s

# ASTRAL

## *A*ll Atlantic Ocean **S**ustainable, Profi**T**able and **R**esilient **A**quacu**L**ture

GA no **863034**

Research and Innovation Action (RIA)

Start date: 1st September 2020. End date: 30th August 2024

# D4.1. Best practices for zero waste

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 863034

**Document administration**

| | |
|---|---|
| **Deliverable number** | D4.1 |
| **Deliverable name** | Best practices for zero waste |
| **WP number** | 4 |
| **Deliverable due date** | 28.02.2022 |
| **Submission date** | 02.03.2022 |
| **Dissemination levels** | Public |
| **Lead beneficiary** | FURG |
| **Responsible** | Luis Poersch |
| **Contributors** | Luis Poersch, Alessandro Cardoso – FURG<br>Brett Macey, John Bolton, Mark Cyrus, Marissa Brink– UCT<br>Adrian Macleod, Kati Michalek – SAMS *<br>Pauline O'Donohoe, Joanne Casserly – Marine Institute * |
| **Internal reviewers** | Thierry Chopin; Preethi Surendran (NORCE) |

**Document history and changes**

| Version | Date | Author | Description |
|---|---|---|---|
| 1.0 | 15/01/2022 | Luis Poersch | First draft for circulation in WP4 |
| 2.0 | 18/02/2022 | Reviewers | Second draft |
| 3.0 | 25/02/2022 | Coordination | Third draft |
| 4.0 | 02/03/2022 | Luis Poersch | Final document |

**Evidence of accomplishment**

| |
|---|
| Report |

# Contents

# List of Figures

# 1 Summary

The WP4 aims to develop more efficient systems to produce aquatic organisms, with minimal production of effluents and better use of resources. Several experiments were carried out in open ocean, semi-closed and closed IMTA systems. Trials for waste reduction have been focussing on the closed and semi-closed IMTA systems as nutrient in- and out flow can be followed to assess nutrient dynamic. In Brazil, the possibility of combining oysters and tilapias as filters of organic matter in an integrated culture with Pacific white shrimp in bioflocs was investigated. In this same system, the effect of introducing seaweeds to reduce of nitrogen and phosphorus concentrations in the water was also assessed. In South Africa, tests were carried out in a commercial abalone culture with the aim of increasing water recirculation to 75%, with the water being treated by macroalgae, which reduces nutrient concentrations and are used as food for abalones. In Ireland, integrated farming of fish, scallops, lobsters, urchins, oysters and macroalgae is being developed in open sea. In a similar environment condition, different species of macroalgae and native oysters are integrated in Scotland. In Brazil IMTA-lab the results observed in this period indicate that it is possible to control the nutrient concentrations with plants (seaweed) in the shrimp culture. Regarding the total suspended solids generated in shrimp culture in biofloc system, oysters were not as effective as tilapias in removing particulate matter (bioflocs). Microorganisms present in the bioflocs and probiotics helped to keep the water quality, transforming toxic nitrogen compounds (ammonia and nitrite) into less toxic ones (nitrate). In addition, microorganisms serve as food for shrimp and tilapia, improving the feed conversion rate. In South Africa IMTA-lab, during the abalone production process, so far, the integration with seaweed has provided a reduction in nutrient concentrations, external feed inputs and greater water recirculation (50% of the total volume), reducing considerably the emission of effluents to the environment. In addition to the benefits on water quality, seaweed serve as supplementary feed for the abalone, making the system less dependent on external supply. The project is still in progress and experiments are underway to further increase recirculation, further reducing energy consumption and effluent emissions.

# 2 General description

ASTRAL aims to develop new, sustainable, profitable and resilient value chains for integrated multi-trophic aquaculture (IMTA) production within the framework of existing, emerging and potential Atlantic markets. The general idea is to increase the circularity by 50-60% compared to monoculture baseline aquaculture and will provide a circular business model, boosting revenue diversification for aquaculture producers increasing profitability by at least 30%.

The actions proposed in the WP4 task 4.1 are strongly related to WP2. The activities developed in the different labs will drive the best configuration to improve the water reuse, waste reduction, improve the feed conversion rate and provide the best biomass relations between species, driving towards zero waste.

The ASTRAL project involves different partners that are focusing their studies on four models of integrated aquatic organism production, named integrated multi-trophic aquaculture. The first case study is in Ireland and the second in Scotland. These case studies are carried out in open ocean areas (Figure 1) with water flow depending on oceanographic conditions.

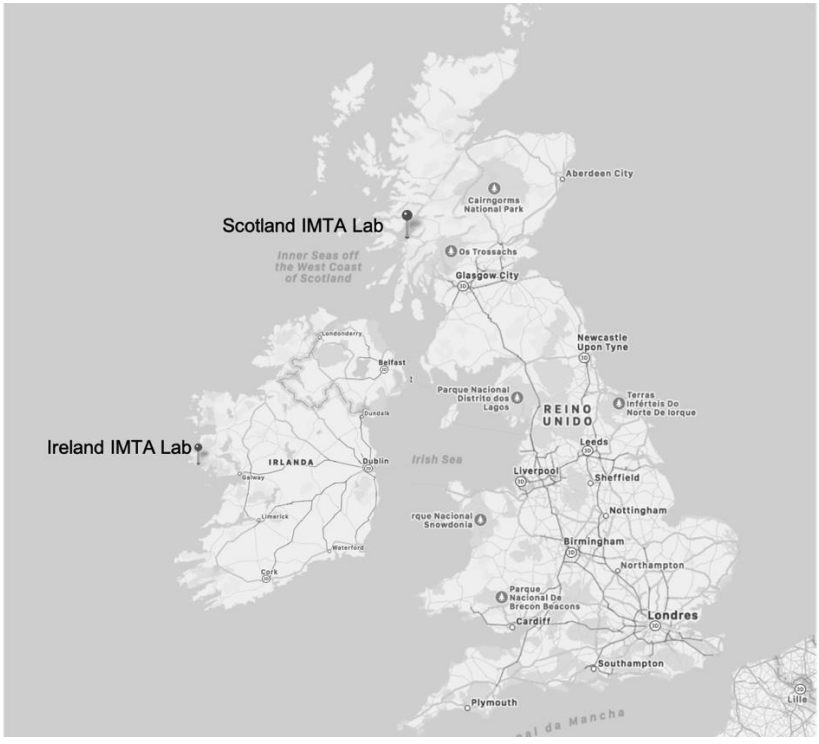



Figure 1: Case studies in Europe

The Irish IMTA Lab, Lehanagh Pool is an open water system that produces a number of species at different trophic levels (Figure 2). This method creates synergistic interactions between the production species to bio-remediate the generated waste products. Seaweed and filter-feeders such as bivalves and other invertebrates assimilate and extract dissolved nutrients and suspended particulate organic matter generated by the higher trophic species (e.g. fish). Atlantic salmon and lumpfish will have a daily input of feed, thus generating waste in the form of uneaten food and faecal matter. This nutrient rich waste can enhance the growth of the lower trophic species (i.e. invertebrates and seaweeds etc.) allowing for nutrient recycling. New species such as lobster and sea urchins will be trialled at this site to assess growth performance.


Figure 2: Salmon production in net tanks integrated with seaweeds, urchins and scallops.

The Scottish Association for Marine Science (SAMS) in Oban operates two experimental open coastal farms on the Scottish west coast. The main site Port-a-Bhuiltin (56° 29.176 N, 5° 28.315 W) has a lease area of 30 hectares with a one-hectare (100m x 100m) submerged tensioned grid system currently deployed. The site provides the infrastructure to develop and test a wide range of culture approaches seeking to efficiently combine seaweed and shellfish culture (Figure 3).

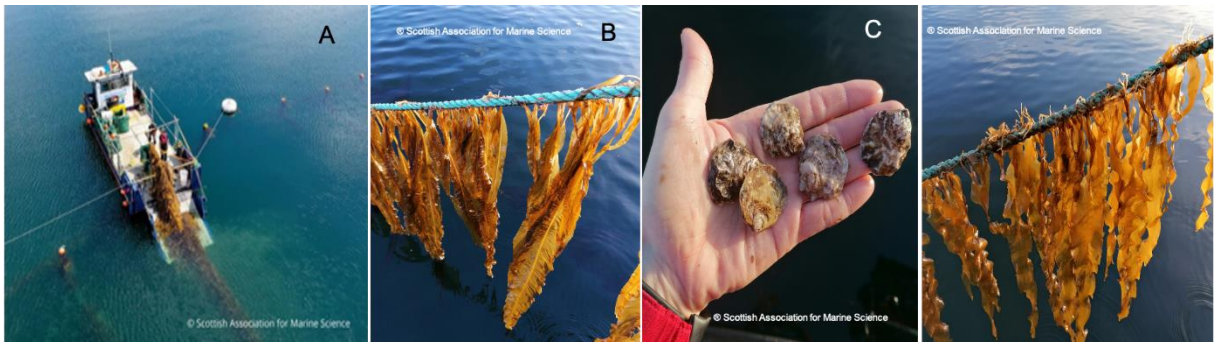



Figure 3: Seaweed production integrated with European flat oyster in Scotland. Seaweed production system (A); *Alaria esculenta* (B); native oyster (C) and *Saccharina latissima* (D).

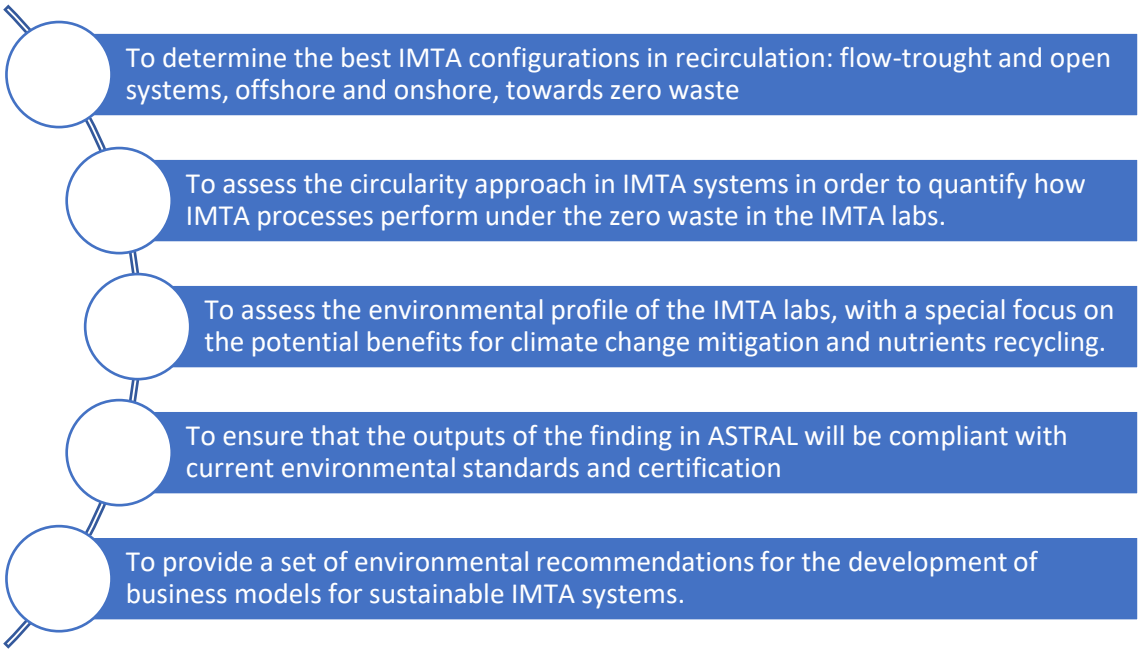
Further two case studies are taken place on in the South Atlantic (Figure 4) using land-based production. In the South Africa the commercial farm produces abalone (*Haliotis midea*) in raceway tanks. The effluent of these tanks is used to grow Ulva in large D-shaped paddle raceways. Ulva, in turn, is used as additional feed to abalone, and 50% of the water from the Ulva systems is re-circulated back to the abalone tanks. The Ulva removes nitrogen (as ammonia) excreted by the abalone, enabling partial re-circulation, and there are two circular processes: nitrogen and water. Higher recirculation rates move closer to Zero Waste. This IMTA process will be adapted to produce a new high value species, the sea urchin *Tripneustes gratilla*.


Figure 4: Case studies in Brazil and South Africa

In Brazil, the Federal University of Rio Grande – FURG produce shrimp in raceways with biofloc technology system (BFT), in which, no water renewal is necessary, and the water treatment occurs with the integration with other organisms. The bacteria transform ammonia and nitrate in biofloc biomass where the tilapia and oyster control the organic material (biofloc) and seaweeds and halophytes are responsible for consuming the nitrate and phosphate.

It should be noted that the species used in the different case studies have distinct characteristics and production cycles. These different case studies reflect the volume of results presented here. Therefore, the species with a shorter cycle (shrimp and tilapia) and that are produced at higher temperatures, required a larger number of experiments in these first 18 months of the project (two summer periods in the southern hemisphere).

## 2.1. WP4 OBJECTIVES



# 3 IMTA in Brazil

Federal University of Rio Grande
Institute of Oceanography
Marine Aquaculture Center

## 3.1 Facility description

Federal University of Rio Grande - FURG is located in Rio Grande do Sul State – southern Brazil (Figure 5A). FURG has approximately 15,000 undergraduate and graduate students currently enrolled. FURG has it vocation focussed on coastal and oceanic ecosystems. It is the pioneer and ranks among the top universities regarding studies of Oceanography (including mariculture), in Brazil. Professors and researchers at the Institute of Oceanography have vast national and international experience, attending and organizing conferences and conducting important research in the field of mariculture as can be seen from their list of publications in peer-reviewed journals.

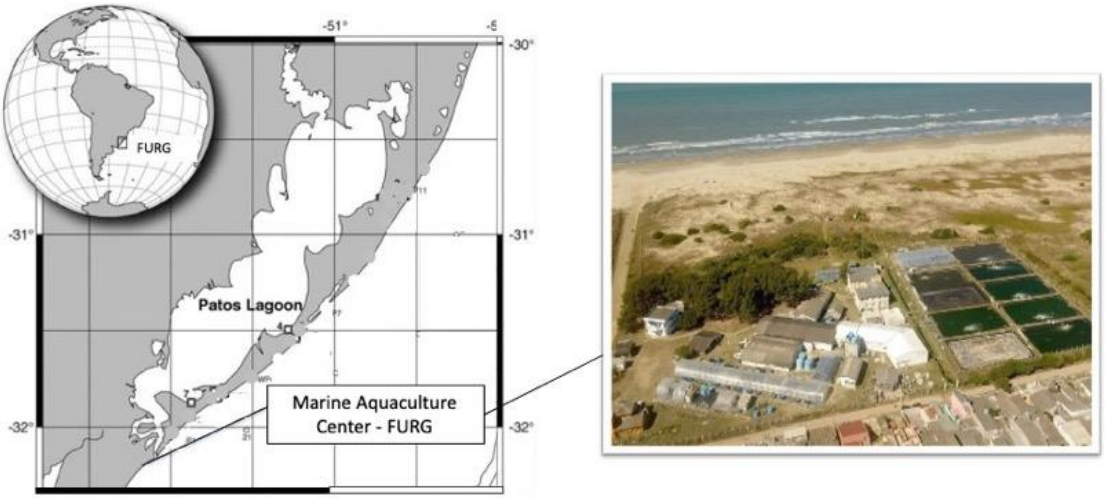



Figure 5: Location of Federal University of Rio Grande- FURG in southern Brazil (A) and the Marine Aquaculture Center (B) (32°12'16S, 52°10'38W).

The Marine Aquaculture Centre (Figure 5B) is located 300 meters away from the shoreline and has 9 experimental shrimp ponds (600 $m^2$ each), 3 greenhouses for research with shrimp production in bioflocs, 1 pilot commercial size greenhouse for shrimp production (2 tanks with 237 $m^2$ each) and 1 multi-trophic greenhouse (6 systems with 3 tanks each). In addition, the laboratory has a shrimp maturation sector (6 tanks with 10 ton each), shrimp hatchery sector with commercial size tanks (8 tanks), sectors of live food production (Artemia and phytoplankton) and water quality laboratory.

Figure 6: Shrimp ponds (600 $m^2$ each), experimental greenhouse (12 tanks with 35 ton), maturation room (6 tanks with 10 tons), pilot commercial size greenhouse (500 $m^2$)

For all experiments in the IMTA greenhouse (Figure 7), the shrimp were kept in high densities (350 to 550 shrimp/$m^3$) with biofloc formation being stimulated by organic fertilization. For each 1 mg of ammonia in the water it is added 15 mg of carbon (molasses) to keep the C:N ratio of 15:1. Water was pumped from the shrimp tank to the raceway where the oysters and fish were responsible for filtering out suspended particles in the system. Then the water was driven into the seaweed tank from where it flowed back into the shrimp raceway through a central bottom pipe. The seaweeds were responsible to consume $CO_2$, nitrate and phosphate.

The choice of the species to be used and their biomass ratios are key factors for maintaining the system's water quality. Thus, it is not necessary to change the water and it can and must be used for several production cycles. Previous studies on literature and trials in our facilities indicate better animal performance when water was reused from the production system, as there were no nitrogen peaks and pH variations. When we reuse water with biofloc from the beginning of the cycle, natural food was provided 24 hours a day, 7 days a week, hence lowering FCR, and reducing the food costs considerably.

Figure 7: IMTA greenhouse with shrimp tanks (left) and fish and seaweed tanks (right).

The proposed IMTA system effectively integrates the expertise of the FURG researchers and their partners to develop a new technology that incorporates different species. The resultant integrated

and multi-trophic system is potentially more biologically efficient, more sustainable as the environmental profile is better and the system is more economically competitive than the four cultivation systems maintained separately.

### 3.1.1 Species

Shrimp - *Litopenaeus vannamei*

The Pacific white shrimp *Litopenaeus vannamei* is a native species from the Eastern Pacific and it is distributed from Mexico to northern Peru, presenting great resilience and easy adaptation to different culture systems. It also presents one of the best zootechnical indexes (low FCR, resistant to management, fast growing, can be produced in high densities and show high survival) and great market acceptance. Besides achieving high growth and survival rates, it has lower nutritional requirements in terms of protein when compared to other penaeid species.

These characteristics make this organism very easily adaptable to different types of systems, including IMTA. It serves as the main species in our system, being the most productive and of highest aggregated value in our region.

Figure 8: Shrimp after harvest (left and centre) and shrimp tank with biofloc (brown water).

Fish - *Oreochromis niloticus*

Tilapia (*Oreochromis niloticus*) presents some characteristics that make it an ideal species for culture in an IMTA system based on the BFT system. This species has a high tolerance for suspended solids, moderate dissolved oxygen, can be maintained in high stocking densities and can be produced in intermediate salinity. In addition, it is an omnivorous species, presenting filtering structures and a digestive system that allows it to have an organic extractive potential, feeding on natural productivity of the BFT system, and removing the excess of total suspended solids, being used as a bioremediation agent in IMTA systems.

Figure 9: Tilapia fingerlings (left), fish biometry during experiment (center) and tilapia close to commercial size.

Molluscs – *Amarilladesma mactroides*, *Crassostrea gigas* and *Crassostrea gasar*

Sessile filtering organisms are also important in the biological removal of total suspended solids in the IMTA system. Many of these organisms have a high added value in many places; however, the characteristic of the IMTA system associated with bioflocs makes their adaptation very difficult in some cases.

In the case of the mussel *Amarilladesma mactroides*, a local and very abundant species, its insertion in the system was not possible because it needs a sandy substrate in which it can remain partially buried, which is not possible in a biofloc system. The species also showed little tolerance to moderate values of total suspended solids (TSS) in some initial tests, and its use as a filtering bivalve in our system was discarded.

Figure 10: Molluscs used in the experiments. *Amarilladesma mactroides* (left), *Crassostrea gigas* (center) and *Crassostrea gasar* (right).

Regarding oysters, the first species to be tested was the Pacific oyster *Crassostrea gigas*. Despite its high added value, it is an unpromising species to our system because of its little tolerance to the presence of SST and high temperatures. Due to these results, the mangrove oyster, *Crassostrea gasar* was tested instead. Since it is a native species that grows in mangrove areas, it was provided to be more tolerant to culture conditions of high TSS values and high temperatures, with promising results

in preliminary tests. Therefore, this species was chosen to be one of the filtering organisms to assist in the removal of TSS in our IMTA system

Seaweed - *Ulva fasciata* and *Ulva flexuosa*

Besides the accumulation of particulate matter in the system, another problem to be solved in IMTA systems is the accumulation of nutrients, in particular nitrogen compounds and phosphorus. Thus, two native species of macroalgae were used in our tests. The species *Ulva fasciata* and *Ulva flexuosa* occur in the region and are easily collected and brought to the laboratory to be acclimated and introduced in the IMTA system to absorb nitrogen and phosphate compounds.

The tests carried out with the two species showed promise in the removal of these compounds, especially phosphorus, from the system. Phosphorus presents slower accumulation in the system when compared to nitrogen compounds that are always present due to the action of nitrifying bacteria present in the system. As these are local species, the use of one or another species will depend on the availability in the environment at the time of collection.

Figure 11: Pictures from the experiments with *Ulva sp* in different structures cultivation.

### 3.1.2 Understanding the shrimp production evolution

#### Shrimp monoculture

The culture of marine shrimp in extensive pond systems uses large coastal areas for production. This type of culture is always linked to processes that use high rates of renewal, leading to the introduction of large amounts of effluent into adjacent environments, and increasing the risk of eutrophication therein. Because they are low technology and low-cost culture, they are still widely used around the world. Despite their low yields, the added value of the products still makes this viable for many producers.

As these systems are in coastal areas, they are much more susceptible to the introduction of pathogens. They are set in very extensive areas in which the presence of birds and other crustaceans introduce pathogens that may lead to great losses in production, since it is not possible to create barriers that keep these animals outside the limits of the farm. That is the reason why systems that use smaller areas for production, with higher productivity rates, have been developed in recent years.

## Shrimp in BFT system

The biofloc system is another culture technology that has been adopted in shrimp farming. This system gives the shrimp monoculture a new direction with respect to aquaculture sustainability. Due to a better maintenance of water quality, this system can reduce water use by up to 90% compared to traditional systems (table 1), allowing culture at high stocking densities reducing the use of land and optimizing the use of artificial food. This is possible through the increase of the Carbon:Nitrogen ratio in water, with the use of organic carbon sources and constant aeration, stimulating the production of microorganisms that will both work on the metabolization of nitrogenous compounds and serve as natural food for reared animals.

Table 1: Shrimp production in different systems

| | Monoculture | Biofloc | Multi-trophic |
|---|---|---|---|
| **Water volume to produce 1kg of shrimp (L)** | 60,000 | 300 | 100 |
| **Feed Conversion Rate (FCR)** | 1.6 | 1.3 | ? |
| **Density (shrimp/m$^3$)** | 30 | 550 | 450 |
| **Biomass production (kg/ha)** | 2,000 | 50,000 | 45,000 |
| **Water renewal (% of volume/day)** | 10-20 | 2 | ? |

Shrimp production in the biofloc system has numerous economic advantages, but there are still some environmental limitations. The minimal or zero water exchange system with high densities of shrimp favours the accumulation of organic matter during production. This accumulation produces an excess of suspended solids, which increases the biological oxygen demand due to the high concentration of microorganisms present in biofloc, that also consume oxygen. Therefore, it is recommended that excess total suspended solids (TSS) in the BFT system should be removed through mechanical processes, for filtration and clarification.

Clarification efficiently removes excess of suspended solids in the biofloc system that are normally discarded as effluents. Therefore, the technique of integrating species in the biofloc system is to take advantage of this excess of microbial protein in the form of suspended solids to feed another species and thus transform effluent into animal protein, making the system even more sustainable.

The generation of effluents with high nutrients is yet another problem related to super-intensive aquaculture. Feeding in intensive monocultures boosts the discharge of nitrogen and phosphorus rich effluents of high polluting potential. These problems have raised concerns among many countries about the risks of environmental impacts of this activity.

To succeed in the production of marine shrimp, the water quality must always be maintained in ideal conditions, thus, numerous variables are measured and controlled. This management is extremely important to avoid the emergence of diseases and to safeguard the survival of the farmed animals. Due to the intensification of this activity, some factors, such as increased stocking densities, water used for stocking, and feed management are related to the occurrence of bacterial diseases. In shrimp hatchery, bacterial diseases are the main causes for massive losses in production. This pathology is caused by bacteria of the genus Vibrio, and it can be characterized as local or systemic infection, affecting all organs and tissues, especially the hepatopancreas.

An alternative for the control of pathologies for successful production is the use of probiotics. Probiotics are defined as live microorganisms added to food that benefits the host by improving its intestinal balance. There are different mechanisms of action of probiotics: competitive exclusion of pathogenic bacteria, source of nutrients, enzymatic contribution to digestion, influence on water quality, and improvement of immune response. The use of probiotics generates bacteriostatic control, better productivities, with increased weight gain, more efficient feed conversion and increased survival.

## Multi-trophic system

The Integrated Multi-Trophic Aquaculture (IMTA) aims for the integration of species with different trophic levels in the culture system, in order to take advantage of the residues from the production of one species for the culture of other species. Even organisms that occupy the same trophic level in the system can also be produced at IMTA if they make use of different food webs in that system. This variety in the combination of organic and inorganic extractive species gives IMTA advantages in

terms of bio-mitigation and economic gain, consequently more sustainability, when compared to monocultures.

The commercial expansion of IMTA has not been easy. While the biological and environmental advantages of this practice are generally accepted, the barriers to its adoption have been mainly economic and regulatory. Thus, studies on a pilot scale, which aim to integrate the super-intensive production of Pacific white shrimp (*Litopenaeus vannamei*) and Nile tilapia (*Oreochromis niloticus*) in the biofloc system, are extremely necessary for the development of integrated multitrophic cultures at a large scale.

There are some studies reporting the integrated culture of shrimp and tilapia, though in low production densities and in clear water systems. More recently, a study of integrated culture of shrimp and tilapia in an experimental scale, and this work serves as a first step towards a future IMTA using BFT technology.

The species that make up the system must necessarily be from different trophic niches so that there is a complementarity in feeding demand. Thus, it assumes that the feed inserted into the system, may not be used in a trophic level, but will be used in another, so that only little nutrient is unused and discarded in the natural environments, and thus reducing the risk of eutrophication. There are three groups of pre-defined organisms for the composition of the system: the fed species, the organic extracting and the inorganic extracting species.

Considering the structure of constitution of multitrophic systems, it is beneficial to elaborate a system that associates multitrophic culture with the biofloc system to give options for adjustments to the situation of increased SST and yet obtaining a system with greater productivity. Nevertheless, the simple intention of inserting a new species into an already established cultivation system is a matter of great complexity, as it demands an understanding of EACH species requirements and the dynamics of the culture environment. When it comes to BFT to receive a new species, the situation is even more delicate, given the complexity of the system.

### System Design (multitrophic greenhouse or IMTA greenhouse)

Super-intensive production in biofloc systems is a sustainable and biosecure alternative to intensive culture systems which rely on expensive filtration systems (RAS) or high-water exchange rates (flow-through systems). The biofloc is composed of uneaten feed, faeces, secretions, and their associated

algal, bacterial, and microplankton communities. Therefore, this biofloc provides the substrate for microbial nitrification of toxic ammonia to nitrate, in the same production tank, and it eliminates the need for external treatment or water exchange, improving economic and environmental sustainability. In addition to providing water quality, biofloc particles can be consumed directly by culture species such as shrimp, tilapia and oysters, thereby making more efficient use of the nutrients contained within the feed more efficient and hence lowering total feed costs (Figure 12).

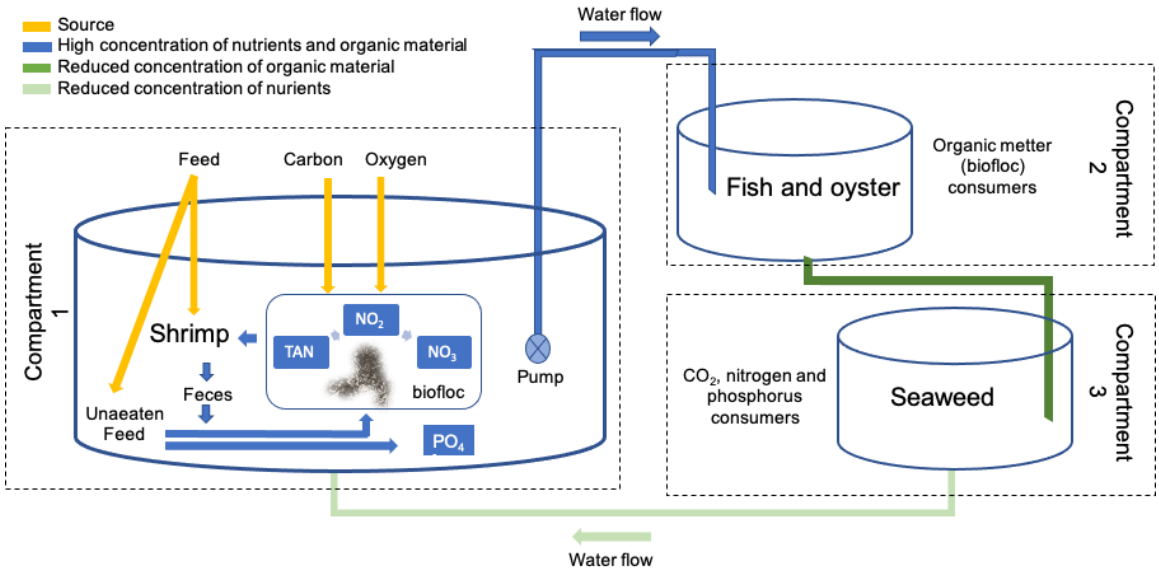



Figure 12: Schematic drawing of the research unit containing 6 systems with 3 compartments each one.

### 3.1.3 Experiments

#### Experiment 1

**Determine the best mollusc species for the IMTA-BFT system**

This first work aimed to specify characteristics of intensive culture systems (BFT and IMTA), as well as potential species of bivalve molluscs to compose the production and, finally, to analyse the possibility of establishing a model that integrates the two systems.

A literature review was carried out in order to gather information that provides relevant theoretical bases for the structuring of the biofloc and multitrophic systems. In addition, a survey of information was gathered on biological and cultivation characteristics of species of bivalve molluscs to compose the system.

RESULTS AND DISCUSSION

INSERTION OF NEW SPECIES

The simple purpose of inserting a new species in a productive system is a matter of great complexity, as it demands an understanding of the species requirements and the dynamics of the culture environment. Considering that the system to receive new species is the BFT, the situation becomes even more delicate. The introduction of a new species in an already established production, such as the marine shrimp *L. vannamei*, may bring some environmental changes to the crops and compromise its performance. Some points to consider are:

- Oxygen demand by the inserted species;
- Interaction of new species with the structure, microorganisms and target species of the main system;
- Acceptance of the characteristics of the biofloc system by the new inserted species; and
- Adequacy of the culture facilities required by the inserted species.

In order to choose species for the bioflocs system, they must support:

- high suspended solids (> 200mg / L);
- moderate dissolved oxygen (3–6mg / L);
- high concentrations of nitrogen compounds; and
- high stocking density.

They must also have their biology receptive to flake conditions, such as:

- omnivorous eating habits;
- presence of adequate filtering structures; and
- an adaptable digestive system.

The use of native species or species that are already found in the area has the advantage of better adaptation to the culture place, as they present characteristics like the environment in which the organism is inserted. The species to be placed in the system must also present biological requirements that are compatible with the characteristics of the culture site where it will be inserted. In the present case, the characteristics of the biofloc culture system are well defined. Therefore, molluscs that tolerate conditions similar to those of biofloc cultivation should be selected.

Besides the biological requirements, the organisms inserted in the system must have other advantages for their choice, including market acceptance and sale price. Another important factor is

the ecosystem service provided by the species. For bivalve molluscs, it is known that they feed by filtering of particulate matter suspended in the water. Aquaculture of filter-feeding molluscs takes advantage of the natural productivity of water, transforming primary productivity into animal protein of high nutritional quality. The most ecologically efficient cultures are those that produce lower trophic level organisms such as bivalve molluscs.

In Brazil the species of bivalve molluscs produced are oysters, scallops and mussels. In the southern region of Brazil, a species that has historically stood out as an accepted and consumed marine resource is white shellfish *Amarilladesma mactroides*. Considering that the white shellfish and oysters of the genus Crassostrea, *C. gigas* and *C. gasar*, are well known in this region, they were selected species for this study.

*Amarilladesma mactroides* (Reeve, 1854).
The marine bivalve *Amarilladesma mactroides* is synonymous with *Mesodesma mactroides* popularly known as marisco branco in Brazil and as almeja amarilla in Uruguay and Argentina. The species feeds upon material suspended in water by filtration, like many bivalves. Its body is laterally compressed, with thin and fragile shells, reaching up to 8 cm; it has two large siphons, an inhalant and an exhalant, which regulate the water flow of water directing it to the gills, which filter microparticles suspended in the water column (organic particles and phytoplankton).

The proposed culture system for infaunal bivalves should be composed of a layer of sand, as the sandy substrate is essential for the clams. Studies proved the effectiveness of the long-line culture system with basket structures with a sandy where the clams are placed.

Thus, its insertion in the IMTA-BFT culture system requires an improvement of the system, with the placement of a sandy layer for the maintenance of the animals. If the suspended basket model is taken as a reference or even adopting the layer of sand at the bottom of tanks with canvas or liners, the dynamics triggered in the water by the intense aeration could cause a disruption in the system. The air bubbles can produce an excavation of substrate, spraying sand grains into the water column and dismantling the substrate for the clams.

The need for sand in the culture facility deserves special attention in the planning and implementation of the BFT system because it can affect the culture structure already established for that system, with high dynamics and requirements for the development of microorganisms.

Thus, at the moment the white clam *A. mactroides* cannot be considered for insertion in the integrated culture with shrimp in BFT. Their organisms are not yet adapted to cultivation environments as they need a layer of sand for their growth and do not cope with high organic loads. Such characteristics can be a barrier to successful production.

*Crassostrea gigas*

The Pacific oyster receives this name due to its natural occurrence in East Asia, with greater predominance in Japan, Korea and China. It is the most important species of oyster produced in aquaculture systems. The *Crassostrea* genus occupies the first place in the list of the most produced molluscs in the world. *C. gigas* alone occupies the seventh position in the ranking. Due to its rapid growth potential, the species has been adopted in different regions of the world.

It is a non-native species in Brazil and is currently cultivated in the state of Santa Catarina, accounting for more than 90% of the national oyster production, thanks to a technological package for its culture that was introduced in the state in the 1980s. It is found in marine farms, having its introduction facilitated, at first, by the cold waters found in southern Brazil and over time by acclimatization of the species at higher temperatures.

*Crassostrea gasar*

The mangrove oyster *Crassostrea gasar* is naturally found in estuarine environments, having mangrove systems as typical habitats, where it lives fixed in the roots of the mangrove trees or in rocks. This species is widely distributed and can be found on the west coast of Africa, from Senegal to Angola and in South America, from French Guiana to Southern Brazil. Its distribution occurs naturally in rocky shores, in mangrove roots in the intertidal region and occurs from Santa Catarina to Pará usually found forming clusters in the mangrove ecosystem.

It is the most important native oyster species of commercial interest, being extracted mainly from the natural environments and has been gradually inserted in the production of the largest Brazilian oyster producing state. The results of research on the species confirm the zootechnical potential of *C. gasar*, mainly due to its rapid growth, which may make it an option for farming in the national sector.

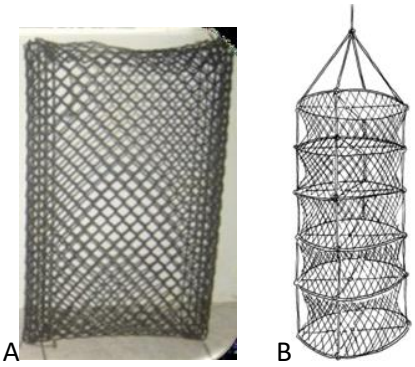
It is a species considered a large species, reaching up to 20 cm in height. In general, it is found in the mid-coastal and infra-coastal of shallow and protected estuaries.

In culture structures, they accept high population density is accepted, as shown in a study who defined the density of 25 dozen oysters/m2 among those evaluated. Another study described the culture of the species in north-eastern Brazil and suggested that its production occurs in marine areas if salinity remains above 5. Organisms inhabiting estuaries tolerate frequent salinity fluctuations by adopting behavioural and physiological strategies. Thus, estuarine oysters have their own characteristics, which allow the maintenance of osmotic concentrations of their body fluids at acceptable levels for a good physiological development.

Some research was done with the species to analyses its performance in different salinities and observed that the oyster *C. gasar* can survive at salinities between 8 and 34. The species grows well in both the marine and estuarine environments, although the estuarine region has been more favourable for its culture and the species cannot survive in water with salinity above 40.

Culture structures for oysters

Oyster farming can be divided into three main phases: initial culture (seeds), intermediate culture (young) and definitive culture (adult). Oysters can be confined in lanterns and pillows (Figure 13) in fixed or floating culture systems. The equipment used to accommodate the oysters differs between the different production locations, due to the adjustments made by the producers themselves. What defines the type of equipment generally used is the environmental condition of the growing location.



A B

Figure 13: Culture equipment used to accommodate the oysters. A) pillow and B) lantern.

## Conclusions

In our initial tests, the *Crassostrea gigas* oysters did not adapt satisfactorily to the culture systems, while *Crassostrea gasar* showed encouraging initial results that will lead us to inclusion of this species in future IMTA works associated with the biofloc system. Oyster mortality varied from 70 to 100% for *C. gigas* and from 30 to 60% for *C. gasar*, mainly at high temperatures and TSS above 250 mg/L, common in shrimp production in BFT system.

Experiment 2

**Integrated multitrophic culture of shrimp and tilapia in biofloc system**

The objective of this study was to evaluate the effect of different fish stocking densities in the integrated super-intensive culture of white shrimp *L. vannamei* and Nile tilapia *O. niloticus* reared in BFT system to promote the maintenance of TSS at the appropriate levels for shrimp culture, through the consumption of the excess of biofloc by the fish.

**Material and Methods**

*Culture Conditions*

The study was carried out at the Marine Aquaculture Centre, Federal University of Rio Grande (EMA-FURG), southern Brazil (32°12'16S, 52°10'38W) in a greenhouse enclosed tanks used for IMTA studies.

Before the experiment started, an inoculum of 20% of the total volume of the shrimp tank, with mature bioflocs, was used in all treatments. Organic fertilization was carried out by manipulating the C: N ratio to 15:1. The carbon source was sugar cane molasses, containing 37% of organic carbon.

During the study, the shrimp were fed twice a day (9:00 am and 5:00 pm) with species-specific commercial feed, containing 38% crude protein (Poty Active 38, 1.6 mm, Guabi®, Campinas, SP, Brazil). The fish were fed twice a day (09: 00 h and 17: 00h) with Guabitech Mirim QS commercial feed (1.0 mm) at the beginning of the experiment, adjusting the feed for Guabitech omnivorous QS (2-3 mm / 5-5 mm), throughout the experimental period, as the fish grew. The fish were underfed, initially offering 2% of the fish biomass in feed to stimulate the consumption of bioflocs. This value was adjusted throughout the culture, reaching 1% of the biomass at the end of the experiment. The shrimp were stored in the experimental tanks with an initial weight of 0.96 ± 0.1 g. Tilapias, with an initial weight of 7.17 ± 3.15 g, were previously acclimated to the salinity of the experiment and later stored in the experimental units.

*Experimental Design*

The experiment lasted 78 days and was performed in two treatments, in triplicate: 1) T35 – integrated culture of shrimp (550 shrimp $m^{-3}$) and tilapia at a stocking density of 35 fish $m^{-3}$; and 2) T65 - integrated culture of shrimp (550 shrimp $m^{-3}$) and tilapia at a stocking density of 65 fish $m^{-3}$).

*Culture system*

The BFT-IMTA recirculation system includes a circular tank of 10 $m^3$, where the shrimp were stored, and another similar tank of 4 $m^3$ of useful volume, stocked with tilapia. With the use of submersible pumps (SB 2700, Sarlo better, Brazil), the water was pumped from the shrimp tank to the tilapia tank through a PVC pipe (40 mm in diameter), and returned to the shrimp tank with gravity, through piping (40 mm) installed 20 cm from the surface of the tank. The recirculation system operated for 24 hours, with an average flow of 965.6 ± 92.8 L $h^{-1}$. Aeration was provided by a 4HP blower connected to the air distribution system by means of microperforated hoses. Each recirculation system contained a cylindrical-conical clarifier made of fiberglass with a useful volume of 150 L. A shade was installed on the greenhouse cover to attenuate 70% of the luminosity to avoid phytoplankton blooms and favour the heterotrophic nature of the system.

*TSS monitoring*

Total suspended solids (TSS) were kept at 500 mg $L^{-1}$ according to (Gaona et al., 2017). When the TSS value exceeded this limit, physical clarification was performed in the systems by removing suspended solids in all treatments.

**Results**

*Water quality*

Table 2. Water quality parameters in treatments T35 (540 shrimp $m^{-3}$ + 35 fish $m^{-3}$) and T65 (540 shrimp $m^{-3}$ + 65 fish $m^{-3}$) throughout the experimental period (mean values ± standard deviation).

| | T35 | T65 |
|---|---|---|
| Temperature (°C) | 28.3 ± 1.9 | 28.4 ± 1.8 |
| DO (mg $L^{-1}$) | 6.1 ± 0.6 | 6.0 ± 0.6 |
| pH | 7.5 ± 0.3 | 7.5± 0.3 |
| Alkalinity (mg $CaCO_3$ $L^{-1}$) | 143.6 ±35.2[a] | 134.9 ± 33.2[b] |
| Salinity (g $L^{-1}$) | 14.2 ± 1.1 | 14.4 ± 0.8 |
| TSS (mg $L^{-1}$) | 436.6 ± 140.5 | 457.2 ± 135.5 |
| Turbidity (NTU) | 247.0 ± 81.5 | 240.1 ± 94.2 |
| Clarification time (hours) | 32.0 ± 18.3 | 52.0 ± 18.3 |
| TAN (mg $L^{-1}$) | 0.01 ± 0.01 | 0.01 ± 0.01 |
| Nitrite (mg $L^{-1}$) | 0.6 ± 0.6[a] | 1.1 ± 1.5[b] |
| Nitrate (mg $L^{-1}$) | 68.7 ± 53.7 | 70.3 ± 53.5 |
| Orthophosphate (mg $L^{-1}$) | 2.0 ± 1.5 | 2.2 ± 1.61 |

DO = dissolved oxygen; TSS=total suspended solids; TAN = total ammonia nitrogen. Different letters in the same line represent significant differences ($p < 0.05$) among treatments after t- test.

*TSS dynamics*

The TSS concentrations and turbidity did not show statistical differences ($p > 0.05$) between treatments. The management of total suspended solids (clarification) was necessary in both treatments, in order to maintain the TSS values close to 500 mg $L^{-1}$. The clarification was performed during 52 ± 18.3 h in T65 and for 32 ± 18.3h in T35, along all the experimental period (Table 2).

*Growth performance*

Fish weight growth was significantly higher ($p < 0.05$) in T35 than in T65 treatments. The productivity was higher in the T65 treatment. Survival and FCR, were similar in both treatments (Table 2).

For the IMTA system (shrimp + fish), the total final biomass was statistically higher in the T65 treatment. The total productivity of the system as a whole and the FCR showed no statistical differences between treatments (Table 3).

Table 3. Performance of *L. vannamei* and *O. niloticus* (mean values ± standard deviation) during the experimental period.

| | T35 | T65 |
|---|---|---|
| **Shrimp** | | |
| Survival (%) | 75.9±0.2 | 88.2±0.02 |
| Initial weight (g) | 0.9±0.1 | 0.9±0.1 |
| Final weight (g) | 11.5±1.9 | 10.1±0.7 |
| Final biomass (kg) | 73.1±12.2 | 75.4±2.7 |
| WWG (g $week^{-1}$) | 0.95±0.16 | 0.83±0.07 |
| FCR | 1.7±0.2 | 1.6±0.03 |
| Productivity (kg $m^{-3}$) | 6.8±1.3 | 7.0±0.3 |
| **Tilapia** | | |
| Survival (%) | 99.5±0.008 | 100±0.0 |
| Initial weight (g) | 7.1±3.2 | 7.1±3.2 |
| Final weight (g) | $127.4±10.9^{a}$ | $99.6±6.5^{b}$ |
| Final biomass (kg) | 17.8±1.5 | 25.9±1.6 |
| WWG (g $week^{-1}$) | $10.79±0.98^{b}$ | $8.3±0.58^{a}$ |
| FCR | 0.7±0.03 | 0.7±0.04 |
| Productivity (kg $m^{-3}$) | $4.3±0.2^{b}$ | $6.3±0.6^{a}$ |
| **System** | | |
| Final biomass (kg) | $55.5±18.6^{b}$ | $67.3±4.6^{a}$ |
| Productivity (kg $m^{-3}$) | 6.1±1.0 | 6.7±0.2 |
| FCR | 1.7±0.3 | 1.6±0.1 |

WWG = weekly weight growth; FCR = feed conversion rate. Different letters in the same line represent significant differences ($P < 0.05$) in t-test performed among treatments.

The results obtained in the present study leads to conclude that the integrated culture of *L. vannamei* and *O. niloticus* in super-intensive systems using BFT is possible, which allows to reduce fish feeding rates without negatively influencing their growth. When higher densities of tilapia are used (65 fish $m^3$), there is an increase in the total suspended solids concentrations in the water, also increasing the clarification time required to keep this concentration within the suitable levels for the species. This study, on a pilot scale, proves that the IMTA systems of tilapia with *L. vannamei* based on bioflocs diversifies production without compromising the productivity of shrimp.

---

Experiment 3

**Use of different probiotic bacteria in super intensive culture of *Litopenaeus vannamei* in the nursery and grow out phases in clear water and BFT system**

This study aimed to analyse the effect of probiotic bacteria on the control of organic material (water quality) and zootechnical performance of *L. vannamei* in both the nursery and grow out stages in super intensive culture in clear water and BFT system.

**MATERIAL AND METHODS**

The experiment was carried out at the Marine Aquaculture Station of the Oceanography Institute of the Federal University of Rio Grande-FURG, Rio Grande/RS (EMA/FURG).

Nursery phase

The experiment was carried in an experimental room with temperature and light control. The animals came from Aquatec® (Rio Grande do Norte) where they arrived in nauplii stage and went through the hatchery phase performed in our laboratory. Post-larvae with an average weight of 0.012 g (±0.001) were stocked at a stocking density of 2000 shrimp $m^{-2}$. Twelve 150 L tanks were used, three control tanks without probiotics (CW-CTL), three tanks with probiotic in the feed and water (CW-PROB) both in clear water, three tanks without probiotic (BFT-CTL), three tanks with probiotic in the feed and water (BFT-PROB) both in BFT system. The tanks have a bottom area of 0.49 m2, thus, 300 post-larvae were stocked per tank, totalling 3600 animals. The experiment lasted 35 days.

Growout phase

Post-larvae with an average weight of 0.45 g (±0.1) were stocked at a stocking density of 300 shrimp m-2. Twelve 150 L tanks were used, three control tanks without probiotics (CW-CTL), three tanks

with probiotic in the feed and water (CW-PROB) both in clear water, three tanks without probiotics (BFT-CTL), three tanks with biofloc and probiotic in the in the feed and water (BFT-PROB). The tanks have a bottom area of 0.49 $m^2$, thus, 45 post-larvae were stocked per tank, totalling 540 animals. The experiment lasted 63 days.

The commercial probiotic is composed of the following bacteria and their respective concentrations: *Bacillus subtilis* (3.4x$10^9$ CFU g-1), *Lactobacillus plantarum* (1.2x$10^9$ CFU g-1) and *Pediococcus acidilactici* (1.2x$10^9$ CFU g-1), using lactose (639g kg-1) as a carrier. The dosages recommended by the manufacturer were applied in the feed (2.0 kg probiotic/ton of feed) and weekly doses of 1g/ton of water were used in the water. The shrimp were fed twice a day, using commercial Active 40% CP feed (Guabi) in the nursery phase and for the growout phase commercial Active 40% CP feed (Guabi), as recommended by Jory et al (2001). Daily renewals of 50% of the total volume (75L) were made, simulating a clear water culture system.

**RESULTS - NURSERY PHASE**

Water quality parameters

Table 4: Mean (± standard deviation) of physical and chemical water parameters in the different treatments during 35 days of *L. vannamei* culture in the nursery phase.

| Nursery | CW-CTL | CW-PROB | BFT-CTL | BFT-PROB |
|---|---|---|---|---|
| Temperature (°C) | 28.6±0.5 | 27.6±0.2 | 28.5±0.6 | 28.4±0.7 |
| DO (mg $L^{-1}$) | 5.9±0.2 | 6.0±0.1 | 5.8±0.2 | 5.9±0.2 |
| pH | 8.2±0.01 | 8.2±0.0 | 8.2±0.0 | 8.2±0.0 |
| Alkalinity (mg $CaCO_3$ $L^{-1}$) | 205.0±17.2 | 207.0±5.2 | 202.0±13.3 | 207.3±8.6 |
| Total Ammonia Nitrogen (TA - N mg $L^{-1}$) | 2.5±0.5 | 3.0±0.6 | 3.0±0.7 | 2.9±0.6 |
| Nitrite ($NO_{2-}$ - N mg $L^{-1}$ | 2.5±0.8 | 2.0±0.3 | 2.6±0.5 | 1.8±0.5 |
| Nitrate ($NO_{3-}$ - N mg $L^{-1}$) | 1.4±0.2$^a$ | 1.0±0.2$^a$ | 21.1±0.2$^b$ | 19.6±0.5$^b$ |
| Phosphate (P-$PO_4^{-3}$ mg $L^{-1}$) | 0.1±0.0$^a$ | 0.1±0.0$^a$ | 1.3±0.0$^b$ | 1.1±0.0$^b$ |
| Salinity | 30.7±0.2 | 30.5±0.1 | 30.8±0.2 | 31.0±0.5 |
| TSS (mg $L^{-1}$) | 80.7±27.3$^a$ | 89.7±33.6$^a$ | 389.7±99.6$^b$ | 380.7±87.3$^b$ |

Different letters in the same line represent significant differences ($P < 0.05$) in t-test.

The results obtained for shrimp performance are expressed in Table 5.

Table 5: Mean (standard deviation) of zootechnical performance parameters in the different treatments during 35 days of super-intensive rearing of *L. vannamei* in the nursery phase.

| Nursery | CW-CTL | CW-PROB | BFT-CTL | BFT-PROB |
|---|---|---|---|---|
| Initial weight (g) | 0.012±0.001 | 0.012±0.001 | 0.012±0.001 | 0.012±0.001 |
| Final Weight (g) | 0.74±0.2$^{b}$ | 0.84±0.1$^{b}$ | 1.07±0.2$^{a}$ | 1.13±0.1$^{a}$ |
| Survival (%) | 88.89±5.1$^{b}$ | 91.89±7.7$^{a}$ | 99.89±5.1$^{a}$ | 94.89±4.6$^{a}$ |
| FCR | 2.29±0.5$^{b}$ | 1.93±0.1$^{a}$ | 1.88±0.3$^{a}$ | 1.84±0.2$^{a}$ |
| Final biomass (kg) | 0.20±0.0$^{b}$ | 0.23±0.0$^{b}$ | 0.32±0.0$^{a}$ | 0.32±0.0$^{a}$ |
| Productivity (kg m$^{2}$) | 0.40±0.0$^{b}$ | 0.47±0.1$^{b}$ | 0.65±0.1$^{a}$ | 0.65±0.1$^{a}$ |

**RESULTS - GROWOUT PHASE**

Water quality parameters

Table 6: Mean (± standard deviation) of physical and chemical water quality parameters in the different treatments during the 63 days of *L. vannamei* culture in the growout phase.

| Growout phase | CW-CTL | CW-PROB | BFT-CTL | BFT-PROB |
|---|---|---|---|---|
| Temperature (°C) | 28.9±0.6 | 28.8±0.8 | 28.6±0.5 | 28.5±0.6 |
| D. O (mg/L) | 6.0±0.1 | 6.0±0.1 | 5.9±0.1 | 5.9±0.1 |
| pH | 7.9±0.0 | 7.9±0.0 | 7.4±0.0 | 7.4±0.0 |
| Alkalinity (mg $CaCO_3$/L) | 130.5±7.5 | 133.8±13.9 | 130.9±9.0 | 133.5±6.3 |
| TAN (mg/L) | 0.3±0.1 | 0.4±0.0 | 0.4±0.1 | 0.4±0.1 |
| Nitrite (mg/L) | 2.9±1.0 | 3.6±1.1 | 4.3±0.9 | 4.5±1.5 |
| Nitrate (mg/L) | 5.8±0.7 | 5.1±1.7 | 35.3±1.0 | 34.7±0.7 |
| Phosphate (mg/L) | 0.4±0.1 | 0.4±0.1 | 1.4±0.1 | 1.5±0.1 |
| Salinity | 31.9±1.1 | 31.7±1.0 | 32.5±1.2 | 32.5±1.1 |
| TSS (mg/L) | 89.0±19.2 | 94.9±25.1 | 421.4±28.6 | 433.0±23.0 |

The results obtained for zootechnical performance are expressed in Table 7.

Table 7: Mean (standard deviation) of zootechnical performance parameters in the different treatments during 63 days of super intensive culture of *L. vannamei* in the growout phase.

| Growout phase | CW-CTL | CW-PROB | BFT-CTL | BFT-PROB |
|---|---|---|---|---|
| Initial weight (g) | 0.4±0.1 | 0.45±0.1 | 0.45±0.1 | 0.45±0.1 |
| Final weight (g) | 6.5±0.9$^{b}$ | 8.15±0.90$^{a}$ | 8.29±0.89$^{a}$ | 8.44±0.88$^{a}$ |
| Survival (%) | 87.0±0.0$^{b}$ | 91.00±0.01$^{a}$ | 100.00±0.02$^{a}$ | 99.29±0.02$^{a}$ |
| FCR | 1.7±0.03$^{b}$ | 1.4±0.11$^{a}$ | 1.4±0.12$^{a}$ | 1.4±0.20$^{a}$ |
| Weekly weight gain (g) | 1.6±0.1$^{b}$ | 2.01±0.23$^{a}$ | 2.05±0.22$^{a}$ | 2.08±0.28$^{a}$ |
| Final biomass (kg) | 3.05±0.2$^{b}$ | 4.00±0.5$^{a}$ | 4.48±0.4$^{a}$ | 4.52±0.3$^{a}$ |
| Productivity (kg m$^{2}$) | 6.22±0.4$^{b}$ | 8.16±1.02$^{a}$ | 9.14±0.81$^{a}$ | 9.22±0.61$^{a}$ |

The results of the present study indicate that the use of probiotic in the water and feed during super intensive culture of *L. vannamei* shrimp in clear water and BFT system contributed to better zootechnical performance in all growth and survival parameters, hence evidencing its efficacy as a high-performance probiotic, creating a safe environment where lower or zero water renewal rates are required.

---

Experiment 4

**Effects of total suspended solids on the growth, nutrient absorption rate of green macroalgae, *Ulva fasciata* and *Ulva flexuosa*, cultivated in pacific white shrimp, *Litopenaeus vannamei,* effluent in a biofloc system**

This study aimed to evaluate the effects of different concentrations of TSS and culture media on the growth of the macroalgae *Ulva flexuosa* and *Ulva fasciata*, evaluating their absorption of nutrients and nitrogen stored in their tissue during culture in shrimp effluent in a biofloc system.

**Materials and methods**

The experiments were carried out in the laboratory at the Marine Aquaculture Centre (EMA), Institute of Oceanography at the Federal University of Rio Grande (FURG), located at Cassino beach, Rio Grande, Rio Grande do Sul State.

*Ulva flexuosa* and *Ulva fasciata* samples were collected from a shipwreck substrate (Navio Altair), located on Cassino Beach (32° 17.523' S / 52° 15.598' W). The samples were transferred to the laboratory for the removal of epiphytes and associated fauna and then moved to plastic culture units filled with seawater, with constant aeration and 12 hours light: dark photoperiod for acclimatization. The marine shrimp, *Litopenaeus vannamei*, effluent, was obtained from an intensive cultivation raceway system and it was used in all the experiments.

*Experiments on Ulva fasciata*

The experimental design included four treatments with three replicates (4x3). Control group (CONT) included an effluent from a fattening system of shrimp production, where TSS is 370 mg $L^{-1}$. BFT group (BFT) included the cultivation of *U. fasciata* algae in effluent from shrimp fattening in a biofloc system where TSS is 400 mg $L^{-1}$. DEC group (DEC) included the culture of *U. fasciata* algae in effluent from shrimp fattening in a biofloc system, after decantation period, where TSS is 30 mg $L^{-1}$. VS (Von-Stosch Solution) group (VS) included the culture of *U. fasciata* in the standard Von Stosch enrichment solution at a concentration of 10ml $L^{-1}$.

The experiment was conducted for 35 days under controlled temperature conditions. A total of 12-carboy, with 3-liter volume capacity for each carboy, was used for macroalgae culture. Constant aeration was obtained by an air-blower that directed to the air-stones. An artificial lighting controlled by a timer with 12:12hours of light:dark photoperiod.

Experiments on *Ulva flexuosa*

This experimental system consisted of three treatments with three replicates, namely: BFT where *U. flexuosa* grown in fresh biofloc effluent with an initial TSS concentration of 650 mg $L^{-1}$; DEC where *U. flexuosa* grown in biofloc effluent after decantation, with initial SST concentration of 65 mg $L^{-1}$; CON where effluent from fresh biofloc was used without including algae.

The experiment was conducted for 17 days under controlled temperature conditions. Constant aeration, was obtained by an air-blower that directed to the air stones and artificial lighting was controlled by a timer with 12:12 hours of light:dark photoperiod. Nine 4 litres bottles were used for the culture of macroalgae.

*Macroalgae performance*

The growth of macroalgae was measured weekly biomass yields, after the removal of excess water by using a absorbent papers. The following formulas were used to determine the daily growth rate, in terms of percent relative growth rate (RGR) and nutrient removal rate (NRR) of macroalgae: RGR (%.$day^{-1}$): [ln (final weight (g) / initial weight (g)) / (final time / initial time) x 100]

NRR (%): [(Concentration of nutrient in the initial time (mg $L^{-1}$) - Concentration of nutrients in the final time (mg $L^{-1}$)) / (Concentration of nutrient in the initial time) -1] x100 (Du et al, 2013).

*Protein analysis in algae thallus*

The samples for protein analysis were taken from each experimental unit at the beginning and at the end of the experiment with *U. fasciata* and then transferred to the Laboratory of Nutrition of Aquatic Organisms-LANOA. The nitrogen content of the algae was determined by using Kjeldahl titration method according to AOAC (1990). The formula used for converting nitrogen to protein was:

Protein (% of dry weight) = $[\left(\frac{0,1xVolx0,014}{Sample}\right) x\ 5,45]x100$

Where, *Vol* is the volume spent on titration and *Sample* is the weight of the sample (Baethgen and Alley, 1989).

**Results**

*Experiments on U. fasciata*

At the beginning, the macroalgae had an initial weight of 6.22 ± 0.10 g with a density of 2g $L^{-1}$ in the 3 L carboys. There was no significant difference ($p \leq 0.05$) in the final weight of the algae between treatments, showing only an increase in Relative Growth Rate (RGR) in all treatments (Table 8). Therefore, the final weight of the algae was higher ($p \leq 0.05$) than it weights at the beginning of the experiment (Figure 14).

Protein concentrations evaluated at the end of culture and showed a significant difference ($p \leq 0.05$) between the treatment groups. The lower protein value was recorded in the experimental group which macroalgae was grown in von Stosch (VS) enrichment solution (Table 8).

Table 8: Initial and final average weights (mean ± standard deviation), relative growth rates, and protein contents (mean ± sd) of *U. fasciata* growth in the treatments of BFT, DEC and VS.

| | Treatments | | |
|---|---|---|---|
| **Parameters** | **BFT** | **DEC** | **VS** |
| **Initial weight (g)** | 6.24±0.12 | 6.16±0.06 | 6.26±0.11 |
| **Final weight (g)** | 9.13±2.19 | 7.94±1.10 | 8.32±1.82 |
| **RGR (%/day)** | 1.03±0.70 | 0.71±0.41 | 0.76±0.64 |
| **Protein content (%)** | 21.15±0.62 | 22.44±1.31 | 12.40±2.73 |

Different lower-case letters represent a significant difference ($p \leq 0.05$) between treatments after one-way ANOVA with Tukey's post-hoc test. RGR (relative growth rate).

Table 9. Initial water TSS, TAN and nutrient composition (mean ± standard deviation) in the Control, BFT, DEC and VS treatment groups.

| | | Treatments | | |
|---|---|---|---|---|
| **Initial Parameters** | **Control** | **BFT** | **DEC** | **VS** |
| **TSS (mg/L)** | 370.0±0.10 | 400.0±0.10 | 30.0±0.06 | - |
| **TAN (mg/L)** | 0.27±0.00 | 0.12±0.00 | 0.12±0.01 | 0.10±0.02 |
| **Nitrite (mg/L)** | 0.72±0.70 | 0.42±0.70 | 0.35±0.01 | 0.09±0.00 |
| **Nitrate (mg/L)** | 54.00±0.01 | 66.0±0.00 | 60.0±0.10 | 5.00±0.03 |
| **Phosphate (mg/L)** | 7.80±0.00 | 5.6±0.02 | 5.6±0.00 | 1.2±0.00 |

*TSS (total suspended solids), TAN (total ammonia nitrogen)

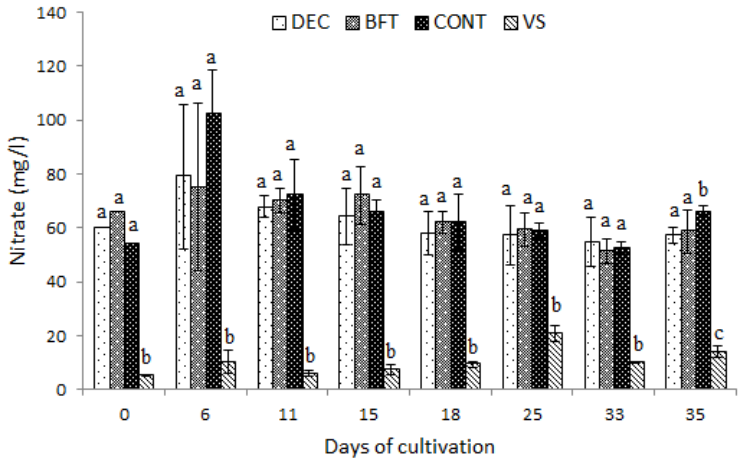



Figure 14 – Weekly nitrate concentrations in the DEC, BFT, CONT and VS treatments, during the 35 days of experimental period. Different lower-case letters on the same day represent a significant difference ($p \leq 0.05$) among the treatment groups after one-way ANOVA with Tukey post-hoc test.

Table 10. Water quality parameters (mean ± standard deviation) in treatments: Control, BFT, DEC and VS, during the 35 days of experimental period.

| | Treatments | | | |
|---|---|---|---|---|
| **Initial Parameters** | **Control** | **BFT** | **DEC** | **VS** |
| **Temperature** | 26.5 | 26.6 | 26.6 | 26.5 |
| **DO (mg/L)** | 7.59 | 7.60 | 7.79 | 7.81 |
| **pH** | 8.35 | 8.35 | 8.36 | 8.37 |
| **Salinity** | 29.42 | 29.24 | 29.11 | 29.27 |
| **Alkalinity (mg $CaCO_3$/L)** | 145.0 | 145.8 | 140.3 | - |
| **TSS (mg/L)** | 210 | 228 | 23 | - |
| **TAN (mg/L)** | 0.12 | 0.09 | 0.1 | 0.09 |
| **Nitrite (mg/L)** | $0.16^a$ | $0.17^a$ | $0.16^a$ | $0.01^b$ |
| **Nitrate (mg/L)** | $66.7^a$ | $64.3^a$ | $62.3^a$ | $10.3^b$ |
| **Phosphate (mg/L)** | $4.62^a$ | $4.54^a$ | $3.40^a$ | $0.96^b$ |

Different letters on the same line indicate significant differences ($p \leq 0.05$) between treatments after one-way ANOVA with Tukey's post-hoc test. DO (dissolved oxygen), TSS (total suspended solids), TAN (total ammonia nitrogen).

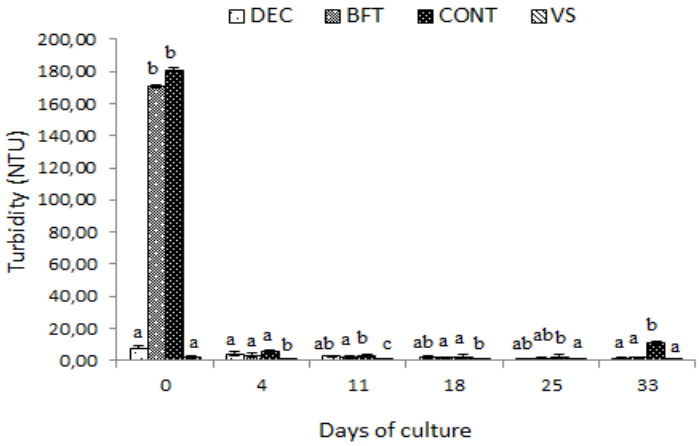



Figure 15 – Weekly Turbidity values in the DEC, BFT, CONT and VS treatments, during the 35 days of experimental period. Different lower-case letters on the same day represent a significant difference ($p \leq 0.05$) among treatments after one-way ANOVA with Tukey test.

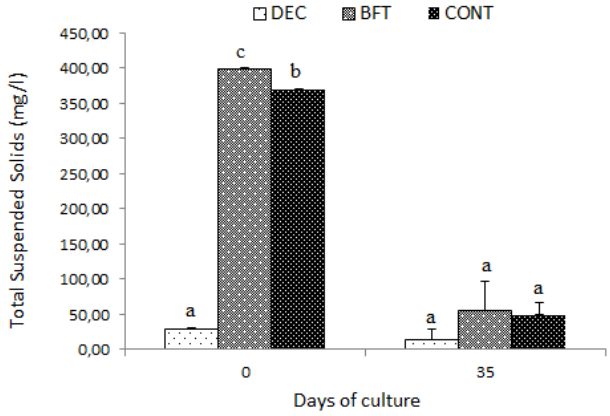



Figure 16 - Concentrations of total suspended solids (TSS) in the DEC, BFT and CONT treatments at the beginning and at the end of the experimental period. Different lower-case letters on the same day represent a significant difference ($p \leq 0.05$) among treatments.

### *3.2 Experiments on U. flexuosa*

At the beginning, the macroalgae had an initial average weight of 12.11 ± 0.14 g with a density of 3 $g.l^{-1}$ in the 4 L carboys. There was no significant difference ($p \leq 0.05$) in the final weight and RGR of the *U. flexuosa* between the treatments (Table 11). The macroalgae in the BFT treatment grew significantly ($p \leq 0.05$) until the first 10 days correlated with the availability of phosphate in the water (Figure 17).

Table 11 - *U. flexuosa* performance in treatments of BFT and DEC, during the 17 days of experimental period.

| Parameters | Treatments | |
|---|---|---|
| | BFT | DEC |
| Initial biomass yield (g) | 12.05 | 12.10 |
| Final biomass yield (g) | 23.91 | 16.60 |
| RGR (%/day) | 3.92 | 3.15 |

RGR (Relative Growth Rate).

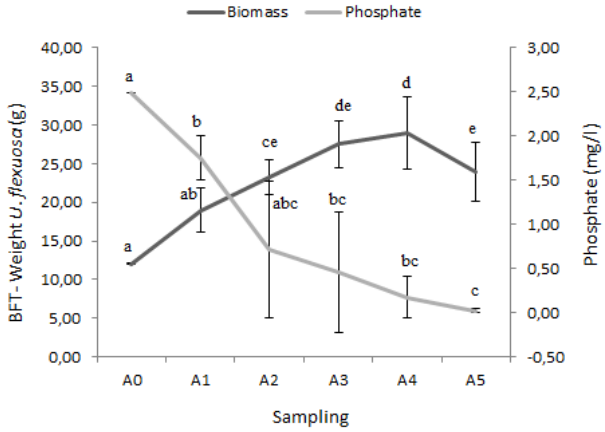



Figure 17 – Weekly biomass yields (g) of *U. flexuosa* and phosphate concentration (mg. $l^{-1}$) in BFT treatment group. Different letters indicate a significant difference ($p \leq 0.05$) among the weeks of the same treatment after performing a one-way ANOVA followed with Tukey's Test.

Table 12 - Initial TSS, TAN and nutrient values (mean ± standard deviation) of the Control, BFT, and DEC during the 17 days of culture.

| Parameters | Treatments | | |
|---|---|---|---|
| | Control | BFT | DEC |
| TSS (mg/L) | 650 | 650 | 55 |
| TAN (mg/L) | 0.08 | 0.09 | 0.08 |
| Nitrite (mg/L) | 0.09 | 0.09 | 0.08 |
| Nitrate (mg/L) | 35 | 36 | 34 |
| Phosphate (mg/L) | 2.5 | 2.6 | 2.6 |

TSS (total suspended solids), TAN (total ammonia nitrogen).

Table 13 - Water quality parameters (mean ± standard deviation) in treatments of BFT (biofloc natural effluent), DEC (biofloc decanted effluent) and CON (fresh effluent without algae), during the 17 days of experimental period.

| Initial Parameters | Treatments Control | BFT | DEC |
|---|---|---|---|
| Temperature | 18 | 17.9 | 18 |
| DO (mg/L) | 8.3 | 8.5 | 8.4 |
| pH | 8.38 | 8.44 | 8.46 |
| Salinity | 29 | 29 | 28 |
| TAN (mg/L) | 0.06 | 0.07 | 0.05 |
| Nitrite (mg/L) | $0.09^{b}$ | $0.07^{a}$ | $0.07^{a}$ |
| Nitrate (mg/L) | $28.3^{b}$ | $27.3^{a}$ | $27.6^{a}$ |
| Phosphate (mg/L) | $2.82^{b}$ | $0.94^{a}$ | $1.08^{a}$ |

Different letters on the same line indicate significant differences ($p \leq 0.05$) among treatments after one-way ANOVA with Tukey's post-hoc test. DO (dissolved oxygen), TSS (total suspended solids), TAN (total ammonia nitrogen).

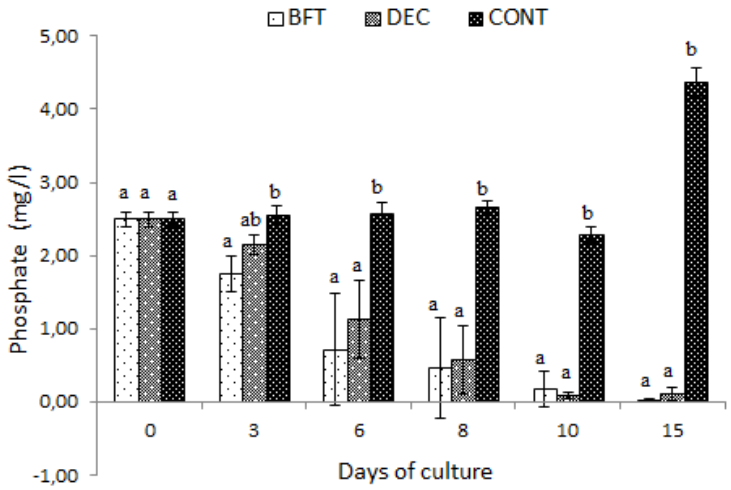



Figure 18 – Weekly Phosphate concentrations in the DEC, BFT and CONT treatments, during the 17 days of experimental period. Different lower-case letters represent a significant difference ($p \leq 0.05$) among treatments after one-way ANOVA with Tukey's test.

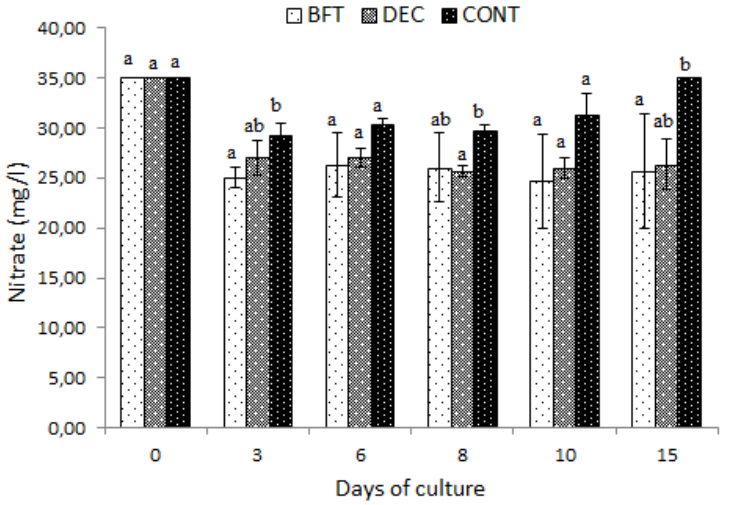


Figure 19 – Weekly nitrate concentrations in the DEC, BFT and CONT treatments, during the 17 days of experimental period. Different lower-case letters represent a significant difference ($p \leq 0.05$) between treatments after one-way ANOVA with Tukey's test.

Based on these results, it can be concluded that the macroalgae *Ulva fasciata* and *Ulva flexuosa* grew when cultivated in shrimp farming effluent in a biofloc system, which can be a substitute for laboratory culture media. The use of the macroalgae *U. flexuosa* as a phytoremediator was also verified in this study, with the absorption of ammonia, nitrate and phosphate from the system, removing 30% of available nitrate over the course of a week and also removing 80% of available phosphate in the same period. The culture of macroalgae in effluent in a biofloc system represents a viable, low-cost and suitable alternative, with the production of a biomass with higher protein content.

### 3.1.4 Conclusions

Based on the results obtained over these 18 months, it was possible to define some parameters that guide the operation of our system, including the definition of the oyster species to be used as filtering bivalve to help controlling biofloc system. In our system the oyster *Crassostrea gasar* showed better adaptability to the characteristics of the system, tolerating TSS concentrations and the high temperatures. We also verified that the use of probiotics in the system led to an improvement in the zootechnical performance of the shrimp and in the water quality of the system, making their use a routine in all experiments performed in our IMTA system.

After defining the Nile tilapia *O. niloticus* as the most suitable fish species for the growing conditions, we determined the fish density that is necessary to keep TSS levels under control, so when associated with shrimp they do not produce more particulate matter that can be biologically removed from the system.

Regarding the uptake of dissolved nutrients, the tested macroalgae proved to be effective in reducing nitrogen and phosphate compounds, keeping them on safe levels for other organisms that integrate the IMTA system.

# 4 IMTA South Africa

## 4.1 Site description

The tasks undertaken by IMTA lab South Africa take place at two sites: (1) the Department of Forestry, Fisheries and the Environment (DEFF) Marine Research Aquarium (MRA) and (2) Viking Aquaculture (Buffeljags abalone farm).

The MRA is a government facility that is dedicated to aquaculture research and is based in Sea Point near the city of Cape Town (33° 55' 6.492'' S, 18° 22' 52.572'' E). The aquarium possesses the most extensive marine aquaculture facilities in the country. The multifunctional facility has wet and dry laboratories; algal culture laboratories; live animal and broodstock holding facilities; hatcheries, larval rearing and grow-out facilities; and fully equipped molecular, parasitology, microscopy and chemistry laboratories. The MRA is the site of the sea urchin (*Tripneustes gratilla*) broodstock holding facility, hatchery and larval rearing facility that produces juveniles to stock the urchin-*Ulva* IMTA at Buffeljags abalone. Laboratory work (sample processing and analysis) and smaller scale research experiments — in a more controlled environment — will also be carried out at this site.


Figure 20: The Marine Research Aquarium, Sea Point, South Africa (A), which is the site of the sea urchin broodstock holding facility, hatchery and larval rearing facility (B).

Buffeljags abalone farm is a commercial aquafarm run by partner Viking Aquaculture. It is ca. 200 km east of Cape Town, situated on a pristine stretch of coastline near the remote settlement of Buffeljags on the Cape south coast (34°45’14.7“ S 19°36’51.9” E). The farm is modern, efficient and environmentally sustainable and is one of the first large commercial abalone farms in South Africa to

consistently recirculate ca. 50% of their seawater by making use of the bio-remediation capacity of *Ulva* (Fig. 21). The farm produces ca. 350 tons abalone and ca. 500 tons of *Ulva* annually.

Figure 21: Aerial overview of Buffeljags abalone farm (a subsidiary of Viking Aquaculture)

### 4.1.1 Species

Species cultivated at IMTA Lab South Africa: South African abalone (*Haliotis midae*), sea lettuce (*Ulva lacinulata*), collector sea urchin (*Tripneustes gratilla*) and Cape urchin (*Parechinus angulosus*).

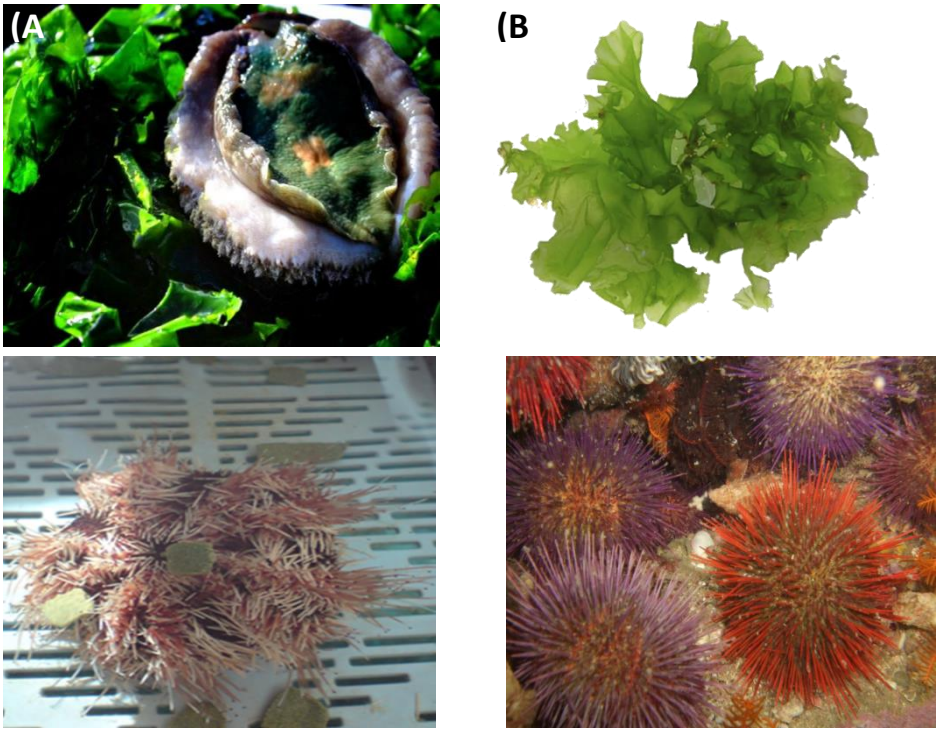


Figure 22: Species cultivated at IMTA Lab South Africa. (A) South African abalone, *Haliotis midae*; (B) sea lettuce, *Ulva lacinulata*; (C) collector sea urchin, *Tripneustes gratilla*; and (D) Cape urchin, *Parechinus angulosus*.

## System Design

*Abalone-Ulva IMTA system*

Buffeljags abalone farm currently has seven modular abalone-*Ulva* IMTA systems, called platforms, which are each composed of four clusters, each consisting of one Ulva paddle-raceway and several abalone tanks (6 rows each made of 7 abalone raceway tanks) (Fig. 23). Effluent water from the abalone tanks in each cluster flows into an adjacent Ulva paddle-raceway and ca. 50% of this bio-remediated water is then recirculated back to the abalone tanks in the same cluster. The remaining 50% replacement seawater is pumped directly from the ocean. Each cluster is regarded as an independent unit, with no sharing of water or fomites between clusters for bio-security reasons. The abalone in each cluster are grown in baskets suspended in sturdy glass fibre tanks. Seawater is circulated through the tanks and Ulva paddle-raceways, ensuring a constant supply of cool, aerated water for the growing abalone. Animals are fed on a combination diet consisting of freshly harvested kelp (*Ecklonia maxima*), farm produced *Ulva lacinulata* and formulated food.


Figure 23: Land-based pump-ashore IMTA system for the production of abalone (*Haliotis midae*) and seaweed (*Ulva lacinulata*) at Buffeljags abalone. The photograph shows the modular abalone-Ulva systems, arranged as seven platforms on the farm, with each platform composed of four clusters, each consisting of one Ulva paddle-raceway and several abalone tanks.

*Urchin-Ulva IMTA system*

The urchin-Ulva IMTA system at Buffeljags abalone farm has 5 × 8000 L glass fibre tanks (Fig. 24A) that are interconnected with a 14000 L Ulva paddle-raceway (Fig. 24B) and a 14000 L sump. The system is fitted with a drum-filter, protein skimmers and a heat-pump to maintain a constant temperature of ca. 25°C. The entire system is housed within a tunnel to conserve heat.

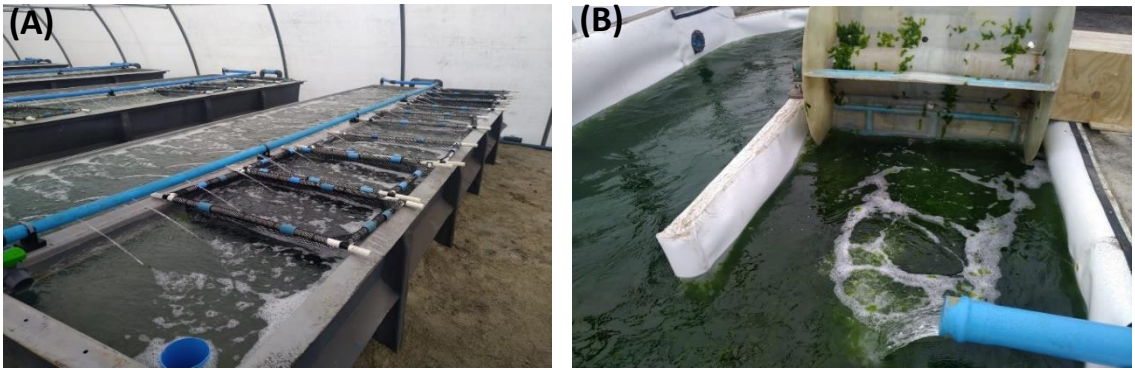



Figure 24: Land-based pump-ashore IMTA system for the production of sea urchin (*Tripneustes gratilla*) and seaweed (*Ulva lacinulata*) at Buffeljags abalone. The photograph shows (A) the sea urchin tanks and (B) the Ulva paddle-raceway systems.

Effluent water exiting each of the urchin tanks flows into the Ulva paddle-raceway, after passing through the drum-filter to remove larger particulates. The bio-remediated seawater exiting the Ulva paddle-raceway enters the sump before being recirculated back to the sea urchin tanks. The water replacement in this system, with fresh incoming seawater from the adjacent ocean, is 50% per day. The sea urchins in each tank are grown in baskets suspended in sturdy glass fibre tanks, with 22 baskets suspended in each glass fibre tank. Seawater is circulated through the tanks and Ulva paddle-raceways, ensuring a constant supply of heated, aerated water for the growing sea urchins. Animals are fed on a combination diet consisting of freshly harvested farmed *Ulva lacinulata* and formulated food containing 20% *Ulva* (Cyrus *et al*., 2014).

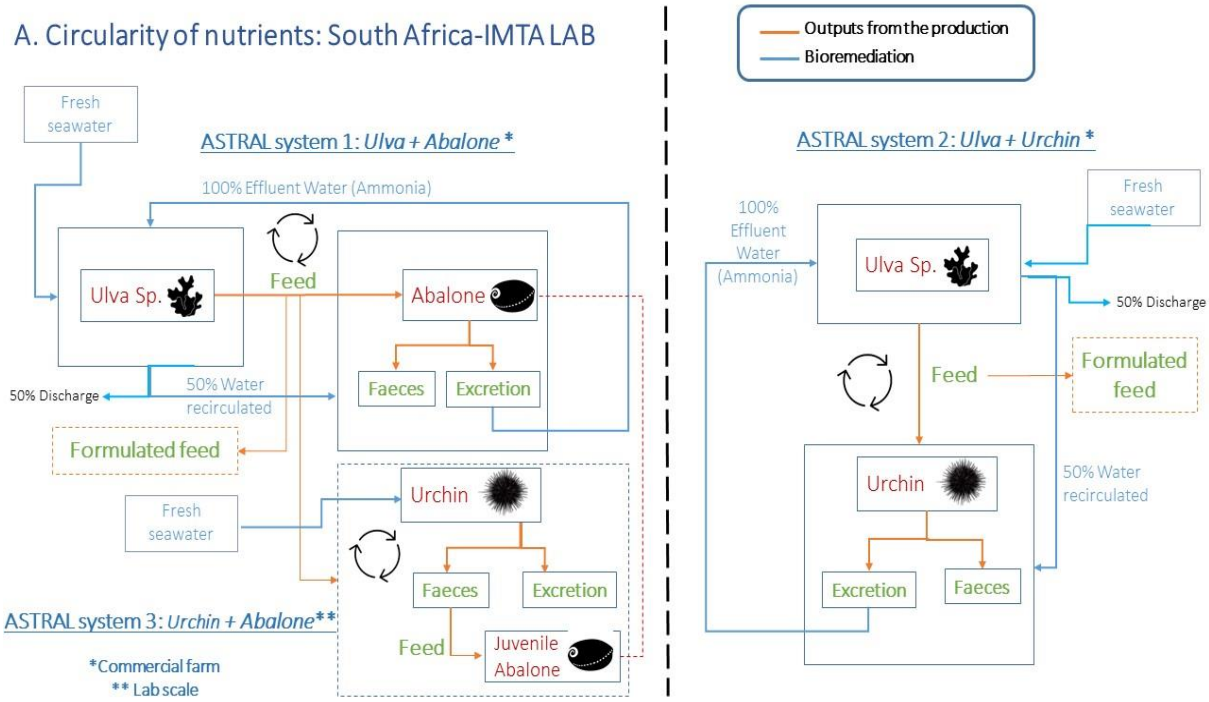



Figure 25: Schematic of the circulation of nutrients within the 3 experimental systems at Buffeljags Abalone farm.

### 4.1.2 Experiment

**Monitoring the physical and chemical parameters of the abalone-Ulva IMTA system with increasing recirculation rates**

*Aim*

Bufflejags abalone currently operates the abalone-Ulva IMTA system with 50% recirculation. This study was conducted to determine the effects of increasing recirculation rates, increasing recirculation from 50% to 75% and finally to 100% recirculation, on the physical and chemical parameters of the abalone-*Ulva* IMTA system. The microbial community of the IMTA operating under different recirculation rates will also be assessed to provide critical information on biosecurity, species health and system health (see deliverable 2.1, Task 2.3).

*Methodology*

A trial was conducted to determine the effects of increasing recirculation rates on the physical and chemical parameters of the abalone-Ulva IMTA system at Buffeljags abalone farm. Monitoring also included an assessment of the microbial community to provide critical information on biosecurity, species health and system health (see deliverable 2.1, Task 2.3). Three abalone-Ulva clusters

(Clusters 2.1, 2.2 & 3.1) at Buffeljags abalone were selected for this study, each containing approximately 15 tons of abalone and 3.5 tons of Ulva.

The 50% recirculation trial started on the 20th of July 2021 (**M11**) and ran for approximately one month before increasing the recirculation in the system to 75% on the 25th of August 2021 (**M12**). The 75% recirculating was maintained for a period of one month, ending on the 27th of September 2021 (**M13**). During the 50% and 75% recirculation trials, pH, temperature and dissolved oxygen (DO) was monitored daily, whereas samples for nutrient (phosphate, nitrate, nitrite and ammonia) analysis were collected once a week in the morning (08H00) and afternoon (13H00). The latter samples were immediately frozen until being analysed at the laboratories in Sea Point (Cape Town). Hobo pH/temp data loggers were also deployed in each cluster — in one of the abalone raceway — for continuous monitoring of pH and temperature, both critical variables in the IMTA. The 100% recirculation trial commenced on the 8th of November 2021 (**M15**) and the systems were maintained at full recirculation for ca. 3 days. During the 100% recirculation trial, the system was intensively monitored (once every 2 hours) to ensure optimal water quality and welfare for animals. Comprehensive water quality assessments were conducted in the IMTA systems during 100% recirculation. The pH, temperature and DO were recorded once every two hours, whereas nutrients (TAN & FAN) were recorded *in situ* (on the farm) once every four hours. OxyGuard Handy Polaris hand-held pH, temperature and DO meters were utilised for measuring these parameters. Total ammonia nitrogen (TAN) was recorded *in situ* using a Lovibond® MD 600 Photometer and the recorded TAN, pH, temperature, and salinity values were used to calculate the free un-ionized ammonia (FAN) using a free ammonia calculator (https://www.hamzasreef.com/Contents/Calculators/FreeAmmonia.php). In addition to the *in situ* nutrient measurement, water samples were collected simultaneously from each system once every 4 hours for nutrient measurements at the laboratories in Sea Point, to provide validation of the readings recorded *in situ* on the farm.

At the chemistry laboratory in Sea Point, total ammonia nitrogen (TAN) was measured in duplicate and absorbances were adjusted for turbidity arising from reagent addition to sample water by the addition of the citrate and phenol reagents only, to the standard solutions. A blank correction was made for the spectrophotometric absorbances using low nutrient seawater which had been treated with the same reagents as the samples, to attempt to account for the matrix effect and scattering of light caused by the cuvette. TAN was measured using a modified version of the spectrophotometric Grasshoff (Grasshoff *et al.*, 1999) method, where the modification involves lower sample and reagent volumes but maintains mass ratios of approximately 25:1 phenol to available chlorine (chlorine

available for reaction at all oxidation levels), recommended in the Grasshoff method. A calibration curve method was not utilised in the assays. Rather, a single accurately formulated calibration standard in the mid-range of expected concentrations (12 µM) was tested in each batch processed, together with a nutrient free seawater sample as reference samples. Reference samples were measured spectrophotometrically following reagent addition, giving an absorbance value which was then used as a 'factor' to calculate concentrations of unknown samples (Probyn *et al.*, 2017). The recorded TAN, pH, temperature, and salinity values were used, as described above, to calculate the free un-ionized ammonia (FAN).

*Preliminary results*

Data loggers in the 100, 75 and 50% recirculation trials recorded that there is a diurnal fluctuation in temperature and pH under all scenarios. Average temperature with in the 100% recirculating system shows a dramatic increase form normal operation (50% recirculation) over the study period with an increase of ± 5$^{o}$C, while systems run at 75% do not show an increasing trend and remain similar to the 50% recirculating system parameters (Figure 26). The pH within the systems shows a shift in the average trend towards higher pH as one increase recirculation from 50 – 75 – 100%.

The *in situ* data measurements from the 100% recirculation trial indicates that there is a diurnal fluctuation in temperature, DO and pH for all clusters maintained at 100% recirculation, as well as the control cluster maintained at 50% recirculation (Fig. 27) — with all values increasing from sunrise until midday and then decreasing again overnight. These findings are suggestive of a diurnal fluctuation in temperature, DO and pH for all clusters. Temperature increases are likely due to solar radiation, while DO and pH are largely affected by Ulva respiration and photosynthesis.

The uptake of $CO_2$ or $HCO_3^-$ by Ulva in the system decreases the concentration of $H^+$ ions and increase pH, while releasing $O_2$ that contributes to the increase in DO. Conversely, respiration at night by the abalone — which are also more active and feed at night — and Ulva lowers the DO and releases $CO_2$, which decreases the pH of the system. Nutrient data over the 3-day period indicated that Ulva was able to sufficiently maintain both TAN and FAN bellow harmful levels for the first 2 days of the 3 day trial (Fig. 28). TAN and FAN levels started to increase rapidly, particularly in cluster 3.1 on day 3, and the trial was abandoned to ensure no detrimental effects were experienced by the abalone.

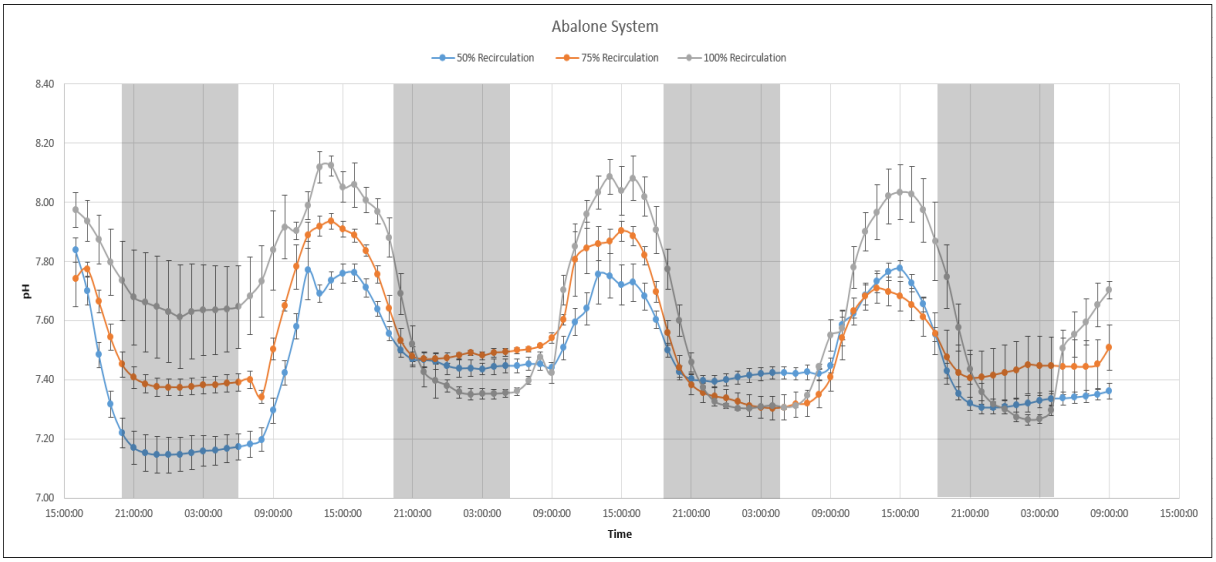



Figure 26: Average temperature recorded across the 3 recirculation sinarios with 3 clusters (2.1, 2.2 & 3.1) running at 50, 75 & 100% recirculation a 62 hour period.

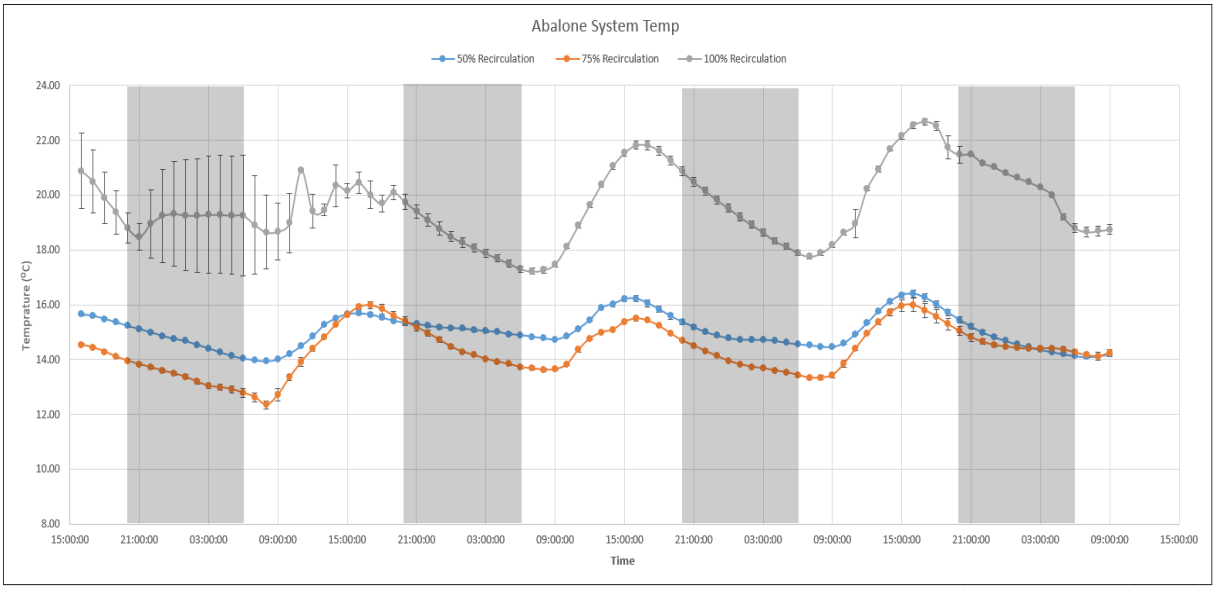



Figure 27: Average pH recorded across the 3 recirculation sinarios with 3 clusters (2.1, 2.2 & 3.1) running at 50, 75 & 100% recurculation a 62 hour period.

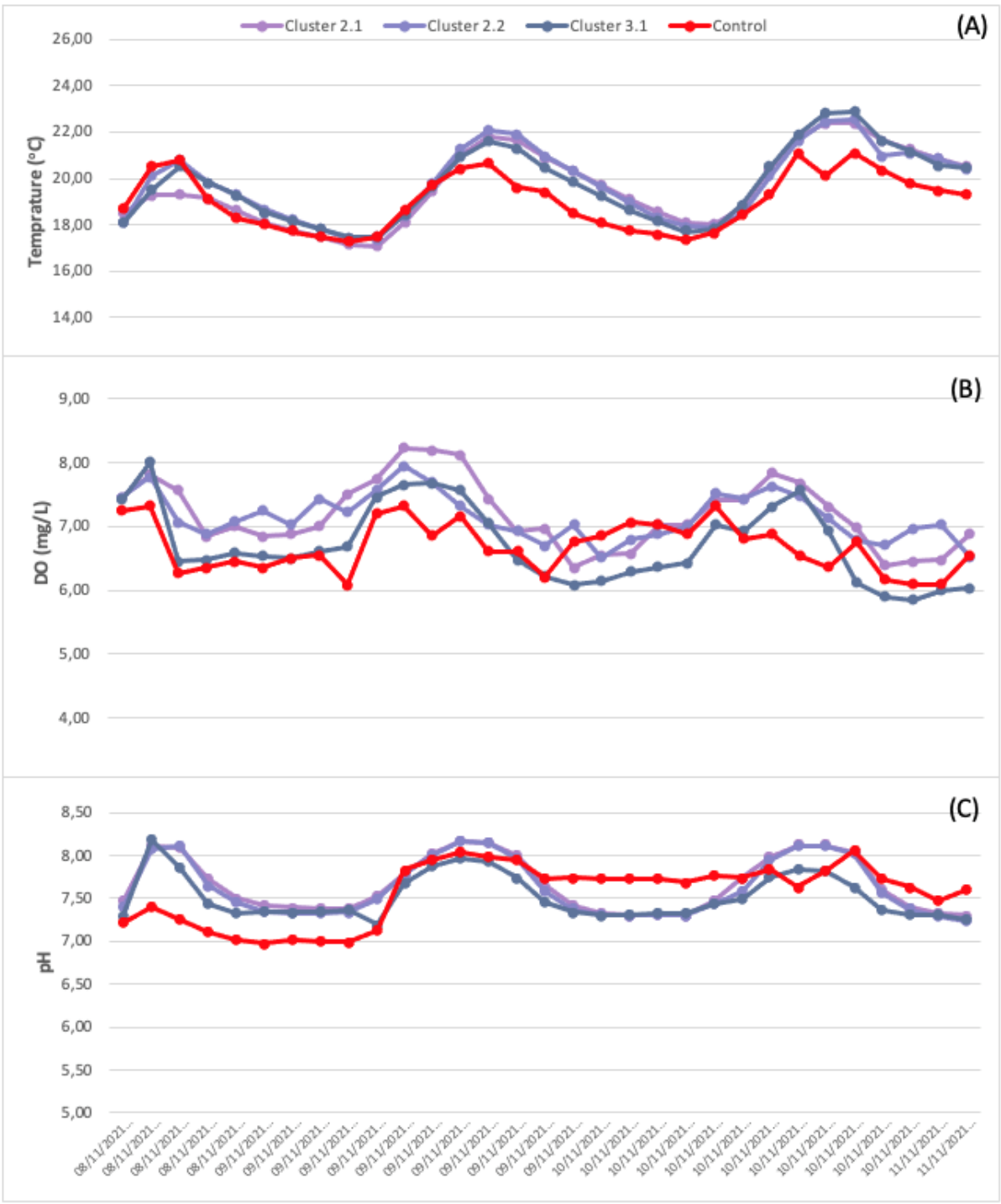



Figure 28: Temperature, Dissolved Oxygen (DO) and pH recorded across the 3 clusters (2.1, 2.2 & 3.1) running at 100% recirculation and the Control cluster running at 50% recirculation over a 62-hour period

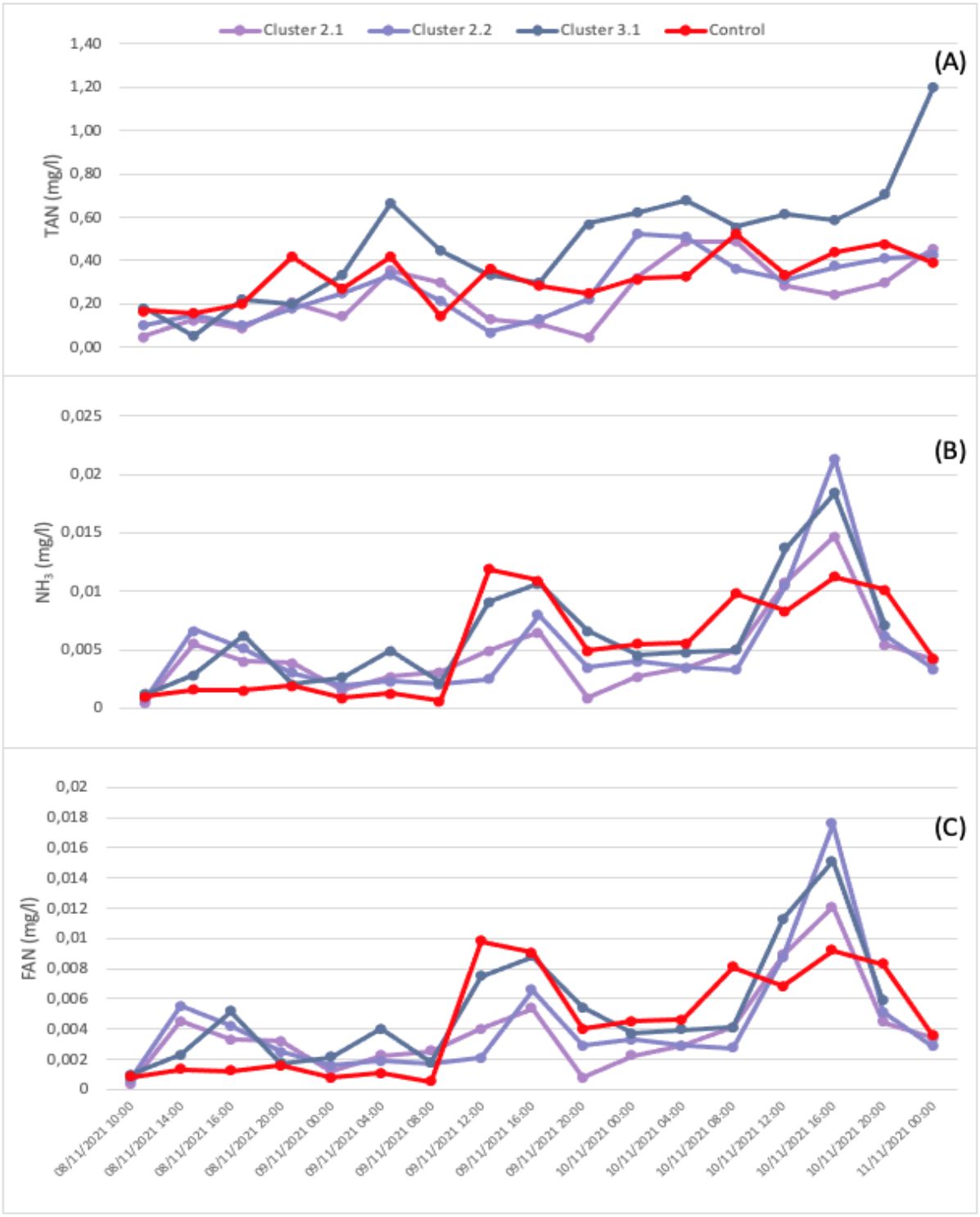



Figure 29: A: Total ammonia Nitrogen (TAN), B: Ammonia ($NH_3$), and C: Free Ammonia Nitrogen (FAN) recorded across the 3 clusters (2.1, 2.2 & 3.1) running at 100% recirculation and the Control cluster running at 50% recirculation over a 62-hour period.

**Monitoring the physical and chemical parameters of the sea urchin-Ulva IMTA system with varying recirculation rates**

This part of the project is still to be conducted once systems functionality and stocking densities have been finalised in the pilot urchin-Ulva system.

### 4.1.3 Conclusions

The study was conducted to determine the effects of increasing recirculation rates on the physical and chemical parameters of the abalone-*Ulva* IMTA system, and much of the data is still being processed to fully understand the effects and address the key goals of the research. Preliminary analyses of the data from the 100% recirculation trial appears to indicate that it is possible to run these systems for short periods of time (±2 days) without any water replenishment, however this is only possible due to the synergistic effect of the *Ulva* raceway that not only removes excess ammonium but also reduces the pH of the system thereby reducing the amount of FAN in the system. Although decreasing pH is potentially problematic in itself, it is advantageous in terms of modulating the free ammonia concentrations. The pH is the most significant influencer of the dynamic equilibrium of dissolved ammonia nitrogen in seawater and lower pH yields lower unionised ammonia fractions. Much of the literature concerned with integrated aquaculture often mentions pH 'rebalancing' as one of the bio-remediatory characteristics of seaweed, which is true in that during photosynthesis the pH will increase in Ulva raceway water as seen during the day, and this increase in pH towards a normal seawater pH of 8.2 will likely be beneficial to abalone; however it will also increases the fraction of unionised ammonia present in the water when operating at 100% recirculation and should be noted.

Nutrient data collected weekly from the trails conducted using 50% and 75% recirculation rates over 2 months periods are still being analysed as well as samples collected during the 100% recirculation trial, and will be presented in the deliverable 4.2, which is related to circularity.

# 5. Next steps

The IMTA production is a dynamic process and still requires studies to maximize production. New challenges arise every day, such as the introduction of new species and changes in biomass relationships, which can alter the dynamics of nutrients and organic matter. In this sense, adjustments in the production systems should be made to improve the production performance, hence making it possible to achieve the goals of "zero waste" emissions and improve the circularity. For such, the following experiments are currently underway:

- To improve the shrimp FCR and make the use of feed more efficient. The preliminary results obtained in December and January led to FCR of 1.2 for shrimp and 0.6 for tilapia. With reduced FCR we aim to minimize production costs, water use, TSS, and to achieve zero waste;
- Experiments with substitution of fishmeal (0, 25, 50, 75, and 100%) by "poultry meal" (fishmeal analogue). Brazil is one the most important chicken producers in the world and the sub-products (chicken feathers, blood, and viscera) can be an alternative protein source to prepare the shrimp and fish feed. This experiment is related to WP 4.2 with emphasis in circularity;
- To include all species in a pilot-scale and find the best biomass ratio among candidate species identified in the first 18 months in order to determine the nutrient balance and economic viability; and
- To establish the best configurations to produce abalone, seaweed, and urchins with water recirculation on a commercial scale.

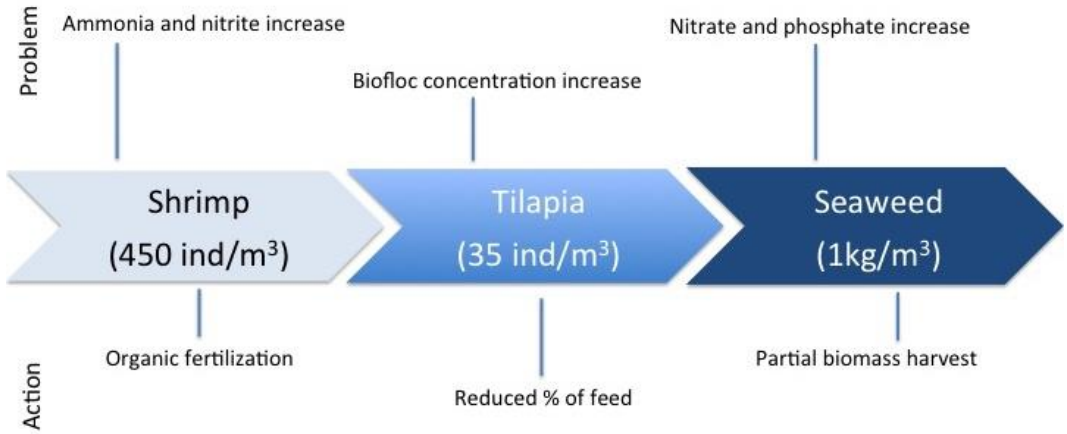
# 6. General conclusions

During these first months of the project, experiments have been carried out in different countries, according to local or regional interests, seeking the integrated culture of commercial species in different configurations that allow minimum effluent input to the environment, maximum efficiency of food use and minimum environmental impact.

Research has shown that nutrients and total suspended solids can been mitigated in absolute terms, as seen with the reductions in nitrogen and phosphorus from the shrimp and abalone farms. The new technological developments associate to species integration driving to zero waste.

Applied scientific research is needed to develop practical solutions to environmental problems. Results of experiments carried out in closed IMTA systems (Brazil) showed the possibilities to produce shrimp in super-intensive densities integrated to tilapias and seaweed with no water renewal or in other words, with zero waste production.

The experiments carry out in Brazil used the same water in 4 shrimp cycles (3 months each) and 2 tilapia production cycles (6 months each) with biofloc technology system. During the shrimp production the biofloc formation was stimulated by organic fertilization to control the ammonia and nitrite (toxic compounds) concentration in the water. Thus, the key procedure to keep the system working well is the biofloc (TSS) control, where tilapia play an important role. When the feed amount is reduced to 1% of the biomass, fishes eat more biofloc and control the concentration close to 200 mg/L. This TSS concentration permits the light penetration in the water and the seaweed reduces the nutrients concentration in the water.



Considering 80% of survival at salinity 20 and the species described, it is possible to produce 43 ton of shrimp/hectare/cycle, 35 ton of tilapia/cycle and 0.8 ton of seaweed/cycle.

The inclusion of oyster *Crassostrea gasar* in the system did not bring a positive effect on the water quality, but it can be used regarding the products diversification in the farm.

The microorganisms present in bioflocs and probiotics helped to maintain the water quality in good conditions, served as food for shrimp and tilapia, reduced the feed conversion rate and, mainly, allowed the reuse of water for several cycles.

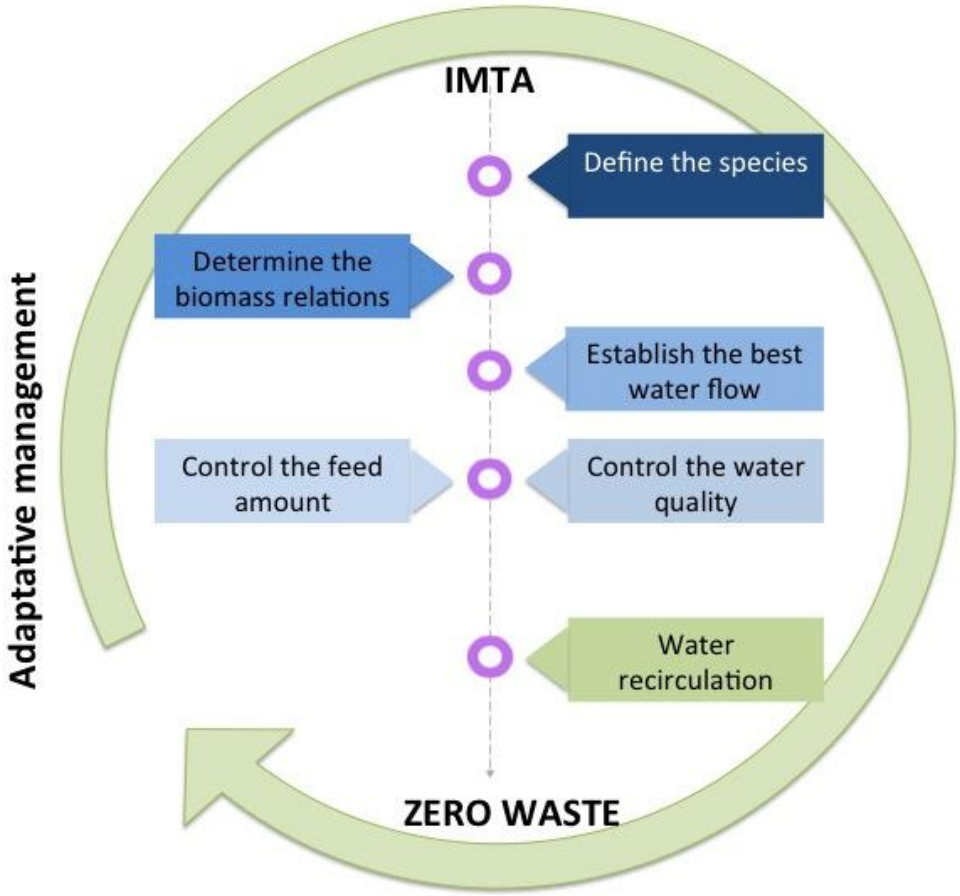
Experiments carried out in South Africa's commercial abalone farm have shown that the integration with algae provided a reduction in nutrient concentrations and allowed greater water recirculation. The results are encouraging and indicate the possibility of water reuse of around 75%. This is made possible by the integration of marine algae that consume dissolved nutrients to generate biomass. As a result, the system is less dependent on external power and considerably reduces the impact of effluents on the environment.

Preliminary analysis indicates the possibility of increasing the farm's standard operational recirculation to 100% recirculation for short periods (3-4 days) of time. This is especially important when the farm is unable to pump seawater when toxic microalgae blooms occur.

Open systems have no physical barriers and solid, suspended and dissolved wastes are released into the environment, so it is not possible to formulate strategies towards zero waste in the sense that can be defined for land-based recirculating aquaculture systems. However, open systems can also be optimized in terms of bioremediation and infrastructure materials, under a circularity approach (task 4.2). The latter will also allow assessing the effects of improvements on feeding in close and semi-close systems as the continuation of the zero waste approach adopted in task 4.1.

The case studies in Brazil and South Africa can be considered good examples of responsible aquaculture, where the management reduces the effluent production. The activities reported here are not intended to indicate one or another production system. Both systems, closed (Brazil) and semi-closed (South Africa), are options to be adopted. The integrated abalone and macroalgae production model may be interesting for investors from countries such as Argentina and Chile, in South America, which have adequate environmental conditions to produce this prized mollusc.

Likewise, European countries that already produce marine shrimp, such as Spain, Portugal, Italy and France, could produce shrimp using biofloc technology, integrated with fish and algae. This would increase production close to the European market, with reduced feed costs, in small areas, with no chemicals or antibiotics and basically with zero waste production.




Using the IMTA system, the farmer can diversify the production; reduce food costs, and add value to products obtained without effluent emissions (green certificate).

# 7. References

Biswas, G., Kumar, P., Ghoshal, T. K., Kailasam, M., De, D., Bera, A., Mandal, B., Sukumaran, K., & Vijayan, K. K. (2020). Integrated multi-trophic aquaculture (IMTA) outperforms conventional polyculture with respect to environmental remediation, productivity and economic return in brackishwater ponds. *Aquaculture*, *516*, 734626. https://doi.org/10.1016/j.aquaculture.2019.734626

Boyd, C. E., D'Abramo, L. R., Glencross, B. D., Huyben, D. C., Juarez, L. M., Lockwood, G. S., McNevin, A. A., Tacon, A. G. J., Teletchea, F., Tomasso, J. R., Tucker, C. S., & Valenti, W. C. (2020). Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. *Journal of the World Aquaculture Society*, *51*(3), 578–633. https://doi.org/10.1111/jwas.12714

Briggs, M. R. P., & Funge-Smith, S. J. (1994). A nutrient budget of some intensive marine shrimp ponds in Thailand. *Aquaculture and Fisheries Manage*, *25*(8), 789–811. https://doi.org/10.1111/j.1365-2109.1994.tb00744.x

Brunetto, L. J. (2018). Cultivo da ostra *Crassostrea gasar* em diferentes densidades. Dissertação (Mestrado) - Universidade Federal de Santa Catarina, Florianópolis, 71 p.

Christo, S.W., Absher, T.M., 2006. Reproductive period of *Crassostrea rhizophorae* (Guilding, 1828) and *Crassostrea brasiliana* (Lamark, 1819) (Bivalvia: Ostreidae) in Guaratuba Bay, Paraná, Brazil. *J. Coast. Res.* vol. 2, 1215–1218.

Gaona, C. A. P., de Almeida, M. S., Viau, V., Poersch, L. H., & Wasielesky, W. (2017). Effect of different total suspended solids levels on a *Litopenaeus vannamei* (Boone, 1931) BFT culture system during biofloc formation. *Aquaculture Research*, *48*(3), 1070–1079. https://doi.org/10.1111/are.12949

Gaona, C. A. P., Serra, F. da P., Furtado, P. S., Poersch, L. H., & Wasielesky, W. (2016). Biofloc management with different flow rates for solids removal in the *Litopenaeus vannamei* BFT culture system. *Aquaculture International*, *24*(5), 1263–1275. https://doi.org/10.1007/s10499-016-9983-2

Krummenauer, D., Peixoto, S., Cavalli, R. O., Poersch, L. H., & Wasielesky, W. (2011). Superintensive culture of white shrimp, *Litopenaeus vannamei*, in a biofloc technology system in Southern Brazil at different stocking densities. *Journal of the World Aquaculture Society*, *42*(5), 726–733. https://doi.org/10.1111/j.1749-7345.2011.00507.x

Krummenauer, D., Samocha, T., Poersch, L., Lara, G., & Wasielesky, W. (2014). The reuse of water on the culture of pacific white shrimp, *Litopenaeus vannamei*, in BFT system. *Journal of the World Aquaculture Society*, *45*(1), 3–14. https://doi.org/10.1111/jwas.12093

Lara, G., Honda, M., Poersch, L., & Wasielesky, W. (2017). The use of biofilm and different feeding rates in biofloc culture system: the effects in shrimp growth parameters. *Aquaculture International*, *25*(5), 1959–1970. https://doi.org/10.1007/s10499-017-0151-0

Lazoski, C. (2004). Sistemática molecular e genética populacional de ostras brasileiras (*Crassostrea* spp.). Tese (Doutorado em genética) – Universidade Federal do Rio de Janeiro, Rio de Janeiro. 150 p.

Liu, W. chang, Luo, G. zhi, Li, L., Wang, X. yong, Wang, J., Ma, N. nian, Sun, D. chuan, & Tan, H. xin. (2017). Nitrogen Dynamics and Biofloc Composition using Biofloc Technology to Treat Aquaculture Solid Waste Mixed with Distillery Spent Wash. *North American Journal of Aquaculture*, *79*(1), 27–35. https://doi.org/10.1080/15222055.2016.1223769

Loureiro R.R., Reis, R.P., Critchley, A.T. (2010). In vitro cultivation of three *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) variants (green, red and brown) exposed to a commercial extract of the brown alga *Ascophyllum nodosum* (Fucaceae, Ochrophyta). *J Appl Phycol* 22:101–104

Loureiro, R.R., Reis, R.P., Berrogain, F.D., Critchley, A.T. (2012). Extract powder from the brown alga *Ascophyllum nodosum* (Linnaeus) Le Jolis (AMPEP): a "vaccine-like" effect on *Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva. *J Appl Phycol* 24:427–432

Melo, C. M. R., Silva, F. C., Gomes, C. H. A. M., Solé-Cava, A. M., & Lazoski, C. (2010). C*rassostrea gigas* in natural oyster banks in southern Brazil. *Biological Invasions,* 12(3), 441–449. https://doi.org/10.1007/s1053 0-009-9475-7

Mizuta, D.D., Silveira, N., Fischer, C.E., Lemos, D. (2012). Interannual variation in commercial oyster (*Crassostrea gigas*) farming in the sea (Florianópolis, Brazil, 27° 44' S; 48° 33' W) in relation to temperature, chlorophyll a and associated oceanographic conditions. *Aquaculture* 366, 105-114. https://doi.org/10.1016/j.aquaculture.2012.09.011

Muangkeow, B., Ikejima, K., Powtongsook, S., & Gallardo, W. (2011). Growth and nutrient conversion of white shrimp *Litopenaeus vannamei* (Boone) and Nile tilapia *Oreochromis niloticus* L. in an integrated closed recirculating system. *Aquaculture Research*, *42*(9), 1246–1260. https://doi.org/10.1111/j.1365-2109.2010.02713.x

Muangkeow, B., Ikejima, K., Powtongsook, S., & Yi, Y. (2007). Effects of white shrimp, *Litopenaeus vannamei* (Boone), and Nile tilapia, *Oreochromis niloticus* L., stocking density on growth, nutrient conversion rate and economic return in integrated closed recirculation system. *Aquaculture*, *269*(1–4), 363–376. https://doi.org/10.1016/j.aquaculture.2007.04.002

Poersch, L. H.S.; Brunson, J.; Gaona, C.; Richardson, J.; Pitts, K.; Leffler, J. W. (2021). Pacific white shrimp, red drum and tilapia integrated in a biolfloc system: use of tilapia as a consumer total suspended solid. *Journal of The World Aquaculture Society*, v. 3, p. 1-10.

Ray, A. J., Lewis, B. L., Browdy, C. L., & Leffler, J. W. (2010). Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. *Aquaculture*, *299*(1–4), 89–98. https://doi.org/10.1016/j.aquaculture.2009.11.021

Rego, M. A. S., Sabbag, O. J., Soares, R., & Peixoto, S. (2017). Financial viability of inserting the biofloc technology in a marine shrimp *Litopenaeus vannamei* farm: a case study in the state of Pernambuco, Brazil. *Aquaculture International*, *25*(1), 473–483. https://doi.org/10.1007/s10499-016-0044-7

Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S., Page, F., Reid, G., Szemerda, M., Sewuster, J., & Boyne-Travis, S. (2007). Integrated multi-trophic aquaculture (IMTA): A potential strategic choice for farmers. *Aquaculture Economics and Management*, *11*(1), 99–110. https://doi.org/10.1080/13657300701202767

Santhana Kumar, V., Pandey, P. K., Anand, T., Bhuvaneswari, G. R., Dhinakaran, A., & Kumar, S. (2018). Biofloc improves water, effluent quality and growth parameters of *Penaeus vannamei* in an intensive culture system. *Journal of Environmental Management*, *215*, 206–215. https://doi.org/10.1016/j.jenvman.2018.03.015

Samocha, T.M. 2019. Sustainable Biofloc Systems for Marine Shrimp. Academic Press, Elsevier. 10.1016/C2018-0-02628-6

Tendencia, E. A., Dela Peña, M. R., & Choresca, C. H. (2006). Effect of shrimp biomass and feeding on the anti-*Vibrio harveyi* activity of *Tilapia* sp. in a simulated shrimp-tilapia polyculture system. *Aquaculture*, *253*(1–4), 154–162. https://doi.org/10.1016/j.aquaculture.2005.08.004

Tendencia, E. A., Dela Peña, M. R., Fermin, A. C., Lio-Po, G., Choresca, C. H., & Inui, Y. (2004). Antibacterial activity of tilapia *Tilapia hornorum* against *Vibrio harveyi. Aquaculture*, *232*(1–4), 145–152. https://doi.org/10.1016/S0044-8486(03)00531-3

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A. H., & Fang, J. G. (2009). Ecological engineering in aquaculture - Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture*, *297*(1–4), 1–9. https://doi.org/10.1016/j.aquaculture.2009.09.010

Yuan, D., Yi, Y., Yakupitiyage, A., Fitzimmons, K., & Diana, J. S. (2010). Effects of addition of red tilapia (Oreochromis spp.) at different densities and sizes on production, water quality and nutrient recovery of intensive culture of white shrimp (*Litopenaeus vannamei*) in cement tanks. *Aquaculture*, *298*(3–4), 226–238. https://doi.org/10.1016/j.aquaculture.2009.11.011

Wasielesky, W., Froes, C., Fóes, G., Krummenauer, D., Lara, G., & Poersch, L. (2013). Nursery of *Litopenaeus vannamei* Reared in a Biofloc System: The Effect of Stocking Densities and

Compensatory Growth. *Journal of Shellfish Research*, *32*(3), 799–806. https://doi.org/10.2983/035.032.0323