Effect of water irrigation volume on Capsicum frutescens growth and plankton abundance in aquaponics system

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Abstract. This study aimed to understand Capsicum frutescens growth and plankton abundance in aquaponics culture. A Completely Randomized Design (CRD) with six treatments in triplicates comprising of treatment A (positive control using organic liquid fertilizer), B (negative control without fertilizer), C (drip irrigation aquaponics with a water debit of 100 ml/day/plant), D (drip irrigation aquaponics with a water debit of 150 ml/day/plant), E (drip irrigation with a water debit of 200 ml/day/plant), and F (drip irrigation aquaponics with a water debit of 250 ml/day/plant) was applied. The water used in treatments C, D, E, and F contained comet fish feces as fertilizer. C. frutescens growth and plankton abundance were observed. Analysis was conducted using analysis of variance for plant productivity and descriptive analysis for plankton abundance and water quality. The results of this study showed that the highest plant growth was seen in plants receiving F treatment with 50 ml/day drip irrigation. However, no significant difference was found when compared to the positive control with organic artificial fertilizer. Eleven types of phytoplankton and six types of zooplankton were found, with Stanieria sp. as the most abundant phytoplankton and Brachionus sp. and Epistylis sp. as the most abundant zooplanktons.

1. Introduction
Aquaponics system is one of the culture technologies that combine fish and plant cultures. This system excels because of its nature as an environmentally friendly system with more productive culture environment, producing fish and plants with a higher quality without using chemicals as fertilizer, pesticide, or herbicide [1].

One of the well-known aquaponics systems is the drip irrigation system. This system is known as a system that can save water through minimization of water loss that enables the system to be used in agricultural areas with limited water source. This creates an opportunity to broaden the market of the plants cultured using this system, including Capsicum frutescens, to include household, domestic, and export markets [2]. Currently, the demand for C. frutescens is quite high, i.e. around 4 kg/capita/year [3].

To meet the demand of the market for C. frutescens is not easy, especially with the low production of chili in Indonesia. Challenges faced in increasing the chili production in this country includes pest and disease attacks exaggerated by damaged soil due to toxic contamination of chemicals from the use of inorganic fertilizers or excessive use of pesticides. Hence, proper fertilizing process that pays more attention to soil conservation is needed [4].
Nutritional needs of plants can be satisfied using fish metabolism waste, in addition to the use of fertilizers. This can be attained by combining fish culture and plant culture in an aquaponics system. Both fish for consumption and ornamental fish can be used for this purpose.

In an aquaponics system, the waste produced by the fish is used as the fertilizer for the plants [5]. Fish produces 80–90% of ammonia through osmoregulation process. Fish feces and urine contain around 10–20% of total nitrogen [6]. The nutrients contained in the fish waste will be different, depending on the feed used. A preliminary study has shown that comet fish fed with feed containing 35% of protein and a feeding rate of 5% of its body weight produces feces with 3.40% of N, 6.08% of P2O5 and 0.05% K2O, C. frutescens absorbs 70 kg·ha⁻¹ N, 16 kg·ha P2O5, and 92 kg·ha⁻¹ K2O [7]. By this in mind, this study focused on the relationship between the nutrients produced in comet fish feces and the growth of C. frutescens in a drip irrigation aquaponics system.

2. Materials and Methods

2.1. Time and place of study
This study was performed in the period of March to June 2016 at the Aquaculture Wet Laboratory, Ciparanje, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Indonesia. The water quality measurement was performed at the Aquatic Resources Management Laboratory of Universitas Padjadjaran and a preliminary testing was performed in the Soil Fertility and Plant Nutrition Laboratory of the Faculty of Agriculture, Universitas Padjadjaran, Indonesia.

2.2. Materials and instruments
The main instruments used during this study were aerator, concrete pond, plankton net, and water quality measurement tool (Lutron pH-207). The materials used were (a) fish, i.e. 5-month-old of Carassius sp. with an average length of 9 cm and average weight of 6 g, (b) three-week-old C. frutescens’s seeds, (c) cocopeat as the growing media (JM Tani), (d) FFF 999 commercial feed (PT Central Pangan Pertiwi) with a crude protein, fat, fiber, ash, and maximum water contents of 35%, 2%, 3%, 13%, and 12%, respectively (e) Cobra organic liquid fertilizer which was used every 10 days (Bharti Bio Chem) with the following compositions: 5.60% N, 2.70% P2O5, 3.23% K2O, 0.3% C-Org, 0.18% CaO, 0.20% MgO, 0.70% SO4, 0.21% NH4, 0.03% B, 0.04% Cl, 33.17 ppm Zn, 40.38 ppm Cu, 0.2 ppm Mo, 67.34 ppm Mn, and 75.27 ppm Fe, (f) reagent solutions comprising of MnSO4 and H2SO4 reagents for dissolved oxygen titration, 0.5 Nestler and 1 ml Siegnet for ammonia testing, SnCl and NH4 molybdate for phosphate testing, and phenol and NH4OH 10% for nitrate testing (Sigma).

2.3. Procedure

2.3.1. Aquaculture pond preparation
The culture pond used in this study was a concrete pond of 3 m × 2 m × 1.1 m. The preparation of the ponds included pond cleaning after drying and then water filling.

2.3.2. C. Frutescens planting
The planting of C. frutescens was started by seed selection and seeding. C. frutescens seeds were soaked in warm water for 30 minutes to clean the seed and the seeding was done using trays. Seeding was performed to select seeds, separating normal from abnormal or diseased ones. In addition, seeding was also used to prepare the seeds until they were strong enough to be planted in a bigger site. The C. frutescens seed was considered ready to be planted in a bigger growing media when it grew four or more leaves.
2.3.3. Drip irrigation aquaponics system
In this study, the culture pond was a concrete pond of $3 \times 2 \times 1.1$ m with a total density of comet fish of 430 fishes, and a water depth of 60 cm. The pond was equipped with aerators for oxygen supply and a clear plastic roof to prevent rain water falling into the aquaponics media. Polybags were filled with coco peat and \textit{C. frutescens} plants were planted in the bags that were then arranged around the pond with a space of 20 cm between each polybag. The water from the culture media was pumped and distributed through a PVC pipe that was installed to match the form of the pond (figure 1).

\textbf{Figure 1.} Schematic aquaponics design used in this study.

2.4. Steps of study
A three month period was assigned for this study. Fishes were fed three times a day with a feeding rate of 5% of the total fish biomass. Fish weighing was done every two weeks. Water quality measurement that included Dissolved Oxygen (DO), temperature, pH, phosphate, nitrate, ammonia, and plankton abundance measurement was conducted once every 7 days. This study used an outdoor location; hence, the sun was used as the source of light. Data on \textit{C. frustescens} were collected by measuring the height of the plant and the number of leaves throughout the study period.

2.5. Design experiment
This study was an experimental study using Completely Randomized Design (CRD) that consisted of six triplicate treatments. The treatments in this study were:
- Treatment A = positive control (use of organic liquid fertilizer)
- Treatment B = negative control (without organic liquid fertilizer)
- Treatment C = irrigation volume of 100 ml/day/plant
- Treatment D = irrigation volume of 150 ml/day/plant
- Treatment E = irrigation volume of 200 ml/day/plant
- Treatment F = irrigation volume of 250 ml/day/plant

2.6. Observed parameters

2.6.1. Comet fish feces content analysis
Feces analyzed in this study was the feces of Comet fish that fed with 5% of feeding rate. Analysis of total Nitrogen, P$_2$O$_5$ content, and K$_2$O content in feces samples were conducted. The nitrogen in the feces was measured using Kjehdal method while the P$_2$O$_5$ and K$_2$O contents were measured using
Olsen method and spectrophotometry, respectively [8]. The results were then compared to the nutrient requirement of the plant using the basic formula of fertilization [9].

\[
\text{Recommended dose of fertilizer plot} = \text{Plot size} \times 1 \text{ ha size}^{-1} \times \text{Dose} \tag{1}
\]

Notes:
Per plot = per plant
Plot size = polybag size (30 × 30)
1 ha size = soil/cocopeat mash

2.6.2. C. frustescens Plant Growth
Plant height was measured using a ruler while the number of leaves, flowers, and fruits were counted manually. Fruit produced was weighed using a scale in the end of research.

2.6.3. Water Quality
The measurement of water quality included temperature, pH, dissolved oxygen (DO), nitrate, phosphate, and ammonia level measurements. The parameter measurement was performed once every seven days since the beginning of the study until the end of the study through titration and spectrophotometry methods.

A pH meter (LUTRON PH-207) was used to measure pH and a mercury thermometer was used to measure temperature. The Dissolved Oxygen (DO) was measured through titration by applying 21 drops of O₂ reagent, 21 drops MNSO₄, and 42 drops of concentrated H₂SO₄ on the sample that was then placed into the Erlenmeyer flask (50 ml) for titration until the color changed. Nitrate, phosphate, and ammonia contents were measured using a spectrophotometer (Thermo Scientific Genesys 20).

2.6.4. Phytoplankton and Zooplankton Densities
Sampling was done using a plankton net 25 mikron size. Phytoplankton samples was stored at around 5 °C to slow down physical and chemical reactions that will cause deterioration of species cell structure. Before counting, samples was acclimatized at room temperature for 12 hours for obtaining random distribution of species in counting chamber as well as for preventing air bubble formation. Sample volume of 1 ml was observed using Sedgewick-Rafter Counting Chamber method [10]. Sample was observed under microscope to determine plankton density using the following formula [11]:

\[
N = n \times Vr \times Vo^{-1} \times Vs^{-1} \tag{2}
\]

Notes:
N = number (cell·m⁻³)
n = number of identified cells
Vr = volume of filtered water (50 ml)
Vo = volume of water observed in counting chamber (1 ml)
Vs = volume of filtered water (25 ml)

3. Results and Discussion

3.1. Chemical content of Comet fish feces
The Comet fish feces contained 3.40 % N, 6.08 % P₂O₅, and 0.05 % K₂O. Based on the values, the N and P levels in the water were calculated and adjusted to the plant’s requirement.
Table 1. Comparison of comet fish chemical contents and the nutrient requirement for *C. frutescens*.

| Content | Feces (%) | Calculation result (ppm) | Plant’s Requirement (ppm) |
|---------|-----------|--------------------------|---------------------------|
| N       | 3.4       | 1.19                     | 0.303                     |
| P       | 6.08      | 2.128                    | 0.263                     |
| K       | 0.05      | 0.0175                   | 0.169                     |

Comet fish cultured in this study was 2.5 kg with an average weight of 6.72 ± 3.23 g. Based on that stocking density, the disposed feces in the water was estimated to be 110 g. Hence, it was estimated that the nitrogen and phosphate levels in the water were 1.19 ppm and 2.128 ppm, respectively (table 1) The nutrient requirement was estimated to be 0.303 nitrogen, 0.263 phosphate, and 0.169 ppm potassium.

However, based on the water quality test performed once every seven days, the nutrient content in the comet fish culture media could not meet the total need of *C. frutescens*. This has inhibited the plant to grow well. The difference in the water quality test result and calculation result was suspected as the result from the challenge in the nitrogen cycle in the culture pond or the small amount of feces in the body of water, making the dissolved organic materials became less. The inadequate amount of nutrients received by the plants has led to low and abnormal-like growth. Furthermore, according to [12], plants that do not receive enough nitrogen will slow growth with pale leaves. If the deficiency is very severe, the leaves will turn color into light green and yellow [9]. This is in line with the results of this study where the *C. frutescens* plant grew slowly and had yellowish leaves (figure 2).

![Figure 2. *C. frutescens*’ leaves that lacks of nutrient (left) and well-nourished leaves (right).](image)

Furthermore, the limited amount of nutrients in the *C. frutescens* planting media was also influenced by the use of coco peat as the planting substrate. When coco peat is used, the water distributed through the irrigation drops cannot hold for a long time because of higher porosity than that of soil. This, eventually, will reduce the amount of nutrients that can be used by the plant.

3.2. *C. Frutescens* growth

Plant growth can be identified from the increase in the stem length (plant height), number of leaves, number of flowers, and fruit weight. Chili plant has two phases of growth, i.e. vegetative phase and generative phase. The vegetative phase is marked by increased plant height and increased number of leaves while the generative phase is marked by flower and fruit growth.
3.2.1. **Plant height**

The vertical growth is identified from the increased height of plant’s stem. This growth is also considered as the characteristics of the vegetative phase. The increase in plant height for each treatment ranged between 15–27 cm during the three months of study (figure 3).

![Vertical growth of *C. frutescens* planted in aquaphonics system with various irrigation volume.](image_url)

**Figure 3.** Vertical growth of *C. frutescens* planted in aquaphonics system with various irrigation volume. (A) Positive control; (B) Negative control; (C) Irrigation volume of 100 ml/day/plant; (D) Irrigation volume of 150 ml/day/plant; (E) Irrigation volume of 200 ml/day/plant; (F) Irrigation volume of 250 ml/day/plant.

Based on the above chart, the lowest vertical growth was seen in treatment A, which used organic fertilizers, as the positive control. This was because the plants in treatment A were attacked by aphids more often than those in other treatment groups. The plants in treatment B, which was the negative control without any fertilizer, were higher than the plants in treatment A, which was around 25 cm high. It was suggested that the water in Treatment B group was mostly used to increase the plant height.

Treatment F group, which was the group that used the highest amount of dripped water produced the highest height of 27 cm. It was suggested that the amount of nutrient absorbed by the plants in this group was higher due to the bigger volume of water. The followings are the results of the statistical testing using analysis of variance (table 3).

Based on the statistical analysis, the plant growth did not show any significant difference between treatments. The nutrient with the most influence towards vertical growth is phosphate. In plants, phosphate is used to form roots, accelerate fruit maturation, and strengthen the plant stem [13]. This study showed that the phosphate content in the pond and in the irrigation drip tap during the study ranged between 0.025–2.187 mg·L⁻¹ and 0.077–0.797 mg·L⁻¹, respectively. The phosphate content in the comet fish culture pond was adequate for plant growth.

3.2.2. **Leaf growth**

During this study, the leaf growth ranged between 8–66 leaves. The lowest leaf growth was seen in treatment B and the highest leaf growth was seen in treatment A, where Treatment B showed a higher vertical growth compared to the Treatment A. This was caused by the priority in water use which, in
Treatment B, was prioritized more on vertical growth than on adding leaves. Below is the chart depicting leaf growth during the study (figure 4).

**Figure 4.** Average number of leaf of *C. frutescens* planted in aquaponics system with various irrigation volume. (A) Positive control; (B) Negative control; (C) Irrigation volume of 100 ml/day/plant; (D) Irrigation volume of 150 ml/day/plant; (E) Irrigation volume of 200 ml/day/plant; (F) Irrigation volume of 250 ml/day/plant.

Based on the chart, it is obvious that the highest leaf growth was seen in treatment A group. In Treatment B group, the leaf growth was reduced because the number of leaves shed was higher due to inadequate nutrition. Among the treatment groups, Treatment F group was found to be the group with the most leaves, which was proportional to the vertical growth of this group.

The statistical analysis results showed that the leaf growth in Treatment A was not significantly different from Treatment F but significantly different from treatment B, E, and D while treatment F was not significantly different from treatment B, E, and D. This was apparent from the fact that treatment A had the highest average leaf growth, which was followed by treatment F. Hence, control treatment showed a better result compared to other treatments because the plants in treatment A group received adequate nutrition from the artificial fertilizer while the plants in treatment F received more nutrition compared to other treatments because the number of drips and water volume were higher. The most influential compound for leaf growth is nitrogen, which is in line with the result of this study where treatment F group had the biggest nutrient-containing water volume, meaning that more N was provided and more leaves were produced. Lack of nitrogen will make the leaves turn yellowish and slow down the growth, making the leaves weak and easily shed [13].

### 3.2.3. Flower growth

Flower is one of the characteristics of the generative (reproductive) phase in a plant. In this study, not all treatment groups produced flowers. The number of flowers is presented in the following table (table 2).
Table 2. Average flower number of *C. frutecens* planted in aquaphonics system with various irrigation volume.

| Treatment               | Number of Flowers |
|-------------------------|-------------------|
| Control (+) (A)         | 24 ± 1.18         |
| Control (-) (B)         | -                 |
| 100 ml (C)              | -                 |
| 150 ml (D)              | 9 ± 0.7           |
| 200 ml (E)              | 5 ± 0.7           |
| 250 ml (F)              | 15 ± 1.15         |

Flowers were only found in Treatment A, D, E, and F groups during the study period. The highest number of flowers was seen in Treatment A where the number of flowers reached 24 and the lowest was in Treatment E with only five flowers. The flowering phase influences the fruit growth. Not all flowers will produce fruit because some flowers shed and fail to form fruit. Several factors influence flower growth, including temperature and humidity. Chili (*C. frutescens*) needs an annual average air temperature between 18–30 °C and air humidity around 60–80 % [14]. In this study, the air temperature reached 32 °C during day time and the humidity never reached more than 30 %.

Air temperature is an important factor that very much influences the physiological activities through the biochemical reaction rate. Each physiological process, such as photosynthesis or respiration, has a certain temperature limit. Above the optimum temperature, biochemical reactions slowly reduce and enzymes go through denaturation or become inactive. Air temperature also influences flower growth, fruit formation, hormone balance, maturation and aging rates, quality, result, and how long the product is consumable [15].

3.2.4. Fruit growth

The main product component of *C. frutescens* culture is the fruit. The fruit of *C. frutescens* can be harvested after 80–90 days or two or three months after planting. The fruit is initially green then turns into red in several weeks. The following table lists the fruit growth during the study (table 3).

Table 3. Fruit growth of *C. frutescens* planted in aquaphonics system with various irrigation volume.

| Treatment               | Number of Fruits | Weight (gr) |
|-------------------------|------------------|-------------|
| Control (+) (A)         | 6 ± 0.63         | 2.3         |
| Control (-) (B)         | -                | -           |
| 100 ml (C)              | -                | -           |
| 150 ml (D)              | 3 ± 0.43         | 1.4         |
| 200 ml (E)              | -                | -           |
| 250 ml (F)              | 2 ± 0.24         | 1.2         |

From the data in table 3, it is apparent that the highest number of fruits was seen in treatment A group with six chilies, followed by treatment D with three chilies and treatment F with two chilies. The fruit growth may also influence the final weight of the plant. The more fruit produced, the higher the possibility that the fruit weight is higher. The plants in treatment A and D groups experienced faster fruit growth than the plants in treatment F; therefore, the number of fruits and weight were higher than those in treatment F.

The difference in flower and fruit growth was also seen in treatment D, which was suspected to have undergone a stress phase, or a phase where the plant feels threatened by the environment. This might be due to the lack of water. Lack of water makes plants divert their energy into flower growth and,
eventually, produce fruits. This happens when the amount of water absorbed is not enough for photosynthesis process because of the closing of stomata [16]. Treatment F received more water but the nutrition was not fully adequate that the amount nutrition absorbed by the plants was used to prolong the vegetative phase of the plants, making them entered the generative phase later than the plants in treatment D.

3.3. Plankton abundance
Besides the artificial feed, natural feed was also available for the comet fish in the form of planktons. Planktons grew in the water because the water contains phosphate. Phosphate is one of the factors that influence plankton growth in the water because planktons absorb nutrition in the water, and may make the nutrition not sufficient for the plant growth. Nitrate and phosphate are important nutrients in plankton growth and metabolism. In general, plankton is one of the indicators to evaluate the quality and fertility level of water [11]. Results of the plankton observation are shown below (table 4).

| PHYTOPLANKTON    | CLASS                        | TOTAL NUMBER OF INDIVIDUALS |
|------------------|------------------------------|-----------------------------|
| Synendra sp      | Bacillariophyceae            | 4                           |
| Phormidium sp    | Cyanophyceae                 | 16                          |
| Paramecium sp    | Oligohymenophoreae           | 2                           |
| Chlorella sp     | Chlorophyceae                | 8                           |
| Scenedesmus sp   | Chlorophyceae                | 26                          |
| Eudorina sp      | Chlorophyceae                | 26                          |
| Oscillatoria sp  | Cyanophyceae                 | 2                           |
| Tribonema sp     | Xanthophyceae                | 4                           |
| Chlorsterium sp  | Chlorophyceae                | 4                           |
| Euglena sp       | Euglenaphyceae               | 10                          |
| Stanieria sp     | Cyanophyceae                 | 1,240                       |

Table 5. Zooplankton abundance in comet fish culture media (cell·ml⁻¹).

| ZOOPLANKTON      | CLASS   | TOTAL NUMBER OF INDIVIDUALS |
|------------------|---------|-----------------------------|
| Brachiomus sp    | Eurotatoria | 10                          |
| Tetrahymena sp   | Ciliophora | 2                           |
| Plumatella sp    | Phylolaeata | 6                           |
| Monostyla sp     | Monogononta | 2                           |
| Rotifera sp      | Eutrotatoria | 2                          |
| Epistylis sp     | Ciliata  | 8                           |

Table 4 shows that most of the phytoplanktons seen was the Stanieria sp. This phytoplankton is a Cyanophyta plankton, which is also categorized as cyanobacteria. The cyanobacteria planktons also play a role in adding organic materials so that other organisms can grow. In water ecosystem, cyanobacteria act as producers for other organisms such as zooplanktons, small fishes, or small shrimps due to their ability in photosynthesis.

The most abundant zooplanktons were Brachiomus sp and Epistylis sp. The later one is known as one of the diseases in fish. The presence of planktons can be beneficial if the plankton in the water by adding nutrients for the plants through the microbial decomposition of dead planktons, producing compost that is beneficial for plants.
4. Conclusion
1. The nutrient contents produced in this drip aquaponics system include 0.032–1.133 ppm of nitrogen and 0.077–0.736 ppm of phosphate. This shows that the nutrient components in this system are not adequate to meet the nutrient requirement of *C. frutescens*, making the growth represented by plant height, number of leaves, number of flowers, and number of fruits lower than the positive control treatment.
2. The best result is gained through 250 ml/day/plant of drip water; however, the result is not better than the result in the positive control treatment.
3. The most frequently found phytoplankton is *Stanieria* sp., which is a *Cyanophyta*, while the most abundant zooplanktons are *Brachionus* sp. and *Epistylis* sp.

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