

Performance of Climbing Perch (*Anabas testudineus*) and Bok Choy (*Brassica chinensis*) in Aquaponics Systems Using Nutrient Film Technique in Indonesian Small-scale Livestock

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ABSTRACT

The Nutrient Film Technique (NFT) in aquaponics is a closed aquaculture system favored by Indonesian aquaculturists due to its environmental friendliness and can be applied on a small scale. Climbing perch (*Anabas testudineus*) has the potential to be cultured in this system as small-scale livestock. This research aims to determine the performance of the NFT aquaponics system of *Anabas testudineus* and *Brassica chinensis* with different stock densities. A completely randomized design within four stock density treatments and five replications was applied to this study. The results showed that the specific growth

rate, survival rate, and feed conversion rate (FCR) of *A. testudineus* differed significantly across treatments ($p < 0.05$). The best performance of specific growth rate ($1.96 \pm 0.15\%$), FCR (1.31 ± 0.13), and survival rate ($88 \pm 4.69\%$) were shown in the second treatment (50 fish/tank). On the other hand, the fourth treatment (100 fish/tank) yielded the tallest *B. chinensis* at 20.7 ± 0.90 cm and a leaf number of 10.68 ± 0.28 . Higher fish stocking density resulted in a slower fish

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growth rate but a faster plant growth rate. It can be concluded that the aquaponics system of NFT with a density of 50 fish/tank could be applied to small-scale livestock. The amount of organic matter that plant roots can use as nutrients is the factor that determines the growth rate of *B. chinensis*.

Keywords: Aquaponics, climbing perch, Nutrient Film Technique, stocking density

INTRODUCTION

Anabas testudineus is an economically important fish with high nutritional value in South and Southeast Asia, including Indonesia (Agustinus & Minggawati, 2020; Ahmadi et al., 2021; Khatun et al., 2019). In Indonesia, this fish is the main ingredient for processing local typical food on Borneo island, where the total demand in 2017 was more than six thousand tons (Lemae & Lasmi, 2019). The increasing demand causes the availability of this fish at consumption size to decrease (Hidayat et al., 2016). The climbing perch can be cultured in closed system cultivation, although Indonesian farmers still rarely cultivate this species. In conventional aquaculture, *A. testudineus* has slow growth (Ahmed et al., 2015; Kohinoor et al., 2009) due to several factors, including sex, genetics, age, water quality, stocking density, and feeding type (Ahmed et al., 2015; Susila, 2016). Technically, a closed system culture is more environmentally friendly, especially due to the minimized use of water, land, and total organic matter wasted on the environment (Hu et al., 2015; Nuryadi et al., 2009).

Aquaponics is combined aquaculture and hydroponics in a single system (Anantharaja et al., 2017; Resh, 2022), it can maintain water balance by the plants (Ebeling & Timmons, 2012; Hu et al., 2015), continuously reduce the waste produced by fish through low-level organisms. However, low dissolved oxygen (DO) hindered closed systems such as aquaponics. Hence, only certain species could survive (Uddin et al., 2016). This system allows for cultivating *A. testudineus* fish with low or medium stocking densities (Kohinoor et al., 2007; Uddin et al., 2016). *Anabas testudineus* has an extra organ (labyrinth) that allows them to live in low DO levels of 0.12–3.80 mg/L (Akbar et al., 2016; Maidie et al., 2015).

However, water quality, including DO, affects fish's survival and growth (Putra et al., 2016). Increased stocking density is a profitable method of intensifying cultivation. However, information about the cultivation of this fish in aquaponics systems with high stocking densities is still lacking. This research cultured *A. testudineus* in a closed system with different stocking densities to determine the best growth rate, survival rate, and feed efficiency due to a lack of information on the subject. Research related to *A. testudineus* in aquaponics systems has been reported by combining it with other plants such as malabar spinach (*Basella alba*) or cum spinach (*Spinacia oleracea*) (Anantharaja et al., 2017; Subhasmita et al., 2021).

However, the aquaponic combination between *A. testudineus* and bok choy (*Brassica chinensis*) has not been reported.

Bok choy, or Chinese cabbage, is a plant with high demand for consumption and good nutritional content (Wu et al., 2019). The use of *B. chinensis* in aquaponics has been widely practiced, such as tilapia or bonylip barb fish, because these plants do not need large spaces for cultivation (Albani et al., 2023; Hadiroseyani et al., 2023). This research is expected to be able to determine the performance of climbing perch (*A. testudineus*) and bok choy (*B. chinensis*) in aquaponics systems using NFT in Indonesian small-scale livestock. NFT is an aquaponic system that continuously flows water over the plant's root from the fish tank (Setiawan, 2018). NFT is used in this study for its benefits, such as being widely used, uniform nutrient concentration, faster plant growth, and adequate water supply (Wibisono & Kristyawan, 2021). Furthermore, correlations between fish production parameters and water quality, as well as plant growth, were investigated.

MATERIALS AND METHODS

Time and Place

This research was carried out in a small-scale closed system rearing tank at Airlangga University, Banyuwangi Campus, East Java, from February to April 2021 and was conducted in accordance with Law No. 18 of 2002 on the National System for Research, Development, and Application of Science and Technology of the Republic of Indonesia. The research was conducted with the approval of the School of Health and Life Sciences, Universitas Airlangga

(Letter of Assignment from the Academic Vice Director of the School of Health and Life Sciences, Universitas Airlangga, 193/UN3.1.16/KP/2021).

Fish Origin and Husbandry

Approximately 1,250 *A. testudineus* seeds with an average weight of 3.13 ± 0.09 g from fish farmers in Demak, Central Java, Indonesia, were used in this study. The biomass was maintained for sixty days in plastic buckets (water volume 70 L). The temperature of the water and DO was maintained between 24.7–28.9°C and 4.12–5.25 mg/L. At the same time, the pH parameters ranged from 7.2 to 7.4. Commercial fish feed (PF 1000™, Prima Feed, Indonesia) was used during rearing (Table 1).

Table 1
The commercial fish feed content in this study

| Content | Percentage (%) |
|---------|----------------|
| Protein | 39 |
| Fat | 5 |
| Fiber | 6 |
| Ash | 12 |
| Water | 10 |

Note. Size = 1.3-1.7 mm

Research Design

A completely randomized design (CRD) was used to treat differences in stocking density. Four treatments and five replications were performed, where the first treatment (T1) was filled with 25 seeds stocking density, the second treatment (T2) had 50 seeds, the third (T3), and the fourth treatment (T4) had

75 and 100 seeds, respectively. A bucket with 50 L of water volume was used as the cultivation medium.

Preparation of Nutrient Film Technique

A total of 20 buckets were perforated on the sides to keep the water level constant, and then 10 net pots with a hanging model were assembled in a plastic bucket using a wire in a circle, and the net pot was filled with activated charcoal and rockwool. Following washing and drying, each container was filled with 50 L of water, and *Nitrobacter*,

as a probiotic (Nitro-Bac, Indonesia), with a density of 2×10^{14} as much as 0.01 ppm was added (Beauty et al., 2012). Furthermore, up to 50% of the net pot is filled with charcoal and rockwool. Activated charcoal is added to absorb dissolved gases, heavy metals, and odors in the water. The aquaponic system used in this study was the NFT system since the water continuously flowed over the roots of *B. chinensis* (Figure 1). NFT is an aquaponic system that continuously flows water over the plant's root from the fish tank (Setiawan, 2018).

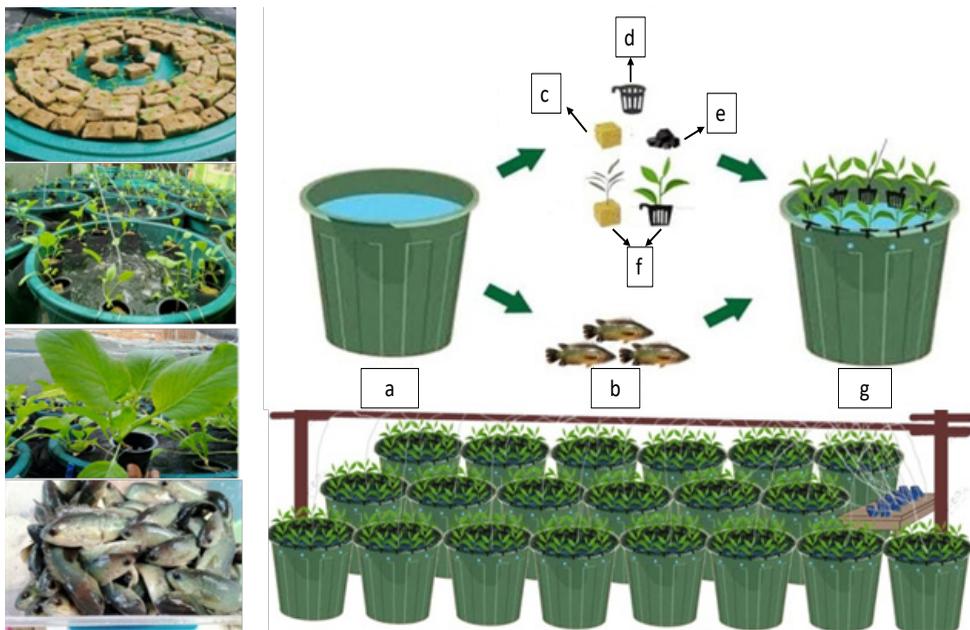


Figure 1. Site of close system aquaponics: a = floating aquaponics design; b = fish of *Anabas testudineus*; c = rockwool media; d = net pot as rockwool media; e = charcoal; f = combination of the rockwool and charcoal in the net pot for *Brassica chinensis* installation; g = establishment of the aquaponic system

Animal and Plant Preparation

Before being stocked in each tank, the fish were acclimatized for physiological

adjustments to the new environment. Meanwhile, the *B. chinensis* seeds (Garuda Seed, Indonesia) were grown on wet

rockwool media perforated with water and kept in a closed container until germinated. After germinating, the seed was sowed in direct sunlight for 1–2 weeks to allow roots to develop before being transferred to a net pot when it has four leaves.

Fish Observation Parameters

The fish observation parameters were survival rate, feed conversion rate (FCR), and specific growth rate determined using the formula according to Uddin et al. (2016). The fish's weight is determined using a scale (CAS MWP 300). The formulas were followed:

$$\text{Survival rate (\%)} = \frac{\text{Initial number of fish} - \text{Final number of fish}}{\text{Initial number of fish}} \times 100\%$$

$$\text{Feed conversion rate (FCR)} = \frac{\text{Feed fed (g)}}{\text{Fish weight gain}}$$

$$\text{Specific growth rate (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Day(s) of culture}} \times 100\%$$

Plant Observation and Water Quality

Water quality parameters such as pH (AMTAST EC910, USA) and temperature (AMTAST EC910, USA) were checked daily in this study, while ammonia (NH₃), nitrite (NO₂⁻), and nitrate (NO₃⁻) (Hitachi, Japan), as well as DO (AMTAST EC910, USA) were checked once per week. Furthermore, the *B. chinensis*'s growth was calculated based on the number of leaves that grew, and the growth rate of stem height was measured with a vernier caliper (Kenmaster, Indonesia) with an accuracy of 0.05 mm.

Data Analysis

Duncan's multiple range test (DMRT) and analysis of variance (ANOVA) were used to determine the significant differences between each treatment in production parameters, such as survival rate and growth rate, as well as feed consumption rate. A level of significance of 0.05 was used to compare the differences between treatments. Principal component analysis (PCA) was used to correlate a water quality parameter and a specific growth rate of fish and plants. In addition, the results of the water quality and production parameters were used to determine the similarity of each treatment using clustering analysis.

RESULTS AND DISCUSSION

Specific Growth Rate

The result of the specific growth rate showed *A. testudineus* with different values (Table 2). The weekly growth rate is in Figure 2, with a boxplot of the initial and final length of *A. testudineus*. The first treatment (T1) differed significantly ($p < 0.05$) from the second (T2) and the third (T3). However, T1 was not significantly different ($p > 0.05$) from the fourth treatment (T4). The specific growth rate is the percentage of regular body weight in individuals (Hossain et al., 2012). The parameter was affected by the rate at which organisms consume and convert food into energy. Furthermore, space competition and DO were the limiting factors inhibiting growth (Anantharaja et al., 2017; Hossain et al., 2012; Khatune-Jannat et al., 2012). In this study, the specific growth rate of

A. testudineus was found to be inversely proportional to solid density. T1 had the lowest specific growth rates with 1.35% or 0.06 g/ind/day. These results are the same as the growth rate carried out by Hanafie (2020), 1.14 g/ind/day in a bioflock system with a stocking density of 1 fish/L.

However, in this study, the best specific growth rate is greater than that of Agustinus and Mingawati (2020), as well as Hidayat et al. (2016), with respective values of 1.13, 1.8, and 1.72%. T2 had the highest (1.96%, or 0.12 g/ind/day). The specific growth rate of *A. testudineus* is quite low since it has a negative allometric growth pattern, where length increases faster than body weight (Kumar et al., 2013). Nonetheless, the best

results of this study were lower than the best treatment in Uddin's (2016) study of 2.52%. Furthermore, the high stocking density impacts the amount of feed provided (Lemae & Lasmi, 2019), resulting in high metabolic waste and organic content (Hossain et al., 2012). Fishes could absorb only about 25% of the feed, with the remaining 75% wasted in the water (Akbar et al., 2016). Feed waste mineralized by bacteria into NH₃ is toxic to the environment's waters (Gichana et al., 2018; Nuryadi et al., 2009). In high concentrations, NH₃ can damage gill tissue and reduce appetite (Liew et al., 2013). As a result, most energy is spent on adaptation rather than growth.

Table 2

The growth (mean±standard deviation), feed conversion rate (FCR), and survival rate of Anabas testudineus over eight weeks in a Nutrient Film Technique aquaponics system using Brassica chinensis

| Parameters | Unit | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 |
|------------------------|-------|---------------------------|---------------------------|----------------------------|---------------------------|
| Initial weight | g/ind | 3.04 ± 0.05 | 3.27 ± 0.09 | 3.19 ± 0.04 | 3.13 ± 0.07 |
| Final weight | g/ind | 5.30 ± 0.07 | 7.03 ± 0.12 | 6.31 ± 0.06 | 5.73 ± 0.05 |
| Initial biomass | g | 76.04 ± 1.17 | 163.30 ± 4.60 | 238.95 ± 2.68 | 313.20 ± 6.65 |
| Final biomass | g | 171.51 ± 8.91 | 528.04 ± 42.94 | 693.54 ± 35.69 | 744.14 ± 78.13 |
| Specific growth rate | % | 1.35 ± 0.105 ^a | 1.96 ± 0.15 ^b | 1.77 ± 0.09 ^b | 1.44 ± 0.17 ^a |
| Survival rate | % | 86.40 ± 4.56 ^b | 88.00 ± 4.69 ^b | 84.60 ± 4.38 ^{ab} | 78.40 ± 5.85 ^a |
| FCR | - | 1.77 ± 0.09 ^a | 1.31 ± 0.13 ^c | 1.63 ± 0.09 ^{ab} | 1.54 ± 0.16 ^b |
| Final feed consumption | g | 202.97 ± 18.37 | 513.56 ± 24.88 | 735.69 ± 19.62 | 850.95 ± 48.34 |

Note. Treatments 1-4 = 25, 50, 75, and 100 fish/50 L with a rearing period of 60 days, respectively. Different superscripts in the same column show that there are significant differences ($p < 0.05$)

Survival Rate

There is no significant difference ($p > 0.05$) between the first (T1) and second (T2) treatments (Table 2). Meanwhile, the third (T3) and fourth (T4) treatments differ

significantly. T2 had the highest survival rate, followed by T3, T1, and T4. Decreasing water quality, stress, and disease are all factors that affect survival rates (Hu et al., 2015). The survival rate data indicated the

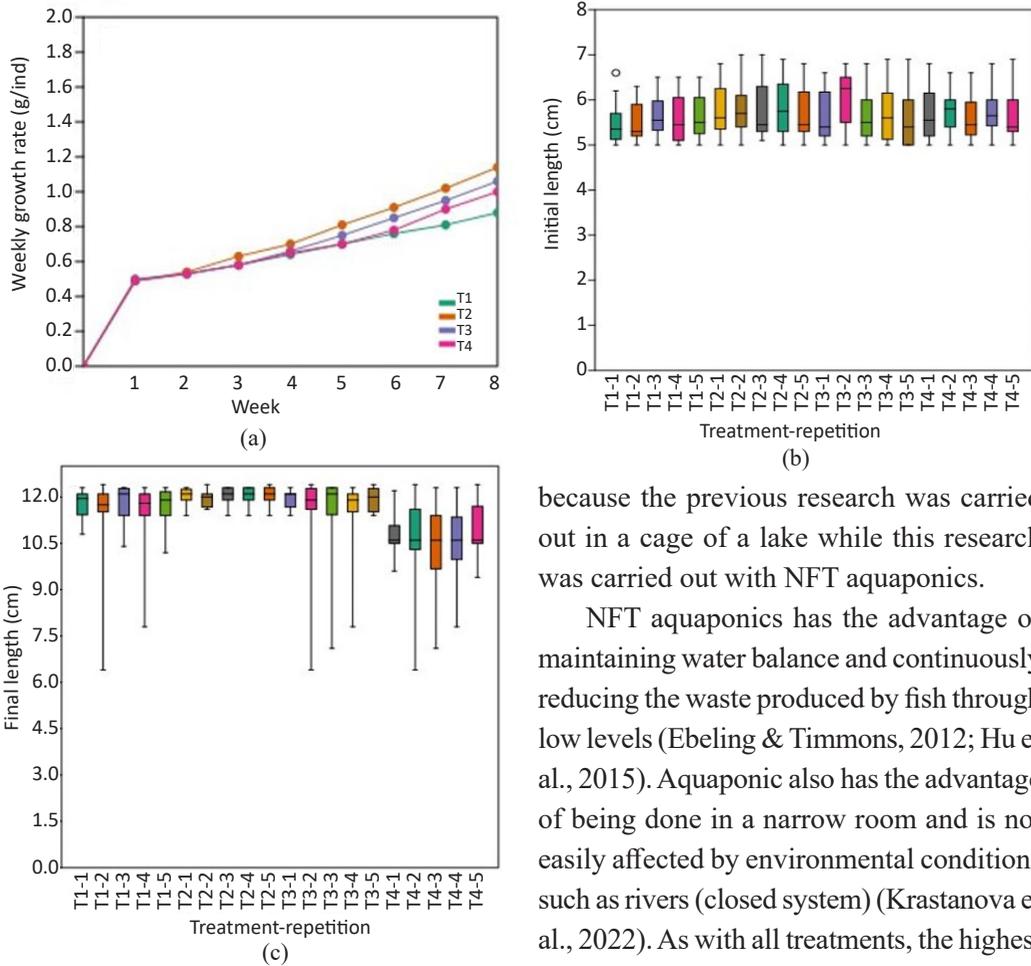


Figure 2. (a) The specific growth rate of *Anabas testudineus*, (b) box plot of the initial length of *A. testudineus*, and (c) box plot of the final length of *A. testudineus*

Note. Different colors in figures b and c are intended to make it easier to read the data. There were 5 biological replicates for 1 treatment. T1–T4 (Treatments 1–4) = 25, 50, 75, and 100 fish/50 L with a rearing period of 60 days, respectively

viability and tolerance of the fish. This study had the highest survival rate of 88%, higher than the research by Uddin (2016) with 83.33%. This result also showed that NFT aquaponics with *B. chinensis* for *A. testudineus* will increase the survival rate

because the previous research was carried out in a cage of a lake while this research was carried out with NFT aquaponics.

NFT aquaponics has the advantage of maintaining water balance and continuously reducing the waste produced by fish through low levels (Ebeling & Timmons, 2012; Hu et al., 2015). Aquaponic also has the advantage of being done in a narrow room and is not easily affected by environmental conditions such as rivers (closed system) (Krastanova et al., 2022). As with all treatments, the highest mortality rate occurred in the second week. It was caused by the increased amount of feed given, as well as the fact that *B. chinensis*' nitrification and filtration processes were not optimal. These findings indicated that each organism has a certain level of tolerance for changes in water quality (Zhang et al., 2019). A concentration of NH_3 greater than 3 ppm is toxic and causes mass death (Akbar et al., 2016; Zhang et al., 2019). However, the subsequent week's improvement in water quality was consistent with forming an ecosystem in the aquaponics system because roots are a medium for developing

nitrifying bacteria, and increasing plant size and number of roots can maximize NO_3^- absorption in water (Gichana et al., 2018; Mantelin & Touraine, 2004).

Feed Conversion Rate (FCR)

Table 2 shows that stocking density was not the only factor that had the most influence on the FCR value, where the best value (T2) is 1.31, followed by T4 and T3. On the other hand, the lowest stocking density (T1) resulted in the lowest FCR, affected by the way *A. testudineus* scavenges for food in groups (Zworykin, 2018). However, the value of the FCR in this study was classified as good for the *Anabas* genus, where the value was still better than that of Akbar et al. (2016) and Kohinoor et al. (2013), with the best FCR of 1.81 and 3.2, respectively. Controlled water quality through plant absorption improves fish appetite. Thus, the aquaponics system also increases the fish growth rate, fresh weight, and feed conversion efficiency (Yang & Kim, 2020).

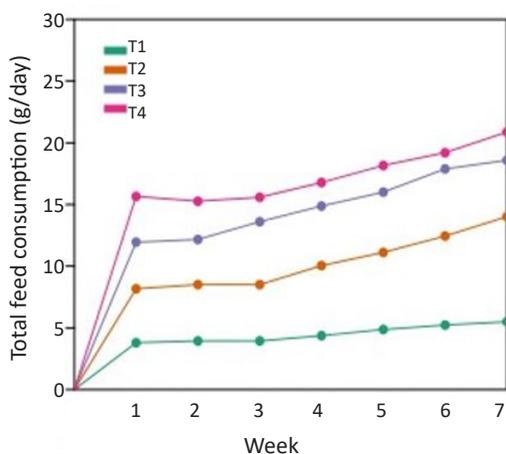


Figure 3. The daily feed consumption per week
Note. T1–T4 (Treatments 1–4) = 25, 50, 75, and 100 fish/50 L with a rearing period of 60 days, respectively

The daily feed consumption per week is shown in Figure 3.

Water Quality Parameters

Table 3 shows that the temperature, pH, DO, NH_3 , NO_2^- , and NO_3^- influence water quality parameters in cultivating *A. testudineus* and *B. chinensis* in the NFT aquaponic system. The average value of water quality parameters is still appropriate for the growth of *A. testudineus*. However, the parameters NH_3 , NO_2^- , and NO_3^- had values that exceeded the quality standard at periods. The metabolism of fish and feed residues causes this condition. Water quality plays an important role in aquaculture because water is a living medium for fish (Gichana et al., 2018). The results of temperature measurements in this study ranged from 27.41 to 27.60°C. This temperature is classified as suitable for the life of *A. testudineus* since temperatures <25°C can reduce fish appetite (Rahmadi et al., 2021; Zworykin, 2018). On the contrary, if the temperature is too high, it will cause the water to become denser, which reduces DO. Furthermore, the oxygen solubility in this study ranged from 4.78 to 5.30 ppm. DO is important in overhauling organic matter (bacteria) and fish respiration (Riedel et al., 2013). The oxygen level is still within the tolerance limits of *A. testudineus* (Akbar et al., 2016). DO solubility < 4 ppm can cause a decrease in appetite and the development of anaerobic bacteria (Mahasri et al., 2018).

On the other hand, the pH measurement in this study was 7.28–7.31. This value is within the optimal limit for the growth of *A.*

Table 3
 Results of measuring the water quality during sixty days of culture in a Nutrient Film Technique aquaponics system

| Parameters | Unit | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 |
|------------------|------|--------------|--------------|--------------|--------------|
| Temperature | °C | 27.41 ± 0.14 | 27.60 ± 0.23 | 27.58 ± 0.18 | 27.50 ± 0.17 |
| pH | | 7.28 ± 1.56 | 7.28 ± 1.56 | 7.28 ± 1.54 | 7.31 ± 1.88 |
| Dissolved oxygen | mg/L | 5.30 ± 0.52 | 4.90 ± 0.62 | 4.83 ± 0.51 | 4.78 ± 0.48 |
| Ammonia | mg/L | 0.06 ± 0.02 | 0.05 ± 0.02 | 0.08 ± 0.02 | 0.09 ± 0.03 |
| Nitrite | mg/L | 0.04 ± 0.01 | 0.04 ± 0.02 | 0.05 ± 0.01 | 0.06 ± 0.01 |
| Nitrate | mg/L | 6.07 ± 1.41 | 7.01 ± 1.29 | 5.63 ± 0.61 | 4.96 ± 0.76 |

Note. Treatments 1–4 = 25, 50, 75, and 100 fish/50 L with a rearing period of 60 days, respectively

testudineus (Akbar et al., 2016). Increasing the pH value affects the performance of nitrifying bacteria, causing high NH₃ and NO₂⁻ values, which are harmful to fish (Hu et al., 2015; Khatune-Jannat et al., 2012). The pH value affects the development and growth of aerobic nitrification bacteria (Anantharaja et al., 2017), whereas probiotic bacteria are agents of decomposing organic matter in water. The accumulation of feed that is not utilized must be able to be decomposed by nitrifying bacteria so that it does not become toxic compounds (NH₃). NH₃ in water is toxic because the ion is uncharged and soluble in fat, so it can easily enter biological membranes, and the threshold limit in aquaculture waters ranges from <0.1 ppm (Liew et al., 2013). Meanwhile, NH₃ concentration in this study ranged from 0.05 to 0.09 ppm, which is still within range. In some fish species, NH₃ concentration >0.07 ppm can damage fish tissue and cause death.

However, the limiting value for NH₃ toxicity depends on the species, size, metal,

and active compound (Liew et al., 2013). The research found that the highest NH₃ concentration value was in the second week. It was suspected that the role of bok choy in absorbing NH₃ was not optimal (Wu et al., 2019). Furthermore, the measurement of NO₂⁻ concentration in the study was 0.04–0.06 ppm. NO₂⁻ is the result of the oxidation of NH₃ in the NO₂⁻ stage carried out by *Nitrosomonas* bacteria (Vadivelu et al., 2007). This value is still within the threshold for *A. testudineus* cultivation. NO₂⁻ concentration increased in the second week because of the low water temperature and the minimal development of NO₃⁻ microbial populations (Maidie et al., 2015). Furthermore, the concentration of NO₃⁻ in this study was 4.96–7.01 ppm. Based on the Indonesian Government Regulation No. 82/2001, this value is classified as safe for fish aquaculture activities (Tallar & Suen, 2016). NO₃ concentrations that exceed the threshold cause eutrophication and stimulate phytoplankton blooms.

Growth of *B. chinensis*

The growth rate of *B. chinensis* showed a significant difference ($p < 0.05$) between the T4 with T1, T2, and T3 (Table 4). On the other hand, there was no significant difference between the three treatments (T1, T2, and T3). The best *B. chinensis* plant height was found in the T4 with 20.7 ± 0.90 cm and the lowest in T1 with a value of 19.18 ± 0.25 cm. The leaf amount also showed a significant difference ($p < 0.05$). There was not a significant difference in T4 (10.68 ± 0.28) with T3 (10.26 ± 0.43), but significantly different with T1 (9.12 ± 0.15) and T2 (10.16 ± 0.32). The best number of *B. chinensis* plants was found in the T4; the lowest was T1. This result indicated that higher fish density would decrease the fish growth but increase the plant growth in aquaponics. It can be caused by a variety

of factors, such as poor seedlings, the lack of light, nutrient availability, and pests (Harahap et al., 2020; Nuryadi et al., 2009; Wang et al., 2022). Nutrient availability is the key factor since higher fish density will increase the nutrients in the water from the feces, fish excreta, or uneaten feed (Bao et al., 2019). The effect of low stocking densities results in organic matter that is not optimal for *B. chinensis* as nutrients (Silva et al., 2017). *Brassica chinensis* is an oriental vegetable that grows well in tropical regions. Its local market price is higher, and it has a fibrous taproot that can reach 3–5 cm (Silva et al., 2017). *Brassica chinensis*' growth rate (Figure 4) is influenced by nutrient availability in water and absorption ability (Harahap et al., 2020; Wang et al., 2022).

Table 4

Measurement of the growth (mean standard deviation) of stem height and number of leaves from *Brassica chinensis* during sixty days of culture in a Nutrient Film Technique aquaponics system

| Parameters | Unit | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 |
|---------------------|------|--------------------|--------------------|--------------------|--------------------|
| Initial stem height | cm | 4.12 ± 0.11 | 4.38 ± 0.11 | 4.22 ± 0.19 | 4.22 ± 0.08 |
| Final stem height | cm | 19.18 ± 0.20^a | 19.22 ± 0.05^a | 19.40 ± 1.18^a | 20.74 ± 0.90^b |
| Initial number | leaf | 3.54 ± 0.09 | 3.86 ± 0.05 | 3.72 ± 0.10 | 3.7 ± 0.14 |
| Final number | leaf | 9.12 ± 0.15^a | 10.16 ± 0.32^b | 10.26 ± 0.43^c | 10.68 ± 0.28^c |

Note. Treatments 1-4 = 25, 50, 75, and 100 fish/50 L with a rearing period of 60 days, respectively. Different superscript letters denote statistically significant treatment differences ($p < 0.05$). Only the final results were examined by statistic

This study found a correlation between water quality parameters and a specific growth rate of fish and plants. PCA presents the relationship of water quality in Figure 5a. The data showed that DO, NH_3 , and NO_2^- affect the performance of *A. testudineus*

while temperature and NO_3^- affect the growth of *B. chinensis*. DO is an important element for growth because oxygen plays a role in respiration and metabolic processes in fish (Little et al., 2020). When the DO concentration decreased, respiration and

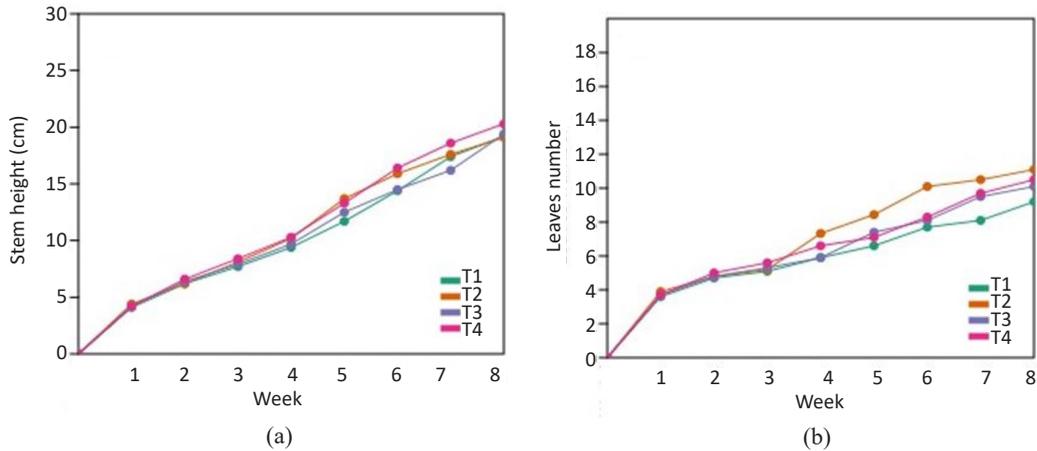


Figure 4. (a) The growth of stem (cm) from weekly sampling during eight weeks, (b) The growth of leaf from weekly sampling during eight weeks

Note. T1–T4 (Treatments 1–4) = 25, 50, 75, and 100 fish/50 L with a rearing period of 60 days, respectively

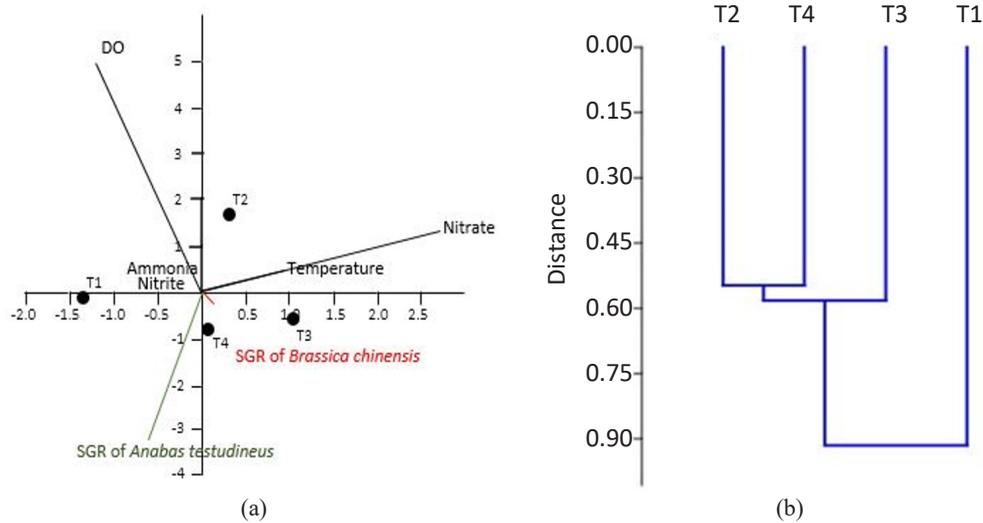


Figure 5. (a) Scatter plot correlation between water quality by principal component analysis; (b) Clustering treatment

Note. SGR = Specific growth rate; T1–T4 (Treatments 1–4) = 25, 50, 75, and 100 fish/50 L with a rearing period of 60 days, respectively

feeding activity also decreased. It causes the growth rate to decrease, and the possibility of disease attacks increases. NH_3 can also affect fish growth because it can cause stress for fish in the waters. When stressed, fish will use the nutrients in their bodies to survive, so their growth is not optimal

(Xu et al., 2021). NO_2^- can reduce growth by increasing methemoglobin formation, disrupting osmoregulation, and changing normal physiology (Ciji & Akhtar, 2020). Temperature can affect the plant since high temperatures cause heat stress and slow growth (Hinojosa et al., 2019). NO_3^-

has a role as a signal molecule for plant metabolism, physiology, growth, and development (Vega et al., 2019).

The results of the water quality and production parameters were used to determine the similarity of each treatment using clustering analysis (Figure 5b). The results showed that T2 and T4 were the best results. However, the growth of *B. chinensis* was not the highest for 50 fish/tank, while the growth of *A. testudineus* was not the highest for 100 fish/tank. Higher fish stocking density resulted in a slower fish growth rate but a faster plant growth rate. Nutrient availability is the key factor since higher fish density will increase the nutrients in the water from the feces, fish excreta, or uneaten feed (Bao et al., 2019). The effect of low stocking densities results in organic matter that is not optimal for *B. chinensis* as nutrients (Silva et al., 2017).

CONCLUSION

Stocking density influenced the growth rate, FCR, and survival rate of *A. testudineus* and *B. chinenses* cultured in closed aquaponics. The treatment with a stocking density of 50 and 100 fish/tank showed the best results. However, the growth of *B. chinensis* was not the highest for 50 fish/tank, while the growth of *A. testudineus* was not the highest for 100 fish/tank. Higher fish stocking density resulted in a slower fish growth rate but a faster plant growth rate. Higher fish stocking density indicated more organic matter. The amount of organic matter absorbed by plant roots is the factor of *B. chinensis*'s growth speed.

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