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Developing an Integrated Multi-Trophic Aquaculture as a solution to the sector's challenges

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“It is not the strongest or the most intelligent who will survive but those who can best manage change.”

Charles Darwin

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Resumo

Sabe-se que atualmente a maioria dos stocks pesqueiros se encontra sobreexplorada e, em alguns casos, os stocks estão mesmo totalmente explorados. Neste sentido, e para responder às necessidades dos consumidores, a aquacultura tem intensificado e diversificado os seus produtos, sendo o setor de produção animal com maior taxa de crescimento anual (6,3%).

No entanto, a aquacultura é responsável por causar diferentes impactos no meio ambiente.

A exploração de stocks de peixe selvagens para a produção de rações e óleo de peixe e a utilização direta de pescado para alimentar peixes de cultivo constitui um dos maiores problemas da aquacultura. Do mesmo modo, a descarga de efluentes com elevados teores de produtos metabólicos é considerada um dos principais causadores de elevadas concentrações de nutrientes e compostos orgânicos nas proximidades das aquaculturas. A necessidade de grandes volumes de água e de grandes áreas, tanto em terra como no mar, causa impactos ao nível dos recursos e habitats naturais. Para além das descargas de nutrientes, também são lançados na natureza compostos químicos, tais como pesticidas, anestésicos e desinfetantes. Para além disso, a fuga de organismos cultivados para o meio selvagem pode ter impactos negativos ao nível da perda de biodiversidade e da transmissão de organismos patogénicos.

De forma a tentar solucionar alguns destes problemas, tem-se proposto o desenvolvimento da Aquacultura Integrada Multi-Trófica (IMTA), uma vez que esta pressupõe a biomitigação dos resíduos da aquacultura. A IMTA é caracterizada pelo facto de os sub-produtos de uma espécie serem reciclados, nas proporções certas, de forma a servirem de alimento para outras espécies do sistema, que utilizam os compostos orgânicos ou inorgânicos produzidos pela primeira.

Nas últimas décadas, tem havido necessidade de otimizar os processos, caudais e a biotransformação de compostos tóxicos, em sistema de aquacultura. O principal objetivo subjacente ao desenvolvimento de modelos matemáticos dinâmicos, nomeadamente modelos multi-espécies, é a possibilidade de maximizar a produção e otimizar a combinação de espécies, de forma a reduzir os impactos ambientais da aquacultura e, consequentemente, minimizar custos de produção. No entanto, a maior parte dos modelos é limitada no que diz respeito à integração do cultivo com o ecossistema, à interação entre espécies e à escala utilizada. O objetivo deste estudo foi desenvolver um modelo matemático que permitisse descrever um sistema de IMTA mais complexo, incluindo três níveis tróficos diferentes: a dourada *Sparus aurata* (principal espécie de cultivo), a poliqueta *Sabella spallanzanii* (o extrator orgânico) e a macroalga vermelha *Gracilaria vermiculophylla* (o extrator inorgânico).

Assim, foi proposto um modelo de IMTA, tendo por base parâmetros como a dinâmica de populações, crescimento, taxas de filtração das poliquetas e das algas, e parâmetros relacionados com a digestão e excreção dos peixes.

S. spallanzanii apresenta uma ampla plasticidade trófica, sendo capaz de se alimentar, não apenas de fitoplâncton, mas também de matéria orgânica dissolvida presente na coluna de água. Além disso, alguns autores já comprovaram a capacidade desta espécie de acumular microrganismos, tanto em condições naturais como experimentais. Assim, a capacidade de *S. spallanzanii* de remover bactérias, como *E. coli* e vibrios presumíveis, e sólidos suspensos totais foi avaliada no sistema de IMTA proposto.

O sistema de IMTA proposto é discutido, sendo apresentada uma proposta de modelo matemático e tendo sido avaliado o potencial dos poliquetas. Os resultados obtidos neste estudo mostraram que a presença das mesmas não afetou a qualidade da água, no que diz respeito à concentração de microrganismos e de sólidos em suspensão. No entanto, devido à elevada complexidade do sistema, será necessário efetuar mais estudos relativamente ao modelo matemático que o descreve, de forma a avaliar também outros parâmetros e combinações de espécies, e que permitam perceber melhor o sistema e a função desempenhada pelos diferentes organismos.

Palavras-chave: sustentabilidade da pesca, impactos da aquacultura, bioremediação, IMTA, modelação em aquacultura, *Sabella spallanzanii*, vibrios presumíveis, *E. coli*

Abstract

It is known that most marine fish stocks are nowadays overexploited or in some cases fully exploited. Thus, aquaculture is responding to consumer needs by intensifying and diversifying the product range, continuing to be the fastest-growing animal-food-producing sector with an average annual growth rate of 6.3%.

However, likewise any other anthropological activity, aquaculture can originate different impacts in the environment.

The exploitation of wild fish stocks for the production of fishmeal and fish oil, and the wild fisheries directed use for cultured fish feeding, constitutes one of the major issues in aquaculture sustainability, as well as the release of metabolic waste products, which is considered one of the most important factors causing organic and nutrient loading in the vicinities of aquatic farms. Inland aquaculture projects are water-intensive in such way that it consumes more water per unit of area than irrigated agriculture. Another natural resource impacted by aquaculture activity is the habitat. Land-based fish farms require land, and cage-based farms occupy areas of the seabed. Besides nutrient loadings, another environmental impact is the chemical input of prescribed compounds (pesticides and drugs), antifoulants, anaesthetics, and disinfectants. Also, accidental releases into natural waters can represent serious impacts in the environment, and risk of transmission of pathogens.

In order to solve some of these problems, Integrated Multi-trophic Aquaculture (IMTA) has been proposed to achieve environmental sustainability through biomitigation of aquaculture wastes. IMTA is a practice in which the by-products from one species are recycled to become inputs for another through the cultivation, in the right proportions, of fed aquaculture species with organic extractive and inorganic extractive aquaculture species.

In the last decades, there has been a growing need to better understand and optimise aquaculture performance, flow rates and transformations of toxic compounds in aquaculture production systems. Thus, dynamic modelling has been developed towards the use of models for analysis and simulation of aquacultures. The main reason to address and develop mathematical models in aquaculture, namely multi-species models, is to maximise the production and optimise species combinations in order to reduce the environmental impacts of aquaculture, and consequently, to minimise costs. However, the most part of the existing models can be limited by lack of integration with the ecosystem, few species interactions and the scale used in the models. This study aimed to develop a mathematical model to describe a more complex land-based IMTA system, with three trophic-levels: the marine fish *Sparus aurata* (main culture/fed species), the polychaete *Sabella spallanzanii* (organic extractor), and the red seaweed *Gracilaria vermiculophylla* (inorganic extractor).

Therefore, an IMTA model was proposed, regarding population dynamics, growth, filtering rates of polychaetes and seaweeds, and parameters related to the digestion and evacuation of fish.

Since *S. spallanzanii* shows a wide trophic plasticity, being able to feed not only on phytoplankton but also on dissolved organic matter present in the water column, and studies showed its capability to accumulate microorganisms under natural and experimental conditions, its use to remove bacterial groups, including *E. coli* and culturable vibrios, and total suspended solids was addressed for the proposed IMTA system.

The development of the proposed IMTA system is discussed regarding its' mathematical modeling, being partially addressed by testing the potential of the polychaetes, that results did not show to affect the water quality to a great extent regarding bacterial abundance and suspension solids. Nevertheless, due to the complexity of the system, more studies should be made concerning the system's modeling in order to assess more parameters, combinations, and characteristics to better understand the system and the possible role played by the different organisms.

Keywords: fisheries sustainability, aquaculture impacts, bioremediation, IMTA, aquaculture modelling, *Sabella spallanzanii*, presumptive vibrios, *E. coli*

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Chapter 1

General introduction

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1.1. Overview of Aquaculture and Fisheries Industry

Fish is considered an important source of proteins, essential micronutrients including vitamins and minerals, and polyunsaturated omega-3 fatty acids. Therefore, fish has a vital positive nutritional impact with evidence of beneficial effects regarding coronary heart disease, stroke, age-related macular degeneration, mental health, high blood pressure, some cancers, rheumatoid arthritis and other inflammatory diseases (Lund 2013). Additionally, fish products are considered a tradable and significantly valuable commodity (Jennings *et al.* 2001).

High-value species such as shrimp, prawns, salmon, tuna, seabass and seabream are highly traded, and low-value species such as small pelagics are exported in large quantities, mainly to developing countries. Particularly in these countries, where total protein intake may be low, fish and fishery products may represent a crucial and affordable source of animal protein (FAO 2012).

Aquaculture is responding to consumer needs by intensifying and diversifying the product range, and, therefore, is expanding in all continents through new areas and species. Many of the species that have registered the highest export growth rates in the last few years are produced by aquaculture (FAO 2012).

In 2012, capture fisheries and aquaculture production supplied the world with about 158 million tonnes of fish and more than 86% (136 million tonnes) was used for human consumption. However, more than half of the marine fish stocks were estimated to be fully exploited (61.3 %), 28.8% overexploited and only 9.9% underexploited in 2011 (FAO 2014). On the other hand, aquaculture continues to be the fastest-growing animal-food-producing sector with an average annual growth rate of 6.3% and 47% of total food fish supply in 2010 compared with only 9% in 1980. The grow-out production reported from aquaculture is almost entirely destined for human consumption, and since the mid-1990s that aquaculture has been the driving force for the growth in total fish production once the global capture production has stabilized (FAO 2012).

World aquaculture is mainly dominated by the Asia-Pacific region, which accounts for 89% in terms of volume and 79% in terms of value, mostly due to China's massive production, which accounts with more than 60% of global production in terms of volume in 2010 and 51% of global value. Other major producers in Asia are India, Vietnam, Indonesia, Bangladesh, Thailand, Myanmar, Philippines, and Japan, and in 2010 up to 65.6% of the production belonged to freshwater aquaculture (FAO 2012).

In Europe, the average annual growth production has slowed substantially to 1.2% since 2000, with a total production of 2,366.354 million tonnes in 2008, 4.5% of total world's production (FAO 2010). Norway has the highest production with more than 850 thousand tonnes in 2008, nearly 40% of the total European production, followed by Spain, France, Italy and the United Kingdom. These 5 countries account for 78% of all aquaculture production within the 34 European countries (European Environmental Agency 2011).

In accordance with the 2002 Strategy for Sustainable Development of European Aquaculture, the purposes of the national fisheries policy regarding aquaculture is to increase production and product diversity, but also to increase product quality in order to improve the competitive position of the sector and promote environmental, economic and social sustainability (Commission of the European Communities 2002).

1.2. Environmental Concerns of Aquaculture

Industrial development and the subsequent population growth have brought the need to retain life-support systems due to the increasing demand on natural resources. Aquaculture, likewise any other anthropological activity, can originate different impacts in the environment. These are usually in accordance with the industrial progress and the level of exploitation of the resources. Some effects can be positive, but some are not in accordance with long-term sustainability of natural ecosystems (Pillay 2004). It is then necessary to discuss and impose conservation and preservation measures in order to promote the rational use of resources and optimise it in a long-term basis. Many of these measures were already discussed and regulated within the European Union (EU) under the Common Fisheries Policy (CFP) in 1992 and 1993. These regulations intend to form a control system and to monitor conservation and resource management, particularly in aquaculture activities that occur in coastal areas. Additionally, during the last 30 years the EU has made efforts concerning the management of environmental impacts of marine aquaculture through the implementation of Directives such as Environmental Quality Objectives (EQOs) and Environmental Quality Standards (EQSs) (Read & Fernandes 2003). Further progress for the protection on natural ecosystems together with the development of aquaculture should be done not only in terms of regulation but also through innovative ideas and concepts in the Industry.

1.2.1. Use of wild resources for the production of farmed organisms

The use of wild resources for the production of farmed organisms is one of the major issues in aquaculture sustainability. This includes the exploitation of wild fish stocks for the production of fishmeal and fish oil and the wild fisheries direct use for cultured fish feeding (Grigorakis & Rigos 2011). The exceptional growth of aquaculture is seen by some as a solution to relieve the pressure on fish stocks and contribute to food security, while others argue that the dependence of aquaculture from the small pelagic fish is unsustainable from an ecological and ethical point of view and will, eventually, represent a delay for the expansion of aquaculture (Natale *et al*. 2013). According to this, some authors assert that aquaculture may in long-term decrease its overall production rather than increase, if the pressure on wild fish stocks used for feed is not reduced (Merino *et al*. 2010).

In 2008, 20.8 million tonnes of world fish production were used for fishmeal and fish oil. Fishmeal and fish oil are mainly produced from small pelagics such as anchoveta, sardine and herrings, and their production fluctuates annually according to the catches of this species (FAO 2012). Fishmeals contain high protein levels and fish oils are characterised by their excellent source of essential fatty acids of the n-3 series. These characteristics make them key ingredients of choice for the production of commercial feeds due to their favourable combination of nutritional value and price (Bendiksen *et al*. 2011). Fishmeal and fish oil also support other industries such poultry and pork farming. However, the majority of total fishmeal production is used in aquaculture, growing from 30% in 2000 to 62% in 2007 (FAO 2012).

Aquafeeds are generally used for feeding omnivorous fishes such as tilapia, catfish and common carp, carnivorous fishes like salmon, trout, eel, seabass, seabream and tuna, and crustacean species such as shrimps, prawns, crabs and lobsters (FAO 2012; Tacon & Metian 2008). Salmon, trout and shrimp farming use nearly 50% of the fishmeal used in aquaculture, although they only provide less than 10% of fish culture production. In 2007, approximately 40% of all aquaculture was firmly dependent on commercial feed, especially for high valuable carnivorous species. The percentage of farms using commercial feeds varies from 100% for salmon and trout to 83% in marine shrimp to 38% in carp farms (Deutsch *et al*. 2007).

The processing waste from commercial fish species used for human consumption is also an important raw material for the production of fishmeal. In the past those residues were simply discarded but nowadays they are used in feed markets in a percentage of about 36% of world fishmeal production in 2010 (FAO 2012).

Countries importing fishmeal are affected by climate variability, making fishmeal availability susceptible to fluctuations. This limited supply and the increasing price of fishmeal has led to the exploration of alternative protein sources. Possible alternatives include terrestrial animal by-products, seafood processing, vegetable proteins and oils, organisms from lower trophic levels and bacterial and algal proteins and oils produced by industrial fermentation technologies (Bendiksen *et al*. 2011). However, it is necessary to evaluate whether these alternatives are economical and can actually be used in commercial aquaculture (Deutsch *et al*. 2007).

1.2.2. Discharges of effluents

Recently, some attention has been dedicated to the effects of discharges of effluents from certain types of aquaculture. Discharges from aquaculture to the aquatic environment can be categorised as: continuous discharges from aquaculture production; periodic discharges from farm activities and periodic discharges of chemicals, mostly veterinary drugs and antifoulants (Read & Fernandes 2003). Aquaculture operations cause the release of metabolic waste products such as faeces, pseudofaeces, excreta, and uneaten food, which is considered one of the most important factors causing organic and nutrient loading in the vicinities of aquatic farms (Grigorakis & Rigos 2011). The organic enrichment causes environmental deterioration of the receiving water bodies and sediments, by increasing water nutrients, in particular nitrogen and phosphorous (Marinho-Soriano *et al*. 2011). Generally, 52–95% of the nitrogen and 85% of the phosphorus input into a marine fish culture system as feed may be lost into the environment through feed wastage, fish excretion and faeces production (Zhou *et al*. 2006b). The amount of uneaten feed relies mostly on the personnel experience and qualifications, feeding management (automatic or hand feeding), and the ingredients comprising the feed (Grigorakis & Rigos 2011; Pillay 2004).

The effluents from shrimp aquaculture are typically enriched in suspended solids; nutrients such as ammonia, nitrate, nitrite; chlorophyll a and biochemical oxygen demand (BOD) (Páez-Osuna 2001). Dissolved nutrients, especially nitrogen and phosphorus, and suspended solids have been considered the most important waste products affecting the quality of the receiving waters and their environment (Pillay 2004). The behaviour of waste released into the water column depends on the hydrographical conditions, bottom topography and the geography of the area. Dissolved products may include ammonia, phosphorus, dissolved organic carbon, as well as dissolved organic nitrogen and dissolved organic phosphorus, and lipids, which may form a film on the water surface. The consequent impacts depend on the rate at which nutrients are diluted before being assimilated by the ecosystem (Read & Fernandes 2003).

Generally, aquatic animals need a high concentration of protein in the feed since their energy production pathway requires the oxidation and catabolism of proteins. Estimates of nutrient retention and potential release by fish into the water are not readily available, and are changing rapidly as feeds, feeding practices, and culture methods evolve. Nitrogen and phosphorous retention range between 10-49% and 17-40%, respectively. Likewise, nitrogen and phosphorous release in faeces range from 3.6% to 35% and 15% to 70%, respectively; and dissolved N and P excretions, range from 37% to 72% and 1% to 62%, respectively (Piedrahita 2003). The trend is to increase nutrient retention and reduce losses as feed quality is improved, such that most N is excreted in the dissolved form (mainly as ammonia) and most P as particulate (Piedrahita 2003). The excreted ammonium coming mainly from protein-rich feed, is oxidized by bacteria to nitrite and nitrate species and, unlike carbon dioxide which is released to the air by diffusion or forced aeration, there is no effective mechanism to release the nitrogenous metabolites. High concentration of ammonium competes for oxygen with aquatic organisms for nitrification. When the oxygen demand is higher than the available, the waters and sediment become anoxic. This can lead to changes in the biological and chemical processes in the sediments and in the ecology of benthic organisms. Excess nitrogen and phosphorous concentration can cause hypernutrification and eutrophication – the two major processes that result from waste discharges from land- or water-based aquaculture farms. Senescence and disintegration of phytoplankton blooms can lead to areas with low dissolved oxygen (Pillay 2004; Paul & Vogl 2011), which can lead to severe reduction of water quality and, consequently, to fish mortality. In the case of restricted exchange environments, there is a risk of high levels of nutrients accumulating in one area. In this case, farm discharges can alter habitats and community structure and lead to disease outbreaks (Jegatheesan *et al*. 2011). Other changes in the water quality in the near of farms due to vigorous flushing of effluents may lead to structure and function of marine ecosystems modifications. Such changes are likely to take place initially in the phytoplankton and phytobenthos, and then propagate through marine food webs. Damage to ecosystem structure can include loss of biodiversity and changes in the "balance of organisms" imply a shift in relative abundances of species' populations (Ferreira *et al*. 2011). Consequently, organic enrichment in sediments will move the ecosystem to the one dominated by bacteria, ciliates and meiofauna (Chávez-Crooker & Obreque-Contreras 2010).

1.2.3. Use of natural resources

1.2.3.1. Use of water resources

Frequent exchange and replacement of water is one of the most common solutions used to remove the excessive nitrogen. However, this approach has restrictions. There are environmental regulations that limit the release of nutrient rich water in the environment, including the concern of introducing pathogens; and pumping vast amounts of water may translate into a high expense (Avnimelech 1999).

Inland aquaculture projects such as ponds are water-intensive in such way that it consumes more water per unit of area than irrigated agriculture (Boyd & Gross 2000). Considering this, it is important to make accurate estimates of water use, and water conservation measures should be discussed (e.g. maintaining storage capacity in ponds equal to the normal, maximum daily precipitation, reduction in seepage beneath dams and through pond bottoms, first harvest without draining ponds and water re-use, etc.). For example, in intensive shrimp aquaculture systems, there is a regular water exchange of 3-30% of the pond volume per day, depending on the local conditions, the stage of grow-out and the feeding cycle (Páez-Osuna 2001; Páez-Osuna *et al*. 1998). The reduction in effluent volume is the most effective water saving method, and not only reduces water consumption but also the potential pollution of pond aquaculture (Boyd & Gross 2000).

1.2.3.2. Habitat impacts and ecological assessment of water bodies

Another natural resource impacted by aquaculture activity is the habitat. Land-based fish farms require land and cage-based farms occupy areas of the seabed. In the case of water-based production systems, as most Mediterranean fish and shellfish farms (i.e. cages, rafts and long-lines), they are sited sensitively and offer little threat in what concerns to the loss of important wildlife habitat (Beveridge 2001).

A small number of inappropriate developments and the intensification of production methods in some traditionally farmed areas have adversely impacted on wildlife. Large-scale shrimp culture has resulted in physical degradation of coastal habitats: mangroves forests and marshes destruction, agricultural and drinking water supplies salinisation, and land subsidence due to groundwater abstraction (Páez-Osuna 2001; World Inventory of Fisheries 2005).

The Water Framework Directive (WFD;2000/60/EC), adopted in 2000 by countries of the European Union, established well-defined objectives to protect all inland and coastal water bodies from existing environmental pressures. The final goal is to achieve at least good ecological quality status in the near future (Ferreira *et al*. 2007). Four groups of environmental pressures were identified based on IMPRESS (2002) and Borja *et al*. (2006): (i) pollution, including urban, industrial, agricultural and aquaculture discharges; (ii) alteration of the hydrological regime, comprising water abstraction; (iii) changes in morphology, including land reclamation and infrastructures; and (iv) biology and its uses, including resource exploitation (e.g. algae exploitation), changes in biodiversity and recreation. The impact assessment entails the identification of pressures, particularly the ones that may result in the failing of an objective - that to a great extend may derive from aquaculture practices as can be noted by the above identified pressure groups, and leading to low classification within the five condition classes defined by the WFD: high, good, moderate, poor or bad (Poikane *et al*. 2014).

1.2.4. Presence of chemical contaminants

Besides nutrient loadings, another environmental impact is the chemical input of prescribed compounds (pesticides and drugs), antifoulants, anaesthetics, and disinfectants (Burridge *et al*. 2010). Particularly, the use of antibiotics may affect non-target species leading to antibiotic resistance and other toxic effects (Cole *et al*. 2009). The prophylactic use of therapeutants and their active persistency in the environment is also a concern (Read & Fernandes 2003). Additionally, many times the use of already banned veterinary medical drugs adds a problem to the regulators and to the environment (Leston *et al*. 2011).

As in all animal food production systems, it is necessary to treat farmed fish for diseases and parasites; although management practices have evolved and fish husbandry has greatly improved over the past years resulting in a reduction in the use of some chemicals, particularly the use of antibiotics in most jurisdictions (Burridge *et al*. 2010).

Recently, with the increasing concerns about ecological impacts of aquaculture, this issue has been developed. Still, scarce information is available on the impacts of these contaminants on the environment.

1.2.4.1. Antibiotics

Bacterial pathogens are the most problematic disease organisms for the aquatic health management causing, in addition, considerable economic losses. Members of the genera *Vibrio*, *Nocardia*, *Aeromonas* and *Streptococcus* are ubiquitous in the environment and therefore common contaminants of aquatic products (Shi *et al*. 2012).

The unprecedented growth of aquaculture brought also hygienic deficiencies in raising methods. The increased stocking densities, lack of sanitary barriers and failure to isolate aquaculture base units with infected animals has raised the possibility of rapid spreading of infection. Consequently, the use of prophylactic (disease prevention) and therapeutic (disease treatment) antibiotics has augmented worldwide, often to compensate the lack of adequate sanitary practices (Zheng *et al*. 2012; Sapkota *et al*. 2008). Antibiotics are then used to kill bacteria or inhibit their growth. This group of natural or synthetic compounds enters the natural environment through faeces and uneaten antibiotic feed. It was estimated that 75% of most the antibiotics in feed are exported to the surrounding environment (Lalumera *et al*. 2004). This can lead to the accumulation of antibiotic residues in ponds, marine sediments, wild fish, and aquaculture products (Sapkota *et al*. 2008).

Drug resistance and sensitivity are the major concerns around the use of antibacterials. Their improper use may result in the resistance of bacterial pathogens to certain drugs. Drug resistance concerns are not exclusively related with animal health, but also with the potential risk of transferring resistance to human pathogens (Schnick 2001). The major problems occur when antibiotics are used prophylactically, in a regular or even daily basis. Bacterial resistance arises and is kept through bacterial DNA mutations or through transfer mechanisms such as conjugation with other bacteria, transduction with bacteriophage and transformation through the uptake of free DNA (Sapkota *et al*. 2008). Resistance genes have been already found in pathogenic *Aeromonas* spp., *Citrobacter* spp*., Edwardsiella* spp., *Photobacterium* spp. and *Vibrio* spp. These genes can be consequently transmitted to bacteria belonging to the terrestrial environment, including animal and human pathogens, as already reported for *Salmonella enterica* serotype *Typhimurium* and *Vibrio cholerae* (Defoirdt *et al*. 2011).

Alternatively to antibacterials there are vaccines for some disease pathogens. However, in some cases they do not work efficiently or vaccines have not been yet developed.

It is then important to use antibiotics in a wise and controlled way once their availability is limited and will remain so due to regulations. In Europe, North America and Japan the regulations on the use of antibiotics are strict and only few antibiotics are licensed for use in aquaculture. However, a large proportion of the global aquaculture production occurs in countries that have no or effective regulations (Leston *et al*. 2011).

1.2.4.2. Parasiticides

Generally, ectoparasites, which have direct life cycles and short generation time, are the ones with the most potential to affect economically the marine aquacultures. Mostly, due to the use of manufactured feed and almost complete elimination of trophic interactions required by some metazoan parasites for their transmission. Parasitic disease may not cause fish mortality, but increases production costs through treatment or reduction in the product quality (Nowak 2007).

Sea lice are ectoparasites that have been causing serious problems in the salmon aquaculture industry. This crustacean parasite can cause skin erosion and haemorrhage, and if untreated can lead to secondary infections. Effective mitigation and management of sea lice infestations often requires treatment with antiparasitic compounds. These compounds are known as major environmental concerns due to their lack of specificity. They can negatively impact sensitive non-target organisms by altering the population structure within the immediate surroundings. Avermectins, pyrethroids, hydrogen peroxide and organophosphates are the classes of therapeutants currently used to treat sea lice infestations. These can be administrated as bath treatments or as additives in feed (Burridge *et al.* 2010).

Sea-cage aquaculture systems are a transmission pathway of many parasite taxa between farmed and wild fish. The increased availability of food and the floating structures that can be used as a refuge from predators are highly attractive to wild fishes, forming large and diverse aggregations in the vicinities (Mackenzie 1999). There are numerous known cases of transmission of pathogens between farmed and wild populations (Torrisen *et al*. 2013). These interactions and increased host densities due to intensive farming may favour increased virulence (Nowak 2007).

Moreover, parasitic crustaceans as well as protozoans and metazoans can act as vectors of bacterial and viral infections (Catalano & Hutson 2010).

1.2.4.3. Metals

Metals and metalloids occur naturally in the environment through several geochemical processes. However, aquaculture can be an additional source of metals via copper-based antifoulants and fish feed that contain various metals in order to fulfil mineral requirements (Sapkota *et al*. 2008). Copper, zinc, iron and manganese are some of the metals present in feed (Burridge *et al*. 2010). Moreover, fish raised in wastewater that contain numerous heavy metals and organic chemicals including polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) can acquire elevated levels of these contaminants in their edible tissues. Individuals consuming these products are vulnerable to the neurotoxic and carcinogenic effects of high exposure to heavy metals.

Copper and zinc have been shown to be significantly elevated near aquaculture sites, especially, in areas where intensive cage aquaculture takes place (Mendiguchía *et al*. 2006; Burridge *et al*. 2010; Russel *et al*. 2011; Chou *et al*. 2002).

Copper-based antifoulants paints are applied to cages and nets in order to prevent the attachment of “epibiota”. Fouling species can decrease water quality, the durability of the nets and reduce their flotation, threatening the stability of suspended culture systems (Burridge *et al*. 2010; Guenther *et al*. 2009).

Metals such as copper have relatively low solubility in water and tend to accumulate in sediments. The critical issue is what fraction of the copper is actually bioavailable so that it can produce toxic effects. Algae, molluscs and crustaceans are the most sensitive groups to copper (Burridge *et al*. 2010). Some studies have also shown that high concentrations of copper can affect phytoplankton diversity (Le Jeune *et al*. 2006; Winner & Owen 1991) and inhibit the reproduction of some phytoplankton species (Brand *et al.* 1986). Copper toxicity combined with other compounds found in sediments such as zinc, silver and organic compounds should also be considered. Zinc is also found in sediments under aquaculture cage sites. Just like copper, it binds to fine particles and sulfides in sediments. When bioavailable, it is usually less toxic than copper. Zinc is used as an additive in aquaculture feed. In some feeds, metal concentrations exceed the dietary requirements. However, some manufacturers changed the form of zinc to a more available form (zinc methionine). Now the levels in some diets are extremely low. Marine algae are particularly sensitive to zinc in water. Invertebrates demonstrated lethal and sub-lethal responses to elevated levels of zinc (Burridge *et al*. 2010). The accumulation of trace metals such as copper and zinc below or in the vicinity of aquaculture sites are a potential risk of toxicity to many benthic organisms (Russel *et al.* 2011).

1.2.5. Conservation and loss of biodiversity

General features of successful invasive species include a widely distributed original range, a broad environmental tolerance, high genetic variability, short generation time, rapid growth and early sexual maturation. Almost all of these characteristics are favoured for species used in aquaculture. Thus, the potential of many aquaculture species to become invasive is high (Diana 2009).

Accidental releases into natural waters can represent impacts in the environment and biodiversity, and a serious risk of transmission of pathogens. Cages, rafts and long-lines are particularly risky in this matter. Release of fish from cages can occur during daily operations like stocking, grading and disease treatment, and also as a result of storms, predator damage, and accidents. The concern is that feral species become established and adversely impact on indigenous biodiversity (Diana 2009). Impacts of non-native on native populations arise from abiotic or biotic interactions, including increased competition and predation, habitat damage, alterations in the water quality, hybridisation and importation of parasites and diseases (Beveridge 2001; Arthur *et al*. 2010). There are also concerns related with the impacts of non-native species focus on interspecific interactions, and those regarding native species focus on intraspecific interactions between partially or fully domesticated types and wild types. It is not clear a priori which option poses less risk to native biota (Arthur *et al*. 2010). Organisms escaping from farms also originate an increasing environmental concern related to genetic pollution. This concept involves the alteration of the natural genetic architecture and microevolutionary processes of wild populations due to the gene flow from farmed conspecifics (Cognetti *et al*. 2006). Escapement is particularly troublesome for Atlantic salmon (*Salmo salar*), since about 94% of all adult fish is reared in cages in the marine environment. Norway recorded an average of 500,000 fish escaping annually since 1992 with upwards of one million fish in 2005. British Columbia reported 26 escape events in a four-year period. Off the coast of Scotland, a single storm event resulted in the release of about 685,000 Atlantic salmon. Recent studies suggest that escapes are genetically affecting populations in Norway, Ireland, and Maine (Tlusty *et al*. 2008). The decline in pink salmon (*Oncorhynchus gorbuscha*) in British Columbia triggered a debate over the role of sea lice derived from salmon farms on wild populations. Many authors argue that salmon farms intensify the level of sea lice in surrounding waters, leading to serious infection of wild juvenile pink and chum salmon (*Oncorhynchus keta*), possibly resulting in increased mortality and thus declines in wild salmon populations (Liu *et al*. 2011). Johansen *et al*. (2011) reviewed disease interaction and pathogen exchange (viral, bacterial and parasitic) between wild and farmed fish in Norway. Atlantic salmon escapement can also represent a threat to the genetic integrity and fitness of wild salmon populations. Farmed salmon have been subjected to selection for economically important traits such as growth, delayed maturation, fat percentage, flesh colour, and disease resistance (Glover *et al*. 2009). It was also showed that farmed salmon strains display reduced genetic variation when compared to wild salmon populations. Offspring of farmed salmon and hybrids display reduced fitness in the wild (Glover *et al*. 2009a; Glover *et al*. 2009b).

Considering the negative ecological consequences that escapes might entail, methodologies and strategies to recapture escapees, especially in the case of large-escape incidents, are highly necessary.

Tlusty *et al*. (2008) studied the potential of acoustic conditioning as method to recall/recapture escaped fish in Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*). Arechavala-Lopez *et al*. (2012) emphasized the importance of local fisheries to reduce the potential effects of escape incidents on natural stocks. Studying the behaviour of the species intended to cultivate, such as the net cage biting and the expression of escape-related behaviours of the Atlantic cod (*Gadus morhua*), is crucial to avoid economic and environmental problems (Zimmermann *et al*. 2012). Glover *et al*. (2009a) studied the use of genetic assignment to identify the farm of origin for escapees in a region where the density of salmon farms is very high. The methods adopted by the authors led to an overall accuracy of self-assignment of 99%. Further studies should be highly encouraged in order to preserve natural resources in a long-term perspective, and also to reduce the economical inconveniences of aquaculture farmers.

1.2.6. Disease Occurrence

Both in aquaculture facilities and in natural aquatic environment, the occurrence of disease is a complex interaction between the host species, disease agents and the environment.

In farming conditions, disease outbreaks are greatly influenced by the susceptibility of the hosts, the virulence of the pathogens and adverse environmental circumstances. Farming practices may favour disease occurrence, like in intensive and semi-intensive production systems characterised by high stocking densities, increased stress of stocks, intensive feeding, and inadequate water exchange. The host species may live healthy normal lives in the continuous presence of pathogens, and only when environmental stresses occur will the balance change, favouring the dominance of the pathogen (Pillay 2004).

Several diseases have emerged as serious economic or ecological problems in aquaculture, and are a significant constraint to the expansion of the industry. The control of endemic diseases imposes severe year-on-year costs on producers. A global estimate of disease losses range about $ 3/4 billion per year (Stabili *et al*. 2010). For example, white-spot syndrome of shrimp (WSS) has cost billions of dollars worldwide. Moreover, the elimination of disease outbreaks, such as ISA (Infectious Salmon Anaemia) in Scotland in 1998/1999, causes unexpected expenditure for both the industry and government (Murraya & Peeler 2005). The marine cage culture of Atlantic salmon in

Chile (Bustos *et al*. 2011), oyster farming in Europe notably France (Segarra *et al*. 2010), and marine shrimp farming in several countries in Asia, South America and Africa have experienced high mortality caused by disease outbreaks in recent years, resulting in partial or sometimes total loss of production; disease outbreaks virtually wiped out marine shrimp farming production in Mozambique in 2011 (FAO 2012).

Even though water is the major recipient of dissolved residues from aquaculture, a considerable portion of the solid material is retained inside the ponds, discharge canals or in the vicinity of the farms. Both the culture medium and coastal habitats where the activity is practiced show high rates of biological activity and organic matter decomposition. Recently, particular emphasis has been directed on development sustainable approaches to coastal aquaculture. In this sense, the promotion of ecological practices to improve the ecosystem health has been closely encouraged, including water recycling, effluent management and biological treatment by integrated culture (Marinho-Soriano *et al*. 2011).

The limits allowed for nutrients concentration in the effluents discharged by aquaculture are expected to become more restrictive in the near future, due to the implementation of the Water Framework Directive, which intend to reduce emissions of hazardous substances to water and contribute to achieving concentrations in the marine environment near background values for naturally occurring substances (Official Journal 327 2000).

1.3. Bioremediation of Aquaculture Wastes

As above referred, the discharge of substantial amounts of polluting effluents containing uneaten feed and faeces constitutes one of the most negative environmental impacts of aquaculture. The organic enrichment causes environmental deterioration of the receiving water bodies and sediments, by increasing water nutrients, in particular nitrogen and phosphorous (Marinho-Soriano *et al*. 2011). Moreover, this organic enrichment also can lead to an increased presence of pathogenic bacteria. Sediments close to aquaculture facilities can become enriched reservoirs of viruses associated with organic detritus.

The improvement in aquaculture waste management is thus a highly desirable objective, in order to decrease potential environmental and economic impacts through disease transmission and water renewal. Therefore, removal of nitrogen and phosphorus from the water column to mitigate eutrophication along with improved wastewater and sediment treatments that reduce the level of organic matter and, consequently, the biological risk, can make aquaculture a sustainable farming practice for the long-term (Chávez-Crooker & Obreque-Contreras 2010).

Recently, bioremediation of water and sediments contaminated by sea cage aquaculture, and of effluents discharged by land-based aquaculture activities, involving the use of many organisms, including bacteria, microalgae, macroalgae and filter-feeding invertebrates, has been discussed. Standard waste treatment methods and other bioremediation techniques may be simultaneously applied, as needed.

Treatment of aquaculture wastes implies the development of sustainable approaches to coastal aquaculture, as the implementation of Integrated Multi-trophic Aquaculture (IMTA) systems, and microbial nitrification and denitrification in sediments (Chávez-Crooker & Obreque-Contreras 2010; Marinho-Soriano *et al*. 2011).

Integrated Multi-trophic Aquaculture strategies combine a number of complementary organisms at a farm site in order to optimise nutrient utilisation and reduce solid waste that goes to sediments. This subject will be developed at Section 4 (IMTA and Trophic Levels in Aquaculture).

Biological nitrification in sediments occurs under aerobic conditions, where two groups of bacteria convert ammonium to nitrite and then to nitrate (NH_3 – NO_2^- – NO_3^-), consuming a great deal of oxygen that can lower dissolved oxygen in the area. On the other hand, biological denitrification in sediments occurs under low oxygen conditions and it is the conversion of fixed nitrogen into N_2 gas, which returns to the atmosphere. A wide range of microorganisms is capable of denitrification reactions, including various bacteria, Archaea and Eukarya. Anaerobic ammonium oxidation is another route to denitrification.

The implementation of water recirculating systems facilitates the technological applications of these biological filters in land-based aquacultures, since the volume of the waste streams becomes more manageable and various treatment options can be considered, such as recirculation loop mainly found in outdoor, IMTA, special reactors under anoxic conditions, and others (Chávez-Crooker & Obreque-Contreras 2010; Rijn 2013).

1.4. IMTA and Trophic Levels in Aquaculture

The use of filter feeding organisms as nutrient (inorganic and organic) extractors has proven to be a valid alternative for nutrient bioremediation. The most frequently tested organisms are molluscs, which filter organic particles, and phytoplankton, and macroalgae which have the capability of inorganic nutrient uptake (Marinho-Soriano *et al*. 2011).

Integrated Multi-trophic Aquaculture (IMTA) has been proposed to achieve environmental sustainability through biomitigation of aquaculture wastes that, as compared to other accompanying methods, has advantages that may include economic stability by product diversification and risk reduction, and social acceptability through better management practices (Troell *et al.* 2009; Barrington *et al*. 2009). Furthermore, IMTA is the only practical remediation approach with a prospect for additional farm revenues by adding commercial crops, while all other biomitigation approaches have generally involved only additional costs to the producer (Troell *et al.* 2009).

IMTA is a practice in which the by-products (wastes) from one species are recycled to become inputs (fertilizers, food and energy) for another through the cultivation, in the right proportions, of fed aquaculture species (e.g. finfish/shrimp) with organic extractive species (e.g. suspension and deposit feeders), and inorganic extractive aquaculture species (e.g. seaweeds) (Troell *et al.* 2009; Barrington *et al*. 2009). One of the differences from the traditional practice of aquatic polyculture is the incorporation of species from different trophic or nutritional levels in the same system. In traditional polyculture, organisms may all share the same biological and chemical processes, with few synergistic benefits; they may, in fact, incorporate a greater diversity, occupying several niches, as extensive cultures (low intensity, low management) within the same pond. However, the “integrated” in IMTA refers to the more intensive cultivation of different species in proximity of each other (not necessarily right at the same location), connected by nutrient and energy transfer through water (Barrington *et al*. 2009).

In the last fifteen years, the integration of seaweed with marine fish culturing has been examined and studied in Canada, Japan, Chile, New Zealand, Scotland and the USA. The integration of mussels and oysters as biofilters in fish farming has also been studied in a number of countries, including Australia, USA, Canada, France, Chile, and Spain. Also, the recent offshore relocation of many coastal finfish farms in Turkey has triggered the interest in IMTA. Recent reviews on IMTA research include a focus on seaweeds, bivalves and crustaceans (e.g. Troell *et al*. 2009).

1.4.1. Seaweeds

The ability of macroalgae to respond to availability of anthropogenic nutrient (nitrogen and phosphorus) input makes them an efficient instrument for bioremediation (Commission Regulation nº 710 2009; Marinho-Soriano *et al*. 2011). Biofiltration by plants, such as algae, is assimilative, and therefore adds to the assimilative capacity of the environment for nutrients. Plants photosynthesize new biomass through solar energy and the excess nutrients, particularly C, N and P. Theoretically, this process recreates an ecosystem, wherein, if properly balanced, plant autotrophy counts on fish or shrimp and microbial heterotrophy, not only with respect to nutrients but also oxygen, pH and CO_2. Algae, particularly seaweeds, are the most suitable for biofiltration because they probably have the highest productivity of all plants and can be economically cultured (Neori *et al.* 2004).

IMTA research along the Atlantic coast is primarily focused on using algae (mainly Rhodophyta) with fish (mainly turbot, *Scophthalmus maximus*, and sea bass, *Dicentrarchus labrax*). Much research is being done using *Gracilaria bursa-pastoris*, *Gracilaria gracilis*, *Chondrus crispus*, *Palmaria palmata*, *Porphyra dioica*, *Asparagopsis armata*, *Gracilariopsis longissima* (Rhodophyta), *Ulva rotundata* and *Ulva intestinalis* (Chlorophyta) as biofilters for use in IMTA units. Using this knowledge, researchers have begun experimental studies where algae have been integrated with sea bass and turbot (Barrington *et al*. 2009).

Recent research on marine IMTA systems in industrialised nations has mostly been developed using experimental and small-scale operations, which it is difficult to extrapolate to larger industrial scale farms (Troell *et al.* 2003). However, some marine IMTA systems, primarily in Asia (China), have been commercially successful at industrial scales, while experimental projects are now scaling up towards commercialization in Canada, Chile, the USA and in some European countries (Troell *et al*. 2009). On the east coast of Canada, in Bay of Fundy, an IMTA combining kelps, such as *Saccharina latissima* and *Alaria esculenta*, with Atlantic salmon and blue mussel, resulted in a substantial increase of kelps and mussels' growth rates. In Sungo Bay (China), a company works at industrial scale, producing the kelp *Laminaria japonica* with scallop (*Chlamys farreri*), abalone (*H. discus hannai*) and blue mussel (*Mytilus edulis*).

The red algae *Gracilaria* spp. and the green algae *Ulva* spp. have been found to be efficient biofilters. *Gracilaria* spp. have been examined for their usefulness by laboratory (using tank) (Zhou *et al*. 2006a; Marinho-Soriano *et al*. 2011; Skriptsova & Miroshnikova 2011; Abreu *et al*. 2011a), outdoor (pond) (Abreu *et al.* 2011b), and field (Zhou *et al*. 2006a; Yang *et al*. 2006; Abreu *et al*. 2009) cultivation experiments.

Ulva spp. have been studied mainly from the viewpoint of the treatment of land-based pond/tank effluent and their usefulness in the coastal IMTA system has not been examined closely except for a few studies conducted in Japan (Yokoyama & Ishihi 2010). An efficient algal-based integrated mariculture farm maintains optimal standing stocks of all the cultured organisms, considering the respective requirements of each for water and nutrients and the respective rates of excretion and uptake of the important solutes by each of them. This allows the profitable use of each of the culture modules with minimum waste (Neori *et al*. 2004). Algae, mainly seaweed, have a large market, and just in 2012 about 23.8 million tonnes valued at US$ 6.4 billion were sold for human consumption, phycocolliods, feed supplements, agrichemicals, nutraceuticals and pharmaceuticals (FAO 2014). Thus, diversifying a culture system, integrating extractive algal culture with fish or shrimp farming, makes sense not only ecologically but also from an economical point of view (Neori *et al*. 2004).

1.4.2. Invertebrates

The reduction of suspended solids and microbial pollution within aquaculture can be achieved by the use of living organisms. Literature also reveals the potential capability of some invertebrates to remediate heavy metals, microbial contaminants, hydrocarbons, nutrients and persistent organic pollutants (Khoi & Fotedar 2012; Stabili *et al.* 2006). Filter-feeding marine macroinvertebrates filter large volumes of water for their food requirements and exert high efficiency in retaining small particles including bacteria (Stabili *et al*. 2010). Detritus feeder species have also been proposed as a means for recycling the particulate organic and inorganic nutrient wastes from fish cage farming (Lander *et al*. 2013).

1.4.2.1. Polychaetes

The Mediterranean polychaete *Sabella spallanzanii* showed ability to filter, accumulate and remove from waste bacterial groups, including human potential pathogens and vibrios (Stabili *et al*. 2010). Licciano *et al.* (2005) calculated the clearance rates and filtration efficiencies for *S. spallanzanii* on *Vibrio alginolyticus*, revealing the ability of sabellids to filter bacteria with high efficiency. Therefore, sabellids are considered suitable to use in aquaculture farms as biofilters, also considering their action in removing suspended solids in wastewaters to which bacteria can be attached. According to the Global Marine Aquarium Database, 11,178 of sabellidae polychaetes, also known as fan worms, were imported from UK between 1991 and 2001 to Indonesia, Philippines, Singapore, Sri Lanka, USA, Brazil, Cuba and Martinique. Thus, additionally to the biofiltering capability of these filter-feeding organisms they are also likely to be traded in the marine aquarium industry. Moreover, Stabili *et al*. (2009) revealed that the mucus of *S. spallanzanii* contains a complex of at least ten major and six minor proteins, one of which displays lysozyme-like activity. The presence of lysozyme indicates an important defending role from bacterial attacks, taking into account that these organisms live in eutrophic environments, like harbours where bacteria, including human pathogens, are abundant. This antibacterial activity can also be explored from a biotechnological perspective.

More studies have been conducted with different polychaetes species. In 2002, Honda and Kikuchi (2002) showed the potential of polychaete *Perinereis nuntia vallata* to ingest and assimilate faecal waste from Japanese flounder, converting about half of the nitrogen ingested into worm body tissue. García-Alonso *et al.* (2008) assessed the possible culture of the ragworm *Nereis diversicolor* using eel sludge as a feed source, suggesting that the use of ragworms in aquaculture could reduce the production of waste and increase the reproductive fitness of cultivated animals. Bischoff *et al.* (2009) cultured *N. diversicolor* in settlement tanks receiving wastewater from a sea bream recirculation system. Moreover, in 2010, Palmer assessed the ability of two intertidal polychaetes, *Perinereis helleri* and *Perinereis nuntia*, cultured in sand beds to remediate wastewater resulting from a prawn farm and produce harvestable polychaetes biomass without supplemental feeding using “polychaete-assisted sand filters”. This study revealed that the total suspended solids and the polychaete filtration process significantly reduced chlorophyll *a* levels. These results demonstrated that it would be possible to reduce the retention times and areas required by the settlement pond used in prawn farms (Palmer 2010). Brown *et al.* (2011) assessed the costs and potential benefits in terms of waste treatment/mitigation and economic return from using *Nereis virens* as a component of an integrated aquaculture system.

The biofiltering efficiency of these polychaetes is influenced by the water flow, the total suspended solids levels, the age-at-stocking, and density are the factors that also influence their survival and growth (Palmer 2010).

1.4.2.2. Sponges

During the past few years, it was proposed for the production of sponge biomass to be used at integrated aquaculture systems and, accordingly, filtering efficiencies and particle uptake in sponges have been studied. The aim was to understand the energy balance of filtering activity, the effects of temperature, and the uptake of microorganisms and/or particles (Milanese *et al*. 2003). Studies have demonstrated the ability of Demospongiae (Porifera) to unselectively filter organic particles within a size range 0.1–50 mm, which includes heterotrophic bacteria, heterotrophic eukaryotes, phytoplankton and detritus, processing the water column within 24h, and retaining up to 80% of the suspended particles (Stabili *et al*. 2006). The utilisation of bacteria by sponges, as observed for sabellids, also suggests an applicative role for bioremediation purposes, considering that bacteria are usually abundant in waters with high amount of organic matter, reaching particularly high densities in areas subjected to aquaculture activities (Stabili *et al*. 2006b). Thereby, a large-scale sponge culture could have a profound effect on the water quality in the vicinity of fish farms, combining the production of sponges with remediation of pollution. At the same time, sponge growth is stimulated, making sponge aquaculture more efficient. Although the idea of integrated sponge/fish aquaculture has been discussed, it has not been applied on a commercial scale. Studies have been conducted on the Mediterranean sponges *Dysidea avara*, *Chondrosia reniformis* (Osinga *et al*. 2010), *Chondrilla nucula* (Milanese *et al*. 2003), and *Spongia officinalis* var. *adriatica* (Stabili *et al*. 2006b) to assess their potential of filtration and integration with maricultures. All these species showed great filtering efficiency and higher growth close to the farm installations.

1.4.2.3. Bivalves

Several studies have showed that bivalves can be potential bio-controllers for fish farm effluents and for other eutrophication sources. Additionally, several authors have found significantly enhanced rates of shellfish, as oysters and mussels, when co-cultivated or grown with salmon (MacDonald *et al*. 2011; Handå *et al*. 2012; Lander *et al*. 2013).

In 1999, it was demonstrated the ability of freshwater mussel *Diplodon chilensis* to reduce chlorophyll *a*, phosphate and ammonia concentrations in tanks with salmon (Soto & Mena 1999). Besides, the effectiveness of the oyster *Saccostrea commercialis* was tested with positive results concerning the reduction of total suspended solids, and total N and P (MacDonald *et al*. 2011). Reid *et al.* (2010) assessed the absorption efficiency of blue mussels, *Mytilus edulis* and *M. trossulus*, on diets of Atlantic salmon particulates (feed and faeces), with results that support the concept of culturing these organisms in close proximity to salmon cages in IMTA systems as a process to remediate the solid waste.

Moreover, bivalves are known to bioaccumulate human pathogens such as *Vibrio* species, hepatitis A virus, human sapovirus and adenovirus. Few studies suggest that there is potential for shellfish to act as reservoirs for finfish pathogens; thus, the integration of shellfish production in fish farms, as in IMTA, could potentially change the infection dynamics for fish pathogens (Pietrak *et al*. 2012). The same author demonstrated the capability of *M. edulis* for bioaccumulating *V. anguillarum* in the digestive gland 2 orders of magnitude above levels observed in the water column. However, if *V. anguillarum* can persist in mussel faecal pellets in sediments for extended periods of time, mussels could generate long-lived *Vibrio* reservoirs in sediments and/or faecal matter. Then, it is necessary to minimise the risk of transmission from re-suspended sediments by sitting farms in locations with sufficient water depth between the bottom of the cage and the benthos at low tide. Therefore, it is reasonable to consider that bivalves can be integrated with fish farming in order to reduce ecological impacts while also having the potential to develop into a valuable crop for the farmers (MacDonald *et al*. 2011).

1.4.2.4. Sea cucumbers

Sea cucumbers are detritus feeders that ingest sediment with organic matter including detritus of plant and animals, and they are considered important processors of surface sediments in many coastal marine systems. Therefore, they would be good candidates for co-culture in IMTA systems (Yokoyama 2013; Slater & Carton 2009).

The japanese common sea cucumber *Apostichopus japonicas* is a valuable species in many parts of Asia and extensively world traded. This organism showed potential to be used in integrated multi-trophic aquaculture systems, being cultured in the water column below fish cages (Yokoyama 2013), with sea urchins in cages (Ito 1995), in abalone tanks (Kang *et al*. 2003), and in hanging scallop lantern nets (Zhou *et al.* 2006a). The California sea cucumber *Parastichopus californicus* has been observed to clean detritus from the oysters and several studies suggest that this species is capable of consuming fouling debris such as fish faeces, excess fish food, and algae, and could turn harmful fouling into a marketable product - sea cucumber biomass (Paltzat *et al.* 2008). Also, Hannah *et al.* (2013) demonstrated that *P. californicus* is well suited to utilise the heavy fraction of waste from a sablefish farm. Nelson *et al.* (2012) assessed the orange-footed sea cucumber, *Cucumaria frondosa*, as a potential extractive species to remove additional particulate organic waste. The results demonstrated that *C. frondosa* exhibit high absorption efficiency (> 80%) when challenged with particulate material of higher organic content, such as salmon food and faeces; and, therefore, it has a great deal potential to become an effective organic extractive IMTA species. Several studies assessed the feeding behaviour of juvenile Australasian brown sea cucumber, *Australostichopus mollis*, when exposed to green-lipped mussel waste. The results showed that green-lipped mussel waste is a suitable artificial diet for juvenile sea cucumbers if provided in sufficient quantities (Slater *et al*. 2009); and indicate that rapid growth can be expected among sea cucumbers cultured beneath mussel farms (Slater *et al*. 2009; Zamora & Jeffs 2011; Zamora & Jeffs 2012). Besides, Slater and Carton (2009) demonstrated that *A. mollis* grazing significantly reduced the accumulation of both organic carbon and phytopigments associated with biodeposition from mussel farms.

Thus, sea cucumbers may have the potential to some extent constrain or, in some cases, even reverse the polluting impacts of coastal bivalve aquaculture (Slater & Carton 2009).

1.5. Conceptual framework of the study

Although aquaculture is the fastest-growing animal-food-producing sector (FAO 2012), it has as several negative impacts on the environment, including the use of wild and natural resources (Grigorakis & Rigos 2011; Páez-Osuna 2001), discharges of effluents (Zhou *et al*. 2006b), release of chemical contaminants (Burridge *et al*. 2010), possible loss of biodiversity (Diana 2009), and disease occurrence (Pillay 2004). These impacts may jeopardize the long-term sustainability of natural ecosystems and of aquaculture industry itself.

It is then necessary to discuss conservation and preservation measures in order to promote the rational use of resources and optimise it in a long-term basis, and to develop more sustainable production techniques. In recent years, integrated multi-trophic aquaculture has been proposed as a more sustainable approach and with more economic benefits.

In the present work, three species from different trophic levels were chosen due to their interesting features and potential.

Concerning biofiltration, seaweeds are very suitable because they have high rates of productivity and can be economically cultured (Neori *et al*. 2004), Thus, the red-algae *Gracilaria* spp. has been addressed in indoor and outdoor aquaculture tanks (Zhou *et al*. 2006a; Marinho-Soriano *et al.* 2011; Skriptsova & Miroshnikova 2011; Abreu *et al*. 2011a); in here the the red-algae *Gracilaria vermiculophylla* was used.

It is also known that the reduction of suspended solids and microbial pollution within aquaculture can be achieved by the use of living organisms. Filter-feeding marine macroinvertebrates filter large volumes of water for their food requirements and are very efficient in retaining small particles (Stabili *et al*. 2010). Due to the mediterranean polychaete *Sabella spallanzanii*'s ability to filter, accumulate and remove bacterial groups, including human potential pathogens and vibrios, and because it is native in Portugal, it was selected to integrate the studied IMTA system.

The gilthead seabream *Sparus aurata* was the fed species of this system. It was chosen as it is one of the main species produced in Portugal in tanks (INE 2014) and the farm techniques are well known and as such was the most adequate to serve as model species and main production for the proposed system.

In **Chapter one**, an overview of the aquaculture and fisheries industry is given and an extensive and overall review on the problems of the sector of aquaculture is given, and then IMTA introduced as a potential approach to address the sectors' challenges in terms of productivity, diversity and sustainability including the current state of the art on the field.

In **Chapter two**, "Modelling for IMTA optimisation", mathematical models are addressed as a key piece of extreme importance, not only to better understand the systems, but also as a tool to extrapolate to larger industrial scale farms, once most studies use experimental and small-scale systems. Here, a set of equations were proposed to develop a mathematical model to describe the projected IMTA system, as well as to optimise species combinations and yields from each trophic level. Also, such a mathematical description of the system also opens the possibility to develop simulators to study the purposed system, comparing it under different conditions. Ultimately, this optimisation may be a means to reduce the negative impacts of aquaculture in the environment while enhancing IMTA performance.

In **Chapter three**, "Filter-feeding polychaete *Sabella spallanzanii* as bioremediator of aquaculture wastes", the bioremediation potential of *S. spallanzanii* – as addressed in previous items – was assessed. For four weeks, their ability to remove bacterial groups, including *Escherichia coli* and presumptive vibrios, and total suspended solids, co-cultured with the marine fish *Sparus aurata* and the highly valued red macroalgae *Gracilaria vermiculophylla*, was evaluated and their potential as a species to be included in IMTA systems discussed.

In **Chapter four**, general conclusions are drawn and future research needs are formulated.

Chapter 2

Modelling for IMTA optimisation

Modelling for IMTA optimisation

2.1. Introduction to modelling in aquaculture

The main reason to address and develop mathematical models in aquaculture, namely multi-species models, is to maximise the production and optimise species combinations in order to reduce the environmental impacts of aquaculture (Duarte *et al*. 2003; Ren *et al*. 2012) and, consequently, to minimise costs.

In the last decades, there has been a growing need to better understand and optimise aquaculture performance, flow rates and transformations of toxic compounds in aquaculture production systems (Jiménez-Montealegre *et al*. 2002). Thus, dynamic modelling has been developed towards the use of models for analysis and simulation of aquacultures (Wik *et al*. 2009).

Most of the simulations have their origin in ecological modelling and often apply to fish ponds (Jiménez-Montealegre *et al*. 2002; Wik *et al*. 2009).

A large number of models applicable to a range of environments and conditions are available.

Several models have been developed to calculate the carrying capacity of farms and to determine environmental effects of bivalve and fish aquaculture (Ren *et al*. 2010). Different definitions of carrying capacity can be found in the literature. One of them considers carrying capacity as the ability of a particular ecosystem to support an organism production in order to maximise production without negatively affecting growth rate (Raillard & Ménesguen 1994; Duarte *et al*. 2003). Byron *et al*. (2011) explain that this concept is more complex, including four types of carrying capacity: physical, production, ecological and social. The large number of possible mathematical carrying capacity models indicates that important aspects may not be adequately modelled, such as the ability of the sites to process the excrement produced by the organisms cultured (Newell 2007). Therefore, Newell (2007) proposed an “ecological carrying capacity model”, in which the standing stock of bivalves enables a maximisation of consumption of phytoplankton, enhancement of nutrient removal and other ecosystem processes, without negatively affecting overall system function. This model implies detailed parameterization of phytoplankton and microzooplankton rates, sediment hypoxia, inorganic nutrient cycling and reduction in turbidity.

As far as the shellfish aquaculture is concerned, one of the limitations that can be observed in some carrying capacity models is related to the fact that they only consider nutrients, plankton, detritus and bivalves (Byron *et al*. 2011). In order to solve this problem, the Ecopath modelling software has been used to model the carrying capacity of bivalve aquaculture (Jiang & Gibbs 2005). Ecopath is a static, mass-balance, ecosystem-based modelling software, originally designed to support fisheries managers to investigate the structure of marine systems subjected to fishing pressure (www.ecopath.org). This modelling approach encompasses the full trophic spectrum, essential to determine the ecological carrying capacity, unlike other shellfish carrying capacity models that are at the production or farm scale and fail to incorporate all trophic levels at the bivalves or higher level (Byron *et al*. 2011). Other mathematical models are available to 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; Ren *et al.* 2010; Filgueira *et al.* 2013).

Regarding fish aquaculture, Stigebrandt *et al.* (2004) developed a model to estimate the holding capacity of sites for fish farming. This estimation, in terms of maximum fish production per month, is made regarding three basic environmental requirements that must be fulfilled, related to the accumulation of organic material and its impact in the benthic fauna, the water quality levels that must be kept high in the net pens, and in the areas surrounding the farm.

Alver *et al.* (2005) proposed a model to estimate larval survival rates of cod in the live feed period. Experiment results indicate that, by monitoring the live feed density in the tank and the average growth rate of the larvae, it is possible to provide a model-based estimator. The accuracy of this model depends, among other factors, on the ability to predict the feed intake rates of the larvae. Moreover, with this estimator it is possible to predict the statistical uncertainty of the estimates.

In aquaculture, one of the most important parameter evaluated is the growth rate of cultured fish species. Some of the best-known models are the von Bertalanffy growth model (Bertalanffy 1938), the logistic model (Ricker 1975), the Gompertz model (Gompertz 1825), and the Schnute model (Schnute 1981). The von Bertalanffy growth model is widely used in aquaculture to predict the individual growth rate of fish through empirical relationships, such as length-weight data, and it is favoured by many because of its simplicity (Baer *et al*. 2011). However, considering a model as an optimal model without testing the others, can lead to incorrect conclusions. Therefore, Baer *et al*. (2011) studied the growth performance of turbot reared in a commercial recirculation system, using different growth models. They concluded that, for turbot reared in these conditions, the Schnute growth model is the most realistic and accurate, reminding the importance of testing several models before choosing one.

To estimate growth, it has been increasingly important to consider fish nutrition, once feed composition affects the growth of marine fish, in particular (Bar & Radde

2009). Mathematical models in animal nutrition enable estimating growth and feed requirements of a livestock production. The fact that nutrition involves complex interactions, substantial costs of experiments and that much information is available, the development of mathematical models presents clear advantages (Dumas *et al*. 2010). Bar and Radde (2009) developed a quantitative dynamic model to predict the growth and body composition of marine fish. This model considers effects of environmental factors, focusing on temperature, growth and metabolic processes involving protein, lipid and central metabolism.

In the last two decades, modelling strategies have been designed in order to predict the dispersion and deposition of organic fish farm waste, usually using the mean settling velocity of faeces and feed pellets (Magill *et al*. 2006). Other models are available to analyse the production and environmental effects of finfish aquaculture (e.g. Cromey *et al.* 2002; Corner *et al.* 2006; Jusup *et al*. 2007; López *et al*. 2008; Skogen *et al.* 2009; Pedersen *et al*. 2012).

The existing models can provide crucial information to support decisions. Nonetheless, these models can be limited by lack of integration with the ecosystem, few species interactions and the scale used in the models (Tsagaraki *et al*. 2011). Most modelling equations have been developed for monocultures, despite the increasing importance of multi-species systems, such as polyculture and IMTA systems (Duarte *et al*. 2003).

Nunes *et al*. (2003) developed a multi-species model for coastal polyculture of the Chinese scallop *Chlamys farreri*, the Pacific oyster *Crassostrea gigas* and the kelp *Laminaria japonica*. The model integrates a bay-scale ecological simulation, upscales the individual processes for scallops and oysters, and simulates the human interaction with the target cohorts over a number of years. This model allows estimating the exploitation carrying capacity and the harvest potential for the target species in the system, and the impacts of different polyculture management strategies on the ecosystem. Also, it may be extended to include a variety of cultivated species and is easily applied to several coastal systems.

Duarte *et al.* (2003) proposed a mathematical model in order to study the carrying capacity of a shellfish polyculture system, comprising the oyster *Crassostrea gigas* and the scallop *Chlamys farreri*, co-cultured with the kelp *Laminaria japonica* at Sungo Bay (People's Republic of China), and to assess their interactions with the ecosystem.

The development of IMTA models provides a quantitative tool to develop and manage the practices involved through mapping energetic pathways between different trophic groups and the environment. Thus, these models are helpful in designing IMTA practices to maximise resource utilization and minimize environmental impacts (Ren *et al*. 2012).

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), in order to assess the quantitative effects of an IMTA combining gilthead bream cages and Pacific oyster (*Crassostrea gigas*) suspended from longlines. This model showed that, when gilthead bream is reared in IMTA, the environmental impact of cultures was substantially reduced. The ecosystem's benefits include a substantial removal of a population equivalent (PEQ) loading equal to 5500 people and, in IMTA, the organic deposition is reduced by about 7%, considering that the shellfish add themselves particulate waste to the culture area due to faeces and pseudofaeces. Also, the combination of this cultures enhanced the oyster production in 20% once the gilthead bream culture provided additional organic detritus as a food supplement. Even the profit from this type of system is over 230% and 68% higher than in finfish and shellfish monoculture, respectively.

Ren *et al*. (2012) developed an IMTA model based on dynamic energy budgets (DEB) considering four trophic groups corresponding to finfish, shellfish, detritivore and primary producer. Parameterization was made by using potential species to integrate IMTA systems, such as salmon, mussels, sea cucumbers and seaweed. This model incorporates benthic and pelagic components, whose interaction is through carbon and nitrogen budgets and nutrient cycling. Other IMTA systems, with the same combination of trophic groups, can use the proposed model for optimizing yields and reducing farm-derived wastes.

In this work, an IMTA model was proposed (Fig. 1), regarding population dynamics, growth, filtering rates of polychaetes and seaweeds and parameters related to the digestion and evacuation of fish.

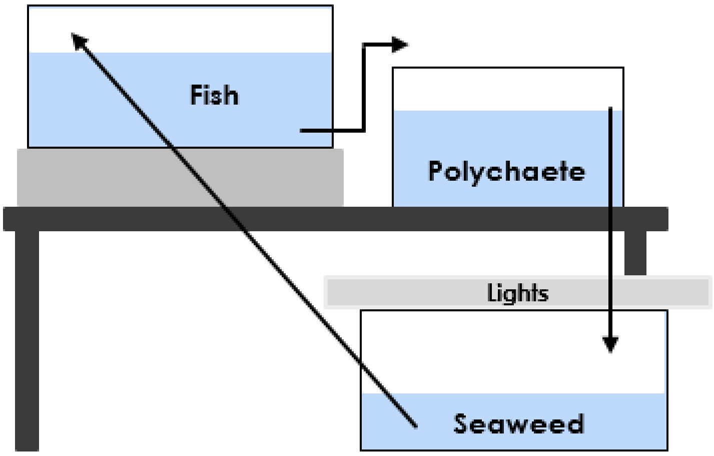

Figure 1 – Indoor integrated multi-trophic aquaculture (IMTA) system for the production of high-value finfish, polychaetes and seaweed. In the proposed IMTA system, by-products from gilthead seabream (*Sparus aurata*), such as excretion and respiration products, are recycled to become inputs for the filter-feeding polychaete *Sabella spallanzanii* and the red seaweed *Gracilaria vermiculophylla*.

2.2. Population dynamics

Survival, as well as growth, determine the yield and is influenced by several parameters, such as water and food quality, energy content of food and stocking density (Shoko *et al*. 2014).

The success and economic benefits of the aquaculture industry depends directly on survival rates of the produced organisms.

2.2.1. Population dynamics modelling

In order to predict and assess the number of reared organisms during the production cycle, it is necessary to develop an equation that describes the **dynamics of population** during that time. Due to mortality, the number of organisms decreases with age, which is commonly expressed as:

$$\frac{dn_*}{dt}(t) = -\delta_* n_*(t), \tag{2.1}$$

where we write ***** to represent the fact that this equation describes both the fish ($*= f$) and the polychaete ($*= p$) population, and, hence, n_* is the number of cultured organisms (fish, n_f, or polychaete, n_p), δ_* a the mortality parameter (for fish, δ_f, or polychaete, δ_p) and, t denotes time.

This is a classical expression applied to model population dynamics due to mortality (Wik *et al*. 2009; Ren *et al*. 2012, for example).

2.2.2. Survival rate

The **survival rate** of fish and polychaetes can be calculated by the formula as follows:

$$Survival\,rate\ (\%) = \frac{n_t}{n_i} \times 100, \tag{2.2}$$

where n_t is the number of cultured organisms at the time t and n_i is the initial number of cultured organisms.

2.3. Growth

Growth can be defined as the weight gain per unit of time. As it was mentioned previously, there are several proposals of mathematical models to describe the weight gain along time, usually modelling the estimation of the mean individual body growth (Baer *et al.* 2011).

Aquaculture is a commercial activity and, therefore, the purpose of farmers is to have economic benefit. The growth performance of the farmed organisms is the factor that most influence the profit (Baer *et al*. 2011). Maximum organisms' growth and production enable the profit maximisation (Shoko *et al*. 2014).

It is crucial to have information about the growth rates and shape of the growth curve, to determine exactly the period of increased growth. This also give information about the optimal moment of harvest the organisms, allowing to maximise the profit and do not waste resources, such as space and feed (Baer *et al*. 2011).

2.3.1. Growth modelling

Growth functions are used to model weight or length (dependent variables), calculated using time as the predictor (independent variable) (Dumas *et al.* 2010).

Modelling the growth of the organisms, as seen before, is essential. If we consider that the organisms are rearing in a multi-species system, such as IMTA, this parameter becomes even more important. The growth is directly involved with rearing densities, feeding rates and waste production. Therefore, it is necessary to develop mathematical dynamic models in order to understand, predict and maximise this parameters and, ultimately, better balance the system.

2.3.1.1. Fish

In the proposed IMTA system, the fish is the only fed species and the main reared organism.

Dumas *et al.* (2010) presented and compared some of the most relevant and applied fish growth models. One of the models described was the thermal growth coefficient (TGC) model, which includes the influence of the temperature in the development rates.

This was the model used in Wik *et al*. (2009) work to express **fish growth.** Wik *et al*. (2009) developed a model to describe a land based recirculation aquaculture system (RAS) which included fish growth and other parameters such as gastric evacuation, feed requirement and nitrogen excretion. One of the characteristics of the proposed IMTA system is the recirculation of water. Moreover, one of the aims of this work was to explore the modelling of a biological wastewater treatment, which is also one of the goals of the proposed IMTA system. Thus, some similarities between the two systems can be found.

Therefore, in this work the function considered to model fish growth was adopted from Wik *et al*. (2009), with a TGC approach:

$$BW_f(t) = \frac{1}{1000}\left(IBW^{1/3} + C_{TG} \cdot T \cdot t\right)^3, \tag{2.3}$$

where BW_f is the unitary fish body weight (kg), IBW is the initial body weight (g), C_{TG} is the Temperature Growth Coefficient, T is the temperature (ºC) and t is time (d).

Hence, multiplying the unitary fish body weight by the total reared organisms, one can obtain the function which estimates the **total fish mass** during the production cycle:

$$m_f(t) = BW_f(t) \cdot n_f(t), \qquad (2.4)$$

where m_f is the fish mass (kg),),BW_f is the unitary fish body weight (in equation 2.3) and n_f is the number of reared organisms (in equation 2.1).

2.3.1.2. Polychaete

In the proposed IMTA system, the polychaete represents the organic extractive species. The polychaete *S. spallanzanii* is able to reduce the bacterial abundance in the aquaculture wastes (Stabili *et al*. 2010) as well as particulate waste (Giangrande *et al*. 2005). Moreover, the produced biomass may have economic value due to the fact that this worm appears to have a very high protein content; thus, it is possible to be utilised as bait, fresh fish food within the same system or treated as fry food for fish (Giangrande *et al*. 2005).

The wide range of models applied to invertebrates indicates a lack of understanding of which models are most suitable and when they should be applied. In the last few years, the developing of unifying approaches has included the metabolic theory of ecology (MTE) to a wide range of processes, such as the allocation of metabolic energy to determine the increase in mass in individual (Hirst & Foster, 2013). This equation (2.5) has the same mathematical form as the von Bertalanffy growth equation:

$$\frac{dBW_p}{dt}(t) = aBW_p(t)^c - bBW_p(t), \qquad (2.5)$$

where BW_p is the mass of a single polychaete (g); and, a, b and c are parameters related to anabolic and catabolic processes and to the cellular structure.

Hirst and Foster (2013) refer that recently a different approach, the West Brown Enquist (WBE) equation, formulated as part of the metabolic theory of ecology, has been proposed as a universal model of growth. Some advantages of this approach are presented, but also some problems with the fit in some classes of invertebrates. Hirst and Foster (2013) tested the fit growth of 58 species of marine invertebrate, comparing the WBE equation with simpler approaches (exponential and power functions). They concluded that the exponential equation was best at modelling changes in mass with time. These exponential functions are a class of the functions described in equation (2.5) ($b = 0$). As the study considered a large number species and not the particular specie considered in the proposed system, equation (2.5) has the advantage of being more general and, eventually, better adapted to describe polychaete mass growth.

Analogously to the expression in equation (2.4), the total polychaete mass is given by:

$$m_p(t) = BW_p(t) \cdot n_p(t), \tag{2.6}$$

where m_p is the total polychaete mass (g), BW_f is the mass of a single polychaete (in equation 2.5) and n_p is the number of polychaetes (in equation 2.1).

2.3.1.3. Seaweed

In the proposed IMTA system, the seaweed represents the inorganic extractive species.

Despite the disparity of growth models for seaweeds, the growth rate unit (% day^{-1}) is used by many authors. With reference to this common unit, the seaweed is said to grow following the theory of geometric progression, where the weight or size increases with a common ratio (Lukeman *et al*. 2012).

For a population subjected to environmental limitation, the most widely used is the logistic model, which assumes that the relative growth rate decreases as the population approaches its environmental carrying capacity.

Considering the classical logistic model, the growth dynamics is given by:

$$\frac{dm_s}{dt}(t) = r\, m_s(t)\left(1 - \frac{m_s(t)}{k}\right), \tag{2.7}$$

where m_s is the seaweed mass (g), r is the fixed per-capita growth rate throughout time (%/d) and k is the carrying capacity (g).

Lukeman *et al*. (2012) applied this growth model approximated by the classical logistic model, for the species *Palmaria palmata*. In this work the main goal was to model harvested shores. So the growth rate parameter r was modified and replaced by a function which models the effect of the time of the year in the growth, with dominating growth in the summer months and dominating decay through frond breakage occurred from August to March. Moreover, the equation was modified to also model the harvesting cycle.

These modifications do not apply to the proposed system, but illustrate how equation (2.7) can be adapted to represent some other variations that can be considered, namely temperature and light intensity.

2.3.2. Aquaculture growth parameters

The **specific growth rates** of fish, polychaetes and seaweed can be estimated by formula as follows (Zhou *et al*. 2006b):

$$SGR = \frac{100\,(\ln W_t - \ln W_0)}{t}, \tag{2.8}$$

where SGR is the specific growth rate (% day^{-1}), W_0 is the initial wet weight (g), W_t is the wet weight (g) at time t since the beginning.

The **mass gain** of fish (and polychaetes) can be estimated by the following formula (9) (Batzina & Karakatsouli, 2014):

$$MG = \frac{100\,(M_{fn} - M_{in})}{M_{in}}, \tag{2.9}$$

where M_{fn} is the mean final body mass (g), M_{in} is the mean initial body mass (g).

The **condition factor (K)** of fish can be calculated using the equation:

$$CF = 100 \times BW \times SL^{-3}, \tag{2.10}$$

where BW is the body mass (g) and SL is the standard length (cm).

Using the next formula (2.11), is it possible to calculate the **coefficient of mass variation** of fish (Batzina & Karakatsouli, 2014):

$$CV = \frac{(100 \times SD)}{M}, \tag{2.11}$$

where SD is the standard deviation and M is the mean body mass (g).

Also, it is possible to make some calculations in order to assess the seaweeds' **productivity**, following the equation (Abreu *et al*. 2011a):

$$P = C \times \frac{W_t - W_0}{Tank\ area}, \tag{2.12}$$

where P is the productivity (g of wet weight m^{-2} week^{-1}); C is the proportion of dry weight to wet weight; W_0 is the initial wet weight (g); W_t is the wet weight (g) at time t.

2.4. Digestion / Evacuation

Generally, nutrients associated with particulate matter from faecal material and uneaten food can be found in wastewater of fish farm industry (Skogen *et al*. 2009). Besides the fact that this constitutes a potential impact on the adjacent environment, uneaten food is an economic disadvantage.

The rate of consumption is one of the most important factors in determining growth rate and is a function of several aspects, such as environmental conditions, species, dietary composition, meal and fish size, and feeding frequency (Riche *et al*. 2004).

Usually, after fish have been fed, waste production increases to a peak after which it decreases monotonically. However, the feed residence time in fish depends on fish size (Wik *et al*. 2009).

2.5.1. Model

It is important to understand the waste production rate of fish, in order no optimise polychaete and seaweed stocking densities, once the output of fish is the input for the other reared organisms.

Wik *et al*. (2009) developed a dynamic model for growth, gastric evacuation, feed requirement and nitrogen excretion, for fish reared in a RAS system. In the present work, an adaptation from this model was made.

The rate of waste compound i leaving each fish, without correction for growth and respiration, is given by:

$$y_i(t) = \gamma_i G(p) F(t), \;\; G(p) = \frac{1}{(1 + p\tau_1)(1 + p\tau_2)}, \tag{2.13}$$

where y_i denotes the production rate (kg/d), γ_i is the proportion of feed converted in waste compound i (kg/kg feed), p is the derivative operator, $G(p)$ is the normalised evacuation rate operator, F is the feeding and τ_1 an, d τ_2 are constants related to the time of digestion and evacuation. For each waste compound to be studied, equation 2.13 describes the corresponding production rate. Some of the waste compounds considered relevant to the system to be explored are represented in Table 1.

Table 1 – Waste production.

i	**Waste compound**
1	Dissolved oxygen
2	Ammonia nitrogen
3	Nitrite nitrogen
4	Nitrate nitrogen
5	Phosphorous
6	Total suspended solids

Equation 2.13, combined with equation 2.4, an evacuation rate signal and a waste production matrix, model the four possible outcomes of an atom in feed: not consumed by the fish, consumed and excreted, consumed and assimilated, or consumed and respired (Wik *et al*. 2009). The waste production matrix is a mathematical tool which describes food content, dispersion of feed lost in water into the modelled compounds and loss by respiration.

2.5.2. Aquaculture parameters

Food conversion ratio of fish can be calculated by the following formula:

$$FCR = \frac{W_f}{W_b} \tag{2.14}$$

where W_f is the fresh weight of fed food (g) (food consumed), and W_b is the production of fresh fish body (g) (mass gain) (Zhou *et al*. 2006b).

2.5. Filtering rate

In order to provide a stressfree environment as much as possible to organisms' normal health and growth performance, physical, chemical and biological standard must be attained. Several factors, including suspended solids, nutrients and potential harmful of toxic elements must be considered (Shoko *et al*. 2014). Poor water quality may have as consequences low production, profit and product quality.

2.4.1. Polychaetes

During the last years, several studies showed that filter-feeding polychaetes accumulate and retain efficiently bacteria from the surrounding environment (Stabili *et al*. 2006a; Stabili *et al.* 2010; Licciano *et al*. 2005) as well as suspended solids (Cavallo *et al*. 2007).

In the proposed IMTA system, polychaetes have the function of clearance of suspensions from the wastewater.

2.4.1.1. Model

Model the filtering rate of the polychaetes is essential to optimise the stocking densities of both fish and polychaetes, since the densities have to be balanced in order to have in the system the right quantity of waste. On one hand, if the polychaetes' density is too high, can occur that they don't have enough food and the productivity would be very low. On the other hand, if the polychaetes' density is too low, they don't have the capability to remove efficiently the waste from the rearing water, having as consequence a poor water quality.

According to Coughlan (1969), to determine the volume of water pumped by a suspension-feeding organism, an indirect method can be used. This method in based on the removal rate of particles from a known volume of suspension and is termed "filtering rate". Coughlan (1969) showed that the 6 equations that had been published, by which the filtering rate can be calculated from the observed depletion of the suspension, are equivalent. According to these equations, the concentration of suspensions can be described by the equation:

$$\frac{dC}{dt} = -C(t)\left(\frac{F_r \cdot n_p}{V} + \alpha\right), \qquad (2.15)$$

where $C(t)$ denotes de concentration of suspensions at the time t, F_r denotes the filtering rate of a single polychaete, V is the volume, α is the rate at which particles settle out of suspension and n_p is the number of polychaetes (in equation 2.1).

Coughlan (1969) considered estimation of filtering rate by suspension-feeding animal, but in a context where there is an initial concentration of suspended particles, which is not being continuously renewed and so, as it is assumed that animals are continuously withdrawing particles, the rate at which particles are removed will progressively decline and therefore concentration is a continuous function represented by a curve like $C(t) = C_0 e^{-at}$.

In the present study, the polychaetes' tank is continuously receiving water with varying levels of suspended particles and so the concentration of the water entering the tank must be considered. Moreover, as water is moving between tanks, the water flow also influences the polychaetes efficiency in the filtering process. Taking in consideration these differences, equation 2.15 was adapted and the following equation is proposed to describe the **filtering rate** of polychaetes, at a certain water flow over the time:

$$\frac{dC}{dt}(t) = Q\big(C_f(t) - C(t)\big) - C(t)\left(\frac{F_r \cdot n_p}{V} + \alpha\right), \qquad (2.16)$$

where $C(t)$ denotes de concentration of suspensions at the time t, $C_f(t)$ denotes the concentration of the water entering the tank at the time , F_r denotes the filtering rate of a single polychaete, V is the volume, α is the rate at which particles settle out of suspension and n_p is the number of polychaetes (in equation 2.1).

In the proposed system the function $C_f(t)$ is obtained from equations 2.4 and 2.13, so that the production rates are converted in concentrations, by considering volume of the tanks and water flow of the system.

2.4.1.2. Aquaculture parameters

Total Suspended Solids can be calculated by filtering a 100 mL sample and applying the following formula (15):

$$TSS = \frac{(FSW - FW)\,1000}{Sample\ volume}, \qquad (2.17)$$

where TSS is the total suspended solids (mg/L), FSW is the average weight of the filter plus the sample (g), and FW is the average weight of the filter (g).

2.4.2. Seaweed

Land-based closed (recirculating) aquaculture systems produce less volumes of wastewater than open systems, but with higher concentrations of N. N waste depends on the intensity of production, being higher in intensive systems (Quintã *et al*. 2015).

It is possible to remediate this through biofiltration by plants, and in this particular case by seaweeds (Neori *et al.* 2004). In the last years, several studies used seaweeds in IMTA and showed the potential of these organisms for bioremediation (Zhou *et al.* 2006a; Troell *et al*. 2009; Marinho-Soriano *et al*. 2011; Abreu *et al.* 2011a).

In the proposed IMTA system, seaweeds have the function of biofiltrate the excess of nutrients from wastewater.

2.4.2.1. Model

In IMTA systems is crucial develop mathematical models in order to predict an optimum N removal, knowing the seaweed N uptake requirements, kinetics and growth.

Ideally, the rate of nutrient uptake by primary producer should be equal to the excretion rate of the principal reared species (Ren *et al*. 2012). Thus, as in the case of polychaetes, optimise the stocking densities of both fish and seaweed is needed to balance the system.

The uptake of dissolved inorganic nitrogen follows a Michaelis-Menten function and is limited by N-quota. Ren *et al*. (2012) proposed a model to describe these processes, including the total uptake of N by seaweed, which is described in an equation (2.18):

$$U_{na} = \frac{NA \cdot T_{emp} \cdot (U_{nha} + U_{noa})}{1 + exp\left(\frac{Q_a - Q_{amax}}{Q_{aoff}}\right)}, \tag{2.18}$$

where U_{na} is the total uptake of N by seaweed (mg N d^{-1}), NA is a function which models the seaweed nitrogen (mg N m^{-3}), T_{emp} is a function for the temperature-dependent rate of seaweed, U_{nha} is a function which describes the potential uptake of ammonium by seaweed (d^{-1}), U_{noa} is a function that represents the potential uptake of nitrate by seaweed (d^{-1}), Q_a is the seaweed N quota (mgN / mgC), Q_{amax} is a parameter related to the maximum seaweed N:C ratio (mgN / mgC) and Q_{aoff} is the seaweed nitrogen uptake parameter (mgN / mgC) (Ren *et al*. 2012). Ren *et al*. (2012) define all the functions which are part of equation 2.18, but they can not be easily described, since their definitions also involve many other functions and parameters. The functions implicitly involved in equation (2.18) can be expressed through other functions, until one gets to a system of differential equations where all the needed parameters can be experimentally calibrated.

2.4.2.2. Aquaculture parameters

The **biofiltering efficiency** (%) of seaweed can be estimated based on nutrient concentrations in recirculated seawater using the following equation:

$$Biofiltering\ Efficiency\ (\%) = \frac{(A - B)}{A} \times 100, \tag{2.19}$$

Where A and B are the initial and final nutrient concentrations (NH_4^+, NO_2^-, NO_3^- and PO_4^{3-}) in the recirculated seawater (Kang *et al*. 2011).

2.6. Conclusion

The importance of mathematical models in aquaculture setup and optimisation towards sustainability and higher revenue, namely IMTA, is unequivocal and may help to understand and resolve the wide range of interactions between cultivated species and between those species and their physical and chemical environment, analysing them in a dynamic manner. In specific, developing mathematical models to apply to IMTA and other multi-species systems is a useful and increasingly necessary tool to balance these systems in terms of productivity, mortality, adequate densities and to control the production cycle, due to their complexity.

This study included the development of a model to mathematically describe the proposed IMTA system. The main focus of this model was to optimise species combinations and yields from each trophic level. Such a mathematical description of the system also opens the possibility of developing simulators to study the purposed system, comparing it under different conditions.

Moreover, this simulator can be adapted to predict the benefits of other species. Equations to describe the filtration ability of other species of invertebrates must be developed, as well as of other main cultures organisms, such as other fish species or shrimp.

Chapter 3

Filter-feeding polychaete *Sabella spallanzanii* as bioremediator of aquaculture wastes

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Filter-feeding polychaete *Sabella spallanzanii* as bioremediator of aquaculture wastes

3.1. Introduction

The fisheries industry is facing a crisis due to the fact that more than half of the marine fish stocks were estimated to be fully exploited (61.3 %) (FAO 2014). In order to respond to consumer needs, aquaculture is intensifying and diversifying the product range, and, therefore, is expanding in all continents through new areas and species (FAO 2012). Thus, aquaculture continues to be the fastest-growing animal-food-producing sector with an average annual growth rate of 6.3% and 47% of total food fish supply in 2010 (FAO 2014).

The intensification of aquaculture, in general, has generated a series of environmental impacts on the ecosystem, particularly in coastal waters, raising concern about how continuing to meet food demand and preserve the environmental quality for a sustainable development of aquaculture (Van Rijn, 1996; Morata *et al*. 2015).

Generally, in aquaculture systems such as fish culture or shrimp farms (e.g.), wastes rich in metabolic products, residual food, faecal matter and residues of prophylactic and therapeutic compounds are discharged without treatment. This can lead to the deterioration of water quality and enhance potential disease outbreaks (Edgar *et al*. 2005; Zaccone *et al*. 2005).

The organic matter coming from the feed is one of the main source of waste in aquaculture and is relatively rich in organic carbon and nutrients such as nitrogen and phosphorus. The release of these compounds may have as consequence the alteration of composition of communities and eutrophication of the surrounding areas (Morata *et al*. 2015).

Also, with the increase of aquaculture practices and stock densities, the excessive release of microbial pathogens from animal waste into the aquaculture environment has become a major concern for this industry. These bacterial pathogen loads represent a significant health hazard to the reared species, once they can cause aquaculture diseases that can widely affect the produced species and surrounding environments. Also, contaminated seafood products may represent a hazard to human health as a result of human consumption (Stabili *et al*. 2010).

In the last few decades, recirculating aquaculture systems have been implemented due to its potential to reduce impacts on the aquatic environment. However, in this type of system, the water quality tends to deteriorate, and despite the utilization of improved filtration methods, small suspended solids tend to accumulate. The urge for more efficient methods has led to the study of filter-feeding organisms as bioremediators. The use of these organisms enables the reprocess of nutrients and organic matter released into the culture system and convert these compounds into biomass. Thus, they can be easily removed and may be a valuable by-product enhancing other species productivity (Giangrande *et al*. 2005).

Moreover, some studies revealed that some invertebrates are potential remediators of heavy metals, microbial contaminants, hydrocarbons and persistent organic pollutants (Stabili *et al.* 2010). In particular, filter-feeding marine macroinvertebrates must filter large volume of water for their food requirements and can also exert high efficiency in retaining small particles including bacteria (Licciano *et al*. 2005).

The most significant factor negatively impacting fish cultures is the incidence of microbial pathologies.

Several pathogenic microorganisms are involved in epizootic outbreaks. For instance, in gilthead seabream cultures, *Pseudomonas* spp., *Photobacterium damselae* ssp. *piscicida*, *Aeromonas salmonicida* and several species of *Vibrio* have been responsible for high mortality rates (Bordas *et al*. 1996). Some of them, such as *Vibrio alginolyticus* and *Vibrio anguillarum*, are the causative agents of fish mortality and important economic losses in gilthead seabream Mediterranean aquaculture (Balebona *et al.* 1998; Esteban *et al*. 1998). Also, in the marine cage culture of Atlantic salmon in Chile (Bustos *et al*. 2011), oyster farming in Europe notably France (Segarra *et al*. 2010), and marine shrimp farming in several countries worldwide have experienced high mortality, resulting in partial or sometimes total loss of production (FAO 2012).

Escherichia coli is often used as an indicator for faecal contamination in waters. Where animal manure, particularly bovine manure, is used as pond fertilizer, there is the risk that pathogenic strains of *E. coli* may be present in pond water (WHO 1997). Also, faecal coliform contamination of shellfish (Sonier *et al.* 2008) and others, such as fish produced in integrated livestock-fish aquacultures (Dang & Dalsgaard, 2012) have been made, and the occurrence of *E. coli* confirmed that may constitute a severe health hazard.

Bivalves are the most common invertebrates used as organic extractive species in systems where species with different trophic levels are co-cultivated and integrated through water transference (integrated multi-trophic aquaculture – IMTA), both in offshore and land-based systems (Lander *et al*. 2013; Reid *et al.* 2010; Pietrak *et al*. 2012). However, other potential species appears of considerable interest, such as ascidians, sponges, and polychaetes (Stabili *et al*. 2006a).

The filter-feeding polychaete *Sabella spallanzanii* (Gmelin, 1791) (Polychaeta, Sabellidae) is a widely distributed mediterranean polychaete, typical of eutrophic environments and colonizing hard bottoms. This species shows a wide trophic plasticity, being able to feed not only on phytoplankton but also on dissolved organic matter present in the water column (Stabili *et al*. 2010). Moreover, studies showed its capability to accumulate microorganisms under natural and experimental conditions (Giangrande *et al*. 2005; Stabili *et al*. 2010).

The aim of the present study was to assess the bioremediation potential of the filter-feeding polychaete *S. spallanzanii*, by its ability to remove bacterial groups, including *E. coli* and culturable vibrios, and total suspended solids, in a IMTA system co-cultured with the marine fish *Sparus aurata* (main product), and the red seaweed *Gracilaria vermiculophylla* (nutrient extractor).

3.2. Material and Methods

3.2.1. The organisms

All water used was natural seawater. During all acclimatization and experimental periods, water quality parameters were daily controlled, including nutrients using kits (API™ Aquarium Pharmaceuticals, United States), salinity, pH, temperature, and dissolved oxygen using a handheld multiparameter probe (YSI Professional Plus, United States).

3.2.1.1. Fish model species – *Sparus aurata*

Specimens of *Sparus aurata* (Linnaeus, 1758) weighing 21.60 ± 4.40 g (mean body weight) were obtained from Instituto Português do Mar e Atmosfera (IPMA) aquaculture facilities (Olhão, Portugal) and transferred to a recirculation aquaculture system where they were acclimated for two months to laboratory conditions.

After the acclimation period, thirty-six organisms (36.71 ± 6.70 g mean body weight) were randomly selected and divided in six groups with six organisms each, and then transferred to the experimental IMTA systems. During the experiment it was used a feeding rate of 3 % body weight every day.

Their survival was assessed, and weekly growth was evaluated, and faeces collected from their intestine for further studies (data to be shown elsewhere).

3.2.1.2. Filter-feeding model species – the polychaete *Sabella spallanzanii*

Specimens of *Sabella spallanzanii* (Gmelin, 1791) weighing 11.75 ± 6.13 g (mean body weight) were hand collected from Olhão coast (Portugal) and maintained in an open aquaculture system for one month prior to experiments. When transferred to aquaculture facilities, all worms were cleaned of any tube epibionts and divided in six sets and inserted in nets in six experimental recirculating aquaculture tanks receiving the wastes from gilthead seabream rearing tanks. Individuals were acclimated for five weeks to laboratory conditions.

After the acclimation period, ninety polychaetes were randomly divided in three sets of thirty polychaetes and inserted into nets in the three experimental IMTA systems (details in further section).

Their survival, growth, bacterial and suspended solids removal by filter-feeding were assessed.

3.2.1.3. Seaweed – *Gracilaria vermiculophylla*

The seaweed *Gracilaria vermiculophylla* (Ohmi) Papenfuss, 1967 (Rhodophyta, Gracilariaceae), obtained from AlgaPlus Lda. (Albergaria-a-Velha, Portugal) – an inland seaweed aquaculture – was cleaned of other organisms and placed in acclimation to laboratory conditions for two months prior to experiments.

After the acclimation period, the seaweed was divided in six similar groups (343.50 ± 0.51 g mean wet weight) – density of 9.81 kg m^{-3} – and placed in the experimental IMTA systems as further explained and growth evaluated weekly.

3.2.2. The experimental IMTA systems and experimental design

Each IMTA systems consisted of three tanks, connected by water transfer: one 90L tank filled with 60L of natural seawater used for fish culture, one 90L tank filled with 65L of natural seawater for polychaete culture and one 90L tank filled with 35L of natural seawater used for macroalgae culture (Figure 2). Water circulated by gravity from the fish tanks to the polychaete tanks and from there to the macroalgae tanks. The water from the macroalgae tanks was then returned to the fish tanks through an electrical pump.

The water flow was, for all tanks, 132.12 ± 12.56 L h^{-1}. A photoperiod of 18:6 h light:dark cycle was used throughout the experiments. Irradiance was 0 lux during the dark period and 3306 lux during the light period in all systems.

Six independent experimental IMTA systems were used for these experiments. Three sets differed from the other three sets by containing polychaetes in the second tank (systems number 1 - 3) while in the other set the second tank was empty (systems number 4 - 6).

The primary tank for fish culture contained a stocking density of 3.67 ± 0.16 kg m^{-3} at the beginning of the experiment. One day before the experiments, the water recirculation was stopped, corresponding to the sampling time -24 (h). At that time, water was sampled for further analysis.

One day later, thirty polychaetes were placed in each of the three tanks (as explained before), and the macroalgae was placed in all systems (third tank), and then the water recirculation was initiated.

One hour after, corresponding to the sampling time 0 (h), samples of 250 mL of water were collected, as well as for all the other sampling times: 24h, 96h, 168h (one week), 336h (two weeks) and 672h (four weeks).

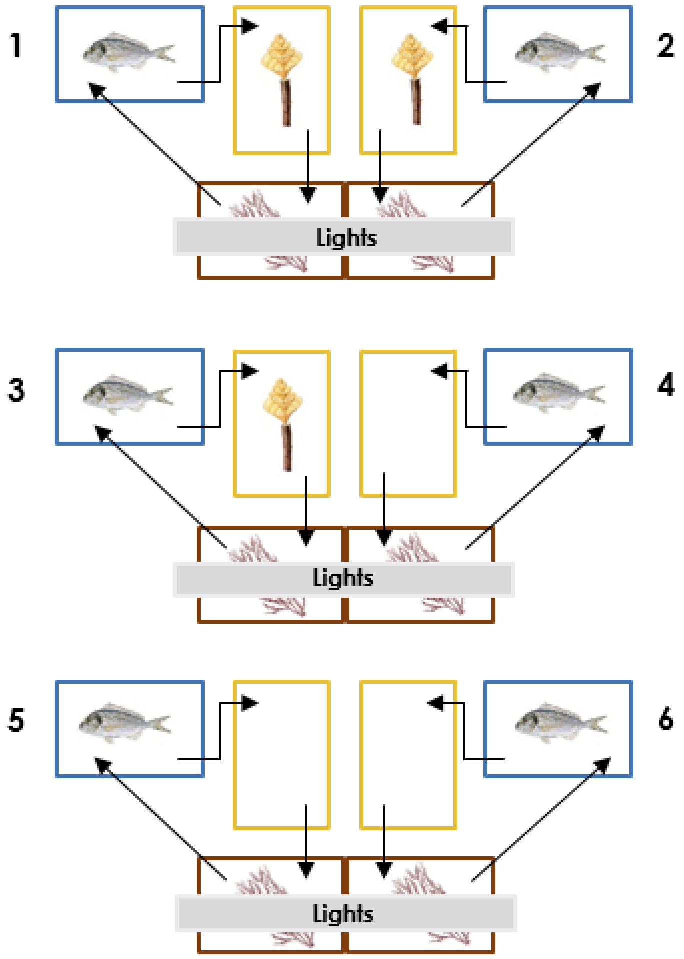

Figure 2 – Experimental integrated multi-trophic aquaculture (IMTA) systems for the production of finfish, polychaetes and macroalgae. The systems are independent, and in each system the tanks are connected through water transfer. In this study, systems number 1, 2 and 3 had polychaetes, while in systems number 4, 5 and 6 polychaetes were absent.

3.2.3. Endpoints measured

3.2.3.1. *Bacteriological analyses*

The bacteriological analyses were performed using the water samples of 250 mL collected at the water entering point of fish tanks, at seven sampling times (hours): -24, 0, 24, 96, 168 (one week), 336 (two weeks) and 672 (four weeks). Every sampling was made six hours after the last fish feeding.

The bacteriological analyses included the quantitative analyses of culturable halophilic vibrios at 22ºC and *Escherichia coli*.

In order to enumerate the culturable vibrios in seawater, 9 mL of serial dilutions of each water sample were filtered on 0.45 µm pore size cellulose filters, in duplicates. Then the filter disks were aseptically placed onto triosulfate-citrate-bile-sucrose-salt agar (TCBS) (VWR, Belgium) supplemented with 2% NaCl, recommended for culturing vibrios. Incubation was carried out at 22ºC for 24 hours and colonies of presumptive vibrios were counted according to the colony-forming unit (CFU) method.

The *E. coli* concentrations were determined by the most probable number (MPN) method, using the Colilert Kit (IDEXX, USA). This kit is based on Defined Substrate Technology. Here, when *E. coli* metabolize Colilert's nutrient-indicator, MUG, the sample fluoresces. Bacterial densities were expressed as MPN 100 mL^{-1}.

3.2.3.2. Total suspended solids removal

The total suspended solids (TSS) quantification was performed using the water collected at the exit of fish, polychaete and algae tanks, with five replicates. There were four sampling times (in hours): 168 (one week), 288, 336 (two weeks) and 672 (four weeks). Similar to what was done for the bacteriological analysis, the sampling was made six hours after the last fish feeding.

Total suspended solids were determined gravimetrically through filtration of 100 mL of seawater, using mixed cellulose filters with 0.45 µm nominal pore size. Filters were weighed, before and after being in the oven at 60ºC for 24h. Removed solids were calculated from the difference in weight (Giangrande *et al*. 2005).

3.2.3.3. Growth parameters

Fish, polychaetes and seaweed were weighed prior to the experiment and after two and four weeks. Their growth rates (Zhou *et al.* 2006b) and mass gain (Batzina & Karakatsouli, 2014) were estimated by the following formula:

$$SGR = \frac{100\ (\ln W_t - \ln W_0)}{t}, \tag{3.1}$$

where SGR is the specific growth rate (% day^{-1}), W_0 is the initial wet weight (g), W_t is the wet weight (g) at time t-24 (for fish) and 0 (for polychaetes and seaweed) and t672.

$$MG = \frac{100\ (M_{fn} - M_{in})}{M_{in}}, \qquad (3.2)$$

where M_{fn} is the mean final body mass (g), M_{in} is the mean initial body mass (g).

3.2.4. Statistical analysis

All data were checked for normality and homoscedasticity. Two-way analysis of variance (ANOVA) was used to assess differences between different sampling time points, concerning bacteriological groups and total suspended solids and between treatments (with polychaete and without polychaete). When differences were found, *Tukey* post-hoc tests were employed. Differences in fish, polychaetes and algae growth parameters were addressed using a *t-students' test*.

Where applicable, results are presented as mean ± SE. For all statistical tests, the significance level was set at $p \leq 0.05$. All statistical tests were performed with Sigma plot 11.0 (Systat Software, Inc. Chicago, IL, USA).

3.3. Results

3.3.1. Environmental parameters

Throughout the experimental period, environmental parameters were measured in all tanks and are shown in Table 2.

Table 2 – Environmental parameters (mean ± SD) measured in the experimental IMTA systems with polychaetes and without polychaetes (Control), during the experimental period.

	Polychaetes	**Control**
T (ºC)	19.94 ± 0.90	19.96 ± 0.88
DO (mg L^{-1})	6.20 ± 0.31	6.25 ± 0.22
pH	7.83 ± 0.07	7.91 ± 0.06
Salinity	36.60 ± 0.43	36.62 ± 0.63
NH_4 – N (mg L^{-1})	0.31 ± 0.14	0.36 ± 0.17
NO_2 – N (mg L^{-1})	2.10 ± 1.89	3.68 ± 1.94
NO_3 – N (mg L^{-1})	62.11 ± 25.79	88.67 ± 47.36

The environmental parameters during the experiment were similar for both Polychaetes and Control treatment, except for nitrite (NO_2-N) and nitrate (NO_3-N) concentrations, which were, in average, higher in the control systems than in the systems with polychaetes.

3.3.2. Growth performance

Survival and growth parameters, such as mass gain and specific growth rate, are shown in Table 3 for the three species, under both Polychaetes and Control conditions.

Table 3 – Mass gain (MG), specific growth rate (SGR), and survival of *Sparus aurata*, *Sabella spallanzanii*, and *Gracilaria vermiculophylla* (mean ± SD) at the experimental IMTA systems after four weeks.

Polychaetes	**n**	**MG (%)**	**SGR (% day $^{-1}$)**	**Survival (%)**
S. aurata	3	0.43 ± 0.09	0.50 ± 0.10	100
S. spallanzanii	3	0.66 ± 0.27	0.23 ± 0.10	96.67 ± 3.33
G. vermiculophylla	3	0.02 ± 0.01	0.18 ± 0.08	-
Control				
S. aurata	3	0.41 ± 0.20	0.48 ± 0.21	100
G. vermiculophylla	3	0.03 ± 0.01	0.31 ± 0.06	-

In all the experimental IMTA systems, *S. aurata* did not present any mortality, and *S. spallanzanii* mortality was low. Also, in the overall, six polychaetes autotomized their tentacular crowns.

Fish growth occurred in both treatments but, no differences were found between treatments (*t-test, p=0,902 for MG and p=0,866 for SGR*), meaning that the presence of the polychaetes did not interfere with the primary culture's growth. The growth of polychaetes, including body and tube, presented a SGR equal to 0.23% day^{-1} (Tab. 3).

Relatively to seaweed's growth, for both Polychaetes and Control treatments, although there seems to be an increased growth in the tanks without the polychaetes comparing to the tanks with polychaetes (SGR of 0.31% day^{-1}and 0.18% day^{-1}, respectively) there were no statistically significant differences (*t-test, p=0,134 for MG and p=0,095 for SGR*).

3.3.3. Bacterial removal

Abundance of presumptive vibrios in the IMTA systems for both Polychaetes and Control treatments, during the experimental time, is shown in Figure 3.

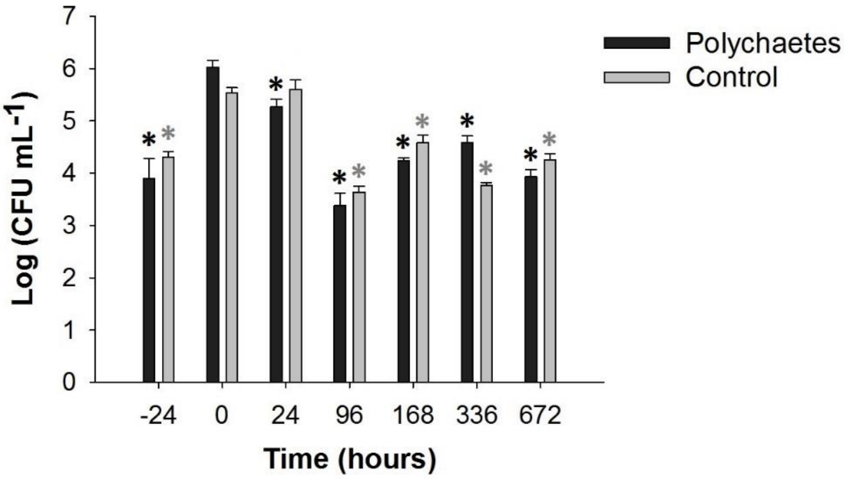

Figure 3 – Abundance of culturable vibrios at 22ºC at the seven sampling times: 24 hours before water recirculation (-24), one hour after starting water recirculation (0) and until the end of the experiment (24h – 672h). Dark bars represent the treatment with Polychaetes and the grey bars the Control systems (with no polychaetes). Results are presented as mean ± SE. * Represents statistical significant differences between sampling times and time 0 (two-way ANOVA, Tukey test, $p<0.05$).

During the system re-circulation stoppage during time -24h and 0h, there was an increase on the amount of presumptive vibrios (ANOVA, Tukey's test: $q=14.522$, $p<0.001$; Fig. 3). After this interruption and when the recirculation was again assured there was a decrease in both Polychaetes and Control treatments, although only statistically significant from 96h onward for Control and 24h onward for the Polychaetes treatment (ANOVA, Tukey's test: $q=11.600$, $p<0.001$; and $q=4.594$, $p=0,042$, respectively), thus with a slightly faster clearance in the treatment with polychaetes. Nevertheless by the end of the experimental period (four weeks), although there was a lesser presumptive vibrio burden in the system with Polychaetes, there were no statistical significant differences between both systems (9.40×10^{3} CFU mL^{-1} in Polychaetes treatment and 1.93×10^{4} CFU mL^{-1} in Control treatment; ANOVA, Tukey's test: $q=1.927$, $p=0.184$).

Abundance of *E. coli* in both Polychaetes and Control treatments, during the experimental time is shown in Figure 4.

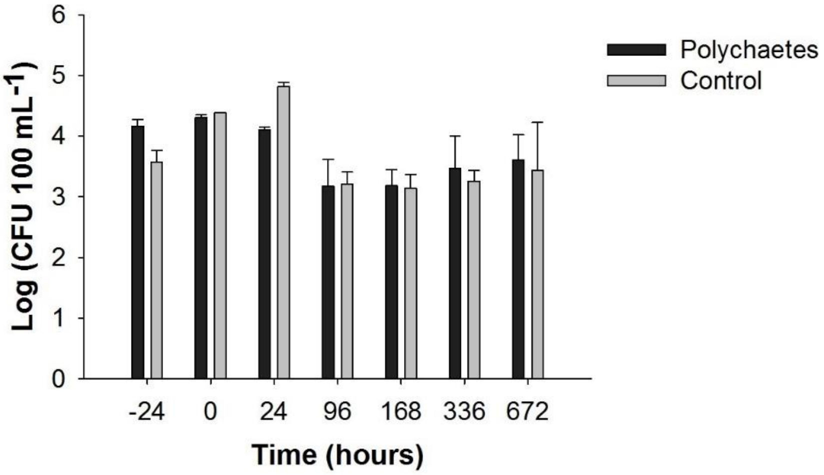

Figure 4 – Abundance of *Escherichia coli* at the seven sampling times: 24 hours before water recirculation (-24), one hour after starting water recirculation (0) and until the end of the experiment (24h-672h). Dark bars represent the treatment with Polychaetes and the grey bars the Control systems (with no polychaetes). Results are presented as mean ± SE. No statistical significant differences between sampling times and time 0 were found (two-way ANOVA, Tukey test, $p<0.05$).

Regarding *E. coli* (Fig. 4), with an initial abundance of 1.56×10^4 CFU 100 mL^{-1}, there were no statistical significant differences in the clearance of *E. coli* between both the Polychaetes and Control treatments and also along sampling times for both treatments (ANOVA, Tukey's test: q=0.225, p=0.875; and ANOVA, $F_{1,6}$=0.0253, p=0,875, respectively).

In the Control treatment, there seems to be an increase of bacteria at 24 hours, with a count of 6.68×10^4 CFU 100 mL^{-1}, but not statistically significant comparing to time point -24h (ANOVA, Tukey's test: q=3.757, p=0.147), but then there was a significant decrease after 96h (ANOVA, Tukey's test: q=4.862, p=0.027). Nevertheless there are no differences at 24h comparing the polychaete and control treatment (ANOVA, Tukey's test: q=2.134, p=0.143).

3.3.4. Suspended solids removal

The mean abundance of total suspended solids (TSS) for both Polychaetes and Control treatments, during the time, is shown in Figure 5. The measurement of this parameter was later decided and as such only times from 288 to 672 are shown.

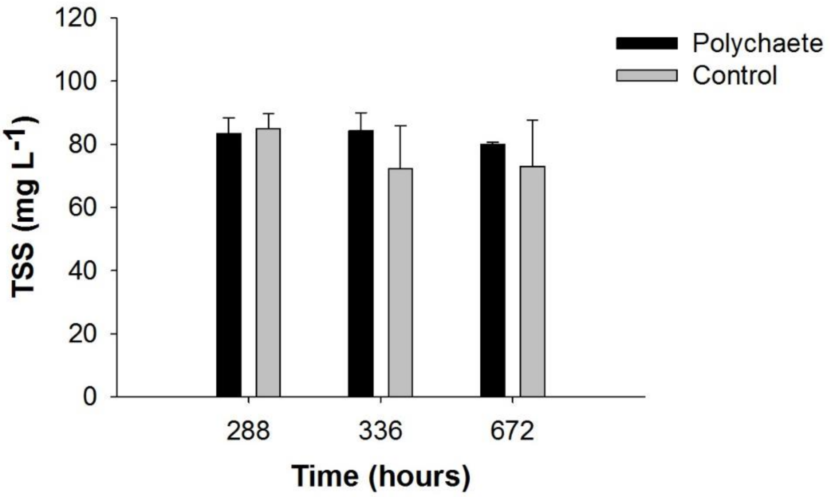

Figure 5 – Abundance of total suspended solids (TSS) at the three sampling times: 288, 336 and 672 hours. Dark bars represent the treatment with Polychaetes and the grey bars the Control systems (with no polychaetes). Results are presented as mean ± SE. No statistical significant differences between sampling times and time 0 were found (two-way ANOVA, Tukey test, $p<0.05$).

Regarding TSS, there weren't statistical significant differences among sampling times and neither between treatments (ANOVA, F=0.408, p=0,674; and F=0.622, p=0,446, respectively), in the water coming out of polychaetes tank.

However, it is possible to observe that, in both Polychaetes and Control treatments, there is a trend to lower the final concentration of TSS.

3.4. Discussion and Conclusions

In an integrated system it is necessary to select species whose optimal abiotic parameters are coincident, in order to provide optimal conditions to the entire system. In this study, for both Polychaetes and Control treatments, environmental parameters in general were similar, except for nitrite (NO_2-N) and nitrate (NO_3-N) concentrations, which were, in average, slightly higher in the control systems (Tab. 2).

Sparus aurata, as well as other fish, is a cold-blooded animal, and therefore body temperature, growth rate, food consumption, feed conversion, and other body functions, is influenced by the temperature of the surrounding water (Azevedo *et al*. 1998). Wild gilthead seabream usually live in environments with temperatures ranging from 11ºC to 23ºC, in winter and summer, respectively, easily coping with these temperature changes (Faggio *et al*. 2014). According to Ibarz *et al*. (2010), this species stop growing at 10ºC and below 13ºC food intake ceases. Faggio *et al*. (2014) studied the growth of gilthead seabream cultivated at temperatures ranging from 13.6 to 23.4 ºC, and demonstrated that a better growth can be obtained with the highest temperatures. Regarding salinity, gilthead seabream tolerate salinities ranging from 8 to 38, obtaining the best growth with a salinity of approximately 28 (Boeuf & Payan 2001). However, Faggio *et al*. (2014) obtained great growth results with a salinity of 40. In relation to dissolved oxygen, this is one of the most important parameter for all fish species. An oxygen concentration of 5 mg L^{-1} is the minimum required by fish during grow-out (Ökte 2002). Concerning ammonia, ammonium ion is relatively nontoxic and predominates when pH is low. Usually, less than 10% of ammonia is in the toxic form when pH is less than 8.0, and this proportion increases dramatically as pH increases (Hargreaves & Tucker 2004).

Sabella spallanzanii can be found in temperatures ranging from 2ºC to 29ºC. Stabili *et al*. (2006a) reported temperature values of about 20ºC at three sampling sites in the coast of Italy, where this species exists in high densities. Other studies were performed with this species, with temperatures of 20ºC (Cavallo *et al*. 2007) and 19.4ºC (Licciano *et al*. 2007) and a salinity of 38 (Stabili *et al*. 2006a; Licciano *et al*. 2007). Moreover, Stabili *et al*. (2010) registered dissolved oxygen concentrations of 8 and 8.5 mg L^{-1} in *S. spallanzanii* cultures.

G. vermiculophylla is a cosmopolitan species and endures a wide range of environmental parameters. Studies have revealed that this species has similar growth rates at a broad range of temperature and light, independently of its life stages (Abreu *et al*. 2011). According to the same study the highest growth rate observed for this species was observed under a mean temperature of 20.17ºC and a pH range of 7.3-8.8.

In the present study, the parameters are included in what was considered by the stated authors as optimal conditions for the selected species, promoting optimal growth performance (Tab. 2), which is aquaculture's main goal – to grow-out the organisms faster than in the wild.

In this study, no differences were found between treatments for gilthead seabream growth, meaning that the presence of the polychaetes did not interfere with the primary culture's growth. Gilthead seabream presented a SGR equal to 0.50 and 0.48 % day^{-1}, and a MG of 0.43% and 0.41%, for the Polychaetes and Control treatments, respectively (Tab. 3).

Sadek *et al*. (2004) studied the growth of gilthead seabream under different diets. With all the diets, the SGR obtained by this author were superior (0.8-0.95% day^{-1}). The same occurred when the results were compared with the study performed by Batzina and Karakatsouli (2014), where a SGR of 1.02% day^{-1} and a MG of 19.07% were obtained.

The low values obtained in this study for the growth parameters of gilthead seabream may be due to the fact that the organisms were under additional stress – due parallel studies done involving the weekly collection of faeces from fish intestine without anesthesia. After this sampling, feeding behaviour might have been modified – reflecting in a lower growth performance.

Concerning to polychaetes, their growth performance is highly dependent on food supply and water movement (Giangrande *et al.* 2005). In the present study, the growth, including body and tube, presented a SGR equal to 0.23% day^{-1} and a MG of 0.66% (Tab. 3). Stabili *et al*. (2010) reported a mean increase of total polychaete biomass of 9.0 mg day^{-1}. Also, Giangrande *et al*. (2005) verified that, in natural conditions, the polychaetes doubled in both length and biomass in two months, suggesting its utilisation and rearing in intensive aquaculture. However, the results may be not comparable, since to the best of our knowledge literature does not exist addressing polychaetes in an integrated three-trophic-levels system. In this system, more and possible unknown factors may have influenced the polychaetes' growth performance – much lower than the reported values.

Moreover, concerning to polychaetes' performance, six autotomies occurred, corresponding to an autotomy rate of 6.67%. Autotomy is the discarding of a body part by an animal, allowing it to reduce the extent of injury, and followed often by regeneration of the lost part – Sabellidae autotomize the tentacular crown. With this crown, polychaetes can create a flow in the surrounding seawater and remove particulate matter and dissolved oxygen from it. Due to this autotomy and regenerating processes, extreme variability in size of the crowns can occur (Kennedy & Kryvi, 1980; Licciano *et al*. 2005). All the polychaetes used in the present study had tentacular crowns at the beginning of the experiment. However, it was not possible to know if the crowns were regenerated and, thus, there is the possibility that they were smaller than they should be, regarding the size of the body. Since it is with this organ that these organisms filter the particulate matter and bacterioplankton, the clearance rates may have been influenced by this, and subsequently, affected growth rates. Also, the occurrence of autotomy and a mortality of 3.33% may suggest the existence of stressors during the experiment period. Kennedy and Kryvi (1980) observed that when specimens of *Sabella penicillus* were roughly handled in aquaria, autotomies occurred. Nevertheless, to our knowledge, no studies about the natural rate of autotomies have been done, and as such it is not possible to fully address this issue concerning natural behaviour and this particular case behaviour. Regarding the seaweeds' growth, there was an increased in weight during the experimental period, for both Polychaetes and Control treatments (Tab. 3). Although with no statistically significant differences, there seems to be a trend for an increased growth in the Control treatment comparing to the Polychaetes treatment (SGR of 0.31% day^{-1}and 0.18% day^{-1}, respectively). Abreu *et al*. (2011) showed a mean minimal RGR value of 1.78% day^{-1}, in less favourable environmental conditions, and a mean maximum value of 6.23% day^{-1}, in more favourable conditions. These values were much higher to the observed in the present study. This could be due to the fact that the density, irradiance or concentrations of carbon and nitrogen were not the most suitable. Moreover, previous studies revealed high N uptake values and a preference for ammonia N sources by *G. vermiculophylla* (Abreu *et al*. 2011). In this experiment, the mean value of ammonia was 2.10 mg L^{-1} and 3.68 mg L^{-1} (for Polychaetes and Control treatment, respectively) (Tab. 2). However, during some days, the values were even equal to 0 mg L^{-1}. In the study performed by Abreu *et al*. (2011), the mean concentration of ammonia was 2.2 mg L^{-1} (and phosphates not measured). Moreover, the difference in growth rates could also be explained by the fact that, in the present study, seaweeds were not kept in constant movement, contrarily to what happened in the author's study; this may have influenced the seaweeds' exposition to light and nutrients in the water. Also, the higher growth rate in the Control treatment, may be related to the fact that the mean value of ammonia was also higher in that treatment than in the Polychaetes treatment.

One of the biggest concerns in aquaculture is the water-born bacterial pathogens that represent a significant bio-hazard for both aquaculture species and human health (Reilly & Käferstein 1997). To prevent aquaculture disease outbreaks and the potential contamination of aquaculture products, bioremediation in as attractive option.

Several studies demonstrated that *S. spallanzanii* is able to reduce the bacterial abundance in the waste from aquacultures, particularly in recirculating aquaculture systems.

Licciano *et al*. (2005) demonstrated that *S. spallanzanii* was extremely efficient in removing *Vibrio alginolyticus* from seawater in experimental tanks, confirming data from field studies. Within the first 30 minutes, polychaetes were able to reduce the bacteria abundance in 70%. However, it is noteworthy that this study was performed in a closed system, without continuously input of bacteria.

Stabili *et al*. (2010) performed a study in which *S. spallanzanii* were cultured in a recirculation aquaculture system and continuously receiving fish wastes. This study showed that this species had ability to remove presumptive vibrios cultured at 22ºC and faecal coliforms, including *E. coli*. The results in the present study were not as conclusive as the results obtained by other authors. After the beginning of the water recirculation, there was a decrease in the presumptive vibrios abundance, more rapidly in the system with polychaetes (Fig. 3). However, at the end of the experimental period, there were no statistically significant differences between treatments, despite of the observation of a lower presumptive abundance in the Polychaetes treatment. Regarding *E. coli* abundance (Fig. 4), there were no statistical significant differences between treatments, and also between the beginning and the end of the experimental period. However, rather than an increase during the experimental period, as observed to the presumptive vibrios abundance, there seems to be an increase of bacteria at 24 hours, followed by a decrease at 96 hours. This happened for both Polychaetes and Control treatments (with a more prominent raise during the 24h for this last one), suggesting that this control of bacteria abundance might have been promoted by other intervener present in both treatments. A common constituent for both systems were the algae. It has been reported that extracts from *Gracilaria* species contain active metabolites or compounds with antiviral, antifungal and antibacterial activities (Kanjana *et al*. 2011; Govindasamy *et al*. 2012). The results obtained by Kanjana *et al*. (2011) indicated that ethanol extracts of *Gracilaria fisheri* had immunostimulant and antimicrobial activity that could protect the black tiger shrimp (*Penaeus monodon*) against *Vibrio harveyi*. Moreover, Eahamban and Antonisamy (2012) showed that *Gracilaria corticata* was a rich source of phytoconstituents which can be isolated for different kinds of biological activities, including antimicrobial.

Another concern of the aquaculture industry is the negative environmental impact caused by the discharge of effluents rich in particulate organic matter, both from coastal and offshore aquacultures (Doglioli *et al*. 2004). Hence, there is an urge for new ways to mitigate the impact of this activity in marine ecosystems.

According to Cavallo *et al*. (2007), Sabellid polychaetes have important characteristics which can be exploited in aquaculture system: they are sedentary, have showed high filtration rates, and the faeces and pseudo-faeces produced are mostly used by them for building up their tubes and not all dispersed in the environment. Lemmens *et al*. (1996) reported that *S. spallanzanii* exhibited *in situ* clearance rates of 1.7-4.0 L g^{-1} h^{-1}, while in experimental systems these rates can increase up to 45 L g^{-1} h^{-1} (Licciano *et al*. 2005). In the study performed by Giangrande *et al*. (2005), removed suspended solids in the experiments with polychaetes were significantly higher than in controls (without polychaetes). Also, this species showed a clearance efficiency of about 40%.

The results of the present study are not in accordance with the previous studies. Statistical significant differences among sampling times and between treatments were not found (Fig. 5). However, it is possible to observe a trend to lower the final concentration of TSS for both treatments, suggesting that in these particular systems, the presence of polychaetes did not affect the water quality regarding suspension solids. These results may be due to the fact that the water flow was not fast enough to keep the particles in suspension or that the majority of particles were too large and, thus, had a high settling velocity. One of the steps of wastewater clearance in land-based aquacultures consists of the decantation of suspended particles in tanks (Cavallo *et al*. 2007). In the present study, the fact that in the Control treatments the polychaetes were absent but the tanks remained empty in the systems may have functioned as a decantation tank, leading to a decrease of suspended solids – and deposited solids was not addressed in the present study.

More studies should be made, in order to quantify the settled particles, allowing the understanding on how this affects the clearance rates of polychaetes. Also, adding systems without fish and study different densities of polychaetes could elucidate their performance in the proposed experimental system. Moreover, study if the antimicrobial properties of the exudates of *Gracilaria* sp. affect the bacterial abundance – in a much higher rate than the presumed polychaete performance – would be useful to better understand the system and the possible role played by the different organisms.

Further, mathematical modelling should be addressed to all the trophic levels, in order to optimise the organisms' performance and, consequently, obtain values of maximum productivity in accordance with the ones obtain in other systems.

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Chapter 4

General conclusions and future perspectives

General conclusions and future perspectives

There are few doubts that IMTA is still in its infancy but presents great prospects towards becoming the aquaculture of the future, with increased production and product diversity, and also with increased quality, promoting environmental, economic and social sustainability.

The addition of complexity in integrated multi-trophic systems can bring several environmental and economic benefits. The integration of filter-feeding organisms capable of uptake potential pathogenic microorganisms and particles from the water column may present an opportunity to decrease disease outbreaks and also control human pathogens. Moreover, together with macroalgae, which already provided evidence of enormous capacity in the assimilation of nutrients that can become toxic for cultivated species, reducing many of the impacts of aquaculture. The use of these bioremediation organisms in co-culture with high valued fish or shrimp species can reduce greatly the water exchange frequency and discharge of effluents as well as decrease the probability of disease occurrence in a symbiosis of environment and economic benefits. Reducing the costs in the treatment of effluents and in water while producing biomass without spending in commercial feed is of great economic advantage.

In the present study, an extensive review on IMTA is presented and some equations to better understand them were proposed, focusing in dynamics population, growth, filtering rate of seaweed and polychaetes and in digestion and evacuation of the fish gilthead seabream. However, as there is scarce information gathered concerning the application of these equations in models applied to IMTA, more work needs to be done to better address the subject. In order to improve the reliability of the model, equations related to parameters such as water flow and settling of the particles, and equations adapted to other species of fish and shrimp, should be developed and seaweed filtration function further tested and, eventually, calibrated.

Regarding the study to assess the bioremediation capability of *S. spallanzanii*, and focusing on part of the potential model, the results were different than the expected, since the presence of the polychaetes in the systems did not seem to promote a significant reduction on bacterial abundance and total suspended solids. However, their presence did not have a negative effect in the systems in general, showing also an increase in growth. This mass growth reveals that both organic and inorganic extractors were being effective in using the main culture's wastes, and thus tackling a major concern of this sort of system that is the production of organic and inorganic wastes. Also, this constitutes an important economic benefit, once organisms are being produced without additional coasts. Nevertheless, further digging into the modelling and testing is needed to achieve maximum productivity of the partial trophic levels that are still far from reported value for other cultures.

Nevertheless, this was a preliminary experiment and a contribution to the topic. Further work should be done, in order to assess other parameters, such as the effect of resuspension of settled particles; autotomy rates and their influence must be studied; and polychaetes' density and water flow should be further explored and modelled for optimal performance.

Moreover, questions such as keeping the seaweeds in constant movement in order to maximise their exposition to light and nutrients in the water. Also regarding this, seaweeds' density should be modelled to obtain an optimal growth and conditions where the nutrients in the water are not limiting. Besides, the experiment gave a new perspective about the potential role of the seaweed in the system, related to its antimicrobial potential.

Concerning IMTA infancy's, great opportunities come along with great challenges as pinpointing the most suitable species to be addressed and combined in this microecosystem, altogether with the need to create models to better assess the densities, and conditions of the culturing for optimum revenue is of overwhelming need for an increasing demand for fish products for a plethora of industries.

Moreover, due to their complexity, it has become more important to develop mathematical models not only to better understand the system, but also to extrapolate the data from laboratorial systems to larger industrial scale farms, and to create simulators allowing the optimisation of species combinations and yields from each trophic level. This optimisation may additionally enable the reduction of negative impacts of aquaculture activities with all the above stated benefits for the sustainable aquaculture of the future.

Chapter 5

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