



Model development to assess carbon fluxes during shell formation in blue mussels

Auteur(s): Alicia Hamer, Edwin Foekema

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Author(s): Alicia Hamer, Edwin Foekema

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Summary

In order to quantify the amount of carbonate, precipitated as calcium-carbonate in the shells of blue mussel (*Mytilus edulis*) in a temperate climate, an existing Dynamic Energy Budget (DEB) model for the blue mussel was adapted by separating shell growth from soft tissue growth. Hereby, two parameters were added to the original DEB-model, a calcification cost [J/mgCaCO₃] and an energy allocation fraction [-], which resulted in the energy allocated for structural growth being divided between shell and meat growth. As values for these new parameters were lacking, they were calibrated by fitting the model to field data.

Calibration results showed that an Energy allocation fraction of 0.5 and a calcification cost of 0.9 J/mgCaCO₃, resulted in the best fit when fitted on 2017 and 2018 field data separately. These values however, show the best fit for data obtained within the first couple of years of the shellfish life, and do not take later years into account. Also it could be discussed that some parameters vary throughout the lifespan of the species. The results were compared to a regular DEB model, where the shell output was calculated through a simple allometric relationship.

It is sometimes assumed that the carbon storage in shell material as calcium carbonate could be regarded as a form of carbon sequestration, with a positive impact on the atmospheric CO₂ concentrations. However, studies on the physical-chemical processes related to shell formation have shown that from an oceanographic perspective, shell formation should be regarded as a source of atmospheric CO₂ rather than a sink. The removal of carbonates, through the biocalcification process, reduces the buffer capacity (alkalinity) of the water to store CO₂. As a result CO₂ is released from the water to the atmosphere when shell material is formed. The actual amount of CO₂ that escapes from the water to the atmosphere as a result of biocalcification depends strongly on local water characteristics.

In this study, the effect of calcification by mussels on the CO₂ flux to the atmosphere is studied using an adapted DEB model where energy costs of calcification are modelled explicitly. The model was subsequently run under two future climate scenarios, (RCP 4.5 and RCP 8.3) with elevated temperature and decreased pH, and the total released CO₂ as a result of shell formation was calculated with the SeaCarb model. This showed growth of mussels, under future climate conditions to be slower, and with that the cumulative shell mass and carbonate precipitated to CaCO₃ to decrease. Yet the amount of CO₂ released, due to biocalcification, increased. This is due to the fact that the amount of CO₂ released/gr of CaCO₃ precipitated will be higher, as a result of the decreased buffering capacity of seawater under future climatic environmental conditions.

In summary the conclusions of the project were:

- Biocalcification (shell formation) of marine organisms, such as bivalves, cannot be regarded as a process resulting in negative CO₂ emission to the atmosphere;
- The actual amount of CO₂ that, due to biocalcification, is released from the water to the atmosphere depends on the physicochemical characteristics of the water, which are influenced by (future) climate conditions;
- Our first model calculations suggest that at future climate conditions mussel's grow rate will be somewhat reduced. While the amount of CO₂ that due to biocalcification, escapes to the atmosphere during its life-time will slightly increase. Making the ratio of g CO₂ release/g CaCO₃ precipitated slightly higher;
- Our model calculations should be considered an exercise rather than a definite prediction of how mussels will respond to future climate scenarios. Additional information/experimentation is strongly needed to validate the model settings, and to test the validity of the above mentioned outcome of the model.

1 Introduction

Bio-based products can contribute to mitigate climate change. Long-term storage of carbon in bio-products and substitution of fossil based products could reduce atmospheric greenhouse gas concentration and contribute in the fight against climate change. Many examples are available for substitution of materials, chemicals and energy sources with bio-based alternatives (e.g. Brockmann *et al.*, 2015; Zhang *et al.*, 2019, Laurens & Nelson, 2020; Carina *et al.*, 2021), however, there is still a lack of integrated insights about the potential reduction of greenhouse gas emissions and the potential scale of applications with long-term carbon storage, as well as their environmental, social and economic effects. To identify and where possible fill in these knowledge gaps, forms the aim of the project *Negative GHG emissions and long-time sequestration through the development of new C-based products* (KB1-3D-1).

As part (WP4) of this project the potential of carbon sequestration in shell material produced by marine shellfish was investigated, thereby assessing the climate-robustness of today's bivalve production (bivalve shells) as well as for future scenario's. A conceptual framework and mass balance approaches for the carbon sequestration potential by shellfish aquaculture and fisheries for marine coastal zones in the Netherlands are presented in Jansen & van den Bogaart (2020). In that report it was pointed out that a better understanding of the process of carbon fixation by shellfish is required, before shellfish can be included in the quantification of carbon dynamics. It is sometimes assumed that the carbon storage in shell material as calcium carbonate could be regarded as a form of carbon sequestration, with a positive impact on the atmospheric CO₂ concentrations. However, studies on the physical-chemical processes related to shell formation (Humphreys *et al.*, 2022; Humphreys *et al.*, 2018; Morris *et al.*, 2019) have shown that from an oceanographic perspective, shell formation should be regarded as a source of atmospheric CO₂ rather than a sink. The actual amount of CO₂ that escapes from the water to the atmosphere as a result of biocalcification depends strongly on local water characteristics (Frankignoulle *et al.*, 1994; Ray *et al.*, 2018).

In order to get a better understanding of the process of shell formation and related calcium carbonate formation during the development of shellfish, an attempt was made to incorporate shell growth into an existing Dynamic Energy Budget (DEB) model for the blue mussel (*Mytilus edulis*). With the model, the amount of CO₂ that is released to the atmosphere as a result of shell formation of a mussel, was estimated under different climate scenario's.

This report briefly describes the relation between shell formation and atmospheric CO₂ (chapter 2); how the DEB model was adjusted to distinguish between the growth of shell and meat, and how the impact of future climate scenario's was included to assess the impact of bivalve shell formation on atmospheric CO₂ under actual and future climate conditions (chapter 3). The results of the calculations (chapter 4) are discussed (chapter 0).

2 Shell formation and atmospheric CO₂

Shells consist of more than 90% calcium carbonate (CaCO₃), 12% of which is carbon. One kg of empty shells thus contains approximately 120 grams of carbon, which corresponds to 440 grams of CO₂. Because calcium carbonate is extremely stable under most conditions, the carbon it contains is fixed for a long time. Therefore, it is sometimes claimed that shellfish aquaculture, by contributing to the formation of shells is a good way to compensate for CO₂ emissions (Tamburini *et al.*, 2022, Martini *et al.*, 2022). This assumption however, does not take into consideration some vital aspects of ocean chemistry. When taking a closer look at all the processes that are involved in the formation of shell from oceanic dissolved carbon, the formation can even be considered a slight source of CO₂ rather than a sink. To understand why some background knowledge about the interaction between atmospheric CO₂, ocean chemistry and the role of shell formation is essential. In the following chapters a simplified description of this complex relation is given, for more details and background information we would like to refer to Humphreys *et al.*, (2018) and Morris *et al.*, (2019).

2.1 Carbon, from air to ocean

Due to human activities, the CO₂ content of the atmosphere has rapidly increased over the last decades, causing a measurable rise in temperature on earth. This greenhouse effect would have been even stronger if about 25% of the CO₂ produced by humans had not been absorbed by the oceans (Middelburg *et al.*, 2020).

At the ocean surface, this absorbed CO₂ leads to ocean acidification. This is the result of hydrogen ions (H⁺) being released during the reaction of CO₂ with water (H₂O), during which carbonates (HCO₃⁻ and CO₃²⁻) are formed (Figure 1). The concentration of CO₂ in air strives for a balance with the concentration of CO₂ in water. The formation of carbonates causes the concentration of CO₂ in the water to decrease, so that more CO₂ can be absorbed from the air. As long as the reaction in Figure 1 moves from left to right, i.e. from CO₂ to carbonate, water can absorb CO₂ from the atmosphere. This will be the case as long as no strong accumulation of H⁺ ions occur.

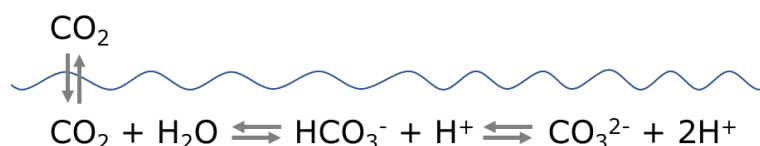


Figure 1 Equilibrium reaction between CO₂ and water (H₂O). The concentration of dissolved CO₂ in the water strives to an equilibrium with the concentration of CO₂ in the air above.

The capacity of water to absorb CO₂ therefore depends strongly on its ability to take up H⁺ ions. This capacity, referred to as the 'alkalinity' of the water, is largely determined by the presence of the dissolved carbonates HCO₃⁻ and CO₃²⁻. These carbonates can, as indicated by the negative charge, absorb 1 and 2 H⁺ ions per molecule, respectively.

Water with a high alkalinity has a surplus of dissolved carbonates in relation to H⁺ ions and can therefore absorb a lot of CO₂. Although dissolved carbonates are formed when CO₂ is absorbed into water, this does not change the alkalinity of the water. After all, in addition to the dissolved carbonates, an equivalent amount of H⁺ ions is also formed (Figure 1Figure 1).



Figure 2 Dissolution of calcium carbonate in water.

The alkalinity of water does increase when calcium carbonate (CaCO_3) dissolves (Figure 2). In this process, a dissolved carbonate molecule is formed and a dissolved calcium ion (Ca^{2+}), but no H^+ ion. While the formed carbonate can take up 2 H^+ ions.

A large part of the deep-sea consists of calcium carbonate. This comes from the remains of prehistoric calcifying foraminifera, algae, shells and corals that easily dissolve under the high water pressure, leading to high alkalinity in the deep sea. The oceans, therefore, potentially have a large capacity to absorb CO_2 , without this leading to serious acidification (Archer, 2005). However, the mixing of the carbonate-rich deep-sea water with the surface water where the CO_2 exchange with the air takes place, is very slow. It is roughly assumed that about 1000 years are needed for complete mixing of the ocean water (Archer, 2005). This is too slow to keep up with the speed at which CO_2 concentrations in the atmosphere/sea surface have been rising in recent decades.

2.2 Carbon from ocean to shell

Shellfish, and coral or calcareous algae alike, produce calcium carbonate from dissolved calcium and carbonate present in the water. Depending on the form of carbonate used, this reaction proceeds according to one of the equations in Figure 3 *Figure 3*.

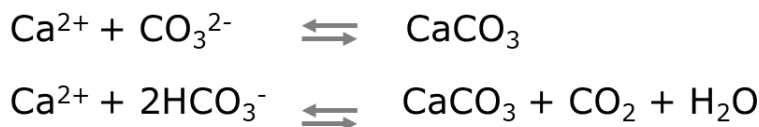


Figure 3. Two chemical reactions in which calcium carbonate (CaCO_3) is formed from calcium (Ca^{2+}) and carbonates (CO_3^{2-} and HCO_3^-) dissolved in water by shellfish, corals and calcifying algae. Note that both reactions result in a reduction of alkalinity, and thus result in reduced CO_2 absorption capacity of the water.

In both equations, after the formation of the shell, 1 carbon atom (or 1 CO_2 molecule) is fixed in CaCO_3 (the shell). The ability of the water to absorb H^+ ions (indicated by the negative charge of the molecules) is reduced from 2 to 0 in both cases. This basically creates an oversaturation of CO_2 , allowing it to escape from the water into the atmosphere.

The capacity to store CO_2 in seawater is thus reduced by the process of shell formation. How much of the CO_2 that is being released by this chemical process actually escapes to the atmosphere depends on the local conditions like alkalinity, temperature and CO_2 concentration in the atmosphere. In order to assess the quantity of CO_2 that will be released to the atmosphere under given conditions the SeaCarb package available in R statistical software (Gattuso *et al.*, 2022) was used to calculate a buffering coefficient based on environmental variables (see chapter 3.2).

It cannot be denied that carbon that is captured in the shell has been withdrawn from the carbon cycle. In the very long term this could have an effect on the amount of CO_2 in the atmosphere. In that case however, we are talking about geological time scales, which are not relevant to mitigate current climate effects. Carbon sequestration in shells can, therefore, not be used to offset¹ CO_2 emissions.

In conclusion, although carbon is sequestered in shells, this has no positive effect on the climate. This is because the carbon that is captured is not extracted as CO_2 from the atmosphere, but from dissolved carbonate and alters the alkalinity of the water. Subsequently, this reduced alkalinity, means shell formation leads to the release of CO_2 from water and ultimately to an increase in atmospheric CO_2 .

¹ A Carbon offset is a way to compensate for your emissions by funding an equivalent carbon dioxide saving elsewhere.

3 The adjusted DEB model

3.1 Dynamic Energy Budget Model

An elaborate description of the de base Dynamic Energy Budget (DEB) model for the blue mussel and its parameters are described in Wijsman (2019). The DEB model describes how an individual organism distributes its energy to maintenance, growth and reproduction. The model for the blue mussel currently only addresses total structural growth and does not differentiate between growth of shell and meat. In order to be able to distinguish the carbon dynamics related to the production of meat from the part that is related to the production of shell, in this report two methods are used to differentiate between shell growth and total structural growth. All parameters and forcing functions were taken directly from Wijsman (2019).

The first method involved running the existing DEB model as usual and calculate the shell weight from the shell length using the allometric relationship from Alunno-Bruscia *et al.* (2001).

$$W_{shell} = 1.8 \cdot 10^{-4} (L_w \cdot 10)^{2.5}$$

In which W_{shell} = shell weight [g], and L_w = the length [cm] of the whole organism.

As this method does not involve energy partitioning between meat and shell, the growth of meat is not affected by the growth of the shell and will proceed in an identical matter to the regular DEB model.

In the second method shell weight was added to the model as a state variable. To differentiate between shell and meat growth an energy allocation parameter, κ_s was added (Table 1) which divides the energy available for structure growth, see Figure 4. Another parameter was added that converts allocated energy [J] into shell growth in mg (E_S , Table 1). To choose the correct values for these parameters, the energy required for shell construction and the amount of energy allocated to shell growth by mussels, was required. However, as data, under the correct environmental circumstances, on both these parameters were unavailable/lacking, values were obtained by fitting model output to field observations (growth of length and weight of both shell and meat). Data used for this was obtained from the INNOPRO project, where mussel growth was monitored for 2 years (2017 and 2018) at 25 locations in the Wadden Sea and the Oosterschelde (Capelle, 2020). The model was fit to the data using the function `ModCost` in R (package: `FME`) and by varying the parameters κ_s and E_S . The best fit was obtained by calculating residuals for each observation i by comparing the modelled and observed value:

$$res_i = Mod_i - Obs_i$$

Then a cost was calculated by taking the square sum of these residuals for each variable. Set parameter values were then iteratively changed obtain a minimisation of this cost.

The calibration using both the datasets of 2017 and 2018 (both individually and together) resulted in the parameters $\kappa_s = 0.5$ and $E_S = 0.9$ J/mg $CaCO_3$. Those parameters were used in this study. In addition several calculations were also run with a much higher calcification cost of $E_S = 11$ J/mg $CaCO_3$, that was derived from Sanders *et al.* (2018), and different partitioning fractions (κ_s). These results are shown in appendix 1.

Table 1 Added parameters to the standard Mussel DEB model of Wijsman (2019) to differentiate between growth of meat and growth of shell.

Parameter	Unit	Description
κ_S	-	Energy allocation fraction to shell growth
$1-\kappa_S$	-	Energy allocation fraction to meat growth
E_s	J/mg CaCO_3	Converts joule to mg CaCO_3

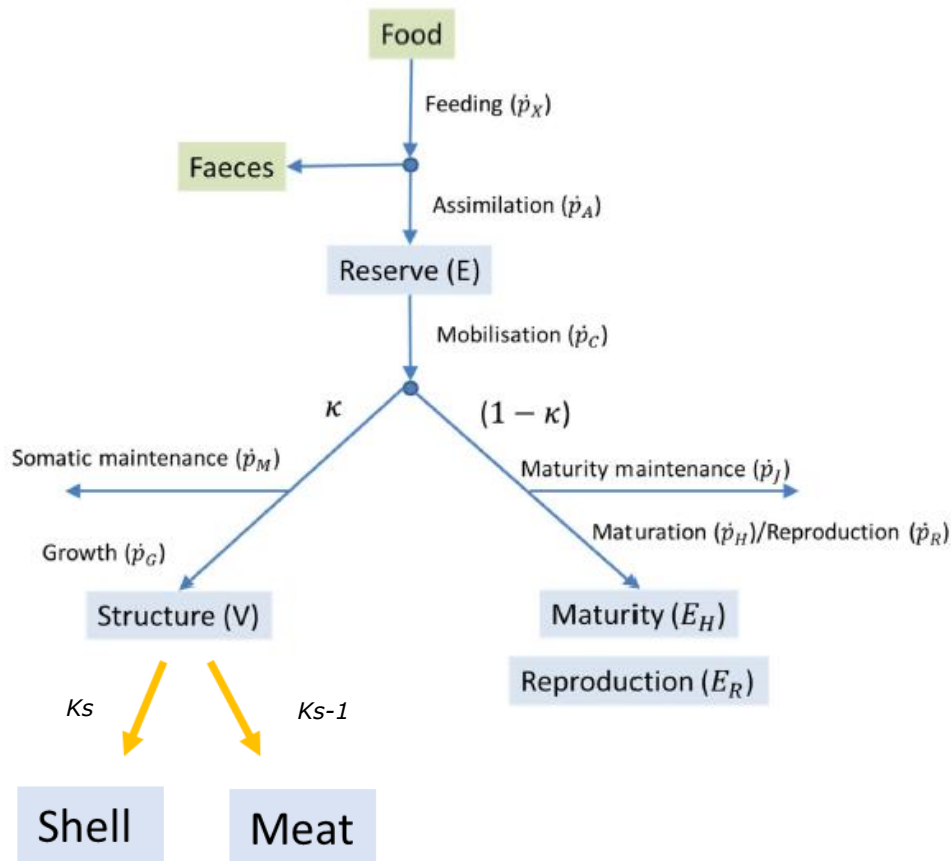


Figure 4 Adapted DEB model in which energy allocation to growth in structure is split into 'Shell' and 'Meat' structure.

3.2 Calculating carbon release

As described in chapter 2 of this report, the production of CaCO_3 (biocalcification) to form a shell reduces the alkalinity of the water and as such reduces the amount of CO_2 that can be absorbed as carbonate in sea water. As a consequence, biocalcification results in a net release of CO_2 from the water to the atmosphere. The ratio of CO_2 released per CaCO_3 precipitated is dependent on the seawater buffering capacity, the alkalinity, and can be expressed as Ψ (Frankignoulle *et al.*, 1994). The "Flag = 21" calculation in the SeaCarb package available in R statistical software was used to calculate Ψ from the pCO_2 , pH, temperature and salinity (Ray *et al.*, 2018; Gatusso *et al.*, 2011). From this value for Ψ , an estimation can be made of the amount of CO_2 released to the atmosphere associated with shell production, by using the following equation:

$$\text{CO}_2 \text{ Release} = \text{Shell Mass} * \Psi * 0.95 * \frac{44.01}{100.0869}$$

Here 0.95 indicates the fraction of CaCO_3 in the total shell mass, and 44.01 and 100.0869 represent the molecular masses of CO_2 and CaCO_3 , respectively.

The seawater buffering capacity, and thus the Ψ value, is affected by changes in environmental conditions, such as salinity, temperature, pH, and pCO_2 making it particularly sensitive to climatic changes (Ray *et al.*, 2018). Hereby, increased temperatures increase the buffering capacity (decreases Ψ value), whereas decreased salinity and decreased pH decrease the buffering capacity (increase Ψ value) of the water (Ray *et al.*, 2018). The decreased buffering capacity due to salinity and pH changes far outweigh the increased buffering capacity resulting from higher water temperatures. With climate change the overall Ψ is expected to increase by at least 17% by the end of this century, which on a large scale can result in significant additional carbon release from shell production (Ray *et al.*, 2018).

3.3 Climate Scenarios

Climate change will affect the biology of shellfish as well as the capacity of the oceans to store CO_2 . In order to test how these factors interact and affect the CO_2 distribution between water and atmosphere during the lifespan of a mussel, the DEB-model and the SeaCarb model were combined, and calculations for future climate scenario were made to assess the overall impact on biocalcification and physical-chemical conditions on the CO_2 cycle.

To describe alternative trajectories in atmospheric greenhouse concentrations from the years 2000 to 2100 the IPCC adopted the Representative Concentration Pathways (RCPs), a set of greenhouse gas concentration trajectories. Using integrated assessment models (IAM's), time series of greenhouse gasses are produced [ppt CO_2 -eq], based on economic and demographic growth, energy consumption and land use trends (IPCC, 2013). The CO_2 trajectory scenarios correspond to different climate scenarios (Figure 5), in which regional/global estimations and predictions can be made for, among others, expected atmospheric and/or oceanic temperature, pH, oxygen and precipitation changes (IPCC, 2013). For our study we used values from RCP 4.5 and RCP 8.5, the latter is characterised by increasing greenhouse gas emissions over time, while in the RCP 4.5 scenario these emissions stabilise shortly after the year 2100.

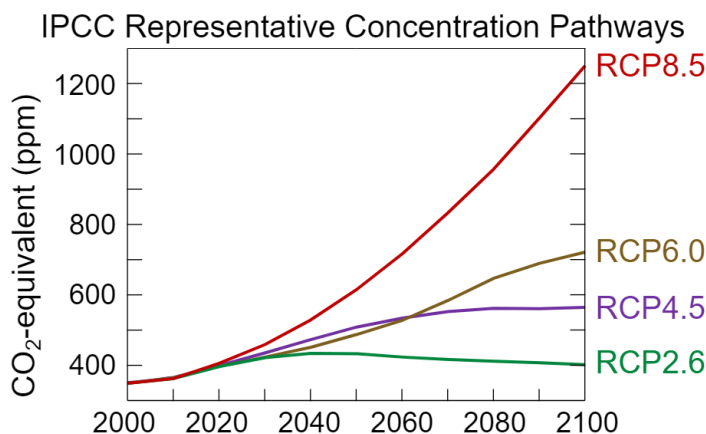


Figure 5 Four representative Concentration Pathways set up by the IPCC (IPCC, 2013)

3.3.1 Temperature

In the DEB model, ingestion rate, assimilation rate, utilisation rate and maintenance rate are all affected by temperature through a so called Arrhenius function (Figure 6). Here the parameters are scaled according to this function, which shows an optimum of $\sim 20^\circ\text{C}$ for this species. So in a temperature range from 1 to 20°C the utilisation rate of incoming energy increases, but the maintenance costs also increase. At higher temperatures, thus beyond the optimum, all processes become less efficient.

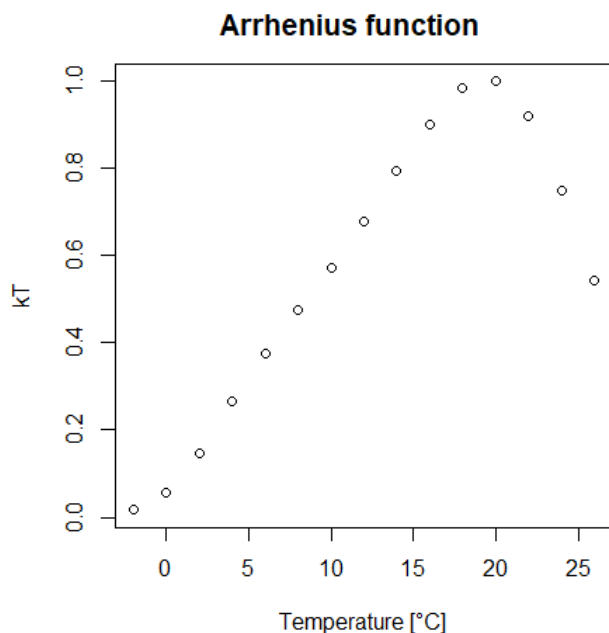


Figure 6 Arrhenius function as used DEB model for the blue mussel (*Mytilus edulis*)

In order to assess the impact of climate change on de shell production of blue mussels, three scenarios, based of temperature data from the 2100 IPCC climate projections, were tested and compared to current reference scenarios, by adding the predicted °C increase to the current temperature input at each timestep in the adjusted DEB-model. This was done by adding it onto the forcing function for temperature described in Wijsman (2019). Hereby, only the direct effect of increased water temperature on the physiology of the organism was taken into consideration. Other or indirect effects that increased temperatures may have on input data, such as on food availability and timing of reproduction were not taken into account.

Table 2 Values used in simulations for the projected average sea surface temperature (SST), pH, pCO₂ and Salinity towards the end of the 21st century under different RCP scenarios in the North Sea (Sources used: IPCC 2013; Ray et al., 2017).

	SST increase [°C]	pH	pCO ₂ [PPM]	Sal [PPT]
Current situation	0	8.1	400	35
RCP 4.5	2	7.9	550	35
RCP 8.5	4	7.7	1200	35

3.3.2 pH

Under the most extreme climate scenario RCP 8.5, the average pH is expected to fall from approximately 8.1 to 7.7 by 2100. However, local and seasonal pH values can differ due to it being so closely linked to biochemical processes, algal growth, and bacterial decay. Since primary production is driven by the availability of nutrients and (sun)light, and bacterial decay by the availability of degradable organic matter and temperature, this pattern may vary greatly between regions and seasons. pH fluctuates throughout the season, especially in coastal areas with a high primary production (such as the Wadden Sea). During spring, when CO₂ consumption via primary production is at its highest, the water contains the lowest levels of CO₂, making it more alkaline (Van Oijen, 2011).

pH is not included in standard DEB models. However, some biological processes are highly dependent on pH levels, especially that of calcifying organisms. Several studies applying DEB models, have proven to be useful in assessing the effects of acidification changes in bivalves. One of these studies (Maynou

et al., 2020), measured the difference in food uptake ('clearance rate' determined by cell density) and growth under different climatic temperature and pH prediction levels, for Manila clams (*Ruditapes philippinarum*). They then re-calibrated model parameters under these different conditions. They found that at lower pH, the volume specific maintenance cost (Pm) decreased, and then used these parameters for future growth predictions.

Due to the reduced maintenance costs, increased growth rates are predicted with lower pH values. These results do not coincide with other studies in which, simulation/observations show slower growth rates at lower pH values. A more likely outcome would be that the maintenance costs for calcifying organisms increase with declining pH values (Klok *et al.*, 2014). Since besides the study of Maynou *et al.* (2020), no data was found how the maintenance costs of marine bivalves respond to changes in pH, we used an arbitrary 10% increase of the maintenance costs for our calculations, for the most extreme climate scenario (RCP 8.5). During the RCP 4.5 simulation, the maintenance cost was not increased.

3.3.3 Salinity

Salinity was kept at a constant of 35ppt as these changes in coastal areas will either be negligible or very local.

3.4 Climate scenario carbon calculations

The adjusted DEB model was used to estimate the shell growth over the life span of a blue mussel. Subsequently the outcome of the different shell growth predictions was inserted into the SeaCarb package and from there the released amount of CO₂ were calculated as described in Ray *et al.*, 2018. The pH, pCO₂, values inserted into the Flag = 21 calculation were set to those corresponding to each RCP scenario according to the IPCC predictions. It should be realised that only shell formation and physicochemical characteristics are taken into account in these calculations. Other processes that also effect CO₂ fluxes, like primary production and respiration, are not included.

4 Results

4.1 Allometric model vs adjusted DEB Model

The results of both the allometric model and the adjusted DEB model, with the optimal parameters obtained, are shown in Figure 7 and Figure 8 respectively. In the allometric model individual mussels seem to initially grow more rapidly in length and weight, yet slow down more rapidly giving the other model chance to catch up.

The largest difference between the two models can be seen in the development of shell weight over time. Here too the allometric model shows a faster growth speed initially, yet the growth speed slows down proportionally to the growth and flattens out at a shell weight of around 10 grams, whereas the adjusted DEB model reached a shell weight of around 17 grams at the end of the modelled 9-year period. Since maximum shell length is similar in both models the DEB model thus predicts a thicker shell than the allometric model. Shell thickness of blue mussels is strongly related to the conditions under which the organisms lives. Turbulent, rough conditions stimulates the animal to create a thicker shell as protection (Briones *et al.*, 2014).

As the climatic conditions, and thus the seawater buffering capacity, were the same in these model runs, the difference in CO₂ released to the atmosphere is purely the result of the amount of CaCO₃ precipitated into shell (Figure 8). This amounts to a cumulative total of approximately 5 g of CO₂ being released from the seawater per individual full grown mussel for the adapted model and approximately 3g being released per full grown mussel for the allometric model, when reaching the same length of approximately 8 cm after the 9 year run.

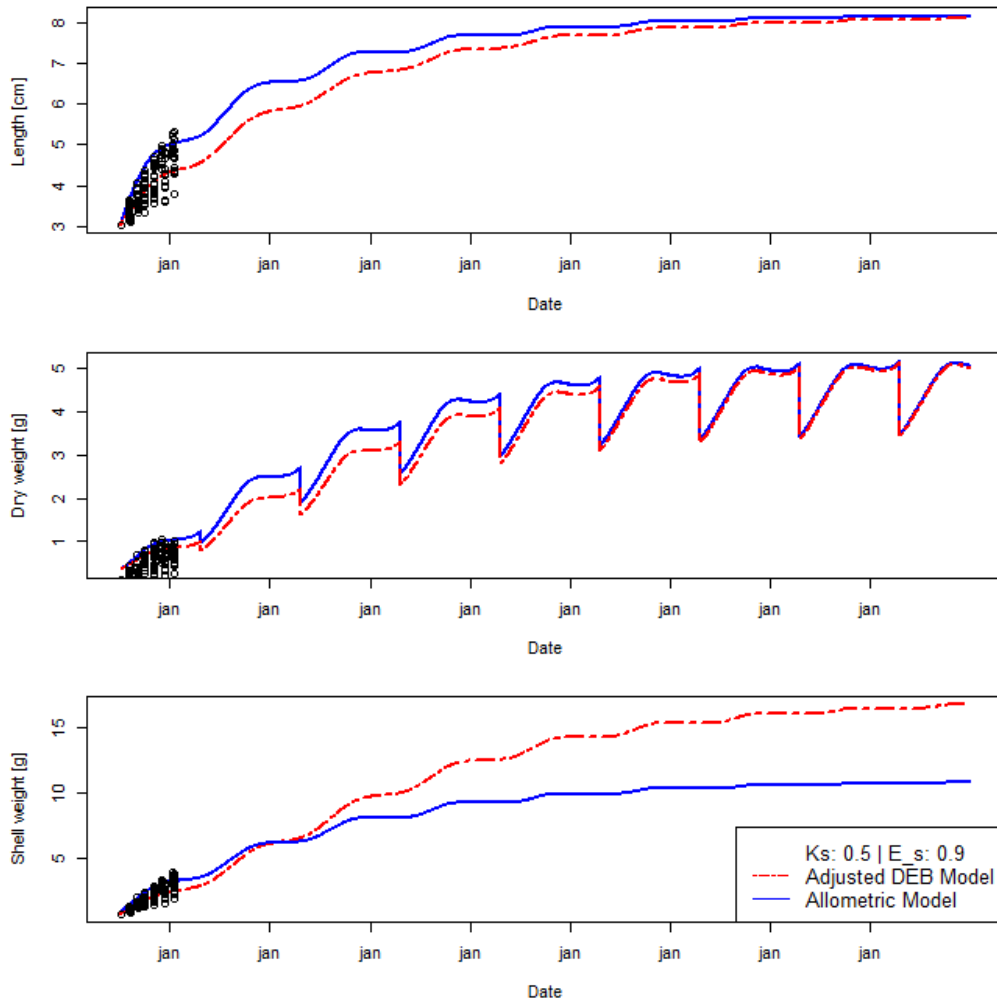


Figure 7 Simulated growth of length, dry tissue weight and shell weight over a 9 year period, when using an allometric model to calculate the shell weight (blue) and the adjusted DEB model (red) when using calibrated parameters for k_s and E_s to minimise the error on the growth data, which is indicated with circles and obtained from Capelle, 2020.

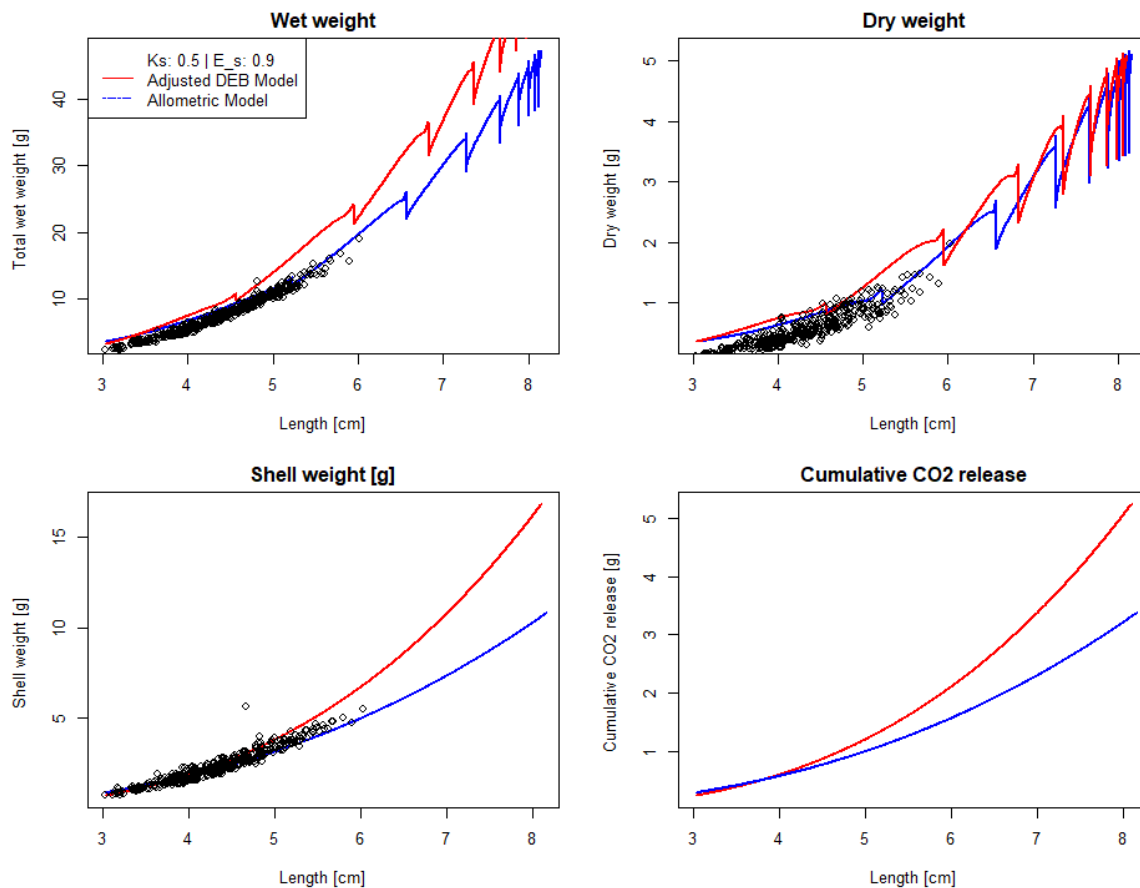


Figure 8 Total wet weight, dry tissue weight, shell weight and cumulative CO₂ release relative to shell length calculated using an allometric model (blue) and the adjusted DEB model (red), when using calibrated parameters for K_s and E_s to minimise the error on the growth data, which is indicated with circles and obtained from Capelle, 2020.

4.2 Climate scenarios

Figure 9 and Figure 10 show the results of our calculations using the adjusted DEB model, with the optimal parameters obtained, under the environmental conditions of the current situation and under RCP scenarios 4.5 and 8.5. Although at higher temperatures the utilisation rate of incoming energy increases for the mussel, the maintenance costs increase as well. Overall this results in a net lower growth rate. This becomes especially apparent when the mussel becomes larger which results in relatively higher maintenance costs. This can be seen in the graph as the models show a diverging trend when time increases.

Our model predicts that (shell)growth of a mussel will be slower under future climate conditions, and with that the cumulative shell mass and carbonate precipitated to CaCO₃ will also decrease. However, the amount of CO₂ that is released to the atmosphere due to shell formation is expected to increase (Figure 10).

This is due to the fact that the amount of CO₂ released/gr of CaCO₃ precipitated increased, as a result of the decreased buffering capacity under future climatic environmental conditions (Table 3) (Ray *et al.*, 2018).

Table 3 Values for attained length, cumulative CO₂ release due to shell formation, and the fraction released during 9 years of life span of a blue mussel .

RCP Scenario	Length [cm]	CO ₂ Release [g]	CO ₂ Release/CaCO ₃ Precipitated	Increase relative to current situation
Current situation	8.096	5.302	0.329	-
RCP 4.5	8.022	5.369	0.342	3.95%
RCP 8.5	7.915	5.577	0.370	12.5%

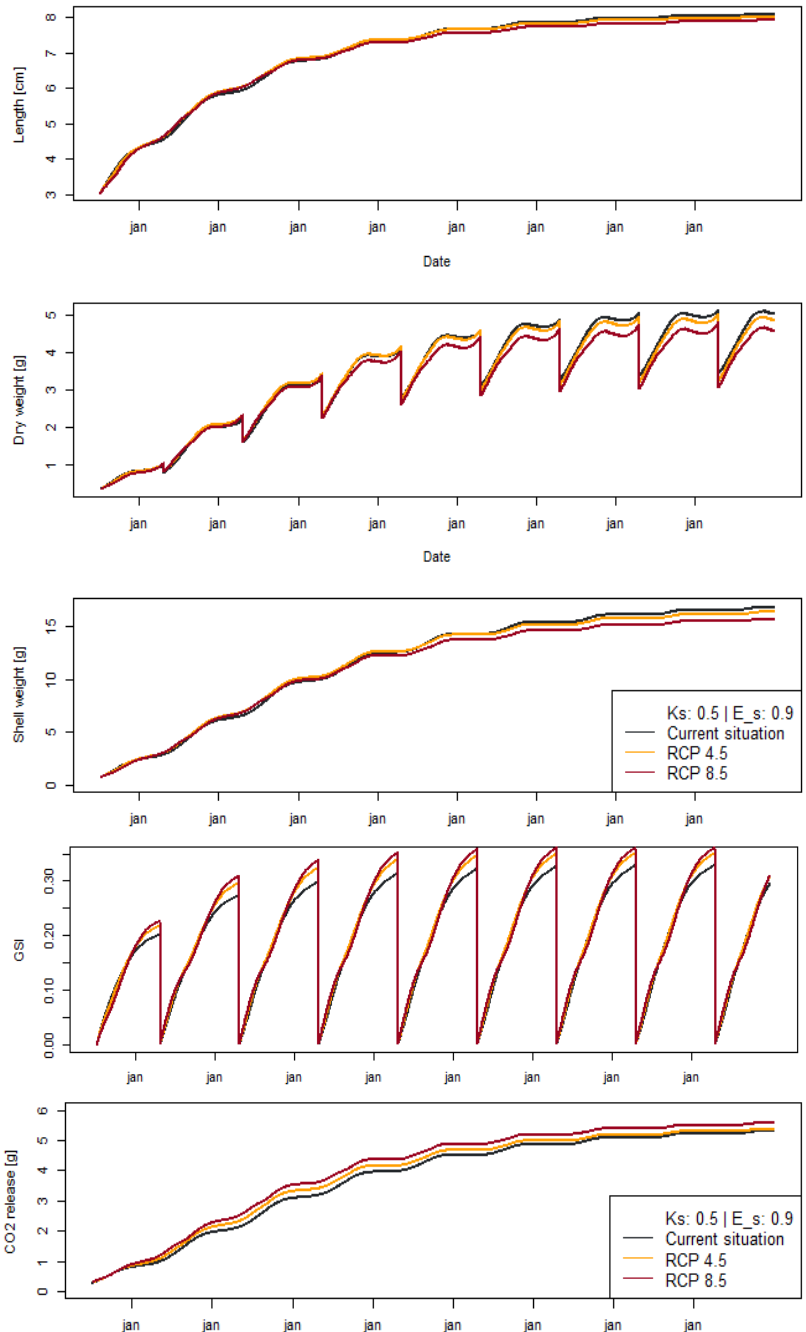


Figure 9 Simulated growth of length, dry weight, shell weight, GSI and cumulative CO₂ release over a 9 year period using the adjusted DEB model with the calibrated parameters for k_s (0.5) and E_s (0.9). Orange and red lines indicate when an increase to the temperature forcing functions was added to simulate the average increase in temperature according to a 4.5 RCP (+2°C) and 8.5 RCP (+4°C) scenario.

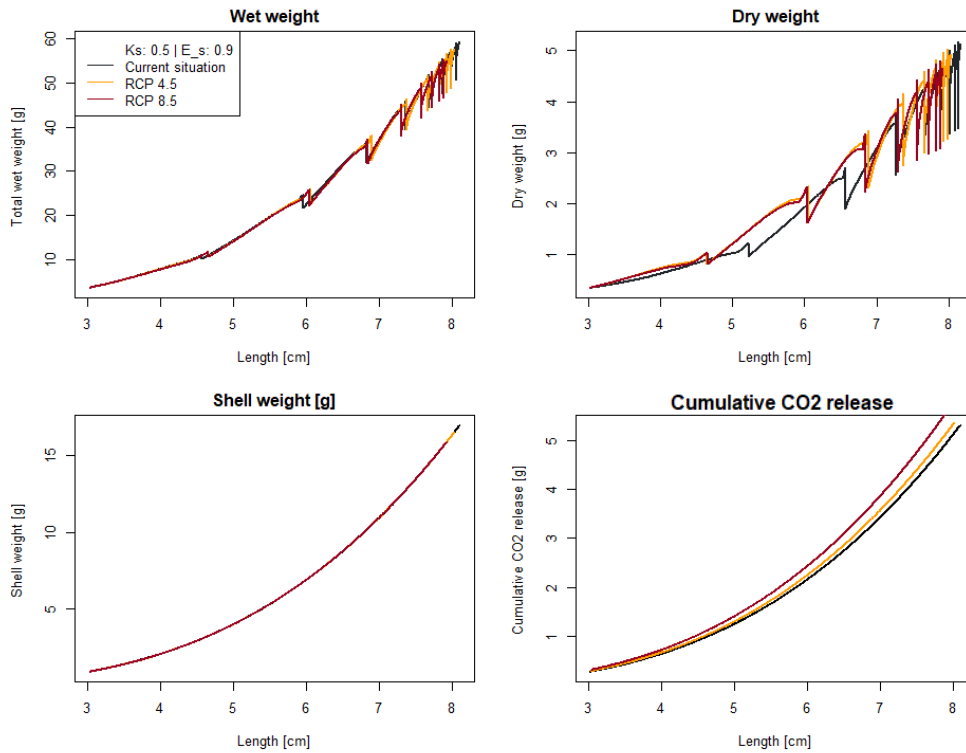


Figure 10 Wet weight, dry weight, shell weight (g) and cumulative CO₂ release due to shell formation (g) as a function of shell length (cm) calculated with the adjusted DEB model with the calibrated parameters for K_s (0.5) and E_s (0.9). Orange and red lines indicate when an increase to the temperature forcing functions was added to simulate the average increase in temperature according to a 4.5 RCP (+2°C) and 8.5 RCP (+4°C) scenario.

5 Discussion

5.1 Shell formation as source for atmospheric CO₂

In various peer reviewed scientific papers (e.g. Aubin *et al.*, 2018; Tamburini *et al.*, 2022), it is stated that shellfish aquaculture results in a negative carbon footprint due to the long-term fixation of carbon in shell material. In other papers (e.g. Humphreys *et al.*, 2018), however, this claim of durable carbon sequestration is rejected. The removal of carbonates, through the biocalcification process, reduces the buffer capacity (alkalinity) of the water to store CO₂. As a result CO₂ is released from the water to the atmosphere when shell material is formed. Among shellfish researchers who study the carbon footprint of shellfish aquaculture (e.g. Álvarez-Salgado *et al.*, 2022), there is a growing recognition that calcification by shellfish leads to a net flux of CO₂ into the atmosphere.

5.2 Assumptions made in our models

The allometric model predicted faster initial growth than the adjusted DEB model, but did not predict differences in ultimate shell length. A more striking difference between both models is the shell weight that is substantially higher in the DEB-model. Since the size of the shells are similar in both model predictions, the higher shell weight means that the DEB model predicts that a thicker shell is being formed. Thickness of the shell, that varies with environmental conditions and cultivation methods, forms an important factor in the amount of CO₂ that is released and need to be taken into account (Briones *et al.*, 2014; Gallardi & Murray 2022). It is important to note that there may be significant variation between data used to calibrate the model, particularly when data are obtained from different regions or cultivation methods. In some cases, this variation may even exceed the differences generated by the climate scenarios themselves. It should also be noted that the allometric regression line used to establish the relationship between shell weight and mussel length in the allometric model, was fitted to mussel growth data collected over a period of only two years (Alunno-Bruscia *et al.*, 2001). However, given that the model is being used to predict over a much longer period, it is possible that this relationship may not be applicable to larger lengths of mussels.

A previous study, conducted at low-salinity Baltic sea conditions, suggested that the energy cost of biocalcification in benthic mussels ranged from 11 to 58 J/mgCaCO₃ (Sanders *et al.*, 2018). These energy costs showed to be highly dependent on salinity: the highest costs (~58 J/mg CaCO₃) were observed at the lowest salinity tested (6 PSU) and the lowest cost (~11 J/mg CaCO₃) was observed at the highest salinity tested (16 PSU). Both salinity values are low compared to the Wadden Sea and the Oosterschelde that roughly ranges between 27 and 32 PSU.

Initially the modified DEB model was run with the lowest biocalcification cost of Sanders *et al.* (2018), of 11 J/mg CaCO₃. This resulted, however, in unrealistically low growth rates being obtained, even when the κ_S was set to be very high (see appendix 1). We therefore assumed it to be unlikely that, under the salinity conditions relevant for our data, the calcification cost for mussels are that high. Subsequently the model was calibrated on INNOPRO data, which resulted in best fit for a κ_S :0.5 and a E_S :0.9 J/mg CaCO₃. However, the INNOPRO dataset only followed the meat and shell development for two early years of the mussels life. Similar to the allometric relationship described earlier, the current model does not include growth data from the entire growth period of the mussel. To obtain more accurate parameters that can represent the entire lifespan of individual mussels, it is recommended that future studies include growth data collected over longer periods of time.

We assumed that the maintenance costs of a blue mussel will increase with 10% when the pH drops from the current 8.1 to 7.7, as in the worst ICP scenario, due to a lack of reliable data available. Without good reference it is unclear if this is a realistic assumption. Cockles that were exposed in mesocosms to

an extreme low pH of 6.95, increased their maintenance cost by more than 100% (Klok *et al.*, 2014). The average seawater pH isn't expected to drop below 7.7 during even the most extreme climate scenario, and certainly won't drop to 6.95 as an annual global average, but it is possible that the 10% increase that we use in this study forms an underestimation. On the other hand organisms like mussels that live in nutrient rich shallow (estuarine) areas are used to deal with fluctuations in pH. In the Wadden sea for instance the seasonal difference between pH values can be as much as 0.4. Which in itself is larger than difference of the current average to predicted future average (Van Oijen *et al.*, 2011). It is therefore clear that for a more accurate prediction of the impact of pH on the growth and shell formation of blue mussels additional experimental data is needed.

Under future climatic conditions (both RCP 4.5 and RCP 8.5) simulated growth was slower and mussels obtained slightly smaller final lengths. Regardless of this reduction growth rate the amount of CO₂ released still increased. This is due to the fact that the amount of CO₂ released/gr of CaCO₃ precipitated increased, as a result of the decreased buffering capacity under future climatic environmental conditions. In this stage our model only covered the direct effect of increased water temperature on the mussel growth. Other effects of increased temperatures such as impact on food availability and seasonal timing, could also affect the outcome of the calculations. In the future this could be assessed by combining the modified DEB model with the DEMO model as has been proposed by Wijsman (2020). Furthermore, in our calculations we also only looked at the CO₂ fluxes related to shell formation, other aspects like respiration and primary production that will also be affected by climate change, and that affect the CO₂ cycle were not taken into account.

Given what's indicated above, it must be made clear that the model calculations presented in this report should be considered an exercise rather than a definitive prediction of how mussels will respond to future climate scenarios.

6 Conclusions

In summary the conclusions of the project were:

- Biocalcification (shell formation) of marine organisms, such as bivalves, cannot be regarded as a process resulting in negative CO₂ emission to the atmosphere;
- The actual amount of CO₂ that, due to biocalcification, is released from the water to the atmosphere depends on the physicochemical characteristics of the water, which are influenced by (future) climate conditions;
- Our first model calculations suggest that at future climate conditions mussel's grow rate will be somewhat reduced. While the amount of CO₂ that due to biocalcification, escapes to the atmosphere during its life-time will slightly increase. Making the ratio of g CO₂ release/g CaCO₃ precipitated slightly higher;
- Our model calculations should be considered an exercise rather than a definite prediction of how mussels will respond to future climate scenarios. Additional information/experimentation is strongly needed to validate the model settings, and to test the validity of the above mentioned outcome of the model.

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Justification

Report C005/23

Project Number: 4315100172

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: Dr. Ir. J.W.M. Wijsman
Colleague scientist

Signature:



Date: 7 maart 2023

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

Signature:



Date: 7 maart 2023

Appendix I: Using values from Sander *et al.* (2018)

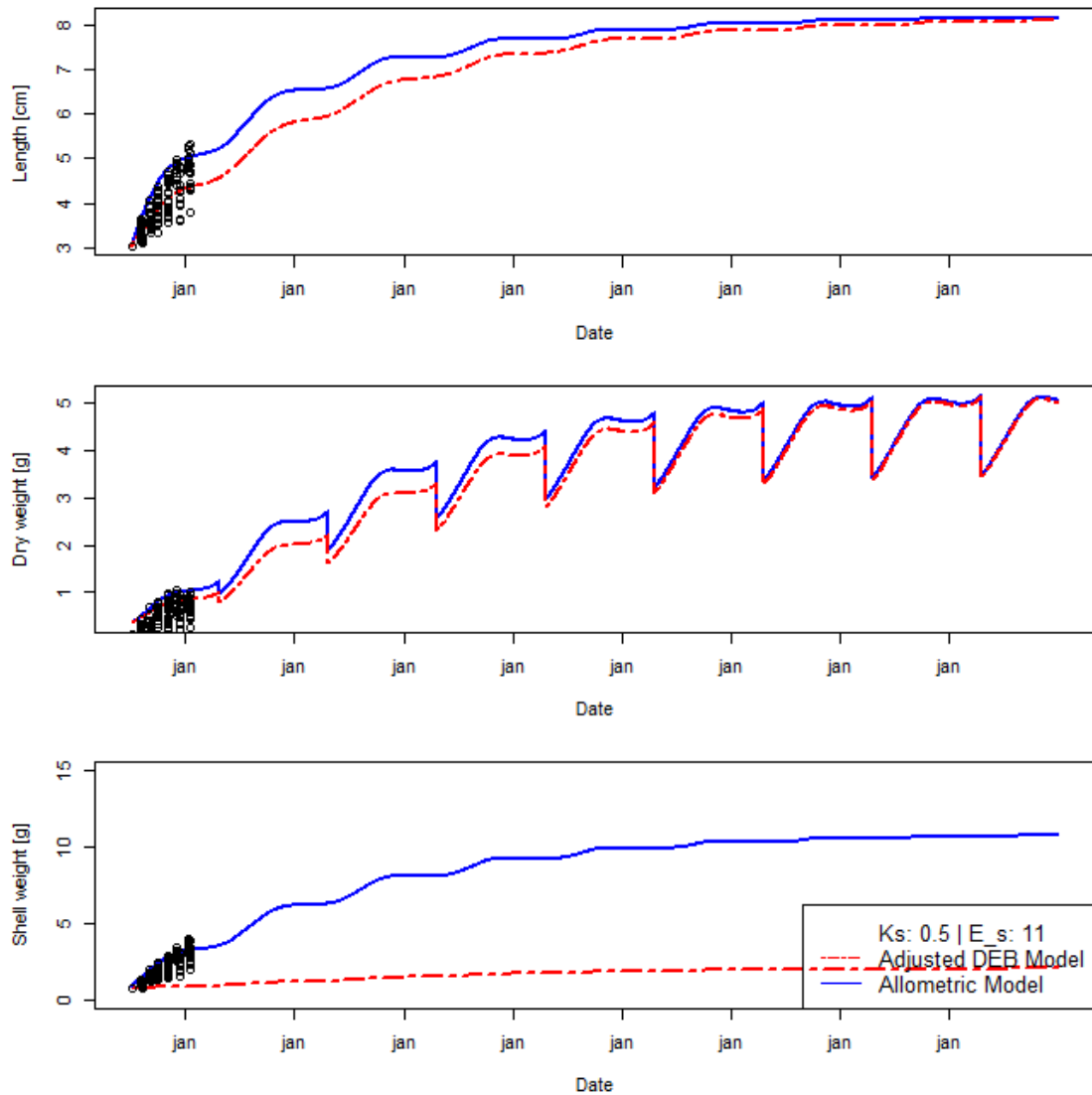


Figure 7 Model results comparison between a base model using the allometric relationship (blue) and a model using the adjusted DEB model described in this paper when the calcification cost value obtained from sanders *et al.* (E_s 11 J/mg $CaCO_3$) was used with an allocation fraction k_s of 0.5.

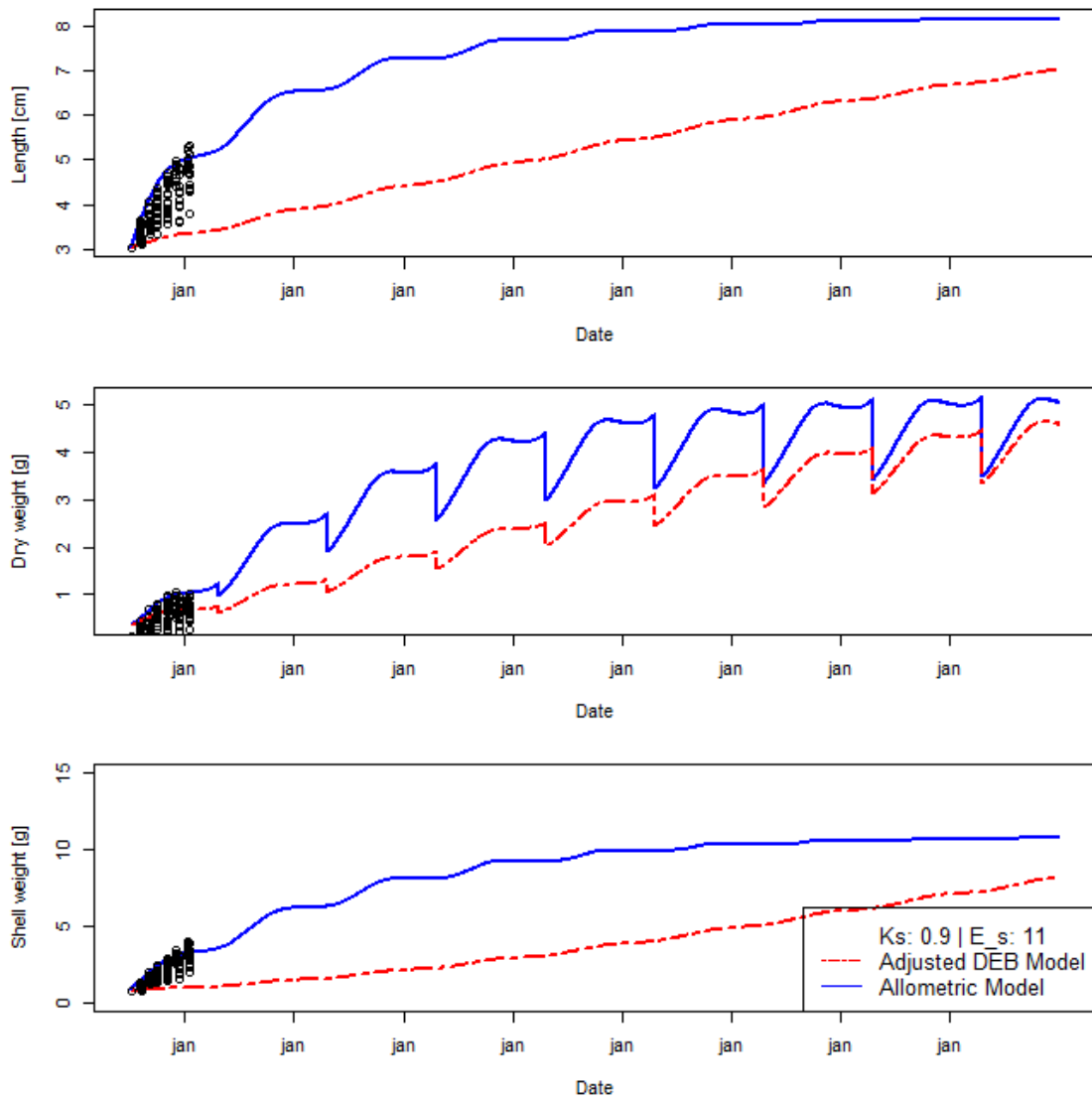


Figure 8 Model results comparison between a base model using the allometric relationship (blue) and a model using the adjusted DEB model described in this paper when the calcification cost value obtained from Sanders et al. (E_s 11 J/mg CaCO_3) was used with an allocation fraction K_s of 0.9.

Wageningen Marine Research
T +31 (0)317 48 7000
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

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