fishes

Article

Enhancing Indoor Culture of Weather Loach (*Misgurnus anguillicaudatus*) and Caipira Lettuce (*Lactuca sativa*) in a Decoupled FLOCponics System

Junseong Park [1], Ju-ae Hwang [1], Jongryeol Choe [1], Donggil Lee [2] and Hyeongsu Kim [3,*]

[1] Advanced Aquaculture Research, National Institute of Fisheries Science, Changwon 51688, Republic of Korea; pjs5420939@gmail.com (J.P.); hjuae1031@korea.kr (J.-a.H.); ht4560@naver.com (J.C.)
[2] Division of Fisheries Engineering, National Institute of Fisheries Science, Busan 48513, Republic of Korea; donggil@korea.kr
[3] Aquaculture Research Division, National Institute of Fisheries Science, Busan 48513, Republic of Korea
* Correspondence: kimk2k@korea.kr; Tel.: +82-55-51-720-2423

Abstract: Interest in aquaponics (AP) is increasing due to its ability to minimize sewage and maximize feed efficiency in fish farming. However, owing to limitations of intensive cultures and a lack of nutrients such as NO_3 for growing crops, AP requires the use of artificial nutrients. Therefore, novel approaches are required to develop AP-intensive culturing methods. An AP system based on biofloc technology (BFT) called FLOCponics (FP) has been recommended. Here, the productivity of the weather loach (*Misgurnus anguillicaudatus*) in the FP system, BFT system, and flow-through systems (FTSs), as well as these systems' effect on Caipira lettuce (*Lactuca sativa*) growth, was analyzed. To compare crop productivity, a hydroponic (HP) bed was installed. The growth rate of *M. anguillicaudatus* showed significant differences, at 51.1 ± 3.69% in the FP system, followed by 24.0 ± 4.16% in the BFT system and −14.3 ± 1.4% in the FTS. Its survival rates were better in the FP system (91.1 ± 2.64%) than in the BFT system (82.1 ± 10.98%) or the FTS (66.8 ± 2.75%) ($p < 0.05$). Total ammonia nitrogen and NO_2^--N concentrations were stabilized in every plot during the experimental period. However, the NO_3^--N concentration continuously increased in the BFT system but decreased in the FP system and was maintained. The shoot weight of the Caipira lettuce was 163.6 ± 8.65 g in the FP system and 149.6 ± 9.05 g in the HP system. In conclusion, FP system can provide a large amount of nutrients and improve the growth performance of both fish and crops in the FP system.

Keywords: weather loach; biofloc; aquaponics; FLOCponics; Caipira lettuce

Key Contribution: The aquaponics system based on biofloc technology (FLOCponics) was more effective than the BFT system and FTS, with higher growth performance and production in both fish and crops.

check for updates

Citation: Park, J.; Hwang, J.-a.; Choe, J.; Lee, D.; Kim, H. Enhancing Indoor Culture of Weather Loach (*Misgurnus anguillicaudatus*) and Caipira Lettuce (*Lactuca sativa*) in a Decoupled FLOCponics System. *Fishes* **2024**, *9*, 150. https://doi.org/10.3390/fishes9050150

Academic Editor: Ronald Kennedy Luz

Received: 17 March 2024
Revised: 17 April 2024
Accepted: 21 April 2024
Published: 23 April 2024

1. Introduction

The weather loach (*Misgurnus anguillicaudatus*), distributed across the Korean peninsula, East Asia, and Europe, serves various purposes, including food, bait, and feed for other pets [1]. However, in South Korea, the amount of catch is declining due to urban expansion and environmental pollution [2].

In South Korea, the majority of the demand for loach is met by importing it from other countries, and most of the morphologically similar related species, Chinese loach (*M. mizolepis*), is imported [3,4]. *M. mizolepis* can hybridize with *M. anguilicaudatus*, and native resources are at risk due to genetic contamination caused by individuals introduced into the drainage system [5–7]. Therefore, in South Korea, aquaculture research is actively being conducted to increase loach production, and aquaculture methods using release and recapture using outdoor fields have been popularized [8,9].

However, in the case of outdoor farming, many problems can occur due to harmful organisms like birds, otters, and so on that prey on loach; the rainy season; or the inflow of contaminated breeding water, and recapture is difficult due to the characteristic of infiltrating the bottom material [10]. Owing to these issues, an increasing number of fish farms are attempting to switch to indoor rather than outdoor farming.

Most indoor breeding systems utilize a flow-through system (FTS), and water quality management is difficult due to high stocking density and substantial feed input [11]. In addition, the characteristics of intensive farming methods contributes to increased effluent and antibiotic problems [12,13]. For indoor loach culture, when producing seeds and rearing them a month after hatching, mortality often occurs for unknown reasons such as water quality and bacteria, so a sustainable, eco-friendly farming method is needed [4,11]. Furthermore, eco-friendly farming practices not only offer high economic viability but also enhance survival rate and water-quality management.

Eco-friendly farming practices, which utilize the natural environment without causing pollution, offer a solution to minimize sewage and maximize feed efficiency [14]. Biofloc technology (BFT), designed specifically to reduce water consumption and pollution, has gained prominence. BFT fosters the growth of microorganisms, particularly heterotrophic bacteria, which play a crucial role in reducing nitrogen components in fish tanks through ammonia assimilation [15–17]. Despite the reduced initial costs of BFT systems compared to recirculating aquaculture systems (RASs), they require advanced management skills for maintaining parameters such as the carbon (C)–nitrogen (N) ratio, dissolved oxygen (DO), temperature, and specific farming species [18–20]. Heterotrophic bacteria form flocs enriched with nutrients, serving as additional nutrient sources for BFT-applied species [21,22]. However, challenges such as the risk of stress and respiratory disturbances in fish due to the excessive addition of bioflocs persist. Additionally, addressing the limit of decreasing nitrate nitrogen (NO_3^--N) concentration is essential, and the availability of BFT rearing water becomes inevitable [23].

Aquaponics (AP), an amalgamation of aquaculture and hydroponics, has also been developed recently. AP represents a technology where waste from cultured freshwater species is utilized to cultivate plants [24,25]. The waste, including feces and feed waste, serves as nutrient sources for plants, which in turn purify the water returned to the culture tanks. The significance of hydroponics and AP is on the rise, but the reliance on artificial nutrients in intensive cultures poses a limitation [16,26,27]. To address this limitation, novel approaches are required for enhancing AP culturing and production methods. The integration of BFT into AP, known as the decoupled FLOCponics system (FP), has been recommended, demonstrating improved fish growth in species such as nile tilapia (*Oreochromis niloticus*) and eel (*Anguilla japonica*) compared to traditional biofloc culture [28,29].

This study aims to improve the method of indoor culturing for *M. anguillicaudatus* and adapting it for the FP system in order to achieve high productivity and sustainable food production. A comparative analysis of various characteristics, including the growth performance of fish, crop production, and water quality, is undertaken across three different aquaculture systems: FP, BFT, and the flow-through system (FTS).

2. Materials and Methods

2.1. Aquaponics System Based on Biofloc Technology—FLOCponics (FP) Design

Three systems for loach farming were used in this study: the FTS (control), which is based on flow-through with two rotations per day; the BFT system, which utilizes biofloc action; and the FP system, which utilizes BFT and aquaponics. The FP system is shown in Figure 1. Every fish tank was aerated (airflow rate: 80 L per minute) to prevent the settlement of bioflocs and to supply sufficient oxygen. The experiments were performed indoors; nine fish tanks (three each for the FP system, BFT system, and FTS; diameter:

1.2 m, height: 1.0 m, capacity: 1 ton and made of fiber-reinforced plastic) were set up using deep water culture (DWC) for crop cultivation with a crop bed (length: 2 m, width: 2 m, depth: 0.6 m, capacity: 2 ton) decoupled system (Figure 1). A total of 3 kg of fish (*M. anguillicaudatus*) was stocked in each tank (2.6 kg/m^3). In the FP system, water in the rearing tank and around the crop bed was circulated using a water pump (25 W, Hyupsin, Seoul, Sedra). To standardize the experimental conditions, the water volume, the amount of water was maintained using two cycles (input volume from fish tank) per day. Each crop bed consisted of 100 pots per bed to cultivate Caipira lettuce (*Lactuca sativa*). For the growth of Caipira lettuce during the experiment period, artificial light was installed indoors (daily average $\geq$ 6000 Lux) under 14L:10D conditions and maintained at 24 $\pm$ 1.0 °C, with zero-water discharge in the BFT and FP systems.

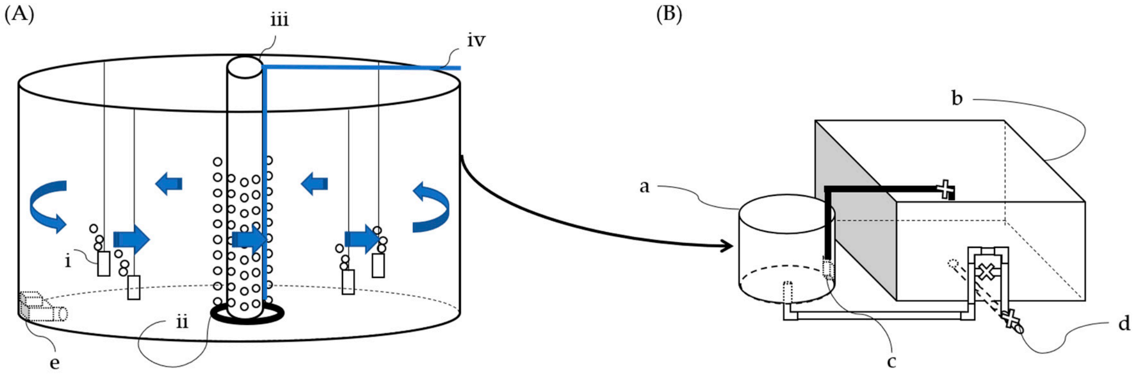

Figure 1. Schematic view of the construction of the fish tank to prevent the settlement of bioflocs (**A**) and the complete FP system for growing crops with cultured fish and a rearing tank (**B**) (a: fish tank, b: crop bed, c: flow pipe, d: discharge pipe, e: pump, i: air stone, ii: air ring, iii: venturi pipe, iv: air hose).

A stocking density of 3 kg (approximately 2500 individuals) of *M. anguillicaudatus* was maintained in each tank, resulting in a stocking density of 2.6 kg/m^3 (Figure 2A). In the FP system, water circulation within the rearing tank and around the crop bed was facilitated using a water pump (25 W, Hyupsin, Seoul, Sedra). The water volume in the system was regulated according to the fish tank's capacity, maintaining a two-cycle-per-day input volume from the fish tank. The crop bed, designed for the cultivation of Caipira lettuce (*Lactuca sativa*), comprised 100 pots (initial weight: 2.0 $\pm$ 0.47 g, height: 45.7 $\pm$ 6.75 mm) per bed. Artificial light was installed indoors to ensure optimal growth conditions for Caipira lettuce, providing a daily average illumination of $\geq$6000 Lux under a 14 h light to 10 h dark photoperiod (Figure 2B). Temperature conditions were maintained at 24 $\pm$ 1.0 °C, with a zero-water discharge policy implemented in both the FP and BFT systems throughout the experimental period.

2.2. Fish and Crop

The weather loach (*M. anguillicaudatus*) juveniles, with a total length of 65.4 $\pm$ 6.78 mm and body weight of 1.27 $\pm$ 0.360 g, were sourced from Goheung-Gun, Jeonnam, South Korea. These fish were reared and carefully managed at the Advanced Aquaculture Research Center, National Institute of Fisheries Science, located in Changwon, South Korea. Throughout the experiment, the fish were fed a commercial feed named "Well-being Miccuri" (composition: crude protein 40%, crude fat 4%, Ca 0.8%, moisture 14%, P 1.8%, crude fiber 5%, crude ash 15%, manufactured by Woosung Institute, Daejeon, South Korea) at a dosage equivalent to 3% of the total weight per day (approximately 90 g).

To maintain optimal conditions for the fish, the water temperature was maintained at 24 $\pm$ 1.0 °C using a 1 kW heater (OKE-HE-100, Sewon OKE, South Korea), and the dissolved oxygen (DO) level was carefully controlled, maintaining approximately 10 mg/L with an oxygen supply system (KMOS-40R, Kumho-Marine, South Korea).

Caipira lettuce (*L. sativa*) was selected for cultivation due to its compatibility with hydroponics (HP) and its high economic value within the Korean food industry. Seeds of

Caipira lettuce (Enza Zaden, Enkhuizen, the Netherlands) were germinated ten days prior to the experiment in a germination chamber at the same location as the fish.

HP system cultivation served as the control method for crop growth, and an artificial nutrient solution (Liquid A: N 2%, K 3.5%, Ca 2%, Fe 0.05%; Liquid B: 1.3%, P 1.5%, K 5%, Mg 0.7%, B 0.05%, Mn 0.01%, Zn 0.002%; Mulfuresiriz, Daeyu Business Limited, Seoul, South Korea) was utilized at a concentration of 1000 ppm for crop cultivation. The electric conductivity (EC) was adjusted to a value exceeding 0.5 dS/cm in both the FP bed (BFT) and HP bed (artificial nutrient solution).

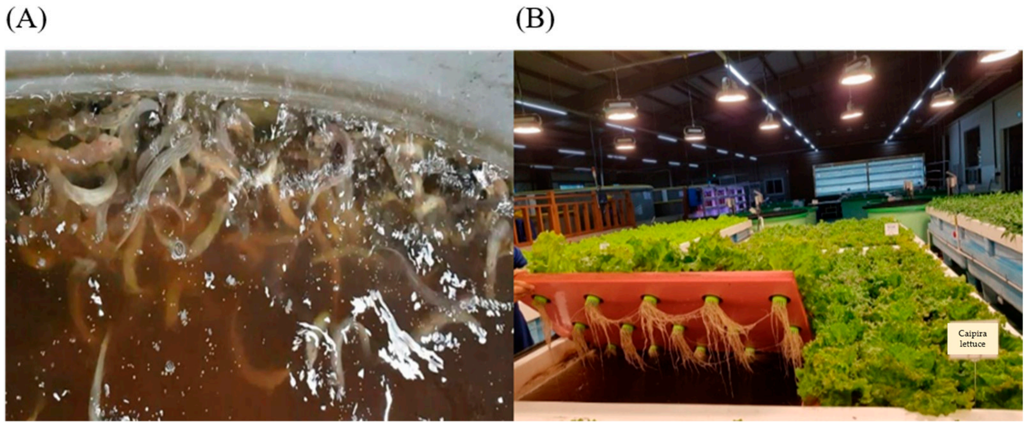

Figure 2. Panoramic photographs ((**A**): weather loach (*M. anguillicaudatus*, (**B**), Caipira lettuce (*L. sativa*)) in an aquaponics system based on biofloc technology (FP).

2.3. Preparing the BFT Rearing Water

The formulation of BFT rearing water was conducted in accordance with the methodology outlined in a previous study for the creation and maintenance of BFT rearing water [30]. Both FP and BFT waters were adjusted to achieve a carbon-to-nitrogen (C:N) ratio calculated using the formula recommended by [31,32]. Inoculation procedures involved the introduction of 30 ppm of microbial additives BFT-ST (*Bacillus* sp., *Cellulomonas* sp., *Rhodopseudomonas* sp., etc.) and BFT-CT (alanine, glutamic acid, glycine, histidine, etc.) provided by ecoTechservice (Gimpo, South Korea). This was supplemented by a daily feed regimen consisting of 90 g (as a nitrogen source) and 39 g of molasses (as a carbon source, EM molasses, Ever-miracle, Jeonju, South Korea).

In the fourth week, when the total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2^--N) levels were stabilized below 1 mg/L, fish were introduced and acclimated for one week prior to the commencement of the experiment. Molasses, a carbon source, was added daily post-feeding to maintain biofloc conditions (Figure 3).

Formula for adding molasses to the inoculation of BFT rearing water:

$$
\begin{gathered}
\text{Carbon (C) in molasses} = 50\% \\
\text{Feed crude protein (CP) (\%)} = 40\% \\
\text{Amount of nitrogen (N) in the feed:} \\
= 0.090\ (\text{kg}) \times 0.40\ (\text{CP\%}) \times 0.155\ (\text{amount of N in CP}) \\
= 0.00558 \\
\text{C/N ratio in the feed:} \\
= 0.090 \times 0.5\ (\text{C} = \text{in the Feed})/0.00558\ (\text{kg}) \\
= 7.76{:}1. \\
\text{Amount of C needed for 15:1} \\
= (0.090 \times 0.5 + \text{molasses} \times 2)/0.00558 = 15 \\
\text{Molasses} = (15 \times 0.00558) - 0.090 \times 0.5 \\
= 0.0387\ \text{kg} \fallingdotseq 39\ \text{g}
\end{gathered}
\quad (1)
$$

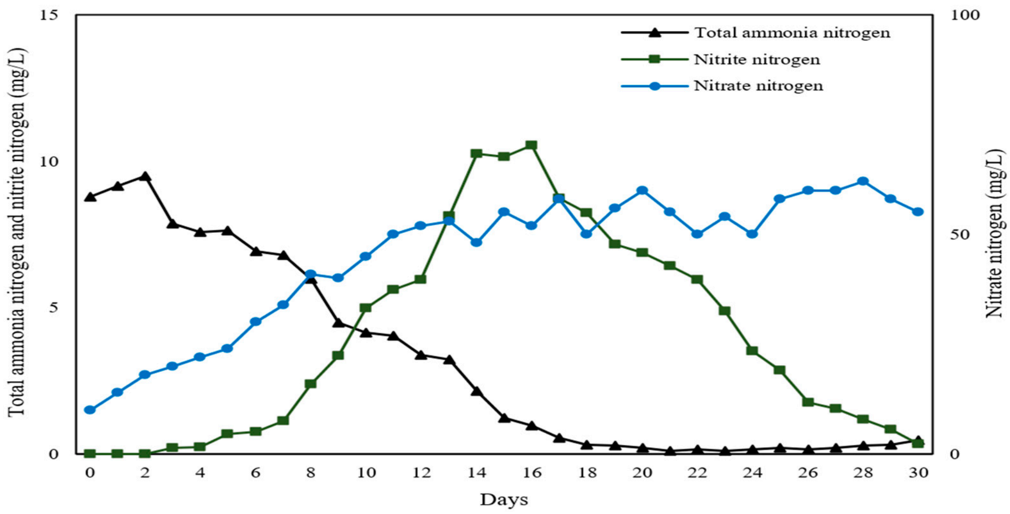

Figure 3. Water quality of nitrogen component concentration (total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^--N), and nitrate nitrogen (NO_3^--N)) in biofloc technology (BFT) rearing water during inoculation.

2.4. Measurements of Water Quality

Water-quality parameters, including water temperature, dissolved oxygen (DO), pH, electric conductivity (EC), and total dissolved solids (TDS), were assessed at three-day intervals utilizing a multi-parameter water-quality device (YSI-650 Inc., Yellow Springs Ins., Yellow Springs, Ohio, USA). Sampling was conducted consistently at 10am before feeding, with readings taken in triplicate.

Additionally, the concentrations of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^--N), and nitrate nitrogen (NO_3^--N) were determined before feeding and analyzed every three days. This analysis was performed using an absorbance photometer (Merck KGaA, Darmstadt, Germany) along with an analytical reagent kit (Merck KGaA, Darmstadt, Germany), employing a colorimetric method.

2.5. Growth Performance and Production of Fish and Crops

At the conclusion of the fourth week of the experiment, the growth performance and production metrics of each experimental group of fish and crops were evaluated. Thirty individuals were randomly selected from each tank, anesthetized with 100 ppm MS-222 (Sigma Aldrich, St. Louis, MO, USA) in a 20 L bucket, and then measured for body weight and total length. Body-weight measurements were conducted using electronic scales (MW-200, CAS, Seoul, Korea) with an accuracy of 0.01 g, while total length measurements were performed using vernier calipers (Mitutoyo electronic, Kawasaki, Japan) with an accuracy of 0.1 mm. Key performance indicators, including weight gain rate (WGR) (Equation (2)), specific growth rate (SGR) (Equation (3)), feed conversion ratio (FCR) (Equation (4)), and survival rate (Equation (5)), were calculated using the respective equations.

$$\text{Weight gain rate} = (\text{final weight} - \text{initial weight})/\text{initial weight} \times 100 \quad (2)$$

$$\text{Specific growth rate} = [\ln(\text{final body weight}) - \ln(\text{initial body weight})]/\text{days} \times 100 \quad (3)$$

$$\text{Feed conversion ratio (FCR)} = (\text{final total weight} - \text{initial total weight})/\text{supplied feed} \quad (4)$$

$$\text{Survival rate (\%)} = (\text{final number of individuals}/\text{initial number of individuals}) \times 100 \quad (5)$$

Similarly, five Caipira lettuce heads were randomly sampled from each plant bed. Total weight and shoot weight were measured using an electronic scale (MW-200, CAS, Seoul, Korea) with an accuracy of 0.1 g, while vernier calipers were employed for precise measurements of total length, shoot length, and leaf length with an accuracy of 0.1 mm.

2.6. Statistical Analysis

The growth performance of fish was analyzed via a one-way ANOVA using SPSS (version 24.0 (SPSS Inc., Chicago, IL, USA) if the data were normally distributed. Tukey's test was used to determine the differences between the treatment groups with homogeneity of variance. The production of Caipira lettuce was analyzed using a t-test for the BF and HP systems. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Growth Performance of *M. anguillicaudatus*

The experiments on the cultivation of *M. anguillicaudatus* over a four-week period in three distinct aquaculture systems (FP, BFT, and FTS) revealed notable differences in growth metrics. The total weight and average body weight were significantly higher in the FP system (4.5 ± 0.11 kg and 1.99 ± 0.734 g, respectively) compared to the BFT system (3.3 ± 0.12 kg and 1.69 ± 0.329 g) and the FTS (2.5 ± 0.04 kg and 1.52 ± 0.432 g) (Table 1; Tukey's test, $p < 0.05$). Furthermore, the WGR, SGR, and FCR demonstrated significant differences in the FP system (51.1 ± 3.69%, 1.6 ± 0.07%, and 1.7 ± 0.12, respectively) in comparison to the BFT system (24.0 ± 4.16%, 1.0 ± 0.08%, and 3.6 ± 0.58, respectively) and the FTS (−15.6 ± 1.35%, 0.7 ± 0.11%, and −5.4 ± 0.46, respectively). Additionally, the survival rate was observed to be lower in the FTS (66.7 ± 2.75%) compared to the FP system (91.1 ± 2.64%) and the BFT system (82.1 ± 10.98%) (Figure 4; Tukey's test, $p < 0.05$).

Table 1. Comparison of the growth performance of *M. anguillicaudatus* in three different aquaculture systems (mean ± SD). The data in rows denoted with different letters are statically different ($p < 0.05$); NS, not significant.

Systems	† FP	‡ BFT	§ FTS
[1] IBW (g)	1.27 ± 0.360 [NS]	1.27 ± 0.360	1.27 ± 0.360
[2] FBW (g)	1.99 ± 0.734 [a]	1.69 ± 0.329 [ab]	1.52 ± 0.432 [b]
[3] ITW (kg)	3.0 [NS]	3.0	3.0
[4] FTW (kg)	4.5 ± 0.11 [a]	3.3 ± 0.12 [ab]	2.5 ± 0.04 [b]
[5] ITL (mm)	65.4 ± 6.78 [NS]	65.4 ± 6.78	65.4 ± 6.78
[6] FTL (mm)	74.0 ± 7.84 [NS]	74.2 ± 3.67	72.4 ± 5.38
[7] WGR (%)	51.11 ± 3.69 [a]	24.00 ± 4.16 [b]	−15.56 ± 1.35 [c]
[8] SGR (%/day)	1.62 ± 0.07 [a]	1.03 ± 0.08 [b]	0.65 ± 0.11 [c]
[9] FCR	1.65 ± 0.12 [a]	3.57 ± 0.58 [b]	−5.43 ± 0.46 [c]
[10] SR (%)	91.06 ± 2.64 [a]	82.12 ± 10.98 [ab]	66.75 ± 2.75 [b]

[1] IBW: initial body weight, [2] FBW: final body weight, [3] ITW: initial total weight, [4] FTW: final total weight, [5] ITL: initial total length, [6] FTL: final total length, [7] WGR: weight gain rate, [8] SGR: specific growth rate, [9] FCR: feed conversion ratio, [10] SR: survival rate. † FP, aquaponics system integrating biofloc technology (BFT) known as FLOCponics; ‡ BFT, biofloc technology; § FTS, flow through systems. Data presented as a mean ± S.D. The data in rows denoted with different letters are statically different ($p < 0.05$). NS, no significant ($p \geq 0.05$).

3.2. Water Quality Analysis in FP, BFT, and FTS

Throughout the four-week experimental period, the water conditions, including temperature, DO, pH, EC, and TDS, were diligently maintained in each fish tank. The water temperature across all three systems was consistently maintained within the range of 23–24 °C, and the DO levels were kept at 8–10 mg/L. pH levels exhibited a decrease from 7.5 to 7.0 across all three systems as the experiment progressed. Notably, EC values varied between systems, increasing from 0.6 to 1.0 in the BFT system and from 0.6 to 0.7 in the FP system, and decreasing from 0.7 to 0.6 in the FTS. TDS displayed a trend similar to EC changes over the course of the experiment.

During the experimental period, water-quality measurements for the FP system, BFT system, and FTS were as follows: water temperature (°C) of 23.4 ± 0.40, 23.6 ± 0.36, and 23.7 ± 0.34; DO (mg/L) of 9.4 ± 0.75, 9.2 ± 0.67, and 9.1 ± 1.10; pH of 7.0 ± 0.33, 7.0 ± 0.40, and 7.4 ± 0.18; EC (dS/cm) of 0.7 ± 0.04, 0.8 ± 0.14, and 0.7 ± 0.04; and TDS (mg/L) of 0.7 ± 0.04, 0.5 ± 0.11, and 0.4 ± 0.02, respectively (Figure 4A–E).

In terms of water-quality parameters, TAN and NO_2^--N in the FP system, BFT system, and FTS were stabilized under 1 mg/L during the four weeks of the experiment. However, TAN decreased from 0.238 ± 0.003 mg/L to 0.157 ± 0.049 mg/L in the FP system, while it increased from 0.238 ± 0.002 mg/L to 0.466 ± 0.138 mg/L in the BFT system. NO_2^--N exhibited an increasing tendency over the experiment duration in both the FP and BFT systems; however, the nitrogen concentration in the BFT system became twice as high as that in the FP system and FTS by the experiment's conclusion. The NO_3^--N level was the highest in the BFT system (66.448 ± 4.331 mg/L), followed by the FP system (48.608 ± 2.947 mg/L) and the FTS (5.758 ± 1.213 mg/L). The nitrogen concentration in the BFT system increased to be two times higher than that in the FP system by the end of the experiment (Figure 4F–H).

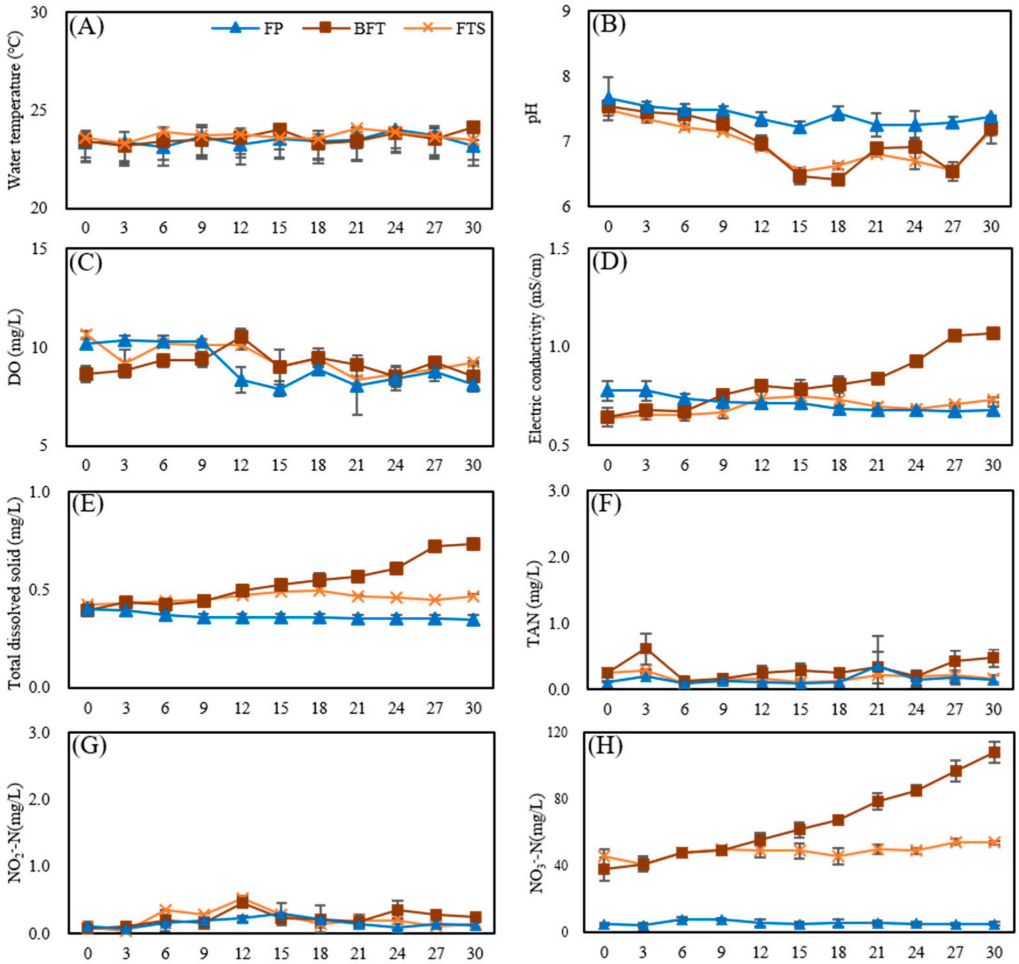

Figure 4. Changes in water quality in three aquaculture systems according to the experimental period ((**A**) water temperature, (**B**) dissolved oxygen, (**C**) pH, (**D**) electric conductivity, (**E**) total dissolved solids, (**F**) total ammonia nitrogen, (**G**) nitrite nitrogen, (**H**) nitrate nitrogen).

3.3. Production of Caipira Lettuce

The findings indicate that Caipira lettuce production in the FP system closely resembled that in the HP system. Specifically, the shoot weight in the FP system (163.60 ± 8.65 g) exceeded that in the HP system (149.60 ± 9.06 g). Other parameters comparing the FP and HP systems showed marginal differences (Figure 5; Tukey's test, $p < 0.05$).

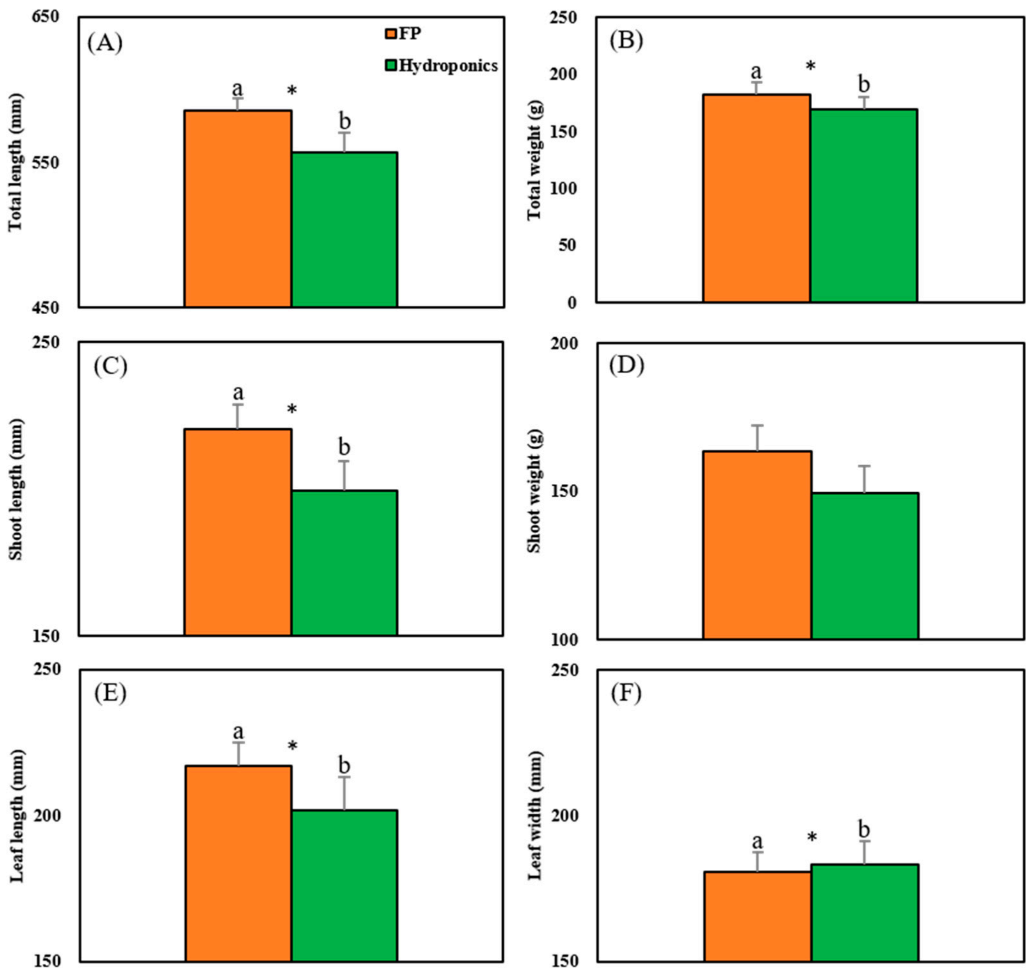

Figure 5. Comparison of crop productivity between the aquaponics system based on biofloc technology (FP) and hydroponics (HP) system after four weeks ((**A**) total length, (**B**) total weight, (**C**) shoot length, (**D**) shoot weight, (**E**) leaf length, (**F**) leaf width). The asterisk (*) with different letters indicates values are statically different ($p < 0.05$).

4. Discussion

We observed notable improvements in the growth and survival rates of *M. anguillicaudatus* within the FP system compared to those in the BFT system and FTS. After four weeks, FTS showed the lowest survival rate of *M. anguillicaudatus* among the three systems. Indoor farming can potentially introduce pathogens through the inflow of water. The influent water is highly influenced by weather and seasonal variations, and environmental factors such as water temperature, DO, and pH are likely to change rapidly. However, in this study, the water temperature, DO, and pH were stabilized, and the levels of ammonia and nitrite remained stable at levels considered safe by a previous study [11]. Despite this, the survival rate within the FTS was very low. Following the introduction of fish into the tank, the TAN levels in the BFT system increased to 0.61 ± 0.236. Subsequently, during the early stages of the experiment, the TAN levels stabilized in both the FP and BFT systems. In contrast, in the FTS group, *M. anguillicaudatus* continuously perished until the end of the experiment, indicating that water FTSs were unsuitable for *M. anguillicaudatus*. Therefore, consistent rearing water with minimal changes in water quality is essential for breeding *M. anguilicaudatus*, as it was hard to adapt to the conditions in freshwater circulation.

Perished individuals showed symptoms of infection with *Aeromonas hydrophyla*, such as mucus hypersecretion and redness, as determined by API 20NE kit (bioMérieux, France) [32]. In addition, there was an increase in aggressive behaviors among *M. anguillicaudatus*, including attacks on other fish and targeting the wound area of injured fish. At the end of the experiment, the total weight of FTS was lower than that at the time of standing, and as in previous studies, it was concluded that a method to reduce mortality during indoor farming is needed. In contrast, the FP and BFT system groups had improved survival rates compared to FTS. Furthermore, the FP and BFT showed that horizontal infection was minimized due to closed farming with no influent or drained water.

The BFT system demands highly skilled operators for effective water-quality management, with the C/N ratio being a crucial aspect [33–35]. The biofloc water, along with its stable water quality, creates favorable conditions for cultivating various species, including carp, catfish, prawns, tilapia, trout, and bass [28,31,36,37]. In South Korea, there is a recognized need for further development in the loach culturing industry [4].

Recently, inland aquaculture species such as eels, tilapia, and catfish have undergone testing in BFT studies aimed at reducing the rearing period and increasing fish production [38,39]. The growth period for *M. anguillicaudatus* extends over 12 to 18 months until harvest. However, indoor farming allows for harvest in four to five months—approximately one-third of the time required for outdoor farming. Despite this, adaptation to indoor conditions has proven challenging for *M. anguillicaudatus*, resulting in low survival rates. BFT consisted of a dominance of heterotrophic bacteria such as *Bacillus* sp. [40]. Previous studies have shown that the probiotic and enzyme activity in BFT can improve the survival rate of culturing fishes [41,42]. This study demonstrated that the growth and survival rates of *M. anguillicaudatus* were higher in the FP and BFT groups than in the FTS group. In the FTS, *M. anguillicaudatus* exhibited a high mortality rate and did not seem to breed. The higher mortality rate in the FTS observed in this study could be attributed to various factors, including direct environmental factors such as water temperature, pH, and dissolved oxygen, as well as indirect environmental factors like water flow, noise pollution, and the availability of shelter. These factors may induce stress responses, impact the metabolic systems of fishes, compromise the immune system, and increase pathogen expression [43].

Many BFT studies focus on growth and water quality [44]. However, in the BFT system, identifying microbial communities and determining their impact on rearing species is crucial [42]. This study represents a limit to the factors of survival rate and microbial composition of *M. anguillicaudatus* in BFT and FP. Fish health can be measured by hematological parameters such as hematology, enzyme activity, hormones, and immunology, and microbial communities of BFT have been researched for BFT target species such as *Oreochromis niloticus*, *Litopenaeus vannamei*, and *Clarias gariepinnus* [38,45–48]. Additionally, it is necessary to identify the factors that improve survival rate through microbial compositions and hematological parameter analysis of *M. anguillicaudatus*. Further research will be conducted to pinpoint the primary contributing factors. This study demonstrates that the BFT or FP system could address issues related to imports and provide effective, productive aquaculture systems for sustainable and eco-friendly cultivation [28,30,36].

Plants require a medium for their roots to absorb nutrients. Recently, HP and aquaponics systems, which use relatively less water, have been reported to be inoculated with artificial nutrients [49]. However, the discharge of sewage containing these artificial nutrients leads to environmental and biological pollution [12]. In an effort to reduce reliance on artificial nutrients, fish were integrated into the HP system, utilizing their feces as feed in an RAS [50]. Nevertheless, RAS incurs high operational costs, and the characteristic of HP systems in providing additional nutrients for crops poses challenges in achieving sustainable productivity [28]. In contrast, the cultivation of biofloc water is reported to contain sufficient macro- and micronutrients for plant growth [36]. The application of BFT in aquaculture does not require artificial nutrients, as BFT generates less wastewater and is less expensive to construct than RAS (BFT: USD 54805.96, RAS: USD 80382.07) [12,20,50]. Therefore, BFT has the potential to enable sustainable and eco-friendly aquaculture. The EC range was above 0.5 dS/cm, indicating sufficient conditions for Caipira lettuce crop growth [36]. The EC and TDS levels in the BFT water with *M. anguillicaudatus* increased during the fourth week. Elevated EC and TDS levels in rearing water create conditions conducive to plant growth, allowing the lettuce to reach commercial size within four weeks [51,52]. HP systems typically require EC levels of over 0.5 dS/cm [36]. The EC and TDS levels of the FP system were lower than those of the BFT system, and this difference contributes to sufficient plant growth.

Additionally, crop productivity, specifically Caipira lettuce production, demonstrated a significant increase in the FP system compared to the HP system. The BFT system harbored beneficial bacteria, including *Bacillus* sp., which are utilized as probiotics for the removal of the nitrogen component and the enhancement of water quality and culture conditions [53]. The observed lower FCR in the FP system in comparison to the BFT system indicates enhanced efficiency, particularly when combined with crop cultivation.

In aquaponics, the metabolic waste generated by aquatic organisms is converted into nitrate through the action of nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*), serving as a vital fertilizer source for crops [54]. Crops play a pivotal role in maintaining nutrient balance and mitigating toxic factors in rearing water [28]. In the nitrogen cycle of the BFT system, the presence of flocs contributes to improved feed efficiency, leading to increased fish growth [55]. The nutrient-rich rearing water in the BFT system, containing nitrogen and phosphorus, facilitates nitrate production, providing ample nutrients [34,39,56].

The lettuce growth results underscore the FP system's capability to provide sufficient nutrients for plant growth, aligning with findings from prior studies [28,57]. These results collectively suggest that the FP system represents a sustainable aquaculture method, leveraging the synergistic relationship between fish and crops to reduce nitrogen levels for the fish while supplying essential nutrients for crop development.

The impact of BFT on growth and economic efficiency has been under study for many years to facilitate the healthy and sustainable production of various species [15,38,56]. Indoor farming of loaches holds promise for increasing production. However, further research is required to ascertain the cause of mass mortality in indoor-cultured loaches. Potential factors include stress, water components, and quality, as well as the microbiota composition of the fish and water.

5. Conclusions

In this study, we focus on the survival rate and production of rearing *M. anguillicaudatus* with indoor culturing. We compared its growth performance and survival rates in three types of aquaculture systems and confirmed the feasibility of an eco-friendly aquaponics method based on biofloc technology, FLOCponics (FP), for growing Caipira lettuce. The results demonstrate an enhanced feed conversion ratio and improved productivity, highlighting the potential of FP as an efficient and sustainable aquaculture system. Common to both FP and BFT, these culture methods are isolated from the outside, minimizing the risk of pathogen invasion from incoming breeding water. Additionally, in the case of FP, crops can be produced from water containing various minerals, which can have a positive impact on economic benefits by generating additional income sources along with the nitrate reduction effect.

Author Contributions: Conceptualization, J.P. and H.K.; formal analysis, J.P., J.C. and J.-a.H.; investigation, J.P., J.C., D.L. and H.K.; resources, J.-a.H.; data curation, J.P., D.L. and J.-a.H.; writing, J.P. and H.K.; visualization, H.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Institute of Fisheries Science, Ministry of Oceans and Fisheries, Republic of Korea (grant number: R2024033).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the National Institute of Fisheries Science (2022-NIFS-IACUC-5).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kim, H.S.; Lee, H.K. Studies on the nutritional value of Loach *Misgurnus mizolepis*. *J. Korean Soc. Food Sci. Nutr.* **1985**, *14*, 296–300.
2. Lee, Y.S.; Kim, K.S.; Lim, S.G.; Im, J.H.; Han, H.G.; Soh, H.Y.; Cheong, C.J. Effect of fertilizer addition on water quality and fingerling production of loach (*Misgurnus naguillicaudatus*) in natural outdoor ponds. *J. Environ. Biol.* **2015**, *36*, 557–563.
3. Kang, K.K.; Han, M.S..; Cho, K.J.; Nam, H.K.; NA, Y.E.; Kim, M.R.; Kim, M.H.; Choe, L.J. *Effet of Agricultural Practices on Mudfish Population in Paddy Fields*; The Korean Society of Environmental Agriculture: Suwon-si, Republic of Korea, 2013; p. 273.
4. Kim, K.S.; Lim, S.G.; Bang, I.C.; Choi, J.; Lee, S.M. Optimal dietary protein and lipid levels for growth of juvenile muddy loach *Misgurnus anguillicaudatus*. *Korean J. Fish. Aquac. Sci.* **2013**, *46*, 371–376. [CrossRef]
5. Lim, H.S.; Kim, M.S.; Seok, Y.S.; Park, S.D.; Lee, H.H. Characterization and DNA Structure Analysis of Replication Origin of *Misgurnus mizolepis*. *Korean J. Fish. Aquac. Sci.* **1996**, *9*, 93–100.
6. Park, I.S.; Kim, B.S.; Im, J.H.; Park, H.M.; Nam, Y.K.; Jeong, C.H.; Kim, D.S. Improved Early survival in backcrosses of male mud loach (*Misgurnus mizolepis*) X cyprinid loach (*M. anguillicaudatus*) hybrids to female cyprinid loach. *J. Aqua.* **1997**, *10*, 363–371.
7. Shin, H.S.; Song, Y.J.; Kwon, S.I.; Eo, J.; Lee, S.H.; Kim, M.H. Monthly change of the length-weight relationship of the loach (*Misgurnus anguillicaudatus*) population in paddy fields by farming practices. *Korean J. Environ. Biol.* **2018**, *36*, 1–10. [CrossRef]
8. Oh, Y.B.; Lee, J.K.; Kim, S.S.; Lim, M.S.; Park, R.K. Studies on the rice-fish farming in rice paddy field 1. Studies on loach culture in rice paddy field. *Res. Rept. RDA(R)* **1991**, *33*, 51–56.
9. Longo, S.B.; Clark, B.; York, R.; Jorgenson, A.K. Aquaculture and the displacement of fisheries captures. *Conser. Biol.* **2019**, *33*, 832–841. [CrossRef] [PubMed]
10. Boyd, C.E.; McNevin, A.A.; Clay, J.; Johnson, H.M. Certification issues for some common aquaculture species. *Rev. Fish. Sci.* **2005**, *13*, 231–279. [CrossRef]
11. Park, N.R.; Sohn, S.G.; Choi, H.S.; Cheong, C.J.; Lee, Y.S. Acute toxicity of Ammonia and Nitrite for Fry Loach. *J. Korean Soc. Environ. Technol.* **2016**, *17*, 71–76.
12. Rajesh, K.R.; Cho, J.Y. Reuse of hydroponic waste solution. *Environ. Sci. Pollut. Res. Int.* **2014**, *16*, 9569–9577.
13. Bondarczuk, K.; Markowicz, A.; Piotrowska-Seget, Z. The urgent need for risk assessment on the antibiotic resistance spread via sewage sludge land application. *Environ. Int.* **2016**, *87*, 49–55. [CrossRef] [PubMed]
14. Van Rijn, J. The potential for integrated biological treatment systems in recirculating fish culture—A review. *Aquaculture* **1996**, *139*, 181–201. [CrossRef]
15. Avnimelech, Y. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture* **1999**, *176*, 227–235. [CrossRef]
16. Diver, S. *Aquaponics-Integration of Hydroponic with Aquaculture*; National Sustainable Agriculture Information Service: Butte, MT, USA, 2006; p. 28.
17. De Schryver, P.; Crab, R.; Defoirdt, T.; Boon, N.; Verstraete, W. The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture* **2008**, *277*, 125–137. [CrossRef]
18. Xu, W.J.; Morris, T.C.; Samocha, T.M. Effects of C/N ratio on biofloc development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. *Aquaculture* **2016**, *453*, 169–175. [CrossRef]
19. Ahmad, I.; Babitha, M.B.; Verma, A.K.; Maqsood, M. Biofloc technology: An emerging avenue in aquatic animal healthcare and nutrition. *Aquac. Int.* **2017**, *25*, 1215–1226. [CrossRef]
20. Betanzo-Torres, E.A.; Pinar-Alvarez, M.A.; Sierra-Carmona, C.G.; Santamaria, L.E.G.; Loeza-Mejia, C.I.; Marin-Muniz, J.L.; Herazo, L.C.S. Proposal of Ecotechnologies for Tilapia (*Oreochromis niloticus*) Production in Mexico: Economic, Environmental, and Social Implications. *Sustainability* **2021**, *13*, 6853. [CrossRef]
21. Hargreaves, J.A. Photosynthetic suspended-growth systems in aquaculture. *Aquac. Eng.* **2006**, *34*, 344–363. [CrossRef]
22. Zhao, B.; He, Y.L.; Zhang, X.F. Nitrogen removal capability through simultaneous heterotrophic nitrification and aerobic denitrification by *Bacillus* sp. LY. *Environ. Technol.* **2010**, *31*, 409–416. [CrossRef]
23. Da Silva, J.L.S.; Cavalcante, D.D.H.; Carvalho, F.C.T.; Vieira, R.H.S.D.F.; Sa, M.V.D.C.; Sousa, O.V. Aquatic microbiota diversity in the culture of Nile tilapia (*Oreochromis niloticus*) using bioflocs or periphyton: Virulence factors and biofilm formation. *Acta Sci. Anim. Sci.* **2016**, *38*, 233–241. [CrossRef]
24. Junge, R.; Konig, B.; Villarroel, M.; Komives, T.; Jijakli, M.H. Strategic Points in aquaponics. *Water* **2017**, *9*, 182. [CrossRef]
25. Knaus, U.; Pribbernow, M.; Xu, L.; Appelbaum, S.; Palm, H.W. Basil (*Ocimum basilicum*) cultivation in decoupled aquaponics with three hydro-components (Grow pipes, Raft, Gravel) and African catfish (*Clarias gariepinus*) production in northern Germany. *Sustainability* **2020**, *12*, 8745. [CrossRef]
26. Seginer, I.; Halachmi, I. Optimal stocking in intensive aquaculture under sinusoidal temperature, price and marketing conditions. *Aquac. Eng.* **2008**, *39*, 103–112. [CrossRef]
27. Solis-Toapanta, E.; Fisher, P.; Gómez, C. Growth rate and nutrient uptake of basil in small-scale hydroponics. *HortScience* **2020**, *55*, 507–514.
28. Hwang, J.A.; Lee, J.H.; Park, J.S.; Choe, J.R.; Lee, D.; Kim, H.S. Effect on Eel *Anguilla japonica* and crop growth by the development of a biofloc technology (BFT) Aquaponics System. *Korean J. Fish. Aqua. Sci.* **2021**, *54*, 418–425.
29. Pinho, S.M.; David, L.H.; Garcia, F.; Portella, M.C.; Keesman, K.J. Sustainability assessment of FLOCponics compared to stand-alone hydroponics and biofloc systems using enerfy synthesis. *Ecol. Indic.* **2022**, *141*, 109092. [CrossRef]
30. Choi, J.Y.; Park, J.S.; Kim, H.S.; Hwang, J.A.; Lee, D.; Lee, J.H. Assessment of water quality parameters during a course of applying biofloc technology (BFT). *J Fish. Mar. Sci.* **2020**, *32*, 1632–1638.

31. Azim, M.E.; Little, D.C.; Bron, J.E. Microbial protein production in activated suspension tanks manipulating C:N ratio in feed and the implications for fish culture. *Bioresour. Technol.* **2008**, *99*, 3590–3599. [CrossRef] [PubMed]
32. Hidalgo, R.B.; Figueras, M.J. Molecular detection and characterization of furunculosis and other *Aeromonas* fish infections. In *Health and Environment in Aquaculture*; IntechOpen Limited: London, UK, 2012; pp. 97–132.
33. Hargreaves, J.A. *Biofloc Production Systems for Aquaculture*; Publication No. 4503; Southern Regional Aquaculture Center (SRAC): Stoneville, MS, USA, 2013.
34. Avnimelech, Y. *Biofloc Technology-A Practical Guide Book*, 3rd ed.; The World Aquaculture Society: Baton Rouge, LA, USA, 2015.
35. Pérez-Fuentes, J.A.; Hernández-Vergara, M.P.; Pérez-Rostro, C.I.; Fogel, I. C:N ratios affect nitrogen removal and nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. *Aquaculture* **2016**, *452*, 247–251. [CrossRef]
36. Food and Agriculture Organization. *Small-Scale Aquaponics Food Production Integrated Fish and Plant Farming*; FAO Fisheries and Aquaculture Technical Paper; Food and Agriculture Organization: Rome, Italy, 2014; pp. 1–19.
37. Park, J.S.; Lee, J.H.; Kim, N.N.; Hwang, J.A.; Kim, H.S. A study on growth performance and water quality of far eastern catfish (*Silurus asotus*) using biofloc technology (BFT) aquaculture. *J. Fish. Mar. Sci. Edu.* **2021**, *33*, 367–375.
38. Abu Bakar, N.S.; Mohd Nasir, N.; Lananan, F.; Abdul Hamid, S.H.; Lam, S.S.; Jusoh, A. Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish, (*Clarias gariepinus*) utilizing Bioflocs technology. *Int. Biodeterior. Biodegrad.* **2015**, *102*, 100–106. [CrossRef]
39. Adriani, Y.; Dhahiyat, Y.; Zahidah, Z.; Zidni, I. The effect of stocking density ratio of fish on water plant productivity in aquaponics culture system. *Nusant. Biosci.* **2017**, *9*, 31–35. [CrossRef]
40. Zokaeifar, H.; Balcazar, J.L.; Saad, C.R.; Kamarudin, M.S.; Sijam, K.; Arshad, A.; Nejat, N. Effects of Bacillus subtilis on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.* **2012**, *33*, 683–689. [CrossRef] [PubMed]
41. Aly, S.M.; Mohamed, M.F.; John, G. Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*). *Aquac. Res.* **2008**, *39*, 647–656. [CrossRef]
42. Kathia, C.M.; del Carmen, M.D.M.; Aida, H.P.; Jorge, C.M.; Felix, A.G.J.; Amadeo, B.M.J. Effect of two probiotics on bacterial community composition from biofloc system and their impact on survival and growth of tilapia (*Oreochromis niloticus*). *Int. J. Fish. Aquat. Stud.* **2018**, *6*, 525–533.
43. Portz, D.E.; Woodley, C.M.; Cech, J.J. Stress-associated impacts of short-term holding on fishes. *Rev. Fish Biol. Fish.* **2006**, *16*, 125–170. [CrossRef]
44. Long, L.; Yang, J.; Li, Y.; Guan, C.; Wu, F. Effect of biofloc technology on growth, digestive enzyme activity, hemotology, and immune response of genetically improved famed tilapia (*Oreochromis niloticus*). *Aquaculture* **2015**, *448*, 135–141. [CrossRef]
45. Tavares-Dias, M.; Mariano, W.S. *Aquicultura No Brasil: Novas Perspectvias*; Pedro & João Editores: São Carlos, SP, Brasil, 2015; Volume 1.
46. Reis, W.G.; Wasielesky, W., Jr.; Abreu, P.C.; Brandao, H.; Krummenauer, D. Rearing of the pacific white shrimp Litopenaeus vannamei (Boone, 1931) in BFT system with different photoperiods: Effects on the microbial community, water quality and zootechnical performance. *Aquaculture* **2019**, *508*, 19–29. [CrossRef]
47. Meenakshisundaram, M.; Suganthам, F.; Muthukuma, C.; Chandrasekar, M.S. Metagenomic characterization of biofloc in the grow-out culture of genetically improved farmed tilapia (GIFT). *Aquac. Res.* **2020**, *52*, 4249–4262. [CrossRef]
48. Hisano, H.; Barbosa, P.T.L.; Hayd, L.A.; Mattioli, C.C. Comparative study of growth, feed efficiency, and hematological and recirculating aquaculture system. *Trop. Anim. Health Prod.* **2021**, *53*, 60. [CrossRef]
49. Rakocy, J.E. Ten guidelines for aquaponics systems. *10th Anniversary Issue. Aquaponics J.* **2007**, *46*, 14–17.
50. Goddek, S.; Schmautz, Z.; Scott, B.; Delaide, B.; Keesman, K.J.; Wuertz, S.; Junge, R. The effect of anaerobic and aerobic fish sludge supernatant on hydroponic lettuce. *Agronomy* **2015**, *6*, 37. [CrossRef]
51. Bailey, D.S.; Ferrarezi, R.S. Valuation of vegetable crops produced in the UVI commercial aquaponics system. *Aquac. Rep.* **2017**, *7*, 77–82. [CrossRef]
52. Hu, Z.; Lee, J.W.; Chandran, K.; Kim, S.P.; Botto, A.C.; Khanal, S.K. Effect of plant species on nitrogen recovery in aquaponics. *Bioresour. Technol.* **2015**, *188*, 92–98. [CrossRef] [PubMed]
53. Boyd, C.E.; Massaut, L. Risks associated with the use of chemicals in pond aquaculture. *Aquac. Eng.* **1999**, *20*, 113–132. [CrossRef]
54. Wongkiew, S.; Hu, Z.; Chandran, K.; Woo-Lee, J.; Khanal, S.K. Nitrogen transformations in aquaponic systems: A review. *Aquac. Eng.* **2017**, *76*, 9–19. [CrossRef]
55. Day, S.B.; Salie, K.; Stander, H.B. A growth comparison among three commercial tilapia species in a biofloc system. *Aqua. Int.* **2016**, *24*, 1309–1322. [CrossRef]
56. Azim, M.E.; Little, D.C. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **2008**, *283*, 29–35. [CrossRef]
57. Delaide, B.; Goddek, S.; Gott, J.; Soyeurt, H.; Jijakli, M.H. Lettuce (*Lactuca sativa* L. var. Sucrine) growth performance in complemented aquaponic solution outperforms hydroponics. *Water* **2016**, *8*, 467. [CrossRef]