oyster bed (in grey) in the northern compartment of the Oosterschelde estuary. Also indicated are the level of mean low water (MLW) and the approximate mean direction of the flood current.

Methods. We placed cages with mussels and oysters at 20 randomly chosen locations in an oyster bed in the northern compartment of Oosterschelde estuary (Figure B7.4.1). Of both species we used two size classes: juveniles and adults. Per location we placed two cages, for oysters and mussels separately (mussels: 6 adults, 40-43 mm, and 7 juveniles, 28-30 mm; oysters: 5 adults, 56-93 mm, and 5 juveniles, 30-48 mm). The animals were marked individually, and measured to the nearest 0.01 mm before placing them in the field. The experiment lasted from August 3 to October 14, 2003. Upon retrieving them from the field, the animals were measured again and their individual shell length increments calculated. Local oyster biomass was determined at each of the 20 locations as ash-free dry weight of the flesh, after drying for 3 days at 70 °C and incineration of the flesh at 520 °C for 4 hours.

Results. Growth of juvenile and adult mussels was negatively related to local oyster biomass (Figure B7.4.2, Table B7.4.1). Growth of juveniles was faster than growth of adults, and negatively related to emersion time and the upstream (during flood tide) distance to the edge of the oyster bed (Table B7.4.1). Growth of juvenile and adult oysters was not dependent on local oyster biomass, emersion time, or distance to the edge (Figure B7.4.2, Table B7.4.1).

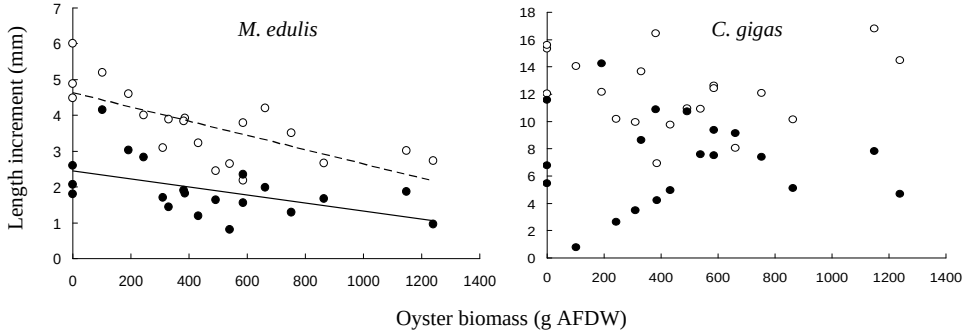


Figure B7.4.2. Shell length increment (in mm) of juvenile (open dots) and adult (filled dots) mussels *M. edulis* and oysters *C. gigas* in relation to local oyster biomass (in g AFDW) in the oyster bed. Each dot represents the average length increment of all juvenile (n = 5-7) or adult (n = 5-6) animals per species in one cage. Growth of juvenile and adult mussels showed a negative relationship with oyster biomass (juvenile: $R^2 = 0.49$, $p < 0.05$; adult: $R^2 = 0.26$, $p < 0.05$). Growth of juvenile and adult oysters showed no relationship with oyster biomass.

Table B7.4.1. Results of a stepwise multiple linear regression with three factors: local oyster biomass, emersion time, upstream (flood) distance to the edge of the oyster bed, tested for growth of juvenile and adult mussels and oysters.

	Factors		
Dependent variable (growth)	Oyster biomass	Emersion time	Distance to the edge upstream (flood)
M. edulis juvenile	$p = 0.0006$ $F = 17.3$	$p = 0.00004$ $F = 19.1$	$p = 0.00001$ $F = 20.6$
M. edulis adult	$p = 0.022$ $F = 6.3$	n.s.	n.s.
C. gigas juvenile	n.s.	n.s.	n.s.
C. gigas adult	n.s.	n.s.	n.s.

Discussion and conclusion. Decreasing growth of both juvenile and adult mussels with increasing local oyster biomass suggests that mussel growth was food-limited. Also the reduced growth of juvenile mussels with increasing emersion time and distance to the edge of the bed suggest food limitation. With an increasing emersion time the animals have less time to feed, and with an increasing distance to the edge of the bed the water will be more depleted of food items by bivalves upstream. Growth of oysters at the same

locations was independent of local oyster biomass, emersion time, and distance to the edge of the bed, and therefore did not appear to be food-limited. Since individuals of both species must have faced similar food levels and hydrodynamic conditions, this suggests that *C. gigas* is affected less by reduced food levels than *M. edulis*.

caged oysters at the same locations showed density-independent growth. This suggests that Pacific oysters either ingest more food, or an additional different type of food (e.g. zooplankton), or utilize the ingested food more efficiently. The first option is supported by e.g. the oyster's large filtration rate and large roughness of oyster beds (section '*Food intake of bivalves in relation to hydrodynamics*'). The second option is supported by results of Riera et al. (2002; 2006) and Dubois et al. (2007) (section '*Diet of filter-feeding bivalves*'). The third option is contradicted by the results of Hawkins et al (1998) (section '*Feeding physiology of filter-feeding bivalves*').

Carrying capacity of the Oosterschelde estuary for filter-feeding bivalves

It is widely acknowledged and demonstrated that bivalve filter-feeders in estuarine ecosystems may have a large effect on phytoplankton communities (see Fréchette et al. 1989; Smaal 1997; Prins et al. 1998; Dolmer 2000). The phytoplankton community in the Oosterschelde estuary seems to have shifted towards smaller species (Geurts van Kessel et al. 2003), probably as a result of an increased filtration pressure (see Noren et al. 1999). After comparing the turn-over time of the phytoplankton in the four different compartments of the Oosterschelde estuary (see Chapter 6) with the estimated time needed for oysters, mussels and cockles to filter the volume of these compartments, Geurts van Kessel et al. (2003) concluded that the carrying capacity may already have been reached in the northern and eastern compartments. The northern compartment showed no increase in biomass of the three dominant bivalves between 1990 and 2000, and the turn-over time of the phytoplankton equaled the estimated filtration
time by bivalves around 2000. A potential food shortage in this compartment cannot directly be compensated by input from the North Sea because of the long residence time of the water in this part of the estuary (although some compensation may occur from the adjacent central compartment). In 2003, this situation appeared to be almost reached in the eastern compartment as well (Geurts van Kessel et al. 2003).

Nowadays both *M. edulis* and *C. gigas* seem affected by reduced food levels in the Oosterschelde estuary. The meat content of commercial mussels appears to be declining (from roughly 25% around 2001 to 18% in 2007, unpublished data of the

Dutch Fish Product Board). The meat content of cultured Pacific oysters (all located in the eastern part of the estuary) is reported to have declined over the past 8 years. The oysters now take six years to reach market size, as opposed to only three years around 2001 (pers. comm. A. Cornelisse, Yerseke). These observations all point to a saturated carrying capacity for bivalve filter-feeders in the Oosterschelde estuary, but do not allow for conclusions on which of the two species is the most vulnerable to reduced food levels. Cultured mussels and oysters in the Oosterschelde estuary are not directly comparable. Oysters are exclusively cultured in the eastern compartment that has seen carrying capacity problems for oyster culture before (Hoek 1902), whereas mussels are mainly cultured in the central en western compartments where food is less limiting (Geurts van Kessel et al. 2003).

In the Dutch Wadden Sea, where *C. gigas* is not (yet) a dominant species, the carrying capacity is not reached (yet) (Brinkman and Jansen 2007).

Competition for space

Oyster reef development

Pacific oysters turn soft substrates into hard substrates by formation of oyster reefs. Thereby, they increase the area suitable for settlement of hard-substrate species and turn former soft-substrate communities into hard-substrate communities. Infaunal species would be expected to be affected negatively by expansion of oyster beds, since the area suitable for settlement decreases, and oyster reefs are difficult if not impossible to recolonize for infaunal species. However, only a limited fraction of the total estuarine intertidal area can be occupied by patches of filter-feeding bivalves, due to limiting physical processes at local scale (e.g. current velocity, mixing) and limiting primary production on a system scale (Heip et al. 1995).

Pacific oysters are strong competitors for space, since persistent oyster reefs can develop very fast once a sufficient amount of hard substrate is present (e.g. shell debris, a mussel bed or a pioneer-stage oyster bed). In the Oosterschelde estuary, oyster reefs mainly developed on hard substrates (e.g. dikes, jetties), former mussel culture plots, and former culture sites for the European flat oyster *O. edulis*. Former culture sites for *M. edulis* may still have contained some mussels or mussel shell debris, offering a hard substrate that stimulated colonization by *C. gigas*. On former culture sites for *O. edulis*, hard substrate was present in the form of low walls constructed of roof tiles. In the Wadden Sea, *C. gigas* also mainly colonizes areas with a relatively high cover of hard substrate, such as mussel beds and (cockle-)shell ridges (Dankers et al.

2006; Nehls et al. 2006; Schmidt et al. 2008). The oysters colonize soft muddy substrates as well, but the speed of reef formation seems dependent on the amount of hard substrate (shell debris in many cases) present (Wijsman et al. 2008).

Most oyster beds in Dutch and German coastal areas do not show a 100% cover of the substrate. Especially younger reefs show an alternation of bare patches and patches of oyster reefs (Figure 7.2). Within the bare patches, soft substrate communities are still present (Van Broekhoven 2005; own unpublished observations).

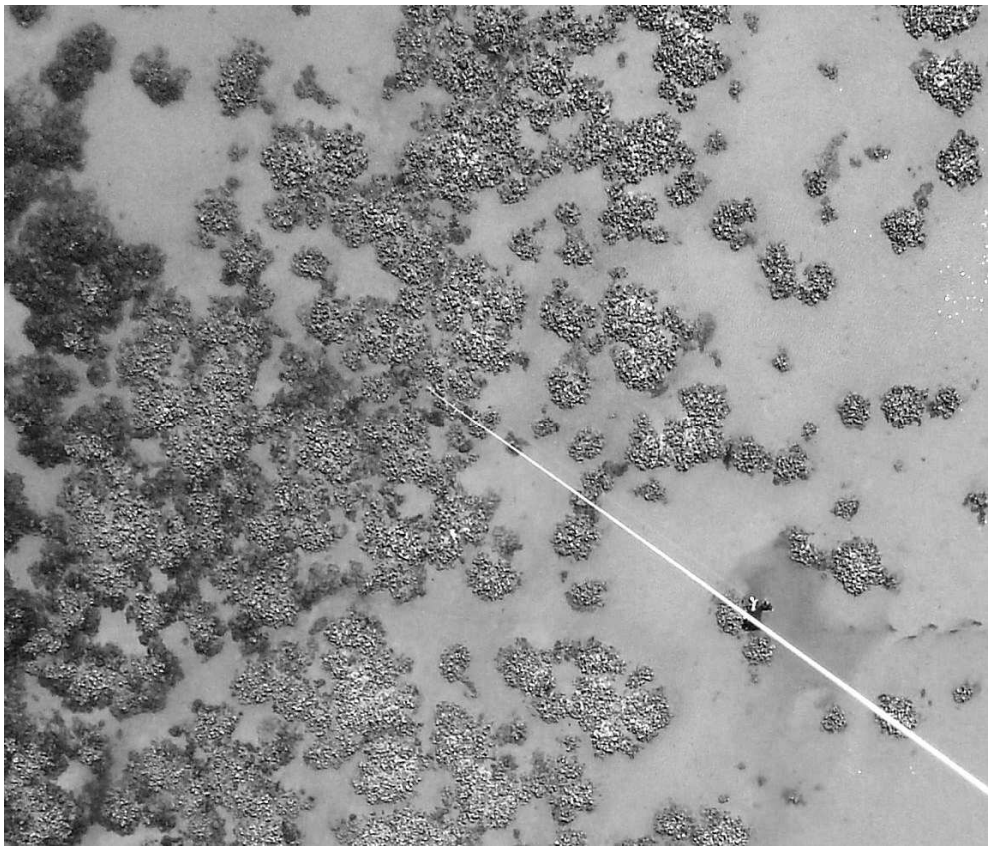


Figure 7.2. Aerial photograph of part of the oyster bed at Neeltje Jans, the Oosterschelde estuary (courtesy Johan van de Koppel). The picture shows patches of oysters, and bare patches in between. In the left part of the picture, the oyster patches are fringed by sea lettuce *Ulva* sp. The person in the lower right corner and her foot prints give an indication of scale. The picture was taken in July 2005, from a height of about 50 metres (with a camera suspended from a blimp (balloon); the white line in the picture is the line holding the blimp, see www.blimppics.com).

Habitat occupation by bivalve filter-feeders

While *C. gigas* are generally found around MLW and below, cockles *C. edule* are found at higher elevations (Table 7.2). Kater et al. (2006) showed that in the Oosterschelde estuary most Pacific oysters occur at locations that are less to not suitable for cockles. In 2002, Pacific oysters occupied less than 5% of locations very suitable for cockles. Significant competition for space between the two species only occurs at locations that are less suitable for cockles (Kater et al. 2006). In the Oosterschelde estuary, the area of locations suitable for cockles is decreasing because the tidal flats are slowly submerging. This is a consequence of the construction of the compartmentalization dams and the storm surge barrier. These reduced the tidal volume, which resulted in a reduced sediment deposition in the intertidal while erosion (due to storms) is still continuing (this phenomenon is called 'sand hunger', Van Zanten and Adriaanse 2008). Competition for space among both species is therefore expected to become relatively more important in the coming decades.

Table 7.2. Habitat occupation of native bivalves dominant in Dutch estuaries, and the introduced Pacific oyster *C. gigas* (From Korringa 1952; Bayne 1976; Hayward and Ryland 1990; Mann et al. 1991; Gosling 2003; De Bruyne 2004).

	Crassostrea gigas	*Mytilus edulis*	*Cerastoderma edule*	*Mya arenaria**	*Macoma balthica*
Tidal range	Low intertidal to subtidal	High intertidal to subtidal	High intertidal to MLW	High intertidal to shallow subtidal (to 200 m depth)	High intertidal to subtidal
Sediment	Attachment to hard surfaces, bed occurrence on any substrate	Attachment to hard and filamental surfaces, bed occurrence on any substrate	Sand, soft mud, gravel	Firm mud / sand	Mud to muddy sand
Salinity	Estuarine to fully marine	Estuarine to fully marine	Estuarine to fully marine	Estuarine	Estuarine to fully marine
Burrowing depth	-	-	< 5 cm	~ 15 cm	5-10 cm
Exposure	Semi-exposed to sheltered	Exposed to sheltered	Semi-exposed to sheltered	Sheltered	Semi-exposed to sheltered

* *Mya arenaria* is not native to Dutch waters, but is included here because its introduction dates centuries back.

Another dominant native burrowing bivalve in Dutch estuaries is the Baltic tellin *Macoma balthica*. This species is found from the high intertidal to the subtidal (Table

7.2). Therefore, it does not occupy the exact same locations as *C. gigas* that is mainly found in the low intertidal to subtidal. The soft-shelled clam *M. arenaria*, introduced centuries ago and therefore treated here as a native species, is found at roughly the same range in tidal elevations as *M. balthica* and therefore also does not have to compete for space with *C. gigas* in the higher intertidal areas.

Although in the Wadden Sea Pacific oysters are colonizing mainly mussel beds, Diederich (2005a) concluded that *C. gigas* and *M. edulis* may co-exist since *M. edulis* settle and grow in oyster beds. She also found that mussels may find a refuge from the invading oyster under a canopy of *Fucus vesiculosus* (forma *mytili* Nienburg). Experimental mussel patches were more often covered by these fucoid algae than oyster patches, and oyster recruitment was poor under a fucoid canopy (Diederich 2005a). Corresponding to Diederich's results (2005a), mussels were also frequently found growing between oysters in the Oosterschelde estuary (Box 7.5). With an increasing oyster biomass in an oyster bed in the northern compartment, numbers of *M. edulis* increased but their condition decreased (Box 7.5). The decrease in condition suggests food limitation, but the increasing numbers suggest good settlement opportunities and/or shelter from predation and environmental extremes. Thus, mussels may find refuge from predation in oyster beds. In the Oosterschelde estuary they are almost exclusively found hidden between the oysters, just above the bottom (Box 7.5). Mussels were also found in oyster beds in the Dutch Wadden Sea by Cadée (2007), who suggested that Pacific oyster beds may facilitate a return of *M. edulis* to tidal flats of the western Dutch Wadden Sea. This can also be hypothesized for the Oosterschelde estuary that has not seen natural intertidal mussel beds since several decades (pers. comm. Aad Smaal).

7.2.2. Larviphagy

Because of their large filtration capacity (Chapter 1, Box 1.3), Pacific oysters were initially expected to predate high numbers of bivalve veliger larvae. In laboratory experiments, *C. gigas* indeed ingested high proportions of larvae of *M. edulis*, but also of its own larvae (Chapter 3). Adult *M. edulis* and *C. edule* also ingested high proportions of *M. edulis* and *C. gigas* larvae (Chapter 3). Larviphagy therefore seems a common phenomenon among bivalve filter-feeders (see also Lehane and Davenport 2004).

Because bivalve filter-feeders retain all particles above a certain threshold size (2 - 6 μm: Møhlenberg and Riisgård 1978; Riisgård 1988; Barillé et al. 1993), they seem unable to filter certain particles selectively. Selection of particles only occurs after

BOX 7.5.
Abundance and condition of wild mussels in an oyster bed

Ingmar Hans and Karin Troost

Introduction. We studied the abundance and condition of mussels *M. edulis* in Pacific oyster beds in the Oosterschelde estuary, in relation to local oyster biomass.

Methods. In an oyster bed in the northern compartment of the Oosterschelde estuary, 20 sampling locations were chosen randomly (the same locations as shown in Box 7.4, Figure B7.4.1). Per location all epibenthos within 0.25 m² was removed and taken to the laboratory. Density and biomass were determined for oysters *C. gigas* and mussels *M. edulis*. Biomass was determined as ash-free dry weight of the flesh as described in Box 7.4. Shell lengths of mussels were measured to the nearest 0.01 mm. Condition indices were determined for mussels in the size range of 28 – 32 mm according to the allometric equation $W = a \cdot L^b$ where W is the biomass (g ash-free dry flesh weight), L is the shell length (mm), 'b' is a constant (2.8 for *M. edulis*) and 'a' represents the condition index (g mm^{-1}).

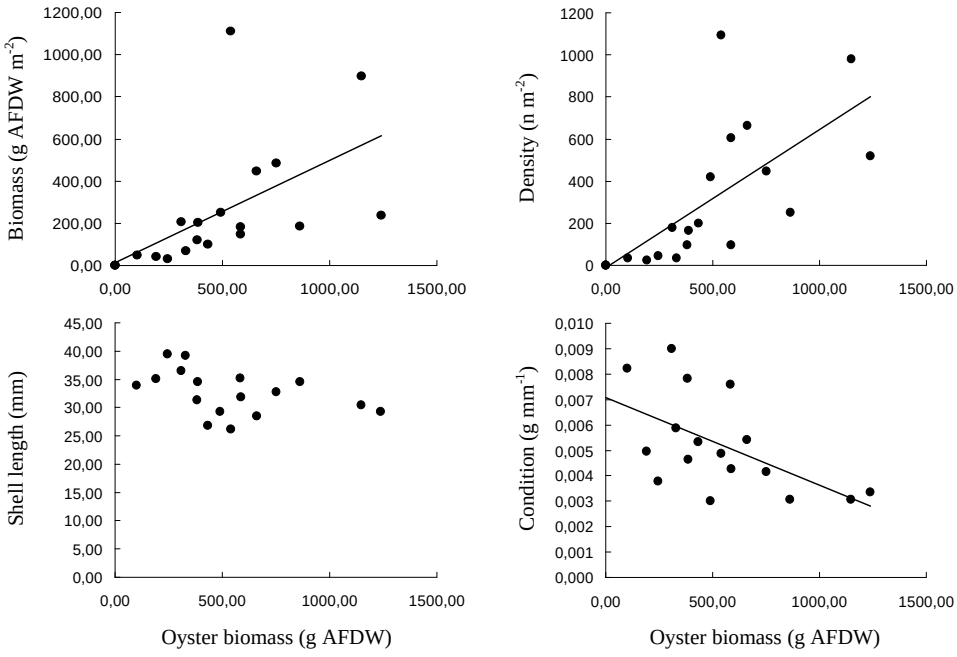


Figure B7.5.1. Biomass (g AFDW m^{-2}), density (n m^{-2}), shell length (mm) and condition (g mm^{-1}) of mussels naturally occurring in an oyster bed, related to local oyster biomass. Significantly related to oyster biomass were: mussel biomass ($R^2 = 0.33$, $p < 0.05$), mussel density ($R^2 = 0.48$, $p < 0.05$) and mussel condition ($R^2 = 0.33$, $p < 0.05$).

Results. Biomass and density of naturally occurring mussels in the oyster bed increased with local oyster biomass and were not dependent on emersion time and upstream (during flood tide) distance to the edge of the oyster bed (Figure B7.5.1, Table B7.5.1). Shell length showed no significant relationship with oyster biomass. Condition of mussels in the length range 28 – 31 mm decreased with increasing oyster biomass (Figure B7.5.1, Table B7.5.1). Up to 1100 mussels per square meter were found in the oyster bed. Most of them were found just above the sediment level, roughly within the first 5 cm.

Table B7.5.1. Results of a stepwise multiple linear regression with three factors: local oyster biomass, emersion time, upstream (flood) distance to the edge of the oyster bed, tested for biomass, density, shell length and condition of naturally occurring mussels *M. edulis*.

	Factors		
Dependent variable	Oyster biomass	Emersion time	Distance to the edge upstream (flood)
Mussel biomass (*g AFDW* m^{-2})	$p = 0.008$ $F = 8.9$	n.s.	n.s.
Mussel density (*n* m^{-2})	$p = 0.0007$ $F = 16.9$	n.s.	n.s.
Mussel shell length (*mm*)	n.s.	n.s.	n.s.
Mussel condition (*g* mm^{-1})	$p = 0.017$ $F = 7.3$	n.s.	n.s.

Discussion. Increasing mussel density and biomass with oyster biomass suggests that the oysters offer a suitable substrate for the mussels and/or offer shelter against predation. A decreasing mussel condition with increasing oyster biomass may be explained by increasing food limitation.

retention, on the gills, labial palps and/or in the stomach and guts (e.g. Shumway et al. 1985; Brillant and MacDonald 2002). Since adult *C. gigas*, *M. edulis* and *C. edule* were shown to routinely filter and ingest larvae of *C. gigas* and *M. edulis* (Chapter 3), bivalve larvae may represent an additional food source for bivalve filter-feeders. Maar et al. (2007) showed that mussels feed on all components of the zooplankton and Wong and co-authors (Wong et al. 2003a; Wong et al. 2003b; Wong and Levinton 2006) showed that zooplankton is assimilated by different species of mussels and that adding zooplankton to the diet significantly contributes to growth (Wong and Levinton 2004). Feeding on zooplankton therefore seems common in bivalve filter-feeders. Differences in filtration risk between zooplankton species will be dependent on their escape capabilities.

A difference in filtration risk was indeed found between larvae of *C. gigas* and *M. edulis*. Larvae of *C. gigas* were filtered approximately 50% less than larvae of *M. edulis* in still water in a laboratory study (Chapter 3). The reduction in filtration rate was not caused by escape reactions of the larvae in response to hydro-mechanical stimuli in the inhalant flow field of the adult bivalves, since both *M. edulis* and *C. gigas* larvae did not respond to a suction current mimicking a bivalve inhalant current in another laboratory study (Chapter 4). Instead, the difference appeared to be caused by *C. gigas* larvae migrating upwards in the water column in response to the presence of an adult filter-feeder on the bottom (Chapter 5). Larvae of *M. edulis* did not show this response but remained distributed homogeneously over the water column whether an adult filter-feeder was present or not.

Effects of larviphagy were confirmed by a field study (Chapter 6). In the water column overlying a dense oyster bed in the northern compartment of the Oosterschelde estuary, abundance of *M. edulis* larvae was reduced but abundance of *C. gigas* larvae was not. Reduction of mussel larvae must have been due to larviphagy by the relatively high filter-feeder biomass in the oyster bed. The results of *C. gigas* larvae were thought to have been influenced by spawning activity of the adult oysters (Chapter 6).

In Chapter 6 the order of magnitude of the mortality rate of bivalve larvae through larviphagy in the Oosterschelde estuary was calculated using a general formula for the calculation of clearance rates. A formula from Riisgård (2001) was modified to calculate the fraction f of all bivalve larvae in the Oosterschelde estuary that are filtered in time t (in days):

$$f = 1 - e^{-CR \cdot t / V} \qquad (7.1)$$

CR is the potential clearance rate of the main bivalve filter-feeders in the estuary (in m^3 day^{-1}, by *C. gigas*, *M. edulis*, *C. edule*) and V is the mean volume of water in the Oosterschelde estuary (2750 million m^3, Nienhuis and Smaal 1994). Assuming a homogeneous distribution of larvae throughout the Oosterschelde estuary, a homogeneous distribution of adult bivalves on the bottom of the estuary, a continuous complete mixing of the estuary and no washing out of larvae to the North Sea with tidal exchange, 95% of the larvae would have been filtered during an average pelagic stage of 20 days (f = 0.95). In this calculation a CR of 398 million m^3 day^{-1} for the year 2000 was used, as estimated by Kater (2003) (Figure 7.3). A larviphagy mortality rate f of 0.95 is in the same order of magnitude as total mortality rates generally estimated or determined for bivalve larvae and larvae of other benthic

invertebrates (Thorson 1950; Rumrill 1990). Therefore, larviphagy appears to contribute significantly to mortality of bivalve larvae in the Oosterschelde estuary. Considering the magnitude of the effect, recruitment is expected to be affected as well. Although oyster spat abundance is mainly attributed to calm weather conditions (pers. comm. A. Cornelisse, oyster farmer) and high water temperatures during spatfall (Diederich et al. 2005), Brandt et al. (2008) could mathematically explain the large-scale invasion pattern of *C. gigas* in the German East Frisian Wadden Sea assuming recruitment dominated by larval supply (over processes at the location of settlement). This supports the hypothesis that recruitment of *C. gigas* itself may also be affected by the estimated high mortality rate due to larviphagy in the Oosterschelde estuary.

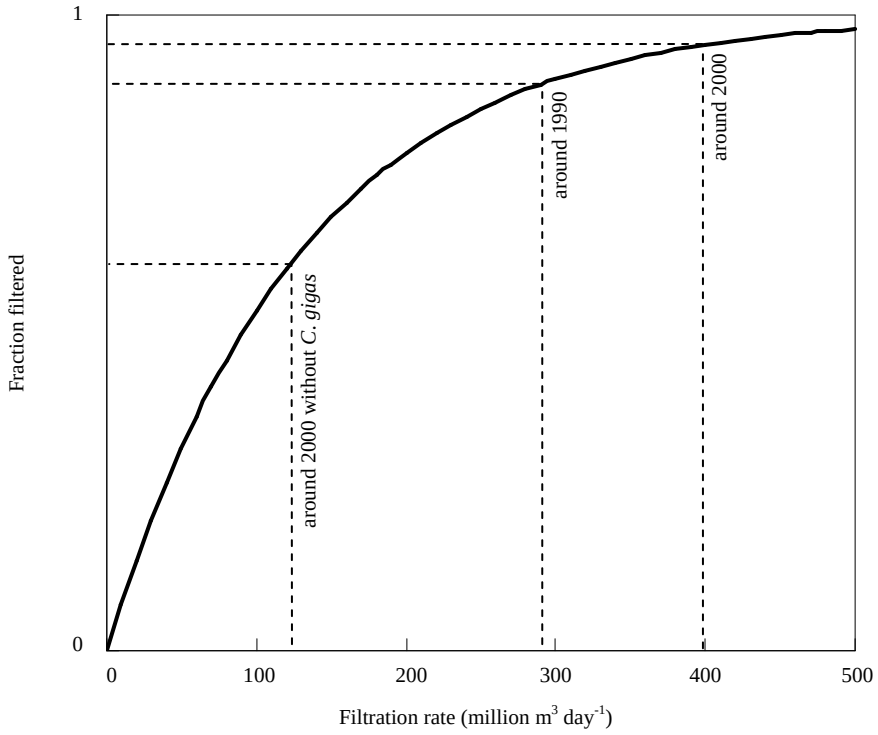


Figure 7.3. Dependence of the fraction *f* of larvae filtered by adult bivalve filter- feeders on the total filtration rate by bivalve filter-feeders in the Oosterschelde estuary, as modelled with Equation 7.1. Three scenarios are emphasized: the situation around 2000, the situation around 1990, and the situation around 2000 but without the filtration capacity of the Pacific oyster stock.

The estimated fraction *f* of larvae filtered was already high in 1990 (*f* = 0.88; *CR* = 289 million m^3 day^{-1}, as estimated by Kater 2003). Not much room was left for a further increase towards the asymptote *f* = 1.00, following the further increase in oyster biomass during the 1990s. This illustrates the high filtration pressure on the estuary. Among the dominant three bivalve species, Pacific oysters are estimated to contribute most to the total filtration pressure. *C. gigas* was estimated to be responsible for roughly 2/3 of the total filtration capacity (by the three dominant species) around the year 2000 (Kater 2003). Around the same year, *M. edulis* and *C. edule* were responsible for a total estimated filtration pressure of 133 million m^3 day^{-1} and in a hypothetical situation without *C. gigas* for an estimated larviphagy mortality fraction *f* of 0.64 (Figure 7.3).

Of course this modelling exercise is only a rough tool in estimating the effects of bivalve filtration pressure on bivalve larval abundance. The aim was to explore the order of magnitude of the effect of larviphagy on larval abundance. Some assumptions had to be made, that are not that representative for the actual situation in the field. Although larvae do seem to be distributed homogeneously vertically over the water column (Chapter 6), larvae are not distributed homogeneously throughout all compartments of the estuary. More larvae are found in areas with higher concentrations of conspecific adults (Chapter 6). How this would influence the estimate of *f* is difficult to grasp since the adult stock of bivalve filter-feeders is not distributed homogeneously over the entire estuary either. Rather, they are concentrated in oyster and cockle beds and on mussel and oyster culture plots. Furthermore, no exchange of bivalve larvae with the North Sea was assumed. If that were included as a dilution factor, *f* would be even higher. Finally, errors in estimates of clearance rates may have been large (see Kater 2003). Clearance rates estimated by Kater (2003) were based on literature values on individual clearance rates and filtration time activity, and empirical values on stock sizes and submersion times. However, even if *CR* would have been overestimated by 50%, the fraction *f* would still be high: 0.67 around 1990 and 0.78 around 2000. This is still of a magnitude that may seriously affect recruitment success among bivalves in the estuary.

Chapter 3 showed that *C. gigas* larvae were filtered 50% less than *M. edulis* larvae in a still-water set-up in the laboratory. Regardless of the reservations in translating this result directly to the field (see discussion section in Chapter 3), for the situation in 2000 this would result in a reduction of *f* from 0.95 to 0.78 for *C. gigas* larvae whereas *f* would still remain 0.95 for *M. edulis* larvae. An increasing stock of *C. gigas* in the Oosterschelde estuary may therefore affect larval abundance and subsequent recruitment of *M. edulis* more strongly than its own larval abundance and recruitment.

A contributing factor is the increased larval production of *C. gigas* with an increasing parent-stock. Potential effects on recruitment remain, however, hypothetical. A study into the effect of the increasing filter-feeder stock in the Oosterschelde estuary (mainly due to the increase in Pacific oyster stock) on larval abundance of *C. gigas* and *M. edulis* showed a decline in larval abundance of oysters but no effect on larval abundance of mussels (Chapter 6). The declining Pacific oyster larval abundance was suggested to be a result of increased larviphagy, possibly in combination with food limitation (reducing the reproductive output of adults and/or reducing the survival of larvae). A trend in larval abundance of mussel larvae may have been undetectable due to the relatively short sampling period of 6 years (vs. 13 years for oyster larvae).

Summarizing, the results for *M. edulis* larvae on bed scale and the results for *C. gigas* larvae on estuary scale do suggest that larviphagy may be an important mortality factor for bivalve veliger larvae. The increasing stock of filter-feeders in the Oosterschelde estuary is therefore expected to reduce abundance of bivalve larvae, but also of other slow-swimming zooplankton species with weak escape capabilities (see Singarajah 1969, 1975; Kiørboe and Visser 1999; Titelman and Kiørboe 2003; see Maar et al. 2007). Eventually, bivalve grazing may exert a top-down control on zooplankton communities through direct grazing on weak escapers and weak swimmers, and on benthic communities through filtration of pelagic larvae. In addition, larvae of *C. gigas* swim faster than larvae of *M. edulis*, and can migrate faster in vertical direction (Chapter 4). This may enable them to more successfully avoid benthic predators, find food-rich water layers (Raby et al. 1994) or transport themselves with the tides into favourable directions (Shanks and Brink 2005).

7.3. Synthesis and conclusions

7.3.1. Causes of *C. gigas*' success

Establishment of *C. gigas* in the Oosterschelde estuary was likely facilitated by the local relative lack of natural predators and low vulnerability to parasites and diseases already present, as well as those introduced from the NW Pacific. The attractiveness of the species for mariculture resulted not only in its initial introduction, but also in repeated introductions that contributed to successful establishment, facilitated by a high genetic variation and phenotypic plasticity. The species also appears highly competitive based on its fast growth, high trophic plasticity, high filtration rates, and its ability to create its own favourable habitat, thereby reducing recolonization opportunities for burrowing bivalves.

Once established, natural range expansion within the Oosterschelde estuary and to other estuaries was fast because of a high dispersal rate, facilitated by a lengthy pelagic larval stage and the large colonization potential of the species. Its life history strategy can be described as largely *r*-selected. Reproductive rates are fast due to a high fertility and fast maturation of adults, and high survival of spat and adults. Survival of larvae appears to be relatively high due to an ability to avoid filtration by adult benthic (bivalve) filter-feeders. The species is furthermore a habitat generalist and colonizes different habitats. It appears to utilize a broad diet and to be highly adaptable to changing food conditions. Establishment in the Oosterschelde estuary and subsequent natural range expansion to other estuaries may additionally have been facilitated by a relatively high level of human-induced disturbance in Dutch estuaries.

Life-history theory predicts a trade-off between 'colonization' and 'establishment' features. Species that have been released from the pressure of diseases or predators in their native habitat have been suggested to reallocate energy previously used for defence into reproduction and growth. Since in Dutch estuaries bottom-living *C. gigas* hardly have any natural predators and are not very vulnerable to parasites and diseases, such a reallocation of energy may have contributed to its fast growth and reproduction. Conclusions on this possibility require a yet to be made detailed comparison between population dynamics of introduced populations in Dutch estuaries and populations in its native habitat in Japan and Korea.

7.3.2. Consequences for native bivalves

Direct effects of the expansion of *C. gigas* on native bivalves were not demonstrated in this thesis. In theory *C. gigas* either: 1) out-competes native bivalves to local extinction; 2) fills an empty niche and does not compete at all with native bivalves; 3) or overlaps partially in niche occupation with native bivalves and they coexist with reduced actual niche breadths. The first option would require 100% niche overlap and a limitation in resources. This thesis shows that resource requirements of *C. gigas* do not overlap 100% with those of native bivalves, which excludes the first option, but also that there is some overlap, which excludes the second option. The possibility that *C. gigas* fills the exact same niche that was left empty by the disappeared *O. edulis*, was already rejected by Cadée (2007) and Reise (1998). Among the many differences between the native and the introduced oyster, *C. gigas* has broader salinity and temperature tolerance ranges, constructs reef structures and is less predated and infected by diseases and parasites (Table 7.3). Whereas *O. edulis* was found at more marine salinities, and was common in the North Sea (e.g. the 'oestergronden' north of the

Wadden Sea islands; Olsen 1883), *C. gigas* is almost exclusively found in estuaries and the coastal Wadden Sea. The third option is the most plausible one; the Pacific oyster may partially fill the niche of *O. edulis*, and may compete in the margins with native bivalves. The introduction of *C. gigas* may have caused a decrease in actual niche breadth of native bivalves (Colwell and Futuyma 1971); they will only compete for resources where their requirements overlap, and only if resources are limiting.

Table 7.3. Comparison between the native European oyster *O. edulis* and the introduced Pacific oyster *C. gigas* (From Korringa 1952, 1976b, 1976a; Buroker 1985; Mann et al. 1991; Reise 1998; Helm et al. 2004; Cadée 2007).

	Crassostrea gigas	*Ostrea edulis*
Tidal range	Low intertidal to subtidal	Low intertidal to subtidal
Sediment	Attachment to hard surfaces, reef formation on any substrate	Sand
Salinity	Mainly in estuaries, tolerance 10 - 34 psu	In open sea and estuaries, tolerance 25 – 34 psu
Low temperature tolerance	-5 °C	-1.5 °C
Density oyster bed	tens to hundreds per m^2, forming reefs	several per m^2, not forming reef structures
Development	Oviparous	Larviparous
Number of eggs/larvae per female per year	1 – 100 x 10^6 (eggs)	0.1 – 1.5 x 10^6 (larvae)
Genetic variation within populations	High	Low
Life history strategy	more on *r*-side	more on *K*-side

Considering competition for space, this indeed occurs in the margins, where habitat requirements overlap. Although expansion of Pacific oyster beds decreases the area suitable for settlement of burrowing bivalves (2 in Figure 7.4), total occupation of the intertidal area by oyster beds is presently only about 6 - 7% of the total intertidal area of the Oosterschelde estuary. Even if Pacific oyster beds will expand further, the total bottom-area occupied by bivalve beds will always stay relatively small because of physical and biological constraints (Heip et al. 1995). Space is therefore not a limiting resource, and Pacific oysters are not expected to completely out-compete other bivalves for space. Species that have exactly the same habitat preferences as *C. gigas* would theoretically be threatened most by the newcomer. Not *C. edule*, because the overlap in habitat occupation has been shown to be far from 100%. In the Wadden

Sea, *C. gigas* mainly colonizes intertidal mussel beds (Reise 1998; Nehls et al. 2006; Schmidt et al. 2008) and therefore does not compete on a significant scale with burrowing soft-sediment species. Although Pacific oysters and mussels do occupy the same habitat in Dutch and German coastal waters, they appear able to co-exist. Expansion of Pacific oyster beds increases the area of hard substrate and hence increases the area suitable for settlement by mussels and other hard-substrate species (3 in Figure 7.4). The structurally complex oyster beds furthermore offer shelter from environmental extremes and from predation (4 in Figure 7.4) to the oysters themselves, to mussels, to other bivalve spat and juveniles, and to other epifaunal species. In the Oosterschelde estuary oyster beds seem to have facilitated a (modest) return of wild mussels to the intertidal. The same is hypothesized for the western part of the Dutch Wadden Sea.

Although Pacific oysters do not appear to feed on exactly the same food items as native filter-feeding bivalves, they do largely filter the same particles from the surrounding water (see section 7.2.1, '*Diet of filter-feeding bivalves*'). This is a case of interference competition rather than actual food competition (Green 1971; Case and Gilpin 1974) although the effect may be the same. Theoretically, this may eventually lead to local disappearance of bivalve species that are least adapted to cope with low food levels in a food-limited system such as the Oosterschelde estuary. Due to the increasing Pacific oyster stock in the Oosterschelde estuary the total bivalve filtration pressure increased, which appears to have caused a shift in the phytoplankton community (section 7.2.1, *'Carrying capacity of the Oosterschelde estuary for filter-feeding bivalves*') and lowered the food availability for bivalve filter-feeders in the estuary (1 in Figure 7.4). It is not clear which of the dominant bivalve species is best adapted to low food conditions. The species with the highest net energy gain (gross energy gain minus metabolic losses) at reduced food levels in such a food-limited system, and/or the species that digests the widest range of particle types from all filtered particles, is expected to have a competitive advantage over other filter-feeders. Although Pacific oysters are less efficient in gaining energy from filtered material than *M. edulis*, this may at least be compensated by their larger filtration capacity and ingestion rate, a greater trophic plasticity and a higher food flux towards an oyster bed due to higher near-bed turbulence levels. They may therefore be better able to cope with lowered levels of preferred food items than *M. edulis*, corresponding with the observed density dependent growth in *M. edulis* but not in *C. gigas* at the same locations at Sylt and in the Oosterschelde estuary. The native bivalves *C. edule* and *M. balthica* may be better adapted to low food levels than *C. gigas* and *M. edulis*, considering their optimal growth in the Wadden Sea while growth of *C. gigas* and *M. edulis* appeared food-limited

(Cardoso et al. 2006). Indeed, *C. edule* appears better adapted to low TPM levels, and because of its longer gut passage time it can extract more nutrients from TPM with low organic content (Hawkins et al. 1998).

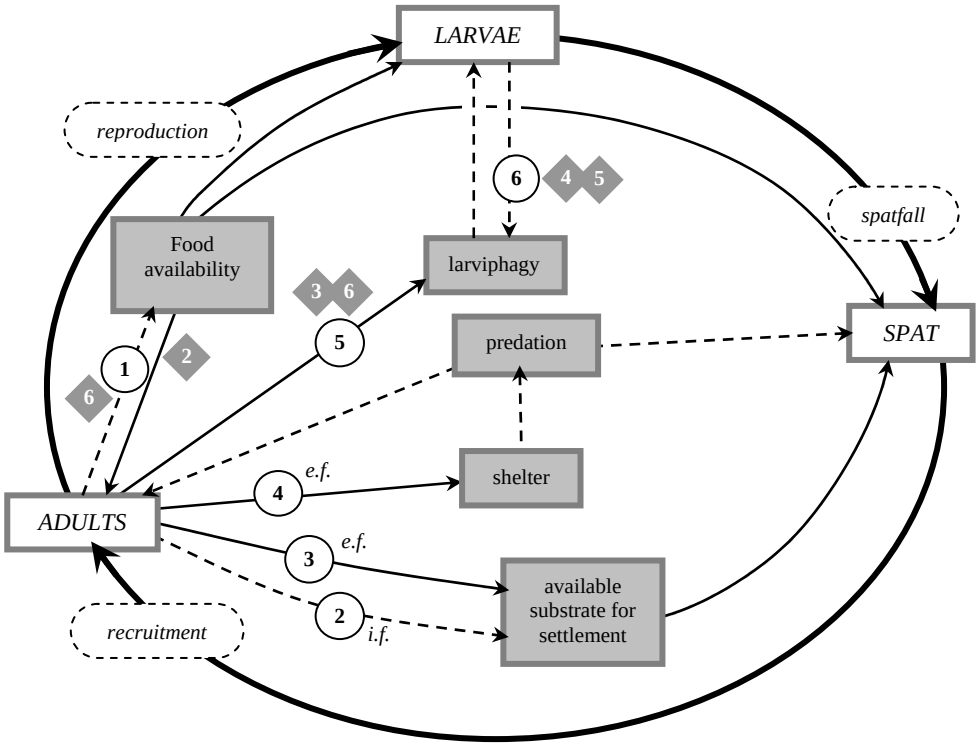


Figure 7.4. Schematic representation of the life cycle of bivalve suspension feeders in general, with the main factors affecting survival, growth and mortality. Effects of different life stages of *C. gigas* on different life stages of any bivalve in Dutch estuaries are indicated with solid arrows representing a positive effect (increase) and dashed arrows representing a negative effect (decrease). Numbers in circles refer to the explanatory text in section 7.3.2. Numbers in grey diamonds refer to the different chapters in this thesis. Where necessary a distinction is made in effects relevant to 'epifaunal bivalves' (*e.f.*) and 'infaunal bivalves' (*i.f.*). This figure was copied from Chapter 1 (Figure 1.4).

The increase in total bivalve filter-feeder stock due to the rapidly increasing Pacific oyster stock not only lowered food levels but also increased the mortality rate of bivalve larvae (5 in Figure 7.4). Mortality due to larviphagy is estimated to be high in the Oosterschelde estuary, but expected to be reduced for *C. gigas* larvae because of

their ability to avoid filtration (6 in Figure 7.4; Chapters 3 and 5). The increased filtration pressure in the Oosterschelde estuary apparently increased the mortality rate of *C. gigas* larvae over the last 13 years (Chapter 6). However, whether mussel larval abundance is also declining, and whether larvae of *M. edulis* indeed suffer higher mortality rates due to larviphagy than *C. gigas* larvae did not become apparent from a 6-year time series (Chapter 6).

In conclusion, competition for space is not expected to lead to replacement of native bivalves. Despite the apparent return of *M. edulis* to the intertidal facilitated by oyster beds, the possibility that natural stocks of *M. edulis* will disappear from the Oosterschelde estuary in the future due to competition (or interference) for food cannot be excluded. *C. edule* seems better adapted to low food levels than both *M. edulis* and *C. gigas* and is therefore not expected to be replaced by *C. gigas*. Recruitment of bivalve filter-feeders in the Oosterschelde estuary is expected to decline with the increasing filter-feeder stock, as a consequence of larviphagy and food limitation for the adult stocks and their larvae. This is, however, not expected to lead to local extinctions. In the Wadden Sea, replacement of native bivalves of any species is considered unlikely at this moment. Since the carrying capacity of the Wadden Sea is not yet reached, food competition and larviphagy are expected to affect bivalve communities on a local scale only.

7.3.3. Consequences for other species

C. gigas has a profound influence on Dutch estuarine ecosystems. It changes conditions for native bivalves locally, by constructing hard-substrate oyster reefs. By its large filtration capacity and high numbers in the Oosterschelde estuary it affects the phytoplankton community and food availability to native bivalves, and mortality of bivalve larvae due to larviphagy. These changes may affect other species at different trophic levels.

Developing oyster reefs change the habitat in such a way that, in general, infaunal species have difficulty to colonize, but colonization by epifaunal hard-substrate species is enhanced (Gutiérrez et al. 2003). Former soft-bottom communities are thus replaced by hard-substrate communities although in the many open spaces within an oyster bed soft-bottom communities are still present (Van Broekhoven 2005; Wijsman et al. 2008). Habitat heterogeneity is enhanced, resulting in a higher species-richness in an oyster bed as a whole, compared to the surrounding tidal flats. The oyster shells represent a large area of hard-substrate settlement opportunities for species that previously only occurred on man-made structures (e.g. dikes and embankments) and

mussel beds. In the shallow water remaining within bare patches in oyster reefs during low tide, many species such as shrimps and gobies can be observed (own unpublished observation). These species find refuge within the oyster bed, but may also serve as prey for shorebirds. Moreover, the oyster reefs may facilitate establishment of other exotics from the same region of origin. For example, Pacific oyster beds in the Netherlands already offer substrate and shelter to the japweed *Sargassum muticum*, wakame weed *Undaria pinnatifida*, the red alga *Heterosiphonia japonica*, the crab *Hemigrapsus penicillatus*, the sea squirts *Botrylloides violaceus* and *Styela clava*, and many more species originating from north-east Asian Pacific coasts (Wolff 2005; Haydar and Wolff in prep.; www.anemoon.org 2008).

Furthermore, since Pacific oysters are hardly eaten by birds in The Netherlands, expansion of *C. gigas* may threaten the food supply of shorebirds if they (partially) replace native bivalves. With expanding oyster reefs, the intertidal area available for foraging birds would be expected to decrease to some extent and with it opportunities to forage on prey such as worms and burrowing bivalves. *C. gigas* is colonizing mussel beds in the Wadden Sea, and if mussel biomass within the now mixed beds is reduced far enough this may have significant consequences for birds that are highly dependent on availability of *M. edulis*. In the Wadden Sea, however, there are no indications yet that *C. gigas* will replace native bivalves completely. Furthermore, development of oyster beds may also have a positive influence on food availability for shorebirds. In the Oosterschelde estuary more intertidal mussels may now be available to foraging birds because of their natural occurrence in expanding oyster reefs on tidal flats. Since the replacement of all mussel culture plots to the subtidal in the 1990s, this may constitute the only availability of mussels to shorebirds such as the oystercatcher *H. ostralegus*. This species was observed to feed on mussels in an intertidal oyster bed (own unpublished observation). However, hardly any studies have been conducted after the suitability of oyster reefs for foraging by shorebirds. In the Oosterschelde estuary, some observations on bird occurrence in oyster reefs and nearby reference sites indicated no apparent differences (Wijsman et al. 2008). Furthermore, mussels may again disappear from the intertidal of the Oosterschelde estuary due to reduced food levels.

Hypothetically, *C. gigas* may change entire Dutch estuarine ecosystems through cascading effects on other trophic levels. The increased filtration pressure in the Oosterschelde estuary due to an increased oyster stock already appears to have affected the phytoplankton community. The oysters exert a top-down control on phytoplankton composition that may in turn affect higher trophic levels in the food

web (e.g. zooplankton → fish → fish-eating birds and seals). Similarly, the oysters might exert directly a cascading effect on zooplankton.

7.3.4. Management aspects

Should feral Pacific oyster beds be managed?

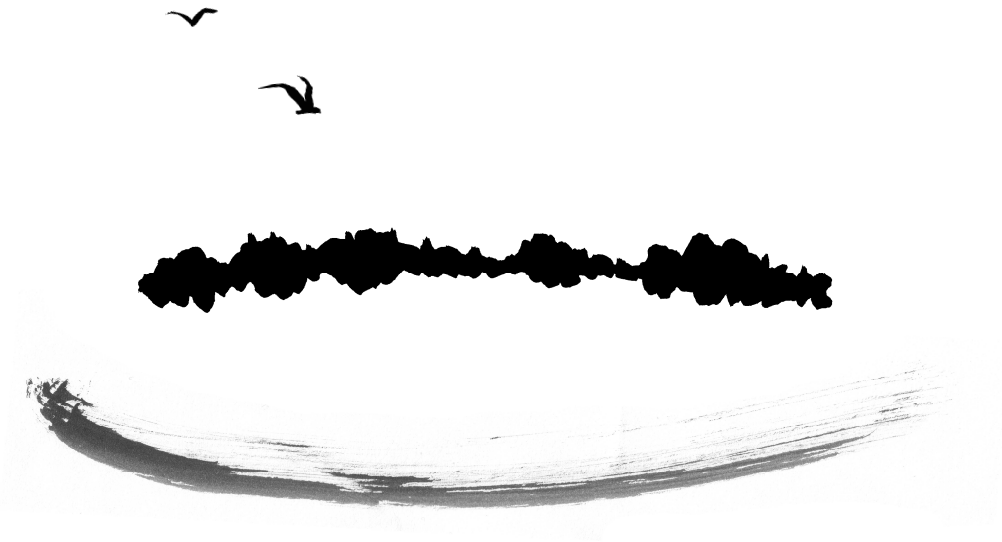
Different stakeholders play important roles in the Oosterschelde estuary. Main interests are the ecological importance of the estuary (it is part of the European 'Natura 2000' network), its economical importance for shellfish farmers, and the protection of dikes against dike-bursts and subsequent flooding. The tidal flats are important foraging grounds for international shorebird populations (Meire et al. 1994; Strucker et al. 2008), and are also important for resting seals (*Phoca vitulina*) (Mees and Reijnders 1994; Strucker et al. 2008). Tidal flats and salt marshes furthermore play an important role in coastal defence, as they reduce wave energy and thereby protect the dikes. As a consequence of the 'sand hunger', tidal flats and salt marshes are slowly eroding. Only 50% of the area of tidal flats is expected to remain in the year 2050, and salt marshes will only remain at sheltered locations (Van Zanten and Adriaanse 2008). Among many potential solutions, Rijkswaterstaat (the government service for transport, public works and water management) is investigating the possibility to use oyster beds as a defence against further erosion of tidal flats. In contrast, instead of creating more oyster beds, shellfish farmers would like to see a reduction in the feral oyster stock, to improve the carrying capacity for cultured mussels and oysters. Feasibility and effects of mechanical removal of oyster beds in the Oosterschelde estuary were studied in 2006 – 2007 (Wijsman et al. 2008). Complete removal of 0.5 km^2 of oyster bed (12.5 million kg total fresh weight, of which roughly 27% live oysters) was highly labour-intensive. Based on the biomass of the removed oysters and their estimated filtration capacity, the experiment was roughly estimated to have lowered the filtration pressure of oysters, mussels and cockles combined by only 0.5%. Oyster beds were estimated to return after three to six years (Wijsman et al. 2008). Therefore, such a solution should be repeated continuously and on a much larger scale in order to have a significant effect on the carrying capacity. This solution appears to conflict with the other two main interests in the area since it has a large impact on benthic communities and may cause further erosion of tidal flats. In addition, a local oyster farmer reported removing 12 million kg total fresh weight of Pacific oysters in 2008, in an attempt to improve food conditions for cultured shellfish (pers. comm. A. Cornelisse). According to him, only the upper 80% of oyster beds

needs to be removed regularly, which would be much less labour-intensive than a complete removal, and would not result in bottom erosion since the lower layer of oyster shell remains in place and can protect the sediments against further effects of 'sand hunger'. On the downside, the more shell debris remains, the faster oysters return. As a result, such an operation has to be repeated more frequently, and may affect benthic ecology more strongly. In conclusion, the feral oyster stock may well be managed in some way in the near future although finding a solution approved by all stakeholders seems a serious challenge.

Should introduced predators be used to control the Pacific oyster population?

Only recently the oyster drills *Ocinebrellus inornatus* and *Urosalpinx cinerea* were found in the Oosterschelde estuary at Yerseke and Gorishoek (Faasse and Ligthart 2007; Goud et al. 2008). These predatory snails were likely introduced accidentally with shellfish imports. They still occur very locally and in low numbers. Of *O. inornatus* adult specimens as well as eggs and juveniles were found (Goud et al. 2008). It is therefore likely that this species will establish itself in the Oosterschelde estuary. Although an increasing population of predatory gastropods may be expected to exert control of oyster stocks, it should also be kept in mind that most of these predators not only feed on *C. gigas* but also on other bivalves. As an example, the veined rapa whelk *Rapa venosa* has a broad dietary preference for (subtidal) bivalve molluscs, including species of the genera *Crassostrea*, *Gouldia*, *Mercenaria*, *Mya*, *Mytilus*, *Ostrea*, *Pecten*, *Pitar*, and *Venus* (Harding and Mann 1999; Mann and Harding 2000). This predatory snail may also be encountered in the Oosterschelde estuary in the near future, as it was already found in the southern North Sea in 2005 (Kerckhof et al. 2006). In the event of an introduction of a predatory gastropod in Dutch coastal ecosystems, there is no way of predicting how stocks of bivalve species will change. Trying to prevent new introductions of any kind is probably the most advisable strategy.

Chapter 8



Nederlandse samenvatting (Dutch summary)

Japanse oesters in Nederlandse wateren: Oorzaken van hun succes en gevolgen voor inheemse schelpdieren

8.1. Inleiding

Aanleiding voor dit onderzoek

Toen de Japanse oester (*Crassostrea gigas*) in 1964 door oesterkwekers in de Oosterschelde werd geïntroduceerd, dacht men nog dat deze soort zich niet voort zou kunnen planten in Nederland. Maar een decennium later, na de oester herhaaldelijk geïmporteerd te hebben, werd de eerste grote broedval geconstateerd. De Japanse oester had zich definitief gevestigd in Nederland. De oester verspreidde zich snel door de hele Oosterschelde, en ook naar andere estuaria. Vooral in de getijdenzone, maar ook in diepere delen, vormde de Japanse oester uitgestrekte oesterbanken (Figuur 8.1). Het werd duidelijk dat de oester een grote invloed zou kunnen hebben op de Nederlandse estuariene ecosystemen. In het ergste geval, zo werd verondersteld, zouden door de uitbreiding van de oester inheemse schelpdieren zoals de kokkel (*Cerastoderma edule*) en de mossel (*Mytilus edulis*) geheel kunnen worden verdrongen en verdwijnen. Dat was de aanleiding voor het onderzoek dat hier gepresenteerd wordt. In dit proefschrift wordt onderzocht waardoor de Japanse oester zich zo snel heeft kunnen vestigen in Nederland en wat daarvan de consequenties zijn voor inheemse tweekleppigen.

Achtergrond

Introducties, invasies en exoten

Door menselijke activiteiten vinden wereldwijd steeds meer invasies van exoten plaats. Exoten zijn soorten die geïntroduceerd zijn in een gebied waar ze van nature niet

voorkwamen. In sommige gevallen betreft een invasie een natuurlijke uitbreiding van het leefgebied, maar in toenemende mate worden deze invasies veroorzaakt door de mens, door bewuste en onbedoelde introducties. Door toenemende introducties door de mens wordt de soortensamenstelling van ecosystemen over de hele wereld steeds meer gehomogeniseerd. De mondiale biodiversiteit wordt er dus door bedreigd. Daarnaast ontwikkelt een klein deel van de veelal per ongeluk geïntroduceerde exoten zich tot een plaag, waarbij de ecologische en/of economische schade groot kan zijn. Exoten die succesvol een nieuw gebied koloniseren, zich daar vestigen en het ontvangende ecosysteem in bepaalde mate beïnvloeden worden invasieve soorten genoemd. Een bekend voorbeeld is de bruine boomslang *Boiga irregularis* die op het eiland Guam in de Stille Oceaan bijna alle inheemse vogels, vleermuizen en reptielen opat. De slang veroorzaakt ook veel economische schade door in electriciteitspalen te klimmen en daar kortsluiting te veroorzaken. Een andere beruchte invasieve exoot is de driehoeksmossel *Dreissena polymorpha*. Deze zoetwatermossel werd per ongeluk geïntroduceerd in de Amerikaanse Grote Meren. De driehoeksmossel verstikt daar inheemse schelpdieren en verandert het hele voedselweb door zijn grote filtratiecapaciteit. Daarnaast verstoppen de mosselen door hun grote aantallen onder andere innamepunten voor koelwater.

Figuur 8.1. Links: oesterbank bij Neeltje Jans, rechts: klomp van aan elkaar gegroeide oesters.

Introducties komen overal voor, ook in het mariene milieu. Hier worden vijf belangrijke routes onderscheiden die gemaakt of veroorzaakt zijn door de mens: 1) opzettelijke introducties, bijv. voor kweek; 2) ontsnappingen uit gevangenschap, bijv. uit aquaria; 3) contaminanten van soorten die worden gehouden en getransporteerd door mensen, bijv. parasieten; 4) verstekelingen in menselijke vervoermiddelen, bijv. in ballastwater van schepen; 5) corridors aangelegd door de mens, bijvoorbeeld via kanaalsystemen. Vervolgens, wanneer een exoot zich in het nieuwe gebied heeft gevestigd, kan de soort zich op natuurlijke wijze verder verspreiden.

De meeste exoten worden geïmporteerd als verstekelingen in ballastwater en op scheepsrompen. Daarnaast vinden ook veel introducties plaats door middel van schelpdiertransporten voor schelpdierkweek, met name oestertransporten. Een berucht voorbeeld is de Bonamia-parasiet (*Bonamia ostreae*) die per ongeluk werd geïntroduceerd met een transport van Europese platte oesters (*Ostrea edulis*) uit Bretagne in 1980. Door deze parasiet is de platte oester bijna geheel verdwenen uit de Nederlandse wateren.

Aantallen introducties worden zwaar onderschat. Dit komt omdat exoten vaak moeilijk herkenbaar zijn. Van sommige soorten is het niet duidelijk of ze inheems of geïntroduceerd zijn. Van de strandgaper *Mya arenaria* werd bijvoorbeeld gedacht dat die inheems was in Nederland, totdat werd aangetoond dat de soort al eeuwen geleden door de Vikingen was geïntroduceerd vanuit Noord Amerika. Het gebeurt ook vaak dat geïntroduceerde soorten nauwelijks te onderscheiden zijn van inheemse soorten. Vaak is moleculair genetisch onderzoek nodig om dergelijke soorten van elkaar te onderscheiden. Ook worden deze technieken vaak gebruikt om aan te tonen waarvandaan een exoot geïntroduceerd is. Soms echter blijken exoten toch niet door mensen geïntroduceerd te zijn, maar is er sprake van een natuurlijke uitbreiding van het leefgebied, bijvoorbeeld door klimaatverandering.

Omdat de aantallen introducties nog steeds toenemen en sommige exoten zich ontwikkelen tot plagen waarbij de ecologische en economische gevolgen groot kunnen zijn, wordt het steeds belangrijker om deze invasies te kunnen voorspellen om ze eventueel te kunnen voorkomen. Het is daarom belangrijk om vanaf het begin te onderzoeken waarom en hoe invasies plaatsvinden, en hoe de mens hier invloed op heeft. Maar vaak is dit niet mogelijk omdat een exoot pas opvalt als die zich al lang gevestigd heeft en in relatief grote aantallen voorkomt. Tegen die tijd kan vaak niet goed meer onderzocht worden hoe de invasie verlopen is en hoe het ecosysteem heeft gereageerd. Waarom de invasie succesvol verlopen is en wat de mechanismen waren wordt dan mogelijk nooit meer achterhaald. Veel literatuur is gewijd aan het opsporen

van algemene wetten bij invasies. Wanneer deze bekend zijn kunnen immers nieuwe invasies beter voorspeld worden.

Hoewel er relatief veel aandacht uitgaat naar exoten met een plaagkarakter zijn het in werkelijkheid maar weinig exoten die grote problemen veroorzaken. Soorten die in een nieuw gebied arriveren moeten veel hindernissen overwinnen. Ze moeten zich vaak aanpassen aan de nieuwe omgeving, bijvoorbeeld aan andere watertemperaturen en andere prooien of predatoren. In het algemeen worden drie stadia onderscheiden in de invasie-ecologie: 1) kolonisatie van het nieuwe gebied; 2) vestiging; en 3) natuurlijke uitbreiding. Succesvolle kolonisten (1) zijn in het algemeen opportunistische soorten met een snelle voortplanting en groei en brede toleranties voor omgevingsfactoren. Voor een succesvolle vestiging in het nieuwe gebied (2) zijn andere eigenschappen van belang. Hiervoor moet een soort goed kunnen concurreren met inheemse soorten en bijvoorbeeld weinig gegeten worden door inheemse roofdieren. Daarnaast geven herhaaldelijke introducties een grotere kans om zich aan te passen en te vestigen in het nieuwe gebied. Ook is genetische variatie van belang. Een hoge genetische variatie geeft een soort meer mogelijkheden om zich snel aan te passen. Herhaaldelijke introducties vergroten de genetische variatie in de geïntroduceerde populatie, en vergroten zo vestigingskansen. Voor een natuurlijke uitbreiding van het leefgebied na vestiging (3) zijn weer vooral eigenschappen van belang die een soort tot een goede kolonist maken.

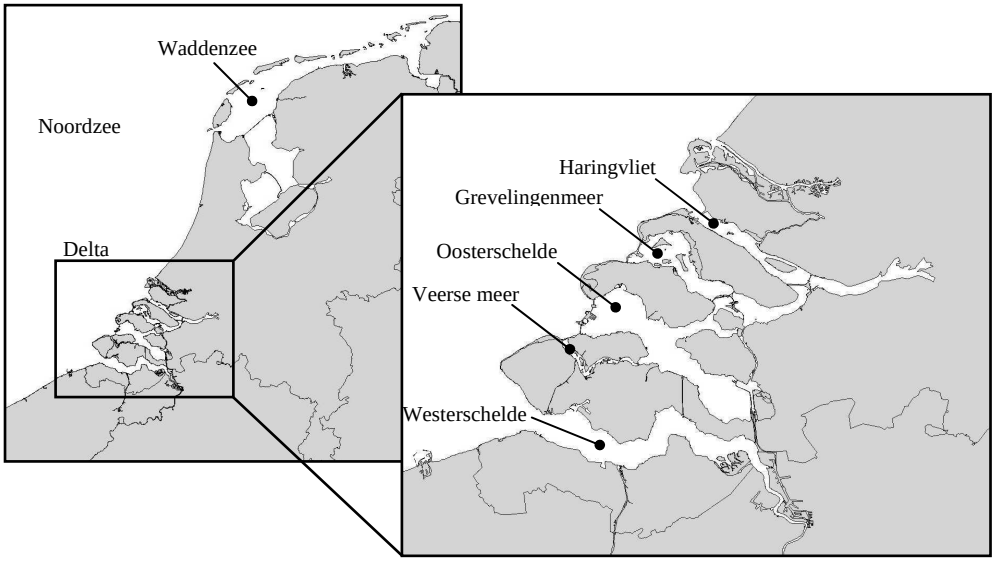
Verder lijken exoten zich gemakkelijker te vestigen in gebieden die verstoord zijn (bijvoorbeeld door vervuiling), in gebieden waar de soortenrijkdom laag is en er mogelijk onbenutte niches zijn of in gebieden met weinig predatoren. Het is echter moeilijk om algemene wetmatigheden aan te wijzen. Als er al regels gevonden worden zijn er steevast vele uitzonderingen. In verschillende gebieden zullen verschillende eigenschappen de doorslag geven.

De Japanse oester is momenteel één van de bekendste, en beruchtste, exoten in Nederland. Terwijl sommigen deze oester zien als een verrijking van de Nederlandse biodiversiteit, zien velen de oester als een vervelende plaag. De oesters bedekken vroegere zandplaten met hun vlijmscherpe schelpen, die verwondingen veroorzaken bij recreanten. Door aangroei van oesterbroed (jonge oestertjes) op gekweekte mosselen en oesters hebben kwekers er meer werk aan om de schelpdieren klaar voor de verkoop te maken. Bovendien is er de mogelijkheid dat de geïntroduceerde oester inheemse schelpdieren wegconcurreert.

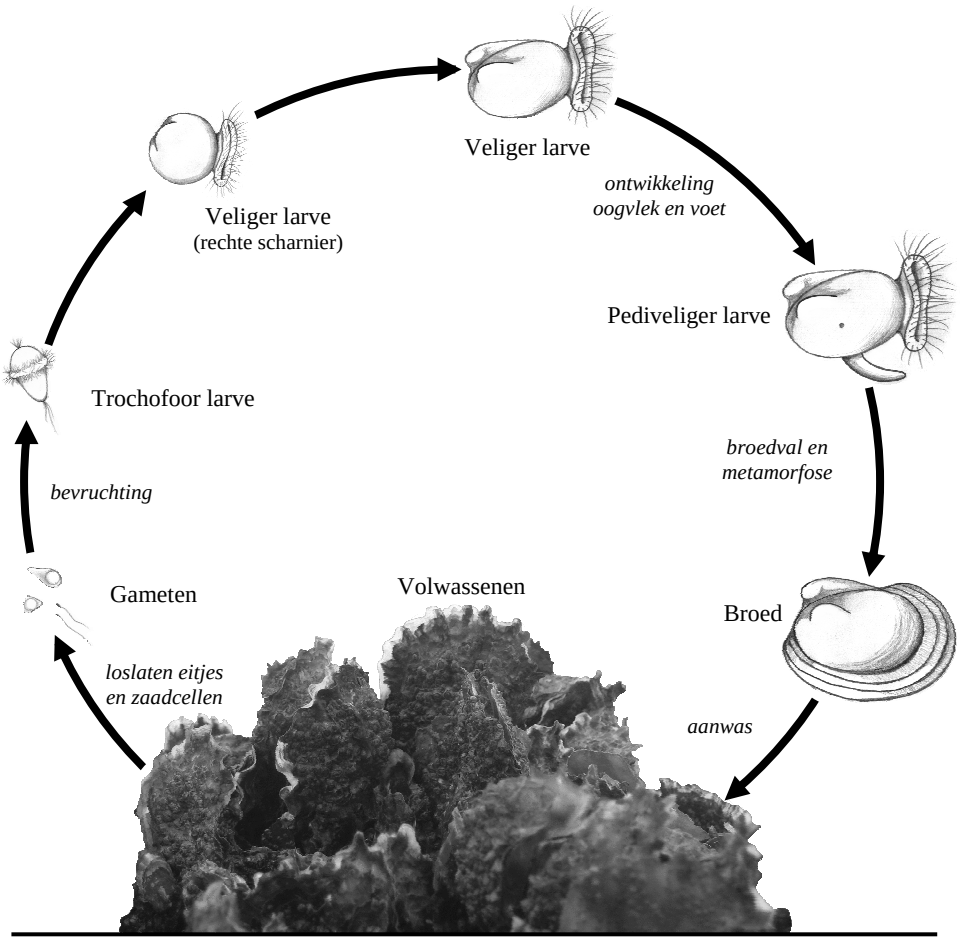
Introductie van de Japanse oester in Nederland en aangrenzende landen

Inheemse oester

Voordat de Japanse oester werd geïntroduceerd, werd in Nederland alleen de Europese platte oester *Ostrea edulis* hier gecultiveerd. Voor 1870 werden alleen natuurlijk voorkomende oesterbanken bevist, maar vanaf dat jaar werden de oesters vooral gekweekt op percelen. Door een combinatie van factoren komt de platte oester nu vrijwel niet meer voor in Nederland. Zeer strenge winters veroorzaakten een hoge sterfte, vooral de winter van 1962-1963. Nederlandse platte oesters werden daarna gemengd met buitenlandse platte oesters wat leidde tot nakomelingen die minder gehard waren tegen koude winters, met weer hoge wintersterfte als gevolg. Verder veroorzaakt de Bonamia-parasiet vanaf diens introductie in 1980 tot op de dag van vandaag sterfte onder oesters die geslachtsrijp worden. De platte oester wordt tegenwoordig alleen nog op beperkte schaal gekweekt in het Grevelingenmeer (Figuur 8.2).



Figuur 8.2. De ligging van het Delta gebied en de verschillende estuaria, waaronder de Oosterschelde, in Nederland.



Figuur 8.3. De levenscyclus van tweekleppige schelpdieren. De Japanse oester is hier als voorbeeld gegeven. De volwassen dieren scheiden aan het eind van de zomer gameten (eitjes en sperma) uit in het omringende water. In het water vinden de gameten elkaar en worden de eitjes bevrucht. De bevruchte eicel ontwikkelt zich eerst tot een trochofoor larve en binnen twee dagen tot een veliger larve. De veliger heeft al een schelp die nog dun en doorschijnend is. Met het 'velum', een uitstulping met trilharen erop, kan de larve zowel zwemmen als foerageren. Na ongeveer 1-2 weken ontwikkelt zich een oogvlek waarmee de larve donker en licht kan onderscheiden. De larve krijgt een gespierde voet en het velum degenereert. Na ongeveer 2-4 weken vestigt de larve zich op een geschikte plek. Een metamorfose volgt waarbij het lichaam zich aanpast aan een leven op de bodem in plaats van zwemmend in het water. De larve heet nu 'broed' en groeit uit tot een juveniel en binnen 1 jaar tot een volwassen, zich voortplantend, individu.

Introductie van de Japanse oester

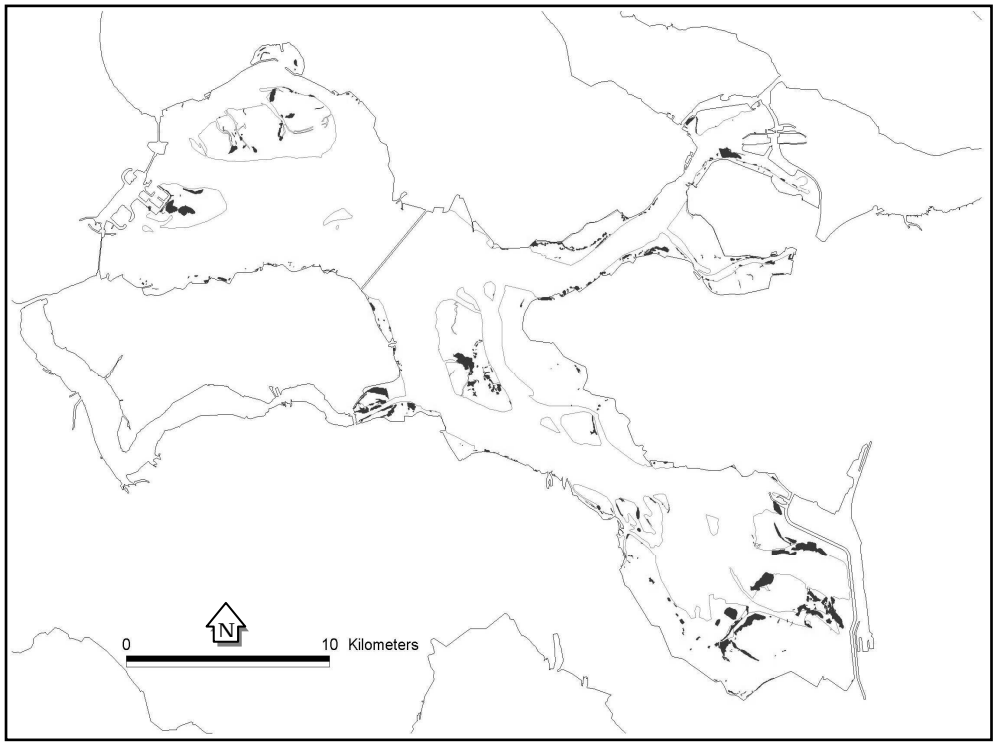
Al voor de strenge winter van 1962-1963 waren oesterkwekers op zoek gegaan naar alternatieven voor de platte oester. Er werd ge-experimenteerd met de Portugese oester *Crassostrea angulata* en de Amerikaanse Atlantische oester *Crassostrea virginica*, maar deze experimenten waren niet succesvol en deze oestersoorten vestigden zich niet in Nederland. In 1964 werd de Japanse oester geïntroduceerd in de Oosterschelde (Figuur 8.2). Door Dr. P. Korringa en J. Bol van het RIVO (Rijksinstituut voor visserij-onderzoek, later opgegaan in Wageningen IMARES) en een oesterkweker werden experimenten gedaan met oesterbroed afkomstig uit Canada waar de Japanse oester eerder was geïmporteerd. Deze experimenten waren zo succesvol dat meer introducties volgden. Verondersteld werd dat deze introducties geen kwaad konden omdat de oester zich toch niet voort zou kunnen planten vanwege te lage watertemperaturen. Bovendien lagen er plannen klaar om de Oosterschelde, in het kader van de Deltawerken, volledig af te sluiten van de Noordzee waardoor het een brak of zelfs zoet meer zou worden. Hierin zouden de oesters niet kunnen overleven. De plannen werden echter gewijzigd. In plaats van een dam werd een stormvloedkering gebouwd waardoor de Oosterschelde zout bleef. De watertemperatuur bleek ook geen belemmering voor de oester, want in 1971 werden oestertjes van ongeveer een jaar oud verzameld in de haven van Zierikzee door F. Kerckhof. In 1975 werd de eerste broedval (zie Figuur 8.3 voor de levenscyclus van tweekleppigen) waargenomen, en in de warme zomers van 1976 en 1982 zou er nog veel meer broed vallen. Daarna schakelden de meeste oesterkwekers over op het kweken van de Japanse oester.

Snelle uitbreiding in Nederland

De natuurlijke broedval van de Japanse oester had tot gevolg dat zich buiten de kweekpercelen over grote oppervlakken oesterbanken gingen vormen. Het RIVO schatte op basis van veldinventarisaties en reconstructies aan de hand van luchtfoto's dat het oppervlak aan oesterbanken in de getijdenzone zich heeft uitgebreid van 0,25 km² rond 1980 tot 8,1 km² in 2003 (Figuur 8.4). Terwijl het oesterbestand zich snel uitbreidde, lieten de bestanden aan mosselen (*Mytilus edulis*) en kokkels (*Cerastoderma edule*) in de jaren '90 een lichte afname zien.

De Japanse oester breidde zijn leefgebied uit naar de Waddenzee, waarschijnlijk met menselijke hulp. In 1983 werden de eerste oesters, van ongeveer 6-7 jaar oud, gevonden bij Oudeschild. Sindsdien breidt de Japanse oester zich gestaag uit in de

Waddenzee. Plaatselijk worden ze aangetroffen in grote dichtheden op dijken en op mosselbanken. In het Grevelingenmeer werd een eerste broedval waargenomen in 1987 en tegenwoordig is de Japanse oester één van de meest dominante schelpdieren in het gebied. In de Westerschelde werd de Japanse oester in de jaren '80 sporadisch aangetroffen, en ook tegenwoordig zijn de aantallen hier nog niet groot en blijven de individuen vrij klein.



Figuur 8.4. Bedekking door oesterbanken in de getijdenzone van de Oosterschelde in 2003 (8,1 km^2; oesterbanken zijn donkere vlekken; Wageningen IMARES). De laagwaterlijn is aangegeven in de kaart als een dunne lijn.

Ontwikkeling in Duitsland

De Japanse oester wordt ook aangetroffen in Duitsland. De oester heeft zich waarschijnlijk vanuit de Nederlandse Waddenzee verspreid naar de Oostfriese Waddenzee in Duitsland waar de eerste oesters werden aangetroffen in 1998. Het

noordelijke deel van de Duitse Waddenzee, de Waddenzee van Sleeswijk-Holstein, is waarschijnlijk gekoloniseerd vanuit een oesterkwekerij op het eiland Sylt. Ook in de Deense Waddenzee en op het eiland Helgoland in de Noordzee worden tegenwoordig verwilderde oesters aangetroffen. In de Duitse Waddenzee worden Japanse oesters vrijwel uitsluitend aangetroffen op mosselbanken in de getijdenzone, met maximale dichtheden van ongeveer 600 oesters per vierkante meter.

Ontwikkeling in België

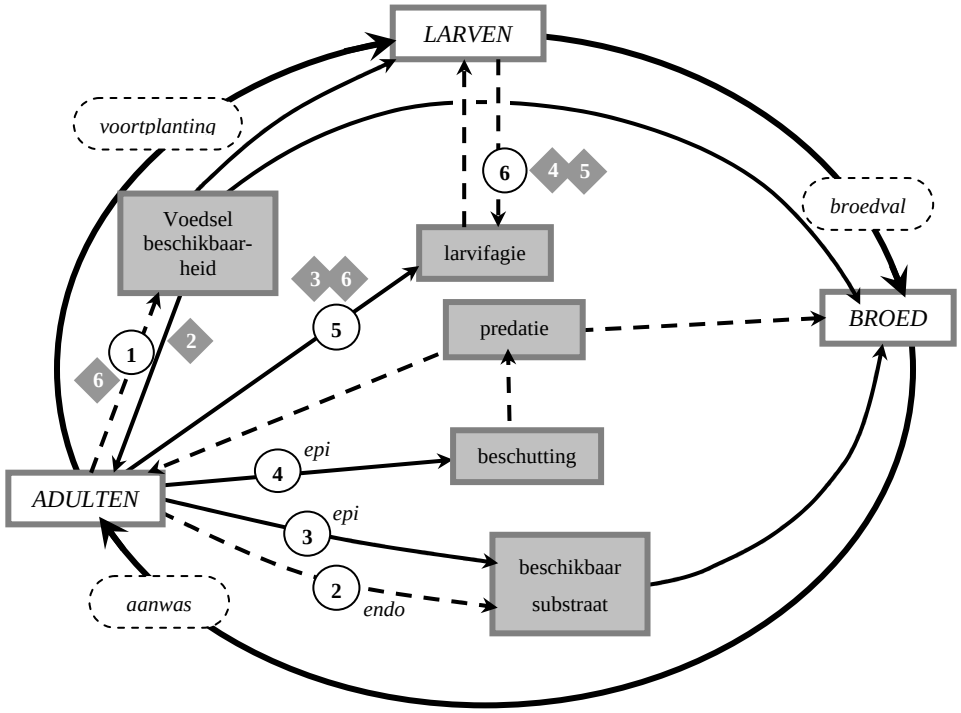
In Belgie wordt sinds 1930 de Spuikom in Oostende gebruikt voor oesterkweek. Er zijn verschillende soorten oesters gekweekt, vanaf 1969 ook de Japanse oester. Verwilderde oesters hebben zich gevestigd in de Spuikom, maar ook daarbuiten. De Japanse oester wordt nu langs de hele Belgische kust aangetroffen. In de havens van Oostende, Nieuwpoort, Zeebrugge en Blankenberge liggen nu oesterriffen.

Doel van het onderzoek

Dit proefschrift richt zich op de oorzaken van de succesvolle vestiging en snelle uitbreiding van de Japanse oester in Nederlandse estuaria en op mogelijke gevolgen voor inheemse tweekleppige filtrerende schelpdieren. In het bijzonder wordt aandacht gegeven aan mogelijke effecten op (en interacties met) de mossel *M. edulis*, omdat dit de enige bankvormende tweekleppige was voordat de Japanse oester zich vestigde. Oorzaken voor de succesvolle vestiging worden gezocht in kenmerken die algemeen worden toegeschreven aan invasieve soorten. Manieren waarop de Japanse oester inheemse schelpdieren zou kunnen beïnvloeden worden schematisch weergegeven in Figuur 8.5. Deze figuur geeft schematisch de levenscyclus weer van filtrerende tweekleppigen in het algemeen, waarbij factoren zijn aangegeven die de verschillende levensstadia positief of negatief kunnen beïnvloeden. De Japanse oester kan middels effecten op deze factoren verschillende levensstadia van zowel zichzelf als andere soorten beïnvloeden.

Omdat Japanse oesters relatief veel water filtreren zal de toename van het oesterbestand mogelijk geleid hebben tot een afname van de beschikbare hoeveelheid voedsel (o.a. ééncellige algen) voor schelpdieren en hun larven (1 in Figuur 8.5). Voedselgebrek kan leiden tot een gereduceerde voortplanting, groei en overleving van volwassen dieren en larven. Verder, omdat de Japanse oester grote riffen vormt en daarmee voormalige zachte sedimenten bedekt met een laag hard substraat, wordt het voor soorten die in de bodem leven (zoals kokkels) moeilijker om zich te vestigen (2 in Figuur 8.5). Voor dit zogeheten endobenthos wordt het geschikte leefgebied

beperkt. Voor soorten die juist boven de bodem leven, en in het bijzonder soorten die vooral op harde substraten leven (zoals de mossel), wordt het areaal aan geschikt leefgebied juist vergroot (3 in Figuur 8.5). Deze zogeheten epi-benthische soorten hebben ook het voordeel van beschutting in een oesterbank. Een oesterbank is een complexe drie-dimensionale structuur waar zowel de oesters zelf, als ook mosselen en andere soorten, beschutting vinden tegen uitdroging, golfwerking en predatie. Sterfte van broed en volwassen dieren wordt daardoor voor epi-benthische soorten mogelijk verlaagd (4 in Figuur 8.5). Daarnaast worden larven van andere schelpdieren, maar mogelijk ook die van de oester zelf, gefiltreerd en gegeten door volwassen oesters. Dit wordt larvifagie genoemd. Larvifagie kan resulteren in een gereduceerde broedval en aanwas (de jaarlijkse toename van het bestand met juvenielen die geslachtsrijp zijn



Figuur 8.5. Schematische weergave van de levenscyclus van tweekleppige filtreerders in het algemeen, met de belangrijkste factoren die overleving, groei en sterfte beïnvloeden. Effecten van verschillende levensstadia van de Japanse oester op ongeacht welke soort van tweekleppige filtreerders in de Nederlandse estuaria zijn aangegeven met pijlen. Ononderbroken pijlen geven positieve effecten (toename) aan en onderbroken pijlen negatieve effecten (afname). Omcirkelde getallen verwijzen naar de tekst. Getallen in grijze ruiten verwijzen naar de verschillende hoofdstukken in dit proefschrift. Waar nodig is aangegeven of effecten gelden voor epi-benthos (*'epi'*; soorten die boven de bodem leven) dan wel endo-benthos (*'endo'*; ingegraven soorten).

geworden) (5 in Figuur 8.5). Dit effect kan echter gecompenseerd worden, of zelfs teniet gedaan, als de larven weten te ontsnappen aan filtratie (6 in Figuur 8.5). Naast de genoemde mogelijke directe effecten van oesters op tweekleppigen, kunnen de oesters ook indirect een concurrentievoordeel hebben ten opzichte van inheemse soorten. Bijvoorbeeld, als de Japanse oester weinig wordt gegeten door roofvijanden kan dit resulteren in een snellere populatiegroei ten opzichte van inheemse soorten die wel in hoge mate gegeten worden.

Dit proefschrift richt zich op twee hoofdvragen:

1. *Oorzaken van succes*: Welke eigenschappen van *C. gigas* hebben bijgedragen aan diens snelle kolonisatie, vestiging en natuurlijke uitbreiding in Nederlandse estuaria?
2. *Gevolgen voor inheemse tweekleppige schelpdieren*: Welke eigenschappen van *C. gigas* beïnvloeden inheemse tweekleppige schelpdieren nadelig en zullen mogelijk zelfs leiden tot het verdwijnen van inheemse soorten?

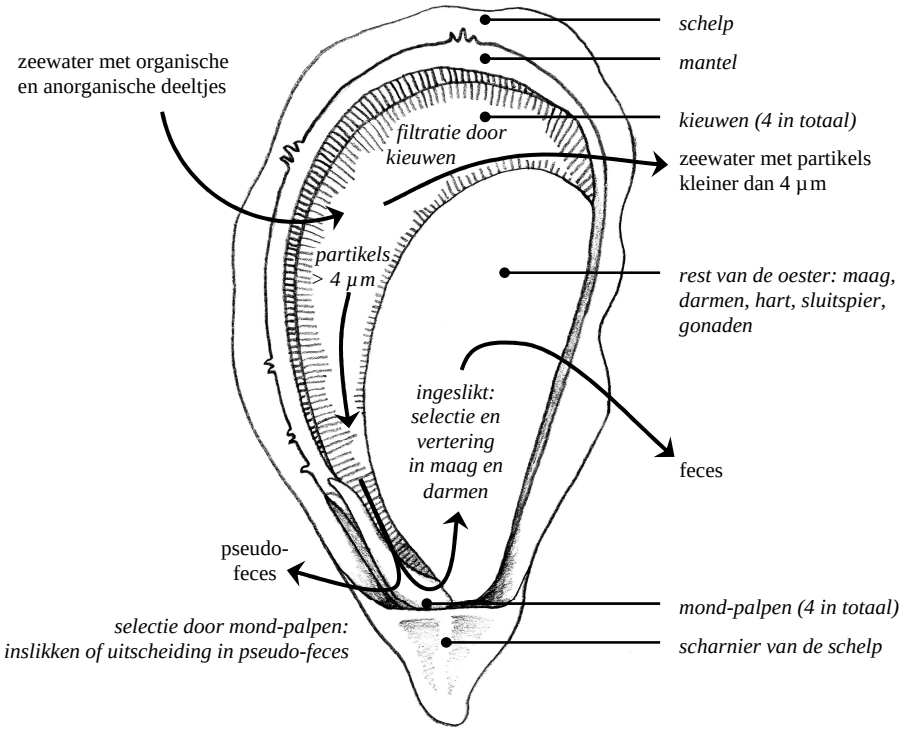
Om de eerste vraag te beantwoorden is vooral literatuuronderzoek gedaan. Voor het beantwoorden van de tweede vraag is literatuuronderzoek gecombineerd met de onderzoeksresultaten die zijn beschreven in Hoofdstukken 2 tot en met 6. Hoofdstuk 2 richt zich op concurrentie om voedsel tussen de Japanse oester, de mossel en de kokkel. De Hoofdstukken 3, 4 en 5 beschrijven laboratoriumonderzoek naar larvifagie en Hoofdstuk 6 gaat over effecten van larvifagie in het veld (de Oosterschelde). Hieronder wordt een samenvatting gegeven van elk onderzoekshoofdstuk.

8.2. Samenvatting van het uitgevoerde onderzoek

Eigenschappen van waterstromen voor voedselopname

Hoofdstuk 2 laat de resultaten zien van onderzoek naar eigenschappen van waterstromen voor de voedselopname opgewekt door drie soorten filtrerende tweekleppige schelpdieren met verschillende lichaamsbouw, *Crassostrea gigas*, *Mytilus edulis* and *Cerastoderma edule*, in relatie tot voedselconcurrentie (hoe tweekleppige filtreerders voedsel opnemen is geïllustreerd in Figuur 8.6). Dit werd onderzocht omdat verschillen in de waterstromen kunnen resulteren in verschillen in voedselopnamen, wat kan leiden tot voedselconcurrentie. Ten eerste kunnen door een grotere instroomsnelheid meer grote deeltjes (bijvoorbeeld langzaam zwemmend dierlijk plankton) worden aangetrokken en gefiltreerd. Daarnaast kunnen soorten met een stromingsveld dat verder de waterkolom in reikt ook uit hogere waterlagen eten.

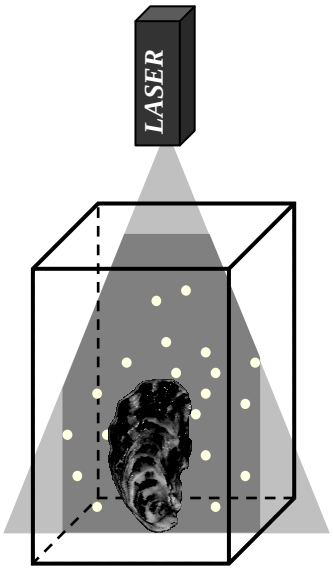
Met hun uitstroom, die doorgaans veel geconcentreerder en sterker is dan de instroom, kunnen tweekleppigen de mate van turbulentie in het omringende water vergroten waardoor de toestroom van vers (nog ongefiltreerd) water naar de bank wordt verhoogd. Bovendien werkt turbulentie als achtergrondruis voor snel zwemmend dierlijk plankton; hoe hoger de ruis, hoe minder goed ze de filtrerende schelpdieren kunnen detecteren en hoe eerder ze ten prooi vallen en als voedsel dienen voor de schelpdieren.



Figuur 8.6. Een schematische illustratie van hoe tweekleppige filtreerders (zoals de oester) foerageren (voedsel vergaren en eten). Zeewater met daarin allerlei rondzwevende partikels worden gefiltreerd ('gezeefd') over de kieuwen. Alles groter dan een bepaalde maat wordt vastgehouden op de kieuwen. De minimum maat is meestal 4-7 µm (micrometer). De vastgehouden partikels worden over de kieuwen met trilhaartjes naar de mond getransporteerd. Vóór de mond bevinden zich de mond-palpen die de partikeltjes sorteren; goedgekeurde partikels (meestal met een hoog organisch gehalte) worden ingeslikt en afgekeurde partikels worden uitgescheiden als pseudo-feces. Op ingeslikte partikels vindt in de maag nog een selectie plaats. Niet goed verteerbare deeltjes worden versneld uitgescheiden in de feces. De resterende partikels worden verteerd en geassimileerd (er worden bouwstenen en energie uit opgenomen) in de maag en darmen, en het onverteerbare deel uitgescheiden als feces.

De patronen in waterstromen voor de voedselopname naar de dieren toe, dus de instroom, werden voor de drie soorten in kaart gebracht met behulp van 'Digital Particle Image Velocimetry' (DPIV) en 'Particle Tracking Velocimetry' (PTV). Deze methoden houden het volgende in:

DPIV: Het te bestuderen dier werd in een aquarium met bewegingloos zeewater gezet (Figuur 8.7). Algen werden toegevoegd om het dier tot eten, en dus filtreren, aan te zetten. Om waterbewegingen zichtbaar te maken werden hele kleine witte plastic deeltjes toegevoegd (∅ 25 – 50 micrometer). Om alleen de deeltjes, en dus de waterbeweging, in een twee-dimensionaal vlak zichtbaar te maken werd een 2D laser-veld (dikte ongeveer 0,5 mm) geprojecteerd daar waar de waterstroming onderzocht moest worden (Figuur 8.6). De beweging van de partikeltjes in het twee-dimensionale vlak werd gefilmd met een digitale camera, en achteraf geanalyseerd met software ('Swift') ontwikkeld door de Rijksuniversiteit Groningen (E.J. Stamhuis). Het hele stromingspatroon werd zo in kaart gebracht.

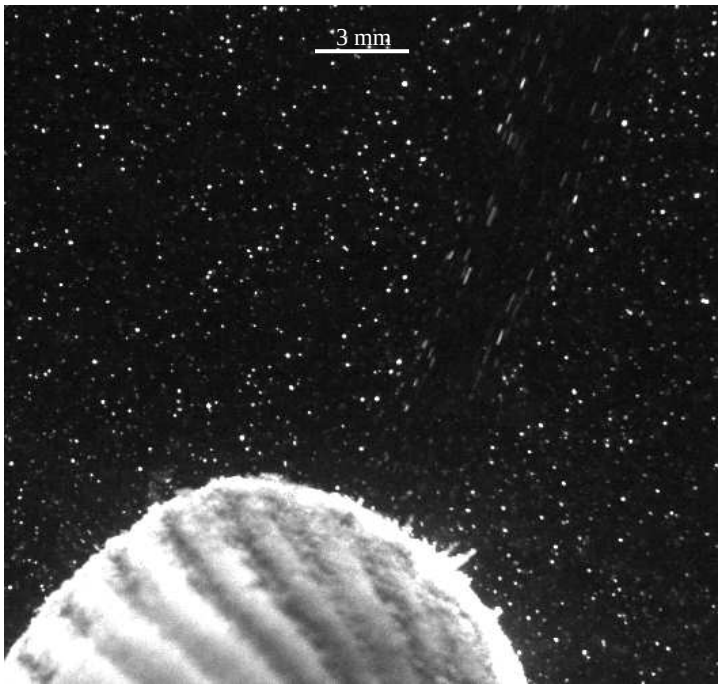


Figuur 8.7. Opstelling voor DPIV en PTV. Een oester is in het zeewater-aquarium geplaatst. In het zeewater zitten witte plastic partikels die de waterbeweging zichtbaar maken. Alleen de partikels in het 2-dimensionale laser-veld lichten op en worden gefilmd.

PTV: Om waterstroomsnelheden vlakbij de instroomopening van het dier te bepalen werd gebruik gemaakt van dezelfde camerabeelden. Nu werd echter een andere analyse toegepast. Met het softwareprogramma Didge© 2.3b1 (A.J. Cullum, Creighton University, Omaha, NE, USA) werden de trajecten afgelegd door

individuele deeltjes geanalyseerd. Beeldje voor beeldje werd de positie van een aantal deeltjes digitaal vastgelegd. Hieruit werden nauwkeurig snelheden berekend.

De uitstroom kon niet met deze methoden geanalyseerd worden omdat de dieren de deeltjes uit het water filterden (precies zoals ze ook voedsel uit het water filtreren). Dit houdt in dat de uitstroom leeg was. Dit is te zien in Figuur 8.8 als een zwarte (dus lege) waterstraal in een omgeving die bezaaid is met witte partikeltjes. Richtingen van uitstroom konden wel bepaald worden uit de beelden omdat de uitstroom zo goed zichtbaar was. De snelheid van de uitstroom werd geschat met literatuurwaarden voor debieten (dus hoeveel water de soorten filtreren per tijdseenheid) en de grootte van de uitstroomopeningen die opgemeten werd in de gefilmde beelden. Beide zijn variabel in de tijd en met de lichaamsgrootte van het dier. De snelheden van uitstroom werden zo gemodelleerd voor een spectrum aan debieten en doorsneden van de uitstroomopening.



Figuur 8.8. De uitstroom van een schelpdier, in dit geval een kokkel. De witte stipjes zijn plastic partikeltjes die worden verlicht door het laser-veld. De uitstroom is zichtbaar als een zwarte pluim, geflankeer door witte streepjes (partikeltjes in het omringende water die meegetrokken worden met de snelle uitstroom).

De resultaten lieten zien dat er, ondanks duidelijke verschillen in lichaamsbouw tussen de Japanse oester, de mossel en de kokkel, slechts kleine verschillen zijn in patronen van hun waterstromen voor voedselopname. Zo waren de instroomsnelheden iets lager voor de oester. Naar verwachting leidt dit niet direct tot verschillen in voedselopname en heeft het niet direct consequenties voor voedselconcurrentie. De uitstroom was duidelijk sneller bij de oester, maar de oriëntatie van de waterstraal was horizontaal (parallel aan de bodem) in plaats van verticaal (weg van de bodem, zoals bij de mossel en de kokkel). Wat voor consequenties dit uiteindelijk heeft voor de stroom van voedsel naar de dieren is niet duidelijk.

Larvifagie bij inheemse tweekleppigen en *C. gigas*

Hoofdstuk 3 laat resultaten zien van onderzoek naar het voorkomen en de mate van larvifagie bij inheemse tweekleppigen en de geïntroduceerde oester. Zoals eerder beschreven wordt het eten van larven van tweekleppigen door volwassen tweekleppigen 'larvifagie' genoemd. In laboratorium-experimenten werd ten eerste onderzocht of larven van de Japanse oester en de inheemse mossel (*M. edulis*) worden gefiltreerd door volwassen Japanse oesters, mosselen en kokkels (*C. edule*). Ten tweede werd onderzocht of Japanse oesterlarven in mindere mate worden gefiltreerd dan mossellarven. Ten slotte werd ook onderzocht of gefiltreerde larven worden opgegeten of uitgescheiden in pseudofaeces (zie Figuur 8.6 voor hoe tweekleppigen eten en verteren).

Larvifagie

Om de onderzoeksvragen te beantwoorden werden volwassen oesters, mosselen en kokkels in individuele graaskamers (emmers) gelegd. Deze waren gevuld met zeewater. Aan het water werden ééncellige algen toegevoegd. Gedurende een periode van gemiddeld twee uur, waarin de schelpdieren actief aan het filtreren waren, werden verschillende watermonsters genomen om te bepalen hoeveel algen al weggefiltreerd waren. Hieruit werd de 'clearance rate' bepaald, wat een maat is voor de tijd die een schelpdier nodig heeft om een bepaald volume van alle zwevende deeltjes te ontdoen. Vertaald naar het Nederlands zou men kunnen spreken van 'graassnelheid', de snelheid waarmee de deeltjes worden afgegraasd. Deeltjes die groot genoeg zijn om door de kieuwen vastgehouden te worden,en die niet weg zwemmen, worden met een efficiëntie van 100% gefiltreerd. Voor deeltjes die wél weg kunnen zwemmen heeft

een schelpdier meer tijd nodig om het watervolume van alle deeltjes te ontdoen. Vanuit deze redenatie werden ook graassnelheden bepaald met oesterlarven en mossellarven in verschillende experimenten. Vervolgens werden de graassnelheden voor larven vergeleken met de graassnelheden voor algen.

Snelheden waarmee mossellarven werden afgegraasd waren gelijk aan die voor algen, wat betekent dat de mossellarven zich gedroegen als 'inerte' deeltjes. Ze hebben dus niets gedaan om filtratie te ontwijken. Oesterlarven daarentegen wél, want snelheden waarmee oesterlarven werden afgegraasd waren gemiddeld twee keer zo laag als graassnelheden voor algen. De schelpdieren hadden dus twee keer zoveel tijd nodig om alle oesterlarven te filtreren dan alle mossellarven.

Het lot van gefiltreerde larven

In een volgend experiment werden oesterlarven en mossellarven met een pipet in de instroom van volwassen individuen van alle drie soorten gebracht. Van sommige individuen werd 5 minuten later de maaginhoud verwijderd en geanalyseerd. Van andere individuen werden na 15 minuten de pseudofaeces verzameld en geanalyseerd. In zowel maaginhoud als pseudofaeces werden larven geteld. Gefiltreerde larven werden, zo bleek, vooral ingeslikt en vervolgens verteerd. Slechts een klein deel van de larven werd uitgescheiden in pseudofaeces.

Conclusie

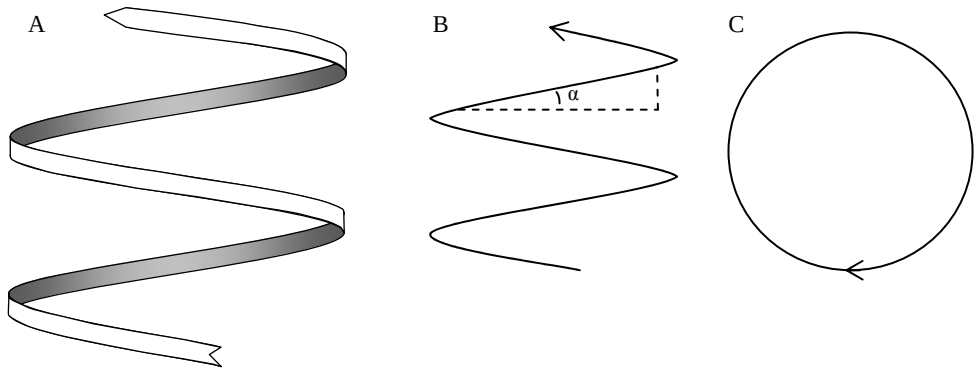
Concluderend worden zowel oesterlarven als mossellarven gefiltreerd door volwassen tweekleppige filtreerders, waarbij Japanse oesterlarven de helft minder worden gefiltreerd dan mossellarven. Het merendeel van de larven dient als voedsel voor de volwassen schelpdieren.

Kunnen larven ontsnappen aan filtratie door volwassen schelpdieren?

In Hoofdstuk 4 werd onderzocht of larven van de Japanse oester en de inheemse mossel kunnen ontsnappen aan de instroom van volwassen schelpdieren. De resultaten zouden hopelijk verklaren waarom eerder werd gevonden dat oesterlarven de helft minder worden gefiltreerd dan mossellarven.

Deze keer werden niet volwassen schelpdieren gebruikt maar werd de instroom voor voedselopname nagebootst door met een automatische pipet via een slangetje water aan te zuigen uit een bakje met zeewater. Eerst werd gecontroleerd of het

resulterende stromingspatroon wel leek op dat van een tweekleppige. Dit werd onderzocht met DPIV (zoals beschreven onder 'Hoofdstuk 2'). Vervolgens werd het water ververst en werden er larven (oester- of mossellarven) aan toegevoegd. Deze larven reflecteerden het laserlicht goed en hun bewegingen binnen het twee-dimensionale laser-veld waren goed te volgen en achteraf te analyseren middels PTV (ook beschreven onder 'Hoofdstuk 2'). Het laserveld doorsneed steeds de instroomopening, zowel horizontaal als verticaal in verschillende experimenten.

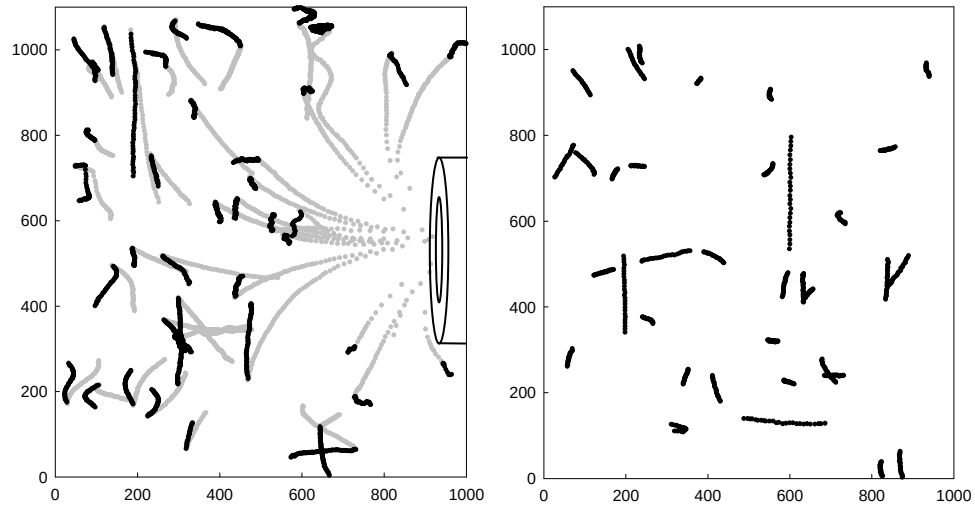


Figuur 8.9. Opwaarts spiraalvormig zwempatroon van veliger larven. A: overzicht, B: zij-aanzicht (α = hoek waaronder de larve naar boven zwemt), C: bovenaanzicht.

Larven van tweekleppigen zwemmen meestal in een bepaald patroon: in een spiraal omhoog (Figuur 8.9) waarna ze zich weer laten zinken door even niet te zwemmen. Als ze verstoord worden zouden ze kunnen reageren door de schelp te sluiten en snel naar beneden te zinken, of juist door sneller omhoog te zwemmen. Voor schelpdierlarven is nooit onderzocht of ze een stromingsveld, of veranderingen daarin, kunnen detecteren. Voor roeipootkreeftjes ('copepoden') wel. Zij kunnen bepaalde hydromechanische signalen detecteren, zoals een plotselinge versnelling of een bepaalde schuifspanning (wrijving tussen waterlagen met verschillende stromingssnelheden). Deze treden op bepaalde afstanden van de instroomopening op. Daarom werd in de experimenten speciaal gelet op consequente veranderingen in het zwemgedrag van de schelpdierlarven op bepaalde afstanden vanaf de instroomopening. Uit de gefilmde beelden werden de zwemtrajecten van een aantal larven gedigitaliseerd door de positie van de larven beeldje voor beeldje vast te leggen. Dit werd gedaan voor larven die zich in een stromingsveld bevonden en voor larven die zich in stilstaand water bevonden. Hieruit werden berekend: de gemiddelde

verticale verplaatsingssnelheid (de waterbeweging niet meegerekend) en de gemiddelde absolute zwemsnelheid. Ook werden de zwempatronen bekeken, en werd gezocht naar verandering in zwemgedrag die erop zouden kunnen wijzen dat de larven het stromingsveld herkenden en probeerden te ontsnappen.

Ten eerste werd aangetoond dat oesterlarven sneller zwemmen dan mossellarven, ook bij dezelfde lichaamsgrootte. Oesterlarven zwommen in een horizontaal vlak met snelheden tot 6 mm per seconde, en mossellarven tot zo'n 2 mm per seconde. Larven lijken continu met maximale snelheden te zwemmen en ze passen hun verplaatsingssnelheid in verticale richting aan door de hellingshoek van de spiraal aan te passen.



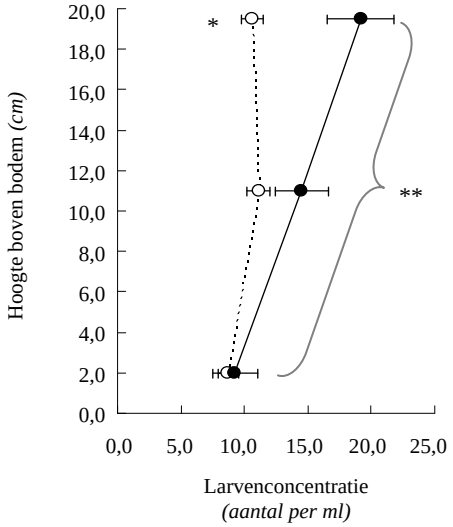
Figuur 8.10. Beweging van oesterlarven in een stromingsveld (links) en in stilstaand water (rechts). De figuren geven allebei weer wat de posities van larven waren in een opeenvolging van zo'n 30 gefilmde beelden (30 beelden per seconde). In de linker figuur geven de grijze sporen de beweging van de larven weer zoals die werd gefilmd. Ze werden naar de buis toe gezogen. De waterbeweging werd hiervan afgetrokken, wat resulteerde in de zwarte sporen die laten zien hoe de larven zich werkelijk hebben gedragen. Over het algemeen zijn typisch spiraalvormige bewegingen te zien met verschillende hoeken van opwaarts zwemmen. In de rechter figuur zijn sporen te zien van larven die in stilstaand water bewogen. Op de assen zijn het aantal pixels in X en Y richting weergegeven (schaal: 1000 px = 30 mm). De buis is 12.8 mm dik.

Verder werd geen enkele reactie op de gesimuleerde instroom bespeurd bij zowel oester- als mossellarven. Ze gedroegen zich in het stromingsveld precies hetzelfde als in bewegingloos water (Figuur 8.10). Het is waarschijnlijk dat ze het stromingsveld

niet kunnen herkennen. Er zal dus een andere verklaring gevonden moeten worden voor de resultaten uit het vorige hoofdstuk.

Ontsnappen larven aan larvifagie door naar boven te zwemmen?

In Hoofdstuk 5 wordt uiteindelijk een verklaring gegeven voor de waarnemingen uit Hoofdstuk 3 dat oesterlarven minder worden gefiltreerd dan mossellarven. Weer werden experimenten uitgevoerd met individuele graaskamers, maar deze keer alleen met volwassen oesters. De oesters mochten gedurende twee uur eten van toegevoegde ééncellige algen. Hierbij kwamen er metabolieten (producten van het metabolisme, zoals bijvoorbeeld ammonia en CO_2) in het water terecht. Vervolgens werden de oesters dichtgebonden met elastiekjes zodat ze niet meer een waterstroom voor de



Figuur 8.11. Gemiddelde concentratie van oesterlarven (van 4 emmers) op drie verschillende hoogtes boven de bodem in emmers met (zwarte stippen) en zonder (witte stippen) volwassen oester op de bodem, 90 minuten na aanvang van het experiment (de balken geven de variatie tussen de verschillende emmers (standaardfouten) weer). De enkele asterisk geeft een statistisch significant verschil weer tussen gemiddelde concentraties in emmers met en zonder een oester. De dubbele asterisk geeft een statistisch significant verschil weer tussen de bovenste en onderste waterlagen in emmers met een oester.

voedselopname konden opwekken en ook niet larven konden filtreren. Daarna werden oesterlarven of mossellarven toegevoegd aan het water. Na het water voorzichtig geroerd te hebben om de larven gelijk te verdelen, werden de graaskamers

met rust gelaten. Op twee tijdstippen werden met een pipet watermonsters genomen van drie verschillende waterdieptes (bodem, oppervlak en precies er tussenin): 50 en 90 minuten nadat de larven waren toegevoegd. Larven werden geteld in de watermonsters en hieruit werd de verticale verdeling over de waterkolom bepaald op twee tijdstippen na aanvang.

Mossellarven bleken hun verticale verdeling niet veranderd te hebben in reactie op de aanwezigheid van een volwassen oester; ze bleven gelijkmatig verdeeld. Oesterlarven waren na 50 minuten ook nog gelijkmatig verdeeld, maar na 90 minuten hadden ze zich meer richting het wateroppervlak verplaatst (Figuur 8.11). Hoewel de mossellarven niet reageerden, lijken de oesterlarven de volwassen oesters chemisch herkend te hebben. Ze hebben de volwassen oesters mogelijk 'geroken', als het ware, en zijn vervolgens omhoog gaan zwemmen. Het experiment is echter maar eenmaal uitgevoerd voor elke larvensoort en daarom gelden de resultaten niet als hard bewijs. Ze sluiten wél heel goed aan bij de experimenten uit Hoofdstuk 3. Daarom denk ik dat de resultaten een verklaring bieden voor het feit dat oesterlarven minder werden gefiltreerd dan mossellarven door alle drie soorten tweekleppigen.

Effecten van larvifagie op larvenaantallen in de Oosterschelde

Uiteindelijk werd in het veld onderzocht of het toegenomen bestand aan tweekleppige filtreerders in de Oosterschelde een effect heeft gehad op larvenaantallen van Japanse oesters en mosselen. Deze larven werden jarenlang gemonitord door het RIVO, en later Wageningen IMARES, ten behoeve van de schelpdierkweek. Effecten van larvifagie werden onderzocht op drie schaalniveaus. Onderstaand worden per schaalniveau methoden, resultaten en conclusies besproken.

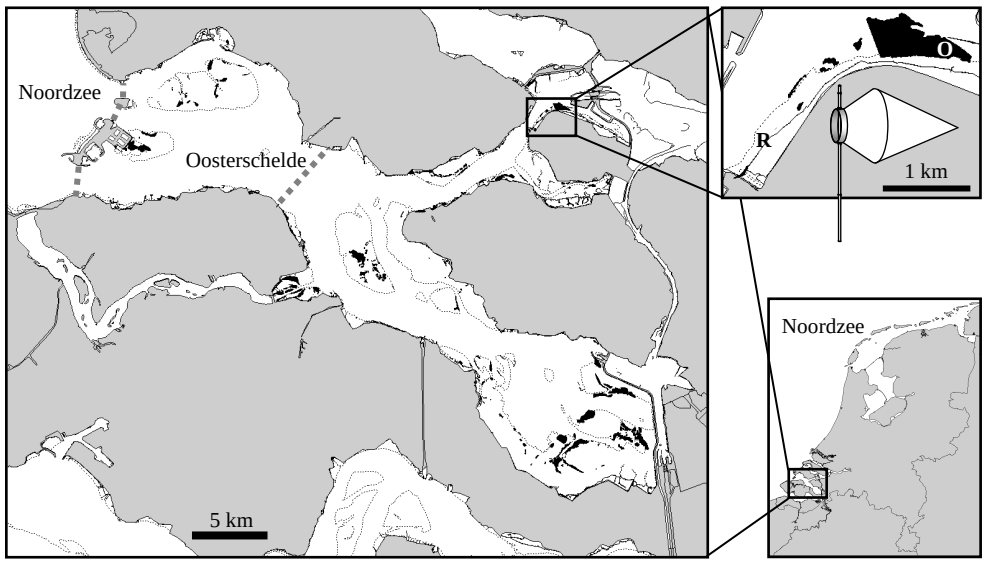
Schaalniveau: individuele schelpdieren

In aanvulling op literatuurgegevens werd ten eerste bevestigd dat in de Oosterschelde individuen van *C. gigas* en *M. edulis* ook daadwerkelijk larven filtreren en opeten. Volwassen oesters en mosselen werden in kooitjes in de Oosterschelde gehangen en maagonderzoek wees uit dat ze inderdaad oester- en mossellarven inslikken.

Schaalniveau: oesterbank

Ten tweede werden effecten van larvifagie onderzocht op het schaalniveau van een oesterbank. Planktonnetjes die vrij rond konden draaien op bamboestokken werden

geplaatst in een oesterbank in de noordelijke tak van de Oosterschelde (bij Anna Jacobapolder, Figuur 8.12) en op een nabije referentielocatie zonder oesters (bij het voormalige veerhaventje aan het Zijpe). De netjes stonden ongeveer 30 cm boven de bodem. Gedurende één getijdencyclus filterden de netjes (met een maaswijdte van 60



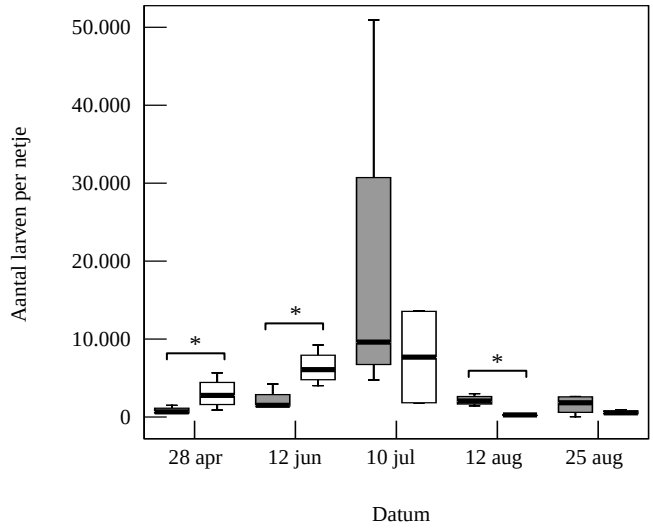
Figuur 8.12. Lokatie van het veld-experiment met plankton-netjes. Links is de Oosterschelde weergegeven, met oesterbanken in de getijdenzone als zwarte vlekken. Rechtsboven is ingezoomd op de experimentele lokatie nabij Anna Jacobapolder bij de ingang van het Slaak. De letter 'O' geeft de oesterbank weer en de letter 'R' de referentie-lokatie. Ook is weergegeven hoe een plankton-netje eruitziet.

micrometer) het doorstromende zeewater, eerst op de vloedstroom en later op de ebstroom. 's Avonds werden de netjes geplaats en de volgende ochtend werden ze opgehaald en in het laboratorium uitgespoeld. Alle schelpdierlarven uit de netjes werden geteld. De netjes werden gebruikt in april, juni, juli en tweemaal in augustus (2003). De vangsten lieten zien dat in april en juni, toen er vooral mossellarven in het water aanwezig waren, er minder larven werden gevangen boven de oesterbank dan boven de referentielocatie (Figuur 8.13). In juli en augustus, toen er juist veel oesterlarven in het water zaten, was er bij twee van de drie vangsten (1 in juli, 2 in augustus) geen verschil in aantallen te zien tussen de beide locaties. Op één dag echter, op 12 augustus, werden juist meer larven boven de oesterbank gevonden dan boven

de referentielocatie. Verschillen in aantallen konden niet worden verklaard door verschillen in stromingssnelheden tussen beide locaties. Aantallen mossellarven waren waarschijnlijk gereduceerd door de filtratie-activiteit van de oesters in de oesterbank, dus door larvifagie. De resultaten van oesterlarvenaantallen waren moeilijker te verklaren. Er bestond een sterk vermoeden dat de resultaten waren beïnvloed doordat de oesters in de oesterbank zich in juli hadden voortgeplant en dus nieuwe larven hadden geproduceerd. Daardoor konden geen conclusies worden getrokken betreffende het plaatselijke effect van larvifagie op oesterlarven-aantallen.

Schaalniveau: het estuarium Oosterschelde

Tenslotte werden effecten van larvifagie op het schaalniveau van de gehele Oosterschelde onderzocht. De toename van het totale bestand aan dominante filtrerende tweekleppigen (Japanse oester, mossel en kokkel) sinds 1990 werd bepaald door per jaar de bestanden van de drie soorten op te tellen. De mossel en de kokkel zijn allebei soorten die commerciëel interessant zijn. Daarom worden de bestanden



Figuur 8.13. Aantallen larven gevangen in plankton-netjes boven de oesterbank (grijs) en de referentie-lokatie (wit). Het gemiddelde aantal van 4 netjes is steeds gegeven (de dikke balk geeft het gemiddelde aantal, de lengte van de balk geeft een idee van de variatie tussen netjes, de spreiding rond het gemiddelde). Waar het aantal gevangen larven statistisch gezien significant verschilde tussen beide lokaties is dit aangegeven met een asterisk.

daarvan gemonitord door Wageningen IMARES (destijds nog RIVO). Ook van Japanse oesters op kweekpercelen bestaan overzichten en daarnaast zijn de bestanden aan verwilderde Japanse oesters deels bepaald en deels geschat door het RIVO. Ten behoeve van de kweek van schelpdieren zijn aantallen en ontwikkelingsstadia van mossellarven gemonitord in de periode 1998-2003 en van oesterlarven in de periode 1994-2006. Trends in het aantalsverloop van oesterlarven en mossellarven werden onderzocht en gerelateerd aan de toename van het bestand aan tweekleppige filtreerders in de Oosterschelde. Oesterlarven bleken in aantallen af te nemen over de periode van 13 jaar, wat overeenkomt met een toename in het bestand aan filtreerders. Mossellarven lieten geen duidelijk trend zien, maar de beschikbare gegevens bestrijken ook een veel kortere periode, namelijk 6 jaar. In zo'n korte periode is het veel moeilijker om een trend te herkennen omdat de effecten van natuurlijke variaties tussen jaren relatief groter zijn. De afname in oesterlarvenaantallen kan rechtstreeks door larvifagie veroorzaakt zijn, mogelijk in combinatie met effecten van voedselgebrek bij de larven. Door de toenemende aantallen filtreerders lijkt het beschikbare voedsel in de Oosterschelde inmiddels namelijk op te raken.

Model-berekeningen

Modelmatige berekeningen lieten tenslotte zien dat de sterfte onder larven in de Oosterschelde door larvifagie ruwweg geschat kan worden op 95%. Dit is aanzienlijk en ongeveer even hoog als de schatting voor de totale natuurlijke sterfte onder larven en juvenielen van ongewervelde bodemdieren die al eerder door andere onderzoekers werd gegeven.

8.3. Synthese en discussie

In Hoofdstuk 7 heb ik een overzicht gegeven van de mogelijke oorzaken voor het succes van de Japanse oester in Nederlandse estuaria en van de mogelijke gevolgen voor inheemse schelpdieren. Het eerste deel, de bespreking van eigenschappen van de Japanse oester die bijgedragen kunnen hebben aan de succesvolle vestiging en aansluitende natuurlijke verspreiding naar nieuwe gebieden, is vooral gebaseerd op literatuuronderzoek. Het tweede deel, de bespreking van gevolgen voor inheemse schelpdieren middels larvifagie en concurrentie om voedsel en ruimte, is vooral gebaseerd op de onderzoeksresultaten uit de Hoofdstukken 2 tot en met 6. Uiteindelijk zijn alle bevindingen samengebracht en de onderzoeksvragen beantwoord.

Oorzaken voor het success van de Japanse oester

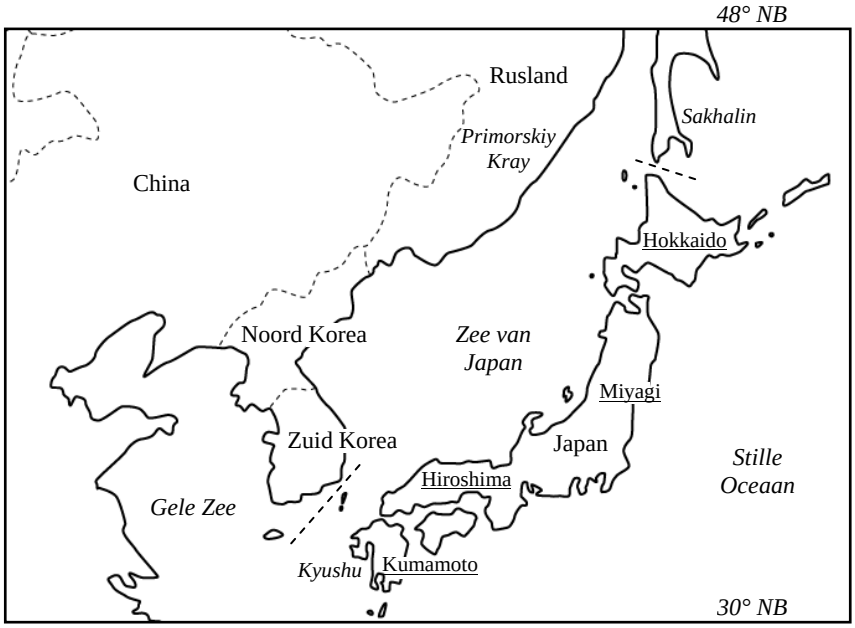
Vestiging van de Japanse oester in Nederland

Bij de kolonisatie van de Oosterschelde door de Japanse oester, dus de eerste introductie, speelden eigenschappen van de oester zelf geen rol. De kolonisatie werd geheel mogelijk gemaakt door oesterkwekers die de oester herhaaldelijk hebben geïmporteerd. Door de herhaalde introducties heeft de geïntroduceerde populatie van Japanse oesters een hoge mate van genetische diversiteit behouden, waardoor de soort zich gemakkelijker aan kon passen aan de nieuwe omgeving. Een grote genetische variatie kan daarbij gezien worden als een grote variatie in gereedschappen om zich aan te kunnen passen. Daarnaast hebben tweekleppige schelpdieren een relatief groot vermogen om zich 'fenotypisch' aan de omgeving aan te passen. Zo kunnen ze bijvoorbeeld de bouw van hun voedings-organen (o.a. kieuwen) aanpassen aan het voedselaanbod om dat zo efficiënt mogelijk te kunnen verwerken.

Los van deze eigenschappen is het de vraag of de Japanse oester zich wel zo sterk aan moest passen. Het klimaat in het gebied van herkomst is namelijk vrij vergelijkbaar met het klimaat in Nederland. De Japanse oester is afkomstig uit het gebied ronde de Zee van Japan, tussen ongeveer 30° en 48° noorderbreedte (Figuur 8.14). In het verleden zijn Japanse oesters uit vier verschillende Japanse regio's, namelijk Miyagi, Kumamoto, Hiroshima en Hokkaido, geëxporteerd naar elders in de wereld. In Nederland zijn vooral oesters uit de prefecturen Miyagi en Kumamoto geïntroduceerd. De Kumamoto oesters bleken later een andere soort te zijn, namelijk *Crassostrea sikamea*. Wat hiermee uiteindelijk in Nederland gebeurd is, is niet duidelijk. Waarschijnlijk hebben ze zich niet gevestigd. De Miyagi en Hokkaido oesters waren afkomstig uit een relatief koel klimaat in Japan, lijkend op het klimaat in Nederland. Uiteindelijk vertonen de Japanse oesters in Nederland waarschijnlijk vooral Miyagi-eigenschappen.

De Japanse oester kon zich verder waarschijnlijk zo snel vestigen in de Nederlandse estuaria omdat er relatief weinig roofvijanden aanwezig zijn en omdat de Japanse oester niet zo bevattelijk is voor aanwezige parasieten en ziektes. De mate van opname door vogels in Nederland lijkt niet heel anders dan in Japan. Japanse oesters lijken in beide gebieden slechts weinig door vogels gegeten te worden. De mate van predatie door ongewervelde bodemdieren lijkt in Nederland echter veel lager te zijn. In Japan en elders in noord-oost Azië zijn met name platwormen (Turbellaria) en borende roofslakken ('oyster drills') een grote bedreiging voor volwassen oesters en

hun broed. Japanse oesters die vanuit Japan in Nederland terecht zijn gekomen (hetzij direct, hetzij indirect via verschillende generaties en andere landen zoals Frankrijk en Canada) zijn dus als het ware bevrijd van roofvijanden. Wel zijn zeer recent (2007-2008) voor het eerst roofslakken uit de Verenigde Staten (*Urosalpinx cinerea*) en noord-oost Azië (*Ocinebrellus inornatus*) in de Oosterschelde aangetroffen. Deze komen echter vooralsnog zeer lokaal en in zeer lage aantallen voor.



Figuur 8.14. Gebied van herkomst van de Japanse oester. De namen van de vier prefecturen waarvandaan oesters zijn geëxporteerd zijn onderstreept.

De Japanse oester in Nederland is niet vrij van parasieten en ziektes. Japanse oesters worden zowel geïnfecteerd door parasieten en ziektes die al in Nederland aanwezig waren (de borstelworm *Polydora* sp., de schimmel *Ostracoblabe implexa*) als ook door parasieten en ziektes die ze meegenomen hebben uit noord-oost Azië of andere oestercultiverende landen (de roeipootkreeftjes *Mytilicola orientalis* en *Myicola ostreae*, de bacterie *Nocardia crassostreae*). De Japanse oester wordt niet geïnfecteerd door de Bonamia-parasiet (*Bonamia ostreae*) die onder de inheemse platte oester *Ostrea edulis* zoveel sterfte veroorzaakt.

Verder is de Japanse oester een 'ecosysteemingenieur', wat inhoudt dat de soort zijn leefgebied aanpast aan zijn eigen eisen en daarbij de hele levensgemeenschap beïnvloedt en verandert. De Japanse oester doet dit door riffen te bouwen. Op plaatsen waar eerst kale zand- of slikplaten waren met alleen levensgemeenschappen van zachte bodems komen nu oesterbanken voor die ten eerste zeer complex van structuur zijn en ten tweede levensgemeenschappen van harde substraten met zich meebrengen. Door de complexe en 'ruwe' structuur zijn er meer mogelijkheden ontstaan voor de vestiging van oesterlarven, maar ook van allerlei andere soorten bodemdieren. Larven hebben over het algemeen wat luwe plekjes nodig om vanuit het stromende water een 'voet' aan de grond te kunnen krijgen om zich te kunnen vestigen. Luwe plekjes zijn volop aanwezig in een oesterbank, in tegenstelling tot een kale bodem. Verder biedt de complexe drie-dimensionale structuur van een oesterbank beschutting tegen uitdroging, golfwerking en roofvijanden. Wederom niet alleen voor de oesters zelf, maar ook voor andere soorten. Daarnaast beïnvloedt de ruwheid van een oesterbank de stroming van het water over de bodem, waardoor transport van voedseldeeltjes (zoals ééncellige algen) naar de oesterbank bevorderd kan worden. Door het vormen van een oesterbank zorgen de oesters dus voor een sterke toename in vestigingsmogelijkheden voor hun larven, een verhoogde overleving van hun broed, juvenielen en volwassen oesters, en waarschijnlijk ook voor een grotere toestroom van vers voedsel.

Natuurlijke verspreiding naar nieuwe locaties

De Japanse oester heeft zich na vestiging zeer snel verspreid, zowel in Nederland als ook elders in noord-west Europa. Dit is waarschijnlijk toe te schrijven aan het hoge verspreidings-potentieel van de Japanse oester in combinatie met eigenschappen die over het algemeen worden toegeschreven aan succesvolle kolonisten.

De Japanse oester heeft een hoog verspreidingspotentieel door een groot aantal nakomelingen en een lange larvale fase. Japanse oesters zijn zeer vruchtbaar. Ze produceren grote hoeveelheden eicellen (tot meer dan 50 miljoen per oester) die in het water worden bevrucht door spermacellen (Figuur 8.3). Dit resulteert in een zeer groot aantal larven. Deze larven kunnen enkele weken overleven in het water, terwijl ze enkele tot tientallen kilometers worden meegevoerd met zeestromingen. Hierdoor kunnen Japanse oesters zich snel over grote afstanden uitbreiden, ook al is de sterfte onder larven en broed zeer groot.

Verder zijn eigenschappen van belang die in het algemeen worden toegeschreven aan succesvolle kolonisten. Veel van dergelijke eigenschappen vallen onder een '*r*-

geselecteerde' levensstrategie. Dit houdt met name in: grote aantallen nakomelingen en een snelle groei. '*r*-Strategen' hebben vaak snel opeenvolgende generaties; ze zijn vaak relatief kortlevend en snel groeiend en hebben vaak veel nakomelingen waaronder de sterfte groot is. Ze kunnen in korte tijd een gebied koloniseren. Hier tegenover staan '*K*-strategen' die meer investeren in hun weinige nakomelingen en in een lang leven. Dergelijke soorten hebben juist weinig nakomelingen die echter intensiever worden verzorgd en een lager sterftepercentage kennen. De Japanse oester heeft veel kenmerken van een *r*-strateeg: veel nakomelingen en een snelle groei van larven, broed, juvenielen en volwassenen. Door een snelle groei kunnen ze waarschijnlijk ook sneller ontsnappen aan roofvijanden, door al snel te groot voor consumptie te worden. De overleving van oesterbroed lijkt mede hierdoor groter te zijn dan die van inheemse tweekleppigen. Daarnaast planten Japanse oesters zich meestal in het jaar na hun geboorte al voort.

Voorts vertoont de Japanse oester veel eigenschappen van een 'habitat generalist'. Dit staat in tegenstelling tot een 'specialist' en houdt in dat de oester zich in veel verschillende habitats kan handhaven en onder verschillende abiotische omstandigheden. Zo kunnen ze zich vestigen op verschillende ondergronden. In eerste instantie vestigen ze zich op harde substraten zoals stortstenen onderaan dijken, maar ze onwikkelen zich ook tot uitgestrekte banken op zandige en slikkige ondergrond. Daarbij vestigen ze zich eerst op kleine stukjes hard substraat zoals schelpresten. Vervolgens vestigen ze zich op elkaar en zo kan zich snel een hele oesterbank ontwikkelen. Dit proces wordt versneld doordat ze zich bij voorkeur vestigen op en nabij soortgenoten (Hoofdstuk 7, Box 7.2). Verder is de Japanse oester tolerant voor een grote variatie aan abiotische condities. Dit kan mogelijk deels worden verklaard doordat de Japanse oester afkomstig is uit een uitgestrekt gebied met een grote variatie aan temperaturen en zoutgehaltes. Japanse oesters kunnen watertemperaturen tot 40°C overleven en luchttemperaturen (tijdens droogvallen bij laagwater) tot -5°C of zelfs lager afhankelijk van het zoutgehalte van het water dat dan in hun schelp is ingesloten. Groei vindt plaats bij temperaturen tussen 10 en 40°C en bij zoutgehaltes tussen 10 en 30‰, voortplanting bij temperaturen tussen 16 en 30°C en zoutgehaltes tussen 10 en 30‰ en larven kunnen overleven bij temperaturen tussen 18 en 35°C en zoutgehaltes tussen 19 en 35‰.

Over het algemeen wordt de mogelijkheid om een eerder gekoloniseerde locatie te herbevolken gezien als een eigenschap van een succesvolle kolonist. Ook hier beantwoordt de Japanse oester aan. Door oesterriffen te bouwen leggen ze een groot oppervlak aan geschikt habitat neer. Als plaatselijk alle oesters doodgaan, bijvoorbeeld door extreme hitte, blijft de oesterbank als structuur liggen. In het volgende jaar kan

de gehele bank bij een goede broedval van larven afkomstig uit andere oesterbanken alweer herbevolkt worden. Dit wordt vergemakkelijkt doordat de Japanse oester zich snel over grote afstanden kan verspreiden middels het larvale stadium en doordat de larven zich bij voorkeur vestigen op of nabij soortgenoten.

Doordringbaarheid van ontvangend ecosysteem

Hoe doordringbaar waren de Nederlandse estuaria eigenlijk voor een exoot zoals de Japanse oester? De doordringbaarheid van ecosystemen voor exoten wordt volgens de literatuur vooral bepaald door de mate van verstoring, de soortenrijkdom en de afwezigheid van roofvijanden. Eerder is al besproken dat de Japanse oester in Nederland relatief weinig te duchten heeft van roofvijanden, dus dit zal zeker een rol gespeeld hebben.

Hoewel de mate van verstoring van ecosystemen moeilijk te kwantificeren is, zou verstoring een rol gespeeld kunnen hebben bij de vestiging van de Japanse oester in Nederland. De Nederlandse estuaria kennen allemaal enige vorm van verstoring. Zo veroorzaken de Deltawerken nog steeds doorgaande veranderingen in de Oosterschelde. Hoewel de Oosterschelde ten tijde van de eerste gedocumenteerde grote broedval, in 1975, nog in open verbinding met de Noordzee stond, waren er al veranderingen opgetreden door de afsluiting van het huidige Veerse Meer in 1960, van het Grevelingenmeer in 1964 en van het Haringvliet en rivierwater afkomstig van de Maas en Rijn in 1969. Verder zijn er veel menselijke activiteiten in de Oosterschelde, zoals visserij, scheepvaart en schelpdierkweek, die invloed uitoefenen op het ecosysteem. Het Grevelingenmeer werd ook sterk beïnvloed door de Deltawerken; het veranderde van een estuarium met getijdewerking naar een stagnant brak meer. Hoewel de Westerschelde nog steeds in open verbinding met de Noordzee staat, is hier sprake geweest van sterke vervuiling, wordt er veel gebaggerd en is er veel scheepvaartverkeer naar de havens van Antwerpen, Gent, Terneuzen en Vlissingen. De Waddenzee werd in de jaren ’60, ’70 en ’80 vervuild met water vanuit de sterk vervuilde Rijn (via de Noordzee). Daarnaast zijn er nog steeds geo- en hydromorfologische veranderingen gaande als gevolg van de bouw van de Afsluitdijk in 1932. Hierdoor zijn ook zoutgehalten in de westelijke Waddenzee veranderd. Tenslotte zijn de kusten langs alle Nederlandse estuaria in de afgelopen decennia, zelfs eeuwen, ingrijpend veranderd door de aanleg en versterking van zeeweringen.

Tenslotte lijken exoten zich over het algemeen in ecosystemen met een lage soortenrijkdom gemakkelijker te kunnen vestigen, mogelijk omdat er meer ‘ecologische niches’ (de plaats die een soort in het voedselweb heeft) beschikbaar zijn

waardoor er minder geconcurreerd hoeft te worden. Met name in de brakke overgangszones (5 – 20‰) van zoet naar zout in estuaria, waar de soortenrijkdom laag is, worden relatief veel exoten aangetroffen. Dit kan echter geen rol gespeeld hebben in de Oosterschelde, omdat de Oosterschelde juist zeer soortenrijk is. Ten eerste is de rijkdom aan inheemse soorten hier groot, en ten tweede worden er tegenwoordig ook veel niet-inheemse soorten (exoten) aangetroffen, die met name via schelpdierimporten zijn geïntroduceerd. Daarnaast komt de Japanse oester meer voor bij hogere zoutgehaltes dan in de soortenarme brakke zone.

Gevolgen voor inheemse schelpdieren

Theoretisch zijn er drie mogelijkheden met betrekking tot concurrentie tussen Japanse oesters en inheemse tweekleppigen:

1- Concurrentie met de Japanse oester leidt tot lokale uitsterving van inheemse tweekleppigen;
2- De Japanse oester bezet een lege niche en concurreert in het geheel niet met inheemse tweekleppigen;
3- De niche die wordt bezet door de Japanse oester overlapt gedeeltelijk met de niches van inheemse tweekleppigen en ze kunt naast elkaar voortbestaan met gereduceerde niche-omvang (bijv. bandbreedte van voorkomen in de getijdenzone).

De eerste optie is niet aan de orde. Hiervoor zouden de niches 100% moeten overlappen en de hulpbronnen (voedsel, ruimte) beperkt moeten zijn. Niches overlappen niet voor 100%. Door literatuuronderzoek is gevonden dat de tweekleppigen, inheems en uitheems, niet exact op dezelfde locaties in de getijdenzone worden aangetroffen en dat ze niet exact hetzelfde voedsel benutten. Niches overlappen echter wel degelijk, waarmee ook de tweede optie wordt uitgesloten. Door andere onderzoekers werd al eerder aangetoond dat de Japanse oester niet exact dezelfde niche bezet als die door de inheemse oester *O. edulis* werd bezet. De derde optie blijft over. De Japanse oester bezet zijn eigen niche, mogelijk deels de niche die werd opengelaten toen *O. edulis* verdween, en concurreert in de marges met inheemse tweekleppigen. Ze concurreren alleen met elkaar waar niches overlappen en alleen als de hulpbronnen beperkt zijn.

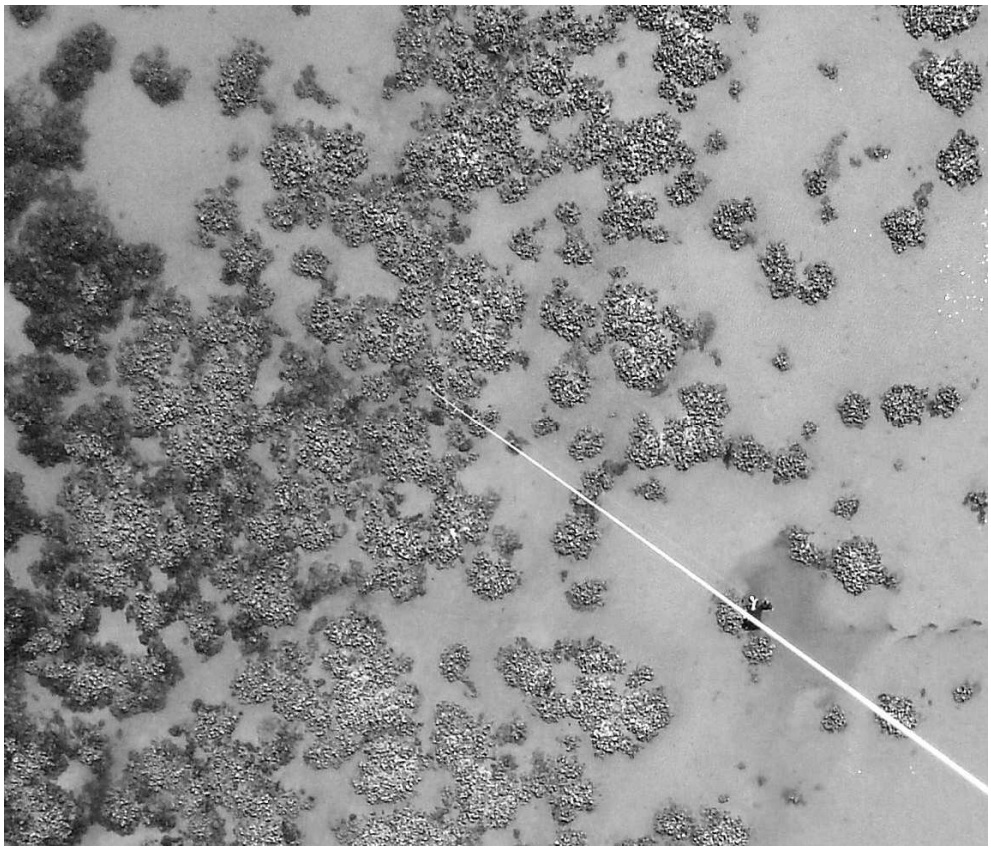
Concurrentie om ruimte

In de marges, waar de habitatvereisten overlappen, vindt concurrentie om ruimte met inheemse schelpdieren plaats. De Japanse oester wordt met name laag in de getijdenzone aangetroffen terwijl de meeste inheemse soorten (kokkel *C. edule*, nonnetje *Macoma balthica* en strandgaper *Mya arenaria*) meer verspreid voorkomen, onder andere hoog in de getijdenzone waar vrijwel geen oesters zitten. Als een oesterbank zich eenmaal heeft ontwikkeld is herkolonisatie door de inheemse zacht-substraatsoorten niet meer mogelijk, behalve in open plekken die in de meeste oesterbanken voorkomen (Figuur 8.15). Omdat Japanse oesters niet op exact dezelfde plaatsen zitten als de inheemse endobenthische (ingegraven) tweekleppigen zullen deze niet geheel weggeconcurreerd worden. In de Oosterschelde doet zich echter de bijzondere situatie voor dat de platen langzaam lager worden. Als gevolg van de Deltawerken (de stormvloedkering en het afkoppelen van de Oosterschelde van Markiezaat en Krammer-Volkerak) vindt geen opbouw van platen meer plaats, alleen nog maar erosie tijdens stormen. Sediment van de platen verdwijnt daarbij in de geulen. Dit wordt 'zandhonger' genoemd. De verlaging van de platen resulteert in een groter oppervlak geschikt leefgebied voor de oester, ten koste van leefgebied voor met name de kokkel. Concurrentie om ruimte tussen Japanse oesters en kokkels zal dan een grotere rol gaan spelen. Echter, het totale oppervlak dat in de getijdenzone van de Oosterschelde is bezet door oesterbanken bedraagt slechts 6-7% van het totale oppervlak aan intergetijdengebied. In het algemeen geldt voor estuaria dat het totale oppervlak bezet door banken van tweekleppigen altijd relatief klein is en zal blijven. Dat komt door verschillende beperkingen die door het milieu worden opgelegd, zoals lokale getijdenstromingen en menging van het water (noodzakelijk voor verversing van het voedselaanbod) en op grotere schaal de primaire productie (de voedselproductie, die grenzen stelt aan het totale bestand aan schelpdieren dat in een systeem kan leven).

Voor mosselen *M. edulis* is de situatie anders omdat dit een soort is die bovenop het sediment leeft, net als de Japanse oester. Uitbreiding van het areaal aan oesterbanken levert een toename op van geschikt substraat voor vestiging door allerlei hard-substraat soorten, waaronder de mossel. Naast een geschikt oppervlak voor vestiging bieden de complexe structuren van oesterbanken beschutting tegen predatie en milieu-extremen zoals uitdroging en golfwerking, voor de oesters zelf maar ook voor andere soorten die in de oesterbank leven. In de Oosterschelde heeft het zich uitbreidende oppervlak aan oesterbanken gezorgd voor een bescheiden terugkeer van de mossel naar de intergetijdenzone. Natuurlijke mosselbanken kwamen namelijk al

decennia niet meer voor in de Oosterschelde, en commerciële mosselen werden alleen nog op percelen onder de laagwaterlijn gelegd. Mosselen en oesters blijken prima naast elkaar te kunnen leven in gemengde banken in zowel de Duitse als Nederlandse Waddenzee, en ook de Zeeuwse Delta. In de Oosterschelde werden nabij Sint Annaland mosselen in een oesterbank gevonden, met dichtheden tot 1200 mosselen per vierkante meter (Hoofdstuk 7, Box 7.5). Wel nam de conditie van de mosselen in de oesterbank af met een toenemende oesterdichtheid.

Ruimte is blijkbaar niet een beperkende factor voor de inheemse schelpdieren en Japanse oester. Concurrentie om ruimte zal daarom niet leiden tot ingrijpende nadelige effecten op inheemse schelpdieren.



Figuur 8.15. Een oesterbank (bij Neeltje Jans) van bovenaf gezien (foto door Johan van de Koppel). Klompen van aaneengegroeide oesters zijn te zien, met daartussen open plekken. Links in de foto is te zien dat de oesterklompen worden omringd door zeesla. De persoon rechtsonder en diens voetstappen geven een idee van de schaal. De foto werd in juli 2005 genomen, vanaf een hoogte van 50 meter met een camera hangend aan een ballon (een 'blimp', zie www.blimppics.com; de witte lijn is het touw waarmee de ballon wordt vastgehouden).

Concurrentie om voedsel

Tweekleppige schelpdieren eten vooral ééncellige algen, en daarnaast ook allerlei ander voedzaam materiaal dat rondzweeft in het water, zoals bijvoorbeeld kleine resten van zeewieren en klein dierlijk plankton. Zolang de deeltjes maar groot genoeg zijn om door de kieuwen vastgehouden ('gezeefd') te kunnen worden, klein genoeg zijn om ingezogen te worden, en niet kunnen ontsnappen, kunnen ze dienen als voedsel voor filtrerende schelpdieren.

Hoe schelpdieren voedsel opnemen

De schelpdieren kunnen niet kiezen welke deeltjes ze wel en welke ze niet willen vasthouden. Selectie vindt pas later plaats. De ingevangen deeltjes worden over de kieuwen, middels trilhaartjes, naar de mond verplaatst (Figuur 8.8). Vlak voor de mond aangekomen vindt een selectie plaats door de mondpalpen. Deeltjes met voldoende voedingswaarde (organisch gehalte) worden zoveel mogelijk ingeslikt, maar deeltjes die niet voldoen worden uitgescheiden, verpakt in slijm. Deze uitgescheiden pakketjes worden 'pseudofaeces' genoemd. De ene soort selecteert efficiënter dan de andere. In de maag vindt een volgende selectie plaats. Deeltjes die niet goed verteerbaar zijn worden sneller door de darmen afgevoerd en uitgescheiden in de faeces.

Overige deeltjes worden verteerd. Eerst in de maag en verder in de darmen. De vertering van organisch materiaal wordt hier 'absorptie' genoemd. Ook hierin is de ene soort efficiënter dan de andere. Uiteindelijk worden de niet-verteerbare componenten ook uitgescheiden in de faeces.

Verschillen tussen soorten

Verschillende soorten tweekleppigen laten verschillende efficiënties zien in de selectie en absorptie van organische deeltjes. Ze kunnen echter ook verschillen in de mate waarin ze het voedselaanbod in het water om hen heen verversen (middels stromingspatronen en de structuur van de bank), in de hoeveelheid water die ze kunnen filtreren per uur, hoe ze herfiltratie beperken (het opnieuw filtreren van reeds leeg-gefiltreerd water) en in het invangen van dierlijk plankton. Al deze verschillen worden hieronder besproken.

Hydrodynamica

Uit de resultaten van Hoofdstuk 2 is gebleken dat verschillen in stromingspatronen voor voedselopname tussen Japanse oesters, mosselen en kokkels waarschijnlijk geen verschil maken in de concurrentie om voedsel. Hoewel dit nog niet is onderzocht, is het aannemelijk dat verschillen in ruwheid tussen oesterbanken, mosselbanken en kokkelbanken wél consequenties hebben voor voedselconcurrentie. Oesterklompen in oesterbanken zijn enkele decimeters hoog. Mosselbanken zijn tegenwoordig enkele centimeters hoog. Kokkels leven ingegraven in het zand. Een oesterbank geeft dus veel meer extra ruwheid aan de bodem dan een mosselbank, die weer meer ruwheid geeft dan een kokkelbank. Ruwheid veroorzaakt turbulentie in het water dat over de bodem stroomt. Turbulentie zorgt voor menging van het water en verversing van het voedselaanbod in leeg-gefiltreerde waterlagen vanuit hoger gelegen waterlagen. Hoe meer turbulentie, hoe sterker de verversing en hoe meer voedsel er beschikbaar is voor de schelpdieren. Dit is aangetoond voor andere soorten schelpdieren dan Japanse oesters. Er is echter nog nooit een vergelijking gemaakt tussen Japanse oesters en inheemse Nederlandse schelpdieren. Theoretisch gezien lijken oesters een voordeel te hebben vanwege de grote ruwheid van de banken. Turbulentie kan nog een ander voordeel hebben. Hoe turbulenter het water, hoe moeilijker dierlijk plankton de waterstromingen van de schelpdieren op kan merken. Er is dan veel achtergrondruis. Een grotere turbulentie kan ertoe leiden dat meer dierlijk plankton gefiltreerd en gegeten kan worden.

Voedselkeuze

Studies in de Oosterschelde en de Franse baaien van Mont Saint Michel en Veys hebben aangetoond dat filtrerende schelpdieren, waaronder de Japanse oester, niet exact hetzelfde voedsel eten. Ze zijn dus niet per se concurrenten voor hetzelfde voedsel. Daarnaast leek de Japanse oester zich beter aan te kunnen passen aan het lokale voedselaanbod (dat sterk kan wisselen onder invloed van o.a. het getij) dan de mossel. Verder werken de kieuwen van de Japanse oester iets anders dan die van inheemse tweekleppigen. De inheemse soorten kunnen alle deeltjes groter dan 4 micrometer vasthouden. Bij oesters ligt deze grens iets hoger: 4-6 micrometer. Daarbij, als het gehalte aan zwevende stof in het water boven een bepaalde grens komt, verandert deze grens bij de Japanse oester tot 12 micrometer. Als er bijvoorbeeld veel kleine slibdeeltjes in het water zitten zorgt deze eigenschap ervoor dat de kieuwen van de Japanse oester niet verstopt raken, maar ook dat alle ééncellige

algen kleiner dan 12 micrometer niet meer gegeten kunnen worden. Tenslotte werd in de vorige alinea al uitgelegd dat Japanse oesters mogelijk meer dierlijk plankton kunnen vangen dan inheemse schelpdieren.

Japanse oesters en inheemse schelpdieren eten dus niet exact hetzelfde voedsel en zouden daarom mogelijk niet elkaars concurrent voor voedsel zijn. Echter, zoals al eerder is uitgelegd, kunnen filtrerende schelpdieren niet kiezen welke deeltjes ze filtreren. Selectie geschiedt pas later, als de deeltjes al uit het water zijn verwijderd en dus niet meer als voedsel voor andere soorten kunnen dienen. In wat ze filtreren uit het water overlappen Japanse oesters voor een groot deel met inheemse schelpdieren, afgezien van bijvoorbeeld sommige soorten dierlijk plankton of hele kleine deeltjes ten tijde van een hoog slibgehalte. Daarom concurreren ze wel degelijk om voedsel, hoewel het waarschijnlijk toepasselijker zou zijn om van 'interferentie' te spreken.

Fysiologie

Filtrerende schelpdieren kunnen de efficiëntie van selectie en absorptie (vertering) aanpassen aan het gehalte aan zwevende stof in het water en aan het organische gehalte van de zwevende stof. Soorten met een relatief snelle voedselinname en efficiënte stofwisseling (selectie, absorptie) zullen relatief veel energie ter beschikking hebben voor groei en voortplanting. Deze soorten hebben een concurrentievoordeel ten opzichte van minder efficiënte soorten. De Japanse oester blijkt een minder efficiënte absorptie te hebben dan de mossel, waardoor de oester netto minder energie haalt uit het gefiltreerde voedsel. Hierin lijkt de mossel dus een sterkere concurrent te zijn.

Groei

Een concurrentievoordeel zou zich moeten uiten in een relatief hogere groei bij een laag voedselaanbod. Hoewel op basis van verschillen in fysiologie verwacht zou worden dat de mossel een sterkere concurrent is dan de Japanse oester, wordt dit toch tegengesproken door studies in de Duitse Waddenzee en in de Oosterschelde. In de Duitse Waddenzee werd onder oesters en mosselen die op dezelfde plaatsen leefden een dichtheidsafhankelijke groei gevonden bij mosselen maar niet bij oesters. Dit wijst erop dat de groei van mosselen was beperkt door voedselconcurrentie, maar groei van oesters niet. Hetzelfde werd gevonden in de Oosterschelde. Hier werd de groeisnelheid bepaald aan oesters en mosselen in kooitjes, die bij verschillende dichtheden van oesters in een oesterbank waren geplaatst. De mosselen lieten een

afnemende groeisnelheid zien bij een toenemende oesterdichtheid, maar de oesters groeiden overal even snel (Hoofdstuk 7, Box 7.4). Mogelijk compenseren een hogere filtratiesnelheid en een groter vermogen om zich aan te passen aan veranderingen in het lokale voedselaanbod de lagere absorptie-efficiëntie van de oester in vergelijking tot de mossel ruimschoots.

Draagkracht van de Oosterschelde

Concurrentie om voedsel kan alleen leiden tot het lokaal uitsterven van minder sterke concurrenten als het voedsel op raakt. Dit is nog lang niet aan de orde in de Waddenzee maar wel in de Oosterschelde. Indicaties daarvoor zijn ruimschoots aanwezig. Zo vond er een verschuiving plaats naar kleinere algensoorten, wat een algemeen effect is van een hoge 'graas'druk (filtratiedruk). Ook nam de primaire productie (de aanmaak van nieuwe algen) af waardoor het voedselaanbod voor schelpdieren werd verlaagd. Rijkswaterstaat heeft in 2003 geschat dat er in de noordelijke tak en de kom van de Oosterschelde al bijna evenveel algen werden gefiltreerd als dat er dagelijks werden geproduceerd. Daarnaast zijn gekweekte mosselen magerder geworden sinds 2001 (gegevens van de mosselveiling te Yerseke) en verdubbelde de tijd die oesters nodig hebben om te groeien tot consumptieformaat (pers. meded. A. Cornelisse). Hieruit kan nog steeds niet goed afgeleid worden welke van de twee soorten een sterkere concurrent is, omdat mosselen en oesters in verschillende compartimenten van de Oosterschelde worden gekweekt en hun groei dus niet direct vergelijkbaar is. Oesters worden gekweekt in de kom van de Oosterschelde (oostelijk van Yerseke) waar rond 1900 ook al draagkrachtproblemen waren met de inheemse platte oester (*O. edulis*). Mosselen worden vooral gekweekt in het centrale en westelijke deel, waar meer voedsel beschikbaar is.

Larvifagie

Vanwege hun grote filtratiecapaciteit werd aanvankelijk aangenomen dat Japanse oesters veel larven van andere soorten opeten. Inderdaad laten de resultaten van Hoofdstuk 3 zien dat Japanse oesters in hoge mate larven van de mossel eten. Echter, ze bleken ook hun eigen larven te eten, en mosselen en kokkels bleken evengoed larven van de mossel en de Japanse oester te filtreren. Er werd geen enkel verschil gevonden in de mate waarin Japanse oesters en inheemse schelpdieren larven filterden. Wel verschillend was de mate waarin Japanse oesterlarven en mossellarven werden gefiltreerd: Japanse oesterlarven de helft minder dan mossellarven. Dit is niet

te verklaren door een grotere vaardigheid van oesterlarven om waterstromen van de schelpdieren te herkennen of betere ontsnappingsreacties daarop (Hoofdstuk 4), maar waarschijnlijk wel doordat oesterlarven volwassen schelpdieren chemisch kunnen herkennen ('ruiken') en vervolgens naar boven zwemmen (Hoofdstuk 5). De laboratoriumresultaten van de Hoofdstukken 3, 4 en 5 werden bevestigd door veldonderzoek (Hoofdstuk 6). Mossellarven werden in grote aantallen gefiltreerd door een oesterbank bij de ingang van het Slaak in de noordelijke tak. Voor oesterlarven kon dit niet goed aangetoond worden, waarschijnlijk doordat de oesters in de oesterbank zich in dezelfde periode hebben voortgeplant. Verder werd gevonden dat larvenaantallen van de oester een afname vertoonden in de periode 1994-2006, parallel aan een toename van het totale bestand aan filtrerende schelpdieren in de Oosterschelde. De afname in larvenaantallen zou direct door larvifagie veroorzaakt kunnen zijn, mogelijk in combinatie met negatieve effecten van voedselgebrek op de produktie en overleving van larven. Middels modelberekeningen werd geschat dat door larvifagie momenteel zo'n 95% van schelpdierlarven de dood vindt.

Of een dergelijke hoge sterfte vervolgens vertaald wordt in een meetbaar afnemende aanwas van de schelpdierbestanden is niet geheel zeker. Aanwas wordt ook wel rekrutering genoemd. Hieronder wordt verstaan: het aantal jongen dat na de broedval (Figuur 8.3) de geslachtsrijpe leeftijd bereikt. Van het aantal larven dat geproduceerd wordt overleeft vaak minder dan 1% tot rekruut. Over het algemeen wordt aangenomen dat het broedvalsucces van de Japanse oester wordt bepaald door weersomstandigheden tijdens de broedval en niet zozeer door het aantal larven. Bij een onderzoek in de Duitse Waddenzee werd echter wél een relatie aangetoond tussen rekrutering en larvenaantallen. Samen met de modelmatig geschatte sterfte van 95% door larvifagie geeft dit aan dat de aanwas van Japanse oesters (en andere filtrerende tweekleppigen) wel degelijk beperkt kan worden door larvifagie. Waarschijnlijk bepaalt een samenspel tussen weersomstandigheden, voedselaanbod en sterfte (voor een groot deel door larvifagie) het broedvalsucces en de mate van aanwas.

De resultaten van gecontroleerde laboratoriumexperimenten beschreven in de Hoofdstukken 3, 4 en 5 laten zien dat oesterlarven in staat zijn volwassen schelpdieren op te merken en opwaarts weg te zwemmen om zo larvifagie te vermijden. Er valt niet met zekerheid te zeggen of de oesterlarven buiten in de Oosterschelde in dezelfde mate kunnen ontsnappen omdat daar allemaal complicerende factoren zijn (zoals stroming en turbulentie). Maar als we aannemen dat ook in het veld oesterlarven de helft minder worden gefiltreerd dan mossellarven, dan zou de sterfte door larvifagie 17% lager zijn voor oesterlarven dan voor mossellarven. Voor mossellarven kon

echter nog geen verband aangetoond worden met de toenemende filtratiedruk, mogelijk omdat er een tijdserie van slechts 6 jaar beschikbaar was (Hoofdstuk 6).

Conclusies

Concurrentie om ruimte met de Japanse oester is geen grote bedreiging voor inheemse schelpdieren. Door de uitbreiding van oesterbanken is de mossel zelfs geherintroduceerd in de getijdenzone van de Oosterschelde. Toch kan niet uitgesloten worden dat inheemse schelpdieren zoals de mossel mogelijk verdwijnen uit de Oosterschelde. Door het sterk toegenomen bestand aan filtrerende tweekleppigen, voornamelijk als gevolg van de uitbreiding van de Japanse oester, lijkt de draagkracht voor filtreerders inmiddels bereikt. De kokkel lijkt beter aangepast aan een laag voedselaanbod dan de mossel en oester (gebaseerd op literatuuronderzoek), en zal daarom naar verwachting niet uit het systeem verdwijnen. De mossel lijkt minder goed aangepast, en natuurlijke bestanden kunnen daardoor mogelijk verdwijnen. Aanwas van schelpdieren zal in de Oosterschelde mogelijk afnemen met een toenemend bestand aan filtrerende tweekleppigen, door een combinatie van larvifagie en voedselgebrek. Larvifagie zal echter niet leiden tot het lokaal verdwijnen van soorten. In de Waddenzee wordt vooralsnog het lokaal verdwijnen van soorten als gevolg van invasie door de Japanse oester zeer onwaarschijnlijk geacht. Omdat de draagkracht van de Waddenzee nog niet is bereikt zullen larvifagie en voedselconcurrentie inheemse schelpdieren (en de Japanse oester zelf) alleen zeer lokaal beïnvloeden.

Dankwoord

Mijn eerste woord van dank gaat uit naar Pauline Kamermans. Zij is gedurende de hele periode mijn grote steun en toeverlaat geweest. Pauline, dankjewel voor je hulp, inzet en begrip. Wim Wolff bedank ik voor een zeer adequate begeleiding op afstand en voor de prettige omgang. Hoewel we niet heel veel contact hadden heb ik mij altijd door jou gesteund gevoeld. Eize Stamhuis ben ik zeer erkentelijk voor diens hulp bij de experimenten in Haren en voor diens onuitputtelijke enthousiasme en originele ideeën.

Ik was gezegend met een groot aantal begeleiders. Naast Wim, Pauline en Eize waren dat Peter Herman en Aad Smaal die ik bedank voor het kritisch lezen van sommige hoofdstukken. Alle begeleiders tesamen, inclusief mijn 'externe' begeleider Carlo Heip, wil ik bedanken voor hun positieve inbreng tijdens de gezamenlijke evaluatiebijeenkomsten. Luca van Duren heb ik gevraagd om hulp bij het tweede hoofdstuk en ik kreeg meer dan waar ik op hoopte (getuige haar co-auteurschap). Ontzettend bedankt, Luca!

Ik heb met veel plezier gewerkt bij het RIVO-CSO, later IMARES, in Yerseke. Ik wil iedereen daar bedanken voor de fijne werksfeer, voor de leuke uitjes samen, de gezellige barbeques en het samen zwemmen in de Oosterschelde. Ik wil in het bijzonder bedanken: Emiel en Ad voor de ondersteuning (en geklier) in het lab, Marnix mijn kamergenootje voor het fijne gezelschap, Ainhoa voor vriendschap en veel hulp met de larven, Divera voor GIS-hulp, Jack voor allerlei praktische hulp, Jaap voor alle mooie verhalen over het Zeeland van vroeger, Jeanet voor de warmte en hulp met allerlei zaken en Jeroen Wijsman en Joke voor hulp met oester-, mossel- en kokkeldata. Cees, Cees, Cecile en Suze uit IJmuiden wil ik verder nog bedanken voor hulp en ondersteuning en Norbert en André van Texel voor het uitje naar de oesterbank bij de Cocksdorp.

Alle studenten die ik heb begeleid bedank ik voor hun hulp en gezelschap. Ik vond het erg leuk om jullie (Edzard, Ingmar, Wouter, Rick, Anne, Peter-Paul, Ronald, Jobine en ook Willemijn en Arno) iets te leren en heb daar zelf ook heel veel van opgestoken. Paul en Laura wil ik verder nog bedanken voor hulp met de ADCP in het veld. Tjeerd Bouma bedank ik voor hulp en het uitlenen van de ADCP. Els Sandée wordt bedankt voor hulp en vrolijke babbeltjes bij mijn korte verblijf in de *Kêête*. Mijn mede-*Kêête*-bewoners bedank ik voor hun 'multiculti' gezelschap en de gezamenlijke dineetjes.

Hoewel ik het grootste deel van de tijd in Yerseke heb doorgebracht voelde ik me in Haren altijd helemaal thuis bij de vakgroep Mariene Biologie. Ik wil dan ook alle collega's daar bedanken, met name Gezien en Joukje en mijn mede-AiO's en OiO's.

In het bijzonder bedank ik Deniz Haydar die ongeveer tegelijkertijd begon met haar promotie-onderzoek. Deniz, dankjewel voor je vriendschap, steun en gekeet!

De pannenkoeken-eters bedank ik voor hun vreetzame gezelschap in het *Anker*. Met name Johan van de Koppel en Sandra van de Wiele met wie het allemaal begon.

Delta Project Management en het RIKZ en Dienst Zeeland van Rijkswaterstaat bedank ik voor de fijne werkplek tijdens het afronden van mijn promotie-onderzoek. Ik kon hier part-time werken, daarbij veel beleids-ervaring opdoen en daarnaast mijn proefschrift afmaken. Door mijn werk voor Rijkswaterstaat Zeeland werd in de laatste fase van het schrijven mijn blik op de Zeeuwse Delta enorm verbreed. Gesprekken met Aylin, Dick, Peter en Eric hebben hier een hoop aan bijgedragen.

Verder heb ik veel te danken aan de bemanningen van verscheidene schepen van het ministerie van LNV, de *Schollevaar*, de *Valk*, de *Kokhaan*, de *Krukel* en de *Harder*. Bedankt voor gezellige tijden aan boord, voor het kokkels spitten, mosselen verzamelen, informatie, koffie en een neutje. Ik ben verder verschillende mensen van de schelpdiersector dankbaar voor hulp, medewerking en het ter beschikking stellen van oesters voor onderzoek. Ik richt met name een woord van dank aan Aard Cornelisse, KJ van IJsseldijk, Prins en Dingemanse en de gebroeders Praet. Stichting Het Zeeuwse Landschap en Vereniging Natuurmonumenten wil ik bedanken voor hun toestemming om onderzoek uit te voeren in hun natuurgebieden.

The following people also meant a lot to me and I want to thank them for a multitude of things: Joana Cardoso, Susanne (*oyster girl*) Diederich, Karsten Reise, Andreas Schmidt, Philipp May, Francis Kerckhof, Arnon Uppabullung, Mark Lenz, the PhD students at Sylt, and Marianne Alunno-Bruscia (and family!).

Tot slot een woord van dank aan mijn familie. Ik bedank vooral mijn ouders voor onophoudende steun en interesse. Ook mijn schoonfamilie wil ik bedanken en met name Ernst en Marja die ontzettend veel interesse hebben getoond in mijn onderzoek en het hele proces. Met mijn grootste schatten wil ik eindigen: Daniël die me heel veel heeft geholpen in late uurtjes in veld en lab en voor wie het niet altijd gemakkelijk was om te leven met een bezeten promovenda, en ex-kater en ontspanningstherapeut Muizemeur die zo goed kon luisteren (althans dat verbeeldde ik me dan toch).

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