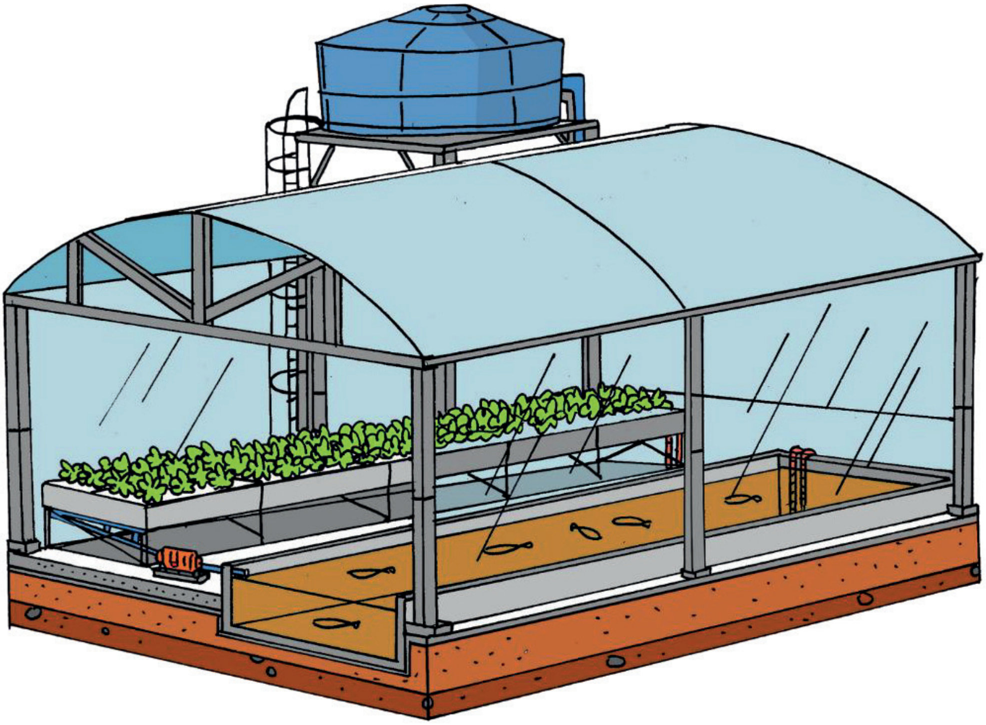
FLOCponics

The integration of biofloc technology with plant production



Sara M Pinho

Propositions

1. On-demand coupling is the only way to achieve optimal fish and plant growth in FLOCponics.
   (this thesis)

2. FLOCponics solely flourishes in large commercial applications.
   (this thesis)

3. High energy-efficiency is key for developing sustainable, intensive aquaculture.

4. Sustainable food production systems will always be a utopia if their products remain economically unappealing.

5. The quality of science is compromised when researchers spread non-peer-reviewed findings on social media.

6. Long-term professional excellence is merely achieved by emotional intelligence.

Propositions belonging to the thesis, entitled

FLOCponics: The integration of biofloc technology with plant production

Sara M Pinho
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FLOCponics:

The integration of biofloc technology with plant production

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Chapter 1

General Introduction

1.1 Background

Feeding the world with sufficient, safe, and nutritious food is a global challenge (Fitton et al. 2019; Fróna et al. 2019). Aquaculture, the production of aquatic organisms, is one of the ways to provide healthy food for the growing population (Thilsted et al. 2016; Fiorella et al. 2021). With an annual production of 114.5 million tonnes in 2018, aquaculture is the fastest growing animal production activity in the world (5.3% from 2001-2018) (FAO 2020). Aquaculture offers social and, when well-managed, environmental benefits (Aubin et al. 2019), besides producing half of the fish consumed nowadays by human population (FAO 2020). For many years this activity has been contributing to food security, livelihoods, employment generation, and poverty alleviation (Hambrey 2017). On the environmental front, compared to other food production methods, aquaculture demands lower volume of water per kg of food produced (e.g., 0.4 m^3 of water per kg of fish produced vs 15.5 m^3 of water per kg of beef produced) (Joyce et al. 2019), contributes very little to the global anthropogenic GHG emissions (MacLeod et al. 2020) and certain cultures can efficiently sequester carbon (Duarte et al. 2017).

Aquaculture contributions are expected to continuously grow through intensive production methods. However, most intensive fish production worldwide is carried out in conventional ponds or in cages monoculture systems with concerning managements (Ahmed et al. 2019; FAO 2020). Such conventional monoculture systems are mostly founded on unsustainable linear economy principles of "take, make, consume, dispose, and pollute" (Boyd et al. 2020). The use of feed, land and water should (and can) be much more optimized than what has been done in the conventional systems (Joyce et al. 2019; Boyd et al. 2020). Also, sometimes the negative environmental impacts, linked to the discharge of nutrient-rich effluent into the environment or the excessive use of chemicals (e.g., antibiotics, pesticides) to achieve high productivity overcome the aforementioned benefits (Henares et al. 2020; Lulijwa et al. 2020). Thus, for aquaculture to continually supply fish in the long term, it is essential to promote and develop technologies and innovative system designs based on sustainable approaches and circular principles.

Circular economy relies on looping systems to "reduce, reuse, recycle, and recover" resources (Stahel 2016; Regueiro et al. 2021). Circular aquaculture basically is a system in which waste is recovered and reused as a resource to feed other process and production methods, promoting sustainable development (Regueiro et al. 2021). Circular systems approaches are aligned with what is expected from sustainable aquaculture practices. In general, there is no completely sustainable food production method; instead, a gradient between sustainable and unsustainable systems is usually accepted (Valenti et al. 2018). More sustainable aquaculture systems are those grounded on well-balanced systems in terms of environmental, social and economic returns and impacts, where the extraction of natural resources does not overcome the capacity of ecosystem regeneration (Valenti et al. 2018; Boyd et al. 2020). Additionally, diversification of the overall production is also seen as a sustainable practice. Diversification can be achieved by producing species from different trophic levels, as seen in, for instance, integrated multitrophic aquaculture or integrated agri-aquaculture systems (Shi et al. 2013; Thomas et al. 2021). Another possibility for aquaculture diversification is increasing the range of species produced, aiming to meet local market and cultural specificities, and avoiding environmental and genetic problems related to the introduction of alien species. Promising aquatic food production system, such as biofloc-based culture (Avnimelech 2015; Bossier and Ekasari 2017) and aquaponics (integration of fish-plant production) (Goddek et al. 2015; Yep and Zheng 2019), are based on these concepts of circular economy and sustainability.

1.2 Biofloc-based fish production

Biofloc technology (BFT) has been applied as an environmental-friendly approach to promote aquaculture sustainability (Bossier and Ekasari 2017; David et al. 2021b). With BFT, feed nutrients are efficiently used, and low quantities of water and land are required to produce fish intensively (Emerenciano et al. 2017; Dauda 2020). In general, biofloc-based aquaculture systems are characterised by turbid soft brown water, as a result of accumulation of microbial communities and solids due to minimal water exchange and absence of high-cost equipment and filters (Browdy et al. 2012; Hargreaves 2013). By requiring low in-outflow of water, BFT is suitable for fish producers who seek water savings, maximal biosecurity and minimal negative environmental impacts linked to effluent discharge (Avnimelech 2015).

The “bioflocs” are aggregates that include microorganisms (bacteria, fungi, flagellates, protozoans, ciliates, algae and others), and particulate organic matter such as faeces and uneaten feed (Hargreaves 2013; Avnimelech 2015). Under suitable conditions, such microorganisms grow *in situ* in the fish tanks and play important roles in biofloc-based culture (Martínez-Córdova et al. 2015; Dauda 2020). In this thesis, we explored two central roles of bioflocs for fish culture, i.e., maintaining the water quality and serving as supplementary feed for the cultured animals.

Concerning water quality maintenance, the built-up of solids and toxic nitrogenous compounds (i.e., ammonia and nitrite) are usually the most concerning issues in closed aquaculture systems (Bao et al. 2019; Espinal and Matuli 2019). The growth and dynamic interactions of diverse microorganisms in the biofloc-based system, essentially heterotrophic and nitrifying bacteria, mitigate such problems. Heterotrophic bacteria degrade organic residues, using the organic carbon as an energy source, and assimilate the nitrogenous compounds in the water into bacteria biomass (Ebeling et al. 2006; Emerenciano et al. 2017). To properly grow, this bacteria community generally demands mixed and aerated water with a balanced carbon:nitrogen ratio of 10-20:1, which is typically maintained in biofloc-based culture (Crab et al. 2012; Samocha and Prangnell 2019). Heterotrophic bacteria grow faster and consume the substrates more efficiently than nitrifying bacteria, being crucial to prevent the negative effect of high ammonia and nitrite levels in the first months of production (Ebeling et al. 2006; Ray and Lotz 2014). On the other hand, nitrifying bacteria obtain energy through the oxidation of inorganic nitrogen, transforming ammonia into nitrite and then oxidise it to nitrate (Ebeling and Timmons 2012; Rurangwa and Verdegem 2015). Nitrate is the nitrogen form less toxic for cultured animals and the most required form for plants in integrated agri-aquaculture systems (Zou et al. 2016; Ru et al. 2017). Nitrifying bacteria communities take approximately 1-2 months to establish, and in the long-term it is the ultimate sink of most of the nitrogen added to the system (Luo et al. 2020). Other microorganisms can also affect the water quality and nitrogen pathway in BFT, e.g., algae in open environments without light limitation (Luo et al. 2020; Abakari et al. 2020). The predominance of each group of microorganisms varies and will depend on several factors, such as the target species, the production management, and the inputs used (Martínez-Córdova et al. 2015).

In terms of the nutritional benefits of BFT, bioflocs are rich protein-lipid natural food constantly available and complement the dietary requirements of, for example, tilapia reared in BFT systems (Bossier and Ekasari 2017; Moreno-Arias et al. 2018; Mugwanya et al. 2021). Tilapia is an omnivorous species with adequate morphological structures to take advantage of bioflocs as a food source. As a result of this, it is the most investigated and reared fish species in BFT systems

(Emerenciano et al. 2021). Recycling nutrients and organic matter by the overall biofloc community promotes a complex heterotrophic food web, based on a microbial-loop that includes the cultured fish (Figure 1.1) (Crab et al. 2012; Emerenciano et al. 2013; Martínez-Córdova et al. 2017). As the microorganism community in BFT is variable, the nutritional composition of biofloc biomass will also be variable. Despite that variability, the consumption of bioflocs by fish has demonstrated many positive effects on zootechnical performance compared to filter-based recirculating aquaculture systems (RAS). For instance, an increase in animal growth rate and survival and a decrease in feed conversion ratio have been reported in biofloc-based tilapia culture (Azim and Little 2008; Luo et al. 2014; Long et al. 2015; Nguyen et al. 2021; Saseendran et al. 2021). In addition, BFT allows for the application of management strategies that reduce expenses and negative impacts of aquaculture, with an emphasis on the possibility of reducing the protein levels of the diets used (Azim and Little 2008; Mansour and Esteban 2017; Sgnaulin et al. 2021). Smaller dietary protein levels provide significant savings on feed costs and favours effluents low in nitrogen and phosphorus (Emerenciano et al. 2013).

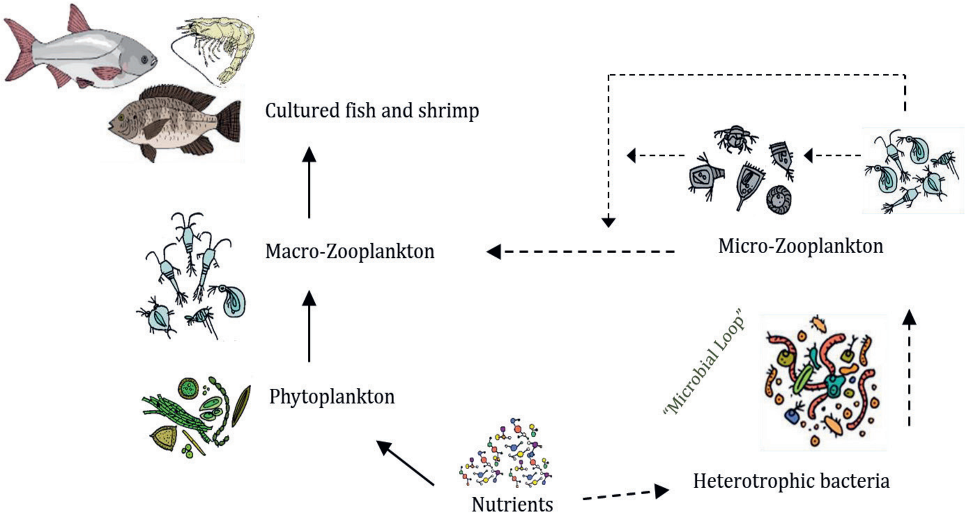

Figure 1.1. Simplified representation of the heterotrophic food web promoted in biofloc-based culture.

Over the past years, BFT has been successfully used in all stages of tilapia farming (Emerenciano et al. 2021). Several studies support the segmentation of the tilapia production process in multi-phases and the use of biofloc-based culture for the nursery phase, i.e., the production of tilapia juveniles weighing approximately 1 to 30g (Brol et al. 2017; Alvarenga et al. 2018; Durigon et al. 2019; Sgnaulin et al. 2020; Pinho et al. 2021). The investment in nurseries structures in a closed and controlled environment, as maintained in BFT, is in many ways advantageous. Compared to single fish stocking (0.5 - 1.0 g until final weight), the nursery phase opens the possibility of effective control in terms of feeding management, water quality parameters, batch size homogeneity, and survival (Durigon et al. 2019; Sgnaulin et al. 2020). Moreover, tilapia juveniles efficiently consume bioflocs as supplementary food (Alves et al. 2017; Correa et al. 2020), boosting the nutritional advantages of BFT mentioned above. In the case of dietary protein reduction, levels up to 28% crude protein (CP) seem to be enough to achieve high growth performance in BFT (Silva et al. 2018; Hisano et al. 2020). While in systems with minimal natural food available, such as in RAS and cages, the recommended crude protein for tilapia juveniles varies between 30 and 40% (Hafedh 1999; Neto and Ostrensky 2015). It is essential to point out that, for tentative reduction in the CP level or any other alternative nutritional management in BFT, the bioflocs must be maintained in enough amounts, i.e., for tilapia juveniles, the recommended volume of biofloc is at least 5 mL L^{-1} or total suspended solids levels of 100 mg L^{-1} (Hargreaves 2013; Emerenciano et al. 2017). Similarly, physical-chemical water parameters, like temperature, pH, and oxygen, must be within comfort ranges for the cultured species.

Biofloc-based culture meets some conditions expected from a sustainable aquaculture system, for example, the responsible use of resources such as water, land and feed, and intensive animal production with minimal effluents discharge (Crab et al. 2012; Avnimelech 2015; Bossier and Ekasari 2017). Yet, there are still possibilities for commercial expansion of tilapia farming and improving the efficiency of the BFT systems. Examples are (but not limited to) diversifying the system in terms of target species produced (Walker et al. 2020) and improving of the use of resources by reusing the nutrient accumulated in the water or solids (e.g., nitrate and phosphorus) to nourish other crops (Quintã et al. 2015; Pinheiro et al. 2017). Integrating BFT with soilless plant production, a variation of aquaponics, can address both issues.

1.3 Aquaponics systems

Aquaponics is an integrated agri-aquaculture technology that combines tank-based production of aquatic animals with hydroponics. Such a system involves microbiological processes to transform nutrient-rich effluent from aquaculture into useful resources for plant nutrition and irrigation (Baganz et al. 2021) (Figure 1.2). Aquaponics systems are based on reusing and recycling resources to produce healthy food, with minimal or no chemicals (fertilisers, pesticides, antimicrobials) (Lennard and Goddek 2019). Thus, compared to conventional aquaculture and hydroponics systems, aquaponics has many advantages and has been developed to be a more sustainable and circular food production process (Joyce et al. 2019).

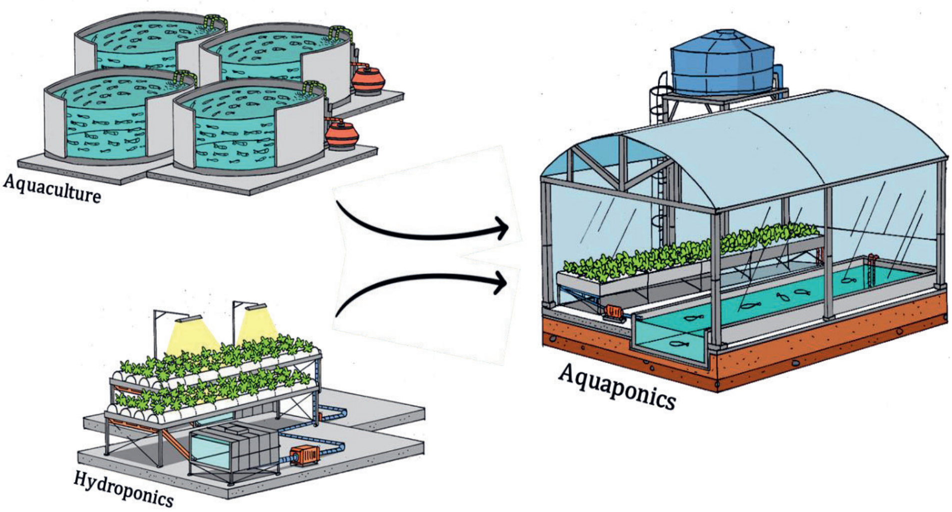

Figure 1.2. Illustration of an aquaponics system, integrating aquaculture and hydroponics system.

The diversity of products offered (fish and plants) and the large variety of cultured plant species are also positive points of aquaponics from a sustainable perspective. Many species of leafy vegetables, flowers, fruits, and garnishes have been successfully produced in aquaponics systems (Love et al. 2015; Bailey and Ferrarezi 2017; Pinho et al. 2018). For animal production, only a limited number of species has been reported, e.g., tilapia, catfish and salmonids (Love et al. 2015; Mchunu et al. 2018;

Yep and Zheng 2019). This limitation exposes a promising scenario to improve aquaponics diversification considering the extensive catalogue of fish species suitable for aquaculture.

The target fish and plant species and their environmental and nutritional needs set out how the aquaponics systems will be operated and designed. In the past years, most studies have used a rule of thumb for system designs, based on the ratio between the amount of fish feed and plant cultivation area so-called feeding rate ratio (Rakocy et al. 2004; Al-Hafedh et al. 2008; Lennard 2012). The ratio, such as suggested by Rakocy et al. (2006), of 60-100 g of feed per m^2 of plants is typically used for this purpose. However, that rule of thumb is too general for commercial applications, as it does not consider basic elements or the complex interaction of biotic and abiotic parameters that occur in the system. A simple way to see the problem of such an approach is that when the 20-100 g rule is applied, usually, the feed composition is not considered. Consequently, it does not take into account that changes in, for instance, protein content in the feed affects the N concentration in the water and its availability for plants. Oppose to using rules of thumb, recent studies have used dynamic models to size, understand, and optimize aquaponics production (Karimanzira et al. 2016; Lastiri et al. 2016, 2018b; Dijkgraaf et al. 2019). These models require some general assumptions to simplify and make simulations feasible. Even so, they consider a much more comprehensive range of variables than the rule of thumb and are powerful tools to find optimal management and operational strategies (Keesman et al. 2019).

The coupling between subsystems and the changes on the type of aquaculture subsystem also affect overall system performance. Even though aquaponics is all about integrating aquaculture and hydroponics production, the coupling of the subsystems can be either permanently or on-demand (Baganz et al. 2021). In permanently coupled layouts, water and nutrient are constantly shared between the aquaculture and hydroponics subsystems. Most of aquaponics worldwide operates as permanently coupled systems. On the other side, the subsystems can also run independently in so-called on-demand coupled aquaponics. This layout is based on the plants' demands for water and nutrients; thus, the effluent of aquaculture flows to the hydroponics subsystem only if necessary (Goddek et al. 2016, 2019). On-demand coupled aquaponics is gaining attention, mainly for commercial production, because it allows to provide optimal conditions for each subsystem, facilitating the management and resulting in high productivities (Kloas et al. 2015; Goddek et al. 2016). The classification "permanently" and "on-demand" is quite new, and it comes to replace respectively the labels "coupled" and "decoupled" aquaponics layouts (Baganz et al. 2021). As the new nomenclature was suggested when some chapters of this thesis had been already published, both ways to name the aquaponics layouts will appear in the following chapters.

Regarding the type of aquaculture subsystem traditionally used, it consists of a recirculating aquaculture system (RAS) with fish tanks, mechanical filters and biofilter (Rakocy et al. 2006; Lennard and Goddek 2019). In RAS, the microbial processes needed to transform fish effluent usually occur in the biofilter, colonised by nitrifying bacteria (Ebeling and Timmons 2012; Espinal and Matuli 2019). In aquaponics systems, however, the RAS is coupled to the hydroponics subsystem, in which plants are exposed to the mineral nutrient solution (Lennard and Goddek 2019), while in stand-alone hydroponics systems the nutrient for plant growth come from a balanced fertiliser (Maucieri et al. 2019). In traditional aquaponics using RAS, the feed leftovers and by-products of fish metabolism are the primary sources of nutrients for fish and plant (Eck et al. 2019). When the aquaculture effluent does not meet all plants nutritional requirements, which is frequently the case, mineral fertilizers are supplemented (Rakocy et al. 2006; Eck et al. 2019; Goddek et al. 2019). Variations in the aquaculture subsystem can be made by adding other loops to produce non-target aquatic organisms, such as algae (Addy et al. 2017) or duckweed (Sarubbi 2017). Furthermore, another alternative and new approach for the aquaculture subsystem that we will focus on from now on is replacing the RAS with BFT in so-called FLOCponics systems.

1.4 Systems' efficiency and sustainability

Recent studies have used different methods to assess whether emerging food production systems are indeed a sustainable alternative for conventional systems. Moreover, the assessment methods are also applied to investigate how efficient, in terms of resource uses and waste avoidance, the emerging systems are. Dynamic systems modelling and emergy synthesis are examples of such methods, and both were used in this thesis.

1.4.1 Dynamic system modelling

Considering the long time necessary to design and build a complete technological system, some researchers evaluated the dynamics of the systems through mathematical modelling. Modelling studies based on mass balances, represented by ordinary differential equations, have been applied to numerically simulate the consequences of possible variations in the system or understand and describe a new production system even before its conception (Goddek and Keesman 2018; Keesman et al. 2019).

Modelling studies have been performed in aquaponics research as a valuable tool to understand the complex interactions and evaluate the system's efficiency in terms of resource use and losses (Lastiri et al. 2016, 2018a; Keesman et al. 2019; Goddek and Keesman 2020). Goddek et al. (2016), Yogev et al. (2016), Dijkgraaf et al. (2019), and Goddek and Körner (2019) presented theoretical designs for the dimensioning of on-demand aquaponics production based on data available in the literature. Based on calculations, they predicted the quantities of water, nutrients (N and P), fish, plants and organic matter generated or required by the system. Karimanzira et al. (2017) showed that modelling the complex interactions in aquaponics systems supports the most efficient decisions regarding fish diet composition, feeding rates, harvesting time and nutrient releases. All these studies have shown that, in addition to projecting results and scaling commercial layouts, modelling allows to direct management strategies to enable long-term success. Up to now, only a few studies applying differential equations and general mass balances in biofloc-based culture were reported (Avnimelech et al. 1995; Avnimelech 2007; Hoang et al. 2020), and none of these were related to FLOCponics production.

1.4.2 Emergy synthesis

Emergy synthesis (ES) is an efficient method to measure sustainability, assist problem identification, and discuss sustainable solutions for food production systems (Hau and Bakshi 2004; Garcia et al. 2014; David et al. 2018). This method includes the environmental, economic, social, and institutional dimensions of sustainability and allows the rational use of natural resources (Brown and Ulgiati 2004b; Amaral et al. 2016).

ES is based on the biocapacity of planet Earth in providing different resources to sustain the production systems functioning over the years, named as a "donor side" approach (Odum 1996; Brown and Ulgiati 2004a). ES accounts for and classifies all the energy needed directly or indirectly from economic and environmental sources to generate goods and services (Odum 1996; Brown and Ulgiati 2016). ES provides technical-scientific support for the planning and adoption of sustainable production systems to ensure the long-term success of the activity. In addition to evaluating production systems in operation, emergy synthesis is a suitable tool to predict whether systems that have not yet been widely spread or are at their initial stage of development will succeed over time, thus supporting further decision making and public policy (Campbell 1998; Zhan et al. 2020; Zhao et al. 2020). Concerning aquaculture sustainability, ES has been used to assess how sustainable using alternative feeding management, different intensification degrees and integration with other cultures are (David et al. 2021a).

1.5 Research aims

As expected for systems that are under development, such as the FLOCponics system, many questions still need to be answered to understand its technical feasibility, efficiency, and sustainability. For example, (1) "What are the opportunities and drawbacks of FLOCponics?", (2) "To what degree do the productive results achieved in on-demand coupled FLOCponics outperform other production systems?", (3) " To what degree is it possible to take advantage of the nutritional benefits of biofloc for tilapia production in an on-demand coupled FLOCponics system?", (4) "How efficient will it be to transform a biofloc-based fish farm into a FLOCponics production in terms of productivity and resource use?", (5) "How and to what degree do FLOCponics systems affect the sustainability of the stand-alone systems?", (6) "What does make a fish species suitable for aquaponics, and how does it contribute to the potential to diversify FLOCponics systems?". Considering that a new food production system will probably not succeed in the long term unless the status quo is clear, and its main outcomes surpass the traditional systems concerning the efficiency of using resources and producing food. Answering these questions becomes crucial for further understanding and for evaluation whether FLOCponics will contribute to sustainable food supply.

The overall aim of this thesis is to investigate and discuss the technical feasibility, efficiency and sustainability of on-demand coupled FLOCponics for tilapia juveniles and lettuce production. Based on the gaps identified in the previous sections and research questions presented above, the following specific objectives were formulated:

(1) To identify the status quo of FLOCponics, highlight current FLOCponics challenges and give directions for further research.

(2) To determine the technical feasibility of producing tilapia juveniles and lettuce in on-demand coupled FLOCponics compared to traditional (RAS-based) on-demand coupled aquaponics, hydroponics and biofloc-based monoculture systems.

(3) To determine how the reduction of protein content in the fish diet affects fish and plant growth, dietary nutrient use by fish and water quality in on-demand coupled FLOCponics system.

(4) To investigate the efficiency of on-demand coupled FLOCponics system in terms of resource use and amount of food produced and discuss the overall advantages and disadvantages of FLOCponics compared to biofloc-based fish culture.

(5) To assess and discuss the sustainability of biofloc-based fish culture with and without integrating with hydroponic plant production and provide insights on how to improve the sustainable character of food production in such systems.

(6) To identify suitable fish species for aquaponics and discuss their applicability to diversify FLOCponics' products.

1.6 Thesis approach and outline

To achieve the aforementioned research objectives, the thesis comprises seven chapters. After this general introduction, **Chapter 2** reviews and analyses the FLOCponics research regarding the system setups, water quality and nutrient recycling, and productive results of plants and fish achieved so far (objective 1). We also identify economic and environmental aspects and discuss the gaps, opportunities, and challenges of FLOCponics systems. Besides analysing the papers that reported the use of FLOCponics systems, an extensive list of journal papers was revised to offer an overview of biofloc-based aquaculture and aquaponics systems and support the critical review.

Chapter 3 reports the findings of the experiment designed to address objectives 2 and 3. In this experiment, fish and plants were cultured in on-demand coupled FLOCponics, traditional aquaponics, biofloc-based monoculture, and/or hydroponics systems (objective 2), and four fish diets were formulated, produced and tested in FLOCponics systems (objective 3). The experiment design allowed data collection for statistically analysing the fish's zootechnical and nutritional efficiency parameters, plant growth data, and the physical-chemical parameters of the water in the aquaculture and hydroponic subsystems of all treatments.

Chapter 4 follows a modelling approach to investigate the efficiency of FLOCponics systems compared to biofloc-based fish culture (objective 4). In this study, we combine empirical data from the conducted experiments with mass balances of these systems. Also, assumptions and simplifications are explicitly specified for building the first mathematical model of a FLOCponics system.

In **Chapter 5,** we compare the sustainability of biofloc and hydroponics as monocultures with their integration in a FLOCponics system, based on the previews experimental results (objective 5). Emergy synthesis was the method used due to its strong scientific-based characteristics in quantifying the sustainability of food production in different economic, social and environmental locations and supporting decision-makers in having more sustainable systems (Chen et al. 2017).

Chapter 6 reviews fish species suitable for aquaponics as a mean to improve the diversification of integrated agri-aquaculture systems. We focused on South American species since the continent hosts the most extensive biodiversity of fish globally, and several of them have been identified as potential for aquaculture (Valladão et al. 2018). This chapter also presents the characteristics that make a fish species sustainable for each system layouts (permanently or on-demand coupled). The feasibility for producing the revised fish species in FLOCponics was generally discussed in Chapter 7.

Lastly, **Chapter 7** discusses the main findings and explores their implications. We also present the general conclusions and suggest recommendations for future research in the FLOCponics field.

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Chapter 2

FLOCponics: The integration of biofloc technology with plant production – a review

This chapter is based on:

Pinho SM, Lima JP, David LH, Emerenciano M, Goddek S, Verdegem MCJ, Keesman KJ, Portella MC (2021) FLOCponics: The integration of biofloc technology with plant production. Reviews in Aquaculture 1–29. https://doi.org/10.1111/raq.12617

Abstract

FLOCponics is an alternative type of aquaponics that integrates biofloc technology (BFT) with soilless plant production. The aims of this paper are to present a detailed overview of the FLOCponics system's designs and performance, discuss their sustainability, highlight the current challenges, and give directions for future research. Data sources include papers containing the keywords bioflocs and hydroponics, aquaponics, and/or plant production. In view of the small number of publications and the lack of standardization in experimental design and system setup, it was concluded that FLOCponics is still in its initial research stage. With respect to the animal and plant yields in FLOCponics, inconsistent results were found. Some investigations presented better or similar yield results in this system compared to traditional cultures, while others found the opposite. One of the key challenges of using FLOCponics is the effective control of solids. Refining the system's design was the main recommended improvement. Moreover, this paper highlights that the commercial application of FLOCponics will require extensive research that clarifies its technical and economic aspects, originating from experimental or pilot-scale setups with characteristics similar to commercial production. This review provides and discusses information that can be useful for the effective development of FLOCponics, guiding further research to make FLOCponics commercially feasible and thus contributing to sustainable aquaculture production.

2.1 Introduction

The global demand for safe and healthy food has increased significantly in the last few years due to world population growth, projected to reach 9.7 billion people in 2050 (UN 2019). Providing them with healthy food is a major global challenge, especially in the current scenario of natural resource scarcity (FAO 2018; Conijn et al. 2018). Many countries still face problems with hunger while others are trying to address their high rates of population obesity and malnutrition (Byerlee and Fanzo 2019). Hence, investment and research into sustainable food production technologies that produce nutritious food and consume fewer natural resources are needed (Pretty et al. 2010; Ickowitz et al. 2019; Boyd et al. 2020). Modern aquaculture systems, for example, can contribute to the production of fish for a healthy human diet in a more sustainable way (Thilsted et al. 2016; FAO 2020).

In recent years, aquaculture has been the fastest growing animal production activity and has increasingly contributed to the fish supply worldwide (FAO 2020). There are several ways to classify aquaculture systems, ranging from the degree of intensification and the use of feed to water renewal or the environment where the farm is installed (Tidwell 2012). Most of the global aquaculture volume is produced in semi-intensive pond systems or intensively in cages (FAO 2020). The pond and cage systems in general require a low degree of technology and, when well-managed, are efficient for fish production (Masser 2012; Tucker and Hargreaves 2012). However, in some situations where proper management is not carried out, that is, no treatment of the effluents occurs or the carrying capacity of the environment is neglected (Boyd 2003; Turcios and Papenbrock 2014; Henares et al. 2020; Boyd et al. 2020), eutrophication of waterbodies might result (Joyce et al. 2019). In addition, these traditional pond and cage aquaculture systems depend on large volumes of water, extensive areas of land, and/or in some critical scenarios the use of antibiotics to achieve high productivity (Boyd and Gross 2000; Lulijwa et al. 2020; Boyd et al. 2020). All these environmental problems undermine aquaculture's sustainability (David et al. 2018; Ahmed et al. 2019).

In order to ensure that the growth of aquaculture does not occur in a disordered way, which will consequently affect its full development, new technologies and management strategies have been proposed to adapt aquaculture to sustainable production methods (David et al. 2018; Ahmed and Thompson 2019). Sustainable aquaculture systems are those that enable maximum production per volume with minimum negative environmental impact and less use of resources (Van Rijn 2013). In this sense, in the last decade an increased number of studies have been seen which focused on closed aquaculture systems which require low volumes of water and minimize effluent discharge. Examples of these types of systems are the recirculating aquaculture systems (RAS) and those using biofloc technology (BFT) (Ebeling and Timmons 2012; Khanjani and Sharifinia 2020). RAS is a filter-based aquaculture system where water is constantly recirculated and partially reused (Verdegem 2013). For this, mechanical filters are used to remove the solid wastes and biofilters, colonized by nitrifying bacteria, are required to convert the toxic metabolic wastes from fish (ammonia is oxidized into nitrite and then to nitrate) and to purify the water (Ebeling and Timmons 2012; Rurangwa and Verdegem 2015). BFT is a closed aquaculture system based on the microbial-loop concept, where the growth of a specific microbial community, such as heterotrophic and nitrifying bacteria, is stimulated in the fish and/or shrimp tanks (Avnimelech 2015; Emerenciano et al. 2017; Samocha 2019; Boyd et al. 2020). Compared to the traditional low-technology aquaculture systems, RAS and BFT offer the advantage of producing aquatic animals in a controlled environment, with a high degree of water reuse and predictable harvesting schedules (Ebeling and Timmons 2012; Avnimelech 2015). However, these systems are highly dependent on electricity for adequate operation, and specialized labor. Besides that, RAS and BFT are usually employed in monocultures and do not reuse the leftover nutrients to nourish other species in traditional configurations (Badiola et al. 2018; Walker et al. 2020).

Integrated multi-trophic aquatic systems are recognized as a modern and more sustainable production method (Goddek et al. 2019a; Boyd et al. 2020). Multi-trophic systems combine the culture of fed species with extractive species, aiming to simulate a natural ecosystem. By this mix of species, the accumulated nutrients and by-products from the fed culture are used by the extractive species for their own growth (Nederlof et al. 2019; Boyd et al. 2020). Nutrient reuse allows the minimization of the environmental impacts of food production, reduction of the costs of fertilizers and water, and contributes to the development of circular food production (Bohnes et al. 2019; Reid et al. 2020). Moreover, the integration of systems and species with different trophic functions increases the variety of products offered and provides food security for local consumers (Kyaw and Ng 2017; Gott et al. 2019).

Aquaponics is an example of an integrated agri-aquaculture system which combines aquatic animal and vegetable production (Lennard and Goddek 2019). The most common and traditional aquaponics system configuration integrates freshwater RAS and hydroponics systems in one loop (König et al. 2018; Yep and Zheng 2019). However, aquaponics is a research field under development and variations on the common one-loop configuration are frequently being proposed to improve the efficiency in the food production process (Kotzen et al. 2019). Examples of different system designs are: decoupled aquaponics systems (Kloas et al. 2015; Goddek et al. 2016), multi-loops aquaponics systems (Goddek et al. 2019b; Goddek and Keesman 2020), algaeponics systems (Addy et al. 2017), maraponics systems (Kotzen and Appelbaum 2010), and the use of biofloc technology (Kotzen et al. 2019) or FLOCponics systems, as recently named by Pinho et al. (2021b).

FLOCponics is defined as the integration of biofloc-based aquaculture with hydroponics (Pinho et al. 2021b). Thus, FLOCponics is an alternative type of aquaponics system where RAS is replaced by a system based on BFT. Kotzen et al. (2019) presented a brief overview of the research carried out on the integration of BFT and plant production. However, they do not provide detailed information about the productive results reached or a critical discussion of the challenges and contributions of such integration to sustainable food supply. The aims of this paper are to: (i) present FLOCponics systems, the justifications for its employment, and an overview of the technical results that have been achieved so far; (ii) discuss the economic and environmental aspects of these systems and the relevance of its development to the food supply; and (iii) highlight current FLOCponics challenges and give directions for further research.

To achieve the aforementioned aims, this review is structured into a further six sections. Firstly, a brief overview of biofloc technology and aquaponics is given in *sections 2.2* and 2.*3*. Then, FLOCponics systems are presented in *section 2.4*. This section is divided into subsections in which a theoretical background is introduced, and information regarding the system setups, water quality and nutrient recycling, and productive results of plant and fish achieved in FLOCponics research are detailed. The main potential technical-economic, social and environmental characteristics of FLOCponics are shown in *section 2.5*. Lastly, the challenges of FLOCponics are discussed in *section 2.6* and the final remarks are presented in *section 2.7*.

2.2 Biofloc Technology

Biofloc Technology (BFT) was developed in the 1970s, by the French Research Institute for Exploitation of the Sea (IFREMER) (Emerenciano et al. 2013; Samocha 2019). Their aim was to improve the productive performance of aquatic animals and solve problems of disease outbreaks in marine shrimp farming (Emerenciano et al. 2013; Samocha 2019). The promising results of BFT were disseminated and, due to its flexibility, such technology is also currently applied in fish farms. Biofloc-based culture is characterized by the presence of specific microbial communities, which enable the intensive and biosafe culture of aquatic organisms (Verdegem and Bosma 2009; Crab et al. 2012; Avnimelech 2015). The growth of heterotrophic bacteria is stimulated by the manipulation of the carbon:nitrogen (C:N) ratio, normally ranging from 10 to 20:1, with constant water movement and aeration and minimal water exchanges (Browdy et al. 2012; Avnimelech 2015) In addition to heterotrophic bacteria, chemoautotrophic bacteria and planktonic organisms, mainly microalgae, copepods, cladocera, protozoa and rotifers, are also frequently reported in biofloc cultures (Ray et al. 2010b; Martínez-Córdova et al. 2015; Brol et al. 2017). The predominance of each group of microorganisms will depend on the target shrimp/fish species, the productive management, and the inputs used (Martínez-Córdova et al. 2015; Emerenciano et al. 2017). Such predominance will define the BFT trophic level, usually categorized as photoautotrophic (algae-based system), chemoautotrophic (based on nitrifying bacteria), heterotrophic (based on heterotrophic bacteria), or mixotrophic systems (Avnimelech 2015; Samocha 2019).

Under proper operation of the system, biofloc microbial aggregates confer several benefits to aquaculture production. Suitably operating a biofloc-based system means, in general, providing the water quality and nutrients required for the growth of the target species and microorganisms (Minaz and Kubilay 2021). In in situ BFT, the microorganisms are constantly available, rich in nutrients, and complement the nutritional requirements of the reared animals (Hargreaves 2013; Bossier and Ekasari 2017; Wasielesky et al. 2020). Consequently, BFT allows for the application of nutritional management strategies which reduce expenses and the negative impacts of aquaculture, for instance, the reduction of fish meal and protein levels in the diets used (Ballester et al. 2010; Moreno-Arias et al. 2018; Sgnaulin et al. 2020, 2021). The biofloc microbiota also confers stability on the system and maintains water quality by recycling the nutrients, incorporating ammonia excreted by organisms into bacterial biomass and promoting the microbial-loop (Krummenauer et al. 2014; Emerenciano et al. 2017). In addition, BFT contributes to minimizing the occurrence of diseases. An improvement in the nutritional and immunological status of the animals through the consumption of bioactive compounds in the bioflocs, and a reduction in the presence of pathogens, has already been reported (Browdy et al. 2012; Dauda 2020). Recent research has also demonstrated the positive effect of BFT on gut microbiota (Li et al. 2018) and on health and enzymatic activity (Durigon et al. 2019).

Biofloc technology has been employed in aquaculture farms and research centers worldwide. In recent years, the number of publications has significantly increased. A total of 138 articles about “biofloc” were published between 2001 and 2010, and this number increased to 635 between 2011 and 2019 (source: ScienceDirect 2020). There are already several reviews and overviews on this topic. The papers range from the definition and detailed explanation of BFT (Hargreaves 2006; Crab et al. 2012; Avnimelech 2015) to more specific subjects, such as the profile of microorganisms usually found (Martínez-Córdova et al. 2017) and their positive effect on water quality (Emerenciano et al. 2017; Luo et al. 2020; Robles-Porchas et al. 2020), animal health (Dauda 2020) and nutrition (Emerenciano et al. 2013; Hargreaves 2013; Martínez-Córdova et al. 2015; Nevejan et al. 2018; Sánchez-Muros et al. 2020). Most research articles on BFT evaluate the production of Pacific white shrimp *Litopenaeus vannamei* (Wasielesky et al. 2006, 2020; Krummenauer et al. 2014; Samocha 2019) and tilapia *Oreochromis spp.* (Azim and Little 2008; Durigon et al. 2019; Emerenciano et al. 2021), although some studies have already shown the suitability of BFT for other species (Walker et al. 2020).

The benefits of BFT are numerous and well known. However, it is a complex system (Avnimelech 2015), not applicable to all aquaculture species (Sgnaulin et al. 2018), and commercially should be applied with proper technical supervision. Some examples of BFT disadvantages in relation to other aquaculture technologies are: (i) the need for intensive monitoring of the physical-chemical parameters of the water; (ii) continuous dependence on electricity; and (iii) the need for specialized labor (Walker et al. 2020; Boyd et al. 2020). Moreover, the accumulation and high (toxic) concentration of nutrients, such as nitrate and phosphate as a result of high fish/shrimp stocking density and low water renewal (Crab et al. 2012; Pinheiro et al. 2020), may affect the efficiency and stability of the system in the long-term. In this sense, its integration with hydroponic vegetable production (in a FLOCponics system) could be an alternative to minimize these problems (Kotzen et al. 2019).

2.3 Aquaponics

In aquaponics systems, aquaculture effluents are transformed by nitrifying bacteria into bioavailable nutrients for plants, supporting almost full feed utilization and plant growth (Wongkiew et al. 2017; Yildiz et al. 2017; Paudel 2020). In aquaponics, nutrients are recycled and low volumes of water are used (Lennard and Goddek 2019), which reduces the negative environmental impacts usually associated with low efficiency in the use of natural resources in conventional food production (Cohen et al. 2018).

To make agri-aquaculture integration viable, a basic layout including some indispensable components is required. An aquaponics system basically consists of aquatic organism tanks and filters (mechanical and biological), which make up the recirculating aquaculture system, connected to hydroponic beds (Lennard and Leonard 2006). Changes in this layout can be found depending on the adopted production scale, i.e., whether it is for hobby, small-scale (semi-commercial) or large-scale (commercial) production. Small-scale production is usually low-cost and flexible in terms of materials used and species produced, while commercial aquaponics needs high investment, labor and upgrading (Palm et al. 2018). Different design, greenhouse environment, management, and type of hydroponic bed are often reported for large production systems (Love et al. 2015; Palm et al. 2018). The objective of the entrepreneur and the requirements of the reared species will define which layout should be used.

Many potential species can be produced in aquaponics depending on the employed system design (Pinho et al. 2021a). For the success of aquaponics, the aquaculture species must have suitable characteristics for production in intensive recirculating aquaculture systems. They should be rustic and tolerate high stocking densities, handling, and a wide range of physical-chemical water parameters (Yep and Zheng 2019). Although there are some reports on the culture of other aquatic organisms, the production of fish, mainly tilapias (*Oreochromis spp.*), catfish (order Siluriformes) and salmonids, are predominant in aquaponics farms (Superior Fresh; Love et al. 2015; Mchunu et al. 2018; Yep and Zheng 2019). Regarding the plants, in general, those that are produced in hydroponics systems thrive in aquaponics. Plant production in aquaponics is directly related to the nutritional characteristics of fish/shrimp feed and the rate of nutrient mineralization by microorganisms (Goddek et al. 2015; Eck et al. 2019). Besides that, plant growth frequently depends on extra fertilization to better meet its nutritional requirements (Eck et al. 2019; Maucieri et al. 2019). In contrast to coupled systems, meeting the nutritional requirements and water conditions for each loop (aquaculture, hydroponics and filters) is possible in decoupled systems due to the individualization of the productive units (Monsees et al. 2017b; Goddek et al. 2019b). It is worth noting that the terms coupled and decoupled aquaponics systems were recently renamed as “permanent coupled” and “on-demand coupled” systems, respectively (Baganz et al. 2021). However, even though these new nomenclatures should be used in further studies, in the present paper, the system layouts were referred coupled and decoupled as labelled in the reviewed papers. Regardless of the design employed or species grown, aquaponics is recognized as offering a wide variety of products that ensure safe and healthy food. This is mainly because minimal or no chemicals such as pesticides and antibiotics are used (Kyaw and Ng 2017; Joyce et al. 2019).

Although aquaponics is an emerging food production technology, several articles have already been published about it. Goddek et al. (2015) presented a detailed review on the characteristics and opportunities of aquaponics. They also discussed the challenges for commercial aquaponics production and the trade-offs between the needs of fish, filter-bacteria and plants in a coupled system. These trade-offs and the dynamics of the decoupled system were discussed in depth by Goddek et al. (2016). After the publication of 160 articles between 2015 and 2019, Yep and Zheng (2019) updated the general trends of aquaponics and showed that research focused on system design, hydroponics components, fish species, plant species, and microflora has increased. Besides these topics, others relating to and focused on aquaponics production have also been investigated and reviewed. For example, studies on economic viability (Bosma et al. 2017; Stadler et al. 2017; Quagrainie et al. 2018; Greenfeld et al. 2019), sustainability (Forchino et al. 2017; Maucieri et al. 2018; Körner et al. 2021), simulation and predictions through mathematical models (Karimanzira et al. 2016; Lastiri et al. 2016; Estrada-Perez et al. 2018; Keesman et al. 2019), use of aquaponics as an educational tool (Junge et al. 2019), applicability of multi-loop aquaponics systems (Yogev et al. 2016; Baganz et al. 2020), and application of other aquatic animal species (Kotzen et al. 2019) are also found in the literature. In most of these papers, it is emphasized that aquaponics systems carry great potential to overcome some of the technical and environmental challenges of the agricultural and aquaculture sector.

Some fields of aquaponics still require research and must be improved in order to exploit their full potential. For example, a few studies have recently been developed on how the nutrients of RAS water-sludge can be recycled and used for plant production (Monsees et al. 2017a; Delaide et al. 2019). Each aquaponics system and species reared need specific water parameters, nutrient balance and pest management. Meeting these specifications is usually the main technical challenge faced by traditional coupled systems (Palm et al. 2019; Stouvenakers et al. 2019). In addition, commercial aquaponics is highly dependent on specialized labor, due to the need for multi-disciplinary knowledge to run the system (Goddek et al. 2015; Love et al. 2015; Forchino et al. 2017).

2.4 FLOCponics

2.4.1 Background

Aquaponics and biofloc-based aquaculture are considered environment-friendly approaches to food production. Both are intensive aquaculture systems with a strong focus on nutrient recycling and water saving (Rocha et al. 2017; Boyd et al. 2020). FLOCponics shares these characteristics. By adopting the principles of aquaponics and bioflocs, FLOCponics can become an additional means to reduce the challenges of the global sustainable food supply. Recently, the term "FLOCponics" was proposed by Pinho et al. (2021b) to identify and unify the systems that have been called "BFT+hydroponics", "BFT+aquaponics" or "BFT+plant production". All these terminologies were used in the search for papers in the ScienceDirect, Google Scholar and Scopus databases, and papers published until September 2020 were considered. The reference lists presented in the articles found were cross-referenced in our review, i.e., these lists were checked in order to find the papers that were not discovered at first. In total, twenty-two papers were found and reviewed, of which 4 were theses and 18 were articles published in peer-review journals (3 of them were found by cross-referencing).

In general, the twenty-two papers found theoretically justified the use of FLOCponics systems by their potential to combine and maximize the advantages of BFT and traditional aquaponics using RAS and/or to minimize their limitations. High nutrient use efficiency and reduction of waste are examples of strengths of aquaponics that can be potentialized in FLOCponics systems (Rocha et al. 2017; Pinheiro et al. 2017). Furthermore, the FLOCponics researchers usually state that adding hydroponics production to a BFT farm may expand economic diversity by producing additional value-added products (plants) and reduce the negative environmental impacts of biofloc-based production, such as the accumulation of nitrate and phosphorus in BFT culture and its discharge through solids management (Poli et al. 2019; Luo et al. 2020; Emerenciano et al. 2021). From an agri-aquaculture production point of view, it is also expected that BFT brings relevant benefits. For example, the improved zootechnical performance reported in BFT compared to RAS cultures (Luo et al. 2014; Guemez-Sorhouet et al. 2019) and the positive effects of BFT on animal nutrition and health (Dauda 2020) suggest that FLOCponics may offer an advantage. Regarding plant growth, the main characteristics that make BFT effluent a promising fertilizer are: (i) the high concentration of nutrients; (ii) the diversity of microorganisms, which are constantly recycling nutrients and may increase their availability or help their absorption by the plants; and (iii) the low investment in filters for water treatment (Emerenciano et al. 2013; Pinho et al. 2017; Pinheiro et al. 2020). Although the authors presented many theoretical advantages of using FLOCponics, some of them were not yet fully proved.

The overview of the objectives and general findings of these papers are described in Supplementary Material (Table S2.1). The details and specific results related to plant and animal growth as well as the system designs and nutrient insights are described in the next subsections. In addition to the twenty-two papers found, three other peer-review articles that reported on the use of BFT effluent for the production of plants in soil were found (Joesting et al. 2016; Doncato and Costa 2018; de Souza et al. 2018). However, they do not fit the definition proposed here for FLOCponics (BFT + hydroponics). Because of this, these articles were not considered in the descriptions and discussions of the system.

2.4.2 System setups

The employed designs of FLOCponics systems are summarized in Table 2.1. Most of the experiments were run in coupled system configurations and only 30% used decoupled (on-demand coupled) systems (Figure 2.1). In coupled configuration, the water and nutrients are fully recirculated between all subsystems (BFT, optional filters, and hydroponics). For decoupled FLOCponics systems, the respective subsystems are seen as stand-alone systems and the water and nutrients are directed from BFT, to filters (optional use) and end-up in the hydroponics subsystem. No study compared or evaluated the possible effects of coupled and decoupled configurations on production in FLOCponics systems. Different types of hydroponics subsystems are employed, in which the Nutrient Film Technique (NFT) and Deep Water Culture (DWC) were mostly used (Figure 2.1). NFT comprises shallow channels where the plants are allocated. A thin layer of nutrient solution flows through these channels to partially irrigate the roots of the plants. In DWC plants are produced in floating supports on tanks filled with nutrient solution (Goddek et al. 2015; Maucieri et al. 2019). No experiment was reported that assessed whether the type of hydroponics system affects the efficiency of FLOCponics systems in terms of food production and nutrient use. In view of this lack of data, it is still unknown which type of hydroponics subsystem works better in FLOCponics.

Table 2.1. Overview of system setups in FLOCponics research.

Reference	**System configuration**	**HP subsystem**	**BFT subsystem**	**Filters**	† **HP : BFT : FT volume**	‡ **Water flow (L min^{-1})**
Barbosa (2017)	Coupled	DWC: 3 tanks of 120 L each	Fish tank: 500 L	(2ni) Treatment with filters: 18 L settling tank, bag filter and 70 L biofilter and sump	0.72 : 1.0 : 0.18 (with filter) 0.72 : 1.0 : 0.0 (without filter)	8.0
Castro-Castellón et al. (2020)	Coupled	DWC: Floating structure on top of the fish tank (Ø 100 cm)	Fish tank: 125 L	-	-	-
Castro-Mejía et al. (2020) Martínez-Meingüer (2020)	Coupled	NFT: pipelines measuring 10 cm in diameter and 1 m in length	Fish tank: 160 L	-	0.19 : 1.0 : 0.0	-
Lenz et al. (2017)	Coupled	DWC: 9 tanks of 72 L each	Fish tank: 500 L	(2m) Two settling tanks (100 and 10 L), a bag filter, 60 L biofilter and 150 L sump	1.30 : 1.0 : 0.64	1.1
Neto (2017) Pinheiro et al. (2017, 2020) Silva (2016)	Coupled	NFT: 0.3 - 0.8 m^2 placed on top of the shrimp tank	Shrimp tank: 800 L	(2p) 40 L settling tank	0.02 - 0.06 : 1.0 : 0.05	3.0
Pinho et al. (2017)	Coupled	DWC: 9 tanks of 150 L each	Fish tank: 500 L	(2ni) 100 L settling tank, 60 L biofilter, and 100 L sump	2.70 : 1:0 : 0.52	8.0

Pinho et al. (2021b)	Coupled	DWC: 3 tanks of 150 L each	Fish tank: 500 L	(2a) Two settling tanks (100 and 10 L) and 70 L sump	0.90 : 1:0 : 0.36	8.0
Poli et al. (2019)	Coupled	NFT: 0.3 m^2 placed on top of the shrimp tank	Fish tank: 90 L Shrimp tank: 800 L	-	0.02 : 1.0 : 0.0	10.8
Rocha et al. (2017)	Coupled	(1) DWC: 700 L	[1] Fish tank: 300 L	-	2.33 : 1.0 : 0.0	10.8
Zidni et al. (2019)	Coupled	NFT	Fish tank: 375 L	-	-	-
Blanchard et al. (2020)	Decoupled	Other: 16 buckets of 11 L each	Fish tank: 102,000 L	(2nr) Two settling tanks connected in a series (1500 L each)	0.002 : 1.0 : 0.03	Plants received between 6 and 8 L of biofloc effluent every day
Doncato and Costa (2021)	Decoupled	NFT: pipelines measuring 10 x 5 cm and 3 m in length connected to a 450 L sump	Shrimp tank: 40,000 L	(2ni) 500 L settling tank, bag filter (50 µm) and 3000 L reservoir	- : 1.0 : 0.04	13.1
Fimbres-Acedo et al. (2020a, b)	Decoupled	NFT: pipelines measuring 7.6 cm in diameter connected to a 250 L sump	Fish tank: 1000 L	(2nr) 300 L settling tank, 1000 L AEMBR and bag filter (5 µm)	0.25 : 1.0 : 1.30	HP subsystems received water from the BFT subsystem in the beginning and middle of the experiment
Martinez-Cordova et al. (2020)	Decoupled	NFT: pipelines measuring 4 cm and 6 m in length	Fish tank: 400 L	-	-	Every three days the water from the HP subsystem was discharged, and replaced with water from the fish tanks, with or without bioflocs.

Pickens et al. (2020)	Decoupled	Other: 0.32 m^2 pots filled with grade perlite and a steel cable trellis system running the length of the greenhouse	Fish tank: 100,000 L	(2ni) Two settling tanks connected in a series (1900 and 1000 L)	0.001 : 1 : 0.015	0.06 in increasing times of irrigation per day throughout the experiment
Rahman (2010)	Decoupled	DWC: 24 buckets of 6 L each	Fish tank: 125,000 L	(2nr) Settling tank	0.001 : 1.0 : -	The water in each plant bucket was completely exchanged every day

– No data available or component not present. † Water volume ratio between the subsystems, based on the initial volume without considering water exchanges. ‡ Water flow in the hydroponics subsystem. (1) Hydroponics bed and BFT tank shared the same 1000-L tank, which was separated by a 0.26-mm net. (2) Management of the settled biofloc/sludge: returns to the BFT subsystem through a) airlift system; p) pump; or m) manual; nr) collected for further analysis or did not return to BFT subsystem; and ni) no information provided. HP: Hydroponics. BFT: Bioflocs technology. FT: Filters. DWC: Deep water culture. NFT: Nutrient film technique. AEMBR: Aerobic mineralization bioreactor.

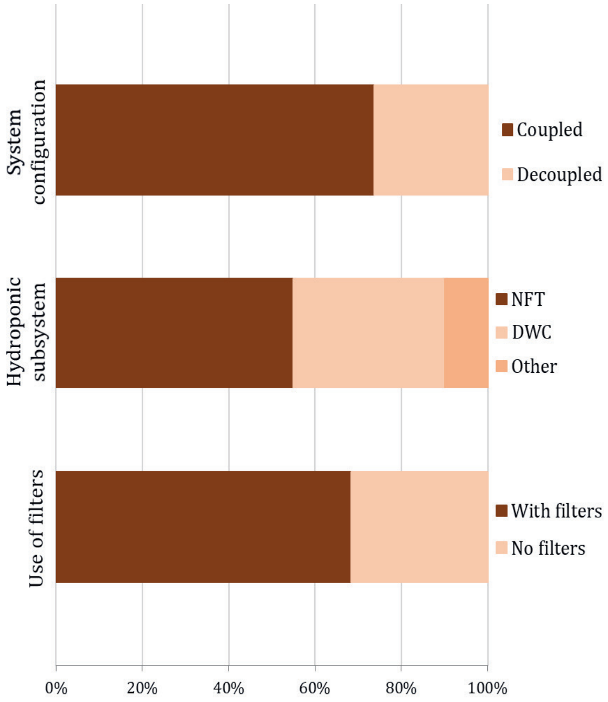

Figure 2.1. Proportion of the use of different system setups in FLOCponics research. DWC: Deep Water Culture. NFT: Nutrient Film Technique.

With respect to the aquaculture subsystem, tanks with different volumes have been used, varying from 125 - 1000 L to more than 100,000 L. The high volumes of fish tanks (>100 m^3) were reported by Rahman (2010), Blanchard et al. (2020), Pickens et al. (2020) and Doncato and Costa (2021). These authors took the effluent from BFT tanks daily or weekly, streaming the water for plant production in decoupled systems. In addition, a remarkable feature was the use of artificial substrates in the shrimp tanks by Silva (2016), Neto (2017), and Poli et al. (2019). These authors did not test the effects of the substrates on FLOCponics production, they were used as a management usually recommended for shrimp growth in BFT (Moss and Moss 2004; Schveitzer et al. 2013; Olier et al. 2020) The adoption of substrates has been proposed to increase the surface area of the tank and favor the growth of periphyton (Martínez-Córdova et al. 2015). Periphyton-based aquaculture brings advantages such as serving as a complementary food for the cultivated animals and assisting in the cycling of nutrients (Azim et al. 2005). Studies on the use of substrates in FLOCponics systems should be carried out to better understand its effect on animal and plant growth, as well as on the quality and amount of nutrients available for the hydroponics subsystem.

In BFT production, the use of simple settling tanks is often needed to control the solids concentration in the fish/shrimp tanks (Ray et al. 2010a; Avnimelech 2015; Gaona et al. 2016). A high concentration of solids can negatively impact the operation of the system since it can result in higher oxygen demand. The recommended range of solids concentration for the production of tilapia and shrimp in biofloc-based systems are 5 to 50 and 5 to 15 mL L^{-1}, respectively, usually measured as volume of bioflocs in Imhoff cones (Hargreaves 2013; Emerenciano et al. 2017). The use of filters in FLOCponics research seems to be optional and varies according to each investigation. In total, 65% of FLOCponics systems employed some type of filters between the BFT and hydroponics subsystems (Figure 2.1). Settling tanks were always present in the filter systems and extra biological filters in 23% (Table 2.1). In general, only information about the total volume and type of filter used in the FLOCponics filter system has been reported so far. Unlike in biofloc-based systems without integration, the use of filters in FLOCponics was intended to try to avoid the flow of particulate matter to the hydroponics subsystems as such particulates may impair plant growth. Except for the systems run by Fimbres-Acedo et al. (2020a, b) and Doncato and Costa (2021), all the others constantly recirculated the water through the filters and 46% of them used some mechanisms to return the decanted biofloc/sludge to the BFT subsystem. Fimbres-Acedo et al. (2020a, b) employed a decoupled system where the hydroponics subsystems received water from the BFT subsystem only in the beginning and middle of the experiment. At these moments, the water from the BFT subsystem was pumped to the 300-L settling tank and left to settle for 24 hours. Subsequently, the supernatant was transferred to a 1000-L aerobic mineralization bioreactor (AEMBR), filtered with a 5-µm bag filter and then directed to the hydroponics subsystem. Doncato and Costa (2021) directed the water from the BFT tanks to the settling tank and bag filters and then to the hydroponics subsystems once a week. With this procedure, the authors managed to reduce the concentration of suspended solids between the affluent and effluent of the filters by 71%. The frequent use of filters in FLOCponics indicates that the BFT management should focus on providing inorganic nutrients to the hydroponics subsystem instead of directing the microbial flocs to it.

A lack of standardization in the proportions of water volumes of the hydroponics, BFT, and filter subsystems was detected among the reviewed papers (Table 2.1). A wide variation was also observed in the water flow through the hydroponic beds, varying from 0.06 to 13.1 L min^{-1}, and in the strategies to direct the water from BFT to hydroponics subsystems in decoupled systems. The lack of a standard among the system setups points out that FLOCponics is still in its initial stage. It further indicates a research gap related to the dimensioning of hydroponics and filter subsystems in relation to the BFT tanks. The implications of this lack of standardization are discussed in *section 2.6.*

In general, simple greenhouses covered with transparent plastic polyethylene and a shading net (20-50% of light retention) were home to most of the experimental FLOCponics systems. These structures tend to have low effectiveness in climate control. Rocha et al. (2017), Castro-Mejía et al. (2020), Castro-Castellón et al. (2020), Martínez-Meingüer (2020), and Pickens et al. (2020) reported different structures. Castro-Mejía et al. (2020), Castro-Castellón et al. (2020), Martínez-Meingüer (2020) carried out the experiments in an indoor lab using LED light to support plant growth. Pickens et al. (2020) used greenhouses equipped with environmental controls for year-round production. Rocha et al. (2017) did not use a greenhouse or any covered structure to run their low-cost FLOCponics systems.

2.4.3 Water quality and nutrient recycling

One of the main characteristics of biofloc-based systems is the ability of BFT microorganisms to recycle nutrients and maintain ideal water quality for the reared animal species (Emerenciano et al. 2017). Phytoplankton, nitrifying bacteria and heterotrophic bacteria contribute to ammonia-nitrogen cycling by converting the toxic ammonia-nitrogen to nitrate or assimilating it into bacteria biomass (Ebeling et al. 2006; Avnimelech 2015). All these types of nitrogen conversion usually happen at the same time and the predominance of one depends on the nutrient management of the system (Hargreaves 2006; Dauda 2020). Additionally, the physical-chemical parameters of the water must meet the requirements of these microorganisms. In particular, high levels of dissolved oxygen (DO) and alkalinity, as well as a high C:N ratio, should be provided. Detailed information about the water quality required for BFT microorganism growth and the standard values of water parameters that must be maintained in the fish or shrimp tanks in BFT systems can be found in Avnimelech (2015), Emerenciano et al. (2017) and Samocha and Prangnell (2019).

The results of the experiments run in FLOCponics systems and focused on animal production (Table S2.1) showed that most of the physical-chemical water quality parameters remain within the acceptable ranges for fish or shrimp production. An exception was the volume of bioflocs (total suspended solids), which was lower than recommended. For example, Lenz et al. (2017) and Pinho et al. (2017, 2021b) reported, respectively, 2.6 to 4.9 mL L^{-1}, 0.2 mL L^{-1}, and 0.2 to 0.95 mL L^{-1} as mean values of volume of bioflocs in tilapia culture, which are below the minimum recommended of 5 mL L^{-1} (Hargreaves 2013). However, these low values seemingly did not affect the maintenance of water quality and nitrogen recycling by the microorganisms. Based on that, it is reasonable to state that the relation between microbial activity and volume of biofloc in FLOCponics, and even in BFT monocultures, is highly variable and still unclear.

For plants, some physical-chemical parameters of water often seem to be non-ideal, mainly regarding the pH and suspended solids values in the coupled FLOCponics systems. The recommended pH range for hydroponics production is generally between 5.5 and 6.5 to ensure high nutrient availability for plant uptake (Tyson et al. 2004). Despite that, most of coupled FLOCponics systems reported so far were run with pH close to neutrality. In a decoupled system, Blanchard et al. (2020) evaluated the effect of four pH levels (5.0, 5.8, 6.5 and 7.0) on nutrient availability in the hydroponics subsystems. The authors showed there were no overarching effects on plant growth that would demand pH regulation in the FLOCponics system. With respect to suspended solids in water, a very low concentration of solids must be maintained in the hydroponics subsystems to avoid the deposit of bioflocs in the plant roots and consequently the impairment of the breathing process and the absorption of nutrients by plants (Rakocy 2012). However, high solids concentration in the hydroponics tanks have been reported in FLOCponics systems (Kotzen et al. 2019; Emerenciano et al. 2021; Pinho and Emerenciano 2021). Keeping biofloc concentration in the fish tanks at appropriate levels for animal production and at the same time maintaining low solids concentration in the hydroponics subsystems seems to be one of the trade-offs of coupled FLOCponics.

The input of nutrients and their transformation by microorganisms are as important as providing ideal conditions of water quality for all subsystems. In traditional aquaponics, most of the nutrients that nourish plants are expected to come from the RAS effluent (Palm et al. 2018), and should also be the case in FLOCponics. The addition of organic and inorganic carbon sources to regulate the heterotrophic community and water alkalinity, respectively, may offer extra nutrients in FLOCponics as compared to RAS, where feed is commonly the only source of nutrients in the aquaculture subsystem. Both procedures are often required to promote the growth of BFT microorganisms (Avnimelech 2015; Emerenciano et al. 2017). Table 2.2 compares the nutritional management and sources of nutrients used in FLOCponics research. No standardization of these factors among the studies was found, probably due to the different species used, animal size, maturation stage of the bioflocs, and carbon source. Hydroponic fertilizers were used only in four studies (Rahman 2010; Castro-Mejía et al. 2020; Martínez-Meingüer 2020; Doncato and Costa 2021). It should be noted that little data is provided on the profile of macro- and micro-nutrients of the nutrient sources. The information is usually limited to the dietary protein content and the type of carbon source used.

Given the aforementioned lack of detailed information on the characteristics of the source of the nutrients fed to the FLOCponics systems it is hard to predict how many nutrients will be available for plant production. In addition, the rate of nutrient recycling and nutrient uptake by the BFT microorganisms are still unclear, which makes predictions very uncertain. Analyzing nutrient content on the plant biomass is a way to estimate which nutrients have been minimally provided. Additionally, recent studies have evaluated the macro- and micro-nutrients available in the water and the solid portion (visible biomass) of FLOCponics systems, in an attempt to minimize uncertainty in predictions (Pickens et al. 2020; Fimbres-Acedo et al. 2020b; Doncato and Costa 2021; Pinho et al. 2021b). In general, lower concentrations of nutrients in FLOCponics water as compared to hydroponic solutions have been found (Pickens et al. 2020). On the other hand, when compared to traditional aquaponics using RAS, higher concentrations of P, K, Ca, S, and Fe were found and seem to be associated with the practice of external carbon addition (Doncato and Costa 2021; Pinho et al. 2021b). Moreover, high concentrations of nutrients in the solid portion of the decanted bioflocs, which are not bioavailable for plants, was also reported (Rahman 2010; Blanchard et al. 2020; Fimbres-Acedo et al. 2020b). Fimbres-Acedo et al. (2020b) suggested that these solids could be mineralized, enhancing nutrient availability. Studies have recently been carried out to mineralize RAS-sludge via bioreactors and successfully use its effluent as fertilizer in multi-loop aquaponics (Goddek et al. 2016). The use of mineralized solids/bioflocs biomass to nourish plants in FLOCponics has not yet been well reported.

Using plants as a filter to remove nutrients from BFT water is one of the approaches related to nutrient recycling that has been investigated in FLOCponics research. In these studies, the focus has been on N and P recovery and their transformation into plant biomass. Silva (2016), Pinheiro et al. (2017, 2020) and Poli et al. (2019) analysed the recovery of N and P from marine BFT effluent by halophyte plants. Their results showed that 24.1-39.3% of N and 14.8-19.4% of P from the total feed input can be removed as a result of the integration of shrimp and plant production. It is important to mention that both nutrients normally accumulate in BFT water (Pinheiro et al. 2017; Luo et al. 2020). At high concentrations, they can be toxic for the reared animal or, when discharged into natural water bodies, they can be potential causes of water eutrophication (Ahmad et al. 2017).

Table 2.2. The main source of nutrients in FLOCponics.

Reference	**Feed**			**BFT maintenance**			**Other**
	Frequency (times per day)	Amount	Composition (%)	C:N ratio	Carbon source	† Method	Alkalinity correction
Barbosa (2017)	3	2.2% of fish biomass, 2 g feed day^{-1} plant^{-1}	CP: 32	15:1	White sugar	Based on daily input of N-feed, added once a day	-
Blanchard et al. (2020)	2	Until satiation	CP: 36 Cfat: 6 Cfib: 3.5 P: 0.9	-			-
Castro-Castellón et al. (2020)	2	5% of fish biomass	-	-	Coffee, Yucca, Moringa or Macroalgae	0.1% of fish biomass, added once a day	-
Castro-Mejía et al. (2020) ‡	-	5% of fish biomass	CP: 35 Cfat: 10	-	Moringa	0.01% of fish biomass	-
Doncato and Costa (2021) ‡	2	1.5% of shrimp biomass	CP: 35 P: 1.5 Ca: 1.5- 3.0	-			-
Fimbres-Acedo et al. (2020a, b)	5	1.2-fold daily protein intake	CP: 40.6	13:1	Unrefined sugar	Based on daily input of N-feed, five times per day only in heterotrophic tanks	-
Lenz et al. (2017)	3	2.7% of fish biomass, 1 g feed day^{-1} plant^{-1}	CP: 32	14:1	Powder molasses	Based on daily input of N-feed, 17 g day^{-1} once a day	10 g Calcium oxide (CaO) were added in all fish tanks in the beginning of the experiment
Martinez-Cordova et al. (2020)	-	Until satiation	CP: 30	The fish tanks were supplied with bioflocs produced in an external single bioreactor, fertilized with Triple 17 (17% N, 17% P, and 17% K). Wheat and amaranth seeds were supplied as nucleation substrates at the			-

				beginning of the trial or when suspended solids were below 20 mL L^{-1}.			
Martínez-Meingüer (2020) ‡	-	5% of fish biomass	CP: 35 vs CP: 47.5	-	Moringa	0.1% of fish biomass	-
Neto (2017)	4	-	CP: 35	The addition of organic carbon was not necessary since the BFT was already in a chemotrophic stage and a nitrification process was established.			Calcium hydroxide (Ca(OH)^{2}) was added when the alkalinity was under 120 mg L^{-1}
Pickens et al. (2020)	2	13% of fish biomass	CP: 36 Cfat: 6 Cfib: 3.5	The biofloc system used in this study was managed strictly as an autotrophic system with no supplemental carbon inputs			Calcium hydroxide was applied after each feeding to maintain a target pH of 6.8 to 7.0
Pinheiro et al. (2017)	4	-	CP: 35	The addition of organic carbon was not necessary since the BFT was already in a chemotrophic stage and a nitrification process was established.			Calcium hydroxide was added when the alkalinity was under 120 mg L^{-1}, at a rate of 20% of the daily feed intake
Pinheiro et al. (2020)	4	11 to 3% of shrimp biomass	CP: 38	The addition of organic carbon was not necessary since the BFT was already in a chemotrophic stage and a nitrification process was established.			Calcium hydroxide was added when the alkalinity was under 120 mg L^{-1}, at a rate of 20% of the daily feed intake
Pinho et al. (2017)	3	5% of fish biomass, 2 g feed day^{-1} plant^{-1}	CP: 22	15:1	Powder molasses	Based on daily input of N-feed, added once a day	-
Pinho et al. (2021b)	4	12% to 6% of fish biomass	CP: 36	20:1	Powder molasses	Based on daily input of N-feed, added once a day	-
Poli et al. (2019) - *Shrimp*	4	11 to 2.7% of shrimp biomass	CP: 35	No organic carbon was used because the ammonia did not reach levels above 1 mg L^{-1}			Calcium hydroxide was added daily at a ratio of 25% of the total feed

- *Tilapia*	1	1% of fish biomass	CP: 38				when the alkalinity was below 150 mg L^{-1}
Rocha et al. (2017)	3	3% of fish biomass	CP: 32 Cfib: 5.5 Ca: 0.5-2.5 P: 0.6 Fe:0.03	20:1	Molasses, wheat bran and rabbit diet	Water from an *ex situ* BFT was directed to each replicate twice over the experiment	-
Silva (2016)	4	-	CP: 35	-			Calcium hydroxide was added when the alkalinity was under 120 mg L^{-1}, at a rate of 20% of the daily feed intake
Zidni et al. (2019)	4	5	-	-	Molasses and flour	Water from an *ex situ* BFT was directed to each replicate once a day	-

– No information available. † Method used for adding the carbon source. ‡ Commercial hydroponic fertilizer was used. CP: Crude protein. Cfib: Crude fiber. Cfat: Crude lipid content. Ca: Calcium. P: Phosphor. Fe: Iron. C:N ratio: carbon and nitrogen ratio. BFT: biofloc technology. N-feed: Nitrogen content on animal feed.

2.4.4 Productive results

Only the experiments that statistically analyzed the plant and fish or shrimp growth and provided sufficient data to compare the productive performance were considered in the descriptions below. In general, the FLOCponics studies were conducted mainly by aquaculture researchers. Despite this, twenty-four trials were performed to evaluate plant production (Table 2.3) and twelve trials tested animal growth (Table 2.4) in FLOCponics systems.

2.4.4.1 Plant production

The use of nutrient-rich effluents from BFT to nourish hydroponic plants is a key point in FLOCponics systems. However, the studies carried out so far have not reached a consensus as to whether FLOCponics has a positive or negative effect on plant yields. To achieve conclusive results on the effect of BFT effluent on plant production, plant growth in this system should be compared with crops in hydroponics, traditional aquaponics using RAS and/or soil-based agricultural methods. At the same time, standardizing the composition of nutrients inputted in all systems might also be done during this comparison. Some of the reviewed papers compared FLOCponics to hydroponics and/or traditional aquaponics, but none of them to soil-based methods. In the studies that compared FLOCponics with other systems, the amount and composition of nutrients offered to the hydroponics subsystem were not the same in all treatments/systems. Eight trials were conducted to evaluate a type of management in FLOCponics and did not compare it to other production systems. Table 2.3 gives an overview of the experimental design and general results related to plant growth in FLOCponics.

Table 2.3. Overview of the experimental designs and results of plants growth in FLOCponics research.

Reference	**Trial**	**Species**	**Period** days	**Density** plant m^{-2}	† **Treatments**	**Yield** kg m^{-2}	**General results**
Barbosa (2017)	1	Smooth lettuce (*Lactuca sativa L.*)	14	16	FLOCponics system with filters (mechanical and biological) vs FLOCponics system without filters	0.87 - 0.90	No statistical difference was found between the treatments for lettuce growth.
	2	Crispy lettuce (*Lactuca sativa L.*)	14	16		0.27 - 0.28	Higher amount of biofilm in the roots of plants grown in FLOCponics without filters was found.
Lenz et al. (2017)	1	Smooth lettuce (*Lactuca sativa L.*)	28	20	Two factors: Water salinity (0 and 3 ppt) vs lettuce variety	0.53 - 1.13	No statistical interaction between plant and salinity factors was found. Greater lettuce growth was seen at salinity 0. Red lettuce was the one that best adapted to culture in brackish water.
		Crispy lettuce (*Lactuca sativa L.*)				0.78 - 1.29	
		Red lettuce (*Lactuca sativa L.*)				1.06 - 1.21	
Pinho et al. (2017)	1	Butter lettuce (*Lactuca sativa L.*)	21	20	Traditional aquaponics vs FLOCponics systems	1.5 - 1.9	Higher plant growth was found in the FLOCponics system for all varieties. Butter lettuce presented the highest growth values, followed by the crispy and the red lettuce varieties.
		Crispy lettuce (*Lactuca sativa L.*)				1.0 - 1.4	
		Red lettuce (*Lactuca sativa L.*)				0.3 - 0.6	
Pinho et al. (2021b)	1	Butter lettuce (*Lactuca sativa L.*)	23	20	Two factors: Technology (traditional aquaponics and FLOCponics) vs plant cycle	2.6 - 2.96	There was a statistical interaction between the technology employed and the production cycle in all parameters of plant growth. Similar plant growth was found in the first cycle between both technologies, while higher plant growth was seen in traditional aquaponics in the second cycle.
	2	Butter lettuce (*Lactuca sativa L.*)	23	20		1.8 - 2.62	

Rahman (2010)	1	Lettuce (*Lactuca sativa L.*)	28	25	FLOCponics using unsettled effluent vs FLOCponics using settled effluent vs hydroponics systems	1.71 - 3.48	No statistical difference was found between lettuce produced in FLOCponics with and without solids removal, while a difference was seen between them and the hydroponics systems.
	2	Lettuce (*Lactuca sativa L.*)	28	25		0.19 - 3.85	Same results as trial 1 were found.
	3[f]	Lettuce (*Lactuca sativa L.*)	28	25		3.60 - 3.80	The growth and quality of lettuce produced in FLOCponics with and without solids removal and hydroponics were similar.
	4[f]	Lettuce (*Lactuca sativa L.*)	28	25		3.90 - 4.00	Same results as trial 3 were found.
Rocha et al. (2017)	1	Lettuce (*Lactuca sativa L.*)	46	20	Traditional aquaponics vs FLOCponics vs hydroponics systems	0.21 - 1.53	Lettuce growth was lower in the hydroponics systems, and similar in the traditional aquaponics and FLOCponics systems
Fimbres-Acedo et al. (2020b)	1	Lettuce *(Lactuca sativa)*	35	75	Different biofloc trophic levels (chemo, hetero and photoautotrophic) in FLOCponics systems vs hydroponics systems	4.07 - 27.35	Wet weight of lettuce leaves produced in hydroponics was higher than in all treatments using FLOCponics.
	2	Pak-choi *(Brassica rapa subsp. Chinensis)*	35	75		23.18 - 36.90	Pak-choi cultured in chemotrophic FLOCponics presented lower wet weight than in all other treatments.
	3	Rocket *(Eruca sativa)*	35	75		1.06 - 9.90	Higher wet weight was found in chemotrophic FLOCponics.
	4	Basil *(Ocimum basilicum)*	35	75		6.82 - 12.16	No statistical difference was found between the treatments.
	5	Spinach *(Spinacia oleracea)*	35	75		0.91 - 4.39	Higher wet weight was found in chemotrophic FLOCponics.
Blanchard et al. (2020)	1	Cucumber *(Cucumis sativus L.)*	60	13	Four pH levels (7.0, 6.5, 5.8, vs 5.0) during USA spring	8.43 - 8.66 ‡	The growth rate of the cucumber was different between the treatments, with higher values at pH 7. No statistical difference was found for yields.

	2	Cucumber *(Cucumis sativus L.')*	60	13	Four pH levels (7.0, 6.5, 5.8, vs 5.0) during USA summer	9.50 - 9.70 ‡	No statistical difference was found between the treatments.
Pickens et al. (2020)	1	Cherry tomato cvs. “Favorita” *(Solanum lycopersicum var. cerasiforme)*	149-157	3.2	Two factors: nutrient source (FLOCponics and hydroponics system) vs harvest time (before and after fish harvest)	5.5 -11.4 ‡	Statistical difference in tomato yield was found only after fish harvest, when the results in the hydroponics systems were higher.
	2	Cherry tomato cvs. “Goldita” *(Solanum lycopersicum var. cerasiforme)*	149-157	3.2	Two factors: nutrient source (FLOCponics and hydroponics system) vs harvest time (before and after fish harvest)	4.3 - 10.5 ‡	Statistical difference in tomato yield was found for both harvest times, when the results in the hydroponic system were greater.
Martinez-Cordova et al. (2020)	1	Jalapeño pepper (*Capsicum annum*)	48	2.5	Traditional aquaponics vs FLOCponics systems	4.0 – 4.25 ‡	No statistical difference was found between the treatments for jalapeño pepper growth.
Neto (2017)	1	*Sarcocornia ambigua*	83	100	Two proportions of feed per m^{-2} of plant: 50 vs 100 g feed per m^{-2}	0.9 - 1.14	Statistical difference was found for final biomass with better results in the treatment with 50 g feed per m^{-2}. No difference was found for final yield.
Pinheiro et al. (2020)	1	*Sarcocornia ambigua*	57	40	Four water salinities: 8, 16, 24 vs 32 ppt	0.38 - 0.61	No statistical difference was found between the treatments.
Silva (2016)	1	*Sarcocornia ambigua*	70	100	Four different periods of water pumping in the hydroponic beds over a day: 6, 12, 18 vs 24 h	1.1 - 1.9	No statistical difference was found between the treatments for lettuce growth.

† When FLOCponics is not mentioned in the treatments column, it indicates that this was the only system used. ‡ Yield was calculated as a sum of marketable fruit. ᶠ Extra fertilizer was added into the hydroponics subsystems in the FLOCponics systems.

Most of the FLOCponics research evaluated the production of lettuce or salicornia (Table 2.3). Leafy vegetables such as lettuce have also been widely used in traditional aquaponics systems, mainly due to their low nutritional requirement and fast production cycle (Diver 2006; Rakocy 2012). Among the trials that cultured lettuce and compared their growth in FLOCponics to other production systems, 19% found better results in FLOCponics, 13% in traditional aquaponics, 25% in hydroponics, and in 44% of the trials no differences between the systems were observed. For those that evaluated a specific factor in the FLOCponics systems, the results of Barbosa (2017) and Rahman (2010) should be highlighted. They evaluated lettuce production using BFT effluents either treated with filtering devices or not, and no differences in plant growth were found in either study. However, the authors emphasized the presence of solids/bioflocs on plant roots, mostly when filters were not used, and suggested that efficient mechanical filters should be developed to avoid this solids accumulation. In this same study, Rahman (2010) also evaluated the effect on lettuce growth of adding fertilizer supplementation to the hydroponics subsystems of the FLOCponics treatments. The author reported that due to the extra fertilizer supplementation the lettuces grew similarly in the hydroponics and FLOCponics systems. Salicornia is a halophyte plant with high market value (Quintã et al. 2015). The studies that cultured this species did not compare FLOCponics to other production systems. Most of them focused on the benefits of integrating salicornia production and BFT. It is important to mention that findings reported by Doncato and Costa (2021) were not considered in Table 2.3, since the authors did not provide sufficient numerical data. Despite this, their findings bring useful insights about the use of fertilizers in marine FLOCponics, by showing that plants grown with mineral fertilizers added to the water outperform those where mineral fertilizers were added directly to the leaves, or were not added at all.

With respect to other plant species, Fimbres-Acedo et al. (2020b) demonstrated that plant performance (lettuce, pak-choi, rocket, basil, and spinach) can be affected by the BFT trophic level. Their results highlighted the importance of investigating how suitable the species are for a given production situation. Tomato and cucumber were also reported in FLOCponics studies (Table 2.3). For tomato, Pickens et al. (2020) compared its growth in FLOCponics to hydroponics and also before and after fish harvest, i.e., in one treatment fish and tomatoes were harvested at the same time (117 days) and in the other tomato cultivation continued for another 40 days after harvesting the fish, and consequently with no more feed intake. The authors showed that, after harvesting the fish, the nutrients in the water were not sufficient to nourish the tomatoes remaining in the FLOCponics system, resulting in lower tomato yield compared to the hydroponics system. For cucumber, Blanchard et al. (2020) showed that the leaf elemental composition was within the recommended ranges even though the nutrient concentrations in the BFT effluent would be considered low. The production of aromatic herbs and pepper was also investigated in a FLOCponics system, but only preliminary results have been published so far (Castro-Mejía et al. 2020; Martinez-Cordova et al. 2020).

In addition to the yields presented in Table 2.3, special attention should also be paid to crop quality due to its key role in market competitiveness and consumer perception (Goddek et al. 2015). Additional analysis such as visual characteristics, composition of nutrients, and indicators of stress were carried out in FLOCponics studies and demonstrated promising results. Pinheiro et al. (2017, 2020) and Silva (2016) evaluated the total phenolic compounds and antioxidant activity of *Sarcocornia ambigua* and, according to their results, FLOCponics culture conditions did not induce high plant stress. For the visual characteristics of the plants, some investigations showed positive effects of BFT or no visual symptoms of nutrient deficiencies (Pinho et al. 2017; Pickens et al. 2020), while others found the opposite (Barbosa 2017; Lenz et al. 2017; Pinho et al. 2021b). Visual symptoms of nutrient deficiencies are usually identified by irregular leaf development, discoloured leaves, or burned leaves.

In general, the undesirable visual characteristics or poor plant growth sometimes found in FLOCponics research have been related to: (i) the presence of solids/bioflocs on plant roots; (ii) high water pH (>7), affecting the bioavailability of nutrients in the form absorbable by plants; (iii) nutrient imbalance; (iv) the consumption of available nutrients in water by the BFT microorganisms, even though there is a lack of precise information regarding their role on nutrient recycling/removal; and (v) lack of waste management and nutrient optimization through solids/bioflocs reuse or remineralization (Rocha et al. 2017; Lenz et al. 2017; Pickens et al. 2020; Fimbres-Acedo et al. 2020b; Pinho et al. 2021b). All of these constraints relating to FLOCponics must be addressed and taken into account in further research. Some alternative solutions for these problems are discussed in *section 2.6.2*.

2.4.4.2 Animal production

The main zootechnical parameters evaluated in FLOCponics experiments, as well as the species, duration and densities used, are presented in Table 2.4. Most studies were conducted with Nile tilapia

(*O. niloticus*) or Pacific white shrimp (*L. vannamei*), except in those of Castro-Castellón et al. (2020) and Rocha et al. (2017) who cultured *Melanochrimis sp* and South American catfish (*Rhamdia quelen*), respectively. Tilapia and Pacific white shrimp are the most popular species in biofloc-based cultures (Avnimelech 2015). This is mainly because both species show tolerance to less than ideal environmental conditions, such as a high concentration of suspended solids and nitrogenous compounds in water, and due to morphological adaptations, which allow them to take advantage of bioflocs as a complementary food (Emerenciano et al. 2013; Walker et al. 2020). Tilapia in the nursery phase with initial weight varying between 0.3 and 4.1 g was the most used (Poli et al. 2019; Fimbres-Acedo et al. 2020a). Only Fimbres-Acedo et al. (2020a) reared fish in growth-out phase, harvesting tilapia between 445 and 520 g. However, in shrimp culture, the growth-out phase was carried out, where shrimps with an initial weight of 1.4 g were produced until they reached approximately 12 g.

The investigations on the growth performance of aquatic organisms in FLOCponics have evaluated diverse variables (Table S2.1). The treatments have tested, for instance: (i) different input of nutrients by varying the carbon source (Castro-Castellón et al. 2020) or the trophic levels of the BFT (Fimbres-Acedo et al. 2020a); (ii) different water salinities (Lenz et al. 2017; Pinheiro et al. 2020); (iii) the influence of the integration of BFT with hydroponics (Pinheiro et al. 2017; Poli et al. 2019); (iv) the effect of specific management for plant production on shrimp performance (Silva 2016; Neto 2017); and (v) the effect of traditional aquaponics using RAS compared to FLOCponics systems on fish and plant growth (Rocha et al. 2017; Pinho et al. 2021b). Within these studies (Table 2.3), only Fimbres-Acedo et al. (2020a), Martínez-Meingüer (2020), Martinez-Cordova et al. (2020) and Pinho et al. (2021b) found statistical differences in animal growth between the treatments. Fimbres-Acedo et al. (2020a) observed a positive effect of algae-based photoautotrophic treatment over chemotrophic and heterotrophic treatments in both nursery and growth-out phases. Martínez-Meingüer (2020) observed that tilapia fed with 35% crude protein and no fertilizer supplementation outperformed those using higher dietary protein (47.5% crude protein) and fertilizer supplementation in FLOCponics system. Martinez-Cordova et al. (2020) showed benefits for tilapia yield and feed conversion ratio when received bioflocs from an *ex situ* BFT. Pinho et al. (2021b) compared the production of tilapia juveniles in traditional aquaponics and FLOCponics systems and found higher final weight, higher specific growth rate and lower feed conversion ratio in FLOCponics. Interestingly, the authors pointed out that the mean volume of bioflocs in the fish tank was lower than the recommended for BFT culture and potentially impacted the fish performance, which could have been even better if the *in situ* natural food availability was higher. The same trend of a low volume of bioflocs and its impact on fish growth was observed by Rocha et al. (2017), also running coupled systems. However, in contrast to Pinho et al. (2021b), the authors did not find statistical differences between aquaponics and FLOCponics for *Rhamdia quelen* production. Both investigations suggested that improvements in system design could optimize BFT and hydroponics integration.

In terms of yields, the current studies revealed that the system's carrying capacity needs to be optimized in FLOCponics. For example, for tilapia, the 23 kg m^{-3} reported by Fimbres-Acedo et al. (2020a) is far below the 70 kg m^{-3} able to be produced in the growth-out phase in commercial aquaponics with RAS (Rakocy 2012) or the maximum of 50 kg m^{-3} in BFT (Emerenciano et al. 2021). Meanwhile, in the nursery phase, the values between 7.8 to 8.7 kg m^{-3} achieved (Lenz et al. 2017; Pinho et al. 2021b) are within the expected range in BFT systems, i.e., between 8 to 10 kg m^{-3} (Emerenciano et al. 2021). For shrimp culture, the recommended initial densities for the growth-out phase are 270 to 530 juveniles per m^{-3} to achieve marketable shrimp (>18 g) and yields of 5 to 9 kg m^{-3}. The experiments with shrimp in FLOCponics used similar stocking densities; however, the yields obtained were lower, ranging from 2.1 to 2.8 kg m^{-3} (Neto 2017; Pinheiro et al. 2017). As mentioned above and in the previous sections, when a hydroponics system is connected to BFT tanks the solids/bioflocs in the system are affected. Reducing the volume of bioflocs makes scarce the *in situ* natural food and might change the microbial activity, which is probably the reason for the reported lower yields in FLOCponics compared to biofloc-based monoculture. The current results suggest that improvement of carrying capacity and system design could solve both yield performance and solids management, boosting FLOCponics outcomes, and making them more comparable to commercial aquaponics with RAS.

Table 2.4. Overview of the zootechnical results found in FLOCponics research.

Reference	Species	Period days	Density animal m^{-3}	Initial weight g	Final weight g	SGR % g day^{-1}	FCR	Yield kg m^{-3}	Survival %
Fish culture									
Castro-Castellón et al. (2020)	*Melanochrimis sp*	120	800	0.8	7.6 - 8.0	1.8 - 1.9	-	5.9 - 6.3	98 - 100
Lenz et al. (2017)	*Oreochromis niloticus*	28	90	67.5	94.8 - 98.3	1.2 - 1.3	2.0 - 2.2	8.3 - 8.7	97.8
Pinho et al. (2021b)†	*Oreochromis niloticus*	46	300	1.1	34.9 - 36.7	7.4 - 7.6	0.8 - 0.9	7.8 - 8.0	96.5 - 98.8
Fimbres-Acedo et al. (2020a) †	*Oreochromis niloticus*	140	180	0.3	33.8 - 54.2	3.3 - 3.6	1.3 - 1.4	4.8 - 9.1	96.6 - 100
	Oreochromis niloticus	133	55	60.0	445.4 - 520.2	1.5 - 1.6	1.5 - 1.7	21.4 - 23.3	95.0 - 100
Martinez-Cordova et al. (2020) †	*Oreochromis niloticus*	56	75	2.1	89.7 - 120.3	6.7 - 7.2	1.1 - 1.4	5.4 - 7.4	80.4 – 82.6
Martínez-Meingüer (2020) †	*Oreochromis niloticus*	140	156	-	257.2 - 333.6	1.8 - 2.3	-	-	-
Rocha et al. (2017)	*Rhamdia quelen*	46	83	19.5	31.1	1.0	1.1 - 1.3	2.9	100.0

Fish and shrimp culture									
Poli et al. (2019) ‡	*Oreochromis niloticus*	57	444	4.1	11.4 - 11.5	1.8	0.16	5.1	87.5 - 91.3
	Litopenaeus vannamei	57	312	1.2	14.1 - 14.6	4.3 – 4.4	1.7	4.4 - 4.6	88.0 - 89.3
Shrimp culture									
Neto (2017)	*Litopenaeus vannamei*	83	250	1.4	12.9 - 13.3	2.7	1.7	2.8	85.7 - 87.1
Pinheiro et al. (2017)	*Litopenaeus vannamei*	73	250	1.4	11.6 - 11.7	2.9	1.7	2.1	72.5 - 74.5
Pinheiro et al. (2020)	*Litopenaeus vannamei*	57	300	1.6	11.5 - 12.7	3.4 - 3.6	1.6 - 2.0	2.3 - 3.0	56.3 - 84.0
Silva (2016)	*Litopenaeus vannamei*	73	250	2.4	10.9 - 11.2	2.1	1.7	2.4	85.2 - 87.3

† Statistical differences were found. ‡ Polyculture focused on shrimp production. Tilapia were fed only once a day with 1% of fish biomass, stimulating tilapia to seek natural food in the bioflocs. SGR: specific growth rate. FCR: feed conversion ratio.

2.5 Sustainability aspects

New technologies have recently been developed to lead aquaculture to more sustainable practices. Being sustainable means that aquaculture systems must be technically viable and economically profitable, aiming to supply human needs with respect to safe and healthy food for present and future generations (Santillo 2007; Valenti et al. 2018; Boyd et al. 2020). Economic assessments of medium and long-term aquaculture projects can provide data for the implementation of management strategies that will contribute to the resilience and longevity of the business (Sabbag et al. 2007). In addition to biological, technical, and economic aspects, understanding the social and environmental impacts of a new production system from a systemic point of view through sustainability assessments is important to provide a basis for the development of appropriate public policies fostering a sustainable growth of the activity (Shang 1990; Garcia et al. 2014; David et al. 2018; Valenti et al. 2018).

Sustainability assessment methodologies such as the ecological footprint (Folke et al. 1998; Gyllenhammar and Hakanson 2005; Madin and Macreadie 2015), emergy synthesis (Vassallo et al. 2007; Lima et al. 2012; Garcia et al. 2014; David et al. 2018, 2021), life cycle analysis (LCA) (Gronroos et al. 2006; Aubin et al. 2006, 2009; Santos et al. 2015; Medeiros et al. 2017) and indicators of sustainability (Valenti et al. 2018) have been used to measure the sustainability of aquaculture. For aquaponics production, studies using LCA have shown that the main environmental impacts of aquaponics are related to infrastructure, electricity and feed (Boxman et al. 2015; Forchino et al. 2017; Maucieri et al. 2018). On the other hand, low water use and the possibility to be adopted as a tool to promote educational, cultural, leisure and tourism values, and landscape improvement are positive aspects usually linked to aquaponics systems (König et al. 2018; Junge et al. 2019). For biofloc-based production, Belettini et al. (2018) evaluated the carbon footprint of commercial shrimp production using LCA and showed that electricity is also a key impacting factor in BFT, while feed has a minor impact. Sustainability assessments of FLOCponics systems were not found in the literature. The lack of these analyses is probably due to their need for a large and detailed database, which is not yet available for FLOCponics systems.

Even though no results from a sustainability assessment are available, FLOCponics has been presented as an example of a new technology with the potential to minimize some unsustainable characteristics of conventional aquaculture (Emerenciano et al. 2021). By replacing the RAS by BFT in a food production system already known to be eco-friendly, some positive aspects of biofloc-based systems and traditional aquaponics can be maximized and some of their limitations reduced. Moreover, the possibility of producing a mix of food products in a small urban area and close to the consumer, causing low environmental impact and generating social benefits, are the main sustainable advantages of the FLOCponics systems. In addition, the fact that these foods are healthy, free of pesticides, and offered to the consumer in a wide variety (fish and vegetables), makes FLOCponics a highly relevant system on the food production field. The main technical-economic, social and environmental characteristics that may justify the recognition of FLOCponics as a sustainable system are summarized in Table 2.5.

Table 2.5 reveals that most of the characteristics of FLOCponics are related to the technical-economic category. At this moment, the main focus of FLOCponics research has been on technical aspects and only one study evaluated the economic feasibility of this system. Castilho-Barros et al. (2018) simulated a theoretical commercial-scale FLOCponics system with shrimp (*Litopenaeus vannamei*) integrated with halophyte *S. ambigua* and calculated its profitability. According to these authors, the high market value of these species made the system economically viable, even in pessimistic business plans. They also identified that FLOCponics requires high implementation costs, expensive operating equipment, and highly skilled labor. It is hasty to draw conclusions about the profitability of FLOCponics based only on hypothetical results with specific scenarios, products and markets. However, the three items with the highest costs identified by these authors seem to compose a pattern as they are also the main weaknesses identified for traditional aquaponics (Quagrainie et al. 2018; Baganz et al. 2020), biofloc-based monocultures (Walker et al. 2020; Boyd et al. 2020) and FLOCponics (Table 2.5). It should be noted that, if the productive potential of FLOCponics is proved, all these costs may be diluted by the highest biomass produced and then this economic issue can be tackled. For example, the electricity cost per kg of food produced in FLOCponics systems will certainly be lower than in biofloc-based monoculture. In addition, the adoption of renewable energy sources such as wind, solar and biogas produced through biodigesters, and the use of infrastructures and equipment with a long useful life would be viable alternatives to further improve the sustainable characteristics of the FLOCponics systems.

Food production systems will always somehow impact the environment, thus those that achieve high yield with minimal negative impact should be encouraged (David et al. 2021). Determining the trade-off between the benefits and costs of FLOCponics and evaluating the sustainability of real systems are still needed. For these purposes, a larger technical and economic database of FLOCponics must be produced and then analyzed through sustainability assessments.

Table 2.5. The main potential technical-economic, social and environmental characteristics of FLOCponics.

Characteristic	Technical-economic	Social	Environmental
Positive			
Low water use	X		X
Diversification of production	X		
Efficient use of feed	X		X
Constant nutrient recycling	X		X
Low or zero effluent disposal	X		X
Educational and leisure tool		X	
Promotion of the local economy	X	X	
Prevents species escape	X		
No use of pesticides	X	X	X
Reduced land use	X	X	X
Use of non-productive areas	X		X
Proximity to the consumer	X	X	X
Diluted cost per biomass produced	X		
Low investment in filters	X		X
Improved animal nutrition and health	X	X	
Negative			
Need for skilled labor	X	X	
High cost of equipment	X		
High dependence on electricity	X		X
Low generation of direct jobs	X	X	
Low widespread technology	X		
Intensive control of water parameters	X		
Unpredictability of available nutrients	X		

2.6 Challenges of FLOCponics systems

FLOCponics is a complex and multidisciplinary food production system, which requires in-depth knowledge in diverse areas such as microbiology, limnology, ecology, aquaculture, engineering, agronomy and hydroponics. Given this complexity, and due to the fact that only a few investigations have been conducted so far, information gaps on FLOCponics need to be addressed by new research.

At this initial stage of scientific research, identifying and discussing the challenges and pointing out the opportunities of FLOCponics may guide future studies and then lead to the efficient development of this system. Currently, the challenges of FLOCponics are technical issues, which affect its sustainable and economic aspects. The same trend occurred in BFT, but nowadays it has been fully developed and commercially applied. The main identified challenges and opportunities of FLOCponics are outlined and described below.

2.6.1 System setup

The crucial points that need to be adjusted in FLOCponics are the design and engineering of the systems. The layout of FLOCponics systems must be designed to provide the best conditions possible for the production of aquatic animals and plants and the maintenance of BFT microorganisms. The main issue identified is related to keeping suspended solids in the water at suitable concentrations for plant and fish production. As stated in the sections above, plant growth seems to be limited by the excess of solids in FLOCponics systems. On the other hand, trying to avoid solids in the hydroponics subsystem has resulted in a decrease in the amount of in situ food/bioflocs for the animals. Alternatives to solve this problem are the development of mechanical filters that efficiently separate the solids and the liquid fraction of the BFT effluent, and then return the bioflocs to the aquaculture subsystem and direct the water and nutrients to the hydroponics subsystem. Examples of filters that need to be investigated in FLOCponics are bag-filters with backwash technology, drum filters, or even sedimentation tanks with well-planned biofloc return flow. Additionally, the frequency of their use and the water flow into these filters should be set. It is necessary to highlight that all these filters can be used in coupled FLOCponics systems as well. However, as in all coupled systems there will always be trade-off between plant and animal requirements (Goddek et al. 2016; Monsees et al. 2017b), so the employment of a decoupled layout is highly recommended.

Another challenge of FLOCponics systems that needs to be addressed is the high variation of the setups used. For instance, the wide range of water flow rates and volumes of the subsystems (Table 2.1) indicate that the water velocity and dilution of nutrients available for the plants are totally different among the investigated FLOCponics systems. It could generally be said that the BFT tank can have any dimension, while the hydroponics and filters subsystem should be carefully designed according to the amount of nutrients and solids that will come from the BFT tank. Because of the lack of standardization in the system setups, it is hard to compare the results found and reach concrete conclusions about the efficiency of FLOCponics in producing food.

Based on the findings pointed out in this paper, further studies should focus on: (i) improving the mechanical filters; (ii) defining the ideal proportion of the subsystem volumes based on the nutritional needs of the targeted plant species; (iii) setting the water flow rate in order to promote greater nutrient uptake and recycling, by adjusting it to the hydroponics subsystem; (iv) assessing the differences between the coupled and decoupled layout with reference to the productive capacity of FLOCponics; and (v) understanding whether the type of hydroponic bed, i.e., NFT and DWC, affects plant growth in FLOCponics. All of these investigations must be conducted to develop systems with the potential to be applied commercially. The economic viability of the proposed solutions should also always be considered.

2.6.2 Plant nutrition, health and production

The success of soilless plant production is directly dependent on the optimal quantity and quality of the nutrients being available in the water. The physical-chemical parameters of the water and the quantity of each macro- and micro-nutrient must be in accordance with the requirement of each plant species. In addition to nutrients, other variables also influence plant growth, e.g., environmental parameters such as irradiance, photoperiod, temperature, and humidity (Goddek et al. 2015; Maucieri et al. 2019). Meeting plant needs is generally a challenge in coupled aquaponics using RAS (Goddek et al. 2015) and seems also to be the case in FLOCponics. The critical points related to plant growth identified in the FLOCponics research were outlined in the *section 2.4.3.1.* All of them somehow affect the uptake of nutrients by plants and can reduce plant quality.

The improvement of the engineering aspects of FLOCponics systems should minimize or even solve some of these problems, which are mainly related to solids control. Furthermore, the use of decoupled layouts will certainly enable pH regulation at ideal levels for each subsystem and the addition of specific minerals directly into the hydroponics subsystem. In contrast to commercial hydroponics which utilize fully formulated fertilizers, in FLOCponics the production costs might be reduced as only specific nutrients would be required due to a wide range of nutrients already available in BFT effluent. For this purpose, detailed information on the quantity of nutrients in the feed and carbon source are required. Additionally, it is highly recommended to deepen the studies on the profile of micro-nutrients present in the process water of the BFT system, given their effect on plant biological processes such as photosynthesis (Maucieri et al. 2019). Comparing the differences in the quality and diversity of the micro-nutrients in the FLOCponics systems and those used in balanced hydroponic fertilizer will clarify whether there is deficiency of specific nutrients. This may enable the design of specific supplementation protocols for each plant species, and, thus, achieve high productivity and quality of vegetables.

Recovering and transforming nutrients from solid biofloc fractions into bioavailable forms through a mineralization process may change future perspectives about the need for extra fertilization in FLOCponics (Blanchard et al. 2020; Fimbres-Acedo et al. 2020b). Since a minimum concentration of bioflocs should be kept in the aquaculture subsystem to promote animal growth, the amount and frequency of solids/biofloc removal that will be directed to the remineralization unit, as well as which process will be used, need to be precisely defined. Defining an efficient biofloc remineralization process might be a win-win situation for fish/shrimp production and water treatment research fields. This is mainly because high animal growth performance is reached by constantly removing excess bioflocs/solids (Ray et al. 2010a; Gaona et al. 2016), and the harvested bioflocs may be relatively carbon-rich, and consequently a desirable substrate for anaerobic bioreactors.

For those that wish to run a coupled FLOCponics system, the tolerance intervals of water quality and overall nutrients concentration for the cultured animals, biofloc microorganisms, and vegetables must be investigated. A key variable in coupled layouts that needs attention is the pH (Goddek et al. 2015, 2019b). While BFT microorganisms work properly at neutral pH (Emerenciano et al. 2017), the plants commonly cultured in hydroponics system (e.g., lettuce, basil, tomato, and cucumber) grow better at pH ranging between 5.5 and 6.5 (Tyson et al. 2004; Goddek et al. 2015; Yep and Zheng 2019). The effect of neutral pH on plant growth was poorly evaluated and discussed by the studies that ran coupled systems. Finding alternative plant species that required neutral-alkaline pH conditions may be a way to minimize pH issues and run a coupled FLOCponics system successfully. From this perspective, examples of crops that could be investigated in further research are swiss chard, broccoli, head cabbage, and mint (FAO 2014).

The influence of nutrient uptake by BFT microorganisms on the availability of nutrients for plant production is yet unclear. At this moment, the results have indicated that running a mixotrophic or chemoautotrophic BFT would be the best option for FLOCponics systems (Lenz et al. 2017; Fimbres-Acedo et al. 2020b) due to the expected predominance of nitrifying communities (higher concentration of nitrate in water) instead of a heterotrophic-based medium. Another approach related to BFT microorganisms that must be clarified is whether a thin flocs biofilm on plant roots has the potential to boost or harm the nutrient uptake by the plants. The effect of BFT microorganisms on FLOCponics production clearly needs further investigation.

2.6.3 Animal nutrition and production

The main issue for animal production in FLOCponics is to maintain an optimum amount of in situ food/bioflocs in the aquaculture tanks. Once the aforementioned improvements in the system design are implemented, the full nutritional advantages of flocs would be achieved. Some of the reported nutritional advantages of using BFT instead of RAS are: (i) reduced feed conversion ratio (Wasielesky et al. 2006; Megahed and Mohamed 2014; Ray and Lotz 2017); (ii) replacement of fish meal by alternative protein sources (Scopel et al. 2011; Bauer et al. 2012; Sousa et al. 2019); and (iii) a reduction of dietary protein content (Azim and Little 2008; Ballester et al. 2010; Jatobá et al. 2014; Panigrahi et al. 2020). Studies aiming to assess the applicability of these nutritional strategies should be carried out, since they may reduce feed costs and the environmental footprint of FLOCponics. Moreover, these studies should be run in intensive densities to achieve higher yields.

Only a few animal species have suitable characteristics to be intensively produced in BFT and consequently in FLOCponics systems. Although several studies have shown the viability of other species (Walker et al. 2020), biofloc technology is commonly applied to Nile tilapia and Pacific white shrimp culture. Both species are widely reared and contribute to the food supply worldwide. On the one hand, the scarce production of other species with high market value is a limitation of FLOCponics, on the other hand, it is always good to produce well-known products when new technologies are being developed.

2.6.4 Practical applicability of FLOCponics

To date, FLOCponics research has been mainly led by aquaculture researchers who normally seek to find solutions to problems directly related to biofloc-based monocultures, i.e., the accumulation of nutrients in water and high production costs. The authors have justified using FLOCponics as a way to reuse these nutrients, increase farm profitability by growing other products with market value, and dilute the costs with inputs, electricity and labor. Thus, at first glance, FLOCponics seems to be more applicable for farmers who already apply BFT. A practical example of this is the fact that some commercial BFT farmers have been testing and applying the principles of FLOCponics. Unfortunately, the results held by the private sector are often not shared with the general public.

FLOCponics will probably be an alternative option for the traditional aquaponists or the investor who wants to start an integrated agri-aquaculture farm only when the technical barriers are solved. For instance, a broad range of knowledge is still required to understand the best way to run a FLOCponics system and to maximize its results. Moreover, the choice of the food production system that will be used must take into account several factors, such as market demand, climate, producer experience, technical knowledge, the cost and availability of inputs, among others. Even if the expected positive potential of FLOCponics is proved, a systemic analysis of the whole production scenario should be done aiming to provide guidance as to which system will be most suitable for a given situation.

Most of traditional aquaponics systems are operated at a small-scale run for personal hobby or family subsistence (Palm et al. 2019). FLOCponics tends to be the opposite of this. To support the complexity of BFT, a basic infrastructure and a significant investment are likely suited to only medium and large commercial-scale scenarios. Based on that, it is reasonable to state that FLOCponics will rarely be employed as a backyard system. This highlights the necessity to improve and standardize system designs for real production situations. Moreover, technological management supported by studies of modelling and forecasting inputs and outcomes will play an important role in developing FLOCponics, especially in medium to larger scaled farms. Modelling FLOCponics systems is a subject to be investigated; then, it was not explored in this paper.

Finally, it should be mentioned that as FLOCponics is a novel and emergent system, some papers were published after the settled literature search period for this review (Saseendran et al. 2021; Ayipio et al. 2021; Pinho et al. 2021c) and many others are expected to be published in the next few years. It is, however, noteworthy that our group has been advancing research in this field and recently published the results of a study in which decoupled layout allowed reduction of critical issues related to FLOCponics systems, leading to similar lettuce growth and an 8% reduction in the Nile tilapia dietary crude protein compared to decoupled aquaponics using RAS (Pinho et al. 2021c).

2.7 Final remarks

This review has identified that FLOCponics research is still in its initial stage, which is shown by the small number of papers published so far and the lack of standardization in experimental designs and system setups. At this stage, there are still some inconsistencies regarding the results of animal and plant yields in the FLOCponics systems. For example, 38% of the studies showed worse plant growth in FLOCponics compared to hydroponics or traditional aquaponics. The other 62% highlighted that improvements in the system design are necessary to achieve better plant yields, even though they reported higher or similar results in FLOCponics. An important contribution of this paper was examining the main challenges of FLOCponics systems and suggesting future research to tackle them (*sections 2.4* to *2.6*). Among the points discussed, the effective control of solids in order to guarantee a suitable concentration for the hydroponics and aquaculture subsystems was highlighted as the main challenge. For this purpose, it is highly relevant that further investigations determine the ideal management and design of the filtering systems, and the feasibility of decoupled FLOCponics systems.

In terms of applicability, the FLOCponics system is likely to be applied in the short-term by farmers who already operate BFT, adapting their structures to receive the hydroponics subsystem. For BFT production, FLOCponics seems to primarily increase the sustainable character of biofloc-based monocultures by recovering nutrients and expanding product diversity, rather than promoting higher animal growth performance. The integration of BFT with plant production fits with the circular economy concept and might contribute to social licenses and farm diversity. The further commercial application of FLOCponics requires research that provides a solid database, originating from experimental setups with characteristics similar to those of commercial production. In future research, assessing the economic, social-educational, and environmental impacts of FLOCponics in an urban setting should be considered, making easier the delivery of products from producer to consumers, with a minimum of middlemen. Lastly, it is expected that the data presented and discussed in this paper will provide guidance and technical support for further FLOCponics development, boosting both research and commercial application, and thus contributing to sustainable aquaculture and plant production.

Supplementary material

Table S2.1. Overview of FLOCponics papers.

Reference	**Animal species**	**Plant species**	**Objective**	**Main outcomes**
Barbosa (2017)	Tilapia (*Oreochromis niloticus*)	† Two varieties of lettuce (*Lactuca sativa L.*)	Evaluate the effect of using filters (mechanical and biological) on the production of lettuce and tilapia in FLOCponics during two 14-day trials.	The use of filters interconnecting the BFT and hydroponics subsystems did not affect plant growth in the first trial, while in the second their use benefited plant growth by reducing the amount of solids in the lettuce roots.
Blanchard et al. (2020)	Tilapia (*Oreochromis niloticus*)	† Cucumber (*Cucumis sativus L. 'Delta Star'*)	Determine the effects of pH (5, 5.8, 6, and 7) on nutrient concentrations in water and leaves and cucumber growth in a decoupled FLOCponics system with minimal solids removal during two seasonal 60-day trials	Availability of macro- and micro-nutrients were affected by pH levels. However, they did not have a practical effect on cucumber growth rate over the two growing seasons. Elemental analysis of leaf tissues was within the recommended ranges even though nutrient concentrations in the BFT effluent would be considered low compared to hydroponic solutions.
Castilho-Barros et al. (2018)	Pacific white shrimp (*Litopenaeus vannamei*)	*Sarcocornia ambigua*	Perform a commercial-scale economic assessment by using a theoretical model to evaluate marine FLOCponics production in Brazil.	The economic indices showed that the integrated production of shrimp and *S. ambigua* in FLOCponics is economically viable for the specific conditions evaluated.
Castro-Castellón et al. (2020)	African cichlid (*Melanochromis sp.*)	Cherry tomato (*Lycopersicon esculentuim var. cerasifonne*)	Evaluate four different carbon sources (coffee, moringa, macroalgae and yucca) on plant and fish production in the FLOCponics system for 120 days.	Fish and tomato produced using coffee and moringa were the ones that presented greater lengths and weights, respectively.

Castro-Mejía et al. (2020)	Tilapia (*Oreochromis niloticus*)	Coriander (*Coriandrum sativum*), Dill (*Anethum graveolens*), Parsley (*Petroselinum crispum*)	A preliminary evaluation of tilapia and aromatic plants production in the FLOCponics system for 160 days.	Preliminary insights about the management and production of aromatic plants in FLOCponics.
Doncato and Costa (2021)	Pacific white shrimp (*Litopenaeus vannamei*)	† *Sarcocornia neei Lag., Apium graveolens L., Paspalum vaginatum Sw.*	Evaluate the effects of micronutrient supplementation, directly in the water and by foliar spraying, on the growth and biomass production of different halophyte plants in saline FLOCponics.	Water from a FLOCponics system provides the required micronutrients for *S.neei* growth. Micronutrient supplementation in water positively affected the concentrations of iron, manganese and molybdenum, and increased *P. vaginatum* growth. Due to the poor development of *A. graveolens*, the responses to micronutrient additions were not evaluated. Foliar spraying was not effective in improving halophyte growth.
Fimbres-Acedo et al. (2020a, b)	† Tilapia (*Oreochromis niloticus*)	† Lettuce (*Lactuca sativa*), pak-choi (*Brassica rapa subsp. Chinensis*), rocket (*Eruca sativa*), basil (*Ocimum basilicum*), spinach (*Spinacia oleracea*)	Evaluate the production of five plant species in different biofloc trophic levels (chemotrophic, heterotrophic and photoautotrophic) in decoupled FLOCponics.	The effluents generated in BFT culture at different trophic levels were able to produce all tested plant species. Pak-choi was the more suitable for heterotrophic BFT effluents, while rocket and basil for chemotrophic and photoautotrophic effluents.
Lenz et al. (2017)	Tilapia (*Oreochromis niloticus*)	† Three varieties of lettuce (*Lactuca sativa L.*)	Evaluate the use of effluents from brackish BFT (3 ppm) for the production of lettuce in FLOCponics for 28 days.	The yield of lettuces grown in freshwater FLOCponics was higher than in brackish water. Crisp and red varieties showed tolerance to salinity, which did not occur with the smooth variety. In relation to plant visual characteristics, red variety produced in brackish FLOCponics had the highest score, presenting leaves with higher integrity and intense coloration.

Martinez-Cordova et al. (2020)	† Tilapia (*Oreochromis niloticus*)	† Jalapeño pepper (*Capsicum annum*)	A preliminary comparation of tilapia-pepper production in FLOCponics and aquaponics system for 56 days. Additionally, the final effluent of both systems were used to fertilizer a soil-based culture of bell pepper.	The productive performance of tilapia was better in biofloc-based tanks. For the peppers, no differences in plant yield were observed between the evaluated systems.
Martínez-Meingüer (2020)	† Tilapia (*Oreochromis niloticus*)	† Tomato (*Lycopersicon esculentum*)	A preliminary evaluation of the use of two commercial diets and extra fertilizer to produce tilapia and tomato in the FLOCponics system for 140 days.	The use of extra fertilizer and the diet with 35% of crude protein (CP) resulted in higher tomato growth. For fish production, higher tilapia weight was found when fed with 35% of CP and no use of fertilizer.
Neto (2017)	† Pacific white shrimp (*Litopenaeus vannamei*)	† *Sarcocornia ambigua*	Assess the FLOCponics production of *S. ambígua* and *L. vannamei* under different ratios of feed per m^2 of plant (50 and 100 g per m^2) and its influence in the quality of the culture's water and in the productive performance of the cultivated organisms.	The proportion of 50 g feed per m^2 of plants was recommended for the FLOCponics production, as it resulted in higher final biomass of *S. ambigua* compared to 100 g feed per m^2. In addition, the growth of shrimp did not differ between the proportions of feed tested.
Pickens et al. (2020)	Tilapia (*Oreochromis niloticus*)	† Cherry tomato cvs. "Favorita" and "Goldita" (*Solanum lycopersicumvar.cerasiforme*)	Evaluate the FLOCponics effluent as a nutrient solution for cherry tomato culture and compare its production with a hydroponics system, before and after fish harvest.	Before fish harvest, few differences in plant yield were observed between those produced in FLOCponics or hydroponics for the cherry tomato 'Favorita', while differences were seen between treatments for the tomato 'Goldita' with greater results in hydroponics system. After fish harvest, both cultivars grew better in the hydroponics system. Low concentration of nutrients were seen in FLOCponics effluents, despite no visual symptoms of nutrient deficiencies being observed throughout the experiment.

Pinheiro et al. (2017)	† Pacific white shrimp (*Litopenaeus vannamei*)	† *Sarcocornia ambigua*	Evaluate the use of nitrogen and production of the halophyte *S. ambigua* and shrimp in a FLOCponics system compared to shrimp reared in BFT, as well as the antioxidant activity and total phenolic compounds in plants.	The integration of shrimp and *S. ambigua* production improved the use of nitrogen in the system and did not affect shrimp growth. The results also showed that *S. ambigua* culture in FLOCponics may be a promising source of natural antioxidants for human consumption.
Pinheiro et al. (2020)	† Pacific white shrimp (*Litopenaeus vannamei*)	† *Sarcocornia ambigua*	Evaluate the relation of water salinity (8, 16, 24 and 32 psu) in the productive performance of Pacific white shrimp and *S. ambigua* cultured in a FLOCponics system.	The salinity between 16 and 24 psu was recommended for the integrated production of *L. vannamei* and *S. ambigua* in FLOCponics, since the performance of the shrimp was not impaired, and the growth of the plants and the removal of nitrogen and phosphate compounds were favored in this salinity range.
Pinho et al. (2017)	Tilapia (*Oreochromis niloticus*)	† Three varieties of lettuce (*Lactuca sativa L.*)	Assess the use of BFT effluent to nourish three varieties of lettuce (red crispy, butter and crispy) produced in FLOCponics during a 21-day period compared to those grown in traditional aquaponics.	The productive performance of lettuce cultured with BFT effluent was better than in traditional aquaponics. Regarding the lettuce varieties tested, butter lettuce presented the best growth results.
Pinho et al. (2021b)	† Tilapia (*Oreochromis niloticus*)	† Lettuce (*Lactuca sativa L.*)	Compare the productive parameters of Nile tilapia juveniles and butter lettuce grown in FLOCponics to those grown in a traditional aquaponics system during two 23-day trials.	The visual characteristics and growth performance of lettuce grown in FLOCponics were lower than those grown in traditional aquaponics, mainly in the second trial. The zootechnical performance of the tilapia juveniles was better in FLOCponics.
Poli et al. (2019)	Tilapia (*Oreochromis niloticus*) and pacific white shrimp (*Litopenaeus vannamei*)	*Sarcocornia ambigua*	Evaluate the water quality parameters and production of an integrated multitrophic aquaculture (IMTA) system applied to shrimp, tilapia and *Sarcocornia ambigua* in FLOCponics compared to a polyculture of shrimp and tilapia in BFT.	The IMTA in the FLOCponics system resulted in a higher yield of all products than in BFT. However, the presence of *S. ambigua* did not affect nitrogen and phosphorus use, despite reducing the amount of nitrate.

Rahman (2010)	Tilapia (*Oreochromis niloticus*)	† Lettuce (*Lactuca sativa L. 'Charles'*)	Compare the production of lettuce nourished by BFT effluent without solids management, BFT effluent with solids management, and commercial hydroponic solution during four 28-day trials.	Plants cultured with a commercial hydroponics solution grew better than those in FLOCponics systems. The presence of suspended solids was a limiting factor for lettuce growth.
Rocha et al. (2017)	† Silver catfish (*Rhamdia quelen*)	† Lettuce (*Lactuca sativa L.*)	Evaluate the production of *L. sativa* in hydroponics, traditional aquaponics, and FLOCponics using minimum infrastructure during a 46-day period.	The use of silver catfish effluent to nourish lettuces, in traditional aquaponics and FLOCponics, improved their growth when compared to those produced in hydroponics.
Silva (2016)	Pacific white shrimp (*Litopenaeus vannamei*)	† *Sarcocornia ambigua*	Evaluate the production of phenolic compounds and antioxidant activity of *S. ambigua* exposed to different periods of water stress, i.e. irrigation periods of 6, 12, 18 and 24 h per day, in a FLOCponics system.	*S. ambigua* cultured with 12 hours of daily irrigation resulted in higher production of bioactive compounds without affecting the productivity of plants and shrimp.
Zidni et al. (2019)	Catfish (*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*)	Water spinach	Determine the effect of different proportions of catfish and tilapia densities on water quality when integrated with water spinach production in a FLOCponics system.	The results presented were not sufficient to show a relationship between fish densities and water quality.

† Main product focused on the experiment.

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Chapter 3

Decoupled FLOCponics systems as an alternative approach to reduce the protein level of tilapia juveniles' diet in integrated agri-aquaculture production

This chapter is based on:

Pinho SM, Lima JP, David LH, Oliveira MS, Goddek S, Carneiro DJ, Keesman KJ, Portella MC (2021) Decoupled FLOCponics systems as an alternative approach to reduce the protein level of tilapia juveniles' diet in integrated agri-aquaculture production. Aquaculture 543:736932. https://doi.org/10.1016/j.aquaculture.2021.736932

Abstract

Decoupled FLOCponics (DFP) is a promising aquaponics approach which takes advantage of the nutritional benefits of biofloc technology (BFT). Enabling the use of less protein in the fish diets is one of the benefits of BFT. The effect of the reduction of protein content, and consequently the input of nitrogen, on fish and plant production in DFP systems has not yet been investigated. This study was designed to investigate and evaluate the production of lettuce and tilapia juveniles in a DFP system using different levels of crude protein (CP) in the fish diets. The zootechnical performance of tilapia juveniles and lettuce growth in the DFP system were evaluated, using different diets containing 24, 28, 32, and 36% CP. Fish production in DFP systems was compared to those reared in traditional decoupled aquaponics systems (DAPS) and in biofloc-based systems (BFT), both fed with 32% CP diet. The experimental period of tilapia juvenile production lasted 56 days. Lettuce production in two cycles was also performed in DFP systems with different CP levels and their growth was compared to those in DAPS and hydroponics systems, as control treatments. In *Cycle 1*, the seedling phase was evaluated in a 14-day trial. In *Cycle 2*, the final production phase was performed for 21 days until harvest. The physical-chemical parameters of the water were monitored in the aquaculture and hydroponic subsystems. High mortality of fish occurred in DFP-36 in the middle of the experiment, thus this treatment was discontinued. The results showed that tilapia reared in DFP and fed with 24 and 28% CP (DFP-24 and DFP-28) grew similarly to those in DAPS fed with 32% CP diet. Fish in DFP-32 and BFT fed with 32% CP diet grew similarly and above the other treatments. Additionally, plant growth results showed no differences in both cycles among all treatments. With respect to water parameters, even though there was a higher inflow of nutrients in the treatments with high CP content in the aquaculture subsystem, the mean values of nitrogen compounds and orthophosphate were similar in all treatments. For water parameters in the hydroponics subsystems, only the mean values of pH in *Cycle 1* were statistically different in the plant treatments. The results obtained in this study indicate that using less CP in fish diets to produce lettuce and tilapia juveniles is technically possible and feasible in a decoupled FLOCponics system.

3.1 Introduction

Integrated agri-aquaculture aquaponics systems typically combine a recirculating aquaculture system (RAS) with soilless plant production in hydroponics, based on sharing and reusing nutrients and water (Lennard and Goddek 2019). Aquaponics has become widespread in aquaculture as a way to increase the efficiency of water and feed use and consequently reduce the discharge of nutrient-rich effluents (Joyce et al. 2019; Yep and Zheng 2019). Commonly, between 21 to 30% of the dry matter and 40 to 47% of the nitrogen (N) content in the feed are retained in the biomass of tilapia, and most of the inputted nutrients are discharged into the environment (Verdegem 2013). The discharge of nutrient-rich effluent might result in pollution and eutrophication of waterbodies (Joyce et al. 2019). These problems can be minimized by directing the aquaculture effluents to nourish plants in aquaponics (Enduta et al. 2012; Turcios and Papenbrock 2014).

Most aquaponics systems are run in coupled setups, in which water and nutrients continuously flow through the aquaculture and hydroponics subsystems (Palm et al. 2019; Abusin and Mandikiana 2020). However, a trade-off between the required water quality and required environmental conditions in the respective subsystems has been reported as an issue in coupled aquaponics (Goddek et al. 2019). Decoupled layouts have been proposed to solve this trade-off by separating each subsystem component with a unidirectional flow from the aquaculture to the hydroponics subsystem. This allows for meeting the requirements of all subsystems and achieving high productivity of both fish and plants (Kloas et al. 2015; Goddek et al. 2016a; Monsees et al. 2017). In addition to evaluating different layouts, different aquaponics approaches have recently been tested, as well, seeking to improve the sustainable character of food production (Kotzen et al. 2019).

FLOCponics is a term proposed by Pinho et al. (2021) as an offshoot of aquaponics in which RAS is replaced by a system based on biofloc technology (BFT). BFT aims to manage the water quality in aquaculture systems without the need for costly mechanical and biological filters or for high volumes of water exchange (Emerenciano et al. 2017; Dauda 2020). The growth of specific microbial communities that recycle the nitrogenous waste is directly fomented in the aquaculture tanks (Verdegem and Bosma 2009; Crab et al. 2012; Emerenciano et al. 2013), by providing strong aeration and water movement. Besides that, an external carbon source is added to increase the carbon-nitrogen ratio of the water and to promote the growth of biofloc microbiota (Browdy et al. 2012; Avnimelech 2015). As a result, extra macro- and micro-nutrients are added to the water via the carbon source (Becerril-Cortés et al. 2018; Rocha et al. 2018). Additionally, lower nutrient loss by minimal solids or sludge removal can be linked to BFT when compared to RAS. The higher accumulation of nutrients in FLOCponics water compared to aquaponics using RAS could directly influence plant nutrition. However, the results presented to date have not reached any consensus on the benefit of using BFT effluents for plant growth in FLOCponics systems. Recent studies show positive effects of BFT on lettuce growth (Pinho et al. 2017; Lenz et al. 2018; Rocha et al. 2018), whereas others have observed the opposite effects (Rahman 2010; Fimbres-Acedo et al. 2020; Pinho et al. 2021). The negative results of plant production in FLOCponics were in general related to nutrient imbalances, high water pH, and the presence of bioflocs in the plant roots. The presence of bioflocs probably affected the breathing process and the absorption of nutrients by the plants (Rahman 2010; Rakocy 2012; Fimbres-Acedo et al. 2020; Pinho et al. 2021). In most of the studies, the FLOCponics systems were run in one loop instead of decoupled layouts (Pinho et al. 2017, 2021; Lenz et al. 2018; Rocha et al. 2018). Thus, only sub-optimal conditions for plant growth were achieved.

With respect to the effect of FLOCponics on tilapia production, increased zootechnical performance should be expected when comparing it to conventional aquaponics. This is because the BFT microorganisms are a constant and nutrient-rich source of natural food for the fish (Emerenciano et al. 2013; Bossier and Ekasari 2017; Martínez-Córdova et al. 2017; Becerril-Cortés et al. 2018), resulting in better fish weight gain, survival and feed conversion rate when compared to RAS production (Luo et al. 2014; Long et al. 2015; García-Ríos et al. 2019). The consumption of bioflocs by tilapia juveniles makes it possible to adapt nutritional strategies. For instance, alternative protein ingredients can be used instead of the conventional high-cost fish meal and soybean meal (Sousa et al. 2019; Freccia et al. 2020; Tubin et al. 2020), or the use of diets with low protein content (Azim and Little 2008; Mansour and Esteban 2017; Sgnaulin et al. 2020). Mansour and Esteban (2017) showed that even with a reduction of 30% to 20% of the dietary protein in tilapia reared in BFT, the fish grew more significantly than those cultured in a clear-water system and fed with 30% protein. To date, whether this nutritional benefit of BFT also occurs in FLOCponics has not yet been reported. Evidence of a negative effect of integration with hydroponics on the benefits of using BFT was found (Pinheiro et al. 2017, 2020; Pinho et al. 2017; Lenz et al. 2018). In these studies low volumes of bioflocs were reported for coupled FLOCponics systems, indicating low availability of natural food. Such a low volume of bioflocs occurred because of the need to limit the quantity of solids in the whole system in order to enable plant production (Barbosa 2017; Pinho et al. 2017). By individualizing each subsystem in a decoupled layout, proper management of bioflocs in the fish tanks can be carried out. Given the optimal biofloc volume for fish growth in the fish tanks, subsequently optimal nutritional strategies for plant production can be explored.

Developing technologies that allow the reduction of the amount of protein in tilapia diet, without undermining the system yields, benefits the aquaculture sector in both economic and environmental terms (Bossier and Ekasari 2017; Hisano et al. 2020). This is mainly because the use of low dietary protein may result in: (i) lower feed cost since protein is the most expensive nutrient in fish diets (Jatobá et al. 2014; Hisano et al. 2020); (ii) lower use of fish meal and, on a large scale, minimizing the overexploitation of natural fish stocks (Deutsch et al. 2007); and (iii) decreased input of N and, depending on the production system, less discharge of N into the surrounding environment (Hari et al. 2006; Lazzari and Baldisserotto 2008). This last consequence of reducing the amount of protein may also influence plant production in the integrated system. The effect of using less CP in the fish diet on plant growth must still be understood. It is important to note that the amount of dietary protein required by fish depends on the employed system and the production phase (Neto and Ostrensky 2015; Silva et al. 2018). For instance, tilapia in the nursery phase (1 to 30 g) usually require high dietary CP to ensure optimal growth when they are young and, consequently, to promote rapid growth until harvest. In systems with minimal natural food available, such as in RAS and cages, the recommended CP for tilapia juveniles varies between 30 to 40% (Hafedh 1999; Neto and Ostrensky 2015), whereas 28% CP has been suggested as enough to achieve high growth performance in BFT (Silva et al. 2018).

Consequently, decoupled FLOCponics (DFP) seems to be an alternative approach to take advantage of the nutritional benefits of BFT in integrated agri-aquaculture systems and thus reduce the amount of protein in the diets of tilapia juveniles. Additionally, testing different CP levels in this new system is necessary to indicate the optimal input of N to meet both plant and fish nutritional needs. The aim of the study was, therefore, to investigate and evaluate the production of lettuce and tilapia juveniles in a decoupled FLOCponics (DFP) system using different levels of crude protein (CP) in the fish diets. For this, the zootechnical performance of tilapias in DFP systems receiving diets with 24, 28, 32 and 36% CP were compared to those reared in a traditional decoupled aquaponics system (DAPS) and in BFT, both fed with a 32% CP diet. Two cycles of lettuce production were also performed in DFP systems with the different CP levels. Their growth was compared to those in DAPS and traditional hydroponics systems as control treatments. In addition, the physical-chemical parameters of the water were monitored in the aquaculture and hydroponic subsystems.

3.2 Material and methods

The experiment was carried out in a 100 m^2 aquaponics greenhouse at the Aquaculture Center of São Paulo State University (Caunesp) in Jaboticabal, São Paulo, Brazil, under the authorization of the Committee on Ethics in Animal Use (CEUA FCAV/Unesp – Protocol No. 001123/20). The greenhouse was covered with a 0.15 mm plastic liner. In addition, a shading net that reduces the luminosity by 40% was put onto the greenhouse. The plastic on the sides and shading net on the top of the greenhouse were movable. The plastic was used as complete coverage only on days when the internal temperature of the greenhouse was below 28 °C and during the night, and the shading net on sunny days. The water used to fill the tanks and replace the loss by evaporation came from an artesian well.

3.2.1 Experimental design and diets

A completely randomized experiment was designed to evaluate the production of tilapia juveniles (*Oreochromis niloticus*) and lettuce (*Lactuca sativa*) under different production techniques or subjected to diets with different crude protein (CP) contents. In total, seven treatments were tested: one treatment was a tilapia culture in BFT without integration with lettuce production (BFT); the second was a lettuce hydroponic treatment (HP); and the other five were tilapia culture integrated within two-loop decoupled systems. Of these five, one comprised a traditional decoupled aquaponics system (DAPS) and the other four were DFP systems using different levels of CP. In the two fish control treatments (BFT and DAPS), diets with 32% CP were used, while in the other DFPs the following levels were tested: 24%, 28%, 32%, and 36% CP (Figure 3.1). There were three replications of each fish treatment and six of each plant treatment. From this, the effluent of one aquaculture subsystem was used to nourish two plant tanks. The experiment lasted 56 days. In this period, two cycles of lettuce production and one of tilapia juveniles were performed.

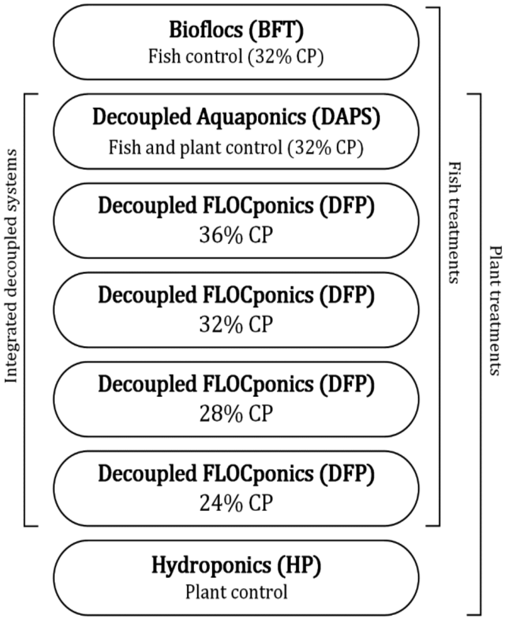

Figure 3.1. Schematic illustration of the experiment design. Three replicates of each fish treatment and six of each plant treatment were run. CP: crude protein

3.2.1.1 Diets

Ingredients usually used in Brazilian commercial feed industries were selected and their nutrient and energy contents were analysed at the Laboratory of Animal Nutrition (LANA) of the Faculty of Agricultural and Veterinary Sciences (FCAV-Unesp, Jaboticabal-SP). After that, four diets for tilapia juveniles were formulated to contain different levels of crude protein according to the tested treatments (Table 3.1). The diets were isoenergetic, isophosphoric and the protein ingredients from animal sources were maintained at 25% of the total protein content. In all diets, the proportion of the protein ingredients from animal sources was set at 3:2:1 for poultry by-product meal, fish meal, and feather meal, respectively. Soybean meal was the most used protein ingredient among the plant-based sources. The choice of these ingredients and their representation in the diet formulation was based on their availability and quality in Brazil. The diets were formulated to meet some nutritional requirements of tilapia juvenile, i.e., a minimum of 35, 5.5, 17.8, and 4 g kg^{-1} of ether extract, methionine, lysine, and phosphorus, respectively, and a maximum of 60 and 80 g kg^{-1} of crude fiber and ash, respectively (Furuya 2010; NRC 2011). Diet ingredients were finely ground and sieved in 0.9 mm mesh and 0.5 to 4 mm feed pellets were processed at the Feed Manufacturing Facility of the FCAV-Unesp.

Table 3.1. Formulas and composition of test diets.

Ingredient (g kg^{-1})	**Diet**			
	24 CP	**28 CP**	**32 CP**	**36 CP**
Fish meal [a]	32.2	38.2	43.6	49.1
Poultry by-product meal [b]	48.6	57.3	65.5	73.7
Feather meal [c]	15.9	19.1	21.8	24.6
Soybean meal [d]	247.3	341.8	431.3	520.4
Corn (grain) [e]	142.8	118.5	93.1	67.0
Wheat meal [f]	142.8	118.5	93.1	67.0
Rice meal [g]	142.8	118.5	93.1	67.0
Broken rice [h]	142.8	118.5	93.1	67.0
Soy oil [i]	37.4	28.7	27.0	28.1
Limestone [l]	9.7	8.1	6.5	5.0
Dicalcium phosphate [m]	20.0	19.0	18.3	17.5
Vitamin-mineral supplement [n]	5.0	5.0	5.0	5.0
Antifungal (Phylax®) [o]	3.0	3.0	3.0	3.0
Antioxidant (BHT) [o]	0.5	0.5	0.5	0.5
Methionine [p]	1.0	0.3	0.0	0.0
Lysine [q]	3.2	0.0	0.0	0.0
Salt	5.0	5.0	5.0	5.0

Total	1000.0	1000.0	1000.0	1000.0
Centesimal composition (g kg^{-1})				
Crude protein	240.0	280.2	320.1	359.9
Ether extract	96.1	85.8	81.8	80.4
Crude fiber	56.1	55.4	54.1	52.6
Ash	58.1	63.3	67.9	72.3
Nitrogen-free extract	50.0	45.7	43.5	38.7
Calcium	13.4	13.4	13.4	13.4
Phosphorus	6.6	6.6	6.6	6.6
Gross energy (Mj kg^{-1})	17.0	16.8	16.9	16.9

[a] Guabi Nutrição e Saúde Animal, SP, Brazil. Protein: 533.8; Ether extract: 131.6; Crude energy (Mj Kg^{-1}): 17.2.

[b] Guabi Nutrição e Saúde Animal, SP, Brazil. Protein: 604.2; Ether extract: 139.9; Crude energy (Mj Kg^{-1}): 18.8

[c] Guabi Nutrição e Saúde Animal, SP, Brazil. Protein: 785.7; Ether extract: 115.7; Crude energy (Mj Kg^{-1}): 22.6

[d] Agromix, SP, Brazil. Protein: 462.4; Ether extract: 27.6; Crude energy (Mj Kg^{-1}): 16.4.

[e] FCAV/UNESP, SP, Brazil. Protein: 90.3; Ether extract: 39.8; Crude energy (Mj Kg^{-1}): 16.2.

[f] Agromix, SP, Brazil. Protein: 150.4; Ether extract: 43.7; Crude energy (Mj Kg^{-1}): 16.8.

[g] Agromix, SP, Brazil. Protein: 119.4; Ether extract: 170.9; Crude energy (Mj Kg^{-1}): 17.8.

[h] Agromix, SP, Brazil. Protein: 77.4; Ether extract: 18.8; Crude energy (Mj Kg^{-1}): 15.9.

[i] Agromix, SP, Brazil. Crude energy (Mj Kg^{-1}): 39.1.

[l] Nutreco Brasil, SP, Brazil. Ca: 384.0.

[m] Nutreco Brasil, SP, Brazil. Ca: 245.0; P: 185.0.

[n] Nutreco Brasil, SP, Brazil. Each 1% contains: folic acid (1 mg); pantothenic acid (20 mg); antioxidant (125 mg); choline (150 mg); copper (10 mg); iron (100 mg); iodine (5 mg); manganese (70 mg); selenium (0.15 mg); vitamin A (3,000 IU kg^{-1}); vitamin B (16 mg); vitamin B12 (20 mg); vitamin B2 (8 mg); vitamin B6 (3 mg); vitamin C (350 mg); vitamin D3 (3000 IU kg^{-1}); vitamin E (200 IU kg^{-1}); vitamin K (6 mg); zinc (150 mg); niacin (100 mg); biotin (0.10 mg).

[o] Nutreco Brasil, SP, Brazil.

[p] Nutreco Brasil, SP, Brazil. Crude Energy (Mj Kg−1): 22.8.

[q] Nutreco Brasil, SP, Brazil. Crude Energy (Mj Kg−1): 20.0.

CP: Crude protein.

3.2.2 Systems description

The greenhouse hosted individual aquaponics systems run in decoupled mode with unidirectional flow from the aquaculture to the hydroponics subsystems. In each replicate the effluent from the aquaculture subsystem was supplied to two hydroponics subsystems. Thus, in total 18 aquaculture subsystems and 36 hydroponics subsystems were run. The configuration of the aquaculture subsystems differed from each other according to the aquaculture technology employed (Figure 3.2).

The aquaculture subsystem of the DAPS treatment was run as a recirculating aquaculture system (RAS) and consisted of a circular fish tank (380 L), a radial flow settler (RFS; 100 L), a bag filter (68 µm, 5 L), and a moving bed bioreactor (MBBR; 180L, containing plastic bio balls with a specific surface area of ~ 1000 m^2 m^{-3}). When operated as DAPS, the water was recirculated between the aquaculture units using a pump (1000 L h^{-1}) submerged in the MBBR. The configuration described above was applied in three identical and independent aquaculture subsystems for the DAPS treatment, whereas 15 identical and independent aquaculture subsystems of the biofloc-based treatments were run. These biofloc-based subsystems consisted of a circular fish tank (380 L) and a RFS (100 L). In contrast to DAPS, in the BFT or DFP treatments the water remained in the fish tank and, in DFPs, was periodically directed to the RFS for the collection of the supernatant for plant nutrition (Figure 3.2). The plant nutrition management is detailed below, in *subsection 2.4.1*. The sedimented organic matter (sludge) from the RFS-DAPS was removed weekly. In the hydroponics subsystem, 36 individualized production units in a deep-water culture (DWC) mode named as plant tanks (PTs) were used, totalling 6 PTs for each treatment. The surface of each PT was 0.42 m^2 (volume of 60 L), where 8 plants were accommodated in an expanded polystyrene block with an identical area to each tank.

It should be noted beforehand that high fish mortality occurred in all replicates of DFP-36 treatment after the middle of the experiment. After two days of exceptionally high temperatures (approximately 40 ºC inside the greenhouse), a combination of high nutrient load, high settleable solids (volume of biofloc by Imhoff cones, 100 mL L^{-1}) and high water temperature (31.9 °C) caused a sudden drop in the dissolved oxygen (0.8 mg L^{-1}) in the water at the end of the fortieth day of the experiment and, subsequently, the death of more than 80% of the fish. Thus, this treatment was discontinued, and its results were not analysed and presented.

Aeration was provided by an air blower (2 HP) and distributed in each system by micro-perforated diffusers (AquaDrop Air®, Brazil). Circular pieces of diffuser (16 cm Ø) were placed in the center of each fish tank and a 15 cm length in each MBBR and PT. In each fish tank, a 500 W thermostat heater was used to maintain the water temperature at 27 °C.

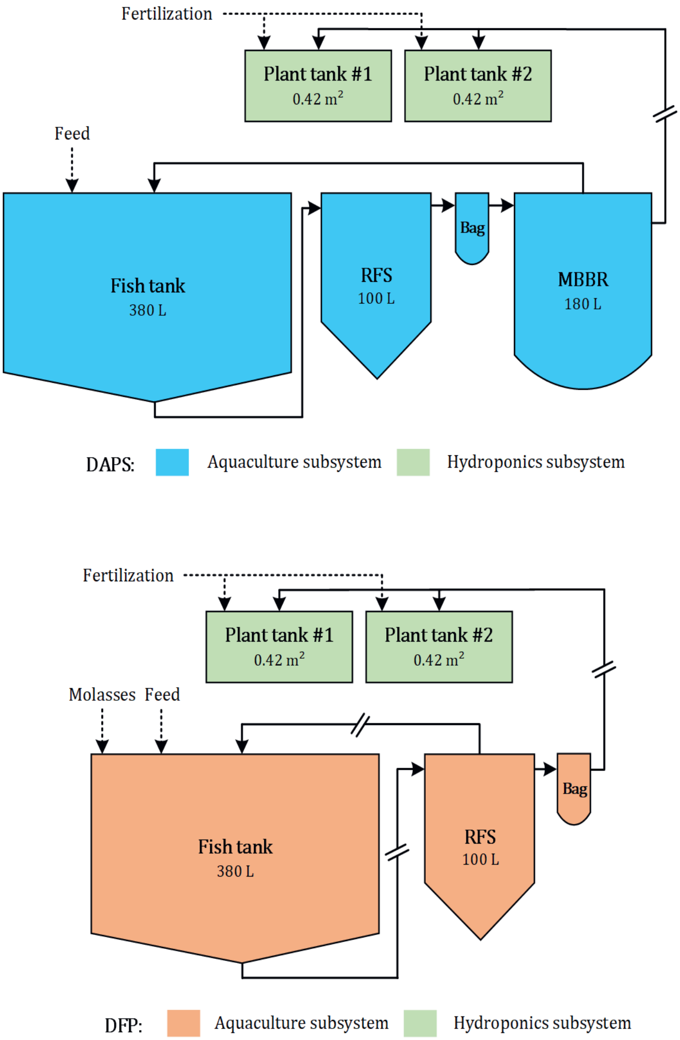

Figure 3.2. Schematic illustration of the experimental devices installed to run as decoupled aquaponics systems (DAPS) or decoupled FLOCponics systems (DFP). Three identical DAPS and 12 identical DFP devices were run. In the hydroponics (HP) and bioflocs (BFT) treatments, only the hydroponics subsystem and the DFP aquaculture subsystem were respectively used, with three replicates each. The aquaculture subsystem of the DAPS systems operated as a recirculating aquaculture system. RFS: radial flow settler. MBBR: moving bed bioreactor. ⫽ Indicates that water flow was manually controlled, i.e., the water did not continuously circulate throughout the tanks and filters where this symbol appears.

Prior to the beginning of the experiment, 75% of the total volume of the aquaculture subsystem was filled with artesian well water. The remaining 25% was filled with water from 60-day mature inoculums from RAS and BFT systems previously performed. These inoculums were used to ensure that the microbial communities of the MBBR and bioflocs were already mature. For the maintenance of the biofloc microorganisms and the C:N ratio of the water at 15:1, liquid molasses was added to the BFT and DFP fish tanks three times a week as a complementary carbon source. The amount of molasses was calculated based on the input of N by fish feed (methodology adapted from Emerenciano et al. (2017)). Calcium hydroxide was added to the fish tanks when the alkalinity was under 80 mg L^{-1}.

3.2.3 Tilapia juveniles production

Nile tilapia (*Oreochromis niloticus*) juveniles were purchased from a commercial hatchery (AQUABEL®) and acclimatized for 7 days after arriving at Caunesp. In all treatments, 114 masculinized juveniles (1.42 ± 0.03 g) were stocked, totaling an initial biomass of 0.43 kg m^{-3} and density of 300 fish m^{-3} in each fish tank. After 4 weeks of culture, the number of fish per tank was managed in order to readjust the densities. Thus, 70 fish per tank were kept, resulting in a density of 184 fish m^{-3} and a calculated biomass of 3.6 kg m^{-3}.

During the 56-day trial, the fish were hand-fed with the test diets four times a day at 08:30, 11:00, 14:30 and 18:00 h. The amount of feed was calculated based on the percentage of body weight recommended by a commercial feed industry (Raguife®), ranging from 12 to 5% according to the average fish weight. A sample of at least 20% of the total number of fish in each tank was weighed weekly to adjust the amount of feed in all treatments.

At the end of the experiment, all tilapia juveniles were counted and weighed and 15 fish from each tank were individually measured. Final individual body weight (g), total fish length (cm), weight gain (g), yield (kg m^{-3}), specific growth rate (SGR), feed conversion ratio (FCR) and survival (%) were assessed. Under the assumption of exponential growth, SGR is defined as: $(\mathrm{Ln}(W_f) - \mathrm{Ln}(W_0))\ /\ (t_f - t_0) \times 100\%$ (% day^{-1}), where t_0 and t_f are initial and final time; W_0 and W_f are the initial and final body weight. Zootechnical performance data and/or fish body composition were used to calculate the protein-use efficiency indices as follows: protein efficiency ratio (PER = mean weight gain / mean crude protein intake), protein productive value (PPV = $[(CP_f \times W_f) - (CP_0 \times W_0)]$ / crude protein intake x 100%) (%), and crude protein on weight gain ($CPwg = [(CP_f \times W_f) - (CP_0 \times W_0)]$ /mean weight gain x 100%) (%), where CP_0 and CP_f are the initial and final crude protein content; W_0 and W_f are the initial and final body weight.

The protein content of the diets and fish body were determined to evaluate protein use efficiency. For this, three different samples were taken: (i) 30 individuals from the initial fish population; (ii) 10 from each repetition per treatment at the end of the experiment; and (iii) 15 g of each diet. The fish were anesthetized and euthanized. Subsequently, the whole bodies were weighed, packed and frozen at -20 °C for later analysis. The frozen fish were ground, homogenized in a meat grinder (C.A.F, model 22S) and lyophilized (Freeze Dryer Edwards, model Pirani 501). The lyophilized matter was used to determine the percentage of dry matter and crude protein (Leco Nitrogen/Protein in Organic Samples, model FP528), according to the methodology of A.O.A.C. (2000). The same analyses were applied to determine the composition of the diets. All analyses of the proximal composition of the tilapia tissue were made in duplicate.

3.2.4 Lettuce production

Two trials of butter lettuce (*Lactuca sativa*) production in different phases were carried out. In *Cycle 1*, the seedling phase was evaluated in a 14-day trial. For this, hydroponic seedlings at 7 days after sowing (d.a.s) and 0.59 ± 0.08 g were grown until 21 d.a.s. In *Cycle 2*, the final production phase was performed, in which new hydroponic seedlings at 21 d.a.s. and 2.04 ± 0.57 g were planted and cultivated for 21 days until harvest. In both cycles, 8 plants were distributed in each hydroponics subsystem with a density of 19 plants m^{-2}. The weight (roots and shoot) of four lettuces in all plant tanks were recorded once a week. These four lettuces per tank were selected randomly at the beginning of the trials and the same were weighed weekly throughout each trial.

At the end of *Cycle 1*, all plants were weighed and the following growth parameters were evaluated: leaf and root height (cm), total wet weight (g), total dry weight (g), number of leaves per plant (-), productivity (g m^{-2}) and specific growth rate. At the end of *Cycle 2*, the following growth parameters were evaluated for seven lettuces from each plant tank: leaf and root heights (cm), wet leaf and root weights (g), dry leaf weight (g), number of leaves per plant (-), and productivity (g m^{-2}). Also, in both cycles, a visual analysis was applied to identify the non-marketable plants. Plants that contained up to 33% of abnormalities on the leaf surface, i.e., with a yellowish color, burns or wrinkles, were considered non-marketable (methodology adapted from Pinho et al. (2017)). For the control of plant pests, twelve traps (ColorTrap, Isca®, Brazil) were distributed through the greenhouse. A visual scan of the presence of plant pests or diseases was performed daily on all plants and no sign of them was seen during the trials.

3.2.4.1 Lettuce nutrition

Prior to the beginning of both trials, 50% of the total volume of each plant tank was filled with water from the aquaculture subsystem and, for the remaining 50%, artesian well water was used. The tanks of HP treatment were only filled with artesian well water. In the DAPS plant tanks, the water was collected from the upper-middle portion of the MBBR of the DAPS aquaculture subsystem. In the DFPs treatment, the water of each DFP fish tank underwent a decantation and filtration process before being directed to the plant tanks. This means that the water was pumped into the RFS and remained there for 20 min until the biofloc particles were decanted. After that, the RFS supernatant was directed to a bag filter (68 µm) and then to the PTs. The initial volume of water taken from each aquaculture subsystem, which was used to supply two PTs (2 x 30 L each), was replaced with artesian well water. Between the two cycles of plant production, all the PTs were emptied, cleaned and all the aforementioned procedures for filling the PTs were repeated.

After filling the PTs, the electrical conductivity (EC) was measured in each PT and a complete commercial fertilizer (Dripsol Folhosas®, concentrated 100 times) was added until the EC reached 1.2 mS cm^{-1} in *Cycle 1* and 1.7 mS cm^{-1} in *Cycle 2*. The commercial fertilizer was composed of 22.5% N, 9% P, 30% K, 4% Mg, 18.5% Ca, 6% S, 0.15% Fe, 0.085% Zn, 0.05% Mn, 0.015% Cu, 0.004% Mo, and 0.003% B, as informed by the manufacturer. The volumes of fertilizer solution (VA_{fs}) needed to reach these ECs were calculated using the following equation, derived by us: $VA_{fs} = (EC_s - EC_{PT}) \times V_{PT} / EC_{cfs}$; where EC_{cfs} is the electrical conductivity of the concentrated fertilizer solution; EC_s is the electrical conductivity standardized for each plant cycle; EC_{PT}: registered electrical conductivity in the plant tank; and V_{PT}: volume of water in the plant tank. When the EC in all PTs was stable, the seedlings were planted.

For plant nutrition during the experiment, water from each aquaculture subsystem or well water (HP treatment) was added manually to the PTs, at the proportion of 2% of the initial volume of the PT since it was the estimated volume of water evaporation in the PTs. In the DFP systems, the biofloc decantation and filtration procedures were always carried out and the decanted bioflocs returned to the fish tanks, except for samples collected in the beginning, middle and end of the experiment. In both plant cycles, the commercial fertilizer solution was added according to the equation above and only if the registered EC values were below the expected ranges. In *Cycle 1*, the EC was maintained between 1.1 and 1.3 mS cm^{-1} and a unidirectional water flow between fish and plant tanks occurred once a day on alternate days. In *Cycle 2*, the EC was between 1.6 and 1.8 mS cm^{-1} and a unidirectional water flow occurred once a day, six days per week. Aiming to maintain the pH in the PTs at between 5.5 and 6.5, diluted phosphoric acid (1:1) was added when the pH exceeded 6.5.

3.2.5 Environmental conditions and physical-chemical parameters of the water

Temperature and relative humidity were monitored daily at 11am at five points, one outside and four inside the aquaponics greenhouse. The water quality parameters such as settleable solids (volume of biofloc by Imhoff cones), pH, electrical conductivity (EC), total dissolved solids (TDS), temperature and dissolved oxygen (Horiba U-52G) were monitored daily in all fish tanks. Concentrations of total ammonia nitrogen, nitrite, nitrate, orthophosphate, and alkalinity in all fish tanks were measured once a week (Koroleff 1976; Golterman et al. 1978; Mackereth et al. 1978). In all PTs, temperature, EC, and pH were monitored daily. The presence or absence of solids in the PTs was also checked daily.

3.2.6 Statistical analysis

Once the premises of normality (Levene's test) and homogeneity of variances (Shapiro-Wilk's test) were fulfilled, water quality, zootechnical performance, protein-use efficiency, and lettuce growth performance data in each plant stage were analyzed by means of one-way ANOVA. For all data related to fish production and water quality in the aquaculture subsystems, three replicates were considered. The productive data of plants and water quality in the hydroponic subsystems were evaluated with six replications per treatment. Significant differences among the treatments were detected using Tukey's test. All data were analyzed at 5% significance level. Descriptive statistics were also used for water quality parameters in the aquaculture and hydroponics subsystems.

3.3 Results

Relative humidity and temperature were similar inside and outside the greenhouse (Figure 3.3). However, they varied widely during the course of the experiment. Mean relative humidity was 42.3 ± 13.8 and 44.1 ± 14.7% inside and outside, respectively, with minimum values of 22.2 and 21.6% and maximum of 73.9 and 74.0%. Temperature mean values were 34.2 ± 4.1 and 33.2 ± 4.99 °C inside and outside, respectively, with minimum values of 25.9 and 25.1 ºC and maximum of 41.3 and 40.9 °C.

Table 3.2 shows the descriptive statistics of the water quality parameters in the aquaculture subsystems. DO, pH, EC, and TDS were the values that significantly differed amongst the treatments. For all these parameters, mean values in DFP-32 and BFT were always statistically similar ($p>0.05$), as well as in DFP-24 compared to DAPS values. The variations of nitrogenous compounds and orthophosphate over the experiment are presented in Figure 3.4. In all biofloc-based treatments (BFT and DFPs), accumulation of settleable solids in the fish tanks was observed during the experiment. The mean values of ammonia nitrogen varied widely without a clear pattern throughout the experiment, mainly in DFP-28. For nitrite, there is also a notable variation. The nitrite concentration in the DAPS treatment shows contrasting behaviour compared to the nitrite concentrations in the DFP and BFT treatments. For nitrate and orthophosphate, a tendency to decrease and accumulate, respectively, was found. For the water quality parameters in the hydroponics subsystems, the descriptive statistics are presented in Table 3.3. Only the mean values of pH in *Cycle 1* were statistically different ($p<0.05$) in the plant treatments. No solids were seen in the plant tanks during the plant cycles. The commercial fertilizer solution was only added in the first two days of each plant cycle regardless of the treatment, i.e., there was no need to add fertilizer during the cycles since the registered EC values were not below the expected ranges. The total volume of fertilizer for each plant tank in *Cycles 1* and *2* were, respectively, 316.2 and 366.2 mL in HP, 221.4 and 318.9 mL in DAPS, 208.1 and 314.9 mL in DFP-32, 209.8 and 315.9 mL in DFP-28, and 213.6 and 322.0 mL in DFP-24.

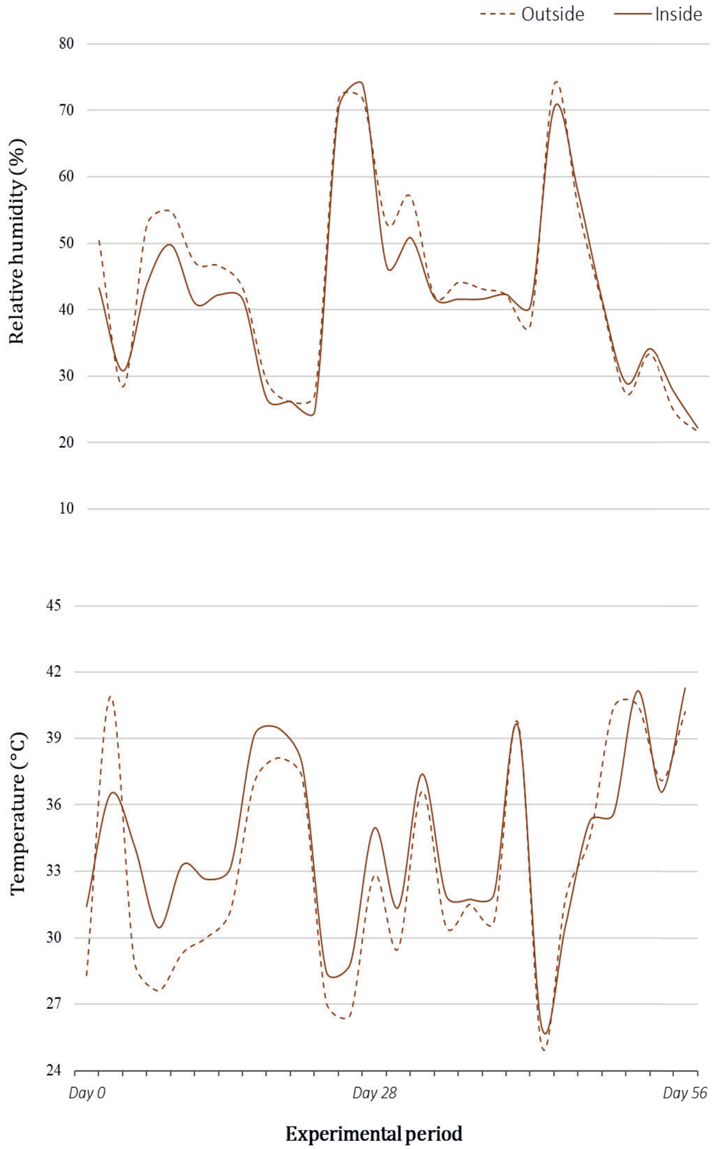

Figure 3.3. Mean values of relative humidity and temperature inside and outside the aquaponics greenhouse over the 56-day experimental period.

Table 3.2. Mean, standard deviation, maximum and minimum values of water quality parameters in the aquaculture subsystems during the 56-day experimental period.

Parameter	**DPF - 24**	**DPF - 28**	**DFP - 32**	**BFT - 32**	**DAPS - 32**	***p*-value***
Temperature	27.49 ± 0.25	27.70 ± 0.25	27.71 ± 0.32	27.75 ± 0.18	27.12 ± 0.12	0.117
(°C)	22.3 30.2	23.9 30.2	24.1 30.5	24.5 31.3	24.1 29.8	
Dissolved oxygen	7.07 ± 0.32^{a}	6.44 ± 0.32^{ab}	6.19 ± 0.21^{b}	6.61 ± 0.08^{ab}	6.87 ± 0.32^{ab}	0.024
(mg L^{-1})	4.8 8.6	4.3 8.6	3.5 8.5	3.7 8.4	4.2 8.7	
pH	7.22 ± 0.04^{a}	7.15 ± 0.04^{abc}	7.14 ± 0.02^{bc}	7.07 ± 0.02^{c}	7.17 ± 0.02^{ab}	0.002
	6.8 7.6	6.5 7.6	6.6 7.6	6.3 7.7	6.8 7.5	
Electrical conductivity	0.39 ± 0.01^{c}	0.45 ± 0.01^{b}	0.48 ± 0.01^{ab}	0.50 ± 0.02^{a}	0.41 ± 0.01^{c}	<0.001
(mS cm^{-1})	0.3 0.6	0.3 0.6	0.3 0.8	0.3 0.8	0.3 0.5	
Total dissolved solids	0.26 ± 0.01^{c}	0.30 ± 0.01^{b}	0.31 ± 0.01^{ab}	0.32 ± 0.01^{a}	0.27 ± 0.01^{c}	<0.001
(mg L^{-1})	0.2 0.5	0.2 0.5	0.2 0.5	0.2 0.5	0.2 0.4	
Settleable Solids	30.37 ± 5.95	27.82 ± 5.95	29.64 ± 5.54	36.87 ± 5.01		0.213
(mL L^{-1})	4.3 106.7	5.0 106.7	5.7 94.7	6.0 150.0		
Alkalinity	85.43 ± 3.51	87.14 ± 3.51	79.71 ± 13.90	86.57 ± 13.94	71.62 ± 2.08	0.138
(mg L^{-1})	66.7 101.3	64.3 101.3	41.3 121.7	54.3 140.0	58.0 92.3	
Ammonia Nitrogen	0.29 ± 0.05	0.42 ± 0.05	0.30 ± 0.03	0.36 ± 0.08	0.30 ± 0.10	0.494
(mg L^{-1})	0.0 0.7	0.1 0.7	0.1 0.7	0.1 1.5	0.0 1.4	
Nitrite	0.27 ± 0.07	0.30 ± 0.07	0.40 ± 0.04	0.35 ± 0.03	0.16 ± 0.05	0.386
(mg L^{-1})	0.0 0.7	0.0 0.7	0.0 0.7	0.0 0.7	0.0 0.6	
Nitrate	0.74 ± 0.04	0.70 ± 0.04	0.72 ± 0.09	0.70 ± 0.09	0.63 ± 0.04	0.065
(mg L^{-1})	0.3 0.9	0.1 0.9	0.0 0.9	0.0 0.9	0.1 0.9	
Orthophosphate	2.22 ± 0.04	2.09 ± 0.04	1.79 ± 0.11	1.82 ± 0.11	2.31 ± 0.04	0.863
(mg L^{-1})	0.3 4.9	0.5 4.9	0.5 4.0	0.5 4.2	1.2 4.1	

* Means followed by different letters in the same line indicate statistical differences (one-way ANOVA at 5% significance level). DFP: decoupled FLOCponics system. BFT: bioflocs system. DAPS: decoupled aquaponics system.

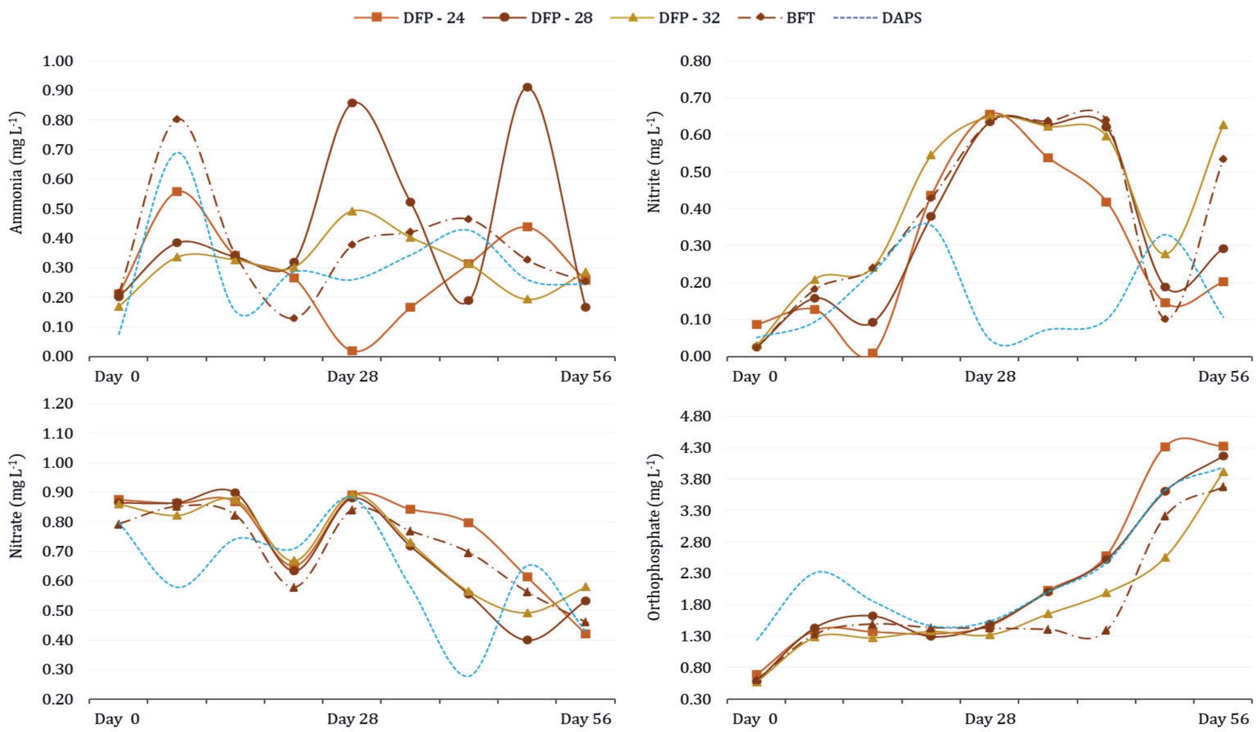

Figure 3.4. Variations of nitrogenous compounds (ammonia nitrogen, nitrite, and nitrate) and orthophosphate over the 56-day experiment. DFP: decoupled FLOCponics system. BFT: bioflocs system. DAPS: decoupled aquaponics system.

Table 3.3. Mean, standard deviation, maximum and minimum values of water quality parameters in the hydroponics subsystems during each plant cycle.

Parameter	**DFP - 24**	**DFP - 28**	**DFP - 32**	**DAPS - 32**	**HP**	***p*-value***
Cycle 1 - Seedling production phase						
Temperature (°C)	23.74 ± 0.29 23.33 24.21	23.81 ± 0.22 23.41 24.03	23.83 ± 0.16 23.59 24.02	23.75 ± 0.32 23.35 24.25	23.78 ± 0.26 23.40 24.03	0.623
Electrical conductivity (mS cm^{-1})	1.27 ± 0.04 1.18 1.35	1.31 ± 0.02 1.26 1.40	1.34 ± 0.02 1.30 1.42	1.31 ± 0.06 1.21 1.42	1.39 ± 0.14 1.06 1.53	0.344
pH	6.95 ± 0.13^{b} 6.77 7.12	6.87 ± 0.10^{ab} 6.70 6.97	6.93 ± 0.14^{b} 6.71 7.05	6.59 ± 0.22^{a} 6.29 6.78	6.73 ± 0.27^{ab} 6.21 6.93	0.009
Cycle 2 - Final production phase						
Temperature (°C)	23.44 ± 0.22 23.14 23.70	23.51 ± 0.21 23.16 23.76	23.47 ± 0.22 23.19 23.78	23.49 ± 0.19 23.27 23.79	23.43 ± 0.23 23.20 23.71	0.699
Electrical conductivity (mS cm^{-1})	1.72 ± 0.14 1.45 1.98	1.67 ± 0.05 1.52 1.88	1.67 ± 0.07 1.46 1.97	1.70 ± 0.03 1.42 1.91	1.66 ± 0.06 1.51 1.85	0.700
pH	6.64 ± 0.24 6.34 7.05	6.55 ± 0.10 6.44 6.71	6.66 ± 0.13 6.48 6.80	6.51 ± 0.05 6.44 6.58	6.46 ± 0.08 6.36 6.57	0.079

* Means followed by different letters in the same line indicate statistical differences (one-way ANOVA at 5% significance level). DFP: decoupled FLOCponics system. DAPS: decoupled aquaponics system. HP: hydroponics control system.

The zootechnical performance and protein-use efficiency of tilapia juveniles are presented in Table 3.4. Survival, feed conversion ratio, and lengths were similar in all treatments ($p>0.05$). The other zootechnical parameters were similar for BFT and DFP-32 means, and both treatments resulted in statistically higher growth performance ($p<0.05$) compared to DFP-24, DFP-28, and DAPS. The protein-use efficiency parameters also significantly differed among the treatments. In general, these parameters were significantly higher in the systems that used bioflocs, mainly in DFP-24, compared to DAPS.

Table 3.4. Mean ± standard deviation of productive performance of tilapia juveniles during the 56-day experimental period.

Parameter	**DFP - 24**	**DFP - 28**	**DFP - 32**	**BFT - 32**	**DAPS - 32**	***p*-value***
Zootechnical						
Final weight (g)	28.06 ± 2.04^{b}	29.63 ± 0.63^{b}	34.61 ± 1.66^{a}	34.76 ± 1.06^{a}	28.26 ± 0.50^{b}	< 0.001
Weight gain (g)	26.63 ± 2.05^{b}	28.2 ± 0.67^{b}	33.19 ± 1.60^{a}	33.37 ± 1.10^{a}	26.84 ± 0.57^{b}	< 0.001
SGR (% g day^{-1})	5.23 ± 0.13^{b}	5.33 ± 0.04^{b}	5.6 ± 0.08^{a}	5.61 ± 0.05^{a}	5.25 ± 0.03^{b}	< 0.001
Productivity (kg m^{-3})	4.88 ± 0.07^{b}	4.99 ± 0.18^{b}	5.86 ± 0.32^{a}	6.1 ± 0.28^{a}	4.71 ± 0.08^{b}	< 0.001
Total length (cm)	11.52 ± 0.34	11.66 ± 0.27	12.18 ± 0.25	12.1 ± 0.47	11.67 ± 0.13	0.098
Standard length (cm)	9.63 ± 0.29	9.7 ± 0.2	10.18 ± 0.25	10.16 ± 0.42	9.85 ± 0.24	0.127
FCR	1.07 ± 0.05	1.06 ± 0.11	1.02 ± 0.08	0.89 ± 0.03	1.05 ± 0.14	0.183
Survival (%)	96.59 ± 2.32	95.15 ± 0.57	90.31 ± 13.41	98.39 ± 0	95.4 ± 1.65	0.566
Protein-use efficiency						
PER	3.82 ± 0.19^{a}	3.3 ± 0.36^{ab}	2.98 ± 0.25^{b}	3.41 ± 0.11^{ab}	2.93 ± 0.41^{b}	0.021
PVV (%)	55.82 ± 2.93^{a}	50.71 ± 3.90^{ab}	48.32 ± 5.69^{ab}	58.43 ± 2.60^{a}	40.11 ± 5.70^{b}	0.004
CPwg (%)	14.65 ± 0.93^{ab}	15.43 ± 1.29^{ab}	16.17 ± 1.04^{ab}	17.14 ± 1.25^{a}	13.7 ± 0.24^{b}	0.017

* Means followed by different letters in the same line indicate statistical (one-way ANOVA at 5% significance level). DFP: decoupled FLOCponics system. BFT: bioflocs system. DAPS: decoupled aquaponics system. SGR: specific growth rate. FCR: feed conversion ratio. PER: protein efficiency ratio. PPV: protein productive value. CPwg: crude protein on weight gain.

Table 3.5 displays the results of lettuce growth parameters in *Cycle 1* and *2*. Regardless of the plant growth phase, no significant differences were found amongst the treatments for all parameters. The marketable plants represented 83% of the seedlings produced in all treatments in *Cycle 1*, whereas in *Cycle 2*, 100% of the harvested lettuce could be traded. Figures 3.5 and 3.6 show the lettuce growth curves. The growth trend lines were represented, according to the highest R^2 achieved, by a polynomial regression in *Cycle 1* (Figure 3.5) and an exponential regression in *Cycle 2* (Figure 3.6).

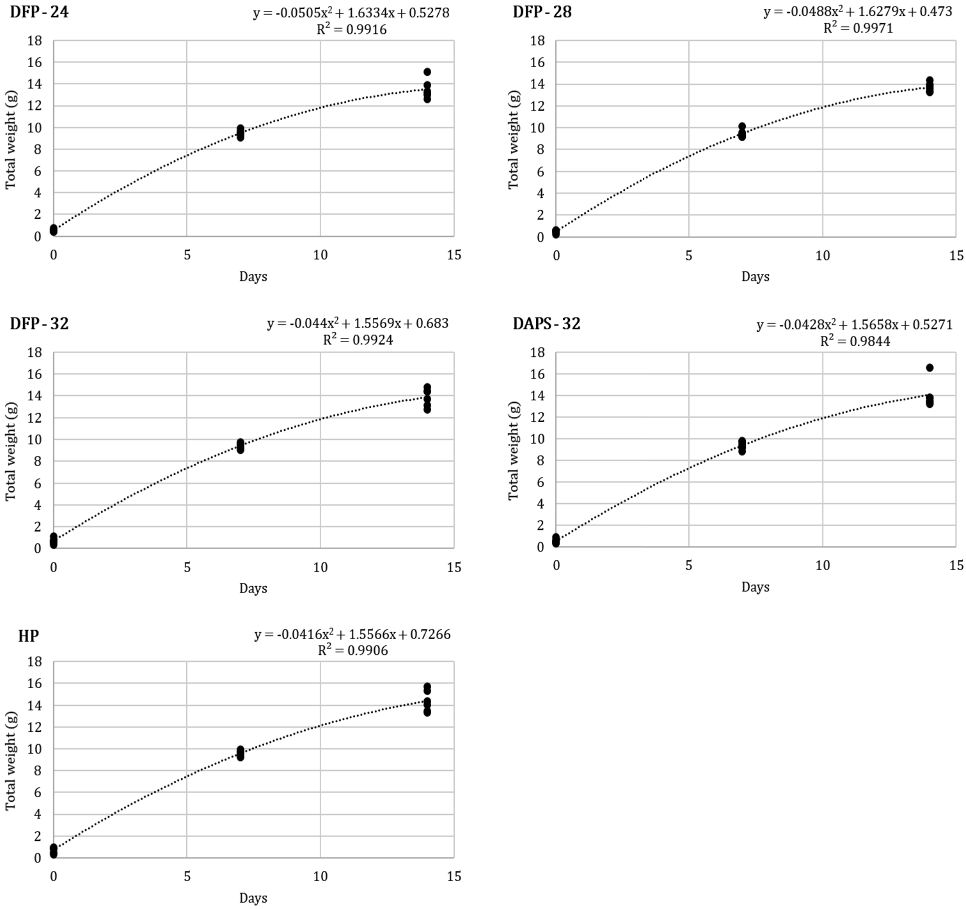

Figure 3.5. Growth curves of lettuce seedlings in *Cycle 1* (weekly sampling). DFP: decoupled FLOCponics system. DAPS: decoupled aquaponics system. HP: hydroponics control system.

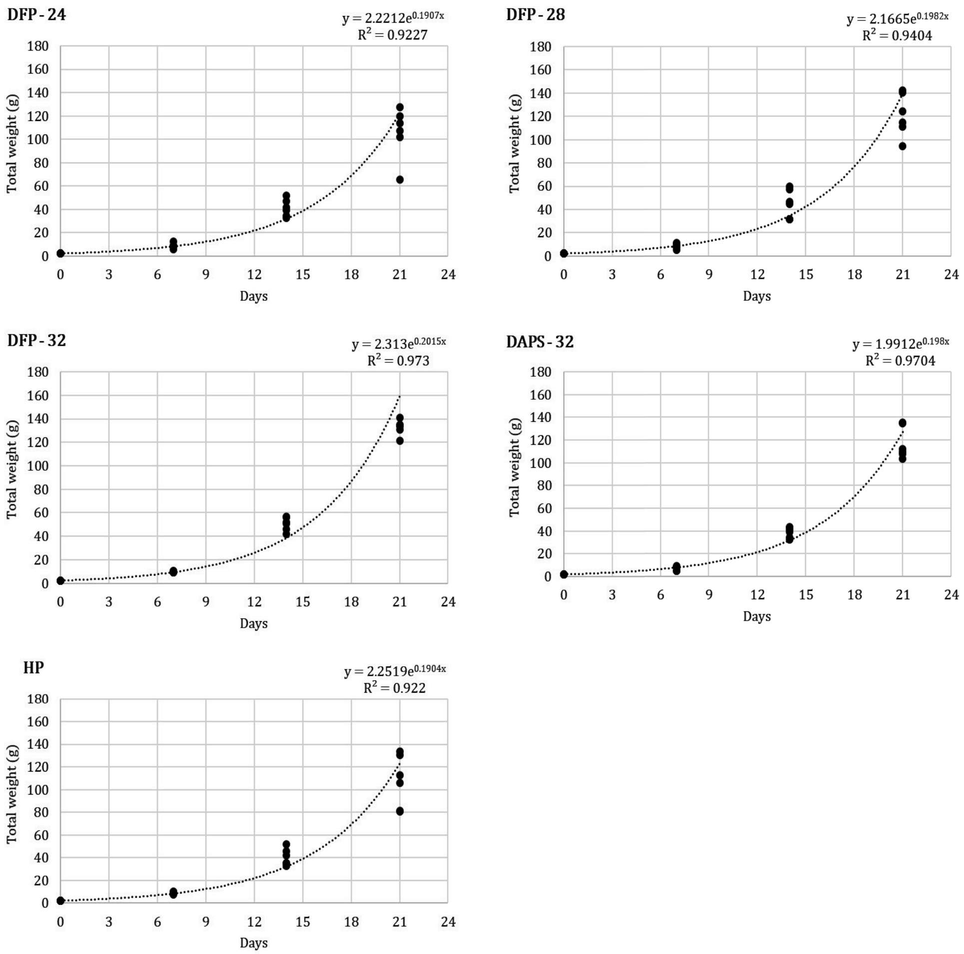

Figure 3.6. Growth curve of lettuce in *Cycle 2* (weekly sampling). DFP: decoupled FLOCponics system. DAPS: decoupled aquaponics system. HP: hydroponics control system.

Table 3.5. Mean ± standard deviation of the growth performance of lettuce during the seedling phase (*Cycle 1*) and final production phase (*Cycle 2*).

Parameter	**DFP - 24**	**DFP - 28**	**DFP - 32**	**DAPS - 32**	**HP**	***p*-value***
Cycle 1 - Seedling production phase						
Leaf height (cm)	9.76 ± 0.46	10.29 ± 0.47	9.80 ± 0.91	10.24 ± 0.75	10.55 ± 0.52	0.190
Root height (cm)	19.14 ± 2.78	20.61 ± 1.58	18.77 ± 1.93	19.58 ± 2.09	20.71 ± 2.18	0.428
Total wet weight (g)	13.50 ± 0.90	13.71 ± 0.42	13.86 ± 0.83	14.05 ± 1.27	14.36 ± 0.98	0.561
Total dry weight (g)	0.79 ± 0.06	0.73 ± 0.07	0.78 ± 0.10	0.83 ± 0.10	0.86 ± 0.06	0.066
Productivity (g m^{-2})	251.22 ± 16.69	254.99 ± 7.83	257.95 ± 15.47	261.41 ± 23.63	267.24 ± 18.15	0.541
Number of leaves	8.98 ± 0.50	9.15 ± 0.41	9.00 ± 0.56	9.00 ± 0.38	8.98 ± 0.60	0.975
Cycle 2 - Final production phase						
Leaf height (cm)	26.20 ± 2.45	27.87 ± 1.73	28.59 ± 0.60	27.89 ± 0.60	27.10 ± 2.51	0.215
Root height (cm)	33.43 ± 5.76	36.46 ± 4.05	34.74 ± 2.71	36.61 ± 2.57	38.29 ± 5.69	0.208
Wet leaf weight (g)	88.45 ± 20.67	101.98 ± 17.53	112.60 ± 4.41	99.21 ± 13.46	90.85 ± 22.06	0.121
Wet root weight (g)	5.23 ± 1.53	5.32 ± 1.14	6.14 ± 1.04	5.53 ± 0.82	5.31 ± 0.91	0.618
Dry leaf weight (g)	1.35 ± 0.17	1.64 ± 0.63	1.48 ± 0.39	1.30 ± 0.26	1.47 ± 0.11	0.542
Productivity (kg m^{-2})	1.68 ± 0.38	1.90 ± 0.33	2.09 ± 0.08	1.85 ± 0.25	1.69 ± 0.41	0.225
Number of leaves	27.94 ± 2.83	29.21 ± 1.78	29.98 ± 1.12	29.07 ± 1.47	29.28 ± 2.15	0.514

* One-way ANOVA at 5% significance level. DFP: decoupled FLOCponics system. DAPS: decoupled aquaponics system. HP: hydroponics control system.

3.4 Discussion

FLOCponics systems were run in a decoupled layout with the aim of enabling proper management of each subsystem; thus, taking advantage of the nutritional benefits of the biofloc-based culture to produce tilapia juveniles and lettuce. The findings of this study suggest that some critical points usually associated with FLOCponics systems were addressed by individualizing the aquaculture and hydroponic subsystems. For instance, the difficulty of maintaining a low concentration of solids in the hydroponics subsystems and, at the same time, providing a sufficient amount of bioflocs in the fish tanks (i.e. higher than 5 mL L^{-1}, Hargreaves 2013), has been reported as an issue of coupled FP systems (Lenz et al. 2018; Kotzen et al. 2019; Pickens et al. 2020; Pinho et al. 2021). Another challenge of coupled FP is regulating the water pH within the appropriate range for fish, bioflocs and plant growth (Lenz et al. 2018; Pinho et al. 2021). The trade-offs related to solids concentration and water pH were tackled in the present study through the use of decoupled FLOCponics (DFP).

The physical-chemical parameters of the water were monitored in each subsystem of all treatments to interpret the production results. Except for the maximum values of settleable solids (volume of bioflocs by Imhoff cone), the other results for water quality in the aquaculture subsystems were within the acceptable range for tilapia (El-Sayed 2006) and also for BFT and RAS microorganisms (Ebeling and Timmons 2012; Emerenciano et al. 2017). The mean results of settleable solids were within the recommended range of 5 to 50 mL L^{-1} for tilapia (Emerenciano et al. 2017; Khanjani and Sharifinia 2020); however, in some measurements the values exceeded 100 mL L^{-1}. No issues regarding a high amount of solids in the aquaculture subsystem have been reported so far in the research on FLOCponics. As already mentioned, low concentrations of bioflocs have usually been indicated as a drawback of coupled FLOCponics systems (Pinho et al. 2021). Hargreaves (2013) stated that the values of settleable solids above the recommended value of 50 mL L^{-1} do not favor fish growth or nutrition, but might result in oxygen depletion and a higher electricity demand in biofloc-based cultures. The high amount of settleable solids in the fish tanks, associated with the unexpected increase in environmental temperature, was probably the main factor that caused the sudden drop in DO and the unviability of the DFP-36 treatment. For the other treatments, the DO values were always higher than 3 mg L^{-1} despite the recorded values of settleable solids. The accumulation of solids was probably a result of the methodology adopted for regulating the C:N ratio, in which the addition of the carbon source was performed periodically based on the amount of N inputted by the feed. Given the need for minimizing the risks associated with high solids concentrations, it is recommended to test the methodology based on the concentration of ammonia in the water to regulate the C:N ratio (Browdy et al. 2012; Pinheiro et al. 2020) or to remove and reuse the solids (Fimbres-Acedo et al. 2020) in FP systems.

The differences found for the mean values of DO, pH, EC and TDS in the aquaculture subsystem were a result of the different input of N and carbon source (molasses) in each treatment. The input of the carbon source seems to be the main factor in these results, since in the DAPS the mean values of pH, EC and TDS were distinct from those recorded in the BFT and DPF-32, even though all of them received the diet with 32% crude protein. For the nitrogenous compounds and orthophosphate results (Figure 3.4), it is hard to conclude whether or how the dietary protein or integration with plant production affected the variation of these nutrients during the experiment. Further studies with a focus on the nutrient flows between the BFT and hydroponic subsystems and the carrying capacity of DFP systems are still required to understand the efficiency of recovering nutrients from the BFT effluents by plants.

In the hydroponic subsystems, except for the pH values in *Cycle 1*, the other parameters of water quality remained within the expected ranges in both cycles. The pH values should remain between 5.5 and 6.5 to enable higher bioavailability of nutrients, whether they come from the aquaculture subsystems or from the extra commercial fertilizer (Tyson et al. 2004). In *Cycle 1*, the pH was above the recommended range in all treatments and the highest values were recorded in the DFP treatments. Phosphoric acid was added in the plant tanks to regulate the pH when it exceeded 6.5. Another factor that interfered with the pH was the buffering in biofloc-based cultures. Despite these issues with pH, they exerted no negative effects on the growth of lettuce seedlings (Table 3.5).

As expected, the results for tilapia growth demonstrated that the well-known benefits of BFT for juvenile nutrition are also found in the DFP systems. Tilapia juveniles fed with 32% CP and grown on both biofloc-based treatments (BFT and DFP-32) grew 22.7% more than those in DAPS also fed with 32% CP. Luo et al. (2014), Long et al. (2015), and Hisano et al. (2019) showed the same tendency of improved zootechnical performance for tilapia grown in BFT compared to RAS, although both were fed with the same amount of CP. They indicated the uptake of the microbial bioflocs as a complementary feed by tilapia as the main reason for these results. Not finding differences in FCR amongst the treatments is somewhat surprising, since better feed conversion is usually related to biofloc-based culture compared to RAS (Azim and Little 2008; Long et al. 2015). Nevertheless, the results of PER (3.83), PPV (55.82%), and CPwg (14.65%) show the highest efficiency in using the dietary protein in the fish produced in the biofloc-based system (mainly DFP-24) compared to DAPS

(32% CP) with 2.93, 40.11% and 13.70%, respectively. These results suggest that even in an integrated system the *in situ* food present in biofloc-based systems is used by tilapia juveniles to complement their dietary protein needs. The similar results for tilapia growth in DAPS and in DFP fed with 8% lower CP (DFP-24) reinforce this statement.

The zootechnical results of tilapia fed with lower CP suggest positive economic and environmental implications of DFP. Since protein is usually the most expensive component in the diets (Jatobá et al. 2014; Hisano et al. 2020), the use of lower CP levels will result in lower feed costs. Furthermore, the reduced need for filters in the DFP system compared to DAPS also indicates that FLOCponics might bring economic advantages for the producers. From an environmental point of view, the dependence on feeds is an aquaculture issue (David et al. 2020). Reducing the dietary CP level may mitigate the negative impact of feed on aquaculture sustainability due to the lower need for protein-rich ingredients and lower concentration of N excreted into the natural environment.

With respect to the question of whether DFP might enable lettuce production in comparable yields to DAPS and traditional hydroponics, this study found no differences amongst the treatments for the growth parameters in the seedling (*Cycle 1*) and final production phase (*Cycle 2*). Interestingly, to achieve these similar yields, less commercial fertilizer was required in the DFP-32 compared to the other treatments. In *Cycle 1*, the volume of fertilizer added to a DFP-32 plant tank was approximately 51.9% and 6.4% lower than in HP and DAPS, respectively. In *Cycle 2,* these differences between DFP-32 to HP and DAPS dropped to 16.3% and 1.3%, respectively. Another important finding was that reducing the amount of N in the fish diet in DFP systems did not affect lettuce growth in either cycle. The higher volume of fertilizer added to the DFP-24 and DFP-28 compared to DPF-32 seems to have compensated for the reduction in the amount of N. Nevertheless, in both treatments, the volumes of fertilizer were lower or similar to those added to the DAPS plant tanks.

The use of conventional dosages of a commercial fertilizer in the hydroponics subsystem could have hindered lettuce growth, as a result of nutrient imbalances in the water. However, the nutritional management employed in this study seemed to have facilitated lettuce production in both cycles and in all treatments. In spite of this, knowing the specific nutrients that need to be supplemented in the hydroponics subsystems, based on the profile of nutrients in the BFT effluents and on the requirements of the crop, might result in less fertilization dependence, and possibly even greater plant production. For the purpose of developing a specific fertilizer scheme for DFP systems, efforts to constantly characterize the profile of macro- and micro-nutrients in BFT water and to adjust the formulations of the fertilizer according to the dynamics of the BFT will be needed.

The curves for lettuce growth presented in Figures 3.5 and 3.6 may be used to predict production in the hydroponic subsystem according to the experimental conditions employed. A notable finding from these curves is the tendency of the seedlings' growth rate in *Cycle 1* to decrease. Possible explanations for this decrease might be that the nutritional management employed in *Cycle 1* was sub-optimal, leading to growth limitations. Optimal nutritional management could have been adopted. For instance, EC of 1.7 $mS.cm^{-1}$, as in *Cycle* 2, instead of 1.2 $mS.cm^{-1}$ and a higher frequency of water flow between fish and plant tanks could have led to exponential growth. Regardless of this growth behavior, the seedlings weighed approximately 14 g at the end of *Cycle 1*, while the seedlings of the same age (21 d.a.s) purchased from a commercial hydroponic producer for *Cycle 2* weighed only 2 g. This difference between the final weight obtained in *Cycle 1* in all treatments compared to that achieved by the commercial producer indicates that the management used in this study was more suitable for seedling production than that commonly used in local farms. It should be noted that the present study was carried out under passively controlled climatic conditions, and the results of lettuce growth are directly related to the environmental conditions shown in Figure 3.3.

The findings of this study may be useful for producers who already apply the concepts of BFT and seek to increase the sustainable character of their farms. Transforming a BFT farm into a decoupled FLOCponics farm may result in an increased variety of marketable products and a reduction of the overall cost per kg of food produced. Moreover, the integration of BFT and hydroponic production in a decoupled layout seems to allow the reuse of nutrients from the feed and the reduction of the amount of dietary protein, thus minimizing the environmental impacts of aquaculture production. Certainly, several research questions have yet to be answered in order to develop and consolidate decoupled FLOCponics systems and also to make it an option for those who produce in hydroponics or conventional aquaponics systems. Some examples for further investigation have been mentioned throughout this paper, such as: (i) in-depth understanding of the nutrient flows and the carrying capacity of each subsystem; (ii) development of a specific fertilizer to be applied in the hydroponics subsystem taking into account the nutrients available in the BFT effluent; and (iii) alternative solutions for minimizing the accumulation of solids in the aquaculture subsystem, whether by regulating the C:N ratio or by collecting the solids and reusing it for another purpose. The reuse of the solids through a mineralization process seems to be a promising option given the substantial amount of nutrients in the BFT solids (Blanchard et al. 2020; Fimbres-Acedo et al. 2020). Positive impacts of using the effluent of a RAS-sludge mineralization as extra fertilizer for plant production in decoupled aquaponics has been reported (Goddek et al. 2016b), and this might also be the case for mineralized bioflocs. In addition, economic and sustainability assessments which consider the local climate, market, and target species must be performed to measure the final applicability of the proposed system.

3.5 Conclusions

The results obtained in this study indicate that decoupled FLOCponics (DFP) is a promising technology to produce lettuce and tilapia juveniles using less CP in the fish diets when compared to traditional decoupled aquaponics. Tilapia cultured in DFP and fed with 24 and 28% CP grew similarly to those in DAPS fed with 32% CP diet, suggesting that even after the integration with hydroponics the consumption of microbial bioflocs led to an 8% reduction in the amount of CP. The non-interference of the integration with plant production on the nutritional benefit of BFT was also corroborated by the results of fish growth in DFP-32 and BFT fed with 32% CP diet. Both grew similarly and above the other treatments. Additionally, plant growth results showed no differences in both production cycles (seedling and final growth) among all treatments. Less volume of commercial fertilizer was required in DFP-32, followed by DFP-28, DFP-24, DAPS, and HP.

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Chapter 4

Towards improved resource use efficiency in biofloc-based fish culture

This chapter is based on:

Pinho SM, Lima JP, Portella MC, Keesman KJ. Towards improved resource use efficiency in biofloc-based fish culture. (Submitted)

Abstract

FLOCponics is an integrated agri-aquaculture system, in which water and nutrients from a biofloc-based fish culture are reused to fertilize soilless plants. In this paper, we conducted the first modelling study focused on FLOCponics to investigate and discuss whether the integration of biofloc-based culture with soilless plant production increases the efficiency of food production in terms of resource use and by how much. For this purpose, we modelled and compared the water, nitrogen and solid balances in a biofloc-based monoculture and FLOCponics system, using experimental data for calibration, for a simulation period of five years. Moreover, changes in the planting area of the FLOCponics system were simulated until the most suitable size was found. The results indicate that FLOCponics is 10% and 27% more efficient in using water and nitrogen, respectively, than stand-alone biofloc system. Also, the integrated system results in a reduction of 10% in the amount of solids discharged. Optimization of the planting area with respect to key model outputs led to an improved FLOCponics system, where the hydroponics size is expanded by a factor of 3.2. The findings presented in this study support the hypothesis that integrating a biofloc system with hydroponics makes biofloc-based fish culture more efficient in terms of resource use and wastes avoidance.

4.1 Introduction

Given the growing pressure to achieve sustainable aquaculture, production systems have been developed to improve resource use efficiency and minimize waste discharge (Ahmed and Thompson 2019; Naylor et al. 2021). While still most aquaculture farmers use monoculture production systems that highly depend on non-renewable resources, the modern trend of aquaculture research focuses on boosting systems that reuse the inputted water and nutrients to grow multiple organisms (Kerrigan and Suckling 2018; Boyd et al. 2020; David et al. 2021). Integrated agri-aquaculture and biofloc-based culture are examples of such production systems (Zajdband 2011; Browdy et al. 2012; Betanzo-Torres et al. 2021).

Biofloc technology (BFT) has been used in intensive fish and shrimp production, enabling high animal yields on small land areas and minimal water discharge (Emerenciano et al. 2013; Khanjani and Sharifinia 2020). Biofloc-based culture is characterized by the growth of specific microorganisms, usually *in situ* in the fish tank, for improving water quality, disease prevention, and waste treatment (Crab et al. 2012; Mugwanya et al. 2021). The microorganisms, especially heterotrophic and nitrifying bacteria, play an important role in the organic matter degradation and nitrogen cycle (Emerenciano et al. 2017). Moreover, these microorganisms are a nutrient-rich supplementary source of food for the cultured fish (Martínez-Córdova et al. 2017; Sousa et al. 2019; Sgnaulin et al. 2021).

By demanding reduced quantities of feed, land, and water, biofloc-based culture has been labelled as a sustainable aquaculture approach (Bossier and Ekasari 2017). Nevertheless, the accumulation of solids and nutrients in the rearing tanks, potentially causing negative impacts if discharged into the environment, is frequently reported (El-Sayed 2021; Mugwanya et al. 2021). Such accumulation occurs, because BFT is usually applied in closed system setups with high fish density and also because BFT demands the input of extra nutrients through a carbohydrate source to regulate the C:N ratio in the water to support the growth of microorganisms (Hargreaves 2013; Walker et al. 2020). Another point of concern is that most biofloc-based farms produce a single marketable species, commonly tilapia (*Oreochromis spp.*) or marine shrimp (*Litopenaeus vannamei*) (Samocha 2019; Walker et al. 2020; Dauda 2020). Restricting the use of BFT to monocultures is an issue, as the use of resources per kg of food produced seems to be sub-optimal. As a solution to this, the excess of nutrients in the BFT water could be reused as fertilizer to nourish other crops (Pinho et al. 2017; Pinheiro et al. 2020).

In the case of integrated agri-aquaculture systems, water and nutrients wasted from aquaculture are reused to produce vegetables with marketable value (Zajdband 2011; Nederlof et al. 2019). FLOCponics is an example of an integrated agri-aquaculture system that combines biofloc-based production with hydroponics, a soilless plant production method (Kotzen et al. 2019; Pinho et al. 2021a). Recent studies have reported higher or similar animal growth in FLOCponics than in biofloc-based culture without integration (Pinheiro et al. 2017; Pinho et al. 2021c) or conventional aquaponics (Rocha et al. 2017; Fimbres-Acedo et al. 2020a; Martinez-Cordova 2020; Saseendran et al. 2021). In conventional aquaponics, the aquaculture subsystem is operated as a recirculating clear-water system, instead of using BFT. With respect to plant growth, in FLOCponics promising results have also been achieved compared to hydroponics (Rocha et al. 2017; Pinho et al. 2021c) or conventional aquaponics (Pinho et al. 2017; Rocha et al. 2017; Martinez-Cordova et al. 2020; Saseendran et al. 2021), mainly for lettuce production. Positive results of plant growth were especially found when the FLOCponics system was operated in a decoupled layout (Pinho et al. 2021c), recently renamed as on-demand coupled layout (Baganz et al. 2021). In this layout, the aquaculture and hydroponics subsystems are run partially independent from each other, where water and nutrients flow from the BFT tank to mechanical filters and end up in the hydroponics subsystem (Fimbres-Acedo et al. 2020b; Pinho et al. 2021c).

Since FLOCponics shares the principles of integrated agri-aquaculture systems, it is expected that FLOCponics will be more sustainable and efficient than biofloc-based monoculture. However, it is still unknown what the appropriate water and nutrients use in FLOCponics is and whether the outputs from FLOCponics are sufficient, given the demanded resource inputs to integrate BFT and hydroponics successfully. Most studies on FLOCponics carried out so far focused primarily on the productive performance of the system and a comprehensive evaluation of the systems' efficiency in terms of resource use has not yet been reported (Pinho et al. 2021b). To fill this gap, the use of mathematical models, based on balances described by ordinary differential equations, is very suitable to understand the dynamic behaviour of a FLOCponics system and its efficiencies. Model-based studies have been widely applied for this purpose in conventional decoupled aquaponics systems (Kloas et al. 2015; Goddek et al. 2016; Karimanzira et al. 2016; Yogev et al. 2016; Estrada-Perez et al. 2018; Dijkgraaf et al. 2019; Keesman et al. 2019; Körner et al. 2021). Furthermore, modelling is a valuable tool to support the development of management practices to optimize resource utilization in modern agri-aquaculture systems (Karimanzira et al. 2016; Lastiri et al. 2016, 2018; Goddek and Keesman 2020). In addition to evaluating existing systems, modelling studies have also been useful to represent and simulate a system that does not commercially exist yet or when it would be too costly and take too long to run experiments needed to fully evaluate and understand a specific system (Goddek and Keesman 2018; Keesman et al. 2019). The current status of FLOCponics systems includes both cases, supporting the use of dynamic modelling to evaluate such systems.

Given these considerations, the objective of this study was to investigate and discuss whether the integration of BFT with hydroponics production increases the efficiency of biofloc-based fish production and by how much. For this purpose, we built a model based on mass balances and consecutive laws and compared the water and nitrogen use per kg of food produced in a biofloc-based monoculture (without plant production) and in FLOCponics system.

4.2 Method

We combined empirical data from conducted experiments with mass balances of biofloc and FLOCponics systems. The system designs are based on an experimental set-up we operated at the Aquaculture Center of Unesp (Caunesp), Jaboticabal, SP, Brazil, described in detail by Pinho et al. (2021c). In the experiments, we compared the production of tilapia juveniles (*Oreochromis niloticus*, 1 to 30 g) and lettuce (*Lactuca sativa*) in biofloc and FLOCponics systems in small-experiment systems setups. To make the model output more comprehensive, in the present model-based study, we considered a ten times larger systems setup than the one described in Pinho et al. (2021c), but using the same ratio between the volume of each compartment and the initial conditions (e.g., fish and plant densities, initial live material weights, production cycle periods, nutrient concentration in the water, feed composition, etc.). The compartment sizes used in our models are presented in Table 4.1. The initial conditions and other parameters are presented in the Supplementary Materials.

Table 4.1. Compartment sizes of the biofloc and FLOCponics systems.

Compartment	Size	Comments
Fish tank (FT)	4 x 3.80 m^3	With 300 fish m^{-3}
Radial flow settler (RFS)	4 x 1 m^3	Operated under demand
Bag filter	4 x 0.05 m^3 , 68 μm	Volume and nutrient retention neglected, not modelled
Mixing tank (MT)	Non-fixed volume	MT receives the nutrient water from all FTs and is where the plant fertilizer is added.
Deep water culture (DWC)	33.6 m^2 - 8 m^3	With 19 plants m^{-2}

4.2.1 *Description of the systems and processes*

In the biofloc system (Figure 4.1), four fish tanks each coupled with a radial flow settler (RFS) were considered. Water remains in the fish tank most of the time, and it is only directed to the RFS to reduce the solids concentration when the total suspended solids (TSS) exceed a level of 500 mg L^{-1}, as recommended for biofloc-based fish culture (Hargreaves 2013; Emerenciano et al. 2017). When the RSF is used, recirculating water enters and leaves the RFS at a specific rate of 0.792 m^3 h^{-1}. Each RFS is operated until the TSS reaches 100 mg L^{-1} in a variable time interval. In this study a TSS settling efficiency of 70% was assumed. In both biofloc and FLOCponics systems, the TSS concentration was assumed to be equal to the bioflocs biomass concentration (Ekasari et al. 2014).

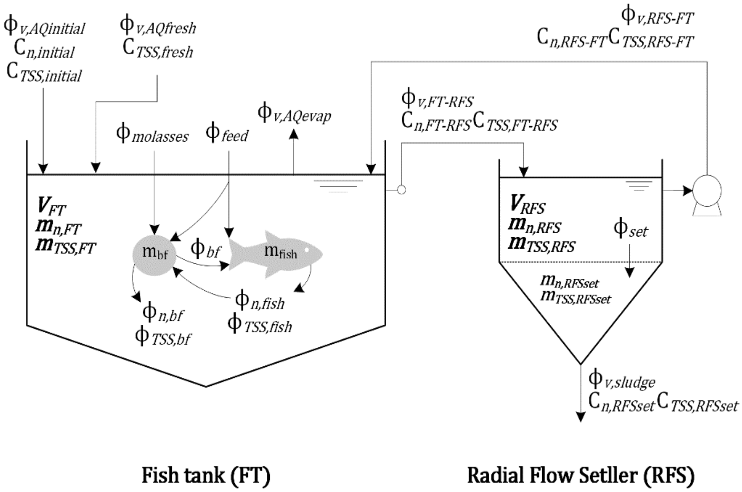

Figure 4.1. Model of biofloc system.

In the FLOCponics system (Figure 4.2), the aquaculture and hydroponics subsystems are operated as separated loops in a decoupled (on-demand) system layout. The aquaculture subsystem comprises four fish tanks, each one with an RFS and a bag filter. The hydroponics subsystem consists of a mixing tank linked to the hydroponic deep-water culture bed.

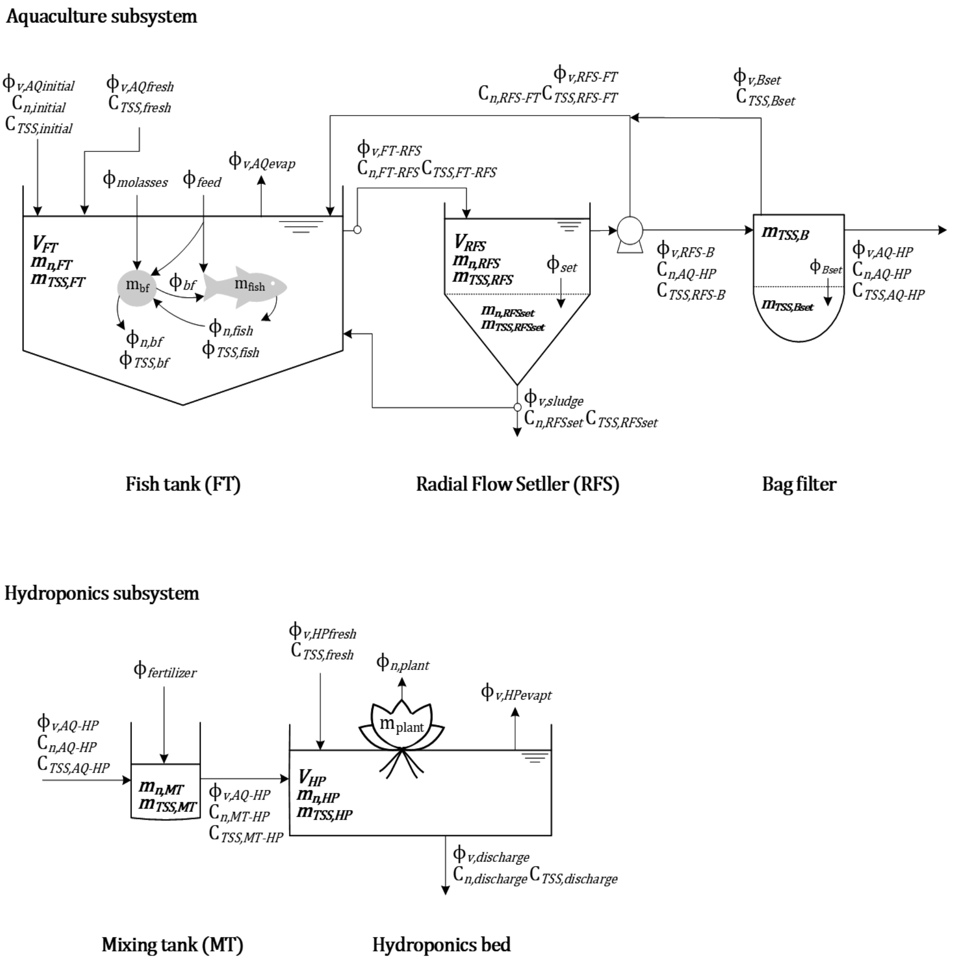

Figure 4.2. Model of FLOCponics system.

Contrary to the biofloc system, in FLOCponics, RFS and bag filter are used every time the water from the aquaculture subsystem is directed to the hydroponics subsystem, aiming to reduce the TSS concentration in the effluent of the aquaculture subsystem. The water from the fish tank is directed to the RFS until filling it, remaining there for 15 min to settle the TSS, considering a settling efficiency of 90% (called FLOCponics RFS procedure). Then, the supernatant nutrient water is pumped through the bag filter, with a solid retention efficiency of 60%, and subsequently to the hydroponics subsystem at a variable rate. When the TSS concentration in the fish tank is lower than the maximum recommended, the solids settled in the RFS return to the fish tank. On the other hand, when the TSS concentration in the fish tanks in the FLOCponics system surpasses 500 mg L^{-1}, the TSS is firstly removed following the FLOCponics RFS procedure and then, if needed, the RFS is operated as described for the biofloc system until TSS concentration reaches 100 mg L^{-1}. The solids retained in the bag filter always return to the fish tank. After the start of the plant production, the RFS and bag filter are operated only once a day. Before the plant production starts, the RFS works as described for the biofloc system.

The water flow from aquaculture subsystem to hydroponics subsystem depends on the water and nutrient uptakes by plants, which are highly dependent on the local and plant species-specific daily evapotranspiration rate. The reference evapotranspiration rate was calculated applying the FAO Penman-Monteith Equation (Allen et al. 1998), for lettuce production in Jaboticabal, SP, Brazil (Figure 4.3, for details, see Supplementary Materials Tables S4.1 and S4.2). The nutrient uptake by lettuce for growth was assumed to be proportional to the evapotranspiration (Dijkgraaf et al. 2019). Even though the FAO Penman-Monteith equation gives a rough estimate of the evapotranspiration, it has been used in aquaponics modelling studies as a simplified and useful equation (Goddek and Keesman 2018; Dijkgraaf et al. 2019).

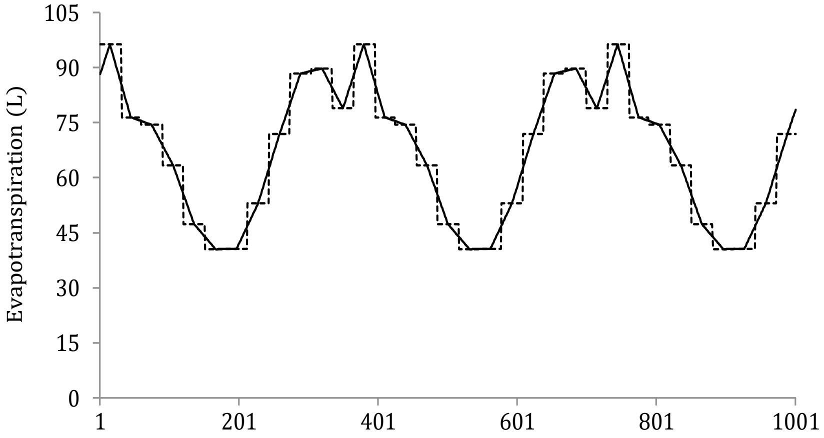

Figure 4.3. Evapotranspiration in the described situation for Jaboticabal, SP, in which day 0 is January 1st, 2019. Evapotranspiration is calculated monthly (dashed line) and smoothed (solid line) for daily interpolation.

In both systems, fish production starts at day 0. Fish is stocked in the four tanks in a staggered sequenced space by approximately 27 days. Each tank is harvested when the tilapia juveniles reach 30 g, i.e., once every 56 days. The fish tanks are filled at a rate of 3.8 m^3 day^{-1} with inoculum from a mature biofloc before starting the production, in which the microbial community was already established. The use of inoculum allows a maintenance management strategy where the external carbon source (molasses) is added only if the concentration of total ammonia nitrogen (TAN) in the fish tank reaches critical values (Avnimelech 2015; Emerenciano et al. 2017). Optimal management and water quality conditions were assumed for fish production.

Lettuce production starts when the fourth fish tank is firstly stocked, on day 82. The length of each complete lettuce growth cycle was set at 35 days. Also, for plant production optimal management was assumed with nutrient concentrations and environmental variables within optimal conditions for lettuce production, resulting in ideal and constant lettuce growth.

4.2.2 Description of the mathematical model

The mathematical model was described in terms of a set of ordinary differential equations. The models were solved numerically in Microsoft Excel™ with a step size Δt of 1 day. Mass balances were set up for water volume (V), nutrients (m_n) and total suspended solids (m_{TSS}) in both systems. The mass balances were expressed in terms of inflows and outflows, and V, m_n and m_{TSS} are the state variables and model outputs (Table 4.2). Figures 4.1 and 4.2 illustrate the functionality of each system in terms of flows. Nitrogen (N) was the nutrient focused on in this study. However, carbon balances were also set-up as a sub-model to calculate bioflocs biomass growth. The auxiliary equations and the values of the parameters used in the models are detailed as supplementary materials (Tables S4.3 and S4.4). The biofloc and FLOCponics systems were simulated for a period of five years (1825 days).

Table 4.2. Mass balances for the models of the biofloc and FLOCponics systems (see also Figures 4.1 and 4.2).

System	**d/dt**	$\boldsymbol{\phi_{in}}$	$\boldsymbol{\phi_{out}}$
Biofloc	V	$\phi_{v,AQinitial} + \phi_{v,AQfresh}$	$\phi_{v,sludge} + \phi_{v,AQevap}$
	m_n	$C_{n,AQinitial}\phi_{v,AQinitial} + \phi_{n,feed} + \phi_{n,molasses} + \phi_{n,bf\_prod}$	$C_{n,RFSset}\phi_{v,sludge} + \phi_{n,bf\_cons}$
	m_{TSS}	$C_{TSS,AQinitial}\phi_{v,AQinitial} + C_{TSS,fresh}\phi_{v,AQfresh} + \phi_{TSS,MicrobProd}$	$C_{TSS,RFSset}\phi_{v,sludge}$
FLOCponics	V	$\phi_{v,AQinitial} + \phi_{v,AQfresh} + \phi_{v,HPinitial} + \phi_{v,HPfresh}$	$\phi_{v,sludge} + \phi_{v,AQevap} + \phi_{v,HPevapt} + \phi_{v,discharge}$
	m_n	$C_{n,AQinitial}\phi_{v,AQinitial} + \phi_{n,feed} + \phi_{n,molasses} + \phi_{n,bf\_prod} + \phi_{fertilizer}$	$C_{n,RFSset}\phi_{v,sludge} + \phi_{n,bf\_cons} + C_{n,HPdischarge}\phi_{v,discharge} + \phi_{n,plant}$
	m_{TSS}	$C_{TSS,AQinitial}\phi_{v,AQinitial} + C_{TSS,fresh}\phi_{v,AQfresh} + \phi_{TSS,bf} + C_{TSS,fresh}\phi_{v,HPfresh}$	$C_{TSS,RFSset}\phi_{v,sludge} + C_{TSS,HPdischarge}\phi_{v,discharge}$

Determining fish growth is necessary to quantify the resources going into the systems. The average values found in the experiment for individual fish growth, survival, feed input, and uptake were used to model fish growth and nutrient release. The fish growth model is shown in the Supplementary Material. The specific parameters related to fish feed and growth can be found in Table S4.5.

Regarding the water balances, in both systems, the volume was considered constant over time, thus $dV/dt = 0$. Consequently, freshwater inflow was needed to compensate for the outflows. The evaporation in the biofloc system or aquaculture subsystem of the FLOCponics system was calculated by Penman's equation, using the same weather variables for the calculation of the evapotranspiration in the hydroponics subsystem, as described in Supplementary Materials Tables S4.1 and S4.2. As minimal water exchange is expected in the biofloc-based culture, no water is wasted after harvesting. The water is discharged through solids management (sludge removal), or, in the case of the FLOCponics system, also when the concentration of TSS in the hydroponics subsystem was higher than the maximum TSS level of 50 mg L^{-1}. It was assumed that all water volume of the hydroponics subsystem is discharged if the solid concentration trespasses a predefined threshold. At the beginning of the plant production or each time that the water from the hydroponics subsystem is discharged, the hydroponics subsystem is filled half with water from the aquaculture subsystem and half with freshwater (same procedure as in the experiment).

For nitrogen, besides the general balances described in Table 4.2, sub-models for TAN, un-ionized ammonia (NH_3) and nitrate (NO_3) were also implemented (presented in auxiliary equations, Table S4.3). The biofloc microbial community is directly involved in the assimilation, ammonification, and nitrification of the nitrogen that enters the systems, affecting the amount of N from the aquaculture subsystem to the hydroponics subsystem. It was assumed that light incidence in the fish tanks is limited. Thus, the effect of algae in the N pathway was neglected. N-fertiliser is added in the mixing tank when the amount of N from the aquaculture subsystem is not enough to reach the minimum N concentration required for lettuce. In general, the ideal nutrient concentration varies a lot in hydroponics culture. We considered as ideal a range of N concentration in the hydroponics subsystem from 100 to 200 mg L^{-1} (Jones 2005).

The TSS balances were mostly based on the bioflocs biomass growth in the fish tanks. Monod equation was used to model the bioflocs growth as a function of substrates consumption (equations and parameters are detailed in Tables S4.3 and S4.4). We included as limiting substrates in the bioflocs growth equation the organic C (following the same mass balances as for total N) and inorganic N, since all biofloc-based studies present the bioflocs microbial communities as a function of the C:N ratio of the water (Browdy et al. 2012; Avnimelech 2015; Emerenciano et al. 2021). The discharges of sludge and hydroponics subsystem water were the main outflows of TSS, both procedures described in the previous paragraphs.

Some assumptions were set to make the models feasible, based on literature information and our experience in running biofloc-based systems and modelling studies. The main assumptions are:

a. Density of water is assumed to be constant ($\rho_{water} = 1.00$ kg L^{-1}).
b. RFS is modelled to settle TSS at a rate linearly related to its concentration, while all the other tanks are assumed to be well mixed.
c. Water parameters are within optimal values for tilapia juveniles and lettuce growth. All water parameters are constant, except for TSS and N concentrations that dynamically change over time.
d. Water retention by fish is neglected.
e. Reactions only take place in the fish tank as a result of the bioflocs microorganisms' growth and their role in the N pathway.
f. Negligible volume of pipes and transportation times between the compartments.
g. The fish tanks are sufficiently aerated thus oxygen does not become a limiting factor.

h. Freshwater, feed and molasses compositions are constant. N concentration in the freshwater is zero, based on measurements during the experiment.
i. Water entering the systems through feed, molasses and fertilizer is neglected, since their dry matter contents are extremely high.

4.2.3 Key performance indicators

Key performance indicators (KPI) were chosen to evaluate relevant model outputs. The resource use efficiencies were calculated following the equation described by Dijkgraaf et al. (2019), given the input streams and waste streams. The inputs considered for Water Use Efficiency (WUE) are the initial water volume and freshwater that enter each system and the wastes are the sum of disposed water during discharge and water present in the waste sludge. The inputs considered in the Nitrogen Use Efficiency (NUE) are N that enters through the initial water (inoculum), feed, molasses and fertilizer. The N waste also includes the discharge of sludge and the sum of disposed N during discharge of the hydroponics subsystem water. The main output of the TSS balances is the total solids discharged. Other important indicators considered as KPI are the ratio of water or nitrogen inputs per kg of food produced, and the discharge of water, nitrogen or solids (TSS) per kg of food produced. The relation between the N that entered the hydroponics subsystem via the aquaculture subsystem or fertilizer was also chosen as a KPI.

4.2.4 Scenario simulations and sensitivity analysis

Changes in the planting area of the FLOCponics system was simulated until the most suitable size was found. The hydroponics subsystem size was considered suitable when it results in the highest WUE and NUE values without compromising the water quality parameters. The critical constraint set for the hydroponics subsystem area was that the concentration of un-ionized ammonia in the fish tank should not be higher than 1 mg L^{-1}. From doing this scenario simulation, we expect to propose a system design that reuses most of the resources from the aquaculture subsystem and thus an improved FLOCponics system.

The normalized sensitivities were also computed to measure the effect of changing specific model inputs/parameters on the key model outputs (KPI) (Tomovic 1963). The sensitivity analysis was performed for the following parameters: bacteria growth yield, coefficient of organic nitrogen degradation, coefficient of solids settling in the RFS_{FP} and RFS_{BFT}, the maximum and minimum concentrations of TSS in the fish tank, coefficient of N excreted by fish, and the minimum concentration of N required by plant.

4.3 Results

The production of tilapia juveniles with a final weight of 30 g was simulated for a five-year period. The total fish biomass that is harvest during this period is equal to 4253 and 4046 kg in the biofloc and FLOCponics systems, respectively. Figure 4.4 presents the fish growth simulated in both systems for the first three years. After the first three years, most of the simulated variables reach a steady oscillation. Thus, all graphics are plotted for three years, while the total production, resource demands and waste discharge, and the KPI values are given for the total simulation period of five years. The different frequencies of fish biomass in the biofloc and FLOCponics system in Figure 4.4 are a result of the lowest average survival value in the FLOCponics system observed during the experiment. For lettuce production, from day 82 to 1825, 3751 kg of lettuce is produced in the FLOCponics systems. These values are all in line with what we expected on the basis of data from the experiment.

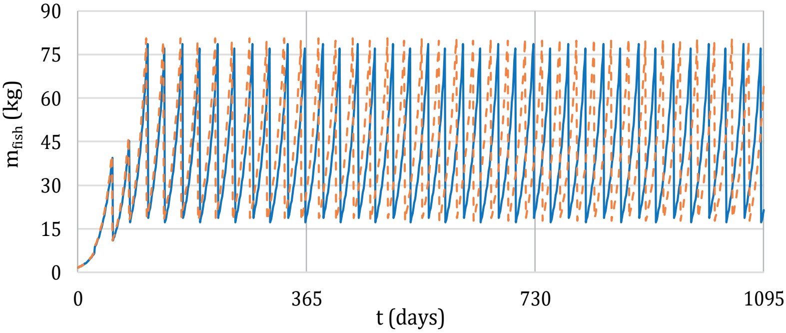

Figure 4.4. Fish biomass growth in the biofloc (dashed orange line) and FLOCponics (solid blue line) systems, for a simulation period of 3 years.

The water volume required to produce the nominal fish biomass in the biofloc system is 135.5 m^3, of which 52% is used to replace the losses for evaporation. While the fish and plant biomass production in the integrated system demands 281.3 m^3 of water, 28% and 42% of this volume are due to evaporation and evapotranspiration, respectively. On the other hand, 41.7 and 61.5 m^3 of water is discharged, respectively, in the biofloc and FLOCponics system. In the biofloc system, 186 kg of nitrogen is inputted, and 110 kg is wasted. Higher amounts of N enter the FLOCponics system (207 kg) than in the biofloc system. However, the calculated N waste is 94 kg. In both systems and under nominal conditions, the concentration of NH_3 in the fish tanks does not reach 0.5 mg L^{-1}. The dynamics of TAN and nitrate in the fish tanks are shown in Figure 4.5. The mass of solids discharged is higher in the stand-alone system, 421 kg compared to 380 kg in the FLOCponics system. Figure 4.6 shows the variation in the amount of solids discharge for both systems in the first three years of production.

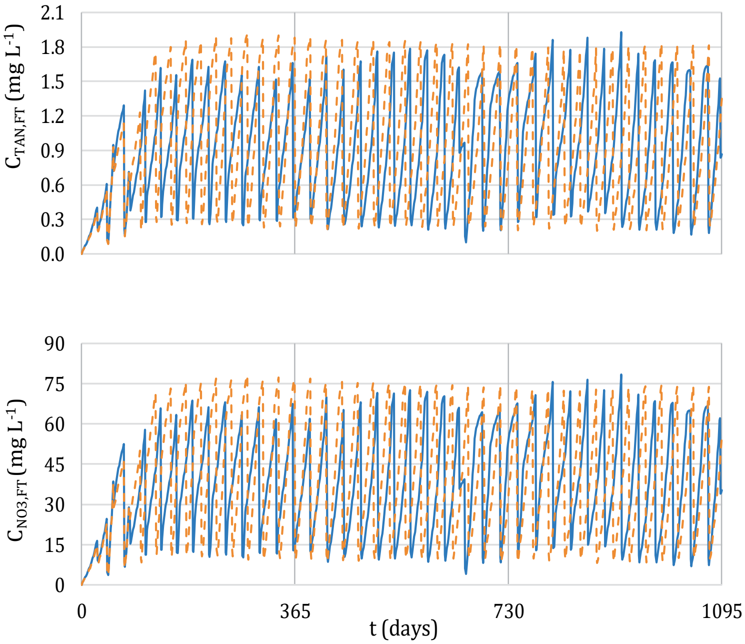

Figure 4.5. Concentration of TAN and NO_3 in the fish tanks of the biofloc (dashed orange line) and FLOCponics (solid blue line) systems, for a simulation period of 3 years.

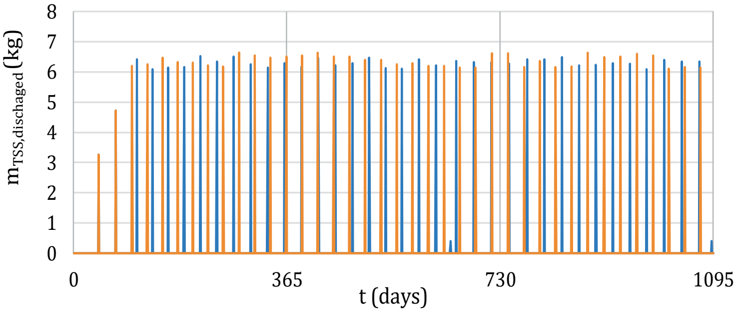

Figure 4.6. Solids discharged (kg) in the biofloc (solid orange line) and FLOCponics (solid blue line) systems, for a simulation period of 3 years.

In terms of resource use efficiency, the WUE and NUE over three years are shown in Figures 4.7 and 4.8. The jumps in Figure 4.7 around day 665 in the FLOCponics system result from the need to discharge all water volume of the hydroponics subsystem as the TSS concentration trespassed the predefined threshold. The KPI results are presented in Table 4.3. FLOCponics outperformed the biofloc-based fish monoculture for most of the KPI evaluated. In addition to the relevant model outcomes for the nominal biofloc and FLOCponics systems, Table 4.3 also presents the KPI found for the improved FLOCponics system where the planting area was increased. Simulations for different planting areas reveal that it can be expanded up to 3.2 times compared to the nominal FLOCponics system (from 34 to 109 m^2) without compromising the water quality parameters for fish and plant growth.

The results of the sensitivity analysis are presented in Supplementary Materials, in Table S4.6 to S4.13. In general, the normalized sensitivity values indicate that variations in the chosen parameters do not impact the different model outputs too much, except for k_{RFSset_FP}, TSS_{max}, $k_{N,fishexcret}$ and $C_{N,required}$ with some of the absolute normalized sensitivities larger than 0.5 indicated in bold in Tables S4.6-S4.13.

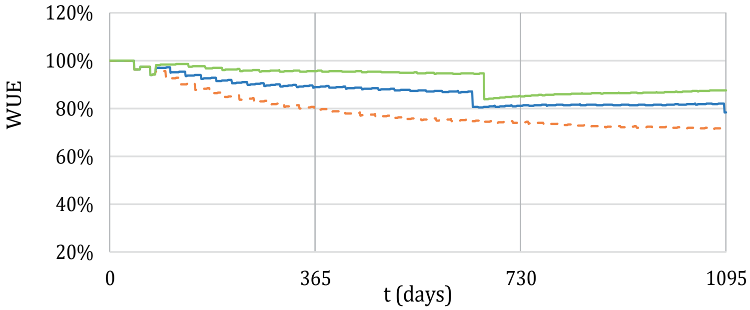

Figure 4.7. Water use efficiency (WUE) of the biofloc (dashed orange line) and FLOCponics systems in the nominal situation (solid blue line) and the improved FLOCponics system (solid green line).

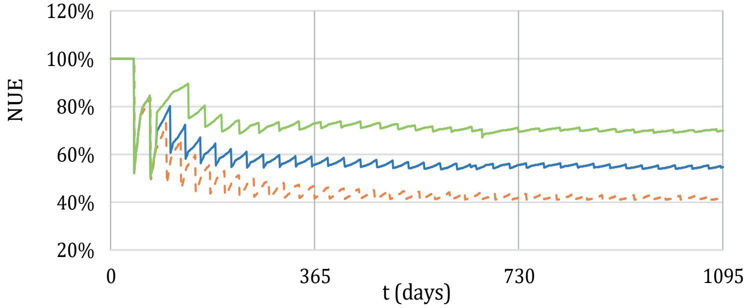

Figure 4.8. Nitrogen use efficiency (NUE) of the biofloc (dashed orange line) and FLOCponics systems in the nominal situation (solid blue line) and the improved FLOCponics system (solid green line).

Table 4.3. Key performance indicators of the biofloc and FLOCponics systems in the nominal situation and for the improved FLOCponics system.

Performance indicator	**Biofloc**	**FLOCponics**	**Improved FLOCponics***	**Improved / nominal**
Total solids discharged (kg)	421.9	386.5	300.9	78%
Average WUE	76%	84%	89%	107%
Average NUE	45%	57%	71%	125%
L water input per kg food produced	32.67	36.15	37.60	104%
L water wasted per kg food produced	9.82	7.97	6.61	83%
kg N input per kg food produced	0.04	0.03	0.01	53%
kg N wasted per kg food produced	0.03	0.01	0.004	36%
% N-fertilizer		58%	60%	104%

* Planting area of the nominal FLOCponics system expanded 3.2 times (from 34 to 109 m^2). WUE: Water use efficiency. NUE: Nitrogen use efficiency.

4.4 Discussion

In this study, we presented the first mathematical model of a FLOCponics system to investigate and describe the resource uses and waste discharge in this system, propose an improved system design, and discuss its efficiency compared to a stand-alone biofloc system. The results presented in this model-based study support the hypothesis that integrating soilless plant production with a biofloc-based fish culture will improve the efficiency of water and nitrogen uses and lower the discharge of solids compared to a stand-alone biofloc system, and also by how much. Moreover, for a simulation period of five years, the amount of food produced in the nominal FLOCponics system was 83% higher than in the biofloc system.

Both biofloc and FLOCponics systems were modelled to simulate the nursery phase of tilapia production, focusing on rearing tilapia juveniles suitable for the grow-out phase. The implementation of nurseries presents many advantages compared to direct stock (1 g juveniles) and provides an efficient segmentation of tilapia farming, i.e., maximizes the rotation of farm facilities and allows better feeding management and disease control (Durigon et al. 2019; Sgnaulin et al. 2020). It is important to highlight that the fish growth model used in this study was calibrated using experimental data and is specific for the phase simulated. Also, we assumed optimal conditions for fish and plant growth. Thus, the effects of variations in, for example, water temperature, pH, alkalinity or the feed composition, were not considered. Nevertheless, all results in the previous figures are in line with what we expected from the experiments. The phase specificity of the fish growth model and the assumption of optimal conditions limit the flexibility of the models to be replicated in situations that present different conditions. Even so, the models we applied serve to address the primary purposes of this study, to describe and compare the efficiency of FLOCponics and biofloc systems.

In terms of water use, the higher volume of water demanded in the FLOCponics was mostly caused by the need to refill the aquaculture subsystem with fresh water to replace the 160 m^3 lost by evapotranspiration in the hydroponics subsystem. Prior studies have noted the determinative effect of the evapotranspiration rate on the water use and design of on-demand aquaponics systems (Goddek et al. 2016; Dijkgraaf et al. 2019), which was also the case for our FLOCponics system. Although still high volumes are required in the FLOCponics system, the water waste is lower than in the biofloc system. As a result, WUE is higher in the integrated system and even better in the improved FLOCponics system.

Compared to other aquaculture production systems, the water uses per kg of food produced in FLOCponics and biofloc system are remarkable. While in the biofloc-based systems the volume of water required per kg of food produced range from 32 to 36 L kg^{-1}, in intensive recirculating aquaculture system the values are around 500 L kg^{-1} (Verdegem et al. 2006). The average WUE values found in our study for both systems also support the premise that biofloc-based systems efficiently use water (Jatobá et al. 2019; Cao et al. 2020). Dijkgraaf et al. (2019) modelled a RAS-based on-demand aquaponics system with an additional loop to mineralize the fish sludge and reuse it as fertilizer for plants. The volume of solids discharged and water losses are expected to decrease when reusing the solids within the system. Even so, these authors reported WUE values around 65-77% in the three-loop RAS-based aquaponics, while in our study the values range from 76% in the biofloc system to 89% in the improved FLOCponics system. We did not include the third loop in the FLOCponics system due to the lack of data to support such models. However, reusing the solids waste in FLOCponics systems is an important topic that should be explored in further research.

4

For nitrogen, FLOCponics also stand as more efficient than the biofloc system, even though an additional N-fertilizer was needed to meet the lettuce needs. The NUE of FLOCponics could be even higher. In the same study described above, Dijkgraaf et al. (2019) reported NUE values of 99% in the simulated three-loop aquaponics system. Our best result for NUE was 71% in the improved FLOCponics system, showing that there is still room to improve FLOCponics design and also emphasizing the need to investigate the mineralization of solids from the biofloc-based systems. With respect to the contribution of the aquaculture effluent on lettuce N-nutrition, Lastiri et al. (2018, 2016) modelled RAS-based on-demand coupled aquaponics systems for tilapia-tomato production and found that 25% of N in the hydroponics subsystem would come from the aquaculture subsystem. Our findings indicate that the biofloc-based aquaculture subsystem can provide approximately 40% of the N required by lettuce. The difference between our results and the value reported by Lastiri et al. (2018, 2016) can be due to many reasons, as the differences in the system designs, species requirements, environmental conditions, but also as a result of the higher concentration of nutrients in biofloc-based water.

In this study, we evaluated only one decision variable to improve the FLOCponics performance, the planting area. However, many other variables can be explored, as for example, but not limited to, the fish stocking density, fish growth phase, plant species, abiotic variables, and settling tank configuration. Another point to consider is that we focused only on the N balance to derive conclusions about the system efficiencies. The reason for restricting our simulations to N was because most studies explore the role of the biofloc microorganisms on the N pathway, and just a few report the role of biofloc on transforming or consuming other important nutrients, such as P, K and C. It should be also noted that numerous variables may affect the model outcomes. Nevertheless, the present study is a pioneer in quantifying and describing the water and nitrogen uses and solids waste in FLOCponics systems. Our models can be used as a benchmark and starting point for future studies on FLOCponics production.

4.5 Conclusions

The present model-based study quantitatively demonstrates the efficiency of FLOCponics in producing tilapia juveniles and lettuce compared to the stand-alone biofloc system. The model outputs show that water and nutrient use efficiencies are higher in FLOCponics than in biofloc system, by 10 and 27%, respectively. For solids discharged, FLOCponics lower it by 10% compared to the biofloc system. We also evaluated changes in the planting area of the FLOCponics system to propose an improved system. The simulations revealed that the FLOCponics system under study could be even more efficient by expanding the planting area up to 3.2 times the nominal area of 33.6 m^2 (Table 4.1).

Supplementary material

i. Reference Evapotranspiration and Evaporation rate

Table S4.1. Greenhouse properties used as input data for the FAO Penman-Monteith Equation.

Parameter	**Unit**	**Value**
Wind speed at 10m	m s^{-1}	1.49
Greenhouse glazing transmittance	%	0.75
Shading factor	%	0.40
canopy reflection coefficient		0.23
Altitude	m	615
Latitude	°	-21
Minute	′	-14

Table S4.2. Calculated reference evapotranspiration and evaporation rate per month and the weather data (2019) from Jaboticabal-SP.

	Max temp. °C	Min temp. °C	Max RH %	Min RH %	Average SR MJ m^{-2} d^{-1}	ET_0 mm d^{-1}	ET_C mm d^{-1}	Evap mm d^{-1}
January	32.51	20.45	90.83	39.76	23.94	4.69	2.87	2.07
February	30.64	19.75	93.88	49.04	19.06	3.77	2.28	1.54
March	30.87	19.73	94.23	45.86	19.29	3.66	2.21	1.76
April	30.30	18.68	92.54	43.75	17.83	3.10	1.88	1.97
May	28.79	16.38	91.87	42.48	15.04	2.31	1.41	2.02
June	27.50	14.03	86.86	34.48	15.12	1.96	1.21	2.44
July	27.22	12.15	83.17	28.78	15.29	1.95	1.21	2.55
August	29.23	14.10	81.20	30.27	17.14	2.55	1.58	2.52
September	33.07	17.99	78.25	27.79	20.10	3.46	2.14	2.68
October	34.04	19.07	82.94	28.33	23.09	4.25	2.63	2.65
November	31.35	25.88	91.33	41.92	21.00	4.39	2.67	1.65
December	30.30	20.20	93.82	48.42	19.31	3.89	2.35	1.48

Weather data provided by the AgroClimatological Station of Unesp. Temp.: temperature. RH: relative humidity. SR: Solar radiation. Evap: Evaporation in the aquaculture system/subsystem, a calculation based on the Penman method. Evapotranspiration calculation based on FAO Penman-Monteith method.

ii. Auxiliary equations and general parameters

Table S4.3. Auxiliary equations that support the mass balances (Table 4.2) for the biofloc and FLOCponics systems.

Auxiliary equation	**Description**	**Unit**	**Eq.**
$\phi_{v,AQinitial} = V_{FT} / t_{fill,AQ}$	Volume of inoculum to fill each fish tank	$m^3\ day^{-1}$	(S1)
$\phi_{v,AQfresh} = \phi_{v,evap} + \phi_{v,sludge} + \phi_{v,AQ\text{-}HP}$	Volume flow of freshwater in the aquaculture subsystem	$m^3\ day^{-1}$	(S2)
$\phi_{v,sludge} = m_{TSS,RFSset} / ((1 - k_{DM,sludge}) / k_{DM,sludge})\ \Delta t$	Outflow of water thought sludge discharge, if $C_{TSS,FT} > TSS_{max}$ else 0	$m^3\ day^{-1}$	(S3)
$m_{TSS,RFSset\_BFT} = C_{TSS,FT}\ \ \phi_{v,FT\text{-}RFS\_BFT}\ k_{set,RFS\_BFT}\ h_{RFS\_BFT}$	Mass of TSS settled in the biofloc system	g	(S4)
$h_{RFS\_BFT} = (C_{TSS,FT} - TSS_{min})\ V_{FT}\ n_{FT} / C_{TSS,FT}\ k_{set,RFS\_BFT}\ \phi_{v,FT\text{-}RFS\_BFT}$	Time that the radial flow settler (RFS) operates in the biofloc system	h	(S5)
$m_{TSS,RFSset\_FP\text{-}1} = C_{TSS,FT}\ \ \phi_{v,FT\text{-}RFS\_FT}\ k_{set,RFS\_FP}$	Mass of TSS settled in the RFS of the FLOCponics system, if $\phi_{v,AQ\text{-}HP} > 0$ else 0	g	(S6)
$m_{TSS,RFSset\_FP\text{-}2} = C_{TSS,FT}\ \ \phi_{v,FT\text{-}RFS\_BFT}\ k_{set,RFS\_BFT}\ h_{RFS\_FP}$	Mass of TSS settled in the RFS of the FLOCponics system if $C_{TSS,FT} > TSS_{max}$ else 0	g	(S7)
$h_{RFS\_FP} = ((m_{TSS,FT} - m_{TSS,RFSset\_FP\text{-}1}) - (TSS_{min}\ V_{FT}\ n_{FT})) / C_{TSS,FT}\ k_{set,RFS\_BFT}\ \phi_{v,FT\text{-}RFS\_BFT}$	Time that the RFS operates in the FLOCponics system if $C_{TSS,FT} > TSS_{max}$	h	(S8)
$\phi_{v,HPinitial} = (V_{HP} / 2) / t_{plant}$	Volume flow of freshwater in the hydroponics subsystem	$m^3\ day^{-1}$	(S9)
$\phi_{v,HPfresh} = \phi_{v,dilution}$	Volume flow of freshwater in the hydroponics subsystem if $C_{N,HP} > C_{N,max}$, same equation described by Dijkgraaf et al. (2019). In our case, $\phi_{v,dilution} = 0$	$m^3\ day^{-1}$	(S10)
$\phi_{v,HPdischarge} = V_{HP} / t_{discharge}$	Outflow of water from the HP_{sub} due to excess of $m_{TSS,HP}$, $t_{discharge} = 1$ if $C_{TSS,HP} > TSS_{max,HP}$ else 0	$m^3\ day^{-1}$	(S11)
$\phi_{N,feed} = \phi_{feed}\ k_{DM,feed}\ k_{N,feed}$	Amount of N-feed entered	$g\ day^{-1}$	(S12)
$\phi_{feed} = FCR\ \Delta m_{fish} / \Delta t$	Amount of feed entered	$g\ day^{-1}$	(S13)
$\phi_{N,molasses} = \phi_{molasses}\ k_{N,molasses}$	Amount of N-molasses entered	$g\ day^{-1}$	(S14)
$\phi_{molasses} = (m_{TAN,FT/\ \Delta t}) / (k_{C,Csource}\ MC / CN_{het})$	Amount of molasses entered based on Avnimelech (2015)	$g\ day^{-1}$	(S15)
$\phi_{N,bf\_prod} = \phi_{Ammonification} + \phi_{nitrification}$	Nitrogen production by the biofloc microorganisms	$g\ day^{-1}$	(S16)

$\phi_{Ammonification} = (m_{Norg,FT}\, k_{N,degrad}) / \Delta t$	Ammonia production due to organic nitrogen degradation by the biofloc (heterotrophic) microorganisms	g day^{-1}	(S17)
$\phi_{N,bf\_cons} = \phi_{assimilation} = u_{bf} * C_{TSS,FT} / Y_{x/N}$	Nitrogen consumed by the biofloc (heterotrophic) microorganisms	g day^{-1}	(S18)
$Y_{x/N} = Y_{x/N} / CN_{maint}$	Bacterial growth yield based on nitrogen consumption	-	(S19)
$u_{bf} = u_{max} (C_{C,FT}/(C_{C,FT} + K_C)) (C_{Ninog,FT}/(C_{Ninog,FT} + K_N))$	Bioflocs biomass growth rate	g day^{-1}	(S20)
$m_{N,inong} = m_{TAN} + m_{NO3}$	Mass of inorganic nitrogen in the FT	g	(S21)
$dm_{TAN}/dt = ((C_{TAN,initial}\, \phi_{v,AQinitial}) + \phi_{Ammonification} + \phi_{N,fishexcretion}) - (\phi_{Assimilation} + \phi_{volatilization} + \phi_{nitrification} + \phi_{TAN,sludge})$	Daily change in the mass of TAN in the FT	g day^{-1}	(S22)
$\phi_{nitrification} = (m_{TAN,FT}\, k_{nitrif}) / \Delta t = m_{NO3} / \Delta t$	Nitrate production by the biofloc (nitrifying) microorganisms	g day^{-1}	(S23)
$\phi_{N,fishexcretion} = \phi_{N,feed}\, k_{feed,eaten}\, k_{N,fishexcret}$	Nitrogen excreted by fish	g day^{-1}	(S24)
$\phi_{volatilization} = 0.16*((m_{Ninorg,FT})/(1+(10^\wedge(9.25-pH_{FT})))/\Delta t$	Amount of volatile nitrogen in the FT	g day^{-1}	(S25)
$dm_{NH3}/dt = (m_{TAN}\, k_{UIA}) / \Delta t$	Daily change in the mass of Un-ionized Ammonia in the FT	g day^{-1}	(S26)
$\phi_{TSS,bf} = dm_{TSS,bf}/dt = u_{bf}\, m_{TSS,FT}$	Bioflocs biomass growth in the FT	g day^{-1}	(S27)
$\phi_{N,fertilizer} = (C_{N,required} - C_{N,HP})\, V_{HP} / \Delta t$	Amount of nitrogen entered in the HP_{sub} to meet the minimum concentration of N required by lettuce	g day^{-1}	(S28)
$\phi_{N,plant} = \phi_{v,HPevapt}\, C_{N,HP}$	Nitrogen uptake by plant	g day^{-1}	(S29)

Table S4.4. General parameters used to model the biofloc and FLOCponics systems.

Parameter	Value	Unit	Description
Δt	1	day	Time step
k_{set,RFS_BFT}	0.7	kg kg^{-1}	Solids settling efficiency in the RFS, based on Mendez et al. 2021 for $\phi_{v,FT\text{-}RFS} = 0.022$ cm s^{-1}
$k_{DM,sludge}$	0.01	kg kg^{-1}	Dry matter per mass of sludge removed, experiment result
$TSS_{max,AQ}$	500	mg L^{-1}	Maximum concentration of TSS in the FT, based on (Hargreaves 2013; Emerenciano et al. 2017)

$TSS_{min,AQ}$	100	mg L^{-1}	Minimum concentration of TSS in the FT (Hargreaves 2013; Emerenciano et al. 2017)
$\phi_{v,FT\text{-}RFS\_BFT}$	0.792	m^3 h^{-1}	Water flow rate from the FT to RFS in the biofloc system
$\phi_{v,FT\text{-}RFS\_FT}$	1	m^3 day^{-1}	Water flow rate from the FT to RFS in the FLOCponics system
k_{set,RFS_FP}	0.9	-	Solid settling efficiency, estimated parameter based on experimental observations
$TSS_{max,HP}$	50	mg L^{-1}	Maximum concentration of TSS in the hydroponics subsystem
$C_{N,AQinitial\_TAN}$	0.2	mg L^{-1}	Initial concentration of TAN
$C_{N,AQinitial\_NO3}$	0.8	mg L^{-1}	Initial concentration of Nitrate
$C_{N,AQinitial\_Norg}$	2	mg L^{-1}	Initial concentration of organic nitrogen
$C_{TSS,AQinitial}$	144	mg L^{-1}	Initial concentration of TSS
$t_{fill,AQ}$	1, 27, 55, 82	day	Time when each fish tank is filled with inoculum
MC	0.6	-	Microbial efficiency (Avnimelech 2015)
CN_{het}	10	kg kg^{-1}	Carbon nitrogen ratio suitable for heterotrophic bacteria (10-20:1)
CN_{maint}	6	kg kg^{-1}	Carbon nitrogen ratio for maintaining microbial community in an established biofloc-based culture (Avnimelech 2015)
$k_{N,degrad}$	0.08	kg kg^{-1}	Coefficient of organic nitrogen degradation (Avnimelech et al. 1995)
k_{nitrif}	0.976	kg kg^{-1}	Coefficient of TAN oxidation by nitrifying bacteria (Ebeling et al. 2006)
u_{max}	0.086	day^{-1}	Maximum biofloc biomass growth rate
k_C	0	-	Coefficient of C half saturation
k_N	0.2	-	Coefficient of N half saturation
$Y_{x/C}$	1.34	kg kg^{-1}	Bacterial growth yield based on C consumption
pH_{FT_BFT}	7.07	-	pH in the fish tanks of biofloc system, average value observed in the experiment
pH_{FT_FP}	7.14	-	pH in the fish tanks of FLOCponics system, average value observed in the experiment
k_{UIA}	0.25	kg kg^{-1}	Coefficient of TAN conversion in un-ionized ammonia
$C_{TSS,fresh}$	15	mg L^{-1}	Concentration of TSS in the freshwater
t_{plant}	82	day	First day of plant production
$C_{N,min}$	100	mg L^{-1}	Minimum concentration of N in the HP_{sub}
$C_{N,max}$	200	mg L^{-1}	Maximum concentration of N in the HP_{sub}
k_{setB}	0.6	kg kg^{-1}	Solid retention efficiency in the bag filter, estimated parameter based on experimental observations

iii. Fish growth calibration and feed input

The total mass of fish was calculated according to the individual fish biomass (Eq. S30) multiplied by the number of fish in each fish tank (Eq. S31). Fish growth was modelled based on the exponential functions found in the experiment from the weekly measurements of fish weight in the biofloc and FLOCponics systems. The number of fish was calculated following the equations described by Karimanzira et al. (2016), the value of the first-order mortality coefficient (k_{mort} day^{-1}) was based on the fish survival noted in the experiment. The amount of feed input was calculated according to the fish mass multiplied by the average values of feed conversion ratio (FCR) experimentally found for each system. The data related to fish growth performance, feed composition and feed use was based on experiment observations.

$$W_t = W_0 \, \mathrm{e}^{k_{fishgrowth} \, t} \quad \text{(S30)}$$

$$n_t = n_0 \, \mathrm{e}^{k_{mort} \, t} \quad \text{(S31)}$$

Table S4.5. Parameters related to fish growth performance and feed content and uptake.

Parameter	**Value (kg/kg)**	**Description**
Fish growth		
FCR	0.89 (BFT) 1.02 (FP)	Feed conversion ratio
$k_{fishgrowth}$	5.61 (BFT) 5.60 (FP)	Fish growth coefficient (based on specific growth rate)
k_{mort}	2.85E-4 (BFT) 1.79E-3 (FP)	Fish mortality coefficient
Feed and molasses		
$k_{N,feed}$	0.053	N content in feed dry mass
$k_{dry,feed}$	0.959	Dry matter in feed mass
$k_{C,feed}$	0.500	C content in feed dry mass
$k_{N,molasses}$	0.007	N content in molasses dry mass
$k_{C,molasses}$	0.500	C content in molasses dry mass
Fish metabolism		
$k_{water,fish}$	0.800	Water content in fish mass
$k_{dry,fish}$	0.200	Dry matter in fish mass
$k_{feed,uneaten}$	0.180	Mass of uneaten feed per mass of feed inputted
$k_{feed,eaten}$	0.820	Mass of eaten feed per mass of feed inputted
$k_{C,release}$	0.500	Mass of C-feed unretained per mass of feed inputted

$k_{N,release}$	0.590	Mass of N-feed unretained per mass of feed inputted
$k_{N,fishexcret}$	0.294	Mass of N indigestible fraction per mass of N in dry feed
$k_{N,indfeed}$	0.116	Mass of N excreted per mass of N in dry feed
$k_{N,fish}$	0.094	Mass of N per mass of dry matter of fish harvested

iv. Biofloc growth rate calibration

The model for bioflocs biomass growth is presented in Eq. S4.27 and is related to the substrate concentration available through the Monod equation and the maximum biomass growth rate (μ_{max}). The μ_{max} values used in the models were calibrated using the ordinary least-squares method (using Excel Solver tool to minimize the Sum of Squared Errors between measured and predicted values, Figure S4.1), given an exponential model of bioflocs biomass growth and data of daily TSS concentrations in the fish tanks, collected in the experiment. It was assumed that N and C concentrations were not limiting in the experiment, as a proactive approach was followed to stimulate the bioflocs microorganisms' growth by regularly adding the external C source (molasses) based on the amount of N that enters the system through the feed (Avnimelech 2015; Emerenciano et al. 2017; Pinho et al. 2021c).

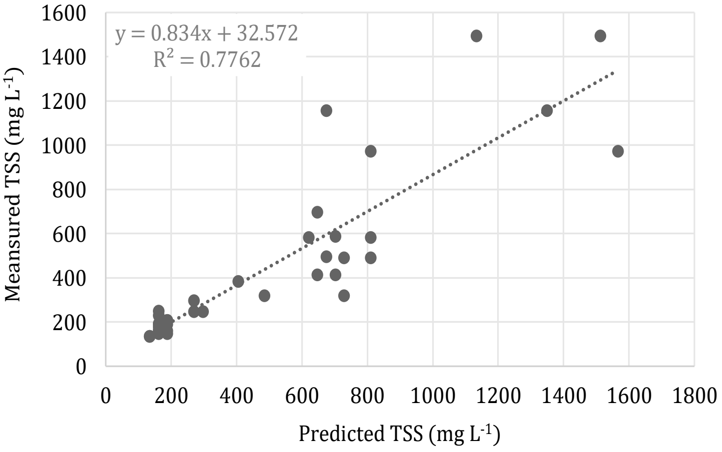

Figure S4.1. Correlation between the measured and predicted TSS values used to calibrate the maximum biomass growth rate (μ_{max}) used in the bioflocs growth model.

v. Sensitivity analysis results

Table S4.6. Key performance indicator values under changes in the bacteria growth yield ($Y_{x/c}$ - nominal value: 1.3), along with the corresponding values of the normalized sensitivity coefficients (last two columns; absolute values >0.5 in bold).

Performance indicator	**BFT**	**FP**	**BFT**	**FP**	**BFT – *S***	**FP – *S***
	-25%		**+25%**			
Total solids discharged (kg)	414.96	375.36	428.46	393.74	0.064	0.097
Average WUE	75.8%	83.8%	75.2%	83.5%	-0.015	-0.008
Average NUE	45.2%	57.1%	45.0%	57.6%	-0.011	0.016
L water input per kg food produced	31.72	36.01	32.03	36.24	0.020	0.013
L water wasted per kg food produced	9.66	7.83	9.97	8.06	0.064	0.059
kg N input per kg food produced	0.04	0.03	0.04	0.03	0.006	-0.006
kg N wasted per kg food produced	0.03	0.01	0.03	0.01	0.005	0.018
% N-fertilizer		60.5%		55.7%		-0.167

Table S4.7. Key performance indicator values under changes in the coefficient of organic nitrogen degradation ($k_{N,degrad}$ - nominal value: 0.08), along with the corresponding values of the normalized sensitivity coefficients (last two columns; absolute values >0.5 in bold).

Performance indicator	**BFT**	**FP**	**BFT**	**FP**	**BFT – *S***	**FP – *S***
	-25%		**+25%**			
Total solids discharged (kg)	415.61	379.37	429.08	392.69	0.064	0.070
Average WUE	75.8%	83.8%	75.2%	83.3%	-0.015	-0.012
Average NUE	45.2%	57.0%	44.9%	57.5%	-0.014	0.017
L water input per kg food produced	31.73	36.06	32.04	36.23	0.020	0.009
L water wasted per kg food produced	9.67	7.88	9.99	8.05	0.064	0.043
kg N input per kg food produced	0.04	0.03	0.04	0.03	0.011	-0.008
kg N wasted per kg food produced	0.03	0.01	0.03	0.01	0.011	-0.034
% N-fertilizer		61.5%		53.1%		-0.288

Table S4.8. Key performance indicator values under changes in the coefficient of solids settling in the RFS_{FP} (k_{RFSset_FP} - nominal value: 0.9), along with the corresponding normalized sensitivity values.

Performance indicator	**BFT**	**FP**	**BFT**	**FP**	**BFT – *S***	**FP – *S***
	-10%		**+10%**			
Total solids discharged (kg)		382.12		386.19	na	0.054
Average WUE		77.1%		87.6%	na	**0.631**
Average NUE		57.3%		57.2%	na	-0.004
L water input per kg food produced		40.18		33.09	na	**-0.983**
L water wasted per kg food produced		12.00		4.90	na	**-4.496**
kg N input per kg food produced		0.03		0.03	na	-0.081
kg N wasted per kg food produced		0.01		0.01	na	-0.133
% N-fertilizer		53.5%		60.7%	na	**0.623**

Table S4.9. Key performance indicator values under changes in the coefficient of solids settling in the RFS_{BFT} (k_{RFSset_BFT} - nominal value: 0.7), along with the corresponding values of the normalized sensitivity coefficients (last two columns; absolute values >0.5 in bold).

Performance indicator	**BFT**	**FP**	**BFT**	**FP**	**BFT – *S***	**FP – *S***
	-25%		**+25%**			
Total solids discharged (kg)	420.94		420.94		0.000	na
Average WUE	75.6%		75.6%		0.000	na
Average NUE	45.1%		45.1%		0.000	na
L water input per kg food produced	31.85		31.85		0.000	na
L water wasted per kg food produced	9.80		9.80		0.000	na
kg N input per kg food produced	0.04		0.04		0.000	na
kg N wasted per kg food produced	0.03		0.03		0.000	na
% N-fertilizer						na

Table S4.10. Key performance indicator values under changes in the maximum concentration of TSS accepted in the fish tank (TSS_{max} - nominal value: 500), along with the corresponding values of the normalized sensitivity coefficients (last two columns; absolute values >0.5 in bold).

Performance indicator	**BFT**	**FP**	**BFT**	**FP**	**BFT – *S***	**FP – *S***
	-25%		**+25%**			
Total solids discharged (kg)	357.03	332.28	487.86	440.51	**0.622**	**0.569**
Average WUE	78.3%	86.1%	73.0%	81.4%	-0.140	-0.112
Average NUE	44.9%	57.0%	45.1%	57.8%	0.008	0.029
L water input per kg food produced	30.37	34.44	33.41	37.86	0.191	0.189
L water wasted per kg food produced	8.31	6.26	11.36	9.68	**0.622**	**0.866**
kg N input per kg food produced	0.04	0.03	0.04	0.03	0.009	0.007
kg N wasted per kg food produced	0.03	0.01	0.03	0.01	0.025	-0.051
% N-fertilizer		58.9%		55.9%		-0.104

Table S4.11. Key performance indicator values under changes minimum concentration of TSS accepted in the fish tank (TSS_{min} - nominal value: 100), along with the corresponding values of the normalized sensitivity coefficients (last two columns; absolute values >0.5 in bold).

Performance indicator	**BFT**	**FP**	**BFT**	**FP**	**BFT – *S***	**FP – *S***
	-25%		**+25%**			
Total solids discharged (kg)	345.29	313.66	484.35	444.52	0.330	0.344
Average WUE	78.9%	86.8%	73.1%	81.0%	-0.077	-0.070
Average NUE	44.9%	58.3%	45.2%	57.2%	0.006	-0.018
L water input per kg food produced	30.09	34.21	33.33	37.91	0.102	0.103
L water wasted per kg food produced	8.04	6.02	11.27	9.73	0.330	0.469
kg N input per kg food produced	0.04	0.03	0.04	0.03	-0.022	0.014
kg N wasted per kg food produced	0.03	0.01	0.03	0.01	-0.032	0.052
% N-fertilizer		46.0%		62.7%		0.288

Table S4.12. Key performance indicator values under changes in the coefficient of N excreted by fish ($k_{N,fishexcret}$ - nominal value: 0.29), along with the corresponding values of the normalized sensitivity coefficients (last two columns; absolute values >0.5 in bold). along with the corresponding normalized sensitivity values.

Performance indicator	**BFT**	**FP**	**BFT**	**FP**	**BFT – *S***	**FP – *S***
	-10%		**+10%**			
Total solids discharged (kg)	415.50	374.67	428.19	393.58	0.151	0.249
Average WUE	75.7%	83.7%	75.3%	83.5%	-0.032	-0.017
Average NUE	47.8%	59.3%	42.3%	55.4%	-0.611	-0.344
L water input per kg food produced	31.73	36.00	32.02	36.24	0.046	0.033
L water wasted per kg food produced	9.67	7.82	9.97	8.06	0.151	0.152
kg N input per kg food produced	0.04	0.03	0.04	0.03	0.028	-0.014
kg N wasted per kg food produced	0.02	0.01	0.03	0.01	**0.519**	**0.576**
% N-fertilizer		61.2%		54.9%		**-0.537**

Table S4.13. Key performance indicator values under changes in minimum concentration of N required by plant ($C_{N,required}$ - nominal value: 80), along with the corresponding values of the normalized sensitivity coefficients (last two columns; absolute values >0.5 in bold).

Performance indicator	**BFT**	**FP**	**BFT**	**FP**	**BFT – *S***	**FP – *S***
	-25%		**+25%**			
Total solids discharged (kg)		380.20		380.20	na	0.000
Average WUE		83.6%		83.6%	na	0.000
Average NUE		56.8%		57.8%	na	0.051
L water input per kg food produced		36.07		36.07	na	0.000
L water wasted per kg food produced		7.89		7.89	na	0.000
kg N input per kg food produced		0.03		0.03	na	0.087
kg N wasted per kg food produced		0.01		0.01	na	0.030
% N-fertilizer		47.1%		65.3%	na	**0.936**

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Chapter 5

Sustainability of food production in biofloc-based systems: from stand-alone to integrated agri-aquaculture system

This chapter is based on:
Pinho SM, David LH, Garcia F, Portella MC, Keesman KJ. Sustainability of food production in biofloc-based systems: from stand-alone to integrated agri-aquaculture system. (Submitted)

Abstract

FLOCponics is an intensive integrated agri-aquaculture system that combines biofloc-based aquaculture with hydroponics. Since research on FLOCponics is in its early stage of development, and many aspects of this system still need to be explored, the objective of this study was to assess and discuss the sustainability of a FLOCponics system and compare it to stand-alone biofloc and hydroponics cultures. For this, we conducted an experiment-based study by applying emergy synthesis to assess the sustainability of tilapia juveniles and lettuce production in FLOCponics, biofloc and/or hydroponics systems. The results indicate that the resources from the larger economy were the inputs with the greatest contribution in all systems. Overall, most of the emergy indicators are similar for all systems, suggesting that FLOCponics, biofloc and hydroponics systems use low amounts of natural renewable resources, cause moderate environmental load, and lead to environmental stress seven times higher than the contribution to the economy. The unit emergy values are different for each system, indicating that, under the conditions evaluated, FLOCponics is more efficient than hydroponics and less efficient than a biofloc system. Further improvements that must be made to increase the efficiency of FLOCponics and explore its full potential for sustainable food provision are also pointed out.

5.1 Introduction

Integrated Agri-AquaCulture (IAAC) systems have been labelled as sustainable and efficient means to produce food (FAO 2020; Boyd et al. 2020). The rationale of IAAC is based on recovering the waste of one sub-activity (agriculture or aquaculture) and reuse it as an input to another (Zajdband 2011). The integration of plant culture with aquatic animal production mainly aims to improve the use of nutrients introduced into the system, optimise water use, prevent waste discharges, diversify production, and provide food security for local consumers (Lennard and Goddek 2019; Farrant et al. 2021). IAAC is an ancient practice employed for local and small-scale producers, predominantly in Asia. In the last decades, however, there has been a shift towards modern and intensive IAAC systems to meet the growing demand for sustainable food provision (Edwards 2003).

FLOCponics is such a modern integrated agri-aquaculture system in its initial stage of development (Pinho et al. 2021b). FLOCponics combines the intensive production of aquatic organisms using biofloc technology with the production of vegetables in hydroponics systems (Kotzen et al. 2019; Pinho et al. 2021a). Biofloc aquaculture systems are based on promoting the growth of specific microbial communities *in situ* in the fish tank to intensify and increase the biosecurity of fish and shrimp production (Browdy et al. 2012; Avnimelech 2015; Dauda 2020). The microorganisms are responsible for maintaining water quality and serving as food for the cultivated organisms, decreasing the need for water renewal and the use of commercial feed (Emerenciano et al. 2017; Martínez-Córdova et al. 2017; Mugwanya et al. 2021). Hydroponics is a soilless plant production method. When operated as a stand-alone system, the typical way to supply water and nutrients required by plants in hydroponics is from a balanced nutrient commercial solution (Maucieri et al. 2019). Aiming at the improvement of its efficiency and reduce the environmental impact of both systems, FLOCponics uses the excess of nutrients from biofloc to nourish hydroponics plants (Pinho et al. 2017; Pinheiro et al. 2017; Emerenciano et al. 2021). In terms of productive results, Pinho et al. (2021c) reported recently that FLOCponics is technically viable and provides similar fish and vegetable growth performance to stand-alone biofloc and hydroponics systems. Opposed to other FLOCponics studies that did not compare FLOCponics to the stand-alone systems (Pinho et al. 2017; Rocha et al. 2017; Lenz et al. 2017; Pickens et al. 2020), Pinho et al. (2021c) presented a robust experimental design to draw such conclusions since they simultaneously evaluated fish and vegetable growth in these production systems under the same climate conditions and using the same type of resources.

Despite having a great potential for improving the use of resources and reducing the environmental impact compared to stand-alone systems, FLOCponics seems to demand several other resources to make the integration feasible. For example, FLOCponics would require a high investment in technology, infrastructure, equipment, and qualified/specialized labour to successfully produce fish and plants. Also, the high demand for electricity to maintain constant aeration in the biofloc fish-tanks may be an issue in FLOCponics (Walker et al. 2020; Pinho et al. 2021b; David et al. 2021b). Such drawbacks make the sustainable character of FLOCponics systems a question that needs to be investigated. The sustainability of food production systems has been investigated using scientifically reliable methods. Some studies have been conducted to measure the sustainability of biofloc (Vieira et al. 2021; David et al. 2021b) and hydroponics systems (Romeo et al. 2018; Martin and Molin 2019), but none was found for FLOCponics.

Emergy synthesis (ES) is one of the scientific sustainability assessment methods that has been used to evaluate food production systems. ES is a biophysical method based on a “donor side” approach, which means the biosphere capacity to provide resources to maintain the system running over the years (Odum 1996; Brown and Ulgiati 2004a). ES recognizes the differences in energy quality according to their position in the hierarchical energy transformation network, which allows it to account for all energy flows required directly and indirectly from economic and environmental sources to produce goods and services (Odum 1996; Brown and Ulgiati 2016). ES converts all energy input flows into a single unit of 'solar emjoules' (sej), allowing the comparation of different energy flows and establishing indicators for environmental performance assessment of different production systems (Odum 1996). More than providing a simple sustainability diagnosis, emergy synthesis usually provides clear information on where, when, and sometimes how to improve existing production systems to achieve higher degrees of sustainability (David et al. 2021a). A recent study assessed the sustainability of a tilapia biofloc-based farm using ES (David et al. 2021b), showing that the biofloc-based farm had potentially sustainable characteristics and could be even more sustainable if the electricity use was optimized. In addition to evaluating case studies, ES is also a valuable tool to predict, through simulations, whether systems that have not yet been commercially implemented or are at an early stage of development will be sustainable over time, thus supporting further decision making (Campbell 1998; Zhan et al. 2020; Zhao et al. 2020). Such a possibility of investigating systems before their implementation makes ES an appropriate method to assess the sustainability of FLOCponics systems.

Since research on FLOCponics is beginning, and many aspects of this system still need to be explored, the objective of this study was to investigate, assess and discuss the sustainability of the FLOCponics system as a potentially sustainable alternative for stand-alone biofloc or hydroponics cultures before such an integrated system become widely spread. This investigation will lead to a perspective of what troubling points need to be covered in the FLOCponics research field before they turn into a commercial scale problem. For this purpose, we used emergy synthesis to compare the sustainability of hydroponics and biofloc systems to the sustainability of the integrated FLOCponics system, based on previous experimental results.

5.2 Methods

5.2.1 General information and data collection

Sustainability assessment was conducted on three different food production systems: a hydroponics, biofloc, and FLOCponics system. The data used in this theoretical experiment-based study came from Pinho et al. (2021c). Pinho et al. (2021c). compared the production of fish and vegetables in these three systems under the same environmental conditions. The fish produced was Nile tilapia (*Oreochromis niloticus*) in the juvenile phase (1 to 30g), and lettuce (*Lactuca sativa*) was the vegetable cultivated from seedling until reaching commercial size. Since each production system has its own characteristics, the experimental devices were built following such characteristics and thus they had different productive areas and times (production cycle period). To present a fair comparison between the systems, all data were standardized to one square meter in one year of production (Table 5.1).

Table 5.1. Technical characteristics of the hydroponics, biofloc, and FLOCponics systems for tilapia juveniles and lettuce production.

Item	**Unit**	**Hydroponics**	**Biofloc**	**FLOCponics**
Area	m^2	0.84	1.44	2.28
Initial water supply	m^3 $year^{-1}$	2.09	0.48	0.60
Replacement water	m^3 $year^{-1}$	0.75	0.77	0.76
Electricity consumption	kWh $year^{-1}$	948	1486	2436
Stocked fish	unit $year^{-1}$	-	1955	1955
Initial average weight of fish	kg $fish^{-1}$	-	0.001	0.001
Final average weight of fish	kg $fish^{-1}$	-	0.030	0.030
Vegetable seedlings	unit $year^{-1}$	330	-	330
Feed	kg $year^{-1}$	-	50.3	57.0
Compound fertilizer	g $year^{-1}$	2546	-	2189
Molasses	kg $year^{-1}$	-	1.95	1.95
Skilled labour	h $year^{-1}$	182.5	182.5	365
Non-skilled labour	h $year^{-1}$	365	365	547.5
Effluent treated	m^3 $year^{-1}$	2.09	0.25	0.25
Vegetables produced	kg $year^{-1}$	31.76	-	39.21
Fish produced	kg $year^{-1}$	-	63.16	57.94
Fish production	cycles $year^{-1}$	-	6.5	6.5
Vegetable production	cycles $year^{-1}$	17.4	-	17.4

A 100 m^2 greenhouse at the Aquaculture Center of São Paulo State University (Unesp) in Jaboticabal, São Paulo, Brazil (21º14’05”S, 48º17’09”W), was used. The greenhouse was covered with a 1.5 mm plastic liner and a shading net to reduce the luminosity by 40%. The plastic on the sides of the greenhouse was movable to allow the regulation of the temperature inside the greenhouse through wind circulation. The water used in all systems for the initial supply of the tanks and replacing the evapotranspiration losses came from an artesian well. The insolation, wind and evapotranspiration were calculated based on the weather dataset provided by the AgroClimatological Station of Unesp (Campus Jaboticabal-SP), monthly average values for 2019.

The electricity used to keep the aeration and pumping systems running came from the municipal grid. The aeration was provided by an air blower and distributed in each system by micro-perforated diffusers. A power generator was available as a backup for eventual power failures. Since constant aeration and water movement are crucial in the fish tank of biofloc and FLOCponics systems, the generator was considered equipment for both systems. Other equipment included, for use in all systems, was a multiparameter required to daily monitor the physical-chemical parameters of the water (e.g., temperature, pH, electrical conductivity, etc.). Skilled labour was required to control critical operations in all systems, such as deciding when and how much fertilizer or extra carbon source should be supplemented. Non-skilled labour performed all other hand-operated activities. We included in all systems a waste treatment system to treat the discharged water (rich in nutrients and solids) before it becomes an environmental problem (David et al. 2021b). As each system will have a different amount of effluent/waste, the treatment system has been sized to be compatible with each of them.

5.2.2 Systems description

The information relevant to the emergy synthesis of each system is presented in the next subsections (for more details, see Pinho et al. (2021c)).

5.2.2.1 Hydroponics

In the hydroponics system, deep-water culture (DWC) was used for lettuce production, where the vegetables were accommodated in floating structures in tanks filled with nutrient solution. In the experiment, two hydroponics tanks with a surface of 0.42 m^2 (60 L) each represented a replicate. Thus, in the present study, a total area of 0.84 m^2 was considered for lettuce production. Lettuce seedlings, 21 days after sowing, were planted in a density of 19 lettuces per m^2 and cultivated for 21 days until harvest.

At the beginning of each lettuce production cycle (every 21 days), the hydroponics tanks were emptied, cleaned, and filled with artesian well water. The emptying was done by pumping, and we considered that the discharged water was properly treated in a waste treatment system before being discharged into the environment. After filling the tanks and during the cycles, the electrical conductivity (EC) of the water was measured and a compound commercial fertilizer (Dripsol Folhosas®, concentrated 100 times) was added until the EC reached 1.7 mS cm^{-1}. Such management of emptying and filling the tanks between the cycles is common in hydroponics, as it is economically cheaper and easier to start the cycle with a balanced solution instead of analysing all nutrients that remained in the water and supplement only those deficient.

The scope boundaries of the hydroponics system are defined in the diagram presented in Figure 5.1. All diagrams presented in this study were designed following the methodology proposed by Odum (1996).

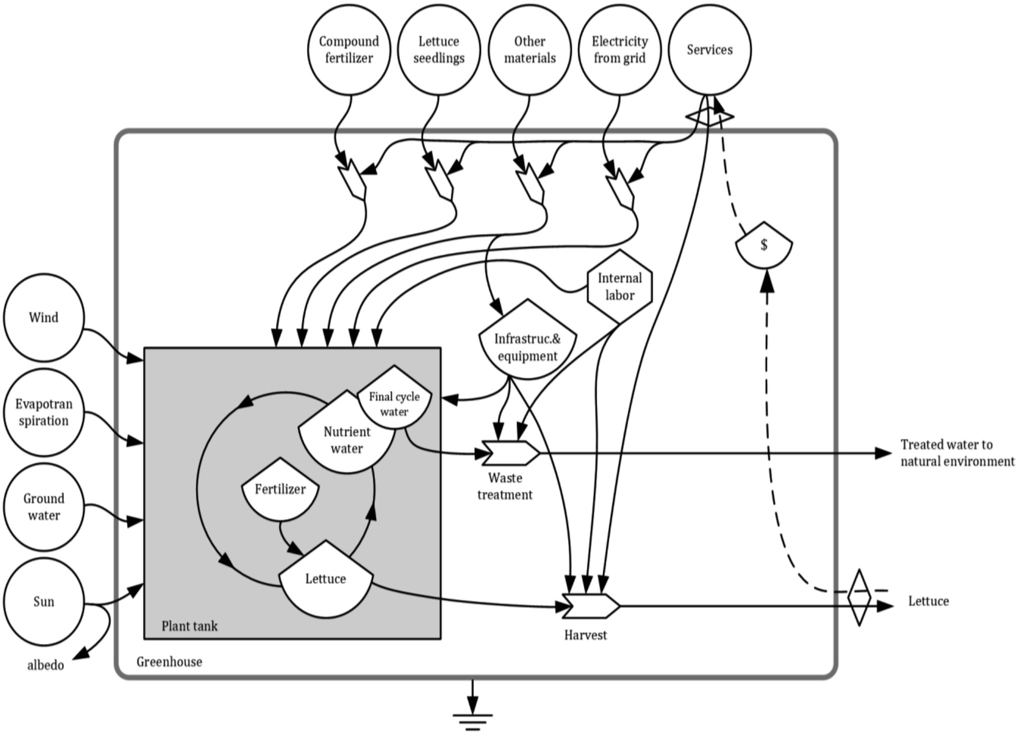

Figure 5.1. Energy diagram of the hydroponics system to produce lettuce.

5.2.2.2 Biofloc

The biofloc system consisted of a circular fish tank (380 L) and a radial flow settler (decanter, 100 L). Tilapia juveniles were hand-fed with a diet containing 32% crude protein, four times a day, in a production cycle of 56 days. Liquid molasses, as an external carbon source, was added to the fish tank three times a week to maintain a C:N ratio in the water of approximately 15:1. The regulation of the C:N ratio is part of biofloc-based cultures routine to maintain the biofloc microorganisms (Emerenciano et al. 2017). Water quality parameters were constantly monitored to guarantee optimal conditions for fish growth and maintaining the desired microorganism community. During the experiment, no water or waste was discharged. However, since control of solids that accumulate in the biofloc water is usually required, we estimated that the sludge (water with an extremely high concentration of solids) discharged is up to 10% of the fish tank volume in each cycle of fish production. The scope boundaries of the biofloc system are defined in the diagram presented in Figure 5.2.

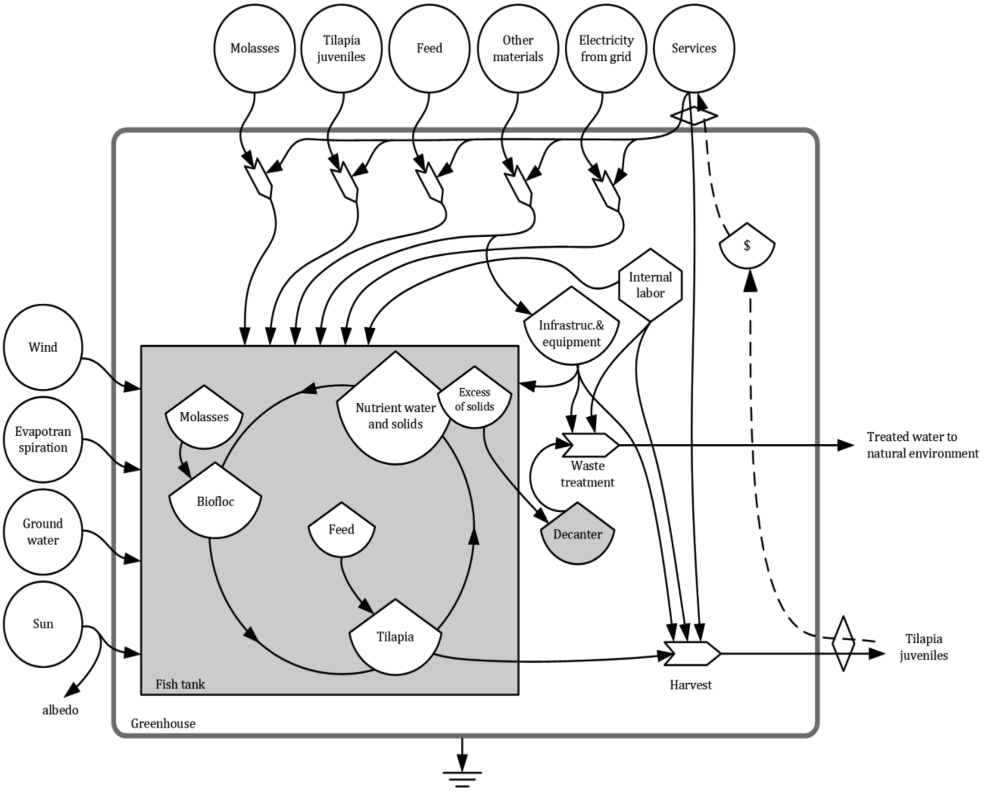

Figure 5.2. Energy diagram of the biofloc system to produce tilapia juveniles.

5.2.2.3 FLOCponics

FLOCponics was run in an on-demand coupled system layout (also called decoupled system) and consisted of a fish tank, a radial flow settler (decanter), a bag filter and two hydroponics tanks. In this layout, the water and nutrients were not constantly shared between the aquaculture biofloc-based and hydroponics subsystems. Instead, the nutrient-rich water from the biofloc subsystem flows to the hydroponics depending on the vegetables' demands for water and nutrients (Pinho et al. 2021b). Thus, once a day, the water from the fish tank underwent decantation and filtration process in the radial flow settler and bag filter, respectively, before being directed to the plant tanks. The volume of water streamed from the biofloc to the hydroponics subsystems was equal to the evapotranspiration losses in the hydroponics tanks.

The same feeding and C:N ratio management for fish culture were performed in the biofloc and FLOCponics systems. We also assumed the same amount of sludge discharge in both systems. In the hydroponics subsystem, the effluent from the biofloc subsystem was the main resource for lettuce nutrition and irrigation. Yet, when the electrical conductivity of the hydroponics tank water did not reach the desired value (approximately 1.7 mS cm^{-1}) the same compound commercial fertilizer was added. The scope boundaries of the biofloc system are defined in the diagram presented in Figure 5.3.

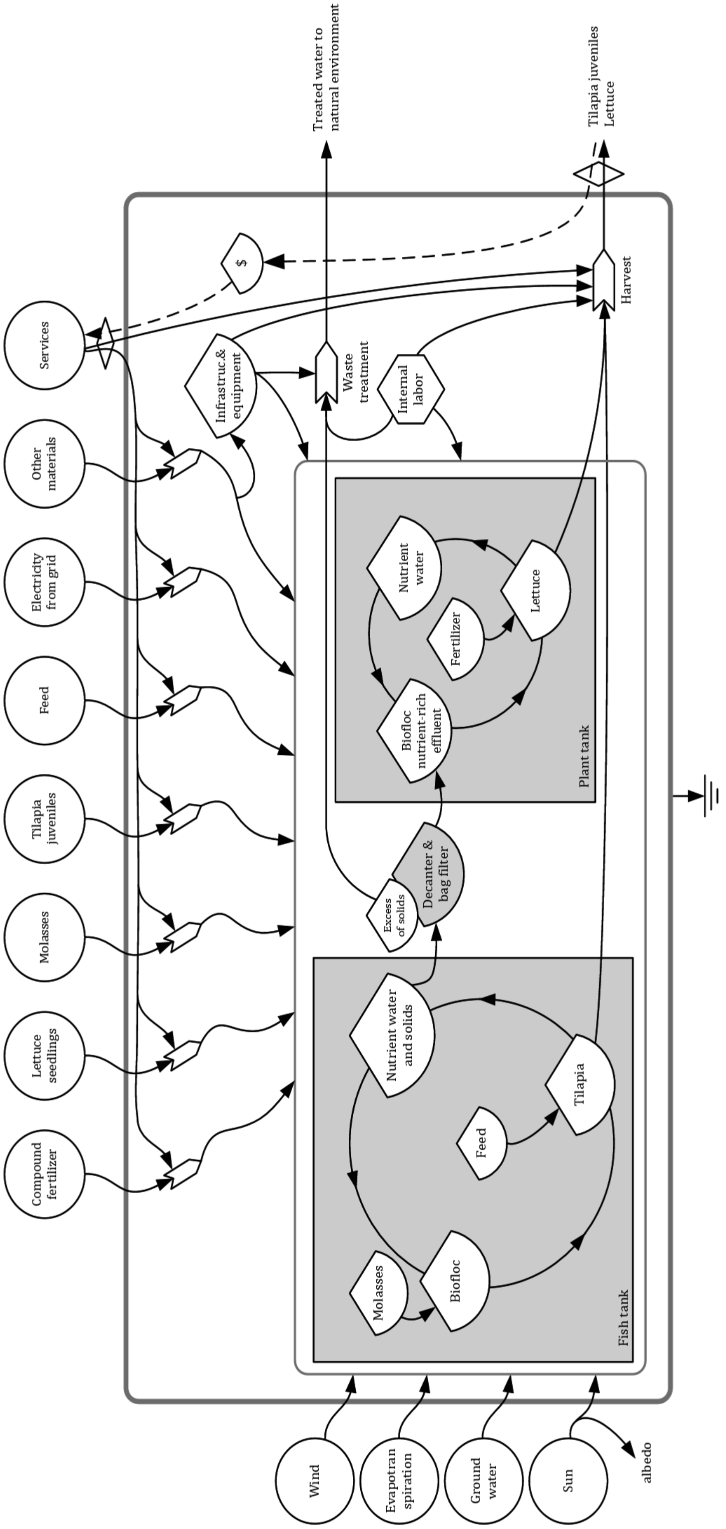

Figure 5.3. Energy diagram of the FLOCponics system to produce tilapia juveniles and lettuce.

5.2.3 Emergy synthesis

The emergy synthesis (ES) was the method chosen to measure the sustainability of the hydroponics, biofloc, and FLOCponics systems. ES methodology includes the spatiotemporal boundaries of the analysed production system and its emergy baseline, identifying all the necessary resources inputs and classifying them into renewable, non-renewable and resources from the larger economy. After this classification, inputs were quantified and converted into solar emjoules (sej, emergy unit). Subsequently, the emergy flow, system outcomes, and emergy indicators were calculated (Brown and Ulgiati 2004b). In this study, all UEVs (Unit Emergy Value) used are according to the 1.20E+25 sej year^{-1} baseline (Brown et al. 2016).

The emergy indicators allow identifying and discussing the distinctions among the three production systems in terms of functional characteristics, including environmental sustainability, resource utilization efficiency, production pressure load on the resource, degree of renewability of the system, and the origin of the resources it is based on. The indicators also support the choice of the system with the best environmental performance, identification of which managements harm the system's sustainability and suggestions for alternatives. The emergy indicators used in this synthesis considered the partial renewability of each input to properly measure the sustainability of the systems (see formulas in Table 5.2) (David et al. 2021a). The Unit Emergy Value (UEV) represents the quantity of energy embodied in the output. This indicator measures the amount of emergy necessary to produce a certain amount of energy. Since UEV is defined by the inverse relation of efficiency, the higher the UEV, the lower the system efficiency. Renewability (%R) shows the fraction of renewable resources in relation to the total emergy used. This indicator is used to determine the degree of sustainability of production systems. Emergy Yield Ratio (EYR) is the ratio between the total emergy and the emergy resources from the larger economy. EYR measures how much an investment enables a production system to exploit local resources to further contribute to the economy. Emergy Investment Ratio (EIR) assesses how the ecosystem responds to an investment of resources from the larger economy. EIR permits comparing alternatives inputs that use the same natural resource. The environmental loading ratio (ELR) measures the stress that the system causes on the environment. A value below 2 indicates low stress, values from 2 to 10 a moderate stress, and values above 10 indicate high stress on the ecosystem. The emergy sustainability index (ESI) is the ratio between EYR and ELR. This indicator shows the potential contribution of a resource or process to the economy per unit of environmental loading generated.

Table 5.2. Emergy indicators used in this synthesis study and their formulas.

Indicator		**Formula**
UEV	Unit Emergy Value	Emergy/Output
%R	Renewability	100*(R+Mr+Sr)/Y
EYR	Emergy Yield Ratio	Y/F
EIR	Emergy Investment Ratio	(Mn+Sn)/(R+N+Mr+Sr)
ELR	Environmental Loading Ratio	(N+Mn+Sn)/(R+Mr+Sr)
ESI	Emergy Sustainability Index	EYR/ELR

R: renewable natural resources; N: non-renewable natural resources; F: Resources from the larger economy; Mr: renewable materials; Mn: non-renewable materials; Sr: renewable services; Sn: non-renewable services; Y: total emergy. The lowercase letters r and n mean, respectively, renewable, and non-renewable fractions of material and services.

5.3 Results

The FLOCponics system has the highest emergy demand (1.35E+15 sej m^{-2} $year^{-1}$), followed by biofloc (1.31E+15 sej m^{-2} $year^{-1}$), and hydroponics (1.32E+15 sej m^{-2} $year^{-1}$) (see Supplementary Materials Tables S5.1, S5.2, S5.3 and the calculations in Tables S5.4, S5.5, and S5.6). Resources from the larger economy account for more than 50% of emergy demanded in all systems, due to the equipment used to measure the physical-chemical parameters of the water (multiparameter) and maintain constant aeration in the tanks (air blower) (Figure 5.4). Electricity was the second input that demanded large amounts of emergy (>30%) in all systems. Overall, on the one hand, most of the emergy indicators were similar for all systems (Table 5.3). On the other hand, the unit emergy values (UEV) differ (Table 5.3). The UEV found for hydroponics were 10^4 times higher than the UEV of FLOCponics and biofloc, while FLOCponics was almost twice the value of biofloc system.

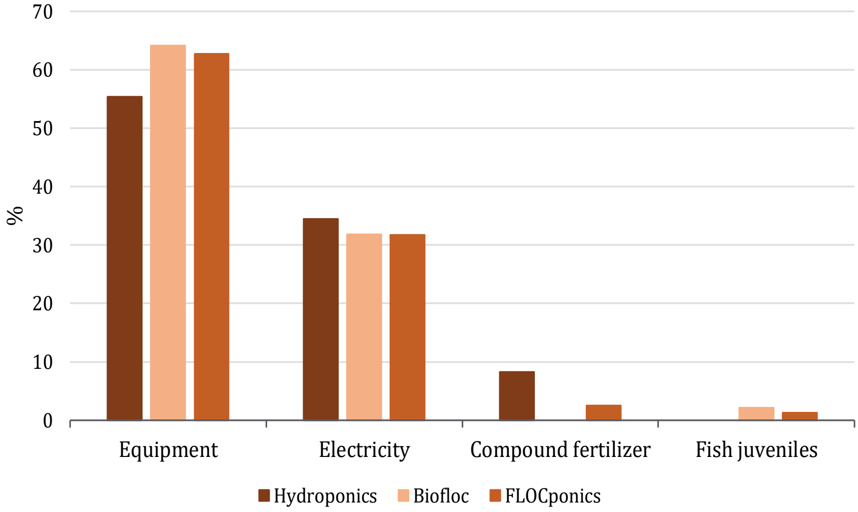

Figure 5.4. Contribution of the main inputs to the total emergy flow for the different systems.

Table 5.3. Emergy indicators found for the different systems evaluated.

Emergy indicators		**Hydroponics**	**Biofloc**	**FLOCponics**
UEV	Unit emergy values (sej J^{-1})	5.55E+10	1.42E+06	2.54E+06
%R	Renewability	23.6	21.7	21.7
EYR	Emergy yield ratio	1.0	1.0	1.0
EIR	Emergy investment ratio	3.1	3.6	3.6
ELR	Environmental loading ratio	3.1	3.6	3.6
ESI	Emergy sustainability index	0.3	0.3	0.3

5.4 Discussion

Recent research has focused on developing FLOCponics systems as a promising modern integrated agri-aquaculture system to improve the sustainable character of biofloc-based aquaculture and hydroponics food production. However, no scientific-based evidence has been presented to date to support (or deny) the narrative that FLOCponics is a more sustainable food production. This study is the first step to fill this gap, as we applied emergy synthesis to assess the sustainability of producing tilapia juveniles and lettuce in FLOCponics systems compared to stand-alone biofloc and hydroponics systems.

The emergy indicator results are a reflection of the particular management practices adopted in each production system (Brown and Ulgiati 1997, 2004c). Yet, even with different amounts and types of input and distinct operational characteristics, the findings of this theoretical experiment-based study show that the three evaluated systems have similar emergy performance. Such similarity reveals that the reuse of nutrients and water in FLOCponics was not enough to boost the sustainability of the integrated system compared to stand-alone hydroponics and biofloc systems when running on small scale (experimental) systems setups. The effect of the systems' size can be seen in the renewability results. The renewability values found for all systems (~23%) are lower than the 33% recently reported for tilapia fingerlings production in a commercial scale biofloc-based aquaculture farm, also located in São Paulo, Brazil (David et al. 2021c). It should be expected that, by expanding the system to commercial size, the emergy demand for equipment would be optimized, and the use of renewable resources would increase, such as electricity from hydropower, both positively affecting the renewability result of all evaluated systems. Another possibility to increase renewability is changing the source of water, for instance, from groundwater to springwater or rainwater. Springwater and rainwater are considered renewable water sources, as nature's effort to replenish them is lower than groundwater. Thus, in FLOCponics and other food production systems, replacing groundwater by these other sources should be encouraged.

The other emergy indicators suggest that FLOCponics, biofloc and hydroponics systems use a low amount of natural renewable resources (EIR), cause a moderate environmental load on the input sources (ELR) and an environmental stress seven times higher than the contribution to the economy (ESI). All these results rely on the fact that the evaluated systems highly depend on resources from the large economy (EYR). The dependence on resources from the large economy has been a recurrent finding in emergy synthesis of intensive food production systems (David et al. 2021a). This is because intensive food production systems consistently require this type of resources for infrastructure (e.g., fish tanks, filters, greenhouse, etc.), equipment, specialized labour, and especially electricity (Ghamkhar et al. 2020). Our results show that the high contribution of these inputs to the total emergy flow is also a concern for intensive integrated biofloc-based agri-aquaculture systems.

In emergy synthesis, the efficiency is measured by the production system's ability to incorporate energy into the product through the production process, given as unit emergy value (UEV). Although most of the emergy indicators were similar between the systems, the UEVs found suggest a different path. Compared to the hydroponics system, FLOCponics is much more efficient due to embodied energy in the fish produced. On the other hand, FLOCponics is not as efficient as the stand-alone biofloc system. The difference between the UEV of FLOCponics (2.54E+06 sej J^{-1}) and biofloc (1.42E+06 sej J^{-1}) systems are much smaller compared to the values found for hydroponics (5.55E+10 sej J^{-1}). Still, the UEVs results stress the need for improvements in the FLOCponics subsystem before applying it on a large scale.

A relevant point for discussion is how to increase the efficiency of FLOCponics. Mainly considering that, apart from the UEVs, the overall emergy performance of FLOCponics was similar to stand-alone systems. The UEV results indicate the need for improvements. In general, replacing industrial resources with organic or natural resources will positively affect the sustainability of any food production system (Oliveira Neto et al. 2018), and it will also be the case for FLOCponics. In terms of practical solutions, based on the emergy synthesis outputs and literature review, we identified the following points that must be taken into account in further FLOCponics research to explore its full potential. Firstly, testing and validating different plant species with higher energetic values will very likely lower the UEV of FLOCponics. Lettuce is the main plant produced in freshwater FLOCponics studies (Pinho et al. 2021b), however, it is not capable of incorporating significant amounts of energy through the production process. Examples of plants to be tested are tomato, broccoli, spinach, among others. We speculated how a tomato production of 60 kg m^{-2} $year^{-1}$ (equals to 5.27E+07 J m^{-2} $year^{-1}$) would affect the UEV of FLOCponics and found a 10% reduction in the UEV compared to lettuce production in the same system setup, indicating improved efficiency. Secondly, the system design and operation must be optimized such that the resources in excess are wisely used. Investigating several system configurations may require costly investments in trials and analysis. Thus, a reasonable way to find optimal management and operation strategies is by applying mathematical models. Modelling has been widely used to predict and simulate complex food production systems aiming at achieving maximum efficiency in the use of resources (Lastiri et al. 2018; Keesman et al. 2019). Lastly, transforming the accumulated and discharged nutrient-rich solids from the biofloc subsystems into valuable co-products has a high potential to improve FLOCponics sustainability. Reusing the biofloc waste will, at first glance, decrease the need for a treatment system. Moreover, recent studies have shown that mineralized aquaculture sludge (solids) can be reused as fertilizer for plant nutrition (Delaide et al. 2019). Consequently, this nutrient recovery will decrease resource input from the large economy and boost FLOCponics systems circularity. Recovery of high-valued compounds from the biofloc waste could also be interesting to boost circularity. However, this route may need more advanced treatments and thus higher costs, but it may finally lead to higher sustainability indicators and probably higher profits.

All the proposed changes and further investigations to support the sustainable development of FLOCponics should not be costly and negatively affect the productive performance of the system. Additionally, the improvements must consider the characteristics of each location, such as the origin of the resources, the regional market of fish and vegetable species, and the technologies and professional know-how available. A higher economic benefit/cost of producing food in the FLOCponics system may be expected than in the stand-alone systems due to the higher variety and amount of biomass grown. Nevertheless, when FLOCponics moves beyond the initial stage of development, economic analyses should be combined with sustainability assessments to measure whether and how the system could be profitable.

In conclusion, from an emergy synthesis point of view, integrating tilapia production in a biofloc system with hydroponics lettuce culture is as sustainable as the stand-alone systems, except by the systems' efficiency. The UEV found for FLOCponics was 78% higher than for the biofloc system, indicating that improvements still need to be made. We must emphasize that the results presented are restricted to the conditions evaluated (experimental/small-scale). Nevertheless, our findings are not trivial since they provide valuable insights regarding the (un)sustainable aspects of FLOCponics and direct further research to improve the system's emergy performance. FLOCponics can be considered a promising sustainable food production approach, mainly considering that it is a system under development and, as indicated above, there are still many opportunities for improvements.

Supplementary Materials

Table S5.1. Emergy table of **hydroponics system.**

Note	Item	Unit	Amount (unit m^{-2} yr^{-1})	UEV (sej unit^{-1})	Emergy (sej m^{-2} yr^{-1})	Emergy (%)
Renewable natural resources (R)						
1	Sun	J	4.20E+09	1.00E+00	4.20E+09	0.0
2	Wind	J	1.38E+05	8.00E+02	1.11E+08	0.0
3	Evapotranspiration	J	3.56E+06	2.88E+04	1.02E+11	0.0
Total (R)					1.07E+11	
Non-renewable natural resources (N)						
	None	-	-	-	-	
Resources from the larger economy (F)						
	Renewable materials (Mr)					
4	Electricity from grid	J	2.76E+09	1.12E+05	3.09E+14	23.5
	Materials for the greenhouse					
5	Iron	g	6.30E+01	3.56E+09	2.24E+11	0.0
	Non-renewable materials (Mn)					
6	Electricity from grid	J	1.30E+09	1.12E+05	1.45E+14	11.0
7	Groundwater	m^3	3.52E+00	1.04E+12	3.66E+12	0.3
8	Vegetable seedlings	kg	6.29E+06	5.96E+04	3.75E+11	0.0
9	Compound fertilizer	g	3.03E+03	3.57E+10	1.08E+14	8.2
	Materials for the greenhouse and waste treatment					
10	Iron	g	3.70E+01	3.56E+09	1.32E+11	0.0
11	Plastic	g	3.13E+03	4.19E+09	1.31E+13	1.0
	Renewable services (Sr)					
12	Skilled labor	h	3.95E+04	2.10E+07	8.28E+11	0.1
13	Non-skilled labor	J	7.89E+04	3.27E+06	2.58E+11	0.0
	Non-renewable services (Sn)					
14	Skilled labor	h	2.20E+05	2.10E+07	4.62E+12	0.4
15	Non-skilled labor	J	4.40E+05	3.27E+06	1.44E+12	0.1
16	Equipment	USD	1.30E+02	5.60E+12	7.29E+14	55.4
Total (N+F)					1.32E+15	
Total emergy (Y=R+N+F)					1.32E+15	
Outputs (O)						
17	Vegetables	J	2.37E+04			

Table S5.2. Emergy table of **biofloc system.**

Note	Item	Unit	Amount (unit m^{-2} yr^{-1})	UEV (sej $unit^{-1}$)	Emergy (sej m^{-2} yr^{-1})	Emergy (%)
Renewable natural resources (R)						
1	Sun	J	4.20E+09	1.00E+00	4.20E+09	0.0
2	Wind	J	1.38E+05	8.00E+02	1.11E+08	0.0
3	Evapotranspiration	J	3.65E+06	2.88E+04	1.05E+11	0.0
Total (R)					1.10E+11	
Non-renewable natural resources (N)						
	None	-	-	-	-	
Resources from the larger economy (F)						
	Renewable materials (Mr)					
4	Electricity from grid	J	2.53E+09	1.12E+05	2.82E+14	21.6
	Materials for the greenhouse					
5	Iron	g	6.30E+01	3.56E+09	2.24E+11	0.0
	Non-renewable materials (Mn)					
6	Electricity from grid	J	1.19E+09	1.12E+05	1.33E+14	10.2
7	Groundwater	m^3	8.95E-01	1.04E+12	9.31E+11	0.1
8	Fish juveniles	J	3.98E+07	7.15E+05	2.84E+13	2.2
9	Feed	J	5.07E+05	9.96E+04	5.05E+10	0.0
10	Molasses	kg	1.95E+00	4.87E+12	9.50E+12	0.7
	Materials for the greenhouse and waste treatment					
11	Iron	g	3.70E+01	3.56E+09	1.32E+11	0.0
12	Plastic	g	2.13E+03	4.19E+09	8.93E+12	0.7
	Renewable services (Sr)					
13	Skilled labor	J	2.30E+04	2.10E+07	4.83E+11	0.0
14	Non-skilled labor	J	4.60E+04	3.27E+06	1.51E+11	0.0
	Non-renewable services (Sn)					
15	Skilled labor	J	1.28E+05	2.10E+07	2.70E+12	0.2
16	Non-skilled labor	J	2.57E+05	3.27E+06	8.40E+11	0.1
17	Equipment	USD	1.50E+02	5.60E+12	8.38E+14	64.2
Total (N+F)					1.31E+15	
Total emergy (Y=R+N+F)					1.31E+15	
Outputs (O)						
18	Fish	J	9.18E+08			

Table S5.3. Emergy table of **FLOCponics system.**

Note	Item	Unit	Amount (unit m^{-2} yr^{-1})	UEV (sej $unit^{-1}$)	Emergy (sej m^{-2} yr^{-1})	Emergy (%)
Renewable natural resources (R)						
1	Sun	J	4.20E+09	1.00E+00	4.20E+09	0.0
2	Wind	J	1.38E+05	8.00E+02	1.11E+08	0.0
3	Evapotranspiration	J	7.21E+06	2.88E+04	2.08E+11	0.0
Total (R)					2.12E+11	
Non-renewable natural resources (N)						
	None	-	-	-	-	
Resources from the larger economy (F)						
	Renewable materials (Mr)					
4	Electricity from grid	J	2.62E+09	1.12E+05	2.92E+14	21.6
	Materials for the greenhouse					
5	Iron	g	6.30E+01	3.56E+09	2.24E+11	0.0
	Non-renewable materials (Mn)					
6	Electricity from grid	J	1.23E+09	1.12E+05	1.38E+14	10.2
7	Groundwater	m^3	9.47E-01	1.04E+12	9.85E+11	0.1
8	Vegetable seedlings	kg	2.32E+06	5.96E+04	1.38E+11	0.0
9	Fish juveniles	J	2.51E+07	7.15E+05	1.80E+13	1.3
10	Feed	J	3.63E+05	9.96E+04	3.61E+10	0.0
11	Molasses	kg	1.95E+00	4.87E+12	9.50E+12	0.7
12	Compound fertilizer	g	9.60E+02	3.57E+10	3.43E+13	2.5
	Materials for the greenhouse and waste treatment					
13	Iron	g	3.70E+01	3.56E+09	1.32E+11	0.0
14	Plastic	g	2.13E+03	4.19E+09	8.93E+12	0.7
	Renewable services (Sr)					
15	Skilled labor	J	1.62E+05	2.10E+07	6.10E+11	0.0
16	Non-skilled labor	J	4.36E+04	3.27E+06	1.43E+11	0.0
	Non-renewable services (Sn)					
17	Skilled labor	J	1.62E+05	2.10E+07	7.95E+11	0.1
18	Non-skilled labor	J	2.43E+05	3.27E+06	7.95E+11	0.1
19	Equipment	USD	1.52E+02	5.60E+12	8.49E+14	62.7
Total (N+F)					1.35E+15	
Total emergy (Y=R+N+F)					1.35E+15	
Outputs (O)						

20	Vegetables	J	1.72E+01
21	Fish	J	5.32E+08

Table S5.4. Calculations for **hydroponics system**.

Note	Item	Value	Unit	Reference
1	**Sun**			
	Insolation	7.00E+09	J m^{-2} year^{-1}	Unesp (2021)
	Solar transmittance coefficient	60.00%	%	Pinho et al. (2021c)
	Annual flow	4.20E+09	J m^{-2} year^{-1}	
	UEV	1.00E+00	sej J^{-1}	By definition
	Emergy	4.20E+09	sej m^{-2} year^{-1}	
2	**Wind**			
	Density of air	1.30E+00	kg m^{-3}	
	Drag coefficient	1.00E-03		
	Wind velocity	1.50E+00	m s^{-1}	Unesp (2021)
	Time	3.15E+07	s	
	Annual flow	1.38E+05	J m^{-2} year^{-1}	
	UEV	8.00E+02	sej J^{-1}	Brown and Ulgiati (2016)
	Emergy	1.11E+08	sej m^{-2} year^{-1}	
3	**Evapotranspiration**			
	Transpiration	7.50E+02	kg m^{-2} year^{-1}	
	Gibbs free energy	4.74E+03	J kg^{-1}	
	Annual flow	3.56E+06	J m^{-2} year^{-1}	
	UEV	2.88E+04	sej J^{-1}	Asgharipour et al. (2020)
	Emergy	1.02E+11	sej m^{-2} year^{-1}	
4	**Electricity from grid**			
	Consumption	9.48E+02	kWh year^{-1}	
	Conversion	3.60E+06	J kWh^{-1}	
	Renewable fraction	68.00%	%	Giannetti et al. (2015)
	Annual flow	2.76E+09	J m^{-2} year^{-1}	
	UEV	1.12E+05	sej J^{-1}	Giannetti et al. (2015)
	Emergy	3.09E+14	sej m^{-2} year^{-1}	
5	**Iron**			
	Quantity	1.00E+03	g m^{-2}	

	Renewable fraction	63.00%	%	Oliveira et al. (2018)
	Annual flow	6.30E+01	g m^{-2} year^{-1}	
	UEV	3.56E+09	sej g^{-2}	Odum (2002)
	Emergy	2.24E+11	sej m^{-2} year^{-1}	
6	**Electricity from grid**			
	Consumption	9.48E+02	kWh year^{-1}	
	Conversion	3.60E+06	J kWh^{-1}	
	Non-renewable fraction	32.00%	%	Giannetti et al. (2015)
	Annual flow	1.30E+09	J m^{-2} year^{-1}	
	UEV	1.12E+05	sej J^{-1}	Giannetti et al. (2015)
	Emergy	1.45E+14	sej m^{-2} year^{-1}	
7	**Groundwater**			
	Initial water supply	2.09E+00	m^{3} year^{-1}	
	Replacement water	8.70E-01	m^{3} year^{-1}	
	Annual flow	3.52E+00	m^{3} m^{-2} year^{-1}	
	UEV	1.04E+12	sej m^{-3}	Buenfil (2001)
	Emergy	3.66E+12	sej m^{-2} year^{-1}	
8	**Vegetable seedlings**			
	Seedlings	3.30E+02	kg	
	Seedlings weight	0.001	kg unit^{-1}	
	Conversion	1.60E+07	J kg^{-1}	Nan et al. (2020)
	Annual flow	6.29E+06	J m^{-2} year^{-1}	
	UEV	5.96E+04	sej J^{-1}	Zhang et al. (2007)
	Emergy	3.75E+11	sej m^{-2} year^{-1}	
9	**Compound fertilizer**			
	Annual flow	3.03E+03	g m^{-2} year^{-1}	
	UEV	3.57E+10	sej g^{-1}	Chen et al. (2020)
	Emergy	1.08E+14	sej m^{-2} year^{-1}	
10	**Iron**			
	Quantity	1.00E+03	g m^{-2}	
	Non-renewable fraction	37.00%	%	Oliveira et al. (2018)
	Annual flow	3.70E+01	g m^{-2} year^{-1}	
	UEV	3.56E+09	sej g^{-1}	Odum (2002)
	Emergy	1.32E+11	sej m^{-2} year^{-1}	
11	**Plastic**			
	Annual flow	3.13E+03	g m^{-2} year^{-1}	

	UEV	4.19E+09	sej g^{-1}	Odum (2002)
	Emergy	1.31E+13	sej m^{-2} $year^{-1}$	
12	**Skilled labor**			
	Man-hours	1.83E+02	hours $year^{-1}$	
	Conversion to kcal	2.85E-01	kcal $year^{-1}$	
	Conversion to J	4.19E+03	J $kcal^{-1}$	
	Renewable fraction	15.20%	%	Giannetti et al. (2015)
	Annual flow	3.95E+04	J m^{-2} $year^{-1}$	
	UEV	2.10E+07	sej J^{-1}	Oliveira et al. (2018)
	Emergy	8.28E+11	sej m^{-2} $year^{-1}$	
13	**Non-skilled labor**			
	Man-hours	3.65E+02	hours $year^{-1}$	
	Conversion to kcal	2.85E-01	kcal $year^{-1}$	
	Conversion to J	4.19E+03	J $kcal^{-1}$	
	Renewable fraction	15.20%	%	Giannetti et al. (2015)
	Annual flow	7.89E+04	J m^{-2} $year^{-1}$	
	UEV	3.27E+06	sej J^{-1}	Oliveira et al. (2018)
	Emergy	2.58E+11	sej m^{-2} $year^{-1}$	
14	**Skilled labor**			
	Man-hours	1.83E+02	hours $year^{-1}$	
	Conversion to kcal	2.85E-01	kcal $year^{-1}$	
	Conversion to J	4.19E+03	J $kcal^{-1}$	
	Non-renewable fraction	84.80%	%	Giannetti et al. (2015)
	Annual flow	2.20E+05	J m^{-2} $year^{-1}$	
	UEV	2.10E+07	sej J^{-1}	Oliveira et al. (2018)
	Emergy	4.62E+12	sej m^{-2} $year^{-1}$	
15	**Non-skilled labor**			
	Man-hours	3.65E+02	hours $year^{-1}$	
	Conversion to kcal	2.85E-01	kcal $year^{-1}$	
	Conversion to J	4.19E+03	J $kcal^{-1}$	
	Non-renewable fraction	84.80%	%	Giannetti et al. (2015)
	Annual flow	4.40E+05	J m^{-2} $year^{-1}$	
	UEV	3.27E+06	sej J^{-1}	Oliveira et al. (2018)
	Emergy	1.44E+12	sej m^{-2} $year^{-1}$	
16	**Equipment**			
	Depreciation	1.30E+02	USD m^{-2} $year^{-1}$	

	UEV	5.60E+12	sej USD^{-1}	Giannetti et al. (2018)
	Emergy	7.29E+14	sej m^{-2} year^{-1}	
17	**Vegetables**			
	Total production	3.78E+01	kg m^{-2} year^{-1}	
	Conversion to kcal	1.50E-01	kcal kg^{-1}	
	Conversion from kcal to J	4.18E+03	J kcal^{-1}	
	Annual flow	2.37E+04	J m^{-2} year^{-1}	

Table S5.5. Calculations for **biofloc system**.

Note	Item	Value	Unit	Reference
1	**Sun**			
	Insolation	7.00E+09	J m^{-2} year^{-1}	Unesp (2021)
	Solar transmittance coefficient	60.00%	%	Pinho et al. (2021c)
	Annual flow	4.20E+09	J m^{-2} year^{-1}	
	UEV	1.00E+00	sej J^{-1}	By definition
	Emergy	4.20E+09	sej m^{-2} year^{-1}	
2	**Wind**			
	Density of air	1.30E+00	kg m^{-3}	
	Drag coefficient	1.00E-03		
	Wind velocity	1.50E+00	m s^{-1}	Unesp (2021)
	Time	3.15E+07	s	
	Annual flow	1.38E+05	J m^{-2} year^{-1}	
	UEV	8.00E+02	sej J^{-1}	Brown and Ulgiati (2016)
	Emergy	1.11E+08	sej m^{-2} year^{-1}	
3	**Evapotranspiration**			
	Transpiration	7.71E+02	kg m^{-2} year^{-1}	
	Gibbs free energy	4.74E+03	J kg^{-1}	
	Annual flow	3.65E+06	J m^{-2} year^{-1}	
	UEV	2.88E+04	sej J^{-1}	Asgharipour et al. (2020)
	Emergy	1.05E+11	sej m^{-2} year^{-1}	
4	**Electricity from grid**			
	Consumption	1.49E+03	kWh year^{-1}	
	Conversion	3.60E+06	J kWh^{-1}	

	Renewable fraction	68.00%	%	Giannetti et al. (2015)
	Annual flow	2.53E+09	J m^{-2} year^{-1}	
	UEV	1.12E+05	sej J^{-1}	Giannetti et al. (2015)
	Emergy	2.82E+14	sej m^{-2} year^{-1}	
5	**Iron**			
	Quantity	1.00E+03	g m^{-2}	
	Renewable fraction	63.00%	%	Oliveira et al. (2018)
	Annual flow	6.30E+01	g m^{-2} year^{-1}	
	UEV	3.56E+09	sej g^{-2}	Odum (2002)
	Emergy	2.24E+11	sej m^{-2} year^{-1}	
6	**Electricity from grid**			
	Consumption	1.49E+03	kWh year^{-1}	
	Conversion	3.60E+06	J kWh^{-1}	
	Non-renewable fraction	32.00%	%	Giannetti et al. (2015)
	Annual flow	1.19E+09	J m^{-2} year^{-1}	
	UEV	1.12E+05	sej J^{-1}	Giannetti et al. (2015)
	Emergy	1.33E+14	sej m^{-2} year^{-1}	
7	**Groundwater**			
	Initial water supply	4.80E-01	m^{3} year^{-1}	
	Replacement water	8.09E-01	m^{3} year^{-1}	
	Annual flow	8.95E-01	m^{3} m^{-2} year^{-1}	
	UEV	1.04E+12	sej m^{-3}	Buenfil (2001)
	Emergy	9.31E+11	sej m^{-2} year^{-1}	
8	**Fish juveniles**			
	Stocked fish	1.96E+03	unit year^{-1}	
	Fish weight	1.40E+00	g	
	Conversion to kcal	5.00E+00	kcal g^{-1}	
	Conversion from kcal to J	4.19E+03	J kcal^{-1}	
	Annual flow	3.98E+07	J m^{-2} year^{-1}	
	UEV	7.15E+05	sej J^{-1}	Brown and Bardi (2001)
	Emergy	2.84E+13	sej m^{-2} year^{-1}	
9	**Feed**			
	Consumed feed	5.03E+01	kg year^{-1}	
	Feed energy	1.45E+04	J kg^{-1}	
	Annual flow	5.07E+05	J m^{-2} year^{-1}	
	UEV	9.96E+04	sej J^{-1}	Brown and Bardi (2001)

	Emergy	5.05E+10	sej m^{-2} year^{-1}	
10	**Molasses**			
	Annual flow	1.95	kg m^{-2} year^{-1}	
	UEV	4.87E+12	sej kg^{-1}	Brown and Ulgiati (2004b)
	Emergy	9.49903E+12	sej m^{-2} year^{-1}	
11	**Iron**			
	Quantity	1.00E+03	g m^{-2}	
	Non-renewable fraction	37.00%	%	Oliveira et al. (2018)
	Annual flow	3.70E+01	g m^{-2} year^{-1}	
	UEV	3.56E+09	sej g^{-1}	Odum (2002)
	Emergy	1.32E+11	sej m^{-2} year^{-1}	
12	**Plastic**			
	Annual flow	2.13E+03	g m^{-2} year^{-1}	
	UEV	4.19E+09	sej g^{-1}	Odum (2002)
	Emergy	8.93E+12	sej m^{-2} year^{-1}	
13	**Skilled labor**			
	Man-hours	1.83E+02	hours year^{-1}	
	Conversion to kcal	2.85E-01	kcal year^{-1}	
	Conversion to J	4.19E+03	J kcal^{-1}	
	Renewable fraction	15.20%	%	Giannetti et al. (2015)
	Annual flow	2.30E+04	J m^{-2} year^{-1}	
	UEV	2.10E+07	sej J^{-1}	Oliveira et al. (2018)
	Emergy	4.83E+11	sej m^{-2} year^{-1}	
14	**Non-skilled labor**			
	Man-hours	3.65E+02	hours year^{-1}	
	Conversion to kcal	2.85E-01	kcal year^{-1}	
	Conversion to J	4.19E+03	J kcal^{-1}	
	Renewable fraction	15.20%	%	Giannetti et al. (2015)
	Annual flow	4.60E+04	J m^{-2} year^{-1}	
	UEV	3.27E+06	sej J^{-1}	Oliveira et al. (2018)
	Emergy	1.51E+11	sej m^{-2} year^{-1}	
15	**Skilled labor**			
	Man-hours	1.83E+02	hours year^{-1}	
	Conversion to kcal	2.85E-01	kcal year^{-1}	
	Conversion to J	4.19E+03	J kcal^{-1}	

	Non-renewable fraction	84.80%	%	Giannetti et al. (2015)
	Annual flow	1.28E+05	J m^{-2} year^{-1}	
	UEV	2.10E+07	sej J^{-1}	Oliveira et al. (2018)
	Emergy	2.70E+12	sej m^{-2} year^{-1}	
16	**Non-skilled labor**			
	Man-hours	3.65E+02	hours year^{-1}	
	Conversion to kcal	2.85E-01	kcal year^{-1}	
	Conversion to J	4.19E+03	J kcal^{-1}	
	Non-renewable fraction	84.80%	%	Giannetti et al. (2015)
	Annual flow	2.57E+05	J m^{-2} year^{-1}	
	UEV	3.27E+06	sej J^{-1}	Oliveira et al. (2018)
	Emergy	8.40E+11	sej m^{-2} year^{-1}	
17	**Equipment**			
	Depreciation	1.50E+02	USD m^{-2} year^{-1}	
	UEV	5.60E+12	sej USD^{-1}	Giannetti et al. (2018)
	Emergy	8.38E+14	sej m^{-2} year^{-1}	
18	**Fish**			
	Total production	4.39E+04	g m^{-2} year^{-1}	
	Conversion to kcal	5.00E+00	kcal g^{-1}	
	Conversion from kcal to J	4.19E+03	J kcal^{-1}	
	Annual flow	9.18E+08	J m^{-2} year^{-1}	

Table S5.6. Calculations for **FLOCponics system**.

Note	Item	Value	Unit	Reference
1	**Sun**			
	Insolation	7.00E+09	J m^{-2} year^{-1}	Unesp (2021)
	Solar transmittance coefficient	60.00%	%	Pinho et al. (2021c)
	Annual flow	4.20E+09	J m^{-2} year^{-1}	
	UEV	1.00E+00	sej J^{-1}	By definition
	Emergy	4.20E+09	sej m^{-2} year^{-1}	
2	**Wind**			
	Density of air	1.30E+00	kg m^{-3}	

	Drag coefficient	1.00E-03		
	Wind velocity	1.50E+00	m s^{-1}	Unesp (2021)
	Time	3.15E+07	s	
	Annual flow	1.38E+05	J m^{-2} year^{-1}	
	UEV	8.00E+02	sej J^{-1}	Brown and Ulgiati (2016)
	Emergy	1.11E+08	sej m^{-2} year^{-1}	
3	**Evapotranspiration**			
	Transpiration	1.52E+03	kg m^{-2} year^{-1}	
	Gibbs free energy	4.74E+03	J kg^{-1}	
	Annual flow	7.21E+06	J m^{-2} year^{-1}	
	UEV	2.88E+04	sej J^{-1}	Asgharipour et al. (2020)
	Emergy	2.08E+11	sej m^{-2} year^{-1}	
4	**Electricity from grid**			
	Consumption	2.44E+03	kWh year^{-1}	
	Conversion	3.60E+06	J kWh^{-1}	
	Renewable fraction	68.00%	%	Giannetti et al. (2015)
	Annual flow	2.62E+09	J m^{-2} year^{-1}	
	UEV	1.12E+05	sej J^{-1}	Giannetti et al. (2015)
	Emergy	2.92E+14	sej m^{-2} year^{-1}	
5	**Iron**			
	Quantity	1.00E+03	g m^{-2}	
	Renewable fraction	63.00%	%	Oliveira et al. (2018)
	Annual flow	6.30E+01	g m^{-2} year^{-1}	
	UEV	3.56E+09	sej g^{-2}	Odum (2002)
	Emergy	2.24E+11	sej m^{-2} year^{-1}	
6	**Electricity from grid**			
	Consumption	2.44E+03	kWh year^{-1}	
	Conversion	3.60E+06	J kWh^{-1}	
	Non-renewable fraction	32.00%	%	Giannetti et al. (2015)
	Annual flow	1.23E+09	J m^{-2} year^{-1}	
	UEV	1.12E+05	sej J^{-1}	Giannetti et al. (2015)
	Emergy	1.38E+14	sej m^{-2} year^{-1}	
7	**Groundwater**			
	Initial water supply	6.00E-01	m^{3} year^{-1}	
	Replacement water	1.56E+00	m^{3} year^{-1}	
	Annual flow	9.47E-01	m^{3} m^{-2} year^{-1}	

	UEV	1.04E+12	sej m^{-3}	Buenfil (2001)
	Emergy	9.85E+11	sej m^{-2} year^{-1}	
8	**Vegetable seedlings**			
	Seedlings	3.30E+02	kg	
	Seedlings weight	0.001	kg unit^{-1}	
	Conversion	1.60E+07	J kg^{-1}	Nan et al. (2020)
	Annual flow	2.32E+06	J m^{-2} year^{-1}	
	UEV	5.96E+04	sej J^{-1}	Zhang et al. (2007)
	Emergy	1.38E+11	sej m^{-2} year^{-1}	
9	**Fish juveniles**			
	Stocked fish	1.96E+03	unit year^{-1}	
	Fish weight	1.40E+00	g	
	Conversion to kcal	5.00E+00	kcal g^{-1}	
	Conversion from kcal to J	4.19E+03	J kcal^{-1}	
	Annual flow	2.51E+07	J m^{-2} year^{-1}	
	UEV	7.15E+05	sej J^{-1}	Brown and Bardi (2001)
	Emergy	1.80E+13	sej m^{-2} year^{-1}	
10	**Feed**			
	Consumed feed	5.70E+01	kg year^{-1}	
	Feed energy	1.45E+04	J kg^{-1}	
	Annual flow	3.63E+05	J m^{-2} year^{-1}	
	UEV	9.96E+04	sej J^{-1}	Brown and Bardi (2001)
	Emergy	3.61E+10	sej m^{-2} year^{-1}	
11	**Molasses**			
	Annual flow	1.95E+00	kg m^{-2} year^{-1}	
	UEV	4.87E+12	sej kg^{-1}	Brown and Ulgiati (2004b)
	Emergy	9.50E+12	sej m^{-2} year^{-1}	
12	**Compound fertilizer**			
	Annual flow	9.60E+02	g m^{-2} year^{-1}	
	UEV	3.57E+10	sej g^{-1}	Chen et al. (2020)
	Emergy	3.43E+13	sej m^{-2} year^{-1}	
13	**Iron**			
	Quantity	1.00E+03	g m^{-2}	
	Non-renewable fraction	37.00%	%	Oliveira et al. (2018)
	Annual flow	3.70E+01	g m^{-2} year^{-1}	
	UEV	3.56E+09	sej g^{-1}	Odum (2002)

	Emergy	1.32E+11	sej m^{-2} year^{-1}	
14	**Plastic**			
	Annual flow	2.13E+03	g m^{-2} year^{-1}	
	UEV	4.19E+09	sej g^{-1}	Odum (2002)
	Emergy	8.93E+12	sej m^{-2} year^{-1}	
15	**Skilled labor**			
	Man-hours	3.65E+02	hours year^{-1}	
	Conversion to kcal	2.85E-01	kcal year^{-1}	
	Conversion to J	4.19E+03	J kcal^{-1}	
	Renewable fraction	15.20%	%	Giannetti et al. (2015)
	Annual flow	2.91E+04	J m^{-2} year^{-1}	
	UEV	2.10E+07	sej J^{-1}	Oliveira et al. (2018)
	Emergy	6.10E+11	sej m^{-2} year^{-1}	
16	**Non-skilled labor**			
	Man-hours	5.48E+02	hours year^{-1}	
	Conversion to kcal	2.85E-01	kcal year^{-1}	
	Conversion to J	4.19E+03	J kcal^{-1}	
	Renewable fraction	15.20%	%	Giannetti et al. (2015)
	Annual flow	4.36E+04	J m^{-2} year^{-1}	
	UEV	3.27E+06	sej J^{-1}	Oliveira et al. (2018)
	Emergy	1.43E+11	sej m^{-2} year^{-1}	
17	**Skilled labor**			
	Man-hours	3.65E+02	hours year^{-1}	
	Conversion to kcal	2.85E-01	kcal year^{-1}	
	Conversion to J	4.19E+03	J kcal^{-1}	
	Non-renewable fraction	84.80%	%	Giannetti et al. (2015)
	Annual flow	1.62E+05	J m^{-2} year^{-1}	
	UEV	2.10E+07	sej J^{-1}	Oliveira et al. (2018)
	Emergy	3.40E+12	sej m^{-2} year^{-1}	
18	**Non-skilled labor**			
	Man-hours	5.48E+02	hours year^{-1}	
	Conversion to kcal	2.85E-01	kcal year^{-1}	
	Conversion to J	4.19E+03	J kcal^{-1}	
	Non-renewable fraction	84.80%	%	Giannetti et al. (2015)
	Annual flow	2.43E+05	J m^{-2} year^{-1}	
	UEV	3.27E+06	sej J^{-1}	Oliveira et al. (2018)

	Emergy	7.95E+11	sej m^{-2} $year^{-1}$	
19	**Equipment**			
	Depreciation	1.52E+02	USD m^{-2} $year^{-1}$	
	UEV	5.60E+12	sej USD^{-1}	Giannetti et al. (2018)
	Emergy	8.49E+14	sej m^{-2} $year^{-1}$	
20	**Vegetables**			
	Total production	1.72E+01	kg m^{-2} $year^{-1}$	
	Conversion to kcal	1.50E-01	kcal g^{-1}	
	Conversion from kcal to J	4.18E+03	J $kcal^{-1}$	
	Annual flow	1.08E+04	J m^{-2} $year^{-1}$	
21	**Fish**			
	Total production	2.54E+04	g m^{-2} $year^{-1}$	
	Conversion to kcal	5.00E+00	kcal g^{-1}	
	Conversion from kcal to J	4.19E+03	J $kcal^{-1}$	
	Annual flow	5.32E+08	J m^{-2} $year^{-1}$	

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Chapter 6

South American fish species suitable for aquaponics: a review

This chapter is based on:

Pinho SM, David LH, Garcia F, Keesman KJ, Portella MC, Goddek S (2021) South American fish species suitable for aquaponics: a review. Aquaculture International 29:1427–1449. https://doi.org/10.1007/s10499-021-00674-w

Abstract

Tilapia and catfish are the most popular fish species in aquaponics. However, they are not well-accepted in all markets, and finding alternative species is important in order to increase the variety of food products and meet market demands. South America has several potential fish species for aquaponics systems. Encouraging the implementation of integrated aquaculture systems by providing information about the production of South American species can help to increase the supply of high-quality food and aquaculture diversification. Thus, data for five South American fish species with potential for aquaponics were compared with existing data for the main traditional warm water species in this system, tilapia and catfish. Moreover, the degree of suitability of the novel species for these systems in terms of zootechnical performance, tolerance to water quality and nutritional composition of fish flesh were discussed. The South American species considered were jundia or silver catfish (*Rhamdia quelen*), yellowtail lambari (*Astyanax lacustris*), pacu (*Piaractus mesopotamicus*), tambaqui (*Colossoma macropomum*) and snook (*Centropomus spp.*). Their description and the tabular comparison with the most traditional aquaponic-cultured species show they are suited for this production system. How suitable they are will depend on the system design, as well as the regional characteristics of the market where they will be produced.

6.1 Introduction

A food production system is considered sustainable when it efficiently uses natural resources to produce high quality food for human consumption (Wunderlich and Martinez 2018; Wilfart et al. 2013). Aquaponics has been recognized as a sustainable food production system, as it reuses a large proportion of its internal waste-streams. It is defined as an integrated multitrophic aquatic food production method, which contains at least one direct connection between an aquaculture and a plant production unit (Lennard and Goddek 2019). Aquaponics is already widely applied in many developed countries (Villarroel et al. 2016; Love et al. 2014).

Food supply in South American countries is mainly based on monocultures; agri-aquaculture integrated food production systems are not yet widespread (Rodrigues et al. 2019; Liu et al. 2018). Monocultures are one of the causes of the overexploitation of both the soil and natural resources (Hampf et al. 2020; Joyce et al. 2019; Castro et al. 2014) and, in the long run, are inefficient in supplying the local population with healthy food. In addition, the social problems faced by many South American countries contribute to their current levels of malnutrition (PAHO 2020) and increase the need for efficient food production systems. In 2017, the annual freshwater fish production per capita in South America was below the worldwide average, 3.2 kg vs 8.2 kg respectively, and the average of vegetable and fruit supply was also low: 51.6 kg per capita in South America vs 135.7 kg per capita worldwide (FAO 2020).

Providing information on the production of South American species in aquaponics systems can encourage the implementation of these systems, and therefore help to minimize the continent's problems related to high-quality food supply and the pressure on natural resources. Compared to cage and pond-based aquaculture systems, both recirculating aquaculture systems (RAS) and aquaponics need lower volumes of water and smaller areas of land to produce fish (Oladimeji et al. 2020; Lennard and Goddek 2019; Martins et al. 2010). In aquaponics, some of the negative effects of fish production, such as nutrient-rich effluent discharge, can be reduced through nutrient reuse by plants and energy/nutrient recovery after waste treatment by implementing additional technologies (Goddek et al. 2019a). Moreover, chemical and antibiotic-free fish and pesticide-free plants are produced, which makes this system useful in promoting food security (Kyaw and Ng 2017).

Regarding the most common aquaponics design (i.e. coupled or one-loop aquaponics system), the process water is fully recirculated between the RAS and the hydroponics unit (Yep and Zheng 2019; König et al. 2018). An alternative configuration is known as the decoupled aquaponics system

(DAPS), where the respective subsystem components can be seen as stand-alone systems. This allows for optimal conditions to produce both fish and plants (Monsees et al. 2017). In addition, multi-loop aquaponics systems have also been introduced (Lennard and Goddek 2019) that can comprise additional loops containing digestion units (Delaide et al. 2019) and/or desalination units (Goddek and Keesman 2018). Such additional loops are added to the system to increase the nutrient and water reuse efficiency of the overall system. For example, nutrient mineralization and mobilization units in the form of bioreactors can be used to reduce the need for additional fertilizers in the hydroponics unit. Desalination technology can be used to extract nutrients from the RAS water in a highly concentrated form and provide it to the hydroponics unit (Goddek and Keesman 2018). Regardless of the chosen design, it is important to make a careful selection of the fish and plant species that will be grown, in order to both optimize nutrient utilisation and achieve maximum profitability.

With respect to the fish species, tilapia (*Oreochromis niloticus*) and several catfish (order Siluriformes) are the most traditional species for aquaponics (Yep and Zheng 2019; Mchunu et al. 2018; Love et al. 2015). However, these species are not well-accepted in all markets. This is because tilapia is usually masculinized with steroid hormone (Joshi et al. 2019; Golan et al. 2014) and there is a high dependence on antibiotics to achieve high yields (Roriz et al. 2017). Also, catfish is known as a fish potentially containing heavy metals as it is conventionally reared using water from contaminated rivers such as the Mekong Delta (Vietnam) and Lake Rukwa (Tanzania) (Mapenzi et al. 2020; Madsen et al. 2015). In general, consumers of these species are concerned that undesirable substances have entered the food chain, especially due to the deposit of residues in the fish flesh (Zhong et al. 2016; Megbowon and Mojekwu 2014). Furthermore, tilapia and the most popular catfish (*Ictalurus punctatus, Pangasius pangasius, Clarias gariepinus*) are exotic in South America countries and, if accidentally released into the environment, could become predators of native species (Padial et al. 2017; Bittencourt et al. 2014).

As stated above, the search for alternative species for aquaponics production is important to meet market demands. This will increase the variety of available food products, allowing farmers to produce species that match local characteristics (Pinho et al. 2017; Goddek et al. 2015), and encourage aquaculture diversification (FAO 2016). The nutritional quality and flesh safety of the fish produced are also important factors when selecting suitable species. Fish is recognized as one of the best animal proteins, being highly digestible and an important source of essential fatty acids (Pal et al. 2018; Smet 2012) and other nutrients for human health (Tilami and Sampels 2018).

South America is home to the largest biodiversity of fish in the world (Reis et al. 2016) and several species have been identified as potential candidates for aquaculture production. In some South American countries, native fish are already widely produced in pond or cage systems (Valladão et al. 2018). However, the feasibility of producing these species in aquaponics is still not well known. In this review, we compare five South American fish species with potential for aquaponics with the main traditional species in this system, i.e., tilapia and catfish. We also discuss the degree of suitability of the novel species for different kinds of aquaponics systems in terms of zootechnical performance, tolerance to water quality, and nutritional composition of fish flesh.

6.2 What makes a fish species suitable for aquaponics?

From a fish production perspective, aquaponics is bound to the same chemical, physical, and biological conditions that occur in RAS (Espinal and Matulic 2019). This means that, just like in RAS, fish species must show some overall characteristics in order to be produced intensively in aquaponics systems, such as a tolerance to both high densities and high levels of total suspended solids and dissolved nutrients (Yep and Zheng 2019). Maintaining fish welfare is also necessary in these systems since it boosts their health and allows the fish to reach their maximum growth potential (Yildiz et al. 2017). In small-scale or hobby aquaponics systems, these are usually the only characteristics considered. However, for commercial productions, some additional specific points must be taken into account and they differ for coupled and decoupled systems.

In coupled systems, the aquaculture, hydroponics, and biological filter units are interconnected; therefore, finding a trade-off between the proper water conditions for each subsystem is required (Palm et al. 2019). The choice of fish species in coupled aquaponics should depend on the crop grown. This is because plants are often the main source of income (Bosma et al. 2017) and, to keep the facility profitable, meeting plant requirements without harming fish growth or filter operation is desired. In these systems, the fish should be rustic and tolerate a wide range of physical-chemical water parameters. The fish should also tolerate high concentrations of macro and micronutrients which are often added to the water as a supplement for plant growth (Yildiz et al. 2017). In coupled aquaponics, the optimal conditions in each subsystem cannot be reached without either harming fish growth and survival or causing the plants to grow very slowly and show nutrient deficits. Achieving good economic system efficiency is a huge challenge when dealing with the trade-offs in terms of temperature, pH, and nutrient concentration (measured in electrical conductivity) (Goddek et al. 2019b).

The range of species that can be produced in DAPS or in decoupled multi-loop systems is, in general, larger than in coupled systems (Figure 6.1). This is due to the possibility of meeting the specific required economic conditions of each loop in decoupled systems, mainly in relation to abiotic factors, such as water and environmental conditions, and to nutrient balances (Danner et al. 2019; Goddek and Körner 2019). Once the requirements of the aquaculture loop are met, fish become an important aquaponics product and the choice of species becomes directly dependent on their market acceptance, production costs, and growth rate. In DAPS, fish species with favourable characteristics for intensive RAS production can be selected, as outlined above.

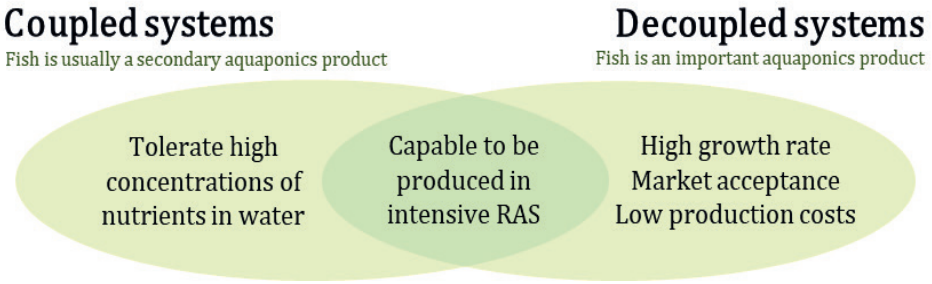

Figure 6.1. The overall characteristics that fish species must have to be productive in coupled and decoupled aquaponics systems.

6.3 South American fish species

The South American continent is recognised for its great potential for aquaculture production. This is due to its water availability, favourable climate conditions, high variety of species (FAO 2018a; Valladão et al. 2018), and access to technical-scientific knowledge, equipment, supplies, and manpower. Valladão et al. (2018) reviewed South American fish for continental aquaculture and described the main fish species, producing countries and systems or used techniques. The potential of such species for aquaponics systems was not evaluated. Based on their characteristics, we consider that the main alternative species that might be interesting for using in freshwater aquaponics systems are the silver catfish jundia (*Rhamdia quelen*), yellowtail lambari (*Astyanax* spp.), pacu (*Piaractus mesopotamicus)*, tambaqui (*Colossoma macropomum*), and snooks (*Centropomus* spp.). The reason to review these species, among several others reared in South America, was due to their market value, nutritional quality for consumption, and/or the large volume produced in conventional systems. A brief description of each one and a tabular overview are presented below. Photos of each species are presented in Figure 6.2. The water quality, zootechnical, and nutritional parameters are shown in Table 6.1. Data related to RAS and aquaponics systems were prioritized. In case no data was found, values from other production systems were reported and identified. With respect to the water quality, value ranges or maximum tolerable values of the main parameters suitable for each species were presented. The zootechnical and nutritional parameters were described according to the maximum values found for intensive cultures. For the stocking density, the values found for intensive cage systems were considered. The market characteristics for each species, such as harvest weight, mean values of nutritional composition for consumers and sale price, are summarised in Table 6.2.

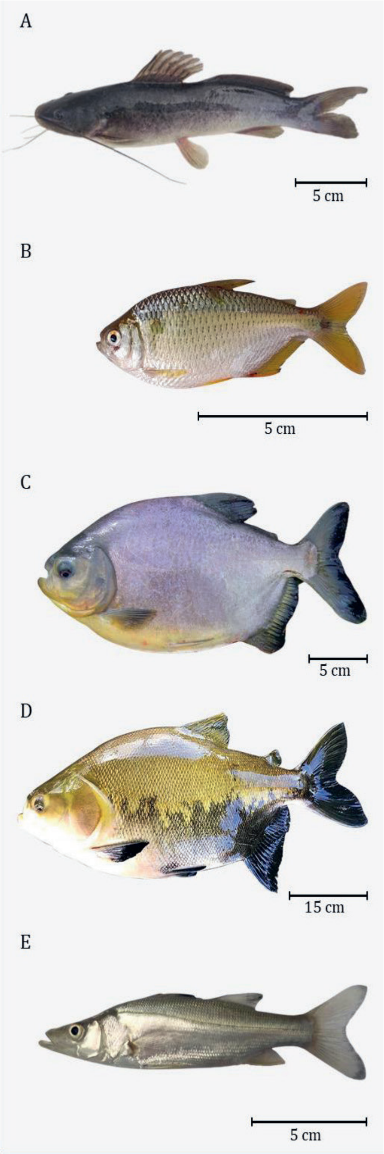

Figure 6.2. The five South American fish species with potential for use in aquaponics systems. A) Jundia (*Rhamdia quelen*) juvenile, 23 cm total length (TL) (photo credit: Eduardo Antônio Sanches). B) Adult yellowtail lambari (*Astyanax lacustris*), 8 cm TL (photo credit: Emerson Durigon). C) Pacu (*Piaractus mesopotamicus*) juvenile, 28 cm TL (photo credit: Eduardo Abimorad). D) Adult tambaqui (*Colossoma macropomum*), 70 cm TL (photo credit: Jenner Menezes). E) Common snook (*Centropomus undecimalis*) juvenile, 14 cm TL (photo credit: Flávio F. Ribeiro).

Table 6.1. Overview of the water quality and productive parameters of the alternative South American fish species that could be considered for use in aquaponics systems compared to traditionally reared species.

Parameter	Units	Traditional Species		South American Species*				
		Tilapia	**Catfish**	**Jundia**	**Lambari**	**Pacu**	**Tambaqui**	**Snooks**
Temperature	°C	27-30 [1 a]	22-28 [1 a]	20-26 [1 a]	25-28 [3 a]	28-32 [1 a]	26-32 [1 a]	25-31 [1 a]
DO	mg/L	4-6 [1 b]	2-6.5 [1 a]	5.6-7 [1 a]	4-8 [1 c]	5.9-8 [1 b]	4-8 [1 b]	4-8 [1 b]
pH	-	7-9 [1 c]	5.6-7.6 [1 a]	5.4-7 [1 b]	6.5-8 [3 a]	6.9-7.8 [1 b]	4-7.3 [3 c d]	6.9-7 [1 b]
Max. Nitrate	mg/L	200 [1 d]	114 [1 b]	86 [1 c]	0.25 [1 c]	1.46 [1 b]	NR	1.5 [1 a]
Max. Ammonia	mg/L	0.1 [1 a]	6.7 [1 a]	3 [1 d]	1 [3 a]	1.32 [1 b]	1.2 [1 e]	0.03 [1 a]
Stocking Density	kg/m^3	70 [1 e]	300 [1 c]	17 [1 d]	14 [1 c]	75 [2 c]	40 [2 f]	60 [2 c]
FCR	-	1.3 - 1.7 [1 f g]	1.0 - 1.3 [1 c d]	1.6 - 1.9 [1 a d]	1.1 - 1.5 [1 c]	1.2- 1.6 [1 b d]	1.1 - 1.7 [1 e g]	1.2 - 1.8 [1 d e]
Feed Habit	-	Omnivore [h]	Omnivore [a]	Omnivore [a]	Omnivore [a]	Omnivore [d]	Omnivore [e]	Carnivore [a]
CP content	%	35 [1 h]	40 [1 a]	39 [1 e f]	32 [1 d]	32 [1 d]	30 [1 e]	45 [1 e]
Reference		[a] El-Sayed (2006) [b] Timmons and Ebeling (2013) [c] Ross (2000) [d] Dalsgaard et al. (2013) [e] Rakocy et al. (2004) [f] Rakocy et al. (2006)	[a] Akinwole and Faturoti (2007) [b] Strauch et al. (2018) [c] Baganz et al. (2020) [d] Rocha et al. (2017)	[a] Piedras et al. (2004) [b] Maffezzolli and Nuñer (2006) [c] Poli et al. (2015) [d] Araújo (2015) [e] Ha et al. (2019) [f] Battisti et al. (2020)	[a] Porto-Foresti et al. (2010) [b] Fonseca et al. (2017) [c] Jatobá and Silva (2015) [d] Moraes et al. (2018)	[a] Santos et al. (2017) [b] Pinho et al. (2017) [c] Bittencourt et al. (2010) [d] Machado-Neto et al. (2016)	[a] Silva and Fujimoto (2015) [b] Costa et al. (2019) [c] Rodrigues (2014) [d] Silva et al. (2007) [e] Oishi et al. (2010) [f] FAO (2019)	[a] Correia et al. (2018) [b] Pinho et al. (2016) [c] Alvarez-Lajonchère and Tsuzuki (2008) [d] David et al. (2019b) [e] Sanches et al. (2011)

[g] Knaus and Palm (2017) [h] Goddek et al. (2016)	[g] Paulino et al. (2018)

[1] Data for closed systems (RAS, aquaponics, or bioflocs systems); [2] data for cage farming; [3] data for pond farming. DO: Dissolved oxygen. FCR: Feed conversion rate. NR: not reported. CP: Crude protein. * Jundia (*Rhamdia quelen*), also known as silver catfish. Lambari (*Astyanax lacustris*), also known as yellowtail lambari, yellowtail tetra or freshwater sardine. Pacu (*Piaractus mesopotamicus*). Tambaqui (*Colossoma macropomum*). Snook (*Centropomus sp.*), also known as robalo.

Table 6.2. Overview of market characteristics of the alternative South American fish species that could be considered for use in aquaponics systems compared to traditionally reared species.

Parameter	Unit	Traditional Species		South American Species*				
		Tilapia	**Catfish**	**Jundia**	**Lambari**	**Pacu**	**Tambaqui**	**Snooks**
Harvest Weight	*kg*	0.6 ^{a}	0.7 ^{a}	0.8 ^{a}	0.02 ^{a}	1.5 ^{a}	2.5 - 3 ^{a}	0.5 ^{a}
*Total Protein***	*%*	20.1 ^{b}	15.5 ^{b}	18.9 ^{b}	16.3 ^{b}	18.2 ^{b}	19.0 ^{b}	22.0 ^{b}
*Total Fat***	*%*	1.7 ^{b}	7.6 ^{b}	4.2 ^{b}	5.7 ^{c}	8.4 ^{b}	2.7 ^{b}	2.2 ^{b}
*n-3/n-6***		0.40 ^{c}	0.49 ^{c}	0.10 ^{c}	0.45 ^{c}	0.28 ^{c}	0.12 ^{c}	2.8 ^{b}
Price	*€/kg*	1.29 ^{d}	1.51 ^{d}	2.36 ^{d}	2.58 ^{d}	1.61 ^{d}	1.14 ^{d}	8.17 ^{d}
Reference		^{a} Goddek et al. (2016) ^{b} SND (2020a) ^{c} Kulawik et al. (2013) ^{d} CEAGESP (2020)	^{a} Akinwole and Faturoti (2007) ^{b} SND (2020b) ^{c} Ng et al. (2003) ^{d} TRIDGE (2020)	^{a} Barcellos et al. (2009) ^{b} Battisti et al. (2020) ^{c} Lazzari et al. (2011) ^{d} CEAGESP (2020)	^{a} Porto-Foresti et al. (2010) ^{b} Furuya et al. (2013) ^{c} Gonçalves et al. (2014) ^{d} CEAGESP (2020)	^{a} Campos et al. (2015) ^{b} Zuanazzi et al. (2013) ^{c} Ramos-Filho et al. (2008) ^{d} CEAGESP (2020)	^{a} Campos et al. (2015) ^{b} FatSecret (2020) ^{c} Filho et al. (2013) ^{d} CEAGESP (2020)	^{a} David et al. (2019b) ^{b} Hauville et al. (2015) ^{c} CEAGESP (2020)

1 € = R$ 4.65 (Jan 2020). * Jundia (*Rhamdia quelen*), also known as silver catfish. Lambari (*Astyanax lacustris*), also known as yellowtail lambari, yellowtail tetra or freshwater sardine. Pacu (*Piaractus mesopotamicus*). Tambaqui (*Colossoma macropomum*). Snook (*Centropomus sp.*), also known as robalo. ** All data for total protein, total fat, and n-3 PUFA to n-6 PUFA (Polyunsaturated Fatty Acids) ratio were obtained from the fillet composition, except for yellowtail lambari which is consumed as a whole and therefore its body composition was reported.

In addition to the aforementioned species, a wide variety of fish should be considered in the future, especially hybrids of the "round fishes", for instance, tambacu (*C. macropomum* x *P. mesopotamicus*), paqui (*P. mesopotamicus* x *C. macropomum*), tambatinga (*C. macropomum* x *P. brachypomus*), which were developed to be reared under different climatic conditions and to present better zootechnical results than the pure species (Hashimoto et al. 2012). However, insufficient productive results about them are available. In the case of the hybrids, their sustainability is uncertain due to lack of knowledge of their impact on the environment and effects on future generations of fish (Hashimoto et al. 2011).

6.3.1 Jundia or silver catfish (Rhamdia quelen)

The jundia (Figure 6.2A) occurs naturally from southeast Mexico to Argentina. It is a suitable species for aquaculture in regions with a temperate or subtropical climate due to its optimal growth in summer and also its ability to withstand the temperatures of the South American winter (Abreu et al. 2016). In addition, jundia presents a high prolific rate, resistance to handling, and high weight gain (Barcellos et al. 2009; Meyer and Fracalossi 2004). Reproductive management of this species is already dominated by the productive sector, with juvenile supply occurring from August to March (Barcellos et al. 2001). Its production has increased markedly because of the absence of intramuscular bones and high acceptance by consumers (Gomes et al. 2000). Under optimal conditions it is possible to produce market size fish (800g) within 8 months (Barcellos et al. 2009). In the last decade, the total production of jundia has been decreasing (FIGIS/FAO 2020), mainly due to the increased production of exotic species, such as carp and tilapia (Baldisserotto 2008). From 2000 to 2010, the average annual volume produced was approximately 1000 tons, while in the following years this average dramatically fell to 15 tons per year (FIGIS/FAO 2020).

No commercial production data of jundia in RAS or aquaponics is available. In experimental systems, the rearing of this species has been carried out for different purposes. Research with jundia in RAS includes evaluations of its reproduction (Goes et al. 2017; Tessaro et al. 2012; Coldebella et al. 2011), larviculture (Sulis-Costa et al. 2013; Uliana et al. 2001), productive management (Battisti et al. 2020; Owatari et al. 2018), nutrition (Yamashita et al. 2020; Ha et al. 2019; Battisti et al. 2017; Gominho-Rosa et al. 2015), and health (Cunha et al. 2018; Tancredo et al. 2015). These articles did not envision evaluating the growth performance of jundia in RAS compared to other production systems. However, their results showed that the species performs well in RAS environmental conditions.

To date, only two reports have been published about jundia reared in aquaponics. Rocha et al. (2017) evaluated the use of biofloc technology on the production of lettuce and jundia in a coupled aquaponics system. They demonstrated that it is possible to produce this fish in aquaponics and that the use of bioflocs did not influence the lettuce growth results. Araújo (2015) studied different feeding rates (7, 12 and 18 g per day) in the integration of jundia and cherry tomato (*Solanum lycopersicum*, var. Cerasiforme) produced in a coupled system. The author reported a difference between the optimum feeding rate for plants (12 g) and for fish (18 g) after 88 days of experiment. These results indicate the importance of continued investigation into jundia in aquaponics, particularly in decoupled systems, where the conditions can be adjusted to allow optimum performance for fish and plants.

6.3.2 Yellowtail lambari (Astyanax lacustris)

Yellowtail lambari (Figure 6.2B), also known as yellowtail tetra or freshwater sardine, is a small (approximately 10 cm) and rustic fish from the Characidae family. The *Astyanax* genus is one of the most specious of the order Characiformes, encompassing more than 100 species distributed over the Neotropical region (Kavalko 2008). The species yellowtail lambari, present in the Upper Paraná basin, was classified for many years as *Astyanax bimaculatus* (Linnaeus 1758). However, the systematic and phylogeny of the *Astyanax* genus was reviewed, and it was found that *Astyanax bimaculatus* did not correspond to only one species, moving the yellowtail lambari to the denomination of *Astyanax altiparanae* (Garutti and Britski 2000; Garutti 1995). Later, the species *Astyanax altiparanae* was considered a synonym of *Astyanax lacustris* (Lütken 1875), which became the valid name of the species (Lucena and Soares 2016). All of these nomenclatures were considered in the literature review.

Yellowtail lambari is a species with a fast life cycle usually produced in semi-intensive rearing ponds (Silva et al. 2011), reaching the commercial size (10-15 g, Sussel 2015) within 3 months (Valladão et al. 2018; Garutti 2003). However, lambari is also suitable for production in intensive systems (Porto-Foresti et al. 2010; Garutti 2003). Yellowtail lambari females develop earlier than males, therefore their production would be more interesting. However, sex separation or manipulation are not commercially applied, and mixed-sex populations have been reared by producers (Fonseca et al. 2017). Regarding its market factors, yellowtail lambari is usually sold per unit and appreciated as snacks or used as live bait, mainly in the Brazilian Southeast region (Valladão et al. 2018). The high demand for lambari in the Brazilian Southeast region probably boosted the local rearing of this species, representing in 2016 more than half of the 595.6 tonnes produced in this country (IBGE 2018). The production chain of lambari was described by Silva et al. (2011), showing that most of the lambari commercialized as snacks still originate from fisheries. The species is widely consumed, highly valued and is considered an alternative fish species for small family-farmers, since on small pieces of land they can obtain a high income (Silva et al. 2011; Fonseca et al. 2017).

Yellowtail lambari is a promising species for aquaponics. Sussel (2015) reported that its culture has been carried out in ponds, cages, RAS, and aquaponics systems. The author points out that natural food must be available to lambari during the first month, and after this period, it should be transferred to closed and intensive production systems. However, to date, only a few studies with yellowtail lambari in RAS have been performed (Moraes et al. 2018; Lira et al. 2018; Jatobá and Silva 2015) and no scientific information about its production in aquaponics have been published. In Brazil, the production of yellowtail lambari in aquaponics systems was initially developed by The Sao Paulo Agency of Agribusiness Technology and was applied in small coupled systems. However, the technology is in its early stages of implementation and the transferring of the production model to commercial scale is still to be achieved (AEAARP 2015).

6.3.3 Pacu (Piaractus mesopotamicus)

Pacu (Figure 6.2C) is one of the most commercially valuable fish in Brazilian fish farming and there is also a huge interest in its production in other South American countries along the Paraná River Basin, such as Paraguay, Uruguay and Argentina (David et al. 2019a; Valladão et al. 2018; Portella et al. 2014; Portella and Dabrowski 2008; Hashimoto et al. 2011). Pacu is valued as a table fish due to its high quality and tasty white meat and as game fish in view of its behaviour in continental sport fishing (David et al. 2019a; Furuya et al. 2008). Its production has been promoted by its being a rustic, omnivorous species with easy acceptance of formulated feed and intensive systems (David et al. 2019a; Nunes et al. 2013; Portella et al. 2012). This allows production of fish of approximately 1.3 kg in the first 12 months (Urbinati and Gonçalves 2005). In aquaculture systems, the reproduction of *P. mesopotamicus* is only possible by hormonal induction, and the supply of juveniles in the South American continent occurs between October and March (Portella et al. 2014; Urbinati and Gonçalves 2005).

Pacu has been mainly reared in Argentina and Brazil, and its production occurs widely via semi-intensive techniques in ponds (Valladão et al. 2018). In 2016, approximately 1,950 tonnes of pacu were produced in Argentina, representing 52% of the total aquaculture production in this country (FAO 2018b), and 11,570 tonnes in Brazil (IBGE 2018). This species is easily adaptable to intensive production in cages (Hilbig et al. 2012;_Bittencourt et al. 2010), however, in ponds, low production density varying from 0.5 kg m^{-2} to 2 kg m^{-2} is usually reported (Valladão et al. 2018). The technical viability of pacu culture in closed systems was described only for larviculture (Jomori et al. 2003) and juvenile production (David et al. 2019a; Machado-Neto et al. 2016) phases, while no information on commercial or experimental production in RAS for the growth-out phase are available._In aquaponics systems, pacu is commonly reported as a species that is already being produced (Yep and Zheng 2019; Martins 2017; Rakocy 2012). Love et al. (2015) interviewed more than 1,000 aquaponists of different scales around the world and found that pacu was among the farmed fish produced. Pinho et al. (2017) evaluated pacu and tilapia growth performance and the use of effluent from each species to produce two varieties of garnish (scallion and parsley) in coupled aquaponics systems. They showed that plant growth was not affected by fish species cultured and that pacu is a viable alternative species for aquaponics production. Fed with a diet containing 32% of CP and reared in an average temperature of 27 ºC, it grew at a specific rate of 2.35 % day^{-1} and showed a feed conversion rate of 1.6 (Pinho et al. 2017).

6.3.4 Tambaqui (Colossoma macropomum)

Tambaqui (Figure 6.2D) is a well-known Amazonian fish, mainly reared and consumed in countries such as Brazil, Colombia, Peru, and Venezuela, although some production of tambaqui is also found in several Asian countries (FAO 2019). In 2016, approximately 142,100 tonnes of tambaqui were produced in these South American countries (FAO 2018a). Currently, it is the number one native species reared in several countries of the South American continent (FAO 2018a). The popularity of tambaqui in the aquaculture sector is due to its fast growth rate, the acceptance of commercial feed, relative resistance to diseases, and tolerance of low water quality (Lima et al. 2019; Oishi et al. 2010). This species is highly prolific; its reproduction is achieved by hormonal induction during the breeding season (Rodrigues 2014), resulting in high availability of tambaqui juveniles (Gomes et al. 2010). Achieving the harvest weight of 3.5 kg is possible within 2 years under general fish farm conditions, although the market size of aquacultured tambaqui in Brazil is around 500 g (Pantoja-Lima 2020) to

2.5-3 kg (Almeida et al. 2016; Campos et al. 2015). Tambaqui flesh is a traditional protein source for the local Amazon population, where its meat is in great demand (Costa et al. 2019), and also appreciated in other regions in the continent. However, like other Characid species, tambaqui has intramuscular bones, which makes its filleting for sale in foreign markets difficult (Perraza et al. 2017). An alternative that has been explored to increase its acceptability is the processing in different cuts, mainly the sale of its ribs (Cartonilho and Jesus 2011). More recently, a program to genetically select tambaqui that do not present these intramuscular bones started in Brazil (Perraza et al. 2017).

The aquaculture sector has invested in technologies to shift tambaqui production from conventional semi-intensive systems to more intensive production systems using RAS (Lima et al. 2019; Silva and Fujimoto 2015). However, only experimental results on the production of this species in RAS have been published so far. As reported for jundia, the investigations carried out with tambaqui used RAS as an experimental system to evaluate other productive management systems or parameters, and they were not specifically designed to evaluate the growth performance of tambaqui in such systems. These studies mainly focused on nutrition (Paulino et al. 2018; Junior et al. 2017; Nwanna et al. 2008), reproduction (Gallego et al. 2017; Maria et al. 2015), management (Dantas et al. 2020; Costa et al. 2019) behaviour (Reis et al. 2019; Barbosa et al. 2009), genetics (Ariede et al. 2020; Silva et al. 2019), and health (Barbas et al. 2020; Paz et al. 2019). Lima et al. (2019) evaluated different stocking densities of tambaqui juveniles in RAS. Although these authors did not compare RAS with other production systems, they contrasted their findings with results reported for pond and cage systems and showed RAS as a potential system for intensive tambaqui production.

No published articles reporting the use of *C. macropomum* in aquaponics were found, except for a few abstracts presented at scientific conferences (Araújo et al. 2017; Cruz et al. 2015; Ibrahim et al. 2015), all of them with anecdotal and inconclusive results. However, researchers from the Brazilian Agricultural Research Corporation (EMBRAPA) described in a technical report the development of compact coupled systems for the aquaponics production of tambaqui and vegetables at the family production level, and in modular systems for large-scale production (Carneiro et al. 2015). They reported satisfactory growth of vegetables and the possibility of reaching the commercial weight of tambaqui in a similar period to that observed in conventional systems.

6.3.5 Snook (*Centropomus sp.*)

The species from genus *Centropomus* present favourable characteristics for aquaculture in recirculating systems, such as fast growth, acceptance of formulated diets, potential for very high biomass yields per unit volume in the nursery and grow-out systems, and high market value (Pinho et al. 2016; Alvarez-Lajonchère and Tsuzuki 2008). The twelve snook species are known as "robalo" in Latin America. The common snook (*Centropomus undecimalis)* (Figure 6.2E) is the fastest-growing snook species and, together with the fat snook (*Centropomus parallelus)*, is the most cultivated species under experimental conditions (Alvarez-Lajonchère and Tsuzuki 2008). They are diadromous, euryhaline, stenothermic, and estuarine-dependent fish found in rivers, estuaries, coastal lagoons and along rocky shores (Mello et al. 2015; Pope et al. 2006). Studies have demonstrated that snook have high osmoregulatory capacity, which allows them to maintain their internal osmotic pressure practically independent of external salinity concentrations (0 to 40 ppt) and to be produced in fresh water (Michelotti et al. 2018; Liebl et al. 2016; Gracia-López et al. 2006). Moreover, they are highly prized for the quality of their meat and valued for sport fishing (Passini et al. 2019). Processing the fillet is easy, with high yield (~ 42%), and its market value is usually high (David et al. 2019b; Cerqueira 2010).

There are still some constraints to the commercial production of snooks, such as their carnivorous habit (requiring diets with high protein content) and difficulties during the hatchery phase. However, the experimental results of reproduction and growth-out are promising (Passini et al. 2019; Michelotti et al. 2018; Alvarez-Lajonchere and Tsuzuki 2008). Researchers from Mexico reported the production of 800 g snook in one year (Sánchez-Zamora et al. 2003). Most of the experiments conducted with snook have been carried out in RAS, and aimed at understanding their reproductive biology (Passini et al. 2019; 2018), nutrition (Michelotti et al. 2020; David et al. 2019b), adequate stocking densities (Sanches et al. 2011), and optimal water parameters, especially temperature and salinity (Michelotti et al. 2018; Mello et al. 2015). However, in the field of aquaponics, snook are still unknown and no reports of their production in these systems were found.

6.4 Discussion

The South American fish species with the potential to be produced in aquaponics have been described. Moreover, the main productive data of these species along with required water quality have been tabulated to enable a comparison with the two most traditionally cultured aquaponic species, tilapia and catfish. Most of these novel species are already known to conventional aquaculture systems. For instance, in the last years, the production of tambaqui and pacu in ponds or cages has been widespread in the American continent (Valladão et al. 2018), as mentioned in the previous species descriptions. However, only a few studies have been carried out to evaluate their production in aquaponics. The reported aquaponics production of South American species was only performed in coupled systems. Among these species, pacu and jundia have been the most evaluated in aquaponics, and encouraging growth results were found in all studies (Pinho et al. 2017; Rocha et al. 2017; Araújo 2015).

Matching the physical-chemical parameters of the water that is tolerated by the respective fish species to those required by plants is a key factor in coupled systems. The optimal ranges or the maximum levels of these parameters for the suggested fish species were reviewed in this study (Table 6.1). In general, fish that tolerate a wide range of water parameters are desirable. More specifically, species are highly suitable for these system designs when (1) they can be reared in water with pH between 5.5 and 6.5, since this is the range when nutrients are mostly available to plants (Resh 2012), and (2) they tolerate high levels of nitrate, which is crucial in determining the plant growing area (Goddek et al. 2016). Given these characteristics, jundia stands out most among the South American species, although the physiological ability of tambaqui to tolerate large pH variations and its better growth in acidic water (Aride et al. 2007) also make it an outstanding species for coupled aquaponics production. The resistance of tambaqui to an acidic environment is due to its adaption to the wide pH range of the Amazonian rivers, home of this species. This fish is naturally found in the Negro River (pH ~ 4.7) and Solimoes/Amazonas River (pH ~ 6.8) (Silva et al. 2013). It is important to highlight that the values in Table 6.1 were obtained from experimental results in different production systems that, in some cases, did not evaluate specifically the parameters mentioned and only reported them as excellent for the species. Because of this, we assert that research designed to investigate the pH toleration level of each species in RAS or aquaponics should be encouraged. New results may show that these species tolerate wider ranges of water parameters and, consequently, have even more potential for production in aquaponics.

For decoupled aquaponics systems or other aquaponics systems where fish is a relevant product, fish zootechnical performance must be considered. Reports of RAS production for all reviewed species are available. However, most of them were cultivated under experimental conditions that did not explore their productive potential. Pacu, snook, and tambaqui stand out as the species that can be grown with the highest stocking density. The density for pacu rearing (75 kg m^{-3}) is higher than that commonly found for tilapia (70 kg m^{-3}) in aquaponics. On the other hand, catfish (*Clarias gariepinus*) culture density in aquaponics is significantly superior (300 kg m^{-3}, Baganz et al. 2020) as it is a rustic species and tolerates poor rearing conditions when compared to the other reviewed species. The production of jundia and yellowtail lambari are usually carried out in densities below 20 kg m^{-3}, three times lower than for tilapia. Research focused on increasing densities should be developed in order to make these species economically competitive in future RAS and aquaponics production. The FCR of lambari is the most desired among the species, ranging from 1.1 to 1.5. However, all the reviewed species show similar FCR and, because protein ingredients are the most expensive components of the diets, it is important to take into account the protein requirement of each species. In this sense, the lowest cost of feed would be for omnivore species, especially tambaqui, because it is the one with the lowest protein requirement. In contrast, although the FCR range for snook culture is comparable to the other species, it is a carnivorous fish and the amount of protein required in the diet would increase production costs.

The choice of fish species will depend on the demand and characteristics of the market where the aquaponic system will be located. For example, if the aim of production is to supply restaurants with differentiated fish cuts, the rearing of tambaqui and/or pacu should be considered. If the local market demands fish as a snack, yellowtail lambari will be the best option. For the supply of fillets, the snook can supply the high-end fish market while the jundia will be a more popular alternative. The market value and production cost of each species must also be taken into account. Table 6.2 shows the fish sale price according to CEAGESP, which is the biggest food warehouse in Latin America and sells fish from fisheries and aquaculture. The price of snook is four times higher than the others; however, its production is not yet at a commercial scale and there is no information on such costs. On the other hand, the sale price of the other novel species is higher or, at least, competitive in relation to the price of the traditional species. It should be noted that these prices are regional and will change according to the local market. Moreover, all these factors mentioned will influence the operational and economic planning of production.

Nutritious healthy food is usually understood as having low fat and high protein content (Jim et al. 2017). In this sense, the protein and fat content of the proposed South American species is more desirable than that reported for catfish, with the exception of pacu flesh which has 8.4% fat compared to 7.6% fat in catfish. On the other hand, protein content in tilapia fillet is surpassed only by snook. In addition to high muscle protein and low fat content, snook can offer an amount of n-3 highly unsaturated fatty acids (HUFA) higher than all other reviewed species. Freshwater tropical fish, generally, present lower concentrations of n-3 HUFA when compared to cold water marine fish. This fact encouraged research into n-3 HUFA supplementation in diets for freshwater fish grown in intensive systems (Stoneham et al. 2018), and this approach may be applicable to aquaponics systems in the future. In contrast to snook, the reported protein composition of yellowtail lambari is low. Although the protein content was analysed in different types of samples, that is, in the fillet for snooks and whole fish for lambari, they indicate the composition of the edible food. In this way, yellowtail lambari, as it is consumed, provides the least protein of the South American species. Nevertheless, it should be considered as an important food for human consumption, since lambari can be a source of minerals, vitamins, and other nutrients for vulnerable populations (Fonseca et al. 2017; Fiedler et al. 2016) and its n-3 to n-6 ratio is similar to the reported values for the traditional aquaponics species, i.e., around 0.40 and 0.49.

Aquaponics is not yet a well-represented food production system in South American countries (Emerenciano 2016). This may be related to the current high availability of fresh water and land in most of these countries, which results in the mistaken impression that incentives for sustainable aquaculture practices are not needed. Another relevant factor is the lack of political incentive, inspection or severe punishment for producers who degrade the environment (Azevedo et al. 2020). All these factors, in addition to the countries' current economic situation, result in low investment in advanced technologies for aquaculture and the predominance of production in conventional methods (ponds and cages). However, the growing need for food production technologies that minimize the use of natural resources should become the driving force for adopting more sustainable methods of production and stimulate the growth of aquaponics in these countries. At this point, knowing the feasibility of producing native fish species as well as encouraging research focused on evaluating the suitability of different regional species of plants in aquaponics is important. Until that happens, the information provided in this review will be useful to increase the variety of products and the satisfaction of different markets in countries where aquaponics is already a reality or is starting to grow.

6.5 Conclusions

The brief description of the South American species and the comparison with the traditionally reared species shows that the five considered species (jundia, pacu, tambaqui, lambari and snook) are suitable for aquaponics production. The degree of their suitability, however, will depend on the system design, i.e., coupled or decoupled systems, as well as the characteristics of the regional market. It is recommended that future research focuses on understanding the optimal or tolerable water parameters and productive management, e.g., density and feeding rate, for each of the five species considered in recirculating aquaculture systems. In addition, practical applications of these species in aquaponics and their economic feasibility should be encouraged.

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Chapter 7

General Discussion

This thesis has explored the integration of biofloc-based aquaculture with soilless plant production in FLOCponics systems as an innovative and alternative approach for food production. The aim of this thesis was to investigate and discuss the technical feasibility, efficiency and sustainability of on-demand coupled FLOCponics for tilapia juveniles and lettuce production. This objective led to the following research questions:

(1) What are the opportunities and drawbacks of FLOCponics?

(2) To what degree do the productive results achieved in on-demand coupled FLOCponics outperform other production systems?

(3) To what degree is it possible to take advantage of the nutritional benefits of biofloc for tilapia production in an on-demand coupled FLOCponics system?

(4) How efficient will it be to transform a biofloc-based fish farm into a FLOCponics production in terms of productivity and resource use?

(5) How and to what degree do FLOCponics systems affect the sustainability of the stand-alone systems?

(6) What does make a fish species suitable for aquaponics, and how does it contribute to the potential to diversify FLOCponics systems?

This final chapter addresses these research questions by synthesising and reflecting on the findings of Chapters 3, 4 and 5, and expanding the discussions brought in the review articles presented in Chapters 2 and 6. Moreover, the opportunities and challenges of FLOCponics are discussed, and future research is recommended all over the text. The chapter ends with an overall conclusion, where each research question is concisely answered.

7.1 Technical feasibility of on-demand coupled FLOCponics

In Chapter 2, we showed that FLOCponics is in its early stage of development, and inconsistencies in the productive performance of fish and plants in this system were identified. While some studies reported similar or better performance in FLOCponics than other production systems, others showed the opposite. The negative results were usually related to operational problems when applying the concept of FLOCponics in a permanently coupled system. The most concerning issue is the accumulation of microbial flocs (solids) in the plant root zone, hindering the full development of plants and fish. This issue happens because passing the flocs from the aquaculture tank to the hydroponic subsystem without proper management considerably decreases the flocs availability to the fish and impairs the absorption of nutrients by plants. The experimental findings reported in Chapter 3 showed that the concerns with solids within the system are handled when running an on-demand coupled layout.

With respect to fish production, the results of Chapter 3 suggest that FLOCponics outperforms traditional (RAS-based) on-demand coupled aquaponics when the same feeding management is applied. These results are in line with previous studies that compared tilapia production in biofloc-based and recirculating aquaculture systems (Azim and Little 2008; Luo et al. 2014; Mansour and Esteban 2017). Even so, it is unlikely that FLOCponics will substitute RAS-based aquaponics. Mainly because aquaponics has been widely disseminated as a backyard system, and when applied for commercial purposes, the focus has usually been on plant production. FLOCponics, on the other hand, seems more likely to be employed only for commercial purposes and primarily focuses on fish and shrimp culture (Chapter 2).

The results of FLOCponics in Chapter 3 reveal that integrating a biofloc system with hydroponics in an on-demand system layout will not affect the benefits of bioflocs for fish production. These findings are relevant from an aquaculture point of view because they indicate that biofloc-based farmers have a clear option to diversify their production and potentially increase the system's circularity without compromising the running production.

From the perspective of plant production, FLOCponics is also technically feasible since similar lettuce growth was seen in FLOCponics, hydroponics and RAS-based aquaponics systems (Chapter 3). However, these positive results in FLOCponics systems are not always the case. Various FLOCponics studies have reported poor plant growth or visual characteristics (details described in Chapter 2). Some procedures we followed in the experimental research led to higher lettuce production. In addition, efficient solids reduction in the water from the aquaculture to the hydroponics subsystems, pH regulation, and fertiliser addition directly to the hydroponics subsystem are also worth mentioning. All these processes were only possible because the subsystems were partially individualised in an on-demand coupled layout.

A positive point for FLOCponics compared to the other production systems mentioned above is that a lower volume of plant fertiliser was required for lettuce production. Nevertheless, there was still a need to add a commercial fertiliser in all systems. Specific nutrient supplementation protocols are needed to optimise fertilisers use in FLOCponics. It is noteworthy that, during the PhD project, an experiment was conducted to investigate lettuce production with and without plant fertiliser supplementation in on-demand coupled FLOCponics. The results of this work were not included in this thesis because the data is still being processed. Nevertheless, it is possible to say beforehand that the preliminary results indicate lower lettuce growth in the treatment without fertiliser, corroborating studies on on-demand RAS-based aquaponics systems that also report dependence on nutrient supplementation in the hydroponics subsystem (Lastiri et al. 2016; Goddek and Keesman 2018). One of the main results that we intend to further present in this study is analysing the gap between the nutrient in the water of the hydroponics subsystem with and without supplementation, bringing new insights into supplementation protocols in FLOCponics.

The findings discussed above were based on tilapia juveniles and lettuce production but could be expanded for other cultures. Such generalisation of the success of different species in FLOCponics is reasonable since, in on-demand coupled FLOCponics systems, the aquaculture and hydroponics subsystems are partially individualised, allowing to provide optimal conditions for both loops. In *section 7.4*, a brief reflection on the fish species suitable for FLOCponics will be given. For plants, we demonstrated in Chapter 5 that exploring other plant species in FLOCponics may increase the system's efficiency from the emergy synthesis point of view. Producing species with high market prices and higher caloric values will surely benefit the sustainable development of FLOCponics and should be encouraged.

7.2 Fish nutrition in FLOCponics system

The benefits of bioflocs for fish production are well-known in the aquaculture field (Avnimelech 2015; Dauda 2020). Still, as identified in Chapter 2, fish nutrition has not been the focus of

FLOCponics research. On the one hand, the lack of studies on fish nutrition seems inexplicable, considering that biofloc-based aquaculture is essentially about maintaining water quality and providing extra food for fish or shrimp culture (Crab et al. 2012; Emerenciano et al. 2021). On the other hand, it may be understandable that solving the previously reported problems with biofloc (*in situ* food) availability in the fish tank would be the primary research aim. As mentioned before, using an on-demand coupled system layout allowed us to handle such biofloc availability issues. Thus, we explored the nutritional benefits of biofloc for tilapia production in FLOCponics by investigating the reduction of crude protein (CP) levels in the fish diet.

The findings presented in Chapter 3 show that tilapia juveniles cultured in on-demand coupled FLOCponics and fed with diets containing 24 and 28% of crude protein (CP) grew similarly to those in RAS-based aquaponics fed with a 32% CP diet. These results suggest that, by keeping the biofloc in the fish tanks, tilapia juveniles could ingest the nutrient-rich bioflocs biomass in FLOCponics systems. The possibility of exploring the nutritional benefits of biofloc-based culture in FLOCponics opens an avenue of opportunities for different feeding management strategies that should be investigated to decrease the environmental impacts and economic costs of this integrated food production system.

Considering fish nutrition, regardless of the production system employed, it is crucial to take into account that the dietary protein contents recommended in scientific studies are not always available in the market. For instance, Brazil is one of the biggest tilapia producers globally and is increasing investments in nursery phase structures for juvenile culture (FAO 2020). Still, it is rare (not to say impossible) to find aquafeed sold for tilapia juveniles (0.5 to 4 mm feed pellets) with less than 32% CP. Such a gap between research and the aquafeed industry may reflect a lack of communication or a profitable-driven behaviour from the entrepreneurs. For the first case, we must establish adequate communication channels and ensure that the information will arrive at the decision-makers in such companies. The second case is more challenging to solve. Perhaps, if biofloc-based aquaculture farmers demand, for example, low-protein diets, the scenario described above may change.

7.3 Sustainability and efficiency of FLOCponics systems

Biofloc, aquaponics and, recently, FLOCponics systems have been labelled as sustainable food production systems. Such a label is justified by the efficiency of these systems to use and reuse the inputs, as water and nutrients, and avoid waste discharge (Bossier and Ekasari 2017; Goddek et al. 2019; Yep and Zheng 2019). The modelling outputs presented in Chapter 4 reinforce the argument that an integrated agri-aquaculture system increases the resource use efficiencies of the stand-alone systems. That is because the efficiency of resource uses and waste avoidance was higher in FLOCponics than in the biofloc system, mainly considering the results of the improved FLOCponics system.

Identifying to what degree a FLOCponics system efficiently uses the resources and management strategies and designs to increase the system's overall efficiency, as shown in Chapter 4, are relevant to boost the sustainability of food production systems. However, the sustainability of a production system is not only about how much and how the inputs are used, it is also dependent on the quality and renewability of these inputs, as confirmed by the results of the sustainability assessment described in Chapter 5. The sustainability assessment performed for FLOCponics reveals that high emergy, linked to the high dependence on infrastructure, equipment and electricity, is demanded to make the system efficient from the perspective of resource use and waste avoidance. Even so, most emergy indicators suggest that FLOCponics is potentially sustainable.

We applied two different methods, dynamic modelling and emergy synthesis, to analyse the FLOCponics from different perspectives. Nevertheless, the results of both analyses pointed out that FLOCponics' sustainability and efficiency are higher than stand-alone systems. The results of Chapters 4 and 5 are complementary, and the analyses performed in both can be even more relevant if combined in a single investigation. For example, applying emergy synthesis to value the dynamic inputs and outputs variations over time of a large scale modelled FLOCponics systems will elevate the discussion about the sustainability of these systems. The combined modelling-emergy approach should be explored in future research.

An important subject that was not included in this thesis is how reducing the protein in the fish diet affects the sustainability and efficiency of FLOCponics. It may be expected that such a reduction will improve the overall system's performance. However, calculations are needed to numerically determine whether and how much changes in the fish diets affect the FLOCponics performance in terms of sustainability and efficiency. Making the models of the FLOCponics system more comprehensive and including the effect of varying the diet compositions are next research steps, not only to investigate and discuss the resource use efficiency due to these variations but also the economic efficiency of FLOCponics systems.

It is worth mentioning that the sustainability and efficiency assessments have limitations, mainly due to the large number of variables involved in the analyses. Even though key data used in both studies were calibrated using experimental data, some model parameters and unit emergy values were obtained from other published studies, from different situations, which increase the uncertainties of the results. Also, these studies are of a theoretical kind and still needs to be verified and validated.

7.4 Fish species suitable for FLOCponics

Chapter 6 reviews the South American fish species suitable for overall aquaponics systems, aiming to diversify the production, attend specific market demands, and encourage the spread of integrated food production systems in this continent. The focus of this review was on overall aquaponics systems and not on FLOCponics systems, because a broad audience could be covered, and, at its conception, the technical feasibility of FLOCponics was still unclear. Given the findings of this thesis regarding FLOCponics viability and the benefits of diversifying the products, it is time to broaden vision and discuss what makes a fish species suitable for FLOCponics.

In general, fish species suitable for biofloc-based culture is also suitable for FLOCponics. Mainly considering that FLOCponics feasibility is shown for on-demand coupled layout and, as discussed in *section 7.1*, the integration with plant production in FLOCponics does not affect the fish growth performance compared to stand-alone biofloc system. Fish species that have succeeded in biofloc systems present some common desirable characteristics, such as filtering feeding habit, resistance to high stocking densities, low sensibility to high levels of NH_4 and NO_2, and/or tolerance to suspended solids (Emerenciano et al. 2013, 2017; Walker et al. 2020). Regarding the five fish species revised in Chapter 6, tambaqui, pacu and judia have been successfully cultured in experimental biofloc-based systems (dos Santos et al. 2020; Hermes et al. 2021; Battisti et al. 2021; Machado et al. 2021; Sgnaulin et al. 2021). In the case of jundia, Rocha et al. (2017) investigated its production in FLOCponics systems and report positive growth results. For robalo and lambari, to the best of our knowledge, there is no scientific report of their culture in biofloc-based systems. Recognizing the market characteristics of alternative species for FLOCponics is as essential as described in Chapter 6 for overall aquaponics systems.

The production of local fish species in FLOCponics systems can be crucial to enhance more sustainable food production in developing countries. In South America, for instance, recirculating aquaculture systems (RAS) are rarely an option for those producers who seek for an intensive and close systems. Biofloc-based systems have been much more popular than RAS in this continent. Thus, given previous practical experiences and knowledge on biofloc systems, FLOCponics seems to more accessible and applicable than RAS-based aquaponics.

7.5 Contributions of FLOCponics for food production

FLOCponics has been developed to maximize the efficiency of food production by reusing water and nutrients from biofloc-based aquaculture to produce plants in a single (two-loops) system setup. From an economic point of view, as discussed in Chapter 2, it is still premature to state whether FLOCponics is feasible or not. However, as FLOCponics is based on the concept of reduce, reuse, and recycle, it is possible to assert that it will contribute for the development of circular economies.

The advancement of FLOCponics research can result in interesting social impacts. From a broad perspective, FLOCponics can bring the production of a mix of fresh and healthy food (fish and plants) close to the consumer. Like stand-alone biofloc systems and aquaponics, FLOCponics will require small land areas and low quantity of inputs to produce a relatively considerable amount of food. Such characteristics make this type of system suitable for implementation in regions usually not destined for food production, such as urban areas, nonarable lands, or regions with an extreme climate. Given the COVID-19 pandemic situation that alerts communities to be as self-sufficient as possible in times of lock down, bringing food production to urban areas seems more desirable than ever. In addition, the use of chemicals in FLOCponics is minimal, making its products healthier than from conventional monocultures.

Reflecting on FLOCponics from a more specific perspective, it may be expected that developing FLOCponics systems contributes to the growth of aquaculture as a representative activity for food supply in the long term. Moreover, scientifically supporting the transition of biofloc-based fish to FLOCponics fish and vegetables production will guarantee more efficient use of resources and a useful end for the accumulated nutrients in the process water.

7.6 Challenges of FLOCponics and recommendations for future research

For all the aforementioned positive impacts of FLOCponics to become a commercial reality, some systems' drawbacks need to be tackled. The challenges of FLOCponics regarding system setup, plant nutrition, health and production, animal nutrition and production, and the practical applicability of FLOCponics are deeply discussed in Chapter 2. Important challenges and research gaps were addressed in this thesis, but several still remain.

The most critical issue of FLOCponics that is referred to in all chapters is finding a proper destination for the solids discharged. In fact, solid wastes are an issue in biofloc systems that persists in FLOCponics systems. Recent research has tested the use of the biofloc biomass, removed from the settling tanks, as ingredients for aquafeed formulations (Shao et al. 2017; Lunda et al. 2020), natural food for other animals (Poli et al. 2019), or fertiliser for soil-based plant production (Joesting et al. 2016; Doncato and Costa 2018). An alternative for handling solid wastes that FLOCponics may make possible is transforming and reusing them as fertiliser for plant production through remineralisation processes. The mineralisation of sludge is a trend to boost the circularity of RAS-based aquaponics (Delaide et al. 2019) and should also be considered in FLOCponics.

Another concerning point that affects the full development of FLOCponics is the lack of knowledge on the effects of bioflocs microbial communities on the concentration of macro and micronutrients in the water. So far, the role of the biofloc microorganism is only well reported on the nitrogen pathway. The understanding of how biofloc microorganisms interact with other nutrients is essential to predict the nutrients available for plant production and propose efficient nutrient supplementation protocols.

The recommendations for future research highlighted in this general discussion chapter, as well as in the other chapters, should be addressed by specialists in the different areas of expertise that FLOCponics comprises. Additionally, FLOCponics research groups worldwide are still small and there are only a few. A communication channel between them would be easy to manage and very advantageous for promoting complementary research and avoiding duplication or lack of standardisation of the studies.

7.7 Overall conclusion

The main findings of this thesis are linked below with each research question:

(1) What are the opportunities and drawbacks of FLOCponics?

- FLOCponics system is likely to be applied in the short-term by farmers who already operate biofloc-based fish culture and seek to improve the system efficiency.
- The current FLOCponics drawbacks are essentially related to systems' design and operation and proper destination of solid wastes.

(2) To what degree do the productive results achieved in on-demand coupled FLOCponics outperform other production systems?

- On-demand coupled FLOCponics outperforms fish yield of RAS-based aquaponics by 24%.
- Similar lettuce yields were found in on-demand coupled FLOCponics, RAS-based aquaponics and hydroponics systems.

(3) To what degree is it possible to take advantage of the nutritional benefits of biofloc for tilapia production in an on-demand coupled FLOCponics system?

- Benefits of bioflocs for fish nutrition are also seen in on-demand coupled FLOCponics systems.
- On-demand coupled FLOCponics leads to 8% reduction in the fish dietary crude protein compared to RAS-based aquaponics.

(4) How efficient will it be to transform a biofloc-based fish farm into a FLOCponics production in terms of productivity and resource use?

- The water and nutrient use efficiencies are higher in FLOCponics than biofloc-based fish monoculture by 10 and 27%, respectively.
- The amount of solids discharged in FLOCponics is lower than biofloc-based fish monoculture by 10%.
- FLOCponics system can be even more efficient by expanding the planting area up to 3.2 times the area used in this thesis, Chapter 4.
- The water and nutrient use efficiencies in expanded FLOCponics are 7 and 25%, respectively, higher than in the nominal FLOCponics system.

(5) How and to what degree do FLOCponics systems affect the sustainability of the stand-alone systems?

- FLOCponics is as sustainable as the stand-alone biofloc and hydroponics systems based on emergy synthesis results.
- The unit emergy value (UEV) in FLOCponics is 104 times lower than in hydroponics system, indicating higher efficiency.
- The UEV of FLOCponics is 78% higher than in biofloc system, indicating lower efficiency.

(6) What does make a fish species suitable for aquaponics, and how does it contribute to the potential to diversify FLOCponics systems?

- In permanently coupled RAS-based aquaponics, the choice of fish species should depend on the crop grown. Thus, fish should be rustic and tolerate a wide range of physical-chemical water parameters and concentrations of nutrients in the water.
- In on-demand coupled RAS-based aquaponics, the choice of the fish species is directly dependent on the fish market acceptance, production costs and growth rate. Fish suitable for RAS production are also suitable for RAS-based aquaponics.
- FLOCponiccs systems are recommended to operate in an on-demand coupled layout, thus, the fish species suitable for the biofloc-based system will also be eligible for FLOCponics.
- The diversification of FLOCponics can be increased by producing alternative fish species, as tambaqui, pacu or jundia.

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Summary

Aquaculture has been responsible for providing healthy food for the growing population. Aquaculture contribution to the global food production scenario is expected to continuously grow through intensive and sustainable production methods, such as biofloc-based culture and aquaponics (integration of fish-plant production). This thesis explored a new aquatic-based food production method, i.e., the integration of biofloc-based aquaculture with soilless plant (hydroponics) production in so-called FLOCponics systems. FLOCponics is a special case of integrated agri-aquaculture, where nutrients from a biofloc-based culture are used to nourish and irrigate plants in a hydroponics subsystem. FLOCponics is quite a new research field, and little is known about fish and plant productivity, resource use efficiency, and sustainability of this integrated system. Developing a better understanding of FLOCponics may bring advantages to food producers and society.

In this context, the overall aim of this thesis was to investigate and discuss the technical feasibility, efficiency and sustainability of on-demand coupled FLOCponics for tilapia juveniles and lettuce production. For that, the following specific objectives were formulated: (1) To identify the status quo of FLOCponics, highlight current FLOCponics challenges and give directions for further research. (2) To determine the technical feasibility of producing tilapia juveniles and lettuce in on-demand coupled FLOCponics compared to traditional (RAS-based) on-demand coupled aquaponics, hydroponics and biofloc-based monoculture systems. (3) To determine how the reduction of protein content in the fish diet affects fish and plant growth, dietary nutrient use by fish and water quality in on-demand coupled FLOCponics system. (4) To investigate the efficiency of on-demand coupled FLOCponics system in terms of resource use and amount of food produced and discuss the overall advantages and disadvantages of FLOCponics compared to biofloc-based fish culture. (5) To assess and discuss the sustainability of biofloc-based fish culture with and without integrating with hydroponic plant production and provide insights on how to improve the sustainable character of food production in such systems. (6) To identify suitable fish species for aquaponics and discuss their applicability to diversify FLOCponics' products.

To achieve the research objectives, the thesis comprises seven chapters. Chapter 1 presents a general introduction containing a brief background on the topics that guide this thesis, including biofloc-

based aquaculture, aquaponics, and the methods to measure the systems' efficiency and sustainability. Chapter 2 critically reviews and analyses the FLOCponics research regarding the system setups, water quality and nutrient recycling, and the productive results of plants and fish. In this chapter, we also identified economic and environmental aspects and discussed the gaps, opportunities, and challenges of FLOCponics systems. In general, FLOCponics systems seem to be applicable in the short-term by farmers who already operate biofloc-based fish culture and seek to improve the system efficiency. An important contribution of this chapter was the identification of current FLOCponics drawbacks that are essentially related to systems' design and operation and proper destination of solid wastes. The review paper presented in Chapter 2 served as a theoretical basis for the following chapters.

An experiment was conducted to investigate and evaluate the production of tilapia juveniles and lettuce in an on-demand FLOCponics system to explore the technical feasibility of it compared to other production systems (objective 2) and to what degree is it possible to take advantage of the nutritional benefits of biofloc for tilapia production in such system (objective 3). For that, tilapia juveniles and lettuce were cultured in on-demand coupled FLOCponics, traditional aquaponics using recirculating aquaculture system (RAS), biofloc-based monoculture, and/or hydroponics systems, and four fish diets were formulated, produced, and tested in FLOCponics systems. The findings of this experimental trial are reported in Chapter 3. We found similar lettuce yields in on-demand coupled FLOCponics, RAS-based aquaponics and hydroponics systems. With respect to fish production, FLOCponics outperforms fish yield of RAS-based aquaponics by 24%. In addition, benefits of bioflocs for fish nutrition were also seen in on-demand coupled FLOCponics systems, leading to 8% reduction in the fish dietary crude protein compared to RAS-based aquaponics. These results indicated that on-demand coupled FLOCponics systems are technically feasible and allow a reduction in the crude protein content in fish diets and thus can be used as an alternative feeding strategy in an integrated system.

Chapter 4 follows a dynamic modelling approach to investigate the efficiency of FLOCponics system compared to stand-alone biofloc-based system. The results showed that, in general, FLOCponics was more efficient in using resources than the biofloc system. The water and nutrient use efficiencies were higher in FLOCponics than in biofloc system by 10 and 27%, respectively. The volume of solids discharged in FLOCponics was 10% lower than in biofloc-based fish monoculture. When performing scenario simulations, the FLOCponics system was even more efficient by expanding the planting area up to 3.2 times in relation to the nominal case. The findings presented in this model-based study support the hypothesis that integrating with hydroponics makes biofloc-based fish culture more efficient in terms of resource use and wastes avoidance.

Assessing the sustainability of food production systems in their early stage of development is essential to identify unsustainable practices and guide further research to avoid them. In Chapter 5, we applied emergy synthesis to compare the sustainability of stand-alone biofloc and hydroponics systems with their integration in a FLOCponics system, based on the previews experimental results. The emergy indicators indicated that FLOCponics is as sustainable as the stand-alone biofloc and hydroponics systems. Additionally, we found that the unit emergy value (UEV) in FLOCponics was 10^4 times lower than in hydroponics system, indicating higher efficiency. On the other hand, FLOCponics was less efficient in converting energy to outputs than biofloc system, as the UEV of FLOCponics was 78% higher. The emergy synthesis of the FLOCponics system pointed out that further improvements must be made to increase the efficiency of the system and explore its full potential for sustainable food provision.

Chapter 6 reviews fish species suitable for aquaponics as a mean to improve the diversification of integrated agri-aquaculture systems. This chapter presented the characteristics that make a fish species sustainable for each system layout (permanently or on-demand coupled). In permanently coupled RAS-based aquaponics, the choice of fish species depends on the crop grown. In on-demand coupled RAS-based aquaponics, the choice of the fish species is directly dependent on the fish market acceptance, production costs and growth rate. In general, fish suitable for RAS production are also suitable for RAS-based aquaponics. The implications of the findings of Chapter 6 on FLOCponics is discussed in Chapter 7. The diversification of FLOCponics can be increased by producing alternative fish species suitable for the biofloc-based systems, as tambaqui, pacu or jundia.

Lastly, Chapter 7 discusses the main findings of the thesis, highlighting the advances in the FLOCponics research field and their social impacts. From a broad perspective, FLOCponics can bring the production of a mix of fresh and healthy food close to the consumer. That is because FLOCponics seems suitable for implementation in regions usually not destined for food production, contributing to increasing food security and fair food distribution. As a final remark, developing FLOCponics to be a representative activity for food supply guarantees more efficient use of resources and contributes to sustainable aquaculture development.

Resumo

Aquicultura tem sido responsável por contribuir para o fornecimento de alimentos para uma população em crescimento. No entanto, para que essa contribuição continue a acontecer, métodos de produção intensivos e sustentáveis precisam ser empregados e desenvolvidos como, por exemplo, os cultivos baseados na tecnologia de bioflocos e aquaponia (integração da produção de peixes e plantas). Esta tese abordou um novo e promissor método de produção de alimentos de base aquática que é a integração da aquicultura utilizando a tecnologia de bioflocos com a produção de plantas hidropônicas, conhecida como FLOCponia. Os sistemas FLOCpônicos são uma inovação aos sistemas integrados de agri-aquicultura, onde os nutrientes do sistema de bioflocos são usados para nutrir e irrigar plantas em um subsistema hidropônico. Pesquisas com sistemas FLOCpônicos estão apenas começando e pouco se sabe sobre a produtividade de peixes e plantas, a eficiência do uso de recursos e sustentabilidade deste sistema integrado. Investigar e desenvolver os sistemas FLOCpônicos trazem vantagens para os produtores de alimentos e para a sociedade.

Nesse contexto, o objetivo geral desta tese foi investigar a viabilidade técnica, a eficiência do uso de recursos e a sustentabilidade dos sistemas FLOCpônicos acoplados sob demanda para a produção de juvenis de tilápia e alface. Para isso, os seguintes objetivos foram formulados: (1) Identificar os trabalhos já realizados com FLOCponia, destacando os desafios atuais, e orientar pesquisas futuras. (2) Determinar a viabilidade técnica da produção de juvenis de tilápia e alface em sistemas FLOCpônicos acoplados sob demanda em comparação à aquaponia tradicional utilizando sistema de recirculação aquícola (RAS), à hidroponia e ao sistema de bioflocos. (3) Determinar como a redução do teor de proteína e o uso de nutrientes na dieta afetam o crescimento dos peixes e das plantas quando cultivados em sistema FLOCpônico acoplado sob demanda. (4) Investigar a eficiência dos sistemas FLOCpônicos em relação ao uso de recursos e quantidade de alimentos produzidos, bem como discutir as vantagens e desvantagens gerais da FLOCponia em comparação ao cultivo de peixes no sistema de bioflocos. (5) Avaliar e discutir a sustentabilidade da produção de peixes nos sistemas de bioflocos com e sem integração com a produção de plantas hidropônicas e, assim, fornecer *insights* sobre como melhorar a sustentabilidade da produção de alimentos em tais sistemas. (6) Identificar espécies de peixes adequadas para a aquaponia e discutir como a diversificação das espécies cultivas pode ser aplicada na FLOCponia.

Para alcançar os objetivos de pesquisa, a tese é composta por sete capítulos. O Capítulo 1 apresenta uma introdução geral contendo um embasamento teórico sobre os tópicos que orientam esta tese, incluindo os sistemas aquícolas de bioflocos, aquaponia e os métodos para medir a eficiência e sustentabilidade destes sistemas. O Capítulo 2 revisa e analisa criticamente as pesquisas com FLOCponia em relação às configurações do sistema, qualidade da água e ciclagem de nutrientes, e os resultados produtivos de plantas e peixes. Nesse capítulo também identificamos aspectos econômicos e ambientais e discutimos as lacunas, oportunidades e desafios dos sistemas FLOCpônicos. Em geral, os sistemas FLOCpônicos parecem ser aplicáveis no curto prazo por produtores que já utilizam a tecnologia de bioflocos para produção de peixes e que buscam melhorar a eficiência da produção. Uma contribuição importante desse capítulo foi a identificação das desvantagens atuais dos sistemas FLOCpônicos. Em geral, a maioria dos problemas enfrentados na FLOCponia está relacionada ao *design,* à operação dos sistemas e ao destino adequado dos resíduos sólidos. O artigo de revisão apresentado no Capítulo 2 serviu como base teórica para os capítulos seguintes.

Um experimento foi conduzido para investigar e avaliar a produção de juvenis de tilápia e alface em sistema FLOCpônico acoplado sob demanda, com foco principal em explorar a viabilidade técnica do mesmo em comparação com outros sistemas de produção (objetivo 2) e entender até quanto é possível aproveitar os benefícios nutricionais dos bioflocos para a produção de tilápia nesse sistema integrado (objetivo 3). Para isso, juvenis de tilápia e alface foram cultivados em sistemas FLOCpônicos acoplados sob demanda, aquaponia tradicional utilizando RAS, monoculturas em sistema de bioflocos e/ou hidroponia. Além disso, quatro dietas para peixes foram formuladas, produzidas e testadas nos sistemas FLOCpônicos. Os resultados deste experimento são relatados no Capítulo 3. Para a produção de alface, produtividades semelhantes foram encontradas nos sistemas FLOCpônicos, aquapônicos e hidropônicos. Com relação à produção de peixes, foi observado um aumento de 24% no crescimento dos peixes na FLOCponia em comparação à aquaponia utilizando RAS. Além disso, os benefícios dos bioflocos para a nutrição dos peixes foram observados em sistemas FLOCpônicos acoplados sob demanda, levando a uma redução de 8% na proteína bruta da dieta dos peixes em comparação ao sistema aquapônico utilizando RAS. Esses resultados indicaram que os sistemas FLOCpônicos acoplados sob demanda são tecnicamente viáveis e permitem a redução do teor de proteína bruta em dietas para peixes.

O Capítulo 4 emprega modelos matemáticos dinâmicos para investigar a eficiência do uso de recursos do sistema FLOCpônico em comparação ao monocultivo de peixes em sistema de bioflocos. Os resultados mostraram que, em geral, o sistema FLOCpônico é mais eficiente no uso de recursos do que o sistema de bioflocos. As eficiências de uso de água e de nutrientes foram maiores na FLOCponia do que no sistema de bioflocos em 10 e 27%, respectivamente. O volume de sólidos descartados no sistema FLOCpônico foi 10% menor do que no de bioflocos. Ao realizar simulações de cenários, o sistema FLOCpônico foi ainda mais eficiente ao expandir até 3,2 vezes a área de plantio em relação ao sistema inicialmente estudado. Os resultados apresentados neste estudo de modelagem confirmam a hipótese de que a integração com a hidroponia torna a produção de peixes em bioflocos mais eficiente no uso de recursos e na prevenção de desperdícios.

Avaliar a sustentabilidade dos sistemas de produção de alimentos em seu estágio inicial de desenvolvimento é essencial para identificar práticas insustentáveis e orientar pesquisas futuras que visem evitá-las. No Capítulo 5, aplicamos a síntese em emergia para comparar a sustentabilidade dos sistemas de bioflocos e hidropônicos com sua integração em FLOCponia, com base nos resultados experimentais. Os indicadores emergéticos indicaram que o sistema integrado é tão sustentável quanto as monoculturas em bioflocos e hidroponia. Além disso, o valor emergético unitário (UEV) no sistema FLOCpônico foi 10^4 vezes menor do que no sistema hidropônico, indicando maior eficiência. Por outro lado, o sistema FLOCpônico foi menos eficiente na conversão de energia em produtos do que o sistema de bioflocos, evidenciado pelo maior UEV do sistema integrado. A síntese em emergia do sistema FLOCpônico apontou que melhorias devem ser feitas para aumentar a eficiência do sistema e explorar todo o seu potencial para o fornecimento sustentável de alimentos.

O Capítulo 6 revisa as espécies de peixes adequadas para a aquaponia como um meio de melhorar a diversificação dos sistemas integrados de agri-aquicultura. Este capítulo apresentou as características que tornam uma espécie de peixe adequada para cada *layout* de sistema (permanentemente acoplado ou acoplado sob demanda). Na aquaponia utilizando RAS permanentemente acoplada com o subsistema hidropônico, a escolha das espécies de peixes depende diretamente do vegetal que será cultivado. Em sistemas aquapônicos acoplados sob demanda, a aceitação do mercado, os custos de produção e a taxa de crescimento são fatores determinantes para escolha das espécies de peixes a serem cultivadas. Em geral, peixes adequados para a produção em RAS também são adequados para aquaponia utilizando RAS. As implicações das descobertas do Capítulo 6 na FLOCponia são discutidas no Capítulo 7. Em resumo, destacamos que a diversidade de

produtos oferecidos pelo sistema FLOCpônicos pode ser aumentada com a produção de espécies de peixes adequadas para os sistemas baseados em bioflocos, como o tambaqui, o pacu ou o jundiá.

Por último, o Capítulo 7 discute as principais conclusões da tese, destacando os avanços nas pesquisas com FLOCponia e seus impactos na sociedade. De uma perspectiva ampla, o sistema FLOCpônico pode aproximar a produção de uma grande diversidade de alimentos frescos e saudáveis ao consumidor. Isso porque o sistema FLOCpônico pode ser implantado em regiões geralmente não destinadas à produção de alimentos, contribuindo para o aumento da segurança alimentar e melhoria da distribuição de alimentos. Por fim, desenvolver os sistemas FLOCpônicos para serem sistemas de produção representativos para abastecimento de alimentos garante utilização mais eficiente dos recursos e contribui para o desenvolvimento sustentável da aquicultura.

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About the Author

Sara Pinho was born on November 25th, 1992, in Laguna-SC, Brazil. In 2011, she started to study Fishing Engineering at Santa Catarina State University. During her BSc education, Sara conducted experiments and collaborated with research projects focused on biofloc-based aquaculture systems, fish nutrition, and aquaponics. Moreover, she carried out a pioneer and simple study to investigate the integration of biofloc-based tilapia production and hydroponics. Such study was the basis for her Master and PhD projects. From 2016 to 2018, she was enrolled as a master's student at the Aquaculture Center of the São Paulo State University (Caunesp). During this period, she published several articles, in one of which the terminology "FLOCponics" was introduced. In 2018, Sara started a joint doctorate at Caunesp and Wageningen University (WU). Here, she continued working on FLOCponics. Besides conducting technical experiments, Sara learned how to apply dynamic modelling and sustainability assessments to understand such a system from a broader perspective and thus propose efficient FLOCponics systems. The results obtained in her PhD period at Caunesp and WU are presented in this thesis.

List of Publications

Pinho SM, Lima JP, David LH, Emerenciano M, Goddek S, Verdegem M, Keesman KJ, Portella MC (2021) FLOCponics: The integration of biofloc technology with plant production. Reviews in Aquaculture 1-29. https://doi.org/10.1111/raq.12617

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- Environmental research in context (2020)
- Research in context activity: ‘Co-organizing workshop on Aquaponics, June 2019 -- Jaboticabal, Brazil’

Other PhD and Advanced MSc Courses

- How to Measure Sustainability in Aquaculture, São Paulo State University (2018)
- Cost Determination and Analysis in Aquaculture, São Paulo State University (2018)
- Project Management and Multidisciplinary Professional Update, São Paulo State University (2018)
- General Concepts of Aquaponics, São Paulo State University (2019)
- Statistics Applied to Aquaculture, São Paulo State University (2019)
- Basic Statistics, PE&RC and WIMEK (2020)
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Oral Presentations

- *South American fish species suitable for aquaponics: a review.* AQUACIENCIA – online congress, 14-16 September 2021, Online

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