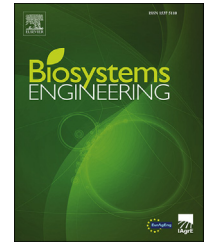


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Research Paper

Modelling FLOCponics systems: Towards improved water and nitrogen use efficiency in biofloc-based fish culture



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FLOCponics is an integrated agri-aquaculture system, in which water and nutrients from a biofloc-based fish culture are reused to fertilise soilless plants. This paper is the first modelling study that focuses on decoupled FLOCponics with the aim of investigating and discussing 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, a biofloc-based monoculture system with a total volume of the fish tanks of 15.2 m³, and a FLOCponics system with similar biofloc system and a planting area of 33.6 m² was modelled. The simulation models of these reference systems were run for a period of five years, and water, nitrogen and total suspended solid balances in both systems were compared. In addition to this, various planting areas of the FLOCponics system were changed step-wise 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 the stand-alone biofloc system. Also, the integrated system results in a reduction of 10% in the amount of solids discharged. Optimisation of the planting area with respect to key model outputs led to an improved FLOCponics system, where the planting area of the system 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.

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Nomenclature	
FP	FLOCponics system
BFT	Biofloc technology or biofloc system
FT	Fish tank
RFS	Radial flow settler
MT	Mixing tank
B	Bag filter
DWC	Deep water culture
AQ _{sub}	Aquaculture subsystem
HP _{sub}	Hydroponics subsystem
C:N	Carbon to Nitrogen ratio
bf	Biofloc
WUE	Water use efficiency
NUE	Nitrogen use efficiency
NSC	Normalised sensitivity coefficients
KPI	Key performance indicators
TAN	Total ammonia nitrogen
NH ₃	Unionised ammonia
NO ₃	Nitrate
TSS	Total suspended solids (mg l ⁻¹)
ET	Evapotranspiration (mm d ⁻¹)
Evap	Evaporation (mm d ⁻¹)
Δt	Time step size (d)
ρ _{water}	Density of water (kg l ⁻¹)
Φ _{v,i-j}	Water flow from tank i to tank j (m ³ d ⁻¹)
Φ _{v,AQinitial}	Volume of inoculum to fill each fish tank (m ³ d ⁻¹)
Φ _{v,AQfresh}	Volume flow of freshwater in the aquaculture subsystem (m ³ d ⁻¹)
Φ _{v,Bset}	Water flow from the solids retained in the bag filter (m ³ d ⁻¹)
Φ _{v,sludge}	Outflow of water through sludge discharge (m ³ d ⁻¹)
Φ _{v,AQevap}	Outflow of water through evaporation (m ³ d ⁻¹)
Φ _{v,HPinitial}	Volume flow of initial freshwater in the hydroponics subsystem (m ³ d ⁻¹)
Φ _{v,HPfresh}	Volume flow of freshwater used for dilution in the hydroponics subsystem (m ³ d ⁻¹)
Φ _{v,discharge}	Outflow of water from the HP _{sub} (m ³ d ⁻¹)
Φ _{v,HPevapt}	Outflow of water through evapotranspiration (m ³ d ⁻¹)
V _i	Water volume in tank i (m ³)
m _{n,i}	Mass of nutrient n in tank i (g)
m _{TSS,i}	Mass of TSS in tank i (g)
m _{n,i-set}	Mass of nutrient n settled in tank i (g)
M _{TSS,i-set}	Mass of TSS settled in tank i (g)
m _{fish}	Mass of fish (g)
m _{bf}	Mass of biofloc (g)
m _{plant}	Mass of plant (g)
Φ _{feed}	Rate of fish feed entered (g d ⁻¹)
Φ _{molasses}	Rate of molasses entered (g d ⁻¹)
Φ _{bf}	Rate of biofloc consumed by fish (g d ⁻¹)
Φ _{n,fish}	Rate of nutrient n released by fish (g d ⁻¹)
Φ _{n,bf}	Rate of nutrient n released by the biofloc microorganisms (g d ⁻¹)
Φ _{N,bf_prod}	Rate of nitrogen consumption by the biofloc microorganisms (g d ⁻¹)
Φ _{N,bf_cons}	Rate of nitrogen production by the biofloc microorganisms (g d ⁻¹)
Φ _{TSS,fish}	Rate of TSS released by fish (g d ⁻¹)
Φ _{TSS,bf}	Biofloc biomass growth rate in the fish tank (g d ⁻¹)
Φ _{set}	TSS settle rate in the RSF (g d ⁻¹)
Φ _{Bset}	TSS settle rate in the bag filter (g d ⁻¹)
Φ _{fertiliser}	Rate of fertiliser entered (g d ⁻¹)
Φ _{n,plant}	Rate of nutrient n uptake by plant (g d ⁻¹)
C _{n,i-j}	Concentration of nutrient n from tank i to tank j (mg l ⁻¹)
C _{TSS,i-j}	Concentration of TSS from tank i to tank j (mg l ⁻¹)
C _{n,initial}	Initial concentration of nutrient n (mg l ⁻¹)
C _{TSS, initial}	Initial concentration of TSS (mg l ⁻¹)
C _{TSS, fresh}	Concentration of TSS in the freshwater (mg l ⁻¹)
C _{n,RSFset}	Concentration of nutrient n in the solids settled in the RSF (mg l ⁻¹)
C _{TSS,RSFset}	Concentration of TSS in the solids settled in the RSF (mg l ⁻¹)
C _{TSS,Bset}	Concentration of nutrient n in the solids settled in the bag filter (mg l ⁻¹)
C _{n,discharge}	Concentration of nutrient n discharged from the hydroponics subsystem (mg l ⁻¹)
C _{TSS, discharge}	Concentration of TSS discharged from the hydroponics subsystem (mg l ⁻¹)
k _{RFSset_FP}	Coefficient of solids settling in the RFS of the FLOCponics system
TSS _{max}	Maximum concentration of TSS in the FT (mg l ⁻¹)
k _{N,fishexcret}	Coefficient of mass of N indigestible fraction per mass of N in dry feed
C _{N,required}	Minimum concentration of N required by plant (mg l ⁻¹)

1. Introduction

Given the growing pressure to achieve sustainable aquaculture, production systems have been developed to improve resource use efficiency and minimise waste discharge (Ahmed & Thompson, 2019; Naylor et al., 2021). While most aquaculture farmers practice monoculture that is highly dependent on non-renewable resources, the modern trend of aquaculture research focuses on boosting systems that reuse water and nutrients to grow multiple organisms (Boyd et al., 2020; David, Pinho, Agostinho, et al., 2021; Kerrigan & Suckling,

2018). Integrated agri-aquaculture and biofloc-based culture are examples of such production systems (Betanzo-Torres et al., 2021; Browdy, Ray, Leffler, & Avnimelech, 2012; Zajdband, 2011).

Biofloc technology (BFT) has been used in intensive farms, enabling high animal yields on small land areas and minimal water discharge (Emerenciano, Gaxiola, & Cuzon, 2013; Khanjani & Sharifinia, 2020; Martinez-Cordova et al., 2022). Biofloc-based culture is characterised by the growth of specific microorganisms, usually *in situ* in the fish tank, for improving water quality, disease prevention, and waste treatment (Crab, Defoirdt, Bossier, & Verstraete, 2012; Mugwanya, Dawood,

Kimera, & Sewilam, 2021). The microorganisms, especially heterotrophic and nitrifying bacteria, play an important role in the organic matter degradation and nitrogen cycle (Emerenciano, Martínez-Córdova, Martínez-Porchas, & Miranda-Baeza, 2017). Moreover, these microorganisms are a nutrient-rich supplementary source of food for the cultured species (Martínez-Córdova, Martínez-Porchas, Emerenciano, Miranda-Baeza, & Gollas-Galván, 2017; Sgnaulin et al., 2021; Sousa, Pinho, Rombenso, de Mello, & Emerenciano, 2019).

By demanding reduced quantities of feed, land, and water, biofloc-based culture has been labelled as a sustainable aquaculture approach (Bossier & Ekasari, 2017; David, Pinho, Keesman, & Garcia, 2021; Pinho, David, Garcia, Portella, & Keesman, 2022). 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 animal density. In addition, 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, Morales Suazo, & Emerenciano, 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*) (Dauda, 2020; Samocha, 2019; Walker et al., 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, BFT effluent could be reused as fertiliser in integrated agri-aquaculture systems (Pinheiro et al., 2020; Pinho, Molinari, Mello, Fitzsimmons, & Emerenciano, 2017).

In integrated agri-aquaculture systems, water and nutrients wasted from aquaculture are reused to produce vegetables with marketable value (Nederlof et al., 2019; Zajdband, 2011). FLOCponics is an example of an integrated system that combines biofloc-based production with hydroponics, a soilless plant production method (Kotzen, Emerenciano, Moheimani, & Burnell, 2019; Pinho, David, Goddek, Emerenciano, & Portella, 2021). Recent studies have reported higher or similar animal growth in FLOCponics than in biofloc-based culture without integration (Pinheiro et al., 2017; Pinho, Lima, et al., 2022) or conventional aquaponics (Fimbres-Acedo, Magallón-Servín et al., 2020; Martínez-Córdova, 2020; Rocha, Biazzezzetti, Stech, & Silva, 2017; Saseendran, Dube, Chandrakant, & Babitha Rani, 2021). In conventional aquaponics, the aquaculture subsystem is typically 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 (Pinho, Lima, et al., 2022; Rocha et al., 2017) or conventional aquaponics (Martínez-Córdova et al., 2020; Pinho et al., 2017; Rocha et al., 2017; 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, Lima, et al., 2022), recently renamed as on-demand coupled layout (Baganz et al., 2022). 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, Servín-Villegas et al., 2020; Pinho, Lima, et al., 2022).

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 how efficient the utilisation of water and nutrients is in FLOCponics 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, David, Goddek, Emerenciano, & Portella, 2021). To fill this gap, the use of mathematical models is very suitable for understanding 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 (Dijkgraaf, Goddek, & Keesman, 2019; Estrada-Perez et al., 2018; Goddek et al., 2016; Goddek & Körner, 2019; Karimanzira, Keesman, Kloas, Baganz, & Rauschenbach, 2016; Keesman et al., 2019; Kloas et al., 2015; Körner et al., 2021; Tarigan, Goddek, & Keesman, 2021; Yogev, Barnes, & Gross, 2016). 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 & 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 resource use efficiency of biofloc-based fish production and by how much. For this purpose, two mathematical models were built and calibrated, using mass balances, consecutive laws and experimental data, with the aim of comparing the water and nitrogen use per kg of food produced in a biofloc-based monoculture (without plant production) and in a decoupled FLOCponics system.

2. Method

Empirical data from experiments were combined with mass balances of biofloc and FLOCponics systems. The system designs were based on an experimental setup we operated at the Aquaculture Centre of Unesp (Caunesp), Jaboticabal, SP, Brazil, which is described in detail by Pinho, Lima, et al. (2021). In the experiments, the production of tilapia juveniles (*Oreochromis niloticus*) were compared in a stand-alone biofloc system and tilapia juveniles and lettuce (*Lactuca sativa*) in a FLOCponics system in small-experiment systems setups. The focus was on the nursery phase of tilapia culture, which

involves producing juveniles weighing approximately 1–30 g, to support the segmentation of the production process into multiple phases. In both systems, heterotrophic brown water biofloc was maintained. To make the model output more comprehensive, in the present model-based study a ten times larger system than the one described in [Pinho, Lima, et al. \(2021\)](#) was considered, 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 model are presented in [Table 1](#). The initial conditions and other parameters are presented in the [Appendices A to D](#).

2.1. Description of the systems and processes

In the biofloc system ([Fig. 1](#)), four fish tanks, each coupled with a radial flow settler (RFS), were considered (see [Table 2](#) and [Appendices B, C and D](#) for modelling details). Water remained in the fish tank most of the time, and it was only directed to the RFS to reduce the solids concentration when the total suspended solids (TSS) exceeded a level of 500 mg l⁻¹, as recommended for biofloc-based fish culture ([Emerenciano et al., 2017](#); [Hargreaves, 2013](#)). When the RFS was used, recirculating water enters ($\phi_{v,FT-RFS}$) and leaves ($\phi_{v,RFS-FT}$) the RFS at a specific rate of 0.792 m³ h⁻¹. Each RFS was operated until the TSS in the fish tank reaches 100 mg l⁻¹ in a variable time interval ([Emerenciano et al., 2017](#); [Hargreaves, 2013](#)). In this study, a TSS settling efficiency of 70% was assumed ([Mendez, Morales, & Merino, 2021](#)). In both biofloc and FLOCponics systems, the TSS concentration was assumed to be equal to the bioflocs biomass concentration ([Ekasari et al., 2014](#)).

In the decoupled FLOCponics system ([Fig. 2](#)), the aquaculture (AQ) and hydroponics (HP) subsystems were operated as separated loops in a decoupled (on-demand coupled) system layout. The aquaculture subsystem comprised four fish tanks, each one with an RFS and a bag filter (B). The hydroponics subsystem consisted of a mixing tank linked to the hydroponics deep-water culture bed.

Contrary to the biofloc system, in FLOCponics, RFS and bag filter were used every time the water from the aquaculture subsystem was directed to the hydroponics subsystem ($\phi_{v,AQ-HP}$), aiming to reduce the TSS concentration in the effluent of the aquaculture subsystem. The water from the fish tank was directed to the RFS ($\phi_{v,FT-RFS}$) until filling it, it remained there for 15 min to settle the TSS, considering a settling efficiency of 90% (called FLOCponics RFS procedure). Then, the supernatant nutrient water was pumped through the bag filter ($\phi_{v,RFS-B}$), with a solid retention efficiency of 60%, and subsequently to

the hydroponics subsystem ($\phi_{v,AQ-HP}$) at a variable rate. The values for RFS settling efficiency and solid retention efficiency of the bag filter were based on experimental observations ([Mendez et al., 2021](#)). When the TSS concentration in the fish tank was lower than the maximum recommended, the solids settled in the RFS return to the fish tank ($\phi_{v,sludge}$). On the other hand, when the TSS concentration in the fish tanks in the FLOCponics system surpassed 500 mg l⁻¹, the TSS was firstly removed following the FLOCponics RFS procedure and then, if needed, the RFS was operated as described for the biofloc system until TSS concentration reached 100 mg l⁻¹. The solids retained in the bag filter always returned to the fish tank ($\phi_{v,Bset}$). After the start of plant production, the RFS and bag filter were operated only once a day. Before the plant production starts, the RFS worked as described for the biofloc system.

The water flow from aquaculture subsystem to hydroponics subsystem ($\phi_{v,AQ-HP}$) 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 by applying the FAO Penman–Monteith Equation ([Allen, Pereira, Raes, & Smith, 1998](#)), for lettuce production in Jaboticabal, SP, Brazil ([Fig. 3](#), for details see [Appendix A](#)). The nutrient uptake by lettuce for growth was assumed to be proportional to the evapotranspiration ([Dijkgraaf et al., 2019](#)). Although the FAO Penman–Monteith equation gives a rough estimate of the evapotranspiration, it has been used in aquaponics modelling studies as a simple and useful equation ([Dijkgraaf et al., 2019](#); [Goddek & Keesman, 2018](#); [Tarigan et al., 2021](#)).

In both systems, fish production started at day 0. Fish was stocked in the four tanks in a staggered sequence for approximately 27 d. Each tank was harvested when the tilapia juveniles reach 30 g, i.e., once every 56 d. The fish tanks were filled at a rate of 3.8 m³ 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 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. The optimal water quality conditions were chosen based on [Emerenciano et al. \(2021\)](#).

Lettuce production started when the fourth fish tank was initially stocked, on day 82. The length of each complete lettuce growth cycle was set at 35 d with initial mass of the lettuce plant of 1.4 g. Also, for plant production, optimal management was assumed with nutrient concentrations

Table 1 – Compartment sizes of the biofloc and FLOCponics systems.

Compartment	Size	Comments
Fish tank (FT)	4 × 3.80 m ³	With 300 fish m ⁻³ , approx. 9 kg m ⁻³
Radial flow settler (RFS)	4 × 1 m ³	Operated under demand
Bag filter	4 × 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 fertiliser is added.
Deep water culture (DWC)	Area 33.6 m ² –Volume 8 m ³	With 19 plants m ⁻²

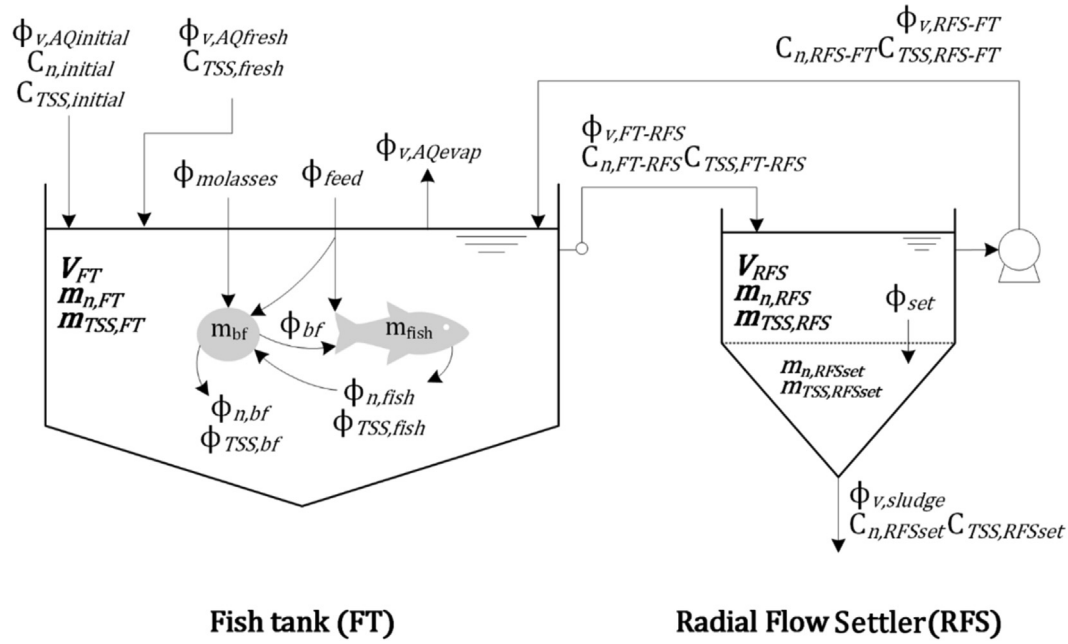


Fig. 1 – Model of biofloc system. The explanations for the abbreviations and variables are presented in the Nomenclature table and Appendices B, C and D.

Table 2 – Mass balances for the models of the biofloc and FLOCponics systems (see also Figs. 1 and 2). The explanations for the abbreviations and variables are presented in the Appendices.

System	d/dt	φ_{in}	φ_{out}
Biofloc	V	$\varphi_{v,AQinitial} + \varphi_{v,AQfresh}$	$\varphi_{v,sludge} + \varphi_{v,AQevap}$
	m_n	$C_{n,AQinitial}\varphi_{v,AQinitial} + \varphi_{n,feed} + \varphi_{n,molasses} + \varphi_{n,bf_prod}$	$C_{n,RFSset}\varphi_{v,sludge} + \varphi_{n,bf_cons}$
	m_{TSS}	$C_{TSS,AQinitial}\varphi_{v,AQinitial} + C_{TSS,fresh}\varphi_{v,AQfresh} + \varphi_{TSS,MicrobProd}$	$C_{TSS,RFSset}\varphi_{v,sludge}$
FLOCponics	V	$\varphi_{v,AQinitial} + \varphi_{v,AQfresh} + \varphi_{v,HPinitial} + \varphi_{v,HPfresh}$	$\varphi_{v,sludge} + \varphi_{v,AQevap} + \varphi_{v,HPevap} + \varphi_{v,discharge}$
	m_n	$C_{n,AQinitial}\varphi_{v,AQinitial} + \varphi_{n,feed} + \varphi_{n,molasses} + \varphi_{n,bf_prod} + \varphi_{fertiliser}$	$C_{n,RFSset}\varphi_{v,sludge} + \varphi_{n,bf_cons} + C_{n,HPdischarge}\varphi_{v,discharge} + \varphi_{n,plant}$
	m_{TSS}	$C_{TSS,AQinitial}\varphi_{v,AQinitial} + C_{TSS,fresh}\varphi_{v,AQfresh} + \varphi_{TSS,bf} + C_{TSS,fresh}\varphi_{v,HPfresh}$	$C_{TSS,RFSset}\varphi_{v,sludge} + C_{TSS,HPdischarge}\varphi_{v,discharge}$

and environmental variables within optimal conditions for lettuce production, resulting in ideal and constant lettuce growth (Dijkgraaf et al., 2019; Goddek et al., 2016; Jones, 2005).

2.2. Description of the mathematical model

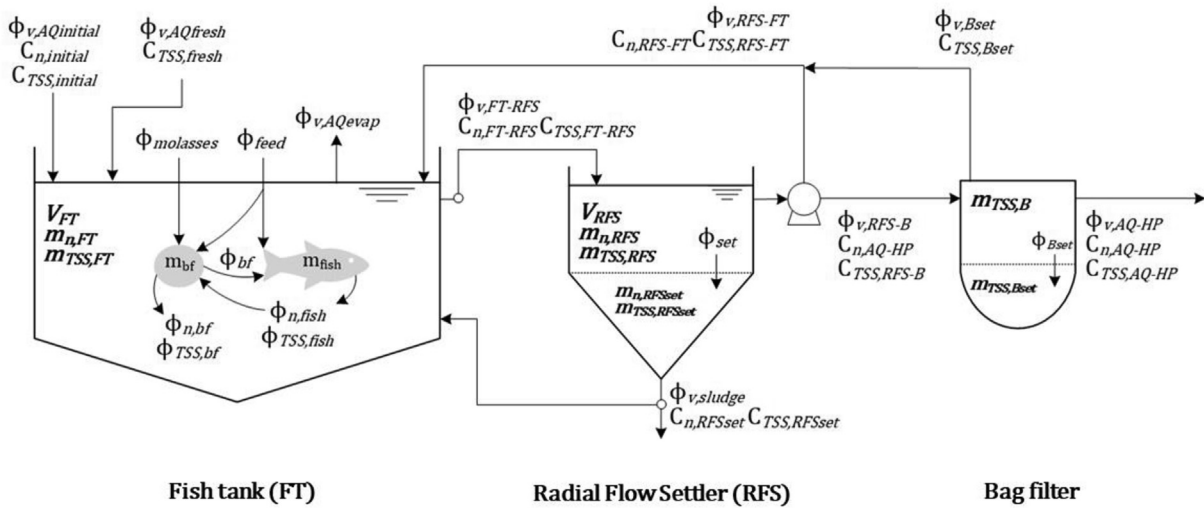
The mathematical model was described in terms of a set of ordinary differential equations, which were solved numerically in Microsoft Excel™ with a step size Δt of 1 day. Mass balances were set up for water volume (V), nutrients (m_n) and total suspended solids (m_{TSS}) in both systems. The mass balances were expressed in terms of inflows and outflows, and V, m_n and m_{TSS} are the state variables and model outputs (Table 2). Figures 1 and 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 setup as a sub-model to calculate bioflocs biomass growth. The auxiliary

equations and the values of the parameters used in the models are detailed in the Appendices (Tables B.1 and B.2). The biofloc and FLOCponics systems were simulated for a period of five years (1825 days). In Section 3, the total production, resource demands and waste discharge, and the key performance indicator (KPI) values are given for the total simulation period of five years, whilst all graphics are plotted for three years.

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 full fish growth model is shown in the Appendices. The specific parameters related to fish feed and growth are based on Pinho, Lima, et al. (2021) and can be found in Table C.1.

Regarding the water balances, in both systems, the volume was considered constant over time, thus $dV/dt = 0$.

Aquaculture subsystem



Hydroponics subsystem

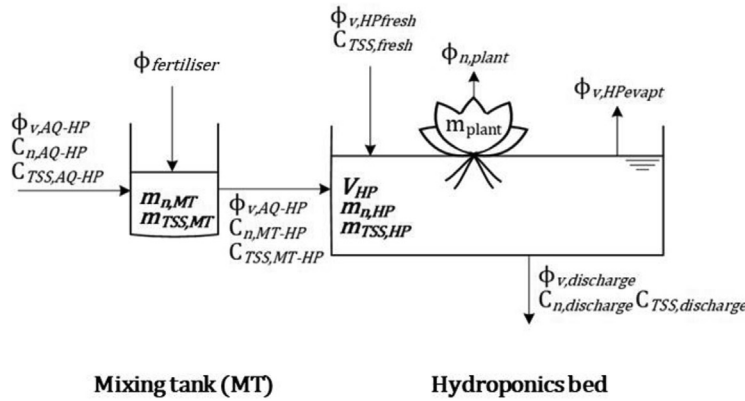


Fig. 2 – Model of decoupled (on-demand coupled) FLOCponics system. The explanations for the abbreviations and variables are presented in the Nomenclature table and Appendices B, C and D.

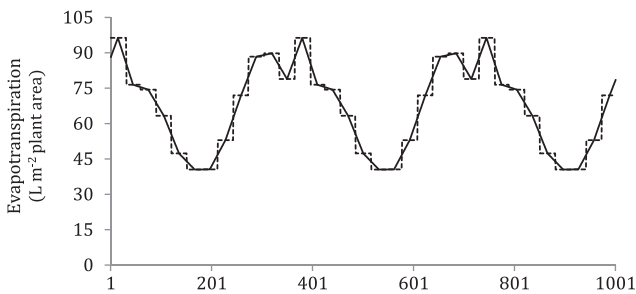


Fig. 3 – Evapotranspiration in the described situation for Jaboticabal, SP, in which day 0 is January 1st, 2019. Evapotranspiration is calculated monthly (dashed line) and smoothed (solid line) for daily interpolation, in 1 m^{-2} plant area.

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 minimal water exchange is expected in the biofloc-based culture, no water is wasted after harvesting. Water was discharged through solids management (sludge removal), or, in the case of the FLOCponics system, it was also discharged 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 the water of the hydroponics subsystem is discharged when the solid concentration trespasses a predefined threshold. At the beginning of the plant production, or each time that the water from the hydroponics subsystem was discharged, the hydroponics subsystem was 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 2, sub-models for TAN, un-ionised ammonia (NH_3) and Nitrate (NO_3) were also implemented (presented in auxiliary

equations, Table B1, and adapted from (Avnimelech, 2015; Lastiri et al., 2016). The biofloc microbial community is directly involved in the assimilation, ammonification, and nitrification of the nitrogen that enters the systems (see Eqs. B.18, B.17, and B.23, respectively), affecting the amount of N from the aquaculture subsystem to the hydroponics subsystem. The production (ϕ_{n,bf_prod} , Eq. B.16) or consumption (ϕ_{n,bf_cons} , Eq. B.18) of N by the biofloc microbial community was included in the balances as inputs and outputs, respectively. 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 concentrations varies a lot in hydroponics culture. Regarding the ideal N concentrations in the hydroponics subsystem, a range from 100 to 200 mg l⁻¹ was chosen (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 B.1 and B.2). We included as limiting substrates in the bioflocs growth equation both the organic C (following the same mass balances as for total N) and inorganic N, since all biofloc-based studies present the growth of bioflocs microbial communities as a function of the C:N ratio of the water (Avnimelech, 2015; Browdy et al., 2012; Emerenciano et al., 2021). The discharges of sludge ($\phi_{v,sludge}$, Eq. B.3) and hydroponics subsystem water ($\phi_{v,discharge}$, Eq. B.11) were the main outflows of TSS, both procedures described in the previous paragraphs.

To limit the model complexity, the following assumptions were made, based on literature information and our experience in running biofloc-based systems and modelling studies.

- a. Density of water is assumed to be constant ($\rho_{water} = 1.00 \text{ kg l}^{-1}$).
- 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 fertiliser is neglected, since their dry matter contents are extremely high.

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 fertiliser. 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 relationship between the N that entered the hydroponics subsystem via the aquaculture subsystem or fertiliser was also chosen as a KPI. The KPIs were presented for the nominal biofloc and FLOCponics systems defined in the previous sections and the simulations described below.

2.4. Sensitivity analysis and scenarios simulations

The normalised sensitivities were computed to measure the effect of changing specific model inputs/parameters on the key model outputs (KPI) (Tomovic, 1963). The sensitivity analysis was performed with respect to 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.

With the purpose of improving the FLOCponics system performance, changes in the planting area were 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-ionised ammonia in the fish tank should not be higher than 1 mg l⁻¹. From these scenario simulations, we expect to find a close to optimal system design that reuses most of the resources from the aquaculture subsystem and thus leading to an improved FLOCponics system. In addition, simulations were performed to quantify the effect of changing the most sensitive parameter on the optimal planting area for the FLOCponics system, according to the criteria described above.

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

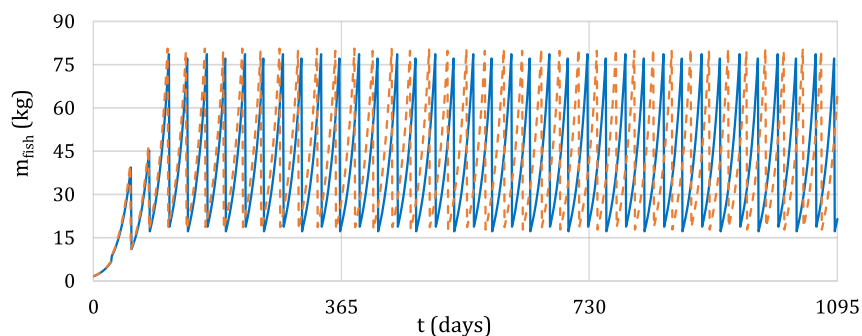


Fig. 4 – Fish biomass growth in the biofloc (dashed orange line) and FLOCponics (solid blue line) systems, for a simulation period of 3 years. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

that is harvested during this period is equal to 4253 and 4046 kg in the biofloc and FLOCponics systems, respectively. Figure 4 presents the fish growth simulated in both systems for the first three years. After the first three years, most of the simulated variables reached a steady oscillation. For lettuce production, 3751 kg of lettuce is produced in the FLOCponics systems from day 82 to 1,825, approximately 75 kg harvested per lettuce production cycle. These values are all in line with what we expected on the basis of data from the experiment.

The water volume required to produce the nominal fish biomass in the biofloc system is 135.5 m³, of which 52% was used to replace the losses for evaporation. While the fish and plant biomass production in the integrated system demands 281.3 m³ of water, 28% and 42% of this volume were due to evaporation and evapotranspiration, respectively. On the other hand, 41.7 and 61.5 m³ of water was discharged, respectively, in the biofloc and FLOCponics system. In the

biofloc system, an input of 186 kg of nitrogen was used, and 110 kg was wasted. In the FLOCponics system, higher amounts of N (207 kg) than in the biofloc system were needed. However, the calculated N waste was 94 kg. In both systems and under nominal conditions, the concentration of NH₃ 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 Fig. 5. In general, the peak of TAN and NO₃ in the biofloc system is most of the time higher than in the FLOCponics system. The mass of solids discharged was higher in the stand-alone system, 421 kg compared to 380 kg in the FLOCponics system. Figure 6 shows the variation in the amount of solids discharge for both systems in the first three years of production.

In terms of resource use efficiency, the WUE and NUE over three years are shown in Figs. 7 and 8. The steps in Fig. 7 around day 665 in the FLOCponics system result from the need to discharge all water from the hydroponics subsystem

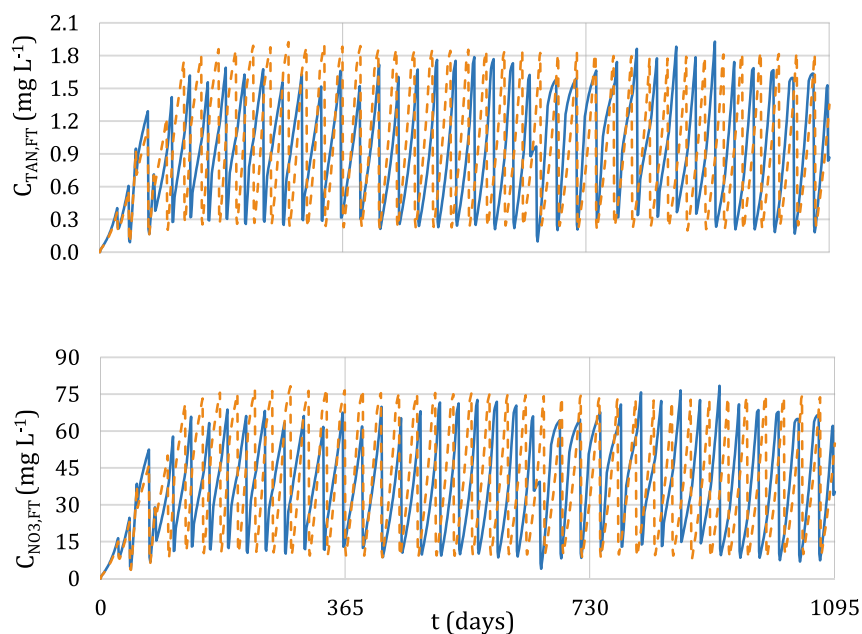


Fig. 5 – Concentration of TAN and NO₃ in the fish tanks of the biofloc (dashed orange line) and FLOCponics (solid blue line) systems, for a simulation period of 3 years. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

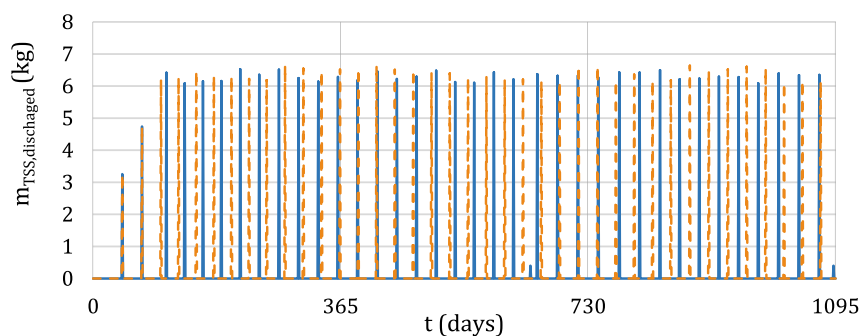


Fig. 6 – Solids discharged (kg) in the biofloc (dashed orange line) and FLOCponics (solid blue line) systems, for a simulation period of 3 years. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

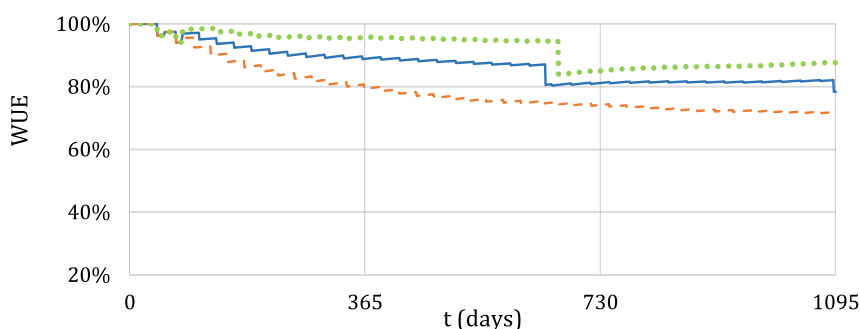


Fig. 7 – Water use efficiency (WUE) of the biofloc (dashed orange line) and FLOCponics systems in the nominal situation (solid blue line) and the improved FLOCponics system (dot green line). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

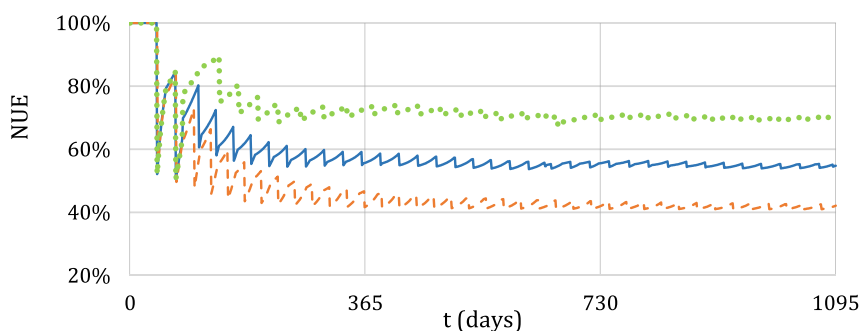


Fig. 8 – Nitrogen use efficiency (NUE) of the biofloc (dashed orange line) and FLOCponics systems in the nominal situation (solid blue line) and the improved FLOCponics system (dot green line). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

as at that time instant the TSS concentration trespassed the predefined threshold. The KPI results are presented in Table 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 3 also presents the KPI found for the improved FLOCponics system where the planting area was increased. Simulations for different planting areas revealed that it can be expanded up to 3.2 times compared to the nominal FLOCponics system (from 34 to 109 m²) without compromising the water quality parameters for fish and plant growth.

The results of the sensitivity analysis are presented in Appendix E, in Tables E.1 to E.8. In general, the normalised 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 normalised sensitivities larger than 0.5 indicated in bold in Tables E.1–E.8. Based on the normalised sensitivity values, the coefficient of solids settling in the RFS_{FP} (k_{RFSset_FP}) was defined as the most sensitive parameter in the FLOCponics system's model (Table E.3). Thus, the planting area extension was computed again using different k_{RFSset_FP}

Table 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 ^a	Improved: nominal
Total solids discharged (kg)	421.9	386.5	300.9	–22%
Average WUE	76%	84%	89%	6%
Average NUE	45%	57%	71%	25%
Water input (l) per kg food produced	32.67	36.15	37.60	4%
Water wasted (l) per kg food produced	9.82	7.97	6.61	–17%
kg N input per kg food produced	0.04	0.03	0.01	–67%
kg N wasted per kg food produced	0.03	0.01	0.004	–60%
% N-fertiliser		58%	60%	3%

^a Planting area of the nominal FLOCponics system expanded 3.2 times (from 34 to 109 m²). WUE: Water use efficiency. NUE: Nitrogen use efficiency.

Table 4 – Key performance indicators of the FLOCponics systems when varying the coefficient of solids settling ($k_{RFS_{set_FP}}$) and the planting area.

Performance indicator	FLOCponics			
	0.90^a	0.90^a	0.81	0.99
Coefficient of solids settling ($k_{RFS_{set_FP}}$)	0.90^a	0.90^a	0.81	0.99
Planting area (m ²)	34 ^a	109	102	116
Total solids discharged (kg)	386.5	300.9	290.1	314.7
Average WUE	84%	89%	81%	95%
Average NUE	57%	71%	70%	73%
Water input (l) per kg food produced	36.15	37.60	42.1	32.9
Water wasted (l) per kg food produced	7.97	6.61	11.26	1.83
kg N input per kg food produced	0.03	0.01	0.01	0.01
kg N wasted per kg food produced	0.01	0.004	0.005	0.004
% N-fertiliser	58%	60%	95%	64%

^a Nominal values. WUE: Water use efficiency. NUE: Nitrogen use efficiency.

values ($\pm 10\%$ of the nominal value). The results of this sensitivity analysis reveal that the planting areas can be expanded to 102 and 116 m² when the RFS_{FP} operates at a coefficient of solids settling of 81% and 99%, respectively. As shown in Table 4, a FLOCponics system that was run under the conditions of $k_{RFS_{set_FP}}$ equal to 99% and a planting area of 116 m² is more efficient in using water and nitrogen than the other scenarios simulated in the present study.

4. Discussion

In this study, the first mathematical model of a FLOCponics system was presented, an improved system design proposed, and its efficiency compared to a stand-alone biofloc system discussed. The results of 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.

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., maximises the rotation of farm facilities and allows better feeding management and disease control (Durigon, Almeida, Jerônimo, Baldisserotto, & Emerenciano, 2019; Sgnaulin et al., 2020). From a commercial viewpoint, the 30-g fish produced in biofloc-based systems can be sold to grow-out farmers or transferred to other commercial institutions where they can be reared until harvest. It is important to highlight that the fish growth mathematical model used in this study was calibrated using experimental data and is specific for the phase simulated. Consequently, the differences in fish biomass production between the biofloc and FLOCponics systems, as shown in Fig. 4, can be attributed to the lower average survival rate observed during the experiment in the FLOCponics system. Also, optimal conditions for fish and plant growth were assumed, i.e., 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 very different conditions. Even so, the models we built and applied serve to address the primary purpose 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 m³ 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 decoupled (on-demand coupled) aquaponics systems (Dijkgraaf et al., 2019; Goddek et al., 2016), which was also the case for our FLOCponics system. Although still high volumes were required in the FLOCponics system, the water waste was lower than in the biofloc system. As a result, WUE was 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 system, the volume of water required per kg of food produced ranges from 32 to 36 l kg⁻¹, in intensive recirculating aquaculture systems

the values are around 500 l kg^{-1} (Verdegem, Bosma, & Verreth, 2006). The average WUE values found in our study for both systems also support the premise that biofloc-based systems efficiently use water (Cao, Abakari, Luo, Tan, & Xia, 2020; Jatobá, Borges, & Silva, 2019). Dijkgraaf et al. (2019) modelled a RAS-based decoupled (on-demand coupled) aquaponics system with an additional loop to anaerobically digest the fish sludge and reuse the supernatant of this process as fertiliser 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. The third loop was not included 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.

For nitrogen, FLOCponics also stand as more efficient than the biofloc system, even though an additional N-fertiliser 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 emphasising the need to investigate the mineralisation of solids from biofloc-based systems. With respect to the contribution of the aquaculture effluent on lettuce N-nutrition, Lastiri et al. (2016, 2018) modelled RAS-based decoupled (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. (2016, 2018) can be due to many reasons, such as the differences in the system designs, species requirements, and environmental conditions, but also as a result of the higher concentration of nutrients in biofloc-based water. It is worth mentioning that nitrate is the form of nitrogen preferred by lettuce. In biofloc-based fish production, nitrate accumulates over time (Luo, Xu, & Meng, 2020). Reusing such nitrate-rich effluent for plant nutrition in FLOCponics leads aquatic food production to circularity.

In this study, only one decision variable was evaluated to improve the FLOCponics performance, which is 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 in transforming or consuming other nutrients (Martins et al., 2017; Sumitro, 2021), such as P, K, and Ca, which are also essential for lettuce nutrition. It is known that the external source of carbon, used to support the growth of biofloc

microorganisms, will provide extra nutrients such as P, K, Ca, S, and Fe to the FLOCponics system compared to a RAS-based aquaponics system (Pinho, David, et al., 2021). This knowledge, however, is insufficient to model and predict the concentration of these nutrients from the biofloc-based aquaculture subsystem to the hydroponics subsystems. Therefore, the following steps for advancing the research field on optimising FLOCponics systems are to unravel the role of biofloc microorganisms in other nutrient pathways and, consequently, determine the need for adding specific inorganic fertilisers to achieve a target nutrient profile and concentration in the hydroponics nutrient solution. Even though there are numerous variables that affect the behaviour of a biofloc-based system and plant production, the present study is pioneer in quantifying and describing the water and nitrogen uses and solids waste in FLOCponics systems. Our FLOCponics mathematical model can be used as a benchmark and starting point for future studies on FLOCponics production.

5. Conclusions

The present model-based study quantitatively demonstrates the efficiency of FLOCponics in producing tilapia juveniles and lettuce compared to a 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 lowered it by 10% compared to the biofloc system. Changes in the planting area of the reference FLOCponics system were evaluated 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 .

Ethical approval

Not applicable.

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ORCID iD authorship contribution statement

Sara M Pinho: Conceptualization, Methodology, Formal Analysis, Data Curation, Writing – Original Draft Preparation, Writing – Review & Editing, Visualisation. Jéssica P de Lima: Data Curation, Writing – Review & Editing, Visualisation. Nurhayati Br Tarigan: Formal Analysis, Writing – Review &

Editing, Visualisation. Luiz H David: Writing – Review & Editing, Visualisation. Maria C Portella: Writing – Review & Editing, Visualisation, Supervision. Karel J Keesman: Methodology, Writing – Review & Editing, Visualisation, Supervision. All authors have read and agreed to publish this version of the manuscript.

Data availability statement

All data used for this study is presented in the main text or appendices.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Reference Evapotranspiration and Evaporation rate

Table A.1 – Greenhouse properties used as input data for the FAO Penman–Monteith Equation.

Parameter	Unit	Value
Wind speed at 10 m	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 A.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. ET: Evapotranspiration. Evap: Evapotranspiration in the aquaculture system/subsystem, a calculation based on the Penman method. Evapotranspiration calculation based on FAO Penman–Monteith method.

Appendix B. Auxiliary equations and general parameters

Table B.1 – Auxiliary equations that support the mass balances (Table 2) for the biofloc and FLOCponics systems.

Auxiliary equation	Description	Unit	Eq.
$\varphi_{v,AQinitial} = V_{FT}/t_{fill,AQ}$	Volume of inoculum to fill each fish tank (FT)	$m^3 d^{-1}$	(B.1)
$\varphi_{v,AQfresh} = \varphi_{v,evap} + \varphi_{v,sludge} + \varphi_{v,AQ-HP}$	Volume flow of freshwater in the aquaculture subsystem	$m^3 d^{-1}$	(B.2)
$\varphi_{v,sludge} = m_{TSS, RFSset}/((1 - k_{DM,sludge})/k_{DM,sludge}) \Delta t$	Outflow of water through sludge discharge, if $C_{TSS,FT} > TSS_{max}$ else 0	$m^3 d^{-1}$	(B.3)
$m_{TSS, RFSset_BFT} = C_{TSS,FT} \varphi_{v,FT-RFS_BFT} k_{set, RFS_BFT} h_{RFS_BFT}$	Mass of TSS settled in the biofloc system	g	(B.4)
$h_{RFS_BFT} = (C_{TSS,FT} - TSS_{min}) V_{FT} n_{FT}/C_{TSS,FT} k_{set, RFS_BFT}$	Time that the radial flow settler (RFS) operates in the biofloc system	h	(B.5)
$\varphi_{v,FT-RFS_BFT}$			
$m_{TSS, RFSset_FP-1} = C_{TSS,FT} \varphi_{v,FT-RFS_FT} k_{set, RFS_FP}$	Mass of TSS settled in the RFS of the FLOCponics system, if $\varphi_{v,AQ-HP} > 0$ else 0	g	(B.6)
$m_{TSS, RFSset_FP-2} = C_{TSS,FT} \varphi_{v,FT-RFS_BFT} k_{set, RFS_BFT} h_{RFS_FP}$	Mass of TSS settled in the RFS of the FLOCponics system if $C_{TSS,FT} > TSS_{max}$ else 0	g	(B.7)
$h_{RFS_FP} = ((m_{TSS,FT} - m_{TSS, RFSset_FP-1}) - (TSS_{min} V_{FT} n_{FT}))/C_{TSS,FT} k_{set, RFS_BFT} \varphi_{v,FT-RFS_BFT}$	Time that the RFS operates in the FLOCponics system if $C_{TSS,FT} > TSS_{max}$	h	(B.8)
$\varphi_{v,HPinitial} = (V_{HP}/2)/t_{plant}$	Volume flow of freshwater in the hydroponics subsystem	$m^3 d^{-1}$	(B.9)
$\varphi_{v,HPfresh} = \varphi_{v,dilution}$	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, $\varphi_{v,dilution} = 0$	$m^3 d^{-1}$	(B.10)
$\varphi_{v,discharge} = V_{HP}/t_{discharge}$	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	$m^3 d^{-1}$	(B.11)
$\varphi_{N,feed} = \varphi_{feed} k_{DM,feed} k_{N,feed}$	Amount of N-feed entered	$g d^{-1}$	(B.12)
$\varphi_{feed} = FCR \Delta m_{fish}/\Delta t$	Amount of feed entered	$g d^{-1}$	(B.13)
$\varphi_{N,molasses} = \varphi_{molasses} k_{N,molasses}$	Amount of N-molasses entered	$g d^{-1}$	(B.14)
$\varphi_{molasses} = (m_{TAN,FT}/\Delta t)/(k_{C,Csource} MC/CN_{het})$	Amount of molasses entered based on Avnimelech (2015)	$g d^{-1}$	(B.15)
$\varphi_{N,bf_prod} = \varphi_{Ammonification} + \varphi_{nitrification}$	Nitrogen production by the biofloc microorganisms	$g d^{-1}$	(B.16)
$\varphi_{Ammonification} = (m_{Norg,FT} k_{N,degrad})/\Delta t$	Ammonia production due to organic nitrogen degradation by the biofloc (heterotrophic) microorganisms	$g d^{-1}$	(B.17)
$\varphi_{N,bf_cons} = \varphi_{assimilation} = u_{bf} * C_{TSS,FT}/Y_{x/N}$	Nitrogen consumed by the biofloc (heterotrophic) microorganisms	$g d^{-1}$	(B.18)
$Y_{x/N} = Y_{x/N}/CN_{maint}$	Bacterial growth yield based on nitrogen consumption	–	(B.19)
$u_{bf} = u_{max} (C_{C,FT}/(C_{C,FT} + K_C)) (C_{Ninog,FT}/(C_{Ninog,FT} + K_N))$	Bioflocs biomass growth rate	$g d^{-1}$	(B.20)
$m_{N,inorg} = m_{TAN} + m_{NO3}$	Mass of inorganic nitrogen in the FT	g	(B.21)
$dm_{TAN}/dt = ((C_{TAN, initial} \varphi_{v,AQinitial}) + \varphi_{Ammonification} + \varphi_{N,fishexcretion}) - (\varphi_{Assimilation} + \varphi_{volatilisation} + \varphi_{nitrification} + \varphi_{TAN,sludge})$	Daily change in the mass of TAN in the FT	$g d^{-1}$	(B.22)
$\varphi_{nitrification} = (m_{TAN,FT} k_{nitrit})/\Delta t = m_{NO3}/\Delta t$	Nitrate production by the biofloc (nitrifying) microorganisms	$g d^{-1}$	(B.23)
$\varphi_{N,fishexcretion} = \varphi_{N,feed} k_{feed, eaten} k_{N,fishexcret}$	Nitrogen excreted by fish	$g d^{-1}$	(B.24)
$\varphi_{volatilisation} = 0.16 * ((m_{Ninorg,FT})/(1 + (10^{(9.25 - pH_{FT})}))) / \Delta t$	Amount of volatile nitrogen in the FT	$g d^{-1}$	(B.25)
$dm_{NH3}/dt = (m_{TAN} k_{UIA})/\Delta t$	Daily change in the mass of Un-ionised Ammonia in the FT	$g d^{-1}$	(B.26)
$\varphi_{TSS,bf} = dm_{TSS,bf}/dt = u_{bf} m_{TSS,FT}$	Bioflocs biomass growth in the FT	$g d^{-1}$	(B.27)
$\varphi_{N, fertiliser} = (C_{N,required} - C_{N,HP}) V_{HP}/\Delta t$	Amount of nitrogen entered in the HP_{sub} to meet the minimum concentration of N required by lettuce	$g d^{-1}$	(B.28)
$\varphi_{N,plant} = \varphi_{v,HPevapt} C_{N,HP}$	Nitrogen uptake by plant	$g d^{-1}$	(B.29)

Table B.2. General parameters used to model the biofloc and FLOCponics systems.

Parameter	Value	Unit	Description
Δt	1	d	Time step
k_{set, RFS_BFT}	0.7	kg kg ⁻¹	Solids settling efficiency in the RFS, based on Mendez et al., 2021 for $\phi_{v,FT-RFS} = 0.022 \text{ cm s}^{-1}$
$k_{DM,sludge}$	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 (Emerenciano et al., 2017; Hargreaves, 2013)
$TSS_{min,AQ}$	100	mg l ⁻¹	Minimum concentration of TSS in the FT (Emerenciano et al., 2017; Hargreaves, 2013)
$\phi_{v,FT-RFS_BFT}$	0.792	m ³ h ⁻¹	Water flow rate from the FT to RFS in the biofloc system
$\phi_{v,FT-RFS_FT}$	1	m ³ d ⁻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	d	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, Mozes, Diab, & Kochba, 1995)
k_{nitrif}	0.976	kg kg ⁻¹	Coefficient of TAN oxidation by nitrifying bacteria (Ebeling, Timmons, & Bisogni, 2006)
u_{max}	0.086	d ⁻¹	Maximum biofloc biomass growth rate based on own experiment
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-ionised ammonia
$C_{TSS, fresh}$	15	mg l ⁻¹	Concentration of TSS in the freshwater
t_{plant}	82	d	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

Appendix C. Fish growth calibration and feed input

The total mass of fish was calculated according to the individual fish biomass (Eq. C.1) multiplied by the number of fish in each fish tank (Eq. C.2). 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 ($k_{mort} \text{ d}^{-1}$) 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.

$$W_t = W_0 e^{k_{fishgrowth} t} \tag{C.1}$$

$$n_t = n_0 e^{k_{mort} t} \tag{C.2}$$

Table C.1 – 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
$k_{\text{fishgrowth}}$	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		
$k_{\text{N,feed}}$	0.053	N content in feed dry mass
$k_{\text{dry, feed}}$	0.959	Dry matter in feed mass
$k_{\text{C,feed}}$	0.500	C content in feed dry mass
$k_{\text{N,molasses}}$	0.007	N content in molasses dry mass
$k_{\text{C,molasses}}$	0.500	C content in molasses dry mass
Fish metabolism		
$k_{\text{water, fish}}$	0.800	Water content in fish mass
$k_{\text{dry, fish}}$	0.200	Dry matter in fish mass
$k_{\text{feed, uneaten}}$	0.180	Mass of uneaten feed per mass of feed inputted
$k_{\text{feed, eaten}}$	0.820	Mass of eaten feed per mass of feed inputted
$k_{\text{C,release}}$	0.500	Mass of C-feed unretained per mass of feed inputted
$k_{\text{N,release}}$	0.590	Mass of N-feed unretained per mass of feed inputted
$k_{\text{N,fishexcret}}$	0.294	Mass of N indigestible fraction per mass of N in dry feed
$k_{\text{N,indfeed}}$	0.116	Mass of N excreted per mass of N in dry feed
$k_{\text{N,fish}}$	0.094	Mass of N per mass of dry matter of fish harvested

Appendix D. Biofloc growth rate calibration

The model for bioflocs biomass growth is presented in Eq. B.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 minimise the Sum of Squared Errors between measured and predicted values, Fig. D.1), given an exponential model of bioflocs biomass growth and data of daily TSS concentrations in the fish tanks, collected in the experiment. The TSS concentration in the fish tanks was assumed as equal to the bioflocs biomass. 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, Lima, et al., 2021).

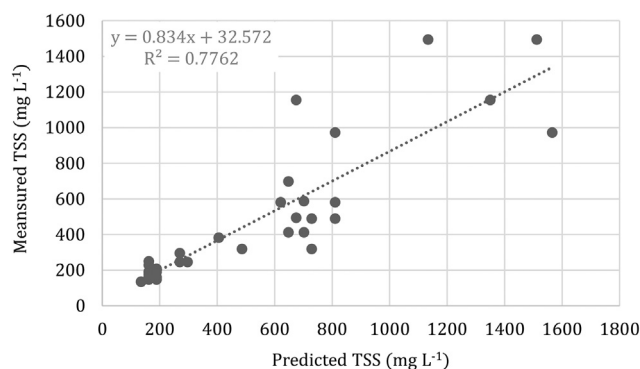


Fig. D.1 – Correlation between the measured and predicted TSS values used to calibrate the maximum biomass growth rate (μ_{max}) used in the bioflocs growth model.

Appendix E. Sensitivity analysis results

Table E.1 – 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 normalised sensitivity coefficients (last two columns; absolute values > 0.5 in bold).

Performance indicator	BFT		FP		NSC	
	–25%		+25%		BFT	FP
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
Water input (l) per kg food produced	31.72	36.01	32.03	36.24	0.020	0.013
Water wasted (l) 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-fertiliser		60.5%		55.7%		–0.167

BFT: biofloc system. FP: FLOCponics system. WUE: Water use efficiency. NUE: Nitrogen use efficiency. NSC: normalised sensitivity coefficients.

Table E.2 – 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 normalised sensitivity coefficients (last two columns; absolute values > 0.5 in bold).

Performance indicator	BFT		FP		NSC	
	–25%		+25%		BFT	FP
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
Water input (l) per kg food produced	31.73	36.06	32.04	36.23	0.020	0.009
Water wasted (l) 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-fertiliser		61.5%		53.1%		–0.288

BFT: biofloc system. FP: FLOCponics system. WUE: Water use efficiency. NUE: Nitrogen use efficiency. NSC: normalised sensitivity coefficients.

Table E.3 – Key performance indicator values under changes in the coefficient of solids settling in the RFSFP (k_{RFSset_FP} – nominal value: 0.9), along with the corresponding values of the normalised sensitivity coefficients (last two columns; absolute values > 0.5 in bold).

Performance indicator	BFT		FP		NSC	
	–10%		+10%		BFT	FP
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
Water input (l) per kg food produced		40.18		33.09	na	–0.983
Water wasted (l) 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-fertiliser		53.5%		60.7%	na	0.623

BFT: biofloc system. FP: FLOCponics system. WUE: Water use efficiency. NUE: Nitrogen use efficiency. NSC: normalised sensitivity coefficients.

Table E.4 – 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 normalised sensitivity coefficients (last two columns; absolute values > 0.5 in bold).

Performance indicator	BFT		FP		NSC	
	–25%		+25%		BFT	FP
Total solids discharged (kg)	420.94	420.94	420.94	420.94	0.000	na
Average WUE	75.6%	75.6%	75.6%	75.6%	0.000	na
Average NUE	45.1%	45.1%	45.1%	45.1%	0.000	na
Water input (l) per kg food produced	31.85	31.85	31.85	31.85	0.000	na
Water wasted (l) per kg food produced	9.80	9.80	9.80	9.80	0.000	na
kg N input per kg food produced	0.04	0.04	0.04	0.04	0.000	na
kg N wasted per kg food produced	0.03	0.03	0.03	0.03	0.000	na
% N-fertiliser						na

BFT: biofloc system. FP: FLOCponics system. WUE: Water use efficiency. NUE: Nitrogen use efficiency. NSC: normalised sensitivity coefficients.

Table E.5 – 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 normalised sensitivity coefficients (last two columns; absolute values > 0.5 in bold).

Performance indicator	BFT		FP		NSC	
	–25%		+25%		BFT	FP
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
Water input (l) per kg food produced	30.37	34.44	33.41	37.86	0.191	0.189
Water wasted (l) 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-fertiliser		58.9%		55.9%		–0.104

BFT: biofloc system. FP: FLOCponics system. WUE: Water use efficiency. NUE: Nitrogen use efficiency. NSC: normalised sensitivity coefficients.

Table E.6 – 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 normalised sensitivity coefficients (last two columns; absolute values > 0.5 in bold).

Performance indicator	BFT		FP		NSC	
	–25%		+25%		BFT	FP
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
Water input (l) per kg food produced	30.09	34.21	33.33	37.91	0.102	0.103
Water wasted (l) 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-fertiliser		46.0%		62.7%		0.288

BFT: biofloc system. FP: FLOCponics system. WUE: Water use efficiency. NUE: Nitrogen use efficiency. NSC: normalised sensitivity coefficients.

Table E.7 – 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 normalised sensitivity coefficients (last two columns; absolute values > 0.5 in bold), along with the corresponding normalised sensitivity values.

Performance indicator	BFT		FP		NSC	
	–10%		+10%		BFT	FP
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
Water input (l) per kg food produced	31.73	36.00	32.02	36.24	0.046	0.033
Water wasted (l) 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-fertiliser		61.2%		54.9%		–0.537

BFT: biofloc system. FP: FLOCponics system. WUE: Water use efficiency. NUE: Nitrogen use efficiency. NSC: normalised sensitivity coefficients.

Table E.8 – 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 normalised sensitivity coefficients (last two columns; absolute values > 0.5 in bold).

Performance indicator	BFT		FP		NSC	
	–25%		+25%		BFT	FP
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		
Water input (l) per kg food produced	36.07	36.07	na	0.000		
Water wasted (l) 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-fertiliser	47.1%	65.3%	na	0.936		

BFT: biofloc system. FP: FLOCponics system. WUE: Water use efficiency. NUE: Nitrogen use efficiency. NSC: normalised sensitivity coefficients.

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