Blue carbon by marine bivalves

Perspective of Carbon sequestration by cultured and wild bivalve stocks in the Dutch coastal areas

Author(s): Henrice Jansen & Lisanne van den Bogaart

Wageningen University &
Research report C116/20

WAGENINGEN
UNIVERSITY & RESEARCH

Blue carbon by marine bivalves

Perspective of Carbon sequestration by cultured and wild bivalve stocks in the Dutch coastal areas

Author(s): Henrice Jansen & Lisanne van den Bogaart

Wageningen Marine Research
Yerseke, December 2020

Wageningen Marine Research report C116/20

WAGENINGEN
UNIVERSITY & RESEARCH

Keywords: carbon capture, shellfish, climate, sustainability, circularity

This report can be downloaded for free from https://doi.org/10.18174/537188
Wageningen Marine Research provides no printed copies of reports

Wageningen Marine Research is ISO 9001:2015 certified.

Photo cover: H.M. Jansen

Wageningen Marine Research, an institute within the legal entity Stichting Wageningen Research (a foundation under Dutch private law) represented by Dr. ir. J.T. Dijkman, Managing director

KvK nr. 09098104,
WMR BTW nr. NL 8113.83.696.B16.
Code BIC/SWIFT address: RABONL2U
IBAN code: NL 73 RABO 0373599285

Contents

Extended summary

Climate ambitions and circularity

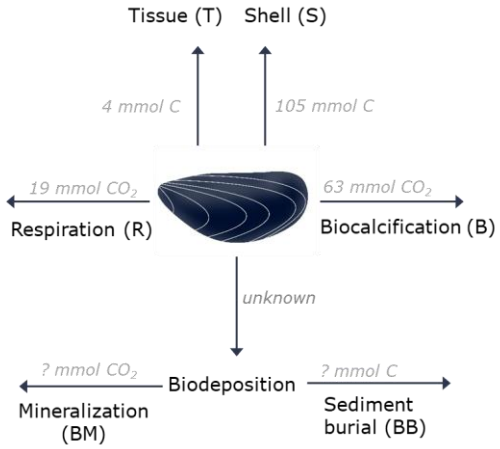
The ocean plays a vital role in the Earth's carbon cycle, and it sequesters a significant part of carbon emitted to the atmosphere by today's fossil fuel burning. However, the uptake of anthropogenic CO_2 by the oceans modifies water chemistry (ocean acidification). Ambitious targets to reduce CO_2 emission and sequester carbon have been outlined in response to climate change. These ambitions highlight the need for a circular approach to enhance long term terrestrial and marine carbon sinks together with the need to increase the use of carbon storage in bio-based products. Marine CO_2-removal techniques, such as production and harvest of bivalves, are suggested. The aim of this study is to provide perspective on 'blue carbon' through carbon sequestration potential (CSP) in shell material of wild and cultured bivalves (shellfish) in the Netherlands.

Bivalves in the Netherlands

Bivalves such as mussels, oysters and clams occur naturally in the Dutch waters (Wadden Sea, Oosterschelde, North Sea). Both total stock as well as the relative contribution of each species to the entire stock is highly variable between years. Fisheries of wild stocks include mussel seed, cockle, *Spisula* and razor clam. Besides wild stocks, mussels and oysters are also cultivated in the Eastern Scheldt and Wadden Sea. The most common technique used in mussel cultivation in the Netherlands is bottom cultivation. Mussel seed is traditionally transferred from wild mussel seed banks to cultivation plots, but more recently a development towards mussel seed collectors has taken place. On average 50 000 tonnes of mussels are harvested per year. Cultivation of oysters (European flat oyster and Pacific oyster) only occurs in the Delta region. The total amount of cultivated oysters is estimated at 3 000 tonnes per year.

Blue carbon – Carbon sequestration in bivalves

Bivalves store carbon in tissue and shell material, and unlike any other animal the structural biomass of the shell is often larger than the meat product itself. Tissue and shells may each have different food, feed or industrial uses. Examples of products include shellfish meat (potential to substitute other protein sources), nature conservation (reefs, carbon storage when building up) and bio-based products (e.g. construction, cement, isolation, bioplastic filler, etc.). The contribution of bivalves in carbon sequestration is debated in literature, and different approaches are used to quantify carbon capture (see table and figure). Some argue that harvesting shells from the marine system is an option for long term carbon sequestration as it locks away carbon in solid mineral form (approach 1). Others argue that CO_2 released through respiration and biocalcification (approach 2) and (pseudo)faeces production and mineralization (approach 3) should also be included. Additionally it has been suggested to split metabolic processes between shell and tissue production and thereby only allocate 10% to shell formation (approach 2b, 3b).



Carbon sequestration by the blue mussel industry

Based on the suggested approaches and scales (individual, population and entire industry) we have estimated the carbon sequestration potential through shells for the largest shellfish aquaculture sector in The Netherlands, the mussel industry (see table below). Depending on which method is chosen, carbon sequestration by mussel shells result in a positive value (max ~1000 g C per individual per production cycle) or in a net release (minimum -500 g C). This equals to respectively 4000 or -7600 tonnes C y^{-1} when these numbers are extrapolated to the entire Dutch mussel sector. This indicates that mussel shells can be regarded both a net sink or a source of carbon, depending which biological processes are included in the budget calculations. The lack of consensus on the approach and scale

applied to estimate carbon budgets of shell material demonstrates why the relevance of CO_2 sequestration in shellfish is still controversial. Important here is to realize that shells are a co-product of shellfish production (food), and partitioning of carbon fluxes between shell/tissue is therefore essential (as proposed in approach 2b, 3b). Carbon budgets of shell and tissue can however only be evaluated separately if both products are fully utilized. If this is not the case, all fluxes have to be allocated to the main product, in this case food production. Furthermore, irrespective of partitioning methods, accurate estimates of carbon dynamics in shellfish production are required, and the mass balance approaches applied in this study all have limitations. We therefore recommend to develop ecosystem models that are explicit in time and space, but more importantly integrate carbon kinetics at individual and ecosystem level. Interactions with phytoplankton populations and benthic-pelagic coupling can significantly alter the CO_2 cycle, but are neglected in current estimates. We thus argue that an ecosystem approach accounting for the trophic interactions of bivalves is needed to provide a rigorous assessment of the role of bivalves as a potential CO_2 sink, especially in situations with high density aquaculture production of bivalves when carrying capacity issues become relevant.

Table: Carbon sequestration potential (CSP) for the blue mussel estimated by means of different approaches

Approach	Individual mussel (g C per production cycle)	Population on cultivation plot (kg C ha^{-2} y^{-1})		Dutch mussel industry (tonnes C y^{-1})	European mussel industry (tonnes C y^{-1})
		*Entire population**	*Harvested population*		
1a. Mass balance of shell material	1030	630	630	4284	50400
1b. Mass balance of shell material, correcting for biocalcification		-202	251	1714	
2a. Mass balance of individuals, correcting for CO_2 fluxes (Biocalcification & Respiration)	-541	-1115	-330	-2246	
2b. Same to 2a with partitioning between shell/tissue (only 10% respiration allocated to shell)	317	-293	194	1321	
3. Mass balance of individuals, including all metabolic processes	Unknown due to data unavailability				

* *Entire population includes individuals that die of at a certain stage during the production cycle*

Carbon storage in wild bivalve stocks

Carbon sequestration in wild stocks is essentially different from aquaculture production as most wild stocks are not harvested. Fisheries of shellfish in the Netherlands is of minor importance in terms of volumes compared to aquaculture production. The shell to tissue ratio indicates how much biomass is stored in shell material relative to the total weight, this varies between 50% and 85% depending on for example the species. Only looking at the carbon stored in shell and tissue material, and neglecting the metabolic processes, shows that on average at least 100 thousand tonnes carbon is locked away in shellfish (tissue+ shell) that live in the Dutch marine coastal zones. Due to the erratic spawning events that characterize shellfish, the stock sizes varies significantly from year to year.

Perspective on Blue carbon

Carbon sequestration in shells locks away carbon which is unique for animal protein production, and it is therefore often compared to primary producers such as seaweed (marine) or forest (terrestrial) production. Just taking account for shell material, and not tissue (approach 2b), shows that carbon capture is somewhat in the lower range but still in the same order of magnitude compared forestry. Translating to theoretical monetary values (CO_2 equivalents) indicates that carbon sequestration in shells only resembles a fraction of annual market revenues. As shells are now considered to be a waste product, the development of biobased products might add another (commercial) value to shellfish production, which at the same time may substitute fossil sources, e.g. in cement applications, adding to climate ambitions.

1 Introduction

1.1 Circularity and climate smart solutions in food production and bio-based economy

Global demand for food and bio-based products grows at a fast pace, and have led to the vision that natural resource use and emissions associated with modern systems can and should be substantially reduced by shifting towards a circular food system. To move towards a circular and climate-neutral society, implies minimizing the input of finite resources, encourage the use of regenerative ones, prevent leakages of natural resources (e.g. carbon), and stimulate the reuse and recycling of resources in a way that adds highest value (de Boer and van Ittersum 2018).

Furthermore, ambitious targets to reduce CO_2 emission and sequester carbon have been outlined in response to climate change (Williamson 2016). In order to fulfil the aim to sequester carbon and comply with the circularity ambitions, there is a need to enhance long term terrestrial and marine carbon sinks together with the need to increase use of bioenergy and carbon capture and storage (BECCS) of bio-based products. Nowadays many products are used in short cycles, such as food, fuel or paper. In this manner, they do not contribute to long term carbon sequestration, nor do they substitute other materials that cause high GHG emissions (like steel, aluminium, concrete) or require non-renewable resources (limestone for cement industry). Climate smart solutions are thus necessary to transform the current food and bio-based production systems to more circular and climate-neutral systems.

Circular and Climate-neutral is one of the KB-programmes (Knowledge Base) to develop new knowledge within WUR. Under this program the subtheme 'Negative GHG emissions and long-time sequestration through development of new C-based products (KB1-3D-1)' is developed. At large the objectives of this subtheme are to (i) provide insight into the potential of different biomass flows and biobased products to capture and store carbon through application and substitution of other materials, and (ii) develop approaches and methodologies to assess this potential, cost effectiveness and impacts on natural capital of different biomass flows, for different terrestrial and marine case studies. The current report provides perspectives on the case study of carbon sequestration by wild and cultured bivalves (shellfish) in the Netherlands. At a later stage of the project this will be used to evaluate the climate-robustness of today's bivalve production as well as for future scenario's against other bio-based terrestrial products such as wood and fibres.

1.2 Carbon sequestration

Over the past decades a whole set of CO_2-removal techniques have been proposed for terrestrial and marine systems (Williamson 2016). Yet, a critical evaluation whether they could work at the scale needed is essential for further development.

Terrestrial carbon sequestration

A key example for active carbon sequestration in terrestrial production is Climate Smart Forestry (Yousefpour, Augustynczik et al. 2018). A common way to evaluate carbon sequestration in forests and wood based products is to map the carbon storage during production (in trees, soil, and leaves) and subsequently the 'decay or half-life time of wood-based products' (e.g. Masera et al., 2003). In the context of circularity and carbon sequestration ambitions, the aim is to stimulate carbon fixation, for example by species selection or forest management, and to develop bio-based products of high quality with long life times. At present, carbon sequestration evaluation and management has been further evolved for terrestrial production compared to marine (production) systems.

Marine carbon sequestration (Blue Carbon)
One of the proposed techniques to sequester carbon is to enhance ocean productivity through increased marine photosynthesis and CO_2 drawdown from the atmosphere (Williamson 2016). Initially the discussions focussed on enhancing primary production (phytoplankton) through ocean fertilization, but that idea has been abandoned due to reverse effects on ecosystem functioning and doubts on the actual longer term carbon capture potential (Lampitt, Achterberg et al. 2008, Strong, Chisholm et al. 2009, Williamson, Wallace et al. 2012). More recently, other ocean-based CO_2-removal techniques, such as through the production and harvest of seaweeds or shellfish (bivalves) have been proposed (Tang, Zhang et al. 2011, Sondak, Ang et al. 2017). The latter is the focus of the current report.

Bivalves store carbon in tissue and shell material, which each may have different food, feed or industrial uses. Examples of products include shellfish tissue (potential to substitute or addition for other protein sources), nature conservation (reefs, carbon storage when building up), shells may have different end-users (e.g. construction, cement = substitution lime stone, isolation, bioplastic filler). There is an increasing interest in the development processing steps to refine shells, which could further add to the (circular) bio-economy of bivalve production.

Additional to the fixation of carbon, bivalves exert several functions (ecosystem services) in the ecosystem (Smaal, Ferreira et al. 2019), which should also be considered when evaluating the potential for developing Climate Smart/Robust Bivalve Culture. It is also highlighted by Williamson (2016) that a crucial component of the feasibility for carbon sequestration techniques is the non-climatic impacts that large-scale CO_2-removal could have on ecosystems and biodiversity (natural capital). It goes beyond the scope of this report to review insights and gaps in our understanding of the influence of CO_2-removal techniques on the marine ecology, but where relevant we will include ecosystem responses (e.g. primary production) that have direct interaction with the carbon dynamics.

1.3 Aim of this study

The objective of the current report is to provide a perspective on the carbon sequestration potential by bivalve aquaculture and fisheries for marine coastal zones in the Netherlands. It also highlights the discussion on the approaches used to quantify carbon sequestration in marine bivalves.

The current report is centred around the following case studies:

1) Case study I. mussel aquaculture: with the aim to quantify C-fixation dynamics for mussel aquaculture at the scale of one cultivation plot, and at the scale of the entire industry
2) Case study II. wild bivalve stocks: with the aim to quantify the carbon stored in wild populations

The report is structured as follows: Chapter 2 provides insight in bivalve stocks in the Netherlands (culture dynamics, population sizes and annual variability of wild stocks). A general (theoretical) overview of the processes involved in carbon sequestration by marine bivalves is presented in Chapter 3. These processes are subsequently used to estimate carbon dynamics in mussel aquaculture (Chapter 4) and translated to wild stocks (Chapter 5). Finally, we provide perspective on the potential for (blue) carbon capture by marine bivalves and discuss the possibilities for Climate Smart Bivalve aquaculture. As the current approaches include coarse mass balance approaches, we also provide recommendations on how to integrate carbon kinetics in dynamic modelling in order to provide more accurate estimates in time and space for current and future production areas/scenario's.

2 Marine bivalve populations in the Netherlands

Marine bivalves are molluscs whose bodies are enclosed by a shell consisting of two hinged valves, such as mussels and oysters (Figure 1). About 8 000 species of marine bivalves exist, including brackish water and estuarine species. They can roughly be divided in infaunal species, that live in the sediment, and epifaunal or epibenthic species that live on top of the sediment. Bivalves, such as mussels, filter food called seston from the water that consists of micro-algae and other small organic particles. They may occur in high densities accumulated in patches or beds. Epibenthic bivalves such as mussels and oysters can form hard, reef-like structures that provide a range of ecosystem services (van der Schatte Olivier, Jones et al. 2018). For example, the bivalves may act as eco-engineers creating a barrier against wave-action, provide habitat for other species and regulate the water quality through filtration. Bivalves such as mussels, oysters and clams occur naturally as wild stock in the Dutch coastal zones. Mussels (blue mussel) and oysters (European flat oyster and Pacific oyster) are also cultivated in the Eastern Scheldt and Wadden Sea. With an estimated production of over 60 000 tonnes in shellfish yearly, bivalve aquaculture is the largest aquaculture sector in the Netherlands (FAO 2015).

Figure 1 Mytilus edulis (left) and Crassostrea gigas (right). From Bos, 2016 (https://images.wur.nl/digital/collection/coll18/id/1404/rec/15) and Bos, 2014 (https://images.wur.nl/digital/collection/coll18/id/53/rec/4).

2.1 Aquaculture

Mussel cultivation

The most common technique used in mussel (*M. edulis*) cultivation in the Netherlands is bottom cultivation. Traditionally mussel seed is transferred from wild mussel seed bed to cultivation plots on shallow mud flats, where the seed remains and grows until it reaches consumption size (Kamermans and Smaal 2002). Over the past decade a development towards mussel seed collectors has taken place to reduce the impacts of mussel seed fishing on benthic systems and to ensure a steady supply of seed (Kamermans and Capelle 2019). Seed mussel collectors (SMC) consist of substrate in the form of nets

or ropes attached to a framework that is kept afloat by buoys. Mussel larvae settle on the substrate in spring and early summer and are harvested late summer. After harvesting the mussels are immediately dispersed onto cultivation plots. Capelle and Van Stralen (2017) estimated that approx. 25% more mussels are present in the Wadden Sea in a situation when mussel aquaculture takes place, compared to a situation without mussel cultivation activities. In the Delta region the mussel cultivation sector almost entirely relies on the introduction of mussel seed from external stocks, that either originated from seed beds in the Wadden Sea or from SMC in the Delta region, since natural spat fall (formation of mussel seed beds) is limited if not absent in the Delta region.

The production cycle of mussels is approximately two to three years. During this period the mussels are placed on bottom cultivation plots where they attach themselves to the bottom or each other using 'byssus' threads. Throughout the cultivation cycle they are transferred to other plots two or three times, either to grow bigger mussels by reducing densities and/or to place them in sheltered areas in winter or in areas typically used for grow-out. Other activities during production include predation control such as removal of star fish. Mussel cultivation can be characterized as an extractive type of aquaculture in which no additional food nor medicines are added to the cultivation plots. Instead, the cultivated mussels completely rely on local environmental conditions such as food availability and temperature for growth. The annual harvest of mussels mainly takes place during summer and autumn when the mussels are fished using nets. On average 50 000 tonnes of mussels are harvested per season (FAO 2015). However yields fluctuate yearly depending on local environmental conditions such as food availability, weather conditions, predation and disease. As an alternative to bottom cultivation mussels are grown in rope or longline culture. In this technique mussels are grown on submerged ropes attached to buoys. The mussels from longline cultures grow faster and typically have a thinner shell and require a different processing technique than bottom cultured specimen.

Oyster cultivation

Two types of oysters are cultivated in the Netherlands. The European flat oyster (*Ostrea edulis*) that occurs naturally in Dutch waters and the Pacific oyster (*Magallana gigas* (before *Crassostrea gigas)*) that was introduced in the 1960s. Pacific oysters mainly occur in the Eastern Scheldt. Due to the parasite Bonamia the flat oysters have disappeared from the Eastern Scheldt and are mainly found in Lake Grevelingen. Cultivation of oysters only occurs in the Delta region. The oysters are traditionally grown on cultivation plots in the Eastern Scheldt and Lake Grevelingen in a semi-natural way (Mol 2019). Reproduction of oysters takes place in summer. In order to catch the oyster larvae, large amounts of empty mussel shells are dispersed on the cultivation plots. These shells provide substrate for the oyster larvae to settle on. After one year the small oysters are collected and transported to a different plot. During this process the mussel shell breaks off, allowing more space for the oysters to grow. Over the next two years the oysters are transported to different plots. During this process the thin growth edges of the oysters break off which benefits the growth of a more desirable deeper-shaped cup. Regular maintenance of the oyster plots may reduce loss due to predation by the oyster driller, a predatory snail that eats the tissue of the oyster after drilling a hole in the oyster shell. When the oysters are at least three years old they reach consumption size. The oysters are harvested using nets and are sorted by size using a shaking device that allows the smaller oysters to be placed back on the plots, while the larger oysters are placed in a basin to clear the sand from the oyster gut. After a week or two they are ready for consumption. Since 2001 the yearly production of Pacific oysters in the Netherlands has fluctuated between 20 and 35 million individuals. The flat oyster production steadily increased from 1 million individuals in 2001 to 7.5 million in 2017 (Mol 2019). The total amount of cultivated oysters is estimated at 3 000 tonnes per year.

Additionally, both European flat and Pacific oyster larvae are cultivated in hatcheries under controlled conditions. When the oyster brood is several millimetres long it is placed in nurseries until the oysters reach the required size (1 to 3 cm). The oyster seed is then placed on cultivation plots for grow-out or, alternatively, in oyster baskets. Off-bottom cultivation is an alternative cultivation method where oysters are placed on tables in baskets or trays. The off-bottom cultivation method prevents loss from predation by the oyster driller that is mainly found on the sediment. Besides oyster drillers the oyster sector has been heavily impacted by the herpesvirus that mainly affects the Pacific oyster. The European flat oyster is not affected by the herpesvirus but suffers from a small parasite called Bonamia.

2.2 Wild stocks

Population dynamics

All wild shellfish stocks are monitored on an annual basis by the WOT programs (Wettelijke Onderzoeks Taken; Figure 2). These programs have initially (1990) been set-up to monitor commercially important species (mussels, oysters, cockles) and since 2004 all stocks are investigated. For most species both abundance (number of individuals) as well as total biomass in investigated. Some species that live deeper in the sediment, are however, less well sampled and often the animals brought to deck are damaged or only pieces of one individual are collected. From these species no biomass estimates are available. This is e.g. the case for *Ensis* and otter shell (*Lutraria lutraria*) in the coastal zone; these two species contributed for 13% to the total number of bivalves in 2018 (Perdon, Troost et al. 2019).

Figure 2 Overview of sampling stations included in the WOT surveys (Wadden Sea, Coastal Zone, Eastern Scheldt, Western Scheldt)

Though all species are monitored (and included in the database) they are not all reported on. Annual stock assessments are only estimated for the commercially relevant species (Figure 3), while stock assessments of the additional species are only available (by literature) for some sub-areas in case they have been analysed in other studies (Figure 4):

- Wadden Sea: for the intertidal zone of the entire basin data is available for mussel, oyster and cockle beds (Figure 3). For sub-areas Marsdiep and Vliestroom data has been analysed for all species, both for the intertidal (sandflat) and subtidal (permanently submerged) zones for the period 1992-2016, where information of mussels on culture plots are only available from 2004 (see Jansen et al., 2019; Figure 4).
- Eastern Scheldt: Annual stock estimates for natural mussel, oyster and cockle beds in the intertidal zone (Figure 3). Annual estimates for all species, including cultured mussels and oysters, have been reported on for the period 1992-2016 in Jansen et al. (2019) (Figure 4).
- Coastal zone: Annual stock estimates are only available for the commercially relevant species (*Spisula*, *Ensis*, otter shell, striped venus clam, banded wedge-shell, Figure 3).

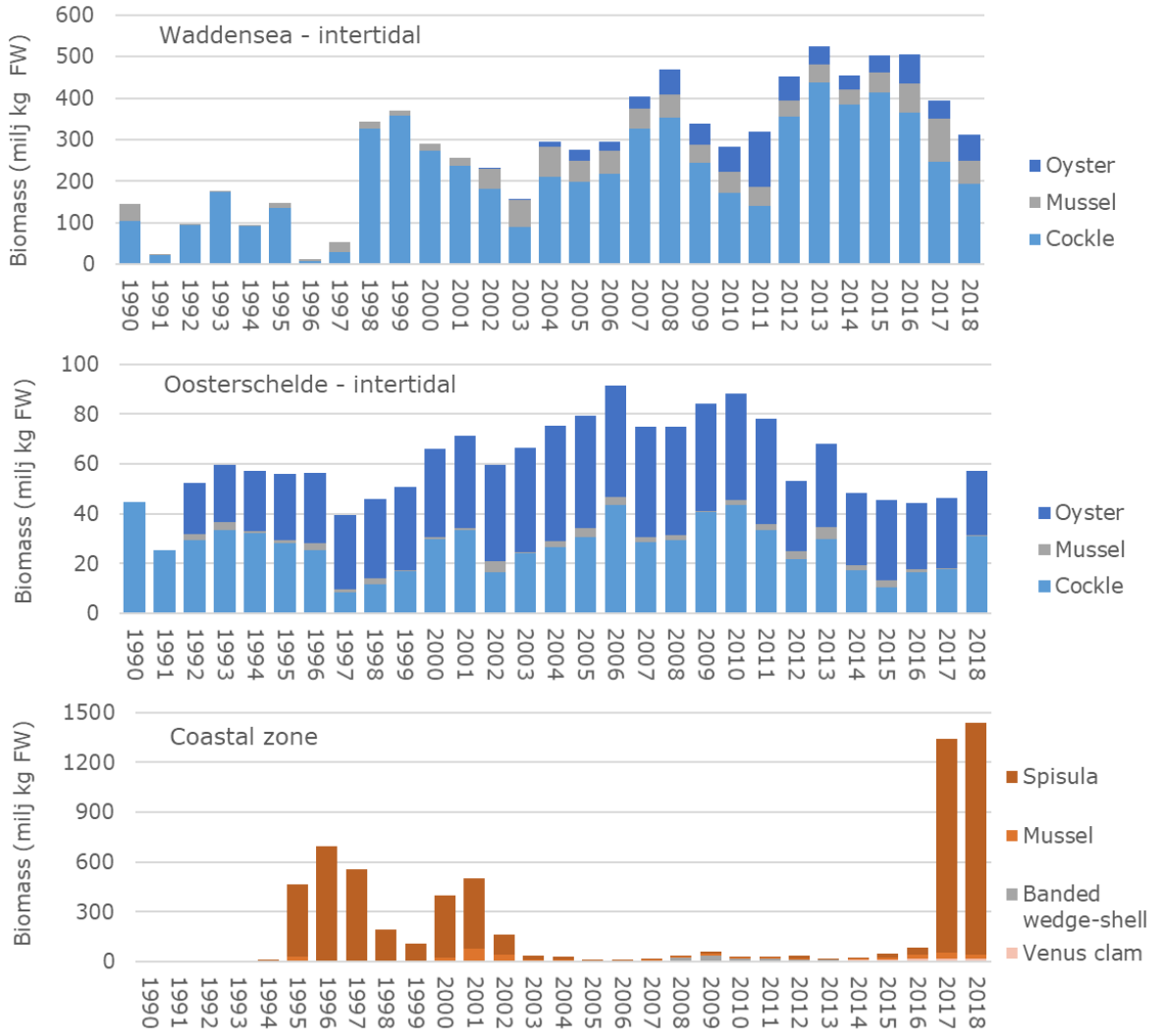


Figure 3 Annual stock estimate of commercial relevant species in the Eastern Scheldt, the entire Wadden Sea, and the Coastal zone (Troost et al., 2019, Perdon et al. 2019). For the Coastal zone biomass of Ensis and otter shell is not included as only numbers (and not biomass estimates) are available.

Time series indicate a large variability between years, both in terms of total stock as well as the relative contribution of each species to the entire stock (Figure 3, Figure 4). Shellfish are generally characterized by recruitment success which only occurs once every so many years (van der Meer, Dankers et al. 2019). The stock size of cockles in the Wadden Sea is largely driven by a few distinctive recruitment successes that occur every 5-8 years, while in the Eastern Scheldt cockle recruitment is low but relatively stable.

The relative abundance of each species varies by area: in Eastern Scheldt cultured mussels dominate the bivalve stock, in the Wadden Sea mussels (wild), *Mya arenaria* and cockles are most abundant, while in the coastal zone *Ensis* and more recently *Spisula* dominate.

The Marsdiep and Vliestroom are the only areas in the Wadden Sea where mussel culture is situated. So 100% of the cultured stock is presented in Figure 4. But also the cockles dominate the total bivalve population in these areas. Out of the entire cockle stock situated in the intertidal zone of the Wadden Sea (Figure 3), approximately 34% is situated in these two sub-areas. Cockles are species that occur predominantly on tidal flats, and are less abundant in the subtidal areas.

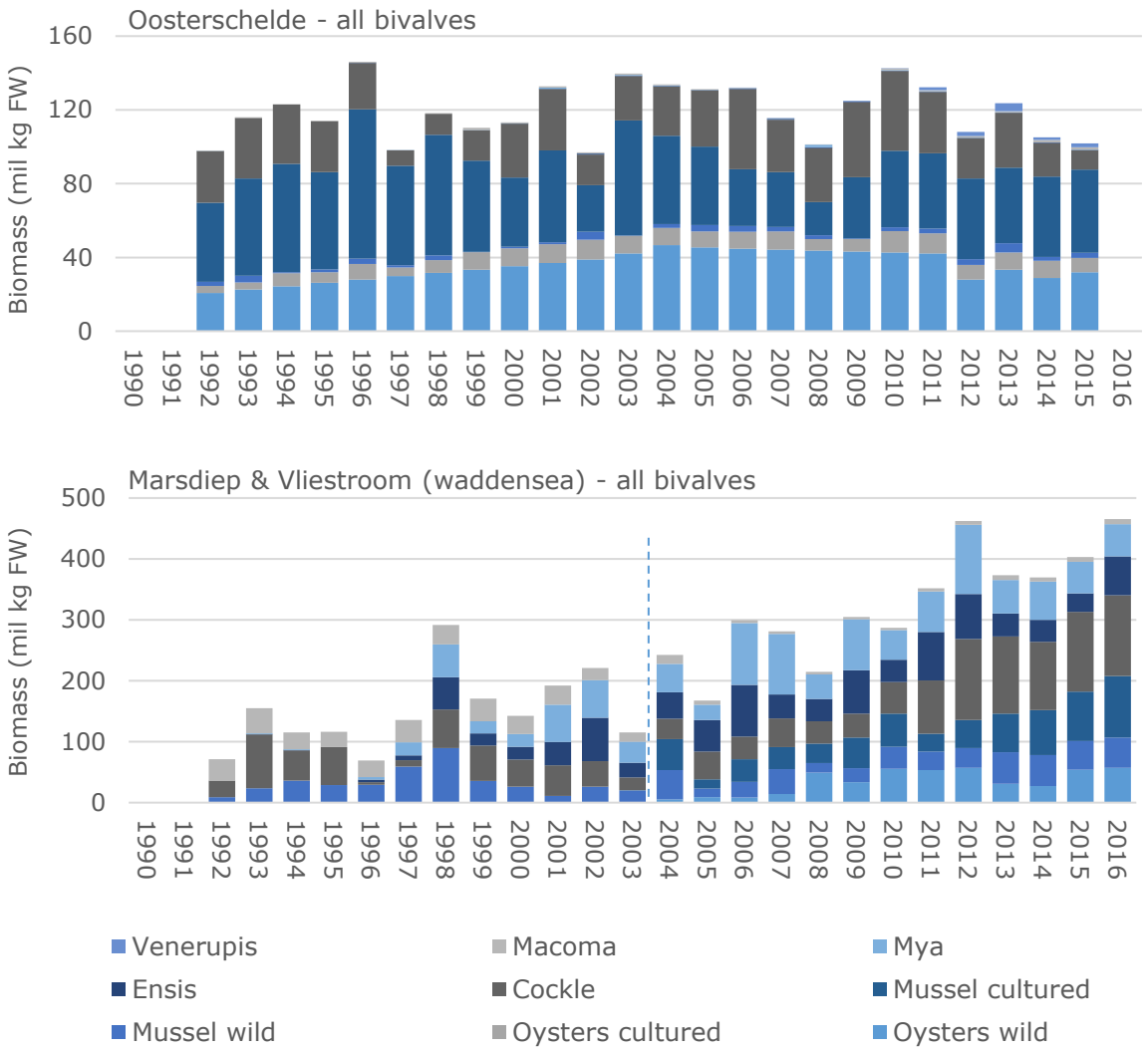


Figure 4 Population dynamics of all bivalve stocks (cultured and natural) in the Eastern Scheldt, Wadden Sea (only Vliestroom and Marsdiep area). In the Wadden Sea stock estimates from the cultured plots are available since 2004. Data from Jansen et al. (2019).

Fisheries

Fisheries of marine bivalve wild stock in the Netherlands include mussel seed (*Mytilus edulis*), cockles (*Cerastoderma edule*), *Spisula* (*Spisula subtruncata*) and razor clam (*Ensis spp.*). Mussel seed is mainly fished on wild mussel banks in the Wadden Sea and they are then relocated on bottom plots for further grow out (see 2.1). A dredge is used to collect the mussel seed which clump together by means of the seeds' byssus threads. The total mussel seed catch in the Wadden Sea varies per year (fluctuates between 140 000 to 1140 000 tonnes total wet weight including shell over the period 1992-2017) and was 958 000 tonnes in 2017 (Troost, Perdon et al. 2017).

Cockles are fished in the Wadden Sea, Western Scheldt, Eastern Scheldt and Voordelta (Van Asch, van den Ende et al. 2014). In the Wadden Sea cockles may only be fished by hand using a net attached to a rake. In the Delta region, cockles may be fished by hand or mechanically by means of a suction device. Only when stock assessments reach densities of 50 cockles per m^2 2.5% of the stock assessment of cockles can be fished. Over the last 20 years the manual catch of cockles in the Wadden Sea has fluctuated between approx. 10 and 1 300 tonnes of cockle tissue, with very low catch numbers around 2001-2003 and highest around 2013-2015 (Agonus 2017). Due to low stock cockles have not been fished in the Delta region since 2006 (Van Asch, van den Ende et al. 2014).

Spisula stock assessments have recently increased in Dutch coastal zones (WMR data) and for the first time in 20 years *Spisula* or surf clams can be fished again in these areas. *Spisula* are fished using an adapted fish trawl equipped with suction devices that are connected to water pumps. Because *Spisula* is buried several centimetres deep in the sediment, a layer of sediment containing *Spisula* is pumped into the fish trawl and then sucked onto the deck. On deck the shells are sorted by size using a sorting device or sieve. Small shells and other organisms are discarded.

The *Ensis* or razor clam fisheries consist of small scale fishery activities (4 vessels) in the Dutch coastal zone. To catch razor clams an adapted fish trawl is used equipped with suction devices and airlifts that are connected to water or air pumps. The quota for *Ensis* is 8 000 tonnes fresh weight. In 2012 the total catch of *Ensis* was 3 385 tonnes fresh weight (Agonus 2013).

3 Carbon kinetics in marine bivalves

This chapter aims to describe the inorganic and organic carbon fluxes involved in bivalve growth for both cultured and wild stocks. At individual level the following processes are important in the carbon cycling: (i) formation of the shell through deposition of calcium carbonate. During this process carbon is fixed in the shell, but at the same time CO_2 is released (paragraph 3.1), (ii) CO_2 is released through respiration (catabolism of ingested material) and organic C is stored in tissue material (paragraph 3.2). These processes depend on environmental conditions, and may vary between wild and cultured stocks (paragraph 3.3). Based on these processes we will outline different approaches used to calculate the carbon sequestration potential (CSP, paragraph 3.4). In Chapter 4 we will apply these approaches to calculate the potential contribution of the Dutch shellfish sectors (fisheries and aquaculture) to carbon capture.

3.1 Shell formation

Calcification

The shell is an exoskeleton that offers protection against predators and adverse environmental conditions. The shell is formed by the deposition of calcium carbonate ($CaCO_3$). Calcification can be described by the formula (Mistri and Munari 2012):

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + CO_2 + H_2O \qquad \text{(formula 3.1)}$$

Calcification tends to drive CO_2 from the ocean to the atmosphere, although HCO_3^- is consumed during the process (Suzuki 1998). It is likely that these forms will reach an equilibrium again, shifting from one form to the other. It is however unknown how this exactly works out at ecosystem scale as this process also depends on environmental conditions such as pH, temperature, salinity and alkalinity (Dickson 2010, Mackenzie and Andersson 2013). Approach 1 (Table 1) only considers the sequestration of carbon in shell material through the formation of calcium carbonate ($CaCO_3$), while approaches 2 and 3 also consider the release of CO_2 during this process (i.e. biocalcification).

Carbonate system

The release of CO_2 during calcification induces a shift in the seawater carbonate system (Filgueira, Byron et al. 2015):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \qquad \text{(formula 3.2)}$$

In solution, the carbon system comprises four different species: dissolved carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) (Gattuso, Pichon et al. 1995). If the carbonate system is open to the atmosphere, the partial pressure of CO_2 (pCO_2) in the water has to be kept equal to the atmospheric pCO_2 (Frankignoulle, Pichon et al. 1995). Oceanic uptake of CO_2 (as a result of elevated atmospheric pCO_2) leads to a decrease of CO_3^{2-}, while in the ocean this CO_3^{2-} has a buffering effect. The decrease of CO_3^{2-} is compensated by the HCO_3^- pool, and this process leads to the release of H^+ molecules (formula 3.2) (Zeebe, Zachos et al. 2008), leading to a decrease in seawater pH (acidification). For each mole of $CaCO_3$ precipitated, nearly 0,6 moles of CO_2 are liberated in buffered seawater and 1 mole in freshwater (Ware, Smith et al. 1992). This difference is due to the low buffering capacity of freshwater compared to seawater (Frankignoulle, Pichon et al. 1995).

BOX 1: Microstructure of shells

Most molluscs have developed an external calcified structure to support their tissue and to protect themselves against predators. Detailed studies on the biomineralization process of bivalves were conducted and published by Petit et al. (Petit, Davis et al. 1978, Petit, Davis et al. 1979, Petit, Davis et al. 1980, Petit, Davis et al. 1980) and Petit (1981) on fresh-water Unionidae. According to their studies, bivalve shells can be divided into three primary layers: the outer layer known as periostracum, consisting mainly of conchiolins; a middle layer designated as the prismatic layer, mainly calcitic; and the inner layer called the nacreous layer, consisting mainly of aragonite crystals (Chen, Fan et al. 2005, Yao, Xia et al. 2014).

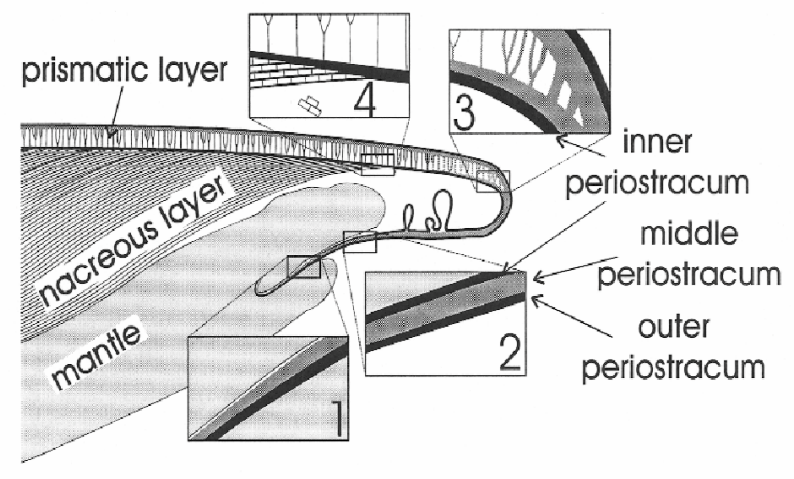


Figure 5. Petit et al.'s model for shell biomineralization in Amblema (Unionidae) (Checa et al., 2000). The periostracal groove secretes a thin outer periostracum and a thicker middle periostracum (inset 1). When this periostracum is extruded, the inner surface of the outer mantle fold secretes a third layer (the inner periostracum) below the middle periostracum (inset 2). The inner periostracum later separates from the middle periostracum and develops folds, which disappear before the whole periostracum reflects at the shell margin. During reflection, the middle periostracum becomes vacuolized and forms a cavernous structure called the antrum (inset 3). In time, antrum cavities become filled with aragonite and originate the outer prismatic layer. The inner periostracum also foliates and becomes mineralized, giving rise to the inner nacreous shell.

The periostracum is an organic protein layer protecting the shell from the outside (Yao et al., 2014). The periostracum is initiated as a pellicle by basal cells located in the periostracal groove (between the outer and middle mantle folds). The pellicle increases in thickness, forming the outer periostracum. A thicker multi-layered middle periostracum is secreted inside the outer periostracum (Figure 5, inset 1). Upon extrusion from the periostracal groove, the inner periostracum is secreted by the mantle epithelium below the middle periostracum (Figure 5, inset 2). The inner periostracum later separates from the middle periostracum and becomes highly folded and looped, but these loops disappear before reflection of the periostracum at the shell edge. During reflection of the periostracum, the middle periostracum becomes vacuolized and forms a cavernous structure (Figure 5, inset 3). The cavities become filled with calcite and originate the prismatic layer. The prismatic layer is mostly an array of parallel prisms, adjacent structural units and may be composed of either aragonite or calcite (Carter & Clark, 1985). In most marine bivalves, the prismatic layer is calcitic (Checa et al., 2005; Lutts et al., 1960), with the exception of the trigoniacean shell which is aragonitic (Weiner et al., 1976). The prismatic layer contains organic matrices consisting of chitin and different proteins (Yao et al., 2014).

The inner periostracum thickens at the shell edge and nucleation of aragonite needles begin (Figure 5, inset 4). These later pack into typical nacreous bricks. Organic proteinaceous layers can be released into the inner periostracal layer, serving as templates for crystallization of nacreous layers (Yao et al., 2014). The nacreous layer consists of interdigitated polygonal calcium carbonate tablets, each covered by an organic membrane; the interlamellar sheets (Jacob et al., 2008). The inorganic aragonite looks like "brick" and the organic biopolymer looks like "mortar" or "adhesive". The model by Bevelander and Nakahara (1969) explains formation of the nacreous layer by precipitation of $CaCO_3$ into pre-existing organic compartments and heterogeneous nucleation of $CaCO_3$ in the organic matrix. In contrast, the model of Schäffer et al. (1997) postulates nacre formation by growth through mineral bridges across the pre-existing organic interlamellar sheets. Both models agree on the assumption that the interlamellar sheets form first and that this organic matrix mediated the crystallization of aragonite or calcite (Jacob et al., 2008).

3.2 Metabolic processes

Most bivalves are filter feeders, which means they pump water over the gills and sieve out the food particles (phytoplankton and other small food items present in the water column). Food particles are ingested, and subsequently used for tissue growth or maintenance, or expelled as (pseudo)faeces. The gills not only serve for feeding, but also for respiration when O_2 is assimilated and the metabolic waste product CO_2 released. Faeces may be buried in the soil (long term storage) or partly be mineralized by bacteria and through this process CO_2 is been formed and released in the water column. Through these processes bivalves interact with both the inorganic (CO_2) and organic carbon sources. Approach 2 only considers CO_2 release through respiration (3.2.1), while approach 3 considers all processes.

Respiration

Like all organisms bivalve catabolism of ingested organic matter results in CO_2 release (=respiration) and production of water (formula 3.3). Rates at which respiration occur depend for example on temperature (as bivalves are ectotherms), species, or maturity stage.

$$CH_2O+O_2 \rightarrow CO_2+H_2O \qquad \text{(formula 3.3)}$$

Food acquisition and tissue growth

Marine bivalves are heterotrophic organisms and they need to consume, among other elements, organic carbon to sustain their growth and their metabolism. Most bivalves are filter feeders, using their gills to capture particulate food from the water, while pumping water along the gills. Food items are then transported to the labial palps, where food is funnelled to the mouth where digestion begins.

Bivalves consume different organic particles, with phytoplankton being the dominant food source. Other food sources may include small zooplankton, but also detritus and other particles (e.g. silt) present in the water column. The size of the food items that may be captured varies between species. It is generally considered that mussels and oysters feed on the same food source varying between 4-6000 µm in size (Rahman, Henderson et al. 2020) recently showed that cockles selected for both large and small food and fed more efficiently on small particles compared to both oysters and mussels.

Bivalves can filter considerable amounts of water; mussels can filter up to 5 L per hour, and oysters even up to 25 litres. Jansen et al. (2019) showed that it takes less than 12 days for all bivalve stocks in the Eastern Scheldt to filter the entire water volume of the estuary.

After passage through the gut, food items are either assimilated and used for tissue maintenance, growth or reproduction.

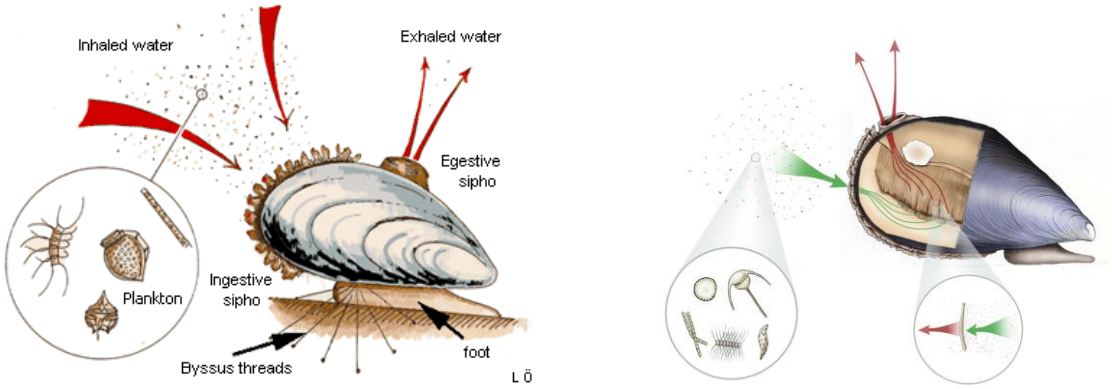


Figure 6 Overview of bivalve feeding by an example of the blue mussel (source right: https://www.kimberly-andrews.com/filter-feeding-in-a-mussel.html)

Biodeposit production and mineralization

Bivalves are special compared to other animals in the sense that they not only expel food items that are not assimilated in the intestines (faeces), but they may also expel low quality food before digestion (pseudofaeces). When food concentration is abundant, bivalves will select high quality food items for digestion, while low quality food items (such as silt) will be packed in mucus and be expelled before entering the intestines. The food concentration threshold before pseudofaeces production is initiated is generally considered around ~ 3-6 mg SPM L-1 depending on body size (SPM=Suspended Particulate Material((Widdows et al., 1979).

Faeces and pseudofaeces are collectively called biodeposits, and contain organic carbon. Biodeposit production represents a significant pathway in bivalve nutrient cycling. Carbon content in biodeposits depends on concentration and type of the food source and varies therefore between seasons and systems. Once expelled, the labile fraction of biodeposits is mineralized by bacteria. During this process CO_2 is being released. The refractory material (carbon) is buried in the sediment and can be considered as a long time store of carbon (Jansen 2012).

3.3 Differences between systems and production types

Wild versus cultured populations

The carbon sequestration potential is different for harvested versus non-harvested (wild) populations, given that harvesting represents a net extraction from the ocean (Filgueira, Strohmeier et al. 2019). The shells of wild bivalves will eventually dissolve in seawater, while those of cultured stocks will end up in land fills or specific applications (spat collection, isolation etc.).

Cultured populations are often found in higher densities, and given their ideal growing condition, growth rates of cultured populations are suggested to be higher than for wild populations. For the Wadden Sea Capelle et al. (2017) indeed showed that mussels on culture plots grew faster than wild mussels(Figure 7). Steenbergen et al. (2005) demonstrated furthermore that mussel cultured on bottom plots are different from wild mussels in terms of tissue versus shell ratios. In the Wadden Sea mussel cultured on bottom plots have generally higher tissue to shell weight ratios (Figure 8), and they concluded that (i) mussels on bottom plots have a high tissue:shell ratio due to higher tissue yields, (ii) wild mussels on tidal flats have a heavy shell and hence a low tissue:shell ratio, and (iii) wild mussels in the subtidal areas have both the thinnest shells and the least amount of tissue. The way bivalves allocate energy to shell and tissue will influence the carbon balance of each product.

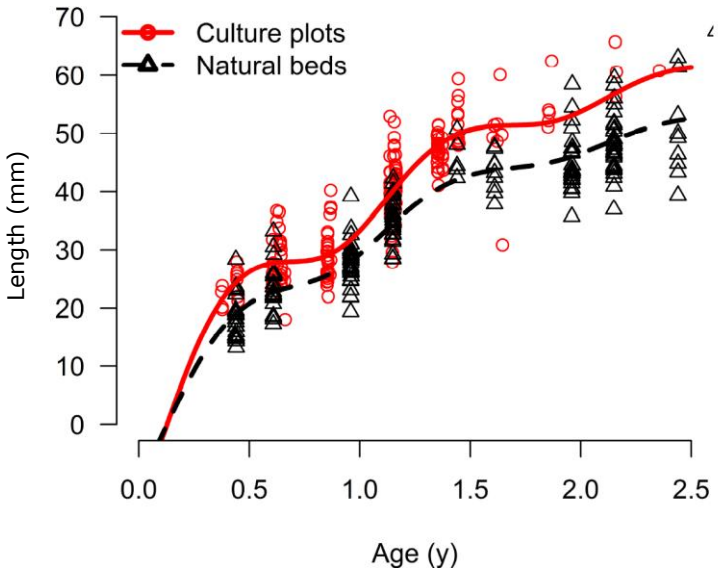


Figure 7 Growth of mussels at bottom plots (cultured) and natural beds (wild). (Source: Capelle et al., 2017)

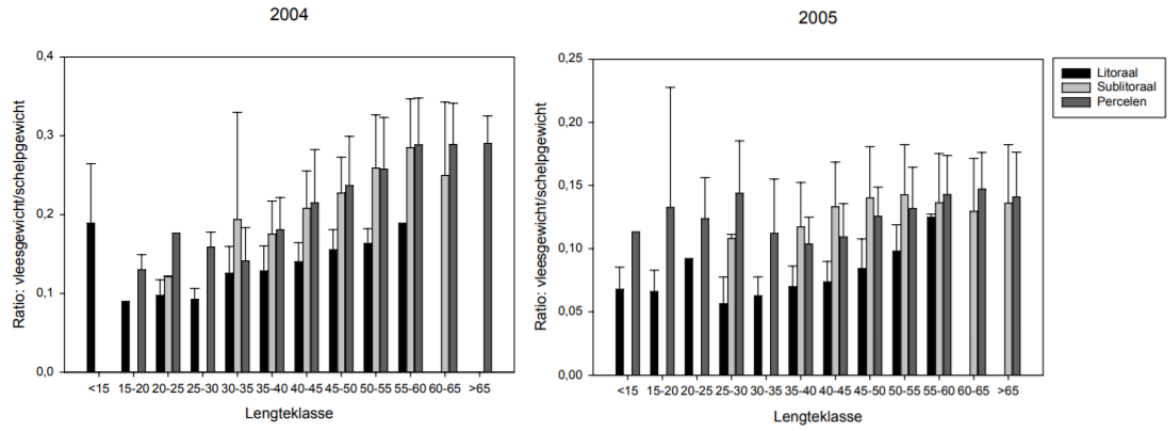


Figure 8 Tissue to shell ratio for mussels on bottom plots (Dutch: perceel), and wild mussels in the intertidal (litoraal) and subtidal (sublitoraal) in the Dutch Wadden Sea (Source: Steenbergen et al., 2005)

Another study by Rodhouse et al. (1984) compared the carbon and nitrogen budget of wild mussels growing in the intertidal shore with mussels cultured on ropes (suspended) (Figure 9). The allocation of carbon to shell material was relatively similar (8% for cultured and 11% for wild mussels). A striking difference was the conclusion that wild mussels allocate 57% of their carbon budget to gamete output, while in suspended culture, mussels allocated this was only 22%. Another important difference between the wild and cultured mussels is the time to reach the same cumulative production: for cultured mussels it took approximately 1.5 year, while for wild mussels the same production was reached after 6 to 7 years. This is considerably different from the above mentioned study by Capelle et al. (2017) who also showed different growth rates, but which were not that extreme between wild and cultured populations (Figure 9).

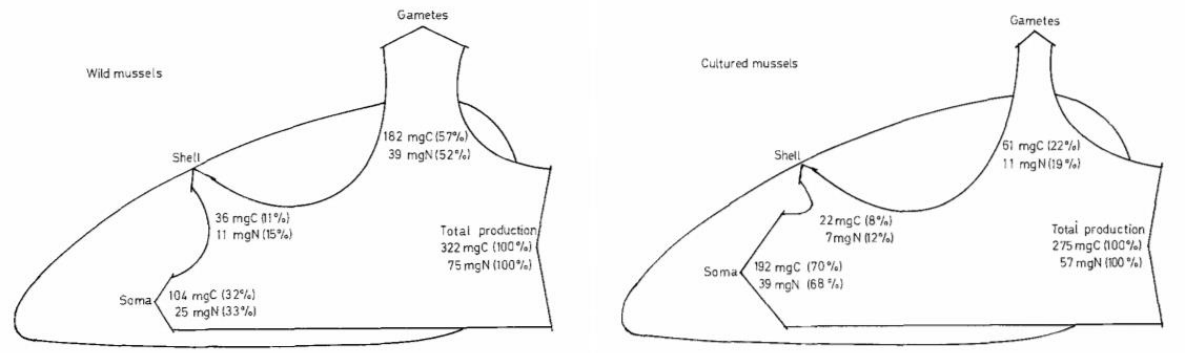


Figure 9 Carbon and nitrogen allocation in wild (intertidal shore) and cultured (suspended) mussels (Source: Rodhouse et al., 1984). Data are expressed for an average individual mussel.

Bottom versus suspended aquaculture

Mussels cultured in suspended cultures tend to have lighter shells than those in natural populations, which could be related to the feeding conditions in aquaculture environment promoting faster growth and thinner shells, but also to the reduced predation pressure. In the study by Rodhouse et al. (1984) presented above it could not be distinguished whether difference in growth and nutrient allocation were a result of wild/culture or intertidal/suspended.

It has recently been shown for mussel cultivation in the UK, that carbon, nitrogen and phosphorus stored in shell and tissue vary significantly between mussels cultured on ropes (suspended) and mussels cultured on bottom plots (pers. comm. Van der Schatte Olivier, 2019). Per kg of mussels in suspended cultures the carbon in tissue is higher, while the amount of carbon in shell is lower. Yet, when adding these values up it shows that carbon removal is relatively similar between the two types of culture.

3.4 Approaches to estimate Carbon Sequestration Potential (CSP) in bivalves

The contribution of bivalves in carbon sequestration is debated in literature, and the controversy stems from the fact that different approaches are used to quantify carbon capture. Carbon sequestration in shells by bivalves has so far been described in three different ways:
(1) As described in paragraph 3.1 bivalves are typically characterised by two shells that protect the animal (tissue) from the surrounding marine system. Shells contain on average 12% carbon and removing them from the marine system, through harvest, is often judged as an option for long term carbon sequestration, in a way similar as commonly done for terrestrial plant biomass (mass balance approach of the harvested product).
(1b) Ray et al (2018) thereby suggests that carbon sequestered in shells should be corrected for CO_2 release during shell formation (biocalcification, paragraph 3.1)
(2) Munari et al. (2013) argue that shells cannot be part of a carbon trading system as the CO_2 released through metabolic processes (paragraph 3.2) and shell formation (paragraph 3.1) is larger than the carbon sequestered in shell material.
(3) Filgueira et al. (2019) further includes biodeposition and mineralization of biodeposits to the organism level approach suggested by Muniari et al (2013).
(2b/3b) Filgueira et al. (2015, 2019) also argue that bivalves are primarily cultured with the aim of producing food, not sequestering CO_2 in their shells, therefore the main ecosystem good provided by bivalve aquaculture is tissue production, and shells should be considered as by-products of this human activity. This reasoning provides justification for dividing up respired CO_2 between tissue and shell when constructing a specific bivalve CO_2 budget for potential use of bivalve shells in the carbon trading system.

Table 1 Approaches to quantify carbon sequestration potential (CSP) by bivalves
Overview of processes considered within each approach

Approach	Processes involved	Formula
1. Mass balance of shell material	Focus on shell material only	CSP = [Shell formation]
1b. Mass balance of shells, corrected for biocalcification *(Ray et al 2018)*	Focus on shell formation, including sequestration and biocalcification (see 3.1.1)	CSP=[Shell formation]- [biogenic calcification]
2. Mass balance of individual bivalves, corrected for CO_2 fluxes *(Munari et al. 2013)*	Focus on carbon storage in shell material versus CO_2 releases through biogenic calcification (see 3.1.1) and respiration (see 3.2.1)	CSP = [Shell formation] - [biogenic calcification] - [respiration]
3. Mass balance of individual bivalves, including on all metabolic processes *Filguerira et al. (2015, 2019)*	Focus on all processes involving C (organic and inorganic). See 3.1.1, 3,2 and 3.3.	CSP = [Shell formation] + [tissue production] + [feces production] - [biogenic calcification] - [respiration] – [mineralized feces]
2b/3b. mass balance for shell and tissue separately *Filguerira et al. (2015, 2019)*	Allocate carbon flux from respiration to tissue (90%) and shell(10%) production	CSP = [Shell formation] - [biogenic calcification] - [10%respiration]

4 Case study: Carbon sequestration by mussel culture in The Netherlands

4.1 Approach

This chapter aims to describe carbon fixation by the current mussel production in the Delta and Wadden Sea. This is done at three levels: one individual specimen (section 4.2), a cultivation plot (section 4.3), and the entire industry (section 4.4). For each step the carbon sequestration potential has been estimated based on the three approaches outlined in Chapter 3.

For the individual estimates we followed the approach outlined by Filgueira et al. (2019) to estimate carbon cycling processes by individual mussels. These carbon budgets were then coupled to population dynamics for mussel populations on cultivation plots to include growth and loss terms (Capelle, Van Stralen et al. 2017). Finally the estimates for a cultivation plot are extrapolated to a tonnes of harvested mussels and multiplied by the annual harvest of the mussel industry.

4.2 Individual budget

This section provides a carbon balance model for individual mussels in the Netherlands (Figure 10), based on bottom cultivation in The Wadden Sea with an average production cycle of 2 years.

Carbon stored in mussel tissue and shell material

The average size of individual mussels after 2 years is 12.3 gram following Figure 7 (Capelle, Van Stralen et al. 2017). This is slightly lower compared to average size of mussels at the auction. While harvested the quality of mussels is defined by the tissue to shell ratio; the higher the tissue yield, the better the product. Tissue yields vary with season and location, with an average yield of harvested mussels of 30% (Jansen et al., 2019). The method to define tissue ratio might however underestimate the real content as the mussels are first boiled so it is easier to remove the tissue content. It is however unknown to what extend this might vary from fresh material, we therefore use the ratio mentioned above. Using this ratio divides the total weight into 8.6 gram shell and 3.7 gram tissue. To convert total weight (wet weight including shell, WW) to shell free dry weight (SFDW) and ash free dry weight (AFDW), and from length to Wet weight conversion factors obtained by Capelle (unpublished data[1]) were applied:

$WW=0.0002 * L^{2.7767}$

$AFDW=0.0000005*WW^{3.6058}$

$SFDW=AFDW/0.9$

These ratios may vary by area and season, and therefore also vary slightly from general conversion factors reported by Ricciardi & Bourget (1998; WW>SFDW=6.6% and WW>AFDW=4.6%).

The average carbon content in shell material of mussels is 12%, resulting in ~1030 mg carbon per individual mussel. The carbon content in shells is based on molar weight of $CaCO_3$, of which C forms 12%. We hereby neglect the organic matrix in shell, which is assumed to form a minor part of the total weight. When wet weight of the shells is determined, the shell is still wet and the matrix might contain water. It is therefore expected that 12% is an overestimate of the true carbon content in shell material (based on wet weight estimates). Following Ware et al. (1992) this indicates that during the process of shell formation 52 mmol CO_2 is formed through biocalcification (see also paragraph 3.1), which represents a carbon flux of ~620 mg C. Carbon content in tissue varies throughout the season (113-

[1] Data on mussel growth (wet weight, dry weight, ash free drwy weight and shell length) is collected during a standardized monitoring program. Set-up is described in Capelle et al 2020, background data is unpublished

623 mg C per g DW), with an average of 45% (Smaal and Vonck 1997). This results in an average of ~300 mg carbon in tissue per individual mussel.

Respiration

CO_2 release through respiration is often derived from oxygen consumption. Oxygen consumption depends on size of the animal, reproductive state, water temperature and food supply. Smaal & Vonck (1997) determined oxygen consumption rates for adult mussels in the Eastern Scheldt over 2 years and reported an average rate of 0.42 mg O_2 h^{-1} g^{-1} DW. In the same study the determined allometric scaling coefficients, these are used to correct for the fact that smaller individuals are relatively more active compared to larger individuals. Respiration rates can therefore be calculated using the following formula: $0.42*W^{0.7}$, where W indicates the weight of the animal (expressed in gram DW, excluding shell). Van Broekhoven et al. (2014) defined respiration rates specifically for mussel seed in the Eastern Scheldt: $0.315*W^{0.765}$µmol h^{-1} g^{-1} AFDW. Finally, to convert oxygen to CO_2 a Respiratory Quotient (RQ) of 0.85 is used (Hawkins and Bayne 1985).

Growth of individual mussels varies throughout the season (Figure 7; Capelle et al. 2017). To calculate the average respiration rates, the production cycle is therefore divided in four sections:

(1) the first 150 days, which is the period when mussel seed is either grown on suspended ropes, or on natural beds. Length at day 150 is 25 mm, which corresponds to 5 mg SFDW (4.5 mg AFDW)
(2) from 150 to 365 days which represented the period when they are transferred to the bottom plots (day 150) up to an age of 1 year. Mussels are transferred to the bottom plots in autumn, and in the following months low growth is realized due to low food availability and low temperatures. Length at day 365 (year=1) is 33 mm, which corresponds to 104 mg SFDW
(3) the following half year (1 to 1.5 years) represents spring and summer when almost linear growth is observed . Length at year=1.5 is 50 mm, which corresponds to 380 mg SFDW
(4) followed by the next autumn/winter (1.5-2 years) with low growth again. Length at year=2 is 53 mm, which corresponds to 825 mg SFDW.

For section 1 respiration rates published by Van Broekhoven et al. (2014) were applied, and for section 2-4 we used the respiration rates by Smaal and Vonck (1997), assuming linear growth between start and end of each section. Respiration (O_2) was converted to CO_2 release using a RQ of 0.85. This resulted in cumulative respiration in the subsequent sections of 0.02, 12, 25, and 43mmol CO_2, respectively. Adding all sections, totals to 79 mmol CO_2 (or 3493 mg CO2 , which is similar to~ ~950 mg C) released per individual during the entire production cycle of 2 years.

Biodeposit production and remineralization

There is relatively little information available on production and breakdown (mineralization) of faeces and pseudofaeces produced by shellfish. Filgueira et al. (2019) however show that biodeposits are important fluxes in carbon dynamics. The estimates provided in this section are therefore largely based on literature values reported for other cultivation areas. Estimates should therefore be considered as an order of magnitude, and values are also not included in Figure 10.

Biodeposit production estimates are unknown for mussels the Dutch waters. As food concentrations are relatively high in the Eastern Scheldt and especially in the Wadden Sea, it can be assumed that pseudofaeces is continuously produced. A review of several cultivation areas worldwide reported maximum biodeposition rates of 116 mg g^{-1} DW d^{-1} (Jansen et al., 2019). The carbon content in faeces and pseudofaeces of mussel seed in the Eastern Scheldt (52 and 55 mg g^{-1}; Van Broekhoven et al., 2014) was shown to be on the lower end of what is reported in other area's (Jansen et al., 2019). During dissolution and bacterial breakdown approximately 32% of the carbon in faeces is released as CO_2, while rates for breakdown of pseudofaeces are largely unknown (Jansen et al., 2019). These rates are based on the remineralization of labile material in the biodeposits, which takes places within weeks (Jansen, Strand et al. 2011). At longer time scales, like an entire production cycle, the total breakdown will be larger when (part of the) refractory material will also decompose.

Combining these estimates, results in approximately ~850 mg C released as result of biodeposit production and subsequent remineralization (in the form of CO_2). Assuming that the remaining biodeposits are buried in the sediment results in a sink of 1 240 mg C. But as being said this is a rough

number based on maximum biodeposition rates. How this related to actual numbers by mussels in the Dutch waters is unknown. But it does indicate this can be a significant flux, as was also addressed by Filgueira et al. (2019).

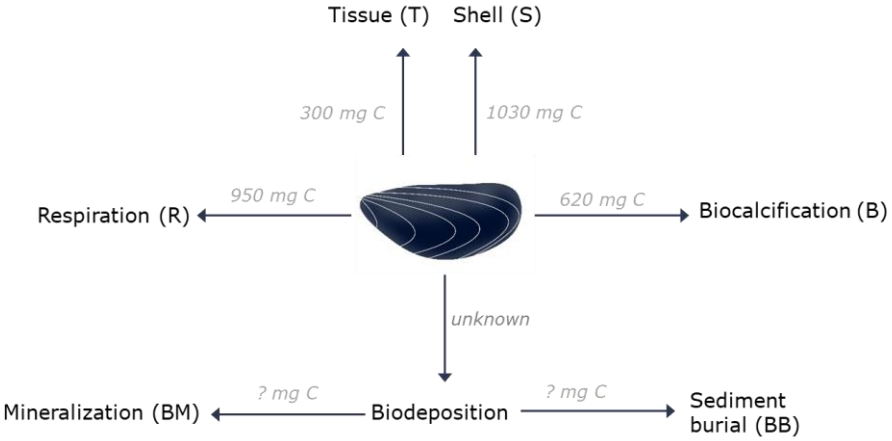


Figure 10 Overview of carbon fluxes for an individual mussel during one production cycle.

Carbon sequestration potential (CSP)

In Chapter 3 it was outlined that carbon capture potential can be estimated following different approaches (see Table 1). Applying the numbers as obtained above (Figure 10) results in a carbon capture potential varying between -540 and 1030 mg carbon per individual during one production cycle (2 years; Table 2), suggesting mussels can be a sink of carbon (approach 1 and 2b) or a source of CO_2 (approach 2).

These estimates are different from Filgueira et al. (2019) who provides a case study for carbon capture by the Norwegian mussel culture, showing that CO_2 is produced applying approach 2b. A major difference between their study and the Dutch case as outlined here is the total shell weight. Bottom cultivation results in thicker shells, and thus more carbon (pers. comm. Van der Schatte Olivier, 2019). Dry tissue weight of harvested mussels in the Dutch and the Norwegian case are comparable, while the shell weight of the Dutch mussel is ~3 times higher compared to the Norwegian mussels.

These estimates do also not account for intermediate die off of mussels during the production cycle. This will be evaluated in the next section.

Table 2 Carbon capture estimates for an individual mussel
Mass balances based on different approaches

Approach (see Table 1)	Formula	Individual mussel (mg C per production cycle)
1. Mass balance of shell material	CSP = [Shell formation]	1 030
1b. Mass balance of shell material, corrected for biocalcification	CSP=[Shell formation] - [biogenic calcification]	412
2. Mass balance of bivalves, corrected for CO_2	CSP = [Shell formation] - [biogenic calcification] - [respiration]	-541
2b. mass balance for shell separately, 10% energy allocation to shell production	CSP= [Shell formation] - [biogenic calcification] - [10% respiration]	317
3. Mass balance of bivalves, including all metabolic processes	CSP = [Shell formation] + [tissue production] + [faeces production] - [biogenic calcification] - [respiration] – [mineralized faeces]	Unknown (due to data unavailability of biodeposit production)

4.3 Scale of a cultivation plot

Biomass of mussels on a cultivation plot is a combination of mussel growth and loss factors such as mortality. Mortality is caused by (i) physical factors, e.g. transfer from seed bed to plot (seeding) and intermediate harvest or thinning/relaying to reduce density, (ii) predation by starfish, crabs and/or birds, and (iii) other natural mortality, e.g. smothering, diseases or food depletion. Capelle et al. (2017) showed the biomass development on cultivation plots and natural beds, demonstrating that farm management lead to better survival and/or growth on the plots.

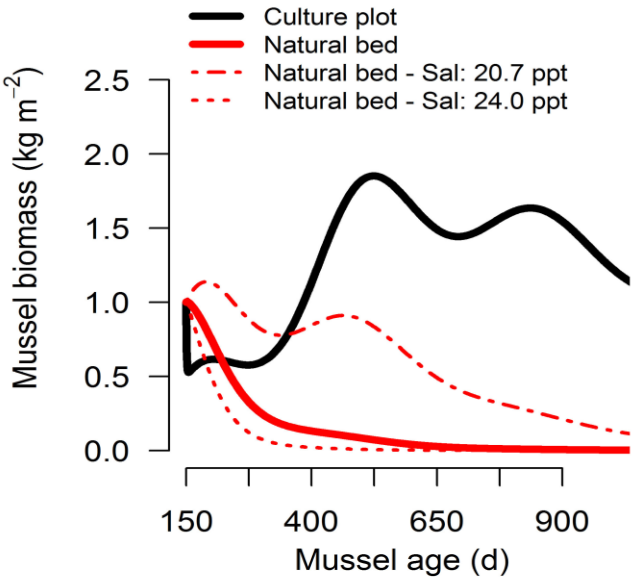


Figure 11 Simulation of mussel biomass (source: Capelle et al., 2017)

Shell and Tissue

During the end of the production cycle the density of mussel on a cultivation plot is approximately 1.5 kg m^{-2} (Figure 11). Assuming a harvest size of 53 mm (=12 gram WW) per individual (see paragraph 4.2; Figure 7) results in a density of 122 mussels m^{-2} that will be harvested. Following Figure 10 this translates in respectively ~125 and ~40 gram C capture per m^{-2} for shell and tissue material of the harvested mussel. Biocalcification (CO_2 release during shell formation) would have resulted in 75 gram C release per m^{-2} during the production of these mussels. These values should be corrected for the duration of the production cycle (2 years) to obtain estimates by year (see Table 2).

There is a significant number of mussels that die during the production cycle: initial seeding density is approximately 657 individuals per m^{-2} (1 kg m^{-2}; 25mm/1.5 gram indiv), while only 122 individuals are harvested per m^{-2}. This material (carbon source) remains in the system. The tissue material will quickly decompose, and the shell material will dissolve during a longer time span, or will be buried in the sediment.

A rough calculation demonstrates that for each kg harvested mussel, approximately 1.2 kg is lost throughout the production cycle (or 1.8 kg lost per 1.5 kg harvest from 1 m^{-2}; Table 3). Per m^{-2} this results in 150 g C in shell and 50 g C in tissue (over 2 years). Figure 11 shows biomass development from the moment mussel seed is transferred from seed beds or seed mussel collectors (SMC) to the bottom plots. This figure demonstrates that initial mortality directly after seeding is high (handling losses) and mussel biomass reduces by half in during a couple of days (Figure 11). Thereafter the mortality is more stable (Table 3).

Table 3 Population dynamics of a cultivation plot

Estimates of individual mussel growth and loss terms of a bottom plot population in relation to carbon dynamics

	150 d	1 year	1.5 year	2 year	Prod. cycle
Individual length (mm)[I]	25	33	50	53	
Individual weight (ww in gr)[II]	1.5	3.3	10.4	12.3	
Biomass[III] (in kg m^{-2})	1 (or 0.5)	0.9	1.8	1.5	
Density[IV] (in no indiv m^{-2})	657 (or 328)	273	173	122	
Loss (indiv m^{-2})	Unknown	383 (or 328+ 55)	101	50	534
Loss (kg m^{-2})	Unknown	0.88 (or 0.13+0.5)	0.63	0.57	1.8
Harvest (indiv m^{-2})					122
Harvest (kg m^{-2})					1.5
Respiration harvested mussels (g C m^{-2})	0.02	17	36	63	116
Respiration entire population, incl losses (g C m^{-2})	0.12	43	65	75	183

*[I] Figure 7; [II] conversion WW=0.0002*L^2.7767 (Capelle unpublished); [III] From Figure 11; [IV] Combining data from Figure 7 and Figure 11.*

Respiration

Development of density of mussel seed on ropes and seed beds is unknown. We here assume a loss term of 0, which underestimates the actual carbon dynamics. Seeding densities (1 kg m^{-2}; 657 individuals) are simply extrapolated to the first 150 days to gain respiration fluxes for mussel seed prior to bottom cultivation, and any mortality during the first 150 days is thus excluded. This results in an average CO_2 release of 10 mmol m^{-2}.

Respiration rates of the mussels during the cultivation on the bottom plot (from day 150 to year 2) are calculated in steps of half a year, as done above for the population estimates. Thereby we multiplied average density during a single time step (Table 3) by average individual respiration rates for a given size during same time step (§4.2). Due to the large loss term of mussels on bottom plots, it varies almost a factor 2 if respiration rates are calculated for the harvested population only, or if also the individuals are included up to the moment they disappear (Table 3; Table 4).

Table 4 Carbon fluxes of a cultivation plot

Estimated carbon capture and CO_2 release of a cultivation plot. Fluxes are split between harvested biomass and biomass lost during the production cycle (mortality).

	Harvested mussels		Mussels lost during production cycle		Total	
	Per prod. cycle	*Per year*	*Per prod. cycle*	*Per year*	*Per prod. cycle*	*Per year*
Biomass (g m^{-2})	1500	750	1800	900		
Carbon fluxes (in g C m^{-2})						
Shell sequestration (S)	126	63	151	76		
Biocalcification (B)	75	38	90	45	166	83
Tissue (T)	41	20	50	24		
Respiration (R)	116	58	66	33	183	91
Biodepsoit production	unknown					
Biodeposit remineralization (BM)	unknown					
Biodeposit sediment burial (BB)	unknown					

Carbon sequestration potential (CSP)

In the previous sections it has already been outlined that the carbon sequestration potential can be calculated in different ways (Table 1). The current paragraph also indicates it makes a large difference whether only the harvested product is considered or whether loss terms during the production cycle are included (Table 5). Filgueira et al. (2019) included a general loss term of 15% of mussel drop off. It is unclear if loss terms were included in Munari et al. (2013). Both studies were based on suspended cultures, and mortality is expected to be lower compared to bottom cultivation.

Mussels are primarily produced for food and hence the production cycle is 2 years to reach market size. Biomass development on a cultivation plot (Figure 11) quickly shows that in the context of carbon sequestration, the potential is higher when mussels are harvest earlier during the production cycle: total biomass of mussels only increases by half (from 1 kg m^{-2} seeding density to 1.5 kg m^{-2} harvested mussels) over a time span of 2 years.

Table 5 Carbon sequetration potential (CSP) estimates for a cultivation plot

Mass balances based on different approaches. $CSP_{harvest}$ refers to calculations only taking into account the harvested biomass, CSP_{entire} refers to calculations including biocalcification and respiration of the entire population (also the fraction lost throughout the production cycle: $CSP=S_{harvest}-B_{entire}-R_{entire}$)

Approach (see Table 1)	Formula	Cultivation plot (g C m^{-2} per year)
1. Mass balance of shell material	CSP= [Shell formation]	CSP=63
1b. Mass balance of shell material, corrected for biocalcification	CSP= [Shell formation] - [Biogenic calcification]	$CSP_{harvest}$= 25 CSP_{entire}=-20
2. Mass balance of bivalves, focus on CO_2	CSP= [Shell formation] - [Biogenic calcification] - [Respiration]	$CSP_{harvest}$ = -33 CSP_{entire} = -112
2b. mass balance for tissue and shell separately	10% energy allocation to shell production	$CSP_{harvest}$ =19 CSP_{entire} = -29
3. Mass balance of bivalves, focus on all carbon fluxes	CSP= [Shell formation] + [tissue production] + [feces production] - [biogenic calcification] - [respiration] – [mineralized feces]	Unknown (due to data unavailability of biodeposit production)

4.4 Scale of the entire Dutch mussel industry

Total production of mussels in the Netherlands varies from year to year, with an average of 51 million kg per year over the last five years (Figure 12).

Table 4 and Table 5 show the carbon fluxes and carbon capture potential based per square meter (m^{-2}) in order to compare to other studies (section 4.3). To upscale these values to the entire sector we first calculated the carbon capture by one tonnes of harvested product using the general conversion factors of 70% shell weight and 12% carbon content in shell material(section 4.2) , and multiplied these with the annual production to obtain an estimate of carbon capture based on shell material only (approach 1; Table 6). Subsequently carbon capture potential for the other approaches are calculated based on the relative difference between the approaches.

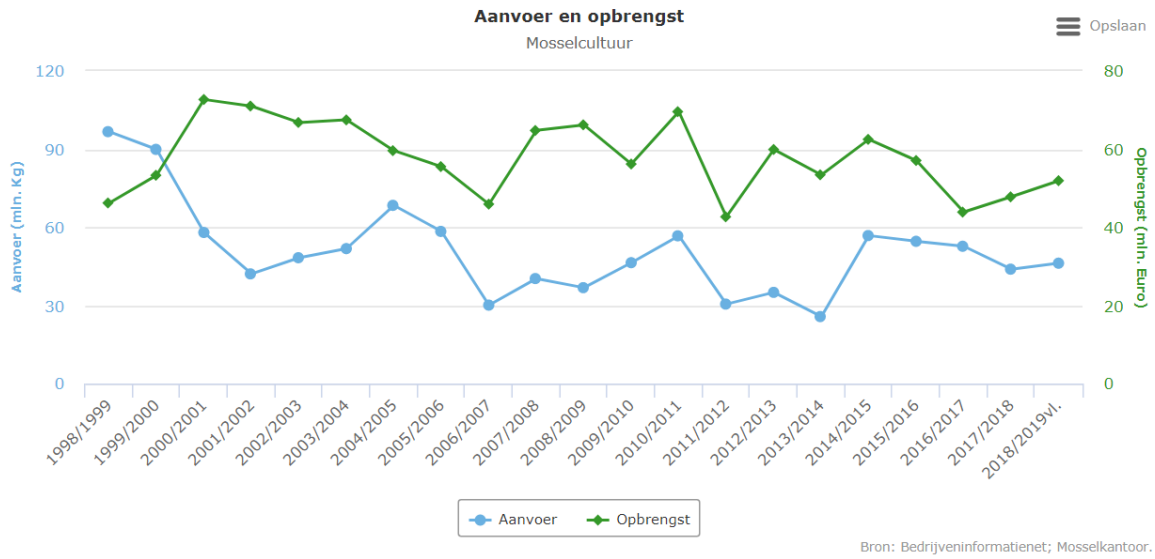


Figure 12 Annual production of mussels in the Netherlands (i.e. blue mussel). Blue line indicates the market volume and the green line represent the revenues (source: https://www.agrimatie.nl)

Upscaling to the entire sector then results in carbon capture estimates varying between 4 300 and -7 600 tonnes Carbon per year (Table 6), depending on the approach chosen. This shows again the importance of how to calculate the carbon sequestration potential.

To value the ecosystem service of mussel farming in the carbon cycle, carbon sequestration can be converted to CO_2 equivalents. Assuming 25€ per ton of sequestered CO_2[2], and a maximum of 15 689 tons of CO_2 sequestered, leads to a maximum value of ~400 thousand euro. This represents ~0.75% of the annual market revenues of the sector (Figure 12).

Table 6 Carbon sequestration potential estimates for the Dutch mussel industry
Mass balances based on different approaches

	C capture per tonnes mussels (kg C tonnes^{-1} y^{-1})	Annual C capture mussel industry (tonnes C y^{-1})	CO_2 equivalents per tonnes mussels (kg)	CO_2 equivalents for the Dutch mussel industry (kg)
1. Mass balance of shell material	84	4284	308	15689
1b. Mass balance of shell, minus biocalcification				
Harvested stock:	34	1714	123	6279
Entire stock:	-27	-1374	-99	-5034
2. Mass balance of bivalves, focus on CO_2				
Harvested stock:	-44	-2246	-161	-8231
Entire stock:	-149	-7600	-546	-27849
2b. mass balance for tissue and shell separately				
Harvested stock:	26	1321	95	4840
Entire stock:	-39	-1996	-143	-7315
3. Mass balance of bivalves, focus on all carbon fluxes	unknown			

[2] https://ember-climate.org/data/carbon-price-viewer/ : Daily EU ETS carbon market price, average 2019=25€ per ton CO_2

4.5 Estimates for the mollusc industry in Europe

Mollusc industry in Europe

In Europe, production of molluscs (excluding cephalopods) varies from year to year (Figure 13). From 2014 until 2018, the average of the total output of European mollusc production (excluding cephalopods) was 901 thousand tonnes live weight. In 2018, the total output was 970 thousand tonnes. The most important mollusc species captured and produced in Europe in 2018 were mussels (sea mussel, nei, blue mussel, Mediterranean mussel) and oysters (European flat oyster, Pacific cupped oyster, Flat and cupped oysters nei).

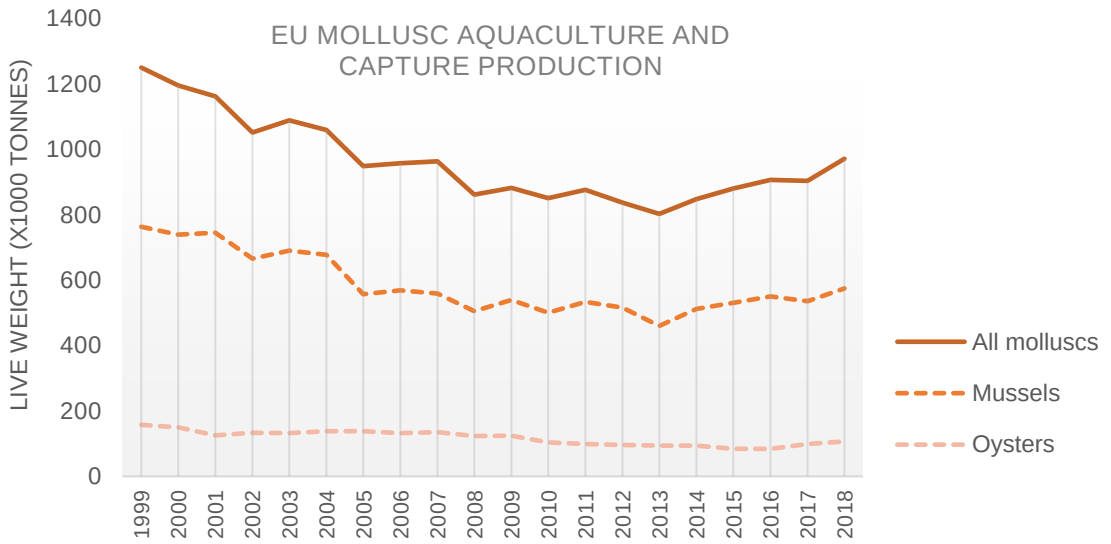


Figure 13 Annual harvest of molluscs in Europe. (source: FAO. 2020. Fishery and Aquaculture Statistics. Global production by production source 1950-2018 (FishstatJ). Criteria for selection include: Species: species by main group Mollusca excluding the cephalopods (FAOSTAT group); Country by continent: Europe; All FAO major fishing areas; Aquaculture (freshwater, marine and brackish water) and capture production). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2020. www.fao.org/fishery/statistics/software/fishstatj/en). Remark: some numbers of 2018 are estimated by the FAO from available sources of information.

The European market for mussels is estimated to be slightly below 600 thousand tonnes per year. Aquaculture is the main source of mussels and is responsible for over 90% of total landings. Three countries in Europe are responsible for two thirds of all European mussel production (Figure 14). Spain is by far the largest producer with 280 thousand tonnes in 2018, representing nearly 50% of all European production. The main producer after Spain is Italy with 63 thousand tonnes (11%), followed by France with 57 thousand tonnes (10%).

The European market for oysters is estimated to be around 100 thousand tonnes per year. France is by far the largest producer with 85 thousand tonnes in 2018, representing around 80% of all European production (Figure 14). Most of the oysters produced in France are Pacific oysters (99%). The main producer after France is Ireland with 10 thousand tonnes (10%), followed by Portugal with 3 thousand tonnes (less than 5%).

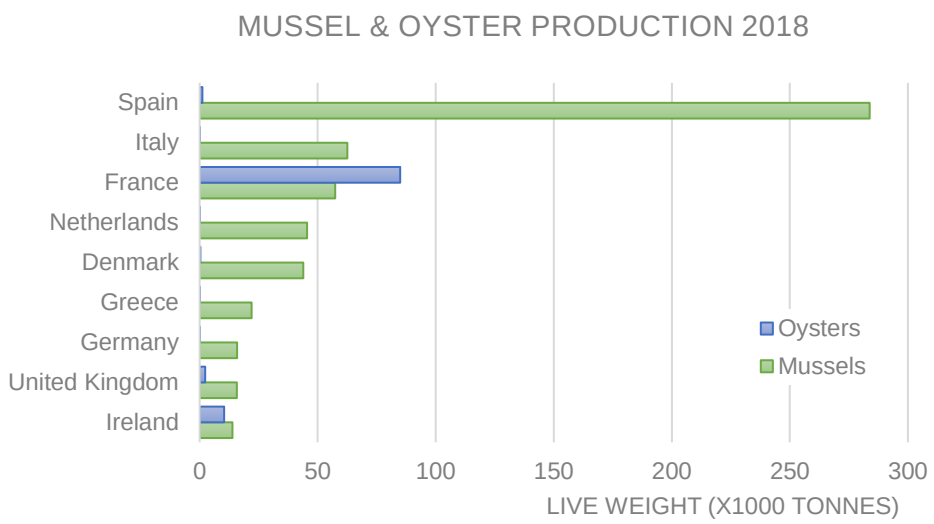


Figure 14 Annual production of oysters in Europe. Blue line indicates the market volume in tonnes in 2018 (source: FAO. 2020. Fishery and Aquaculture Statistics. Global production by production source 1950-2018 (FishstatJ). Criteria for selection include: Species: European flat oyster, Pacific cupped oyster, Cupped oysters nei (i.e. "not elsewhere included", which are oysters that have not been identified by species or family), Flat and cupped oysters nei . For mussels: blue mussel, Mediterranean mussel, sea mussels nei (i.e. "not elsewhere included", which are mussels that have not been identified by species or family),; Country by continent: Europe; All FAO major fishing areas; Aquaculture (freshwater, marine and brackish water) and capture production). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2020. www.fao.org/fishery/statistics/software/fishstatj/en). Remark: some numbers of 2018 are estimated by the FAO from available sources of information.

Carbon sequestration by the European mussel industry

It is difficult to extrapolate the estimates obtained for the Dutch mussel industry to European production, as production techniques and environmental conditions largely vary. This will affect the carbon sequestration potential. CSP is therefore not calculated based on all 3 approaches. Only considering the carbon removed by harvest of shell material (approach 1) results in a value of 50 thousand tonnes carbon (or 184 thousand tonnes CO_2). Using carbon ETS carbon prices (25 € per ton of sequestered CO_2) indicates a maximum 1.3 million Euro.

5 Case study: Carbon stored in wild stocks

5.1 Approach

Shellfish also occur naturally in the marine system, and some populations are fished/exploited (extraction from ocean). There is a difference in wild and cultivated stocks that is relevant for carbon sequestration: not only are growth rates of cultured stocks generally higher, but mussel cultured in (suspended) cultures also tend to have lighter shells than those in natural populations. Wild populations that are not exploited remain in the marine system and shells will eventually dissolve again. In that case there will only be a temporary storage but no long term sequestration of carbon.

This chapter will give an overview of the carbon stored in wild bivalve populations, based on annual stock estimates (see chapter 2) and shell and tissue carbon content of individual species.

5.2 Differences in carbon storage between species

The thickness and shape of shells in relation to the tissue weight (shell to tissue ratio) varies between species: e.g. the pacific oyster is known for a thick uneven shaped shell, while the razor clam has a relatively thin shell. The shell:meat ratio is important in estimating the carbon capture potential (see also chapter 3).

The tissue composition and shell:meat ratios vary between areas, habitats, seasons, reproductive state and other environmental variables. It goes beyond this study to take these factors into consideration. We therefore use generic conversion factors based on:

- Calculations from MWTL data collected in several areas along the Dutch coast (see Annex 1)
- Ricciardi & Bourget (1998) provide conversion factors for many species, including shellfish
- Other literature sources

Table 7 Conversion factors for several shellfish species
Shell to meat ratios & WW to DW ratios used in further calculations (Table 9)

	Shell: meat ratio	Total WW: tissue DW
Blue mussels – Wild (*Mytilus edulis*)	51%[I]	10.7%[I]
Blue Mussels - cultured (*Mytilus edulis*)	70%[II]	6%[IV]
Pacific oysters (*Magallana gigas*)	70%[I]	3.3% [I]
Pacific oysters – cultured (*Magallana gigas*)	85%[III]	
Common cockle (*Cerastoderma edule*)	73%[I]	5.1% [I]
Razor clams (*Ensis spp.)*	54% [I]	11.5% [I]
Gaper clams (*Mya spp.*)	55% [I]	8.3% [I]
Baltic macoma (*Limecola balthica*)	51% [I]	7.9% [I]
Japanese carpet shells (*Ruditapes philippinarum*)	71% [I]	6.2% [I]

[I] MWTL survey data (see Annex 1); [II] Jansen et al., 2019; [III] Capelle et al., 2017; [IV] Capelle unpublished

It is expected that the shell structure of all species is relatively similar: the shell is composed of a small organic matrix and predominantly consists of calcium carbonate ($CaCO_3$). We therefore assume that the carbon content in all shellfish species is approximately 12%. Tissue carbon content is unknown for most

species: Jansen et al. (2019) provides data for mussel species (*Mytilus spp*). Data for mussels in the Dutch coastal water show an average carbon content of 45% per DW (Smaal and Vonck 1997). The eastern oyster (C*rassostrea virginica*) cultivated in the US show similar a carbon content of 44.5% per DW (Higgins, Stephenson et al. 2011). For non-commercial species the tissue content is less known. As a general rule of thumb we here use the value of 45%.

5.3 Carbon Mass balance of Dutch bivalve stocks

Figure 15 and Table 9 provide an overview of the carbon biomass stored in shellfish populations in the Dutch marine waters (Eastern Scheldt, Wadden Sea, Coastal zone).

The coastal zones seems to represent a minor role in the total carbon storage, despite the relative large area (in comparison to the Eastern Scheldt and Wadden Sea). We should however take into account that shellfish stock may vary a lot between years. Data presented in Figure 15 and Table 9 is based on average annual shellfish stock in the year 2010-2015. Figure 3 shows that these were years with a relatively low stock. In 2017 and 2018 we observed a massive S*pisula* population, which represents on its own >145 thousand tonnes Carbon (30-40x times higher than shown in Figure 15 based on the 2010-2015 estimates).

The Eastern Scheldt and the 'Marsdiep and Vliestroom areas in the Wadden Sea' are the areas where mussel aquaculture is practiced. These are also the areas for which we have stock estimates of all shellfish (tidal and subtidal). The sum of carbon storage in shellfish for these areas is 55 thousand tonnes carbon (40 thousand tonnes carbon in shell material). On an annual basis 4 thousand tonnes of carbon is removed through mussel harvest (shell material only; Table 6). Which represents 10% of the total carbon storage in shellfish stocks in these areas.

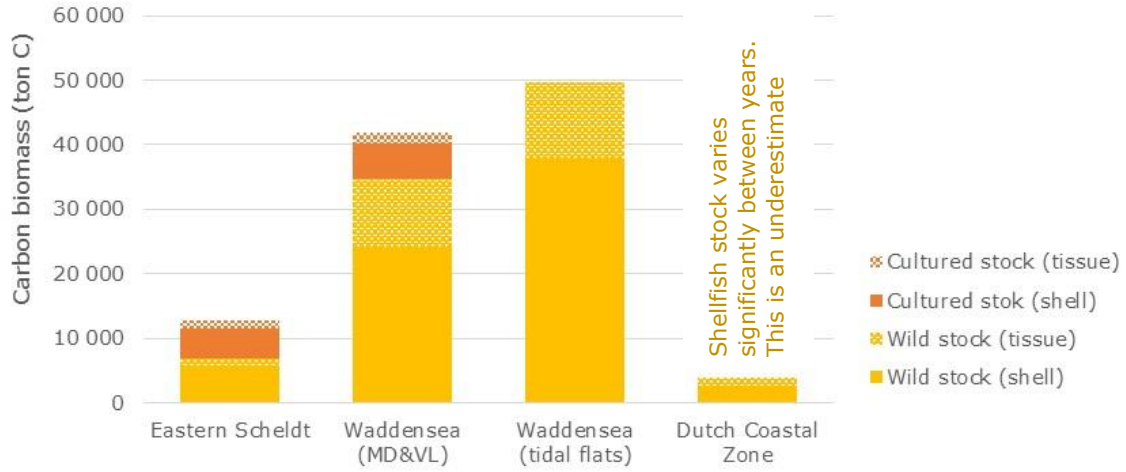


Figure 15 Carbon biomass stored in the most abundant shellfish species in the Dutch estuaries and coastal zones (see also Table 9 and chapter 2 for stock estimates). We differentiate between cultured and wild stocks, and between carbon stored in shell material and in tissue material. Note that there are no estimates for the entire shellfish stocks in the Wadden Sea, we therefore provide 2 different datasets (1- based on the stock estimate for the Marsdiep and Vliestroom area, this includes both subtidal and tidal stocks and can be seen as a total stock estimate for these areas. 2- Stock estimates of all shellfish on the tidal flats in the entire Wadden Sea (thus excluding subtidal stocks).

Table 8 Shellfish biomass and carbon storage in Dutch coastal waters and estuaries

Average biomass (± SD) of the most abundant shellfish species in the period 2010-2016 (in tonnes Fresh Weight; see §2.1 and §2.2), and related total carbon content stored in the shell material of these species using conversion factors provided in Table 8.

Species	Biomass (million kg)				Carbon biomass (tonnes C)											
	Eastern Scheldt	Wadden Sea (Marsdiep Vliestroom)	Wadden Sea (tidal areas)	Dutch Coastal Zone	Eastern Scheldt			Wadden Sea (Marsdiep & Vliestroom)			Wadden Sea (tidal areas)			Dutch Coastal Zone		
					total	shell	tissue	total	shell	tissue	total	shell	tissue	total	shell	tissue
Blue mussels (*Mytilus edulis*) – Wild	3.0± 6.4	42.6 ± 9.3	71.8 ± 14.9	5.8 ± 4.8	324	185	139	4581	2611	1971	7711	4394	3317	622	354	267
Blue mussels (*Mytilus edulis*) – Cultured	42.6 ± 1.8	64.2 ± 23.7	n.a.	n.a.	4727	3577	1150	7123	5391	1733						
Pacific Oyster (*Magallana gigas*) - Wild	34.5 ± 6.4	47.9 ± 13.0	63.5 ± 33.7	n.d.	3385	2875	510	4699	3991	708	6229	5291	938			
Pacific Oyster (*Magallana gigas*) – Cultured	9.5 ± 1.6	n.a.	n.a.	n.a.	1105	965	140	n.a.								
Common cockle (*Cerastoderma edule*)	26.1 ± 11.7	110.6 ± 30.5	324 ± 118	n.d.	2878	2277	601	12183	9638	2545	35729	28265	7464			
Razor clam (*Ensis*)	0.2 ± 0.1	51.2 ± 20.6	n.d.	n.d.	25	14	11	5977	3338	2639						
Sand gaper (*Mya areanaria*)	0.1 ± 0.1	64.5 ± 22.5	n.d.	n.d.	15	9	5	6660	4258	2403						
Baltic tellin (*Macoma baltica*)	0.9 ± 0.3	6.6 ± 1.7	n.d.	n.d.	88	56	33	630	397	233						
Japanse carpet shell - *(Venerupis)*	1.9 ± 1.4		n.d.	n.d.	220	166	54	0	0	0						
Surf clam (*Spisula subtruncata*)				14.2 ± 13.1	0	0	0	0	0	0				1504	1024	480
Striped venus clam (*Chamelea striatula*)				8.8 ± 4.6	0	0	0	0	0	0				935	636	298
Banded wedge-shell (*Donax vittatus*)				8.8 ± 6.8	0	0	0	0	0	0				934	636	298
Total					**12767**	**10124**	**2643**	**41854**	**29624**	**12230**	**49669**	**37950**	**11719**	**3995**	**2651**	**1344**
Wild stock					6935	5582	1354	34731	24233	10498	49669	37950	11719	3995	2651	1344
Cultured stock					5832	4542	1290	7123	5391	1733						

6 Perspectives on blue bivalve carbon

6.1 Approaches to evaluate carbon sequestration potential

This report has provided insight in the carbon dynamics in marine bivalves. It has also shown that quantifying the carbon sequestration potential (CSP) is not straight forward and is still under debate in the international literature. These discussions can generally be separated in three themes: (i) The scale required to evaluate carbon sequestration through shell/bivalve production, (ii) Carbonate balance in seawater, and (iii) Shells as a by-product of food production

Approaches: shell, individual, population or ecosystem scale

In this report we have shown three approaches currently proposed in literature to calculate the carbon sequestration potential by marine bivalves. The approaches vary in scale and complexity and provide different outcomes showing both positive and negative carbon capture estimates, indicating that bivalves can be seen as a sink or a net source of CO_2 to/from the atmosphere (Table 10 for the case of the blue mussels in The Dutch waters, Table 11 adapted from Filgueira et al. 2019 for other study areas). These tables highlight that the value of carbon sequestration highly depends on the approach used in calculations. To summarize:

- Approach 1: is based on shell content only and is the most simple method. This approach has been described by numerous papers to be a sink for atmospheric CO_2 (Tang, Zhang et al. 2011, Humphreys, Daniels et al. 2018), as it locks away carbon in solid mineral form (Morris and Humphreys 2019). It always results in positive values for carbon sequestration.
- Approach 2a: a whole-organism (individual) perspective proposed by Munari corrects the carbon assimilation in shell material for CO_2 produced during shell formation (calcification) and from respiration. Following this approach bivalves are always a net CO_2 source to the atmosphere.
- Approach 2b: is essentially similar to approach 2a but focusses on the shell processes only, it therefore attributes only 10% of CO_2 produced by the animal to shell formation. Using this approach results in small but positive values, indicating that bivalves are a sink of carbon.
- Approach 3: adds to the whole-organism perspective where biodeposit (faeces and pseudofeces) production and decomposition is also included. As many factors are included, data is often lacking to calculate these processes.

Table 9 Summary of carbon sequestration potential of an individual mussel, a population on a cultivation plot, and for the entire Dutch mussel sector
Mass balances based on different approaches

	Individual mussel (g C per production cycle)	Population on cultivation plot (kg C ha^{-2} y^{-1})		Dutch mussel industry (tonnes C y^{-1})
		Entire population	*Harvested population*	
1. Mass balance of assimilation in shell material (gross)	1030	630	630	4284
2. Mass balance of individuals, correcting for CO_2 fluxes (nett)	-541	-1200	-540	-3660
2b. Same to 2a with partitioning between shell/tissue	317	-300	173	1179
3. Mass balance of individuals, including all metabolic processes	Unknown due to data unavailability			

These approaches indicate that the role of marine bivalves in the CO_2 cycle so far has been evaluated based on individual approaches. However Filgueira et al. (2015) argue that this neglects important ecosystem interactions that occur at the population level, and processes that go beyond individual scale are not included. They suggest that a fully coupled ecosystem evaluation models (Approach 4) are needed to truly evaluate carbon dynamics related to bivalve aquaculture.

An important aspect of such ecosystem models is the interaction with phytoplankton, which is a process that is neglected in all of the approaches stated above. Shellfish facilitate atmospheric-CO_2 drawdown via filtration and rapid biodeposition of carbon-fixing primary producers (phytoplankton). Phytoplankton is the most favoured food source for bivalves. Phytoplankton are autotrophic free floating micro-algae that grow given enough inorganic nutrients and light. During this process CO_2 is used and assimilated in the phytoplankton cells. In many marine waters primary production (growth of phytoplankton) is nitrogen limited (e.g. Oosterschelde), and in some cases phosphate limited (e.g. North Sea). The grazing of bivalves on phytoplankton is called 'negative feedback', but excretion and regeneration of nutrients (predominantly nitrogen and CO_2) by bivalves may lead to enhanced primary production in nutrient limited areas/seasons. This latter process is called 'positive feedback'. Prins et al. (1995) have previously shown that shellfish populations do perform a positive feedback in the Eastern Scheldt during periods when nitrogen is limited (summer), indicating that primary production is higher in a system with shellfish compared to a system where shellfish populations are much lower. From this perspective, the CO_2 released from calcification, respiration and microbial mineralisation of faeces does not necessarily escape to the atmosphere but may be (partly) taken up by enhanced photosynthetic activity. An increased bivalve stock, may thus increase the turnover of nutrients and thereby stimulate primary production. It could thereby act as a reservoir to store nutrients in an solid form. This can only continue until the threshold when carrying capacity is reached and subsequently overgrazing results in a decrease of the primary production, indicating that food sources is grazed before it can reproduce.

Regeneration of nutrients and availability for phytoplankton populations depends on the stoichiometry, which means the relative ratio between the nutrients C:N:P. Jansen et al. (2019) expressed turnover of nutrients by shellfish populations as a function of nutrients assimilated by food uptake. They indicated that 36-44% of carbon in the food is again released as CO_2, while for nitrogen and phosphorus this fraction is generally lower (11-39% and 0-52% respectively). This shows that relatively more carbon is released compared to nitrogen and phosphorus. How this translates to uptake by phytoplankton varies between areas and seasons.

How exactly these processes relate to and interact with carbon sequestration should be evaluated with simulation models and cannot be answered with simple mass-balance approaches as presented in this report. Such models can vary from individual 0-D models to test specific hypothesis, for example on nutrients interaction, or can be based on fully coupled 3-D ecosystem models. In section 4.3 we showed that carbon sequestration potential also changes significantly when coupling individual based estimates to population dynamics. As shellfish populations, both in wild and aquaculture stocks, are characterised by high mortality indicates that some part of the population contributes to carbon dynamics in the ecosystem, but is not available for carbon capture through harvest. Carbon budgets at population level are not straightforwardly comparable as it is often unknown whether estimates for the entire stock or only for the harvested stock are included. The fate and rates of decaying bivalves shells then become relevant, yet little is known about that and is therefore not included in mass balance approaches presented here. In general is dissolution rates of shells controlled by both extrinsic factors (water chemistry and movement, pH, mechanical abrasion) and intrinsic factors (species, shell size, $CaCO_3$ crystal size and mineralogy, chemical composition and structure of organic matter) (Strayer and Malcom 2007) and may vary between 2 to 70% per year (Driscoll 1970) Ilarri et al. (2015). No specific information on decay rates for blue mussel shells is available.

The above indicates that greater understanding is required before shellfish can be included in the quantification of carbon capture. We thereby follow Filgueira et al (2015) who advocates that an ecosystem approach accounting for the trophic interactions of bivalves is an essential requisite for providing a reliable assessment of the role of bivalve shells in the carbon cycle, especially in situations with high density aquaculture production of bivalves when carrying capacity issues become relevant.

Table 10 Carbon sequestration (CSP) by marine bivalves

Overview of shell formation and metabolic processes for marine bivalves based on a literature review (adapted and supplemented from Filgueira et al 2019, all expressed in gC m^{-2} $year^{-1}$), and related carbon sequestration estimates based on the three different approaches (see Table 1)

Species	Sequestration (S)	Biocalcification (B)	Respiration (R)	Tissue Growth (T)	Biodeposit burial (BB)	Biodeposit mineralization (BM)	Reference	Carbon Sequestration Potential for each approach (gC m^{-2} $year^{-1}$)				
								1 (S)	2a (S-B-R)	2b (S-B-10%R) shell	3a (S-B-R +T+B-BM)	3b (S-B-R +T+B-BM) shell
Potamocorbula amurensis	23.9	18.0	37.0				Chauvaud et al. (2003)	23.9	-31.1	2.2		
Mytilus edulis (sheltered)	3.8	2.3	1.9				Hily et al. (2013)	3.8	-0.4			
Mytilus edulis (semiexposed)	129.2	77.4	44.3				Hily et al. (2013)	129.2	7.6			
Mytilus edulis (exposed)	45.0	27.0	19.6				Hily et al. (2013)	45.0	-1.6			
Crassotrea gigas (sheltered)	286.8	172.0	11.9				Hily et al. (2013)	286.8	103.0			
Chlamys farreri	78.1	54.0	71.7				Jiang et al. (2014)	78.1	-47.6	7.2		
Crassostrea gigas	15.5	11.1	32.7				Lejart et al. (2012)	15.5	-28.3	3.3		
Ruditapes philippinarum	98.2	66.7	272.4				Mistri and Munari (2012)	98.2	-241.0	27.2		
Arculata senhousia	46.0	11.7	50.4				Munari et al. (2013)	46.0	-16.1	5.0		
Mytilus galloprovincialis	1639.2	1041.6	2253.6				Munari et al. (2013)	1639.2	-1656	225		
Mytilus edulis	**62.9**	**37.7**	**91.4**	**20.3**			**This study**	**62.9**	**-33.0**	**19.4**		
Mytilus edulis (suspended) Expressed per m rope	273	164	1064	218	737	177	Filgueira et al. (2019) under the assumption of *500 indiv rope^{-1}*	273	-955	2.7	-177	61

Carbonate balance in seawater

An important process in all approaches to evaluate carbon sequestration potential is 'calcification'. As shell formation consumes CO_3^{2-} and generates CO_2, this will induce a new distribution of the four different carbon forms in the carbonate system (see section 3.1). The release of CO_2 has basically the same effect as CO_2 uptake from the atmosphere: it leads to a decrease of CO_3^{2-} and increase of H^+ ions. In the approaches presented in the current report (chapter 2 and 3) we have used a factor of 0.6 for CO_2:$CaCO_3$ release during the calcification process, indicating that 0.6 mol CO_2 is released in the water column during the production of 1 mol $CaCO_3$. In colder temperatures this ratio might actually be higher, suggesting that CO_2 sequestration potential is lower at colder temperatures: 0.6 at 25°C, 0.7 at 15°C and 0.8 at 5°C (Frankignoulle, Canon et al. 1994, Morris and Humphreys 2019). Another environmental factor that is negatively correlated to the CO_2:$CaCO_3$ ratio is salinity (Frankignoulle, Canon et al. 1994).

An increase of CO_2 concentrations in the water column can in turn have a negative effect on marine calcifiers, including bivalves, as the carbonate ion (CO_3^{2-}) is one of the building blocks of $CaCO_3$ and changes in its concentration can affect the ability of calcifying organisms to precipitate $CaCO_3$ (Gazeau, Quiblier et al. 2007). This demonstrates that although carbon is assimilated in bivalve shells, and can eventually be harvested for food and bio-based products, the water chemistry may change in such a way that is negative for bivalve growth. In the ocean this process is however neglectable in comparison to all other carbon processes and the large buffering capacity.

Shells are a by-product of seafood production

While discussing carbon sequestration by bivalves and thereby their role in a circular economy, an important aspect to be considered is the final destination of the shellfish (Alonso, Álvarez-Salgado et al. 2021). Bivalves are, and will be, primarily be produced to supply food, not to produce shells and thereby sequester CO_2. As of today, shells are therefore a waste product. The aim of circular economies is to re-use waste streams. In that respect offers the bivalve shell remarkable opportunities to lock carbon in a mineral form during prolonged periods. Approach 2b includes this aspect by partitioning respiration costs between shell (10% of energy) and tissue (90% of energy), and Filgueira et al. (2019) thereby argue to assess GHG emission for tissue production and shell production separately.

By-products from the food industry are found in other sectors too. For example leather is an intermediate industrial product from cattle raising with numerous applications in downstream sectors (Joseph and Nithya 2009). The raw material in the production of leather is a byproduct of the meat industry. When estimating the environmental burdens in terms of GHG emissions of leather products are only calculated based on the downstream processes such as tanning and finishing of leather as well as the electricity production and transportation required in the life cycle. Cattle raising and feed production are considered outside the boundary of the system for leather production, and are fully attributed to meat production. In this study the partitioning of environmental burdens between meat and leather is only performed during the slaughter process. Here they used the total market value share of rawhide (14%) to divide between products. This is not exactly similar as partitioning is performed for processing steps, but it shows that distinguishing between primary and by-products can be done based on different methods.

Furthermore, in the discussion on carbon sequestration by shellfish and the debate on which processes to include in calculations it is interesting to look at the LCA methods commonly applied for food production. These LCAs generally do not account for the large amounts of CO_2 are emitted from lifestock production systems through plant, animal, and microbial respiration. This is considered as biogenic, because the carbon was originally extracted from the atmosphere by plant fixation and now is returned to the atmosphere. With no long-term effect on the atmosphere, this source is normally ignored in modelling farm emissions (Rotz 2018). This is particularly the case for poultry production, while for dairy production CO2 emission by the animals might become more relevant, especially considering the enteric fermentation processes (Blonk and Luske 2008).

6.2 Extent of the carbon sequestration potential by marine bivalves

National, European and global magnitude of blue carbon by bivalve aquaculture

The national production of blue mussels accounts for an average of 51 thousand tonnes per year (over the last five years), resulting in a maximum of 4 thousand tonnes sequestered carbon, or 16 thousand tonnes CO_2 equivalents (Table 6). As highlighted above, this number can vary significantly depending on the method used to calculate the carbon sequestration potential. For the European mussel production these numbers equal to maximum 45 thousand tonnes C, and 166 thousand tonnes CO_2 (Table 7). Mussel aquaculture is the major form of shellfish production in the Netherlands (85% mussels, 5% oysters), and within Europe it accounts for 60% of the total mollusc/shellfish production. Filgueira et al. (2019) demonstrated based on the global annual production of cultured bivalves (~14 $x10^6$ tons, including clams, cockles, oysters, mussels and scallops www.fao.org reporting 2015 data), results in a shell by-product of $\sim 7 \times 10^6$ tonnes. Assuming a 12% carbon content 840 thousand tonnes carbon (3080 thousand tonnes CO_2). This might again, differ based on how to estimate the Carbon sequestration potential.

Besides aquaculture stocks large amounts of carbon are stored in wild bivalve populations. Some of these stocks are fished, but most remain in the ecosystem. In the Netherlands exploitation of wild stocks through fisheries can be neglected compared to aquaculture production. Yet, the amount of carbon locked away in shell material is several times higher compared to the harvest. Besides fisheries for human food, there is another type of fishery specifically focussing on shell material (died shellfish). These shells are used in several applications such as for poultry grit, roads, and isolation.

Future shellfish production in the Netherlands

Within the last decade, interest has grown in investigating the potential for larger-scale aquaculture operations in the open ocean (Buck, Ebeling et al. 2010). However, the North Sea is a crowded place and the development of offshore aquaculture intersects with other maritime activities, such as fishing, shipping and offshore wind energy. This results in competing claims for space. On the one hand, this competition sets boundaries to the development of offshore aquaculture. On the other hand, this can create new opportunities for smart combinations of activities (Jansen et al., 2016). The Dutch government has come with a The National Climate Agreement to combat climate change, where multiuse plays an important role. One of the results of the National Climate Agreement is the knowledge and innovation agenda for climate and energy "innovate with a mission" (March 2019). In this document, the development of substantial claim for seaweed production (in combination with nature) is mentioned. Seaweeds may sequester significant amounts of carbon in the oceans (Krause-Jensen and Duarte 2016) and may therefore have the potential to offset greenhouse gas emissions. In the present study, we have also shown that bivalves can potentially function as a carbon sink. With potential options to monetarize or obtain social acceptance.

A spatial distribution model adapted for the Dutch North Sea conditions demonstrated that offshore mussel production in wind farms can be profitable (Jansen, Van Den Burg et al. 2016). Jansen et al. demonstrated this with a case-study, where a hypothetical mussel farm was designed using longlines between monopiles. The estimated production per ha offshore is expected to be 24 tonnes per ha, which is 1.5 times higher than for bottom plots. Mortality due to predation pressure from starfish and crabs is expected to be lower on longlines compared to bottom cultivation (Dolmer 1998, Leonard, Bertness et al. 1999). Furthermore, suspended mussels tend to have thinner shells than bottom mussels, which can also be related to reduced predation pressure (Christensen 2012). The thinner shells in suspended mussels are also a result of faster growth rates when the mussels are suspended in the water column (Garen, Robert et al. 2004). The tissue composition and shell:tissue ratios are important in estimating the carbon capture potential of shellfish, but these values vary between areas, habitats, seasons, reproductive state and other environmental variables. For mussel cultivation in the UK, it has been shown that suspended cultures have a higher carbon content in the tissue, but a lower carbon content in the shell compared to bottom plots (pers. Comm. With Van der Schatte Olivier, 2019). A study by Kamermans et al. demonstrated that mussels collected from offshore buoys have an average tissue yield of 25-30% (Kamermans, Soma et al. 2016). In comparison: in the Wadden Sea and the Eastern

Scheldt mussels are harvested with an average tissue yield of 22-34%, indicating that the differences might not be substantial (Kamermans, Soma et al. 2016).

Bivalves versus other marine and terrestrial production

Regardless of the approach applied to estimate carbon sequestration, and how to partitioning between food (shellfish tissue) and biobased (shell) products, it is of interest to compare how shell(fish) relates to other biobased products. Based on the information from our study it can be concluded that 2.3 tonnes CO_2 ha^{-1} y^{-1} is harvested by shell material, this can be regarded as a gross value for carbon sequestration by shellfish. Corrected for biocalcification and respiration the nett value is -2.0 tonnes CO_2 ha^{-1} y^{-1}. (or 0.6 tonnes CO_2 ha^{-1} y^{-1} applying approach 2b and thus focussing on shell material only). Gross sequestration by marine seaweed production is 15 tonnes CO_2 ha^{-1} y^{-1} , assuming production of 10 ton DW ha^{-1} and a Carbon:DW conversion of 0.4 (Duren, Poelman et al. 2019). For terrestrial forest production the nett carbon sequestration varies between 3-12 tonnes CO_2 ha^{-1} y^{-1} (Nabuurs and Mohren 1995). Note that animal production (shellfish) is here compared with marine and terrestrial primary producers.

6.3 Carbon Credits and Biobased products

To value the ecosystem service of mussel farming in the carbon cycle, carbon sequestration can be converted to CO_2 equivalents. Assuming 48€ per ton of sequestered CO_2 (World Bank, 2016 *in* Filgueira et al., 2019), and a maximum of 15 689 tons of CO_2 sequestered in the Dutch mussel industry, leads to a maximum value of ~750 thousand euro. This represents ~1.5% of the annual market revenues of the sector (Figure 12).

This is just a measure for carbon capture in shells, so valuing the natural capital. At present the shells hardly represent a commercial value. Developing biobased products including shell material, will add another (commercial) value to shellfish production. Recycling shells has potential application in various fields. Possible products of shell material include construction material, soil conditioner, calcium supplements, adsorbents and artificial bone (Yao, Xia et al. 2014). Already many experiments have been conducted on recycling shells and a great deal of effort has been spent in developing applications for shell waste. Shellfish producers are interested as this could potentially lead to additional income out of what is now called a waste product.

6.4 Recommendations for further work

Dynamic modelling

The current study represents a first inventory of the potential for blue carbon sequestration by bivalves. This was based on some key figures and general assumptions. However, it becomes clear that greater understanding is required before shellfish can be included in the quantification of carbon dynamics. To provide better estimates, dynamic models should be developed for carbon fixation in shellfish aquaculture. In order to do so DEB models should be extended to include carbon parameters, and coupled to population dynamic models for mussel plots, and subsequently integrated in ecosystem models to include processes beyond the individual mussels. To feed the models with process and validation data additional lab and/or mesocosm experiments might be required.

These models will provide better estimates of carbon fixation and are spatially and temporally more explicit. Furthermore, the potential for C-fixation through mussel aquaculture in new production areas can be explored via scenario analysis (North Sea), as well as C-fixation by oyster reefs and oyster aquaculture. If relevant the scope will be widened to European and/or global scales.

Carbon dynamics in marine waters are closely linked to nitrogen (N) and phosphorus (P) dynamics. Carbon sequestration should therefore always be evaluated together with N and P. This could not only result in ecological benefits, but extraction of these nutrients might also contribute to an economic benefit (Ferreira and Bricker 2019).

Trade-offs

Furthermore, more emphasis should be addressed towards effects of stimulating C-fixation through shellfish production on natural capital (e.g. biodiversity) and how to value this in the sequestration perspective. The perspective of new cultivation areas in the North Sea, not only poses potential for climate robust production but also asks for an evaluation of the maximum production potential without causing negative environmental impacts (trade-offs), for example based on carrying capacity.

7 Conclusions

The question to what extent bivalve stocks and bivalve production contributes to climate ambitions is not one that is straightforward to answer as different approaches are used to quantify carbon sequestration potential, in addition to the connection between food (tissue) and bio-based products (shells) which requires allocation of carbon fluxes between products.

The commercial shellfish production in Holland is dominated by the blue mussel production. Based on the approaches currently proposed in literature is was shown that carbon sequestration by mussel shells may result in a positive value (max ~1000 g C per production cycle) or in a net release (minimum -500 gC per production cycle), indicating a threefold difference in the way it is estimated. This equals to respectively 4000 or -3500 tonnes C y^{-1} when we translate these numbers to the entire Dutch mussel sector. We however argue that none of these approaches are sufficient to address all/essential ecosystem processes and therefore suggest to apply the ecosystem approach to evaluate carbon fluxes related to shellfish production. These models should at least include interactions with phytoplankton populations, but also all metabolic processes related to bivalve growth (such as feeding, respiration, growth, (pseudo)faeces production and decomposition). This is more complex than the approaches presented in the current report, but we feel this is required for accurate estimates and insight in carbon dynamics. Furthermore, the proposed models should include population dynamics, as numbers indicated above are solely based on individual/organism level. It was shown that carbon sequestration potential may vary significantly if population effects are included, and might even define whether the sequestration estimate is positive or negative (Table 10).

The approaches discussed in this report focus on carbon sequestration of the shell. It is however impossible to separate shell production from shellfish (food) production. While discussing the role of shellfish in a circular economy an important aspect to be considered is therefore the final destination of the shellfish. In one way or the other carbon fluxes have to be attributed to a certain product, in this case tissue or shell. Filgueira et al. (2019) suggested to allocate CO_2 fluxes in a ratio of 10%/90% to

Table 11 Partitioning of carbon fluxes between food and biobased products
Scenarios for tissue (food) and shell (biobased) production

Scenario	Carbon fluxes allocated to Food (tissue)	Carbon fluxes allocated to Biobased products (shell)
1. Shellfish only produced for food production	100% of metabolic fluxes* 100% of shell formation fluxes** 100% of ecosystem processes***	n.a.
2. Shellfish produced for food production, all waste shells are re-used in by-products	90% of metabolic fluxes 90% of ecosystem processes	10% of metabolic fluxes 100% of shell formation fluxes 10% of ecosystem processes
3. Shellfish only produced for shells	n.a.	100% of metabolic fluxes 100% of shell formation fluxes 100% of ecosystem processes

** Metabolic fluxes include the net results of respiration (CO_2), growth (POC), (pseudo)faeces production (POC) and decomposition (CO_2)*

*** Shell formation includes net result of shell deposition ($CaCO_3$) and biocalcification (CO_2)*

**** Ecosystem processes include the net effect on phytoplankton populations (CO_2 assimilation)*

shell and tissue production based on energy allocation to produce shells (approach 2b, 3b). In other sectors divisions are for example based on market value, or the by-products is seen as a waste product and therefore all carbon fluxes are allocated to the main product, in this case shellfish tissue for food production. Following the approach by Filgueira et al. results in the different scenario's (Table 12). This shows that if shells are produced for shell material only (scenario 3), the carbon sequestering will be low/negative as all metabolic fluxes also have to be taken care for. The most simple approach actually is to allocate all fluxes (including carbon sequestration in shells) to food production, as is done for other sectors too. To comply to circularity ambitions, one should of course aim for re-use of shell material (what is now a waste product). In this way shell material may substitute fossil sources (e.g. lime stone). To change a fossil source for a renewable will contribute to CO_2 emission reductions.

8 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. This certificate is valid until 15 December 2021. The organisation has been certified since 27 February 2001. The certification was issued by DNV GL.

References

Agonus, 2013. Passende beoordeling Ensisvisserij Natura 2000 gebieden Voordelta, Vlakte van de Raan en Noordzeekustzone, Leiden, p. 45.

Agonus, 2017. Habitattoets handmatige kokkelvisserij Waddenzee, Leiden, p. 66.

Alonso, A.A., Álvarez-Salgado, X.A., Antelo, L., 2021. Assessing the impact of bivalve aquaculture on the carbon circular economy. Journal of Cleaner Production 279, 123873.

Bevelander, G., Nakahara, H., 1969. An electron microscope study of the formation of the nacreous layer in the shell of certain bivalve molluscs. Calcified tissue research 3, 84-92.

Buck, B.H., Ebeling, M.W., Michler-Cieluch, T., 2010. Mussel cultivation as a co-use in offshore wind farms: potential and economic feasibility. Aquaculture Economics & Management 14, 255-281.

Capelle, J.J., Van Stralen, M., 2017. Invang van mosselzaad in MZI's. Resultaten 2016. Wageningen Marine Research, Wageningen, p. 30. Report number: C044/17.

Capelle, J.J., Van Stralen, M.R., Wijsman, J.W., Herman, P.M., Smaal, A.C., 2017. Population dynamics of subtidal blue mussels Mytilus edulis and the impact of cultivation. Aquaculture Environment Interactions 9, 155-168.

Carter, J.G., Clark, G.R., 1985. Classification and phylogenetic significance of molluscan shell microstructure. Studies in Geology, Notes for a Short Course 13, 50-71.

Chauvaud, L., Thompson, J.K., Cloern, J.E., Thouzeau, G., 2003. Clams as CO2 generators: the Potamocorbula amurensis example in San Francisco Bay. Limnology and Oceanography 48, 2086-2092.

Checa, A., 2000. A new model for periostracum and shell formation in Unionidae (Bivalvia, Mollusca). Tissue and Cell 32, 405-416.

Checa, A.G., Rodríguez-Navarro, A.B., Esteban-Delgado, F.J., 2005. The nature and formation of calcitic columnar prismatic shell layers in pteriomorphian bivalves. Biomaterials 26, 6404-6414.

Chen, B., Fan, J.H., Wang, J., Peng, X., Wu, X.L., 2005. Research of nanostructure of bivalva shell, Journal of Metastable and Nanocrystalline Materials. Trans Tech Publ, pp. 83-86.

Christensen, H.D., Per & Petersen, Jens & Tørring, Ditte, 2012. Comparative study of predatory responses in blue mussels (Mytilus edulis L.) produced in suspended long line cultures or collected from natural bottom mussel beds. HELGOLAND MAR RES. 66. 10.1007/s10152-010-0241-0.

de Boer, I.J., van Ittersum, M.K., 2018. Circularity in agricultural production. Wageningen University & Research.

Dickson, A.G., 2010. The carbon dioxide system in seawater: equilibrium chemistry and measurements. Guide to best practices for ocean acidification research and data reporting 1, 17-40.

Dolmer, P., 1998. Seasonal and spatial variability in growth of Mytilus edulis L. in a brackish sound: comparisons of individual mussel growth and growth of size classes. Fisheries Research 34, 17-26.

Driscoll, E.G., 1970. Selective bivalve shell destruction in marine environments; a field study. Journal of Sedimentary Research 40, 898-905.

FAO, 2015. Fishery and aquaculture statistics [Global capture production 1950–2013] (FishStatJ). FAO Fisheries and Aquaculture Department, Rome.

Filgueira, R., Byron, C., Comeau, L., Costa-Pierce, B., Cranford, P.J., Ferreira, J., Grant, J., Guyondet, T., Jansen, H., Landry, T., 2015. An integrated ecosystem approach for assessing the potential role of cultivated bivalve shells as part of the carbon trading system. Marine Ecology Progress Series 518, 281-287.

Filgueira, R., Strohmeier, T., Strand, Ø., 2019. Regulating services of bivalve molluscs in the context of the carbon cycle and implications for ecosystem valuation, Goods and Services of Marine Bivalves. Springer, Cham, pp. 231-251.

Frankignoulle, M., Canon, C., Gattuso, J.P., 1994. Marine calcification as a source of carbon dioxide: Positive feedback of increasing atmospheric CO2. Limnology and Oceanography 39, 458-462.

Frankignoulle, M., Pichon, M., Gattuso, J.-P., 1995. Aquatic calcification as a source of carbon dioxide, Carbon sequestration in the biosphere. Springer, pp. 265-271.

Garen, P., Robert, S., Bougrier, S., 2004. Comparison of growth of mussel, Mytilus edulis, on longline, pole and bottom culture sites in the Pertuis Breton, France. Aquaculture 232, 511-524.

Gattuso, J., Pichon, M., Frankignoulle, M., 1995. Biological control of air-sea CO2 fluxes: effect of photosynthetic and calcifying marine organisms and ecosystems. Marine Ecology Progress Series 129, 307-312.

Gazeau, F., Quiblier, C., Jansen, J.M., Gattuso, J.P., Middelburg, J.J., Heip, C.H., 2007. Impact of elevated CO2 on shellfish calcification. Geophysical research letters 34.

Hawkins, A., Bayne, B., 1985. Seasonal variation in the relative utilization of carbon and nitrogen by the mussel Mytilus edulis: budgets, conversion efficiencies and maintenance requirements. Marine ecology progress series. Oldendorf 25, 181-188.
Higgins, C.B., Stephenson, K., Brown, B.L., 2011. Nutrient bioassimilation capacity of aquacultured oysters: quantification of an ecosystem service. Journal of environmental quality 40, 271-277.
Hily, C., Grall, J., Chauvaud, L., Lejart, M., Clavier, J., 2013. CO2 generation by calcified invertebrates along rocky shores of Brittany, France. Marine and freshwater research 64, 91-101.
Humphreys, M.P., Daniels, C.J., Wolf-Gladrow, D.A., Tyrrell, T., Achterberg, E.P., 2018. On the influence of marine biogeochemical processes over CO2 exchange between the atmosphere and ocean. Marine Chemistry 199, 1-11.
Ilarri, M., Souza, A., Sousa, R., 2015. Contrasting decay rates of freshwater bivalves' shells: Aquatic versus terrestrial habitats. Limnologica 51, 8-14.
Jacob, D., Soldati, A., Wirth, R., Huth, J., Wehrmeister, U., Hofmeister, W., 2008. Nanostructure, composition and mechanisms of bivalve shell growth. Geochimica et Cosmochimica Acta 72, 5401-5415.
Jansen, H., Kamermans, P., Glorius, S., van Asch, M., 2019. Draagkracht van de Oosterschelde en westelijke Waddenzee voor schelpdieren: evaluatie van veranderingen in de voedselcondities en schelpdierbestanden in relatie tot de mosselkweek in de periode 1990-2016. Wageningen Marine Research.
Jansen, H.M., 2012. Bivalve nutrient cycling: nutrient turnover by suspended mussel communities in oligotrophic fjords.
Jansen, H.M., Strand, Ø., Strohmeier, T., Krogness, C., Verdegem, M., Smaal, A., 2011. Seasonal variability in nutrient regeneration by mussel Mytilus edulis rope culture in oligotrophic systems. Marine Ecology Progress Series 431, 137-149.
Jansen, H.M., Van Den Burg, S., Bolman, B., Jak, R.G., Kamermans, P., Poelman, M., Stuiver, M., 2016. The feasibility of offshore aquaculture and its potential for multi-use in the North Sea. Aquaculture International 24, 735-756.
Jiang, Z.J., Fang, J.G., Han, T.T., Mao, Y.Z., Li, J.Q., Du, M.R., 2014. The role of Gracilaria lemaneiformis in eliminating the dissolved inorganic carbon released from calcification and respiration process of Chlamys farreri. Journal of applied phycology 26, 545-550.
Joseph, K., Nithya, N., 2009. Material flows in the life cycle of leather. Journal of Cleaner Production 17, 676-682.
Kamermans, P., Capelle, J.J., 2019. Provisioning of Mussel Seed and Its Efficient Use in Culture, in: Smaal, A.C., Ferreira, J.G., Grant, J., Petersen, J.K., Strand, Ø. (Eds.), Goods and Services of Marine Bivalves. Springer, Cham, Switzerland.
Kamermans, P., Smaal, A.C., 2002. Mussel culture and cockle fisheries in the Netherlands: Finding a balance between economy and ecology. J Shellfish Res 21, 509-517.
Kamermans, P., Soma, K., van den Burg, S., 2016. Haalbaarheid mosselteelt binnen offshorewindparken in de Nederlandse kustzone. IMARES Wageningen UR.
Krause-Jensen, D., Duarte, C.M., 2016. Substantial role of macroalgae in marine carbon sequestration. Nature Geoscience 9, 737-742.
Lampitt, R.S., Achterberg, E.P., Anderson, T.R., Hughes, J., Iglesias-Rodriguez, M., Kelly-Gerreyn, B.A., Lucas, M., Popova, E., Sanders, R., Shepherd, J., 2008. Ocean fertilization: a potential means of geoengineering? Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 366, 3919-3945.
Lejart, M., Clavier, J., Chauvaud, L., Hily, C., 2012. Respiration and calcification of Crassostrea gigas: contribution of an intertidal invasive species to coastal ecosystem CO 2 fluxes. Estuaries and coasts 35, 622-632.
Leonard, G.H., Bertness, M.D., Yund, P.O., 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, Mytilus edulis. Ecology 80, 1-14.
Lutts, A., Grandjean, J., Grégoire, C., 1960. X-ray diffraction patterns from the prisms of mollusk shells. Archives Internationales de Physiologie et de Biochimie 68, 829-831.
Mackenzie, F.T., Andersson, A.J., 2013. The marine carbon system and ocean acidification during Phanerozoic time. Geochemical Perspectives 2, 1-3.
Masera, O.R., Garza-Caligaris, J., Kanninen, M., Karjalainen, T., Liski, J., Nabuurs, G., Pussinen, A., de Jong, B.H., Mohren, G., 2003. Modeling carbon sequestration in afforestation, agroforestry and forest management projects: the CO2FIX V. 2 approach. Ecological modelling 164, 177-199.
Mistri, M., Munari, C., 2012. Clam farming generates CO2: A study case in the Marinetta lagoon (Italy). Marine pollution bulletin 64, 2261-2264.
Mol, A., 2019. Aanvoer Japanse oesters stabiliseert, aanvoer platte oesters neemt toe. Wageningen University & Research, Wageningen, pp. Agrimatie - informatie over de agrosector.
Morris, J.P., Humphreys, M.P., 2019. Modelling seawater carbonate chemistry in shellfish aquaculture regions: Insights into CO2 release associated with shell formation and growth. Aquaculture 501, 338-344.

Munari, C., Rossetti, E., Mistri, M., 2013. Shell formation in cultivated bivalves cannot be part of carbon trading systems: a study case with Mytilus galloprovincialis. Marine environmental research 92, 264-267.
Perdon, K., Troost, K., van Zwol, J., Van Asch, M., van der Pool, J., 2019. Schelpdierbestanden in de Nederlandse kustzone in 2019. Stichting Wageningen Research, Centrum voor Visserijonderzoek (CVO).
Petit, H., 1981. Survol de la minéralisation chez les Unionidae. Haliotis 11, 181-195.
Petit, H., Davis, W.L., Jones, R., 1979. Morphological studies on the periostracum of the fresh-water mussel Amblema (uniondae): Light microscopy, transmission electron microscopy, and scanning electron microscopy. Tissue and Cell 11, 633-642.
Petit, H., Davis, W.L., Jones, R.G., 1978. Morphological studies on the mantle of the fresh-water mussel Amblema (Unionidae): scanning electron microscopy. Tissue and Cell 10, 619-627.
Petit, H., Davis, W.L., Jones, R.G., 1980a. A scanning electron microscopic study of the inorganic and organic matrices comprising the mature shell of Amblema, a fresh-water mollusc. Tissue and Cell 12, 581-593.
Petit, H., Davis, W.L., Jones, R.G., Hagler, H., 1980b. Morphological studies on the calcification process in the fresh-water mussel Amblema. Tissue and Cell 12, 13-28.
Prins, T., Escaravage, V., Smaal, A., Peeters, J., 1995. Nutrient cycling and phytoplankton dynamics in relation to mussel grazing in a mesocosm experiment. Ophelia 41, 289-315.
Rahman, M., Henderson, S., Miller-Ezzy, P., Li, X., Qin, J., 2020. Analysis of the seasonal impact of three marine bivalves on seston particles in water column. Journal of Experimental Marine Biology and Ecology 522, 151251.
Ray, N. E., et al. (2018). "Consideration of carbon dioxide release during shell production in LCA of bivalves." The International Journal of Life Cycle Assessment volume 23: 1042-1048.
Ricciardi, A., Bourget, E., 1998. Weight-to-weight conversion factors for marine benthic macroinvertebrates. Marine ecology progress series 163, 245-251.
Rodhouse, P., Roden, C., Hensey, M., Ryan, T., 1984. Resource allocation in Mytilus edulis on the shore and in suspended culture. Marine Biology 84, 27-34.
Schäffer, T.E., Ionescu-Zanetti, C., Proksch, R., Fritz, M., Walters, D.A., Almqvist, N., Zaremba, C.M., Belcher, A.M., Smith, B.L., Stucky, G.D., 1997. Does abalone nacre form by heteroepitaxial nucleation or by growth through mineral bridges? Chemistry of Materials 9, 1731-1740.
Smaal, A., Vonck, A., 1997. Seasonal variation in C, N and P budgets and tissue composition of the mussel Mytilus edulis. Marine Ecology Progress Series 153, 167-179.
Smaal, A.C., Ferreira, J.G., Grant, J., Petersen, J.K., Strand, Ø., 2019. Goods and services of marine bivalves. Springer Nature.
Sondak, C.F., Ang, P.O., Beardall, J., Bellgrove, A., Boo, S.M., Gerung, G.S., Hepburn, C.D., Hong, D.D., Hu, Z., Kawai, H., 2017. Carbon dioxide mitigation potential of seaweed aquaculture beds (SABs). Journal of Applied Phycology 29, 2363-2373.
Steenbergen, J., Breen, V., Jol, J., 2005. LNV bestek mosselen en eidereenden Deelproject 3: een vergelijking van de kwaliteit van mosselen op percelen en in het wild. RIVO.
Strayer, D.L., Malcom, H.M., 2007. Shell decay rates of native and alien freshwater bivalves and implications for habitat engineering. Freshwater Biology 52, 1611-1617.
Strong, A., Chisholm, S., Miller, C., Cullen, J., 2009. Ocean fertilization: time to move on. Nature 461, 347-348.
Suzuki, A., 1998. Combined effects of photosynthesis and calcification on the partial pressure of carbon dioxide in seawater. Journal of Oceanography 54, 1-7.
Tang, Q., Zhang, J., Fang, J., 2011. Shellfish and seaweed mariculture increase atmospheric CO2 absorption by coastal ecosystems. Marine Ecology Progress Series 424, 97-104.
Troost, K., Perdon, K.J., van Zwol, J., Jol, J., Van Asch, M., 2017. Schelpdierbestanden in de Nederlandse kustzone in 2017. Wageningen Marine research, IJmuiden, p. 38.
Troost, K., van den Ende, D., van Asch, M., van Stralen, M., 2019. Ontwikkeling en verspreiding van schelpdieren en andere bodemdieren in het sublitoraal van de westelijke Waddenzee in de periode 1992-2017. Wageningen Marine Research.
Van Asch, M., van den Ende, D., Brummelhuis, E.B.M., Troost, K., 2014. Het kokkelbestand in de Nederlandse kustwateren in 2014. Wageningen Marine Research, Yerseke, p. 47.
van Broekhoven, W., Troost, K., Jansen, H., Smaal, A., 2014. Nutrient regeneration by mussel Mytilus edulis spat assemblages in a macrotidal system. Journal of Sea Research 88, 36-46.
van der Meer, J., Dankers, N., Ens, B.J., van Stralen, M., Troost, K., Waser, A.M., 2019. The birth, growth and death of intertidal soft-sediment bivalve beds: No need for large-scale restoration programs in the Dutch Wadden Sea. Ecosystems 22, 1024-1034.
van der Schatte Olivier, A., Jones, L., Le Vay, L., Christie, M., Wilson, J., Malham, S.K., 2018. A global review of the ecosystem services provided by bivalve aquaculture. Reviews in Aquaculture 1, 23.
Waldbusser, G.G., Steenson, R.A., Green, M.A., 2011. Oyster shell dissolution rates in estuarine waters: effects of pH and shell legacy. Journal of Shellfish Research 30, 659-669.

Ware, J.R., Smith, S.V., Reaka-Kudla, M.L., 1992. Coral reefs: sources or sinks of atmospheric CO 2? Coral reefs 11, 127-130.
Weiner, S., Lowenstam, H., Hood, L., 1976. Characterization of 80-million-year-old mollusk shell proteins. Proceedings of the National Academy of Sciences 73, 2541-2545.
Widdows, J., Fieth, P., Worrall, C., 1979. Relationships between seston, available food and feeding activity in the common mussel Mytilus edulis. Marine Biology 50, 195-207.
Williamson, P., 2016. Emissions reduction: scrutinize CO 2 removal methods. Nature 530, 153-155.
Williamson, P., Wallace, D.W., Law, C.S., Boyd, P.W., Collos, Y., Croot, P., Denman, K., Riebesell, U., Takeda, S., Vivian, C., 2012. Ocean fertilization for geoengineering: a review of effectiveness, environmental impacts and emerging governance. Process Safety and Environmental Protection 90, 475-488.
Yao, Z., Xia, M., Li, H., Chen, T., Ye, Y., Zheng, H., 2014. Bivalve shell: not an abundant useless waste but a functional and versatile biomaterial. Critical Reviews in Environmental Science and Technology 44, 2502-2530.
Yousefpour, R., Augustynczik, A.L.D., Reyer, C.P., Lasch-Born, P., Suckow, F., Hanewinkel, M., 2018. Realizing mitigation efficiency of European commercial forests by climate smart forestry. Scientific reports 8, 1-11.
Zeebe, R.E., Zachos, J.C., Caldeira, K., Tyrrell, T., 2008. Carbon emissions and acidification. Science 321, 51-52.

Justification

Report C116/20
Project Number: 6224103800

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved: Ing. M Poelman MSc.
Colleague scientist

Signature: 

Date: January 26th 2021

Approved: Dr. ir. T.P. Bult
Director

Signature: 

Date: January 26th 2021

Annex 1 Estimating conversion factors wild shellfish

Tools engaged towards the estimation of carbon storage capacity in selected species

The quantification of the variables required by the estimation of the carbon storage capacity relies on different approaches going from general relationships to specific direct measurements:

- Data on Flesh/shell ratio directly measured on individuals collected in the context of Netherlands benthos monitoring surveys (MWTL, PMR).
- Allometric relationships related to secondary production and conversion factors as reported by Brey (2001, 2012) with regular updates brought by the author on the online version of his "Virtual Handbook".

Differentiations based on areas, years and seasons could be expected to produce more specific patterns than when using generic descriptors as proposed here. However making further selection based on the spatio-temporal scales would also contribute to increase the relative contribution of i.-other factors that are not explicitly defined in the metadata and ii.- individual variation as result of the decrease in the number of observations.

The blurring associated with the relative increase of these unintended sources of variations is expected to annihilate the gain in accuracy aimed with the selections based on areas, years and seasons. The same reasoning holds for the use of the general P/B relation as established by Brey based on a literature compilation instead of specific measurements being obtained in conditions that are generally not exhaustively described in the literature source nor exactly matched in the data to be converted. These considerations have led to the present choice of generic and robust above specific and sensitive descriptors.

The variables such as individual carbon content, shelf/flesh ratio and growth rate ratio are obtained from a combination between these different approaches as described in the following and summarized in Table 9.

Individual body mass with distinction of tissue and shell

The data available from the Dutch benthos monitoring programs consists in individual observations on macrofauna mostly collected during the 90's in the Dutch Delta waters and Voordelta. Weighing's are performed on batch of individuals gathered by size where individuals are wet weighed (WW=sum of shell and flesh), transferred in crucibles, weighed a.-after drying (48 hours, 60°C) and b.-after burning (2 hours 550°C). The ash free dry weight (AFDW) is thereafter estimated as the difference before and after burning. The strength of the relation between the ash free dry weights and the measured lengths as shown in Table A1**Error! Reference source not found.** witnesses the consistency of these measurements.

Separate estimates of shell and flesh are however missing from these data but might be estimated through an approximation for the shell free wet weight (SFWW) as described further:

1. A first option could be the use of standard "tissue yields", the ratio of tissue to WW (whole weight including shell). Average values are available from the literature as 18% for clams , 22.1% for mussels and 10.8% for oysters as cited in review by Olivier et al. (2018).
2. A second option could make use of the measurement of AFDW, calculating first the shell free dry flesh weight (SFDW) from the AFDW and second the SFWW from the SFDW using the following ratio's cited in published review: AFDW/SFDW≈0.83 (Ricciardi & Bourget, 1998) and

SFDW/SFWW≈0.18 (Hulscher 1974, 1982, Kersten & Visser 1996). This leads to a ratio between AFDW and SFWW of *ca* 0.15.

Table A1.-Ratio's (average ± 95% confidence interval) between the estimates for Shell Free Wet Weight obtained from the AFDW and from the Tissue yield methods.

SFWW2 [AFDWmethod] / SFWW1 [TISSUE_YIELDmethod]						
Mussels	Oysters	Cockles	Razor clams	Sand gapers	Baltic tellins	Japanese carpet shells
1.48 ±0.16	2.85 ±0.43	1.32 ±0.08	2.16 ±0.03	1.40 ±0.06	2.43 ±0.04	3.07 ±0.68

The ratio's between the estimates for Shell Free Wet Weight (SFWW) show for all species higher values (x1.3 till x2.4) for estimates obtained from the AFDW than from the Tissue yield methods. This discrepancy might be possibly ascribed to the fact that the wet weights used for the calculation via the Tissue yield method were obtained from animals after fixation and therefore with very few water remaining within the shell.

Additionally the tissue yield method have two main drawbacks concerning the taxon specificity of the SFWW/WW ratio and the assumption made about the relative weight of the shells, what occurs to be the current variable that is questioned by the present study. On the other hand, whereas the estimate of shell free wet weight from the ash free dry weight is based on two successive conversion steps (AFDW->SFDW->SFWW) what could result in an accumulation or errors, it relies on robust relations about the relative ash and water content of shellfish tissue that are independent from assumptions about species and/or the relative weight of the shell. Therefore the choice has been made to draw the estimate of the shell weight from the wet weight (WW) directly available from the monitoring data and the shell free wet weight (SFWW2) obtained from the ash free dry weight (AFDW) also available from the monitoring data. This computation leads to estimates of shell weights that show a consistent relation with the individual weight expressed as AFDW or SFWW (Table A2).

Growth rate ratio as function of individual body mass

Brey (2012) proposes a model (Multi-Parameter Artificial Neural Network) to calculate the P/B ratio as function of the individual body mass, mean annual temperature, water depth, taxonomic classis and lifestyle information. For the present exercise we will hold fixed values for temperature (10°C) depth (10 m) and taxonomic classis (Mollusca).

The individual body mass required for this calculation is obtained from literature and direct measurements as mg AFDW/ind. after conversion to Joules (1 mg AFDW=17,55 J) according to Gnaiger & Bitterlich (1984) and Sadava & Orians (2000) as referred in Brey (2012).

An example of the predicted P/B obtained with the model by Brey (2012) is given in Figure 16 for a marine herbivorous sessile mollusc living at 10 m depth for year average temperature of 10 °C corresponding to our study area. The relation between the P/B ratio and the individual body mass as predicted by the model by Brey (2012) is adequately described with a segmented regression, linear for individual biomass below 1.5 mg and negative power for body mass larger than this value (Figure 16). The relation shown in Figure 16 depicts the importance of knowledge on individual body mass for any prediction about production.

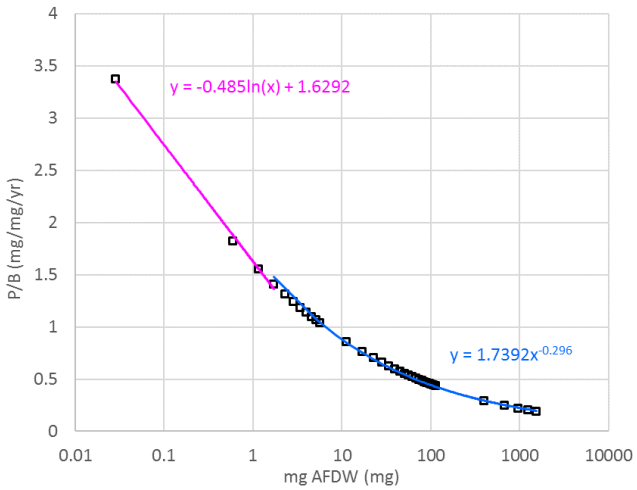


Figure A1.-P/B (mg/mg/yr) as function of the individual body mass (AFDW, mg) calculated with use of the model by Brey (2012) for a marine herbivorous sessile mollusc living at 10 m depth for year average temperature of 10 °C below (linear) and above (power function) 1.5 mg AFDW.

Estimated variables towards an estimation of carbon storage capacity in selected species

Consistent relation has been shown for the seven species selected for this evaluation between the individual weight (AFDW) and shell length, shell length and shell weight and between individual weight (AFDW) and P/B (mg/mg/yr). Therefore we can propose to estimate the shell weight accumulated within one year op basis van de shell length after conversion into individual weight used for the calculation of the annual yield in biomassa to be eventually converted into the shell weight accumulated within one year.

The different relations that have been parameterized here (Table A2) are suitable for the calculation of shell weight (accumulation) based on measurements available from monitoring programs such as individual weights (WW, SFWW, AFDW) or shell lengths. Eventually the shell and tissue weights either directly measured or calculated are converted into carbon using factors available from the literature as indicated in table A2.

Table A2.-Table including shell/tissue ratios

Species	y=log(mgAFDW) x=log(mm)	y=log(mgShell) x=log(mgAFDW)	y=log(mgShell) x=log(mgWW)
Mussels	y=2.913x-2.163	y=0.846x+1.194	y=0.959-0.143
Japanese oysters	y=2.535x-2.010	y=1.109x+1.0416	y=1.041x-0.352
Cockles	y=3.131x-2.098	y=0.908x-1.391	y=0.995x-0.1226
Razor clams	y=2.962x-2.970	y=0.881x+0.871	y=0.995x-0.249
Sand gapers	y=3.115x-2.4034	y=0.889x+1.206	y=0.975x-0.159
Baltic tellins	y=3.037x-2.033	y=1.027x+0.781	y=1.057x-0.439
Japanese carpet shells	y=2.580x-1.454	y=1.096x+1.051	y=1.0233x-0.223

The relations shown in Table A2 are established on data acquired from different areas, years and seasons. These differences combined with individual variations are responsible for the spread observed around the regressions calculated here.

Wageningen Marine Research
T +31 (0)317 48 09 00
E: marine-research@wur.nl
www.wur.eu/marine-research

Visitors' address
- Ankerpark 27 1781 AG Den Helder
- Korringaweg 7, 4401 NT Yerseke
- Haringkade 1, 1976 CP IJmuiden

With knowledge, independent scientific research and advice, **Wageningen Marine Research** substantially contributes to more sustainable and more careful management, use and protection of natural riches in marine, coastal and freshwater areas.

Wageningen Marine Research is part of Wageningen University & Research. Wageningen University & Research is the collaboration between Wageningen University and the Wageningen Research Foundation and its mission is: 'To explore the potential for improving the quality of life'