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# Transformation of phosphorus in an experimental integrated multitrophic aquaculture system using the media filled beds method in plant cultivation

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ABSTRACT: The objective of this study was to trace the transformations of phosphorus in an integrated multitrophic aquaculture (IMTA) system and to determine whether the method of plant breeding influenced the dynamics of these changes. In the experiment, the media filled beds (MFB) method of plant cultivation was applied. Fish tanks were stocked with 200 common carp *Cyprinus carpio*, and hydroponic terraces were planted with 49 zantedeschia *Zantedeschia* sp. bulbs. Water samples were taken directly from the fish tanks immediately after each type of filtration (mechanical, biological and hydroponic). The basic forms of orthophosphates in the IMTA system included some $H_2PO_4^-$ ions but mostly $HPO_4^{2-}$. The higher supply of reactive phosphorus that occurred over time in the experiment may have contributed to a decrease in calcium ion concentration due to the formation of $Ca_3(PO_4)_2$ and $CaHPO_4$ salts, thus inactivating some of the phosphorus available to plants. Phosphorus may have also been inactivated in the sediment due to the formation of $Mg_3(PO_4)_2$ and $MgHPO_4$ salts after decreasing the concentration of calcium ions as a result of their precipitation in sediments. Mineralization of organic matter took place under aerobic conditions. Organic matter was a source of biogenic substances in the IMTA system. Experimental results showed that IMTA systems have significant potential to reduce phosphorus in aquaculture wastewater and thus provide a good environment for fish farming by improving water quality.

KEY WORDS: Integrated multitrophic aquaculture · IMTA · Media filled beds · MFB · Transformations of phosphorus compounds

## 1. INTRODUCTION

In view of the growing global human population and the consequent requirement to increase food production, there is a need to seek economically viable and environmentally safe solutions for food production. One of the problems that must be addressed is the availability of phosphorus for fertilizer production, as phosphorus is a key nutrient in the developing agricultural industry. The extraction and processing of phosphate is a highly energy-intensive process that results in a wide range of adverse environmental effects (Cerozi & Fitzsimmons 2017). From this perspective, the use of phosphorus-rich aquaculture waste for irrigation and soil fertilization makes both economic and environmental sense.

In aquaculture, widely used recirculating closed-circuit systems, such as the recirculating aquaculture system (RAS), are becoming increasingly popular worldwide. These types of systems can be improved through integration with hydroponic farming, thus providing additional economic and ecological benefits (Palm et al. 2018). The integration of aquaculture and hydroponic plant breeding has been analyzed

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many times using different system layouts, plant species and species of aquatic organisms. The most suitable and effective aquacultural practice involves closed recirculation systems integrated with hydroponic systems, i.e. integrated multitrophic aquaculture (IMTA), because nutrient concentrations can be sufficiently maintained for proper plant growth and development (Seawright et al. 1998).

According to Cerozi & Fitzsimmons (2017), the transformation of phosphorus and its budget in IMTA systems is not fully understood. The dynamics of phosphorus metabolism in IMTA systems have been described by Seawright et al. (1998), but according to that study, the amount of phosphorus in the fish, plants and sediments was higher than the amount of phosphorus supplied with the feed. Phosphorus dynamics were also discussed by Cerozi & Fitzsimmons (2017), who created a model which can be used as a tool to maximize the use of phosphorus in the system and minimize the amount of phosphorus in sediments. In their experiment, they showed that phosphorus use by fish comprised 42.3 % of the phosphorus available in the feed and that phosphorus use by plants amounted to 29.4 %. They also found that 13.1 % of the phosphorus was not available for the fish or plants.

The concentrations of different nutrients in IMTA systems vary depending on the production technology adopted (Seawright et al. 1998). These include 3 hydroponic systems classified according to growth medium types. In the nutrient film technique (NFT), plants are grown in long, narrow plastic channels with a 1 % gradient through which a thin layer of water constantly flows. The deep water culture (DWC) method is also widely used in aquaponic systems. In this system, plants float on plastic sheets in 30 cm deep tanks filled with water. The roots of the plants grow directly in the continuously flowing aerated water, and the rafts of plants floating on the water move as the plants grow (Love et al. 2015, Forchino et al. 2017). Finally, the media filled beds (MFB) system is probably the simplest and most common technique for small- and medium-scale systems. In this system, a terrace is filled with a special substrate that provides extra space for biofiltration and thus for efficient plant growth. This type of bed is flooded with water from the RAS system, in this way the nutrients necessary for the proper growth of plants are provided. Because of the mechanical support for the roots of the plants, this substrate can be used to grow various species of plants (Somerville et al. 2014, Engle 2015, Li et al. 2019).

Most IMTA systems are designed on a nitrogen budget, which does not guarantee that other nutrients, such as phosphorus, reach levels for sufficient plant growth and development. Phosphorus deficiency will inhibit plant growth, while excess phosphorus can lead to antagonistic interactions with microelements, especially zinc (Barben et al. 2010).

Taking into account a number of aspects related to the functioning of the IMTA system, such as production technology, the availability of nutrients for both fish and plants and ecological aspects, this study was undertaken to determine the intensity of phosphorus compound transformation in the experimental multitrophic aquaculture system using the MFB method of plant breeding.

## 2. MATERIALS AND METHODS

### 2.1. Description of the experimental IMTA system

The experimental IMTA system was set up in the laboratory of the Department of Aquatic Bioengineering and Aquaculture of the West Pomeranian University of Technology, Szczecin, Poland, and had a volume of 5 m$^3$ and was run for 21 weeks from February to the end of May. The system consisted of 3 polyethylene rearing fish tanks, each with a capacity of 1 m$^3$, a mechanical filter (0.5 m$^3$) filled with 0.1 m$^3$ of CALDNESS K1 mobile resin and a biological filter comprising a nitrification chamber (0.5 m$^3$) filled with 0.1 m$^3$ of Mutag BioChip mobile medium with a protective active area of 3000 m$^2$ m$^{-3}$. The entire IMTA system was initiated 3 wk before the start of the experiment to allow the biofilm on the biological bedding to develop naturally, using water from another system in operation. The entire system, including the water in the breeding tanks and the biofilter bed, was aerated using a Hi-Blow blower, which forced compressed air into membranes installed in the tanks. The remains of uneaten food and faeces were removed through an outflow located centrally at the bottom of the tanks, transferring contaminated water to the mechanical filter. The mechanical filter was rinsed every 2–3 d, depending on its condition.

The hydroponic terraces (0.5 m$^3$) were made of PVC foam sheets filled with perlite (420 l) used as a medium for planting in the MFB method. Tricolor lights (GROW LED UFO 90 W) were placed above each terrace. The lighting regime was 12 h light:12 h dark, with the plants illuminated by full light and the fish tanks remaining in the twilight. Water loss by evaporation, transpiration and sludge removal was compensated for by adding mains water at 1.5–2.0% of the system volume over the experiment.

### 2.1.1. Fish tanks

Each experimental tank was stocked with 200 common carp *Cyprinus carpio* L. The mean initial weight of each fish was 40 g. The fish were fed twice a day throughout the experiment; the daily feed ration was adjusted every 2 wk depending on the body weight of the fish. The feed used was initially the commercial feed Aller Primo, comprising 37% protein content, 12% fat, 32.5% nitrogen-free extracts (NFE) and 7% ash, with a total energy of 19.6 MJ and digestible energy of 16 MJ per kg. For practical reasons, this feed was replaced after 1 mo by the identically composed Aller Primo Float, as the use of recirculating floating feeds in the system reduced the risk of water quality deterioration caused by unused feed and gave a better level of feeding, allowing for better ongoing control of the condition of the fish. During the experiment, water temperature was maintained at an average of 22°C, forming optimal conditions for carp breeding.

### 2.1.2. Hydroponic terraces (filters)

At the beginning of March, the hydroponic terraces were planted with zantedeschia (*Zantedeschia* Spreng.) bulbs at a density of 49 plants terrace$^{-1}$. The bulbs were planted in the perlite medium, maintaining a distance of 10 cm from the edges of the tank and 20 cm between the bulbs. Water samples were taken once a week beginning in February, when the fish were introduced into the rearing tanks, until the end of May, when the flowers from the hydroponic terraces were harvested. Water samples from the fish tanks were taken from a half-tank depth, directly at the water outlet of the filters and the fish were transferred to the breeding in the Fisheries Experimental Station in Nowy Czarnów. A diagram of the IMTA system experiment is shown in Fig. 1.

## 2.2. Physico-chemical analyses of water

The concentrations of biogenic phosphorus compounds were measured in 3 replicates using a PHARO 300 MERCK UV-VIS spectrophotometer. Reactive phosphorus (mg dm$^{-3}$) and total phosphorus (mg dm$^{-3}$) were analysed at $\lambda$ = 882 nm. Organic phosphorus (mg dm$^{-3}$) was calculated from the difference between total phosphorus and reactive phosphorus, and dissolved oxygen concentration (mg$O_2$ dm$^{-3}$) was determined using the Winkler method. Other parameters measured included pH, water temperature, alkalinity, bicarbonate, calcium and magnesium. Organic matter loading of the experimental IMTA system was calculated by determining the chemical oxygen demand (COD). Moreover, the ratio of bicarbonate ions to carbonate ions was calculated. All analyses used standard methods (APHA 1999).

The percentage of reduction after each filter was calculated, and results are presented as mean, minimum and maximum values. Results were analysed statistically using Statistica v.13.3 software. The significance of the correlation coefficients was tested using an R-Spearman non-parametric test; results were considered significant at $p < 0.05$.

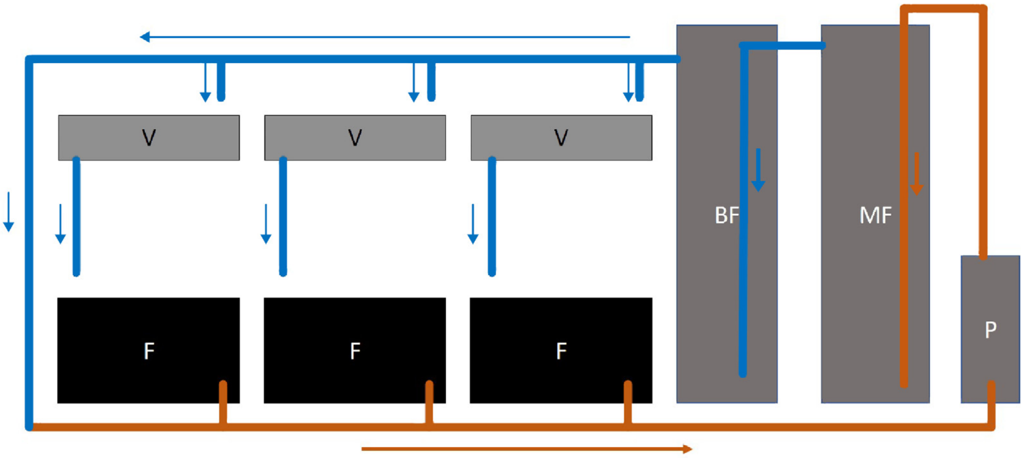

Fig. 1. Water cycle in the experimental system. V: hydroponic terraces (filters); F: fish tanks; BF: biological filter; MF: mechanical filter; P: tank with pump. Arrows indicate direction of water flow. Orange line: water after fish tanks and mechanical filter; blue line: water after biological and hydroponic filters

### 2.3. Calculation of phosphorus balance

The equation expressing the phosphorus balance during the experiment is:

$$P_{\text{feed}} = \Delta P_{\text{fish}} + P_{\text{water}} + P_{\text{faeces}} + P_{\text{plants + sediments}} \quad (1)$$

where $P_{\text{feed}}$ is the amount of phosphorus in the feed, $\Delta P_{\text{fish}}$ is the increase in phosphorus accumulated in the fish during the experiment, $P_{\text{water}}$ is the amount of phosphorus accumulated in the water and $P_{\text{faeces}}$ is that in the fish faeces. All variables are given in the unit 'kg per kg'.

From Eq. (1), we obtain the amount of phosphorus in the plants and sediments:

$$P_{\text{plants + sediments}} = P_{\text{feed}} - (\Delta P_{\text{fish}} + P_{\text{water}} + P_{\text{faeces}}) \quad (2)$$

$\Delta P_{\text{fish}}$ can be evaluated considering the mass of the fish at the beginning ($M_{\text{fish}}^{(\text{begin})}$) and end ($M_{\text{fish}}^{(\text{end})}$) of the experiment and the concentrations of the phosphorus in the fish bodies (p) measured experimentally ($p_{\text{fish}}^{(\text{begin})}$) and ($p_{\text{fish}}^{(\text{end})}$):

$$\Delta P_{\text{fish}} = p_{\text{fish}}^{(\text{end})} \cdot M_{\text{fish}}^{(\text{end})} - p_{\text{fish}}^{(\text{begin})} \cdot M_{\text{fish}}^{(\text{begin})} \quad (3)$$

Phosphorus supplied to the RAS ($P_{\text{feed}}$) comes from the feed ($M_{\text{feed}}$) and amounts to:

$$P_{\text{feed}} = p_{\text{feed}} \cdot M_{\text{feed}} \quad (4)$$

All concentrations of the phosphorus: $p_{\text{fish}}$, $p_{\text{feed}}$ and $p_{\text{water}}$ are given in unit 'kg per kg', with the exception of $p_{\text{feaces}}$, which is taken from the data given by the feed producer in 'kg per 100 kg'. The concentration of the phosphorus is related to the average fish mass $M_{\text{fish}}^{(\text{average})}$:

$$M_{\text{fish}}^{(\text{average})} = \frac{1}{2}(M_{\text{fish}}^{(\text{begin})} + M_{\text{fish}}^{(\text{end})}) \quad (5)$$

and is equal to:

$$P_{\text{faeces}} = \frac{p_{\text{faeces}}}{100} \cdot M_{\text{fish}}^{(\text{average})} \quad (6)$$

while $P_{\text{water}}$ is determined based on the concentration of phosphorus measured experimentally after the experiment ($p_{\text{water}}$):

$$P_{\text{water}} = p_{\text{water}} \cdot V_{\text{water}} \quad (7)$$

## 3. RESULTS

### 3.1. Breeding

Fish were weighed every 3 wk and the feed conversion ratio (FCR) determined. During the experiment, the weight gain of fish was almost 7-fold. The greatest increase in fish weight was observed after the system had stabilized the FCR coefficient showed a downward trend, while in the second phase it stabilized at a constant level (Table 1).

Table 1. Average weight of common carp and calculated feed conversion ratio (FCR) during the IMTA experiment

| Week | Weight (g) | FCR |
|---|---|---|
| 0 | 40.0 | – |
| 3 | 57.0 | 1.52 |
| 6 | 80.0 | 1.28 |
| 9 | 110.0 | 1.20 |
| 12 | 150.0 | 1.14 |
| 15 | 200.0 | 1.15 |
| 18 | 268.0 | 1.14 |

### 3.2. Variability in the physical and chemical parameters of the water

Reactive phosphorus concentrations showed an upward trend during the experiment, both in the water of the fish tanks and in the water from the individual filters, ranging from 0.07–3.29 mg dm$^{-3}$ (Fig. 2). In the final 2 weeks, a slight decrease in concentration to about 2.90 mg dm$^{-3}$ was noted at all measurement points (Fig. 2). No significant differences in reactive phosphorus concentrations were observed among the filters (Fig. 2). For the concentrations of this form of phosphorus, an average reduction level was determined in the water after each filter (Table 2, Fig. 3). The observed change in the reduction of reactive phosphorus concentrations in the water after each filter compared to the water after the fish tanks was statistically significant, which indicates that after each filtration step, the concentration of reactive phosphorus was lower than in the water of the fish tanks (Table 3).

The highest concentration of organic phosphorus (5.53 mg dm$^{-3}$) was recorded on the last day of the experiment in the water from the fish tanks; this amount was twice as high as the previous determinations (Fig. 2). In the hydroponic terrace water samples, the concentration of organic phosphorus was characterized by a similar variability to the biological and mechanical filter samples (Fig. 2). During the experiment, the concentration of organic phosphorus was reduced after all filters, and the levels of reduction fluctuated significantly (Table 2, Fig. 3). The correlation coefficients for the reductions in organic phosphorus for all filters were statistically significant (Table 3).

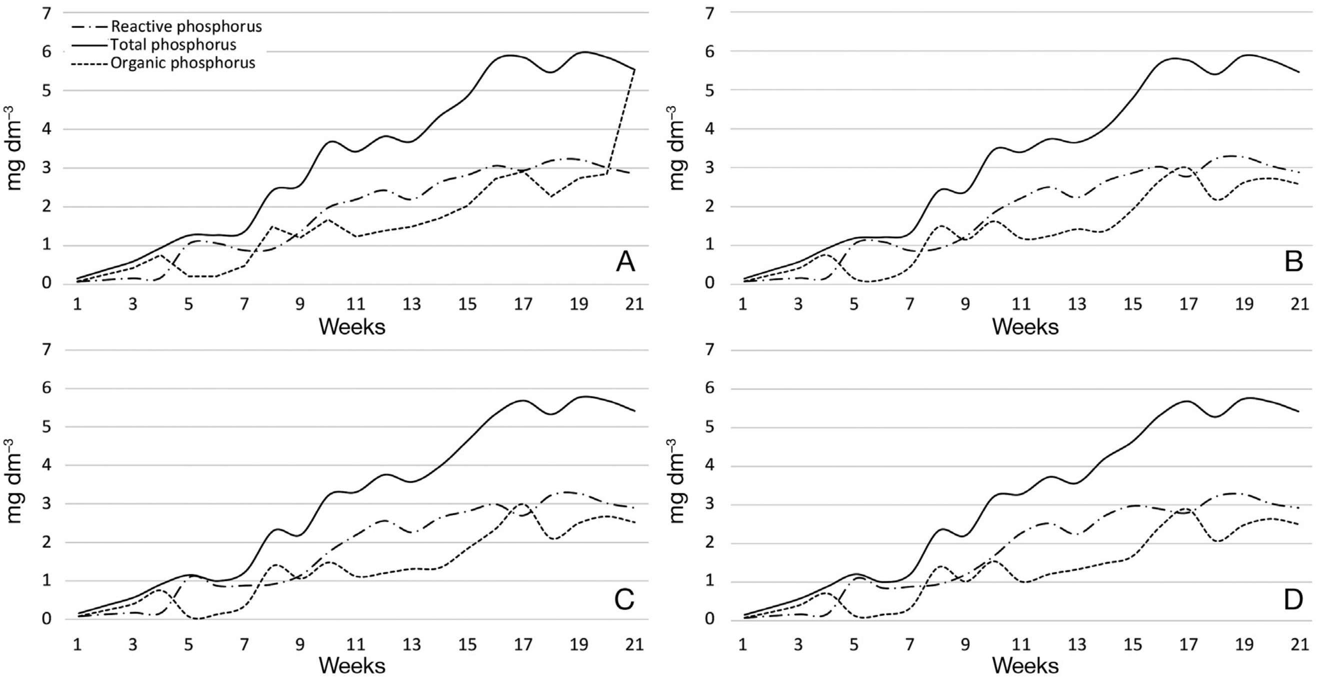

Fig. 2. Variability in average concentrations of phosphorus in the IMTA system during the 21 wk experiment in water (A) from fish pools and after (B) mechanical filtration, (C) biological filtration and (D) hydroponic filtration

Table 2. Average, maximum and minimum reduction of phosphorus and organic matter in the aquaculture system water after 3 types of filtration

| Parameter | Average reduction (%) | Maximum reduction (%) | Minimum reduction (%) |
|---|---|---|---|
| **Reactive phosphorus** | | | |
| Mechanical filter | −0.3 | −8.8 | 2.9 |
| Biological filter | −2.3 | −19.1 | 5.3 |
| Hydroponical filter | −1.2 | −21.0 | 5.2 |
| **Organic phosphorus** | | | |
| Mechanical filter | −9.4 | −53.3 | 2.7 |
| Biological filter | −15.4 | −74.6 | 5.9 |
| Hydroponical filter | −14.2 | −54.8 | 8.0 |
| **Total phosphorus** | | | |
| Mechanical filter | −2.2 | −7.4 | 0.0 |
| Biological filter | −5.6 | −21.8 | 0.0 |
| Hydroponical filter | −5.4 | −21.2 | 0.3 |
| **Organic matter** | | | |
| Mechanical filter | −13.8 | −64.3 | 2.3 |
| Biological filter | −21.3 | −52.1 | −3.8 |
| Hydroponical filter | −26.6 | −61.4 | −11.7 |

Total phosphorus concentrations ranged from 0.15–5.96 mg dm$^{-3}$. A decrease in total phosphorus concentration to an average of 5.45 mg dm$^{-3}$ was recorded at the end of the experiment (Fig. 2). The average reduction after biological filtration was −5.6%, while the water filtered by the hydroponic filter had an average reduction of −5.4% (Table 2, Fig. 3).

Dissolved oxygen concentrations showed a downward trend during the experiment, both in the water from the fish tanks and after filtration (Fig. 4A). The highest dissolved oxygen concentrations were observed in the initial phase of the experiment, when levels above 16.0 mg dm$^{-3}$ were recorded (Fig. 4A). After the 3rd week of the experiment, the amount of dissolved oxygen gradually decreased, reaching a minimum of 2.0 mg dm$^{-3}$ in the last week of the experiment (Fig. 4A).

pH remained stable throughout the experiment, with an average value of 7.47 in the fish tank water, 7.43 after filtering with the mechanical filter, 7.48 after the biological filter and 7.46 after the hydroponic filter (Fig. 4B). The highest pH values were recorded in the first 2 weeks of the experiment, followed by a decrease to a stable level between 7.0 and 7.5 until the end of the experiment (Fig. 4B).

The highest value of general alkalinity was observed in the first 3 weeks of the experiment (Fig. 4C). Then a gradual decrease of alkalinity was observed up to the 9th week of the experiment, when it reached a minimum value (Fig. 4C). An increase in alkalinity to a level above 2.50 mval dm$^{-3}$ was observed in the 14th week of the experiment; alkalinity remained at this level until the end of the experiment (Fig. 4C).

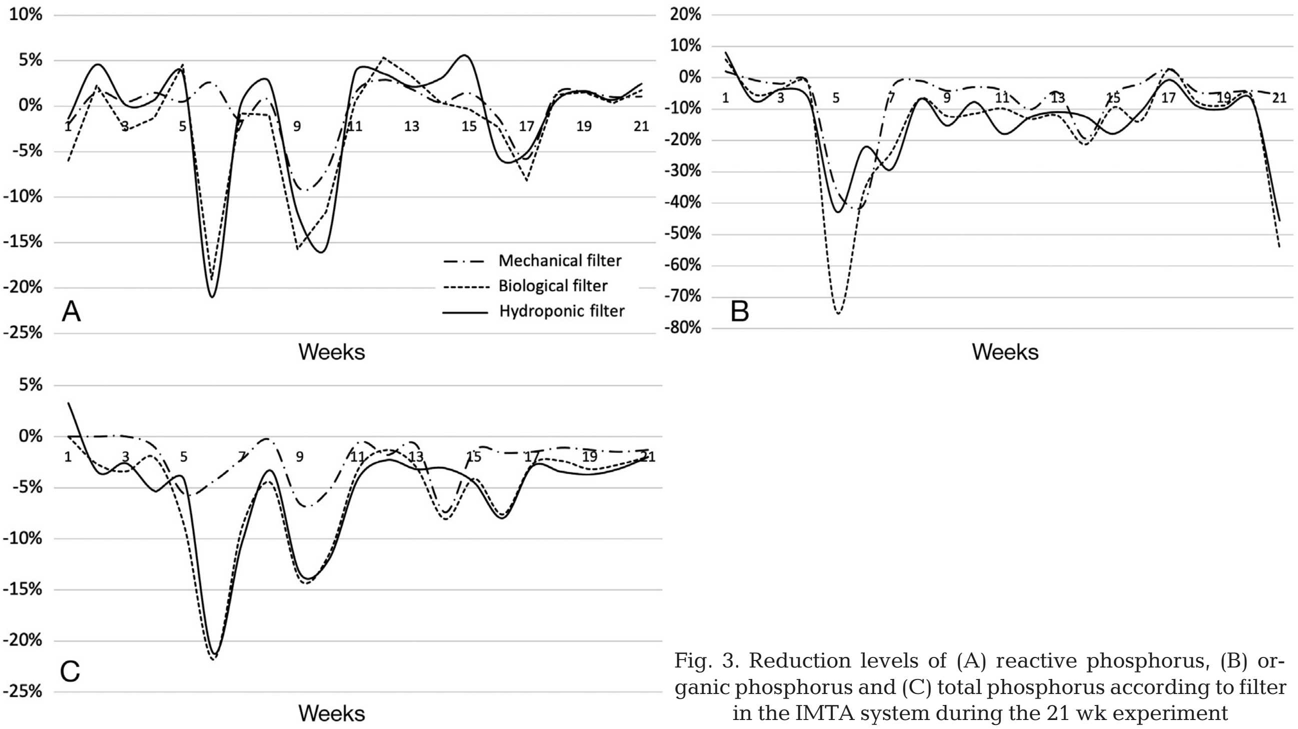

Fig. 3. Reduction levels of (A) reactive phosphorus, (B) organic phosphorus and (C) total phosphorus according to filter in the IMTA system during the 21 wk experiment

Table 3. Correlation coefficients based on non-parametric R-Spearman tests for reduction level of reactive phosphorus, organic phosphorus and organic matter according to filter method. COD: chemical oxygen demand. *$p < 0.050$

| Filter | Mechanical | Biological | Hydroponical |
|---|---|---|---|
| **Reactive phosphorus** | | | |
| Mechanical | – | 0.566* | 0.514* |
| Biological | 0.566* | – | 0.787* |
| Hydroponical | 0.514* | 0.787* | – |
| **Organic phosphorus** | | | |
| Mechanical | – | 0.772* | 0.802* |
| Biological | 0.772* | – | 0.902* |
| Hydroponical | 0.802* | 0.902* | – |
| **COD** | | | |
| Mechanical | – | 0.751* | 0.589* |
| Biological | 0.751* | – | 0.851* |
| Hydroponical | 0589* | 0.851* | – |

Bicarbonate concentrations ranged from 61.6–164.0 mg dm$^{-3}$ (Fig. 5A). The highest concentrations of bicarbonates were observed in the initial phase of the experiment (values peaked up to 4 times in the first week) when they were above 140.0 mg dm$^{-3}$, followed by a decrease. The lowest bicarbonate concentrations were observed twice: in the 6th week of the experiment and between the 8th and 10th weeks of the experiment. After 10 weeks, the bicarbonate concentrations ranged between 100.0 and 140.0 mg dm$^{-3}$ (Fig. 5A).

The highest concentrations of calcium ions were recorded during the first 9 weeks of the experiment (Fig. 5B). In the 10th week, concentrations decreased rapidly and continued to decline in the following weeks until the last week of the experiment (Fig. 5B).

Variability in the concentrations of magnesium ions followed a different pattern. The mean concentration was above 95.0 mg dm$^{-3}$, both in the water from the fish tanks and after all the filters. Magnesium ion concentrations ranged from 70.3–144.0 mg dm$^{-3}$ (Fig. 5C) and remained at a constant level until the 8th week of the experiment. In the 9th week, there was a single increase in magnesium ion concentrations to a maximum value (Fig. 5C). In the following weeks, concentrations returned to the values observed in the first 8 wk of the experiment (Fig. 5C).

The ratio of bicarbonate ions to calcium ions ranged from 0.4–3.2 (Fig. 6). The ratio declined to a minimum in the 9th week and then increased to a maximum value at the end of the experiment (Fig. 6).

Correlation coefficients were determined to illustrate the role of calcium ions in the ratio of bicarbonate to calcium ions. All relationships were statistically significant, which indicates the high affinity of calcium ions to bicarbonate ions (Table 4). Calcium ions combine with bicarbonate ions to form calcium bicarbonates in the system, which results in the loss of calcium ions from the system in the water from the fish tanks and after each filtration.

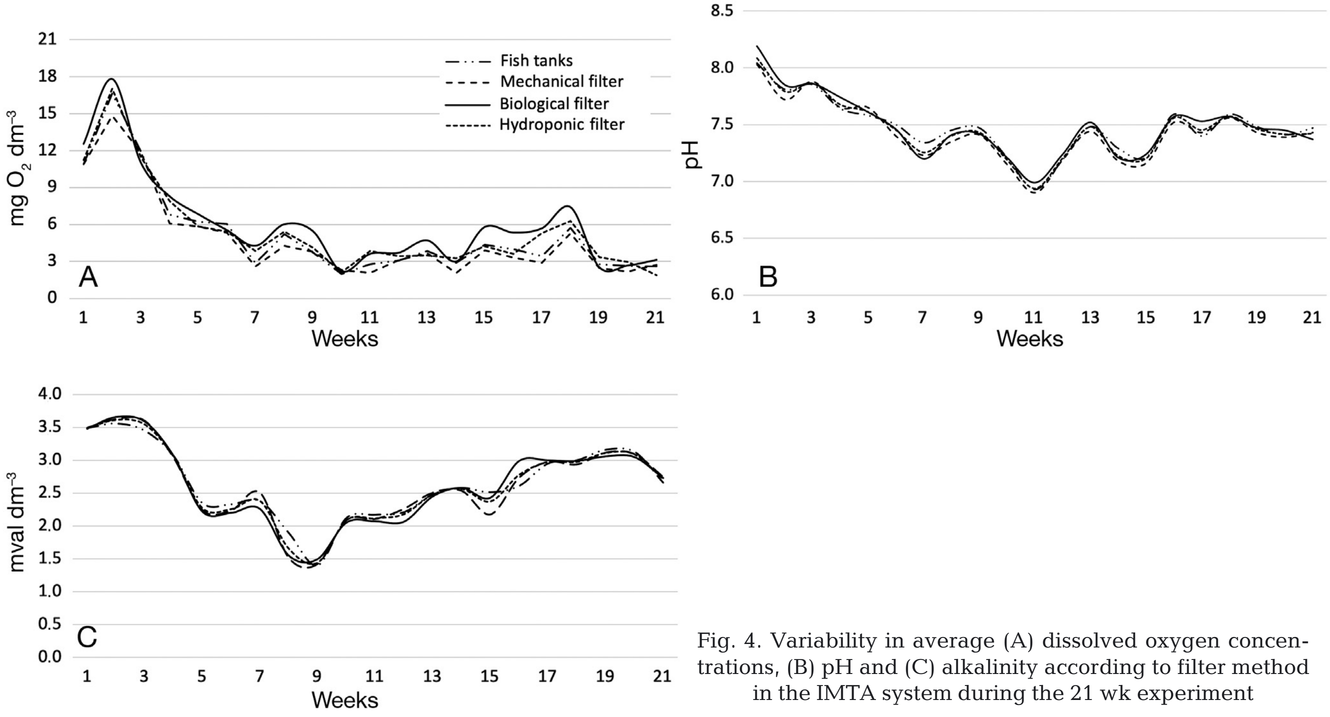

Fig. 4. Variability in average (A) dissolved oxygen concentrations, (B) pH and (C) alkalinity according to filter method in the IMTA system during the 21 wk experiment

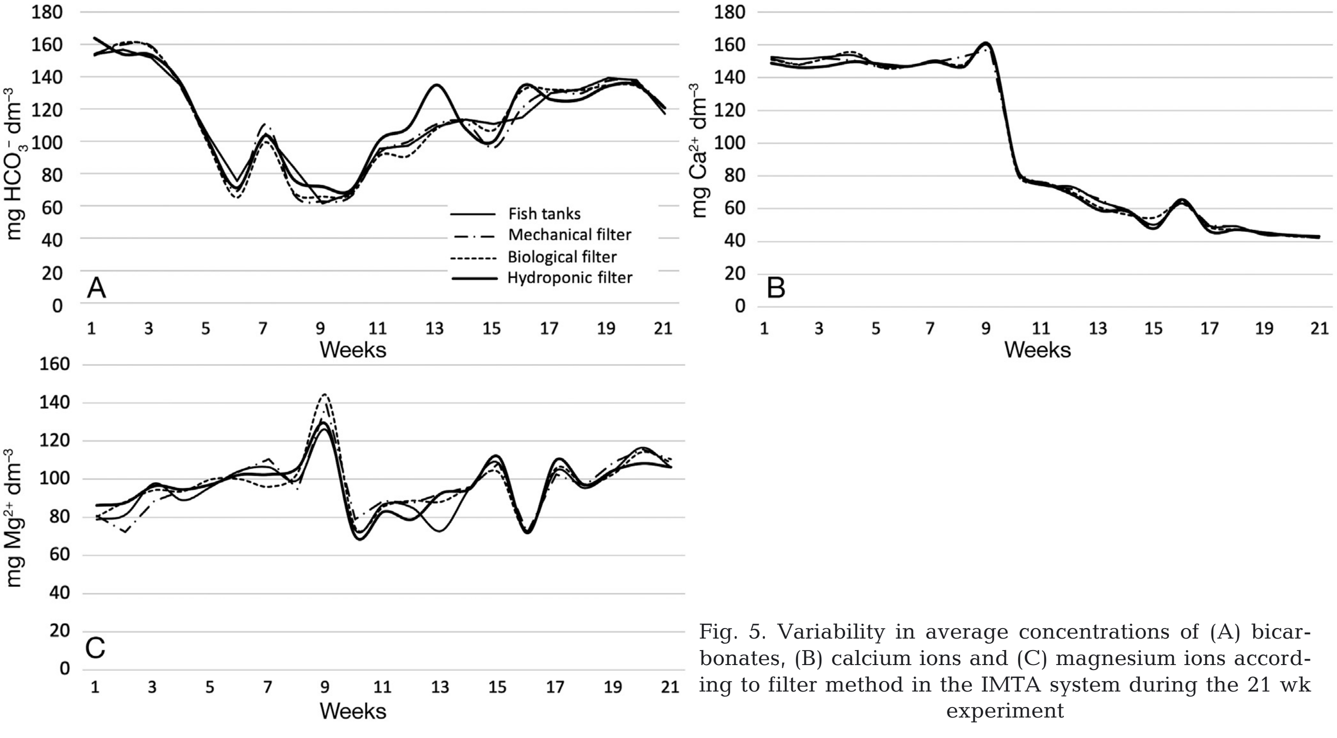

Fig. 5. Variability in average concentrations of (A) bicarbonates, (B) calcium ions and (C) magnesium ions according to filter method in the IMTA system during the 21 wk experiment

COD values ranged from 22.6–199.3 $mgO_2$ dm$^{-3}$ (Fig. 7). Lower index values were observed in the first 5 weeks of the experiment (Fig. 7). Between Weeks 5 and 12, an increase in the index value to a maximum was observed (Fig. 7). In the 13th and 15th week, there was a decrease in the amount of organic matter in the system (Fig. 7). After the 15th week, an upward trend was observed.

The reduction of organic matter after all filters fluctuated significantly, and the overall tendency indi-

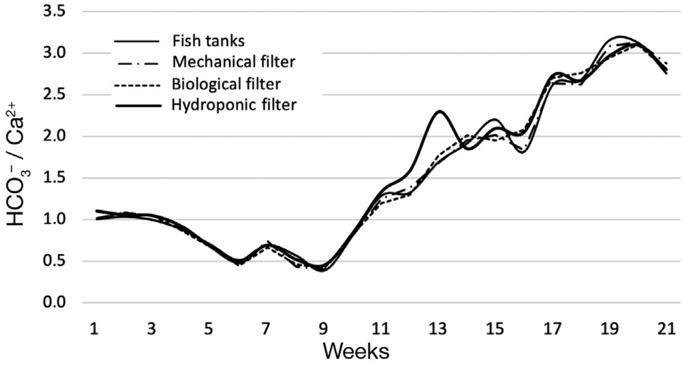

Fig. 6. Variability in the ratio of bicarbonates to calcium ions ($HCO_3^-/Ca^{2+}$) according to filter method in the IMTA system during the 21 wk experiment

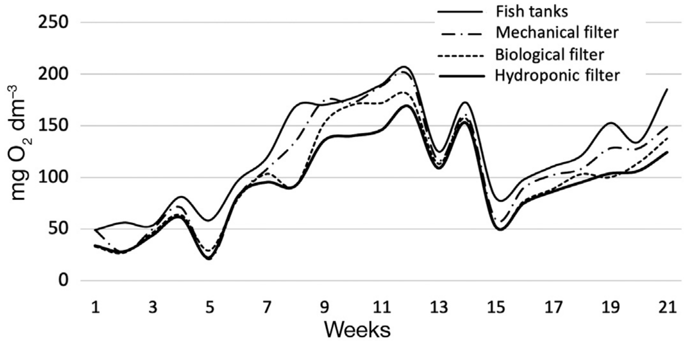

Fig. 7. Average organic matter (chemical oxygen demand) according to filter method in the IMTA system during the 21 wk experiment

cates an increase in organic matter during the experiment (Table 2). On the other hand, there was less organic matter in the water after each filter relative to the amount of organic matter in the water after the fish tanks. This was confirmed by the significant correlation coefficients (Table 3).

The relationship between the concentration of calcium ions and reactive phosphorus is shown in Fig. 8. Until the 9th week of the experiment, when the concentration of reactive phosphorus remained below 1.5 mg $dm^{-3}$, calcium ion concentrations remained above 140.0 mg $dm^{-3}$. After the 9th week, there was a sharp decrease in calcium ion concentrations and an increase in reactive phosphorus. This trend continued until the end of the experiment (Fig. 8).

### 3.3. Total phosphorus balance in the experimental multitrophic aquaculture system

We used the equations in Section 2.3 as well as data obtained from the experiment and provided by the feed producer to obtain the following values: $M_{fish}^{(begin)} = 24.27$ kg, $M_{fish}^{(end)} = 159.95$ kg, $M_{feed} = 165.841$ kg, $V_{water} = 5000$ $dm^3$, $p_{feed} = 1\,\%$, $p_{fish}^{(begin)} = 0.25\,\%$, $p_{fish}^{(end)} = 0.27\,\%$, $p_{water} = 5.46 \cdot 10^{-6}$ kg $dm^{-3}$ and $p_{faeces} = 0.39$ kg. From these values, we calculated the amount of phosphorus accumulated in the plants and sediments as $P_{plants + sediments} = 0.947$ kg, which is 57 % of the phosphorus supplied to the RAS.

The amount of phosphorus in the sediments can be estimated based on the phosphorus reductions obtained after individual filtration. The maximum reduction of phosphorus after the hydroponic filter was 21.2 %, so it can be assumed that the amount of phosphorus accumulated from circulation in plants was just 21.2 % (Table 2). However, we can assume that not all of the available phosphorus was used by the plants. Therefore, it was assumed that only half of the 21.2 % of available phosphorus was accumulated in the plants. On this basis, the following result was obtained: $P_{plants} = 10.6\,\%$ and $P_{sediments} = 46.4\,\%$.

Fig. 9 shows the balance of total phosphorus delivered with the feed in the experimental IMTA system.

## 4. DISCUSSION

### 4.1. Transformation of phosphorus in an integrated aquaponic system

Phosphorus is a basic macroelement necessary for the growth and development of living organisms. It occurs in forms that are strongly dependent on envi-

Table 4. Correlation coefficients based on non-parametric R-Spearman test for a relationship between the ratio of bicarbonate ions to calcium ions and the concentration of calcium ions. All correlation coefficients were statistically significant for $p < 0.050$

| Parameter | $HCO_3/Ca$ fish tanks | $HCO_3/Ca$ after mechanical filter | $HCO_3/Ca$ after biological filter | $HCO_3/Ca$ after hydroponic filter |
|---|---|---|---|---|
| ($mgCa^{2+}$ $dm^{-3}$) fish tanks | −0.868 | −0.864 | −0.862 | −0.854 |
| ($mgCa^{2+}$ $dm^{-3}$) after mechanical filter | −0.902 | −0.904 | −0.890 | −0.884 |
| ($mgCa^{2+}$ $dm^{-3}$) after biological filter | −0.879 | −0.874 | −0.871 | −0.866 |
| ($mgCa^{2+}$ $dm^{-3}$) after hydroponic filter | −0.917 | −0.918 | −0.913 | −0.905 |

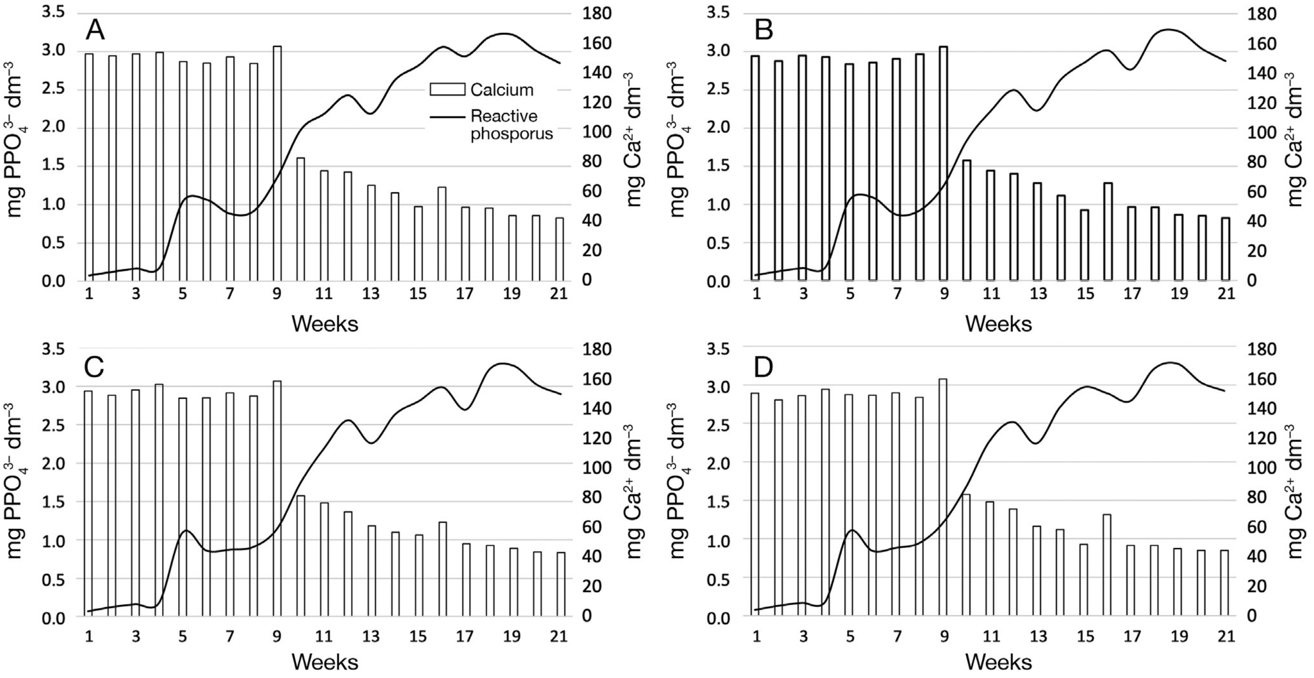

Fig. 8. Relationship between average concentrations of reactive phosphorus and average concentrations of calcium ions in the IMTA system during the 21 wk experiment in water (A) from fish pools and after (B) mechanical filtration, (C) biological filtration and (D) hydroponic filtration

ronmental pH. In the root zone, this element can be found as $PO_4^{3-}$, $HPO_4^{2-}$ and $H_2PO_4^{-}$, where the latter 2 ions are the main forms of phosphorus taken up by plants. Thus, when pH is maintained at a level below 7.5, the largest amount of phosphorus is available for plants (De Rijck & Schrevens 1997, Prabhu et al. 2007, Resh 2013). In our experiment, the pH was maintained at a level that allowed the formation of phosphorus in forms available to the plants; hence, based on the previously discussed results of pH determinations, we me that $HPO_4^{-2}$ dominated in the system, with a small amount of $H_2PO_4^{-}$. The appropriate pH of the water is important not only to ensure the presence of phosphate forms available to plants but also to create appropriate conditions for the proper course of biochemical processes in the system (Espinal & Matulić 2019).

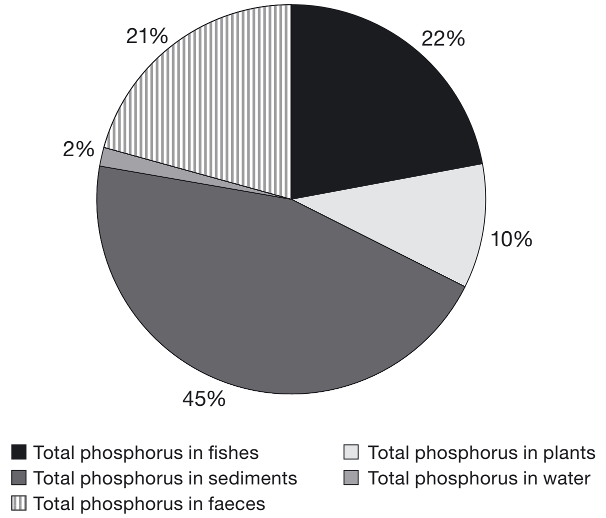

Fig. 9. Balance of total phosphorus supplied by the fish feed in the experimental integrated multitrophic aquaculture system

The primary source of phosphorus in an aquaponic system is fish feed (Eck et al. 2019). A stable and relatively low value of the FCR coefficient indicates optimal use of feed by the fish; the unused part of the feed is certainly a source of phosphorus in the IMTA system, even though phosphorus can be a limiting element and can impede plant growth (Graber & Junge 2009). We assume that a ratio of 1:15 nitrogen: phosphorus is optimal for building organic matter in the primary production process. The results from calculations obtained in our experiment showed phosphorus was a limiting element because the nitrogen: phosphorus ratio was 1:4.9.

According to Rafiee & Saad (2005), fish can use up to 15% of the phosphorus available in their feed. Cerozi & Fitzsimmons (2017), on the other hand, pointed out that in hydroponic lettuce production, the amount of phosphorus supplied from fish tanks can be both sufficient and insufficient, depending on the growth phase of the lettuce. Even 100% of the phosphorus that is supplied from fish farms can be used to produce plant biomass. Graber & Junge (2009) observed a phosphorus use efficiency (PUE) of 50%, Schmautz et al. (2016) found a 32% PUE and Cerozi & Fitzsimmons (2017) reported a PUE of 29.4%.

In our experiment, a 21% reduction of phosphorus was achieved. Thus, the reduction level obtained in our experiment was lower than previously described reduction levels. It can be assumed that the lower PUE was related to the MFB method of plant cultivation, where hydroponic terraces were filled with perlite. The filling of the hydroponic terraces and the oxygen conditions in the experiment may have contributed to the formation of insoluble phosphorus compounds such as hydroxyapatites and their deposition in the sediments that formed at the bottom of hydroponic tanks; hence the inferior absorption of orthophosphates by plants using the MFB method. In addition, at relatively high calcium concentrations in the system, phosphorus may be excluded from circulation by forming $Ca_3(PO_4)_2$ or $CaHPO_4$ salts at neutral pH (Yep & Zheng 2019). Phosphorus precipitation may also occur through the formation of the salts $Mg_3(PO_4)_2$ and $MgHPO_4$ (Palm et al. 2018).

A higher supply of reactive phosphorus in the experiment may have contributed to a decrease in calcium ion concentration as a result of the formation of $Ca_3(PO_4)_2$ or $CaHPO_4$ (Fig. 8). In the second phase of the experiment, reactive phosphorus precipitated in the form of $Mg_3(PO_4)_2$ and $MgHPO_4$. An important factor preventing the coupling of orthophosphates with calcium ions is the general alkalinity of the water and the associated concentration of carbonates and bicarbonates (Cerozi & Fitzsimmons 2017). The speciation of carbonates in the water is strongly pH-dependent and is similar to that of orthophosphates. At high pH, carbonate ions dominate and, along with calcium ions, will form calcium carbonate. At a lower pH, calcium bicarbonate will be formed. It should be assumed that the presence of bicarbonates at the level presented in this experiment also allowed binding of calcium ions with bicarbonates. A deficit of bicarbonates relative to calcium ions occurred between Weeks 5 and 11 of the experiment (Fig. 6). This was also confirmed by an increase in the concentration of reactive phosphorus after 6 wk of the experiment Therefore, after the 6th week, there was more reactive phosphorus available for the plants because calcium was bound to bicarbonates. After the system stabilized, the supply of reactive phosphorus increased. The balance of phosphorus supplied to the circulation with the feed clearly shows that both fish and plants use orthophosphates, although a significant amount is excluded from circulation in the form of sediment.

The water pH also affects the possibility of phosphorus assimilation by plants. According to Yildiz et al. (2017), higher pH contributes to the creation of phosphorus forms that are not accessible by plants. With a stable level of pH, however, the main reasons for lower absorption of reactive phosphorus by plants were presumably the oxygen conditions and the formation of $Ca_3(PO_4)_2$ and $CaHPO_4$ salts. Thus, loss of phosphorus from the cycle may have been mainly due to its entrapment in the resulting sediment. According to Schneider et al. (2005), 30–65% of the phosphorus from fish feed is lost due to chemical transformations, leading to the formation of phosphorus that is not accessible by plants and which is then removed from circulation by mechanical filtration. Yogev et al. (2016) estimated these losses to reach as much as 85%. A similar situation occurred in our experiment, where the precipitation of phosphorus in sediments was estimated at 45%. Achieving stable conditions in the circulation and equilibrium between the concentrations of individual parameters increases the availability of reactive phosphorus, which can be better absorbed by plants. Eck et al. (2019) proposed using additional containers to the aquaponic system that would form a trap in which the phosphorus could be retained and then converted by aerobic and anaerobic processes into a form available to the plants and subsequently returned to the system. Despite the relatively high phosphorus losses, PUE was generally higher than nitrogen use efficiency (NUE) (Cerozi & Fitzsimmons 2017, Yep & Zheng 2019). Similar results were also obtained in this experiment, where NUE averaged 16.6% (Tórz et al. 2021) and PUE averaged 21%.

The basic form of organic phosphorus in ecosystems is insoluble phytates. Enzymatic transformations with the use of phytases result in the release of these organic forms of orthophosphates, which are available to plants. Bacteria play a significant role in enzymatic transformations of phytates, showing significant activity in the rhizosphere. Increasing available phosphorus can be achieved through biochemical processes. Microorganisms also contribute to the control of pathogens (Jorquera et al. 2008, Lennard & Goddek 2019). Considering the method of plant breeding in our experiment (i.e. MFB), it should be assumed that in this environment in the rhizosphere there could also be both microorganisms producing phytases (increasing the pool of available phosphorus in the system) and microorganisms controlling pathogens; therefore, to better understand the transformations of phosphorus in the IMTA systems, further experiments should be conducted that also take the microbiological aspect of the system into account.

Aerobic conditions play a decisive role in processes related to the transformation of biogenic compounds. Improvements in aquaponic systems can be achieved by introducing other microorganisms into the system. Fang et al. (2017) proposed introducing microalgae into the biological filter to increase NUE by 13.8%, increase dissolved oxygen and reduce nitrogen losses associated with $N_2O$ emissions. Moreover, microalgae can use the $CO_2$ produced by the bacteria, and they also contribute to a reduction in ammonium nitrogen in the water (Gilles et al. 2014). If there is an excessive accumulation of algae in the system, the microalgae can be a source of food for the fish, which is an additional benefit of their introduction into the system. Improving oxygen conditions through the use of microalgae can also contribute to improving phosphorus metabolism in the system to prevent loss of the forms of phosphorus available to the plants. The proposed solutions are interesting and promising and require further research.

### 4.2. Circulation of organic matter in an IMTA system

Accumulation of organic matter (feed residue, fish droppings, microorganisms developing in the system) can adversely affect aquaponic systems. Some organic matter is necessary because it ensures the constant presence of nutrients in circulation (resulting from mineralization of the organic matter), but an excess can be harmful. Too much organic matter in the form of sludge creates conditions for an anaerobic environment and thus for the development of anaerobic bacteria that decompose the organic matter, contributing to the formation of carbon dioxide, ammonia or methane, which are harmful to fish (Yep & Zheng 2019). Moreover, if sediment accumulates around the roots of the cultivated plants, the resulting anaerobic environment restricts penetration of oxygen into the root system, which is necessary for the proper uptake of nutrients by the plants (Yep & Zheng 2019). Anaerobic conditions can also contribute to the formation of undesirable compounds such as geosmin and 2-methylisoborneol produced by *Streptomyces*, for which organic matter containing phosphorus provides a desirable environment (Rurangwa & Verdegem 2015). Although these microorganisms are rarely found in aquaponics systems, primarily due to the aerobic conditions, the formation of compounds such as geosmin and 2-methylisoborneol can occur when there is a significant accumulation of organic sediment (Yep & Zheng 2019).

The increase in organic matter was related to the course of the experiment — along with the growth and development of the fish, the system was enriched with organic substances, and dissolved oxygen was consumed (Figs. 4A & 7). As mentioned earlier, there was no depletion of oxygen in the system at any time, so it should be assumed, based on Yep & Zheng (2019), that no undesirable compounds such as geosmin or 2-methylisoborneol were formed, which confirms that these substances occur sporadically in aquaponic systems. The highest reduction of organic matter occurred after the biological and hydroponic filters, so it is most likely that in these filters, mineral forms of biogenic substances were released from the organic matter via biochemical processes. Thus, it can be concluded that the amount of organic matter found allowed for the constant presence of nutrients in circulation.

Depending on the manner of aquaponic breeding, variable amounts of both organic nitrogen and organic phosphorus can be obtained (Rakocy 2012, Rakocy et al. 2017, Yep & Zheng 2019). This does not change the fact that the amount of organic matter provided access to biogenic substances in the experiment. Without the presence of the organic matter and its mineralization, the aquaponic system would need to be enriched with the nutrients necessary for plant growth and development. Another important value of the presence of organic matter is that the microorganisms involved in its decomposition are antagonistic to the pathogens of the plant root systems and thus allow for healthy development of these systems (Yep & Zheng 2019). Mineralization of organic matter occurs throughout the entire system, whether in the mechanical, biological or hydroponic filters. As mentioned earlier, organic phosphorus is also a source of nutrients in aquaponic systems, and its reduction from the system is the result of mineralization and transformation into forms available to plants.

### 4.3. The calcium and magnesium cycle in an IMTA system

In aquaponic systems, biogenic macro- and microelements that may not be present in sufficient quantities for proper plant growth and development are calcium, potassium, magnesium and iron (Seawright et al. 1998). Reared fish usually have minimal requirements for these elements, hence their amounts are low in production feed. Calcium is important for the proper growth and development of plants. It is involved in the construction of cell walls, the permeability of cell membranes and is an important element in cell division. The availability of calcium also makes plants more resistant to bacterial and fungal infections (Liu et al. 2014). A deficit of calcium will manifest in reduced plant growth or deformation of the edges of young leaves (Maucieri et al. 2019). In turn, magnesium is part of chlorophyll, and a symptom of magnesium deficit is browning of the older leaves (Maucieri et al. 2019). As mentioned earlier, the primary source of all nutrients (including macro- and microelements) in the aquaponic system is the remains of feed and fish excrement. An ideal situation would be if all feed added to the system was consumed by the fish. However, typically, part of the feed supplied (less than 5%) is not eaten by the fish and becomes a source of nutrients in the system (Yogev et al. 2016). The amount of feed left uneaten in an aquaponic system can be estimated from the FCR coefficient. Even a stable and relatively low value of the FCR does not guarantee full utilization of the feed, which makes it a source of all nutrients. The chemical composition of fish faeces depends on the fishes' diet, and it also affects the quality of the water in circulation (Goddek et al. 2015). The retention of nutrients such as macroelements in the system is closely related to the species and size of the fish, the method of feeding, the chemical composition of the feed and the water temperature in the system (Schneider et al. 2005). At higher temperatures, for example, fish metabolism is accelerated, resulting in a higher amount of nutrients in the form of dissolved salts in fish faeces (Turcios & Papenbrock 2014). According to Rafiee & Saad (2005), fish consume an average of 26.8% of the calcium and 20.3% of the magnesium available in the feed. The ionic forms of the macroelements available to the plants are formed by the mineralization of organic matter. However, individual macroelements may interact with each other and form insoluble forms that are not available to the plants. The balance between the available and unavailable macroelements is determined by the pH of the water. A well-known process in aquaponic systems is the precipitation of calcium in the form of $Ca_3(PO4)_2$, $CaHPO_4$ and $CaCO_3$, as mentioned earlier (Peng et al. 2018). We assumed that the decrease in the concentration of calcium available for plant growth during our experiment was caused by the formation of insoluble salts. In the first phase, insoluble salts were formed from a combination of calcium and orthophosphates (Fig. 8). In the next phase, calcium bicarbonates could also be formed. Similar dependencies were described also by Cerozi & Fitzsimmons (2017). Calcium is more readily absorbed by plants at pH > 7 (Lennard & Goddek 2019). Considering that pH was kept above 7 during our experiment, it can be assumed that the pH of the water caused proper absorption of calcium by the plants. Villarroel et al. (2011) reported that in aquaponic cultures, magnesium concentration is about 4.0 mg $dm^{-3}$, while the optimal magnesium concentration for proper plant growth ranges from 20–50 mg $dm^{-3}$ (Sonneveld & Voogt 2009). Over the entire experimental cycle, magnesium concentrations significantly exceeded the level observed by Villarroel et al. (2011), so it was available in sufficient quantity for plant development. As in the case of calcium, better assimilation of magnesium occurs at pH > 7, so in this case, the water pH caused proper absorption of magnesium by the plants. As presented in Villarroel et al. (2011), magnesium, similarly to calcium, can also be removed from circulation in the form of magnesium bicarbonates. In the present study, after the concentration of calcium ions had decreased, magnesium bicarbonates could be precipitated; however, this process did not significantly affect the availability of magnesium for plants, as during the whole experiment the concentration of magnesium ions was above 50 mg $dm^{-3}$.

## 5. CONCLUSIONS

The basic forms of orthophosphates in the IMTA system were $H_2PO_4^-$ and $HPO_4^{2-}$. A higher level of reactive phosphorus, which occurred over the course of the experiment, may have contributed to a decrease in calcium ion concentration due to the formation of $Ca_3(PO_4)_2$ or $CaHPO_4$, and thus inactivated part of the phosphorus available to the plants. Phosphorus may have been inactivated in the sediment due to the formation of $Mg_3(PO_4)_2$ and $MgHPO_4$ in the second phase of the experiment. The water pH was maintained at 6.90–8.18, ensuring the efficiency of biochemical processes in the system and the proper use of nutrients by the plants. These conditions were also suitable for carp breeding. The amount of organic matter gradually increased throughout the experiment, reaching a maximum of 204 $mgO_2\,dm^{-3}$ at the and of the experiment. Mineralization of organic matter took place under aerobic conditions. Organic matter was a source of biogenic substances in the IMTA. The range of general alkalinity and the observed bicarbonate concentrations suggest that the decrease in calcium ion concentrations was also caused by the formation of $CaCO_3$ and $Ca(HCO_3)_2$. Magnesium ions did not show such a significant affinity for bicarbonate ions. Concentrations of calcium and magnesium ions were maintained at an optimal level for proper plant growth.

This experiment showed that achieving stable conditions in the aquaponic cycle and in the balance between the concentrations of individual parameters increases the availability of reactive phosphorus, which can be better absorbed by plants. Our experiment also showed that the selected method of plant breeding has an impact on the transformation of phosphorus in the aquaponic systems.

## LITERATURE CITED

APHA (American Public Health Association) (1999) Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC

Barben SA, Hopkins BG, Jolley VD, Webb B, Nichols BA (2010) Optimizing phosphorus and zinc concentrations in hydroponic chelator-buffered nutrient solution for Russet Burbank potato. J Plant Nutr 33:557–570

Cerozi BS, Fitzsimmons K (2017) Phosphorus dynamics modelling and mass balance in an aquaponics system. Agric Syst 153:94–100

De Rijck G, Schrevens E (1997) pH influenced by the elemental composition of nutrient solution. J Plant Nutr 20: 911–923

Eck M, Körner O, Jijakli MH (2019) Nutrient cycling in aquaponics system. In: Goddek S, Joyce A, Kotzen B, Burnell GM (eds) Aquaponics food production systems. Springer, Cham, p 231–246

Engle CR (2015) Economics of aquaponics. Publication No. 5006. Southern Regional Aquaculture Center (SRAC), Stillwater, OK. http://osufacts.okstate.edu

Espinal CA, Matulić D (2019) Recirculating aquaculture technologies. In: Goddek S, Joyce A, Kotzen B, Burnell GM (eds) Aquaponics food production systems. Springer, Cham, p 35–77

Fang Y, Hu Z, Zou Y, Zhang J, Zhu Z, Nie L (2017) Improving nitrogen utilization efficiency of aquaponics by introducing algal–bacterial consortia. Bioresour Technol 245: 358–364

Forchino AA, Lourguioui H, Brigolin D, Pastres R (2017) Aquaponics and sustainability: the comparison of two different aquaponic techniques using the Life Cycle Assessment (LCA). Aquacult Eng 77:80–88

Gilles S, Ismiño R, Sanchez H, David F and others (2014) An integrated closed system for fish–plankton aquaculture in Amazonian fresh water. Animal 8:1319–1328

Goddek S, Delaide B, Mankasingh U, Ragnarsdottir KV, Jijakli H, Thorarinsdottir R (2015) Challenges of sustainable and commercial aquaponics. Sustainability 7: 4199–4224

Graber A, Junge R (2009) Aquaponic systems: nutrient recycling from fish wastewater by vegetable production. Desalination 246:147–156

Jorquera M, Martinez O, Maruyama F, Marschner P, de la Luz Mora M (2008) Current and future biotechnological applications of bacterial phytases and phytase-producing bacteria. Microbes Environ 23:182–191

Lennard W, Goddek S (2019) Aquaponics: the basics. In: Goddek S, Joyce A, Kotzen B, Burnell GM (eds) Aquaponics food production systems. Springer, Cham, p 113–143

Li C, Zhang B, Luo P, Shi H and others (2019) Performance of a pilot-scale aquaponics system using hydroponics and immobilized biofilm treatment for water quality control. J Clean Prod 208:274–284

Liu S, Hou Y, Chen X, Gao Y, Li H, Sun S (2014) Combination of fluconazole with non-antifungal agents: a promising approach to cope with resistant *Candida albicans* infections and insight into new antifungal agent discovery. Int J Antimicrob Agents 43:395–402

Love DC, Uhl MS, Genello L (2015) Energy and water use of a small-scale raft aquaponics system in Baltimore, Maryland, United States. Aquacult Eng 68:19–27

Maucieri C, Nicoletto C, van Os E, Anseeuw D, Van Havermaet R, Junke R (2019) Hydroponic technologies. In: Goddek S, Joyce A, Kotzen B, Burnell GM (eds) Aquaponics food production systems. Springer, Cham, p 77–110

Palm HW, Knaus U, Wasenitz B, Bischoff AA, Strauch SM (2018) Proportional up scaling of African catfish (*Clarias gariepinus* Burchell, 1822) commercial recirculating aquaculture systems disproportionally affects nutrient dynamics. Aquaculture 491:155–168

Peng L, Dai H, Wu Y, Peng Y, Lu X (2018) A comprehensive review of phosphorus recovery from wastewater by crystallization processes. Chemosphere 197:768–781

Prabhu AS, Fageria NK, Berni RF, Rodrigues FA (2007) Phosphorus and plant disease. In: Datnoff LE, Elmer WH, Huber DM (eds) Mineral nutrition and plant disease. The American Phytopathological Society, St. Paul, MN, p 45–55

Rafiee G, Saad CR (2005) Nutrient cycle and sludge production during different stages offered tilapia (*Oreochromis* sp.) growth in a recirculating aquaculture system. Aquaculture 244:109–118

Rakocy JE (2012) Aquaponics — integrating fish and plant culture. In: Tidwell JH (ed) Aquaculture production systems. John Wiley & Sons, Ames, IA, p 343–386

Rakocy JE, Masser MP, Losordo TM (2017) Recirculating aquaculture tank production systems: aquaponics — integrating fish and plant culture. Publication No. 454. Southern Regional Aquaculture Center (SRAC), Stillwater, OK

Resh HM (2013) Hydroponic food production: a definitive guidebook for the advances home gardener and the commercial hydroponic grower, 7th edn. CRC Press, Boca Raton, FL

Rurangwa JW, Verdegem MCJ (2015) Microorganisms in recirculating aquaculture systems and their management. Rev Aquacult 7:117–130

Schmautz Z, Loeu F, Liebisch F, Graber A, Mathis A, Griessler Bulc T, Junge R (2016) Tomato productivity and quality in aquaponics: comparison of three hydroponic methods. Water 8:533

Schneider O, Sereti V, Eding EH, Verreth JAJ (2005) Analysis of nutrient flows in integrated intensive aquaculture systems. Aquacult Eng 32:379–401

Seawright DE, Stickney RR, Walker RB (1998) Nutrient dynamics in integrated aquaculture–hydroponics systems. Aquaculture 160:215–237

Somerville C, Cohen M, Pantanella E, Stankus A, Lovatelli A (2014) Small-scale aquaponic food production: integrated fish and plant farming. Technical Paper No. 589. FAO, Rome

Sonneveld C, Voogt W (2009) Plant nutrition of greenhouse crops. Springer, Dordrecht

Tórz A, Burda M, Półgęsek M, Sadowski J, Nędzarek A (2021) Biochemical transformations of nitrogen compounds in the integrated multi-trophic aquaculture the using media filled beds in plant cultivation. Aquaculture 533:736141

Turcios AE, Papenbrock J (2014) Sustainable treatment of aquaculture effluents — What can we learn from the past for the future? Sustainability 6:836–856

Villarroel M, Alvariño JMR, Duran JM (2011) Aquaponics: integrating fish feeding rates and ion waste production for strawberry hydroponics. Span J Agric Res 9:537–545

Yep B, Zheng Y (2019) Aquaponic trends and challenges — a review. J Clean Prod 228:1586–1599

Yildiz HY, Robaina L, Pirhonen J, Mente E, Dominguez D, Parisi G (2017) Fish welfare in aquaponics systems: its relation to water quality with an emphasis on feed and faeces — a review. Water 9:13

Yogev U, Barnes A, Gross A (2016) Nutrients and energy balance for a conceptual model of three loops off grid, aquaponics. Water 8:589

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