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Effects of breeding density, feeding cycle, and light environment on the artificial culture of *Poecilobdella manillensis*

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Abstract

To provide a scientific basis for artificial culture, the effects of different breeding densities, feeding cycles, and light environments on the growth regulation of *Poecilobdella manillensis* were studied. After *P. manillensis* were cultured at breeding densities of 250 ~ 3250 leeches m^{-2}; feeding cycles of 2 ~ 16 days; and a light environment with noise or a light-free environment without noise, the weight gain rate (RWG), specific growth rate (SGR), feed conversion rate (FCR), and total content of the effective component (TCEC) were measured for 64 days. The results showed that the RWG and SGR presented the same growth indexes, which decreased with the increase in breeding density and the lengthening of the feeding cycle. In the light environment, the RWG and SGR of *P. manillensis* were lower than in the dark environment. The TCEC of groups 2 d and 4 d were significantly higher than in the other feeding cycle experiment groups. From these results, we advise that the optimal breeding density for artificial culture is 1750 leeches m^{-2} and the feeding cycle is 4 days; in the dark and quiet environment, *P. manillensis* grew better.

Keywords: Artificial culture, Specific growth rate, Feed conversion rate, Total content of effective component

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Introduction

Poecilobdella manillensis Lesson is subordinate to Arhynchobdellida, Hirudinidae, and *Poecilobdella* Blanchard (Yang, 1996), which, according to our experimental research, is usually located in Guangdong, Guangxi, Hainan and Fujian Provinces in China and in the tropical areas of Southeast Asia that are 500 meters below sea level, such as the Philippines and Vietnam. Therefore, it is part of the characteristic resources of medicinal animals in the region of Guangxi, China. *P. manillensis* Lesson are cultivated by many farmers using two models: one is indoor pond farming, the other is field aquaculture that imitates the wild environment of *P. manillensis* Lesson.

It is widely known that the culture of animals is affected by some anthropogenic and environmental factors, such as density, feeding cycle, light and noise. Many studies have been conducted to test the effects of different breeding densities on changes of fish physiology, food utilization, fish growth and mortality (Soderberg and Krise, 1986; Jørgensen *et al*., 1993; Björnsson, 1994; Sergio *et al*., 2006). Feeding frequency is another important variable and has gradually become a problem that aquatic animal culture has been forced to face. The correct feeding strategy can promote growth and feed utilization in fish. By contrast, wasting feed and the labor force in fish farming could be effectively reduced by following feeding rhythms of fish for maximum benefit (Hossain *et al*., 2001; Wu *et al*., 2015). The lighting conditions around the habitat in which fish live have significant effects on their ability to search for food, feeding, growth and survival (Dey and Damkaer, 1990; Tamazouzt *et al*., 2000; Carton, 2005; Jirsa *et al*., 2009). Similarly, sound has a very large impact on the lives of many aquatic animals. It plays a key role in communication among organisms under water, including mating and delivering the danger signals (Popper and Hastings, 2009). However, noise, defined as "unwanted sound" can cause stress and damage hearing (Clark, 1991; Wysocki *et al*., 2006), even affect the metabolic rate and survival of fish (Codarin *et al*., 2009; Wale *et al*., 2013). Therefore, breeding density, feeding cycle, light and sound have become an important variable in the artificial culture of aquatic animals.

Modern research has shown that the bufrudin isolated from leeches are widely used for the treatment of myocardial disease and the thrombolytic events (Teh *et al*., 2011). This type of resource has been widely utilized with the thorough research on medicinal materials form leeches, diversified products related to bufrudin have been developed, which causes a constantly increasing market demand for leeches. In addition, the wild resources of leeches were seriously affected by the changes of habitat environment (Davies and Mcloughlin, 1996). As a result, the price of leeches is constantly rising. Therefore, the artificial culture of leeches for medicinal materials has been a widespread focus. At present, the

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genetics (Liu *et al*., 2016a; Liu *et al*., 2016b) of *P. manillensis* have been mainly studied. Few studies on *P. manillensis* have referred to the artificial culture of *P. manillensis*. This study aims to explore the growth and feeding rhythm of *P. manillensis* under different conditions of breeding density, feeding cycle, and light environment, and combine production with antithrombin activity to provide scientific guidance for the breeding of *P. manillensis* and to make the artificial culture of *P. manillensis* more efficient, intensive and precise.

Materials and methods

Experimental materials and conditions

P. manillensis used in experiments was field-collected from the Qinzhou in the Guangxi Zhuang Autonomous Region of China. Active and healthy *P. manillensis* (body length: 2.5 - 3.0 cm, maximum body width: 0.3 - 0.6 cm) that weighed about 0.75 g were selected and cultured in cement pools (area of bottom 1 m^2, height: 0.6 m, water depth: 0.3 m) which was equipped with water inlet and floor drain device to exchange water to keep pH between 6.50 and 8.50, the concentrations of NO_2 and NH_4 below 0.1 mg L^{-1} and 0.2 mg L^{-1}, respectively, and the dissolved O_2 concentration greater than 5.5 mg L^{-1}. The water used in the study was aerated for 48 h before infusing into breeding pool. All pools were disinfected for 45 days before the start of experiments and gauze was pasted around the top of every pool to prevent the leeches from escaping. The experiment cycle was set for 64 d. During the experimental period, the animals were fed with sufficient fresh bovine blood that was poured into pig gut casing. This pig gut casing was placed on the bottom of the breeding pool, *P. manillensis* would adhere to it to feeding. The animals were fed once every four days (except for the feeding cycle experiment). All experiments were conducted in June 2016 to August 2016 and under room temperature.

Experimental groups

In the experiment on the effects of breeding density on *P. manillensis*, seven breeding density dealing groups were set: B_1, B_2, B_3, B_4, B_5, B_6 and B_7. Initial densities were: 250, 750, 1250, 1750, 2250, 2750, and 3250 leeches m^{-2}, respectively. In the experiment on the effects of the feeding cycle on *P. manillensis*, six feeding cycle dealing groups were set: F_1, F_2, F_3, F_4, F_5, and F_6. The feeding cycle were: 2 d, 4 d, 6 d, 8 d, 12 d, and 16 d, respectively, and the breeding density was set at 1250 leeches m^{-2}. In the experiment on the effects of light environment on *P. manillensis*, two environments L_1 and L_2 were set: a room with light and noise and a room with dark and quiet, and the breeding density was also set at 1250 leeches m^{-2}. Three replicates per group were used.

Examination of antithrombin activity

Eight days after experiments, 200 g of *P. manillensis* were chosen from each experimental group and dried in the sun for the detection of antithrombin activity. The antithrombin activity was measured according to the

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Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia Editorial Committee, CPEC, 2015). The measuring process was as following:

One gram of a powder of *P. manillensis* was precisely weighed through a number 3 sifter, and 5 ml of 0.9% sodium chloride solution was added into a beaker. Then, the mixture was leached for 30 minutes after being fully stirred, and the supernatant was put into a test tube (8 mm ×38 mm) for centrifuging. After 10 minutes of centrifuging, 100 µl of supernatant and 200 µl of Tris-HCl buffer (prepared as needed) containing 0.5% (cattle) fibrinogen (calculating as solid) were precisely added into a glass tube and agitated gently. After 5 minutes of a warm bath, a thrombin solution (40 units ml^{-1}) was added dropwise once per minute, 5 µl per addition, into the glass tube, which was in a water bath kettle (37°C±5°C); simultaneously, the glass tube was shaken until solidification. The volume of thrombin solution consumed was recorded and calculated according to the following formula:

$$\mathbf{U} = \frac{C_1 V_1}{C_2 V_2}$$

In this formula, U is the unit of thrombin activity for every g;

C_1 is the concentration of the thrombin solution;

C_2 is the concentration of the sample solution;

V_1 is the volume of the thrombin solution consumed for the condition of solidification; and

V_2 is the volume of the sample solution added into test tube.

Measurements and calculations of parameters

The average Initial Weight (IW) and average Final Body Weight (FBW) were measured at the beginning of experiments and eight days after experiments, respectively. Weight Gain Rate (RWG) and Specific Growth Rate (SGR) were calculated as the following formulas: RWG=100×(FBW-IW)/IW and SGR=100×(Ln FBW-Ln IW)/(t_2-t_1), respectively. Where t_1 is the time at which the experiment begins, and t_2 is the time at which the experiment finishes. The feed intake parameters were noted after every feeding, Feed Conversion Rate (FCR), Total Feed Intake per *P. manillensis* (TFI), Average Feed Intake per unit time (AFI), Average Feed Intake Rate per unit time (AFIR) were calculated as these following formulas: FCR=F/[n (FBW-IW)], TFI=AFI×t, AFI=F/n/t and AFIR =AFI/[(IW+FBW)/2]×100; where n is the amount of *P. manillensis*, F is the total intake of *P. manillensis* during the experimental period, and t is the total number of feeding times. All pools were inspected daily for recording survival rate. Survival Rate (SR) was calculated at the end of experiments as the next formula: SR=100×(Nt-N_0)/N_0; where Nt is the amount of *P. manillensis* at the beginning of the experiment. N_0 is the amount of *P. manillensis* at the end of the experiment. The area was measured as 1 m^2 when calculating production, Production (P) and Total Content of the Effective Component (TCEC) were

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calculated using the following formulas: P=den×SR×FBW×S and TCEC=den×SR×FBW×U; where S is the culture area, den is the breeding density and U is the antithrombin activity per g.

Data analysis

All data were recorded in Excel and analyzed using a one-way ANOVA in SPSS 19.0. Duncan's multiple range tests were conducted among different experimental groups. The results of the analysis based on the significance level $p<0.05$ are presented in the form of "mean ± standard error (SE)".

Results

Effect of breeding density on the growth of P. manillensis

After breeding for 64 days, the SR of the group at 250 leeches m^{-2} reached 86.67%, the highest level, which did not significantly differ from the results of the groups at 750, 1250, 1750 leeches m^{-2} ($p<0.05$) but was clearly higher than the groups at 2250, 2750, 3250 leeches m^{-2} ($p<0.05$). The SR of the group at 3250 leeches m^{-2} was 53.59%, which was the lowest.

Table 1 shows that the FBW, RWG, and SGR of *P. manillensis* decreased as breeding density increased. When the density was 250 leeches m^{-2}, the FBW, RWG, and SGR reached their maximum. A regression analysis indicated that a linear relationship exists between the breeding density and the SGR ($p<0.05$), defined by the regression equation:

$y=-0.0003x+5.3966(R^2=0.993)$.

When the density was 3250 leeches m^{-2}, the indexes of FCR and AFIR per unit time reached their maximum. The TFI per *P. manillensis* was highest and reached 51.57 g at the density of 250 leeches m^{-2}, which was significantly different from the results of the higher breeding density groups of 2250, 2750, 3250 leeches m^{-2} ($p<0.05$).

The antithrombin activity increased as the breeding density increased. When the density was 2250, 2750, 3250 leeches m^{-2}, the antithrombin activity was significantly higher than in the other experimental groups ($p<0.05$), and there was no significant difference between them. The antithrombin activity in all seven breeding gradients reached the standard of The Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia Editorial Committee, CPEC, 2015).

Table 1: Growth and feeding indexes of *Poecilobdella manillensis* cultured at different breeding densities.

Index	250 (leech m^{-2})	750 (leech m^{-2})	1250 (leech m^{-2})	1750 (leech m^{-2})	2250 (leech m^{-2})	2750 (leech m^{-2})	3250 (leech m^{-2})
IW(g)	0.74±0.00	0.74±0.01	0.75±0.00	0.75±0.01	0.75±0.00	0.74±0.01	0.76±0.00
FBW(g)	$22.53±1.67^{a}$	$21.12±1.44^{ab}$	$19.24±0.75^{bc}$	$17.60±0.67^{cd}$	$15.94±0.23^{de}$	$14.75±1.20^{e}$	$14.32±0.24^{e}$
RWG(%)	$2938.21±217.25^{a}$	$2748.41±198.33^{ab}$	$2457.54±90.68^{bc}$	$2256.95±112.51^{cd}$	$2039.01±42.93^{de}$	$1880.14±149.51^{e}$	$1793.16±35.34^{e}$

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Table 1 continued:

SGR(%)	5.33±0.11[a]	5.23±0.11[ab]	5.06±0.06[bc]	4.94±0.08[cd]	4.79±0.03[de]	4.66±0.12[ef]	4.59±0.03[f]
SR(%)	86.67±12.47[a]	85.56±10.99[a]	83.33±2.49[a]	77.14±3.08[a]	57.78±5.52[b]	59.39±9.22[b]	53.59±10.44[b]
TFI(g)	51.57±6.75[a]	47.85±0.67[ab]	44.30±5.29[ab]	42.99±5.21[ab]	38.88±2.28[b]	39.18±3.20[b]	38.51±1.35[b]
AFIR(%)	27.59±1.61[a]	27.47±1.53[a]	27.86±4.42[a]	29.31±3.43[a]	29.11±1.40[a]	31.66±2.08[a]	31.97±1.65[a]
FCR	2.36±0.13[a]	2.36±0.14[a]	2.41±0.39[a]	2.55±0.30[a]	2.56±0.12[a]	2.80±0.19[a]	2.84±0.15[a]
AT(U)	155.73±1.86[e]	162.84±2.03[d]	169.59±0.70[c]	174.31±1.45[b]	179.66±2.01[a]	181.86±2.05[a]	181.98±0.58[a]

[1]Different superscripts in each row indicate significant differences between treatments (p<0.05)
[2]IW: Initial weight, FBW: Final Weight, RWG: Weight Gain Rate, SGR: Specific Growth Rate, SR: Survival
[3]TFI: Total Feed Intake per individual *P. manillensis*, AFIR: Average Feed Intake Rate per unit time, FCR: Feed Conversion Rate, AT: Antithrombin Activity

Effect of feeding cycle on the growth of P. manillensis

After breeding for 64 days, the SR of all groups was greater than 80%, and there were no significant differences among these experimental groups.

Table 2 shows that the FBW, RWG, and SGR of *P. manillensis* decreased as the feeding cycle increased.

The AFIR per unit time of the group 16 d was significantly higher than the other experimental groups, which reached 92.57%. When the feeding cycle was 2 d, the TFI per *P. manillensis* was 40.91 g, which was the maximum and was significantly different from the other groups (p<0.05). The FCR of each experimental group had no significant differences (p<0.05).

The antithrombin activity increased as the feeding cycle lengthened.

Table 2: Growth and feeding indexes of *Poecilobdella manillensis* cultured at different feeding cycles.

Index	2 d	4 d	6 d	8 d	12 d	16 d
IW(g)	0.72±0.02	0.73±0.01	0.71±0.02	0.72±0.02	0.73±0.01	0.71±0.03
FBW(g)	16.25±1.42[a]	13.37±1.73[b]	9.42±0.84[c]	7.02±0.93[d]	4.75±0.30[de]	3.33±0.22[e]
RWG(%)	2154.27±148.48[a]	1729.58±246.44[b]	1227.20±79.67[c]	874.53±111.76[d]	547.79±41.30[e]	370.86±20.80[e]
SGR(%)	4.86±0.10[a]	4.53±0.22[b]	4.04±0.10[c]	3.55±0.18[d]	2.92±0.10[e]	2.42±0.07[f]
SR(%)	87.33±1.89[a]	87.33±1.25[a]	87.67±2.62[a]	83.33±2.49[a]	84.67±5.25[a]	88.67±3.40[a]

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Table 2 continued:

TFI(g)	40.91±2.43[a]	35.25±1.40[b]	23.14±1.24[c]	15.52±2.40[d]	11.60±0.33[e]	7.44±0.20[f]
AFIR(%)	15.16±1.47[f]	31.70±3.83[e]	41.66±1.69[d]	50.01±2.44[c]	70.73±3.35[b]	92.57±7.00[a]
FCR	2.65±0.27[a]	2.84±0.39[a]	2.67±0.13[a]	2.46±0.09[a]	2.90±0.19[a]	2.86±0.25[a]
AT(U)	197.19±8.69[e]	211.37±7.61[d]	217.48±5.97[d]	242.85±5.03[c]	353.39±3.23[b]	420.41±2.05[a]

[1]Different superscripts in each row indicate significant differences between treatments (p<0.05)

Effect of light environment on the growth of P. manillensis

After breeding for 64 days, the SR, FCR, and AFIR per unit time of the two groups showed no significant differences (p<0.05).

Table 3 shows that indexes such as the FBW, RWG, SGR, and TFI per *P. manillensis* were significantly higher under the dark and quiet environment (p<0.05), while the antithrombin activity was significantly higher under the light environment (p<0.05).

Table 3: Growth and feeding indexes of *Poecilobdella manillensis* cultured in different light environments.

Index	room with light and noise	room without light and noise
IW(g)	0.74±0.00	0.75±0.01
FBW(g)	18.55±0.10[b]	20.77±0.68[a]
RWG(%)	2398.02±6.82[b]	2656.43±96.16[a]
SGR(%)	5.03±0.00[b]	5.18±0.05[a]
SR(%)	79.33±1.24[a]	81.00±1.12[a]
TFI(g)	50.52±3.28[b]	58.15±3.19[a]
AFIR(%)	32.72±1.95[a]	33.74±0.78[a]
FCR	2.84±0.17[a]	2.90±0.06[a]
AT(U)	198.59±0.51[a]	193.44±1.47[b]

[1]Different superscripts in each row indicate significant differences between treatments (p<0.05)

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Effects of breeding density, feeding cycle, and light environment on the production and total content of the effective component of P. manillensis

Table 4 shows that the P of the breeding density groups 1750, 2250, 2750, 3250 leeches m^{-2} was significantly higher than that of the low density groups (250, 750 leeches m^{-2}) after breeding for 64 days, and there were no significant differences among the 1750, 2250, 2750, 3250 leeches m^{-2} experimental groups (p<0.05). P decreased as the feeding cycle increased. When feeding cycle was 2 d, P reached its maximum, which was significantly higher than in the other experimental groups (p<0.05). P under the light and noisy environment was significantly lower than that in the group with no light and a silent environment (p<0.05).

After breeding for 64 days, the TCEC of groups 2 d and 4 d were significantly higher than other feeding cycle experimental groups, and there was no significant difference between them (p<0.05). Similarly, groups in the light environment had a lower TCEC than that in the no light environment (p<0.05).

Table 4: Production and TCEC of *Poecilobdella manillensis* cultured at different factors.

Index		P(g)	TCEC(U m^{-2})
Breeding density(leech m^{-2})	250	4842.33±519.14[c]	752929.07±82190.63[c]
	750	13495.26±1475.47[b]	2197615.32±242956.09[b]
	1250	20021.08±390.94[ab]	3395142.79±54907.01[a]
	1750	23733.98±856.26[a]	4137029.09±152233.64[a]
	2250	20748.1±2238.93[a]	3732046.97±440296.51[a]
	2750	24395.81±5479.27[a]	4426583.69±959880.50[a]
	3250	25003.97±5209.37[a]	4547602.63±935290.27[a]
Feeding Cycle (d)	2	17728.5±1408.02[a]	3507756.40±435666.95[a]
	4	14604.50±2004.57[b]	3086012.83±420344.61[a]
	6	10349.83±1208.02[c]	2257924.82±318438.01[b]
	8	7298.00±897.14[d]	1776478.89±250916.98[b]
	12	5042.75±638.85[de]	1782435.53±229793.45[b]
	16	3689.75±215.68[e]	1551619.83±97599.72[b]
Light Environment	room with light and noise	18397.73±289.10[b]	3653731.52±65643.06[b]
	room without light and noise	21025.18±486.80[a]	4066749.48±82490.22[a]

[1]Different superscripts in each row indicate significant differences between treatments (p<0.05)
[2]P: Production, TCEC: Total Content of Effective Component
[3]The area was measured as 1 m^2 when calculating production

Discussion

Effect of breeding density on the growth of P. manillensis

The breeding density, as an element of environmental stress, has been proven to cause various important effects on the culture of fish (Leatherland and Cho, 1985; Schreck *et al*., 1985; Mollah *et al*., 2009). After being fed under different density conditions, the mean

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individual growth rate of rainbow trout reached a maximum at the lowest density and fell to the minimum at the highest density (Holm *et al*., 1990); after being fed for a period of 8 weeks, the mean weight of g*reat sturgeon* increased as the density, which ranged from 1-8 kg m^{-2}, decreased (Rafatnezhad *et al*., 2008). Our growth results are consistent with the aforementioned research. Therefore, it can be concluded that a lower density is appropriate for the culture of *P. manillensis.*

During the experiment, each feeding has an ideal effect. The feeding *P. manillensis* could achieve satiety which means that they would stop feeding after eating for a period of time, while a lower TFI per *P. manillensis* was observed in groups of high density (2250, 2750, 3250 leeches m^{-2}). We suggested that the reason is probably due to the growing competition for space and social interaction among the *P. manillensis* under high density conditions, which affect the ability and opportunity to search for food and movement to food (Refstie and Kittelsen, 1976; Lambert and Dutil, 2001). The FCR, representing the weight of food consumed causing an increase in weight of 1 g, is also called the conversion coefficient. In this study, the FCR of the high-density groups (2250, 2750, 3250 leeches m^{-2}) was higher than that of the low-density groups (250, 750, 1250 leeches m^{-2}), which explains why less food was consumed by the low-density groups than by the high-density groups for the same weight gain. The reason is probably that in the high-density situation, *P. manillensis* uses too much energy to produce physiological and biochemical changes to allow it to adapt to environmental stress, which influences its growth. As a result, weight gain is slow.

Effect of feeding cycle on the growth of P. manillensis

Previous research showed that feeding cycle had a remarkable effect on the growth and feed utilization in fish (Ruohonen *et al*., 1998; Dwyer *et al*., 2002; Salama, 2008). At present, there are few studies on the culture of *P. manillensis*, Cheng *et al*. (2015) researched the effect of feeding cycle on the larvae of *P. manillensis* and found that the feeding cycle was negatively related to SGR. Thus, studies on the feeding cycle in the process of *P. manillensis*' growth have an important and realistic significance on the culture of *P. manillensis*. Zhou *et al*. (2003) demonstrated that growth rate of *Gibel carp* significantly increased when the feeding cycle shortened. Atlantic sturgeon feeding twelve times per day can grow better than the one only feeding eight times per day (Roustaian *et al*., 2015). Similarly, *channel catfish* fed to satiation two times per day showed greater growth than the groups fed to satiation one time per day (Andrews and Page, 1975). Our growth results are consistent with the aforementioned research. In this study, the low feeding frequency groups (12 d and 16 d) showed a higher AFIR per unit time than that of the high feeding frequency groups (2 d and 4 d). The

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reason is likely that there is only a short interval between the two feedings in the groups with high feeding frequency, and feeding *P. manillensis* fresh animal blood once can satisfy their metabolic needs for a certain period as the result of the large capacity organ for food storage (Cheng *et al*., 2015). Thus, the groups of high feeding frequency consume less animal blood for metabolism than the low feeding frequency groups before the next feeding. Therefore, the high feeding frequency groups (2 d and 4 d) have less desire to feed than the low feeding frequency (12 d and 16 d) in the later period. In addition, their weight gain is greater than in the low feeding frequency groups (12 d and 16 d), and consequently, the AFIR per unit time is lower.

Effect of light environment on the growth of P. manillensis

Han *et al.* (2005) suggested that medium light intensity ranging from 74-312 lx is beneficial to the growth of catfish. *Brachymystax lenok* can grow and develop well under lower light intensities (Liu et *al*., 2011), while other species have higher growth rates at intense light levels, such as sea bass larvae and Atlantic cod larvae from the Northeast Grand Banks (Barahona-Fernandes, 1979; Puvanendran and Brown, 1998). The auditory system plays a role in guiding the responses of the body, which can accept different acoustic signals from around the environment in which animals are living and deliver information about mating, food and danger (Scholik and Yan, 2001). When fishes are exposed to higher noise levels, some negative effects, including low growth rate, increasing metabolic rate, even hearing loss, could be induced (Lagardère, 1982; Regnault and Lagardère, 1983; Wysocki *et al*., 2007).

In this study, *P. manillensis* in the group without light are put in a closed room that is dark and silent; for the group with light, they are put inside a room with light scattering and noise made by human activities and machines. This research showed that the *P. manillensis* in the situation without light and noise can grow and feed better than those in the situation with light and noise. According to our field work, *P. manillensis* mainly live throughout the year in paddy fields, ditches and ponds that are shady and moist, which correspond to the conditions of this study. Therefore, the dark environment without noise benefits *P. manillensis*' consumption and growth. Further studies are needed on the most suitable light intensity and the bearable noise threshold for *P. manillensis*.

The production and total content of the effective component of P. manillensis

Antithrombin, an important physiological serine protease inhibitor in the plasma coagulation system, combines with heparin to inhibit the activities of thrombin and the formation of thrombosis (Quinsey *et al*., 2004; Johnson *et al*., 2006). Hirudin-like polypeptides can be isolated from *P. manillensis*, and hirudin is a natural antithrombin substance (Krstenansky

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and Mao, 1987; Rydel *et al*., 1990; Scacheri *et al*., 1993). Thus, the strength of antithrombin is one of the main indices that measures *P. manillensis* quality. This study indicated that the antithrombin activity of *P. manillensis* increased and the FBW of *P. manillensis* decreased with the increase of the breeding density and feeding cycle. Thus, we can conclude that the antithrombin activity has a negative correlation with the FBW of *P. manliness* which is consistent with the results of the research of Gou *et al*. (2016), who determined that antithrombin activity significantly decreased with an increase in weight.

In this study, although the P value of high feeding frequency groups (2 d and 4 d) was higher than that in low feeding frequency groups (12 d and 16 d), the antithrombin activity was clearly lower than in the low feeding frequency groups. Similarly, although the P value under the environment without light is higher, the antithrombin activity is significantly lower than that in the environment with light. Therefore, considering the P value of *P. manillensis* together with quality, we have created an index: the Total Content of the Effective Component (TCEC), which can be used as an integrated evaluation index for guiding the artificial culture of *P. manillensis*. In this study, when the breeding densities were 1250, 1750, 2250, 2750, 3250 leeches m^{-2}, their TCEC was much higher than those from low breeding densities (250, 750 leeches m^{-2}), and there were no significant differences among these breeding densities. The costs of *P. manillensis* larvae themselves and their feeding increased with the increase in breeding density, and the maximum economic benefits may not occur under high densities. The TCEC of the 2 d feeding frequency reached the maximum and showed no significant difference with the 4 d feeding frequency. Similarly, the costs of labor and feeding accordingly increased with the shortened feeding cycle, which also limits the maximum economic benefits. A higher TCEC could be created in an environment without light and noise. From the results mentioned above, we concluded that 1750 leeches m^{-2} and 4 d is likely the optimal breeding density and feeding cycle for culturing *P. manillensis* and appropriately reducing the light intensity is beneficial for the TCEC of *P. manillensis*.

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