DEVELOPMENT OF A MODEL FOR EVALUATING AND OPTIMIZING THE PERFORMANCE OF INTEGRATED MULTITROPHIC AQUACULTURE (IMTA) SYSTEMS

A THESIS SUBMITTED TO THE UNIVERSITY OF STIRLING FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

by

FANI LAMPRIANIDOU

UNIVERSITY OF STIRLING, INSTITUTE OF AQUACULTURE

NOVEMBER 2015

In loving memory of Herta

ABSTRACT

Earth's population is expected to reach 9 billion by 2050. Ensuring food security for the growing world population is one of today's society's major challenges and responsibilities. Aquatic products have the potential to contribute significantly in the growing population's dietary requirements. Since increasing the pressure on most natural fish stocks is now widely agreed not to be an option, the aquaculture sector needs to grow. The challenge is to increase aquaculture production without depleting natural resources or damaging the environment but also in a financially sustainable way.

Integrated Multitrophic Aquaculture (IMTA) is one method of sustainable aquatic production. Integrating bioremediatory organisms that extract particulate organic matter or dissolved inorganic nutrients with monocultures of fed species has the potential of reducing the particulate and soluble waste loads from effluents, whilst producing a low-input protein source that may also increase the farm income. IMTA is a viable solution for mitigating the environmental impact of waste released from fish farms. The fish waste is exploited as a food source for lower trophic, extractive organisms giving an added value to the investment in feed.

Studies up to now have shown that under experimental conditions as well as in small-scale commercial studies, various filter-feeding, deposit-feeding and grazing species can ingest fish waste particles. The aim now is to achieve IMTA optimization, where extractive organisms can ingest most of the finfish waste food and excretions. Any such design is likely to be complex incorporating a multidisciplinary approach, and therefore to date a reason why most studies have failed to prove the environmental and economic benefits of IMTA. Consequently, the aim of this study is to develop ways of selecting an ideal combination of species for a specific locality, manage the cultures in a way that ensures the maximum nutrient recycling feasible per unit of area; and ensure high growth rate of the extractive organisms while being financially beneficial. The approach taken was a combination of investigative literature reviews, computer modelling work and small-scale growth trials to determine the relative growth of extractive organisms fed fishfeed and waste, followed by the

development of a systems-based model of interaction and growth efficiency for combinations of organisms within an IMTA system.

This study starts by investigating, with small-scale laboratory experiments, the potential of two organic extractive species, the lugworm, *Arenicola marina* and the sea urchin, *Psammechinus miliaris*, as organic extractive components of IMTA systems. Their ability to consume and assimilate salmon faeces was evaluated as well as their remediation efficiency. This was done by comparing the carbon, nitrogen and phosphorus content of the pellet-faeces mixture to that of the sea urchin faeces and sea urchin gonad content. Their growth, gonadosomatic index (GSI) (for the sea urchins), tissue carbon, nitrogen and phosphorous content were compared between seaweed diets and a diet consisting of a mixture of salmon faeces and feed pellets. The results showed statistically significant gonad carbon content for the sea urchins fed with faeces. Similarly, statistically significant higher phosphorous content was found in the tissues of the lugworms fed with the mixture of salmon faeces and pellets than in the lugworms of the other two groups.

The subsequent and main phase of this study was the development of a model for optimising IMTA performance. The modelling process included model development, run, optimization and risk assessment. The IMTA model developed consisted of Atlantic salmon *Salmo salar*, the sea urchin *Paracentrotus lividus* and the macroalgae *Ulva* sp.. It simulates the growth as well as the uptake and release of nitrogen by these organisms under environmental conditions of a hypothetical site on the west coast of Scotland. The aim of the model was to maximize the potential of IMTA in terms of productivity and to reduce the amount of nutrients that are released in the environment, and thus to contribute towards a more sustainable and productive form of aquaculture.

The IMTA model developed can be re-parameterised to simulate the growth and nutrient uptake of different species and the growth and nutrient uptake under different environmental conditions. This capacity of the model was used in order to do a comparative study of the nitrogen bioremediation potential of three different invertebrate species, cultivated as part of an IMTA. These species were the lugworm (*Arenicola marina*), the blue mussel (*Mytilus edulis*)

and the purple sea urchin (*Paracentrotus lividus*). The results of this comparative study showed that weight for weight, *M. edulis* is more efficient in removing POM than *P. lividus* that is in turn better than *A. marina* with regard to the amount of nitrogen they can assimilate. But in terms of cultivation area required for the production of the same total biomass, *P. lividus* was better at removing POM followed by *M. edulis* and then by *A. marina.*

Acknowledgements

I would like to express my gratitude to my supervisors Prof. Trevor Telfer and Prof. Lindsay Ross for all their support and for trusting me to steer my research in the right direction. I would also like to thank the University of Stirling and the Marine Alliance for Science and Technology (MASTS) for their financial support.

I am thankfull to all the people that have made these last few years so enjoyable. In particular my office mates and friends Cai, Lynne, Joly, Neil, Olga, Taslima, Aliya and Giuseppe.

Finally, I would like to thank my father for his endless support and devotion and Chris for always being there for me and bearing with me during the challenging phases of the PhD.

Table of Contents

List of Figures

List of Tables

List of Abbreviations and Acronyms

DEB	Dynamic Energy Budget
DIN	Dissolved Inorganic Nitrogen
FCR	Feed Conversion Ratio
GIS	Geographic Information Systems
IMTA	Integrated Multitrophic Aquaculture
NS	Normalised sensitivity
PAR	Photosynthetic Active Radiation
POM	Particulate Organic Matter
PON	Particulate Organic Nitrogen
RAS	Recirculating Aquaculture System
TGC	Thermal-unit Growth Coefficient
ww	Wet Weight
dw	Dry Weight
AFDW	Ash-Free Dry Weight
GSI	Gonadosomatic Index
OOP	Object-oriented programming
SASI	Seaweed-based integrated aquaculture suitability index

CHAPTER 1

Introduction

1.1 Curent state of global marine resources and increasing demand for aquaculture

The world's population rose from 3 billion in the early 1960s to 7.3 billion in 2015. At the same time, the average per capita annual seafood consumption also increased. In 2015 the consumption of fish within the EU was 23 kg per capita, increasing from 22 kg in 1989 (FAO, 2015a). The increase is much higher per capita outside Europe, increasing from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (FAO 2014). But even then finfish and shellfish contributed only 17% of the global animal based food supply in 2010 (Waite et al. 2014).

The ever-increasing demand for seafood as a food source cannot be met by capture fisheries. In 2011 it was appraised that the majority of marine fish stocks were fully exploited (61.3%), with a further 28.8% overexploited and only 9.9% underexploited (FAO, 2014). Capture fisheries production is static while the global demand for seafood products is rising, leading to the growth of the aquaculture sector. In 2012, the combined amount of fish derived from capture fisheries and aquaculture production was 158 million tonnes consisting of 58% capture fisheries and 42%, aquaculture. Of the total more than 86% was used for human consumption (FAO, 2014) (Figure 1-1).

Aquaculture is expanding and is one of the fastest-growing animal-food-production sectors with an average annual growth rate of 6.3%. It supplied 47% of total food fish in 2010 compared with only 9% in 1980. To meet the upcoming demand for seafood products it is anticipated that aquaculture production will need to increase by 50% over the next 40 years (FAO, 2012). This will involve considerable expansion while facing the reality of competing demands on aquatic resources, including space.

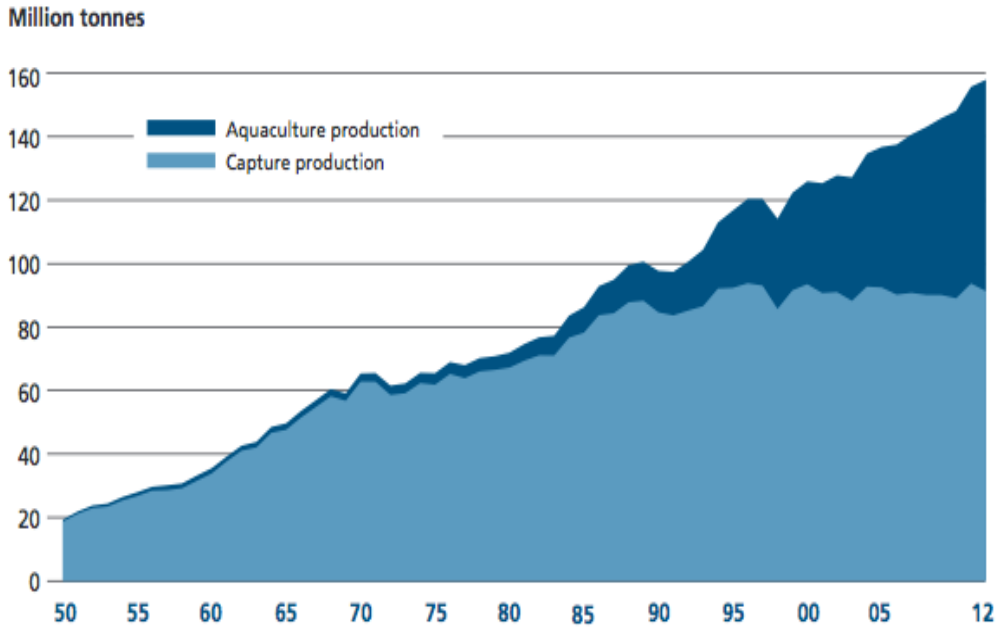


Figure 1-1: World capture fisheries and aquaculture production (FAO, 2014).

1.2 Environmental impact of aquaculture wastes

Cage aquaculture can release a considerable amount of biogenic waste such as organic and inorganic nutrients, in particulate and soluble forms, that are generated during the production process. The effect of the waste output is defined by the husbandry, feeding technique and site selection. In detail it depends on the feed composition, digestibility, feed conversion coefficient, the farm size/tonnage and the production stage as well as by the site's bathymetry and hydrography (Corner et al. 2006).

The local environmental impact of a farm depends on the region's biological assimilative capacity; which is defined by the regional hydrodynamic conditions, by the physical, chemical and biological characteristics of the receiving ecosystem and on additional release of waste products from other sources (e.g. urban and rural human settlements and sewage effluents, agricultural/industrial runoffs, precipitations, etc.).

Particulate organic waste released from fish farms alters sediment chemistry, increases the biochemical oxygen demand, changes composition and productivity of benthic communities and may lead to an increase of pathogenic bacteria (Brown et al. 1987; Chávez-Crooker and Obreque-Contreras, 2010). Kutti et al. (2007) estimated that the average carbon biodeposition rate at a *Salmo salar* (Atlantic salmon) farm is 17.65 $gC\ m^{-2}\ d^{-1}$. A portion of the solid waste is re-disperesed and another is remineralized and along with dissolved fish metabolic wastes, they are dispersed within the receiving water body.

The dissolved inorganic waste released from fish farms is converted to inorganic N and P by bacteria and is then in a form suitable for uptake by photosynthetic organisms. In large amounts the waste may lead to hypernutrification, change the N/P ratios and trigger the development of algal blooms (Gowen and Bradbury, 1987; Kaartvedt et al. 1991; Wu, 1995). However, Wu (1995) states that there has been no sound scientific evidence of a red tide incident occurring due to fish farming. The impact of the fish farm waste is more pronounced when the assimilative capacity of the receiving water body is surpassed in which case phenomena such as eutrophication, harmful algal blooms and the spread of diseases might arise. Fed species aquaculture may lead to eutrophication, which effects can range from local effects in the immediate surroundings of the fish farm to the contribution to large-scale eutrophication, e.g., in the entire Baltic Sea (Rönnberg and Bonsdorff, 2004). However, for most larger scale eutrophication effects, the anthropogenic nutrient load is in most cases dominated by agricultural runoff and municipal sewage (Enell, 1995). It is difficult to distinguish the source of emissions that lead to eutrophication since such phenomena are usually caused by a combination of sources. Although aquaculture is usually not the cause of severe eutrophication, it is still a significant source of nutrients. For example, Hall et al. (1992) showed that phosphorus concentrations in the farm emissions are typically a magnitude higher than in unaffected sediments.

1.2.1 Quantification of salmon aquaculture nutrient release

Sanderson et al. (2008) estimated that at a salmon monoculture facility, less than 70% of the N and 80% of the P is lost. Similarly, Wang et al. (2012) estimated that approximately 62% of the nitrogen, 70% of the phosphorus and 70% of the carbon input is lost to the environment. This corresponds to 397, 50 and 9.3 kg of C, N and P per tonne ww of fish produced, respectively. Similarly, Mente et al. (2006) estimated that the average dissolved N released per year per tonne of fish produced is 43 kg and Sanderson et al. (2008) estimated that the total N discharged by Scottish salmon farms per tonne of fish produced is 45–48 kg of which 35–45 kg is dissolved N. Angel et al. (2005) estimated that from the total nutrient content in the feed, 9% of the N, 42% of the P and 15% of the C descend to the seafloor as particulate matter. Wang et al. (2012) showed that 45% of feed N consumed was excreted as DIN corresponding to 36 kg DIN t^{-1} of salmon produced which agrees with the DIN release rate by salmon farms in Scotland (35 to 45 kg N t^{-1} fish produced; Davies 2000; Sanderson et al. 2008) (Table 1-1).

Global aquafeed supply for mariculture for 2008 was 29.2 Mt and is expected to reach 51 Mt by 2015 and 71 Mt by 2020. Most fish farms utilize feed with 36% protein content and thus 5.76% nitrogen content and 67% of the nitrogen excreted is as ammonia–N, then 4% of the total feed quantity is released to the sea as ammonia–N (TAN). According to the above calculations, annually 2.5 Mt and by 2020 3.4 Mt of ammonia-N will be released in the sea. The above estimation represents the average nutrient release, since the nutrient output depends on the fish species, the nutrient content of the feed, the feeding strategy, the feed particle properties and on other variables. For example, the global salmonid feed conversion ratio (FCR) in 2003 was about 1.3 (Reid et al. 2009) and at the Norwegian salmon industry the FCR is as low as 1.0 (Islam 2005; Wang et al. 2012). For sea bass and sea bream, most of the nitrogen excreted is in the form of urea ($CO(NH_2)_2$) (41%) and ammonium (NH_4^+) (26%), and 22% of phosphorus is released as phosphate (PO_4^{3-}) (Lupatsch and Kissil, 1998; Tsapakis et al. 2006). The nutrient release from salmon mariculture systems

is not consistent throughout the year, Wang et al. (2012) showed that 67% of the total biogenic waste was released within the warmer half of the year. The variation in waste release is not only seasonal. For example salmonids feeding behaviour favours two feeding periods per day, which leads to soluble and particulate waste peaks (Reynolds, 2005).

Table 1-1: Salmon monoculture waste release.

Nutrients	N	P	C
Feed nutrient content (% of dry weight)	7.2[1]	1.2[3]	51[2]
Total percentage of feed nutrients released (%)	62[4]	70[4]	70[4]
Feed nutrient assimilation efficiency (%)	85[4]	50[3]	80[2]
Percentage of feed nutrients released as DIM (%)	45[4]	18[4]	-
Percentage of feed nutrients released as POM (%)	15[4]	44[4]	19[4]
Percentage of feed nutrients released as DOM* (%)	3[4]	8[4]	3[4]
Percentage of feed nutrients that descend to the seafloor as particulate matter (%)	9[5]	42[5]	15[5]
Feed nutrient content per tonne of salmon produced (kg)	80[4]	13.3[4]	565[4]
Total nutrients released per tonne of salmon produced (kg)	50[4]	9.3[4]	397[4]
POM released per tonne of salmon produced (kg)	14[4]	6.85[4]	127[4]
DOM released per tonne of salmon produced* (kg)	2.1[4]	1[4]	19[4]
DIM released per tonne of salmon produced (kg)	36[4]	2.45[4]	-

[1] Gillibrand et al. 2002 [2]; Corner et al. 2006 [3]; Reid et al. 2009 [4]; Wang et al. 2012 [5]; Angel et al. 2005

* produced from POM

1.3 Sustainable aquaculture and integrated multitrophic aquaculture (IMTA)

The development of the aquaculture industry is limited by resources, such as water, land, fishmeal, and by other factors, such as environmental pollution (Naylor et al. 2000; Westers, 2000). In order to minimize the environmental impacts, the aquaculture sector should advance in a sustainable manner. Sustainable growth could be achieved by

biotechnological approaches such as DNA vaccines and genetic manipulation techniques or by the development of more sustainable management practises such as improved feed management, husbandry innovation and developing true oceanic (i.e. offshore) aquaculture. The present study focuses on a sustainable management method for environmentally responsible aquaculture practise, Integrated Multitrophic Aquaculture (IMTA).

1.4 IMTA; what is it?

IMTA is the practice which combines, in the appropriate proportions, the cultivation of fed aquaculture species (e.g. finfish/shrimp) with organic extractive aquaculture species (e.g. shellfish/herbivorous fish) and inorganic extractive aquaculture species (e.g. seaweed) to create balanced systems for environmental sustainability (biomitigation) economic stability (product diversification and risk reduction) and social acceptability (Figure 1-2 and Figure 1-3) (Barrington et al. 2009). A well–designed IMTA system is a way of developing environmentally sound aquaculture practices and resource management through a balanced ecosystem approach.

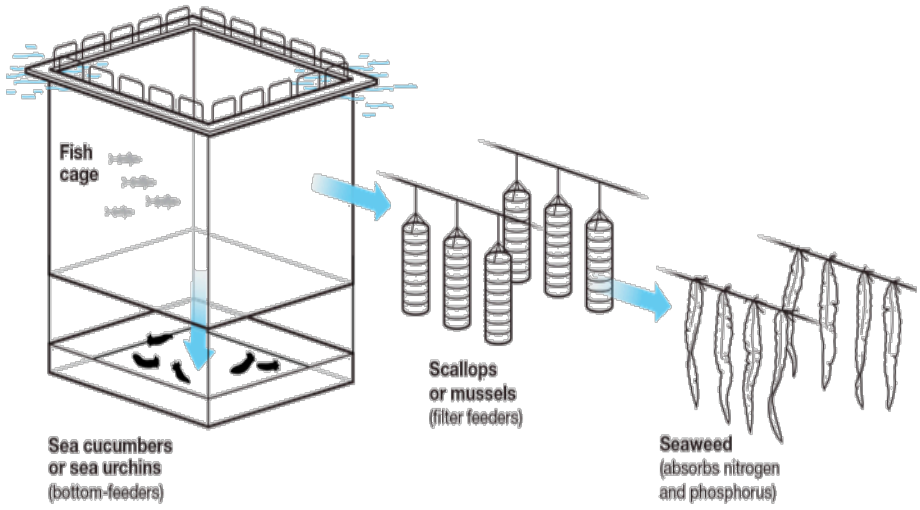


Figure 1-2: Typical offshore IMTA setup (Joel and Bourne, 2014).

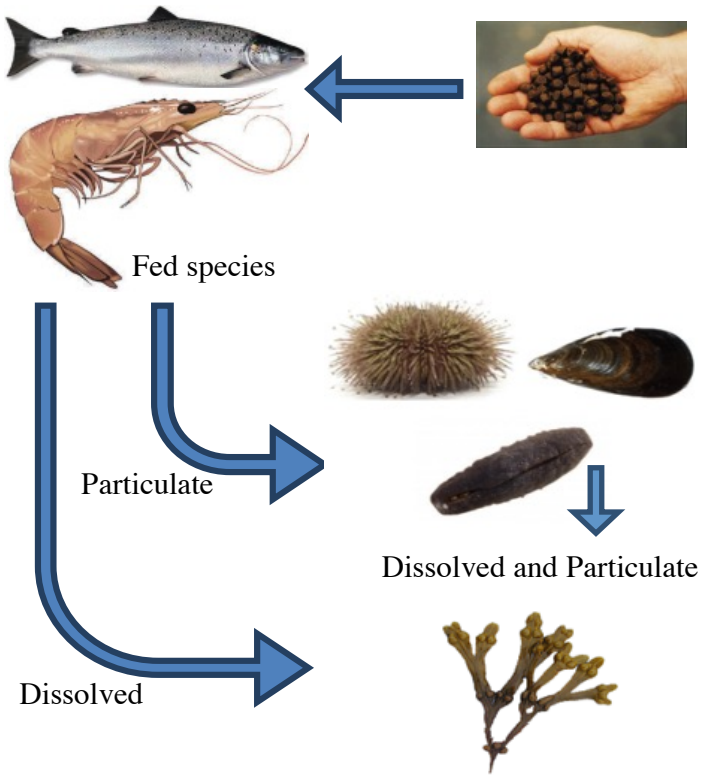


Figure 1-3: Diagrammatic representation of nutrient and particulate matter exchange within an IMTA system.

1.4.1 IMTA benefits for the cultured species

IMTA does not only benefit the environment but it also provides mutual benefits to the cultured organisms. The extractive organisms benefit from finfish waste often leading to higher growth rates (for macroalgae: Ruokolathi, 1988; for oysters: Ferreira et al. 2012) and the filter feeding extractive organisms can ingest finfish parasites and viruses. Bartsch et al. (2013) estimated that blue mussels (*Mytilus edulis*) and Atlantic sea scallops (*Placopecten magellanicus)* consumed copepodids, such as the free-swimming stages of sea lice with a clearance rate of 18 and 38% $hour^{-1}$ (Bartsch et al. 2013). Simarly, mussels can filter infectious salmon anaemia virus particles from the water column and are likely to inactivate them and other viruses (Bouchard et al. 2014; Skar and Mortensen, 2007). Cultivation of seaweeds in the proximity of fish cages not only counterbalances nutrient inputs but also other metabolic aspects, such as dissolved oxygen, acidity and CO_2 levels.

1.5 IMTA benefits

1.5.1 IMTA environmental benefits

It is widely accepted that the excessive nutrient and organic enrichment caused by intensive fish mariculture can impact the surrounding environment. The build-up of nutrients and particulates can lead to anoxic sediment under the sea cages and changes in benthic communities. The nutrient reduction benefits of IMTA have been clearly shown at closed systems, where nutrient uptake rate estimations are rather straightforward. Buschmann et al. (1994) found that for each 100 t of salmon 92 t of *Gracilaria* can remove as much as 90–95% of ammonium released from the fish tank. Such precise estimations have not yet been made for open-water mariculture systems and extrapolations can lead to misleading results. An extrapolation for open sea IMTA that was made by Troell et al. (1997), who estimates that if 227 t of fish were co-cultured with 10 t ww of *Gracilaria*, then 258 t ww year^{-1} *Gracilaria* would be produced and the harvest of the *Gracilaria* produced would remove 1020 kg nitrogen and 374 kg phosphorus year^{-1} and thus this way 6.5% of the dissolved inorganic nitrogen and 27% of the dissolved phosphorus of the effluents released by the salmon farm would be removed.

1.5.2 IMTA economic benefits and economic potential

Apart from the environmental benefits, IMTA production has the potential to generate more profit than monoculture (Nobre et al. 2010). IMTA is essentially the only aquaculture waste remediation method that could increase farm revenues, without involving signifcant additional costs to the producer (Troell et al. 2009). The economic benefits of IMTA can be achieved due to economic diversification by producing other value-added crops thus increasing the profitability per cultivation unit for the aquaculture industry (although farming different species increases the risk and uncertainty of production (Buschmann et al. 2008b; Chopin, 2010; Chopin 2011). In this way products may gain access to more lucrative markets; and product sales might increase due to consumer preference for sustainably produced seafood (FAO, 2006). For example, salmon grown in IMTA

systems (such as WiseSource™ Salmon in Canada) can reach a higher market price (Waite et al. 2014). There is already the tendency for consumers to pay more for sustainably produced seafood (Roheim et al. 2011). However, campaigning would be necessary to increase consumer preference for IMTA–produced seafood, explaining to consumers the risks and benefits of IMTA, since they might be concerned about the food safety (Chopin, 2011; Bunting and Shpigel, 2009).

The scientific research of the last three decades has showed that extractive organisms cultured as part of IMTA systems; achieve higher growth rates than they would if they were cultured in a monoculture under similar environmental conditions (for shellfish: Handå et al. 2012; Lander et al. 2013)). The studies that failed to achieve higher growth rates were performed under environmental conditions unsuitable for IMTA (i.e. Chesuk et al. 2003). Higher growth rates are strongly associated with higher potential for profit.

Even if it becomes widely accepted that IMTA is an environmentally favourable system of food production, still the diversification of monocultures to IMTA farms will depend on the profitability of the system. It is possible that private financial incentives for the adoption of IMTA production technologies at the site level and other financial incentives for the wider promotion of IMTA will be necessary. The financial limitations of IMTA can be decreased if the value of biomitigation is acknowledged and assessed (Chopin, 2010; Bunting and Shpigel, 2009). A way to achieve this would be if non-IMTA operations had to pay for discharge (Neori et al. 2007), which could be based on the waste cost function of nutrients extracted (Ferriera et al. 2008; Musango et al. 2007). Alternatively IMTA farmers could increase their profits via carbon trading (Holdt et al. 2006) and potentially using nutrient credits. The current financial success of intensive fish mariculture in Britain is associated with the fact that the nitrification of the environment involves little monetary cost to the growers. However, if the cost of water treatment and general environmental costs were internalized then the financial benefits of monoculture would be significantly smaller.

Most of the published IMTA studies do not carry out financial estimations, however many support the idea that some forms of IMTA can increase the farm profits. For example Buschmann et al. (2001) and Chopin et al. (2001) stated that the integration of *G. chilensis* with fish farms has the potential of increasing the company's profitability. Buschmann et al. (1994) stated that if alongside with 100 t of salmon 92 t of *Gracilaria* are produced using the effluents released from the fish tank then the total sales value would increase by 18% (additional profit 110,000 US$) and by 10% if the infrastructure and maintenance costs are included (Buschmann et al. (1994). In 1997 the price for *Gracilaria* was 1 US$ kg^{-1} dw (Troell et al. 1997). Today seaweed can be bought for 2-3 US$ kg^{-1} dw at the international online retail store Alibaba. Moreover, growing seaweed or invertebrates as part of an IMTA leads to a reduction of costs because there is no need to provide them additional fertilizer/feed for the duration of the grow-out cycle.

Seaweed has a large market, in 2012 about 23.8 Mt valued at US$ 6.4 billion were sold for human consumption, phycocolloids, feed supplements, agrichemicals, nutraceuticals and pharmaceuticals (FAO, 2014). Biofuel production is also a promising use for seaweed, but at the moment production costs are high; e.g. the estimated cost of bioethanol production from macroalgae is $0.50/kg (dw) compared to $0.16/kg from corn (Aitken et al. 2014).

The energy put into the aquaculture systems in the form of fish feed is a valuable resource that should not be wasted. Especially because the main expense at modern intensive monoculture is feed, which accounts for about half the expense of operating a fish farm (Neori et al. 2004). The difference in expenses and profit between a salmon monoculture and an IMTA system depends to a large extend on the species that are selected as IMTA components.

1.6 Setting up an IMTA system

1.6.1 IMTA limitations

The nutrient removal capacity is closely related to the growth performance, thus by achieving high growth rates high bioremediation efficiency is also achieved. When the environmental conditions favour primary production then filter feeding extractive organisms are also favoured since the nutrients released from the sea cages will lead to a phytoplankton increase, which can be used as a food source for the filter feeders (as seen in Chesuk et al. 2003).

Increased growth of the extractive organisms and reduced fish waste loading cannot always be achieved when organic extractive organisms and/or primary producers are suspended in proximity to finfish aquacultures. In order to achieve the economic and environmental benefits of an IMTA, there needs to be a specific design encompassing the site's and the selected organisms' characteristics. In principal, optimisation of IMTA open-water cultures (e.g. cage cultures) can be achieved through manipulations in extractive culture densities, culture depth and relative position to fish cages, species choice and harvesting frequencies. Presently, due to the lack of in depth knowledge regarding interactions within IMTA systems, the placement of the extractive organisms is mainly driven by availability of space as opposed to optimal design for maximum nutrient recycling (Hughes and Kelly, 2001). Moreover, every IMTA system setup and composition needs to be site specific, taking into account the natural characteristics of each site like the ambient seston and phytoplankton of each site, the hydrographic conditions etc.

The site's hydrographic processes need to be studied, because they dictate the flow of dissolved nutrient and particulate plumes (Newell and Richardson, 2014). So they will define how best the IMTA production systems should be designed/configured in order to take full advantage of these waste-dispersion routes. At the same time, the influence of the extractive cultures (at commercial production scale) on the waste streams should be also taken into account (Hardstein and Stevens, 2005). The

movement of the sea cages can also influence the deposition of particulate waste (Corner et al. 2006). Proximity to the fish cages, density of the grow-out structures (nets, cages, trays), vertical and horizontal orientation with respect to the flows, within-production unit densities, and spatial/temporal integration of multi-species/multi-year classes within each type of IMTA system are all issues that need to be addressed in order to ensure continual and optimal system performance. At the target site, the particulate organic matter (POM) derived from the farm should contribute significantly to ambient POM levels for extended periods and the nature of the waste particles (shape, density and settling speed) should be suitable for the selected organisms. The concentration and duration of organic waste pulses should be also investigated.

The appropriate nutrient and POM extractive species need to be selected so as that they can ingest aquaculture waste, have fast assimilation and growth rate, and can store excess feed and thus take advantage of nutrient pulses. The location specific site/receiving ecosystem environmental characteristics and their temporal and spatial variability need to be studied prior to the set-up of an IMTA system.

These environmental characteristics are:

- Current velocity. Low current velocity (< 10 cm s^{-1}) has been suggested as preferable when the IMTA includes filter feeders, so that POM produced by the fish and by the organic extractive organisms can be recycled through the filter feeders (Mazzola and Sara, 2001).
- Bathymetry suitable for IMTA design. Developments should not exceed the receiving ecosystem carrying capacity.
- Abiotic conditions such as temperature and pH suitable for all the cultured species.
- Potential background nutrient enrichment that would contribute to the feed availability for the extractive organisms.
- It is advantageous when algal (micro and macroalgal) growth is nutrient limited. Meaning that no other parameter (e.g. photoperiod,

light intensity, temperature, salinity, competition of fouling organisms and other factors affecting seaweed growth and uptake capacity) is limiting algal growth. For example, nutrient limited phytoplankton growth is common in the Aegean Sea.

The daily to seasonal fluctuations of most of these environmental parameters should also be taken into account since they might by affecting the extractive organisms directly and indirectly via changes in nutrient availability. The seasonal requirements and performance trends of the extractive species should be also taken into account, thus data from entire annual cycles should be used. It is important to know the seasonal performance of seaweed. Both the seaweed nutrient uptake rate and areal yield vary seasonally, usually being highest in summer.

A sub-optimally designed IMTA might not succeed in decreasing the environmental impacts of a finfish monoculture and moreover it could magnify them or create other problems. Some examples of these problems are the following:

- The use of organic extractive organisms such as bivalves can lead to additional nitrification of the water column, because most of the organic material ingested by the organic extractive organisms returns to the water column as nutrients (Nizzolli et al. 2005). This might lead to eutrophication, since during low light conditions high phytoplankton density may deprive oxygen from the fish. Hence one must ensure that availability of dissolved nutrients is not such that can allow phytoplankton to reach very high concentrations.
- The extractive cultures may interfere with the water movement, changing the particle dispersal patterns and reducing the water flow through the sea cages (Hardstein and Stevens, 2005).
- Depletion of phytoplankton and zooplankton caused by the filter feeders may impact the food web (Newell, 2004).
- Pseudofaeces produced by filter feeders may collect on the sediment impacting benthic communities (Kaiser et al. 1998). Pseudofaeces creation is a specialized mechanism employed by filter-feeding

bivalve mollusks in order to reject ingested particles that cannot be used as food. The rejected particles are wrapped in mucus, and are expelled without having passed through the digestive tract.

- The co-cultures may impact the health and growth of the finfish. For example, shellfish are bioaccumulating organisms and they may also increase disease risk on farms by serving as reservoirs for fish pathogens. For example, the blue mussel (*Mytilus edulis*) can accumulate the infectious pancreatic necrosis virus and transmit it, at low frequencies, to Atlantic salmon smolts that are grown within the same IMTA systems (Molloy et al. 2013). Farming different species within the same system can increase the exposure to pathogen. Pietrak et al. (2012) found that mussels bioaccumulate and shed harmful bacteria. Conversely, Molloy et al. (2011) found that bivalves are not hosts but they can consume parasites such as *Lepeophtheirus salmonis*.
- The tissue/flesh quality of the extractive organisms may be inferior to that produced in a monoculture of the same species. Troell et al. (1997) concluded that agar yield from seaweed cultured near the fish cages was lower but its quality was expected to be higher due to its high tissue nitrogen content. In general the organisms cultured near the fish cages have higher protein content (e.g. protein content of *Ulva lactuca* more than 34% of dry weight in Schuenhoff et al. 2003). The main problem affecting seaweed quality near sea cages is epiphytic growth. Depending on the environmental conditions, mainly on the current strength, high epiphytic growth can be observed even 100 m away from sea cages (e.g. Abreu et al. 2009).

1.6.2 Practical considerations

1.6.2.1 Placement of seaweed at IMTA sites

The major environmental parameters that influence the growth of macroalgae are temperature, light and availability of nutrients (Lobban and Harrison, 1997). Growth has a positive relation with temperature (e.g. for Gracilaria: Friedlander and Levi, 1995; Abreu et al. 2011) and irradiance

levels need to be within specific limits (species specific) in order to achieve maximum growth rates. Algae can make use of the nutrients released from sea cages only if they are nutrient limited or if they are capable of luxury nutrient uptake.

The seaweed nutrient uptake can be limited by the physical arrangement of the IMTA system, water currents, nutrient concentrations, light and temperature conditions, stocking densities and bio–fouling.

It has been shown that seaweed production is enhanced by the presence of fish farms (Troell et al. 1997; Fei et al. 2002). Weston (1986) estimated that ammonia concentration is significantly higher within a 40 m perimeter of the sea cages. Troell et al. (1997) report that *Gracilaria chilensis* cultivated at 10 m from salmon cages has up to 40% higher SGR than at 150 m and 1 km distance. Similarly, Abreu et al. (2009) achieved higher productivity at 100 and 800 m than 7 km away from the farm. On the other hand, at the same study, the productivity was higher at 800 m than at 100 m due to high epiphytic growth at 100 m. The last result could indicate that part of the soluble effluents from the sea cages spread for at least 800 m or alternatively that the environmental conditions were more favourable for seaweed growth near the 800 m sampling area. The hypothesis that part of the waste spread up to 800 m from the sea cages at high enough concentrations to enhance seaweed growth can also be supported by the fact that the mean current speed at the IMTA site of that study was relatively low (2.4–7.6 cm s^{-1}).

The seaweed does not only need to be cultured at the right distance and position in relation to the sea cages but also at the appropriate depth. The optimal distance and position from the sea cages is primarily defined by the hydrodynamic conditions, while the culture depth is primarily dependent on the seaweed species and stocking density.

However, the depth that the seaweesd can be cultured at depends also on the photosynthetic pigments of each species. As light enters the water column, the longer (red and infrared) waves are absorbed, near the surface.

Detrital particles and dissolved organic matter absorb principally at shorter wavelengths, and phytoplankton absorbs light at two peaks corresponding to the action of chlorophyll. However, each photosynthetic pigment (chlorophyll *a*, *b*, and *c*, fucoxanthin, peridin, etc.) has its own absorption patterns in the spectrum (Valiela, 1995). Macroalgae have different arrays of photosynthetic pigments, in comparison with phytoplankton that is mainly composed by chlorophyll *a*. There exist three main groups of macroalgae, green (Chlorophyta and Charophyta), red (Rhodophyta) and brown algae (Phaeophyta). The Chlorophyta absorb mainly in the red and blue wavelengths of the spectrum due to presence of chlorophylls *a* and *b* as well as carotenoids such as xantophophylls and carotenes. Phaeophyta contain chlorophyll *a* and *c,* as well as fucoxanthin pigments and use the green and yellow wavelengths more efficiently. Rhodophyta contain chlorophyll *a* and water soluble pigments in phycobiliproteins that allow absorption of light in the blue and green wavelengths (Valiela, 1995) enabling them to photosynthesise at greater depths, where these wavelengths still exist. Red seaweeds are very suitable for IMTA because they can survive in low light conditions where green seaweeds could not.

Zhou et al. (2006) concluded that the optimum depth for *Gracilaria lemaneiformis* thallus growth is 1–2 m. Similarly, Troell et al (1997) found that *Gracilaria chilensis* cultured at 1 m depth presented significantly higher growth rate than at 3 and at 5 m deep. It is broadly agreed that higher seaweed productivity can be achieved in the first couple of meters of the water column. In some cases though, high irradiance levels can lead to photoinhibition, especially at low stocking densities (Mata et al. 2006). But this is not the case for all species, for example *Gracilaria vermiculophylla,* has high resistance to high irradiance levels (Abreu et al. 2011).

Apart from the irradiance levels the stocking density has also a significant effect on the depth of the photic zone and thus should be considered when deciding on the culture depth. For example, at tanks seeded with 7 kg m^{-2} *Gracilaria*, less than 15% of the irradiance reached a depth of 15 cm (Abreu et al. 2011). Light is not needed for nitrogen assimilation; in low light levels

algae may use nitrogen to form pigments rather than for growth (Lobban and Harrison, 1997).

1.6.2.2 How many extractive organisms should an IMTA have?

One of the most challenging parts of an IMTA model is deciding on the appropriate amount of extractive organisms that should be cultured. It is clear that large-scale cultivation of extractive organisms would lead to a higher degree of nutrient removal. However, the amount of space available for growth of extractive organisms is limited for a number of reasons. These include: competing demands for space from multiple users, large-scale cultures have a visual impact, high algal culture densities over a large area can alter the currents and might lead to low dissolved oxygen concentrations during the night and finally practical issues also hinder the extractive culture growing area. Moreover, high densities of extractive cultures might limit the growth rate of the extractive species. The extractive cultures should allow extractive species to be exposed to farm nutrients, self-shading should be avoided and the culture size must be realistic from a management point of view. Also, the area directly under the sea cages may not always be usable, especially during the summer months when high mortality rates at bottom cultures occur (e.g for sea cucumber in Yu et al. 2011).

Kautsky et al. (1996) estimated that for the assimilation of the nitrogen released by a salmon mariculture farm, co-cultured *Gracilaria* covering an area 150 times that of the fish cages is required, and similarly for the assimilation of the released phosphorus an area 25 times as large as that of the fish cages. Abreu et al. (2009) estimated that for the assimilation of the released nitrogen, the seaweed culture would need to take up 100 times as much space as the fish culture. These two estimations are similar if one considers that feed nitrogen content and feed loss have significantly decreased in the time between these two publications. In detail, Abreu et al. (2009) calculated that 1 km^2 of *Gracilaria chilensis* cultured around a salmon fish farm producing 1500 t of fish that release 65 t of nitrogen $year^{-1}$ could remove 100% of nitrogen released from that fish farm. These calculations

were made using the maximum nitrogen uptake results acquired during the summer months of this study (9.3 g m^{-1} longline $month^{-1}$) as the uptake rate for half of the year and for the other half they used an uptake rate of 1.9 g m^{-1} longline $month^{-1}$. This hypothetical farm would consist of 2500 lines all placed at 1 m depth and with a density of 1.7 kg m^{-2}. Abreu et al. (2011) estimated from a land-based experiment that *Gracilaria vermiculophylla* could remove 221 g m^{-2} $month^{-1}$ of carbon and 41 g m^{-2} $month^{-1}$ of nitrogen.

Consequently, the predicament is how to achieve significant bio-filtration in limited space. In order to achieve significant bio-filtration we need to use organisms that can absorb and assimilate large quantities of nutrients and to achieve constant maximum growth rate of these organisms. One way of utilising space more efficiently is based on taking advantage of the 3-dimensional nature of the marine environment by culturing different species at various depths. For example, seaweed with different light intensity requirements can be cultured at different depths and benthic organisms (such as sea cucumbers or polychaetes) can be cultured on the seafloor. A way to achieve maximum growth rates at all time is by altering the seaweed species between the seasons, in order to take advantage of temperature and irradiance level changes and achieve continuous maximum seaweed productivity. For example, in the summer seaweed sensitive to high irradiance levels can be cultivated deeper to avoid pigment photo-destruction or can be replaced by another species.

1.7 Investigation of possible IMTA extractive species, choosing the most suitable extractive species

Inorganic nutrients such as DIN and DIP are readily available for phytoplankton and macroalgae (Troell et al. 2003, 2009). Large particles (faeces and uneaten feed) sink rapidly and may accumulate in sediments on the seafloor (Cromey et al. 2002, Olsen and Olsen 2008) where they may be consumed by detritus–eating animals. Small particles of waste remain in suspension and they can be consumed by zooplankton or by filter feeders (Olsen and Olsen 2008, Troell et al. 2009).

One of the most important steps towards the creation of an efficient IMTA system is the selection of the best possible combination of native extractive species suitable for each IMTA site. The selection of a seaweed species can be based on its physiological characteristics, ecological properties (biofiltration capacity, biochemical composition and growth rate), on its market value and on the marketability of the final product. Kang and colleagues (2013) developed an index for the selection of the most suitable seaweed species for seaweed-based IMTA aquaculture, the seaweed-based integrated aquaculture suitability index (SASI). This index was developed using data available in the literature as well as data acquired from physiological experiments performed for the purpose of that study. The index takes into account the economic value and market standing, physiological characteristics and nutrient–removal efficiency of the seaweed species (NH_4 uptake rate, maximal uptake rate (V_{max}), half saturation value (K_m), tissue chl a, tissue nitrogen content), whether or not it can be cultivated easily and whether it has been already used as a component of IMTA systems. The results of that study showed that from the six seaweed species examined, *Undaria pinnatifida*, *Porphyra yezoensis* and *Ulva compressa* scored the highest according to the SASI index, while *Gracilaria incurvata, Eckonia cava* and *Undaria pinnatifida* scored lower.

The potential of an organism as an organic extractive species within IMTA sites depends primarily on its efficiency to capture and convert particles. Species are selected due to specific culture performance traits, for these traits quantitative information needs to be available, with respect to nutrient uptake rate, reduction efficiency and secondary considerations (e.g. yield and protein content). For the organic extractive species that we select the following need to be known from other studies:

- Determine whether it can consume the size and consistency of particles released from the fish farm and with the current speed of the specific locality
- Its absorption/ingestion rate and assimilation efficiency (Biofiltration potential)

- Its relationship to the quality of particulate material present at the site
- The time necessary to convert food to faeces (gut passage time)
- Growth rate
- Culture practicalities
- Marketability and market value
- Suitability of species to the site (e.g. within its optimal temperature range)
- Quantity and quality of available data in the literature

1.7.1 Seaweed species as IMTA inorganic extractive species

Seaweed cultivation on a commercial scale is relatively new (it only started to expand after the 70s) and limited in Europe. However, global seaweed production accounts for 24% of the total quantity of aquaculture (fresh and marine) worldwide (FAO 2012). Most seaweed cultivation occurs in Asia (99.6%), with China accounting for 58.4% of the total seaweed biomass produced (FAO 2012).

Seaweeds are a good source of nutrients, they contain high levels of protein (up to 47% of dry weight) (Darcy-Vrillon, 1993), polysaccharides (30–71% of dry weight) (Jensen, 1993), low levels of lipids (1.5 and 3.3% of dry weight) and some contain mineral elements such as calcium (e.g. *Ulva*; MacArtain et al. 2007) and magnesium (Fleurence et al. 2012).

The major commercially important seaweed species are: edible brown seaweeds (*Laminaria spp., Undaria spp., Hizikia spp.*), edible red seaweeds (*Porphyra spp., Palmaria palmata*), agar-containing red seaweeds (*Gelidum spp.* and *Gracilaria spp.*) and Carrageenan-containing seaweeds (*Chondrus crispus*) (FAO, 2015b). A more detailed description of some of these species follows below.

1.7.1.1 Porphyra

Porphyra is used extensively in food it is commonly known as nori in Japan, zicai in China and “purple laver” in Great Britain. It is used in the food industry as the seaweed wrapping around the Japanese ‘sushi’. It is also a

major source of taurine that controls blood cholesterol levels (Tsujii et al. 1983), and is a staple in health food diets (Mumford and Miura, 1988). *Porphyra* contains high levels of proteins (25–50% depending on the species) 75% of which is digestible, significant amounts of vitamins (A,B_1,B_2,B_6,B_{12},C, niacin and folic acid), trace minerals, dietary fibre and is low in sugars (0.1%) (Noda, 1993; FAO, 2003). It contains large amounts of the amino acids alanine, glutamic and glycine (FAO, 2003). It also serves as a preferred source of the red pigment r-phycoerythrin, which is utilised as a fluorescent 'tag' for fluorescence in situ hybridisation (Mumford and Miura, 1988).

Porphyra requires constant availability of nutrients, thus in *Porphyra* monocultures during the summer time when temperate waters are nutrient depleted, fertilizers need to be added. *Porphyra* has been proved to be able to assimilate aquaculture–derived nutrients (Chopin et al. 1999; Carmona et al. 2006; Pereira et al. 2008). Consequently, integrating *Porphyra* with fish cultures does not only mitigate the environmental impact of the fish farm but also some nutrient inputs, otherwise necessary, are avoided. *Porphyra* cultures achieve high levels of production and nutrient accumulation due to their physiology. *Porphyra* blades are thin with 1 or 2 layers of cells (flat sheet blades), which are all involved in nutrient absorption. Flat sheet is the most productive morphotype (Littler and Arnold, 1982) and thallus thickness is negatively correlated with the maximum rate of photosynthesis (Enriquez et al. 1995). *Porphyra* has a high growth rate; the production cycle from seeding to first harvest in net culture lasts less than 40 days (Merrill, 1989). This permits repeated harvesting of a net-grown crop every 9 to 15 days (Chopin et al. 1999). Using mechanical harvesters, multiple harvests can be taken from a single seeding. *Porphyra* can store nitrogen weighing as much as up to 6% of its dry weight (Pereira and Yarish, 2010). *Porphyra* has higher phycoerythrin and phycocyanin contents when cultivated in the proximity of salmon cages (Chopin et al. 1999). The production and processing of *Porphyra* is advanced and its biology is well understood; this enables the manipulation and control of its aquaculture i.e. there is no need to rely on natural seeding. The technical problem with its cultivation is it

relies on the conchocelis sporophyte stage growing in bivalve (usually old oyster) shells to produce conchospores to seed nets (Mumford, 1990).

1.7.1.2 Gracilaria

Gracilaria species can be used for agar production, human consumption, and as feed for other high-valued aquaculture organisms, such as abalone (Chopin et al. 2001; Neori et al. 2004; Fei, 2004). Many studies have shown that *Gracilaria* can effectively remove nutrients through utilization of excess nutrients (e.g., N and P) in IMTA systems of fish, scallop, or shrimp co-cultured with algae (Buschmann et al. 1996; Hernández et al. 2006; Jones et al. 2001; Mao et al. 2009; Neori et al. 1998; Troell et al.1997; Yang et al. 2006; Zhou et al. 2006).

The genus *Gracilaria* is an attractive candidate for intensive culture because of its ability to achieve high yields of commercially valuable products and to its ability to withstand a wide range of environmental conditions as; implied by its cosmopolitan distribution. Finally, *Gracilaria* is a very efficient biofilter due to its ability to store nitrogen for later growth (Troell et al. 1997; Pereira et al. 2008; Abreu et al. 2011).

1.7.1.3 Ulva

Species of the genus *Ulva* are good candidates for nutrient bioremediation due to their high biofiltering efficiency (Neori et al. 1996). The specific growth rate of *U. lactuca* is 16-18% day^{-1} (Ale et al. 2011, Bruhn et al. 2011). *Ulva* can be used as a biofuel or as an organic fertilizer (Copertino et al. 2009). It contains up to 3.25 g of calcium per kg of dry weight.

Ulva spp. are annual or pseudo-perennials in that the holdfast portions are perennial and grow new blades each spring (Lobban and Harrison, 1997). The life cycle of *Ulva lactuca* includes a haploid gametophyte phase and a diploid sporophyte generation. It has an isomorphic diplohaplonic life cycle with haplogenotypic sex determination (Lobban and Harrison, 1997).

1.7.2 Organic extractive organisms

The most commercially important invertebrate crops are molluscs and crustaceans, especially oysters (*Crassostrea* spp.), mussels (*Mytilis* spp.), shrimps (*Penaeus* spp., *Macrobrachium rosenbergii, Metapenaeus* spp.), lobsters (*Homarus americanus* and *H. gammarus*), and various crayfish species. However, markets for new species such as sea cucumbers are also emerging, especially in Asia.

1.7.2.1 Sea cucumbers

Sea cucumbers have been consumed in Asia and used in traditional Chinese medicine for hundreds of years, but they are relatively obscure in the western world, although this may be soon changing. Extracts (the compound frondoside A) from the sea cucumber *Cucumaria frondosa* may provide curative and/or preventive treatment options against diseases (including cancer types) (Aminin et al. 2008).

Deposit–feeding sea cucumbers can reduce organic pollution by feeding on sediment with high organic content (Paltzat et al. 2008; Yu et al. 2011). This is why they can thrive near mariculture facilities. *Parastichopus californicus* settles and grows on oyster culture gear (Paltzat et al. 2008), *Australostichopus (Stichopus) mollis* can consume mussel aquaculture waste (Slater and Carton, 2007) and the suspension-feeding sea cucumbers *Cucumaria frondosa* can consume salmon waste (Nelson et al. 2012). MacDonald et al. (2013) concluded that if *Holothuria forskali* individuals are grown to a density of 400–500 g m^{-2} they can process all solid waste produced by a commercial *Dicentrarchus labrax* sea-cage production unit, if appropriate temperature and deposition rate conditions are maintained.

Ahlgren (1998) showed that muscle development of California sea cucumbers reared inside floating net pens at a salmon fish farm was significantly greater than that of sea cucumbers feeding in their natural environment. Yu et al. (2011) give the growth rates of both suspended (0.6% day^{-1}) and bottom (1% day^{-1}) cultures of *Holothuria leucospilota* co-cultured with fish as comparable to or higher than those of sea cucumbers

co-cultured with bivalves (Zhou et al. 2006; Slater and Carton 2007; Paltzat et al. 2008).

Another trait of some sea cucumbers, e.g. *Cucumaria frondosa*, that makes them suitable for IMTA is that they can consume some of the larger organic particles released from the fish farm, which can not be consumed by other extractive organisms such as bivalves (Nelson et al. 2012). Additionally, the absorption efficiency of most sea cucumbers is comparable to that of the mussel *Mytilus edulis* (e.g. 69–85% for *Cucumaria frondosa*) (Nelson et al. 2012) but the assimilation efficiency is more dependent on food quality in mussels than in sea cucumbers (Bayne et al. 1987; Reid et al. 2010; Ren et al. 2012). Finally, sea cucumbers can reduce salmon net fouling through grazing (e.g. *Parastichopus californicus;* Ahlgren, 1998).

1.7.2.2 Sea urchins

Sea urchin gonads (roe) are harvested in many parts of the world as a delicacy (Lawrence, 2001). However, currently natural sea urchin populations are facing increased fishing pressure (Yokota, 2002) and many populations are currently overfished (Conand and Sloan, 1989; Le Gall, 1990).

Sea urchins such as *Paracentrotus lividus* can assimilate fish-farm waste and can achieve high growth and survival rates near salmon cages (Cook and Kelly, 2007b).

1.7.2.3 Bivalves

Bivalves ingest small, suspended particles and initiate the sedimentation of larger particles with high organic content, by ingesting and releasing them in the form of faeces and pseudofaeces (Kautsky and Evans, 1987). Captured particles are rejected before ingestion as pseudofaeces or ingested and excreted as faeces (Haven and Morales-Alamo, 1966; Navarro and Thompson, 1997). Although there is no net addition of organic matter, the larger biodeposits become available as an energy source to microorganisms and ultimately to higher trophic levels such as benthic macroinvertebrates (Yingst, 1976; Dame and Dankers, 1988).

The mussel *Mytilus edulis* has a wide range (43–90%) of extraction efficiencies related to the type of feed that they consume (Reid et al. 2010). When feeding in the natural environment *M. edulis* have an average absorption efficiency of approximately 70% when consuming particulate material between 45 and 90% organic content (Cranford and Hill, 1999).

Mussels are good candidates for IMTA because they have a wide geographic distribution, can be cultured with high stocking density and have relatively high biomass. However, it has been suggested that for the production of mussels, the particulate waste from salmon cages is less important than natural food sources (Wang et al. 2012). Mussel population filtration rates can reach levels known to seriously deplete suspended particulate matter and control phytoplankton production at the coastal ecosystem scale (Grant et al. 2008, Dame, 2011).

Shellfish cultures increase dissolved inorganic nutrient concentrations by increasing remineralisation of particulate organic material and thus it is beneficial if they are co-cultured with inorganic extractive species such as macroalgae that can absorb the nutrients released by both the fed species and the shellfish.

1.8 Review of polyculture and IMTA studies

During the Tang dynasty in China, farmers developed polycultures of carp, pigs, ducks, and vegetables on their small family farms, using the manure from ducks and pigs to fertilize the pond algae grazed by the carp. Carp were later added to flooded paddies, where the fish consumed insect pests and weeds and fertilized the rice before becoming food themselves. Such carp–paddy polyculture sustains China's traditional fish–and–rice diet and is still used on more than seven million acres of paddies in the country (Joel and Bourne, 2014). Traditional inland aquaculture, is integrated with other agricultural activities (e.g., crops and livestock) and wastes (e.g., crop residues, livestock manure) provide the sources of nutrition for the fish, and fish wastes are recycled back into the system to fertilize crops (e.g., rice).

Examples of traditional integrated aquaculture include:

- Integrated agriculture–aquaculture systems (e.g. rice–fish, livestock–fish). Rice and fish may be raised together, or alternated in a rotation.
- Polyculture of fish (e.g., multiple species of carp in one pond) that occupy different spatial and feeding niches in a pond.
- Wastewater-fed integrated peri–urban aquaculture systems (fed from human sewage or industrial effluents).

Traditional Chinese polyculture practices are currently declining. Intensification of both rice and fish farming, especially due to the availability of industrialy–produced fish feed, has led farmers to abandon polyculture (rice–fish farming) and move toward intensive monoculture systems (Garnett and Wilkes, 2014).

The first integrated aquaculture studies examined methods for treating sewage outlets using seaweeds and bivalves (Ryther et al. 1972, 1975; Goldman et al. 1974) and methods for treating effluents from land-based aquaculture systems using seaweeds (Ryther et al. 1975; Harlin et al. 1978). Research on land–based IMTA systems includes the combinations among various types of organisms (Table 1–2).

Table 1-2: Examples of extractive organisms and combinations of extractive organisms that have been used in land–based IMTA systems.

Extractive species	Example references
Bivalves and shrimps	Wang and Jacob, 1991; Jacob et al. 1993; Hopkins et al. 1993; Lin et al. 1993; Nelson et al. 2001
Seaweeds and shrimps	Chandrkrachang et al. 1991; Lin et al. 1992, 1993; Primavera, 1993; Enander and Hasselström, 1994; Phang et al. 1996; Jones et al. 2001
Bivalves and fish	Shpigel and Blaylock, 1991; Shpigel et al. 1993
Algae and molluscs cultivated in fish or shrimp effluents	Wang, 1990; Shpigel et al. 1991; Enander and Hasselström, 1994; Neori et al. 1996, 1998, 2000; Neori and Shpigel, 1999; Chow et al. 2001; Jones et al. 2002; Schuenhoff et al 2003

The studies presented in Table 1–2 have shown that the waste released from aquaculture is suitable for the growth of the co-cultured extractive organisms and thus these organisms have the potential to reduce the amount of waste that intensive fish farming releases into the environement. The nutrient–uptake efficiency varied greatly among these studies, but from the extractive organisms studied, seaweed seems to be the most promising and land–based IMTA systems with seaweeds have been shown to remove more ammonia than traditional biofilters (Cahill et al. 2010). Bivalves seem to be the least effective, or at least the most challenging, biofilters for IMTA (see Chesuk, 2001).

Most of the IMTA studies up to the 1990's have been performed in closed systems, such as tanks and ponds. Within such experimental setups the bioremediation capacity of extractive organisms can reach its maximum and the effluent can be biofiltered up to 100%. The challenge is to achieve high bioremediation in open water systems where the nutrients are diluted and the particulate wastes are being transferred away from the farm. In open-culture systems a range of organisms have been used for filtering fish cage effluents (Table 1–3).

It has been shown that marine IMTA with finfish and shellfish can remove up to 54% of total particulate matter (Reid et al. 2010), and seaweed can remove up to 60% of dissolved inorganic nitrogen and phosphorus (Abreu et al. 2009).

Currently, commercial and experimental IMTA systems are in operation in land–based and marine systems in over 40 countries, including Canada, the United States, the United Kingdom, Chile, South Africa, Israel, Japan, and China (Buschmann et al. 2008a; Soto, 2009; Chopin, 2010; Chopin 2011).

Table 1–3: Examples of extractive organisms and combinations of extractive organisms that have been used in open-water IMTA systems.

Extractive species	Example references
Seaweeds	Hirata and Kohirata, 1993; Buschmann et al. 1996; Troell et al. 1997; Chopin et al. 1999; Abreu et al. 2009
Bivalves	Jones and Iwama, 1991; Troell and Norberg, 1998; Buschmann et al. 2000; Mazzola and Sará, 2001; Cheshuk, 2001; Chesuk et al 2003
Polychaetes	Honda and Kikutchi, 2002; Giangrande et al. 2005), mussels (Sara et al. 2009
Sea urchins	Kelly et al. 1998
Sea cucumbers	Ahlgren, 1998; Zhou et al. 2006; Slater and Carton 2007; Yu et al. 2011
A combination of sea cucumbers and bivalves	Paltzat et al. 2008
A combination of sea urchins, fish, bivalve and seaweed	Chow et al. 2001; Schuenhoff et al. 2003
A combination of salmon, sea cucumbers, mussels and kelp	Nelson et al. 2012

1.9 Objectives of the thesis

The aim of this PhD thesis was the development of a model that could help maximize the potential of IMTA in terms of productivity and biomitigation and thus to contribute towards a more sustainable and productive form of aquaculture.

In detail, the specific aims of this thesis are as follows:

1) To investigate the suitability of the sea urchin *Psammechinus miliaris* and the lugworm *Arenicola marina* as IMTA organic extractive components. This was achieved by studying the growth, their ability to survive and have high–quality flesh with salmon aquaculture waste as their only food source as well as to study their remediation efficiency potential**.** This study and its findings are reported on and discussed in Chapters 3 and 4.

2) The development of a model that enables the user to simulate different scenarios, which I believe is a crucial step towards optimization of IMTA systems. The model developed consists of sub-models that are each based on different types of growth models; the growth models are linked via the exchange of nitrogen among them. This study and its findings are reported on and discussed in Chapter 5. A published manuscript that includes the work of this chapter can be found in the Appendix.

3) To illustrate the potential of the modelling tool (described in Chapter five) by running a comparative study. The comparative study investigated the nitrogen–bioremediation potential of three different invertebrate species, the lugworm *Arenicola marina*, the sea urchin *Paracentrotus lividus* and the mussel *Mytilus edulis*, cultivated as part of an IMTA. The comparison was in terms of the amount of nitrogen removed by a specific biomass of the extractive species or by a specific cultivation area with that species. This study and its findings are reported on and discussed in Chapter 6.

This study has generated a dynamic tool, with a simple user interface. This model can be easily re-parametrized and used as a tool for a evaluating the potential of a specific IMTA set-up consisting of any species combination, or for optimizing the performance, in terms of nitrogen removal and growth rates of the extractive organisms, of an existing IMTA set operation.

CHAPTER 2

Growth models and modelling methodologies

2.1 System Dynamics modelling

2.1.1 Introduction to System Dynamics modelling

The use of models can contribute in decreasing uncertainty about the future or about cause-effect relationships of the system. “A model is a substitute for a real system. Models are used when it is easier to work with a substitute rather than with the actual system.” (Ford, 1999). Mathematical models use equations to represent links within a system; the type of mathematical model used in this study is a computer simulation model.

The objectives of this study were addressed through the application of System Dynamics (SD) modelling. SD is a method that enables better understanding of the behaviour of complex systems over time (MIT, 1997). Dynamic modelling relies on systems that can be described by ordinary differential equations. The unique characteristic of SD in comparison to other approaches to studying complex systems is the use of feedback loops, stocks and flows and time delays that affect the behaviour of the entire system, all the SD building blocks are linked to each other with information links, delayed links or initialization links (Anonymous, 2003). These features of SD modelling are used to support Systems Thinking, which employs SD diagrams to develop systems models based on the four key elements mentioned above (Grossmann, 2015):

- The level (or “stock”) is used for state variables and is represented by a rectangle. A level is a repository where something is accumulated and potentially passed to other components of the system.
- The valve symbol represents flows. Flows into a level increase its content, and flows out of it decrease its content (Figure 2-4).

- Converters or auxiliary variables, represented by circles, are used to calculate interim values and can be paired with flows to create flows with rate (Figure 2-1).
- Constant values are represented with rhomboids (Figure 2-1).

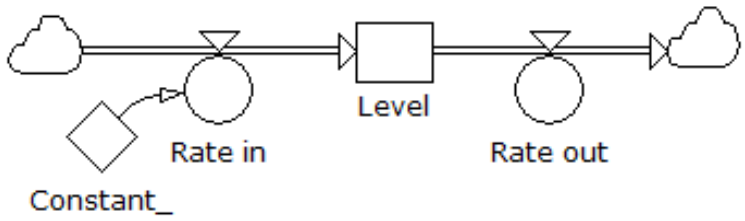


Figure 2-1: Diagrammatic representation of the symbol used in SD modelling software Powersim.

SD modelling is developed using object-oriented programming (OOP), which is a programming paradigm based on the concept of "objects", which are data structures that contain data. In object-oriented programming, computer programs consist of objects that interact with one another (Lewis and Loftus, 2008; Kindler and Krivy, 2011). Examples of objects in Powersim are the "circle," and "square". Many programming languages, such as C++, Java, C#, Perl and Python support object-oriented programming.

2.1.2 Powersim and other software for System Dynamics modelling

Software specifically designed for SD modelling is available from four main vendors: Stella (Iseesystems), Vensim (Ventana Systems Inc), Simile (Simulastics Ltd) and Powersim (Powersim Software AS). Such software offers a flexible approach to modelling by allowing development of models that can be easily shared, used and allow users to adapt available models to their own requirements (Ross et al. 2010).

The SD model-maker platform used in this study was Powersim Constructor 2.8 (Powersim, 2015). Powersim is a graphical interface object-oriented modelling software. It allows users to visualize, conceptualize, build and test system dynamics models. In Powersim all variables are represented as graphical objects, which connect with links or flows (Figure 2-4).

Each link or flow represents the relationship between the variables linked; the relationship is defined using equations. Powersim has been used widely to model ecological systems interactions, for example uptake of nitrogen by marine phytoplankton was modelled, focusing on the interactions between nitrate and ammonium within the environment and organism (Flynn et al. 1997). It has also been used to simulate sustainable shellfish culture in China (Nunes et al. 2003), and for shrimp growth modells to enable aquaculture development (Franco et al. 2006).

Powersim was selected as the SD modelling software for developing the IMTA model, although it is usually used for business modelling, due to its powerful ability to model system dynamics and to its user-friendly interface. Its graphics with the use of object-oriented stocks and flows allow better understanding of the model and can potentially allow various stakeholders, or in general people not familiar with the model, to understand the modelling processes and the steps involved in providing the outcomes. Powersim contains a user interface that allows users to interact with the model without the need for the user to interact directly with the model (Anonymous, 2003). The approachable format of Powersim can aid implementation of the outcomes of the model into actual aquaculture practice due to considerable stakeholder “buy-in” and understanding at each stage of the modelling and management process.

2.2 Existing models used as aquaculture analysis tools

In recent decades numerous dynamic models, most of them deriving from ecological modelling, were developed and used for the simulation and analysis of aquaculture (Wik et al. 2009). These include: EcoWin (Ferreira, 1995), FARM (Ferreira et al. 2007), ASSETS (Bricker, 2003), WinShell (Longline Environment, 2015) and various waste dispersion models such as DEPOMOD (Cromey et al. 2002), COD-MOD (Pillay and Kutty, 2005) and MERAMOD (Cromey et al, 2012).

EcoWin is an object-oriented modelling platform programmed with C++ (Ferreira, 1995). It is used for offshore aquaculture ecosystem models,

mostly for analysis of large systems by breaking the extended areas into boxes (Ferreira, 1995). It has been used for modelling shellfish polyculture and to aid the understanding of the sustainability of current culture practices (Nunes et al. 2003). It has also been used for the analysis of phytoplankton productivity in marine and estuarine systems (Duarte and Ferreira, 1997; Macedo and Duarte, 2006).

FARM is a local-scale resource management tool that simulates shellfish growth and analyses the carrying capacity and environmental effects at farm level. It was initially developed using the STELLA (ISEE Systems), modelling environment. It is used for site and species selection; estimation of biomass produced and feeding requirements; optimisation of culture period; operational optimisation of farming methods and profitability assessment for ecological and economic optimization of farming practices and for environmental assessment of farm-related eutrophication effects. Its main use is the estimation of production capacity while ensuring that it is within the limits of the local ecological carrying capacity (Ferreira et al. 2007).

The FARM model also simulates the concentration of dissolved oxygen. This can be combined with chlorophyll for assessment of environmental impact using the ASSETS method (Bricker, 2003), which is a management level eutrophication-screening model.

WinShell (Longline Environment, 2015) is a model used for determining individual marine and estuarine shellfish growth on the basis of food availability and environmental conditions.

DEPOMOD and its related models, coded in Visual Basic or Borland Delphi 7, are used for local–scale assessment of the environmental effects of marine fish cages by estimating the distribution of particulate waste material within the environment. They are used to support site selection at local scale (Ross et al. 2010) and environmental regulation procedures. While DEPOMOD is specifically used for marine salmonid production (Cromey et al. 2002), CODMOD was developed for cod (Cromey et al. 2009) and

MERAMOD for the Mediterranean fish species and conditions (Cromey et al. 2012).

Newell (2007) developed an 'ecological carrying capacity model', in which a pre-calcuated level of standing stock of bivalves enables maximum consumption of phytoplankton and enhancement of nutrient removal, without impacting the ecological function of the overall system. This model implies that detailed parameterisation of phytoplankton and microzooplankton rates are required with the model outcome suggesting, sediment hypoxia, inorganic nutrient cycling and reduction in turbidity from bivalve culture.

A number of models exist that predict the yield, environmental impact and economic optimisation of shellfish aquaculture (e.g. Brigolin et al. 2009; Ferreira et al. 2009; Giles et al. 2009; Filgueira et al. 2013). A disadvantage of carrying capacity models for shellfish aquaculture is that they only consider nutrients, plankton, detritus and bivalves production (Byron et al. 2011). In order to address broadscale carrying capacity questions related to the structure of the ecosystem, Jiang and Gibbs (2005) used the Ecopath modeling software (Christensen and Pauly, 1992) to model the carrying capacity of bivalve aquaculture, considering the full trophic spectrum.

A large number of models for fish aquaculture have been developed during the last decade these include various fish growth models (e.g. Bar and Radde, 2009), models to estimate the holding capacity of sites for fish farming (e.g. Stigebrandt et al. 2004), fish farm waste dispersion models (e.g. Corner et al. 2006; Magill et al. 2006), fish farm production and environmental effects (e.g. Cromey et al. 2002; López et al. 2008; Skogen et al. 2009; Pedersen et al. 2012).

2.3 IMTA modelling

There is a need for a modeling approach to IMTA; this is because open water experiments are not directly comparable with each other since they are based on site-specific parameters (biomass produced, species, site environmental characteristics) and various researchers have collected and

published different types of data and results. For example, the water body nutrient concentrations have not been measured in all the studies and even in those that they have been, different nitrogen and phosphorus fractions have been measured. An accurate comparison could have been made if at all trials phosphate, which is the form of phosphorus most suitable for seaweed growth (Neori, 1996), and ammonia, one of the preferred nitrogen sources (Carmona et al. 2001), was recorded and available in the literature. Another problem is that results acquired from studies lasting less than a year do not give entirely accurate predictions because seaweeds have both diurnal and seasonal cycles.

The majority of models for aquaculture production are developed for monocultures, despite the increasing importance of multispecies systems, such as polyculture and IMTA systems (Duarte et al. 2003). Polyculture models provide a quantitative tool to develop and manage such systems through mapping energetic pathways between different trophic groups and the environment. Models are helpful in designing IMTA practices with maximum resource utilization and minimum environmental impacts (Ren et al. 2012). Developing mathematical models to apply to IMTA and other multispecies systems can help understand and resolve the wide range of interactions among cultivated species and between those species and their physical and chemical environment. Such models are also useful for estimating the productivity, production parameters (e.g. mortality, stocking density) and to control the production cycle, since the interactions within IMTA systems are complex.

Nunes et al. (2003) developed a multispecies model for coastal polyculture of Chinese scallop (*Chlamys farreri*), Pacific oyster (*Crassostrea gigas*) and kelp (*Laminaria japonica*). The model can estimate the exploitation carrying capacity, the harvest potential of the three species and environmental impact of different management strategies. The model can be extended to other farmed species and would be suitable for several coastal systems and to assess the interactions of the farmed species with the ecosystem. The

uniqueness of this model is that it considers demographics and has the potential to integrate parameters that affect or are affected by stakeholders.

Ferreira et al. (2012) developed a model for gilthead bream (*Sparus aurata*) and integrated it with an existing shellfish model in the Farm Aquaculture Management System (FARM), to assess the quantitative effects of an IMTA combining gilthead bream cages and Pacific oyster (*Crassostrea gigas*) suspended from longlines. Some interesting simulations were run with this model, including aeration and water exchange for ponds and incorporation of metabolic costs in the fish growth models for the comparison of coastal and offshore aquaculture. However, this model does not include an inorganic extractive component.

Ren et al. (2012) developed an IMTA model consisting of three trophic groups: finfish, shellfish, detritivore and primary producer. It was parameterized using salmon, mussels, sea cucumbers and seaweed. The model is based on dynamic energy budgets (DEB). The model incorporates benthic and pelagic components that interact via carbon and nitrogen budgets and nutrient cycling. The model can be used for optimizing yields and reducing farm-derived wastes. This IMTA model is entirely based on the DEB, this is limiting because for some species there exists no data that can be used for a DEB model or there exist more accurate models for predicting their growth (e.g. for salmon).

2.4 Introduction to growth models

2.4.1 Types of fish and invertebrate growth models

There are a number of models to estimate the growth of fish; those commonly used include:

- Simple growth functions. These empirical models predict weight or length using time as the independent variable. They are usually analytical solutions to differential equations that can be fitted to measured growth data by means of non-linear regression analysis (Thornley and France, 2007).

- The von Bertalanffy equation (von Bertalanffy, 1957). The theory is based on the assumption that growth is determined by the difference between anabolism and catabolism. However, this assumption overlooks the role of timing of maturation on the shape of the growth curve (Lester et al. 2004).
- The Thermal–unit growth coefficient (TGC) (Iwama and Tautz, 1981). It is a simple model, widely used in aquaculture. However, it can present errors when the temperature is too far from the optimum for growth and is suitable only for fish species with a specific weight–length relationship (Jobling, 2003). The TGC has the following basic form:

$$TGC = 1000\ \frac{\sqrt[3]{W_t} - \sqrt[3]{W_0}}{T * t}$$

Where, W_i is the initial weight of the smolt, TGC is the thermal growth coefficient, T is the temperature, and t is time in degree days.

- The Specific growth rate (SGR). It is used to estimate the production of fish after a certain period using the following formula:

$$SGR = \frac{\ln(W_t - W_i)}{\mathrm{t}} * 100$$

It does not take into account either the effect of body weight or of temperature, on fish growth. However, there exist tables with different SGR values according to body weight and temperature. The SGR assumes fish weight increases exponentially, an assumption that is only accurate for most young fish cultured for short periods. Thus SGR is principally useful for reporting growth of small fish, but not suitable for larger fish or longer culture periods (Hopkins, 1992).

- The Daily growth coefficient (DGC) (Iwama and Tautz, 1981). The DGC calculated using one-third exponent of an animals weight gain:

$$DGC = \frac{{W_t}^{1/3} - {W_i}^{1/3}}{t} * 100$$

Its main advantage is that at a given temperature, it is independent of fish body weight. This way it eliminates the problem of decline in SGR with increasing body size.

In general these models lack biological interpretation, disregard properties important for many species such as ectothermy, indeterminate growth and variations in growth across life stages. The inclusion of growth variations across life stages in a model is important because as an animal increases in size, the rate of its metabolic activities slows down, and consequently the relative growth rate also declines (Ricker, 1979). Furthermore, an increase in growth is relatively smaller for large fish than for small fish, which makes the relative growth rate unsuitable for comparing growth rates for fish of different sizes (Jobling, 1994). Finally, the relative growth rate is restricted to the length of time for which it was computed and cannot be easily converted to another time period (Hopkins, 1992).

An alternative to simple growth models are bioenergetic growth models. Simple bioenergetic models may be used for physiological and behavioral properties of individual organisms, for population and community dynamics and for ecosystem processes. These models are based on the quantification of the energy exchange induced by metabolic processes in organisms to stay alive, grow and reproduce (Nelson and Cox, 2000). They study the flow and transformation of energy in and between living organisms and between living organisms and their environment. Bioenergetic models are based on the first law of thermodynamics (energy and matter cannot be created or destroyed, but they can be changed from one form to the other) and on the rule that consumption equals with the sum of metabolism, waste and growth. Consequently, bioenergetics is based on energy balance, which is related to the energy flow through living systems via metabolism and is the biological homeostasis of energy in living systems. Energy balance is measured using the energy intake, which equals the sum of the internal heat produced, the external work and storage. Models based on bioenergetics and nutrient metabolism do not only predict growth but also feed requirements and waste outputs.

The Dynamic Energy Budget (DEB) theory (Kooijman, 1986) is based on the same principles as the 'standard' bioenergetics models, but is theory driven rather than data driven (Nisbet et al. 2012). Research in this area started in the 80's and the DEB was first described in Kooijman (1986), but it remains a very active research area.

The DEB is based on a set of distinct assumptions and is presently the simplest model that describes the complete life cycle (embryo, juvenile and adult) of an organism (using only 12 parameters for the standard DEB model) in a variable environment (Sousa et al. 2010; Pecquerie et al. 2011). It predicts both interspecific and intraspecific variation in the energy and mass fluxes in any biologically relevant environment. The disadvantage of this more general model is that it is more abstract. In detail, the model's state variables are not directly measurable, and even the certain observable fluxes, such as respiration rate or heat loss, are usually linear combinations of individually unobservable fluxes (Nisbet et al. 2012).

DEB theory describes the process of substrate uptake and use by individuals (Kooijman, 2008). In DEB theory biomass is portioned in reserves and structures, reserves are necessary to smooth out fluctuations in resource availability and to describe other observed physiological patterns such as respiration and body size scaling (Kooijman, 2008). The DEB model describes the full life cycle of an organism and thus there is the need to differentiate between growth and development. Development describes the stage from the fertilization of the egg to the organism's mature form. Figure 2-2 illustrates the energy flows and state variables of the DEB during the different life stages.

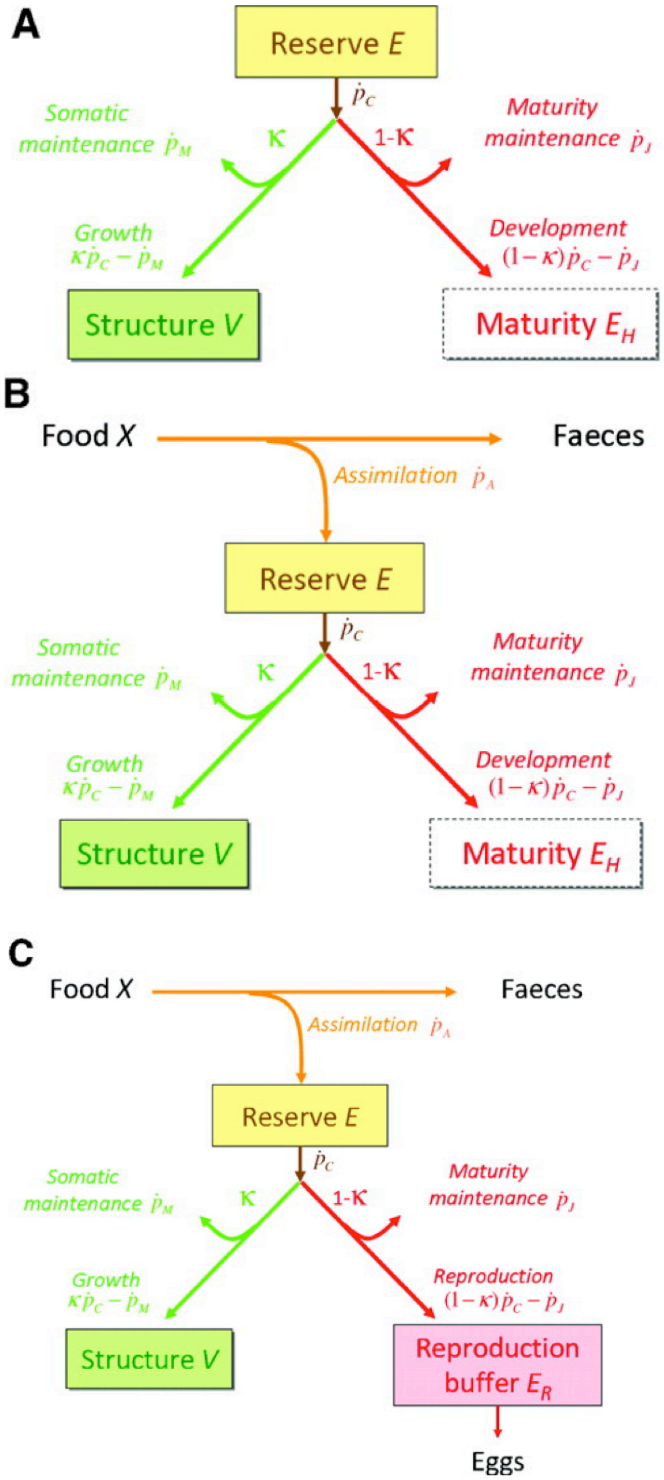


Figure 2-2: Schematic representation of the three life stages of the 'standard' DEB model (Kooijman, 2010). (A) An embryo uses reserve to grow and develop. (B) At 'birth', a juvenile starts feeding, and (C) at 'puberty', an adult starts allocating energy to reproduction.

Because the emphasis of DEB theory is on mechanisms, the standard application of allometric equations is eliminated. The theory also implies rules for co-variation of parameter values among species, “body size scaling relationships”, based on their difference in maximum size, using the zoom factor (z) (Hastings, 2011). For the co-variation of parameters that relate to the physical design of the organism among species the zoom factor is used. The zoom factor is a term introduced to demonstrate how the parameters that affect physical design relate to the maximum size of the organism. The zoom factor is the ratio of the maximum structural length (l_m) of the species of interest to the maximum structural length of a reference species $z = L_m/L_m^{ref}$. Using the zoom factor several predictions for scaling of DEB parameters inter-specifically can be made and the scaling of these parameters leads to apparent covariations such as growth rate, respiration, and life span (van der Veer, 2006; Kooijman, 2010).

There are often some discrepancies between DEB model predictions and observations, they can be interpreted in terms of differences in life-history strategy and specific adaptations of these species to their local environment. It can also be attributed to the fact that the maximum surface-area-specific assimilation rate depends on the food type (van der Veer, 2006). Certain food types have higher energetic content than others and thus organisms raised on *ad-libitum* concentrations of varying quality food can grow at different rates.

Most of the energetic models developed up to date are net-production models based on the Scope for Growth (SFG) concept (Bayne, 1976). SFG is based on the energy balance in steady state conditions and it is basically the difference between energy gained by feeding and energy lost by respiration (van Haren and Kooijman, 1993). When the energy gained by feeding is more than that lost by respiration then the energy is available for growth and reproduction, otherwise there is weight loss due to the utilization of energy reserves (Bayne and Newell, 1983). The main trait that distinguishes the DEB from other net-production models is that DEB models assume that the energy assimilated is first stored in reserves and then used

for maintenance, growth, development, maturity maintenance and reproduction (Pouvreau et al. 2006). The main difference between the DEB and SFG is that SFG does not distinguish storage of energy reserves from 'structural biomass' in its standardization for body weight, this problem is particularly noticeable when modelling seasonal variations of body composition (van Haren and Kooijman, 1993). Another problem in the application of SFG is the way respiration rates are interpreted. This is because part of the respiration measured with a standard conversion to energy is connected with growth, while in the SFG, it is fully assigned to maintenance (van Haren and Kooijman, 1993).

2.4.2 Algal growth models

Macrophyte productivity models usually follow a mass-balance approach. In detail, the standing crop is a function of the rate of biomass production through gross photosynthesis and biomass loss through respiration, plant damage and decay (Carr et al. 1997). The differences among the different models are the environmental factors used to influence growth rates and the algorithms used to describe the relationship of growth with these parameters. In some models co-limiting factors are multiplied and in other the most limiting factor is used. A list of different aquatic plant growth models is shown in Table (2-1).

Some of these models are based on the assumption that growth and uptake rates are equal and, therefore, depend on the external concentration of the nutrient and other assume that uptake kinetic is independent from growth, which is a function of the nutrient content of the cell, Q. The model used in this study is based on Droop's equation (Droop, 1968), which describes the relationship between algal growth rate and the total amount of nutrient per cell (cell quota). Droop (1973) states that the internal nutrient status of an algal cell can be as important or even more important than the concentration of nutrients in the environment. The model developed in this study is based on the implementation of Droop's equation by Solidoro et al. (1997). Similar models include those developed by Coffaro and Sfriso (1997), Duarte and

Ferreira (1997), Alvera-Azcárate and collaborators (2003), and Baird and collaborators (2003).

Table 2-1: List of aquatic plants growth models modelling

Reference	Model description
Scheffer et al. 1993	MEGAPLANT: Model evaluating general aquatic plant laws and new theories; submerged macrophytes in lakes
Collins and Wlosinski, 1989	CE-QUAL-R 1: General macrophyte submodel for reservoirs
Davis and McDonnell, 1997	Species-specific, partitioned biomass model for rooted macrophytes in streams
Wright and McDonnell, 1986	Submerged vegetation in Pennsylvania streams
Titus et al. 1975	WEED: *Myriophyllum spicatum* production model for Lake Wingra, WI
Best, 1981	*Ceratophyllum demersum* growth model in Lake Vechten, the Netherlands
Hootsmans, 1994	SAGAI: *Potamogeton pectinatus* growth model for shallow eutrophic lakes
Toerien et al. 1983	*Salvinia molesta* phosphorus, nitrogen and temperature growth model for fish ponds
Wetzel and Neckles, 1986	*Zostera marina* growth model for lower Chesapeake Bay
Gordon and McComb, 1989	Growth model of *Cladophora montagneana* in a eutrophic Australian estuary
Canale and Auer, 1982	General *Cladophora* biomass model for Great Lakes
Painter and Jackson, 1989	Internal phosphorus *Cladophora* model for Great Lakes
Huisman et al. 2002	Light-limited scalar model
Haario et al. 2001	Algal growth model that includes two nutrients and a temperature dependence
Klausmeier et al. 2004	A model for nutrient-limited growth where nutrient densities are variable, and where intra- and extracellular densities are distinguished
Huisman et al. 2002	Light-limitation growth model
Droop, 1968	Nutrient-limitation growth model

2.5 Choice of growth models used in this study for each IMTA component

The IMTA model developed in this study consists of sub-models that are each based on different types of growth models (Figure 2-3). The choice of growth models was based on what could provide the most accurate predictions for each component of the IMTA, based on the data available in the literature for model parameterization and on the requirements of the overall model.

A DEB model was used for the simulation of invertebrate growth and particulate organic matter uptake and release (Figure 2-3). The reason why a DEB model was used for the organic extractive organisms was to link the environmental variables, mainly food availability and temperature, with feed intake, growth, excretion and faeces production. Furthermore, Larsen et al. (2014) conducted a comparative study of the ability of bioenergetics growth (BEG, Riisgård et al. 2012), DEB, and SFG to predict growth of blue mussels and concluded that the DEB was the best at predicting the mean growth (Larsen et al. 2014).

The TGC was deemed most suitable for simulating the salmon growth (Figure 2-3). The main reasons for choosing the TGC were that it is known that under intensive aquaculture conditions feed is not limiting salmon growth and because salmon is so well studied that data, often with a one-day time resolution, for the TGC and FCR as well as for excretions and faeces production are available. Finally, the TGC could allow comparisons between different culture operations, fish strains, production years, sampling intervals, etc, (Bureau et al. 2000). The DEB was deemed less suitable especially because its basic strength: simulation of the full life cycle, was not relevant since salmon is grown at sea only for a part of its life. The most common alternatives to the TGC, the SGR and DGC, were rejected for the following reasons: The DGC is more accurate than the SGR in predicting weight of fish of different body sizes (Kaushik, 1998), but the major downside of both the SGR and the DGC is that they do not consider temperature (Iwama and Tautz, 1981). This problem does not apply for the

TGC, which is stable over a wide range of temperatures (Iwama and Tautz, 1981; Alanärä et al. 2001). Another strength of the TGC is that is affected by fish size less than the SGR and the DGC (Bureau et al. 2000).

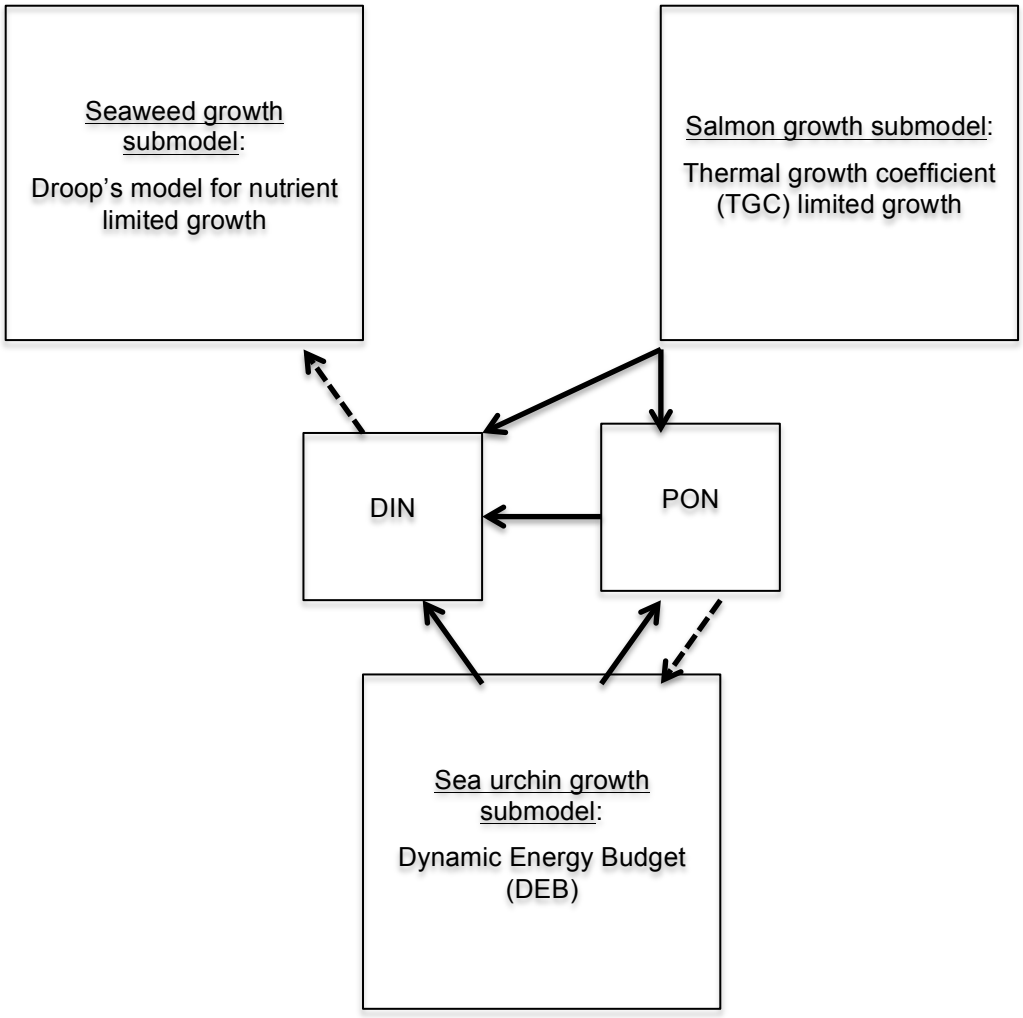


Figure 2-3: Schematic representation of the IMTA model with the three submodels and the modelling methods used for each one.

Although the TGC was selected to simulate salmon growth in this study, it would not be the model of choice for other fish species (e.g. in other possible scenario simulations with the IMTA model), because to date it has only been validated for salmonids (Bureau et al. 2000). Furthermore, when using the daily TGC values as input for the model, it is important that the values used are for the correct feed type, husbandry methods and

environment conditions and for the right season, because all these can influence the TGC (Cho, 1990; Cho and Bureau, 1998; Alanärä et al. 2001).

Droop's model for nutrient limited algal growth was used for the seaweed growth and nitrogen uptake model (Figure 2-3). An alternative to the Droop model would have been the DEB; the reasons for not choosing the DEB are as follows: The standard DEB model assumes isomorphy and has a single reserve and a single structure, which is appropriate for many aspects of the metabolic performance of animals; other organisms typically require more reserves, and some (e.g. plants) also more structures. In detail, in order to simulate the algal growth with a DEB we would need to use a multivariate DEB model with several substrates (nutrients), reserves and structural masses (Kooijman, 2008). For a photosynthetic organism DEB model we also need to consider differentiation of root and shoot, life stages, nutrient acquisition via transpiration, symbioses with animals, fungi, bacteria (e.g. re-mineralisation leaf litter, pollination), multiple reserves (micro-algae). Thus the DEB was not deemed suitable for simulating seaweed growth, since the DEB is more complex for plants (e.g. due to the multiple reserves) and it has not been suggested that it can simulate seaweed growth more accurately than the classical methods.

CHAPTER 3

Preliminary investigation of the consumption and remediation of Atlantic salmon waste by the sea urchin *Psammechinus miliaris* and comparison with dry seaweed diets

3.1 Introduction

Psammechinus miliaris is an echinoid with dorsoventral flattening of the test and color that varies with habitat from deep purplish-brown with no difference between the test and the spines (shallow or littoral zone) to a light green test and vivid purple spine tips (deeper water).

P. miliaris is found all around the British Isles, its geographical distribution ranges from Scandinavia up to Morocco, but it has not been found to date in the Mediterranean Sea (Jackson, 2008). *P. miliaris* populations can be found at the littoral zone exposed on boulders but also up to depths of 100 m (Mortensen, 1943). This species has high tolerance for low temperatures and is found in areas where winter temperatures are just above freezing (Ursin, 1960). It is also able to reproduce in cold waters, for example in the waters around the Faroe Islands, where the summer temperatures seldom exceed 11°C (Ursin, 1960).

In Scotland *P. miliaris* typically occurs in dense populations in sheltered areas of sea lochs on the west coast (Davies, 1989; Holt, 1991). Kelly (2000) estimated that the population density could be up to 352 individuals m^{-2} for littoral populations, but emphasized that the population density can vary greatly. *P. miliaris* individuals are found attached to the undersides of rocks, boulders, seaweed fronds (mainly *Laminaria latissima*) and shallowly buried under gravel on the foreshore (Kelly, 2000).

P. miliaris has been found to settle in large numbers on artificial structures associated with aquaculture such as suspended rope cultures of mussels in Killary Harbour and Bantry Bay in Ireland (Leighton, 1995), on suspended scallop cultures in Loch Fyne, Scotland (Cook, 1999) and on artificial structures deployed adjacent to salmon cages in the Lynne of Lorne, Scotland (Cook et al. 2006). Kelly et al. (1998) and Cook (1999) found that *P. miliaris* grazed on a range of fouling organisms both from salmon cage netting and corrugated PVC collector plates.

The overall aim of this study was to assess the suitability of the sea urchin as an IMTA organic extractive component. Choosing the right combination of species is a crucial step towards the establishment of an IMTA system.

The specific objectives of the trial described here were:

- To investigate the ability of *P. miliaris* to consume salmon waste
- To investigate whether they can survive with salmon waste as the only food source
- To compare the growth rate, gonadosomatic index (GSI), gonad color and nutrient content of the sea urchins fed with the different diets
- To investigate whether the sea urchins had a bioremediation effect, by comparing the element content of the different diets, to the nutrient content of the sea urchin gonads and faeces.

The initial study aimed to use *P. lividus* for both the experimental and modelling part of the work but this was not possible due to difficulties in obtaining *P. lividus* specimens. However, for the purposes of proof of concept the sea urchin, *Paracentrotus lividus*, a sea urchin of the same family (Parechinidae) and with similar biology and ecology to *P. miliaris* is used at the model simulation, described in chapter 6.

3.2 Materials and methods

The sea urchins were fed with the following three different diets:

Diet 1: A mixture of feed pellets and salmon faeces, sourced from a salmon farm with recirculating water and then frozen.

Diet 2: Dried *Laminaria digitata*, collected from the low tide zone at extreme low water tide and oven dried.

Diet 3: Dried *Palmaria palmata*, collected from the middle tide zone and oven dried.

The seaweed species *L. digitata* was chosen because it is an important seaweed species for the diet of *P. milaris* (Kelly, 2000) and the seaweed species *P. palmata* was chosen because *Palmaria* is a candidate genus for IMTA.

3.2.1 Experimental system

The 6-week feeding trial was carried out at a controlled temperature (CT) room at the University of Stirling. On the 28th of June 2013, 27 *P. miliaris* individuals were collected by hand at Millport from the intertidal zone. They were placed in plastic bags together with some moist kelp (*L. digitata*), the bags were filled with oxygen, closed airtight and transported to Stirling University. The sea urchins were transferred to the CT room and placed into nine already prepared experimental tanks, filled with artificial seawater (Figure 3-1). They were starved there for 3 days and acclimatised in the temperature and photoperiod that the experiment was carried out (Figure 3-2). The dimensions of the tanks were 48 x 33 x 25 cm. The sea urchins' weight ranged from 13.08 to 85.95 g. Three sea urchins were randomly allocated in each of the nine tanks, the average sum weight of the three individuals of each tank was 93.52 g, the tank with the smallest sum weight had 77.36 g and the tank with the largest had 109.99 g.

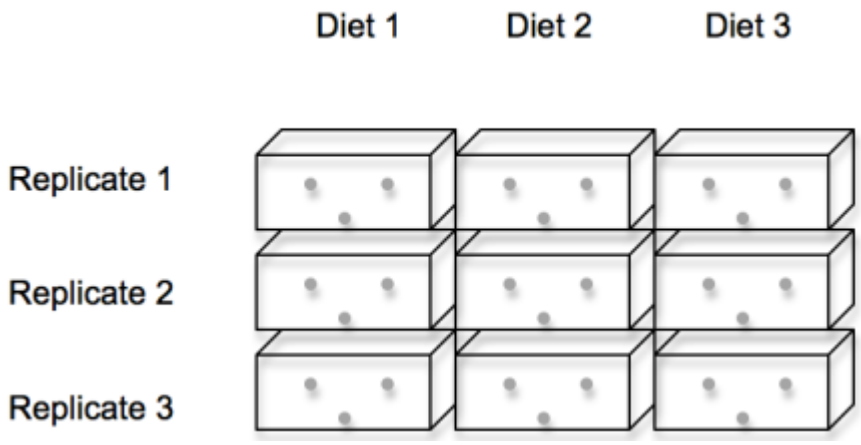


Figure 3-1: The three diet treatments with three replicates each, the grey circles depict the sea urchins.

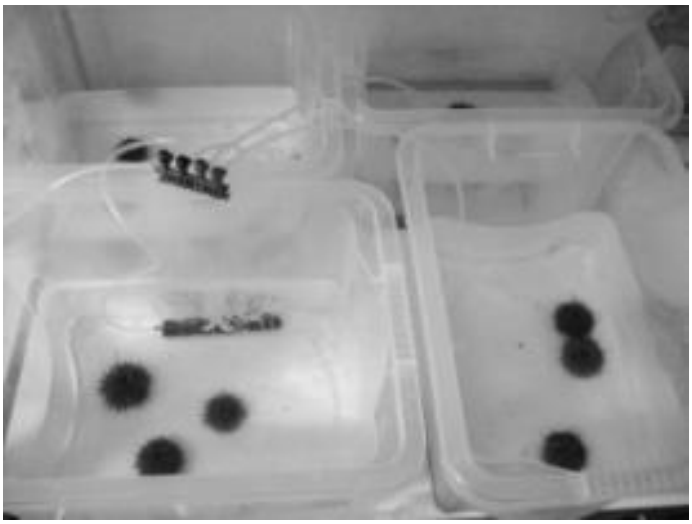


Figure 3-2: Sea urchins in the experimental tanks.

3.2.2 Experimental protocol

The sea urchins were fed every two days for 38 consecutive days. The first five days they were fed with 5% (10% every two days) of the initial body weight and for the remaining of the experiment with 2% (14% every two days) of the initial body weight per tank day^{-1} (See Table 3-1). The reason for reducing the amount of feed was that in the first five days there was a lot of uneaten feed remaining in the tanks and that could lead to high ammonia in the water, since the water was not filtered.

Table 3-1: Amount of feed given to the sea urchins, at each treatment.

Treatment	2%	5%
Salmon-faeces and salmon-pellet mixture	1.87 g ww	4.69 g ww
L. digitata	0.20 g dw	0.52 g dw
P. palmata	0.38 g dw	0.94 g dw

The moisture content of each diet type was calculated so that the same weight of dry mass of each diet was given to the sea urchins. The moisture content was calculated as follows:

$$MC = \frac{W_i - W_f}{W_i} * 100$$

Where *MC* is moisture content W_i is the initial weight and W_f the final weight.

Following the starvation period and prior to the trial's initiation each sea urchin was weighed, measured in length and photographed for photo-identification and measurement of individual growth. The treatment started on the 1st of July when all sea urchins were weighed and measured. Prior to the weight measurement the sea urchins were left on absorbent paper out of the water for 5 minutes in room temperature same as the water temperature (as in Grosjean, 2001), in order to drain the excess water for a more accurate measurement (Figure 3-3).

Each experimental tank was filled with 5 L artificial seawater. In order to keep ammonium levels low, every two days prior to feeding the remaining feed and faeces were siphoned and discarded and 20% of the water was exchanged. The tanks were continuously aerated, held under an artificial 12:12 L/D light regime, the temperature was maintained steady at 12 - 14°C (which is typical for Scottish sea temperature for the time of year). For the duration of the experiment the pH and salinity of each tank were measured every other day. During the course of the experiment water salinity was between 32 and 36 ppt and pH was between 7.4 and 8.1.

Figure 3-3: Prior to weighing the sea urchins they were let to drain the excess water for 5 minutes.

On the 18th day and on the last (38th day on the 7th of August) of the experiment faecal samples were collected from each tank, dried, weighed and frozen for biochemical analysis. Prior to physiochemical analysis, the samples were oven dried at 60^{o}C for 24h. At the end of the trial a 24h starvation took place, and following that all sea urchins were weighed and measured in length for calculation of their individual growth rate. The faeces were collected, by siphoning all the waste from the tanks, less than 24 hours after the end of the experiment in order to ensure that the faeces were fresh with no decomposition. The sea urchin gonads were extracted and oven dried for biochemical analysis. Gonad samples were obtained by cutting around the peristomial membrane and exposing the viscera, gonads were separated from the other organs and blotted dry with paper towel (Figure 3-4). The colour of each gonad (pale/ dark brown, yellow, dark orange) was recorded.

3.2.3 Carbon, Hydrogen, Nitrogen (CHN) analysis

The carbon, nitrogen and hydrogen content of the sea urchin faeces, gonads and sea urchin diet content were estimated using the Perkin Elmer Model CHN/SO analyser, which determined the elemental composition of 1 mg samples.

Figure 3-4: a) Sea urchin dissection b) Gonad of a dissected sea urchin fed with the faeces and waste feed diet.

3.2.4 Specific growth rate (SGR)

The specific growth rate expressed as % per day was calculated:

$$SGR = 100 * \frac{(\ln W_f - \ln W_i)}{t}$$

Where, W_f is the final body weight, W_i is the initial body weight and t is the duration of the experiment (days).

3.2.5 Gonadosmatic Index (GSI)

The GSI of the sea urchins was calculated as a ratio of the gonad mass to the whole-body wet mass:

$$GSI = \frac{Gonad\ weight}{Total\ weight} * 100$$

3.2.6 Video recordings

One of the trial tanks was dedicated to video recordings for the entire duration of the experiment, aiming to show sea urchin feeding mechanisms employed when different feed sources are available. Since sea urchins are nocturnal the camera was equipped with infrared light to be able to record the sea urchin activity when the lights were off. The camera was connected to a laptop computer for continuous streaming of the camera data.

3.3 Results

3.3.1 Sea urchin diet elemental composition

The results of the elemental analysis of the three diet types show that the mixture of salmon faeces and fish pellets had a much higher concentration of carbon and lower concentration of nitrogen and phosphorus than the two seaweed species (Table 3-2). The two seaweed species had similar amounts of the elements carbon and phosphorus, with *L. digitata* having slightly higher concentration of both but *P. palmata* had twice the amount of nitrogen than *L. digitata* and almost seven times as much as the mixture of salmon faeces and pellets (Table 3-2). The moisture content of *P. palmata* and *L. digitata* was estimated to be 79.9% and 88.8%, respectively.

Table 3-2: Elemental content of feed types (% of dry weight).

Diet type	C (%)	N (%)	H (%)	P (%)
Mixture of salmon faeces and pellets	53.3	5.29	3.13	0.8
L. digitata	28.78	7.85	3.03	5.28
P. palmata	25.93	15.46	3.37	4.62

3.3.2 Sea urchin growth

When the three replicates of each diet are grouped, it is shown that the average weight of the diet group fed with faeces and the one fed with *P. Palmata* has increased by the end of the experiment, while the average weight of the individuals fed with *L. digitata* present a non statistiacly significant decrease (Figure 3-5).

The growth was not the same for each sea urchin given the same diet. The growth rates for the *P. palmata* diet ranged from negative to 0.11% day^{-1}, for the *L. digitata* from negative to 0.17% day^{-1} and for the faeces from negative to 0.24% day^{-1} (Appendix Table 1).

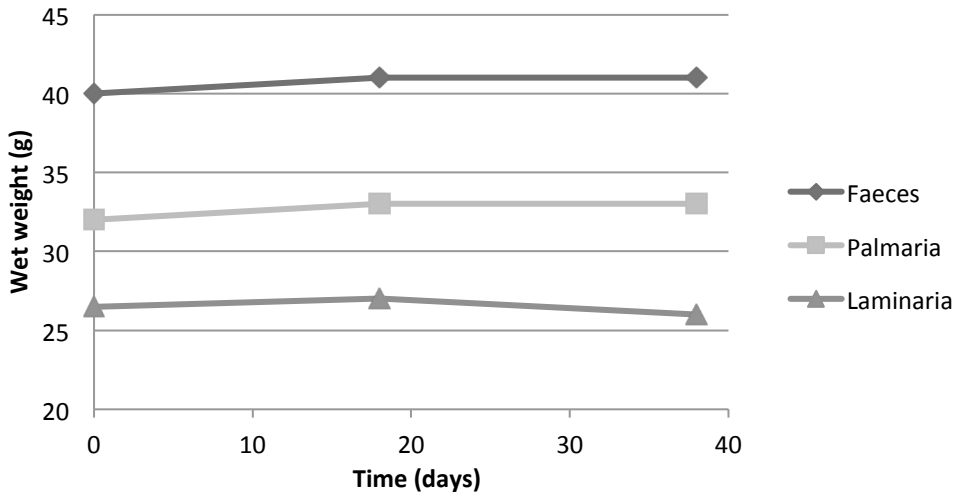


Figure 3-5: Average wet weight (g) of sea urchins fed with the three different diets, on the first, 18th and 38th day of the experiment during the 38-day feeding trial.

One-way Anova was used to analyze the differences between group means and their associated procedures (such as "variation" among and between groups). In detail, the weight gain, increase in test diameter, nitrogen, phosphorus and carbon content (in the gonads and in the faeces) of the three groups of sea urchins that were fed with different diets (*L. digitata, P. palmata*, faeces) were compared.

There was no significant difference between the weight gain (final weight – initial weight) of the sea urchins fed with the three different diets (F= 0.65, p= 0.533).

The carbon and nitrogen content of each sea urchin's gonads was also measured, the average carbon and nitrogen content of each diet treatment group is illustrated in Figure 3-6: Average carbon and nitrogen content in the gonads of the sea urchins of each diet treatment group. 3-6.

There was no statistically significant difference between the means of the three diets for the nitrogen content in the sea urchin gonads (F= 3.23, p= 0.06). However, there was a significant difference between the carbon-content of the sea urchin gonads fed with the three different diets (F= 5.67, p= 0.041). A post-hoc test was performed to identify which of the pairs of treatments are significantly different from each other. The Tukey post-hoc

test shows that there is a significant difference (p=0.035) in the percentage of carbon in the sea urchin gonads between the sea urchins that were fed with *Laminaria* and the sea urchins fed with salmon faeces.

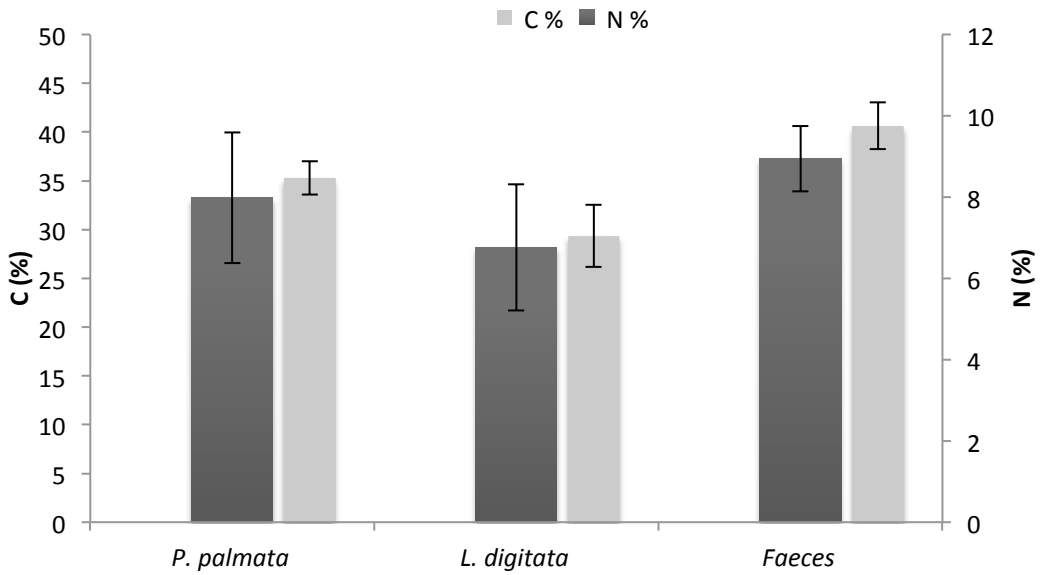


Figure 3-6: Average carbon and nitrogen content in the gonads of the sea urchins of each diet treatment group.

The carbon content of the sea urchin faeces that were collected on the 18th day of the experiment ranged between 9.19% (*P. palmata* diet treatment) and 11.93% (mixture of salmon faeces and pellets diet treatment) (Figure 3-7). The nitrogen content of the sea urchin faeces collected on the 18th day of the experiment ranged between 1.06% (mixture of salmon faeces and pellets diet treatment) and 1.3% (*L. digitata* diet treatment) (Figure 3-7). The nutrient content of the faeces collected on the last day of the experiment was higher than on the 18th day. The carbon content of the faeces collected on the last day of the experiment ranged between 23.29% (mixture of salmon faeces and pellets diet treatment) and 7.73% (*L. digitata* diet treatment) (Figure 3-8). The nitrogen content ranged between 1.7% (*L. digitata* diet treatment) and 3.9% (mixture of salmon faeces and pellets diet treatment) (Figure 3-8).

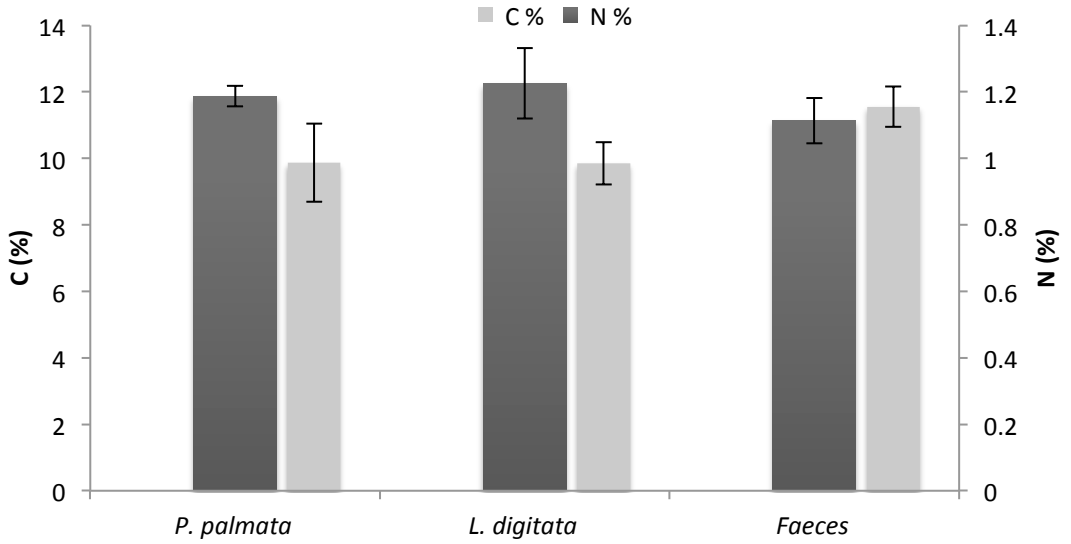


Figure 3-7: Average carbon and nitrogen content of the sea urchins faeces collected on the 18th day of the experiment for the sea urchins of each diet treatment group, the error bars represent the standard deviation.

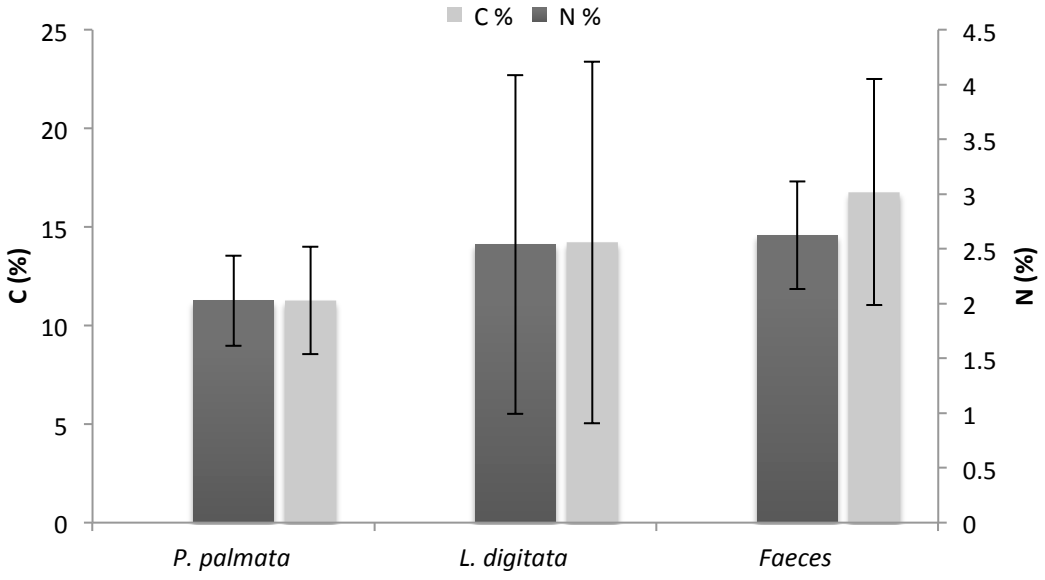


Figure 3-8: Average carbon and nitrogen content of the sea urchins faeces collected on the last day of the experiment for the sea urchins of each diet treatment group, the error bars represent the standard deviation.

There was no significant difference between the three diet groups in the average nitrogen content of the sea urchin faeces collected on the 18th ($F= 2.23$, $p= 0.189$) or on the last day of the experiment ($F= 4.23$, $p= 0.072$).

Similarly there was no significant difference between the three diet groups in the average carbon content of the sea urchin faeces collected on the 18th (F= 0.82, p= 0.484) or on the last day of the experiment (F= 0.04, p= 0.965). The sea urchin faeces had higher nutrient content during the second stage of faecal collection than they did at the first stage (Figure 3-9). By comparing the results of the two faecal collections, it is clear that at the second stage (end of experiment) the faeces collected from almost all the tanks had a higher concentration of both carbon and nitrogen than the faeces collected on the 18th day (Figure 3-9). T-tests were performed to check the significance of these differences. The T-test showed that the nitrogen content of the sea urchin faeces collected on the last day of the experiment from the aquaria of the diet group that was fed with *P. palmata* had significantly higher nitrogen content than the faeces that were collected from the same aquaria on the 18th day of the experiment (F=8.54, p=0.04). Similarly, for the diet group that was fed with the mixture of salmon feed and faeces (F=7.82, p=0.049).

All dietary treatments promoted gonadal growth, the sea urchins fed with faeces had a higher GSI index, but the difference was not significant (Figure 3-10).

The gonads of the sea urchins fed with the different diets varied in colour from light yellow to dark yellow/orange. Most of the sea urchins fed with the mixture of salmon faeces and pellets had gonads with an acceptable appearance for human consumption (Figure 3-11b).

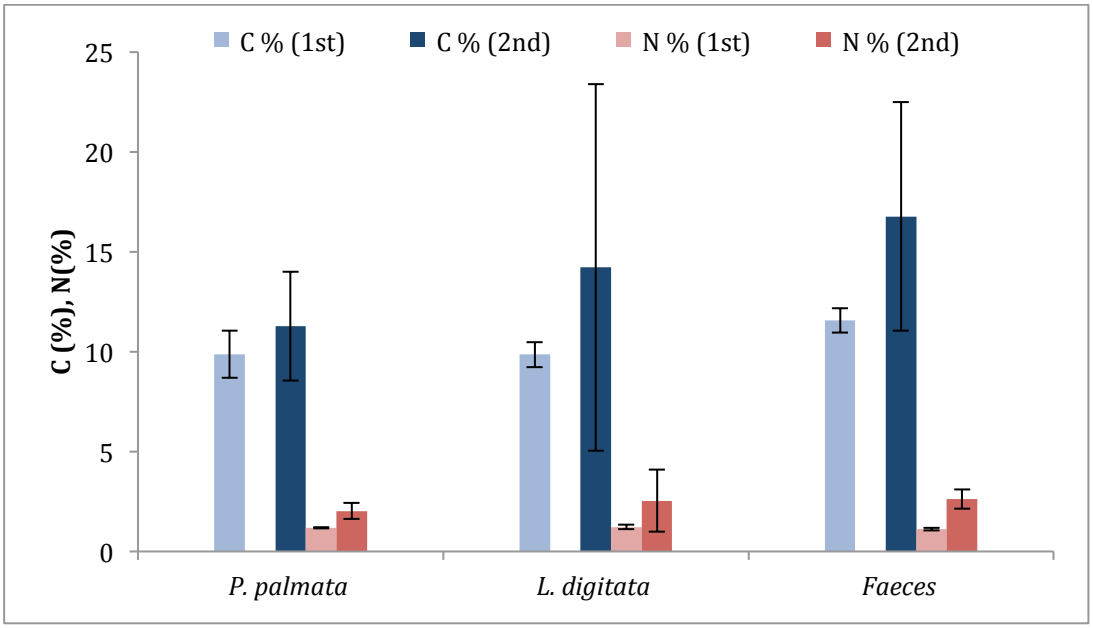


Figure 3-9: Comparative graph of the average carbon and nitrogen content, expressed as percentage of the total dry mass of 1mg samples of faeces, of sea urchin faeces of each treatment group at the two stages of faecal collection (18th and last day of the experiment), the error bars represent the standard deviation.

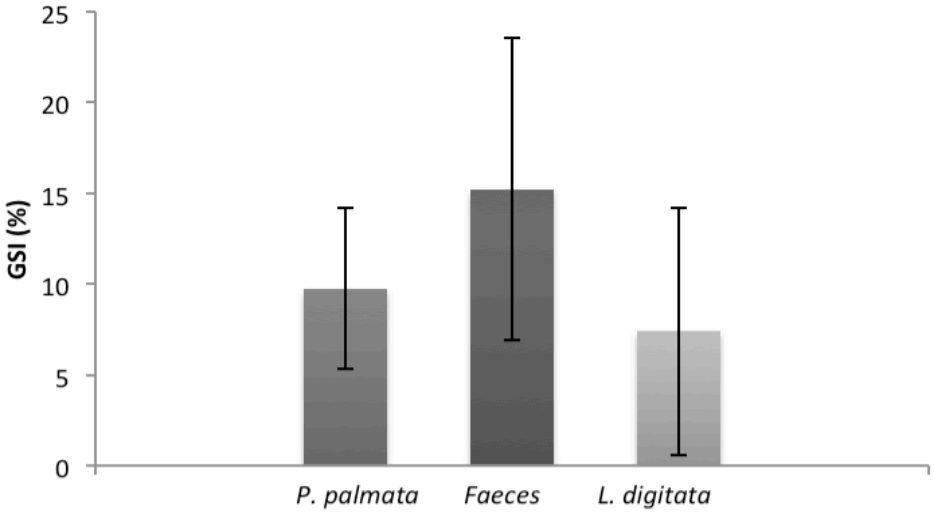


Figure 3-10: The average sea urchins GSI of each diet treatment group, presented as a percentage of body weight, the error bars represent the standard deviation.

(a)

(b)

(c)

Figure 3-11: Sea urchin gonad from a) the *L. digitata* diet group b) the mixture of salmon faeces and pellets diet group c) the *P. palmata* diet group.

3.3.3 Other observations

Sea urchin faeces were first observed on the third day of the experiment, and it was in one of the tanks fed with fish faeces. On the 16th day of the experiment, one sea urchin was found dead, possibly due to stress created from syphoning the water.

3.4 Discussion

It had been shown that *P. miliaris* fed with salmon feed could have enhanced gonadal and somatic growth rates (Cook et al. 1999). Kelly (2002) estimated that the growth rate of *P. miliaris* fed with salmon feed was 1.39 mm month^{-1} and that the growth rate of *P. miliaris* fed with macroalgae was 0.36 mm month^{-1}. This study investigated whether salmon faeces have a similar effect. The data illustrate a statistically non-significant effect of the diet type on sea urchin growth and GSI. However, there was a significant difference among the diets in the carbon content of the sea urchin gonads (higher carbon content in the gonads of the sea urchins that were fed with salmon faeces).

The gonads of the sea urchins fed with the mixture of salmon faeces and pellets had significantly higher concentration of carbon, reflecting the high carbon content of the salmon faeces diet. This indicates that the carbon available in the mixture of salmon faeces and feed pellets is in a form that can be easily assimilated.

The fact that at the second stage (end of experiment) of faeces collection, the faeces collected from almost all the tanks had a higher concentration of both carbon and nitrogen than the faeces collected on the 18th day indicates that all three diets had adequate amount of these two elements for the growth of the sea urchins.

An interesting result is the low nitrogen content of the faeces and gonads of the sea urchin that were fed with *P. palmata*, although the seaweed itself had double the amount of nitrogen than *L. digitata* and more than three times as much as the mixture of salmon faeces and pellets (Table 3-2). This indicates that the nitrogen in this seaweed species is not easily digested and assimilated by the sea urchins and thus *P. palmata* is unsuitable as feed for *P. miliaris*. The condition of the seaweed can also influence the uptake of nutrients by the sea urchin. *P. miliaris* graze on sublittoral beds of detached *S. latissima* and have a different response to fresh and rotting seaweed (Bedford and Moore, 1985). Fresh seaweed is not easily digested so gut

retention times are long, resulting in high protein absorption efficiencies. Conversely, when rotting seaweed is consumed, gut retention time is short so more food passes through the gut. The two feeding mechanisms, though, lead to similar growth rates for individuals fed with fresh and rotten seaweed (Kelly et al. 2013).

The conditions of the experiment were not optimal and the sea urchins were stressed as was indicated by their spontaneous spawning during the first day of the acclimation phase in seven out of nine tanks (Tanks 4 and 6 no spawning). However, this spawning is also within the time frame of their natural spawning period in the Firth of Clyde area, which is between June and August (Elmhirst, 1922). The first weight measurement was taken after the end of the acclimation and thus after the spawning incident. Thus the observed weight loss of some individuals cannot be attributed to weight loss due to spawning. Consequently, due to the sub–optimal conditions the experiment was terminated and there were no follow–up experiments. Sea urchins require very good water recirculation and the removal of water by siphon is stressing them. Also, the sea urchins are stressed when they are taken out of the water, so a scale measuring their weight while they are in the water would be required for minimising stress. Due to infrastructural limitations, the length of this experiment was very short. It is probable that differences among the diet groups would be more evident at an experiment with longer duration.

CHAPTER 4

Preliminary investigation of the consumption and remediation of Atlantic salmon waste by the lugworm *Arenicola marina* and comparison with a fresh seaweed diet

4.1 Introduction

The polychaete *Arenicola marina,* the common lugworm, is an important member of intertidal zone sedimentary communities in northwestern Europe. It is found at European Atlantic coasts from the Mediterranean to the Arctic (Riisgård and Banta, 1998). They are so abundant that they can constitute 30% of the biomass of a sandy beach (Howie, 1984).

A. marina is an iteroparous animal, breeding several times per lifetime but at annual intervals (Clark and Olive, 1973). When fully grown, the lugworm of the coasts of Europe is up to 23 cm long and 1 cm in diameter. They are typically found in sandy sediments in densities of up to 100 individuals m^{-2} (Beukema and de Vlas, 1979). Lugworms, like all animals inhabiting the intertidal zone, have to survive in constantly changing physical conditions and thus present very high environmental tolerances. They feed by ingesting sediment at the end of their 20–25 cm deep, J-shaped burrows and ingested particles are transported to the sediment surface, where they are deposited as faecal mounds (see Flach, 1992). The feeding method is a continuous cycle of ingestion, upwards transport, defecation and burial of particles (Kristensen et al., 2012). This feeding behaviour has a greater ecosystem effect. It impacts the distribution and composition of fauna and macrophytes and modulates important ecosystem processes (Flach, 1992; Volkenborn and Reise, 2006). The ecosystem effect of lugworm feeding depends upon worm size, density, food availability and temperature (Schröer et al., 2009; Valdemarsen et al., 2011); the bioturbation rate may

range from 100 to 600 cm^3 m^{-2} day^{-1} (Riisgård and Banta, 1998). Apart from their important ecosystem effect that results from their feeding behaviour; they are also an important link in many food chains, including those containing commercial fish.

Lugworms can be easily harvested from the intertidal zone. However, digging the intertidal zone for lugworm collection has a negative environmental impact and after digging for lugworm collection the local population needs at least a month to reach their initial density (McLusky, 1983). Lugworm aquaculture could be a viable solution for their high demand. In the United Kingdom, lugworms are cultivated primarily as bait for sea angling. However, cultured lugworms are currently also used as an ingredient of aquaculture feeds, particularly for shrimp and finfish brood stocks (Greenpeace, 2015) and could potentially be used as an ingredient for fish feed for cod, trout and cobia (Wilding et al. 2006). Additionally, their haemoglobin is a potential substitute for human red cells (Zal et al. 2002).

Polychaetes have been suggested as a suitable organic extractive component for IMTA systems. It has been shown that they can contribute to sediment bioremediation (Tsutsumi et al. 2005). Tsutsumi and Montani (1993) initiated a mass culture of the deposit-feeding polychaete *Capitella* sp. directly under sea cages. The polychaete population reached very high densities within three months, which led to considerable decrease of the organic content of the sediment surface under the sea cages. Ragworms (*Nereis virens*) were used at a pilot IMTA project in the Netherlands ('Sealand Sole'). This land-based IMTA systems also includes sole (*Solea solea*), algae and shellfish. The ragworms provide a live food source for the fish as well as being harvested for use as an ingredient for aquaculture feeds (Ketelaars 2007). Ragworms are favoured for aquaculture because they are fast growing compared to other polychaetes such as lugworms, but they are carnivorous in contrast to lugworms that are deposit/detritus feeders and thus are suitable as organic extractive components for IMTA better. Lugworms showed high potential at an investigative study of the potential of IMTA in Galicia (Regional Government of Galicia, 2012). The

aquaculture techniques required for the growth of lugworms are well understood. Olive et al. (2006) published a patent for the aquaculture of *A. marina* using fish–farm waste as foodstuff, using this aquaculture method a 0.5 g lugworm can grow to 5-6 g in 90-120 days.

The trial objectives of this study are:

- To investigate the ability of *A. marina* to consume salmon waste.
- To investigate whether the lugworms can survive with salmon waste as the only food source.
- To compare the growth rate and element content of the lugworms fed with the different diets.
- To investigate whether the lugworms had a bioremediation effect, by comparing the element content of the different diets, to the element content of the lugworm body tissues and faeces.

The overall aim of this study was to assess the suitability of the lugworms as an IMTA organic extractive component. The selection of suitable species is the first step in the design of an IMTA system. Lugworms are used in the comparative modelling study in chapter 6.

4.2 Materials and Methods

4.2.1 Experimental system and trial protocol

The two-week feeding trial was carried out at a laboratory at the University of Stirling. On the 24th of May 2013, 21 *A. marina* individuals were collected by hand at Torryburn, Fife, Scotland (56°3′25.05″N 3°34′14.88″W) from the intertidal zone during low tide. They were placed in plastic containers together with sediment and transported to Stirling University. The lugworms were transferred to the laboratory and placed into a tank that was filled with 5 cm of sediment (as in Olive et al. 2006) collected from the intertidal zone at the same location were the specimens were collected and 5 cm of sea water that was also collected from that site. The lugworms were left in that tank for six days to acclimatise in the temperature and photoperiod that the experiment was carried out.

The mortality was high (40% mortality rate) during the acclimation period, due to injury of the animals at the collection process. The 12 individuals that survived after the acclimation period were distributed randomly over eight 24 L - tanks, with some tanks containing one and some two individuals. The dimensions of the tanks were 48 x 33 x 25 cm. Each tank was filled with 5 cm sediment collected from the same location as the lugworms and there was a 5 cm layer of artificial seawater at 35 ppt above the sediment. The sediment collected from the site was thoroughly mixed (by hand) before being distributed in the eight tanks. For the duration of the experiment the pH and salinity of each tank were measured every other day. During the course of the experiment water salinity was between 32 and 36 ppt and pH was between 7.4 and 8.1. Each tank was well aerated using an air stone and held under an artificial 12:12 L/D light regime. The water temperature was not regulated, daily water temperature measurements showed a water temperature variation from 12 to 20°C.

The lugworms were fed with the following three different diets:

Diet 1: A mixture of feed pellets and salmon faeces, sourced from a salmon recirculating aquaculture system (RAS) water and frozen.

Diet 2: Fresh seaweed of the species *Laminaria digitata.*

Diet 3: No additional feed was given, the lugworms fed on the organic matter that was in the sediment.

Four lugworms were fed with each diet treatment. The lugworms were fed with 2.5 g ww of salmon faeces or seaweed per individual every 5 days. No protocol was found on feed requirements for *A. marina* so it was decided to provide the lugworms with a large amount of feed (50% of average body weight, every 5 days).

On the last day of the experiment, two 5 g sediment samples and two 5 g faecal samples were collected from each tank and placed in a drying oven for 24h at 60°C, to be prepared for physiochemical analysis. Following that all lugworms were weighed and measured and then placed in the drying

oven in order to be prepared for physiochemical analysis. The body lengths were measured to the nearest 1 mm at the beginning (after the acclimation phase) and end of the experimental period. The lengths refer to specimens in a moderate degree of contraction, a condition that they usually adopt a few seconds after being handled. The weight was also measured to the nearest milligram, at the beginning (after the acclimation phase) and end of the experimental period, using a precision digital scale. Prior to weighing, the lugworms were submerged in clean seawater and then left on paper to dry the excess water, in order to remove the sediment that was fixed on their bodies.

4.2.2 CHN analysis and mineral element analysis protocol for phosphorus

The carbon, nitrogen and hydrogen content of the lugworm faeces, body tissues and of their diet content were estimated, at the end of the experiment, using the CHN analyser. The CHN analyser determined the elemental composition of 1 mg dried and crushed samples of tissue of the whole lugworm.

The total Phosphorus of the samples was analysed using the Thermo-Electric ICP-MS. Background correction was achieved using a phosphorus standard (from the chemical supplier BDH), following microwave digestion. In detail, the Mineral Element Analysis Protocol for the phosphorus was as follows. Three 0.1 g replicates of each dried sample were placed in Kjeldahl tubes and were digested with 5 ml nitric acid in a high-pressure Teflon® lined digestion vessel using microwave heating and a feedback program to control temperature and pressure. Analytical solutions were nebulized and the aerosol transported to a plasma where desolvation and excitation occur. A pneumatic nebulization sample introduction was used. Characteristic atomic emission spectra are produced by radio frequency inductively coupled plasma. Spectra are dispersed by a grating spectrometer, and line intensities are measured with a light sensitive detector such as a photomultiplier tube or a charge transfer device and the photocurrents are processed by a computer system. A background correction technique was

used to compensate for variable background emission contribution. The microwave digestion consisted of the following steps. Step 1: from 21 to 190˚C in 10 minutes at 800 W. Step 2: 190˚C for 20 minutes at 800 W. Step 3: from 190 to 21˚C for 30 minutes as a cool–off period. The Kjeldahl tubes were then opened under the laboratory exhaust and their content was emptied in 10 ml volumetric tube that was then filled up to 10 ml with distilled water. From each of the volumetric tubes a 0.4 ml sample was taken and stored in the fridge until further analysis.

The following equation was used for calculating the phosphorus content of the samples using the ICP values obtained from the Mineral Element Analysis for phosphorus:

$$\frac{\mu g\ Phosphorus}{mg\ of\ sample} = \frac{\frac{Sample\ volume}{1000} * ICP\ Value}{Sample\ weight}$$

4.3 Results

4.3.1 Lugworm diet elemental composition

The results of the elemental analysis of the three diet types show that the mixture of salmon faeces and fish pellets had a much higher concentration of phosphorus than *L. digitata,* while *L. digitata* has a higher concentration of carbon and nitrogen than the mixture of salmon faeces and fish pellets (see section 2.5.1.1).

4.3.2 Lugworm growth

The initial length of the lugworms ranged from 5 to 13.5 cm and their weight from 1.98 to 5.8 g. In each tank the average weight ranged from 4.2 to 4.6 g. The growth rate in terms of weight after the 14 days of the experiment was 7.7% for the lugworms that were given no additional feed, 32.2% for those that were fed with salmon faeces and was negative (-13.3%) for those that were fed with seaweed. As it is clear from Figure 4-1 and Figure 4-2 there was a large variation in the growth among individuals of the same diet group. There was no statistically significant difference in the average growth (in length) of the lugworms among the three diet groups (Figure 4-1). Similar

results were obtained for the change in weight, the average weight of the lugworms fed with faeces and those that were provided no feed increased while the average weight of the seaweed diet group decreased (Figure 4-2). In detail, the weight of only five lugworms increased, two of which belonged to the no feed group and the other three in the faeces diet group.

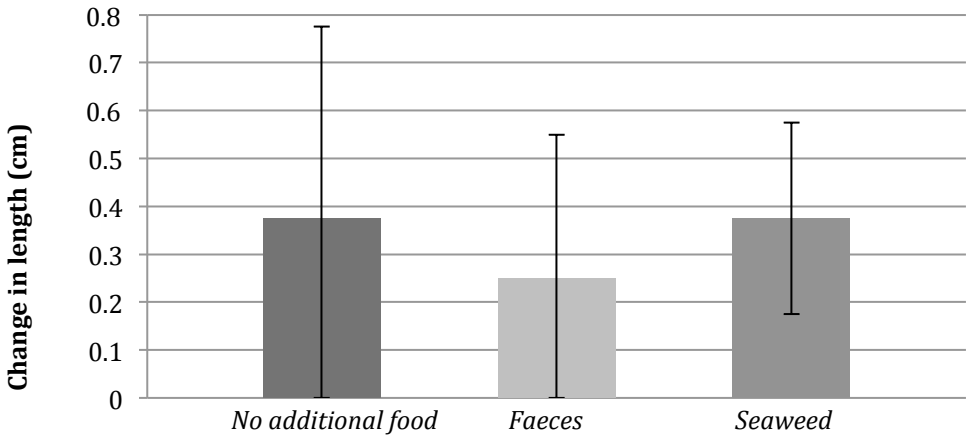


Figure 4-1: Average change in length (final length – initial length) of the lugworms given the three different diets.

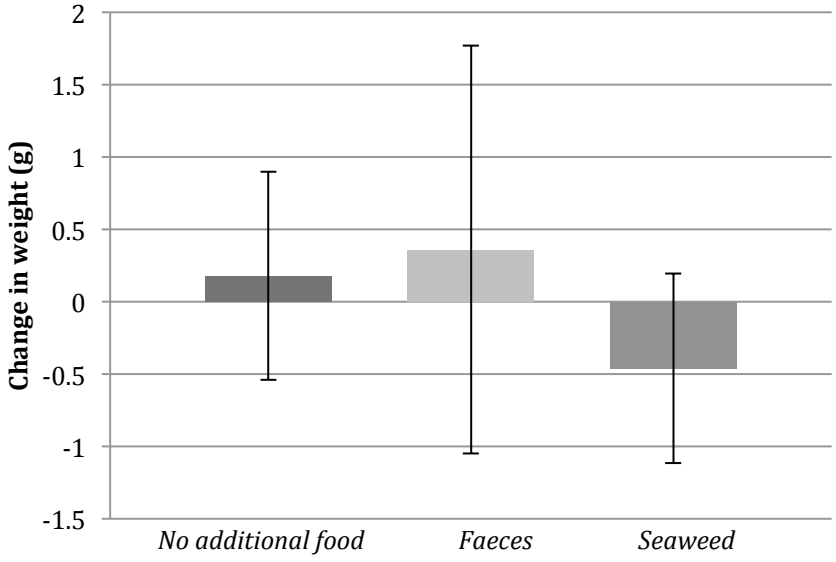


Figure 4-2: Average change in weight (final weight – initial weight) of the lugworms given the three different diets.

The proportional change in weight and length were also examined to investigate any influence of the body size on the growth results (Figure 4-3

and Figure 4-4). There was no statistically significant difference in the percentage growth of the lugworms among the three diet groups (Figure 4-3 and Figure 4-4).

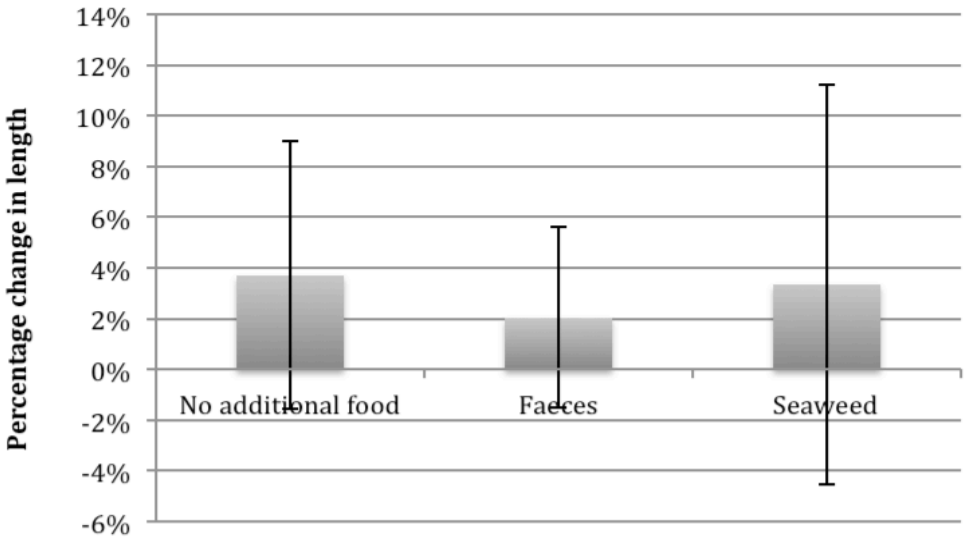


Figure 4-3: Percentage change in length of the lugworms given the three different diets.

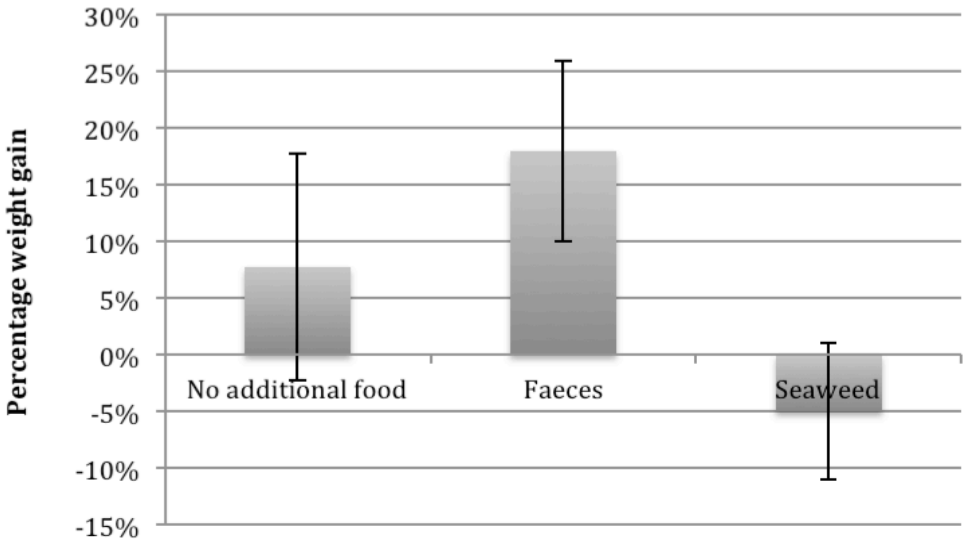


Figure 4-4: Percentage change in weight of the lugworms given the three different diets.

There was no statistically significant difference between the average tissue carbon and nitrogen content of the three diet groups, but there was a difference for the phosphorous ($F= 309.6$, $p< 0.0001$) (Figure 4-5). The post-hoc Tukey test showed that the phosphorous content of the lugworms fed

with the mixture of salmon faeces and pellets was significantly higher than in the lugworms of the other two groups but there was no significant difference between the other two diet groups (Figure 4-5).

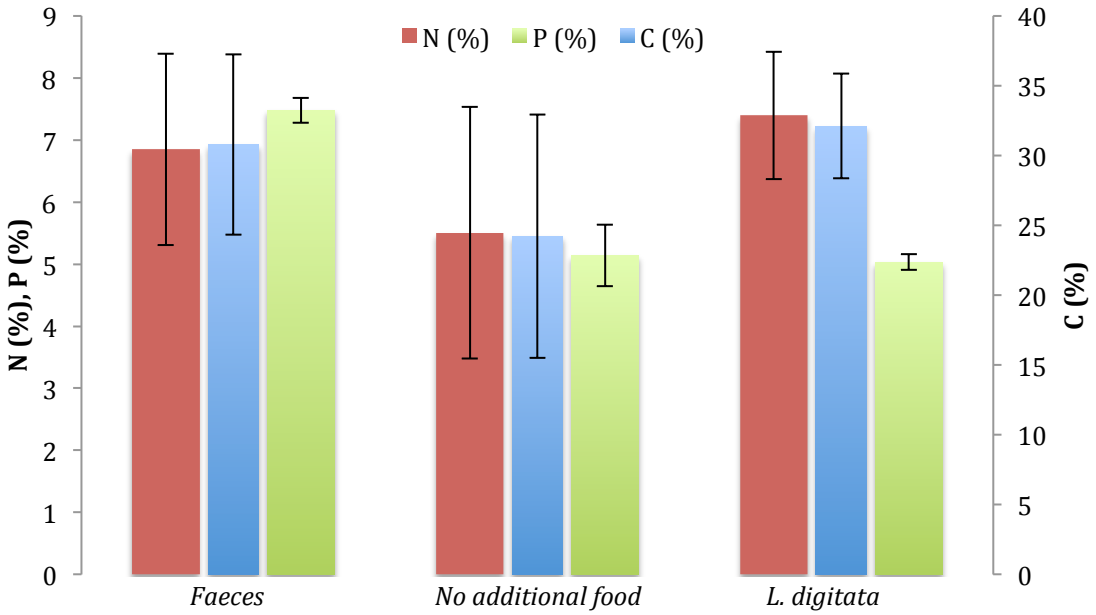


Figure 4-5: The average carbon, nitrogen, phosphorus and hydrogen content of the lugworm body tissues of each diet treatment group.

From Figure 4-6, it is clear that the lugworm faeces with higher nitrogen content were from lugworms that were fed with seaweed. This difference was shown to be significant (F=5.67, p= 0.025). That is in accordance with the high nitrogen content of the sediment in the tanks with the seaweed diet (Figure 4-7) and the high tissue nutrient content of *L. digitata* (Chapter 3, Table 3-2).

A large variation was observed in the sediment samples from each tank (Figure 4-7), there was no significant difference in the amount of phosphorous (F= 0.77, p= 0.511) and carbon (F= 5.12, p= 0.062) but there was a significant difference in the amount of nitrogen in the sediment samples of the different treatments (F= 17.21, p = 0.003).

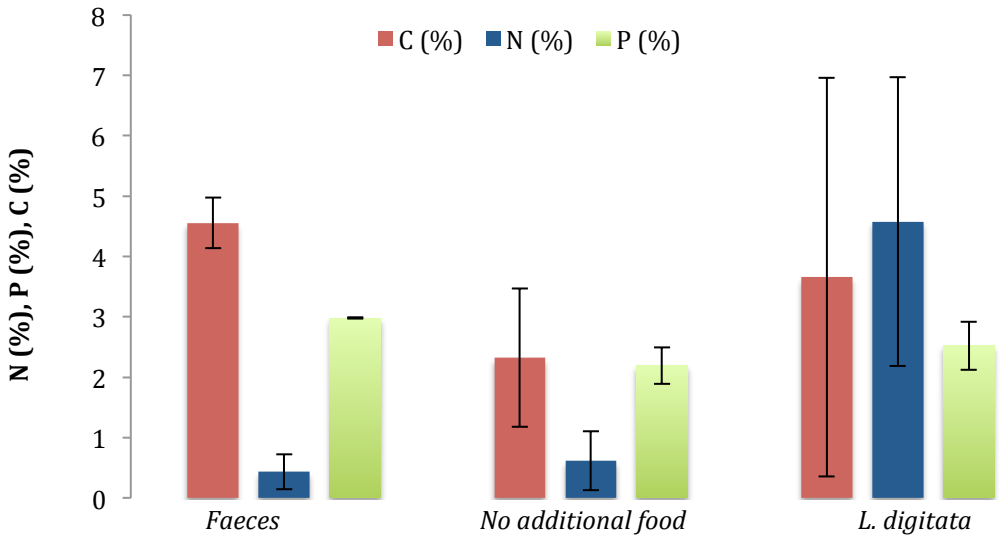


Figure 4-6: The carbon, nitrogen, phosphorus and hydrogen content of the lugworm faeces collected from each tank.

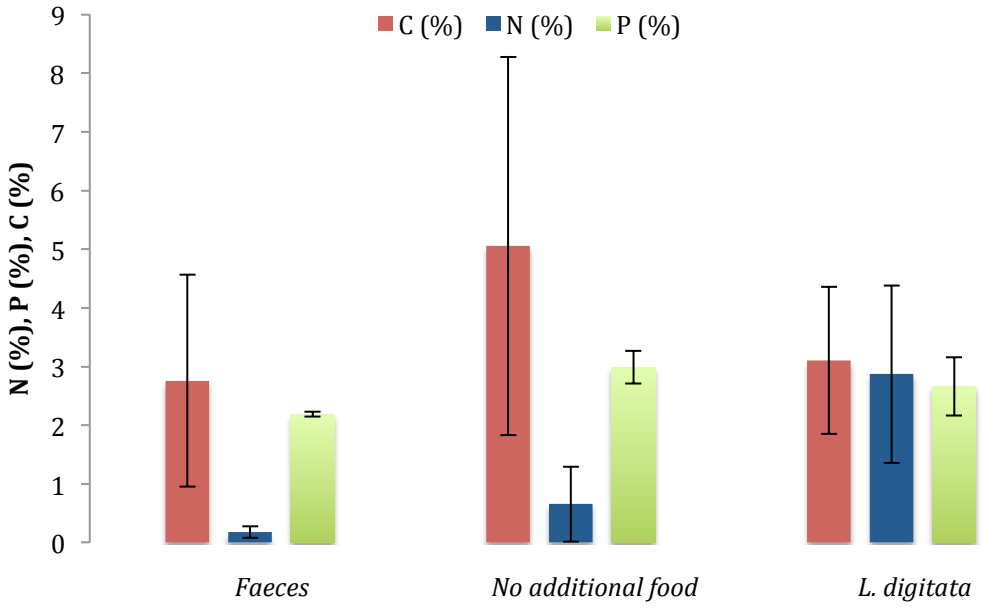


Figure 4-7: The average carbon, nitrogen, phosphorus and hydrogen content in the sediment the tank of each treatment group.

An interesting observation was that at night the lugworms ascended to the surface of sediment and were seen swimming in the water.

4.4 Discussion

The results indicate that the nutrients in salmon faeces are in a form suitable for uptake by the *Arenicola*. The nutrient content of the *Arenicola* body tissues is representative of the diet group they belong in. This is evident by the statistically significant higher phosphorous content in the tissues of the lugworms fed with the mixture of salmon faeces and pellets than in the lugworms of the other two diet groups, as well as by the higher nitrogen and carbon content in the tissues of the diet group that was fed with *L. digitata*.

The very low level of nitrogen (0.4 % of the faeces dry weight) in the faeces of the diet group that was fed with the mixture of salmon faeces and pellets indicates that the amount of nitrogen available in the mixture of salmon faeces and pellets might potentially limit *Arenicola* growth. This is also suggested by the very low amount of nitrogen content available in the sediment samples of the salmon faeces diet group, which indicates that the *Arenicola* extracted most of the nitrogen that was available in their feed.

The *Arenicola* fed with the mixture of salmon faeces and pellets presented on average the highest growth rate (7.7% for the 14 days of the experiment) in comparison to the other two diet groups, although the difference was not statistically significant. At an experiment studying the suitability of *Arenicola* as an IMTA component using substrate contained 25% mud from an aquaculture farm and 75% sand, the lugworms obtained 106% average growth after 39 days (Regional Government of Galicia, 2012). In comparison, the growth rate for this study after 39 days would have been only 23%. The low growth rate as well as the high mortality presented during the acclimation phase could be attributed to high water temperature as well as to damage during the collection process. Wilde and Berghuis (1979) concluded that at 20 ^{o}C (which was also the higher temperature reached in the present study) the mortality could reach up to 50%. High mortality rate has been suggested to be the disadvantage of lugworm aquaculture (Regional Government of Galicia, 2012).

The weight loss presented in the diet groups fed with seaweed was consistent; three out of four individuals of the seaweed diet group presented

a decrease in weight. This suggests that seaweed pieces sinking from the seaweed culture area of an IMTA to the grow-out area of the *Arenicola* is not going to be consumed, at least not before the seaweed is decomposed.

There were two major difficulties in quantifying lugworm growth during these experiments. Firstly, it is difficult to measure their length precisely without injuring them due to their body type. During the measurements the lugworms were stretched softly. A solution to this problem could be measuring the length using image analysis of digital pictures. These problems contribute to the results presented in Figure 4-1 and in Figure 4-2. Measuring the length of the body without the tail would have given a more reliable index of size than does total length of the body, since the tail (posterior achaetous region of the body) varies greatly in its proportion to the rest of the body, being frequently shortened by damage and often almost absent (Newell, 1948).

The swimming behaviour observed during the experiment could because *Arenicola* migrate by swimming. The swimming migration of lugworms occurs in May (Ladle et al. 2015), this experiment was performed at the end of May. It is interesting this behaviour occurred although the worms were in captivity.

This study showed that *Arenicola* could be grown successfully on a diet composed of salmon food and faeces. Polychaetes are robust and widespread, a fact that makes them a potentially suitable class to grow under the fish cages (Serebiah et al. 2015).

CHAPTER 5

A model for optimization of the productivity and bioremediation efficiency of marine Integrated Multitrophic Aquaculture

5.1 Introduction

The constantly increasing demand for seafood, during a period of overexploitation of the fisheries sector, can only be met by sustainable growth of aquaculture. This growth is limited by the environmental impacts and economic requirements of intensive monoculture of fed species. Moreover, rapid and uncontrolled expansion of the aquaculture sector challenges the realization of an Ecosystem Approach to Aquaculture (Soto et al., 2008). It has been proposed that expansion of marine aquaculture in parallel with environmental protection can be achieved using Integrated MultiTrophic Aquaculture systems (IMTA) (Chopin et al., 2001; Neori et al., 2004). IMTA has the potential to be an economically viable solution to the problems of dissolved and particulate nutrient enrichment, since the waste from fed species aquaculture is exploited as a food source by extractive organisms of lower trophic levels giving added value to the investment in feed by producing a low input protein source as well as increasing the farm income. In order to promote more resilient growth of the Scottish aquaculture industry, a draft Seaweed Policy Statement that examines the cultivation of seaweed as part of IMTA systems was introduced in 2013 (Marine Scotland, 2013). Large-scale seaweed cultivation has been suggested as a means to mitigate the nutrient enrichment environmental impact of marine fish farms (Abreu et al., 2009; Wang et al., 2013). As a very large area is required for the cultivation of sufficient seaweed biomass for complete nutrient bioremediation, doubt remains as to whether complete bioremediation by seaweed cultivation is practically feasible (Broch and Slagstad, 2012). However, there is a general agreement that cultivation of seaweed as part of an IMTA is a promising way for partial removal of

dissolved fish farm effluent (Broch et al., 2013; Jiang et al., 2010; Reid et al., 2013; Wang et al., 2013). Similarly, sea urchins can consume sea cage effluent (Kelly et al., 1998; Schuenhoff et al., 2003) and it has been shown that *Paracentrotus lividus* can assimilate fish farm waste and can achieve high growth and survival rates near salmon cages (Cook and Kelly, 2007b).

IMTA systems design needs to encompass the characteristics of both the site and the selected organism, and optimizing synergies requires advanced understanding of the system at a specific site. A major factor restricting the efforts to optimize open water IMTA is the lack of knowledge on how IMTA systems operate, coupled with the lack of data from large–scale extractive cultures and thus comes the need to extrapolate results from small-scale studies (Troell et al., 2003). Due to limited knowledge of IMTA system properties, the placement of the extractive organisms is often driven by availability of space as opposed to nutrient uptake maximization (Hughes and Kelly, 2001).

Lack of knowledge or inaccurate IMTA design might impact the health and growth of the finfish or the surrounding environment or the extractive organism flesh might be of inferior quality. For example, the use of organic extractive organisms can lead to additional nitrification of the water column, because most of the organic material ingested by the organic extractive organisms returns to the water column as nutrients (Nizzolli et al., 2005) and pseudofaeces produced by filter feeders may collect on the sediment impacting benthic communities. Also, the extractive cultures may interfere with the water movement, changing the particle dispersal patterns and reducing the water flow through the sea cages. Farming different species within the same system can increase the exposure to pathogens; mussels for instance bioaccumulate and shed harmful bacteria (Pietrak et al., 2012). Other limitations of open water IMTA include the need for high stocking densities and the need for deployment of the organic extractive organisms lower in water column near the primary source of particulate waste.

The maximum production of an organic extractive species crop is limited by food availability (e.g. Grant and Filgueira, 2011). Increasing crop biomass

beyond this carrying capacity causes food depletion and thus crop production cannot be maximized (Cranford et al., 2013). There needs to be a balance between waste production and uptake, where the waste is sufficient to feed the extractive organisms and concurrently as much of the waste as possible is removed from the ecosystem. An efficient IMTA farm allows the profitable use of each of the culture modules with minimum waste (Neori et al., 2004). In order to achieve this the standing stocks of all the cultured organisms have to be maintained, considering nutrient requirements of each and the rates of excretion and uptake of the important solutes by each of them (Granada et al., 2015).

From a biological point of view, the choice of extractive species in an IMTA system is crucial because their physiological and ecological attributes determine the rate of particle or nutrient consumption and assimilation, their growth rate and capabilities in terms of particle or nutrient removal. Species are chosen based on specific culture performance traits, for which quantitative information needs to be available, with respect to nutrient uptake efficiency and secondary considerations (e.g. yield and protein content). The marketability of the extractive species is largely dependent on the location, with the Western world showing less demand for food species that are low in the trophic chain. Nevertheless, dried seaweed products can always be exported and seaweeds can be processed to produce cosmetics, fertilizers, animal feed, biogas and others.

The environmental benefits, matter and energy flux within an IMTA farm, as well as between the environment and the IMTA system, need to be qualified and quantified prior to the establishment of a marine IMTA system. The aim of this study was to provide a tool for designing IMTA farms at any site by creating a modelling tool that can be used to fine-tune IMTA designs for maximising yields and nutrient removal.

Without a thorough understanding of the dynamics of the system, the environmental and economical benefits of IMTA cannot be achieved. However, field measurements of nutrient and Particulate Organic Matter (POM) concentrations in open-water systems are challenging due to the

highly diluting, dynamic nature of openwater systems, presenting high spatial and temporal variation both diurnally and seasonally. The model described in this study determines the temporal availability of nutrients and POM released by the different IMTA components and thus the amount available for uptake by different groups of extractive organisms. Because of the site specificity of waste distribution, this model focuses on simulation of a virtually closed system, within which the nitrogen is homogenously distributed. The species used in this study are Atlantic salmon (*Salmon salar*), a sea urchin (*P. lividus*) and sea lettuce (*Ulva lactuca*), though it will be possible to re-parameterise the model for a range of different species.

5.2 Model development

The model was implemented using the visual simulation package Powersim™ Constructor Studio 8 (Powersim Software AS, Bergen). An 18-month period time horizon was used, to simulate the at-sea phase of salmon production cycle, which lasts between 14 and 24 months (Marine Harvest, 2012). The model is typically operated with a one-day time step and the model's differential equations are solved using a third order Runge-Kutta integration method. The selected time-step reflects accurately the time–dependent environmental changes (accurate integration) with low computing effort.

An extensive literature review was carried out for model parameterization for *Ulva* (Table 5-1) and for *Paracentrotus lividus* (Add_my_pet, 2014), while the model for *Salmo salar* was parameterized using data acquired from commercial Scottish salmon farms. For the parameters where a range of values was available in the literature, the most representative value was used. It is evident that the inclusion of many proxy variables from the literature propagates uncertainties through the model, affecting the overall model accuracy. Since the model is deterministic, its output is entirely determined by the input parameters and structure of the model. Due to the high structural complexity of the model and high degree of uncertainty in estimating the values of many input parameters, a detailed sensitivity analysis was performed by varying each input parameter by ± 10% and

quantifying the effect on eight output variables (Table 5-2). The selected output variables reflect the objectives of the research with respect to nitrogen bioremediation and yield productivity. Within the sensitivity analysis all model parameters and initial values of state variables (50 input variables) were varied in order to determine the response of the following eight effect variables: harvested seaweed, salmon and sea urchin biomass; nitrogen accumulated by seaweed, salmon and sea urchins; DIN and PON available at the IMTA site at the end of the simulation. The sensitivity analysis results are presented as a normalized sensitivity coefficient (NS) (Fasham et al. 1990):

$$NS = \frac{DV/V_b}{DP/P_b} \quad (1)$$

where, DV = (V_b– V) is the change of a response variable, V_b is the value of a response variable for the base run, V is the value of a response variable for the sensitivity analysis run, DP = (P_b– P) is the change in a model parameter, P_b is the baseline value of a model parameter and P is the value of a model parameter for the sensitivity analysis run.

When the value of NS for a parameter +10% is negative then there is a negative correlation between parameter and effect. When it is negative for a parameter -10% then there is a positive correlation between parameter and effect.

5.3 Model outline

The model determines the nutrient recovery efficiency and biomass production of IMTA based on a baseline simulation; components of the model can be altered or removed for the simulation of particular scenarios. Following re-parameterization, the model can simulate IMTA systems consisting of different combinations of finfish, sea urchin (or other grazing invertebrate) or seaweed species. The present model incorporates a multispecies model consisting of three submodels that interact with each other and with their surrounding environment via nutrient cycling (Figure 5-

1). The submodels consist of growth models for *Salmo salar*, *Ulva* sp. and *Paracentrotus lividus* that interact with each other through modelled nitrogen release and subsequent assimilation (Figure 5-1). A snapshot of the model as seen at the modelling software Powersim can be seen in Appendix Figures 5-1 to 5-4.

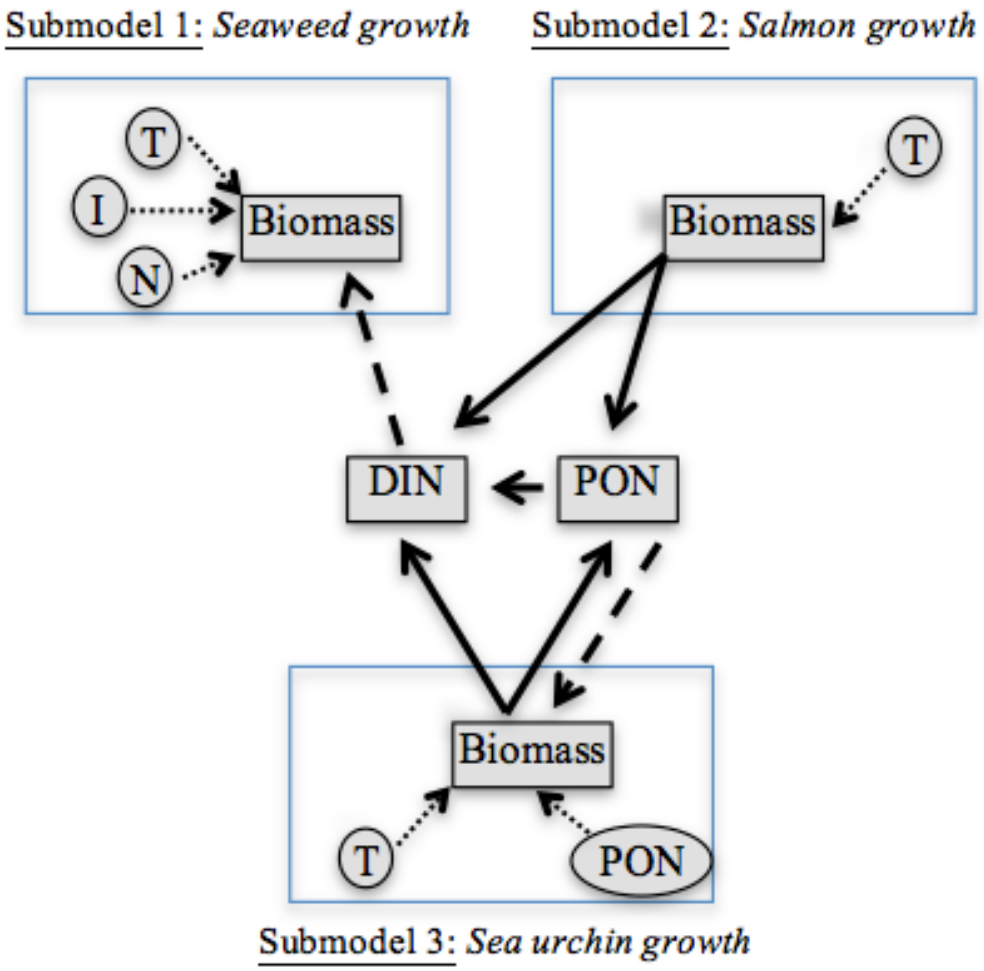


Figure 5-1: Conceptual diagram of the model showing the major state variables (squares) and forcing functions (circles) of each submodel as well as the interactions among the submodels. The dashed lines represent nitrogen assimilation and the solid lines nitrogen release, respectively.

Salmon growth was modelled using the Thermal-unit Growth Coefficient (TGC) (Iwama and Tautz, 1981), the seaweed growth model is based on Droop's model for nutrient-limited algal growth (Droop, 1968) and sea urchin growth was modeled using the Dynamic Energy Budget (DEB) theory (Kooijman 1986).

The TGC is a simple model widely used in aquaculture, based on three basic assumptions, which may be violated under certain conditions (Jobling, 2003). The TGC can present errors when the temperature deviates far from the optimum for growth (Jobling, 2003), but this is not a setback given the temperature range used in the present simulations. For the organic extractive organisms a bioenergetic model was used in order to link the environmental variables, mainly food availability and temperature, with feed intake, growth, excretion and faeces production. For the simulation of salmon growth and nutrient uptake and release, the TGC was preferred to a bioenergetic model because under intensive aquaculture conditions feed is not limiting growth. Furthermore, salmon is well-studied and daily time series data for the TGC and food conversion ratio (FCR) as well as sources of data for excretions and faeces production were available in the literature. Finally, as salmon are grown at sea for only for a part of their production, data are not required for the full life cycle, which is the strength of the DEB approach.

The model includes daily time steps for better understanding of the process affecting the IMTA productivity and nutrient removal efficiency. Due to the dynamic design of the model the bioremediation potential of different production scenarios can be estimated by altering various production parameters of the baseline simulation. These include site-specific environmental conditions (temperature, irradiance and ambient nutrient concentration) and production practices (seaweed harvesting frequency, seaweed culture depth, nitrogen content of feed, initial stocking biomass of extractive organisms etc.). The maximum seaweed and sea urchin biomass that can be sustained at any given time can also be estimated based on the daily amount of nitrogen within the IMTA system that is available for uptake.

The complete model is used to determine the overall ability of the IMTA system to reduce the nutrient and POM waste of fedspecies taking into account the quantity of nutrients and POM that are released and the quantity that could be potentially absorbed/consumed by the extractive organisms if all the waste remained within the virtually closed system. The

only nitrogenous input to the seaweed and sea urchin submodels is the daily waste released to the sea from the salmon submodel. This is used to calculate the amount of particulate (suspended) and dissolved nitrogen released from the salmon farm for a given fish production over 18 months, as well as the potential for decreasing the nutrient released by converting salmon monocultures into IMTA systems. The model considers fish growth and consequent feed input and waste release, and the uptake and release of DIN and PON by the different IMTA components. The growth models are combined with nutrient transfer/cycling and this way the virtually closed system bioremediation efficiency is estimated (Figure 5-1).

5.3.1 Salmon growth submodel

The growth rate of fish fluctuates throughout an individual's life cycle and is mainly influenced by feed availability, temperature and photoperiod (Austreng et al. 1987). Salmon growth was simulated using a thermal growth coefficient (Iwama and Tautz, 1981):

$$TGC = 1000\ \frac{\sqrt[3]{W_t} - \sqrt[3]{W_0}}{T*t} \tag{2}$$

where, W_0 is the smolts initial wet weight, W_t is the fish's wet weight at time t, T is the temperature in °C and t is time in degr ee-days.

Solving for W_t we obtain:

$$W_t = \left[\sqrt[3]{W_0} + \frac{TGC*T*t}{1000}\right]^3 \tag{3}$$

The total salmon biomass was calculated as individual weight multiplied by the number of individuals. The model also accounted for natural mortality, modeled as a time series variable since mortality decreases with fish size, using empirical data from Scottish salmon farms.

The amount of waste released from the salmon farm in the form of excretion, faeces production and feed loss was assumed to be as calculated by Wang et al (2012) for Norwegian salmon farms, with the exception that the feed nitrogen content was set to be 5.76% of the feed weight, since to

date crude protein content is around 36% (Skretting, 2015). We assume that every day of the simulation 2% of feed nitrogen is released in the environment as feed loss, 45% as dissolved excretions and 15% as faeces, while the remaining 38% is assimilated into salmon biomass and removed from the IMTA area when the fish are harvested.

5.3.2 Seaweed growth and nitrogen uptake

Seaweed biomass *(B)* increases with a varying growth rate and decreases due to natural causes and periodic harvesting. The basic processes affecting seaweed biomass form the differential equation 4:

$$\frac{dB}{dt} = (\mu - \Omega) * B - (D + H) * B \quad (4)$$

where, μ is the specific growth rate, Ω the specific decomposition rate, D the loss rate due to environmental disturbance and H the harvesting rate. Biomass is calculated as wet biomass, for the conversion of seaweed wet to dry weight an 8.43 to 1 ratio was used (Angell et al. 2012; Neori et al. 1991). At the baseline simulation, due to lack of data in the literature for the specific decomposition rate and the loss due to environmental disturbance for *Ulva* sp. the term mortality (M) is used, where M= Ω + D. The specific decomposition rate (Ω), was set to be equal to the loss rate due to environmental disturbance (D) (Table 5- 1).

The gross growth rate was defined as a function of water temperature, availability of Photosynthetic Active Radiation (PAR) and nutrient concentration in the water column and in the plant tissues. The joint dependence of growth on environmental variables is defined by separate growth limiting factors, which range between 0 and 1. A value of 1 means the factor does not inhibit growth (i.e. light is at optimum intensity, temperature is optimum and nutrients are available in excess). The limiting factors are then combined with the maximum gross growth rate at a reference temperature as in equation 5 (Solidoro et al. 1997):

$$\mu = \mu_{max(T_{ref})} * f(T) * f(I) * min(f(N), f(P)) \quad (5)$$

where, $\mu_{max(Tref)}$ is the maximum growth rate at a particular reference temperature (T_{ref}) under conditions of saturated light intensity and excess nutrients, $f(T)$, $f(I)$, $f(N, P)$ are the growth limiting functions for temperature, light and nutrients (nitrogen and phosphorus).

The major nutrients required for growth are nitrogen and phosphorus, while carbon is often available in excess and micronutrients such as iron and manganese are only limiting in oligotrophic environments. Typically, in marine ecosystems, nitrogen is the element limiting algal growth (Lobban and Harrison, 1994). Thus in the baseline simulation it is assumed that phosphorus is not limiting, so Eq. 5 becomes:

$$\mu = \mu_{max(T_{ref})} * f(T) * f(I) * f(N) \tag{6}$$

The Photosynthetic response to light is based on Steele's photoinhibition law (Steele, 1962):

$$\frac{P}{P_{max}} = \frac{I}{I_{opt}} exp\left(1 - \frac{I}{I_{opt}}\right) \tag{7}$$

where, P is the photosynthetic response at a given light intensity I (W m^{-2}) for an organism that has a maximum photosynthetic rate P_{max} at the optimal (saturating) light intensity I_{opt} and I is the light intensity at a given depth (z). Light intensity at a given depth is an exponential function of depth, seaweed and phytoplankton standing biomass and is given by:

$$I(z) = I_0\, e^{-kz} \tag{8}$$

where, k is the light extinction coefficient (m^{-1}).

After mathematical integration of the light limitation factor Eq. 8 we obtain:

$$F(I) = \int_0^z \frac{P}{P_{max}} dz = \int_0^z \frac{I(x)}{I_{opt}} exp\left(1 - \frac{I(x)}{I_{opt}}\right) dx = \int_0^z \frac{I_0 e^{-kx}}{I_{opt}} \exp \frac{1 - I_0\, e^{-kx}}{I_{opt}}\, dx = \frac{1}{k} * \exp\left(\frac{1}{I_{opt}}\right) * \left[\exp\left(-\frac{I_0}{I_{opt}} * \exp(-z * k)\right) - \exp\left(-\frac{I_0}{I_{opt}}\right)\right] \tag{9}$$

The temperature limitation factor, like the light, follows an inhibition law.

$$F(T) = q_{10}^{0.1(T-T_{ref})} \tag{10}$$

where, q_{10} is a temperature coefficient and T_{ref} is the reference temperature at which the seaweed growth rate was measured.

The nitrogen limitation factor (Eq. 11) is given by the range of internal nitrogen concentration, with a feedback effect on the uptake function (Aveytua-Alcázar et al. 2008; Coffaro and Sfriso, 1997; Solidoro et al. 1997). It can range between 1, when $N = N_{max}$ and uptake is saturated and 0 when $N = N_{min}$ and maximum uptake rate is possible, all measured in mgN g^{-1} dry seaweed. Internal nitrogen quota/concentration *(N)* refers to the concentrations in algal cells as opposed to external concentrations that refer to the concentration in the water column.

$$F(N) = 1 - \frac{N_{\max} - N}{N_{max} - N_{min}} \tag{11}$$

For calculation of *(N)*, a quota-based model was used developed from Droop's original formula (Droop, 1968):

$$\frac{dN}{dt} = V * F(N) - \mu * N \tag{12}$$

where, *V* is the nitrogen uptake rate (mg g^{-1}dw h^{-1}) and μµ is the specific growth rate.

Nutrient uptake rates *(V)* are proportional to nutrient concentration in the water according to Michaelis–Menten kinetics:

$$V = \frac{V_{max} S}{K_N + S} \tag{13}$$

where, V_{max} is the maximum nitrogen uptake rate under the site's prevailing conditions (mg g^{-1}dw h^{-1}), *S* is the total DIN concentration in the seawater (mg l^{-1}) and K_N is the half-saturation coefficient for nitrogen uptake (mg l^{-1}).

By combining Eqs. 11, 12 and 13 we obtain:

$$\frac{dN}{dt} = \frac{V_{max}\, S}{K_N + S} \frac{N_{\max} - N}{N_{max} - N_{min}} - (\mu * N) \quad (14)$$

The bioremediation effect of IMTA is closely dependent on the biomass of extractive organisms harvested. However, the maximum biomass is restricted by culture practicalities such as the potential alteration of water currents and by the availability of nutrients. The maximum biomass is site and species dependent. For the baseline simulation presented here, the maximum seaweed biomass permitted on site at any given time was set at 35 tonnes wet weight. The area required for the culture of 35 t of *Ulva*, with stocking density of 1.6 kg m^{-2} and two layers of seaweed one at the sea surface and one 3 m deep would be 10,937 m^2. This stocking density was selected because the maximum density permitted to guarantee the greatest uptake of nutrients in *U. lactuca* is 1.9 kg m^{-2} (Neori et al. 1991). The area required for the seaweed culture is used for the estimation of the virtually closed IMTA site's water volume. The virtually closed IMTA volume is estimated by multiplying the average depth with the combined total area that the salmon cages and the seaweed rafts take.

Seaweed is lost due to mortality, harvesting and natural biomass loss (seedling mortality, grazing, epiphytism, sediment abrasion and smothering and removal by wave action). Managing the harvesting rate is of paramount importance for achieving high productivity rates. For optimal results, when the seaweed biomass reaches a predefined level (35 t in the baseline simulation) the seaweed is harvested at regular time intervals. The biomass harvested depends on the forecasted growth and natural mortality rate of the forthcoming days. A discrete flow in the model controls the loss of seaweed biomass due to harvesting; the rate of the flow (harvest rate) is regulated by the following instruction:

IF (start harvesting = 0, 0 ton, IF (current time step * timestep = stoptime - starttime, seaweed biomass, IF (accrued part of 10 days = 1, seaweed

biomass – maximum seaweed biomass, IF (accrued part of 10 days = 0, seaweed biomass – maximum seaweed biomass, 0 ton))))

where, '*start harvesting*' is a level that allows harvesting to start only when the seaweed biomass has surpassed the value of a constant that defined as maximum biomass that can be on site (maximum seaweed biomass). The level 'start harvesting' changes from 0 to 1 when the level '*seaweed biomass*' is equal to or larger than the constant '*maximum seaweed biomass*'. '*Current time step*' is a level that counts the time steps, starting from zero. *Timestep*, *starttime* and *stoptime* are Powersim built-in functions that return the time step of the simulation, the start-time and stop-time of the simulation, respectively. In the final time step all the seaweed in the level *'seaweed biomass'* is transferred to the level *'harvested seaweed'*. '*Seaweed biomass*' is a level that shows the seaweed biomass. *'Accrued part of 10 days'* is a level used for the calculation of 10-day periods. When the value of this level is one, all the seaweed is harvested apart from *'maximum seaweed biomass'*.

The model is effective for perennial seaweed species. However, as the gametophyte stage of *Ulva* lasts only for a few months, frequent reseeding will be necessary at time intervals dependent on the environmental conditions, epiphytic growth or disease. The numerical parameters used in the seaweed model are summarized in Table 5-1.

Table 5-1: Parameterization of constants and time series variables used at the seaweed growth submodel

Variable	Description	Value range in literature	Value used	Units	Reference
μ_{max}	Maximum growth rate	0.8-18	10	% Day^{-1}	Neori et al. 1991; Luo et al. 2012; Perrot et al. 2014
N_{max}	Maximum intracellular quota for N	36-54	50	$mg^{-1}N\ g\ dw^{-1}$	Fujita, 1985; Cohen and Neori 1991; Perrot et al. 2014
N_{min}	Minimum intracellular quota for N	10 to 13	10	$mg^{-1}\ N\ g\ dw^{-1}$	Fujita, 1985; Cohen and Neori 1991; Perrot et al. 2014
T	Water Temperature	Site specific	6.8-13.7*	°C	n/a
q_{10}	Seaweed temperature coefficient	2	2	[1]	Aveytua-Alcázara et al. 2008
I_0	Water-surface light intensity	Site specific	50-190*	$W\ m^{-2}$	n/a
I_{opt}	Optimum light intensity for macroalagae	50	50	$W\ m^{-2}$	Perrot et al. 2014
k	Light extinction coefficient	Site specific	1	m^{-1}	n/a
z	Culture depth	Farm practice	2	m	n/a
V_{max}	Maximum N uptake rate	0.44-2.2	1.32	$mgN\ g^{-1}\ dw\ h^{-1}$	Lapointe and Tenore 1981; Perrot et al. 2014

K_N	N half saturation	0.06-0.55	0.31	mg L^{-1}	Perrot et al. 2014
Wet/Dry	Wet to dry weight ratio	6.7-10.15	8.43	n/a	Neori et al. 1991; Angell et al. 2012
M	Mortality	0.009-0.02	0.015	d^{-1}	Aveytua-Alcázara et al. 2008; Perrot et al. 2014
T_{ref}	Reference temperature for seaweed growth	n/a	15	°C	Neori et al. 1991; Luo et al. 2012; Perrot et al. 2014
Ω	Decomposition rate and natural biomass loss	n/a	M / 2	d^{-1}	n/a
D	Loss rate due to environmental disturbance	n/a	M / 2	d^{-1}	n/a
S	DIN concentration in sea water	Site specific	0.594	mg m^{-3}	n/a

* Time series variable

5.3.3 Sea urchin growth and nitrogen uptake and release

The sea urchin growth submodel is based on the DEB theory (Kooijman, 1986). DEB theory is based on two state variables: structural volume (*V*) and energy reserves (*E*) and on two forcing variables: temperature (*T*) and food density *(X)*. The basic concept of the theory is that from the food ingested a certain amount is released as faeces and the rest is assimilated. All assimilated food enters a reserve compartment.

A detailed description of the DEB can be found at Kooijman (2008). Most of the species-specific parameters used for this DEB model were obtained from (Kooijmann, 2014).

The initial structural length/diameter of sea urchin juveniles was set to 10 mm, a size suitable for successful transfer of hatchery-reared sea urchins to sea (Kelly et al. 1998). At this length *P. lividus* individuals are characterized as sub adults (Grosjean et al. 1998), so in the baseline simulation the DEB model simulates the growth from late juveniles to mature adults. The physical length (L_w) was converted to volumetric length (L), which is the cubic root of the animal's volume:

$$L_w = L/\delta_M \quad (15)$$

where, δ_M is the shape coefficient.

The DEB model starts with the ingestion of PON (*mgN* d^{-1}) by the sea urchins. This is based on ingestion rate (j_x) (mgC d^{-1})) divided by the C/N ratio of the aquaculture waste. Ingestion rate is proportional to the surface area of the structural volume and follows type-II function response depending on the density of PON. The food that is ingested but not assimilated as biomass is released to the environment as faeces or as excretion by diffusion. The DEB model enables estimation of the potential amounts of excretions released by the sea urchins by estimating the daily production of faeces released into the surroundings this is then divided by the C/N ratio in order to calculate the amount of PON and DIN that is in sea

urchin excretions, which is assumed to be immediately added to the PON and DIN pools and is thus available for consumption by the sea urchins and seaweed, respectively. The *P. lividus* N quota (*Q)* was set to 127 mgN mgC^{-1} (Tomas et al. 2005) and sediment N quota (*Qs)* is site specific it was set to 7, which is a representative value for an average Scottish salmon farm site.

For this simulation the notation from Kooijman (2000) was used. All rate variables are dotted above, all variables that are expressed per unit volume and per unit surface area are given between square brackets and braces, respectively. Additionally, the expression (x)+ is defined as: [x]+ = x for x > 0, [x]+ = 0.

Most of the processes described by the DEB model are influenced by the effect of temperature on the metabolic rate *(K(T))* according to Eq. 16:

$$K(T) = K_o\, e^{\left(\frac{T_A}{T_o} - \frac{T_A}{T}\right)} * \left[1 + e^{\left(\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L}\right)} + e^{\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T}\right)}\right]^{-1} \quad (16)$$

where, K_o is the reference reaction rate at 288 K, T_A is the Arrhenius temperature, T_o is the Reference temperature, T_{AL} and T_{AH} are the Arrrhenius temperature at lower and upper boundary, respectively, T_L and T_H are the lower and upper boundary tolerance, respectively and T is the water temperature (simulated as a time series variable). The Arrhenius temperature is used, because it is the typical temperature unit used when dealing with temperature dependent reaction rates.

The DEB model starts with the ingestion of PON (*mgN* d^{-1}) by the sea urchins. This is based on ingestion rate *(j_x)* (mgC d^{-1}) divided by the C/N ratio of the aquaculture waste (Eq. 17). Ingestion rate is proportional to the surface area of the structural volume and follows type-II function response depending on the density of PON.

$$\dot{j}_x = K(T) * f * \{\dot{j}_x\} * V^{2/3} \quad (17)$$

where, *K(T)* is a temperature dependent rate, $\{j_X\}$ is the maximum animal surface area-specific ingestion, *V* is the structural volume and *f* is the functional response that can range between 0 and 1 and is given by:

$$f = \frac{X}{X+X_K} \tag{18}$$

The saturation coefficient (X_H), is analogous to a Michaelis-Menten constant, in this case being the food density at which the ingestion rate is half the maximum. For the calculation of the food density in the environment *(X)*, the concentration of PON is converted to organic carbon concentration.

DEB models assume that the assimilation rate $(\dot{P}_A)$, is independent of the ingestion rate:

$$\dot{P}_A = K(T) * f * \{\dot{P}_{Am}\} * V^{2/3} \tag{19}$$

where, *K(T)* is a temperature-dependent rate, *f* is the functional response, $\{\dot{P}_{Am}\}$ is the maximum animal surface area-specific assimilation and *V* is the structural volume.

The food that is ingested but not assimilated as biomass will be released to the environment as faeces or as excretion by diffusion. The DEB model enables estimation of the potential amounts of faeces released by the sea urchins by estimating the hourly production of faeces released into the surroundings using Eq. 20 for the faeces production in (mgC d^{-1}) and Eq. 21 for the excretion rate in (mgN d^{-1}). Eq. 20 is then divided by the C/N ratio in order to calculate the amount of PON that is in the sea urchin faeces, which is assumed to be immediately added to the PON and DIN pools and is thus available for consumption by the sea urchins and seaweed, respectively.

The faeces production rate is estimated using the following formula:

$$\dot{F} = \dot{J}_x - \dot{P}_A/\mu_{cj} \tag{20}$$

where, j_x is the consumption rate, $\dot{P}_A$ is the assimilation rate and μ_{cj} is the ratio of carbon to energy content.

The excretion rate is estimated using the following formula:

$$\dot{D}_{excr} = \left\{\left[\dot{P}_c - (1 - k_R) * \frac{dE_R}{dt} - \mu_V * \rho * \frac{dV}{dt}\right] * Q + \dot{P}_A * (Qs - Q)_+\right\} / \mu_{cj} \quad (21)$$

where, $\dot{P}_c$ is the catabolic rate, k_R are the reproductive reserves fixed in the eggs, E_R are the reproductive reserves, μ_V is the structural energy quota, ρ is the biovolume density, V is the structural volume, Q is the sea urchin N quota, $\dot{P}_A$ is the assimilation rate, μ_{cj} is the ratio of carbon to energy content and Qs is the sediment N quota (calculated as the ratio of organic nitrogen to organic carbon in the sediment). The *P. lividus* N quota (Q) was set to 127 mgN mgC^{-1} (Tomas et al. 2005) and sediment N quota (Qs) is site specific it was set to 7, which is a representative value for an average Scottish salmon farm site.

The assimilated energy from the food enters the reserve pool. The energy density $[E]$ in an organism may vary between 0 and the maximum energy density $[E_m]$ depending on the food density in the environment.

$$\frac{d[E]}{dt} = \dot{P}_A - \dot{P}_c \quad (22)$$

where, $\dot{P}_A$ is the assimilation and $\dot{P}_c$ the catabolic rate.

The sea urchin catabolic rate) $(\dot{P}_c)$ denotes the energy utilised by the structural body and is given by:

$$\dot{P}_c = K(T) * \left[\frac{[E]}{[E_G] + K * [E]}\right] * \left(\frac{[E_G] * \{\dot{P}_{Am}\} * V^{2/3}}{[E_M]} + [\dot{P}_M] * V\right) \quad (23)$$

where, $K(T)$ is a temperature dependent rate, $[E]$ is the reserves, $[E_G]$ the volume-specific cost of growth, K the catabolic flux to growth and maintenance, $\{\dot{P}_{Am}\}$ the maximum surface area-specific assimilation, V the

structural volume, *[E_M]* the maximum reserve density and $[\dot{P}_M]$ the volume specific maintenance rate.

The rate of maintenance cost of the animals ($\dot{P}_M$) is proportional to the body volume and calculated with Eq. 24. Since the sea urchins will be mature the maturity maintenance *Pj* is also used Eq. 25:

$$\dot{P}_M = K(T) * [\dot{P}_M] * V \tag{24}$$

$$\dot{P}_j = \min(V, V_p) * [\dot{P}_M] * \frac{1-k}{k} \tag{25}$$

where, *K(T)* is a temperature dependent rate, $[\dot{P}_M]$ is the volume specific maintenance rate, V is the structural volume, V_p is the structural volume at puberty and *k* is the catabolic flux to growth and maintenance.

The sea urchin structural volume growth (V) is given by:

$$\frac{dV}{dt} = \frac{(k*\dot{P}_C - \dot{P}_M)_+}{[E_G]} \tag{26}$$

where, *k* is the catabolic flux to growth and maintenance, $\dot{P}_c$ is catabolic rate, $\dot{P}_M$ is the maintenance rate and $[E_G]$ is the volume specific cost of growth.

In this model we are also interested in the body mass (W) of the sea urchins, in order to calculate the total biomass of the stock. To convert volume to dry weight Eq. 27 is used:

$$W = \mathrm{V} * \rho + \frac{(E + E_R * k_R)}{\mu_E} \tag{27}$$

where, V is the structural volume, ρ is the biovolume density, E and E_R are reserves and reproductive reserves, respectively, k_R are the reproductive reserves fixed in the eggs and μ_E is the reserve energy content.

The total biomass was calculated as individual weight multiplied by the number of individuals. Once an individual has reached the volume *(V_p)* at

sexual maturity, a portion of the total energy reserve is stored in the sea urchin reproductive reserves *(E_R):*

$$\frac{dE_R}{dt} = (1 - k) * \dot{P}_c - \dot{P}_j \quad (28)$$

where, k is the catabolic flux to growth and maintenance, $\dot{P}_c$ is the catabolic rate and $\dot{P}_j$ is the maturity maintenance.

The DEB model simulates the process within individuals. However for this model it is necessary to know how a non-reproducing stock *(N)* will decrease in size with time, due to mortality. The decrease of the sea urchin stock size is calculated in Eq. 29 where due to the planktonic nature of sea urchin larvae, it is assumed they will be dispersed from the IMTA site and thus reproduction will represent a net energy loss and restocking of the sea urchins will be necessary. However, the release of the larvae will contribute to restocking the native sea urchin population.

$$\frac{dN}{dt} = -\delta_r * N - \delta_h * N \quad (29)$$

where, δ_r and δ_h are the natural and harvest mortality of sea urchins, respectively. The harvest mortality δ_H was zero and at the last time step of the simulation all sea urchins were harvested, same as in the salmon and seaweed submodels. The natural mortality (δ_r) was set to 0.00102 individuals d^{-1} for sea urchins with test diameter smaller than 2 cm and 0.00056 individuals d^{-1} for sea urchins with test diameter larger than 2 cm (Turon et al. 1995).

During the grow-out stage of *P. lividus* juveniles, the stocking density is approximately 400 individuals m^{-2} (Carboni, 2013). Space is not an issue for the organic extractive component of the IMTA, since for the production of 560,525 individuals only 1,401 m^2 would be required and this area would be directly underneath the fish cages and the seaweed rafts.

5.4 Assumptions and simplifications

The overall model's key assumption is that all nitrogen released by the IMTA components is dispersed homogenously within a quantified water volume defined as the IMTA site water volume (see section 5.2.3). It is also assumed that all the nitrogen available in the IMTA site volume is in a form suitable for uptake. Correspondingly, the model does not take into account the interactions between nitrate and ammonium within the environment and organisms, such as the role of sediment and water in the nutrient dynamics or denitrification. The increase of light limitation due to increased self-shading as the seaweed grows was not considered, neither was the shading caused by phytoplankton. Data from Broch and Slagstad (2012) could be used to derive a seaweed self -shading formula from which an add-on model could be used to simulate the changes in the light extinction coefficient (*k)*, in this study *k* is a constant. In the seaweed growth submodel the biomass loss due to mechanical damage caused by harvesting was not included. It is also assumed that nitrogen is the only nutrient limiting seaweed growth. Additionally, the seaweed biomass used as initial biomass is assumed to have an average $((N_{min} + N_{max})/2)$ amount of intracellular nitrogen (this can be regulated by using nitrogen deprived seedlings). Each seaweed species can contain up to a certain amount of nitrogen in its cells, we define this as the maximum nitrogen quota. When seaweed is harvested it is assumed that the N quota of the harvested seaweed is equal to the maximum N quota due to the high availability of DIN in the virtually closed system. The assumption that the seaweed harvested has this high nitrogen quota might lead to overestimation of the bioremediation efficiency and the effect of lower N quota at harvest was examined in the sensitivity analysis (Table 5-2). From a farm practice perspective, it is assumed that the relative position of the extractive organisms in relation to the fish cages is such that it ensures high O_2 availability for the fish. For the salmon growth model, excretion, faeces production and feed loss were assumed to be a steady proportion of feed input during the 18-month production period while in reality they change as fish grow.

5.5 Production specifications of the baseline simulation

The results presented are from the IMTA baseline simulation, which was parameterized using data acquired from the literature and from commercial salmon farm sites. The environmental data such as monthly variations in seawater temperature and irradiance were acquired from empirical databases for the West coast of Scotland and the production-specific input data from Scottish commercial salmon farm sites (Figure 5-2 and Figure 5-3). Typically, S1 smolts are transferred to sea in spring (April-May), so April is set as simulation time 0. The baseline scenario farm consists of nine 90 m circular salmon cages with the extractive organisms placed in immediate proximity to those cages. The model simulates a farm that produces 1,000 t of Atlantic salmon in 18 months on-growing, a farm size representative of the Scottish industry.

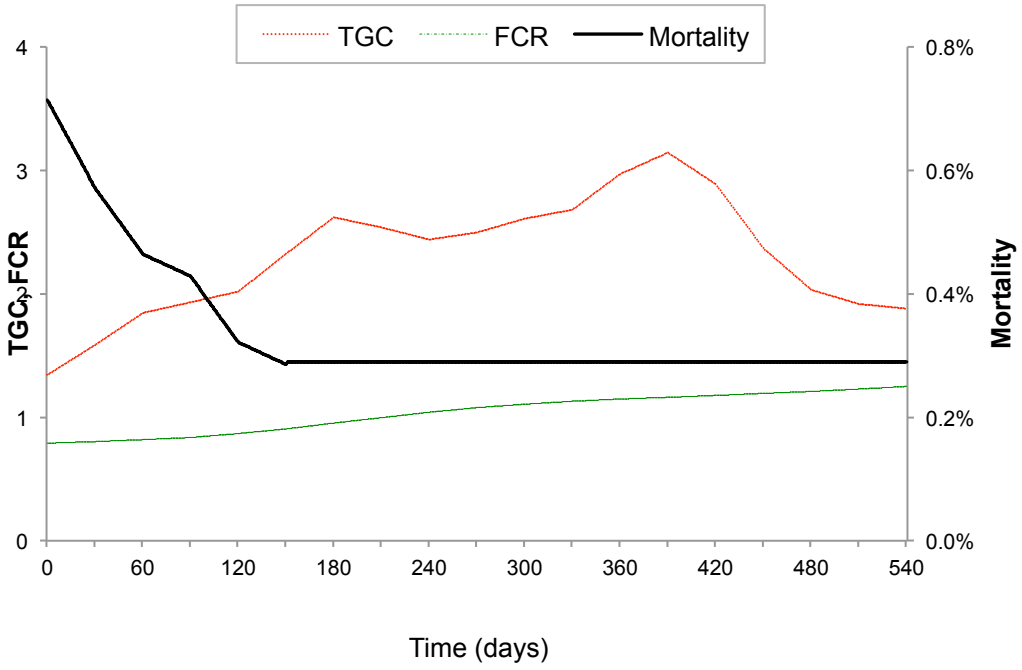


Figure 5-2: Baseline scenario values of the time series variables: TGC, FCR and salmon mortality.

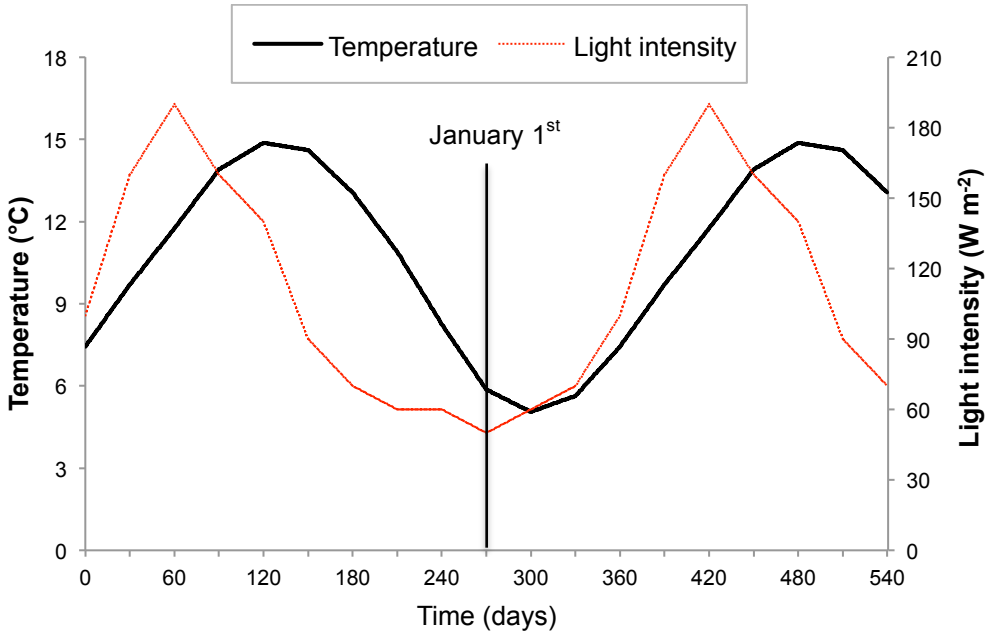


Figure 5-3: Baseline scenario values of the time series variables: water temperature and light intensity.

5.6 Results

5.6.1 Growth performance of IMTA components at the baseline simulation

The baseline simulation run estimated that the mean individual fish biomass after 540 days (18 months) was 3.78 kg (Figure 5-4a) and the salmon stock decreased by 16,500 individuals from 281,000 to 264,000 individuals (Figure 5-4b).

During the 18-month production period, 342 t of seaweed and 20.02 t of sea urchins were produced and harvested as well as the targeted 1,000 t of salmon. The seaweed achieved high growth rates, especially during the summer months (Figure 5-5). The effect of the growth limitation factors on seaweed growth rate is presented in Figure 5-6. The lower seaweed growth rate during the first 300 days (10 months) of the simulation (Figure 5-5) can be mainly attributed to low levels of nitrogen available for uptake (Figure 5-6 and Figure 5-9). It is clear that in the hypothetical baseline model scenario,

during the first 340 days of the simulation seaweed growth is mainly limited by the availability of nitrogen. Temperature limits growth more during the colder months (October – April) while, the effect of light intensity is rather stable throughout the year (Figure 5-6). It should be emphasized here that site– specific shading caused by phytoplankton or seaweed self shading does not contribute to light limitation in the baseline simulation (see section 5.2.5).

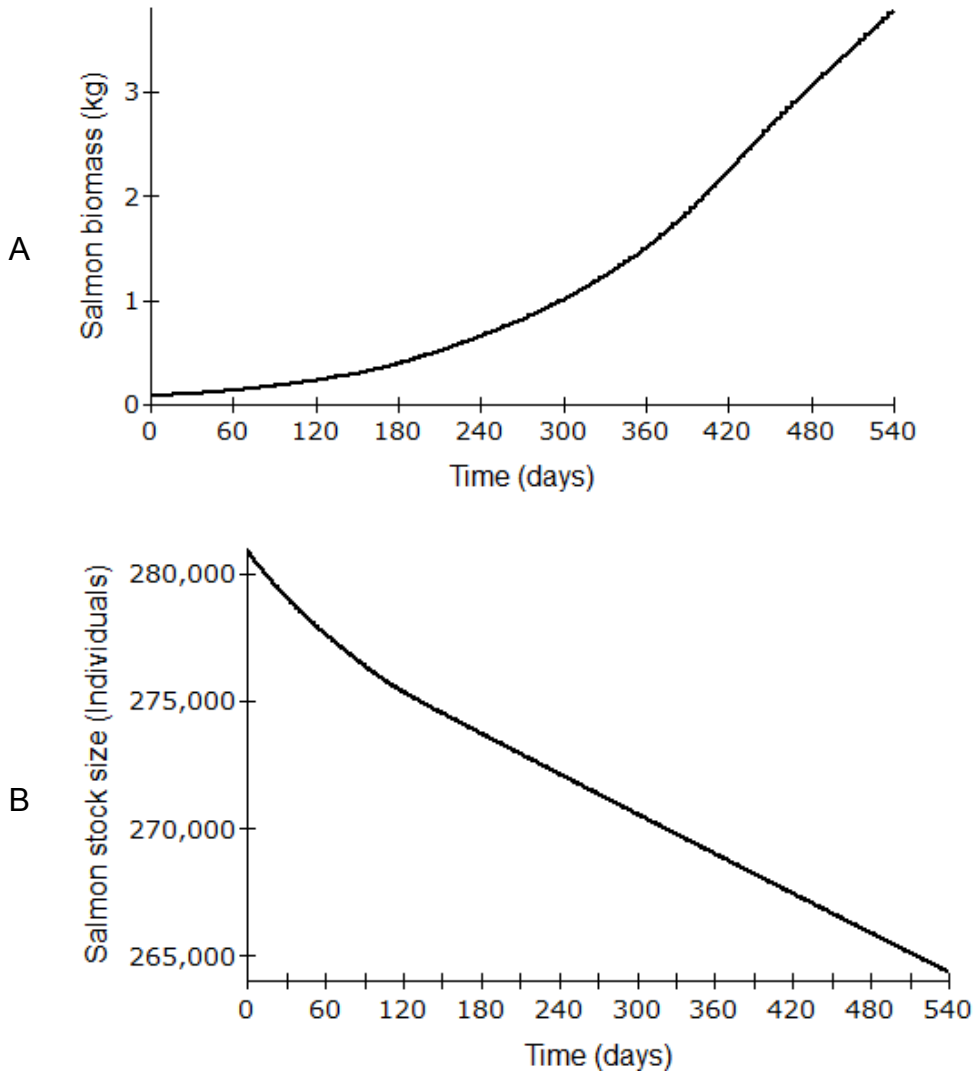


Figure 5-4: Simulated output of the salmon: a) individual average biomass, b) stock size, during the 540 days of culture at sea.

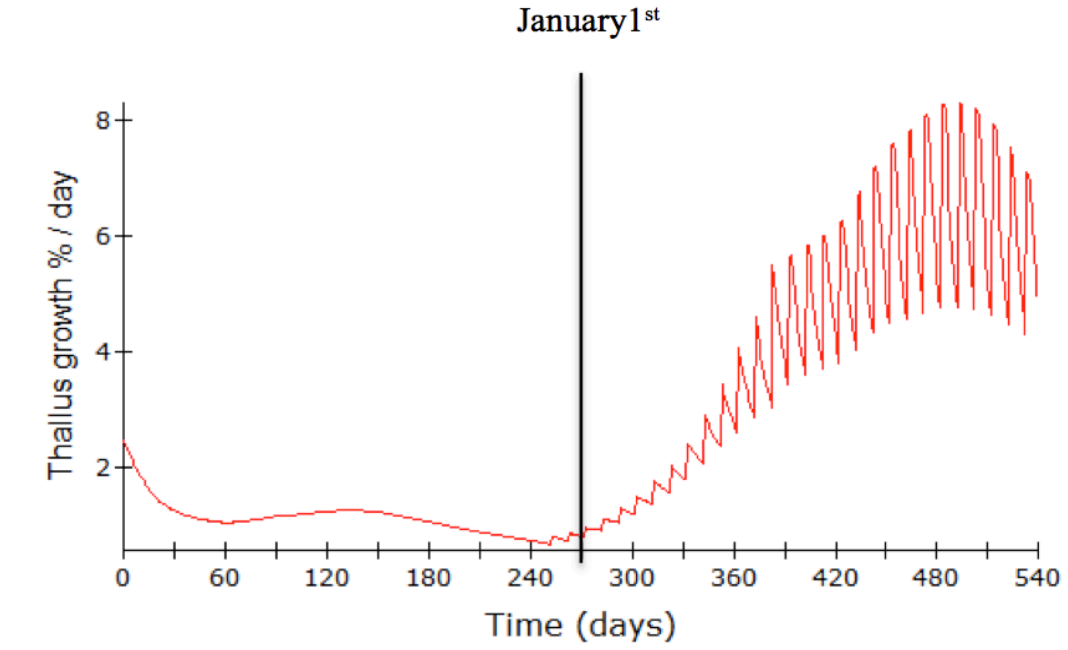


Figure 5-5: Seaweed specific growth rate for *Ulva* sp. under the baseline scenario production conditions.

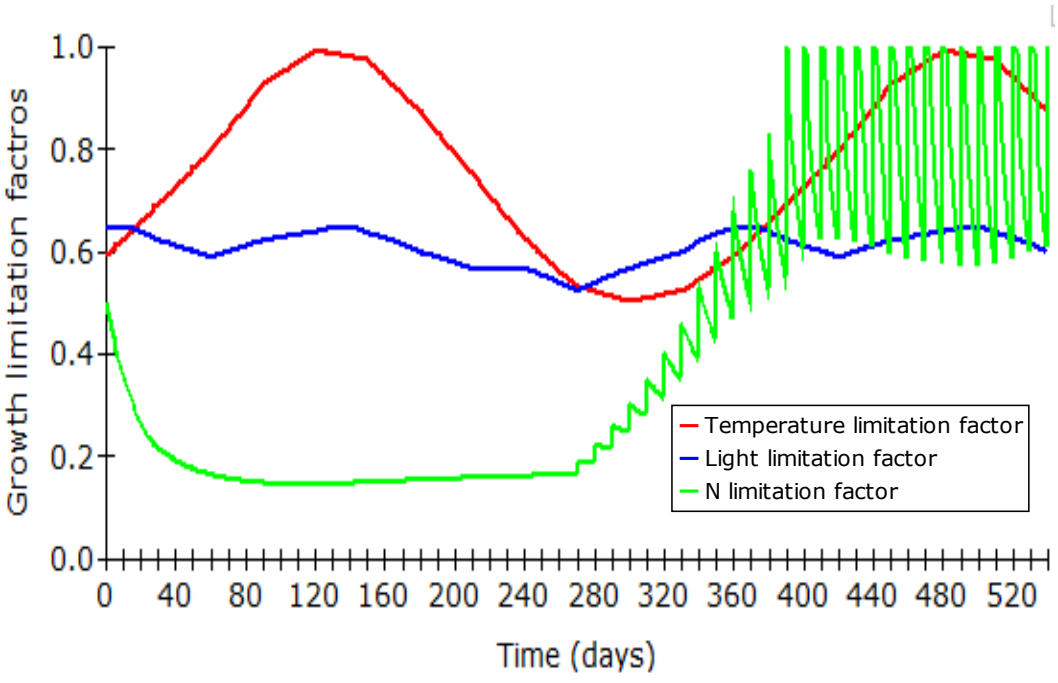


Figure 5-6: Seaweed growth limitation factors, under the baseline scenario production conditions. The limitation factors can vary between 0 and 1; where a value of 1 means that the factor does not inhibit growth.

The model's aim is to achieve high nutrient bioremediation in limited space. Sustaining the seaweed biomass at a high density at all times, using the harvesting instruction (described at section 5.2.3), played an important role in achieving high bioremediation efficiency (Figure 5-7). The first seaweed harvesting occurred 250 days after the simulation start, following which there was enough nitrogen available due to the large size of the fish and the environmental conditions were also favorable for the remaining seven months of the simulation (April – October) (Figure 5-3 and Figure 5-6) thus ensuring constant high growth rate and harvesting at 10-day intervals (Figure 5-7).

At simulation time zero the site was stocked with 827,900 (0.09 t) sea urchins. During the 18-month production period 20.01 t (wet weight) of sea urchins were produced with average test diameter 4.47 cm (Figure 5-8). As a result 0.96 t of nitrogen were assimilated in the sea urchin biomass and removed from the IMTA area via the process of harvesting.

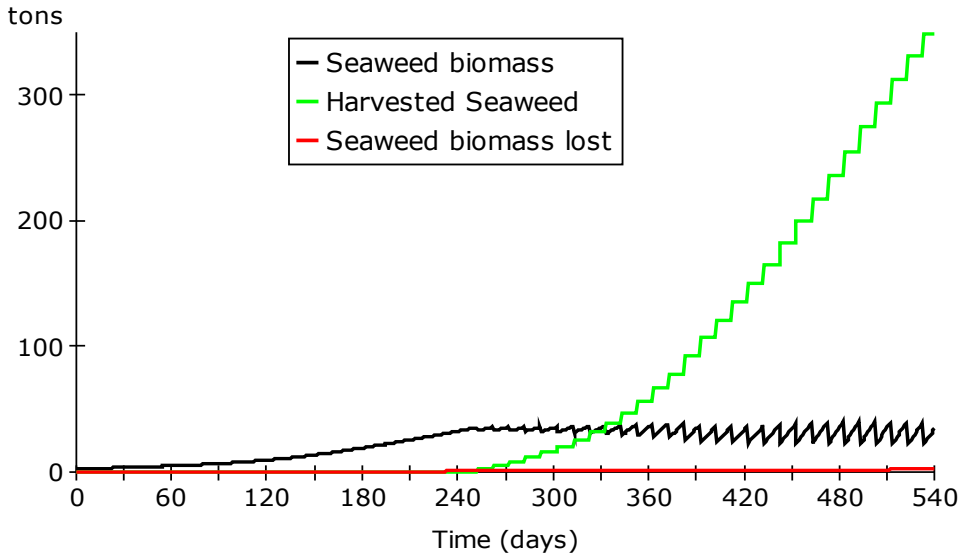


Figure 5-7: Seaweed submodel simulation output for *Ulva* sp. produced under the baseline scenario conditions. It illustrates the biomass change over time, the cumulative amount of seaweed biomass lost due to natural causes and the cumulative amount of seaweed biomass.

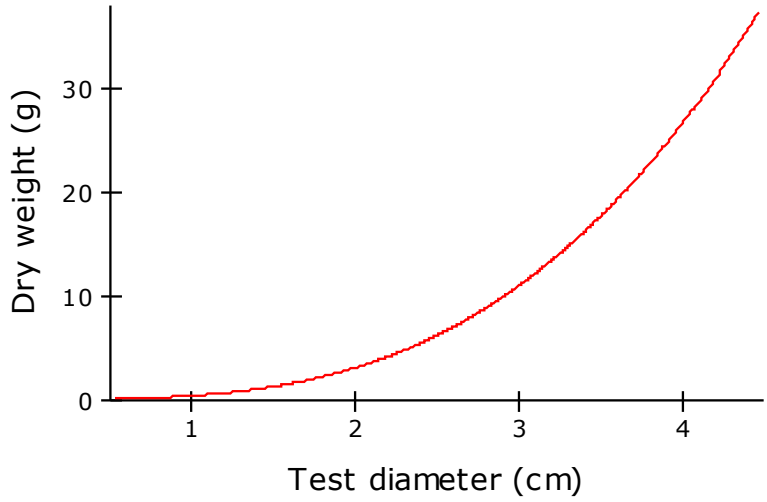


Figure 5-8: Sea urchin submodel simulation output for the length - dry weight relationship of *P. lividus*.

5.6.2 Baseline scenario bioremediation potential

For the production of 1,000 t of salmon with average feed conversion ratio (FCR) of 1.02 and feed nitrogen content 5.76%; the model shows that 65 t of nitrogen are introduced into the system over the 540 day simulated production period. From this 65 t, the fish accumulates only 38% and the remaining 62% (40.2 t) is released into the environment. Under the environmental conditions and production method of the baseline scenario, the total nitrogen released to the environment from the IMTA site would be 45.2% less (22.03 t instead of 40.2 t) than what would have been released from a salmon monoculture farm of the same capacity. In detail, the amount of nitrogen released from salmon monoculture would be 62% of the exogenous nitrogen input but only 34% in the IMTA system since a large proportion of the nitrogenous waste will be assimilated by the extractive organisms and removed from the IMTA area via harvesting (Figure 5-9). Figure 5-9 shows the gradual increase in nitrogen within the IMTA system over the simulated production period.

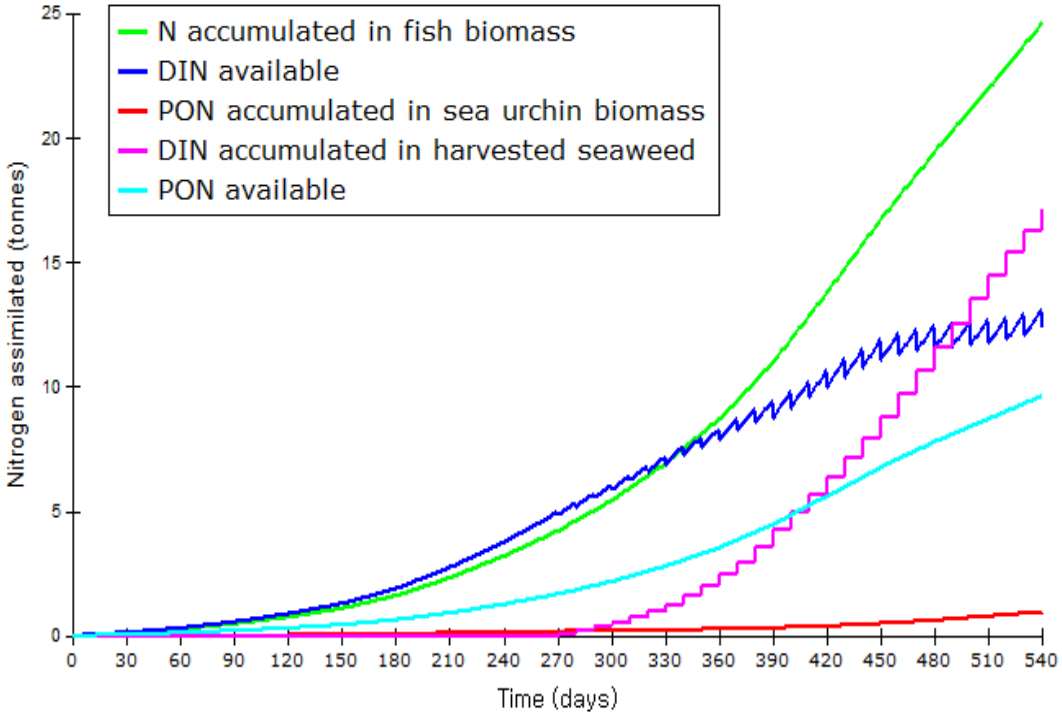


Figure 5-9: Modelled output of cumulative amount of nitrogen assimilated by the different IMTA components and the amount of DIN or PON remaining at the IMTA site area at each time step.

5.7 Sensitivity analysis

All biological, environmental and production parameters were analysed in terms of uncertainty and their relative importance in the model. Due to the large number of input and response variables used in the sensitivity analysis, only the results for the most sensitive parameters (absolute values) are summarized in Tables 5-2. Those parameters are the potential critical assumptions and thus require accurate estimation and/or calibration.

In the salmon submodel, the growth and nutrient uptake is most sensitive to change in the TGC and secondarily to variation in the FCR (Table 5-2; sections a and b).

In the seaweed submodel, all output variables were most sensitive to parameters affecting growth and nutrient uptake either indirectly (through nitrogen uptake and nitrogen content of the seaweed tissues, wet/dry ratio and the culture depth) or directly (through maximum growth rate, temperature and nitrogen input from salmon excretion). These results show

the overall importance of temperature and nitrogen uptake for seaweed growth (Table 5-2; sections c and d). All parameters, apart from the minimum and maximum intracellular nitrogen quota, were positively correlated with the output variables. Also, increasing parameter values mirrored the effect on the model output of decreasing parameter values, which indicates that most parameters affected growth linearly.

In the sea urchin submodel the output variables were most sensitive to parameters related to temperature. Other sensitive parameters included the maximum surface-specific feeding rate, the volume–specific cost of growth and the ratio of carbon to energy content (Table 5-2; sections e and f). Overall, this analysis revealed that the DEB model was most sensitive to increases in the sea urchin's lower boundary tolerance (T_L). Changes in the remaining DEB input variables had little effect on growth (NS < 1).

The most sensitive parameters within the salmon and seaweed sub-models are also the most sensitive to outcomes of the overall model. The most sensitive parameters of the DEB sub-model do not play such an important role within the overall model performance due to the sea urchin biomass being very small in comparison to that of salmon and seaweed (Table 5-2; section g and h).

Table 5-2: Most sensitive parameters (with NS ≥ 1) for the effect variables a) Nitrogen accumulated in harvested salmon b) Harvested salmon biomass c) DIN accumulated in harvested seaweed d) Harvested seaweed biomass e) Nitrogen accumulated in harvested sea urchin

Parameter symbol	Parameter name	Parameter baseline value	Effect for parameter + 10%	NS for parameter +10%	Effect for parameter -10%	NS for parameter -10%
a) Nitrogen accumulated in harvested salmon: effect baseline value is 24.66 tonnes						
TGC	Thermal-unit growth coefficient*	2.33	30.55	2.42	19.61	2.07
FCR	Feed conversion ratio*	1.04	24.92	0.1	20.39	1.73
b) Harvested salmon biomass: effect baseline value is 1000 tonnes.						
TGC	Thermal-unit growth coefficient*	2.33	1,242	2.45	808	1.95
c) DIN accumulated in harvested seaweed: effect baseline value is 17.09 tonnes						
N_{state}	Nutrient state of seaweed at harvest**	10	3.18	-7.93	10.59	3.97
μ_{max}	Max seaweed growth rate	0.13	19.78	1.57	13.71	1.98
T	Water Temperature*	10.89	18.01	0.54	12.96	2.41
V_{max}	Maximum N uptake rate	1.32	19.18	1.22	13.50	2.10
W/D	Wet / dry ratio	8.43	19.19	1.23	13.49	2.10
z	Culture depth	2	19.39	1.35	14.32	1.62

N_{excr}	Nitrogen lost via excretion	0.45	16.80	-0.17	15.09	1.17
d) Harvested seaweed biomass: effect baseline value is 341.84 tonnes						
μ_{max}	Max seaweed growth rate	0.13	395.69	1.58	274.19	1.98
T	Water Temperature*	10.89	360.20	0.54	259.27	2.41
V_{max}	Maximum N uptake rate	1.32	383.68	1.22	269.92	2.11
W/D	Wet / dry ratio	8.43	383.73	1.23	269.88	2.11
z	Culture depth	2	387.89	1.35	286.49	1.62
N_{min}	Min intracellular quota for N	10	303.32	-1.13	358.39	-0.48
N_{max}	Max intracellular quota for N	50	307.66	-1.00	360.90	-0.56
e) Nitrogen accumulated in harvested sea urchin biomass: effect baseline value is 0.96 tonnes						
T	Water Temperature*	10.89	1.119	1.65	0.640	3.33
$\{P_x\}$	Maximum surface-specific feeding rate	578.55	1.248	3.00	0.723	2.47
K_0	Reference reaction rate at 288 K	1	1.229	2.80	0.734	2.35
T_A	*P. lividus* Arrhenius temperature	8000	0.793	-1.74	1.172	-2.21
μ_{cj}	Ratio of carbon to energy content	83.30	0.876	-0.88	1.068	-1.13
f) Harvested sea urchin biomass: effect baseline value is 20.02 tonnes						
TL_{T_L}	*P. lividus* lower boundary tolerance	273	0.08	-9.96	n/a	n/a

T	Water Temperature*	10.89	23.01	1.15	13.37	3.32
$\{P_x\}$	Maximum surface-specific feeding rate	578.55	26.01	2.99	15.00	2.50
K_0	Reference reaction rate at 288 K	1	25.36	2.67	15.39	2.31
T_A	*P. lividus* Arrhenius temperature	8000	16.59	-1.71	24.21	-2.09
$[E_G]$	Volume specific cost of *P. lividus* growth	2748	18.28	-0.87	22.02	-1.00
g) DIN available at the IMTA site: effect baseline value is 12.38 tonnes.						
N_{state}	Nutrient state of seaweed at harvest**	10	23.31	0.22	16.95	0.18
TGC	Thermal-unit growth coefficient*	2.33	18.05	4.64	5.55	5.59
FCR	Feed conversion ratio*	1.04	11.82	-0.45	6.82	4.49
N_{excr}	Nitrogen lost via excretion	0.45	15.60	2.60	10.65	1.40
μ_{max}	Max seaweed growth rate	0.13	9.69	-2.17	15.77	-2.74
$N_{content}$	Nitrogen content in feed	0.057	15.66	2.63	10.59	1.44
T	Water Temperature*	10.89	11.46	-0.74	16.53	-3.35
V_{max}	Maximum N uptake rate	1.32	10.29	-1.69	15.98	-2.91
W/D	Wet / dry ratio	8.43	10.30	-1.68	15.97	-2.90
z	Culture depth	2	10.08	-1.86	15.15	-2.24

N_{min}	Minimum intracellular quota for N	10	14.32	1.57	11.56	0.66
h) PON available at the IMTA site: effect baseline value is 9.65 tonnes						
TGC	Thermal-unit growth coefficient*	2.33	12.07	2.54	7.41	2.35
FCR	Feed conversion ratio*	1.04	9.68	0.03	7.78	1.94
$N_{content}$	Nitrogen content in feed	0.0576	10.70	1.08	8.61	1.07

* Time series variable. The time series parameters where increased/decreased by 10% at each time step

** For the parameter “Nutrient state of seaweed at harvest” we used N_{min} instead of N_{max} at the column labeled as +10% and (N_{min} + N_{max})/2 at the column labeled as -10

5.8 Discussion

The aim of this study was the development of a dynamic tool for relative comparison of IMTA scenarios at a given production site, rather than the generation of absolute bioremediation and production estimates. The model results presented are derived from a baseline simulation, which can be re-parameterised to simulate different scenarios.

Results from similar IMTA studies have shown bioremediation potential of a similar scale to the output generated by the present model. Broch and Slagstad (2012) estimated that 0.8 km^2 of *Saccharina latissima* biomass would be needed to sequester all the waste released from a salmon farm producing 1,000 t a year and Abreu et al. (2009) estimated that a 1 km^2 *Gracilaria chilensis* farm would be needed to fully sequester the dissolved nutrients released from a salmon farm producing 1,000 t a year. Sanderson et al. (2012) estimated that 0.01 km^2 of *S. latissima* could remove 5.3-10% of the dissolved nitrogen released from a salmon farm producing 500 t of salmon in two years. However, the results presented, as the results from any other IMTA model or trial, cannot be directly compared with output from similar studies due to the fact that the productivity of an IMTA farm depends on local environmental characteristics, the species combination used, the duration of the grow out seasons and other factors. Moreover, linear interpolation of results from studies with shorter durations can lead to misestimation of results. Thus a large variance in production and bioremediation results is natural. The results of this study are in the same order of magnitude as the results acquired from the studies mentioned above; however they suggest higher bioremediation potential, possibly largely due to the harvesting method applied. Specifically, it was estimated that 35% of the total nitrogen released from a salmon farm, with the specifications of the simulated scenario, would be accumulated by the 0.01 km^2 of *Ulva* sp. suggesting very high bioremediation efficiency. Aiming to achieve 100% bioremediation (i.e. no available nitrogen above the ambient concentration occurs at any given time), especially without the addition of external feed sources for the extractive organisms and while sustaining the

quality of the extractive organisms, is unrealistic and might only be possible in a fully closed system such as a Recirculating Aquaculture System (RAS). Nonetheless, even at lower bioremediation efficiencies, the model already demonstrates the environmental benefits of IMTA.

The simulated growth for juvenile and adult sea urchins showed good correspondence with literature data (e.g. Cook and Kelly, 2007a), although the reference temperature for which all the DEB constants were calculated was 20°C (Table 5-1) which is significantly higher than the average temperature (11°C) at the modelled IMTA site. The sea urchin growth model output is comparable to the results of Cook and Kelly (2007a) who concluded that *P. lividus*, with an initial 1 cm test diameter, deployed adjacent to fish cages need approximately 3 years to reach market size (>5.5 cm test diameter). The sea urchins will be around one year old when they are deployed and 2.5 years old at the end of the grow–out phase at which point their test diameter will be 4.47 cm. At the end of the 18-month grow-out phase of the salmon, the sea urchins will have reached the lower limit of their target market size. The growth rate achieved in this study was similar to that achieved directly adjacent to the sea cages (Cook and Kelly, 2007a) and higher than that achieved by Fernandez and Clatagirone (1994) (1.41 mm $month^{-1}$) where the sea urchins were fed with artificial feed containing fish meal and fish oil at higher water temperature than this study (5-33°C). After the sea urchins have reached market size, a two to three month period of market conditioning at controlled environment is required (Carboni, 2013; Grosjean et al. 1998).

In the first eight to ten months of the IMTA baseline scenario, seaweed and sea urchin growth is limited by nitrogen (Figures 5-6 and 5-8), since the fish are still small and thus require a relatively low feed input. From the eleventh month onwards mainly light and to a lower extent temperature are limiting the seaweed growth. From that point onwards the seaweed growth rate is high as can be seen in Figure 5-5. For successful high bioremediation efficiency at an IMTA farm, seaweed growth should not be limited by light or temperature but only by nutrient availability. For this reason IMTA systems

could be more efficient in sites further south than the one used for the baseline simulation. It can be seen clearly in Figure 5-9 that there is a constant increase of the residual DIN and PON remaining at the IMTA site. This high waste output particularly during the last months of the salmon production is a challenge for achieving very high bioremediation efficiency. The ratio of salmon to extractive organisms used at the baseline scenario is very low: final salmon to seaweed weight ratio was 2.92 and final salmon-sea urchin ratio was 50). From the perspective of space requirement there is the potential for increase of the amount of sea urchins produced, however the quantity of waste available for consumption by the sea urchins decreases with distance from the sea cages and thus increasing the production would mean that some sea urchins would be potentially too far from the food source. Furthermore, limited market demand for marine invertebrates might also pose limitations.

The results of the sensitivity analysis indicate that the model is robust, since variation of key model parameters by ±10% does not cause unexpected changes in the effect parameters. The various model parameters have a different relative influence on the model's output, both in terms of harvestable biomass and in terms of nitrogen bioremediation. Thus, depending on users' specific study objectives, one should consider the precision with which certain parameter values are determined, and whether further tuning is required. This model sensitivity analysis is a useful means for assessing which are the key parameters that increase model uncertainty. Those parameters with high sensitivity have a big impact on the output of the model (e.g. thermal sensitivity parameters T_L in the sea urchin DEB submodel, T in all the submodels and μ_{max} in the seaweed submodel), and therefore future efforts should focus on methods for improving their estimation. In contrast, because parameters with low sensitivity have little influence on the output of the model, their estimation could be simplified. Consequently, despite the large variability observed in some of the parameters, their relative importance may be minor if their sensitivity is low.

Other polyculture and IMTA models developed, to date, include Nunes et al. (2003); Ferreira et al. (2012); Shi et al. (2011); Ren et al. (2012). The uniqueness of the model developed in this study is that it is a dynamic model developed in a software environment with a simple user interface and thus can be used by anyone prior to the set up of an IMTA system. The model presented here is highly adaptable as all the submodels can function independently. By altering model variables the submodels can simulate growth and nutrient assimilation under different environmental conditions or for different species. Altering the values of constants can also help assess their effect on the IMTA system and in some cases these values can be optimised. For example, all the values related with production practices at the IMTA site, such as seaweed harvesting frequency, maximum seaweed biomass allowed, initial biomass of seaweed or sea urchins, seaweed culture depth and seaweed density, can be optimised for the achievement of higher bioremediation efficiency and/or higher extractive organism production.

The model can be also used for the accomplishment of more general objectives such as optimization of IMTA culture practices (e.g. timing and sizes for seeding and harvesting, in terms of total production), assessment of the role of IMTA in nutrient waste control, and used as input for the evaluation of economic efficiency of various system designs. The present model can be used as a decision support tool for open-water IMTA only after being coupled with waste distribution modelling and environmental sampling for model parameterization. Future versions of the model can link the virtually closed IMTA system to hydrodynamic models for spatial analysis of the waste dispersion and nutrient dilution. Such a model could help develop a balance among the components of the IMTA system and assist in developing an IMTA design for maximum waste uptake in “open environment systems”, as water exchange rate is the key factor influencing the assimilative performance, thus enabling prediction of the effectiveness and productivity of open water IMTA systems.

This chapter has been adapted into a manuscript, which has been published as Lamprilou et al. (2015), the paper is given in Appendix Figure 5.

CHAPTER 6

A comparison of three IMTA candidate extractive species and a comparison of their bioremediation efficiency

6.1 Introduction

IMTA systems consist of a fed species co-cultured with an organic extractive species (usually bivalves) that can utilize the particulate waste or with an inorganic extractive species (usually seaweed) that can utilize the soluble waste or of a combination of species from both groups. Although bivalves are the most common invertebrates used in IMTA, a number of species have properties constituting them suitable prospective organic extractive components.

Marine invertebrate cultivation in Europe is limited to only a few species, with the dominant group being the bivalve molluscs and in particular mussels. However there is potential for cultivation of a large variety of species in invertebrate monoculture or as part of IMTA systems. Total global mussel cultivation is approximately 1,800,000 t, of which 9.8 % is the common blue mussel (*Mytilus edulis*), of which 84% are produced in Europe (FAO 2013).

The high demand for certain marine invertebrate species enables the rapid expansion of new fisheries before scientists and managers can develop strategies to secure the long-term, sustainable use of these resources (Anderson et al. 2008). Such fisheries include the sea urchins (Anderson et al. 2008; Andrew et al. 2012) and sea cucumbers (FAO, 2008). IMTA systems enable fish farmers to diversify into producing additional highly

valuable products while at the same time reducing the increasing worldwide pressure on invertebrate fisheries on wild stocks.

The aim of this study is to compare the suitability of three organic extractive species as IMTA components. For this purpose DEB models were developed for *P. lividus, A. marina* and *M. edulis.* These DEB models were used as sub-models of the IMTA model described in chapter five. The dynamic structure of the model enabled comparisons among the species.

Research on the Dynamic Energy Budget (DEB) theory started 35 years ago; a consistent theory was developed and currently the scientific focus is on parameterizing DEB models for various species. Currently a large amount of data for the parameterization of DEB models is available in the literature. This study compares the bioremediation efficiencies and cultivation area requirements of each candidate species within an IMTA system consisting of salmon and the seaweed *Ulva*. The area requirements of the extractive cultures are an important consideration for various reasons. These include: competing demand for space competing demands for space from multiple users, large-scale cultures have a visual impact, high algal culture densities over a large area can alter the currents and might lead to low dissolved oxygen concentrations during the night, and finally practical issues also hinder the extractive culture growing area. Operationally, the extractive cultures, if in large numbers, are likely to present a real obstacle to normal husbandry routines for salmon (boat access, net changes, bath treatments etc.) and therefore engineering design and incentive is required to assist this process. Extractive organisms grown further from the fish cages might have access to less food, depending of course on the hydrodynamic conditions of the site.

6.2 The organic extractive species

6.2.1 Arenicola marina

Arenicola marina, commonly known as lugworm or sandworm, is a large marine worm of the phylum Annelida. In North Western Europe, lugworms are one of the abundant macrobenthic species of tidal mud flats and can be

found at densities of up to 100-150 individuals m^{-2} (Fish, 1996). They play an important role in many food chains. However, naturally occurring supplies of *Arenicola* are not inexhaustible and collection of marine worms has been recognised as a cause of serious environmental concern (Olive et al. 2006). The negative impact on the environment of the manual turning of sediment for the collection of bait (Fowler, 1999) as well as the pressure caused to the environment by removing such an important species can be mitigated through *Arenicola* aquaculture.

Polychaetes have commercial value, they can be used as fish feed or sold as bait (Olive, 1999). Furthermore, the haemoglobin of *A. marina* has the potential to substitute human red blood cells (Zal et al. 2002), and could be a promising alternative to human blood for transfusions. *A. marina* can also be sold for human consumption, to aquaria and as laboratory specimens, especially for toxicity testing. *A. marina* has strong bioturbation impact through non-selective sediment ingestion and defaecation as well as by irrigating its burrow (Riisgård and Banta, 1998).

Polychaetes are good candidates for IMTA: some species such as *Capitella* are often found in high abundance in the sediment under sea cages. They can play an important role in organic sediment bioremediation (Lu and Wu, 1998). Although, *A. marina* ingests sediment in a non-selective way, selective feeding in the field has been observed in some closely related species (Hylleberg, 1975) and there is indirect evidence for it in *A. marina*. Various polychaete genres such as *Nereis, Arenicola, Glycera* and *Sabella* have been considered as potential IMTA components in marine temperate waters.

Postlarvae (4 to 9 mm long) are present in the plankton during spring and settle on the tidal flats from spring to early summer (Farke and Berghuis, 1979). During the benthic life stage *Arenicola* occupy J-shaped burrows and have a normal lifespan of between 5-6 years (Beukema and de Vlas, 1979; Howie, 1984). The majority of UK *A. marina* populations exhibit epidemic spawning (simultaneous shedding of gametes by a large number of individuals) over a few days during autumn (Betteley et al. 2008). Each

female has an average of 316,000 oocytes with total wet weight of 4 g (Wilde and Berghuis, 1979). The eggs and early larvae develop within the female burrow. At the post larval developmental stage *Arenicola* may actively migrate by crawling from the burrows, swimming in the water column and passive transport by currents. E.g. Günther (1992) suggested that post-larvae of *A. marina* can be transported distances in the range of 1 km.

Wilde and Berghuis (1979) concluded that for the wild population they studied over 10 years, the average annual population mortality was 22%, and the annual recruitment was 20%, reporting that the abundance of the population remained stable. However, Newell (1948) reported 40% mortality of adults after spawning. Wilde and Berghuis (1979) estimated that the mortality of fed *Arenicola* at 10-15 °C is negligible, but increases to 20% over 130 days at 5 °C and at 20 °C the mortality could reach up to 50%.

In the wild, mature *Arenicola* form tubes at depths of 20 to 40 cm (Cowin et al. 2005). However, when cultured *Arenicola* tanks can have 5 cm deep substrate as described by Cowin et al. (2005), who developed the standard methods for growth of these species.

More information on *A. marina* is available in Chapter 4, which investigated its suitability for IMTA.

6.2.2 Mytilus edulis

The blue mussel (*Mytilus edulis*), also known as the common mussel, is a medium-sized edible marine mollusc in the family Mytilidae. Mussels are filter feeders with POM being their major food component (Van Haren and Kooijman, 1993). Mussels can therefore be considered to utilize DIN indirectly through the assimilation of the phytoplankton that directly requires DIN for growth and development (MacDonald et al. 2011). Troell and Norberg (1998) found that the ambient seston concentration is of greater importance in controlling mussel growth in a co-cultivation with salmon, and increases in suspended solids from the fish farm may only contribute significantly during periods of low phytoplankton production. It has also been

shown that mussels can ingest particulates from excess fish food or fish faeces released from fish farms (MacDonald et al. 2011; Handå, 2012). Other studies suggest that when mussels were co-cultured with fish actual assimilation of DIN did not occur in the field (e.g. Navarrete-Mier et al. 2010). Suspended particles in natural conditions are mixtures of organic and inorganic compounds that vary in size. *M. edulis* fully retains particles larger than 4 μm in diameter and retains 50% of the particles of 1 μm in diameter (Vahl, 1972; Mohlenberg and Riisgard, 1978), the ideal particle sizes for maximum retention efficiency range from 30-35 μm (Strohmeier et al. 2012) and the maximum recorded particle size ingested by bivalves is 400 μm (Cefas, 2008). Although, mussels are able to retain particulate matter of a variety of sizes, they still reject a large number of particles in the form of pseudofaeces. The selection process depends on the size and shape of the particles as well as on other physical attributes (Cefas, 2008).

The two most important parameters affecting mussel growth are temperature and food availability. However, at northern latitudes like the west coast of Scotland, temperature is the main limiting factor (Stirling and Okumus, 1995). Consequently, the effect of increased feed availability due to the presence of the fish farm might be less significant in more northern latitudes. The growing season for mussels on the west coast of Scotland is limited from May to October when the temperature is within the optimum range for mussel growth (10 - 20°C), under these conditions the mussels need approximately 24 months to reach market size (5 - 6 cm) (Stirling and Okumus, 1995).

6.2.3 Paracentrotus lividus

P. lividus is a species of sea urchin in the family Parechinidae commonly known as the purple sea urchin. Another member of this family, *P. miliaris*, was investigated in Chapter 3. P. *lividus* is a potential candidate species for IMTA, there is a direct trophic linkage between the urchins and salmon feed, provided the sea urchins are appropriately situated within the trajectory of feed waste, are gaining shelter from the salmon cages and are housed in cages of the correct mesh size. Cook and Kelly (2007b) showed that *P.*

lividus can thrive in proximity to salmon farms and suggested that integration of *P. lividus* with Atlantic salmon is a viable method of culturing this species in Scotland.

P. lividus is gonochoristic and fertilization is external. Spawning is synchronized and triggered by external environmental signals (Spirlet et al. 1998; Spirlet, 1999) in early spring (Allain, 1975; Spirlet et al. 1998) and in some localities in autumn (Crapp and Willis, 1975; Fernandez, 1996). Constant artificial conditions lack the "usual" stressors (low temperature, lighting variation, lower quality or lack of food during winter) that trigger spawning and thus the annual reproductive cycle fades. Under such conditions, the echinoids tend to bypass the growth phase of the gonads and have permanent gametogenesis, giving rise to “flabby” gonads with few nutritive phagocytes.

The minimum market size is a 40 mm diameter (Grosjean et al. 1998). Sea urchin size is evaluated by means of the diameter, which is measured to the ambitus of the test (the hard shell that surrounds the internal organs) considered without spines. The test diameter defined as the diameter without the spines. Grosjean et al. (1998) defined as sub adults the individuals whose size exceeds 10 mm but are below the minimum market size of around 40 mm. They are potentially mature but not large enough for the market. Consequently, their somatic growth performances must be promoted while their gonadal growth should be kept as low as possible to optimize food allocation to the soma. Juvenile's individual growth in test diameter is slow; it accelerates for sub-adults but then scatters for intermediate sizes (15 to 35 mm) (Gosjean, 2001). When echinoids approach asymptotic size, their growth rate drops (Gosjean, 2001). Hence, the initially fastest growing individuals are eventually caught up by individuals with test diameter around the minimal market size. This minimal market size is attained between 1.7 and 3.5 years old (respectively 10% and 90% of the individuals are larger than 40 mm) with a median value of 2.6 years (Gosjean, 2001) (Table 6-1). The maximum recorded age of *P. lividus* in wild populations in the Adriatic Sea is 15.06 years (Tomšić et al. 2010).

Such mature adults can reach a maximum size of up to 70 mm (Grosjean personal obs.).

Table 6-1: *P. lividus* age and mean survival rate (from the original number of embryos) at each rearing stage, in a controlled environment land–based system (Grosjean et al. 1998)

Developmental stage	Age	Mean survival rate (%)
Embryos	4 hours	100
Competent larvae	16 - 25 days	56.4
Postlarvae	competent larvae + 1 days	45.3
Juveniles	postlarvae + 10 days	24.7
Sub-adults	ca. 9 months	1.2
Adults	1.7 - 3.5 years	0.6

6.3 Materials and Methods

6.3.1 Model set-up and parameterisation

The model output presented in this chapter is derived using the model described in chapter 5. The model follows the fate of nitrogen throughout the whole nutrient pathway, from initial nitrogen input via the salmon feed to retention and eventual environmental loading by the extractive organisms. The simulation time zero is April first. An identical IMTA scenario simulation was run for the three different organic extractive species. At each scenario the sub model of the organic extractive species was represented by one of the three species and the rest of the model (as presented in Chapter 5) remained the same. In order to ensure the model outputs were directly comparable, the three models simulated the production of the same total wet biomass for each of the three extractive species and all the other model parameters, such as fish biomass produced and the environmental parameters remained the same.

The invertebrate growth and nitrogen uptake and release is modelled using the “standard” DEB. The “standard” DEB model is the simplest model that describes assimilation, maintenance, development, growth and reproduction

of an organism throughout all stages of its life cycle in a dynamic environment (Sousa et al. 2010). Four state variables define an individual: the structural mass, the reserve mass, the cumulative mass invested into maturity and the mass allocated to the reproduction buffer during the adult stage. In this study a dynamic energy budget-based continuous-time model is used to describe the uptake of the food, storage in reserves and allocation of the energy to growth, maintenance and reproduction. Two forcing variables characterize the environment: the temperature *T* (K) and the food density (x). A discrete-event process is used for modelling reproduction. At a fixed spawning date (triggered at a given date corresponding to literature data) of the year, the reproduction buffer is emptied and eggs with a fixed size and energy content form a new cohort. A detailed description of the DEB model is given in chapter five, section 5.2.4.

From a modeling perspective, reproduction is considered a net loss of energy. Due to the planktonic nature of invertebrate larvae, it is assumed that they will be dispersed from the IMTA site. Some individuals may settle within the IMTA area and in general the larvae will contribute in the restocking of the native populations. The proportion of the larvae that will settle within the IMTA site depends also on the IMTA design. For example, if the *Arenicola* are kept in tanks with sediment and slightly higher walls larvae dispersal could be decreased.

The DEB tracks the exchange of carbon but the model simulates the exchange of nitrogen. In order for the model to simulate the transfer of nitrogen among the different system components, the uptake and release of carbon by the invertebrates is converted to uptake and release of nitrogen, using the appropriate N/C ratio for the tissues of each invertebrate species. The N/C ratio (Tissue N quota, Q) in the sea urchin gonads was set to 0.127 (Tomas *et al*. 2005), for *Mytilus* it was 0.2 (Smaal and Vonck, 1997) and for the *Arenicola* 0.143 (Table 6-2).

The parameter values of the standard DEB model for the three invertebrate species used in this comparative study are given in Table 6-2. The extensive dataset for DEB parameterisation available at “add_my_pet” library (Kooijmann, 2014) is used as the main source for model

parameterization, for the DEB models that simulate invertebrate growth and nitrogen assimilation. The invertebrate models are then linked with other models and with environmental parameters for the simulation of a functioning IMTA system (see chapter 5 Figure 5-1 and Appendix Figures 1 to 4).

The initial structural volume of the organism was used for calculating the initial value of the level that estimates the energy fixed in structural growth (E_v). For example, for a sea urchin with initial test diameter 1.01 cm the value of E_{vo} can be calculated by:

$$E_{vo} = (L * \delta_M)^3 * [E_G] = (1.01 * 0.5251)^3 * 2748 = 409.93\, J$$

where, $[E_G]$ is the volume-specific cost of *P. lividus* growth, $[\delta_M]$ is the shape coefficient and [L] is the structural length.

The quality of the invertebrate products destined for sale was quantified using their condition index. The condition index is the ratio of dry tissue weight to wet tissue weight.

Table 6-2: List of the parameters implemented in the DEB model for the *M. edulis, P. lividus* and *A. marina* submodels. All values, unless indicated, originate from Kooijmann (2014).

Symbol	Description	*P. lividus*	*M. edulis*	*A. marina*	Unit
$\dot{j}_x$	Maximum surface area-specific consumption	6.943	1.018	1.06	mg C cm^{-2} d^{-1}
K_o K_o	Reference reaction rate at 288 K	1	1	1	n/a
T_A T_A	Arrhenius temperature	8000	7022	8000	K
T_o T_O	*R*eference temperature	293	283	293	K
T_{AL} T_{AL}	Arrhenius temperature at lower boundary	50000	45430	50000	K
T_L T_L	Lower boundary tolerance	273	275	273	K
T_{AH} T_{AH}	Arrhenius temperature at upper boundary	190000	31376	190000	K
T_H T_H	Upper boundary tolerance	400	296	296	K
μ_{cj} μ_{cj}	Ratio of carbon to energy content	83.3	83.3	83.3	$J\ mgC^{-1}$
K	Catabolic flux to growth and maintenance	0.801	0.732	0.944	[1]
$[E_G]$ $[E_G]$	Volume–specific cost of growth	2748	4783	2514	$J\ cm^{-3}$
k_R k_R	Reproductive reserves fixed in eggs	0.95	0.95	0.95	[1]
μ_V μ_v	*S*tructure energy quota	500000	100 (j/g)	100 (j/g)	$J\ mol^{-1}$
ρ ρ	Biovolume density of cultured animals	0.105	0.009	0.15	$g\ cm^{-3}$
Q	Tissue N-quota	0.127[a]	0.2	0.143	$mgN\ mgC^{-1}$
V_p V_p	Structural volume at puberty	0.244	0.040	2.972	cm^3

$[\dot{P}_M]$	Volume–specific maintenance rate	16.32	7.749	34.49	J cm^{-3} d^{-1}
μ_E μE	Reserve energy content	22415.9	27795.3	20033.2	$J\ g^{-1}$
$[E_M]$ [EM]	Maximum reserve density	6056.34	1105.39	1810.43	$J\ cm^{-3}$
X_K XH	Half-saturation uptake of food	0.002	0.002	0.002	$gC\ m^{-3}$
Q_s	C : N ratio in sediment	7[c]	7[c]	7[c]	[1]
δ_M δM	Shape coefficient	0.5251	0.294	0.103	[1]
T T	Water Temperature	6.8-13.7[d]	6.8-13.7[d]	6.8-13.7[d]	°C
δ_r δr	Natural mortality	0.00102[e]	0.0003[f]	0.0014[g]	Individuals d^{-1}
δ_H δH	Harvest mortality	0[h]	0[h]	0[h]	Individuals d^{-1}
$\{\dot{P}_{Am}\}$	Maximum surface area specific assimilation	6.85578	79.698	113.586	J cm^{-2} d^{-1}

Rates are given at the reference temperature T_1 = 293 K (= 20 °C). Most of the parameters used in the three models were taken from the "add_my_pet" dataset that can be found at: http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/Species.html. For the mussels Saraiva (2010) http://www.bio.vu.nl/thb/deb/deblab/add_my_pet_old/mydata/html/mydata_Mytilus_edulis.html

[a] Tomas et al. 2005

[b] Smaal and Vonck, 1997

[c] Representative value for an average Scottish salmon farm site

[d] Site-specific time series variable

[e] (Test diameter < 2cm), 0.00056 (test diameter > 2cm) Turon et al. 1995

[f] Wilde and Berghuis, 1979

[g] Karayücel and Karayücel, 1999

[h] Harvest at the last time step of the simulation

6.4 Species–specific parameterisation

6.4.1 Arenicola marina

6.4.1.1 Arenicola marina stocking density

According to Olive et al. (2006) any density of worms may be used, however a density of 100 to 300 worms m^{-2} gives good growth results. According to the depth of the substrate densities of up to 1000 worms m^{-2} may be used (Olive et al. 2006). In this study the model was parameterized to simulate a stocking density of 300 worms m^{-2}. The worms can be placed on trays directly on the sea floor or the trays can be suspended. For the production of 6,699,146 *Arenicola* (50 t wet weight) with a stocking density of 300 worms m^{-2} more than 23,330 m^2 are required if the trays are placed only on the sea floor. However if more levels of suspended trays are used then the area required for assimilating the same amount of waste decreases significantly. For examples if five levels are used then only 4,466 m^2 are required. This is based on the assumption that enough waste for the growth of the *Arenicola* will reach all trays.

6.4.1.2 Arenicola marina species–specific parameterisation

A. marina individuals reach sexual maturity by their second year (Newell, 1948; Wilde & Berghuis, 1979) but may mature by the end of their first year in favorable conditions depending on temperature, body size, and food availability (Wilde & Berghuis, 1979). Consequently, in the model simulation they will reproduce towards the end of their 18-month period at the IMTA site. Reproduction frequency is annual episodic (breed every year but in one discrete period initiated by a trigger) so the reproduction buffer is emptied at a fixed spawning time during the year and a new cohort is formed by eggs of a fixed size and energy content.

At the simulation scenario, the *Arenicola* will be grown until they reach commercial size (5 to 6 g), *Arenicola* of that size can be used for fishing or for food, especially aquaculture (Cowin et al. 2005).

6.4.2 Mytilus edulis

6.4.2.2 Mytilus edulis stocking density

The stocking density per longline is given by the number of mussels per meter rope, the frequency of ropes per longline and the length (how deep in the sea they reach) of the ropes. The longline spacing has a threshold value, below which there is growth reduction and spatial growth variability (Rosland et al. 2011). Similarly, Cubillo et al. (2012) found a negative effect of density on growth of *M. galloprovincialis*. However, in that study the effects of density on growth started when individuals reached sizes around 66 ± 1.3 mm, which is more than the final size of the mussels at the present simulation (6.29 cm). The reduced growth and spatial growth variability is a consequence of reduced water flow and seston supply rate and the increased filtration due to higher mussel densities (Rosland et al. 2011). The spacing threshold is moderated by other farm configuration factors and environmental conditions (Rosland et al. 2011). Using the model developed at Rosland et al. (2011) a more informed decision concerning the stocking density suitable for a specific site can be made. A typical mussel density of 500 individuals m^{-1} vertical rope and a separation distance of 0.5 m per rope attached to the longlines was used for the model, equivalent to a longline stocking density of 1,000 individuals m^{-2}. Consequently, for the production of 529,007 mussels (50 t) 10,529 m^2 are required. The longlines are usually oriented parallel to the main current directions so that water can flow through the channels delimited by the longlines and the vertical ropes (Rosland et al. 2011), but the exact set up should be site specific.

6.4.2.3 Mytilus edulis species specific parameterisation

Karayücel and Karayücel (1999) estimated that the mean cumulative mortality rate of one–year–old rope–grown mussels of the species *M. edulis* in Loch Kishorn (Scotland) over a 15 month–long trial was 15.15%. If the mortality rate in 15 months (455) days is 16.7% then the daily mortality rate is 0.14%.

In this study the initial seed size was 2 cm. In general, the initial size of seed depends on the on-growing method. For example for bottom cultivation the shell length of the seed used is 1-3 cm (one–year–old seed). Mussels on bouchots or long–lines can be harvested after 18-24 months of growth (Beaumont et al. 2007).

6.4.3 Paracentrotus lividus

During the grow-out stage of *P. lividus* juveniles, the stocking density of a sea urchin carpet is approximately 400 individuals m^{-2} (Carboni, 2013). Consequently, for the production of 561,428 sea urchins (50 t), 1,404 m^2 are required. The grow-out method used for open water sea urchin aquaculture varies. For example, Cook and Kelly (2007b) used pyramidal 'pearl' nets (mesh size 5.0 mm; dimensions 40 x 40 x 30 mm), commonly used in the shellfish industry, at a density of 40 individuals per net. This would equivalent to 250 individuals m^{-2} but possibly the spacing between the lanterns both vertically and horizontally in the water column could be manipulated to allow a higher density, provided that enough food for the growth of the urchins reaches all lanterns.

6.5 Comparison of DEB results

The bioremediation performance of the three species was compared weight by weight and by area required for their cultivation, as estimated by the integrated model. The simulation was run twice for each species once to estimate the nitrogen bioremediation during the production and harvest of 50 t wet weight of an invertebrate species, and the second time to estimate the nitrogen bioremediation during the production and harvest of the invertebrate/extractive species using a total area of 2,000 m^2.

6.6 Results

6.6.1 Growth results

6.6.1.1 Arenicola marina

The initial size of the *Arenicola* at simulation time zero is 0.8 cm physical length and 290 mg wet weight. At the end of the simulation the *Arenicola*

grew to market size. The average length was 13.6 cm, the average wet weight 7.46 g and the average dry weight 679 mg (Figure 6-1 and Figure 6-3). The *Arenicola* are normally harvested at the end of their second summer, shortly before the average worm has reached sexual maturity and when their average length is 13.6 cm (Figure 6-1and Figure 6-2).

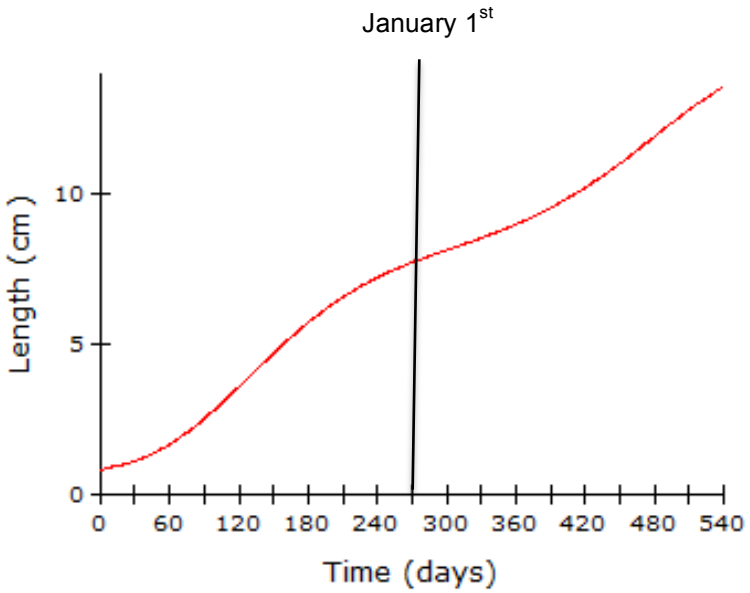


Figure 6-1: Modelled *A. marina* growth in length over the simulation period.

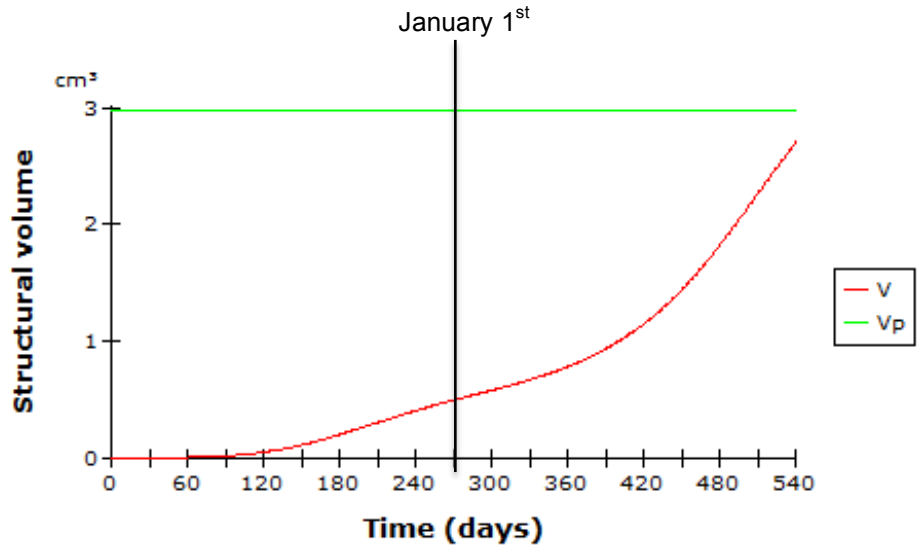
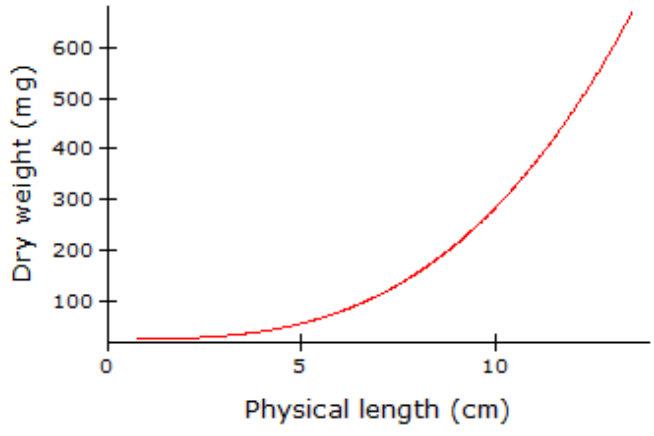
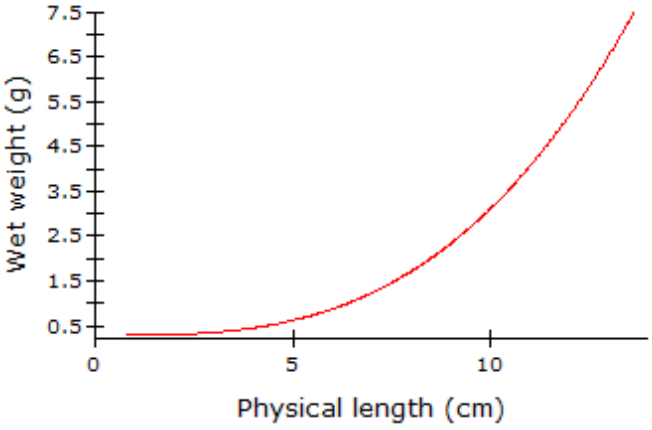


Figure 6-2: *A. marina* structural volume *(V)* during the simulation period and *A. marina* the structural volume at puberty *(V_p)*.



(a)



(b)

Figure 6-3: *A. marina* submodel simulation output for: a) the length - dry weight relationship b) the length - wet weight relationship.

At simulation time zero the only DIN and PON available at the site is that which is naturally available. Under the simulation conditions set for this study the initial level (simulation time zero) of both DIN and PON is set to 10 mg m^{-3}. From time step one onwards, their concentration increases along with the increase of feed input due to the gradual growth of the salmon, up to the end of the simulation (Figure 6-2). At the beginning of the simulation the food concentration (X) is below the level of the half–saturation coefficient (X_H), which leads in a relatively low scaled functional response (f) during the first months of the simulation (Figure 6-3). A few months after simulation time zero, the scaled functional response approaches 1 (Figure 6-3).

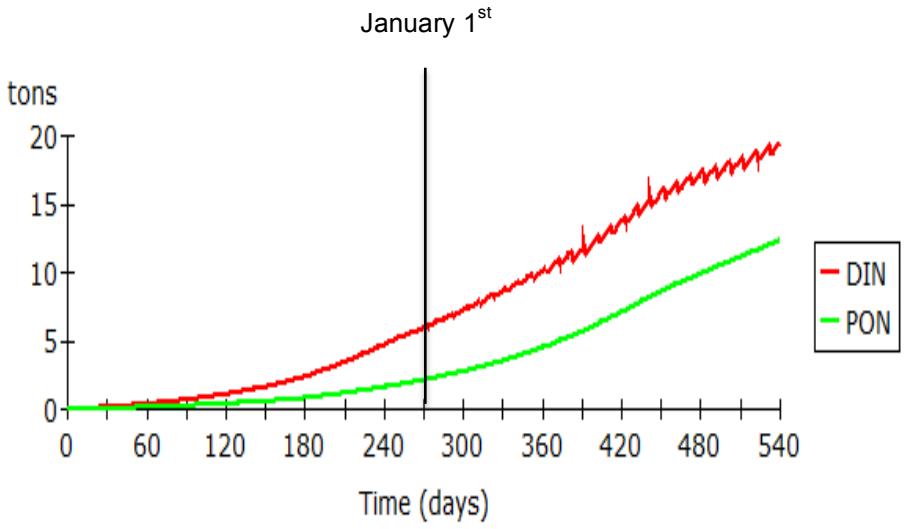
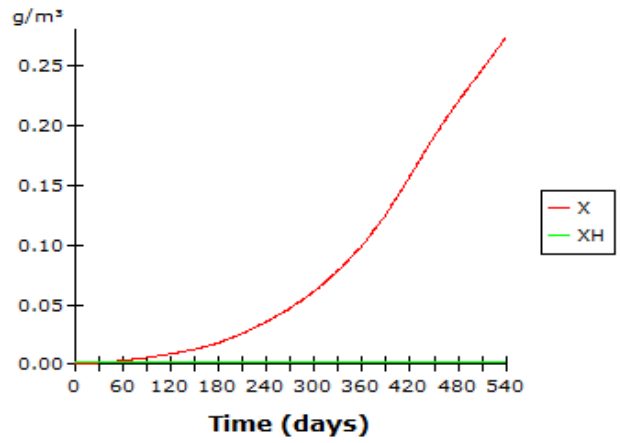
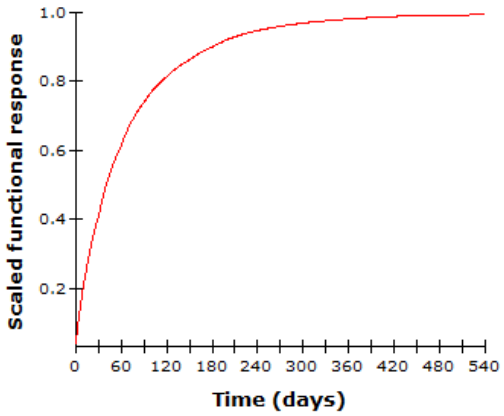


Figure 6-4: DIN and PON available at the IMTA site, for low ambient PON concentration.



(a)



(b)

Figure 6-5: a) The half saturation coefficient *(X_H)* and the food concentration *(X)* under the simulation scenario conditions b) Scaled functional response under the simulation scenario conditions for *A. marina* (low ambient PON concentartion Figure 6-4).

Under the simulation low nutrient conditions set for this study (Figure 6-4), it is clear that in the beginning (first two months) of the simulation the food concentration (X) is below the level of the half saturation coefficient (X_H), which leads to a relatively low-scaled functional response during the first months of the simulation (Figure 6-5). When the PON concentration is high already at simulation time zero (Figure 6-6) then the feed is sufficient for a high growth rate and thus the lugworms achieve a higher final mass (Figure 6-7, Figure 6-8 and Figure 6-9).

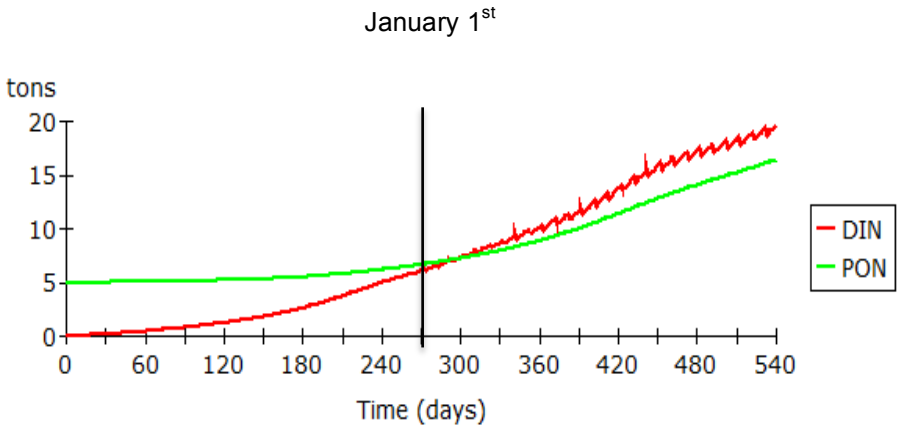
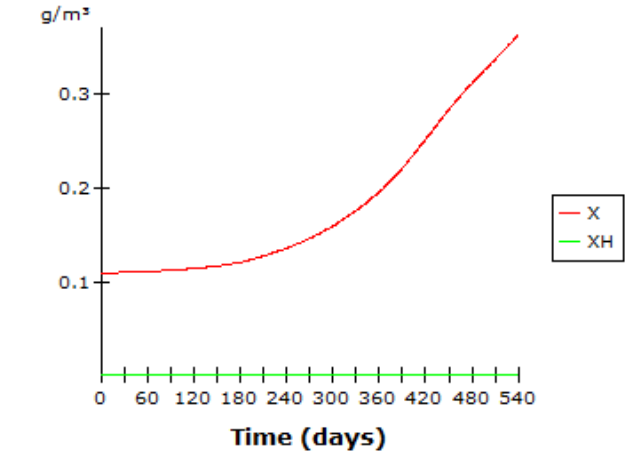
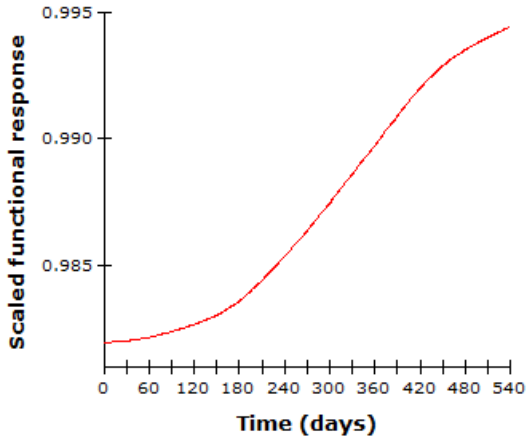


Figure 6-6: DIN and PON available at the IMTA site, for very high ambient PON concentration.

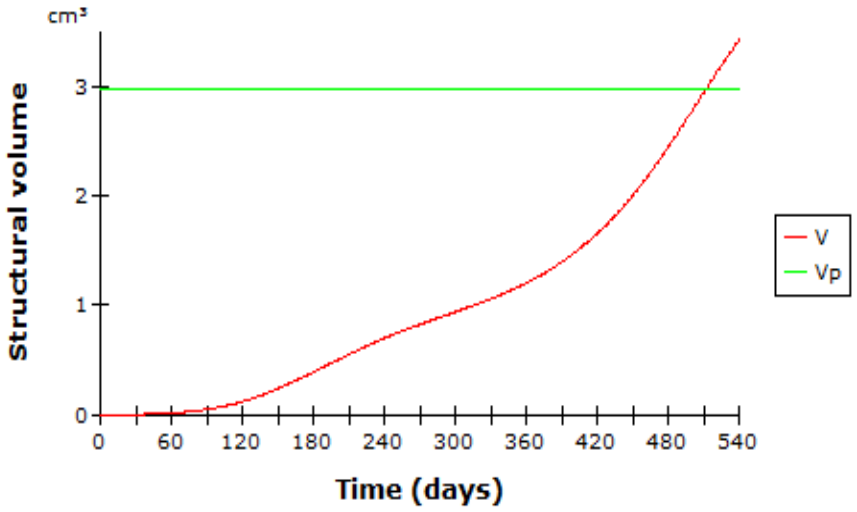


(a)

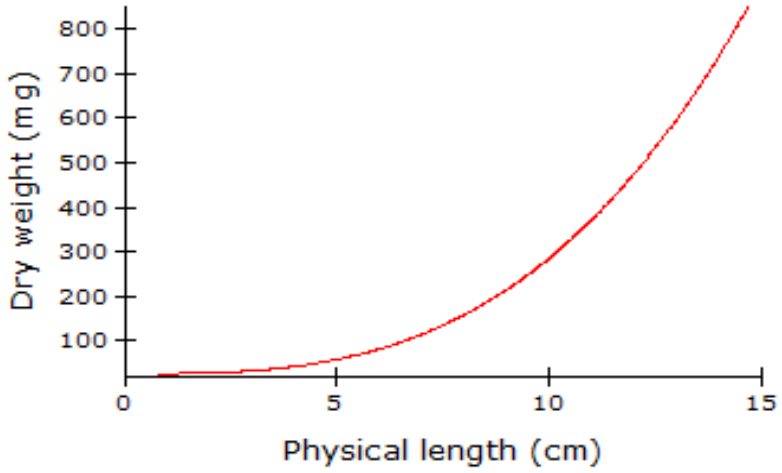


(b)

Figure 6-7: a) The half saturation coefficient *(X_H)* and the food concentration *(X)* b) Scaled functional response, under the simulation scenario conditions, when the PON availability is as shown at Figure 6-6 (high PON availability).

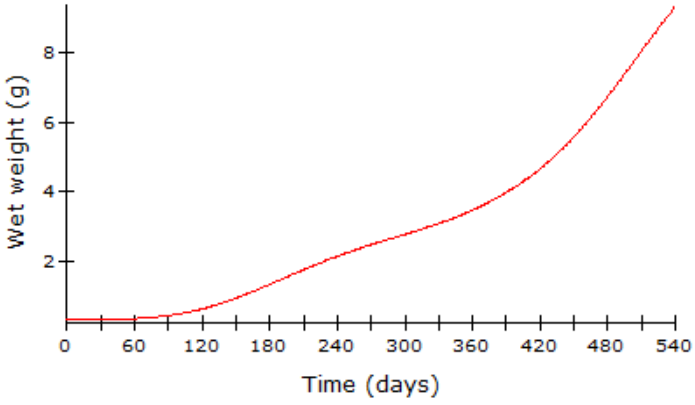


(a)



(b)

Figure 6-8: *A. marina* a) Volume *(V)* and structural volume *(V_p)* b) dry weight to length, under the simulation scenario conditions, when the PON availability is as shown at Figure 6-6 (high PON availability).



(a) (b)

Figure 6-9: *A. marina* wet weight, when PON availability is as shown at Figure 6-6 (high PON availability).

6.6.1.2 Mytilus edulis

At simulation time zero, the only DIN and PON available at the site is that which is naturally available. Under the simulation conditions set for this study, the initial level (simulation time zero) of both DIN and PON is set to 10 mg m^{-3}, as it was set for the lugworms. From time-step one onwards their concentration increases along with the increase of feed input due to the gradual growth of the salmon, up to the end of the simulation (Figure 6-2). The initial size of the mussels at simulation time zero is 2 cm physical length and 0.15 g wet weight. At the end of the simulation the mussels will have grown to market size. The average length will be 6.29 cm; the average wet weight 4.76 g and the average dry weight 1.05 g (Figure 6-11). The mussels are already mature at simulation time zero (Figure 6-12).

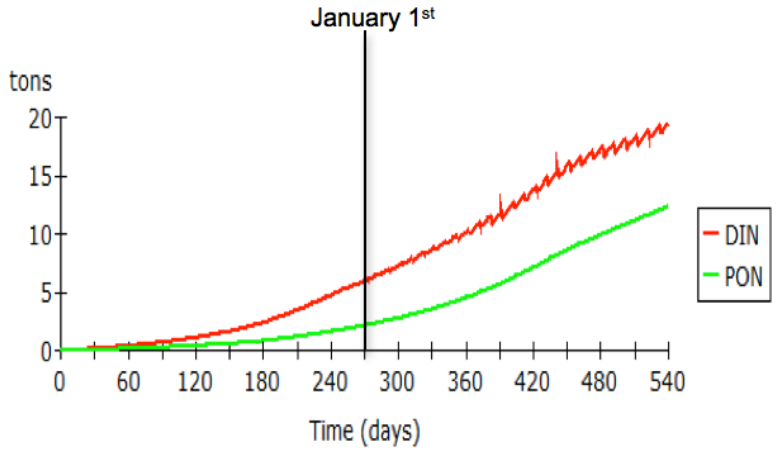


Figure 6-10: DIN and PON available at the IMTA site, for low ambient PON concentration.

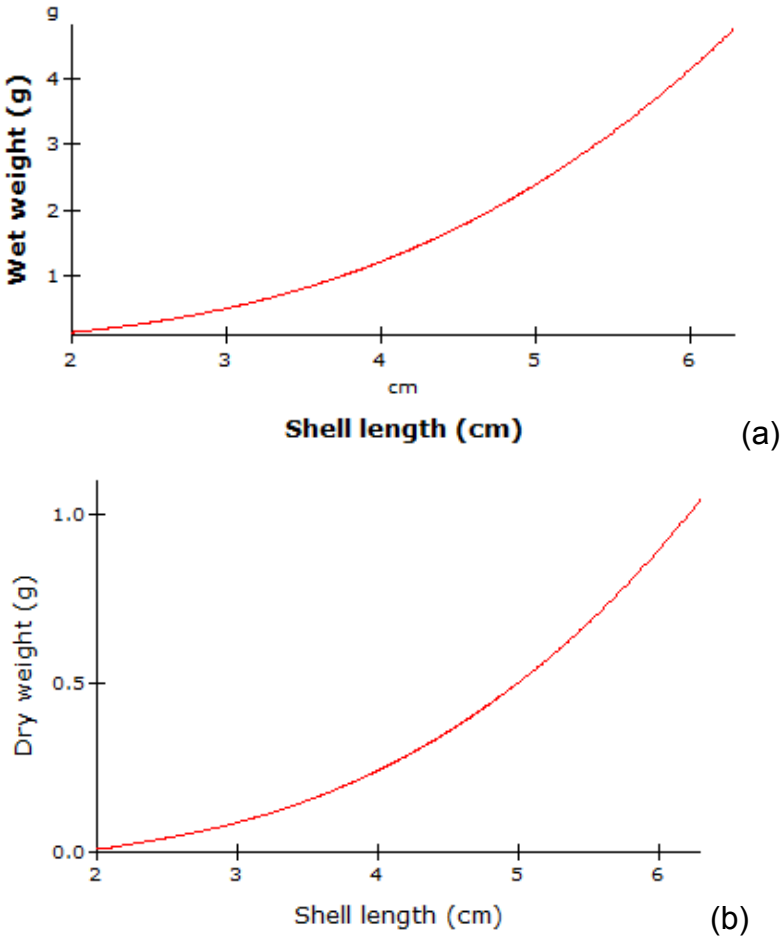


Figure 6-11: a) *M. edulis* physical length relationship to wet weight b) *M. edulis* physical length relationship to dry weight, under the simulation scenario conditions, when the PON availability is as shown at Figure 6-10.

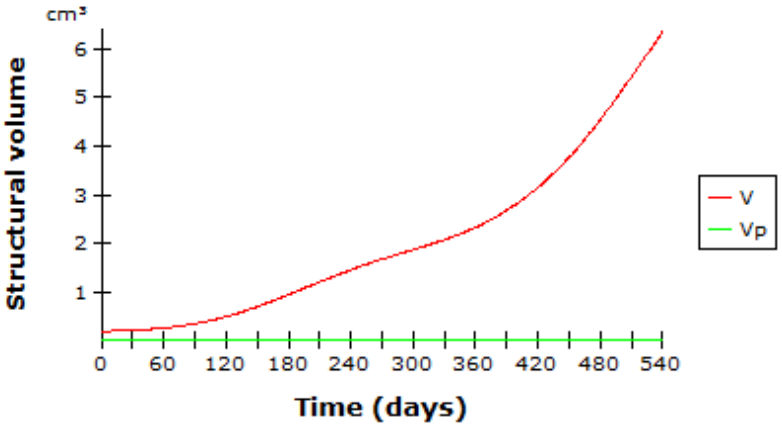
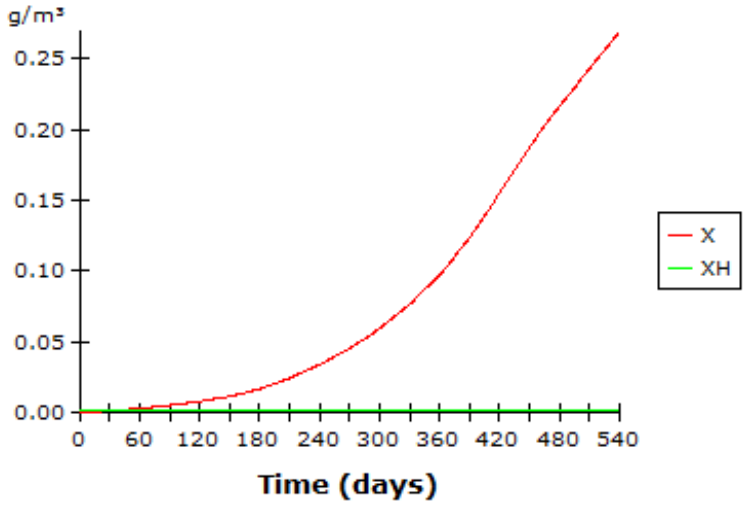
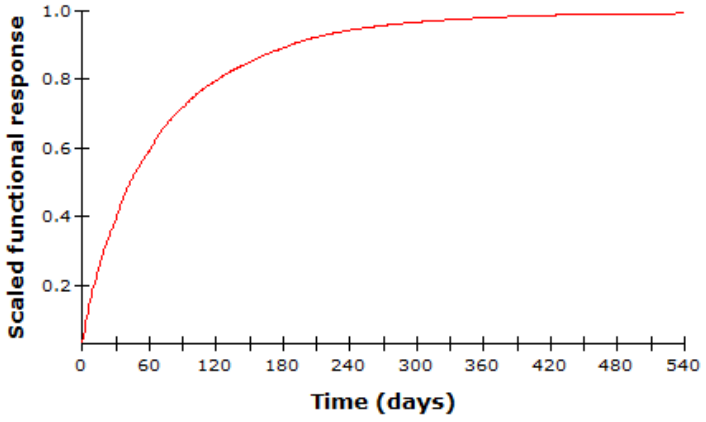


Figure 6-12: *M. edulis* structural volume *(V)* during the simulation period and *M. edulis* the structural volume at puberty *(V_p)*.

Under the simulation low nutrient conditions set for this study (Figure 6-10), it is clear that at the beginning of the simulation the food concentration (X) is below the level of the half saturation coefficient (X_H), which leads in a relatively low-scaled functional response during the first months of the simulation (Figure 6-13). When the PON concentration is high already at simulation time zero (Figure 6-14), then the feed is sufficient for maximum growth rate (Figure 6-15) and thus the mussels achieve a higher final mass (Figure 6-16).



(a)



(b)

Figure 6-13: a) The half-saturation coefficient *(X_H)* and the food concentration *(X)* b) Scaled functional response (*f*) under the simulation scenario conditions for *M. edulis*, when the PON availability is as shown at Figure 6-10.

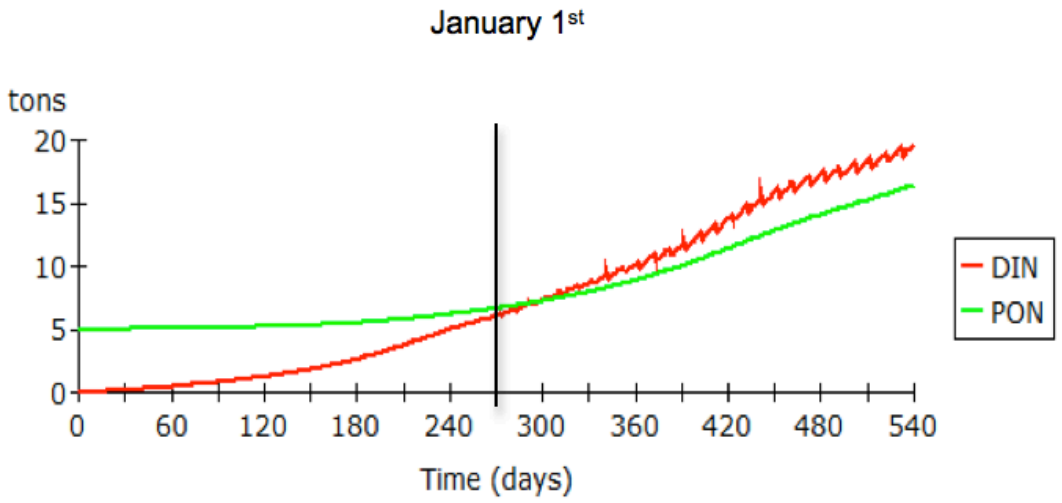
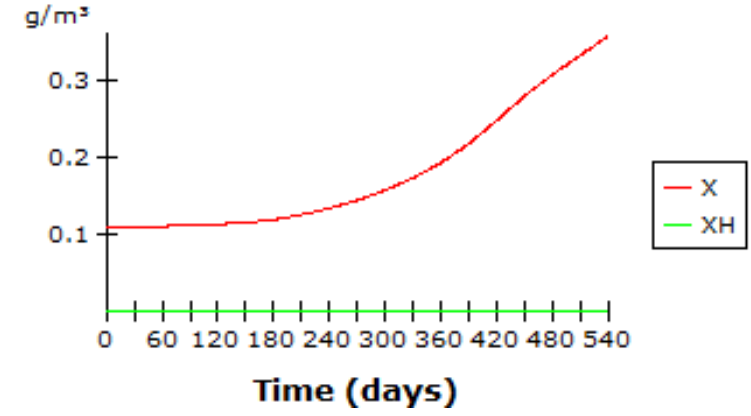
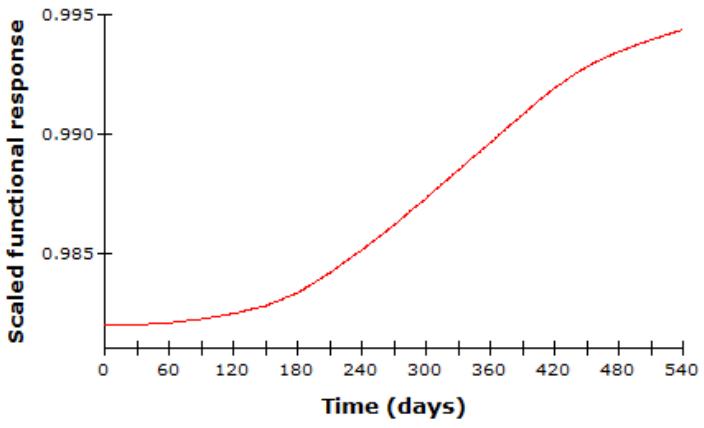


Figure 6-14: DIN and PON available at the IMTA site, for very high ambient PON concentration.

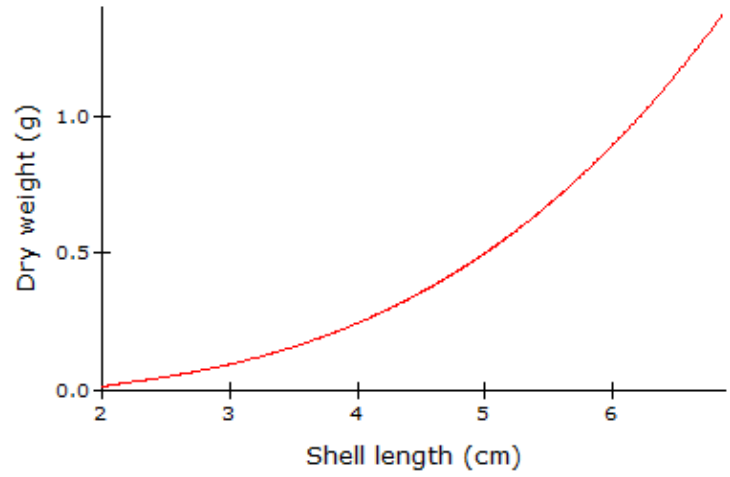


(a)

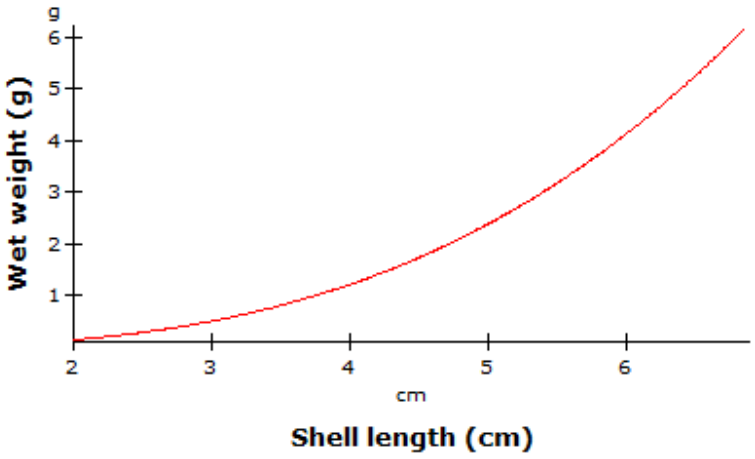


(b)

Figure 6-15: a) The half-saturation coefficient *(X_H)* and the food concentration *(X)* b) Scaled functional response, under the simulation scenario conditions for *M. edulis*, when the PON availability is as shown at Figure 6-14 (high PON availability).



(a)



(b)

Figure 6-16: *M. edulis* a) dry weight *versus* length b) wet weight versus length, under the simulation scenario conditions, when the PON availability is as shown at Figure 6-14 (high PON availability).

6.6.1.3 Paracentrotus lividus

The initial size of the sea urchins at simulation time zero is 1.01 cm physical length and 0.15 g wet weight. At the end of the simulation the sea urchins will have grown to market size. The average test diameter will be 4.47 cm, the average wet weight 87.46 and the average dry weight 37.16 g (Figure 6-17), when the PON availability is as shown in Figure 6-18. The average sea urchin reaches mature size within the first few days of the simulation (Figure 6-19), when the PON availability is as shown in Figure 6-18.

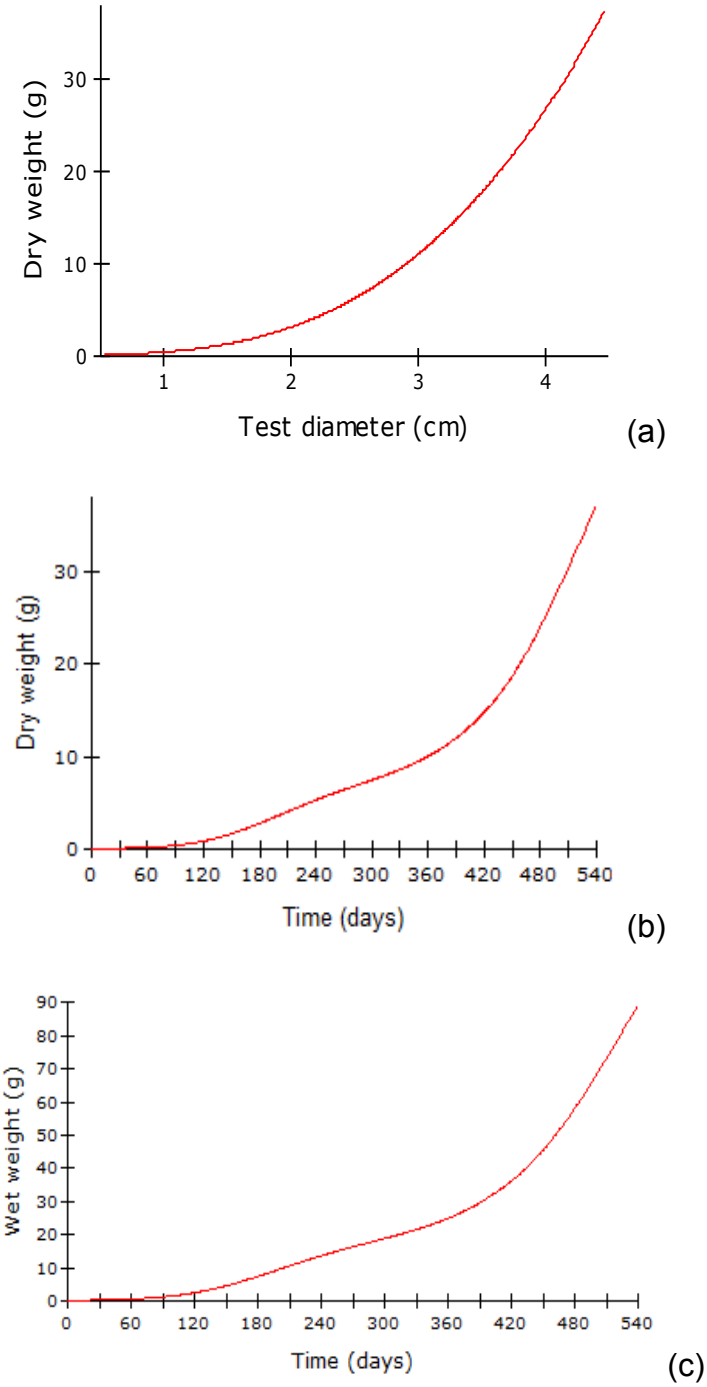


Figure 6-17: *P. lividus* submodel simulation output for: a) the test diameter–dry weight relationship b) dry weight c) wet weight, under the simulation scenario conditions, when the PON availability is as shown at Figure 6-18.

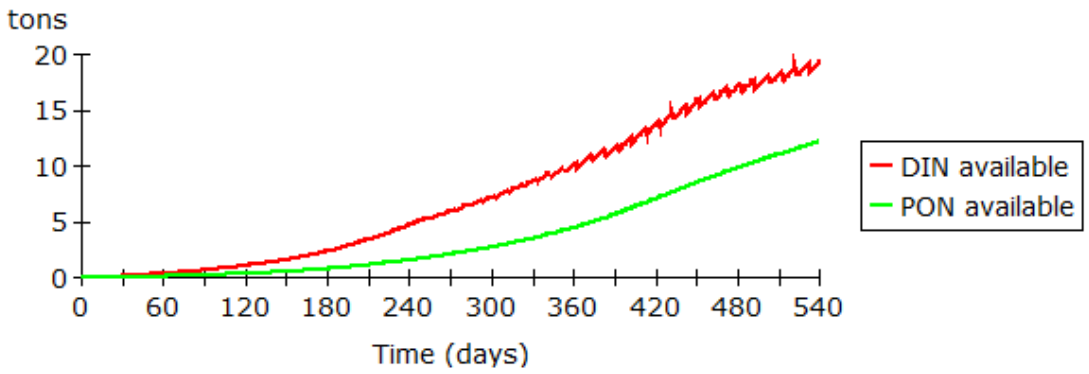


Figure 6-18: DIN and PON available at the IMTA site.

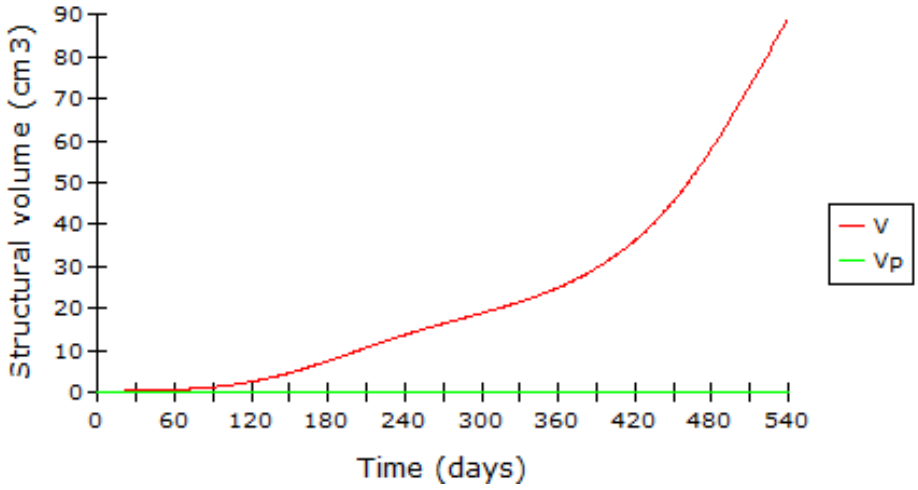


Figure 6-19: *P. lividus* structural volume *(V)* during the simulation period and *P. lividus* the structural volume at puberty *(V_p)*, when the PON availability is as shown at Figure 6-18.

Under the simulation low nutrient conditions set at this study (Figure 6-18), it is clear that the beginning (first 45 days) of the simulation the food concentration (X) is below the level of the half saturation coefficient (X_H), which leads in a relatively low scaled functional response during the first months of the simulation (Figure 6-20). When the PON concentration is high already at simulation time zero (Figure 6-21) then the feed is sufficient for maximum growth rate (Figure 6-22) and thus the sea urchins achieve a higher final mass (Figure 6-23).

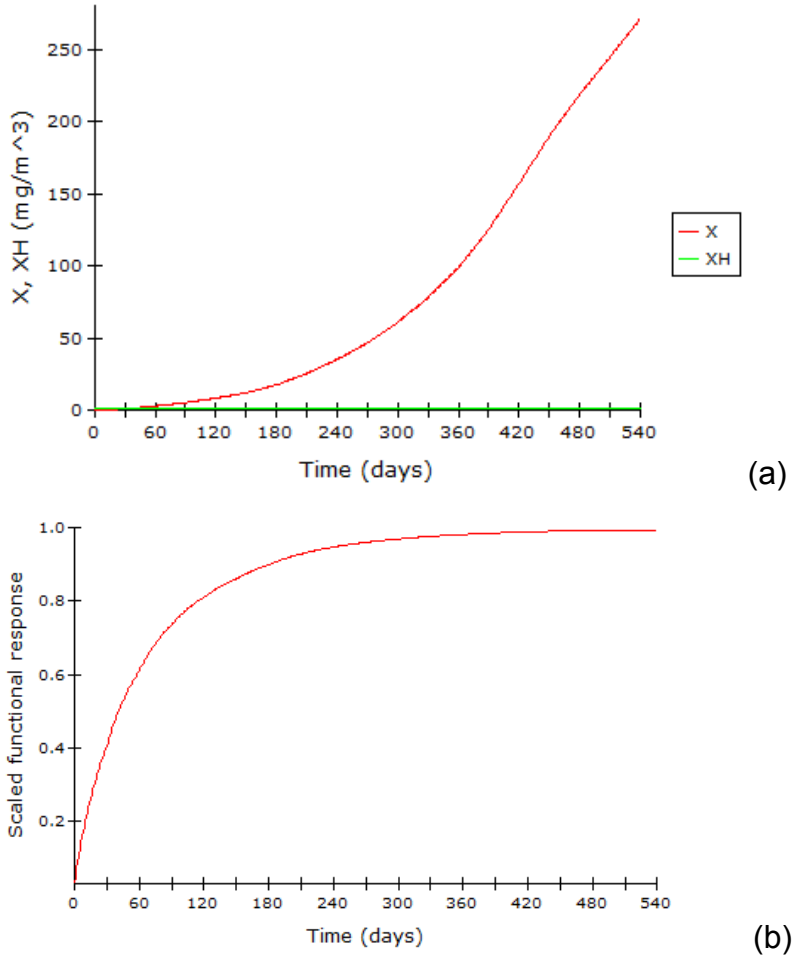


Figure 6-20: a) The half–saturation coefficient *(X_H)* and the food concentration *(X)* b) Scaled functional response, under the simulation scenario conditions for *P. lividus*, when the PON availability is as shown at Figure 6- 18.

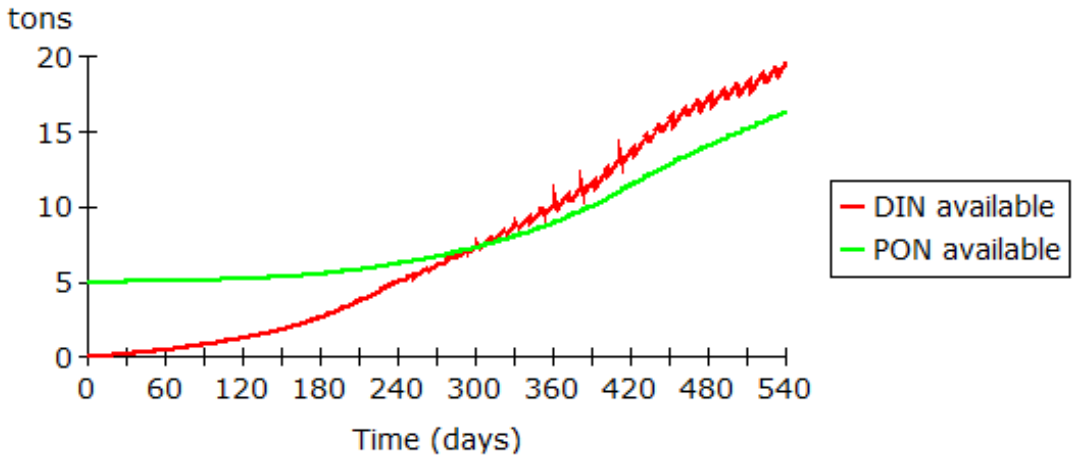


Figure 6-21: DIN and PON available at the IMTA site (high PON availability).

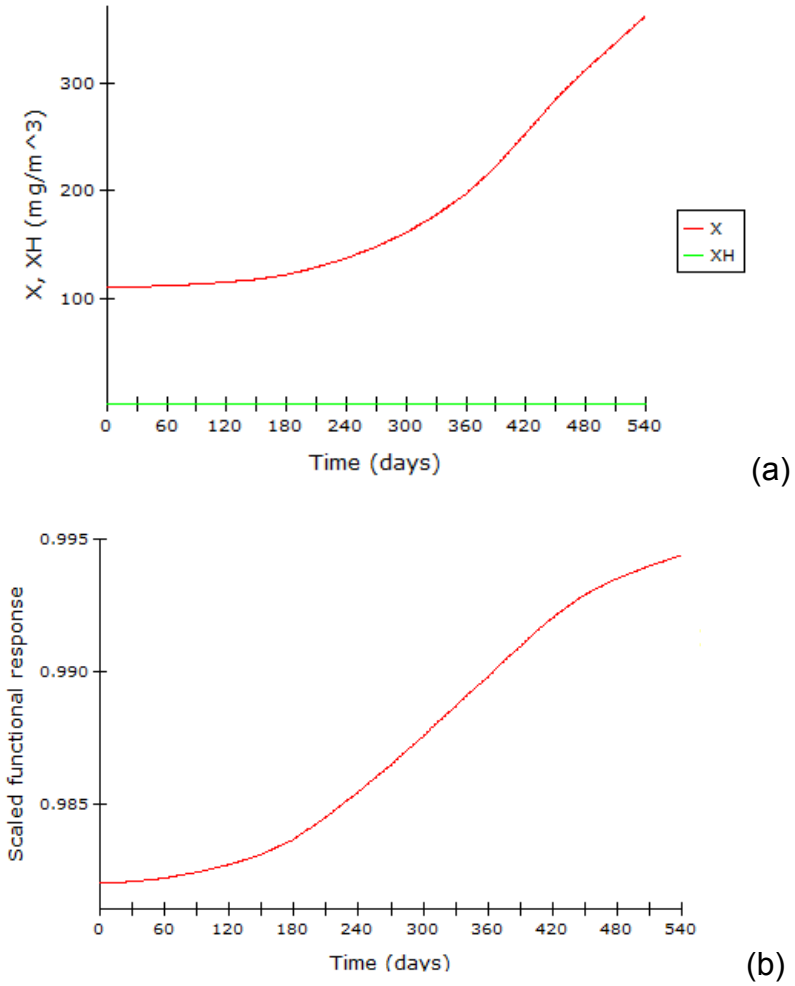


Figure 6-22: a) The half–saturation coefficient *(X_H)* and the food concentration *(X)* b) Scaled functional response, under the simulation scenario conditions for *P. lividus*, when the PON availability is as shown at Figure 6-21 (high PON availability).

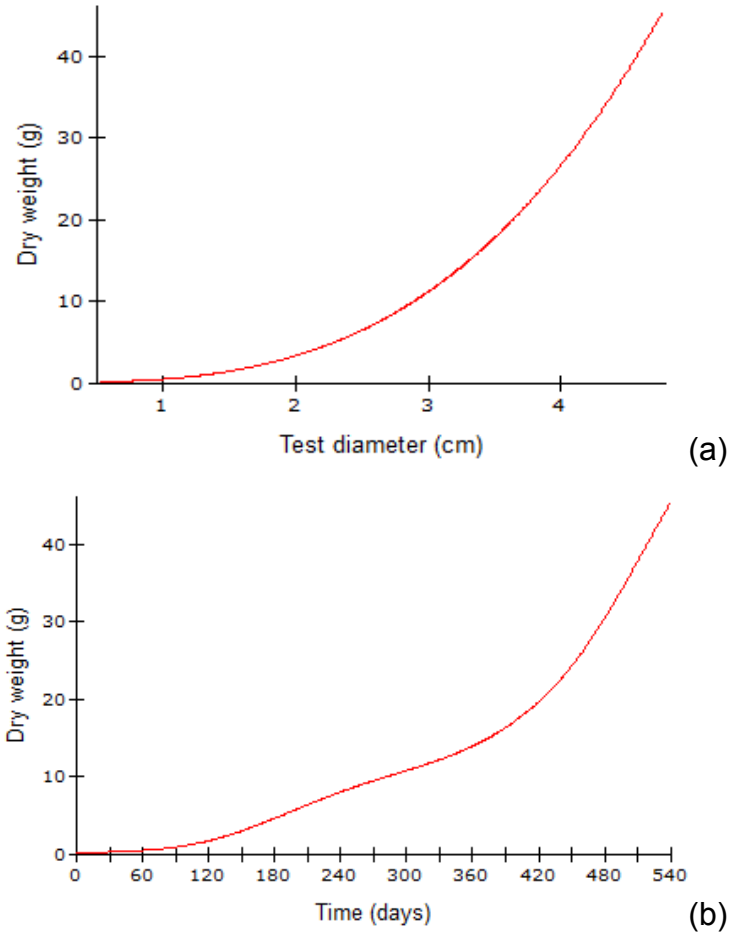


Figure 6-23: *P. lividus* simulation output for: a) the test diameter-dry weight relationship of *P. lividus* b) *P. lividus* dry weight, under the simulation scenario conditions, when the PON availability is as shown at Figure 6-21.

6.7 Comparison of bioremediation results

The three organic extractive species (invertebrates) have different PON assimilation potentials. Table 6-3 illustrates the output of three identical simulation runs for three versions of the IMTA model; the only difference among the IMTA model versions is that they each include a submodel for a different invertebrate species. Each version of the model was run for the production of a 50 t wet weight of invertebrates. The amount of PON assimilated by the organic extractive species as well as the spatial requirements for the production of 50 t of each of the invertebrate species is illustrated in Table 6-3. Similarly, Table 6-5 shows the bioremediation potential of each of the invertebrate species, but this time the comparison is made in terms of the area required for their cultivation instead of the

tonnage produced. Specifically, it compares the bioremediation potential of 2,000 m^2 of each of the invertebrate species.

Table 6-3: PON accumulated and area required for the production of 50 t soft body tissue biomass of three different organic extractive species.

Extractive species	PON assimilated (kg)	Number of individuals	Area required (m^2)	Wet biomass produced (t)	Dry biomass produced (t)
A. marina	260.76	6,699,146	4,466	50	4.55
P. lividus	1,004.64	561,429	1,404	50	20.86
M. edulis	1,268.81	10,529,007	10,529	50	10.97

The stocking densities described in section 6.2 were used as the final stocking densities (the density at the last step of the simulation), since when the invertebrates are smaller the stocking density can be higher because they need less food and take up less space. The model was fitted manually by running several times, each time changing the intitial number of individuals until the final number of individuals/final biomass was the desired one (Table 6-4).

Table 6-4: Initial and final number of individuals of each species, that can be grown in an area of 2,000 m^2.

Extractive species	Stocking density (individuals m^{-2})	Individuals at simulation time zero	Individuals harvested
A. marina	1,500	6,392,606	3,000,000
P. lividus	400	1,179,705	800,000
M. edulis	1,000	2,364,516	2,000,000

This comparison shows that weight for weight, *M. edulis* is 20% more efficient as a bioremediator than *P. lividus,* which is in turn 80% more efficient than *A. marina* with regard to the amount of nitrogen they can assimilate. But when the model was run for the production of invertebrates using the same cultivation area (space for space comparison), instead of the same amount of total biomass (weight for weight comparison), then *P.*

lividus was 85% more efficient as a nutrient bioremediator than *M. edulis* that was in turn 50% more efficient than *A. marina.* Table 6-6 illustrates clearly the difference in the bioremediation potential of the three species by showing the PON bioremediation potential of the three species per tonne wet biomass produced and per square meter.

Table 6-5: Soft body tissue biomass produced and PON accumulated by three different organic extractive species, utilising the same total area.

Extractive species	PON assimilated (kg)	Number of individuals	Area required (m^2)	Wet biomass produced (t)	Dry biomass produced (t)
A .marina	116.92	3,000,000	2,000	22.42	2.04
P. lividus	1,426.45	800,000	2,000	71.02	29.63
M. edulis	245.64	2,000,000	2,000	9.68	2.13

Table 6-6: PON assimilation potential of the three different organic extractive species.

Extractive species	kg PON ton^{-1} wet biomass	kg m^{-2}
A. marina	5.22	0.06
P. lividus	20.09	0.72
M. edulis	25.38	0.12

6.8 Discussion

6.8.1 Validation of results with literature data

6.8.1.1 Arenicola marina

This is the first study that describes the results of a DEB model for *A. marina*, although the DEB constants have already been calculated at the DEBlab's "add_my_pet" dataset.

6.8.1.1.1 Bioremediation potential

The protein content of *A. marina* fed with brewery yeast was found to range between 68-78% of dw, but it varies even more depending on the diet (Olive et al. 2004). According to the above for the production of 10.29 t of dry

Arenicola, the protein content of the *Arenicola* would range between 6997 and 8026 kg. The amount of nitrogen, estimated by dividing the crude protein with 6.25, would range from 1120–1284 kg. The model estimated that 914.1 kg of PON are accumulated in the biomass of *Arenicola* individuals (Table 6-5), which is slightly lower than what was accumulated by the *Arenicola* at Olive et al. 2004, but still within the same scale.

The relationship between wet and dry weight is in agreement with the simulation output, since marine worms typically comprise approximately 80% water (Olive et al. 2004) and 80% of 50 t is 10 t (in the model it is 50 t ww are 10.29 t dw). Similarly, for sedentary polychaetes the Ash Free Dry Weight (AFDW)/ww = 0.014, the AFDW/dw = 0.752 and the dw/ww = 0.177 (Ricciardi and Bourget, 1998). The *A. marina* ash content ranges between 6.8 and 7.8% dw (Olive et al. 2004). At the simulation the dry to wet weight ratio is 0.2489.

6.8.1.1.2 Growth

Newell (1948) studied an *Arenicola* population in British waters. From that study it was concluded that *Arenicola* from eggs spawned in autumn reach a length excluding the tail of 0.8 cm in April (six months old; the starting length in this study) and 4.3 cm excluding the tail by September (10-12 months old; four to six months after simulation time zero, day 120-180). The above is in agreement with this study, as illustrated in Figure 6-1. By the following April and May (1.5 years old; one year after simulation time zero, day 365) the young worms that had reached a length of 4 cm in the previous September will have reached the average length of the adult size group (around 11 cm) (Newell, 1948). Similarly, Flach and Beukema (1994) found that in the Wadden Sea, juveniles grow from about 1 cm to about 5 cm reaching a weight of a few to several tens of mg, up to about 100 mg AFDW from the beginning of their first spring (around simulation time zero) to the end of their first summer (around simulation day 150). The above results are in agreement with the simulation results (Figure 6-1 and Figure 6-3). They reach adult size and weight (several hundreds to a few thousands of mg AFDW) at end of their second summer (Flach and Beukema, 1994). *A.*

marina individuals become sexually mature in their second summer and spawn when they are about two years old (Newell, 1948). In this study the *Arenicola* individuals are harvested shortly before the average individuals reaches puberty (Figure 6-2).

With the methodology developed by Olive et al. (2006) a 0.5 g *A. marina* individual may grow to a 5 - 6 g size within 90 to 120 days. However, under the reduced feed availability of the simulation (Figure 6-4) the *A. marina* individuals need 139 days to reach 0.5 g and then another 199 days to grow to 5 (Figure 6-3). At the beginning of the simulation scenario the growth of *A. marina* individuals is limited by the low availability of PON (Figure 6-2 and Figure 6-3). When the model is re-parameterized for higher initial PON availability (Figure 6-6 and Figure 6-7), then the *Arenicola* grow faster, than they would under the "normal simulation conditions", (Figure 6-1 and Figure 6-3) during the first months of the simulation (Figure 6-8 and Figure 6-9). Under the high feed availability simulation (Figure 6-6 and Figure 6-7) the *Arenicola* need only 115 days to grow to 5 g (Figure 6-5), similarly to the 90-120 days that they need at the Olive et al. (2006). At a site rich in alternative food sources, apart from the fish waste, the growth of the *Arenicola* will not be limited by feed supply and thus they will reach higher final weight. This model simulates the growth of organisms feeding on a single food source (the wastage from the fish cages) the ambient PON is only contributing at the start of the simulation as a starting value of the level.

6.8.1.2 Mytilus edulis

The DEB model has already been used to describe the growth and reproduction of *M. edulis* (i.e. Ross and Nisbet (1990), van Haren and Kooijman (1993) and by Thomas et al (2011)) but in this study its potential as an organic extractive species for IMTA is examined.

6.8.1.2.1 Bioremediation potential

Biochemical compositions of *M. edulis* can show considerable variation. Dare and Edwards (1975) and Pieters et al. (1979) found specific protein contents between 400 and 700 $mg\,g^{-1}$ dw in *M. edulis*, while Pleissner et al. (2012) found specific protein contents between 150 and 330 $mg\,g^{-1}$ dw in

mussels grown in net bags in the sea. From the above we can estimate that the nitrogen content of *M. edulis* soft tissues can range between 24 and 112 $mg\,g^{-1}$ dry weight of soft parts of mussel. Consequently, the nitrogen content of 14,590 kg dw should range between 350 kg and 1,634 kg. This in accordance with the model simulation output, which showed that 1,069 kg of PON will be assimilated by the mussels and removed from the IMTA area when the mussels are harvested (Table 6-5).

6.8.1.2.2 Growth

In Bivalves, the ash-free dry weight (AFDW) to wet weight ratio is 0.055 (Ricciardi and Bourget, 1998). Wyatt et al. (2014) estimated during two three-month long field trials at a grow-out site that the dry to wet weight ratio for *M. edulis* was 0.0743 – 0.1206 for the January to April experiment and 0.0507 – 0.1704 for the April to June experiment. The condition index for *M. edulis* at the simulation was 0.2206. The condition index is higher than that found at Wyatt et al. (2014) possibly due to the higher food availability of an IMTA site in comparison to mussel monoculture, where no additional feed is provided. When the model was run for higher POM concentration so that the scaled functional response (f) was almost 1 (Figures 6-14 and 6-15) then the condition index increases even more and reached a value of 0.2217 (1.37:6.18), showing further improvement of the condition index with increased feed availability.

Van Haren and Kooijman (1993) used data from Borchardt (1985) and Pieters et al. (1979) and concluded that the least squares fitted curve is $W_w = d_w (\delta_m L)^3$ with specific density and shape coefficient $d_w = 1$ g cm^{-3}, $\delta_m{}^3 = 0.03692$. If this formula is applied to the results of this study, with $d_w = 1$ g $cm^{-3}$and $\delta_m = 0.03692$ the wet weight for the final length (6.78 cm) should be 7.92 g instead of 6.25 g that was estimated at this study. However, it is clear that there exists a large variation in the weight-length relationship (Figure 6-24).

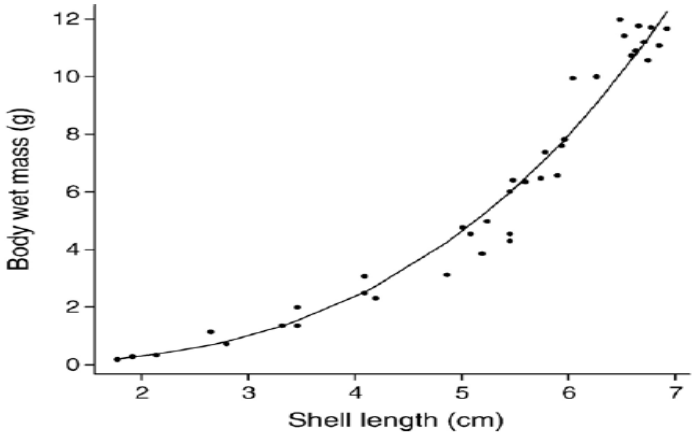


Figure 6-24: The relationship between shell length (L) and wet weight ww for *M. edulis*. Figure after Van Haren and Kooijman, 1993.

6.8.1.3 Paracentrotus lividus

6.8.1.3.1 Bioremediation potential

The crude protein content of *P. lividus* (packed for sale) ranges from 10.6 to 12.2 % of wet weight (Pais et al. 2011). The amount of nitrogen, estimated by dividing the crude protein with 6.25, would range from 1.7 to 2 % of the wet weight. According to the above, the nitrogen content of the gonads of 50 t wet weight (20.86 t dw) harvested at the end of the simulation scenario should be within the range of 850 – 1,000 kg. The model output, that the sea urchins accumulated 1,005 kg of PON, which is 0.5% higher than the maximum value of the range above possibly due to the high availability of nitrogen at the IMTA site. However, other studies have different estimates of tissue nitrogen content. For example, at the Pais et al (2011) study, the *P. lividus* moisture content was calculated to be 73%, according to that 50 t wet weight should be 13.5 t dry weight instead of 20.86 t that was calculated at the simulation. The protein content was 56% of the dry matter at the Pais et al (2011) study, which would means that for 20.86 t the protein content should be 11.68 t so the N content should be 1,869 kg. Similarly, at a study examining the seasonal variations of wild *P. lividus* in Spain it was concluded that the protein content ranged between 36 and 60% of the dry weight (Montero-Torreiro and Garcia-Martinez, 2003). Which would means that for 20.86 t the protein content should be 11.68 t so the nitrogen content should be 1.2 – 2 t.

6.8.1.3.2 Growth

Cook and Kelly (2007b) estimated that over a 12-month growth trial, the SGR of *P. lividus* grown as part of an IMTA system ranged from 0.43 - 0.8% day^{-1} depending on the distance from the salmon cages. The growth rate achieved in this study was similar to that achieved directly adjacent to the sea cages (Cook and Kelly, 2007b) and higher than that achieved by Fernandez and Clatagirone (1994) (1.41 mm $month^{-1}$), where the sea urchins were fed with artificial feed containing fish meal and fish oil at higher water temperature than this study (5 - 33°C).

The growth performance of the sea urchins is also in accordance with Grosjean (2001). At the simulation scenario, the sea urchins will be about one year old when they are deployed at sea and after 18 months will be about 2.5 years old. So according to the graph their average test diameter should be around 3.5 - 4.5 cm and the simulation results indicate that the average test diameter would be 4.46 cm (Figure 6-23 and Figure 6-25).

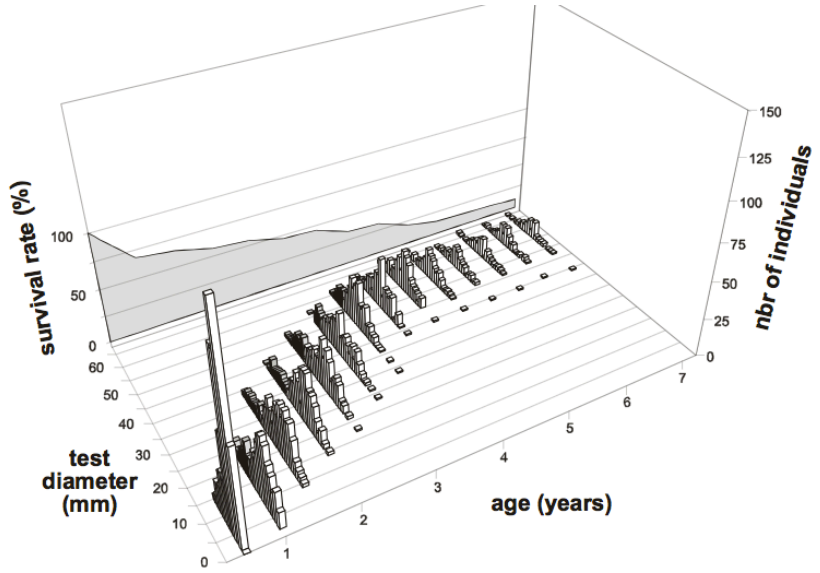


Figure 6-25: Changes with time in the size distribution and survival rate of one fertilization issued from a single larval rearing tank and followed over 7 years. Figure after Grosjean (2001).

6.9 General conclusions from the validation

The simulated growth for juveniles and adults showed good correspondence with empirical literature data, although precision varied with the size metric

considered (e.g. predictions for length were more precise than for wet weight). The reduced precision in predicting weight may be attributed to the fact that weight-at-age data are more scattered than the corresponding length-at-age data (Karasov and Martínez 2007). When looking at the DEB formulas this difference in precision is expected: this is because weight contains contributions from three state variables (including the structural length), each of which could contribute to the overall error (Monarco et al. 2014), while the physical length is only estimated using the structural length.

6.10 Further considerations

The nitrogen uptake potential of the different invertebrates as well as their suitability for IMTA does not only depend on their clearance rate and the possibility to be cultured in high stocking densities. Other parameters such as the particle size that each species selects and their sensitivity (e.g. to sedimentation and to anoxia) are also significant. A detailed analysis on this issue can be found on chapter 1, section 1.6. Sea urchins are the most sensitive to sedimentation, presenting high intolerance, meaning that the population is likely to be killed or destroyed by smothering, and intermediate intolerance to low oxygenation (Jackson, 2008). *Mytilus edulis* has intermediate intolerance to smothering, meaning that some individuals of the species may be killed or destroyed by smothering and the viability of a species population may be reduced, and low intolerance to changes in oxygenation (Tyler-Walters, 2008a). On the other hand *Arenicola marina* is tolerant to smothering and presents only low intolerance to changes in oxygenation (Tyler-Walters, 2008b).

The major strength of the simulation output generated from this study is the fact that the simulation runs for the different species are identical, something that cannot easily be achieve with lab or field trials. The comparative study performed in this chapter is a consistent and straightforward comparative method that can easily be repeated for a large number of potential organic extractive species, due to the large amount of data available at the DEB database (Kooijman, 2014). The large amount of easily accessible data was one of the reasons for choosing the DEB for modelling the organic extractive

component of the IMTA model. A detailed analysis of the reasons for which the DEB was chosen can be found in chapter 2 section 2.5.

The high demand for space is a very important factor constricting the wider adoption of IMTA. Thus knowing which species can perform better in terms of nitrogen removal efficiency per unit of area/volume is of great importance. As it is clear from this study the differences among the three species compared are significant. However, a species that is suggested to be very efficient from the modelling output of this study might not be as efficient under different circumstances. This model is based on a number of assumptions and simplifications (as analysed in chapter 5 section 5.4). Changes in assumptions or simplifications will affect the results. An example of the simplifications that could affect the results is that mussel growth is modelled without including phytoplankton dynamics. Mussels assimilate DIN indirectly via the ingesting of phytoplankton. However, due to the high site-specificity of phytoplankton growth and dispersion patterns a phytoplankton model was not deemed suitable for this study. For a more complete and site specific approach, the current model can be merged with add-on models such as a phytoplankton biomass and hydrodynamics sub-model (e.g. Flynn et al. 2001) as well as with waste dispersion (e.g. DEPOMOD) and nutrient dilution models.

Modelling phytoplankton is meaningful because macroalgae compete with phytoplankton for nutrients and light. The phytoplankton sub-model would be very similar to the seaweed sub-model. Zooplankton grazing, light, temperature or nutrients can limit phytoplankton production. The main difference between the macroalage and phytoplankton model would be that for phytoplankton it is also necessary to take into account hydrodynamic transport and loses due to settling/sinking, while the seaweed biomass depends only on physiological processes. Reproduction does not need to be included because phytoplankton is being transported with the currents and thus has low residence time at the site. Additionally, phytoplankton has only limited time to photosynthesize before sinking to the aphotic zone.

Waste dispersion modelling is crucial but also entirely site specific and requires thorough data collection. Currents play a major role in the success

of IMTA. On-site current flow and cyclic shifts can be measured using Acoustic Doppler Current Profilers (ADCP). Alternatively, GIS, remote sensing and mapping could be useful tools for the spatial and geographic development and management of marine aquaculture. Data on temperature, current velocity, wave height and chlorophyll concentration could be obtained using remote sensing. This environmental data can be incorporated into a GIS database from where they can be used for the creation of map layers that when overlapped form a map composition. Map compositions can be used to visualize and thus examine the suitability of different locations for the development of aquaculture facilities or for more effective management of the sector, while enabling an EAA also in the context of other uses of land or water. GIS can also be used to define whether the environment is suitable for the desired cultured species as well as for the structure of their enclosures and to find the options for accessing the facilities taking into account other uses of the area. Waste dispersion models can also be used to set biomass limits for a specific site depending on its carrying capacity. A waste dispersion model could be used for determining the movement of the waste and thus for placing the extractive organisms in the area where most of the waste moves towards/through (Perez et al. 2002).

Chapter 7

Discussion

Presently, there are large-scale IMTA initiatives in Norway, China and Korea (Waite et al. 2014). The adoption of IMTA by the industry is limited by many factors. A major one of which is the lack of knowledge and skills (or unwillingness to take the risk) to grow new species. Commercial scale IMTA farms are knowledge intensive and difficult to manage as businesses, due to the complexity of growing many species together. Growing new species presents biological and technological challenges both in the growing and processing stages. Fish farmers are used to growing fish, moreover most companies specialise in the production of one species.

Another major factor delaying the adoption of IMTA is that there are no legal frames for IMTA products. Current aquaculture legislation and fish purchasing standards have been formed for monoculture systems. This makes obtaining permits for IMTA farms and selling products produced in IMTA systems challenging (Waite et al. 2014). Fortunately, there are on-going policy reform initiatives in both Europe and North America (Waite et al. 2014). For example, in 2008, the Canadian Shellfish Sanitation Program was amended to recognize IMTA and provide a procedure for registration and management of IMTA sites (Chopin, 2008).

Furthermore, marketing new species is a long process especially when facing the challenge of how consumers would react in the idea of consuming animals or plants that have been essentially fed with fish farm waste.

IMTA is currently commercially implemented by only a handful of companies and thus there is still insufficient confidence in its success. There are still concerns over potentially negative interactions between species and practical problems like the mismatch in production cycles. The potentially high capital costs of starting to cultivate a new species and concerns over operational constraints, such as the need for seaweed hatcheries.

The aim of this study was to develop a tool for optimising the production and nutrient bioremediation efficiency of IMTA. The modelled system can help understand practical difficulties concerning the timing (e.g. mismatch in waste release and waste uptake potential). It can also forecast the fish-extractive organism weight ratio needed for a certain percentage of nitrogen removal. Thus helping to overcome some of the open questions and practical difficulties of IMTA.

This study has generated a dynamic tool, with a simple user interface. The model developed takes into account a variety of factors including farm size, food supply and environmental parameters to calculate the yield of the farm. It allows aquaculture farms to test different production scenarios and thus helps make production decisions, in a cost effective way that meets their goals in terms of biomass production but also in terms of environmental impact. The model can be used as a tool to test potential alterations in the production, by altering key production variables. The prospective farmer can analyze and thus manipulate (via alteration of stocking density, production cycle length, addition of external feed/fertilizer, re-stocking or thinning of crops) the relations between feed availability and extractive organism growth. The generated output concerning the nitrogen removal could also aid aquaculture farms to comply with certification programs and international environmental standards.

Furthermore, the present study is contributing in identifying the scale of one of the major issues that causes apprehension over IMTA, the issue of space. Culturing seaweed requires a lot of space and thus there is the possibility of conflicts for space among different users and the need to take visual impact into account. This model can give a prediction of how much space is needed for a specific nitrogen-bioremediation or seaweed biomass production target.

The model can also generate output that could help with economic optimisation. Model add-ons can also take into account the economics, assisting in developing better management for the farm concerned. Finally, the IMTA model developed in this study could also be used to facilitate obtaining permits for IMTA following an EIA.

Aquaculture has the potential to become a powerful tool for long-term sequestration of atmospheric carbon dioxide and thus potentially play a significant role in reducing greenhouse gases. Algae can be used as a tool to raise pH and make the mariculture of fish and Bivalves less sensitive to corrosive water, since the harvest of algae leads to a net increase in pH. The dissolved CO_2 that is in the seawater can be assimilated by algae or by invertebrates that have carbon-containing hard outer layers (shells, exoskeletons etc.) such as shellfish, crustaceans and molluscs (Casey et al. 2008). When marine invertebrates with carbon-containing hard outer layers ingest the CO_2 that is dissolved in the seawater it may be converted to calcium bicarbonate ($CaCO_3$). The carbon that is assimilated by the extractive organisms is removed from the water and thus the seawater can accumulate more carbon. Via the process of CO_2 sequestration aquaculture farms may earn carbon credits that can be traded, within an emissions trading scheme or with companies or individuals acting outside of an emissions trading scheme. Thus another potential economic benefit of IMTA is carbon trading. The amount of carbon that is removed can be estimated from the total amount of carbon that is total shell/seaweed biomass produced. The shells recovered after the meat has been harvested can be used as paving material for roads, parking lots etc. or for insulating the roots of grape vines at vineyards. These methods provide a purpose for the shells while long-term carbon sequestration is achieved. A forecast of the IMTA's carbon sequestration and nutrient bioremediation potential could also facilitate obtaining permits for the establishment of IMTA farms. The model developed in this study can be adpted to show the exchange of carbon within the IMTA system and thus give an estimate of IMTA's carbon-sequestration potential.

Integrated aquaculture can be a highly efficient system producing negligible amounts of waste while optimizing the use of scarce resources such as land and water. It has the potential of being a successful aquaculture method that contributes in ensuring food security. Polyculture is already used as a resilience strategy and IMTA can play a similar role. For example, shrimp farmers in Thailand grow rice and shrimp, switching between them

depending on prices and rainfall. The extractive species grown in IMTA are lower-value and lower-risk species and this will be even more evident in the coming years as prices for feed and fertilizers as well as energy costs keep rising. It should not be overlooked that IMTA is not suitable for every site, at least not with a site-specific species combination and grow-out set up.

The basic question that needs to be answered is whether IMTA works as a nutrient bioremediation method. I believe the answer is simple: it depends on what we expect from IMTA. Biofiltering 100% of the fish waste is unnecessary and in addition hard to achieve. This study has shown that depbeding on production practices high levels of nutrient bioremediation can be achieved.

References

Abreu, M.H., Varela, D.A., Henríquez, L., Villarroel, A., Yarish, C., Sousa-Pinto, I., 2009. Traditional vs. integrated multi-trophic aquaculture of *Gracilaria chilensis* C.J. Bird, J. McLachlan, E.C. Oliveira: productivity and physiological performance. Aquaculture, 293 (3-4): 211-20.

Abreu, A.H., Pereira, R., Yarish, C., Buschmann, A.H., Sousa-Pinto, I., 2011. IMTA with *Gracilaria vermiculophylla*: productivity and nutrient removal performance of the seaweed in a land based pilot scale system. Aquaculture, 312: 77-87.

Ackefors, H., Enell, M., 1990. Discharge of nutrients from Swedish fish farming to adjacent sea areas. Ambio, 19: 28-35.

Add_my_pet, 2014. <http://www.bio.vu.nl/thb/deb/deblab/add_my_pet_old/Species.htm> [Accessed, 29 January 2015]

Ahlgren, M., 1998. Consumption and assimilation of salmon net pen fouling debris by the red sea cucumber *Parastichopus californicus*: implications for polyculture. Journal of World Aquaculture Society, 29: 133-139.

Aitken, D., Bulboa, C., Godoy-Faundez, A., Turrion-Gomez, G.L., Antizar-Ladislao, B., 2014. Life cycle assessment of macroalgae cultivation and processing for biofuel production. Journal of Cleaner Production, 75: 45-56.

Alanärä, A., Kadri, S. and Paspatis, M., 2001. Feeding management. In: Feed intake in fish. Houlihan, D., Jobling, M. and Boujard T. (Eds.), Blackwell Science, Oxford, UK: 332-353.

Ale, M.T., Mikkelsen, J.D., Meyer, A.S., 2010. Differential growth response of *Ulva lactuca* to ammonium and nitrate assimilation. Journal of Applied Phycology, 23 (3): 345-351.

Allain, J.Y., 1975. Structure des populations de *Paracentrotus lividus* (Lamarck) (Echinodermata, Echinoidea) soumises à la pêche sur les côtes Nord de Bretagne. Revue des Travaux de l'Institut des Pêches Maritimes, 39: 171–212.

Alvera-Azcárate, A., Ferreira, J.G., Nunes, J.P., 2003. Modelling eutrophication in mesotidal and macrotidal estuaries. The role of intertidal seaweeds. Estuarine and Coastal Shelf Science, 57: 715-724.

Aminin, D.L., Agafonova, I.G., Kalinin, V.I., Silchenko, A.S., Avilov, S.A., Stonik, V.A., Collin, P.D., Woodward, C., 2008. Immunomodulatory properties of frondoside A, a major triterpene glycoside from the north Atlantic commercially harvested sea cucumber *Cucumaria frondosa*. Journal of Medicinal Food, 11 (3): 443-453.

Angel, D., Katz, T., Eden, N., Spanier, E., Black K., 2005. Damage control in the coastal zone: improving water quality by harvesting aquaculture-derived nutrients. Strategic Management of Marine Ecosystems 50: 53-64.

Angell, A.R., Pirozzi, I., de Nys, R., Paul, N.A., 2012. Feeding preferences and the nutritional value of tropical algae for the abalone *Haliotis asinine*. PLoS ONE, 7(6), art. No. e38857.

Anonymous, 2003. Powersim studio user's guide. <http://homepage.cem.itesm.mx/alesando/index_archivos/DinamicaDeSistemas/powersim_studio2003_users_manual.pdf> [Accessed, 10 September 2013]

Austreng, E., Storebakken, T., Åsgård, T., 1987. Growth rate estimates for cultured Atlantic salmon and rainbow trout. Aquaculture. 60: 157-160.

Aveytua-Alcázara, L., Camacho-Ibara, V.F., Souzab, A.J., Allenc, J.I., Torres, R., 2008. Modelling *Zostera marina* and *Ulva sp.* in a coastal lagoon. Ecological Modelling, 218: 354-366.

Azevedo, P.A., Cho, C.Y., Leeson, S., Bureau, D.P., 1998. Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). Aquatic Living Resources, 11(4): 227-238.

Baird, M.E., Walker, S.J., Wallace, B.B., Webster, I.T., Parlsow, J.S., 2003. The use of mechanistic descriptions of algal growth and zooplankton grazing in an estuarine eutrophication model. Estuarine and Coastal Shelf Science, 56 (3/4): 685-695.

Bar, N.S., Radde, N., 2009. Long-term prediction of fish growth under varying ambient temperature using a multiscale dynamic model. BMC Systems Biology 3: 107.

Barrington, K., Chopin, T. and Robinson, S. Barrington, K., Chopin, T., Robinson, S., 2009. Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. In D. Soto (ed.). Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper. No. 529. Rome, FAO. pp. 7-46.

Bartsch, A., Robinson, S.M.C., Liutkus, M., Ang, K.P., Webb, J., Pearce, C.M. 2013. Filtration of sea louse, *Lepeophtheirus salmonis*, copepodids by the blue mussel, *Mytilus edulis*, and the Atlantic sea scallop, *Placopecten magellanicus*, under different flow, light and copepodid-density regimes. Journal of Fish Diseases, 36: 361–370.

Bayne, B.L., 1976. Marine Mussels: Their Ecology and Physiology. Cambridge University Press, Cambridge, 506 pp.

Bayne, B.L., Newell, R.C., 1983. Physiological energetics of marine molluscs. In Wilbur KM Saleuddin AS (eds) The Mollusca. Vol 4 Academic Press New York pp 407-515.

Bayne, B.L., Hawkins, A.J.S., Navarro, E., 1987. Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia: Mollusca) in mixtures of silt and algal cells at low concentrations. Journal of Experimental Marine Biology and Ecology, 111: 1-22.

Beaumont, A.R., Gjedrem, T., Moran, P., 2007. Blue Mussel *Mytilus edulis*, Mediterranean mussel *M. galloprovincialis*. In: Svasand T, Crosetti D, Garcia-Vasquez E, Verspoor E (ed) Genetic impact of aquaculture activities in native populations. Genimpact Final Scientific Report pp 62–69.

Bedford, A.P., Moore, P.G., 1985. Macrofaunal involvement in the sublittoral decay of kelp debris: the detritivore community and species interactions. Estuarine, Coastal Shelf Science, 18: 97-111.

Bertalanffy, L. Von., 1957. Wackstum. Küken- thal's Handbuch der Zoologie. Vol. VIII 4 (6). de Gruyter, Berlin.

Best, E.P.H., 1981. A preliminary model for growth of *Ceratophyllum demersum*. L. Verhandlungen des Internationalen Verein Limnologie, 21: 1484-1491.

Betteley, K.A., Watson, G.J., Hannah, L., Bentley, M.G., 2008. Aspects of gametogenesis, oocyte morphology and maturation of the lugworm *Arenicola marina* (Annelida: Polychaeta) in relation to commercialised procedures to extend the breeding season. Aquaculture, 279 (1-4): 131-141.

Beukema, J.J., de Vlas, J., 1979. Population parameters of the lugworm, *Arenicola marina*, living on tidal flats in the Dutch Wadden Sea. Netherlands Journal of Sea Research, 13: 331-353.

Borchardt, T, 1985. Relationships between carbon and cadmium uptake in *Mytilus edulis*. Marine Biology, 85: 233-244.

Bouchard, D.A., Bricknell, I., Pietrak, M., Malloy, S.D., 2014. The interactions of infectious salmon anaemia virus (ISAV) with the blue mussel, *Mytilus edulis*. Articles. Paper 3. <http://digitalcommons.library.umaine.edu/ari_articles/3> [Accessed, 15 October 2015].

Boudouresque, C.F., Verlaque, M, 2007. Ecology of *Paracentrotus lividus*. In: Lawrence J. M. (Editor). Edible sea urchins: Biology and Ecology, 2nd Edition. Elsevier publ., Amsterdam, pp. 243-285.

Bricker, S.B, Ferreira, J.G, Simas, T., 2003. An integrated methodology for assessment of estuarine trophic status. Ecological Modelling, 169 (1): 39-60.

Brigolin, D., Dal Maschio, G., Ramapazzo, F., Giani, M., Pastres, R., 2009. An individual-based population dynamic model for estimating biomass yield and nutrient fluxes through an offshore *Mytilus galloprovincialis* farm. Estuarine, Coastal and Shelf Science, 82: 365–376.

Broch, O., Slagstad, D., 2012. Modelling seasonal growth and composition of the kelp *Saccharina latissima*. Journal of Applied Phycology, 24: 759-776.

Broch, O.J., Ellingsen, I.H., Forbord, S., Wang, X., Volent, Z., Alver, M.O., Handå, A., Andresen, K., Slagstad, D., Reitan, K.I., Olsen, Y., Skjermo, J., 2013. Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway. Aquaculture Environmental Interactions 4: 186-206.

Brown, J.R., Gowen, R.J., McLusky, D.S., 1987. The effect of salmon farming on the benthos of a Scottish sea loch. Journal of Experimental Marine Biology and Ecology, 109: 39-51.

Bruhn, A., Dahl, J., Nielsen, H.B., Nikolaisen, L., Rasmussen, M.B., Markager, S., Olesen, B., Arias, C., Jensen, P.D., 2011. Bioenergy potential of *Ulva lactuca*: biomass yield, methane production and combustion. Bioresource Technology, 102 (3): 2595-604.

Bunting, S.W., Shpigel, M., 2009. Evaluating the economic potential of horizontally integrated land-based marine aquaculture. Aquaculture, 294: 43-51.

Bureau, D.P., Azevedo, P.A., Tapia-Salazar, M., Cuzon, G., 2000. Pattern and cost of growth and nutrient deposition in fish and shrimp: Potential implications and applications. In: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A., Civera-Cerecedo, R. (Eds.), Avances en Nutrición Acuícola V. Memorias

del V Simposium Internacional de Nutricón Acuícola.19–22 Noviembre. Mérida, Yucatán, Mexico, pp. 111–140.

Burridge, L., Weis, J.S., Cabello, F., Pizarro, J., Bostick, K., 2010. Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture, 306: 7-23.

Buschmann, A.H., Mora, O.A., Gómez, P., Böttger, M., Buitano, S., Retamales, C., Vergara, P.A., Gutierrez, A., 1994. *Gracilaria chilensis* outdoor tank cultivation in Chile: use of land-based salmon culture effluents. Aquacultural Engineering, 13: 283-300.

Buschmann, A.H., Troell, M., Kautsky, N., Kautsky, L., 1996. Integrated tank cultivation of salmonids and *Gracilaria chilensis* (Gracilariales, Rhodophyta). Hydrobiologia, 326/327: 75-82.

Buschmann, A.H., López, D., González, M.L., 2000. Cultivo integrado de moluscos y macroalgas en líneas flotantes y en estanques. In: Faranda, F.M., Albertini, R., Correa, J.A. (Eds.), Manejo Sustentable de los Recursos Marinos Bentónicos en Chile Centro-Sur: Segundo Informe de Avance. Pontificia Universidad Católica de Chile, Santiago, pp. 7-16.

Buschmann, A.H., Varela, D.A., Hernandez-Gonzalez, M.C., Huovinen, P., 2008a. Opportunities and challenges for the development of an integrated seaweed-based aquaculture activity in Chile: determining the physiological capabilities of *Macrocystis* and *Gracilaria* as biofilters. Journal of Applied Phycology, 20: 571–577.

Buschmann A.H., Hernández-González M.C., Aranda C., Chopin T., Neori A., 2008b. Mariculture waste management. In Encyclopedia of Ecology, ed. SE Jørgensen, BD Fath, Oxford, UK: Elsevier. pp. 2211-17.

Byron, C., Bengtson, D., Costa-Pierce, B, Calanni, J., 2011. Integrating science into management: ecological carrying capacity of bivalve shellfish aquaculture. Marine Policy, 35: 363-370.

Cahill, P.L., Hurd, C.L., Lokman, M., 2010. Keeping the water clean—Seaweed biofiltration outperforms traditional bacterial biofilms in recirculating aquaculture. Aquaculture, 306: 153-59.

Canale, R.P., Auer, M.T., 1982. Ecological studies and mathematical modelling of *Cladophora* in Lake Huron: V. Model development and calibration. Journal of Great Lakes Research, 8: 112-125.

Carboni, S., 2013. Research and development of hatchery techniques to optimize juvenile production of the edible sea urchin, *Paracentrotus lividus*. PhD. Thesis. University of Stirling, UK.

Carmona, R., Kraemer, G.P., Zertuche, J.A., Chanes, L., Chopin, T., Neefus, C., Yarish, C., 2001. Exploring *Porphyra* species for use as nitrogen scrubbers in integrated aquaculture. Journal of Applied Phycology, 37: 9-10.

Carmona, R., Kraemer, G.P. and Yarish, C., 2006. Exploring Northeast American and Asian species of *Porphyra* for use in an Integrated Finfish-Algal Aquaculture System. Aquaculture, 252: 54-65.

Carr, G.M., Duthie, H.C., Taylor, W.D., 1997. Models of aquatic plant productivity: A review of the factors that influence growth. Aquatic Botany, 59 (3-4): 195-215.

Casey, P.J., Gold, D., Mils N., 2008. Earning carbon credits using aquaculture to sequester carbon dioxide: US20080226527 A1

Cefas, 2008. Literature review on particle assimilation by molluscs and crustaceans. Environment Report RL 10/08. <http://www.cefas.defra.gov.uk/publications/environment/Literature-review-on-particle-assimilation.pdf > [Accessed, 10 October 2015]

Chandrkrachang, S., Chinadit, U., Chandayot, P., Supasiri, T., 1991. Profitable spin-offs from shrimp-seaweed polyculture. INFOFISH Int. 6/91, 26-28.

Chávez-Crooker, P., Obreque-Contreras, J., 2010. Bioremediation of aquaculture wastes. Current Opinion in Biotechnology, 21(3): 313-317.

Cheshuk, B.W., 2001. The potential of integrated open-water mussel (*Mytilus planulatus*) and Atlantic salmon (*Salmo salar*) culture in North West Bay. Tasmania. PhD Thesis. University of Tasmania, Launceston. 281 pp.

Cheshuk, B.W., Purser, G.J., Quintana, R., 2003. Integrated open-water mussel (*Mytilus planulatus*) and Atlantic salmon (*Salmo salar*) culture in Tasmania. Australia Aquaculture, 218: 357-378.

Cho, C.Y., 1990. Fish nutrition, feeds, and feeding: with special emphasis on salmonid aquaculture. Food Reviews International, 6(3): 333-357.

Cho, C.Y., Bureau, D.P., 1998. Development of bioenergetic models and the Fish-PrFEQ software to estimate production, feeding ration and waste output in aquaculture. Aquatic Living Resources, 11(4): 199-210.

Chopin, T., Yarish, C., 1998. Nutrients or not nutrients? That is the question in seaweed aquaculture and the answer depends on the type and purpose of the aquaculture system. World Aquaculture, 29: 31–33.

Chopin, T., Yarish C., 1999. Aquaculture does not only mean finfish monoculture: seaweeds must be a significant component for an integrated ecosystem approach. Bulletin of Aquaculture Association Canada, 99: 35-37.

Chopin, T., Yarish, C., Wilkes, R., Belyea, E., Lu, S., Mathieson, A., 1999. Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. Journal of Applied Phycology, 11: 463– 472.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G., Zertuche-Gonzalez, J., Yarish, C., Neefus, C., 2001. Integrating seaweeds into aquaculture systems: a key towards sustainability. Journal of Applied Phycology, 37: 975–986.

Chopin, T., 2008. “IMTA and Regulations.” Fredericton, New Brunswick, Canada: University of New Brunswick. Accessible at: <http://www2.unb.ca/chopinlab/imta/regulations/index.html>.

Chopin, T., 2010. Integrated multi-trophic aquaculture. In Advancing the Aquaculture Agenda, Paris: OECD. pp. 195–218.

Chopin, T., 2011. Progression of the Integrated Multi-Trophic Aquaculture (IMTA) Concept and Upscaling of IMTA Systems towards Commercialization. Aquaculture Europe, 36 (4): 5-12.

Chow, F., Macciavello, J., Santa Cruz, S., Fonck, O., 2001. Utilization of *Gracilaria chilensis* (Rhodophyta: Gracilariaceae) as a biofilter in the depuration of effluents from tank cultures of fish, oyster, and sea urchins. Journal of World Aquaculture Society, 32: 214-220.

Christensen, V., Pauly, D., 1992. Ecopath II - a software for balancing steady-state ecosystem models and calculating network characteristics. Ecological Modelling, 61:169-185.

Coffaro, G., Sfriso, A., 1997. Simulation model of *Ulva rigida* growth in shallow water of the Lagoon of Venice. Ecological Modelling, 102: 55-66.

Cohen, I., Neori, A., 1991. *Ulva lactuca* biofilters for marine fishpond effluents. Botanica Marina, 34: 475-482.

Collins, C.D., Wlosinski, J.H., 1989. A macrophyte submodel for aquatic ecosystems. Aquatic Botany, 33: 191-206.

Conand, C., Sloan, N.A., 1989. World fisheries for echinoderms. Marine Invertebrate Fisheries, 647-663.

Cook, E.J., 1999. *Psammechinus miliaris* (Gmelin) (Echinodermata: Echinoidea): Factors effecting its somatic growth and gonadal growth and development. PhD Thesis, Napier University, Edinburgh.

Cook, E.J., Bell, M.V., Black, K.D., Kelly, M.S., 2000. Fatty acid compositions of gonadal material and diets of the sea urchin, *Psammechinus miliaris*: trophic and nutritional implications. Journal of Experimental Marine Biology and Ecology, 255(2): 261-274.

Cook, E.J., Black, K.D., Sayer, M.D.J., Cromey, C., Angel, D., Katz, T., Eden, N., Spanier, E., Karakassis, I., Tsapakis, M., Malej, A., 2006. Pan-European study on the influence of caged mariculture on the development of sub-littoral fouling communities. ICES Journal of Marine Science, 63: 637-649.

Cook, E.J., Kelly, M.S., 2007a. Effect of variation in the protein value of the red macroalga *Palmaria palmata* on the feeding, growth and gonad composition of the sea urchins *Psammechinus miliaris* and *Paracentrotus lividus* (Echinodermata). Aquaculture, 270: 207–217.

Cook, E.J., Kelly, M.S., 2007b. Enhanced production of the sea urchin *Paracentrotus lividus* in integrated open-water cultivation with Atlantic salmon *Salmo salar*. Aquaculture, 273(4): 573-585.

Copertino, M.S., Tormena, T., Seeliger, U., 2009. Biofiltering efficiency, uptake and assimilation rates of *Ulva clathrata* (Roth) J. Agardh (Clorophyceae) cultivated in shrimp aquaculture waste water. Journal of Applied Phycology, 21: 31-45.

Corner, R., Brooker, A., Telfer, T., Ross, L., 2006. A fully integrated GIS-based model of particulate waste distribution from marine fish-cage sites. Aquaculture, 258: 299−311

Costanza, R., Gottlieb, S., 1998. Modelling ecological and economic systems with STELLA: Part II. Ecological Modelling, 112: 81-84.

Costanza, R., Voinov, A., 2001. Modelling ecological and economic systems with STELLA: Part III. Ecological Modelling, 143: 1-7.

Cowin, P.B.D., Craig, S, Olive, P.J.W., 2003 Aquaculture of marine worms: WO 2003007701 A3.

Cranford, P.J., Hill, P.S., 1999. Seasonal variation in food utilization by the suspensionfeeding bivalve molluscs *Mytilus edulis* and *Placopecten magellanicus*. Marine Ecology Progress Series, 190: 223-239.

Cranford, P.J., Reid, G.K., Robinson, S.M.C., 2013. Open water integrated multi-trophic aquaculture: constraints on the effectiveness of

mussels as an organic extractive component. Aquaculture Environment Interactions, 4: 163-173.

Crapp, G.B., Willis, M.E., 1975. Age determination in the sea urchin *Paracentrotus lividus* (Lamarck) with notes on the reproductive cycle. Journal of Experimental Marine Biology and Ecology, 20: 157-178.

Cromey, C.J., Nickell T.D., Black, K.D., 2002. DEPOMOD – modeling the deposition and biological effects of waste solids from marine cage farms. Aquaculture, 214: 211-239.

Cromey, C.J., Nickell, T.D., Treasurer, J., Black, K.D., 2009. Modelling the impact of cod (*Gadus morhua* L.) farming in the marine environment – CODMOD. Aquaculture, 289: 42-53.

Cromey, C.J., Thetmeyer, H., Lampadariou, N., Black, K.D., Kögeler, J., Karakassis I., 2012. MERAMOD: predicting the deposition and benthic impact of aquaculture in the eastern Mediterranean Sea. Aquaculture Environmental Interactions, 2: 157-176.

Cubillo, A.M., Peteiro, L.G., Fernández-Reiriz, M.J., Labarta, U., 2012. Influence of stocking density on growth of mussels (*Mytilus galloprovincialis*) in suspended culture. Aquaculture, 342-343: 103-11.

Cuomo, V., Merrill, J., Palomba, I., Perretti, A., 1993. Systematic collection of *Ulva* and mariculture of *Porphyra*: Biotechnology against eutrophication in the Venice Lagoon. International Journal of Environmental Studies, 43: 141–149.

Dame, R.F., Dankers, N., 1988. Uptake and release of materials by a Wadden Sea mussel bed. Journal of Experimental Marine Biology and Ecology 118, 207–216.

Dame, R.F., 2011. Ecology of marine bivalves: an ecosystem approach. CRC Press, Boca Raton, FL.

Darcy-Vrillon, B., 1993. Nutritional aspects of the developing use of marine macroalgae for the human food industry. International Journal of Food Sciences and Nutrition, 44: 23-35.

Dare, P.J., Edwards, D.B., 1975. Seasonal changes in flesh weight and biochemical composition of mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales. Journal of Experimental Marine Biology and Ecology, 18(2): 89-97.

Davies, M., 1989. Surveys of Scottish Sealochs, Lochs A'Chairn Bhain, Glendhu and Glencoul. Marine Nature Conservation Review Report no. 983. JNCC, Peterborough, UK.

Davies, I.M., 2000. Waste production by farmed Atlantic salmon (*Salmo salar*) in Scotland. ICES CM O: 01.

Davis, J.F., McDonnell, A.J., 1997. Development of a partitioned-biomass model for rooted macrophyte growth. Aquatic Botany, 56: 265-276.

De La Cruz-García, C., López-Hernández, J., González-Castro, M.J., Rodríguez-Bernaldo De Quirós, A., Simal-Lozano, J., 2000. Protein, amino acid and fatty acid contents in raw and canned sea urchin (*Paracentrotus lividus*) harvested in Galicia (NW Spain) Journal of the Science of Food and Agriculture, 80 (8): 1189-1192.

Droop, M.R., 1968. Vitamin B12 and marine ecology, IV: the kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. Journal of Marine Biological Association UK, 48: 689–733.

Droop, M.R., 1973. Some thoughts on nutrient limitation in algae. Journal of Phycology, 9: 264-72.

Duarte, P., Ferreira, J.G., 1997. A model for the simulation of macroalgal population dynamics and productivity. Ecological Modelling, 98: 199-214.

Duarte, P., Meneses, R., Hawkins, A.J.S., Zhu, M., Fang, J., Grant, J., 2003. Mathematical modelling to assess the carrying capacity for multi-species culture within coastal waters. Ecological Modelling 168: 109–143.

Dumas, A., France, J. and Bureau, D., 2010. Modelling growth and body composition in fish nutrition: where have we been and where are we going? Aquaculture Research, 41: 161–181.

Elmhirst, R., 1922. Notes on the breeding and growth of marine animals in the Clyde sea area (Millport, Marine Biol. St.) Annual Report. Scottish Marine Biological Association, Oban 1–47.

Enander, M., Hasselström, M., 1994. An experimental wastewater treatment system for a shrimp farm. INFOFISH Int. 4/94: 56–61.

Enell, M., 1995. Environmental-impact of nutrients from Nordic fish farming. Water Science and Technology, 31: 61−71.

Enriquez, S., Duarte, C.M., Sand-Jensen, K., 1995. Patterns in the photosynthetic metabolism of Mediterranean macrophytes. Marine Ecology Progress Series, 119: 243–252.

FAO, 2003. A guide to the seaweed industry. FAO fisheries technical paper 441 pp 74.

FAO, 2006. Food and Agriculture Organization of the United Nations. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome. <http://www.fao.org/fishery/sofia/en > [Accessed, 10 October 2014].

FAO, 2008. Sea cucumbers: A global review of fisheries and trade. Technical Report 516, Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO, 2009. Use of algae and aquatic macrophytes as feed in small-scale aquaculture 531 pp.5 <http://www.fao.org/docrep/012/i1141e/i1141e.pdf.> [Accesed, 4 September 2015].

FAO, 2012. Food and Agriculture Organization of the United Nations. The state of the world fisheries and aquaculture 2012. FAO Fisheries and Aquaculture Department. Rome. FAO 209 pp

FAO, 2013. Food and Agriculture Organization. Aquaculture statistics. <www.fao.org> [Accessed, 10 August 2014]

FAO, 2014. The State of World Fisheries and Aquaculture. Food and Agriculture Organization Of The United Nations, Rome. <http://www.fao.org/3/a-i3720e.pdf> [Accessed, 30 May, 2015]

FAO, 2015a. Future prospects for fish and fishery products. 4. Fish consumption in the European Union in 2015 and 2030. Part 1. European overview. FAO Fisheries Circular No. 972/4, Part 1 <http://www.fao.org/3/a-ah947e.pdf> [Accessed, 10 September 2014]

FAO, 2015b. Commercially important seaweed species. <http://www.fao.org/docrep/006/y4765e/y4765e04.htm>. [Accessed, 10 May 2015]

Farke, H., Berghuis, E.M., 1979. Spawning, larval development and migration behaviour of *Arenicola marina* in the laboratory, Netherlands Journal of Sea Research, 13 (3-4).

Fei, X.G., Lu, S., Bao, Y., Wilkes, R., Yarish, C., 1998. Seaweed cultivation in China. World Aquaculture, 29: 22–24.

Fei, X.G., Tseng, C.K., Pang, S.J., Lian, S.X., Huang, R.K., Chen, W.Z., 2002. Transplant of *Gracilaria lemaneiformis* by raft culture on the sea along fish cages in southern China. Proc. World Aquaculture Society, April 23-27, 2002. World Aquaculture Society, Baton Rouge, LA, USA, p. 219.

Fei, X.G., 2004. Solving the coastal eutrophication problem by large scale seaweed cultivation. Hydrobiologia, 512: 145-151.

Fernandez, C.M., Caltagirone, A., 1994. Growth rate of adult *Paracentrotus lividus* in a lagoon environment: the effect of different diet types, in: David, B., Guille, A., Feral, J.P., Roux, M. (Eds.), Echinoderms Through Time. Balkema, Rotterdam, pp. 655–660.

Fernandez, C., 1996. Croissance et nutrition de *Paracentrotus lividus* dans le cadre d'un project aquacole avec alimentation artificielle (Growth

and nutrition of *Paracentrotus lividus*, within artificial feed project frame). Ph. D. Thesis, Universite de Corse, 278 pp.

Ferreira, J.G., 1995. EcoWin—an object-oriented ecological model for aquatic ecosystems. Ecological Modelling, 79: 21-34.

Ferreira, J.G., Hawkins, A.J.S., Bricker, S.B., 2007. Management of productivity, environmental effects and profitability of shellfish aquaculture — the Farm Aquaculture Resource Management (FARM) model. Aquaculture, 264 (1-4): 160-174.

Ferreira, J.G., Andersson, H.C., Corner, R.A., Desmit, X., Fang, Q., de Goede, E.D., Groom, S.B., Gu, H., Gustafsson, B.G., Hawkins, A.J.S., Hutson, R., Jiao, H., Lan, D., Lencart-Silva, J., Li, R., Liu, X., Luo, Q., Musango, J.K., Nobre, A.M., Nunes, J.P., Pascoe, P.L., Smits, J.G.C., Stigebrandt, A., Telfer, T.C., de Wit, M., Yan, X., Zhang, X.L., Zhang, Z., Zhu, M.Y., Zhu, C.B., Bricker, S.B., Xiao, Y., Xu, S., Nauen, C.E., Scalet, M., 2008. SPEAR: Sustainable Options for People, Catchment and Aquatic Resources. EU 6th Framework Programme. INCO-CT-2044-510706. Final Report. 184 pp. ISBN 978-972-99923-2-2.

Ferreira, J.G., Sequeira, A., Hawkins, A.J.S., Newton, A., Nickell, T., Pastres, R., Fortef, J., Bodoyg, A., Bricker, S.B., 2009. Analysis of coastal and offshore aquaculture: application of the FARM model to multiple systems and shellfish species. Aquaculture, 289: 32-41.

Ferreira, J.G., Saurel, C., Ferreira, J.M., 2012. Cultivation of gilthead bream in monoculture and integrated multi-trophic aquaculture. Analysis of production and environmental effects by means of the FARM model. Aquaculture, 358–359: 23–34.

Filgueira R., Grant J., Stuart R., Brown M.S., 2013. Ecosystem modelling for ecosystem-based management of bivalve aquaculture sites in data-poor environments. Aquaculture Environment Interactions, 4: 117-133.

Fish, J.D., Fish, S., 1996. A Students guide to the seashore. 2nd Ed. Cambridge University Press.

Flach, E.C., 1992. Disturbance of benthic infauna by sediment-reworking activities of the lugworm *Arenicola marina*. Netherlands Journal of Sea Research, 30: 81-89.

Flach, E.C., Beukema, J.J., 1994. Density-governing mechanisms in populations of the lugworm *Arenicola marina* on tidal flats. Marine Ecological Progress Series, 115:139-149.

Fleurence, J., Morançais, M., Dumay, J., Decottignies, P., Turpin, V., Munier, M., Garcia-Bueno, N., Jaouen P., 2012. What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? Trends in Food Science and Technology, 27(1): 57-61.

Flynn, K.J., Fasham, M.J.R., Hipkin, C.R., 1997. Modelling the interactions between ammonium and nitrate uptake in marine phytoplankton. Philosophical Transactions of the Royal Society B: Biological Sciences, 352: 1625-1645.

Flynn, K.J., 2001. A mechanistic model for describing dynamic multi-nutrient, light, temperature interactions in phytoplankton. Journal of Plankton Research, 23 (9): 977-997.

Ford, A., 1999. Modelling the Environment. Island Press, Washington DC, pp 319-352.

Ford, A., 2009. Modelling the Environment 2nd ed. Island Press, Washington DC, p319-352.

Forrester, J., 1990. Principles of systems. Portland: Productivity Press.

Fowler, S.L., 1999. Guidelines for managing the collection of bait and other shoreline animals within UK marine special areas of conservation. Report to English Nature UK Marine SACs Project. 112 pp.

Fox, D.L., Coe, W.R., 1943. Biology of the California sea-mussel (*Mytilus californianus)*. II. Nutrition, metabolism, growth and calcium deposition. Journal of Experimental Zoology, 93: 205-249.

Franco, A.R., Ferriera, J.G., Nobre, A.M., 2006. Development of a growth model for penaeid shrimp. Aquaculture, 259: 268-277.

Fréchette, M., Aitken, A.E., Pagé, L., 1992. Interdependence of food and space limitation of a benthic suspension feeder: consequences for self-thinning relationships. Marine Ecological Progress Series, 83: 55-62

Friedlander, M., Levy, I., 1995. Cultivation of *Gracilaria* in outdoor tanks and ponds. Journal of Applied Phycology, 7: 315-324.

Fujita, R.M., 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. Journal of Experimental Marine Biological Ecology, 92: 283-301.

Garnett, T., Wilkes, A., 2014. Appetite for Change: Social, Economic and Environmental Transformations in China's Food System. Oxford, United Kingdom: Food Climate Research Network.

Giangrande, A., Cavallo, A., Licciano, M., Mola, E., Pierri, C., Trianni, L., 2005. Utilisation of the filter feeder polychaete *Sabella spallanzanii* Gmelin (Sabellidae) as bioremediator in aquaculture. Aquacultur International, 13: 129-136.

Giles, H., Broekhuizen, N., Bryan, K.R., Pilditch, C.A., 2009. Modelling the dispersal of biodeposits from mussel farms: the importance of simulating biodeposit erosion and decay. Aquaculture, 291: 168-178.

Gillibrand, P.A., Gubbins, M.J., Greathead, C., Davies, I.M., 2002. Scottish executive loctional guidelines for fish farming: predicted levels of nutrient enhancement and benthic impact. Scott Fish Res Rep 63/2002, Fisheries Research Services, Aberdeen. <http://www.gov.scot/Uploads/Documents/Report63.pdf> [Accessed, 21 November 2015].

Goldman, J.C, Tenore, K.R., Ryther, J.H., Corwin, N., 1974. Inorganic nitrogen removal in a combined tertiary treatment–marine aquaculture system: I. Removal efficencies. Water Resourses, 8: 45-54.

Gordon, D.M., McComb, A.J., 1989. Growth and production of the green alga *Cladophora montagneana* in a eutrophic Australian estuary and its interpretation using a computer program. Water Resourses, 23: 633-645.

Gowen, R.J., Bradbury, N.B., 1987. The ecological impact of salmonid farming in coastal waters: a review. Oceanography and Marine Biology Review, 25: 563-575.

Gowen, R.J., Bradbury, N.B., Brown, J.R., 1989. The use of simple models in assessing two of the interactions between fish farming and the marine environment. In Aquaculture biotechnology in progress, ed. N. De Pauw, E., Jaspers and N. Wilkins,. European Aquaculture Society, Belgium. pp. 1071-1080.

Granada, L., Sousa, N., Lopes, S., Lemos, M.F.L., 2015. Is integrated multitrophic aquaculture the solution to the sectors' major challenges? – a review. Reviews in Aquaculture: 1753-5131.

Grant, W. E., Pedersen, E.K., Marin, S.L., 1997. Ecology and natural resource management: systems analysis and simulation. John Wiley and Sons Inc. USA.

Grant, J., Bacher, C., Cranford, P.J., Guyondet, T., Carreau, M., 2008. A spatially explicit ecosystem model of seston depletion in dense mussel culture. Journal of Marine Systems, 73: 155-168.

Grant, J., Filgueira, R., 2011. The application of dynamic modeling to prediction of production carrying capacity in shellfish farming. In: Shumway SE (ed) Shellfish aquaculture and the environment. John Wiley & Sons, Chichester, pp. 135-154.

Greenpeace, 2015. Challenging the Aquaculture Industry in Sustainability. <http://www.greenpeace.org/canada/Global/canada/report/2008/6/challenging-aquaculture-industry-on-sustainability.pdf> [Accessed, 5 October 2015]

Grigorakis, K., Rigos, G., 2011. Aquaculture effects on environmental and public welfare – The case of Mediterranean mariculture. Chemosphere, 855: 899-919.

Grosjean, P., Spirlet, C., Gosselin, P., Vaïtilingon, D., Jangoux, M., 1998. Land-based, closed-cycle echiniculture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata): A long-term experiment at a pilot scale. Journal of Shellfish Research, 17 (5): 1523-1531.

Grosjean P., 2001. Growth model of the reared sea urchin *Paracentrotus lividus* (Lamarck, 1816). Ph.D. Thesis. Universite Libre De Bruxelles: Belgium.

Grossmann W. D. Integrated Dynamic Modelling. Available at: <http://oliver.comedia.co.at/fileadmin/Dokumente/uni/sl1/usw1www_skript_grossmann_2.pdf> [Accessed, 10 June 2015]

Haario H., Kalachev L., Laine M. 2009. Reduced models of algae growth. Bulletin of Mathematical Biology, 71: 1626-1648.

Hall P.O.J., Anderson L.G., Holby O., KoUberg S., Samuelson M.O., 1990. Chemical flux and mass balances in a marine fish cage farm. Marine Ecological Progress Series, 61: 61-73.

Hall P.O.J., Holby O, Kollberg S. and Samuelson M.O., 1992. Chemical flux and mass balances in a marine fish cage farm. 4. Nitrogen. Marine Ecological Progress Series, 89, 81-91.

Handå, A., Ranheim, A., Olsen, A.J., Altin, D., Reitan, K.I., Olsen, Y., Altinc, D., Reitan, K.I., Olsen, Y., Reinertsen, H., 2012. Incorporation of salmon fish feed and feces components in mussels (*Mytilus edulis*): implications for integrated multi-trophic aquaculture in cool-temperate North Atlantic waters. Aquaculture, 370-371: 40-53.

Hansell, D.A., Carlson, C.A., 2002. Biogeochemistry of Marine Dissolved Organic Matter, 774 pages, Academic Press.

Hardstein, N.D., Stevens, C.L., 2005. Deposition beneath long-line mussel farms. Aquacultural Engineering, 33: 192-213.

Harlin, M.M., Thorne-Miller, B., Thursby, G.B., 1978. Ammonium uptake by *Gracilaria sp.* (Florideophyceae) and *Ulva lactuca* (Chlorophyceae) in closed system fish culture. In: Jensen, A., Stein, R.J. (Eds.), Proceedings of the Ninth International Seaweed Symposium. Science Press, Princeton, pp. 285-292.

Hastings, A. and Gross, L., 2011. Sourcebook in Theoretical Ecology University of California Press pp 249-258.

Haven, D.S., Morales-Alamo, R., 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. Limnology and Oceanography, 11: 487-498.

Hernández I., Martinez-Aragon J.F., Tovar A., Perez-Llorens J.L. and Vergara J.J., 2002. Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 2. Ammonium. Journal of Applied Phycology, 14: 375-384.

Hernández, I., Pérez-Pastor, A., Vergara, J.J., Martínez-Aragón, J.F., Fernández-Engo, M.A., Pérez-Lloréns, J.L., 2006. Studies on the biofiltration capacity of *Gracilariopsis longissima*: from microscale to macroscale. Aquaculture, 252: 43-53.

Hirata, H., Kohirata, E., 1993. Culture of the sterile *Ulva sp.* in marine fish farm. Israeli Journal of Aquaculture – Bamidgeh, 45: 164-168.

Holby, O., Hall, P.O.J., 1991. Chemical fluxes and mass balance in a marine fish cage farm. Phosphorus. Marine Ecological Progress Series, 70: 263-272.

Holt, R., 1991. Surveys of Scottish Sealochs, Lochs Laxford, Inchard, Broom and Little Loch Broom. Marine Nature Conservation Review Report no. 16. JNCC, Peterborough, UK.

Holdt, S., Moehlenberg, F., Dahl-Madsen, K.I., 2006. Polyculture in Denmark: Status, Feasibility and Future, paper presented at the World Aquaculture Society conference, Florence, Italy.

Holdt, S., Edwards, M., 2014. Cost Effective IMTA: a comparison of the production efficiencies of mussels and seaweed. Journal of Applied Phycology, 26(2): 933-945.

Honda, H., Kikutchi, K., 2002. Nitrogen budget of polychaete *Perinereis nuntia* Vallata fed on the feces of Japanese flounder. Fisheries Science, 68: 1304-1308.

Hootsmans, M.J.M., 1994. A growth analysis model for *Potamogeton pectinatus* L. Geobotany, 21: 250-286.

Hopkins, K.D., 1992. Reporting Fish Growth: a Review of the Basics. Journal of the World Aquaculture Society, 23(3): 173-179.

Hopkins, J.S., Hamilton, R.D., Sandifer, P.A., Browdy, C.L., 1993. The production of bivalve molluscs in intensive shrimp ponds and their effect on shrimp production and water quality. World Aquaculture, 24: 74-77.

Howie, D.I.D., 1984. 'The reproductive biology of the lugworm *Arenciola marina*' Fortschritte der Zoologie, Band 29 - Fischer/Pfannenstiel: Polychaete reproduction, Gustav Fisher Verlag, New York.

Hughes, A.D., and Kelly, M.S., 2001. Integrated Multi-trophic Aquaculture. Scottish Association of Marine Science, p 6 <http://www.sarf.org.uk/cms-assets/documents/28926-823833.current-state-of-integrated-aquacultur> [Accessed, 23 May 2014]

Huisman J., Matthijs H.C.P., Visser P.M., Balke H., Signon C.A.M., Passarge J., Mur. L.R., 2002. Principles of the light-limited chemostat: theory and ecological applications. Antonie van Leeuwenhoek, 81(1-4): 117-133.

Hylleberg, J., 1975. Selective feeding by *Abarenicola pacifica* with notes = on *Abarenicola vagabunda* and a concept of gardening in lugworms. Ophelia, 14: 113-137.

Isee Systems Website (STELLA). 2015. <http://www.iseesystems.com/softwares/Education/StellaSoftware.aspx> [Accessed, 5 March 2015]

Islam, M.S., 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. Marine Pollution Bulleting, 50: 48-61.

Iwama, G.K., Tautz, A.F., 1981. A Simple Growth Model for Salmonids in Hatcheries. Canadian Journal of Fisheries and Aquatic Sciences, 38: 649-656.

Jackson, A., 2008. *Psammechinus miliaris*. Green sea urchin. Marine Life Information Network: Biology and Sensitivity Key Information Sub-programme. Plymouth: Marine Biological Association of the United Kingdom. <http://www.marlin.ac.uk/specieshabitats.php?speciesID=4216> [Accessed, 22 September 2015]

Jackson, A., 2008. *Psammechinus miliaris*. Green sea urchin. Marine Life Information Network: Biology and Sensitivity Key Information Sub-programme.<http://www.marlin.ac.uk/speciessensitivity.php?speciesID=4216> [Accessed, 30 October 2015].

Jacob, G.S., Pruder, G.D., Wang, J.K., 1993. Growth trail with the American oyster *Crassostrea virginica* using shrimp pond water as feed. Journal of World Aquaculture Society, 24: 344-351.

Jiang, W., Gibbs, M.T., 2005. Predicting the carrying capacity of bivalve shell- fish culture using a steady, linear food web model. Aquaculture, 244: 171-185.

Jiang, Z.J., Fang, J.G., Mao, Y.Z., Wang, W., 2010. Eutrophication assessment and bioremediation strategy in a marine fish cage culture area in Nansha Bay. Journal of Applied Phycology, 22: 421-426.

Jobling, M., 1994. Fish bioenergetics. Chapman & Hall, London.

Jobling M., 2003. The thermal growth coefficient (TGC) model of fish growth: a cautionary note. Aquaculture Research, 34: 581-584.

Jones, T.O., Iwama, G.K., 1991. Polyculture of the Pacific oyster, *Crassostrea gigas* (Thurnberg) with Chinook salmon, *Oncorhynchus tschawytscha*. Aquaculture, 92: 313-322.

Jones, A.B., Dennison, W.C. and Preston, N.P., 2001. Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: a laboratory scale study. Aquaculture, 193: 155-178.

Jones, A.B., Dennison, W.C. and Preston, N.P., 2002. The efficiency and condition of oysters and macroalgae used as biological filters of shrimp pond effluent. Aquaculture Research, 33: 1-19.

Kaartvedt, S., Johnsen, T.M., Aksnes, D.L., Lie, U., Svendsen, H., 1991. Occurrence of the toxic phytoplagellate *Prymnesium parvum* and associated fish mortality in a Norwegian fjord system. Canadian Journal of Fisheries and Aquatic Science, 48: 2316-2323.

Kaiser M.J., Laing, I., Utting, S.D., Burnell, G.M., 1998. Environmental impacts of bivalve mariculture. Shellfish Research, 17: 59-66.

Kang, Y.H., Hwang, J.R., Chung, I.K., Park, S.R., 2013. Development of a Seaweed Species-Selection Index for Successful Culture in a Seaweed-Based Integrated Aquaculture System. Journal of Ocean University China, 12(1): 125-133.

Karasov, W.H., Martínez del Rio, C., 2007. Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins. Princeton: Princeton University Press.

Karayücel, S., Karayücel, I., 1999. Growth and mortality of mussels (*Mytilus edulis* L.) reared in lantern nets in loch Kishorn, Scotland. Journal of Veterinary and animal sciences 23: 397-402.

Kaushik, S.J., 1998. Nutritional bioenergetics and estimation of waste production in non-salmonids. Aquatic Living Resources, 11(4): 211-217.

Kautsky, N., Evans, S., 1987. Role of biodeposition by *Mytilus edulis* in the circulation of matter and nutrients in a Baltic coastal system. Marine Ecological Progress Series, 38: 201-212.

Kautsky, N., Berg, H., Buschmann, A., Folke, C., Troell, M., 1996. Ecological footprint, resource use and limitations to aquaculture development. IX Congreso Latinoamericano de Acuicultura, Book of Abstracts: 193.

Kelly, M.S., Brodie, C.C. and McKenzie J.D., 1998. Somatic and gonadal growth of the sea urchin *Psammechinus miliaris* (Gmelin) in polyculture with the Atlantic Salmon. Journal of Shellfish Research, 17: 1557-1562.

Kelly, M.S., 2000. The reproductive cycle of the sea urchin *Psammechinus miliaris* (Echinodermata: Echinoidea) in a Scottish sea loch. Journal Marine Biological Association UK 80: 909-919.

Kelly, M.S., 2002. Survivorship and growth rates of hatchery-reared sea urchins. Aquaculture International, 10(4): 309-316.

Kelly, M.S., Hughes, A.D., Cook, E.J., 2013. Chapter 22 - *Psammechinus miliaris*, In: John M. Lawrence, Editor(s) Developments in Aquaculture and Fisheries Science, 38: 329-336.

Ketelaars, J.M.H., 2007. Sealand Sole: an innovative pilot project on integrated multitrphic aquaculture. Abstract. 10th International Conference on Shellfish Restoration (ICSR), November 12-16, 2007, Vissingen, The Netherlands.

Kindler, E., Krivy, I., 2011. Object-Oriented Simulation of systems with sophisticated control. International Journal of General Systems, 313-343.

Klausmeier, C.A., Litchman, E., Levin, S.A., 2004. Phytoplankton growthand stoichiometry under multiple nutrient limitation. Limnology and Oceanography, 4: 1463-1470.

Kooijman, S.A.L.M., 1986. Energy budgets can explain body size relations. Journal of Theoretical Biology, 121: 269-282.

Kooijman, S.A.L.M., 2008. Dynamic Energy Budget theory for metabolic organization. Third Edition CUP, Cambridge.

Kooijman, S.A.L.M., 2014. Add_my_pet: *Paracentrotus lividus*. URL: http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/html/Paracentrotus_lividus.html [28 January 2015]

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C.O., Banta, G.T., 2012. What is bioturbation? Need for a precise definition for fauna in aquatic sciences. Marine Ecology Progress Series, 446: 285-302.

Kutti, T., Ervik, A., Hansen, P.K., 2007. Effects of organic effluents from a salmon farm on a fjord system. I. Vertical export and dispersal processes. Aquaculture, 262: 367-381

LaBarbera, M., 1989. Analysing body size as a factor in ecology and evolution. Annual Reviews of Ecological Systems, 20: 97-117.

Ladle, M., Casey, H., Gledhill, T., 2015. Operation sea anglers: what fish eat. <http://www.mikeladle.com/chapter2.html> [Accessed, 26 October 2015]

Lander, T.R., Robinson, S.M.C., MacDonald, B.A., Martin, J.D., 2013. Characterization of the suspended organic particles released from salmon farms and their potential as a food supply for the suspension feeder, *Mytilus edulis* in integrated multitrophic aquaculture (IMTA) systems. Aquaculture, 406-407: 160-171.

Lapointe, B.E., Tenore, K.R., 1981. Experimental outdoor studies with *Ulva fasciata* Delile I. Interaction of light and nitrogen on nutrient uptake, growth and biochemical composition. Journal of Experimental Marine Biology and Ecology, 92: 135-152.

Larsen, P.S., Filgueira, R., Riisgård, H.U., 2014. Somatic growth of mussels *Mytilus edulis* in field studies compared to predictions using BEG, DEB, and SFG models. Journal of Sea Research, 88: 100-108.

Lawrence, J.M., 2001. Function of eponymous structures in echinoderms: a review. Canadian Journal of Zoology, 79: 1251-1264.

Le Gall, P., 1990. Culture of echinoderms. In: G. Barnabe (Editor). Aquaculture 1, Ellis Horwood, New York, pp. 443-462.

Leighton, P., 1995. Contributions towards the development of echinoculture in North Atlantic waters with particular reference to *Paracentrotus lividus* (Lamarck). PhD Thesis, National University of Ireland, Galway.

Lester, N.P., Shuter, B.J., Abrams, P.A., 2004. Interpreting the von Bertalanffy model of somatic growth in fishes: the cost of reproduction. Proceedings of the Royal Society of London Series B, 271: 1625-1631.

Lewis, J., Loftus, W., 2008. Java Software Solutions Foundations of Programming Design 6th ed. Pearson Education Inc. ISBN 0-321-53205-8., section 1.6 "Object-Oriented Programming".

Lin, C.K., Ruamthaveesub, P., Wanuchsoontorn, P., Pokaphand, C., 1992. Integrated culture of green mussel (*Perna viridis*) and marine shrimps (*Penaeus monodon*). Book of Abstracts, Aquaculture '92, 21-25 May, Orlando, FL.

Lin, C.K., Ruamthaveesub, P., Wanuchsoontorn, P., 1993. Culture of the green mussel (*Perna viridis*) in waste water from an intensive shrimp pond: concept and practice. World Aquaculture, 24: 68-73.

Littler, M.M., Arnold, K.E., 1982. Primary productivity of marine macroalgal functional-form groups from southwestern North America. Journal of Phycology, 18: 307-311.

Lobban, C.S., Harrison P.J., 1994. Seaweed Ecology and Physiology. CUP, Cambridge.

Lobban, C.S., Harrison, P.J., 1997. Seaweed ecology and physiology. Cambridge University press pp 178, 366.

López, B.D., Bunke, M., Shirai, J.A.B., 2008. Marine aquaculture of Sardinia Island (Italy): ecosystem effects evaluated through a trophic mass-balance model. Ecological Modelling, 212: 292-303.

Lu, L., Wu, R.S.S., 1998. Recolonization and succession of marine macrobenthos in organic enriched sediment deposited from fish farms. Environmental Bulletin, 101: 241-251.

Luo, M.B., Liu, F., Xu, Z.L., 2012. Growth and nutrient uptake capacity of two co-occurring species, *Ulva prolifera* and *Ulva linza*. Aquatic Botany, 100: 18-24.

Lupatsch, L., Kissil, G.W., 1998. Predicting aquaculture waste from gilthead seabream (*Sparus aurata*) culture using a nutritional approach. Aquatic and Living Resources, 11: 265-268.

MacArtain, P., Gill, C.I.R., Brooks, M., Campbell, R., Rowland, I.R., 2007. Nutritional value of edible seaweeds. Nutrition Reviews, 65: 535-543.

MacDonald, B.A., Robinson, S.M.C., Barrington, K.A., 2011. Feeding activity of mussels (*Mytilus edulis*) held in the field at an integrated multitrophic aquaculture (IMTA) site (*Salmo salar*) and exposed to fish food in the laboratory. Aquaculture, 314: 244-251.

MacDonald, C.L.E., Stead, S.M., Slater, M.J., 2013. Consumption and remediation of European seabass (*Dicentrarchus labrax*) waste by the sea cucumber *Holothuria forskali*. Aquaculture International, 21(6): 1279-1290.

Macedo, M.F. Duarte, P., 2006. Phytoplankton production modelling in three marine ecosystems- static versus dynamic approach. Ecological modelling, 190: 299- 316.

Magill, S.H., Thetmeyer, H., Cromey, C.J., 2006. Settling velocity of faecal pellets of gilthead sea bream (*Sparus aurata* L.) and sea bass (*Dicentrarchus labrax* L.) and sensitivity analysis using measured data in a deposition model. Aquaculture, 251: 295-305.

Mao, Y., Yang, H., Zhou, Y., Ye, N., Fang, J., 2009. Potential of the seaweed *Gracilaria lemaneiformis* for integrated multi-trophic aquaculture with scallop *Chlamys farreri* in north China. Journal of Applied Phycology, 21: 649-656.

Marine Harvest, 2012. Salmon farming industry handbook. <http://www.marineharvest.com/PageFiles/1296/2012%20Salmon%20Handbook%2018.juli_h%C3%B8y%20tl.pdf> [Accessed, 30 April 2014]

Marine Scotland, 2013. Draft Seaweed Policy Statement Consultation Paper. <http://www.scotland.gov.uk/Publications/2013/08/6786> [Accessed, 1 June 2014]

Mata, L., Silva, J., Schuenhoff, A., Santos, R., 2006. The effects of light and temperature on the photosynthesis of the *Asparagopsis armata* tetrasporophyte (*Falkenbergia rufolanosa*) cultivated in tanks. Aquaculture, 252: 12-59.

Matos, J., Costa, S., Rodrigues, A., Pereira, R. and Sousa Pinto, I., 2006. Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. Aquaculture, 252: 31-42.

Mazzola, A., Sará, G., 2001. The effect of fish farming organic waste on food availability for bivalve molluscs (Gaeta Gulf, central Tyrrhenian, MED): stable carbon isotopic analysis. Aquaculture, 192: 361-379.

McLusky, D.S, 1983. Distribution and population recovery of *Arenicola marina* and other benthic fauna after bait digging. Marine Ecology Progress Series, 11: 173-179.

Mente, E., Pierce, G.J., Santos, M.B., Neofitou, C., 2006. Effect of feed and feeding in the culture of salmonids on the marine aquatic environment: a synthesis for European aquaculture. Aquaculture International, 14: 499−522.

Merrill, J., 1989. Commercial nori (*Porphyra*) sea-farming in Washington State. In Kain J, Andrews JW, McGregor BJ (eds), Aquatic Primary Biomass-Marine Macroalgae: Outdoor Seaweed Cultivation, Proc. 2ndWorkshop of COST 48, Subgroup 1. COST 48, Commission of the European Communities, Brussels: pp. 90-112.

Metaxa, E., Deviller, G., Pagand, P., Alliaume, C., Casellas, C., Blancheton, J.P., 2006. High rate algal pond treatment for water reuse in a

marine fish recirculation system: water purification and fish health. Aquaculture, 252: 92-101.

MIT System Dynamics in Education Project (SDEP). 1997. MIT SDEP: What is System Dynamics? <http://web.mit.edu/sysdyn/sd-intro/> [Accessed, 22 June 2014].

Møhlenberg, F., Riisgård, H.U., 1978. Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia, 17: 239-246.

Molloy, S.D., Pietrak, M.R., Bouchard, D.A., Bricknell, I., 2011. Ingestion of *Lepeophtheirus salmonis* by the blue mussel *Mytilus edulis*. Aquaculture, 311: 61-64.

Molloy, S.D., Pietrak, M.R., Bricknell, I., Bouchard, D.A., 2013. Experimental transmission of infectious pancreatic necrosis virus from the blue mussel, *Mytilus edulis*, to cohabitating Atlantic salmon (*Salmo salar*) smolts. Applied and Environmental Microbiology, 79(19): 5882-5890.

Monaco, C.J., Wethey, D.S., Helmuth, B., 2014. A Dynamic Energy Budget (DEB) Model for the Keystone Predator *Pisaster ochraceus*. PLoS One 9(8): e104658. doi: 10.1371/journal.pone.0104658.

Montero-Torreiro, M.F., Garcia-Martinez, P., 2003. Seasonal changes in the biochemical composition of body components of the sea urchin, *Paracentrotus lividus*, in Lorbe (Galicia, north-western Spain). Journal of the Marine Biological Association of the United Kingdom, 83: 575-581.

Mortensen, T.H., 1943. A monograph of the Echinoidea. Camarodonta II. CA Reitzel, Copenhagan Otero-Villanueva M, Kelly MS, Burnell G (2004) How diet influences energy partitioning in the regular echinoid *Psammechinus miliaris*; constructing an energy budget. Journal of Experimental Marine Biology and Ecology, 304: 159-181.

Muetzelfeldt, R., Massheder, J., 2003. The Simile visual modelling environment. European Journal of Agronomy, 18: 345-358.

Mumford, T.F., 1990. Nori cultivation in North America: growth of the industry. Hydrobiologia, 204/205: 89-98.

Mumford, T.F., Miura, A., 1988. Porphyra as food: cultivation and economics. In Lembi CA, Waaland JR (eds), Algae and Human Affairs, Cambridge University Press, London, pp. 87-117.

Musango, J.K., de Wit, M.P, Corner, R., Telfer, T., Zang, Z., Yan, X., 2007. Waste cost functions in aquaculture. CSIR Report No: CSIR/NRE/ECO/ER/2007/0065/C. Stellenbosh, South Africa. 29 pp.

Joel, K., Bourne, J.R., National Geographic. 2015. How to farm better fish? <http://www.nationalgeographic.com/foodfeatures/aquaculture/> [Accessed, 22 June 2014].

Navarrete-Mier, F., Sanz-Lázaro, C., Marín, A., 2010. Does bivalve mollusc polyculture reduce marine fin fish farming environmental impact?, Aquaculture, 306(1-4): 101-107.

Navarro, J.M., Thompson, R.J., 1997. Biodeposition by the horse mussel *Modiolus modiolus* during the spring diatom bloom. Journal of Experimental Marine Biology and Ecology, 209: 1-13.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature, 405 (29): 1017-1024.

Nelson, D.L., Cox, M.M., 2000. Principles of Biochemistry, 3rd edition; By. Worth Publishers: New York, ISBN 1-57259-9316.

Nelson, G.S., Glenn, E.P., Conn, J., Moore, D., Walsh, T. and Akutagawa, M., 2001. Cultivation of *Gracilaria parvispora* (Rhodophyta) in shrimp-farm effluent ditches and floating cages in Hawaii: a two phase polyculture system. Aquaculture, 192: 239-248.

Nelson, E.J., MacDonald, B.A., Robinson, S.M.C., 2012. The absorption efficiency of the suspension-feeding sea cucumber, *Cucumaria frondosa*, and its potential as an extractive integrated multi-trophic aquaculture (IMTA) species. Aquaculture, 370-371(11): 19-25.

Neori, A., 1996. The type of N-supply (ammonia or nitrate) determines the performance of seaweed biofilters integrated with intensive fish culture. Israeli Journal of Aquaculture – Bamidgeh, 48: 19-27.

Neori A., Krom M.D., Ellner S.P., Boyd C.E., Popper D., Rabinovitch R. et al. 1996. Seaweed biofilters as regulators of water quality in integrated fish–seaweed culture units. Aquaculture, 141: 183-199.

Neori, A., Cohen, I., Gordin, H., 1991. *Ulva lactuca* biofilters for marine fishpond effluents. II. Growth rate, yield and C:N ratio. Botanica Marina, 34: 483-489.

Neori, A., Ragg, N.L.C., Shpigel, M., 1998. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: II. Performance and nitrogen partitioning within an abalone (*Haliotis tuberculata*) and macroalgae culture system. Aquaculture Engineering, 17: 215-239.

Neori, A., Shpigel, M., 1999. Using algae to treat effluents and feed invertebrates in sustainable integrated mariculture. World Aquaculture, 30: 46-49.

Neori, A., Shpigel, M., Ben-Ezra, D., 2000. A sustainable integrated system for culture of fish, seaweed and abalone. Aquaculture, 186: 279-291.

Neori, A., Msuya, F.E., Shauli, L., Schuenhoff, A., Kopel, F., Shpigel, M., 2003. A novel three-stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculture. Journal of Applied Phycology, 15: 543-553.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M. and Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasising seaweed biofiltration in modern mariculture. Aquaculture, 231: 361-391.

Neori, A., Troell, M., Chopin, T., Yarish, C., Critchley, A., Buschmann, A.H., 2007. The need for a balanced ecosystem approach to blue revolution aquaculture. Environment, 49: 36-43.

Newell G.E., 1948. A contribution to our knowledge of the life history of *Arenicola marina* L. Journal of Marine Biology Association UK, 27 (3): 554-580

Newell, R., 1989. Species profiles: life histories and environmental requirements of coastal fiches and invertebrates (north and mid-atlantic): blue mussel. U.S. Fish and Wildlife Service Biological Report, 82(11.102). U.S. Army Corps of Engineers,TR E1-82-4: 25pp.

Newell, R.I.E., 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: A review. Journal of Shellfish Research, 23(1): 51-61.

Newell, R.I.E., 2007. A framework for developing 'ecological carrying capacity' mathematical models for bivalve mollusc aquaculture. Bulletin Fisheries Research Agency, 19: 41-51.

Newell, C.R., Richardson, J., 2014. The effects of ambient and aquaculture structure hydrodynamics on the food supply and demand of mussel rafts. Journal of Shellfish Research, 33(1): 257-272

Nisbet, R.M., Jusup, M., Klanjscek, T., Pecquerie, L., 2012. Integrating dynamic energy budget (DEB) theory with traditional bioenergetic models. Journal of Experimental Biology, 215: 892-902.

Nizzolli, D., Welsh, D.T., Bartoli, M., Viaroli, P., 2005. Impacts of mussel (*Mytilus galloprovincialis*) farming on oxygen consumption and nutrient cycling in a eutrophic coastal lagoon. Hydrobiologia, 550: 183-198.

Nobre, A.M., Ferreira, J.G., Nunes, J.P., Yan, X., Bricker, S., Corner, R., Groom, S., Gu, H., Hawkins, A.J.S., Hutson, R., Lan, D., Lencarte, Silva J.D., Pascoe, P., Telfer, T., Zhang, X., Zhu, M., 2010. Assessment of coastal management options by means of multilayered ecosystem models. Estuarine, Coastal and Shelf Science, 87(1): 43-62.

Noda, H., 1993. Health benefits and nutritional properties of nori. Journal of applied Phycology, 5: 255-258.

Nunes, J.P., Ferreira, J.G., Gazeau, F., Lencart-Silva, J., Zhang, X.L., Zhu, M.Y., Fang, J.G., 2003. A model for sustainable management of shellfish polyculture in coastal bays. Aquaculture, 219: 257-277.

Olive, P.J.W., 1999. Polychaete aquaculture and polychaete science: a mutual synergism. Hydrobiologia, 402: 175-183.

Olive, P.J.W., Craig, S., Rutherford, G., 2004. Enhanced aquaculture feeds: WO2006016135 A2.

Olive, P.J.W., Craig, S., Douglas, P.B, 2006. Aquaculture of marine worms: US 7,004,109 B2

Olsen, Y., Olsen, L.M., 2008. Environmental impact of aquaculture on coastal planktonic ecosysytems. In: Tsukamoto K, Kawamura T, Takeuchi T, Beard TD Jr, Kaiser MJ (eds) Fisheries for global welfare and environment. Proc 5th World Fisheries Congress 2008, Terrapub, Tokyo, p 181-196.

Painter, D.S., Jackson, M.B., 1989. *Cladophora* internal phosphorus modelling: verification. Journal of Great Lakes Research, 15: 700-708.

Pais, A., Saba, S., Rubattu, R., Meloni, G., Montisci, S., 2011. Proximate composition of edible sea urchin *Paracentrotus lividus* roe commercialised In Sardinia. Biologia Marina Mediterranea, 18(1): 390-391.

Paltzat, D.L., Pearce, C.M., Barnes, P.A., McKinley, R.S., 2008. Growth and production of California sea cucumbers (*Parastichopus californicus* Stimpson) co-cultured with suspended Pacific oysters (*Crassostrea gigas* Thunberg). Aquaculture, 275:124-137.

Pecquerie, L., Johnson, L.R., Kooijman, S.A.L.M., Nisbet, R.M., 2011. Analyzing variations in life-history traits of Pacific salmon in the context of dynamic energy budget (DEB) theory. Journal of Sea Research, 66: 424-433.

Pedersen, L.F., Suhr, K.I., Dalsgaard, J., Pedersen, P.B., Arvin, E., 2012. Effects of feed loading on nitrogen balances and fish performance in replicated recirculating aquaculture systems. Aquaculture, 338-341: 237-245.

Pereira, R., Kraemer, G.P., Yarish, C., Sousa-Pinto, I., 2008. Nitrogen uptake by gametophytes of *Porphyra dioica* (Bangiales, Rhodophyta) under controlled culture conditions. Journal of Phycology, 43(1): 107-118.

Pereira, R., Yarish, C., 2010. The role of Porphyra in sustainable culture systems: physiology and applications. In: Israel A, Einav R, Seckbach J (eds) Seaweeds and their role in globally changing environments, vol 15, Cellular Origin, Life in Extreme Habitats and Astrobiology. Springer, Dordrecht, pp 339-353.

Perrot, T., Rossi, N., Ménesguen, A., Dumas, F., 2014. Modelling green macroalgal blooms on the coasts of Brittany, France to enhance water quality management. Journal of Marine Systems, 132: 38-53.

Peters, R.H., 1983. The ecological implications of body size. Cambridge University Press, Cambridge.

Phang, S.M., Shaharuddin, S., Noraishah, H., Sasekumar, A., 1996. Studies on *Gracilaria changii* (Gracilariales, Rhodophyta) from Malaysian mangroves. Hydrobiologia, 326-327: 347-352.

Pieters, H., Kluytmans, J.H., Zurburg, W., Zandee, D.I., 1979. The influence of seasonal changes on energy metabolism in *Mytilus edulis* (L.). I. Growth rate and biochemical composition in relation to environmental parameters and spawning, in Cyclic Phenomena in Marine Plants and Animals, E. Naylor and R. G. Hartnoll, Eds., pp. 285–292, Pergamon Press, Proceedings of the 13 European Marine Biology Symposium Isle of Man.

Pietrak, M.R., Molloy, S.D., Bouchard, D.A., 2012. Potential role of *Mytilus edulis* in modulating the infectious pressure of *Vibrio anguillarum* on

an integrated multi-trophic aquaculture farm. Aquaculture, 326-29: 36-39.

Pillay, T.V.R., Kutty, M.N., 2005. Aquaculture: principles and practices. Blackwell Publishing Ltd, Oxford. 624pp.

Pleissner, D., Eriksen, N.T., Lundgreen, K., Riisgård, H.U., 2012. Biomass Composition of blue mussels, *Mytilus edulis*, is Affected by Living Site and Species of Ingested Microalgae, ISRN Zoology, doi:10.5402/2012/902152.

Porrello, S., Lenzi, M., Tomassetti, P., Persia, E., Finoia, M.G., Mercatali, I., 2003. Reduction of aquaculture wastewater eutrophication by phytotreatment ponds system: II. Nitrogen and phosphorus content in macroalgae and sediment. Aquaculture, 219: 531-544

Pouvreau, S., Bourles, Y., Lefebvre S., Gangnery, A., Alunno-Bruscia, M., 2006. Application of a dynamic energy budget model to the Pacific oyster, *Crassostrea gigas*, reared under various environmental conditions. Journal of Sea Research, 56(2): 156-167.

Powersim. 2015. <http://www.powersim.com/> [Accessed, 9 February 2015]

Primavera, H.J., 1993. A critical review of shrimp pond culture in the Philippines. Reviews of Fisheries Science, 1: 151- 201.

Read, P., Fernandes, T., 2003. Management of environmental impacts of marine aquaculture in Europe. Aquaculture, 226: 139-163.

Regional Government of Galicia, 2012. Integrated Multi-Trophic Aquaculture. A sustainable, pioneering alternative for marine cultures in Galicia. <http://www.medioruralemar.xunta.es/fileadmin/arquivos/publicacions/MAR/2012/Acuicultura/AMTI-Galicia_EN.pdf> [Accessed, 5 October 2015]

Reid, G.K., Liutkus, M., Robinson, S.M.C., Chopin, T.R., Blair, T., Lander, T., Mullen, J., Page, F., Moccia, R.D., 2009. A review of the biophysical properties of salmonid faeces: implications for

aquaculture waste dispersal models and integrated multi-trophic aquaculture. Aquaculture Research, 40: 257−273.

Reid, G.K., Liutkus, M., Bennett, A., 2010. Absorption efficiency of blue mussels (*Mytilus edulis* and *M. trossulus*) feeding on Atlantic salmon (*Salmo salar*) feed and fecal particulates: implications for integratedmulti-trophic aquaculture. Aquaculture, 299: 165-69.

Reid, G.K., Chopin, T., Robinson, S.M.C., Azevedo, P., Quinton, M., Belyea, E., 2013. Weight ratios of the kelps, *Alaria esculenta* and *Sacharina latissima*, required to sequester dissolved inorganic nutrients and supply oxygen for Atlantic salmon, *Salmo salar*, in Integrated Multi-Trophic Aquaculture systems. Aquaculture, 408/409: 34-46.

Ren, J.S., Stenton-Dozey, J., Plew, D.R., Fang, J., Gall, M., 2012. An ecosystem model for optimising production in integrated multitrophic aquaculture systems. Ecological Modelling, 246: 34-46.

Reynolds, P.J., 2005. Investigation of the growth potential and ecosystem impact of intensively farmed Atlantic salmon fed on experimental diets. Unpublished PhD thesis. University of Stirling.

Ricciardi, A., Bourget, E., 1998. Weight-to-weight conversion factors for marine benthic macroinvertebrates Vol. 163: 245-251.

Ricker, W.E., 1979. Growth rates and models. In: Fish physiology. Hoar, W.S., Randall, D.J., Brett, J.R., (Eds.), Academic Press Inc. Ltd., London, Vol. VIII.

Riisgård, H.U., Berntsen, I., Tarp, B., 1996. The lugworm (*Arenicola marina*) pump: Characteristics, modelling and energy cost. Marine Ecology Progress Series, 138(1-3): 149-156.

Riisgård, H.U., Banta, G.T., 1998. Irrigation and deposit feeding by the lugworm *Arenicola marina*, characteristics and secondary effects on the environment. A review of current knowledge. Vie et Milieu, 48 (4): 243-257.

Riisgård, H.U., Lundgreen, K., Larsen, P.S., 2012. Field data and growth model for mussels *Mytilus edulis* in Danish waters. Marine Biological Research, 8: 683-700.

Roheim, C.A., Asche, F., Santos, J.I., 2011. The elusive price premium for ecolabelled products: evidence from seafood in the UK market. Journal of Agricultural Economics, 62: 655-68.

Rönnberg, C., Bonsdorff, E., 2004. Baltic Sea eutrophication: area-specific ecological consequences. Hydrobiologia, 514: 227-241.

Rosland, R., Bacher, C., Strand O., Aure J., Strohmeier T., 2011. Modelling growth variability in longline mussel farms as a function of stocking density and farm design. Journal Of Sea Research, 66(4): 318-330.

Ross, A. H., Nisbet, R. M., 1990. Dynamic models of growth and reproduction of the mussel *Mytilus edulis* L. Functional Ecology, 4: 777-787.

Ross, L.G., Telfer, T.C., Falconer, L., Soto, D., Aguilar-Manjarrez, J., Asmah, R., Bermúdez, J., Beveridge, M.C.M., Byron, C. J., Clément, A., Corner, R., Costa-Pierce, B.A., Cross, S., De Wit, M., Dong, S., Ferreira, J.G., Kapetsky, J.M., Karakassis, I., Leschen, W., Little, D., Lundebye, A.-K., Murray, F.J., Phillips, M., Ramos, L., Sadek, S., Scott, P.C., Valle-levinson, A., Waley, D., White, P.G. & Zhu, C. 2013. Carrying capacities and site selection within the ecosystem approach to aquaculture. In L.G. Ross, T.C. Telfer, L. Falconer, D. Soto & J. Aguilar-Manjarrez, eds. Site selection and carrying capacities for inland and coastal aquaculture, pp. 19–46.FAO/Institute of Aquaculture, University of Stirling, Expert Workshop, 6–8 December 2010. Stirling, the United Kingdom of Great Britain and Northern Ireland. FAO Fisheries and Aquaculture Proceedings No. 21. Rome, FAO. 282 pp.

Rumohr, H., Brey, T., Ankar, S., 1987. A compilation of biometric conversion factors for benthic invertebrates of the Baltic Sea. The Baltic Marine Biologists Publication No. 9.

Ruokolahti, C., 1988. Effects of fish farming on growth and chlorophyll a content of *Cladophora*. Marine Pollution Bulletin, 1(4): 166-169.

Ryther, J.H., Dunstan, W.M., Tenore, K.R., Huguenin, J.E., 1972. Controlled eutrophication-increasing food production from the sea by recycling human wastes. Bioscience, 22: 144-152.

Ryther, J.H, Goldman, J.C., Gifford, C.E., Huguenin, J.E., Wing, A.S., Clarner, J.P., Williams, L.D., Lapointe, B.E., 1975. Physical models of integrated waste recycling– marine polyculture systems. Aquaculture, 5: 163-177.

SAMS. 2004. Ecosystem Approach for Sustainable Aquaculture (ECASA). <www.ecasa.org.uk> [Accessed, 15 February 2015]

Sanderson, J.C., Cromey, C.J., Dring, M.J., Kelly, M.S., 2008. Distribution of nutrients for seaweed cultivation around salmon cages at farm sites in north–west Scotland. Aquaculture, 278(1-4): 60-68.

Sanderson, J.C., Dring, M.J., Davidson, K., Kelly, M.S., 2012. Culture, yield and bioremediation potential of *Palmaria palmata* (Linnaeus) Weber & Mohr and *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders adjacent to fish farm cages in northwest Scotland. Aquaculture. 354/355, 128−135.

Sapkota, A., Sapkota, A.R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., et al. 2008. Aquaculture practices and potential human health risks: current knowledge and future priorities. Environment International, 34: 1215-1226.

Sara, G., Zenome, A., Tomasello, A., 2009. Growth of *Mytilus galloprovincialis* (mollusca, bivalvia) close to fish farms: a case of integrated multi-trophic aquaculture within the Tyrrhenian Sea. Hydrobiologia, 636:129-136.

Scheffer, M., Bakema, A.H., Wortelboer, F.G., 1993. MEGAPLANT: a simulation model of the dynamics of submerged plants. Aquatic Botany, 45: 341-356.

Schröer, M., Wittmann, A.C., Gruner, N., Steeger, H.U., Bock, C., Paul, R., Portner, H.O., 2009. Oxygen limited thermal tolerance and performance in the lugworm *Arenicola marina*: a latitudinal comparison. Journal of Experimental Marine Biology and Ecology, 372: 22-30.

Schuenhoff, A., Shpigel, M., Lupatsch, I., Ashkenazi, A., Msuya, F.E., Neori, A., 2003. A semi-recirculating, integrated system for the culture of fish and seaweed. Aquaculture, 221: 167-181.

Serebiah, J.S., 2015. Culture of marine polychaetes. In Perumal, S., Thirunavukkarasu A.R., Perumal, P. (Eds.), Advances in marine and brackishwater aquaculture. Springer India, pp. 43–49.

Shi, J., Wei, H., Zhao, L., Yuan, Y., Fang, J., Zhang, J., 2011. A physical-biological coupled aquaculture model for a suspended aquaculture area of China. Aquaculture, 318(3-4): 412-424.

Shpigel, M., Blaylock, R.A., 1991. The pacific oyster, *Crassostrea gigas*, as a biological filter for marine fish aquaculture pond. Aquaculture, 92: 187-197.

Shpigel M., Neori A., Popper D.M., Gordin H., 1993. A proposed model for ‘‘environmentally clean’’ land-based culture of fish, bivalves and seaweeds. Aquaculture, 117: 115-128.

Skar, C.K., Mortensen, S., 2007. Fate of infectious salmon anaemia virus (ISAV) in experimentally challenged blue mussels *Mytilus edulis*. Diseases of Aquatic Organisms, 74:1-6.

Skogen M.D., Eknes M., Asplin L.C., Sandvik A.D., 2009. Modelling the environmental effects of fish farming in a Norwegian fjord. Aquaculture, 298: 70-75.

Skretting (2015) Sustainable Economic Aquafeeds. <http://www.skretting.com/Internet/SkrettingGlobal/webInternet.nsf/wprid/9EE28C6A30408348C125749500466A75/$file/10_05_06_SEA_eng.pdf> [Accessed 4, May 2015]

Slater M.J., Carton A.G., 2007. Survivorship and growth of the sea cucumber *Australostichopus (Stichopus) mollis* (Hutton 1872) in polyculture trials with green-lipped mussel farms. Aquaculture, 272: 389-398.

Smaal, A.C., Vonck, A.P.M.A., 1997. Seasonal variation in C, N and P budgets and tissue composition of the mussel *Mytilus edulis*. Marine Ecology Progress Series, 153: 167-179.

Solidoro, C., Pecenik, G., Pastres, R., Franco, D., Dejak, C., 1997. Modelling macroalgae (*Ulva rıgida*) in the Venice lagoon: model structure identification and first parameters estimation. Ecological Modelling, 94: 191-206.

Soto, D., Aguilar-Manjarrez, J., Hishamunda, N., 2008. Building an ecosystem approach to aquaculture. FAO/Universitat de les Illes Balears Expert Workshop. 7–11 May 2007, Palma de Mallorca, Spain. FAO Fisheries and Aquaculture Proceedings. No. 14. Rome, FAO.

Soto, D., 2009. Integrated mariculture: a global review. Fisheries and Aquaculture Technical Papers No. 529, Food Agric. Organ. UN, Rome, 183 pp.

Sousa T., Domingos T., Poggiale J.C., Kooijman S.A.L.M., 2010. Dynamic energy budget theory restores coherence in biology. Philosophical Transactions of the Royal Society Biology, 365(1557).

Spirlet, C., Grosjean, P., Jangoux, M., 1998. Reproductive cycle of the echinoid *Paracentrotus lividus*: analysis by means of maturity index. Invertebrate Reproduction and Development, 34: 69–81.

Steele, J.H., 1962. Environmental control of photosynthesis in the sea. Limnology and Oceanography, 7:137-150.

Stigebrandt A., Aure J., Ervik A., Hansen P.K., 2004. Regulating the local environmental impact of intensive marine fish farming III. A model for estimation of the holding capacity in the Modelling – Ongrowing fish farm – Monitoring system. Aquaculture, 234: 239-261.

Strohmeier, T., Strand, O., Alunno-Bruscia, M., Duinker, A., Cranford P.J., 2012. Variability in particle retention efficiency by the mussel *Mytilus edulis*. Journal of Experimental Marine Biology 412: 96-102.

Sukhotin, A., Strelkov, P., Maximovich, N., Hummel, H., 2007. Growth and longevity of *Mytilus edulis* (l.) from northeast Europe. Marine Biology Research, 3 (3): 125-167.

Thomas, Y., Mazurié, J., Alunno-Bruscia, M., Bacher, C., Bouget, J.-F., Gohin, F., Pouvreau, S., Struski, C., 2011. Modelling spatio-temporal variability of *Mytilus edulis* (L.) growth by forcing a dynamic energy budget model with satellite-derived environmental data. Journal of Sea Research, 66(4): 308-317.

Thornley, J., France, J., 2007. Mathematical Models in Agriculture: QuantitativeMethods for the Plant, Animal and Ecological Sciences. CAB International, Wallingford, Oxfordshire, UK.

Tignor, W., Myrtveit, M., 2000 'Object Oriented Design Patterns and System Dynamics Components'. Proceedings of the International System Dynamics Society.

Titus, J., Goldstein, R.A., Adams, M.S., Mankin, J.B., O'Neill, R.V Jr., Weiler, P.R. Jr., Shugart, H.H., Booth, R.S., 1975. A production model for *Myriophyllum spicatum*. Limnology and Ecology, 56: 1129-1138.

Toerien, D.F., Cary, P.R., Finlayson, C.M., Mitchell, D.S., Weerts, P.G.J., 1983. Growth models for *Salvinia molesta*. Aquatic Botany, 16: 173-179.

Tomas, F., Romero, X., Turon, X., 2005. Experimental evidence that intra-specific competition in seagrass meadows reduces reproductive potential in the sea urchin *Paracentrotus lividus* (Lamarck). Scientia Marina, 69: 475-484.

Tomšić, S., Conides, A., Radić, I.D., Glamuzina, B., 2010. Growth, size class frequency and reproduction of purple sea urchin,

Paracentrotus lividus (Lamarck, 1816) in Bistrina Bay (Adriatic Sea, Croatia). Acta Adriatica, 51 (1): 67-77.

Tortonese, E., 1965. Echinodermata. Fauna d'Italia. Edittore Calderini, Bologna, 422 pp.

Troell, M., Halling, C., Nilsson, A., Buschmann, A.H., Kautsky, N., Kautsky, L., 1997. Integrated marine cultivation of *Gracilaria chilensis* (Gracilariales, Rhodophyta) and salmon cages for reduced environmental impact and increased economic output. Aquaculture, 156: 45-61.

Troell, M., Norberg, J., 1998. Modelling output and retention of suspended solids in an integrated salmon–mussel culture. Ecological Modelling, 110: 65-77.

Troell, M., Halling, C., Neoric, A., Chopind, T., Buschmanne, A.H., Kautskya, N., Yarishf, C., 2003. Integrated mariculture: asking the right questions. Aquaculture, 226: 69-90.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H., Fang, J.G., 2009. Ecological engineering in aquaculture— potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture, 297: 1-9.

Tsapakis, M., Pitta, P., Karakassis, I., 2006. Nutrients and fine particulate matter released from sea bass (*Dicentrachus labrax*) farming. Aquatic Living Resources, 19: 69-75.

Tsujii, K., Ichikawa, T., Matusuura, Y., Kawamura, M., 1983. Hypercholesterolemic effect of taurocyamine or taurine on the cholesterol metabolism in white rats. Sulfur Amino Acids, 6: 239-248.

Tsutsumi H, Montani S. 1993. Utilization of biological activities of capitellid polychaete for treatment of (Hedoro) (organically enriched sediment) deposited on the marine bottom just below net pen culture. Nippon Suisan Gakkaishi, 59: 1343-1347.

Tsutsumi, H., Kinoshita, K., Srithongouthai, S., Sato, A., Nagata, S., Inoue, A., Yoshioka, M., Ohwada, K., Hama, D., 2005. Treatment of the organically enriched sediment below the fish farm with the biological activities of artificially mass cultured colonies of a small deposit feeding polychaete, *Capitella sp*. Benthos Research, 60(1): 25-37.

Tsutsumi, H., Srithonguthai, S., Inoue, A., Sato, A., Hama, D. 2006. Seasonal fluctuations in the flux of particulate organic matter discharged from net pens for fish farming. Fisheries Science, 72: 119-127.

Turon, X., Giribet, G., López, S., Palacín, C., 1995. Growth and population structure of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats. Marine Ecological Progress Series, 122: 193-204.

Tyler-Walters, H., 2008a. *Mytilus edulis*. Common mussel. Marine Life Information Network: Biology and Sensitivity Key Information Sub-programme.<http://www.marlin.ac.uk/speciessensitivity.php?speciesID=3848> [Accessed, 30,October 2015].

Tyler-Walters, H., 2008b. *Arenicola marina*. Blow lug. Marine Life Information Network: Biology and Sensitivity Key Information Sub-programme.<http://www.marlin.ac.uk/speciessensitivity.php?speciesID=2592> [Accessed, 30 October 2015].

Ursin, E., 1960. A quantitative investigation of the Echinoderm fauna of the central North Sea. Medd Komm Danm Fisk-og Havunders Ser Fisk 24: 204 pp.

Vahl, O., 1972. Efficiency of particulate retention of *Mytilus edulis* (L.). Ophelia, 10: 17-25.

Valdemarsen, T., Wendelboe, K., Egelund, J.T., Kristensen, E., Flindt, M.R., 2011. Burial of seeds and seedlings by the lugworm *Arenicola marina* hampers eelgrass (*Zostera marina*) recovery. Journal of Experimental Marine Biology and Ecology, 410: 45-52.

Valiela, I., 1995. Marine Ecological Processes; Springer-Verlag, New York, 2nd Edition.

Van Haren, R.J.F., Kooijman, S.A.L.M., 1993. Application of a dynamic energy budget model to *Mytilus edulis* (L.). Netherlands Journal of Sea Research, 31: 119-133.

van der Veer, H.W., Cardoso, J.F.M.F., van der Meer, J., 2006. The estimation of DEB parameters for various Northeast Atlantic bivalve species. Journal of Sea Research, 56(2): 107-124.

Volkenborn, N., Reise, K., 2006. Lugworm exclusion experiment: responses by deposit feeding worms to biogenic habitat transformations. Journal of Experimental Marine Biology and Ecology, 330: 169-179.

Waite, R., Beveridge, M., Brummett, R., Castine, S., Chaiyawannakarn, N., Kaushik, S., Mungkung, R., Nawapakpilai, S., Phillips, M., 2014. "Improving Productivity and Environmental Performance of Aquaculture." Working Paper, Installment 5 of Creating a Sustainable Food Future. Washington, DC: World Resources Institute. <http://www.worldresourcesreport.org> [Accessed, 22 June 2015]

Wang, J.K., 1990. Managing shrimp pond water to reduce discharge problems. Aquaculture Engineering, 9: 61-73.

Wang, J.K., Jacob, G.S., 1991. Pond design and water management strategy for an integrated oyster and shrimp production system. Agricultural Engineering Dep., Univ. of Hawaii at Manoa, Honolulu, Hawaii. Aquacultur Engineering, 2: 70-82.

Wang, X., Olsen, L.M., Reitan, K.I., Olsen, Y. 2012. Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquaculture Environment Interactions, 2: 267-283.

Wang, X., Broch, O.J., Forbord, S., Handå, A., Skjermo, J., Reitan, K.I., Vadstein, O., Olsen, Y., 2013. Assimilation of inorganic nutrients from salmon (*Salmo salar*) farming by the macroalgae (*Saccharina*

latissima) in an exposed coastal environment: implications for integrated multi-trophic aquaculture. Journal of Applied Phycology 26(4), 1869-1878.

West, G.B., Brown, J.H., Enquist, B.J., 1997. A general model for the origin of allometric scaling laws in biology. Science, 276: 122-126.

Westers, H., 2000. A Fourteen-Year Dispute About Phosphorous Effluent Contribution and Its Impact on Platte Lake. Recirculating Aquaculture. Roanoke, Virginia. <http://nsgl.gso.uri.edu/vsgcp/vsgcpc00001/2000/4-EPA_DEQ_effluents.pdf > [Accessed, 20 November 2015]

Weston, D.P., 1986. The environmental effects of floating cage mariculture in Puget Sound. School of Oceanography, College of Oceans and Fisheries Sci. University of Washington, Seattle, USA, 148pp.

Wetzel, R.L., Neckles, H.A., 1986. A model of *Zostera marina* L. photosynthesis and growth: simulated effects of selected physical-chemical variables and biological interactions. Aquatic Botany, 26: 307-323.

Widdows, J., Hawkins A.J.S., 1989. Partioning of the rate of heat dissipation by *Mytilus edulis* into maintenance, feeding and growth components. Physiology and Zoology, 62: 764-784.

Wik, T.E.I., Lindén, B.T., Wramner, P.I., 2009. Integrated dynamic aquaculture and wastewater treatment modelling for recirculating aquaculture systems. Aquaculture, 287: 361-370.

WinShell, 2015 <http://www.winshell.de/> [Accessed, 27 May 2015]

Wright, R.M., McDonnell, A.J., 1986. Macrophyte growth in shallow streams: biomass model. Journal of Environmental Engineering, 112: 967-982.

Wu, R., 1995. The environmental impact of marine fish culture: towards a sustainable future. Marine Pollution Bulleting, 31: 159-166.

Xia, L.Z., Yang, L.Z., Yan, M.C., 2004. Nitrogen and phosphorus cycling in shrimp ponds and the measures for sustainable management. Environmental Geochemistry and Health, 26: 245-251.

Yang, H.S., Zhou, Y., Hu, H.Y., Liu, Y., Mao, Y., Zhou, H., Xu, X., Zhang, F., 2006. Bioremediation potential of the macroalga *Gracilaria lema neiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. Aquaculture, 252: 264−276.

Yingst, J.Y., 1976. The utilization of organic matter in shallow marine sediments by an epibenthic deposit-feeding holothurian. Journal of Experimental Marine Biology and Ecology, 23: 55-69.

Yokota, Y., 2002. Fishery and consumption of the sea urchin in Japan. In: Y. Yokota, V. Matranga & Z. Smolenicka (Editors). Sea Urchin: From Basic Biology to Aquaculture. Balkema Publ., Rotterdam, pp. 129–138.

Yu, Z.H., Hu, C.Q., Zhou, Y., Peng P.F., Li H.P., 2011. Survival and growth of the sea cucumber *Holothuria leucospilota* Brandt: A comparison between suspended and bottom cultures in a subtropical fish farm during summer. Aquaculture Research, 44(1): 114-124.

Zal, F., Lallier, F., Toulmond, A., 2002. Utilisation comme substitut sanguin d'une hémoglobine extracellulaire de poids moléculaire élevé. French Patent No.0007031, granted August 2, 2002.

Zhao, Y., Zheng, H., Bai, M. and Lin, X., 2004. Aquaculture. In: An Environmental Impact Assessment of China's WTO accession. A Report by the Task Force of WTO and environment China Council for International Co-operation on Environment and Development, (Ed. IISD), pp. 75-104.

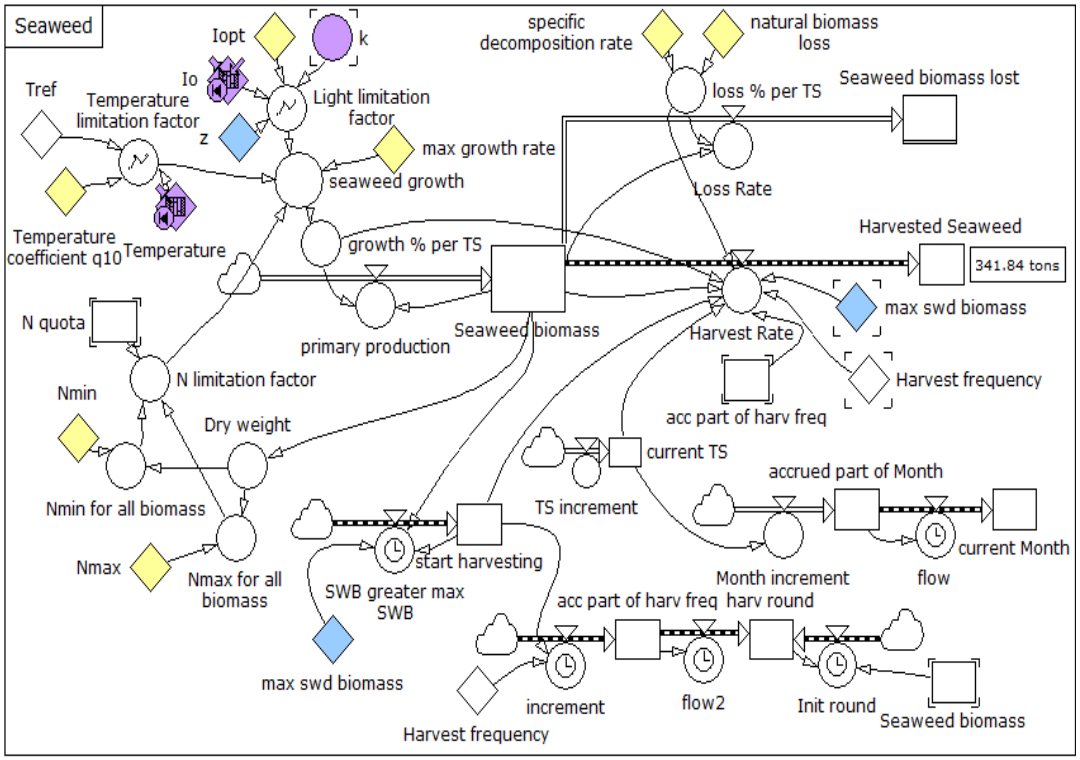
Zhou, Y., Yang, H., Liu, S., Yuan, X., Mao, Y., Zhang, T., Liu, Y., Zhang, F., 2006. Feeding on biodeposits of bivalves by the sea cucumber *Stichopus japonicus* Selenka (Echinidermata: Holothuroidea) and a suspension coculture of filter-feeding bivalves with deposit feeders in lantern nets from longlines. Aquaculture, 256: 510-520.

Appendix

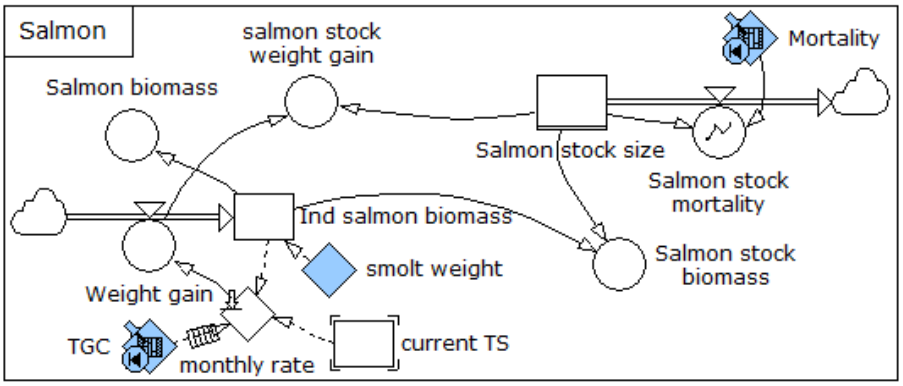
Appendix Table 1: Initial weight and diameter, weight on the 38th day and on the last day of the experiment and gonad weight of each sea urchin, where (P) is for *P. palmata*, (L) is for *L. digitata* and (F) is for the mixture of salmon faeces and pellets.

Tank	Individual	Initial weight	Initial diameter	18/7 weight (day 18)	18/7-initial weight	6/8 weight (day 38)	6/8 weight-initial weight	Gonad weight	SGR
1 (P)	1	30.97	4	31.43	0.46	31.04	0.07	1.76	0.03
1 (P)	2	22.68	3	22.69	0.01	21.72	-0.96	2.86	0.11
1 (P)	3	42.11	4	43.04	0.93	43.6	1.49	3.77	-0.03
tank average		31.92		32.39	0.47	32.12	0.2		0.02
2 (P)	1	46.88	5	47.7	0.82	48.17	1.29	1.9	-0.03
2 (P)	2	43.7	5	44.55	0.85	44.8	1.1	7	-0.01
2 (P)	3	17.07	3.5	17.75	0.68	18.46	1.39	1.77	-0.10
tank average		35.88		36.67	0.78	37.14	1.26		-0.03
3 (P)	1	32.94	4	31.6	-1.34	31.91	-1.03	1.77	-0.03
3 (P)	2	33.39	4	33.6	0.21	33.36	-0.03	4.87	0.02
3 (P)	3	19.88	3	20.65	0.77	20.85	0.97	2.33	-0.03
tank average		28.74		28.62	-0.12	28.71	-0.03		-0.01
4 (F)	1	85.95	7	86.88	0.93	86.65	0.7	11.77	0.01
4 (F)	2	24.04	4	24.8	0.76	23.58	-0.46	6.28	0.13

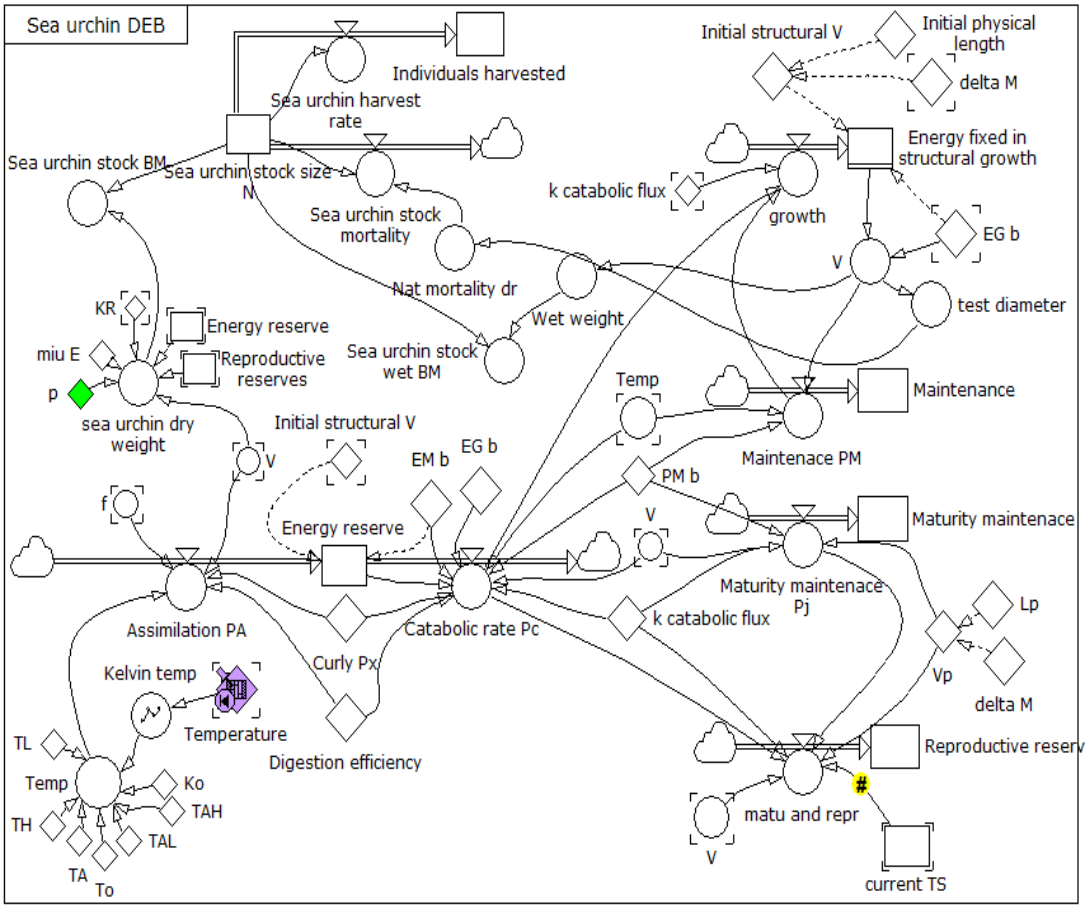
tank average		54.10		55.84	0.85	55.12	0.07		0.03
5 (L)	1	34.9	4.5	34.83	-0.07	33.1	-1.8	1.99	0.13
5 (L)	2	26.34	4	26.73	0.39	26.36	0.02	1.9	0.04
5 (L)	3	19.9	4	20.63	0.73	20.04	0.14	2.03	0.08
tank average		27.05		27.40	0.35	26.5	-0.55		0.09
6 (L)	1	22.96	3	24.96	2	25.34	2.38	4.42	-0.04
6 (L)	2	32.8	3.5	33.29	0.49	34.4	1.6	2.68	-0.09
6 (L)	3	21.6	3	22.65	1.05	22.89	1.29	2.88	-0.03
tank average		25.79		26.97	1.18	27.54	1.76		-0.06
7 (L)	1	13.08	3	14.25	1.17	12.55	-0.53	0.04	0.33
7 (L)	2	41.04	4.5	40.95	-0.09	38.45	-2.59	1.29	0.17
7 (L)	3	34.92	4.5	34	-0.92	n/a	n/a	n/a	n/a
tank average		27.06		27.6	0.54	25.5	-1.56		0.21
8 (F)	1	35.2	5	35.77	0.57	36.17	0.97	4.97	-0.03
8 (F)	2	27.87	4.5	28.96	1.09	28.95	1.08	7.87	0.00
8 (F)	3	24.87	4.5	25.57	0.7	25.61	0.74	3.66	0.00
tank average		29.31		30.1	0.79	30.24	0.93		-0.01
9 (F)	1	21.88	3	23.26	1.38	25.96	4.08	2.29	-0.29
9 (F)	2	60.76	6	60.15	-0.61	61.21	0.45	9.08	-0.05
9 (F)	3	24	3	25.56	1.56	23.3	-0.7	1.77	0.24
tank average		35.55		36.32	0.78	36.82	1.28		-0.04



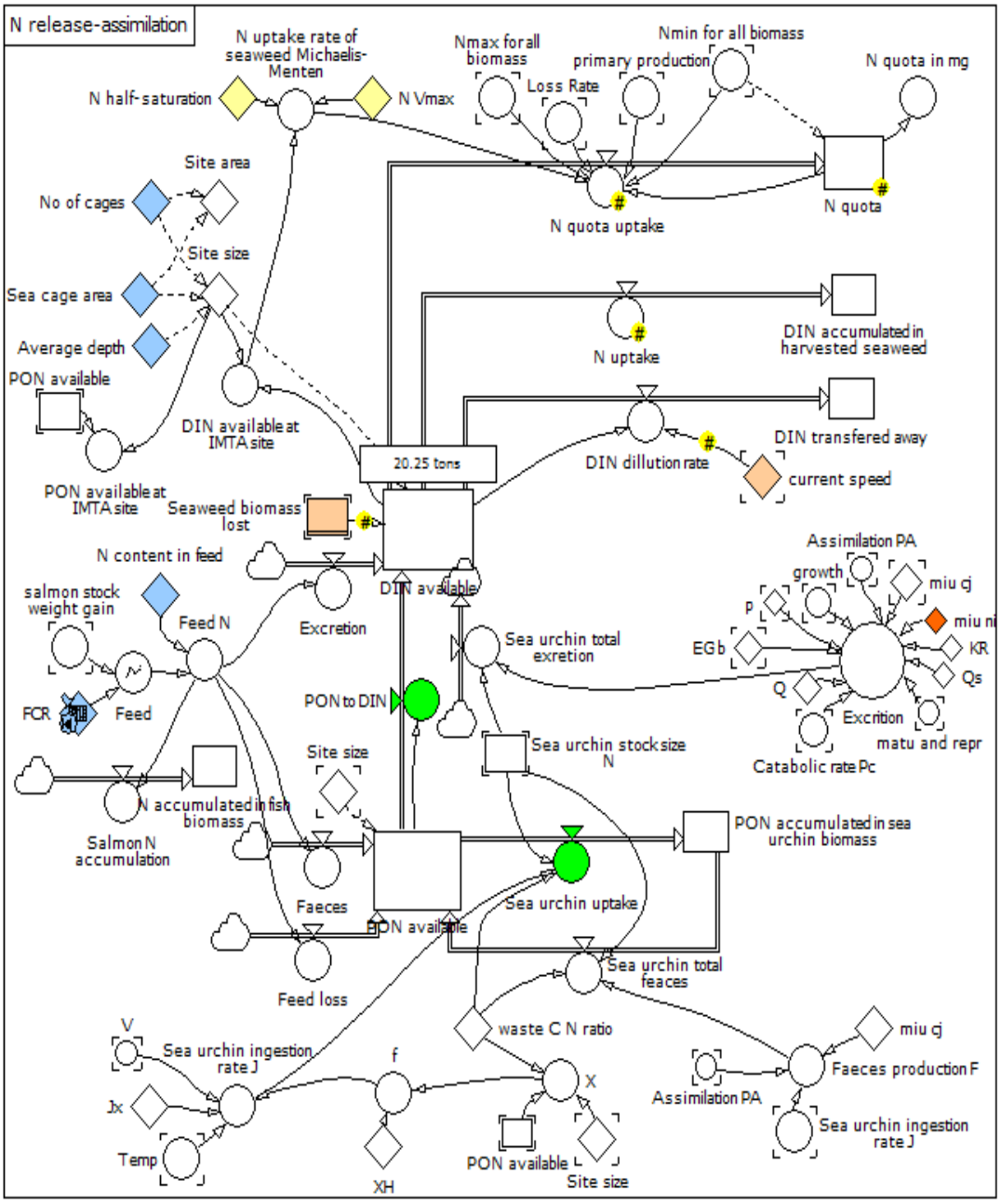
Appendix Figure 1: A snapshot of the seaweed growth and nutrient release and uptake mode as in Powersim



Appendix Figure 2: A snapshot of the salmon growth and nutrient release mode as in Powersim



Appendix Figure 3: A snapshot of the sea urchin growth and nutrient release and uptake mode as in Powersim



Appendix Figure 4: A snapshot of the nutrient release and uptake core of the mode as in Powersim.

Appendix Figure 5: The publication of the research described in chapter 5.

Lamprianidou F, Telfer T, Ross LG. (2015) A model for optimization of the productivity and bioremediation efficiency of marine integrated multitrophic aquaculture, Estuarine, Coastal and Shelf Science 164: 253-264.

Estuarine, Coastal and Shelf Science 164 (2015) 253–264

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

A model for optimization of the productivity and bioremediation efficiency of marine integrated multitrophic aquaculture

Fani Lamprianidou*, Trevor Telfer, Lindsay G. Ross

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK

ARTICLE INFO

Article history:
Received 14 January 2015
Received in revised form
12 June 2015
Accepted 30 July 2015
Available online 4 August 2015

Keywords:
IMTA
Ulva
Paracentrotus lividus
Dynamic energy budget
Nitrogen
Modelling

ABSTRACT

Integrated multitrophic aquaculture (IMTA) has been proposed as a solution to nutrient enrichment generated by intensive fish mariculture. In order to evaluate the potential of IMTA as a nutrient bioremediation method it is essential to know the ratio of fed to extractive organisms required for the removal of a given proportion of the waste nutrients. This ratio depends on the species that compose the IMTA system, on the environmental conditions and on production practices at a target site. Due to the complexity of IMTA the development of a model is essential for designing efficient IMTA systems. In this study, a generic nutrient flux model for IMTA was developed and used to assess the potential of IMTA as a method for nutrient bioremediation. A baseline simulation consisting of three growth models for Atlantic salmon *Salmo salar*, the sea urchin *Paracentrotus lividus* and for the macroalgae *Ulva* sp. is described. The three growth models interact with each other and with their surrounding environment and they are all linked via processes that affect the release and assimilation of particulate organic nitrogen (PON) and dissolved inorganic nitrogen (DIN). The model forcing functions are environmental parameters with temporal variations that enables investigation of the understanding of interactions among IMTA components and of the effect of environmental parameters. The baseline simulation has been developed for marine species in a virtually closed system in which hydrodynamic influences on the system are not considered. The model can be used as a predictive tool for comparing the nitrogen bioremediation efficiency of IMTA systems under different environmental conditions (temperature, irradiance and ambient nutrient concentration) and production practices, for example seaweed harvesting frequency, seaweed culture depth, nitrogen content of feed and others, or of IMTA systems with varying combinations of cultured species and can be extended to open water IMTA once coupled with waste distribution models.

1. Introduction

The constantly increasing demand for seafood, during a period of overexploitation of the fisheries sector can only be met by sustainable growth of aquaculture. This growth is limited by the environmental impacts and economic requirements of intensive monoculture of fed species. Moreover, rapid and uncontrolled expansion of the aquaculture sector challenges the realization of an Ecosystem Approach to Aquaculture (Soto et al., 2008). It has been proposed that expansion of marine aquaculture in parallel with environmental protection can be achieved using Integrated Multi-Trophic Aquaculture systems (IMTA) (Chopin et al., 2001; Neori et al., 2004). IMTA has the potential to be an economically viable solution to the problems of dissolved and particulate nutrient enrichment, since the waste from fed species aquaculture is exploited as a food source by extractive organisms of lower trophic levels giving added value to the investment in feed by producing a low input protein source as well as increasing the farm income. In order to promote more resilient growth of the Scottish aquaculture industry a draft Seaweed Policy Statement that examines the cultivation of seaweed as part of IMTA systems was introduced in 2013 (Marine Scotland, 2013). Large-scale seaweed cultivation has been suggested as a means to mitigate the nutrient enrichment environmental impact of marine fish farms (Abreu et al., 2009; Wang et al., 2013). As a very large area is required for the cultivation of sufficient seaweed biomass for complete nutrient bioremediation, doubt remains as to whether complete bioremediation by seaweed cultivation is practically feasible (Broch and Slagstad, 2012). However, there is a general agreement that cultivation of seaweed as part of an IMTA is a promising way for partial removal

* Corresponding author. Tel.: +44 1786 7878.
E-mail address: fani.lamprianidou@stir.ac.uk (F. Lamprianidou).

http://dx.doi.org/10.1016/j.ecss.2015.07.045
0272-7714/

of dissolved fish farm effluent (Broch et al., 2013; Jiang et al., 2010; Reid et al., 2013; Wang et al., 2013). Similarly, sea urchins can filter sea cage effluent (Kelly et al., 1998; Schuenhoff et al., 2003) and it has been shown that *Paracentrotus lividus* can assimilate fish farm waste and can achieve high growth and survival rates near salmon cages (Cook and Kelly, 2007).

IMTA systems design needs to encompass the characteristics of both the site and the selected organism and optimizing synergies requires advanced understanding of the system at a specific site. A major factor restricting the efforts to optimize open water IMTA, is the lack of knowledge on how IMTA systems operate coupled with the lack of data from large scale extractive cultures and thus the need to extrapolate results from small-scale studies (Troell et al., 2003). Due to limited knowledge of IMTA system properties, the placement of the extractive organisms is often driven by availability of space as opposed to nutrient uptake maximization (Hughes and Kelly, 2001).

Lack of knowledge or inaccurate IMTA design might impact the health and growth of the finfish or the surrounding environment or the extractive organism flesh might be of inferior quality. For example, the use of organic extractive organisms can lead to additional nitrification of the water column, because most of the organic material ingested by the organic extractive organisms returns to the water column as nutrients (Nizzolli et al., 2005) and pseudofaeces produced by filter feeders may collect on the sediment impacting benthic communities. Also, the extractive cultures may interfere with the water movement, changing the particle dispersal patterns and reducing the water flow through the sea cages. Farming different species within the same system can increase the exposure to pathogens; mussels for instance bioaccumulate and shed harmful bacteria (Pietrak et al., 2012). Other limitations of open water IMTA include the need for high stocking densities and the need for deployment of the organic extractive organisms lower in water column near the primary source of particulate waste.

The maximum production of an organic extractive species crop is limited by food availability (e.g. Grant and Filgueira, 2011). Increasing crop biomass beyond this carrying capacity causes food depletion and thus crop production cannot be maximized (Cranford et al., 2013). There needs to be a balance between waste production and uptake where the waste is sufficient to feed the extractive organisms and concurrently as much of the waste as possible is removed from the ecosystem. An efficient IMTA farm allows the profitable use of each of the culture modules with minimum waste (Neori et al., 2004). In order to achieve this the standing stocks of all the cultured organisms have to be maintained, considering nutrient requirements of each and the rates of excretion and uptake of the important solutes by each of them (Granada et al., 2015).

From a biological point of view, the choice of extractive species in an IMTA system is crucial because their physiological and ecological attributes determine the rate of particle or nutrient consumption and assimilation, their growth rate and in capabilities in terms of biofiltration. Species are chosen based on specific culture performance traits, for which quantitative information needs to be available, with respect to nutrient uptake efficiency and secondary considerations (e.g. yield and protein content). The marketability of the extractive species is largely dependent on the location, with the Western world showing less demand for food species that are low in the trophic chain. Nevertheless, dried seaweed products can always be exported and seaweeds can be processed to produce cosmetics, fertilizers, animal feed, biogas and others.

The environmental benefits, matter and energy flux within an IMTA farm as well as between the environment and the IMTA system, need to be qualified and quantified prior to the establishment of a marine IMTA system. The aim of this study was to provide a tool for designing IMTA farms at any site by creating a modelling tool that can be used to fine-tune IMTA designs for maximising yields and nutrient removal.

Without a thorough understanding of the dynamics of the system, the environmental and economical benefits of IMTA cannot be achieved. However, field measurements of nutrient and Particulate Organic Matter (POM) concentrations in open-water systems are challenging due to the highly diluting, dynamic nature of open-water systems, presenting high spatial and temporal variation both diurnally and seasonally. The model described in this study determines the temporal availability of nutrients and POM released by the different IMTA components and thus the amount available for uptake by different groups of extractive organisms. Because of the site specificity of waste distribution, this model focuses on simulation of a virtually closed system, within which the nitrogen is homogenously distributed. The species used in this study are Atlantic salmon (*Salmon salar*), a sea urchin (*P. lividus*) and the sea lettuce (*Ulva lactuca*), though it will be possible to re-parameterise the model for a range of different species.

2. Model development

The model was implemented using the visual simulation package Powersim™ Constructor Studio 8 (Powersim Software AS, Bergen). An 18-month period time horizon was used, to simulate the at-sea phase of salmon production cycle, which lasts between 14 and 24 months (Marine Harvest, 2012). The model is typically operated with a one-day time step and the model differential equations are solved using a third order Runge–Kutta integration method. The selected time-step reflects accurately the time dependent environmental changes (accurate integration) with low computing effort.

An extensive literature review was carried out for model parameterization for *Ulva* (Table 1) and for *P. lividus* (Add_my_pet, 2014), while the model for *S. salar* was parameterized using data acquired from commercial Scottish salmon farms. For the parameters where a range of values was available in the literature, the most representative value was used. It is evident that the inclusion of many proxy variables from the literature propagates uncertainties through the model, affecting the overall model accuracy. Since the model is deterministic, its output is entirely determined by the input parameters and structure of the model. Due to the high structural complexity of the model and high degree of uncertainty in estimating the values of many input parameters, a detailed sensitivity analysis was performed by varying each input parameter by ± 10% and quantifying the effect on eight output variables (Table 2). The selected output variables reflect the objectives of the research with respect to nitrogen bioremediation and yield productivity. Within the sensitivity analysis all model parameters and initial values of state variables (50 input variables) were varied in order to determine the response of the following eight effect variables: harvested seaweed, salmon and sea urchin biomass, nitrogen accumulated by seaweed, salmon and sea urchins, DIN and PON available at the IMTA site at the end of the simulation. The sensitivity analysis results are presented as a normalized sensitivity coefficient (NS) (Fasham et al., 1990):

$$NS = \frac{DV/V_b}{DP/P_b} \quad (1)$$

where $DV = (V_b - V)$ is the change of a response variable, V_b is the value of a response variable for the base run, V is the value of a

Table 1
Parameterization of constants and time series variables used at the seaweed growth submodel.

Variable	Description	Value range in literature	Value used	Units	Reference
μ_{max}	Maximum growth rate	0.8–18	10	% Day^{-1}	Neori et al., 1991; Luo et al., 2012; Perrot et al., 2014
N_{max}	Maximum intracelular quota for N	36-54	50	mg^{-1} N g dw^{-1}	Fujita, 1985; Cohen and Neori 1991; Perrot et al., 2014
N_{min}	Minimum intracelular quota for N	10 to 13	10	mg^{-1} N g dw^{-1}	Fujita, 1985; Cohen and Neori 1991; Perrot et al., 2014
T	Water Temperature	Site specific	6.8–13.7[a]	°C	n/a
q_{10}	Seaweed temperature coefficient	2	2	n/a	Aveytua-Alcázara et al., 2008
I_0	Water surface light intensity	Site specific	50-190[a]	W m^{-2}	n/a
I_{opt}	Optimum light intensity for macroalagae	50	50	W m^{-2}	Perrot et al., 2014
k	Light extinction coefficient	Site specific	1	m^{-1}	n/a
z	Culture depth	Farm practice	2	m	n/a
V_{max}	Maximum N uptake rate	0.44–2.2	1.32	mgN g^{-1} dw h^{-1}	Lapointe and Tenore 1981; Perrot et al., 2014
K_N	N half saturation	0.06–0.55	0.31	mg L^{-1}	Perrot et al., 2014
Wet/Dry	Wet to dry weight ratio	6.7–10.15	8.43	n/a	Neori et al., 1991; Angell et al., 2012
M	Mortality	0.009–0.02	0.015	d^{-1}	Aveytua-Alcázara et al., 2008; Perrot et al., 2014
T_{ref}	Reference temperature for seaweed growth	n/a	15	°C	Neori et al., 1991; Luo et al., 2012; Perrot et al., 2014
Ω	Decomposition rate and natural biomass loss	n/a	M/2	d^{-1}	n/a
D	Loss rate due to environmental disturbance	n/a	M/2	d^{-1}	n/a
S	DIN concentration in seawater	Site specific	0.594	mg m^{-3}	n/a

[a] Time series variable.

response variable for the sensitivity analysis run, $DP = (P_b - P)$ is the change in a model parameter, P_b is the baseline value of a model parameter and P is the value of a model parameter for the sensitivity analysis run.

When the value of NS for a parameter +10% is negative then there is a negative correlation between parameter and effect. When it is negative for a parameter −10% then there is a positive correlation between parameter and effect.

2.1. Model outline

The model determines the nutrient recovery efficiency and biomass production of IMTA based on a baseline simulation, components of the model can be altered or removed for the simulation of particular scenarios. Following re-parameterization, the model can simulate IMTA systems consisting of different combinations of finfish, sea urchin (or other grazing invertebrate) or seaweed species. The present model incorporates an ecosystem model consisting of three submodels that interact with each other and with their surrounding environment via nutrient cycling (Fig. 1). The submodels consist of growth models for *S. salar*, *Ulva* sp. and *P. lividus* that interact with each other through modelled nitrogen release and subsequent assimilation (Fig. 1).

Salmon growth was modelled using the Thermal-unit Growth Coefficient (TGC) (Iwama and Tautz, 1981), the seaweed growth model is based on Droop's model for nutrient-limited algal growth (Droop, 1968) and sea urchin growth was modelled using the Dynamic Energy Budget (DEB) theory (Kooijman, 1986).

The TGC is a simple model widely used in aquaculture, based on three basic assumptions, which may be violated under certain conditions (Jobling, 2003). The TGC can present errors when the temperature deviates far from the optimum for growth (Jobling, 2003), but this is not a setback given the temperature range used in the present simulations. For the organic extractive organisms a bioenergetic model was used in order to link the environmental variables, mainly food availability and temperature, with feed intake, growth, excretion and faeces production. For the simulation of salmon growth and nutrient uptake and release, the TGC was preferred to a bioenergetic model because under intensive aquaculture conditions feed is not limiting growth. Furthermore, salmon is well studied and daily time series data for the TGC and food conversion ratio (FCR) as well as sources of data for excretions and faeces production were available in the literature. Finally, as salmon are grown at sea for only for a part of their production, data are not required for the full life cycle, which is the strength of the DEB approach.

The model includes daily time steps for a better understanding of the process affecting the IMTA productivity and nutrient removal efficiency. Due to the dynamic design of the model the bioremediation potential of different production scenarios can be estimated by altering various production parameters of the baseline simulation. These include site-specific environmental conditions (temperature, irradiance and ambient nutrient concentration) and production practices (seaweed harvesting frequency, seaweed culture depth, nitrogen content of feed, initial stocking biomass of extractive organisms etc.). The maximum seaweed and sea urchin biomass that can be sustained at any given time can also be estimated based on the daily amount of nitrogen within the IMTA system that is available for uptake.

The complete model is used to determine the overall ability of the IMTA system to reduce the nutrient and POM waste of fed-species taking into account the quantity of nutrients and POM that are released and the quantity that could be potentially absorbed/consumed by the extractive organisms if all the waste remained within the virtually closed system. The only nitrogenous input to the seaweed and sea urchin submodels is the daily waste released to the sea from the salmon submodel. This is used to calculate the amount of particulate (suspended) and dissolved nitrogen released from the salmon farm for a given fish production over 18 months, as well as the potential for decreasing the nutrient released by converting salmon monocultures into IMTA systems. The model considers fish growth and consequent feed input and waste release, and the uptake and release of DIN and PON by the different IMTA components. The growth models are combined with nutrient transfer/cycling and this way the virtually closed system bioremediation efficiency is estimated (Fig. 1).

2.2. Salmon growth submodel

The growth rate of fish fluctuates throughout an individual life cycle and is mainly influenced by feed availability, temperature and photoperiod (Austreng et al., 1987). Salmon growth was simulated using a thermal growth coefficient:

$$TGC = 1000\frac{\sqrt[3]{W_t} - \sqrt[3]{W_0}}{T*t} \quad (2)$$

where W_0 is the smolts initial wet weight, W_t is the fish's wet

Table 2
Most sensitive parameters (with NS ≥ 1) for the effect variables a) Nitrogen accumulated in harvested salmon b) Harvested salmon biomass c) DIN accumulated in harvested seaweed d) Harvested seaweed biomass e) Nitrogen accumulated in harvested sea urchin biomass f) Harvested sea urchin biomass g) DIN available at the IMTA site h) PON available at the IMTA site, by descending absolute normalized sensitivity coefficient (NS) for either + or − 10% of the effect parameter's value.

Parameter symbol	Parameter name	Parameter baseline value	Effect for parameter +10%	NS for parameter +10%	Effect for parameter −10%	NS for parameter −10%
a) Nitrogen accumulated in harvested salmon: effect baseline value is 24.66 tonnes						
TGC	Thermal-unit growth coefficient[a]	2.33	30.55	2.42	19.61	2.07
FCR	Feed conversion ratio[a]	1.04	24.92	0.1	20.39	1.73
b) Harvested salmon biomass: effect baseline value is 1000 tonnes						
TGC	Thermal-unit growth coefficient[a]	2.33	1242	2.45	808	1.95
c) DIN accumulated in harvested seaweed: effect baseline value is 17.09 tonnes						
N_{state}	Nutrient state of seaweed at harvest[b]	10	3.18	−7.93	10.59	3.97
μ_{max}	Max seaweed growth rate	0.13	19.78	1.57	13.71	1.98
T	Water Temperature[a]	10.89	18.01	0.54	12.96	2.41
V_{max}	Maximum N uptake rate	1.32	19.18	1.22	13.50	2.10
W/D	Wet/dry ratio	8.43	19.19	1.23	13.49	2.10
z	Culture depth	2	19.39	1.35	14.32	1.62
N_{excr}	Nitrogen lost via excretion	0.45	16.80	−0.17	15.09	1.17
d) Harvested seaweed biomass: effect baseline value is 341.84 tonnes						
μ_{max}	Max seaweed growth rate	0.13	395.69	1.58	274.19	1.98
T	Water Temperature[a]	10.89	360.20	0.54	259.27	2.41
V_{max}	Maximum N uptake rate	1.32	383.68	1.22	269.92	2.11
W/D	Wet/dry ratio	8.43	383.73	1.23	269.88	2.11
z	Culture depth	2	387.89	1.35	286.49	1.62
N_{min}	Min intracellular quota for N	10	303.32	−1.13	358.39	−0.48
N_{max}	Max intracellular quota for N	50	307.66	−1.00	360.90	−0.56
e) Nitrogen accumulated in harvested sea urchin biomass: effect baseline value is 0.96 tonnes						
T	Water Temperature[a]	10.89	1.119	1.65	0.640	3.33
$\{Px\}$	Maximum surface-specific feeding rate	578.55	1.248	3.00	0.723	2.47
K_o	Reference reaction rate at 288 K	1	1.229	2.80	0.734	2.35
T_A	*P. lividus* Arhenius temperature	8000	0.793	−1.74	1.172	−2.21
μ_{cj}	Ratio of carbon to energy content	83.30	0.876	−0.88	1.068	−1.13
f) Harvested sea urchin biomass: effect baseline value is 20.02 tonnes						
T_L	*P. lividus* lower boundary tolerance	273	0.08	−9.96	n/a	n/a
T	Water Temperature[a]	10.89	23.01	1.15	13.37	3.32
$\{Px\}$	Maximum surface-specific feeding rate	578.55	26.01	2.99	15.00	2.50
K_o	Reference reaction rate at 288 K	1	25.36	2.67	15.39	2.31
T_A	*P. lividus* Arhenius temperature	8000	16.59	−1.71	24.21	−2.09
$[E_G]$	Volume specific cost of *P. lividus* growth	2748	18.28	−0.87	22.02	−1.00
g) DIN available at the IMTA site: effect baseline value is 12.38 tonnes						
N_{state}	Nutrient state of seaweed at harvest[b]	10	23.31	0.22	16.95	0.18
TGC	Thermal-unit growth coefficient[a]	2.33	18.05	4.64	5.55	5.59
FCR	Feed conversion ratio[a]	1.04	11.82	−0.45	6.82	4.49
N_{excr}	Nitrogen lost via excretion	0.45	15.60	2.60	10.65	1.40
μ_{max}	Max seaweed growth rate	0.13	9.69	−2.17	15.77	−2.74
$N_{content}$	Nitrogen content in feed	0.057	15.66	2.63	10.59	1.44
T	Water Temperature[a]	10.89	11.46	−0.74	16.53	−3.35
V_{max}	Maximum N uptake rate	1.32	10.29	−1.69	15.98	−2.91
W/D	Wet/dry ratio	8.43	10.30	−1.68	15.97	−2.90
z	Culture depth	2	10.08	−1.86	15.15	−2.24
N_{min}	Minimum intracellular quota for N	10	14.32	1.57	11.56	0.66
h) PON available at the IMTA site: effect baseline value is 9.65 tonnes						
TGC	Thermal-unit growth coefficient[a]	2.33	12.07	2.54	7.41	2.35
FCR	Feed conversion ratio[a]	1.04	9.68	0.03	7.78	1.94
$N_{content}$	Nitrogen content in feed	0.0576	10.70	1.08	8.61	1.07

[a] Time series variable. The time series parameters where increased/decreased by 10% at each time step.
[b] For the parameter 'Nutrient state of seaweed at harvest' we used N_{min} instead of N_{max} at the column labelled as +10% and $(N_{min} + N_{max})/2$ at the column labelled as −10%.

weight at time t, T is the temperature and t is time in degree-days.

Solving for W_t we obtain:

$$W_t = \left[\sqrt[3]{W_0} + \frac{TGC*T*t}{1000}\right]^3 \quad (3)$$

The total salmon biomass was calculated as individual weight multiplied by the number of individuals. The model also accounted for natural mortality, modelled as a time series variable since mortality decreases with fish size, using empirical data from Scottish salmon farms.

The amount of waste released from the salmon farm in the form of excretion, faeces production and feed loss was assumed to be as calculated by Wang et al. (2012) for Norwegian salmon farms, with the exception that the feed nitrogen content was set to be 5.76% of the feed weight, since to date crude protein content is around 36% (Skretting, 2015). We assume that every day of the simulation 2% of feed nitrogen is released in the environment as feed loss, 45% as dissolved excretions and 15% as faeces, while the remaining 38% is assimilated into salmon biomass and removed from the ecosystem

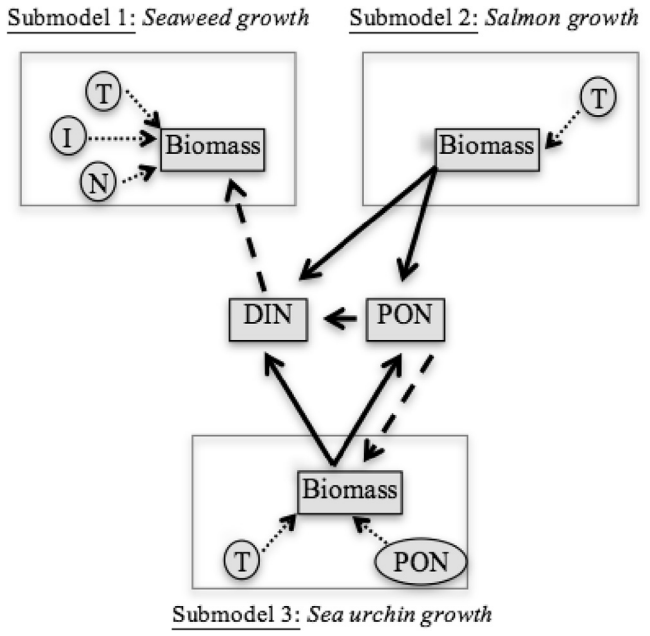


Fig. 1. Conceptual diagram of the model showing the major state variables (squares) and forcing functions (circles) of each submodel as well as the interactions among the submodels. The dashed lines represent nitrogen assimilation and the solid lines nitrogen release. *T, I* and *N* represent temperature, irradiance and nitrogen, respectively.

when the fish are harvested.

2.3. Seaweed growth and nitrogen uptake

Seaweed biomass *(B)* increases with a varying growth rate and decreases due to natural causes and periodic harvesting. The basic processes affecting seaweed biomass form the differential Equation (4):

$$\frac{dB}{dt} = (\mu - \Omega)^*B - (D + H)^*B \tag{4}$$

where μ is the specific growth rate, Ω the specific decomposition rate, D the loss rate due to environmental disturbance and H the harvesting rate. Biomass is calculated as wet biomass, for the conversion of seaweed wet to dry weight an 8.43 to 1 ratio was used (Angell et al., 2012; Neori et al., 1991). At the baseline simulation due to lack of data in the literature for the specific decomposition rate and the loss due to environmental disturbance for *Ulva* sp. the term mortality (M) is used, where $M = \Omega + D$ and $\Omega = D$ (Table 1).

The gross growth rate was defined as a function of water temperature, availability of Photosynthetic Active Radiation (PAR) and nutrient concentration in the water column and in the plant tissues. The joint dependence of growth on environmental variables is defined by separate growth limiting factors, which range between 0 and 1. A value of 1 means the factor does not inhibit growth (i.e. light is at optimum intensity, temperature is optimum and nutrients are available in excess). The limiting factors are then combined with the maximum gross growth rate at a reference temperature as in Equation (5) (Solidoro et al., 1997):

$$\mu = \mu_{max(T_{ref})}{}^*f(T)^*f(I)^*min(f(N), f(P)) \tag{5}$$

where $\mu_{max(Tref)}$ is the maximum growth rate at a particular reference temperature (T_{ref}) under conditions of saturated light intensity and excess nutrients, $f(T)$, $f(I)$, $f(N, P)$ are the growth limiting functions for temperature, light and nutrients (nitrogen and phosphorus).

The major nutrients required for growth are nitrogen and phosphorus, while carbon is often available in excess and micronutrients such as iron and manganese are only limiting in oligotrophic environments. Typically, in marine ecosystems, nitrogen is the element limiting algal growth (Lobban and Harrison, 1994). Thus in the baseline simulation it is assumed that phosphorus is not limiting, so Equation (5) becomes:

$$\mu = \mu_{max(T_{ref})}{}^*f(T)^*f(I)^*f(N) \tag{6}$$

The Photosynthetic response to light is based on Steele's photoinhibition law (Steele, 1962):

$$\frac{P}{P_{max}} = \frac{I}{I_{opt}} exp\frac{1 - I}{I_{opt}} \tag{7}$$

where P is the photosynthetic response at a given light intensity I (W m^{-2}) for an organism that has a maximum photosynthetic rate P_{max} at the optimal (saturating) light intensity I_{opt} and I is the light intensity at a given depth (z). Light intensity at a given depth is an exponential function of depth, seaweed and phytoplankton standing biomass and is given by:

$$I(z) = I_0 e^{-kz} \tag{8}$$

where k is the light extinction coefficient (m^{-1}).

After mathematical integration of the light limitation factor Equation (8) we obtain:

$$\begin{aligned} F(I) &= \int_0^z \frac{P}{P_{max}} dz = \int_0^z \frac{I(x)}{I_{opt}} exp\frac{1 - I(x)}{I_{opt}} dx \\ &= \int_0^z \frac{I_0 e^{-kx}}{I_{opt}} \exp\frac{1 - I_0 e^{-kx}}{I_{opt}} dx \\ &= \frac{1}{k}{}^*\exp\left(\frac{1}{I_{opt}}\right)^*\left[\exp\left(-\frac{I_0}{I_{opt}}{}^*\exp(-z^*k)\right) - \exp\left(-\frac{I_0}{I_{opt}}\right)\right] \end{aligned} \tag{9}$$

The temperature, like the light, limitation factor follows an inhibition law.

$$F(T) = q_{10}^{0.1(T - T_{ref})} \tag{10}$$

where q_{10} is a temperature coefficient and T_{ref} is the reference temperature at which the seaweed growth rate was measured.

The nitrogen limitation factor Equation (11) is given by the range of internal nitrogen concentration, with a feedback effect on the uptake function (Aveytua-Alcázar et al., 2008; Coffaro and Sfriso, 1997; Solidoro et al., 1997). It can range between 1, when $N = N_{max}$ and uptake is saturated and 0 when $N = N_{min}$ and maximum uptake rate is possible, all measured in mgN g^{-1} dry seaweed. Internal nitrogen quota/concentration *(N)* refers to the concentrations in algal cells as opposed to external concentrations that refer to the concentration in the water column.

$$F(N) = 1 - \frac{N_{max} - N}{N_{max} - N_{min}} \tag{11}$$

For calculation of *(N)*, a quota-based model was used developed from Droop's original formula (Droop, 1968):

$$\frac{dN}{dt} = V^*F(N) - \mu^*N \tag{12}$$

where V is the nitrogen uptake rate (mg g^{-1}dw h^{-1}) and μ is the

specific growth rate.

Nutrient uptake rates *(V)* are proportional to nutrient concentration in the water according to Michaelis–Menten kinetics:

$$V = \frac{V_{max}S}{K_N + S} \tag{13}$$

where V_{max} is the maximum nitrogen uptake rate under the site's prevailing conditions (mg g^{-1}dw h^{-1}), S is the total DIN concentration in the seawater (mg l^{-1}) and K_N is the half-saturation coefficient for nitrogen uptake (mg l^{-1}).

By combining Equations (11)–(13) we obtain:

$$\frac{dN}{dt} = \frac{V_{max}S}{K_N + S}\frac{N_{\max} - N}{N_{max} - N_{min}} - (\mu^{*}N) \tag{14}$$

The bioremediation effect of IMTA is closely dependent on the biomass of extractive organisms harvested. However, the maximum biomass is restricted by culture practicalities such as the potential alteration of water currents and by the availability of nutrients. The maximum biomass is site and species dependent. For the baseline simulation presented here, the maximum seaweed biomass permitted on site at any given time was set at 35 tonnes wet weight. The area required for the culture of 35 t of *Ulva*, with stocking density of 1.6 kg m^{-2} and two layers of seaweed one at the sea surface and one 3 m deep would be 10,937 m^2. This stocking density was selected because the maximum density permitted to guarantee the greatest uptake of nutrients in *U. lactuca* is 1.9 kg m^{-2} (Neori et al., 1991). The area required for the seaweed culture is used for the estimation of the virtually closed IMTA site's water volume, which is estimated using the following formula:

'IMTA site volume' = 'Average depth' * 'Number of salmon cages' * 'Sea cage area' + 'raft area' * 'number of rafts' * 'Average depth'.

Seaweed is lost due to mortality, harvesting and natural biomass loss (seedling mortality, grazing, epiphytism, sediment abrasion and smothering and removal by wave action). Managing the harvesting rate is of paramount importance for achieving high productivity rates. For optimal results, when the seaweed biomass reaches a predefined level (35 t in the baseline simulation) the seaweed is harvested at regular time intervals. The biomass harvested depends on the forecasted growth and natural mortality rate of the forthcoming days. A discrete flow in the model controls the loss of seaweed biomass due to harvesting; the rate of the flow (harvest rate) is regulated by the following instruction:

IF (start harvesting = 0, 0 ton, IF (current time step * timestep = stoptime − starttime, seaweed biomass, IF (accrued part of 10 days = 1, seaweed biomass − maximum seaweed biomass, IF (accrued part of 10 days = 0, seaweed biomass − maximum seaweed biomass, 0 ton))))where '*start harvesting*' is a level that allows harvesting to start only when the seaweed biomass has surpassed the value of a constant that defined as maximum biomass that can be on site (maximum seaweed biomass). The level 'start harvesting' changes from 0 to 1 when the level '*seaweed biomass*' is equal to or larger than the constant '*maximum seaweed biomass*'. '*Current time step*' is a level that counts the time steps, starting from zero. *Timestep*, *starttime* and *stoptime* are Powersim built-in functions that return the time step of the simulation, the start-time and stop-time of the simulation, respectively. In the final time step all the seaweed in the level *'seaweed biomass'* is transferred to the level *'harvested seaweed'*. '*Seaweed biomass*' is a level that shows the seaweed biomass. *'Accrued part of 10 days'* is a level used for the calculation of 10-day periods. When the value of this level is one, all the seaweed is harvested apart from *'maximum seaweed biomass'*.

The model is effective for perennial seaweed species. However, as the gametophyte stage of *Ulva*, lasts only for a few months, frequent reseeding will be necessary at time intervals dependent on the environmental conditions, epiphytic growth or disease. The numerical parameters used in the seaweed model are summarized in Table 1.

2.4. Sea urchin growth and nitrogen uptake and release

The sea urchin growth submodel is based on the DEB theory (Kooijman, 1986). DEB theory is based on two state variables: structural volume (V) and energy reserves (E) and on two forcing variables: temperature (T) and food density *(X)*. The basic concept of the theory is that from the food ingested a certain amount is released as faeces and the rest is assimilated. All assimilated food enters a reserve compartment. From there a fixed fraction is spent on maintenance and the rest is spent on maturity or reproduction (Kooijman, 1986). A detailed description of the DEB can be found at Kooijman (2008). Most of the species-specific parameters used for this DEB model were obtained from (Kooijman, 2014).

The initial structural length/diameter of sea urchin juveniles was set to 10 mm, a size suitable for successful transfer of hatchery-reared sea urchins to sea (Kelly et al., 1998). At this length *P. lividus* individuals are characterized as sub adults (Grosjean et al., 1998), so in the baseline simulation the DEB model simulates the growth from late juveniles to mature adults.

The DEB model starts with the ingestion of PON ($mgN\ d^{-1}$) by the sea urchins. This is based on ingestion rate (j_x) (mgC d^{-1}) divided by the C/N ratio of the aquaculture waste. Ingestion rate is proportional to the surface area of the structural volume and follows type-II function response depending on the density of PON. The food that is ingested but not assimilated as biomass is released to the environment as faeces or as excretion by diffusion. The DEB model enables estimation of the potential amounts of excretions released by the sea urchins by estimating the daily production of faeces released into the surroundings this is then divided by the C/N ratio in order to calculate the amount of PON and DIN that is in sea urchin excretions, which is assumed to be immediately added to the PON and DIN pools and is thus available for consumption by the sea urchins and seaweed, respectively. The *P. lividus* N quota *(Q)* was set to 127 mgN mgC^{-1} (Tomas et al., 2005) and sediment N quota *(Qs)* is site specific it was set to 7, which is a representative value for an average Scottish salmon farm site.

The total sea urchin biomass is calculated as individual weight multiplied by the number of individuals. The decrease of the sea urchin stock size, due to mortality, is calculated in Equation (15) where due to the planktonic nature of sea urchin larvae, it is assumed they will be dispersed from the IMTA site and thus reproduction will represent a net energy loss and restocking of sea urchins will be necessary. However, the release of the larvae will contribute to restocking native sea urchin populations.

$$\frac{dN}{dt} = -\delta_r{}^{*}N - \delta_h{}^{*}N \tag{15}$$

where δ_r and δ_h are the sea urchin natural and harvest mortality, respectively. The harvest mortality was zero and at the simulation last time step all sea urchins were harvested, same as in the salmon and seaweed submodels. The natural mortality was set to 0.00102 individuals d^{-1} for sea urchins with test diameter less than 2 cm and 0.00056 individuals d^{-1} for sea urchins with test diameter more than 2 cm (Turon et al., 1995).

During the grow-out stage of *P. lividus* juveniles, the stocking density is approximately 400 individuals m^{-2} (as used in tank cultures; Carboni, 2013). Space is not an issue for the organic extractive component of the IMTA, since for the production of

560,525 individuals only 1401 m^2 would be required and this area would be directly underneath the fish cages and the seaweed rafts.

2.5. Assumptions and simplifications

The key assumption of the overall model is that all nitrogen released by the IMTA components is dispersed homogenously within a quantified water volume defined as the IMTA site water volume (see Section 2.3). It is also assumed that all the nitrogen available in the IMTA site volume is in a form suitable for uptake. Correspondingly, the model does not take into account the interactions between nitrate and ammonium within the environment and organisms, such as the role of sediment and water in the nutrient dynamics or denitrification. The increase of light limitation due to increased self-shading as the seaweed grows was not considered, neither was the shading caused by phytoplankton. Data from Broch and Slagstad (2012) could be used to derive a seaweed self-shading formula from which an add-on model could be used to simulate the changes in k, in this study k is a constant. In the seaweed growth submodel the biomass loss due to mechanical damage caused by harvesting was not included. It is also assumed that nitrogen is the only nutrient limiting seaweed growth. Additionally, the seaweed biomass used as initial biomass is assumed to have an average $((N_{min} + N_{max})/2)$ N quota (this can be regulated by using nitrogen deprived seedlings). When seaweed is harvested it is assumed that the N quota of the harvested seaweed is equal to the maximum N quota due to the high availability of DIN in the virtually closed system. The assumption that the seaweed harvested has this high nitrogen quota might lead to overestimation of the bioremediation efficiency and the effect of lower N quota at harvest was examined in the sensitivity analysis (Table 2). From a farm practice perspective it is assumed, that the relative position of the extractive organisms in relation to the fish cages is such that it ensures high O_2 availability for the fish. For the salmon growth model, excretion, faeces production and feed loss were assumed to be a steady proportion of feed input during the 18 month production period while in reality they change as fish grow.

2.6. Production specifications of the baseline simulation

The results presented are from the IMTA baseline simulation, which was parameterized using data acquired from the literature and from commercial salmon farm sites. The environmental data such as monthly variations in seawater temperature and irradiance were acquired from empirical databases for the West coast of Scotland and the production-specific input data from Scottish commercial salmon farm sites (Figs. 2 and 3). Typically, S1 smolts are transferred to sea in spring (April–May), so April is set as simulation time 0. The baseline scenario farm consists of nine 90 m circular salmon cages with the extractive organisms placed in immediate proximity to those cages. The model simulates a farm that produces 1 000 t of Atlantic salmon in 18 months on-growing, a farm size representative of the Scottish industry.

3. Results

3.1. Growth performance of IMTA components at the baseline simulation

The baseline simulation run estimated that the mean individual fish biomass after 540 days (18 months) was 3.78 kg (Fig. 4A) and the salmon stock decreased by 16,525 individuals from 280,883 to 264,358 individuals (Fig. 4B). During the 18-month production period, 342 t of seaweed and 20.02 t of sea urchins were produced and harvested as well as the targeted 1 000 t of salmon. The seaweed achieved high growth rates, especially during the summer months (Fig. 5). The effect of the growth limitation factors on seaweed growth rate is presented in Fig. 6. The lower seaweed growth rate during the first 300 days (10 months) of the simulation (Fig. 5) can be mainly attributed to low levels of nitrogen available for uptake (Figs. 6 and 9). It is clear that in the hypothetical baseline model scenario, during the first 340 days of the simulation seaweed growth is mainly limited by the availability of nitrogen. Temperature limits growth more during the colder months (October–April) while, the effect of light intensity is rather stable throughout the year (Fig. 6). It should be emphasized here that site specific shading caused by phytoplankton or seaweed self-shading does not contribute to light limitation in the baseline simulation (see Section 2.5).

The aim of the model is to achieve high nutrient bioremediation efficiency in limited space. Sustaining the seaweed biomass at a high density at all times, using the harvesting instruction (described at Section 2.3), played an important role in achieving high bioremediation efficiency (Fig. 7). The first seaweed harvesting occurred 250 days after the simulation start, following which there was sufficient nitrogen available due to the large size of the fish and the environmental conditions were also favourable for the remaining seven months of the simulation (April–October) (Figs. 3 and 6) thus ensuring constant high growth rate and harvesting at 10-day intervals (Fig. 7).

At simulation time zero the site was stocked with 827,900 (0.09 t) sea urchins. During the 18-month production period 20.01 t (wet weight) of sea urchins were produced with average test diameter 4.47 cm (Fig. 8). As a result 0.96 t of nitrogen were

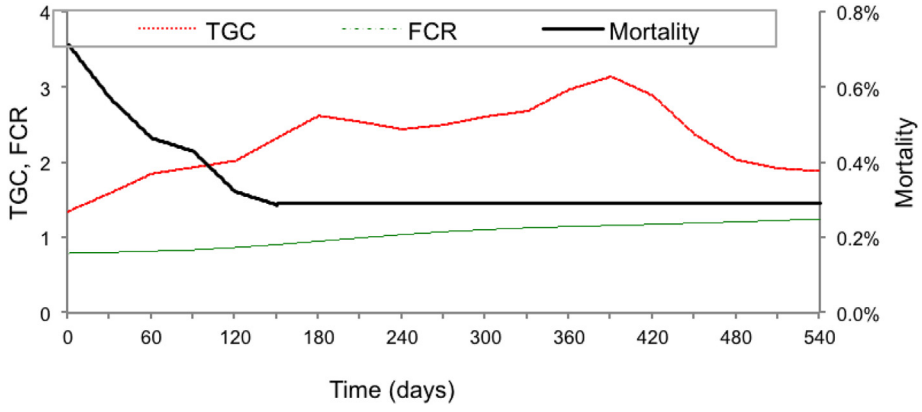


Fig. 2. Baseline scenario values of the time series variables, TGC, FCR and salmon mortality.

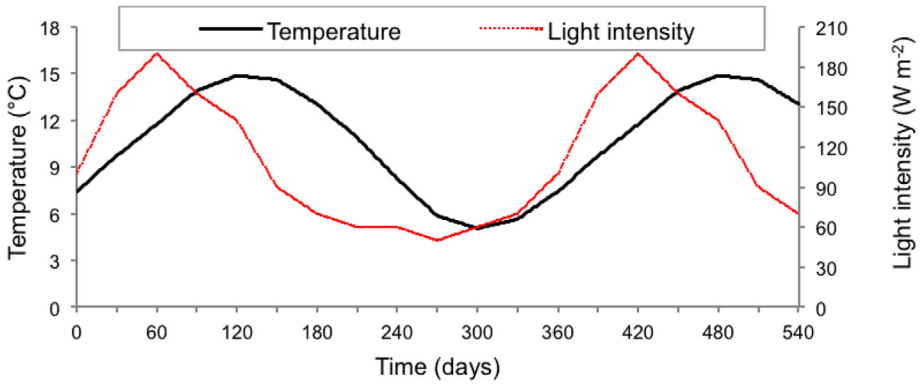


Fig. 3. Baseline scenario values of the time series variables, water temperature and light intensity.

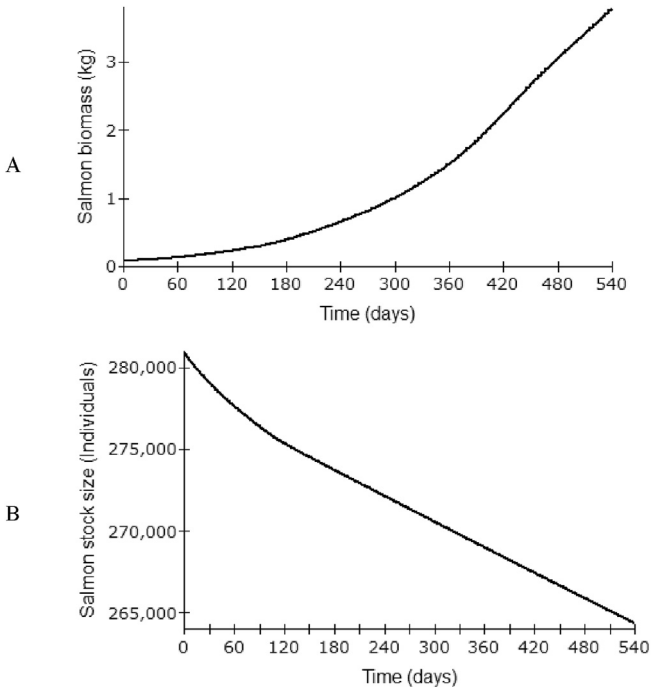


Fig. 4. Simulated output of the salmon: a) individual average biomass, b) stock size, during the 540 days of culture at sea.

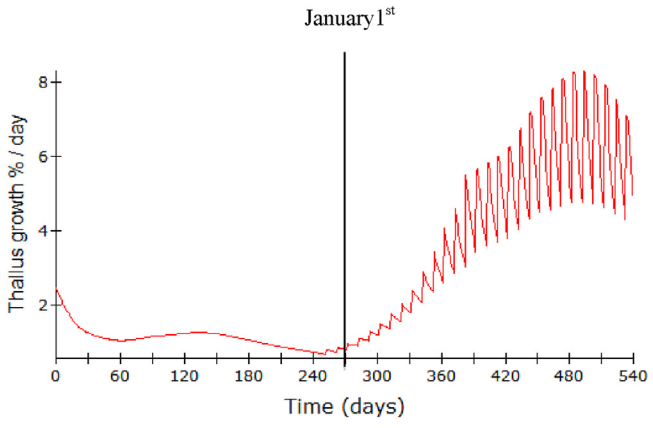


Fig. 5. Seaweed specific growth rate for *Ulva* sp. under the baseline scenario production conditions.

assimilated in the sea urchin biomass and removed from the ecosystem via the process of harvesting.

3.2. Baseline scenario bioremediation potential

For the production of 1 000 t of salmon with an average feed conversion ratio (FCR) of 1.02 and feed nitrogen content 5.76%, the model shows that 65 t of nitrogen are introduced into the system over the 540 day simulated production period. From this 65 t, only 38% is accumulated by the fish and the remaining 62% (40.2 t) is released into the environment. Under the environmental conditions and production method of the baseline scenario the total nitrogen released to the environment from the IMTA site would be 45.2% less (22.03 t instead of 40.2 t) than what would have been released from a salmon monoculture farm of the same capacity. In detail, the amount of nitrogen released from salmon monoculture would be 62% of the exogenous nitrogen input but only 34% in the IMTA system since a large proportion of the nitrogenous waste will be assimilated by the extractive organisms and removed from the ecosystem via harvesting (Fig. 9). Fig. 9 shows the gradual increase in nitrogen within the IMTA system over the simulated production period.

3.3. Sensitivity analysis

All biological, environmental and production parameters were analysed in terms of uncertainty and their relative importance in the model. Due to the large number of input and response variables used in the sensitivity analysis, only the results for the most sensitive parameters (absolute values) are summarized in Table 2. Those parameters are the potential critical assumptions and thus require accurate estimation and/or calibration.

In the salmon submodel, the growth and nutrient uptake is most sensitive to change in the TGC and secondarily to variation in the FCR (Table 2; sections a and b).

In the seaweed submodel, all output variables were most sensitive to parameters affecting growth and nutrient uptake either indirectly through nitrogen uptake and nitrogen content of the seaweed tissues, wet/dry ratio and the culture depth or directly through maximum growth rate, temperature and nitrogen input from salmon excretion. These results show the overall importance of temperature and nitrogen uptake for seaweed growth (Table 2; sections c and d). All parameters, apart from the minimum and maximum intracellular nitrogen quota, were positively correlated with the output variables. Also, increasing parameter values mirrored the effect on the model output of decreasing parameter values, which indicates that most parameters affected growth linearly.

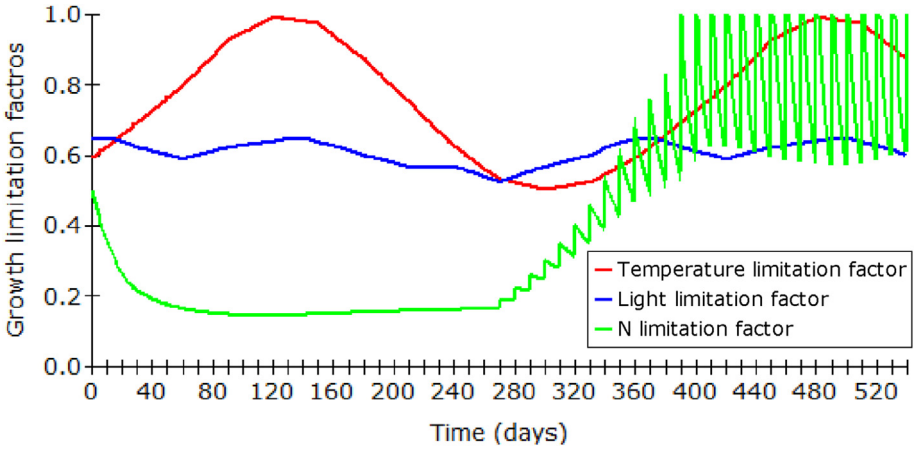


Fig. 6. Seaweed growth limitation factors, under the baseline scenario production conditions. The limitation factors can vary between 0 and 1; where a value of 1 means that the factor does not inhibit growth.

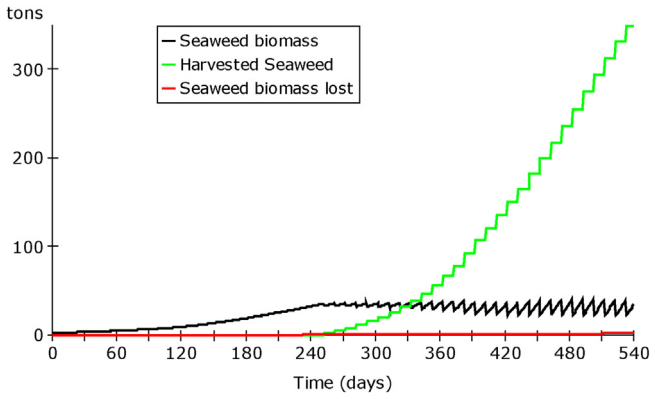


Fig. 7. Seaweed submodel simulation output for *Ulva* sp. produced under the baseline scenario conditions. It illustrates the biomass change over time, the cumulative amount of seaweed biomass lost due to natural causes and the cumulative amount of seaweed biomass harvested.

In the sea urchin submodel the output variables were most sensitive to parameters related to temperature. Other sensitive parameters included the maximum surface-specific feeding rate, the volume specific cost of growth and the ratio of carbon to energy content (Table 2; sections e and f). Overall, this analysis revealed that the DEB model was most sensitive to increases in T_L. Changes in the remaining DEB input variables had little effect on growth (sensitivity < 1).

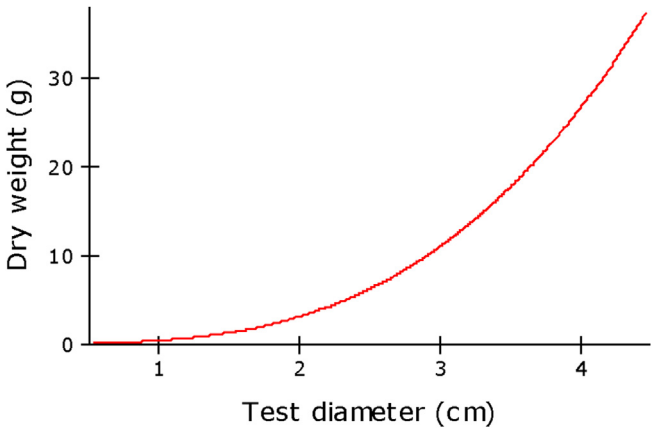


Fig. 8. Sea urchin submodel simulation output for the length – dry weight relationship of *P. lividus*.

The most sensitive parameters within the salmon and seaweed sub-models are also the most sensitive to outcomes of the overall model. The most sensitive parameters of the DEB sub-model do not play such an important role within the overall model performance due to the sea urchin biomass being very small in comparison to that of salmon and seaweed (Table 2; section g and h).

4. Discussion

The aim of this study was the development of a dynamic tool for relative comparison of IMTA scenarios at a given production site, rather than the generation of absolute bioremediation and production estimates. The model results presented are derived from a baseline simulation, which can be re-parameterised to simulate different scenarios.

Results from similar IMTA studies have shown bioremediation potential of a similar scale to the output generated by the present model. Broch and Slagstad (2012) estimated that 0.8 km^2 of *Saccharina latissima* biomass would be needed to sequester all the waste released from a salmon farm producing 1 000 t a year and Abreu et al. (2009) estimated that a 1 km^2 *Gracilaria chilensis* farm would be needed to fully sequester the dissolved nutrients released from a salmon farm producing 1 000 t a year. Sanderson et al. (2012) estimated that 0.01 km^2 of *S. latissima* could remove 5.3–10% of the dissolved nitrogen released from a salmon farm producing 500 t of salmon in two years. However, the results presented, as the results from any other IMTA model or trial, cannot be directly compared with output from similar studies due to the fact that the productivity of an IMTA farm depends on local environmental characteristics, the species combination used, the duration of the grow out seasons and other factors. Moreover, linear interpolation of results from studies with shorter durations can lead to misestimating results. Thus a large variance in production and bioremediation results is natural. The results of this study are in the same order of magnitude as the results acquired from the studies mentioned above; however they suggest higher bioremediation potential, possibly largely due to the harvesting method applied. Specifically, it was estimated that 35% of the total nitrogen released from a salmon farm, with the specifications of the simulated scenario, will be accumulated by the 0.01 km^2 of *Ulva* sp. suggesting a very high bioremediation efficiency. Aiming to achieve 100% bioremediation (i.e. no available nitrogen above the ambient

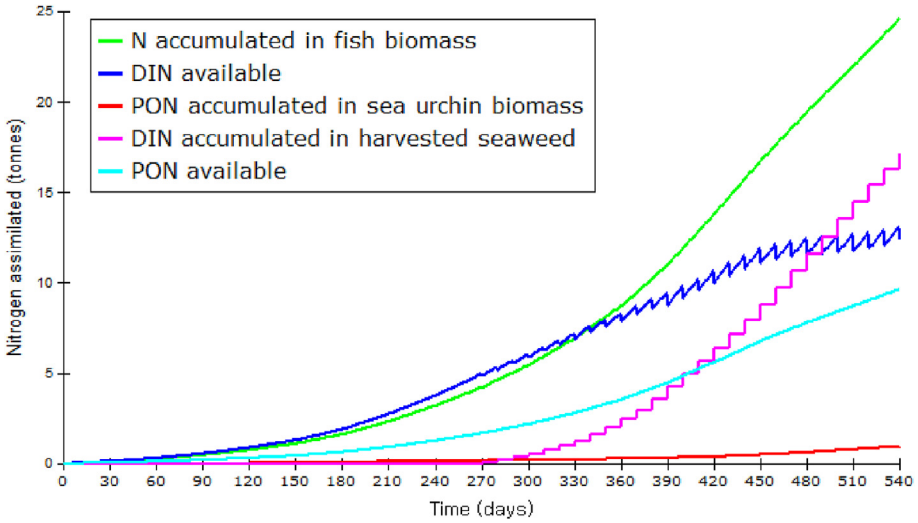


Fig. 9. Modelled output of cumulative amount of nitrogen assimilated by the different IMTA components and the amount of DIN or PON remaining at the IMTA site area at each time step.

concentration occurs at any given time), especially without the addition of external feed sources for the extractive organisms and while sustaining the quality of the extractive organisms, is unrealistic and might only be possible in a fully closed system such as a Recirculating Aquaculture System (RAS). Nonetheless, even at lower bioremediation efficiencies, the model already demonstrates the environmental benefits of IMTA.

The simulated growth for juvenile and adult sea urchins showed good correspondence with literature data (e.g. Cook and Kelly, 2007), although the reference temperature for which all the DEB constants were calculated was 20 °C (Table 1) which is significantly higher than the average temperature (11 °C) at the modelled IMTA site. The sea urchin growth model output is comparable to the results of Cook and Kelly (2007) who concluded that *P. lividus*, with an initial 1 cm test diameter, deployed adjacent to fish cages need approximately 3 years to reach market size (>5.5 cm test diameter). The sea urchins will be approx. one year old when they are deployed and 2.5 years old at the end of the grow out phase at which point their test diameter will be 4.47 cm. At the end of the 18-month grow-out phase of the salmon, the sea urchins will have reached the lower limit of their target market size. The growth rate achieved in this study was similar to that achieved directly adjacent to the sea cages (Cook and Kelly, 2007) and higher than that achieved by Fernandez and Clatagirone (1994) (1.41 mm month^{-1}) where the sea urchins were fed with artificial feed containing fish meal and fish oil at higher water temperature than this study (5–33 °C). After the sea urchins have reached market size a two to three month period of market conditioning at controlled environment is required (Carboni, 2013; Grosjean et al., 1998).

In the first eight to ten months of the IMTA baseline scenario, seaweed and sea urchin growth is limited by nitrogen (Figs. 6 and 8), since the fish are still small and thus require a relatively low feed input. From the eleventh month onwards mainly light and to a lower extent temperature are limiting the seaweed growth. From that point onwards the seaweed growth rate is high as can be seen in Fig. 5. For successful high bioremediation efficiency, at an IMTA farm seaweed growth should not be limited by light or temperature but only by nutrient availability. For this reason IMTA systems could be more efficient in sites further south than the one used for the baseline simulation. It can be seen clearly in Fig. 9 that there is a constant increase of the residual DIN and PON remaining at the IMTA site. This high waste output particularly during the last months of the salmon production is a challenge for achieving very high bioremediation efficiency. The ratio of salmon to extractive organisms used at the baseline scenario is very low, final salmon to seaweed weight ratio was 2.92 and final salmon-sea urchin ratio was 50). From the perspective of space requirement there is the potential for increase of the amount of sea urchins produced, however the quantity of waste available for consumption by the sea urchins decreases with distance from the sea cages and thus increasing the production would mean that some sea urchins would be potentially too far from the food source. Furthermore, limited market demand for marine invertebrates might also pose limitations.

The results of the sensitivity analysis indicate that the model is robust, since variation of key model parameters by ±10% does not cause unexpected changes in the effect parameters. The various model parameters have a different relative influence on the model output, both in terms of harvestable biomass and in terms of nitrogen bioremediation. Thus, depending on the specific study objectives of users, one should consider the precision with which certain parameter values are determined, and whether further tuning is required. This model sensitivity analysis is a useful means for assessing which are the key parameters that increase model uncertainty. Those parameters with high sensitivity have a big impact on the output of the model (e.g. thermal sensitivity parameters T_L in the sea urchin DEB submodel, T in all the submodels and μ_{max} in the seaweed submodel), and therefore future efforts should focus on methods for improving their estimation. In contrast, because parameters with low sensitivity have little influence on the output of the model, their estimation could be simplified. Consequently, despite the large variability observed in some of the parameters, their relative importance may be minor if their sensitivity is low.

Other polyculture and IMTA models developed, to date, include (Nunes et al., 2003; Ferreira et al., 2012; Shi et al., 2011; Ren et al., 2012). The uniqueness of the model developed in this study is that it is a dynamic model developed in a software environment with simple user interface and thus can be used by anyone prior to the setup of an IMTA system. The model presented here is highly adaptable as all the submodels can function independently. By altering model variables the submodels can simulate growth and nutrient assimilation under different environmental conditions or for different species. Altering the values of constants can also help

assess their effect on the IMTA system and in some cases these values can be optimised. For example, all the values related to production practices at the IMTA site, such as seaweed harvesting frequency, maximum seaweed biomass allowed, initial biomass of seaweed or sea urchins, seaweed culture depth and seaweed density, can be optimised for the achievement of higher bioremediation efficiency and/or higher extractive organism production.

The model can be also used to accomplish more general objectives such as: optimization of IMTA culture practices (e.g. timing and sizes for seeding and harvesting, in terms of total production), assessment of the role of IMTA in nutrient waste control and used as input for the evaluation of economic efficiency of various system designs. The present model can be used as a decision support tool for open-water IMTA only after being coupled with waste distribution modelling and environmental sampling for model parameterization. Future versions of the model can link the virtually closed IMTA system to hydrodynamic models for spatial analysis of the waste dispersion and nutrient dilution. Such a model could help develop a balance among the components of the IMTA system and assist in developing an IMTA design for maximum waste uptake in 'open environment systems', as water exchange rate is the key factor influencing the assimilative performance, thus enabling prediction of the effectiveness and productivity of open water IMTA systems.

Acknowledgements

This PhD study to FL was funded by the Marine Alliance of Science and Technology, by the Institute of Aquaculture, University of Stirling and by IKY State Scholarships Foundation of Greece.

References

Abreu, H., Varela, D.A., Henríquez, L., Villaroel, A., Yarish, C., Sousa-Pinto, I., Buschmann, A.H., 2009. Traditional vs. integrated multi-trophic aquaculture of *Gracilaria chilensis* C. J. Bird, J. McLachlan & E. C. Oliveira: productivity and physiological performance. Aquaculture 293 (3–4), 211–220.

Angell, A.R., Pirozzi, I., de Nys, R., Paul, N.A., 2012. Feeding preferences and the nutritional value of tropical algae for the abalone Haliotis asinine. PloS One 7 (6) art. No. e38857.

Austreng, E., Storebakken, T., Åsgård, T., 1987. Growth rate estimates for cultured Atlantic salmon and rainbow trout. Aquaculture 60, 157–160.

Aveytua-Alcázara, L., Camacho-Ibara, V.F., Souzab, A.J., Allenc, J.I., Torres, R., 2008. Modelling *Zostera marina* and *Ulva sp.* in a coastal lagoon. Ecol. Modell. 218, 354–366.

Broch, O., Slagstad, D., 2012. Modelling seasonal growth and composition of the kelp *Saccharina latissima*. J. Appl. Phycol. 24, 759–776.

Broch, O.J., Ellingsen, I.H., Forbord, S., Wang, X., Volent, Z., Alver, M.O., Handå, A., Andresen, K., Slagstad, D., Reitan, K.I., Olsen, Y., Skjermo, J., 2013. Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway. Aquacult. Environ. Interact. 4, 186–206.

Carboni, S., 2013. Research and Development of Hatchery Techniques to Opimize Juvenile Production of the Edible Sea Urchin, Paracentrotus lividus (Ph.D. thesis). University of Stirling, UK.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-González, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds into marine aquaculture systems: a key toward sustainability. J. Phycol. 37, 975–986.

Coffaro, G., Sfriso, A., 1997. Simulation model of *Ulva rigida* growth in shallow water of the Lagoon of Venice. Ecol. Modell. 102, 55–66.

Cohen, I., Neori, A., 1991. *Uloa lactuca* biofilters for marine fishpond effluents. Bot. Mar. 34, 475–482.

Cook, E.J., Kelly, M.S., 2007. Enhanced production of the sea urchin *Paracentrotus lividus* in integrated open-water cultivation with Atlantic salmon *Salmo salar*. Aquaculture 273 (4), 573–585.

Cranford, P.J., Reid, G.K., Robinson, S.M.C., 2013. Open water integrated multi-trophic aquaculture: constraints on the effectiveness of mussels as an organic extractive component. Aquacult. Environ. Interact. 4, 163–173.

Droop, M., 1968. Vitamin B12 and marine ecology. IV. The kinetics of uptake, growth and inhibition in Monochrysis Lutheri. J. Mar. Biol. 48, 689–733.

Fasham, M.J.R., Ducklow, H.W., McKelvie, S.M., 1990. A nitrogen based model of plankton dynamics in the oceanic mixed layer. J. Plankton Res. 48, 591–639.

Fernandez, C.M., Caltagirone, A., 1994. Growth rate of adult *Paracentrotus lividus* in a lagoon environment: the effect of different diet types. In: David, B., Guille, A., Feral, J.P., Roux, M. (Eds.), Echinoderms through Time. Balkema, Rotterdam, pp. 655–660.

Ferreira, J.G., Saurel, C., Ferreira, J.M., 2012. Cultivation of gilthead bream in monoculture and integrated multi-trophic aquaculture. Analysis of production and environmental effects by means of the FARM model. Aquaculture 358–359, 23–34.

Fujita, R.M., 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. J. Exp. Mar. Biol. Ecol. 92, 283–301.

Granada, L., Sousa, N., Lopes, S., Lemos, M.F.L., 2015. Is integrated multitrophic aquaculture the solution to the sectors' major challenges? – a review. Rev. Aquacult. 6, 1–18. http://dx.doi.org/10.1111/raq.12093.

Grant, J., Filgueira, R., 2011. The application of dynamic modeling to prediction of production carrying capacity in shellfish farming. In: Shumway, S.E. (Ed.), Shellfish Aquaculture and the Environment. John Wiley & Sons, Chichester, pp. 135–154.

Grosjean, P., Spirlet, Ch, Gosselin, P., Vaïtilingon, D., Jangoux, M., 1998. Land-based closed cycle echiniculture of Paracentrotus lividus (Lamarck) (Echinoidea: Echinodermata): a long-term experiment at a pilot scale. J. Shellfish Res. 17 (5), 1523–1531.

Hughes, A.D., Kelly, M.S., 2001. Integrated Multi-trophic Aquaculture. Scottish Association of Marine Science, p. 6. http://www.sarf.org.uk/cms-assets/documents/28926-823833.current-state-of-integrated-aquacultur.

Iwama, G.K., Tautz, A.F., 1981. A simple growth model for Salmonids in Hatcheries. Can. J. Fish. Aquat. Sci. 38 (6), 649–656.

Jiang, Z.J., Fang, J.G., Mao, Y.Z., Wang, W., 2010. Eutrophication assessment and bioremediation strategy in a marine fish cage culture area in Nansha Bay. J. Appl. Phycol. 22, 421–426.

Jobling, M., 2003. The thermal growth coefficient (TGC) model of fish growth: a cautionary note. Aquacult. Res. 34, 581–584.

Kelly, M.S., Brodie, C.C., McKenzie, J.D., 1998. Somatic and gonadal growth of the sea urchin *Psammechinus miliaris* (Gmelin) maintained in polyculture with the Atlantic salmon. J. Shellfish Res. 17, 1557–1562.

Kooijman, S.A.L.M., 1986. Energy budgets can explain body size relations. J. Theor. Biol. 121, 269–282.

Kooijman, S.A.L.M., 2008. Dynamic Energy Budget Theory for Metabolic Organization, third ed. CUP, Cambridge.

Kooijman, S.A.L.M., 2014. Add_my_pet: Paracentrotus lividus. URL: http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/html/Paracentrotus_lividus.html (accessed 21.05.14.).

Lapointe, B.E., Tenore, K.R., 1981. Experimental outdoor studies with *Ulva fasciata Delile I*. Interaction of light and nitrogen on nutrient uptake, growth and biochemical composition. J. Exp. Mar. Biol. Ecol. 92, 135–152.

Lobban, C.S., Harrison, P.J., 1994. Seaweed Ecology and Physiology. CUP, Cambridge.

Luo, M.B., Liu, F., Xu, Z.L., 2012. Growth and nutrient uptake capacity of two co-occurring species, *Ulva prolifera* and *Ulva linza*. Aquat. Bot. 100, 18–24.

Marine Harvest, 2012. Salmon Farming Industry Handbook. http://www.marineharvest.com/PageFiles/1296/2012%20Salmon%20Handbook%2018.juli_h%C3%B8y%20tl.pdf (accessed 30.04.14).

Marine Scotland, 2013. Draft Seaweed Policy Statement Consultation Paper. http://www.scotland.gov.uk/Publications/2013/08/6786 (accessed 01.06.14).

Neori, A., Cohen, I., Gordin, H., 1991. Ulva lactuca biofilters for marine fishpond effluents. II. Growth rate, yield and C: N ratio. Bot. Mar. 34, 483–489.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shipgel, M., Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231, 361–391.

Nizzolli, D., Welsh, D.T., Bartoli, M., Viaroli, P., 2005. Impacts of mussel (Mytilus galloprovincialis) farming on oxygen consumption and nutrient cycling in a eutrophic coastal lagoon. Hydrobioligia 550, 183–198.

Nunes, J.P., Ferreira, J.G., Gazeau, F., Lencart-Silva, J., Zhang, X.L., Zhu, M.Y., Fang, J.G., 2003. A model for sustainable management of shellfish polyculture in coastal bays. Aquaculture 219, 257–277.

Perrot, T., Rossi, N., Ménesguen, A., Dumas, F., 2014. Modelling green macroalgal blooms on the coasts of Brittany, France to enhance water quality management. J. Mar. Syst. 132, 38–53.

Pietrak, M.R., Molloy, S.D., Bouchard, D.A., 2012. Potential role of *Mytilus edulis* in modulating the infectious pressure of *Vibrio anguillarum* on an integrated multi-trophic aquaculture farm. Aquaculture 326–29, 36–39.

Reid, G.K., Chopin, T., Robinson, S.M.C., Azevedo, P., Quinton, M., Belyea, E., 2013. Weight ratios of the kelps, Alaria esculenta and Saccharina latissima, required to sequester dissolved inorganic nutrients and supply oxygen for Atlantic salmon, *Salmo salar*, in integrated multi-trophic aquaculture systems. Aquaculture 408/409, 34–46.

Ren, J.S., Stenton-Dozey, J., Plew, D.R., Fang, J., Gall, M., 2012. An ecosystem model for optimising production in integrated multitrophic aquaculture systems. Ecol. Modell. 246, 34–46.

Sanderson, J.C., Dring, M.J., Davidson, K., Kelly, M.S., 2012. Culture, yield and bioremediation potential of *Palmaria palmata* (Linnaeus) Weber & Mohr and *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders adjacent to fish farm cages in northwest Scotland. Aquaculture 354/355, 128–135.

Schuenhoff, A., Shpigel, M., Lupatsch, I., Ashkenazi, A., Msuya, F.E., Neori, A., 2003. A semi-recirculating, integrated system for the culture of fish and seaweed. Aquaculture 221, 167–181.

Shi, J., Wei, H., Zhao, L., Yuan, Y., Fang, J., Zhang, J., 2011. A physical-biological

coupled aquaculture model for a suspended aquaculture area of China. Aquaculture 318 (3–4), 412–424.
Skretting, 2015. Sustainable Economic Aquafeeds. http://www.skretting.com/Internet/SkrettingGlobal/webInternet.nsf/wprid/9EE28C6A30408348C125749500466A75/$file/10_05_06_SEA_eng.pdf (accessed 04.05.15).
Solidoro, C., Pecenik, G., Pastres, R., Franco, D., Dejak, C., 1997. Modelling macroalgae (*Ulva rigida*) in the Venice lagoon: model structure identification and first parameters estimation. Ecol. Modell. 94 (2–3), 191–206.
Soto, D., Aguilar-Manjarrez, J., Hishamunda, N., 2008. Building an ecosystem approach to aquaculture. In: FAO/Universitat de les Illes Balears Expert Workshop. 7–11 May 2007, Palma de Mallorca, Spain. FAO Fisheries and Aquaculture Proceedings. No. 14. Rome, FAO.
Steele, J.H., 1962. Environmental control of photosynthesis in the sea. Limnol. Oceanogr. 7, 137–150.
Tomas, F., Romero, X., Turon, X., 2005. Experimental evidence that intra-specific competition in seagrass meadows reduces reproductive potential in the sea urchin *Paracentrotus lividus* (Lamarck). Sci. Mar. 69, 475–484.
Troell, M., Halling, C., Neoric, A., Chopind, T., Buschmanne, A.H., Kautskya, N., Yarishf, C., 2003. Integrated mariculture: asking the right questions. Aquaculture 226, 69–90.
Turon, X., Giribet, G., López, S., Palacín, C., 1995. Growth and population structure of Paracentrotus lividus (Echinodermata: Echinoidea) in two contrasting habitats. Mar. Ecol. Prog. Ser. 122, 193–204.
Wang, X., Olsen, L.M., Reitan, K.I., Olsen, Y., 2012. Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquacult. Environ. Interact. 2, 267–283.
Wang, X., Broch, O.J., Forbord, S., Handå, A., Skjermo, J., Reitan, K.I., Vadstein, O., Olsen, Y., 2013. Assimilation of inorganic nutrients from salmon (*Salmo salar*) farming by the macroalgae (*Saccharina latissima*) in an exposed coastal environment: implications for integrated multi-trophic aquaculture. J. Appl. Phycol. 26 (4), 1869–1878.