



A bioenergetic model to address carbon sequestration potential of shellfish farming: example from *Ruditapes philippinarum* in the Venice lagoon

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Shellfish are increasingly been looked at as sustainable food sources that provide additional ecosystem services. However, their role as CO₂ sinks or sources is still debated. To quantify it, shell accretion dynamics should be accounted for. However, the shell component is usually calculated with allometric scaling, in most bivalve models. With this study, shell accretion was added to a bioenergetic model of the Manilla clam (*Ruditapes philippinarum*), and a parameter linking assimilated energy towards shell growth (K_{SH}) was calibrated for four sites, where clams from the same cohort were monitored during a transplant experiment. The model was then used to calculate CO₂ fluxes resulting from respiration and shell calcification, taking into account CaCO₃ stocked in the shell and CO₂ emission. The findings show that K_{SH} varied slightly among sites and that clams played a role as a moderate sink of CO₂ over a whole year. CO₂ fluxes were characterized by a seasonal variability: clams were net sources in wintertime and sinks in summer time, suggesting the need of accounting for seasonality and growth should shellfish farming be included in carbon markets.

Keywords: aquaculture, CO₂ fluxes, ecosystem services, manilla clam, shell accretion

Introduction

Along with the increasing emphasis on sustainability, the balance between aquaculture development and good environmental conditions has become a requirement, posing new challenges to both research and commercial practices (Wijsman *et al.*, 2019). Shellfish, particularly bivalves, have long been recognized as “key players” in ecologically sustainable aquaculture (Shumway *et al.*, 2003); a recent review (van der Schatte Olivier *et al.*, 2020) estimated the value of the multiple ecosystem services provided by bivalve shellfish culture at \$6.47 billion worldwide. Nonetheless, attention should be paid to the site-specific ecological and social carrying capacity of shellfish culture (McKindsey *et al.*, 2006; Kluger, 2020), as some negative impacts are also to be expected, for example, space com-

petition with wild species (e.g. Sequeira *et al.*, 2008) with potential repercussions for wild shellfish fisheries, or space competition with other activities such as recreation (e.g. Holden *et al.*, 2019).

One of the ecosystem services that have received attention is the carbon sequestration potential of shellfish (Filgueira *et al.*, 2015). By growing shell material made of CaCO₃, they can act as a sink, but the release of CO₂ from the calcification process needs to be taken into account in a CO₂ budget of shell accretion. Furthermore, respiration is always a CO₂ source. The net balance depends on both physiological processes, linked to water temperature and seston composition (Bayne, 2009), and environmental conditions governing calcification rates (Gazeau *et al.*, 2007). Therefore, understanding and modelling shell growth dynamics, which is likely to differ among locations and culture methods (e.g. on the seabed

vs. suspended cultures, in sheltered vs. exposed sites), is essential for including shellfish aquaculture into climate adaptation schemes. This will also be necessary for including this ecosystem service in spatially explicit methodologies for the selection of allocated zone for aquaculture (FAO, 2019). In fact, there is still an ongoing debate as to whether bivalves can be considered as sinks (Turolla et al., 2019), or source [e.g. *Potamocorbula amurensis* (Chauvaud et al., 2003), *Ruditapes philippinarum* (Mistri and Munari, 2012), *M. galloprovincialis* (Munari et al., 2013), *Crassostrea gigas* (Lejart et al., 2012)] of CO₂. This points to gaps in both field studies and modelling investigations, aimed at elucidating the dynamic of energy allocation and calcification in relation to site-specific environmental forcing. First, growth is site specific due to a dependency on available food and temperature. This site specificity in attainable sizes influences individual respiration rates (linked to weight), which should be considered as an important CO₂ source. Second, the CO₂ emitted during the calcification process depends upon the function ψ , which represents the potential amount of CO₂ released by mollusc calcification. In turn, this function depends on water temperature, pH, alkalinity, and salinity; therefore, this term of the CO₂ budget depends both on shell growth and on site-specific environmental variables (Morris and Humphreys, 2019).

To gain a deeper understanding of growth dynamics, individual models based on ecophysiology are useful tools. Most of the bivalve bioenergetic models include soft tissues as a state variable and provide shell length as an output variable, derived from allometric relationships (e.g. Brigolin et al., 2009; Duarte et al., 2010; Larsen et al., 2014). However, shell growth can be an energetically demanding process, precluding the use of that energy towards other processes (i.e. soft tissue growth, reproduction). In some situations, an organism may invest more in shell growth, resulting in trade-offs with soft tissues, for example, if an individual feels threatened by predators (e.g. Fässler and Kaiser, 2008). Moreover, environmental variables [e.g. Sea Surface Temperature (SST), salinity] may also influence shell thickness and its resistance to breakage (Gizzi et al., 2016), as indicated by latitudinal gradients in shell production costs (Watson et al., 2017). A greater investment towards shell at the expense of soft tissue may also result in a reduction of the condition index (a ratio of tissue weight and shell weight, Lucas and Beninger, 1985), thus influencing market values. Predicting shell weight as an independent variable would therefore be useful not only to solve the dilemma of bivalve molluscs acting as a sink or source (Smaal et al., 2019) in a site-specific context (Filgueira et al., 2015), but also to include the condition index in the set of criteria to be used in site selection study (Sasikumar and Krishnakumar, 2011) and evaluate shellfish productivity under different scenarios of climate change. Only recently, shell production was included into dynamic energy budget models for mussels (e.g. Fuentes-Santos et al., 2019), but there is still lack of applications available for other shellfish species of high ecological and economical interest [e.g. oysters and clams, which make together 50% of molluscs aquaculture (FAO, 2019)]. In this regard, Manila clam (*R. philippinarum*) lends to be included as suitable species for modelling because of its ecological and commercial relevance. Approximately, 4 million tonnes of Manila clam are produced by aquaculture globally (FAO, 2019). Furthermore, this filter-feeding bivalve species represents a high-value seafood product in Europe, mainly produced in the Adriatic Sea of Italy (Scarcella and Cabanelas, 2016), where circa 30.000 tons are being produced yearly (Turolla et al., 2019).

In this work, *R. philippinarum* aquaculture in the Venice lagoon was used as a model system to:

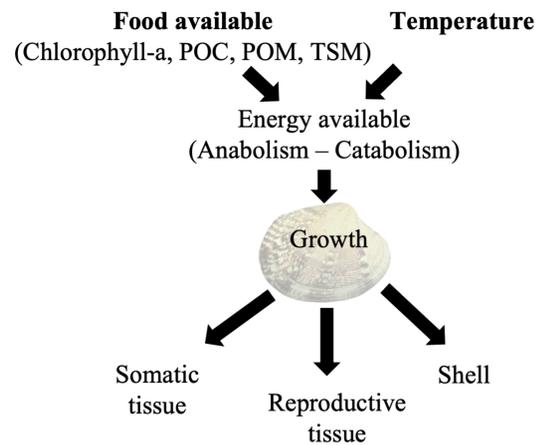


Figure 1. Conceptual diagram representing energy partitioning in the *R. philippinarum* individual model (model equations are provided in Appendix A).

- (1) develop and test an individual bioenergetic model including as state variables somatic tissue dry weight, reproductive tissue and shell weight;
- (2) understand the effect of spatial variability on energy allocation towards shell; and
- (3) estimate the seasonal evolution and spatial variability of CO₂ fluxes associated with clam culture during one year, taken as representative of the local environmental conditions.

Method

Model theory

The individual model is based on the dynamic estimation of the Scope for Growth (SfG), which is given by the difference between net anabolism and fasting catabolism. The SfG was partitioned between soft tissue growth, shell growth, and gonadal mass increment. The model includes three state variables, namely somatic tissue dry weight, gonad dry weight, and shell weight (all in grams). The model equations are presented in Supplementary Table S1 and the model parameters are presented in Supplementary Table S2. The SfG was allocated among the variables (see Figure 1) in accordance with two coefficients: K_R (energy towards reproduction) and K_{SH} (energy towards shell). The energy was allocated in reproductive tissues if environmental conditions were favourable for reproductive investment (based on minimum temperature below which reproduction investment is null). Spawning was treated as a single “event” based on reaching a level of maturity (as indicated by the Gonado–Somatic Index, representing the quantity of reproductive tissue compared to the whole soft tissue weight, here identified based upon experimental works, see Supplementary Table S1). Shell growth was included following (Fuentes-Santos et al., 2019). The energy content of the shell was considered for the inorganic and organic parts using literature data. The parameter “ K_{SH} ,” which quantifies the fraction of SfG allocated into shell growth, was estimated in this study (see model set up).

Samples and experimental sites

Twenty thousand spats of Manila clam (*R. philippinarum*), supplied by Satmar Company (France) in August 2018, were placed in a

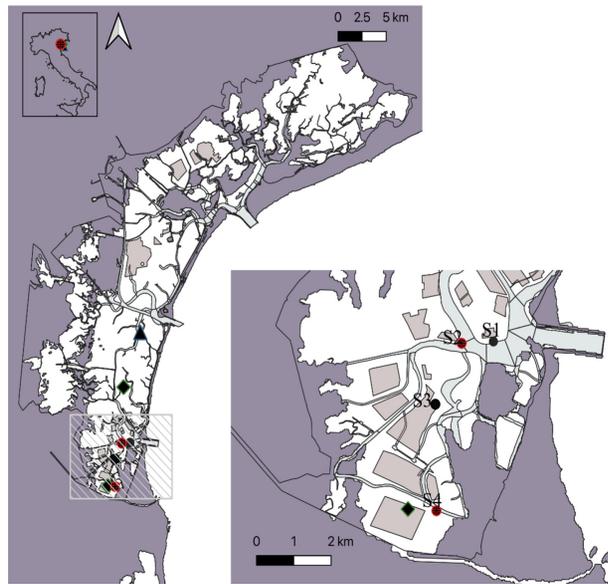


Figure 2. Map of study location. Showing the general area within the Venice lagoon and then a zoomed-in map to show the specific locations of the four sites. Grey shaded areas in the lagoon representing clams concessions. Dots represent experimental sites (S1–S4). Dots circled in red represent locations of the two multiparametric sensors. Black diamonds represent the two ARPAV sampling stations used for POC and TSM and the black triangle represents the SAMANET multiparametric sensor used to obtain data for the missing period (May–September 2019).

farming area S3 (coordinates: 45° 13' 0.00"N; 12° 15' 26.40"E) in the south of the Venice Lagoon covered by protection nets for 1 year. In May 2019, clams were partitioned in four groups (5000 clams each) placed in the lagoon at gradual distances from the Chioggia inlet. In detail, the four experimental sites represented in Figure 2 were the outmost S1 (coordinates: 45° 13' 51.60"N; 12° 16' 35.40"E), S2 (coordinates: 45° 13' 49.20"N; 12° 15' 56.40"E), S3, and the innermost S4 (coordinates: 45° 11' 35.40"N; 12° 15' 31.80"E).

During the monitoring year (May 2019–May 2020), for each sampling time and location, 90 clams were randomly collected in each site manually and using a manual rake for a total of four samplings performed in July 2019, October 2019, February 2020, and May 2020. The measurements done in the laboratory were: total wet weight, length, soft tissue dry weight, shell dry weight, and condition index [as (soft tissue/shell)*100].

Model setup and parameter calibration

In order to calibrate the parameter K_{SH} for each site, the model was run using environmental forcing described below. Model equations were solved using the R package *deSolve* (v 1.28), and the condition index was computed as an output variable. The time series of forcing functions were interpolated using the *approxfun* R function. The function chosen for the minimization was the standardized residual sum of squares error (Equation 1) based on the observed values at time $-i$ of soft tissue weight, W_i , and shell weight, S_i , the model predictions \hat{W}_i , and \hat{S}_i , and their SD , σ_W , and σ_S , calculated over the whole set of observed data. This function was minimized using the *fminbnd* package *Pracma* (v 2.2.9). In order to estimate the statistical distribution of K_{SH} , which is consistent with that of

observations, for each site 100 synthetic time series of soft tissue and shell dry weights were randomly extracted from the joint distribution of the observations, assumed to be normally distributed. From these, 100 values of K_{SH} per site were obtained, so that the mean and SD values of K_{SH} at each site could be estimated.

$$\sum_{i=1}^n \left[\frac{\sum_{i=1}^n \left[\widehat{W}_i, \widehat{S}_i \right] - [W_i, S_i]}{[\sigma_W, \sigma_S]} \right]^2. \quad (1)$$

To obtain estimates of soft and shell tissue growth for the year, simulations were made based on the mean, SD , and ranges of K_{SH} .

Environmental forcing functions

The SfG model requires as input time series of water temperature ($^{\circ}C$), as a regulator of main physiological processes and Chlorophyll-a ($mg\ m^{-3}$), and particulate organic carbon (POC), particulate organic matter (POM), and total suspended matter (TSM), as a proxy for food (phytoplankton and detritus) availability and quality (see Brigolin et al., 2009). The calculation of CO_2 fluxes requires the calculation of a function, Ψ (Morris and Humphreys, 2019), which requires as inputs pH, alkalinity, water temperature, and salinity. This function represents the additional reduction in DIC required, relative to the amount of DIC converted into $CaCO_3$, such that there would be no net change in seawater pCO_2 and can be seen to represent the potential amount of CO_2 released by mollusc calcification. The package *seacarb* (v 3.2.13) in R (Lavigne et al., 2019) was used to calculate it, using flag = 8 which outputs the function Ψ directly. Details of locations and sampling methodologies for the necessary parameters can be found in Table 1.

CO_2 budget

The net flux, CO_{2net} [$gCO_2\ individual^{-1}\ d^{-1}$], was calculated in accordance with the following equation:

$$CO_{2net} = CO_2\ respired + CO_2\ released - CO_2\ in\ shell. \quad (2)$$

The following equation from Turolla et al. (2020) was used to estimate the CO_2 released as a consequence of the calcification process:

$$CO_2\ released = \left(\frac{dS}{dt} \right) \times \psi(\text{temp}(t), \text{sal}(t), \text{pH}(t), \text{alk}(t)) \times \text{prCaCO}_3 \times 0.44, \quad (3)$$

where $\frac{dS}{dt}$ is the growth of shell (in $g\ d^{-1}$), and prCaCO_3 is the proportion of $CaCO_3$ in the shell [species specific, in this case 0.97, based on organic: inorganic ratio (Gouletquer and Wolowicz, 1989), adimensional]. Ψ , the ratio of CO_2 released to $CaCO_3$ precipitated (adimensional), was estimated at each time step (see the paragraph on environmental forcing), as it depends on water chemistry [e.g. increases with increasing partial pressure of CO_2 in seawater (Frankignoulle et al., 1994), which depends on temperature].

The CO_{2eq} stocked in the shell was estimated using the following equation (following Turolla et al., 2020):

$$CO_{2eq\ in\ shell} = C_{shell} \times \left(\frac{dS}{dt} \right) \times 3.66, \quad (4)$$

Table 1. List of environmental forcing required with details on their source, coordinates, sampling frequency, and data processing applied.

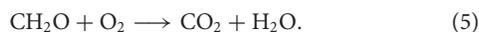
Forcing	Source	Coordinates	Sampling frequency	Notes	Data treatment
Temperature Chlorophyll-a pH Salinity	Multiparametric sensors	S1–S3 sites: 45° 13' 82" N 12° 15' 94" E S4 site: 45° 11' 31" N; 12° 15' 32" E	12 minutes	May 2019–September 2019 data from SAMANET VE-3 45° 19' 32.12" N; 12° 17' 8.63" E (triangle, Figure 1), frequency: 30 minutes September 2019–May 2020 multiparametric sensor buoy deployed at S2	Data averaged to obtain daily means and then spline smoothed with $\text{spar} = 0.4\text{--}0.7$
POC (POM was calculated multiplying this by 5.22 (Brigolin et al. 2019)) TSM	ARPAV campaigns (https://www.arpa.veneto.it/dati-ambientali/open-data/file-allegati/soaml/lag-una-di-venezia/tss-nutr-acqua)	S1–S3 sites: 45° 14' 27" N 12° 16' 15" E S4 site: 45° 11' 36" N 12° 14' 57" E	Quarterly	Data available until 2019	Average of last triennia of available data 2017–2019
Total alkalinity	MeLa surveys MAV (2006) (also in Bandelj et al., 2008; Lovato et al., 2013)	S1–S3 sites station 17B S4 site station 20B	Monthly		Median value

Table 2. Estimated values of K_{SH} , predicted and observed shell and tissue weight estimates at the end of the grow-out period (1 year), and predicted spawning day.

Site	K_{SH} Mean \pm SD (min, max)	Range of tissue weight at the end of growing predicted (observed)	Range of shell weight at the end of growing predicted (observed)	Range of estimated spawning day
S1	0.586 \pm 0.06 (0.463, 0.758)	0.33–0.79 (0.89–1.3)	8.44–9.49 (4.76–11)	August 8–29
S2	0.559 \pm 0.05 (0.412, 0.685)	0.43–0.9 (0.53–0.67)	8.03–9.45 (4.17–8.82)	August 8–September 6
S3	0.583 \pm 0.06 (0.438, 0.758)	0.33–0.85 (0.47–0.87)	8.25–9.49 (4.53–12.8)	August 8–September 2
S4	0.497 \pm 0.06 (0.338, 0.646)	0.48–1.05 (0.65–1.11)	7.32–9.36 (5.95–15.9)	August 10–September 18

where C_{shell} is the estimated carbon content (g) of 1 g of shell (0.117, Turolla et al., 2020) and 3.66 is the ratio of molecular weights used to transform C in CO_{2eq} in shell.

To calculate the CO_2 emitted by respiration, we used model (A7) (see Appendix A), and considered a fixed stoichiometry, according to the general equation accounting for the catabolism of ingested organic matter.



The total flux, expressed as $kg\ CO_2$ per ton^{-1} of harvest, was also estimated for comparisons with other studies. For this purposes, the average total wet weight of individual clams at each site on the last sampling occasion was used to calculate CO_2 flux per unit of weight.

Results

Bioenergetic model

The K_{SH} parameter was estimated for each site and is shown in Table 2.

Figures 3–5 show the comparisons between predicted shell weights, soft tissues, and condition indices and the observed ones from May 2019 to May 2020 at the four sites (S1–S4). Spawning periods predicted are presented in Table 2: during the sampling campaign of July 2019, individuals from S1 and S2 were found to contain enough reproductive material to recognize sex, while for individuals from S3 and S4 this was not the case. However, at all site a decrease in soft tissue weight between July and October campaigns was observed (see Figure 4), consistent with loss of reproductive tissue.

CO_2 budget

In terms of CO_2 sequestration, Figure 6 shows the net flux throughout the year of grow-out, with clams acting as a sink during summer and as a source during winter. This was the period with the least shell growth; thus, the CO_2 fluxes were close to zero (Figure 6a and b). Respiration associated fluxes, on the other hand, had an increasing trend over time. Respiration is linked with the weight of the organism and had the greatest peaks during the intermediate seasons

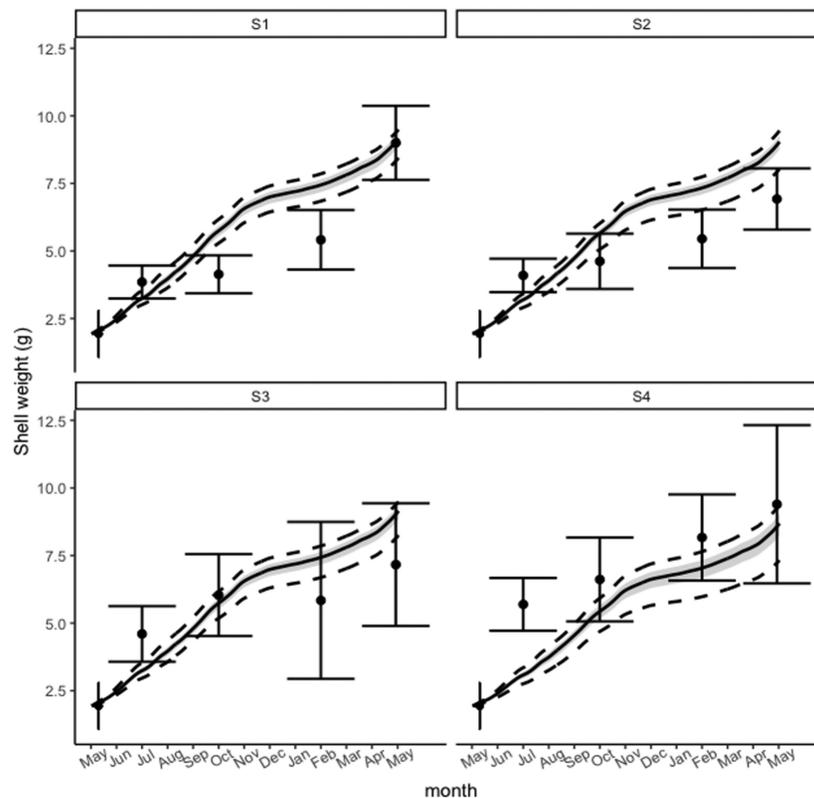


Figure 3. Estimated mean shell weights (full line) with confidence intervals based on the SD (shaded area) and range (dotted lines) of K_{SH} values (dotted lines). Points represent measured mean condition indices at the four sampling occasions with relative SD .

(autumn and spring). Looking at the average monthly CO_2 fluxes (Figure 6, Supplementary Table S3), these were positive (source) from late autumn to early spring (November–March) and negative fluxes (sink) from late spring up to early autumn (April–October). During winter months, the minimum values of fluxes associated with shell growth could be observed (e.g. December fluxes were 28% of September fluxes, for both shell sequestration and emissions related to calcification), while respiration remained similar (in this case the value in December was 93% of the value in September). When fluxes are cumulated for the whole year (Table 3), it can be seen that clams are a sink, but the amount of CO_2 sunk differs amongst the sites.

The average monthly environmental parameters are presented in as Supplementary materials (Supplementary Figure S1 and Table S4).

Discussion

In this study, we developed a bioenergetic individual for *R. philippinarum* based on the approach proposed by Brigolin *et al.* (2009) for *M. galloprovincialis*. The innovation presented in this manuscript is the explicit inclusion of shell weight in the energy budget: this allows one to calculate dynamically the individual CO_2 budget throughout a grow-out cycle. Most parameters were estimated on the basis of the available literature (Solidoro *et al.*, 2000): in this study, a key parameter concerning energy allocation among potentially competing physiological processes, i.e. soft tissue growth, shell growth and reproduction, was estimated with

a translocation experiment. As individuals from the same cohort were transplanted, the experiment allowed the elucidation of site-specific effects, which may be important in some cultivation areas that are heterogeneous. The output showed that clams role as CO_2 sinks is seasonal and that there may be some location effects.

While the model in itself appeared to predict most of the observations of shell and tissue weights, falling within their observed SD , some limitations to the estimates were present. At S1, there was an underestimation of the last observation of soft tissue, which increased greatly from the previous measurement, at S2, there was a minor overestimation of shell, and, and at the remaining sites, there was an underestimation, particularly evident at S4, in the first part of the growing season (July). It should be noted that the forcing function used was not taken directly *in situ* for all sites, with buoys in proximity to two sites (S2 and S4), which were close to the multiparametric buoys for temperature and Chlorophyll-a. Furthermore, POC, POM, and TSM were estimated based on past data (2016–2018) for the area, and POC can have high intra annual variability, from low values of 0.04–0.12 and 0.08–0.19 in 2014 at the two sites to high values of 0.33–0.61 and 0.42–1.4 at the two sites in 2011, based on data spanning 2011–2018. TSM had wider ranges that could be as low as 1 in 2016 and 2018 in the site closer to the inlet and as high at 111 in 2013 in the more internal site. These data were collected as point samples four times in a year rather than being continuous; thus, they may not accurately reflect the reality for the whole sampling period. More frequent sampling of this parameter could be beneficial for more accurate use of the model approach presented here. Furthermore, the low fit in the first period

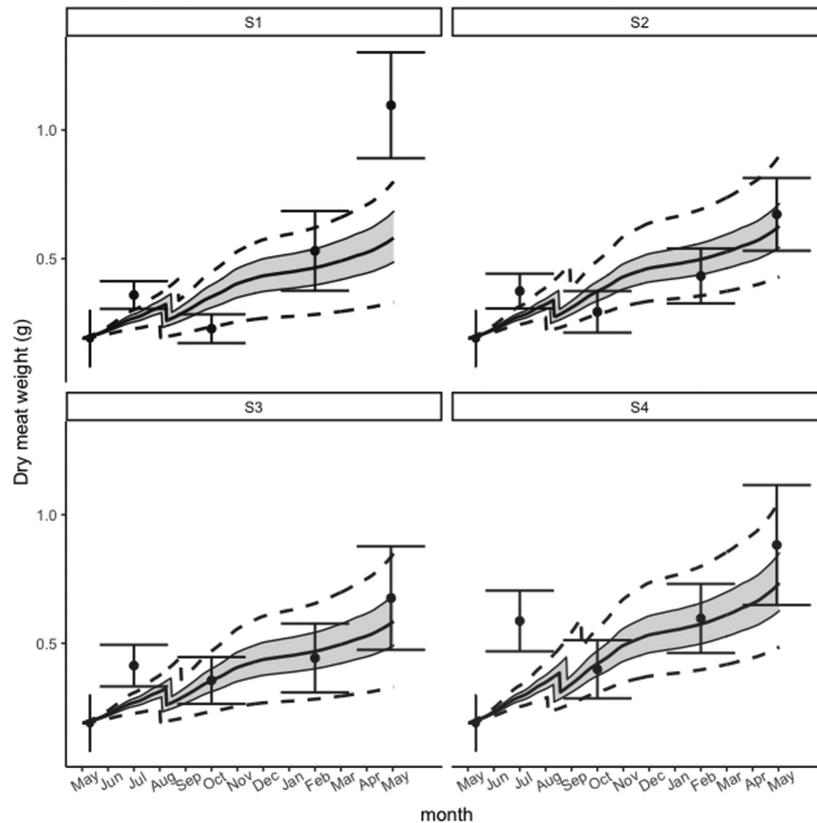


Figure 4. Estimated mean dry meat weight (full line) with confidence intervals based on SD of K_{SH} values (shaded area) and on range of K_{SH} values (dotted lines). Points represent measured mean condition indices at the four sampling occasions with relative SD.

may be due to the initial Chlorophyll-a and temperature coming from the Ve-3 buoy from the SAMANET network, which was the closest available and reflecting most closely the conditions, but was not *in situ*. Last, infaunal bivalves such as clams may also feed from microphytobenthos (Watanabe *et al.*, 2019), but in this study, the benthic part of the food was not quantified. Moreover, temperature refers to water column temperature, which may slightly differ from that experienced by infaunal bivalves: however, *R. philippinarum* inhabits shallow sediment depths and thus water temperature could be considered as a good proxy (Guo and Ma, 2018). More frequent monitoring of biometrics, which was not possible in the present campaign, would also be useful to fine tune the model approach presented in this paper.

Spawning predictions seemed to be accurate in terms of general timing, with all sites predicted to have spawning events around mid-August. During the sampling campaigns, some individuals were found to be mature in July but no mature individuals were found in October, thus spawning happened somewhere in between, as it can also be seen by the weight loss observed between the July and the October sampling. Previous studies have reported spawning between May and August in Turkey (Çolakoglu and Palaz, 2014) and between late July and August in South Korea (Lee *et al.*, 2020). In the present study, a greater weight loss than that predicted by the model could be seen. In the model, the assumption was made of no reproductive tissue at the beginning of the experiment and that a single spawning event takes place. In the future, it would be useful to incorporate field measurements of reproductive tissue dry weight coupled with more frequent sampling, especially in the summer-

autumn period, to obtain a more accurate calibration and understanding of the reproduction dynamics.

In terms of shell growth effort, similar values were estimated at all sites varying between 33 and 76%. This range lies on the upper end of that estimated for mussels by (Fuentes-Santos *et al.*, 2019) (25–52%) and Duarte *et al.* (2010) (30%) with our results being compatible with those concerning young individuals (Fuentes-Santos *et al.*, 2019). In this study, individuals that were still in their initial growing phase were followed; thus, it can be expected that a large part of the energy is invested towards the growth of both shell and soft tissues. Shell costs are dependent upon age, as younger individuals grow faster and deposit larger amounts of CaCO_3 (Watson *et al.*, 2017). A dynamic approach that takes into consideration ontogenetic changes may lead to improving the model prediction, both in terms of soft tissue evolution and CO_2 budget. Furthermore, Sanders *et al.* (2018) studied calcification costs directly in *Mytilus* and showed that total shell production could demand up to 67% of total assimilated energy in certain conditions such as low salinity, highlighting how environmental context can be important for energy investment towards shell formation.

In our study, the shell effort was estimated based on real measurement of shell weight and not based on allometric scaling, which allowed us to find differences amongst sites. Some site-specific factors may explain some of the variation in shell effort. In the S1–S3 sites, for example, a large number of *Hexaplex trunculus* can be observed (CB and IB, pers. obs.). It may be considered as an advantage to grow a thicker shell when feeling threatened (Johnson and Smeed, 2012), clams in these sites put a greater proportion of the total en-

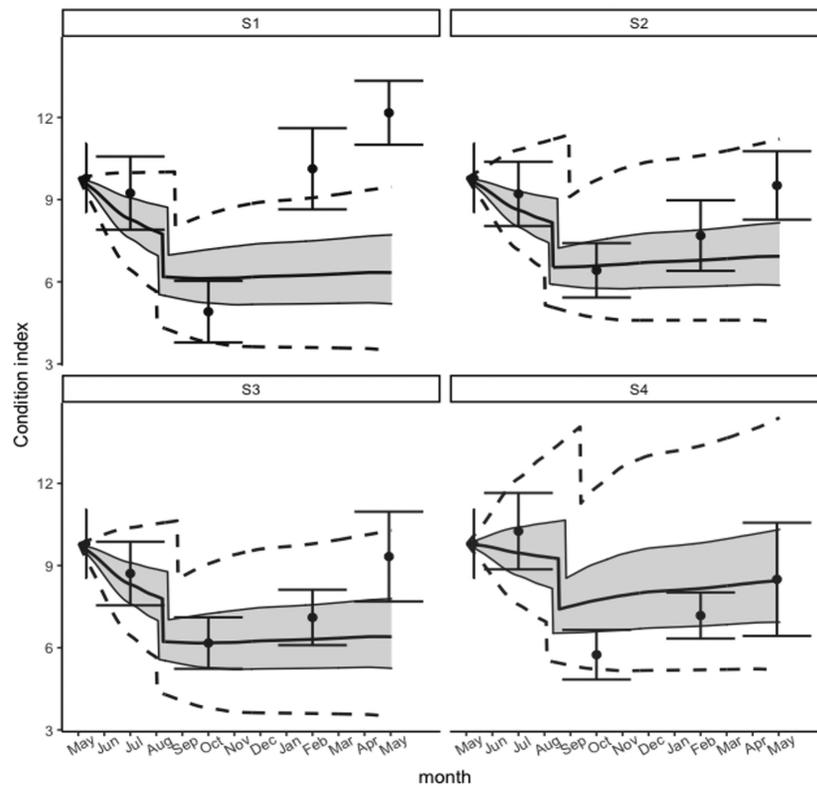


Figure 5. Estimated man condition index (full line) with confidence intervals based on SD (shaded area) and range (dotted lines) of K_{SH} values (dotted lines). Points represent measured mean condition indices at the four sampling occasion with relative SD.

ergy in shell making when compared to S4, where no *H. trunculus* are observed. In S4, however, the shell was the thickest at the end of the experiment, despite a lower investment predicted. This is an area of softer sediment consisting mainly of “silt” and “sandy silt” rather than “silty sand” (from *Atlante della laguna*: <http://cigno.atlantedelalaguna.it/maps/28/view>). Sediment properties are known to influence shell properties in other species: for example, *Mya arenaria* was found growing faster, with wider and thicker shells, in the mud treatment compared to sand and gravel (Newell and Hidu, 1982).

Site differences in shell growth are drivers of slight differences in CO_2 sinking capacities: applying this modelling approach, in the present study, *R. philippinarum* sink capacity varies from 4 to 22 kg of CO_2 per ton of harvested material, which was lower than the 54.5 kg CO_2 per ton estimated by Turolla *et al.* (2020) for clam cultivation in the neighbouring Po delta lagoons. Comparing the two compartments of shell calcification with those of Turolla *et al.* (2020), we obtain similar results for emissions due to the calcification (77–144 vs. 124 kg CO_2 ton⁻¹ of harvest) and lower carbon sequestered (34–63 vs. 88 kg C ton⁻¹ of harvest) however, we had an additional 38–56 kg CO_2 ton⁻¹ of harvest attributed to respiration. Mistri and Munari (2012), in fact, added respiration and argued that in the Po delta, *R. philippinarum* culture acts as a source of CO_2 . For comparison reasons, converting Mistri and Munari (2012) mol CO_2 into grams of wet weight harvest, and considering the differences between final and initial total wet weights of an individual clam in our system, thus considering only the weights increment during the year modelled, we obtain similar values for the shell pro-

cesses (0.0042–0.012 vs. 0.007 mol CO_2 g⁻¹ stored in shell, 0.0026–0.0075 vs. 0.0043 mol CO_2 g⁻¹ emissions from calcification).

Seasonal effects are important. For example, the greatest sink occurs in summer. In summer, there are lower calcification-associated emissions compared to winter, given by lower Ψ values in summer months (0.55) compared to winter months (0.7). Shell mass increments are, however, at their lowest in winter (down to 0.27 g month⁻¹ in December–January, compared to 0.9 g month⁻¹ in autumn when they are at the highest), meaning that emissions are at their lowest then, but this effect is counterbalanced by lower sequestration in the shell. Thus, Ψ fluctuations are as important as growth dynamics. In Turolla *et al.* (2020), the values of Ψ used was an “average value” for seawater (0.6), which is also the average value for the year of our study. Ψ is most sensitive to changes in pH and temperature, and it has a much lower sensitivity to changes in salinity and total alkalinity. Thus, calculating and modelling fluxes continuously can also be useful in understanding their seasonal evolution and predicting effects of climatic changes (including warming and ocean acidification) on the capacity to sequester.

In contrast with CO_2 dynamics associated with shell processes, in this study much lower values for respiration (0.0011–0.0033 compared to 0.019 mol CO_2 g⁻¹) were found when the whole animal (soft tissue and shell) is considered. Respiration values estimated in accordance with Mistri and Munari (2012) were slightly higher (0.0013–0.0038 mol CO_2 g⁻¹) but still lower than those found by these authors. The values of respiration in the studies of Mistri and Munari (2012) for *R. philippinarum* and Munari *et al.* (2013) for *M. galloprovincialis* are, however, higher when compared to other stud-

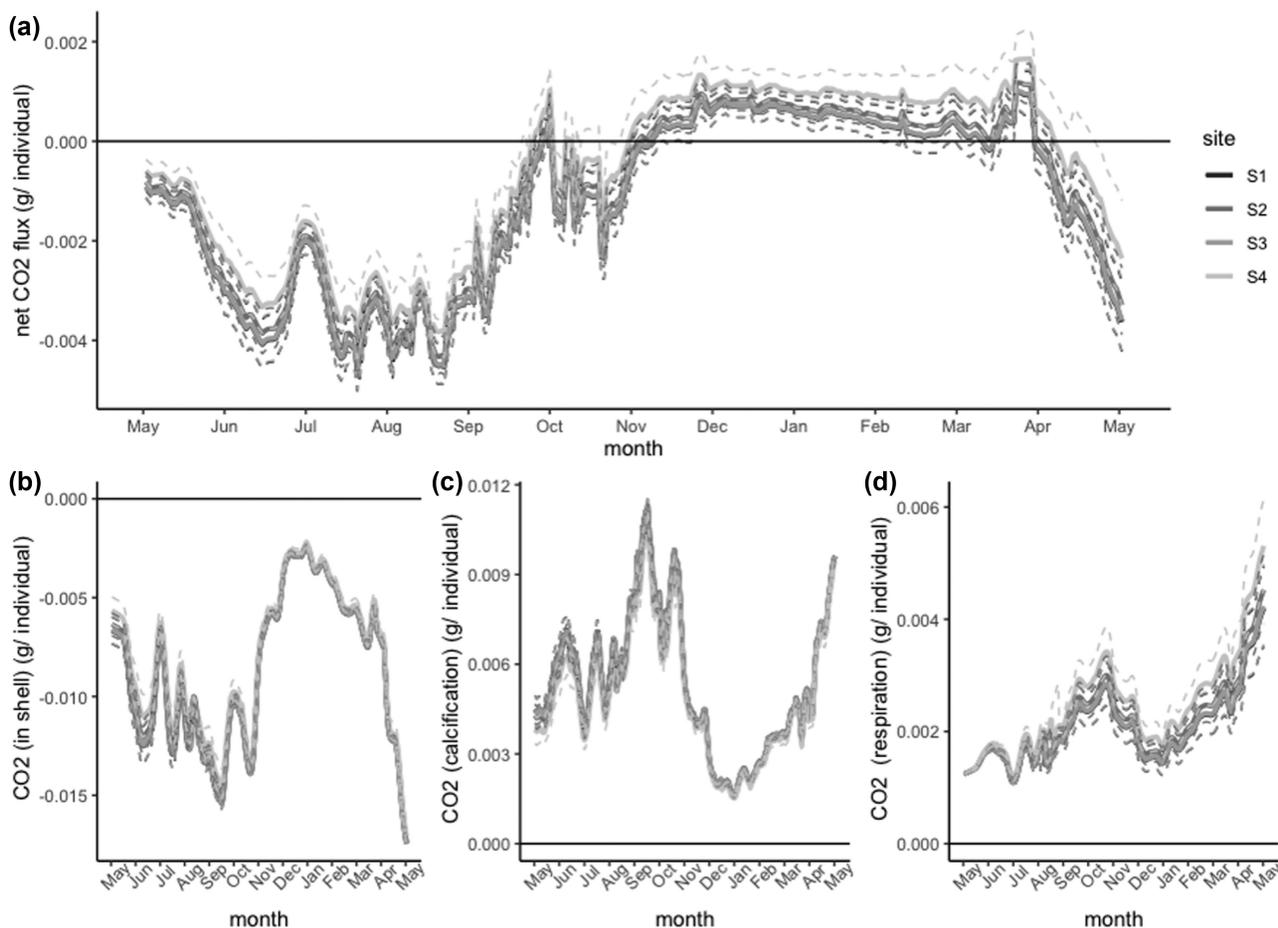


Figure 6. (a) Net CO₂ flux, (b) CO₂ stocked in the shell, (c) CO₂ emitted during calcification process, and (d) CO₂ emissions during respiration, expressed in g ind⁻¹ during 1 year of simulation (May–May). Positive values represent emissions, and negative value represent sinking. Horizontal line at “zero.” Colours represent sites.

ies (Filgueira *et al.*, 2019). The approach used in this study, a bioenergetic model, allows us to calculate more precisely the amount of catabolism that an animal undergoes throughout that period if 1 year under varying temperatures and food, and it is more accurate than simply using estimates. Production estimates should be combined with this individual-based approach in order to assess which of the areas have the biggest possibility of being sinks overall. In the present study, we observed that by individual, S1 had the greatest sink, but by weight, S3 had the greatest sinking potential, suggesting that the site biomass production dynamics are important to be considered. On a population level, sink and source overall may be dependent on mortality dynamics, with some areas having higher mortality rates. Considering that the activity around *R. philippinarum* cultivation can also be a source of CO₂ (estimated by Turolla *et al.*, 2020 at 75 kg CO₂ ton⁻¹), one has to be careful when considering the results of this study in terms of the whole practice, and should instead consider integrating this approach within a whole life cycle assessment.

This study developed a model that allows one to calculate the investment in shell and the output in terms of shell and soft tissue, allowing both biomass yields, in terms of wet weight and shell accretion, to be taken into account for valorizing shellfish cultivation in the carbon market (Filgueira *et al.*, 2015). This should help with site selection for farming areas and restoration zones, for example,

Table 3. Sum of fluxed for the whole period in the four sites, in bold the net flux taking into account what is stored in shell and what is emitted through the calcification processes and respiration.

Site	Net flux g ind ⁻¹	In shell g ind ⁻¹	Calcification g ind ⁻¹	Respiration g ind ⁻¹	kg CO ₂ ton ⁻¹
S1	– 0.33	– 3.12	1.93	0.74	– 17.6
S2	– 0.26	– 3.07	1.89	0.78	– 19.1
S3	– 0.32	– 3.12	1.92	0.74	– 24.4
S4	– 0.08	– 2.9	1.8	0.88	– 3.6

On the left, the net flux is expressed on an individual basis, while on the right, it is scaled up as kg CO₂ in the last year of cultivation per ton of harvested weight.

areas where shell production is higher, making it more suitable for restoration purposes with additional benefits of carbon sequestration, and the areas with thinner shell production, making it more suitable for food production, taking into account the potential of CO₂ sequestration and likely necessities of offsetting eventual emissions through greening practices (e.g. IMTA, Ahmed *et al.*, 2017). Furthermore, this model allows to understand the seasonality of CO₂ sinks and source that can be an important aspect when planning co-productions (e.g. seaweed farming).

In conclusion, while other ecosystem services may need to be considered (e.g. Saurel *et al.*, 2014), this study highlights the complexities of determining the role of shellfish farming as a source or sink, and shows that it is context-dependent. Overall, this research wants to provide a framework to work with, using a site-specific modelling approach to quantification of meat vs. shell production, followed by the estimations of these processes as sinks and sources of CO₂ when allocating space for different activities, in particular, bivalve aquaculture. This framework should be further tested in other contexts and with other species before using it in combination with future climate and trophic scenarios.

Supplementary data

Supplementary material is available at the ICESJMS online version of the manuscript.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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