Modelling the bioremediation of aquaculture waste through an IMTA-system with the sponge *Chondrosia reniformis*

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Abstract

In the last decades, the sea-based aquaculture sector caused environmental problems worldwide. Finfish aquaculture impacts the water through nutrient-rich waste (feed and fish faeces). Integrated multi-trophic aquaculture (IMTA) could be a solution to the growing fish farming waste problem. IMTA combines fed aquaculture species with extractive species, called bioremediators, that can filter waste particulates and take up dissolved nutrients, thereby reducing the negative impact of fish farms on water quality. Sponges are promising candidates to be used as bioremediators, as they possess the ability to process and clear large volumes of water. Sponge cultivation could also provide economic benefits as a bonus to fish production, since sponges produce compounds with potential medical applications, such as collagen.

This study aimed to develop a model that predicts the bioremediation capacity and biomass production of the collagen-producing Mediterranean sponge *Chondrosia reniformis*. This model assumes a nutrient-rich plug flow, which is unidirectional and flows directly through the sponge farm. created model was tested for 27 scenarios over a duration of one year, including the possible combinations of three different carbon and nitrogen inflow concentrations, three ambient water flow velocities, and three sponge biomass coverages. A separate model to estimate fish waste production was made for sea bass (*Dicentrarchus Labrax).* The

Model outcomes showed that large sponge farms under low current velocity were able to reduce ambient nutrient concentrations most, with a decrease in carbon concentration of 31%. Per year per kg of planted sponge biomass, a maximum of 2.69 kg sponge biomass and 28 grams of collagen could be harvested. Furthermore, per year a kg of planted sponge biomass could filter up to 4.99 kg of carbon (C) and 1.08 kg of nitrogen (N). Per tonne of produced sea bass (*D. labrax),* this translated in compensation of 439% of the produced C waste and 467% of the N waste per tonne of planted sponge biomass. To compensate the fish waste of a sea bass pen with a volume of 7069 m^3, 115hectares of sponge farm is needed for C and 108 hectares for N. Possibly

The presented model could be a step towards understanding the potential of bioremediation by sponges in IMTA systems. However, due to limited data availability, many assumptions have been made in the model. Conclusions should, therefore, be interpreted with care. The model has helped to identify possible areas for future research. Further research is needed on how *C. reniformis* and its microbiome adapts to environmental conditions. Especially processes like sponge growth, detritus production, respiration, and nitrification under different nutrient concentrations and current velocities. Secondly, the space needed for sponge farming could potentially be reduced. Future research could evaluate the effect of sponge density on nutrient uptake and growth. Lastly, it would of interest to test if sponge farms could be used to clean up bays that are exposed to low ambient flow and were polluted by cage farming in the past.

Contents

1. Introduction

1.1 Fish farming in the Mediterranean

In the last decade, the aquaculture sector in the Mediterranean Sea has grown rapidly (Sarà et al., 2004; Demirak et al., 2006; Neofitou and Klaoudatos, 2008). Most of the production takes place in the Eastern Mediterranean with Turkey accounting for 38% of the marine Mediterranean production (120 471 tons) (FEAP, 2017). The main fish species grown are sea bass-*Dicentrarchus labrax* (49%) and sea bream-*Sparus aurata* (45%)(Memis et al., 2002; FEAP, 2017). The rapid and uncontrolled expansion has led to environmental problems and conflicts between the aquaculture sectors and other coastal such as tourism and fisheries. (Yucel-Gier et al., 2007; Basaran et al., 2010).

One of the major problems associated with fish farming is the effect of the nutrient-rich waste(uneaten feed and faecal material) on water quality (Fu et al., 2007; Holmer et al., 2008) and sediment composition (Yokoyama et al., 2005; Alongi et al., 2009). Research has repeatedly shown that changes in water and sediment chemistry due to fish farming can change microbial processes, lead to anoxic conditions, cause eutrophication and algal blooms, and affect the benthic flora, fauna and bacterial community dynamics (Sarà et al., 2004; Yucel-Gier et al., 2007; Holmer et al., 2008; Basaran et al., 2010; Price et al., 2015).

Recently, to reduce the conflict, Turkish authorities have urged the aquaculture sector to move offshore (Basaran et al., 2010). The effect of fish farming activities is dependent on coastal hydrology (e.g. ambient flow) and geomorphology (Neofitou and Klaoudatos, 2008). In areas with high water-flushing rates, farm wastes are dispersed while in semi-enclosed areas the dispersal of nutrients is limited (Yokoyama et al., 2004).

1.2 Aquaculture waste

The composition of aquaculture waste is dependent on husbandry, feed composition and digestibility, feeding technique, and site selection. Aquaculture waste is rich in organic matter (OM), carbon (C), nitrogen (N), and phosphorus (P). In fish farming systems in the Mediterranean Sea, 62% of the C, 57-98% of the N and 38-85% of the P input are not converted into fish biomass but instead lost into the environment (Islam, 2005; Karakassis et al., 2005; Wang et al., 2013). It is estimated that this translates into an inflow of 12 000 tons of N and 2 000 tons of P per year in the Eastern Mediterranean alone (Karakassis et al., 2005). An overabundance in organic matter and organic carbon can result in reduced species richness and changes in benthic biomass and abundance (Hyland et al., 2005). Under an increasing OM input, species richness typically decreases, whereas pollution-tolerant, opportunistic species survive, and sensitive species disappear (Hyland et al., 2005). In the study of Hyland *et al*. (2005), the strongest decrease in species richness under increasing TOC concentrations was found for the samples from the Eastern Mediterranean Sea, compared to 6 other coastal regions including the North Sea and the Northern Black Sea. The Eastern Mediterranean is an oligotrophic and diverse ecosystem that might be more vulnerable to eutrophication (Hyland et al., 2005). Accordingly, the benthic species richness might be more sensitive to increased organic carbon and nutrient inflow.

1.3 Integrated multi-trophic aquaculture

Integrated multi-trophic aquaculture (IMTA) could be a solution to the growing fish farming waste problem. IMTA combines fed aquaculture species with extractive species that can filter waste particulates (e.g. bivalves) and take up dissolved nutrients (e.g. macroalgae) (Troell et al., 2009). In such systems, the waste from the farmed species is recaptured and used as a resource by an extractive culture species or is converted into less harmful or useful products. Depending on the species, the extractive culture species could be used as a fertilizer or feed (Kambey and Chung, 2015). IMTA systems have been considered as more sustainable systems since they optimize nutrient usage and reduce the negative impact on water quality (Chá vez-Crooker et al., 2010). Extractive culture species can also be referred to as bioremediators. Bioremediation refers to the capacity of living organisms to remove or detoxify pollutants like heavy metals, microbial contaminants, hydrocarbons, nutrients, and persistent organic pollutants. There are marine animal species that are suitable candidates to use as bioremediators due to, for example, their resistance to toxicity, their ability to accumulate, stabilize or degrade pollutants or due to the ability to also provide economic benefits

(Gifford et al., 2007). Species that are considered suitable bioremediators include molluscs, bivalves such as blue mussels, oysters and clams, macroalgae such as kelps and seaweed, fish, polychaetes and sea cucumbers. Many of these species have already been included in IMTA systems (Langan, 2004; Gifford et al., 2007; Troell et al., 2009; Zhang et al., 2010). Recently, sponges have been considered as promising candidates to be used as bioremediators, which will be described in detail in the following chapter.

1.4 Sponges as bioremediators

Sponges can filter large amounts of water while also retaining a significant percentage of the suspended particles (Osinga et al., 1999; Milanese et al., 2003; Zhang et al., 2010). Sponges are therefore very efficient cleaners, processing volumes of water up to 0.6 cm^3 cm^{-3} sponge s^{-1} (Savarese et al., 1997; Weisz et al., 2008).

Most of the nitrogen excreted by farmed fish is excreted in a dissolved form, mostly ammonium (NH_4) and dissolved organic nitrogen (DON) (Fernandez-Jover et al., 2007). In nitrogen-limited systems, an increased ammonium concentration in the water column is thought to be a key factor in the growth enhancement of phytoplankton species. Some sponges host metabolically diverse and active microbial communities including nitrifying bacteria, which oxidise NH_4 into nitrite (NO_2^-) and nitrate (NO_3^-) species (referred to as NO_x^-) (Yucel-Gier et al., 2008). Microbial nitrification helps sponges to discard NH_4 and NO_2, which can be toxic at high concentrations (Ribes et al., 2015).

Sponge cultivation can also provide economic benefits (Schippers et al., 2012), for example by cultivating collagen-producing species (Osinga et al., 2010) or species containing biomedical compounds (Jha and Zi-rong, 2004). By using species with an economic value, farmers can profit financially while simultaneously reducing their environmental impact (Price et al., 2015). Furthermore, fish feed is proportionally the biggest expense for fish farmers (General fisheries commission for the medditerranean, 2010). By including sponge production, the wasted feed could be transformed into economic value through the sale of collagen, thus reducing the amount on unused feed.

1.5 Modelling

With the ongoing growth of the aquaculture sector, it is important to help farmers to reduce their impact on water quality using scientific knowledge. Providing tools can be an effective way to transfer this knowledge and to help farmers putting theory into practice (Antle et al., 2017). Models can function as useful tools to help in the design of balanced IMTA systems and help farmers to reduce their environmental impact. They can also be used to assess the feasibility and benefit of including extractive species in an IMTA system.

Models have been developed for particular components of IMTA systems including shellfish (Reid et al., 2011), bivalves (Descy et al., n.d.; Shpigel et al., 1993), seaweed (Shpigel et al., 1993; Schuenhoff et al., 2003; Hadley et al., 2015), kelp (Broch et al., 2013) and sea cucumber (Kambey and Chung, 2015). Zijffers (2009) developed a model to test the feasibility of a sponge aquaculture system and to aid in the design of such a system. The focus of this model was on sponge production, but it did not include a bioremediation component. When including bioremediation, the model could potentially estimate the corresponding filtering capacity of the sponges, next to predicting sponge harvest. Furthermore, Zijffers' (2009) model uses the primary production of algae and sedimentation of organic particles as a food source for sponges. Research has shown that dissolved organic carbon (DOC) is a major source of carbon making up 70 to 90% of the total carbon uptake of the investigated sponges (Yahel et al., 2003; De Goeij et al., 2008; Fiore et al., 2017). The remaining percentages of carbon are taken up in the form of particulate organic carbon (POC) (Mueller et al., 2014).

1.6 Aim of this study

Research has been done on sponge aquaculture (Van Treeck et al., 2003; Osinga et al., 2010; Gökalp et al., 2019). However, little is known on the potential and feasibility of including *C. reniformis* in an IMTA system. This study aims to develop a model predicting sponge production and bioremediation capacity of sponges for sea-based IMTA systems. The focus of this study will be on carbon and nitrogen, which in this study will be referred to as "nutrients". The model will be based on the sponge *C. reniformis* in combination with the

production of the European sea bass (and *D. labrax*) in the Mediterranean Sea. *C. reniformis* is a good source of collagen (Pozzolini et al., 2012). Selling the collagen to the biomedical and cosmetic industry potentially makes sea-based *C. reniformis* production commercially interesting (Osinga et al., 2010). Further, data is available on the cultivation of *C. reniformis* (Osinga et al., 1999, 2010; Gökalp et al., 2019) and general processes related to *C. reniformis* like clearance, growth and mortality rates (Ribes et al., 2012; Alexander et al., 2014; Morganti et al., 2017). Lastly, *C. reniformis* has an association with nitrifying bacteria and is a net consumer of ammonia (Schläppy et al., 2010).

With the model this study aims to answer the following research questions:

- How large should a sponge farm be to fully compensate C/N waste of a suspended sea bass farm?
- How is the direct reduction of the C/N concentration by a sponge farm influenced by the ambient flow, C/N concentration, and sponge farm size?
- How much sponge biomass/collagen can be harvested over the course of a year?

The mass balances for C and N under different scenarios will be evaluated to test whether the uptake of nutrients reaches or exceeds the use of nutrients for assimilation. Assimilation in this study will refer to the use of nutrients for growth, respiration, nitrification, and detritus production.

2. Model description

2.1 System description

The production system is based on an imaginary IMTA system with European sea bass (*D. labrax*) and the sponge *C. reniformis.* The fictitious circular fish pen has a diameter of 30 meters, a depth of 10 meters and a volume of 7069 m^3. For the area needed for the fish pen, a square was assumed, leading to a surface area of 900 m^2. The sea bass weights 40 grams at the beginning of the year and approximately 350 grams at the end of the year. The cultivation year starts in spring (March). When the fish are 300 grams the stocking density is 15 kg m^{-3}, leading to 350.000 individual fish being grown. The sponge farm is placed downstream of the fish pen and has a width of 30 meters. An illustration of the system is displayed in Figure 1. For the sponge farm, it is assumed that the sponges are grown in lanterns as used in the study of Kelly et al. (2004) Figure 2). These lanterns 'trap' the sponges, preventing them from moving away, and protect the sponges against predation. The size of the sponge farm is dependent on the scenarios used to run the model and therefore the length of the sponge farm is variable. It is assumed that the lanterns are positioned with 2 meters space between each other, have a diameter of 61 cm, and contain 25 layers. Finally, the system assumes a laminar flow with a constant velocity, which leads the exhalant stream of the fish pen over the sponge farm (plug-flow approach).

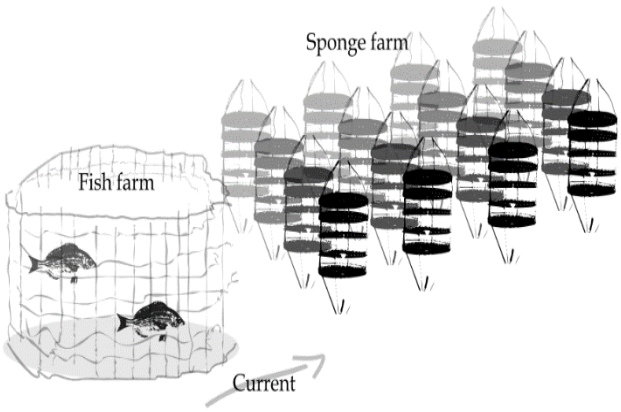

Figure 1 (Above). Sketch of fish farm and sponge farm combination.

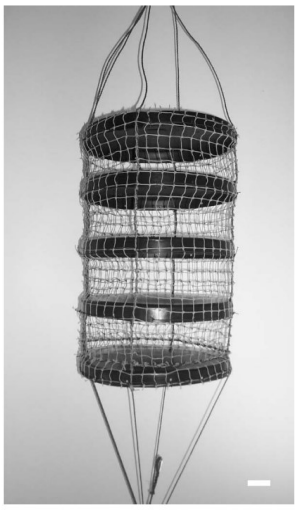

Figure 2 (Right) Design of a lantern for aquaculture of marine sponges as designed for the study of (Kelly et al., 2004). Diameter of the plastic base trays is 61 with 5-cm-high sides facing downwards to provide shadow for the layer below. The sponges are protected against predators by a nylon net with 40mm^2 mesh size. (Scale = 10cm)

2.2 Model description

The model is built in MATLAB R2018a using the extension GRIND 2.0 (Nes, 2008). A separate model was made for the fish farm and the sponge farm. The sponge farm model was not connected to the fish farm but instead used an input nutrient concentration. Due to time constraints, this was an easier solution. In the future, these models could be connected by deciding on a dilution rate for the fish farm waste. The inflow concentration for the sponge farm could then be adjusted by varying different dilution rates, feeding rates, and feed utilization efficiencies. The conceptualisation of both models is shown in Figure 3, with the state variables displayed in rectangles. The differential equations will follow in Section 2.4.

The fish farm model was made to estimate the waste coming from a fish farm. The model for the fish farm stabilised immediately and was run for 365 days. The fish farm component was a dynamic model, including a time series for temperature. This component produces total carbon and total nitrogen based on the feeding rate and the efficiency with which the feed is retained in feed biomass. The sponge farm model was run for 375 days. The modelled values stabilized after 10 simulated days; therefore, these first 10 days were excluded. All input parameters were constant over the whole year. In the model, the sponges, expressed in kg dry weight (DW), take up dissolved organic carbon (DOC), particulate organic carbon (POC), ammonia (NH_4), and dissolved organic nitrogen (DON) and transform this into sponge biomass, detritus, CO_2, and nitrate and nitrite (NO_x), The harvest of collagen is based on the total harvest of sponge biomass. In the model, sponge biomass is expressed in sponge dry weight. Furthermore, part of the POC sinks and settles on the sediment.

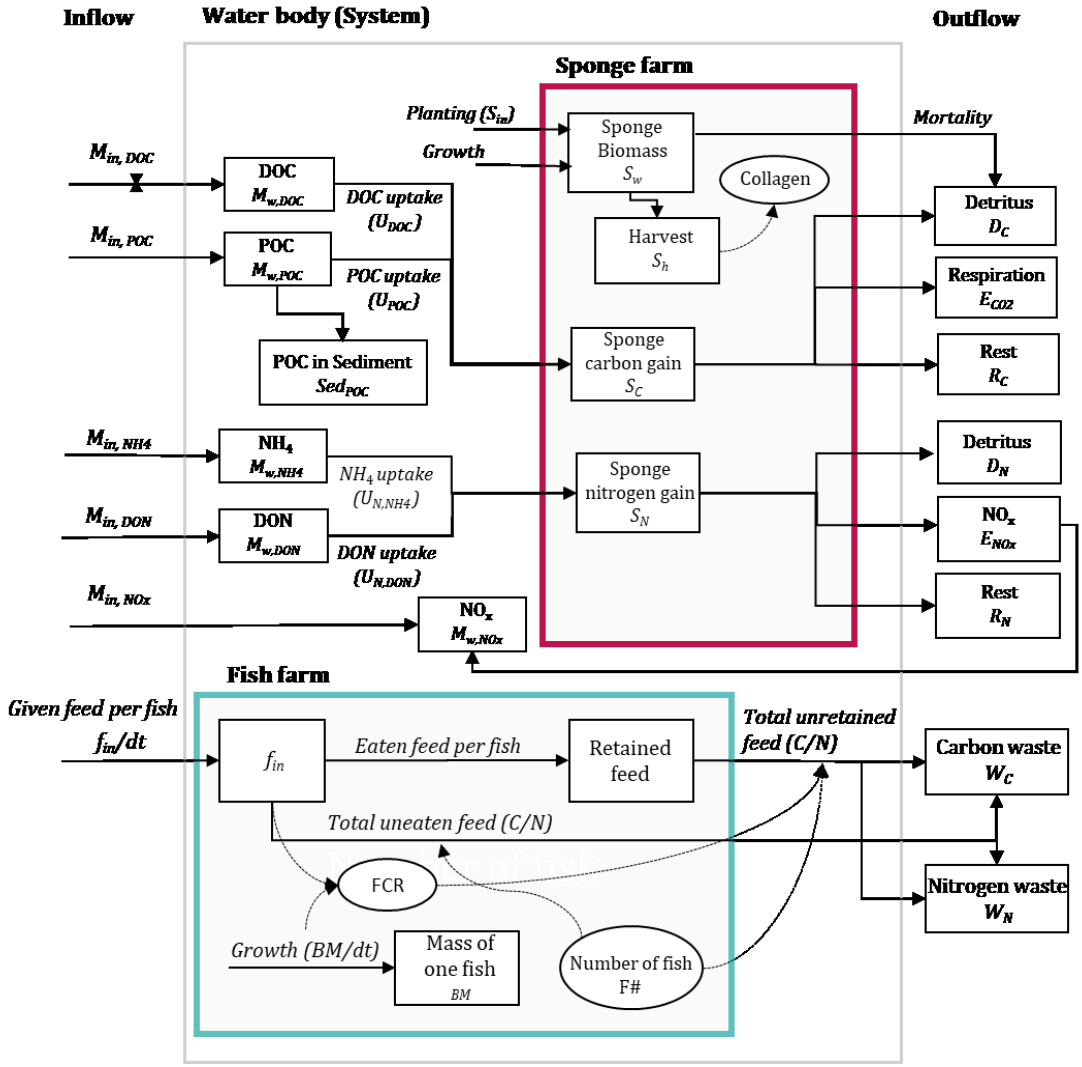

Figure 3. Conceptual model of C and N in the water body (inside blue lines), the sponge farm (inside pink lines) and the fish farm (inside green lines). Square boxes display states and the circles display auxiliary variables, straight arrows display mass flows, and dotted arrows display information flows. Not all auxiliary variables and no parameters are shown.

2.3 Assumptions

The assumptions made in the development of the model are shown in Table 1.

Table 1 Assumptions made for the models for the water component, sponge farm component, fish farm component

	Assumptions
Water	a) Laminar flow; where the velocity and direction of the current are constant
	b) The nutrient inflow concentration is constant
	c) Nutrient concentration is homogenous over the whole water body, where all sponges in the sponge farm experience the same concentration.
	d) Flow velocity refers to a higher inflow of mass per day and is unrelated to the residence time.
	e) Shear stress, re-suspension, and ambient flow do not influence the sedimentation rate of POC
Sponge farm	a) Optimal linear growth can be reached
	b) Temperature and depth do not influence sponge processes (Morganti et al., 2019; Gökalp et al., 2020)
	c) The sponge farm can be planted at once with sufficient supply of sponge biomass
	d) The standing stock in the sponge farm can be maintained on a fixed value, where the harvesting is equal to the growth and occurs daily.
	e) *C. reniformis* consumes DOC, POC, NH_4 and DON (Morganti et al., 2017)
	f) Clearance rates for bacteria are representative for the clearance rates of POC
	g) Sponge CO_2 and NO_X excretion is constant and independent on the nutrient concentration
	h) Ambient flow does not influence pumping rates or energy used for respiration
	i) Cultured sponges smother at high TOC concentrations (Osinga et al., 2010)
Fish farm	a) The number of fish in a fish pen is constant and not dependent on fish size and age.
	b) There is no fish mortality
	c) C and N content of feed the is independent on fish size
	d) The percentage of uneaten feed is independent of fish size
Data	a) Data from *in situ* studies in Spain, Greece, Turkey, and Croatia are comparable
	b) When data for *C. reniformis* was lacking, a comparison could be made with other sponge species.

2.4 Differential equations

2.4.1 Water flow

The model assumes a constant inflow of water, with a homogeneous C/N concentration *[Mi]*. Every timestep (per day, d^{-1}) the concentration of DOC, POC, NH_4, NO_x, and DON in the water ($M_{w,i}$,) declines or increases due to sponge related processes as described in section 2.4.3 (Eq. (C1) and (C2), Appendix C) The waterbody is then replenished back to the initial environmental inflow concentration $[M_i]$ at the start of the next day. The net nutrient change in the water body M_{in} in kg d^{-1} is expressed by Eq. (1). The compound is indicated by subscript *i* (DOC, POC, NH_4, DON and NO_x,).

$$\frac{dM_{in,i}}{\mathrm{d}t} = Q \cdot [M_i] - \frac{M_{w,i}}{dt} \qquad (1)$$

where Q is the volumetric flow rate in L d^{-1}, $[M_j]$ is the inflow concentration in kg L^{-1}, and $M_{w,i}$ is the mass present in the water body in kg after a day. The volumetric flow rate Q is dependent on the ambient flow which differs in the different run scenarios (Eq. (C3), Appendix C).

For POC, the concentration of POC in the water body is not only reduced by sponge related processes, but also by sedimentation. Resuspension and shear stress are not included in the model. The net sedimentation Sed_{POC} in kg d^{-1} is expressed by Eq. (2).

$$\frac{dSed_{poc}}{\mathrm{d}t} = \frac{vs}{h} \cdot \frac{M_{w,poc}}{\mathrm{d}t} \qquad (2)$$

In which *vs* is the settling velocity in m d^{-1}, h is the height of the water column in m and $M_{w,POC}$ is the POC present in the water body in kg.

2.4.2 Sponge planting and harvest

The total kilograms of sponge biomass present in the system, S_b, is affected by the sponge planting, sponge growth, sponge harvesting, and sponge mortality. The change in sponge biomass is described by Eq. (3).

$$\frac{S_b}{dt} = S_{in} + g\,S_{b(t)} - S_h - m\,S_{b(t)} \quad (3)$$

In which S_{in} is the sponge planting in kg d^{-1}, g is the growth rate in d^{-1}, S_b is the sponge biomass in the system at time t, S_h is the sponge harvest in kg d^{-1} and m is the mortality rate in d^{-1}.

The planting and harvesting of sponge biomass are based on the available surface for sponge production where S_{max} is the maximum amount of sponge biomass when 100% of the available surface is covered. S_{max} in kg is calculated by Eq. (4). The number of lanterns is calculated by Eq. (5).

$$S_{max} = \pi \cdot (0.5\,ld)^2 \cdot ll \cdot ln \cdot pa \cdot 10^4 \quad (4)$$

$$ln = \frac{l \cdot w}{(ld+rs)^2} \quad (5)$$

In which ld is the lantern diameter in m, ll is the number of layers per lantern, ln is the number of lanterns, pa is the sponge surface density in g m^{-2}, l is the length of the sponge culture site in meters and rs is the space between the rows in meters.

New sponges are planted to reach the minimal coverage (Eq. (6)). Planting occurs when the total sponge biomass covers less than 50% of the available surface. Daily sponge harvesting starts when 70% of the available surface is covered by sponges. The sponge biomass harvested is based on net growth (Eq. (7)).

$$\frac{S_{in}}{dt} = \begin{cases} S_{max} - S_b, & if\ S_b < 0.5 \cdot S_{max} \\ 0, & otherwise \end{cases} \quad (6)$$

$$\frac{S_h}{dt} = \begin{cases} S_b \cdot (g-m), & if\ S_b \geq 0.7 \cdot S_{max} \\ 0, & otherwise \end{cases} \quad (7)$$

The growth rate and mortality rate are dependent on many aspects such as the attachment method, plate material, plate orientation, light exposure, and TOC concentration (Gökalp et al., 2019, 2020). Reported growth rates of *C. reniformis* vary, where rates of $7.2 \cdot 10^{-3}$ day^{-1} (Osinga et al., 2010), $4.1 \cdot 10^{-3}$ day^{-1}, (Gökalp et al., 2019)$1.8 \cdot 10^{-3}$ day^{-1} (Wilkinson and Vacelet, 1979), and $0.84 \cdot 10^{-3}$ day^{-1} (Garrabou and Zabala, 2001) have been observed. Performed aquaculture trials with *C. reniformis* have given more insight into optimal growth conditions and therefore, for the model, it is assumed that high growth rates and low mortality rates can be achieved (Osinga et al., 2010; Gökalp et al., 2019). Linear growth was assumed. In the model, it was assumed that under optimal circumstances the growth rate would be close to the finding of Osinga *et al.* (2010). Both a shortage and overabundance of TOC could hamper growth. The growth rate, incorporating the effect of TOC concentration, is described by Eq. (8) and the TOC concentration by Eq. (9). The growth rate as expressed by Eq. (8) under different TOC concentrations is displayed in Figure B 2 (Appendix B).

$$g = (2 \cdot g_{max}) \cdot \frac{TOC}{TOC+M_1} \cdot \frac{M_2}{TOC+M_2} \quad (8)$$

$$TOC = \frac{M_{w,DOC}(t) + M_{w,POC}(t)}{Q \cdot 10^{-6}} \quad (9)$$

In which g_{max} is the maximum growth rate in d^{-1}, TOC is the TOC concentration at time t in the water body in mg L^{-1}, and M_1 and M_2 are the TOC concentrations in mg L^{-1} where the growth is half of the maximum growth rate.

In the study of Morganti *et al.* (2017) sponges showed net excretion of DOC at a DOC concentration of ~1 mg L^{-1}. Based on this finding, it was assumed growth would due to limited food availability be induced around 1 mg L^{-1} DOC and therefore M_1 was estimated on 1.1 mg TOC L^{-1}. In conditions of a high nutrient concentration and high disposition of solids, the sponge could deteriorate and die due to smothering (Duckworth et al., 1997; Osinga et al., 2010). In the model, it is assumed that smothering occurs at higher TOC concentrations and limits growth. M_2 was estimated at 6 mg TOC L^{-1}.

Research on *C. reniformis* has shown different mortality rates, with Osinga *et al*. (2010) reporting increased mortality at a fish farm location with high TOC concentrations where the sponges were planted below the fish cages. Gokälp *et al*. (2019) reported the opposite with higher survival under higher TOC concentrations. In the model it is assumed the sponges are next to the fish farm (and not under) and are protected from direct vertical sedimentation due to the layered design (Figure 2). Therefore, the mortality rate of 0.00157 d^{-1} (survival rate of 86% in 13 months) was used based on a *C. reniformis* culture experiment by Gökalp *et al.* (2019).

2.4.3 Sponge food sources

DOC uptake in the model is based on the research of Morganti *et al.* (2017) in which the uptake is dependent on the DOC concentration. The filtrated DOC in kg cm^{-2} d^{-1}, F_{DOC}, is expressed by Eq. (10). The total uptake of DOC (U_{doc}) in kg d^{-1} is expressed by Eq. (11).

$$F_{DOC} = \left((\, 0.06 \cdot \frac{M_{w,DOC}\,(t)}{Q} - (4.5 \cdot 12.0107 \cdot 10^{-9}) \right) \cdot 10^{-3} \cdot 86\,400 \quad (10)$$

$$\frac{dUdoc}{dt} = \begin{cases} \frac{F_{DOC}}{pa} \cdot S_w\,, & if\ \frac{F_{DOC}}{pa} < \mathrm{U}_{max} \\ U_{max} \cdot S_w\,, & otherwise \end{cases} \quad (11)$$

In which U_{max} is the maximum DOC uptake in kg DOC kg d^{-1}. In Eq. (10) 0.006 and 4.5 are from the formula defined by Morganti *et al.* (2017), 12.017 is the molecular weight of carbon (g mol^{-1}) and 86 400 is the number of seconds in a day.

Based on Eq. (10), at low levels of DOC inflow, net excretion might take place. The inflow concentration should, therefore, be chosen with care, until a consensus is reached that sponges excrete DOC under low DOC concentrations. The relationship of Eq. (10) has not been tested for concentrations higher than 1.65 mg L^{-1} DOC. The relationship will likely be different under high DOC concentrations. There is no data available on how *C. reniformis* retains DOC under high DOC concentrations. Fu *et al.* (2007) reported a maximum TOC uptake of 25 mg TOC g^{-1} FW d^{-1} by *Hymeniacidon perleve* under a TOC concentration of 52.9 mg L^{-1}. Based on this finding, the maximum DOC uptake by *C. reniformis,* $U_{max,}$ was estimated on 0.025 kg DOC kg DW^{-1} d^{-1}. For *C. reniformis* a wet to dry weight conversion of 5.59 g DW g FW^{-1} was used (this study).

The uptake of POC is based on the clearance rate measured for bacteria and is calculated by Eq. (12).

$$\frac{dU_{POC}}{dt} = CR \cdot \frac{S_w}{ps} \quad (12)$$

In which *CR* is the clearance rate in L d^{-1} cm^{-3} and *ps* is the sponge density in kg cm^{-3}.

Morganti *et al.* (2017) studied the net difference of different nitrogen species in the water inhaled and exhaled by *C. reniformis*. In this study, 49% of the inhaled NH_4 and 16% of the inhaled DON was taken up. The net uptake of nitrogen is expressed by Eq. (13) where subscript *j* indicates NH_4 or DON.

$$\frac{dU_N}{dt} = \sum_j p \cdot \frac{S_w}{ps} \cdot u_j \cdot \frac{M_{w,j}}{Q * dt} \quad (13)$$

In which u_i is the percentage of retained N.

2.4.4 Detritus, CO_2 and NO_X production

Through the sponge loop, DOM is transferred to higher trophic levels in the form of POM, also referred to as detritus (De Goeij et al., 2013). This process makes the nutrients and energy stored in DOM available to fauna that cannot digest DOM. Next to sponge biomass, the model will give an estimation of the produced detritus. It is thought that sponge detritus is released via rapid proliferation and shedding of sponge cells and via releasing undigested particulate food and metabolic waste products (Alexander et al., 2014; Rix et al., 2017). The particulate detritus production of the sponge is defined by the percentage of DOC and DON transformed into detritus plus the sponge mortality. The assimilation of carbon in detritus, D_c/dt in kg d^{-1}, is expressed by Eq. (14), and the assimilation of nitrogen in detritus, D_N/dt in kg d^{-1}, is expressed by Eq. (15).

$$\frac{dD_C}{dt} = U_{DOC} \cdot dc + m \cdot S_c \cdot snc \quad (14)$$

$$\frac{dD_N}{dt} = U_{DON} \cdot dn + m \cdot S_n \cdot scc \quad (15)$$

In which U_{DOC} and U_{DON} are the assimilated DOC and DON in kg d^{-1}, *dc* is the detritus conversation rate for carbon in kg kg^{-1}, *dn* is the detritus conversion rate for nitrogen in kg kg^{-1}, S_C is the carbon stored sponge biomass in kg, S_N is the nitrogen stored in sponge biomass in kg, s*cc* is the sponge carbon content in kg kg^{-1} and *snc* is the sponge nitrogen content in kg kg^{-1}. Alexander *et al*. (2014) showed in an isotope tracer study for five sponge species, including *C. reniformis*, a conversion efficiency of 11-24%, for $DO^{13}C$ to $PO^{13}C$ and a conversion efficiency of 18-36% for $DO^{15}N$ to $PO^{15}N$. There was no data available on the detritus production for *C. reniformis* specifically, so the midpoint of these percentages was used to estimate detritus production (17.5 and 27%).

The excretion of CO_2 (E_{CO2}) is described by Eq. (16) and the excretion of NO_X (E_{NOx}) is described by Eq. (17).

$$\frac{dE_{CO2}}{dt} = \frac{S_w}{ps} \cdot r \quad (16)$$

$$\frac{dE_{NOx}}{dt} = p \cdot \frac{S_w}{ps} \cdot n \quad (17)$$

In which *r* is the respiration rate in kg cm^{-3} d^{-1}, *p* is the pumping rate in L d^{-1} cm^{-3} and *n* is the nitrification rate in kg L^{-1}.

2.4.5 Mass balance

A mass balance is made up for each nutrient by including an unresolved "Rest"-factor, required to balance the carbon and nitrogen budget. The rest carbon, R_C, is the assimilated carbon through DOC (U_{DOC}) and POC (U_{POC}) minus the carbon used for respiration (CO_2), growth (S_C), and detritus production (D_c) (Eq. (18)). The unresolved nitrogen, R_N, is the assimilated nitrogen through the uptake of nitrogen (U_N) minus the nitrogen used for growth (S_n) and detritus production (D_n) (Eq. (19)). S_C is calculated by Eq. (20) and S_N by Eq. (21).

$$R_C = U_{DOC} + U_{POC} - (CO_2 + S_C + D_C) \quad (18)$$

$$R_N = U_N - (S_N + D_N) \quad (19)$$

$$S_C = g \cdot S_W \cdot sc \quad (20)$$

$$S_N = g \cdot S_W \cdot sn \quad (21)$$

2.4.6 Fish production

The amount of carbon and nitrogen (indicated by subscript *j)* leaving the system (W_j/dt, *g* d^{-1}) is based on the amount of given feed (g d^{-1}) minus the fraction of the given feed that is retained in the fish (g g^{-1}) (Eq. (22)). The given feed is compromised of the feed intake (f_{in}) plus the uneaten feed (f_w). The uneaten feed is a fixed percentage (20%) of the ingested feed. This percentage is a rough estimate, inspired by the study of Ballester-Moltó *et al.* (2017). The feed intake in g d^{-1} is calculated by Eq. (23) from the study of Lupatsch (2005). The retained feed is calculated using the feed conversion ratio (*FCR)* (g g^{-1}) comprising of feed intake by the fish divided by the gain in fish biomass (*BW/dt)*. The fish weight gain in g d^{-1} is calculated by Eq. (24) from the paper of Lupatsch (2005). The fish production in Eq. (25) starts at a fish size of 40 grams when the fish is a few months old and is moved to growing cages ($BW_{t=0}$=40). It is assumed the fish are transferred in spring and therefore the temperature data starts in March. Rad & Sen (2016) reported that it takes about 16 months for sea bass to reach the harvestable weight of 350 grams. Using the calculations of Lupatsch (2005), sea bass would reach harvest size sooner. Therefore, to reduce the growth rate, a factor for farm efficiency (*eff,* 75%) was added to Eq. (25). The number of fish kept, $F_{\#}$, is calculated by Eq. (26) and is based on the fish density of 15 kg/m^3 when the fish are 300 grams.

$$\frac{W_j}{dt} = F_{\#} \cdot f_j \cdot \left(\frac{\frac{f_{in}}{dt}}{1-fw} - \frac{f_{in}}{dt} + \frac{f_{in}}{dt} \cdot \left(1 - \frac{1}{FCR}\right) \right) \quad (22)$$

$$FCR = \frac{f_{in}}{\mathrm{BW/dt}} \quad (23)$$

$$\frac{f_{in}}{dt} = 0.0216 \cdot BW^{0.588} \cdot e^{0.063 \cdot T} \quad (24)$$

$$\frac{BW}{dt} = 0.0196 \cdot BW^{0.517} \cdot e^{0.065 \cdot T} \cdot eff \quad (25)$$

$$F_{\#} = \frac{F_d \cdot \pi \cdot (0.5 \cdot w)^{2} \cdot h_n}{300} \quad (26)$$

In which f_j is the content of nutrient j (C, N) in the feed, T is the water temperature in degrees Celsius, F_d is the fish density in g m^{-3}, and h_n is the height of the fish pen in m.

2.5 Inputs

The model was designed for the conditions of Turkey, the Eastern Mediterranean Sea. If data was not available for this region, data from regions with similar conditions were used (e.g. Greece). Additional data has been collected during the summer of 2019 in Kaş, Turkey in cooperation with M. Gökalp and T. Kooistra. The methodology and results of this data collection have been reported by Kooistra (2019). The model was tested with 27 scenarios in which nutrient inflow concentration, velocity, and sponge farm size were altered in different combinations. The used values can be found in Table 2. The parameters used in the model are given in Table A1, Appendix A.

Table 2 Values for carbon and nitrogen input, velocity, and sponge farm size classified as low, medium, and high which are used as input for the scenarios.

	Nutrient input *(mg L^{-1})*					**Velocity**	**Sponge planting**	
	DOC	POC	NH4	NOx	DON	m s^{-1}	Farm length	Sponge
Low/Small	1.2 Δ *	0.13†	0.0015§	0.0006†	0.056§	0.015	50	± 260
Medium	3Δ	0.34	0.0182*	0.0114§	0.21††	0.15	150	± 770
High/Large	10.24	1.16‡	0.066†	0.075†	0.50††	0.4	500	± 2600

* (Kooistra, 2019), Δ (Gökalp et al., 2019), # (La Rosa et al., 2002), †(Aksu and Kocatas, 2007) ‡ (Basaran et al., 2010), § (Yucel-Gier et al., 2007), $ (Morganti et al., 2017), †† (Porrello et al., 2003)

2.6 Data analysis

The output data were analysed using R (R Core Team, 2020) and Excel with the help of the packages dplyr (Wickham et al., 2018), reshape2 (Wickham, 2017), tidyverse (Wickham, 2019), qwraps2 (DeWitt, 2019), and writexl (Ooms, 2019). With the output of the fish farm model and sponge model, the needed sponge biomass and sponge farm space was calculated manually. Additional packages used for data visualisation were ggplot2 (Wickham, 2016) and ggpubr (Kassambara, 2018).

3. Results

3.1 Model evaluation

A sensitivity analysis of the parameters was done (Monte Carlo, range:0.1, rep: 1000). The total DOC, POC, NH4, and DON in kg in the water body ($M_{w,i}$) were most sensitive for changes in height, width, and velocity. The concentration of NO_X in kg in the water body was most sensitive for changes in pumping rate and sponge density. S_H, S_C, and S_N were most sensitive to the diameter of the lantern, the number of layers per lantern, and the maximum growth rate. R_C and R_N were most sensitive to the collagen percentage and the growth rate G. There was not sufficient data available to validate the model.

3.2 Model outcomes

Model outcomes of the scenarios for collagen harvest, assimilated C, and assimilated N after one year under different nutrient concentrations and different farm sizes are displayed in Table 3.

Table 3. Output data for produced collagen, assimilated carbon and nitrogen in kilogram under different carbon and nitrogen inflow concentrations, ambient flow and sponge farm sizes.

Nutrient inflow concentration	**Ambient flow**	**Farm size**	**Produced sponge biomass** (kg)	**Collagen harvest** (kg)	**Fixed Carbon**				**Fixed Nitrogen**			
					Biomass	*Detritus*	*CO_2*	*Unresolved*	*Biomass*	*Detritus*	*NO_X*	*Unresolved*
Low	Low	Low	6880	62	3099	1278	3436	-2555	949	385	460	-1118
Low	Low	Medium	20417	183	9220	3626	10308	-9128	2823	1119	1379	-3455
Low	Low	High	66100	590	30054	10576	34292	-41211	9201	3407	4589	-12305
Low	Medium	Low	6914	62	3109	1314	3433	-2291	952	391	459	-1099
Low	Medium	Medium	20715	186	9322	3917	10301	-7058	2854	1168	1379	-3312
Low	Medium	High	68749	617	30964	12767	34329	-25531	9479	3847	4594	-11172
Low	High	Low	6906	62	3108	1316	3430	-2270	952	391	459	-1097
Low	High	Medium	20764	187	9345	3948	10317	-6898	2861	1174	1381	-3305
Low	High	High	69036	621	31086	13036	34364	-23770	9517	3893	4599	-11062
Medium	Low	Low	7992	74	3490	6451	3443	24632	1068	853	461	557
Medium	Low	Medium	24211	223	10557	19392	10352	72495	3232	2426	1385	966
Medium	Low	High	80843	745	35220	52140	34481	170981	10782	6892	4614	-1850
Medium	Medium	Low	7962	73	3480	6455	3446	24919	1065	877	461	687
Medium	Medium	Medium	23926	220	10456	19378	10344	74616	3201	2615	1384	1970
Medium	Medium	High	80104	738	34980	64652	34512	246866	10709	8548	4618	5578
Medium	High	Low	7963	73	3481	6459	3448	24952	1066	879	461	697
Medium	High	Medium	23936	220	10459	19399	10355	74875	3202	2633	1386	2058
Medium	High	High	79870	735	34886	64637	34503	248659	10680	8705	4617	6459
High	Low	Low	4454	36	2245	6337	3381	37675	687	1702	452	4744
High	Low	Medium	13714	112	6864	19058	10168	108275	2101	4800	1361	12263
High	Low	High	49376	413	24161	63623	33943	321051	7397	13256	4542	26526
High	Medium	Low	4407	36	2230	6340	3382	38561	683	1756	453	5118
High	Medium	Medium	13259	107	6703	19028	10152	115133	2052	5233	1359	15097
High	Medium	High	44636	363	22506	63511	33884	377589	6890	17056	4534	47541
High	High	Low	4400	36	2227	6336	3380	38598	682	1758	452	5142
High	High	Medium	13229	107	6692	19028	10152	115697	2049	5267	1359	15344
High	High	High	44208	358	22354	63415	33833	382968	6843	17395	4528	49995

3.3 Carbon and nitrogen balances for sponges and sponge biomass production

Figure 4 shows C and N mass balances for different nutrient inflow concentrations in gram per kg sponge weight ($SW_{t=375}$) per day. The mass balances showed that under low nutrient inflow the assimilation exceeded calculated C and N uptake, resulting in a negative rest C pool (R_C: -2.02±0.47 g kg^{-1} d^{-1}) and N pool (R_N = -0.86±0.03 g kg^{-1} d^{-1}). Under medium nutrient inflow and high nutrient inflow, a surplus of C and N was taken up: the average R_C was positive (1.82±0.20 g kg^{-1} d^{-1} and 2.88±0.17 g kg^{-1} d^{-1} respectively) and the average R_N was positive (0.40±0.22 g kg^{-1} d^{-1} and 3.58±0.65 g kg^{-1} d^{-1} respectively). The only exception was for N at the large sponge farm under medium nutrient inflow and low ambient flow (R_N =-0.15 g kg^{-1} d^{-1}, not shown in Figure 4).

Under low nutrient inflow, the largest portion of assimilated C was used for respiration: 2.62±0.00 gr CO2 g kg^{-1} d^{-1} (44.07%). Under medium and high nutrient inflow this was, excluding *Rc*, detritus production: 4.80±0.33 g kg^{-1} d^{-1} (17.00%) and 4.91±0.00 g kg^{-1} d^{-1} (12.87%) respectively (Figure 4A). Under low and medium nutrient concentration the largest portion of N uptake was used for sponge growth: 0.72±0.01 g CO2 g kg^{-1} d^{-1} (52.95%) and 0.81±0.00 g kg^{-1} d^{-1} (37.07%) respectively. Under high nutrient concentration largest portion was used for detritus production: 1.30±0.11 g kg^{-1} d^{-1} (22.52%) (Figure 4B).

Figure 4. Carbon (C) and nitrogen (N) mass balance budgets for sponge uptake and assimilation under different inflow concentrations in gram per kg planted sponge biomass per day. Averages have been shown for different flow rates. Percentage of total uptake or assimilation are shown inside the bars. **A.** Carbon balance with the uptake of DOC (UD_{OC})and POC (U_{POC})and the assimilation of C in body mass (S_C), detritus (D_C) and CO2 (gr per kg sponge per day). **B.** Nitrogen balance with the uptake of NH4 (U_{NH4})and DON (U_{DON})and the assimilation of N in body mass(S_N), detritus (D_N) and NOx (gr per kg sponge per day). For N the scneario with a large sponge farm, medium nutrient inflow and low ambient flow is not shown.

3.4 Carbon and nitrogen fixation

In one year, 2.95±0.05 kg, 4.99±0.16 kg, and 4.59±0.03 kg of C were fixed in sponge biomass, detritus, and CO_2 per kg of planted sponge under low, medium and high nutrient inflow (Figure 5). The highest amount of C was fixed under medium nutrient inflow when sponge biomass production was highest (1.32±0.01 kg C). Under low, medium, and high nutrient inflow 0.68±0.01 kg, 0.89±0.03 kg, and 1.08±0.06 kg of N was fixed in sponge biomass and detritus (Figure 5). The highest amount of N was fixed under high nutrient inflow, due to the high production of detritus (0.64±0.05 kg N).

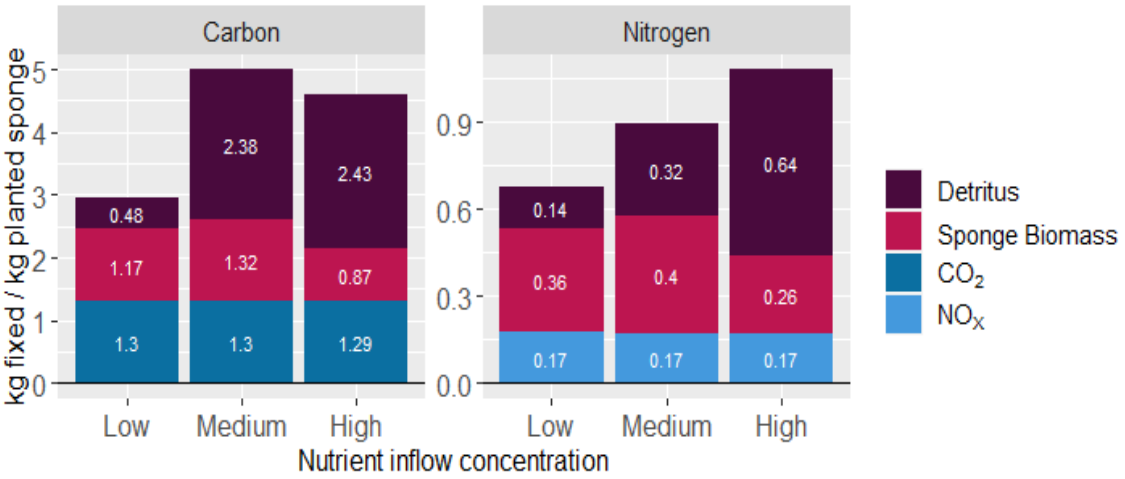

Figure 5. Fixed carbon and nitrogen in sponge biomass and CO_2 in kg per kg planted sponge biomass. An average is shown for different velocities.

3.5 Dimensions of the sponge farm

Per fish, 0.39 kg of C and 0.07 kg of N waste were produced per year. In the whole fish pen 121.36 tonnes of sea bass, $1.38 \cdot 10^5$ kg of C waste, and $0.24 \cdot 10^5$ kg of N waste were produced. Table 4 shows the percentage of fish waste from one fish, a tonne of fish, and a fish pen, fixed in sponge biomass, detritus, and CO_2 under different nutrient inflow concentrations. Fixation of N in NO_X was excluded. Furthermore, it shows the needed sponge biomass and sponge farm space in m^2 to compensate for the waste from one fish, a tonne of fish, and a fish pen under different nutrient inflow concentrations. Based on a surface density *ps* of 0.32 g DW cm^{-2} (Table A1, Appendix A), a lantern width of 0.61 m, and an intern-lantern space of 2 m (Section 2.1), 25 g sponge DW can be cultivated per m^2. One gram of planted sponge biomass (DW) takes up between 0.75% and 1.36% of the waste produced by one fish. To balance the C waste of one fish cultivated to the size of 350 grams, minimally 78.22 grams of sponge are needed and to balance the N waste minimally 73.59 grams of DW sponge biomass is needed. Between 0.23 to 0.39 tons of sponge biomass was needed to balance the C and N waste of a ton of produced fish. To balance the C waste of a fish farm, a minimum of 1279 hectares of sponge farm per m^2 of fish farm was needed. To balance the N waste of a fish farm, a minimum of 1203 hectares of sponge farm per m^2 of fish farm was needed .

Table 4 Percentage of carbon and nitrogen waste fixed in sponge biomass, detritus, and CO_2 per kg of sponge under different nutrient inflow concentrations in one year in % per fish and % per fish pen and sponge biomass and space needed to balance fish cultivation waste.

	Carbon			**Nitrogen**		
	Nutrient concentration			*Nutrient concentration*		
	Low	Medium	High	Low	Medium	High
<u>One fish</u>						
Waste uptake *(% per gr planted sponge DW)*	0.75±0.01	1.28±0.04	1.17±0.01	0.75±0.02	1.08±0.03	1.36±0.07
Sponge needed *(gram DW per fish)*	132.47	78.22	85.14	132.65	92.65	73.59
<u>Ton of fish</u>						

Waste uptake *(% per tonne planted sponge DW)*	259.22±3.99	438.99±13.35	403.32±2.12	258.86±5.24	370.64±11.12	466.63±24.38
Sponge needed *(ton DW per ton produced fish)*	0.39	0.23	0.25	0.39	0.27	0.21
Space needed *(m^2 sponge farm per ton produced fish)*	16057	9482	10320	16079	11230	8920
Fish pen						
Waste uptake ***(%** per tonne planted sponge DW)*	2.14±0.03	3.62±0.11	3.32±0.02	2.13±0.04	3.05±0.09	3.84±0.2
Sponge needed *(ton DW per fish pen)*	46.82	27.65	30.09	46.88	32.74	26.01
Space needed *(m^2 sponge farm per fish pen)*	$1.95 \cdot 10^6$	$1.15 \cdot 10^6$	$1.25 \cdot 10^6$	$1.95 \cdot 10^6$	$1.36E \cdot 10^6$	$1.08 \cdot 10^6$
Ratio fish farm : sponge farm (m_2)	1:2165	1:1279	1:1392	1:2168	1:1514	1:1203

3.6 Bioremediation potential

The highest difference in C and N outflow concentrations was achieved under low flow rates/velocity (Figure 6). Under medium and high ambient flow, the decrease in concentration was low and was smaller than 10%. Under low ambient flow, the net change in C concentration (DOC + POC) was -3.70±2.09% d^{-1}, -10.26±5.65% d^{-1} and -26.11±13.56% d^{-1} for small, medium and large size sponge farms, respectively. For N (NH_4 + DON) this was -7.58±3.97% d^{-1}, -19.26±9.03% d^{-1}, and -42.51±14.58% d^{-} respectively. The nutrient inflow concentration influenced the decline of DOC (Figure 6). Under medium nutrient inflow concentration, the decrease in C concentration was highest: 30.61% (Figure 7). For N, when NO_X was also included, the concentration increased with 2635% under low nutrient concentration and low ambient flow (Figure 7).

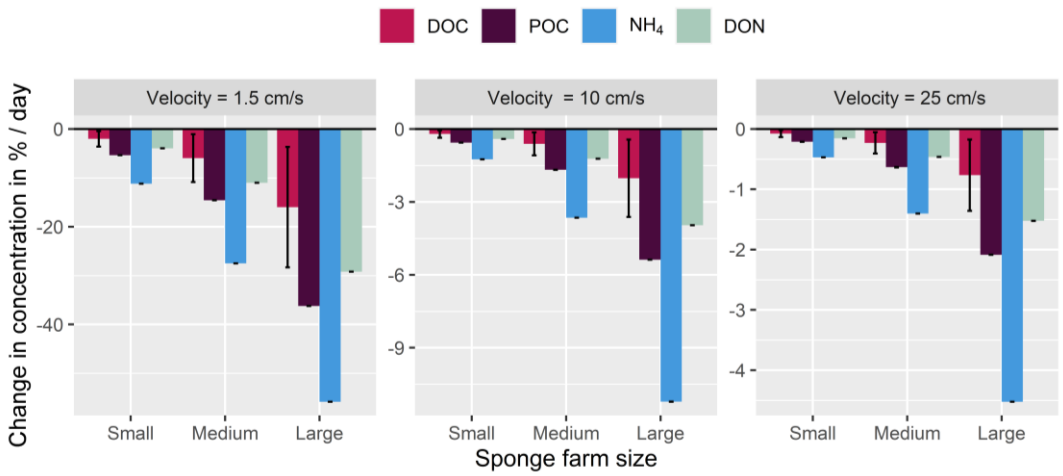

Figure 6. Difference in outflow concentrations of DOC, POC, NH_4 and DON due to sponge uptake under different sponge biomass and velocity. The difference in POC due to sedimentation has not been shown. Means are shown where the error bar shows the variation caused by the nutrient inflow concentration.

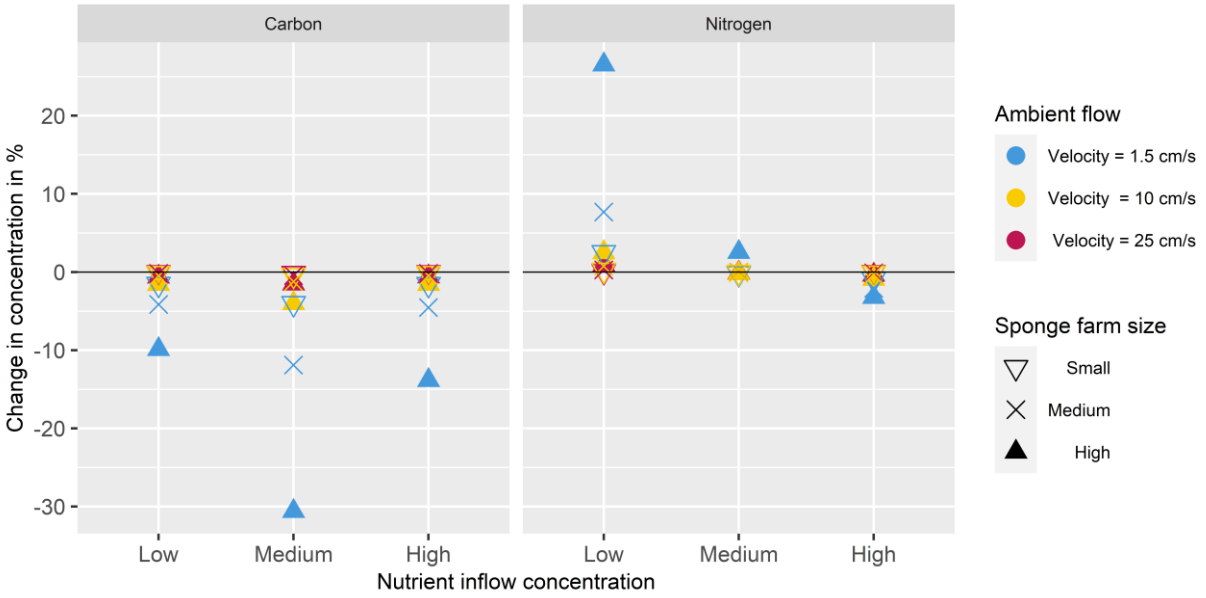

Figure 7. Difference in outflow concentrations of C (DOC + POC) and N (NH_4,DON and NO_X) due to sponge uptake under different nutrient inflow concentrations. The colour symbolises the velocity and the shape the sponge farm size. The difference in POC due to sedimentation has not been shown.

Figure 8A shows the TOC concentration of the water body and Figure 8B the harvested collagen in grams per kg planted sponge biomass and the increase in sponge biomass (%). The biomass increase under low, medium, and high nutrient inflow was 259.50%, 301.14%, and 172.02% respectively. The highest sponge biomass increase was 304.36% for medium nutrient inflow, large farm size, and low velocity. The lowest biomass increase was 168.51% under high nutrient inflow, small farm size, and high velocity. The average collagen harvest under low, medium, and high nutrient inflow was 23.30±0.39, 27.72±0.17 and 14.01±0.69 gr per kg planted sponge biomass respectively.

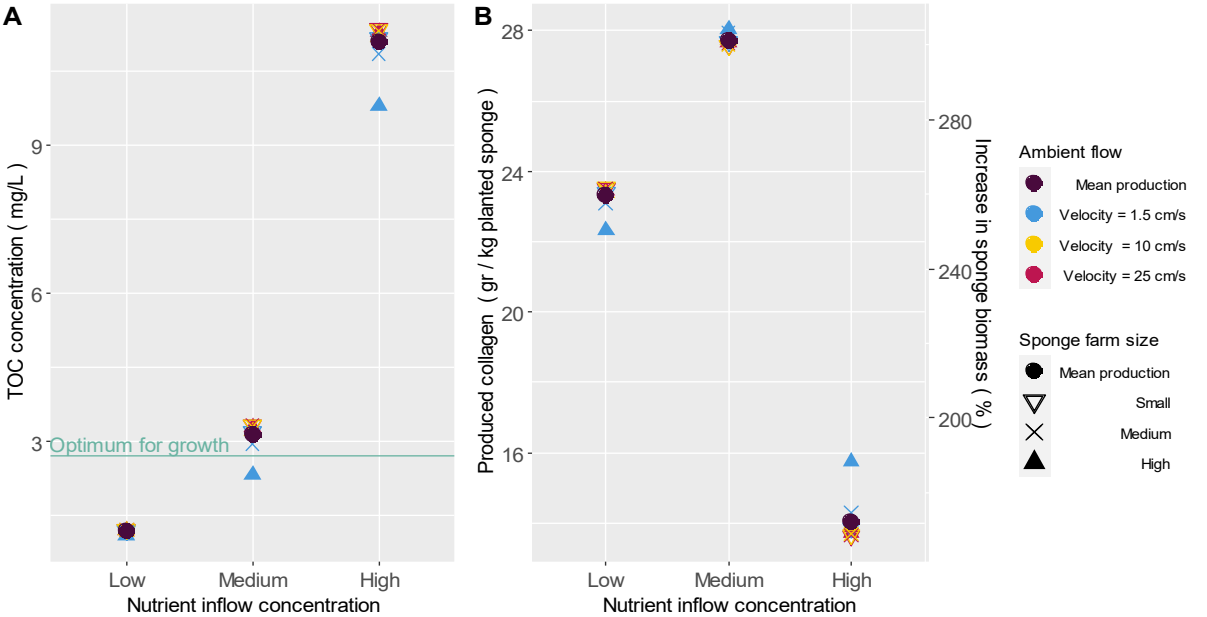

Figure 8. **A.** TOC concentration of the water body under different nutrient inflow concentrations, velocity (colour) and sponge farm size (shape). The green line indicates the optimum TOC concentration for growth set in the model based on Eq. (8). **B.** Harvested collagen in grams per kg planted sponge biomass and increase in sponge biomass in % of planted sponge biomass under different nutrient inflow concentrations, velocity (colour) and sponge farm size (shape).

4. Discussion

As stated in the Introduction, this study aimed to develop a model predicting sponge production and bioremediation capacity of sponges for sea-based IMTA systems with sea bass (*D. labrax)* production. The effect of ambient flow, nutrient inflow concentration, and sponge farm size on bioremediation capacity and sponge production was explored. In the section, the research questions and the C and N mass balances will be discussed. The research questions were: 1) "How large should a sponge farm be to fully compensate C/N waste of a suspended sea bass farm?", 2) "How is the direct reduction of the C/N concentration by a sponge farm influenced by the ambient flow, C/N concentration, and sponge farm size?", and 3) "How much sponge biomass/collagen can be harvested over the course of a year?". Lastly, recommendations for improvements of the model and future research will be discussed.

4.1 Sponge farm size to fully compensate C/N waste

The model calculated that a circular sea bass fish pen with a surface area of 900 m^2 and a volume of 7069 m^3 produced in one year 12.37 tonnes of seabass, $1.38 \cdot 10^5$ kg of C waste, and $0.23 \cdot 10^5$ kg of nitrogen N waste. Feed conversion ratios (FCR) calculated by the model (Figure B 4**,** Appendix B) are in line with the FCR of European seabass and seabream cage farms in the Mediterranean sea, which vary from 1.7:1 to 2.3:1 (Macalister Elliott & Partners LTD, 2009; Rad and Sen, 2016). The waste a sea bass fish pen produces depends on a.o. the given feed, the age of the fish, feeding rate, and the stocking density. The feeding rate, the feed type, and the fraction of uneaten feed generally vary with the size of the fish (Ballester-Moltó et al., 2017). In the model, the type of feed and fraction of uneaten feed was independent of fish size. Therefore, these results should be interpreted with some caution.

The modelled results in relation to nutrient concentrations showed that one tonne of sponge biomass could filtrate up to 439% of the C waste and up to 467% produced cultivating one tonne of sea bass. However, to eliminate the total fish pen waste, producing 121.36 tonnes of sea bass, a minimum sponge biomass of 27.65 tonnes for carbon and 26.01 tonnes for nitrogen. With the assumed sponge production system, a large area would be needed to cultivate this amount of sponge biomass. The results for sponge farm dimensions show that the minimal ratio m^2 fish farm to m^2 sponge farm was 1:1279 to balance carbon waste and 1:1203 to balance nitrogen waste. The maximum needed space was 195 hectares under low nutrient inflow concentrations (ratio fish farm: sponge farm of 1:2168 m^2). Assuming nutrient levels are lower far away from the source of pollution, placing a sponge farm further away from a fish pen greatly reduces efficiency. The needed space might even be larger: maximum growth rates of sponges have been assumed and seasonality of sponge growth has not been considered. Therefore, the model probably shows an overestimation of the sponge capacity. This raises the question of what the ecological impact would be of such a large sponge farm on the system.

Acquiring a large initial stock of sponges sustainably will also pose a challenge. Additionally, it should be kept in mind that the C and N stored in detritus are not harvested and still affect the environment, although in a different form. Lastly, the model calculations are made for only one fish pen (12.37 tonnes) while in 2018 the marine finfish aquaculture sector in Turkey alone already produced 208 463 tonnes (Turkstat, 2018).

The model assumed sponge lanterns with 25 layers and a distance of 2 meters in between lanterns (Figure B 1, Appendix B). Possibly, the needed space could be reduced by adopting a more space-efficient sponge farm design. In the model, due to time and computational constraints, the sponges did influence the nutrient concentration of the whole water body, but not the local concentration available for their direct neighbours. To test the implications of increasing sponge density, a future version of the model could take this into account. Proximity to other sponges might locally reduce the nutrient concentrations. Under high nutrient concentrations, a high density could be beneficial, reducing the risk of smothering. However, under low nutrient concentrations, it could have the opposite effect, leading to starvation. Dependent on the conditions of the sponge plantation, a balance should be found between providing enough space and facilitating the optimal conditions for sponge growth. In the study of Zijffers (2009), the sponges further away from the nutrient source were exposed to a lower availability to food due to the uptake of the sponges in the upstream segment. It is advisable to include this in future versions of the model.

4.1 The influence of the C/N inflow concentration, ambient flow, and sponge farm size on outflow concentration

The highest reduction in DOC, POC, NH_4, and DON concentrations was achieved under low ambient flow and large sponge size (Figure 6). Under medium and high ambient flow and low and medium sponge coverage, the differences in outflow concentrations were minimal (Figure 7). In the model, a high velocity refers to a high daily inflow of mass and water. Other aspects like the effect of the residence time were however not considered. Vogel *et al.* (1977) showed that under higher currents, the rate with which water passes through sponges also increases. However, in the model, the pumping rate was unrelated to flow. Furthermore, the study of Leys *et al.* (2011) showed that the energy used for pumping decreases under increasing velocity. It would be interesting to include these aspects as well in assessing the role of current velocity.

The decline in C concentration was highest under medium nutrient concentration inflow. Under this nutrient concentration, the assumed growth and storage of C in biomass was highest (Figure 5). The inflow concentration had the largest effect on the change in DOC concentration. This can be explained by the assumption that the uptake of DOC is dependent on the DOC concentration (Eq. (10)). It was assumed that under higher DOC concentrations the pumping rate would decrease, and DOC uptake would stabilise up to U_{max} (Eq. (11)). Therefore, also at very high C inflow concentrations, uptake would never exceed U_{max}. The study of Gokalp *et al.* (2020) on *C. reniformis* showed that sponges at a polluted site (3.34±0.86 mg C L^{-1}) pumped less efficiently then sponges at a pristine site (1.37±0.08 mg C L^{-1}). They argue this could be either due to the conditions at the polluted site being close to the threshold of turbidity that *C. reniformis* could sustain, or because the higher food abundance means the sponges did not have to pump as much to obtain the same amount of food. If the first scenario is true, the threshold of *C. reniformis* could be close to 3.34 mg C L^{-1}.

In contrast to the uptake of DOC, the uptake of POC, NH_4, and DON is in the model a fixed percentage of the inhaled C/N. Therefore, for these nutrients under different inflow concentrations, the percentage of change on the outflow concentration was the same. It is likely that also for POC, NH_4, and DON the uptake is dependent on the inflow concentration. In the model, the pumping rate remains constant and is independent of the nutrient concentration or current velocity. Several studies have shown that glass sponges can control the pumping rate and arrest pumping under high velocities or high particulate loads (Gerrodette and Flechsig, 1979; Tompkins-Macdonald and Leys, 2008; Leys et al., 2011). Also, Gokalp *et al.* (2020) showed that nutrient concentration influenced the pumping activity of *C. reniformis*. More data is needed to establish what the relationship is between different nutrient concentrations and nutrient uptake, both for DOC as for POC, NH_4, and DON. This would provide a more accurate foundation for the model and would help to estimate what impact a sponge farm can have on the waste stream of a fish farm for different nutrients.

An increase in total N concentration was observed under low nutrient concentration and low ambient flow (Figure 7). In the model, the excretion of NO_X was constant and independent of the inflow of NH_4 and DON, even though nitrification likely depends on the nutrient inflow concentration. Therefore, at low N concentration and low N uptake, excretion would exceed uptake. The nitrification rate is dependent on many factors, and therefore difficult to estimate. Several nitrifiers have been found in various sections of sponges, with different nitrification rates (Subina et al., 2018). Reported microbial nitrification rates are not only variable between different sponge sections, sponge species, and studies (Schläppy et al., 2010; Morganti et al., 2017; Subina et al., 2018). The activity of the bacterial population is influenced by the pumping activity of the sponge (Schläppy et al., 2010). Furthermore, the pumping rates from sponges are dependent on the environmental conditions and on the bacterial community as well with different pumping rates being observed for high microbial abundance (HMA) sponges and low microbial abundance (LMA) sponges (Weisz et al., 2008). Therefore, it is likely that nitrification through *C. reniformis* is also variable for different environmental conditions. The NO_X excretion in the model was based on Morganti *et al.* (2017). In their study, the excretion of NO_X was higher than the uptake of NH_4. The lack of correlation could indicate that multiple metabolic pathways exist (Zehr and Ward, 2002; Morganti et al., 2017). For example, DON might be oxidised to NH_4, which in turn can be used for nitrification (Ribes et al., 2015). In conclusion, the complex interaction between the sponges, the microbial community, and the environmental conditions are

not yet well understood. Therefore, it will remain a challenge to accurately estimate the excretion of NO_X until further research is done.

4.2 Harvested biomass and collagen

The maximum amount of sponge biomass production and collagen production was reached at the large farm site (15000 m^2) under medium nutrient flow and low ambient flow. Under these conditions, a weight increase of 304.36% $year^{-1}$ was reached with a harvest 71.366 kg of sponge biomass and 745 kg of collagen. Collagen production per kilogram of planted sponge biomass was highest under medium nutrient inflow (Figure 8B). This can be explained by the combination of the TOC-dependent growth rate (Eq. (8)) and the fixed mortality rate used in the model.

In the model, the optimal TOC concentration for maximum growth was set at 2.6 mg L^{-1}. At a medium nutrient concentration, both the medium and large sponge farm lowered the nutrient concentration towards the optimal TOC concentration, resulting in higher collagen production compared to the small sponge farm. The optimal TOC concentration used in this study was a rough estimate. For future research, it would be of interest to find the optimal TOC concentration for the growth of *C. reniformis*. In the design of the sponge farm, this information could help to decide the right distance from the fish pen to supply enough nutrients and prevent smothering by creating space where sedimentation and dilution can sufficiently reduce TOC/POC concentrations.

Based on the environmental conditions, the sponge farm size should be considered. The sponge farm size significantly influenced the TOC concentration under low ambient flow. Under low ambient flow and low nutrient inflow concentration, the large sponge farm even lowered the nutrient concentration to a concentration where a food shortage and reduced growth was assumed (Figure 8A). Under high nutrient inflow, the large sponge farm reduced the TOC concentration the most, but in this case resulting in a higher growth rate and higher collagen production compared to the small and medium sponge farm (Figure 8B).

In the model, to exclude competition for space, it is assumed that part of the sponges is harvested every day (Eq. (7)). When harvesting frequently from the sponges, sponge tissue regeneration is possibly stimulated. The study of Pozzolini *et al.* (2012) showed that after cutting a tissue fragment, the repaired *C. reniformis* sponge tissue contained 8.7-fold higher levels of nonfibrillar collagen mRNA. Nonfibrillar collagen is thought to play a role in the regeneration of sponge tissue and is also thought to be one of the best candidates for future biotechnological applications (Pozzolini et al., 2012). Frequent harvesting could therefore potentially lead to higher collagen contents then now used in the model (0.01 g g-1). However, due to logistics, it might not be feasible to harvest with a high frequency.

4.3 Reflection on the mass balances for *C. reniformis*

In the model, at low nutrient levels, not enough N and C was filtered to compensate the N and C used for assimilation. For N these findings are in line with the findings of Morganti *et al.* (2017). They argue the missing N to balance the N budget might derive from the uptake of particulate detritus. For C, the large unresolved fraction of carbon (*Rc*) indicates that the uptake might have been underestimated, or the assimilation overestimated. Regarding the uptake of C; the clearance rate for POC was based on the uptake of bacteria from experiments performed in Turkey. The clearance rate of POC, which is also composed of C from various planktonic cells, eukaryotic algae, and particulate detritus, could differ from the clearance rate for bacteria alone. The uptake of DOC at low nutrient concentrations was based on the relationship described in the study of Morganti *et al.* (2017). For future research, it would be advisable to verify this relationship in the field.

Regarding the overestimation of the uptake: under low nutrient concentration, the growth rate of *C. reniformis* was around the 0.005 d^{-1} while in the study of Gokalp *et al.* (2019), this was for pristine conditions 0.0028 d^{-1}. Growth might, therefore, be overestimated in the model. Furthermore, Koopmans *et al.* (2010) found that about 90% of the filtered C was used for generating energy for growth, maintenance, reproduction, and pumping and that only 10% of the C was fixed in biomass in *Haliclona oculata*. The model outcomes showed that under low nutrient concentration growth compromises 66% of the filtered C, 39.7% of the assimilated C, 170% of the filtered N and 53% of the assimilated N. Secondly, for *C. reniformis* a respiration rate (2 μmol O_2 cm^{-3} h^{-1}) similar to the respiration rate reported for *Halisarca caerulea* (1.82 to

3.98 µmol cm^{-3} h^{-1}) was found (De Goeij et al., 2008; Kooistra, 2019). In the model, this translated to a respiration value of 0.26 kg C kg^{-1} DW d^{-1}. This equals a fraction of 44% of the assimilated C and 67% of the calculated C uptake. In contrast, de Goeij *et al.* (2008) found that 39-45% of the filtered C was used for respiration for the sponge *Halisarca caerulea*. Possibly, *C. reniformis* used a larger portion of the filtered C for respiration under low nutrient concentrations compared to *H. caerulea,* or also respiration is overestimated.

At medium and high nutrient inflow, the C and N uptake was higher than the calculated assimilation leading to a positive R_C/R_N. Most of the used parameters are based on experiments done under low nutrient concentration. Therefore, the uptake and assimilation of C and N under higher nutrient inflow scenarios are estimations and could, therefore, be over or underestimated. The maximum DOC uptake was based on the maximum uptake of TOC by *Hymeniacidon perleve* at a TOC concentration of 52.9 mg L^{-1}. Following the uptake curve defined by Morganti *et al.* (2017), this maximum was already reached at a DOC concentration of ±2.5 mg L^{-1}. It is possible that *C. reniformis* filters more DOC than *H. perleve* and that the maximum uptake at the tested DOC inflow was higher than now estimated. It is also possible that the total uptake of *C. reniformis* does not increase much more at concentrations higher than 1.65 mg L^{-1}, which is the maximum concentration tested by Morganti *et al.* (2017).

As mentioned earlier, the uptake of C and N might not be overestimated, but the assimilation underestimated. It is probable that part of the filtrated C and N is used for reproduction. However, this was not included in the model as no data was found on the C used for reproduction by *C. reniformis.* The C used for growth was 9% of the filtered C for medium nutrient inflow and 7% for high nutrient inflow, which is in line with the findings of 10% of Koopmans *et al.* (2010) for the sponge *Haliclona oculata.* In de model, the N used for growth was 35.71% of the filtered N under medium nutrient inflow and 13.75% under high nutrient inflow. No data was available on the relative assimilation of N for growth to allow a comparison.

Part of the missing C and N assimilation in the nutrient budgets could have been used for cell turnover. Cell turnover is cell proliferation of choanosome compensated by cell loss, leading to cell recycling/shedding. This cell turnover maintains a healthy population of cells and is hypothesized to be the main underlying mechanism in producing sponge-derived detritus (Alexander et al., 2014). For *C. reniformis*, Alexander *et al.* (2014) found high amounts of cell shedding and a choanocyte proliferation. It is unknown to what extent cell shedding contributes to the sponge detritus production. In rats, proliferation rates decrease during starvation and increase after re-feeding (Goodlad and Wright, 1984). Conceivably, also in sponges the proliferation rate and cell turnover rate is dependent on the nutrient concentration. In the model, detritus production is dependent on the nutrient flow and based on the data collected by Alexander *et al.* (2014). However, these experiments have only been done under one nutrient concentration. Furthermore, the study of Alexander *et al.* (2015) showed that cell proliferation differs for wounded tissue and non-wounded tissue. Possibly, detritus production might also depend on the harvesting style, where sponge biomass harvested by cutting a part of an individual would possibly decrease the detritus production. Lastly, the study of McMurray *et al.* (McMurray et al., 2018) found low detritus production for HMA sponges, and they hypothesise the large uptake of DOC is used for growth instead. For future research, it would be interesting to test if there is a relationship between nutrient inflow concentration, cell turnover, and detritus production.

4.4 Recommendations

Sponges are complex organisms, and much is still unknown about their relationship with their environment. An enhanced understanding of how the environmental conditions affect sponge processes is needed for predicting whether sponge farms can be used for balancing fish farm waste. To improve the reliability of model outcomes, further data collection is especially required on processes like nutrient uptake, growth, cell turnover, detritus production, mortality, and reproduction under different food concentrations. Furthermore, research has shown that temperature, depth, and light influence sponge morphology, growth, and pumping (Wilkinson and Vacelet, 1979; Kooistra, 2019; Gökalp et al., 2020). However, not enough data was available to include this in the model.

In future versions of the model, it could be interesting to directly link the nutrient inflow concentrations to the fish waste. In this case, the distance between the fish farm and the sponge farm, the ambient flow, water

depth, and the sedimentation could determine the dilution of the fish waste, and therefore the nutrient inflow concentration. The current model assumes laminar flow where the sponge farm is positioned at one side of the fish farm and is always exposed to the same ambient flow. However, the direction of flow varies naturally and therefore the fish farm waste is not transported only in one direction. Taking this into account, multiple formations of the sponge farm are possible, including positioning sponge lanterns in half circles or all around the fish farm. In this case, not all compartments of the fish farm are exposed to similar flow rates and nutrient inputs. A spatial component would then be needed to properly assess total sponge growth and total fish waste remediation.

In this study, the effect of a sponge farm on the nutrient outflow concentration was discussed. It would be interesting to evaluate the effect of a decline in C and N concentration on the ecosystem, even when the reduction is only small, Models predicting the effect of fish farms on the environment or the effect of eutrophication (for example on the benthic community or microalgae growth) have already been created (Cromey et al., 2002; Hadley et al., 2015). Linking these models together will give more insight into the potential of sponge farms in IMTA systems. Cooperation with existing models for other extractive species could also be beneficial to optimize the design of an IMTA system. A diverse collection of extractive species could take up more forms of C and N. For example, the addition of the green algae *Ulva lactuca* could take up N that is not retained by sponges and the NOx excreted by sponges (Ben-Ari et al., 2014). Lastly, phosphate could be included as a nutrient in the model.

Possibly sponge farms could be used to clean up polluted bays. Turkey has urged cage aquaculture to move offshore (Basaran et al., 2010). However, remnants of previous fish farming activity in combination with sewage, runoff and coastal development can still expose bays to elevated eutrophication (Yucel-Gier et al., 2013). An example of this is the Gulluk Bay in Turkey (Demirak et al., 2006; Yucel-Gier et al., 2013). For validation of the model it would be interesting to investigate a sponge farm in open water and in a closed bay where current velocities are lower. This could give insight in the role of current velocities on sponge production and bioremediation. Secondly, it might also contribute to understanding where sponge aquaculture would be most beneficial.

The created model could be used for other sponge species, locations and environmental conditions. However, due to the diversity between various sponge species, adaptions may be required based on the sponge species used. Likewise, if a different fish species is used, the model will have to be adapted as the fish waste in this model was estimated based on sea bass production. Although the model is focused on sponges, an extension to other filter feeders should be possible as well.

5. Conclusion

With the ongoing growth of the sea-based aquaculture sector in the Mediterranean, it is becoming increasingly important to reduce the negative environmental impact of the sector. The created model can be used to illustrate the effects of environmental conditions on the bioremediation capacity of *C. reniformis*, and possibly of other sponge species in the future as well.

A tonne of *C. reniformis* biomass could balance maximally 439% of the C waste and 466% of the N waste produced by one tonne of farmed fish. The ratio fish farm to sponge farm was 1:1279 m^2 for C and 1:1203 for N. The needed space increased greatly under low nutrient concentration, to 1:2165 m^2 for C and 1:2168 for N. Therefore, the distance with which a sponge farm is placed to a source of pollution can greatly affect bioremediation efficiency. The created model showed that under low velocity sponges were able to reduce ambient nutrient concentrations most, with a maximum decline of 30.61% in total C concentration. At higher velocities the change in C and N concentrations was minimal. To maximise sponge biomass production and collagen production, the optimal sponge farm size and optimal distance to the source of pollution can be chosen based on the optimal TOC concentration for sponge growth and velocity/ambient flow of the selected area. For *C. reniformis* highest sponge growth, sponge biomass harvest (max 2.69 kg/kg) and collagen harvest (max 28.05 g/kg) were reached under medium nutrient inflow.

This study has given rise to many questions in need of further study. For all tested scenario's gaps were found in the nutrient mass balances. Further research is needed on how *C. reniformis* and its microbiome adapts to environmental conditions and how this influences the uptake and assimilation of C and N.

Especially more data is needed on the effect of environmental conditions on sponge growth, detritus production, respiration, and nitrification rates. Furthermore, with the large sponge farm area needed to balance the fish farm waste, it is unlikely *C. reniformis* farming can fully compensate fish farm waste production. Future research could investigate if the needed space could be reduced by adopting a more space-efficient sponge farm design and how this would affect sponge production and bioremediation. Lastly, it would be interesting to test if sponge farms could be used to clean up bays that are exposed to a low ambient flow and were polluted by cage farming in the past.

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Appendix A

Table A1. Parameters used in the model

	Description	Units	Range	Used	Source
Water					
h	Column height	m		20	Estimated
w	Column width	m		30	Estimated
l	Column length	m	50-500	*Scenario-based*	
v	Sea current speed / velocity	cm s^{-1}	0.5 - 45	*Scenario-based*	(El-Geziry and Bryden, 2010)
[DOC]	Pristine	kg L^{-1} *10^3	0.73 – 1.57	*Scenario-based*	(Morganti et al., 2017)
[DOC]	Fish farm	kg L^{-1} *10^3	Nd- 10.43	*Scenario-based*	(La Rosa et al., 2002)
[POC]	Pristine	kg L^{-1} *10^3	0.132 - 1.1	*Scenario-based*	(Aksu and Kocatas, 2007; Basaran et al., 2010)
[POC]	Fish farm	kg L^{-1} *10^3	0.174 - 1.16	*Scenario-based*	(Aksu and Kocatas, 2007; Basaran et al., 2010)
[NH4]	Pristine	kg L^{-1} *10^3	Nd – 0.04	*Scenario-based*	(Aksu and Kocatas, 2007)
[NH4]	Fish farm	kg L^{-1} *10^3	0.11 – 11.4	*Scenario-based*	(Yucel-Gier et al., 2007; Neofitou and Klaoudatos, 2008)
[NH4]	Pristine	kg L^{-1} *10^3	0.015 - 159.7	*Scenario-based*	(Yucel-Gier et al., 2008)
[NH4]	Fish	kg L^{-1} *10^3	1.5 – 54.6	*Scenario-based*	(Yucel-Gier et al., 2007; Neofitou and Klaoudatos, 2008)
[NOx]	Pristine	kg L^{-1} *10^3	0.0006-0.0114	*Scenario-based*	(Aksu and Kocatas, 2007; Yucel-Gier et al., 2008)
[NOx]	Fish	kg L^{-1} *10^3	0.98 – 90.06	*Scenario-based*	(Aksu and Kocatas, 2007; Yucel-Gier et al., 2007; Neofitou and Klaoudatos, 2008)
[DON]		mg L^{-1} *10^3	0.056 – 0.50	*Scenario-based*	(Porrello et al., 2003; Morganti et al., 2017)
Sedimentation					
g	Gravitational acceleration	m s^{-1}		9.81	
d_p	Average particle diameter	m		10 10^{-6}	(Zijffers, 2009)
ρ_p	Density particle	kg m^{-3}		1200	(Zijffers, 2009) *(assumed)*
ρ_w	Density seawater	kg m^{-3}		1022.77	Calculated based on (Unesco, 1983)
eta/η	Viscosity seawater	Pa		1.01*10^{-3}	
Sponge farm					
mincov	Sponge minimal coverage	%		0.5	Estimated
maxcov	Sponge maximal coverage	%		0.7	Estimated
Ld	Lantern diameter	m2		0.061	(Kelly et al., 2004)
Ll	Number of lantern layers	#		25	Estimated
rs	Space in between rows	m		2	Estimated
Sponge *Chondrosia reniformis*					
Pr	Pumping rate	mL h^{-1} cm^{-3}		12.24	Calculated based on (Morganti, 2015)
Cr	Clearance rate POC	mL h^{-1} cm^{-3}		2.81	(Kooistra, 2019)
N_{NH4}	Uptake of NH4 (%)	%	0.49 – 0.51	0.50	(Ribes et al., 2012; Morganti et al., 2017)
N_{NOx}	Uptake of NOx (%)	%	-1.02 – -0.35	-0.69	(Ribes et al., 2012; Morganti et al., 2017)
N_{DON}	Uptake of DON (%)	%	-0.02 – 0.16	0.070	(Ribes et al., 2012; Morganti et al., 2017)
ps	Sponge density	kg DW cm^{-3}		0.22*10^{-3}	This study
pa	Sponge surface density	kg DW cm^{-2}		0.32*10^{-3}	This study
r	Respiration rate	g d^{-1} cm^{-3}			(Kooistra, 2019)
m	Mortality rate	d^{-1}		0.00157	(Gökalp et al., 2019)
Sc	Carbon content sponge	g g-1 DW		0.342	(Morganti et al., 2017)
Sn	Nitrogen content sponge	g g-1 DW		0.1047	(Morganti et al., 2017)
dc	Detritus conversion carbon	d^{-1}	11-24% in 3h	0.175	(Alexander et al., 2014)
dn	Detritus conversion nitrogen	d^{-1}	18-36% in 3h	0.27	(Alexander et al., 2014)
ColPer	Collagen percentage	g g^{-1}		0.01044	(Pozzolini et al., 2012)
Gmax	Maximum growth rate	d^{-1}	0.0013-0.0057	0.008	Estimated based on (Osinga et al., 2010)
M1	TOC concentration where growth is half	mg L^{-1}		1.2	Estimated based on (Morganti et al., 2017)
M2	TOC concentration where growth is half	mg L^{-1}		6	Estimated
Eauropean sea bass *(Dicentrarchus labrax)*					
hn	Net height	m		10	Estimated
fw	Food waste	g g^{-1}	0.02 – 0.52	0.20	Estimated based on (Ballester-Moltó et al., 2017)
fd	Fish density	g m^{-3}		15.000	(Mente et al., 2012)
fc	Carbon content feed	g g^{-1}		0.41	Table A2
fn	Nitrogen content feed	g g^{-1}		0.07	Table A2
$BW_{t=0}$	Body weight of fish at start	g		40	Estimated

eff	Farm efficiency			0.75	Estimated
T	Temperature	C	16.2-28.2	Table A3	(Www.seatemperature.org, n.d.)

Table A2. Fish feed for gilthead seabream (FAO, 2015)

Component	**Feed content (%)**		**%C**	**C (g g-1 feed)**	**%N**	**N (g g-1 feed)**
	Range	*Used*				
Carbohydrate		20	37	0.074	0	0
Crude Lipid	12-25	17	75	0.128	1.6	0.027
Crude protein	45-60	45	47	0.212	15	0.068
Crude fibre	2-4	3				

Table A3. Temperatures in Kas, Turkey (Www.seatemperature.org, n.d.)

Month	**Temperature** (Celcius)
March	16.6
April	17.2
May	20.3
June	24.1
July	27.1
August	28.2
September	27.0
October	23.9
November	20.6
December	18.2
January	17.1
February	16.2

Appendix B

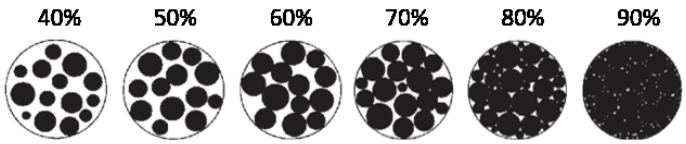

Figure B 1. Top down visualisation of different cover percentages of sponge growth on the growth plates.

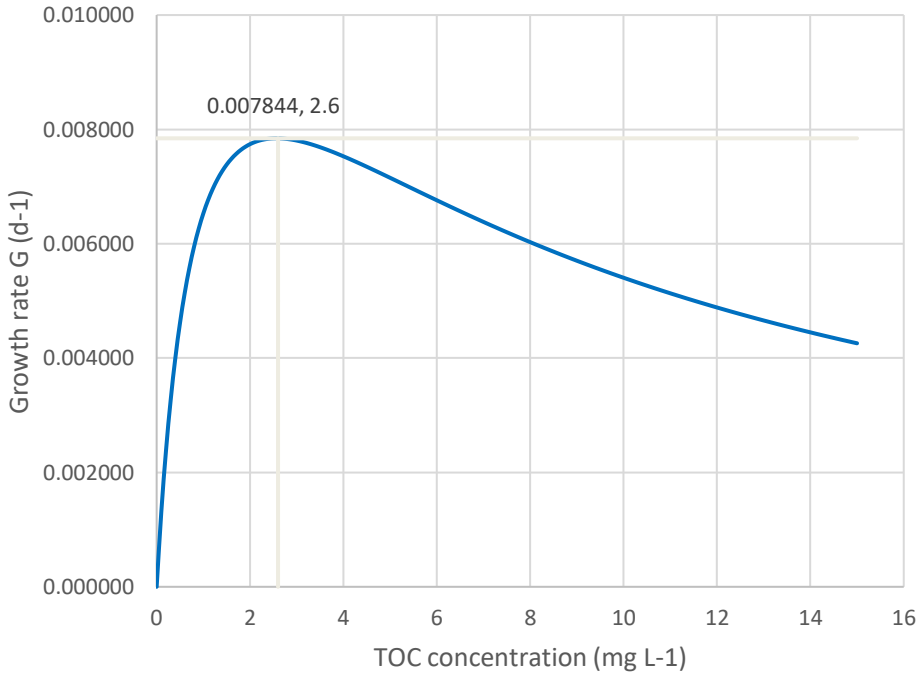

Figure B 2. Growth rate g plotted against TOC concentration based on Eq. (8).

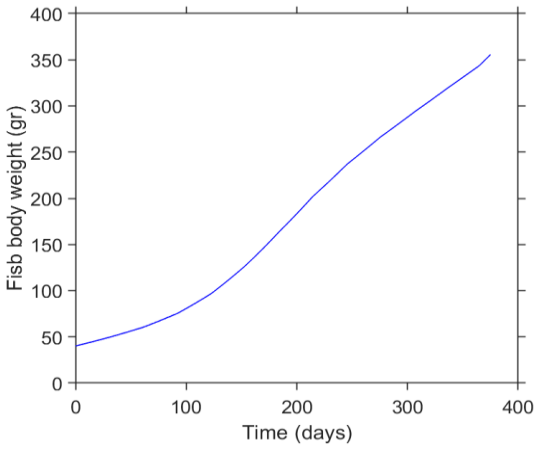

Figure B 3. Fish body weight (BW, Eq. (25) over time.

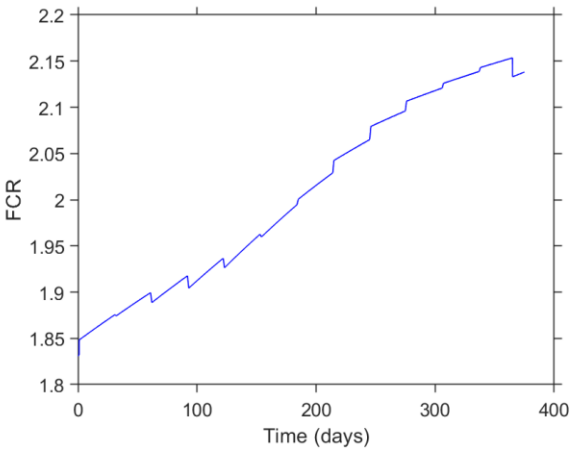

Figure B 4. Food conversion ratio (FCR) over time.

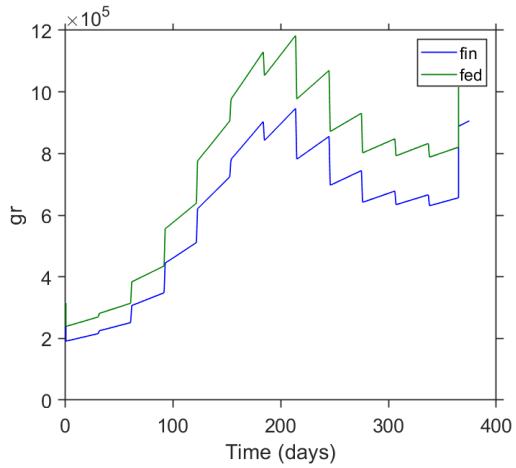

Figure B 5. Given feed (fed, green) and ingested feed (fin, blue) in grams for the fish pen over time.

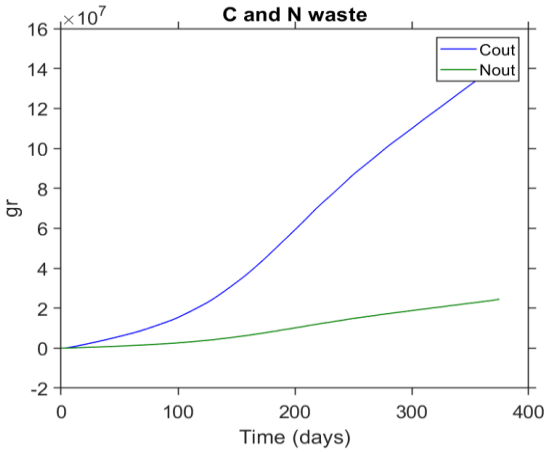

Figure B 6. Total C and N waste produced by the fish pen over time.

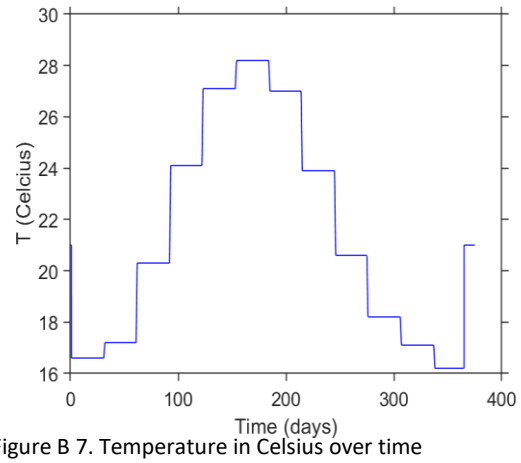

Figure B 7. Temperature in Celsius over time

Appendix C

Clarification M_W

The nutrient content in the water is expressed by Eq. () for the nutrients DOC, POC, NH4 and DON (subscript k) and by Eq (28). For NOx :

$$\frac{dM_{w,k}}{dt} = \frac{M_{w,k}}{dt} - \frac{U_k}{dt} + \frac{dM_{in,k}}{dt} \quad \text{(C127)}$$

$$\frac{dM_{w,NOx}}{dt} = \frac{M_{w,NOx}}{dt} + \frac{E_{NOx}}{dt} + \frac{dM_{in,NOx}}{dt} \quad \text{(C2)}$$

The volumetric flow Q is defined by equation C3;

$$Q = h \cdot w \cdot v \cdot 1000 \quad \text{(C3)}$$

In which h is the water column height in m, w the water column height in m and v the current velocity in m d-1

Fish model (Matlab)

Equations

```
defextern T 21 -n;                    External variable = time series

Fw = pi*w^2*h*Fd*1000;

fi = 0.0216*exp((0.063*T))*BW^0.588;

FCR = fi/gain;

Fnum = Fw/300;

Ec = fc*Featen;

En = Featen*fn;

UnretC = Ec*(1-(1/FCR));

UnretN = En*(1-(1/FCR));

fin = Fnum*fi;

gain = eff*0.0196*(BW^0.517)*exp((0.065*T));

findt = (fi/(1-wf))*Fnum;

UneatenN = (FinTOTAL*fn)-En;

UneatenC = (FinTOTAL*fc)-Ec;

Featen' = fin -Ec -En;

Fc' = Ec -UnretC;

Fn' = En -UnretN;

Nout' = UnretN +UneatenN;

Cout' = UnretC +UneatenC;

BW' = gain;

FinTOTAL' = findt -fin -UneatenN -UneatenC;
```

Parameters:

eff = 0.75;

fc = 0.41; %,g g-1 d-1

Fd = 15; %,kg/m3

fn = 0.07; %,g g-1 d-1

h = 10;

w = 15; %diameter,

wf = 0.2; %feed waste,

Initial values:

BW = 40; %,g

Cout = 0; %,g

Fc = 0; %,g

Featen = 0; %,g

FinTOTAL = 0; %,g

Sponge model

Equations

Q = h*w*v*3600*24*1000;

vs = (((G*(pd*um)^2)*(pp-pw))/(18*eta))*3600*24;

g = (2*gmax)*(TOC/(TOC+Mtoc))*(Mtoc2/(TOC+Mtoc2));

Svol = Sweight*/ps;

P = Pr*Svol;

DOCin = DOCci/1000000*Q;

Smax = ((Ld/2)^2)*pi*layer*Lanterns*(pa*10000);

Smin = Smax*mincov;

Cpoc = CR/1000*24*Svol;

Collagen = ColPer*Sharv;

DOCkgl = DOC/L;

POCin = POCci/1000000*Q;

DOCupt = ((0.06*DOCkgl)-(4.51*10^-9*12.0107))*10^-3;

SedLay = Csoil/(l*w);

TOC = (DOC+POC)*1000000/Q;

Lanterns = round((Parkl*wfish)/(Ld+rowspace)^2);

w = wfish;

Fdoc = DOCupt*3600*24;

U = Fdoc/pa;

L = Q*dt;

nox = 0.45*14.0067*10^-9;

dPOC = POCin-POC;

dCother = (Udoc+Upoc)-((g*Sweight*scc)+dCdet+dCO2);

Upoc = (POC/L)*Cpoc;

dCO2 = r*Svol;

Udoc = iif((Fdoc/pa) < Umax, (Fdoc/pa)*Sweight, Umax * Sweight);

dCdet = (dc*Udoc)+(m*scc*Sweight);

Unh4 = NH4/L*P*nh4;

dNH4 = (NH4i/1000000*Q)-(NH4);

dNdet = (m*Sweight*snc)+(Udon*dn);

Growth = Sweight*g;

Sed = (POC*vs)/h;

dNother = (Unh4+Udon)-((g*Sweight*snc)+Unox+dNdet);

dSharv = iif(Sweight>maxcov*Smax & (g-m) > 0, Sweight*(g-m), 0);

Smort = Sweight*m;

Sin = iif(Sweight<Smin, (Smin-Sweight)*P01, 0);

dDOC = DOCin-DOC;

Udon = DON/L*P*don;

dDON = (DONi/1000000*Q)-(DON);

Enox = P*nox;

dNOx = (NOxi/1000000*Q);

F03 = Unox;

Csponge' = Upoc +Udoc -dCother -dCO2 -dCdet;

Nsponge' = Unh4 +Udon -dNdet -dNother -Unox;

POC' = dPOC -Upoc -Sed;

DOC' = dDOC -Udoc;

Csoil' = Sed;

Cdet' = dCdet;

NH4' = dNH4 -Unh4;

Ndet' = dNdet;

Cother' = dCother;

CO2' = dCO2;

Sweight' = Growth +Sin -dSharv -Sweight*m;

Nother' = dNother;

DON' = dDON -Udon;

Sharv' = dSharv;

NOxw' = Unox +dNOx -NOxw;

NOx' = F03;

Parameters:

ColPer = 0.01044; %Collagen percentage sponge,

CR = 2.808; %Filtration rate (POC),mL h-1 cm-3

dc = 0.175; %Carbon detritus production,

dn = 0.27; %Nitrogen detritus production,

DOCci = 1.07; %Concentration polluted water mgC L-1,mg L-1

don = 0.16; %,d

DONi = 0.056; %DON ambient water,mg L-1

dt = 1; %,d

eta = 0.00101; %Seawater viscosity,Pa

G = 9.81; %Gravitational accileration,m s-2

gmax = 0.008; %Max growth rate,d-1

h = 20; %Collumn height,m

l = 500; %Collumn length,m

layer = 25;

Ld = 0.61; %Lantern diameter,m

m = 0.00157; %Mortality,d-1

maxcov = 0.7; %Harvest treshold % of cover,m2 m-2

mincov = 0.5; %Minimal coverage,

Mtoc = 1.1; %Half TOC,mg L-1

Mtoc2 = 6; %,mg L-1

nh4 = 0.49; %% of N taken up,d

NH4i = 0.0015; %,mg L-1

NOxi = 0.0006; %,mg L-1

pa = 0.00032; %Sponge surface density,kg cm-2

Parkl = 500; %M

pd = 10; %Particle diameter,µm

POCci = 0.13; %,mg L-1

```
pp      = 1200;       %Density particle,kg m-3
Pr      = 11.24;      %Pumping rate,mL h-1 cm-3
ps      = 0.00022;    %sponge density,g cm-3
pw      = 1025.2;     %Density seawater,kg m-3
r       = 0.024021;   %Respiration rate,mg cm-3 h-1
rowspace = 2;         %,m
scc     = 0.342;      %Carbon content sponge,
snc     = 0.1047;     %Sponge n content,
um      = 1e-06;      %um to m,µm m-1
Umax    = 0.025;      %Maximum uptake,kg kg-1 d
v       = 0.015;      %Sea velocity,m s-1
wfish   = 30;         %,m
```

Initial values:

```
Cdet    = 0;          %Carbon in particulate detrittus,kg
CO2     = 0;          %Produced CO2,kg
Cother  = 0;          %Other, Unknown carbon use/production,kg
Csoil   = 0;          %Carbon in sediment,kg
Csponge = 0;          %Carbon in sponge biomass,kg
DOC     = 0;          %DOC in water,kg
DON     = 0;          %,kg
Ndet    = 0;          %Nitrogen in detritus,kg
NH4     = 0;          %N water,kg
Nother  = 0;          %Unknown, nitrogen use/production,kg
NOx     = 0;
NOxw    = 0;          %,kg
Nsponge = 0;          %Nitrogen in sponge biomass,kg
POC     = 0;          %POC water,kg
Sharv   = 0;
Sweight = 0;          %Sponge dry weight,kg
```

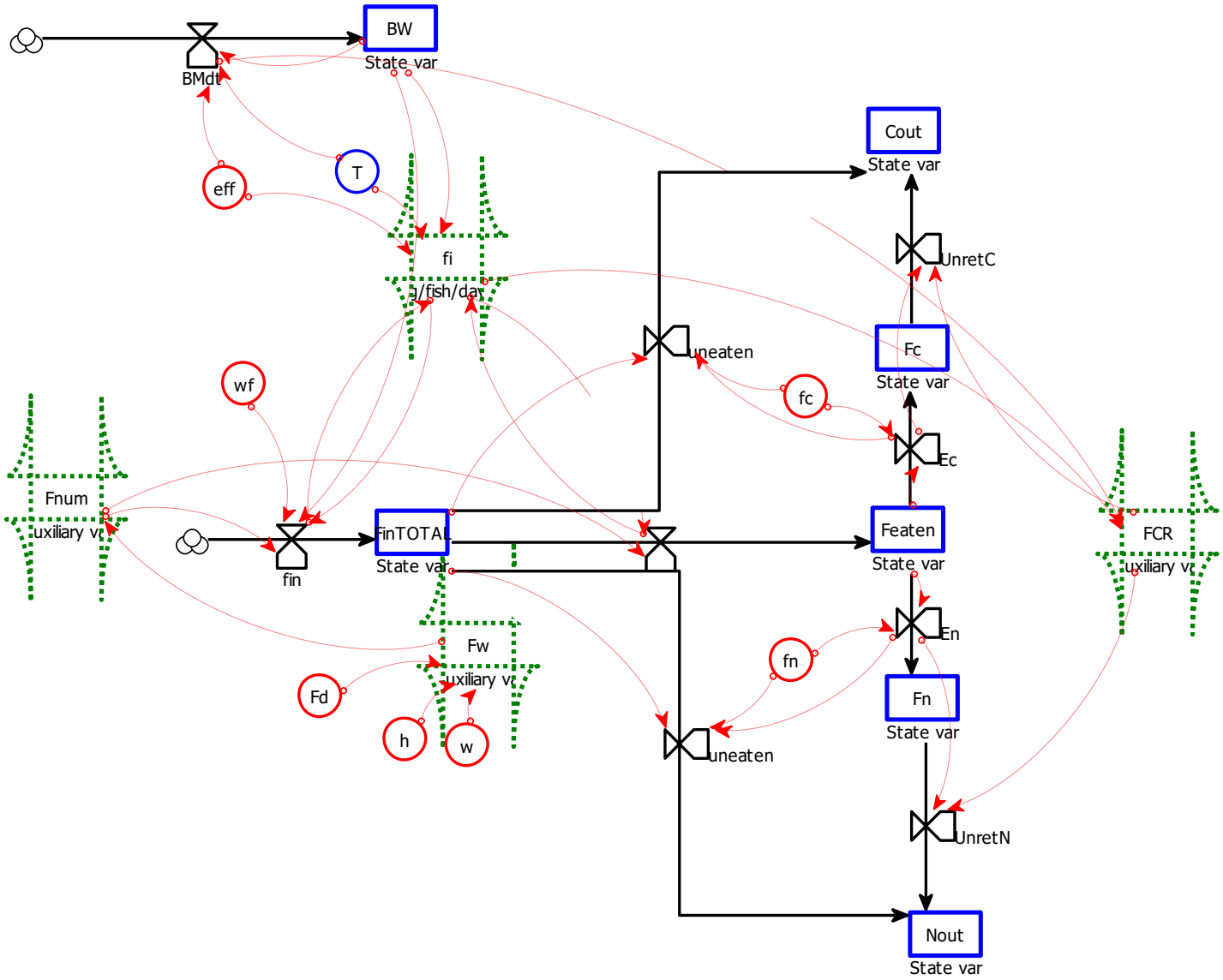

Figure B 8. Forrester diagram of the fish model as displayed in MATLAB by GRIND (van Nes, 2008).

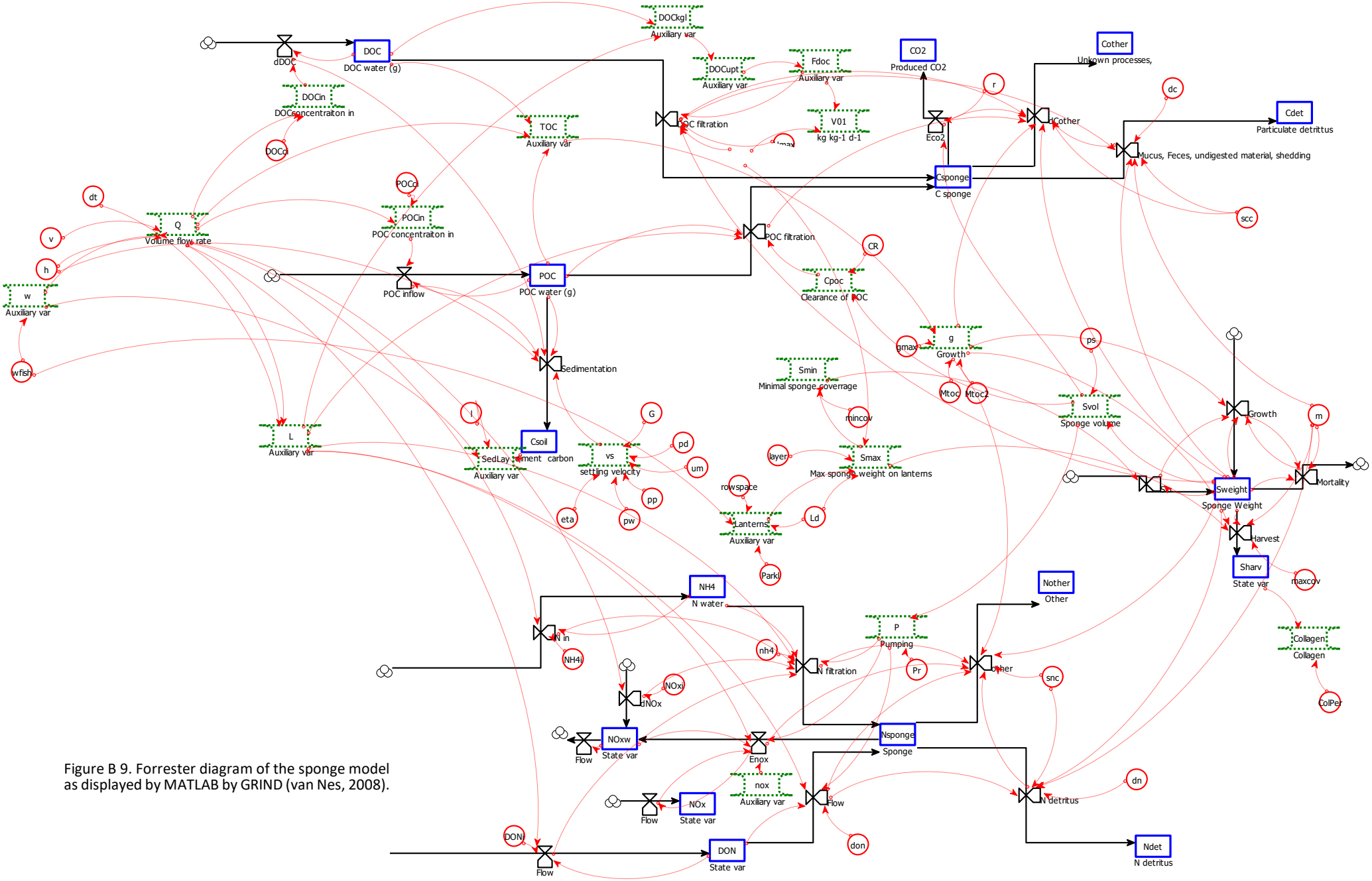

Figure B 9. Forrester diagram of the sponge model as displayed by MATLAB by GRIND (van Nes, 2008).