Effects of flow velocity on water quality and ammonia excretion in recirculating aquaculture system culturing juvenile largemouth bass (*Micropterus salmoides*)

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Abstract: Flow velocity plays an important role in recirculating aquaculture systems (RAS) and the growing practice of culturing juvenile largemouth bass (*Micropterus salmoides*). In this study, the effects of flow velocity on the water quality as well as the ammonia excretion were discussed from the perspective of actual production, and a polynomial model of ammonia nitrogen excretion was established, using the juvenile largemouth bass. Results showed that the range of ammonia nitrogen and nitrite nitrogen decreased with flow velocity increasing, while the number and volume share of large particles increased. According to the polynomial model, compared with the medium flow velocity (11 cm/s, 2.45 body length (bl)/s), the ammonia excretion of juvenile largemouth bass at high (18 cm/s, 4.00 bl/s), and low (4 cm/s, 0.90 bl/s) flow velocity changed faster with time, and the excretion rate peaked at the 6th hour after feeding, earlier than that under medium flow velocity. Therefore, it is suggested to increase the flow velocity at the 5th hour after feeding and then decreased it at the 10th hour, to ensure better water quality in RAS culturing juvenile largemouth bass.

Keywords: flow velocity, recirculating aquaculture system, juvenile largemouth bass, water quality, ammonia excretion
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1 Introduction

The recirculating aquaculture system (RAS), is regarded as one of the industry's top future directions[1,2], which has the advantages of high productivity and efficiency[3], saving water and land[4], and controllable environment and products. Largemouth bass (*Micropterus salmoides*) is popular among farmers because of its quick growth, strong environmental adaptability, and easy fishing[5]. Therefore, largemouth bass farming in RAS has gained popularity due to the quick development of improved culture methods and largemouth bass production.

Water quality is critical to the growth of fish and efficient production in RAS[6,7]. The system water contains various organic compounds and suspended particles, including ammonium, dissolved organic carbon, residual uneaten feed, and manure[8,9]. Some of these substances can be converted to amino acids and then further to ammonia and nitrite nitrogen[10]. The harmful consequences of excess ammonia and nitrite nitrogen on fish include stunted growth, altered tissue structure, and decreased survival[11-13]. Besides, the suspended particles in the water bodies also will affect the function of fish gills[14] and provide more breeding space for pathogens[15,16].

Understanding the effect of water flow velocity is necessary to maintain water quality such as ammonia nitrogen excretion and particle removal in RAS[17]. Franco-Nava et al.[18] found that dissolved nitrogen waste and particulate matter in the water showed the lowest values during the lowest water flow rate. The efficiency of solids removal is in part related to the rate of flow[19], an increase in the inflow rate was followed by an improvement in the self-cleaning attribute of the circular tank[20]. Based on management tolerance for short-term, sub-optimal water quality, Ernst et al.[21] and Wik et al.[22] applied the daily peak-mean ratio of

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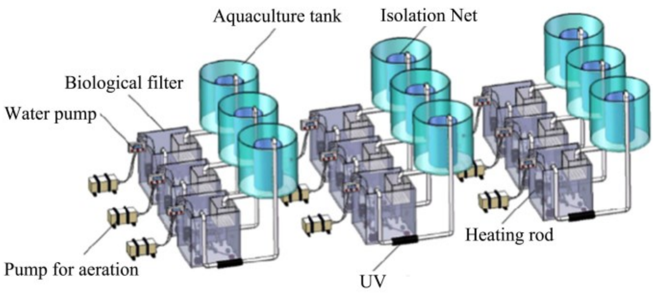
nitrogen content to management variables such as water flow rates of fish farming units. Most existing RAS loading designs based on tank water quality refer to flow rates, however, tank design and management techniques may lead to differences in the velocity field at the same flow rate[23]. This may account for the occurrence of unsuitable flow velocity for fish or low flow zones, and even the risk of excess ammonia and nitrite nitrogen. In addition, related studies on recirculating aquaculture largemouth bass are rarely reported.

Given all that, the effect of flow velocity on water quality and the ammonia excretion rule after feeding in RAS rearing the juvenile largemouth bass were explored in this study, using the actual rearing flow velocities as reference. The results here were expected to provide a theoretical guide and basis for the regulation of the juvenile largemouth bass' flow velocity in RAS.

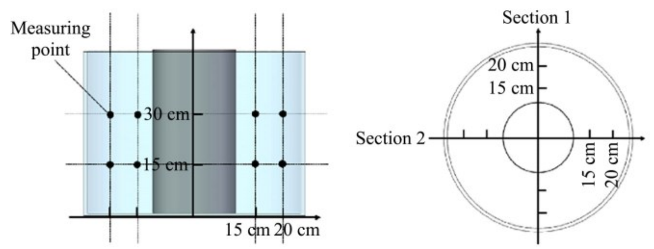
2 Materials and methods

2.1 Recirculating aquaculture system

Three sets of RASs (Figure 1) were designed, with each system consisting of three circular aquaculture tanks (with an inner diameter of 0.52 m and a water depth of 0.4 m), biological filters (with an effective volume of 150 L, and suspended porous filler filling rate of 50%), variable frequency water pump, heating rod, and other units, all of which were located at Zhejiang University (Zhejiang, China). Each tank had an independent water treatment system. The tailwater flowing out of the tank was first biochemically filtered to remove large particles, and then the filtered water flowed back to the tank after aeration, temperature adjustment, and ultraviolet disinfection. Noted that, the biological filter here was isolated and replaced by a reservoir when the 'ammonia excretion rule experiment' was carried out.



a. Schematic diagram of the experimental system



b. Front view of the aquaculture tank c. Vertical view of the aquaculture tank

Figure 1 Recirculating aquaculture experiment system

Before the experiment, the flow velocity of recirculating aquaculture largemouth bass was measured at the Hangzhou Jianguang Blackfish Professional Cooperative, which is the representative of recirculating aquaculture in China. The actual flow velocity was about 11 cm/s, so the velocities in this study were set to 4 cm/s (low flow velocity), 11 cm/s (middle flow velocity), and 18 cm/s (high flow velocity), respectively. And the flow velocity was controlled by adjusting the corresponding pore size of the inlet. Following this operation, the flow rates in different treatments could be kept almost the same. Totally 16 points (Figures 1b, 1c) were selected to measure the flow velocity with the help of a miniature Doppler flow meter (LSH10-1M, Xiamen Boyida Technology Co., Ltd., China). And the mean value of the above measurement points was used to represent the flow velocity.

2.2 Experimental fish

2.2.1 Water quality change trend experiment

Before the experiment, the juvenile fish went through a two-week adaptation transient stage. Afterwards, 720 juvenile fish (initial weight: (8.13±0.54) g) with similar sizes were selected and placed in 9 culture tanks (80 fish/tank) equally. The juveniles were adapted at each flow velocity for one week. During acclimation and trials, culture conditions were the same: feeding was done at 09:00 and 21:00 daily; dissolved oxygen in the tanks was controlled above 6.0 mg/L, ammonia nitrogen below 0.5 mg/L; 12 h of light was provided daily.

2.2.2 Ammonia excretion rule experiment

In this section, 80 juvenile fish (initial weight: (6.25±0.68) g) after acclimation were placed in each of the 9 tanks, and the culture environment was the same as above.

2.3 Experimental method

2.3.1 Water quality change trend experiment

Before the experiment, 20 L of the same mature filler was added to each group of biological filters. During the experiment, all fish were fed two times (at 9:00 and 21:00, respectively) a day, and a total of 3% biomass feed was fed each time. The biochemical cotton was replaced once a day at 21:00. The system did not change water, and only added 100 mL of water to the tank per day to make up for the losses. The pH of the water was maintained at 7.0±0.2 by adding baking soda ($NaHCO_3$).

20 mL water sample was taken from each tank every 30 min to measure ammonia nitrogen and nitrite nitrogen within 12 h after feeding. In addition, ammonia and nitrite nitrogen were measured daily at 8:50 for 12 consecutive days. Ammonia nitrogen was determined by Nessler Method, and nitrite nitrogen by N-(1-naphthyl)-ethylenediamine photometric method. The distribution of water suspended particulate matter was measured using a laser particle size meter (Bettetsize 3000Plus, Bettersize Instruments Ltd., China) on the 2nd, 7th, and 12th days, respectively.

2.3.2 Ammonia excretion pattern experiment

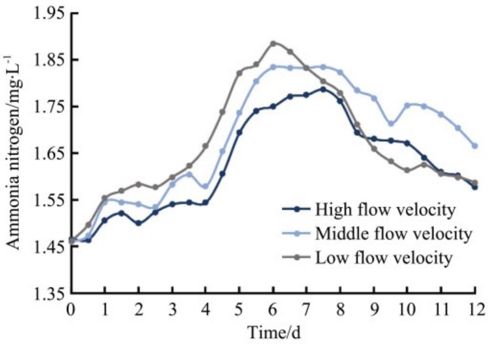
Ammonia nitrogen levels were measured hourly for 12 h after feeding in the same way. In this experiment, the total volume of water in the RAS was 110 L and a 25 L reservoir was used instead of a biofilter. Theory predicted maximum ammonia concentration was about 3.6 mg/L, which was in the safe range[24].

3 Results

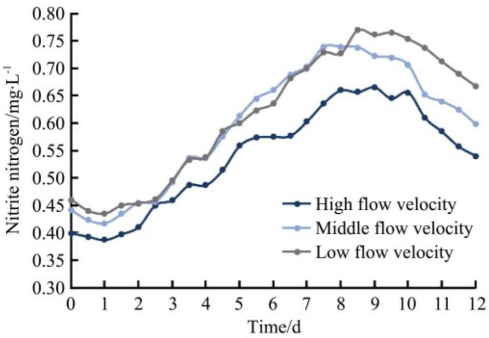
3.1 Water quality change experiment

3.1.1 Changes of ammonia nitrogen and nitrite nitrogen

Within 12 hours of feeding, there was a surge in the excretion of ammonia nitrogen, which then declined (Figure 2a). This implies ammonia excretion rate by fish was first greater than the biofilter degradation rate, and then gradually decreased. It is worth noting that the peak of ammonia excretion occurred later, with a shorter duration, and a smaller peak when the flow velocity was higher. In addition, the peak time of nitrite nitrogen was later than that of ammonia nitrogen at any flow velocity (Figure 2b). This is consistent with the reaction process of nitrogen, in that organic nitrogen is first decomposed to ammonia nitrogen and later converted to nitrite nitrogen.



a. Ammonia nitrogen



b. Nitrite nitrogen

Figure 2 Changes of ammonia nitrogen and nitrite nitrogen within 12 h after feeding under different flow velocities

The content range of ammonia nitrogen and nitrite nitrogen in water within 12 h after feeding is shown in Table 1. All statistical analyzes were performed using SPSS 19.0 software, and the indexes were compared using one-way ANOVA and Tukey's test. Ammonia nitrogen concentrations varied most in the low flow group and the smallest in the high flow group, with a significant difference ($p<0.05$), which was consistent with the variation in tilapia cultured in RAS[25]. During the peak ammonia discharge period after feeding, the ammonia content in the water will significantly change. The greater the change of ammonia nitrogen, the greater the possibility of exceeding the safe range and damaging fish. In addition, there was no significant difference in the maximum variation of water nitrite nitrogen at different flow velocities within 12 h after feeding, however, smaller for the mean value was as the flow velocity increased.

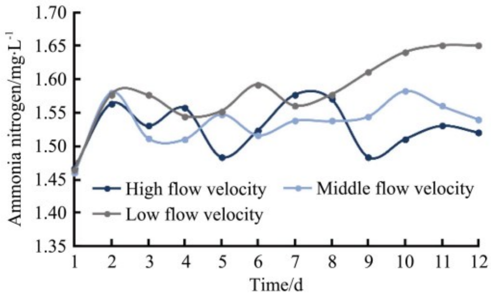
Table 1 Content range of ammonia nitrogen and nitrite nitrogen in waters

Parameters	High flow velocity	Middle flow velocity	Low flow velocity
Ammonia nitrogen/$\mu g \cdot L^{-1}$	357.4±7.1^a	401.9±24.4^b	428.7±30.5^b
Nitrite nitrogen/$\mu g \cdot L^{-1}$	290.0±72.1^a	343.3±55.0^a	366.7±28.8^a

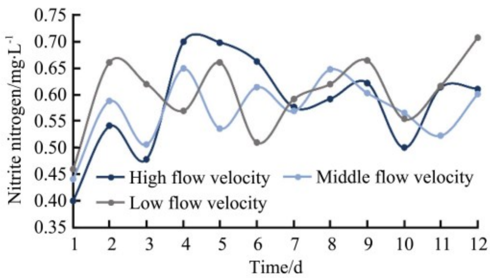
Note: In the same column, different lowercase letters indicate significant differences ($p<0.5$).

It can be seen that the ammonia nitrogen after feeding can be removed from the tank faster through the increase of flow velocity, which therefore could provide a comfortable water environment with proper water nitrogen for fish. According to the results here, the flow velocity after fish feeding in real production should be increased first and then decreased, which then could ensure the safety of water quality as well as the welfare of fish growth, while reducing energy consumption.

The variations in daily water nitrogen content are shown in Figure 3. The ammonia nitrogen at high and medium flow velocity fluctuated in the range of 1.45-1.60 mg/L (Figure 3a), while that at low flow velocity increased with time, and peaked on the 12th day, at 1.65 mg/L. This may be since, at low flow velocity, large particles were deposited at the bottom and could not be removed with the flowing out of the tank. The particles accumulated at the tank bottom for a long time and continuously decomposed to release ammonia nitrogen, resulting in the increase of ammonia nitrogen in the water. There was no significant difference in the trend of nitrite nitrogen content at different flow velocities (Figure 3b).



a. Anmonia nitrogen



b. Nitrite nitrogen

Figure 3 Daily water nitrogen content within 12 d

3.1.2 Variation of particle distribution

The volume (Figure 4) and number (Figure 5) distributions of suspended solids have been shown to vary with flow velocity and time. High flow velocity increased the number and volume share of large particles, regardless of the time. This may be caused by the presence of more large suspended particles at high flow velocities. It is of concern that the effect of time on particles is complex. The proportion of large particle volume increased with time (Figure 4), the opposite was true for small particles, which made up a large proportion of the quantity (Figure 5) but contribute little to the total particle volume. Combining the two, we believed that high flow velocity increased the large particles in the water, but that increasing the time would, on the one hand, result in a general reduction in the particulate matter, i.e. the number of small particles appeared to increase, and on the other hand, there would be an accumulation of large particles that were difficult to discharge from the tank.

The studies[26,27] showed that a higher suspension velocity was needed for bigger particle sizes. At low flow velocity, small particles are suspended in the water, while large particles can be settled better. For culture tanks having bottom suction, a low flow velocity is more conducive to removing particulate matter. However, the particulate matter is discharged from the tank mainly by secondary flow in RAS, and high flow velocity tends to create a more effective secondary flow, allowing more large particles to be suspended in the water and discharged from the tank with the flow, improving filtering. Therefore, variable flow velocities can be operated in the actual feeding process. In the low flow velocity stage, the particles are settled to the bottom and removed by suction, then increase the flow velocity to further filter the water.

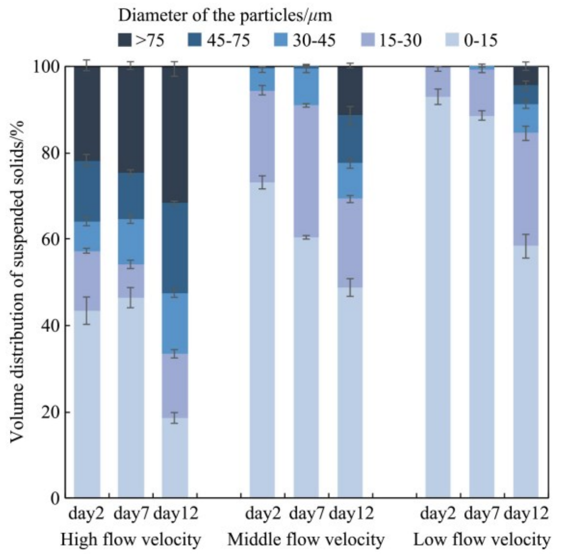


Figure 4 Volume distribution of suspended solids in RAS

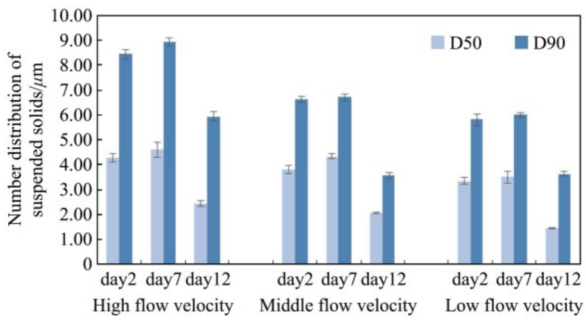


Figure 5 Number distribution of suspended solids in RAS

3.2 Pattern of Ammonia Nitrogen Excretion

3.2.1 Ammonia nitrogen content model at different flow velocities

Ammonia nitrogen content is not only a water quality parameter but also an important indicator of fish physiological metabolism. There is also a certain relationship among the decomposition of particulate matter, metabolism, swimming, and velocity of fish. Therefore, there is a direct or indirect connection between flow velocity and ammonia nitrogen emission. In this study, 18 groups of ammonia content from 0-9 h after feeding were sampled to establish a model, and data from 10-12 h were used to verify the model. The model's accuracy was evaluated by contrasting the anticipated and measured values after the construction of the second, third, and fourth-order polynomials (Figure 6). The coefficient of determination (R^2) and root mean square error (RMSE) were also used for evaluation (Table 2), and the higher the R^2 and the lower the RMSE, the better the model. It can be seen that the third-order polynomial fitting model performs better.

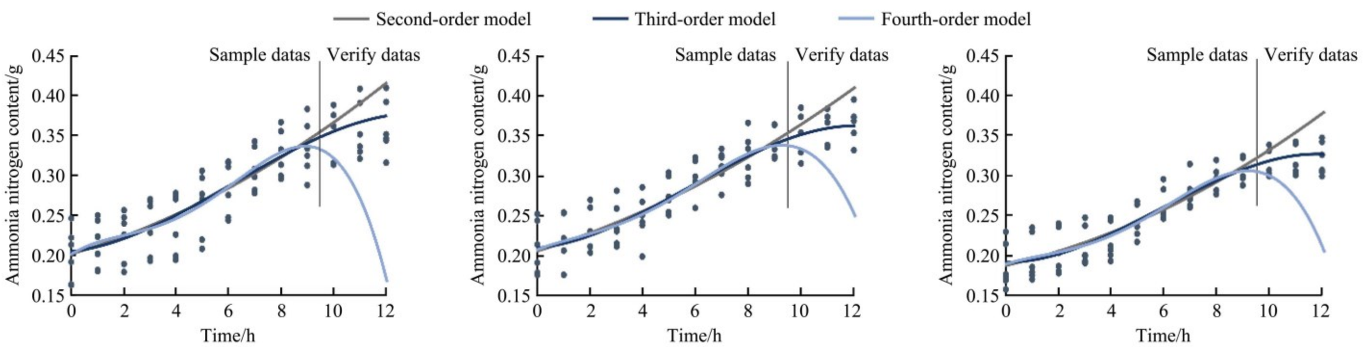
3.2.2 Effect of flow velocity on ammonia nitrogen excretion

The ammonia excretion pattern of juvenile largemouth bass was characterized by third-order polynomial model, within 0-12 h after feeding at different flow velocities as Equation (1). The trend in ammonia content over time was generally consistent at different flow velocities (Figure 7), and all could be divided into three stages based on the rate of ammonia excretion, with a slow rate in the early and late stages and a marked increase in the middle. To further analyze the effect of flow velocity, the derivatives of the excretion model were taken to obtain the rate model. It can be seen that the rate can be fitted into a second-order parabolic model which increases first and then decreases with time.

$$N = P_3t^3 + P_2t^2 + P_1t + P_0 \tag{1}$$

in the equation above, P_0, P_1, P_2, and P_3 are evaluated as the following:

$$P = |P_3 \ \ P_2 \ \ P_1 \ \ P_0| = \begin{vmatrix} P_H \\ P_M \\ P_L \end{vmatrix} = \begin{vmatrix} P_{3H} & P_{2H} & P_{1H} & P_{0H} \\ P_{3M} & P_{2M} & P_{1M} & P_{0M} \\ P_{3L} & P_{2L} & P_{1L} & P_{0L} \end{vmatrix} = \begin{vmatrix} -0.16 & 2.74 & 2.87 & 204.15 \\ -0.13 & 2.25 & 4.26 & 213.04 \\ -0.17 & 2.93 & -0.05 & 189.69 \end{vmatrix}$$



a. High flow velocity b. Medium flow velocity c. Low flow velocity

Figure 6 Ammonia nitrogen content model after feeding

Table 2 Ammonia nitrogen content models by juvenile largemouth bass after feeding

Parameters	Model	Fitting model	Determination (R^2)	Root mean square error (RMSE)
High flow velocity	Second-order	N=0.71t^2+7.58t+185.63	0.891	35.2
	Third-order	N=−0.17t^3+3.01t^2−0.28t+189.93	0.943	20.6
	Fourth-order	N=−0.17t^4+3.01t^3−0.28t^2+189.93t+187.35	0.925	106.6
Middle flow velocity	Second-order	N=0.57t^2+10.00t+209.99	0.910	38.1
	Third-order	N=−0.15t^3+2.63t^2+2.95t+213.85	0.932	32.7
	Fourth-order	N=−0.15t^4+2.63t^3+2.95t^2+213.85t+211.49	0.894	87.4
Low flow velocity	Second-order	N=0.68t^2+9.62t+200.64	0.875	45.2
	Third-order	N=−0.13t^3+2.47t^2+3.49t+203.99	0.935	27.5
	Fourth-order	N=−0.13t^4+2.47t^3+3.49t^2+203.99t+199.66	0.917	76.4

Note: N represents the ammonia nitrogen content in culture water, g; t represents the time after feeding, h.

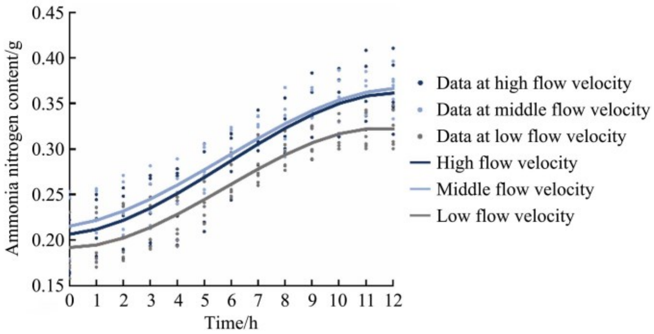


Figure 7 Ammonia nitrogen content model at different flow velocities

The coefficients were analyzed (Table 3) to quantify the effect of flow velocity on ammonia excretion. The absolute value of P_3 can reflect the change in ammonia excretion rate. The smaller the absolute value, the slower the rate changes with time. The value of $-\frac{P_2}{3P_3}$ indicated the time of peak ammonia excretion rate after feeding. The smaller value was presented at an earlier time. The ammonia excretion rate changed similarly with time at high and low flow velocities, both faster than medium flow velocity (Table 3). The peak was reached first in the high flow velocity group at 5.71 h, followed by the low flow velocity group at 5.75 h, and the latest was 5.77 h in the medium flow velocity.

Table 3 Coefficients of ammonia excretion model at different flow velocities

Parameters	High flow velocity	Middle flow velocity	Low flow velocity
P_3	−0.16	−0.13	−0.17
$-\frac{P_2}{3P_3}$	5.71	5.77	5.75

The excretion rate affects the peak time of ammonia nitrogen, which may be related to fish metabolism. The metabolic rate and metabolic waste in fish can be changed as adaptive responses following environmental stressors, like water velocity[28] and feeding[29,30]. In this experiment, the metabolism of juvenile fish in high velocity group would be accelerated, therefore, the ammonia excretion rate varied rapidly with time and peaked at the earliest. This result is similar to findings previously documented for other fish species. Alsop et al.[31] studied the increase in ammonia excretion rate of Nile tilapia as the flow velocity increased from 15 to 45 cm/s; Shrivastava allowed a gradual increase in ammonia excretion efficiency when the fish swam at a speed of 1.5 body length (bl)/s compared to resting[32]. However, some studies have shown that low flow velocity also increases fish metabolism and ammonia discharge. Skov et al.[33] studied the ammonia excretion of rainbow trout at three flow velocities of 0, 0.5, and 1 bl/s, and the results showed that the ammonia discharge decreased with the increase in flow velocity. Kvamme et al.[34] found that the ammonia discharge rate of Atlantic salmon decreased with an increasing flow rate in the range of 0.2-0.5 L/(kg·min). Chen et al.[24] found that the digestive enzyme activity of juvenile largemouth bass was enhanced as the flow velocity increased, which may also lead to enhanced protein uptake and reduced ammonia nitrogen excretion. To date, there is no general consensus as to the effects of flow velocity on the swimming and metabolism of fish species. Many of the apparent discrepancies are generally caused by differences in the fish species chosen and the regime of flow rate utilized[35,36]. Therefore, the internal mechanisms that produced the results in this study have to be explored in depth. In addition, nitrogen also remains in particles. In this experiment, the low flow velocity could not promptly flush the particles out of the tank, causing the decomposition to excrete ammonia nitrogen into the water. This may also cause the ammonia excretion faster change at low flow velocity and reach the peak rate earlier.

Based on the above experiments, it can be seen that the effect of flow velocity on fish ammonia excretion is multifaceted. Firstly, the increased flow velocity improves the fish intake, absorption, and metabolism of food, which affects the rate of ammonia excretion. At the same time, the flow velocity affects the decomposition and discharge of residual feed, manure, and other wastes, which also changes the ammonia nitrogen excretion. Therefore, the flow velocity can still be kept low, and appropriately increased after the 4th-5th hours after feeding and decreased 10th hour, to promote the excretion rate in actual production. This action can avoid the high energy consumption of running at a high flow velocity immediately after feeding, and has a certain engineering significance.

4 Conclusions

1) The reduction in water ammonia was mainly attributed to the increased flow velocity and was to some extent related to the timing of the regulation. When the rate of ammonia discharge was high, a larger flow velocity was chosen, which promoted the flushing of ammonia nitrogen out of the tank.

2) Water flow through tanks promoted solid re-suspension, decreasing retention of particles due to an increase in velocity. In addition, the suspended large particles in the water would gradually increase over time. Time-varying flow velocities were necessary to avoid deterioration of water quality and fish health because of the retention of particulate matter, and to prevent the release of ammonia nitrogen from the particulate matter decomposition.

Acknowledgements

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