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Growth Performance of Climbing Perch *(Anabas testudineus)* in Biofloc System with Different Salinity

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ABSTRACT
The study investigated the effects of different salinity levels in a biofloc system on the growth and survival of climbing perch (*Anabas testudineus*). It employed a completely randomized design with five treatments, each replicated three times. The treatments consisted of maintaining climbing perch in the biofloc system at various salinity levels: 0 g L^{-1} (P0), 2 g L^{-1} (P1), 4 g L^{-1} (P2), 6 g L^{-1} (P3), and 8 g L^{-1} (P4). The results demonstrated varying impacts across the treatments. The most favorable outcomes were recorded in the group maintained in the biofloc system with molasses and a salinity of 6g L^{-1} (P3). This treatment yielded an absolute length growth of 1.40cm, weight growth of 1.78g, a survival rate of 92.52%, feed efficiency of 69.02%, and a floc volume of 10.6mL L^{-1}. Throughout the rearing process, total suspended solids (TSS) ranged from 91 to 145mg L^{-1}, total dissolved solids (TDS) were at 7984.81mg L^{-1}, pH was 7.60, dissolved oxygen was 4.81mg L^{-1}, ammonia levels ranged from 0.020 to 0.077mg L^{-1}, and nitrate levels varied between 2.25 and 11.75mg L^{-1}. In conclusion, salinity influences the growth of climbing perch in the biofloc system, with 6g L^{-1} identified as the most effective salinity level.

INTRODUCTION

Intensive aquaculture farming efficiently produces a dense biomass of fish or shrimp. In such systems, feed residues, organic matter, and toxic inorganic compounds accumulate during rearing. This is inevitable since fish can only store 20-30% of the nutrients in feed, with the rest being excreted and usually accumulating in the water **(Avnimelech, 2007)**. As a result, water quality decreases, leading to increased ammonia levels and high values of suspended solids that cause odor and water turbidity **(Sinaga *et al.*, 2021)**.

Biofloc is an intensive aquaculture technology that utilizes bacteria to break down metabolic waste from feed, thereby improving water quality and forming flocs to serve as natural food for fish **(Fitrani *et al.*, 2015)**. It is emerging as an alternative and effective solution to ensure sustainable aquaculture systems **(Kaya & Genç, 2018)** and can also overcome the problem of aquaculture waste while increasing aquaculture productivity **(Purnomo, 2012)**.

Local fish that have the potential to be developed in biofloc systems include climbing perch (*Anabas testudineus*). According to research by **Fitrani *et al.* (2015)**, climbing perch reared in a biofloc system with a stocking density of 400 fish per m^2 showed better growth and feed efficiency than conventionally reared fish. The potential for developing this fish is very promising, given its adaptability and tolerance to the maintenance environment with low pH and the stability of high consumer demand **(Djauhari *et al.*, 2022)**. Climbing perch can also be maintained in media with a salinity of 0 to 10g L^{-1} **(Akbar, 2012)**. For the best growth performance, climbing perch can be maintained at a salinity of 6- 7g L^{-1} **(Chotipuntu & Avakul, 2010)**.

Salinity, along with temperature, pH, light intensity, and aeration rate, plays an essential role in the biofloc system to create optimal environmental conditions for microorganisms in biofloc formation **(Nurdin, 2018)**. Salinity also significantly affects feed digestibility and fish growth **(Ardi *et al.*, 2017)**. The extent to which salinity affects the growth and survival of climbing perch as a biological indicator of adaptation in the osmoregulation process has yet to be known. Therefore, this study examined the potential of rearing climbing perch in salinity media in a biofloc system. That is important because climbing perch commands a relatively high selling price despite its relatively slow growth **(Ahmad, 2010)**. It is expected that maintaining climbing perch in salinity media using a biofloc system will lead to improving its survival and growth performance.

MATERIALS AND METHODS

Preparation of media for fish-rearing

First, twelve aquarium units, each measuring 35 x 35 x 40 cm^3, were washed and dried. Each aquarium was filled with either freshwater (the control) or saline water at concentrations of 2, 4, 6, and 8g L^{-1}, up to a level of 30cm (volume ±37 L). To initiate bacterial breeding, 3g of salt, 100g of dolomite, 100mL of molasses, and 10mL of bacteria were added per cubic meter of the maintenance media water. The media water was left to stand for seven days to allow the bacteria to develop and dominate the environment.

Stocking of climbing perch

After a week of bacterial inoculation, climbing perch was introduced into the aquariums. The fish measured 4.0 ± 0.5 cm in length and were stocked at a density of one fish per liter **(Fitrani *et al.*, 2015)**, which translates to 400 fish per square meter. Acclimatization of the fish took place over approximately one week.

Carbon addition

Carbon was added using molasses with a C/N ratio of 20, applied daily in the afternoon. The C/N ratio was determined using a formula based on **Najamuddin (2008)**.

Heterotrophic bacteria addition

Heterotrophic bacteria from the commercial probiotic product Probio-7 in liquid form were used. According to the guidelines, 10mL per cubic meter was added to the water daily throughout the rearing period **(Pratama *et al.*, 2019)**. Floc biomass (FB) was measured by weighing a clean and dry filter paper and recording the weight as W_1 (in mg). 100– 500mL of the biofloc water sample was filtered through the pre-weighed filter paper. Dry the filter paper with the retained solids in an oven at 105°C for 2–3 hours. The filter paper was cooled in a desiccator, then weighed again and the final weight was recorded as W_2 (in mg). FB (mg/L) was calculated using the following formula:

$$FB\ (mg/L) = \frac{(W2-W1)x1000}{2aVolume\ sample\ (mL)}.$$

Floc composition was analyzed using a light microscope at 100–400× magnification to identify the types of plankton, protozoa, filamentous bacteria, fungi, or organic particles in the floc. Serial dilutions of the biofloc water sample were performed (e.g., from 10^1 to 10^6). To count the total bacterial colonies, the diluted samples were inoculated onto solid media such as Nutrient Agar or Tryptic Soy Agar using the pour or spread plate method. The plates were incubated at 28–30°C for 24–48 hours. The colonies were counted and the results were expressed as colony-forming units per milliliter (CFU/mL).

Fish rearing

Fingerlings of climbing perch (*Anabas testudineus*) were reared in biofloc media for 45 days. The fish were fed three times a day until they were satiated. The daily amount of feed was calculated to determine the required dosage of molasses for the rearing container. Body weight was measured every 15 days using a sampling method that involved 49 samples per unit of test media using formula growth rate for weight and length (g/day) = [(final weight/length – initial weight/ length)], based on **Effendie (2002)**. Feed efficiency (FE) was calculated using formula: FE= live weight gain (wet weight)/feed consumed (dry weight) × 100. Water levels were monitored daily, and additional water was added when the levels decreased.

Research design

This study used a completely randomized design (CRD) consisting of five treatments with three replications. The treatment with the provision of molasses as a carbon source in the cultivation media with different salinities is as follows:

P0: Maintenance in biofloc system with fresh water (Control)
P1: Maintenance in the biofloc system with salinity 2 g L^{-1}
P2: Maintenance in biofloc system with salinity 4 g L^{-1}
P3: Maintenance in biofloc system with salinity 6 g L^{-1}
P4: Maintenance in biofloc system with salinity of 8 g L^{-1}

Statistical analysis

The data were analyzed statistically on survival rates, absolute growth, feed efficiency, floc volume, and water quality. Water quality parameters included pH, dissolved oxygen, total suspended solids (TSS), total dissolved solids (TDS), ammonia, and nitrate. An analysis of variance was conducted to identify any significant differences. If a significant difference was found, the least significantly different test was performed at a 95% confidence level **(Hanafiah, 2002)**. Additionally, the composition of the floc and the water quality parameters of salinity and temperature were analyzed descriptively.

RESULTS

Floc volume

Measurement of floc volume was conducted every 15 days. The results of floc volume measurements during maintenance are shown in Fig. (1).

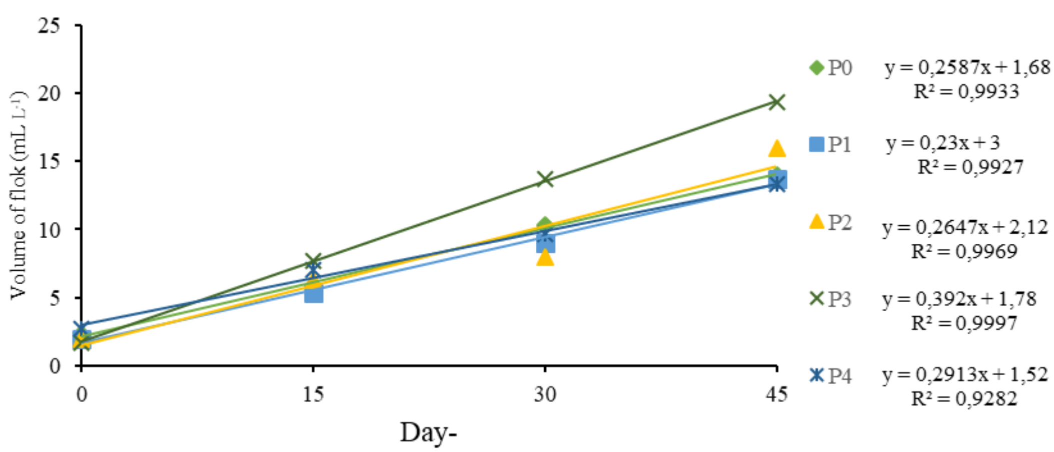

Fig. 1. Floc volume during the study (mL L^{-1})

Floc biomass

Measurement of floc biomass was conducted every 15 days. The results of floc volume measurements during maintenance are displayed in Fig. (2).

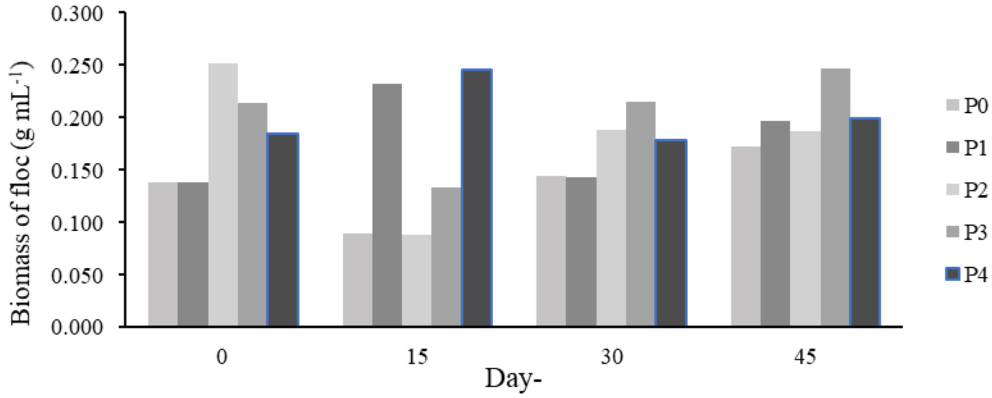

Fig. 2. Floc biomass during the study (g)

Floc composition

Observations of floc composition were recorded every 15 days. The results of these observations during maintenance are presented in Table (1).

Table 1. Flocs composition

Floc-forming microbes	Treatments				
	P0	P1	P2	P3	P4
Arthropoda	✓	-	-	✓	✓
Chordata	-	-	-	✓	✓
Chlorophyta	✓	✓	✓	✓	✓
Cnidaria	-	-	✓	✓	✓
Ochrophyta	✓	✓	✓	-	-
Protozoa	✓	✓	✓	✓	✓
Rotifera	-	✓	✓	✓	✓

Description: (✓) present (-) absent

Total bacterial colonies were calculated every seven days. Table (2) presents the results of the floc composition observation during maintenance.

Table 2. Total bacterial colonies during maintenance

Treatment	Total bacterial colonies (x 10^3 CFU mL^{-1})
P0	31,6 ± 2,98
P1	31,2 ± 2,31
P2	33,4 ± 2,26
P3	34,8 ± 2,03
P4	32,3 ± 1,96

Water quality

Salinity, temperature, and pH

Salinity, temperature, and pH measurements were taken every morning and evening. The results of the salinity, temperature, and pH variance analysis during maintenance are presented in Table (3).

Table 3. Salinity, temperature, and pH levels during the rearing process

Treatments	Salinity (g L^{-1})	Temperature (^{0}C)	pH
P0	0	27,1 – 29,4	7,89 ± 0,20^{NS}
P1	2	26,9 – 29,5	7,81 ± 0,16^{NS}
P2	4	27,0 – 29,5	7,74 ± 0,08^{NS}
P3	6	26,6 – 29,5	7,60 ± 0,16^{NS}
P4	8	27,0 – 29,5	7,60 ± 0,31^{NS}

Note: Numbers followed by similar superscript letters in the same column indicate not significant differences at the LSD α 0,05.

Total suspended solids (TSS)

TSS measurements were taken every 15 days. The results of the analysis of variance of TSS during maintenance are presented in Table (4).

Table 4. Total suspended solids (TSS) analysis during rearing

Day-	TSS (mg L^{-1})				
	P0	P1	P2	P3	P4
0	63,95±1,20^{NS}	71,65±10,39^{NS}	88±0,99^{NS}	89,15±1,63^{NS}	91,20±0,71^{NS}
15	180±48,08^{NS}	102±50,91^{NS}	152±19,09^{NS}	145±18,38^{NS}	129±12,73^{NS}
30	113±21,21^{NS}	123±12,73^{NS}	121±1,41^{NS}	129±4,24^{NS}	125±1,41^{NS}
45	70±56,57^{NS}	108±2,83^{NS}	83±41,01^{NS}	91±21,21^{NS}	77±32,53^{NS}

Note: Numbers followed by similar superscript letters in the same column indicate not significant differences at the LSD α 0,05.

Total dissolved solids (TDS) and dissolved oxygen (DO)

Measurement of TDS and DO was conducted daily. The results of the $LSD_{\alpha\ 0.05}$ further test analysis of TDS and dissolved oxygen values during maintenance are presented in Table (5).

Table 5. Total dissolved solids (TDS) and dissolved oxygen (DO) analysis during maintenance

Treatment	TDS (mg L^{-1})	Dissolved oxygen (mg L^{-1})
P0	900,90 ± 25,24^{a}	5,04 ± 0,07^{b}
P1	2698,32 ± 283,73^{b}	5,07 ± 0,02^{b}
P2	7202,74 ± 313,97^{c}	4,90 ± 0,15^{ab}
P3	7984,81 ± 350,49^{c}	4,81 ± 0,04^{a}

P4	8372,88 ± 220,31[c]	4,81 ± 0,02 [a]
Nilai $LSD_{\alpha\ 0,05}$	711,41	0,203

Note: Numbers followed by different superscript letters in the same column indicate significant differences at the LSD α 0,05.

Ammonia

Ammonia measurements were taken every 15 days. The results of ammonia analysis during maintenance are presented in Table (6).

Table 6. Ammonia analysis results during rearing

Day-	Ammonia (mg L^{-1})					$LSD_{\alpha 0,05}$
	P0	P1	P2	P3	P4	
0	0,035±0,004	0,036±0,01	0,020±0,005	0,020±0,004	0,026±0,003	0-
15	0,071±0,008[a]	0,101±0,007[b]	0,063±0,018[a]	0,075±0,016[a]	0,082±0,006[a]	0,032
30	0,077±0,008[a]	0,085±0,018[a]	0,068±0,006[a]	0,077±0,04[a]	0,133±0,016[b]	0,032
45	0,078±0,030	0,047±0,008	0,062±0,015	0,053±0,010	0,078±0,042	0-

Note: Numbers followed by different superscript letters in the same column indicate significant differences at the LSD α 0,05.

Nitrate

Nitrate measurements were taken every 15 days, and the variance analysis results are presented in Table (7).

Table 7. Results of nitrate analysis during rearing

Day-	Nitrate (mg L^{-1})				
	P0	P1	P2	P3	P4
0	2,70 ± 1,13	3,30 ± 0,42	3,55 ± 0,07	3,50 ± 0,71	3,60 ± 0,57
15	8,85 ± 1,91	7,05 ± 0,21	7,35 ± 0,49	7,75 ± 0,49	7,18 ± 0,81
30	11,20 ± 1,70	10,50 ± 0,99	10,55 ± 1,91	11,75 ± 0,64	8,65 ± 0,78
45	2,00 ± 0,71	2,45 ± 0,64	2,10 ± 0,57	2,25 ± 0,07	2,00 ± 0,14

Survival rate

A research study examined the survival rates of climbing perch. The results of the variance analysis for the survival values of climbing perch are presented in Table (8).

Table 8. Fish survival rates

Treatment	Survival Rate (%)
P0	84,35 ± 5,13
P1	85,03 ± 3,11
P2	85,71 ± 4,08
P3	92,52 ± 3,11
P4	86,39 ± 3,11

The growth performance (length and weight gain)

The absolute length and weight growth of the climbing perch are detailed in Table (9).

Table 9. Length and weight gain

Treatment	Length gain (cm)	Weight gain (g)
P0	0,85 ± 0,02[a]	0,82 ± 0,27[a]
P1	0,86 ± 0,05[a]	1,03 ± 0,22[ab]
P2	0,93 ± 0,08[a]	1,35 ± 0,03[bc]
P3	1,40 ± 0,11[b]	1,78 ± 0,02[c]
P4	0,97 ± 0,09[a]	1,35 ± 0,16[bc]
$LSD_{\alpha\ 0,05}$	0,219	0,466

Note: Numbers followed by different superscript letters in the same column indicate significant differences at the LSD α 0,05.

Feed efficiency

Regarding feed efficiency, the research results on climbing perch rearing media are presented in Table (10), showing the analysis of the variance of climbing perch feed efficiency values.

Table 10. Data and results of feed efficiency values

Treatment	Feed Efficiency (%)
P0	29,79 ± 11,56[a]
P1	47,73 ± 16,31[a]
P2	51,26 ± 11,05[ab]
P3	69,02 ± 6,51[b]
P4	65,87 ± 11,60[b]
$LSD_{\alpha\ 0,05}$	31,74

Note: Numbers followed by different superscript letters in the same column indicate significant differences at the LSD α 0,05.

DISCUSSION

The addition of carbon sources significantly impacts the increase in floc volume. As shown in Fig. (1), the volume of flocs in all treatments tends to increase throughout the maintenance period, starting from day 0, since the carbon source was regularly added. According to **De Schryver *et al.* (2008)**, factors affecting the formation of bioflocs include the provision of probiotics with floc-forming microbial composition, aeration and stirring, carbon sources, and water quality. In addition to probiotics and sufficient organic carbon sources to increase floc volume, water quality factors such as salinity, temperature, light intensity, pH, and aeration rate also play a significant role. Fig. (1) shows that treatment P3, with a salinity of 6 g L^{-1}, has the highest average floc volume.

The formation of bio flocs can be determined based on the biomass expressed in dry weight. As depicted in Fig. (2), the floc biomass in the study varied, with alternating periods of increase and decrease every 15 days. The highest average floc biomass was observed in the P3 treatment with a salinity of 6g L^{-1} at 0.202g mL^{-1}. It can be attributed to the activity and optimal growth of floc-forming microorganisms, such as phytoplankton, at a salinity of 6g L^{-1}. According to **Ahmad *et al.* (2016)**, floc biomass provides a rich source of nutrients and various bioactive compounds that enhance the overall well-being of aquatic organisms.

A successful biofloc system is characterized by small clumps floating in the cultivation medium and a specific bacterial composition in the water. It is suspected that salinity affects the growth of floc-forming microorganisms and bacteria. **Pariakana *et al.* (2021)** have indicated that water salinity has a significant relationship with the presence of bacteria. This is because salinity is closely related to osmotic pressure, which affects bacterial growth **(Arisandi *et al.*, 2017)**.

The data above indicate that the total bacterial colonies in this study ranged from 31.2 to 34.8 x 10^3 CFU mL^{-1}. The highest density of bacterial colonies is found in P3, which is 34.8 x 10^3 CFU mL^{-1}. The result is related to **Widnyana (2016)**. In biofloc technology, the total bacterial colonies ranged from 10^3 to 10^4 CFU mL^{-1} in the indoor system, while the outdoor system had a range of 10^8 to 10^{10} CFU mL^{-1} bacteria. The high value of total bacterial colony density indicates that the provision of probiotics significantly influences the high density of bacteria, which is assisted by its effectiveness in remodeling organic matter **(Adharani *et al.*, 2016)**.

During maintenance, climbing perch can tolerate various salinity levels, temperature, and pH. **Akbar (2012)** stated that salinity can range from 0 to 10 g L^{-1}. **BSN (2016)** reported that the temperature can vary from 25 to 32^0C, while the pH ranges from 3.5 to 8.0. However, it is crucial to note that high salinity levels can lead to the death of climbing perch.

The analysis of variance indicated that different salinity levels in the rearing of climbing perch within the biofloc system did not significantly impact total suspended solids (TSS). TSS measurements fluctuated between days 15 and 45, likely due to the daily addition of carbon sources with a specific carbon-to-nitrogen (C/N) ratio. **Xu *et al.* (2016)** noted that excessive carbon sources with a high C/N ratio can rapidly increase TSS values. According to **Effendi (2003)**, TSS values that do not adversely affect fisheries interests are generally less than 25 mg L^{-1}. In biofloc systems, it is recommended that TSS levels should not exceed 500 mg L^{-1} **(Samocha *et al.*, 2007)**. **De Schyver *et al.* (2008)** also stated that the recommended TSS range in biofloc systems is between 200 and 1000 mg L^{-1}. While suspended and dissolved materials in natural waters are not inherently toxic, excessive concentrations can cause turbidity, which inhibits sunlight penetration into the water column **(Effendi, 2003)**.

The analysis of variance indicates that different salinity levels in the rearing of climbing perch using the biofloc system significantly impact the total dissolved solids (TDS). The LSDα 0.05 test further demonstrates that the P4 treatment, with a salinity level of 8g L^{-1}, is significantly different from the P0 and P1 treatments but not significantly different from the P2 and P3 treatments. The increase in TDS values is attributed to the addition of seawater, which has a high TDS due to its various chemical compounds, leading to higher salinity and electrical conductivity **(Effendi, 2003)**.

Regarding the dissolved oxygen parameter, the analysis of variance shows that varying salinities significantly affect the dissolved oxygen levels in the maintenance of climbing perch within the biofloc system. The dissolved oxygen levels observed in this study are suitable for climbing perch farming. According to **BSN (2016)**, the dissolved oxygen level for climbing perch should be greater than 1mg L^{-1}, while **Effendi (2003)** indicated an optimal range of 3-7 mg L^{-1}.

The results of the LSDα 0.05 further test demonstrated that varying salinities in the climbing perch biofloc system significantly affected ammonia levels from day 15 to day 30, but not on days 0 and 45. The ammonia concentration observed in this study ranged from 0.01 to 0.15mg L^{-1}, suitable for fish farming and within the recommended tolerance level of less than 0.2mg L^{-1}, as outlined by **Effendi (2003)**. It indicates that the ammonia levels did not negatively impact the growth and survival of the climbing perch.

Additionally, the analysis of variance revealed that different salinities in the rearing environment of the climbing perch using a biofloc system did not significantly affect nitrate levels. **Kuhn *et al.* (2010)** suggested that nitrate concentrations up to 75mg L^{-1} could harm livestock. While **Rakocy *et al.* (1997)** stated that the safe nitrate level in fish farming should not exceed 10- 20mg L^{-1}. Therefore, the nitrate levels measured in this study are within the acceptable range for fish farming.

The variance analysis results indicated no significant difference in the survival rates of climbing perch across the various treatments. The highest survival rate during the maintenance phase was observed in the P3 treatment, which reached 92.52%. According to **Chotipuntu and Avakul (2010)**, climbing perch thrive optimally at a salinity level of 6- 7g L^{-1}, whereas **Akbar (2012)** reported that these fish can survive in a salinity range of 0- 10g L^{-1}.

The results of the LSDα 0.05 test demonstrate that varying salinity levels in a biofloc system significantly impact the absolute length and weight of the climbing perch. This study suggests that climbing perch can effectively utilize biofloc as an additional feed source to promote their growth. Salinity plays a crucial role in the fish's osmoregulation system, which can lead to enhanced growth rates. According to **Prayogo (2019)**, maintaining appropriate salinity levels is essential for fish to regulate their fluid balance, stimulating faster growth.

The results of the LSDα 0.05 further test showed that the P3 treatment produced the highest feed efficiency value at 69.02%. It indicates that variations in salinity affect fish feed utilization efficiency. According to **Kursistiyanto *et al.* (2013)**, salinity is a physiological factor influencing feed utilization and growth.

CONCLUSION

In conclusion, salinity affects fish growth in the biofloc system. The treatment with 6g L^{-1} salinity in the biofloc system is the most effective, resulting in an absolute length gain of 1.40cm, absolute weight growth of 1.78g, a survival rate of 92.52%, and a feed efficiency of 69.02%. A biofloc system with 6g L^{-1} salinity can enhance the growth and survival of climbing perch. Based on the research findings, utilizing water with a salinity of 6g L^{-1} is recommended to promote the growth of climbing perch in the biofloc system.

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