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The Open Biotechnology Journal, 2015, *9*, 143-148

The Growth Study of *Perinereis aibuhitensis* in Airlift Recirculating Aquaculture System

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Abstract: For sustainable development of aquaculture industry, it is important to apply biological method and biotechnology to treat the waste of aquaculture. In this paper, an airlift recirculating aquaculture system was designed and polychaete worms were cultured in it. According to the different food level experiments, the growth of *Perinereis aibuhitensis* was tested in each system with different feeding ratios according to food/total worms' weight percentage. It was marked as M1, M2, M3, M4 and M5 respectively. The water parameters were also tested. The results showed that in the M3 groups, the production of *P.aibuhitensis* was the highest being 2.36 g/m^2, while other groups exhibited negative production. Fed on the residual feeds and feces of flounder fish, the mean weight of worms increased to a maximum in M4, which was 1.570g and in M3, it was 0.986g in 40 days. The results provided a novel method to biologically utilize the waste of aquaculture.

Keywords: *Perinereis aibuhitensis*, Growth, Airlift recirculating, Aquaculture system.

1. INTRODUCTION

China promotes aquaculture at large. In the recent years, its aquaculture production has been observed to be the highest in the world [1]. In 2012, China's total aquaculture production was 4,288.36 million tons, of which, mariculture production was 3,033.34 million tons, accounting for more than 70% of the world's mariculture production. However, with the continuous development of aquaculture, environmental problems have been increasingly focused, which have become a major problem restricting the sustainable development of China's aquaculture. Therefore, it is important for healthy development of aquaculture and sustainable use of resources to research the methods of effectively eliminating the rising environmental pollution, and to restore and optimize the breeding environment as soon as possible [2].

Polychaete belongs to Annelida. Polychaete is one kind of benthic polychaetes, which are widely distributed in the coastal beaches and estuarine areas. It has high economic value not only as the best marine bait [3], but also has wide prospects for drug development in the ocean and other areas [4]. As a typical marine benthic fauna of deposited diet, polychaete eats detritus and transforms it into body's energy [5]. It is not only a secondary producer of marine ecosystem, but also is an important part in marine detritus food chain [6]. *Perinereis aibuhitensis*, a kind of dominated benthic fauna, which is widely distributed in China's coast, is used as the fishing bait and bioremediation species to polluted marine sediment according to their absorption and transformation of organic pollutants detritus [7].

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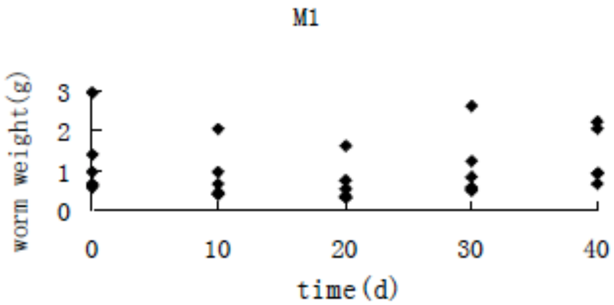
In this study, we constructed an Airlift Recirculating Aquaculture System (ARAS). Polycheate worm *Perinereis aibuhitensis* was cultured with no water exchange. The purpose of this research was to evaluate the role of *P. aibuhitensis* in bioremediation in the marine organic detritus. At the same time, the experiment was also a good trial for the sample recirculating aquaculture system. It was effective for the sustainable development of aquacultural industry and for maintaining the health of marine ecosystem.

2. MATERIALS AND METHOD

2.1. Establishment of the ARAS

The system was constructed with polyvinyl chloride (PVC) materials; its size was 30cm(W)×20cm (H)×25cm(D). In each tank, there were two different layers from the bottom to upwards, which were, the sand layer and the water layer. Two L style PVC pipes were installed in the tanks with one side of the L type pipe inserted in the sand layer and the other side of the L type pipe was kept 3cm higher than the surface of water. The pipe surface in the sand had many holes which were covered with nylon net(diameter:210μm). An air stone was put in the PVC tube with the pipe having a wide mouth, and the water in the pipes was lifted by the air lifting effects through sand layer to the water layer. When the water in the pipes was lifted and outflowed, the water in the tanks flowed in the pipes through the holes. In this way, a self-circulation system was constructed (see Fig. **1**). During the experiment, the circulation velocity of water was 0.2-

0.3L/min and the actual content in the tanks was 5L. Before the experiment, all the pipes and sand were disinfected with sodium hypochlorite. During the experiment period, no water was exchanged.



a. Gas-filled tube; b. Air stone; c. Water (flow) direction; d. Water layer; e. Sand layer; f. L style PVC pipe.

Fig. (1). Diagram of the Airlift Recirculating Aquaculture System (ARAS).

2.2. Animals and Aquaculture for Experiment

The animals for the experiment of *P.aibuhitensis* were selected from their natural intertidal habitats near Dalian city. Prior to the experiment, the worms were temporarily cultured in tanks about 15 days, and the worms in good condition were selected for the experiment. The density of *P.aibuhitensis* was 100 ind/m^2 in each tank. The seawater was collected from worms habitat and the water quality parameters included were; temperature 19±1℃, salinity 30-32, and pH 8.01-8.03. During the experiment, air pump was used continuously and the temperature was controlled by air conditioner. The illumination was 50-100Lx.

The food of *P.aibuhitensis* in the experiment included feces and residual feeds of the Japanese flounder fish farm. The feces and residual feeds were mixed evenly with a small amount of water and sodium alginate, which were then turned to 1-2mm diameter particles by feed mechanism.The particles were preserved in the refrigerator at 4°C, whose nutrient composition is shown in Table **1**.

2.3. Grouping

The worms were fed with five feeding ratio according to the food/worms' weight percentage (0%, 6%, 12%, 18%, 24%). The M1, M2, M3, M4 and M5 were marked in different feeding groups. Each feeding group performed four repeated experiments. The experimental period was 40 days and 20 worms were dug up from the sediment and weighed in each ten days.

2.4. Test Methods of Water Chemistry Parameters

The methods of water chemistry parameters used the Chinese specification for marine monitoring—Part 4: Seawater analysis [8] in which the total nitrogen was tested by potassium persulfate oxidation-ultraviolet spectrophotometry method, ammonia was determined using the hypobromite method. Nitrate and nitrite were tested by cadmium column reduction and spectrophotometry method and hydrochloric acid naphthyl ethylenediamine photometric method, respectively.

2.5. Calculation and Statistical Analysis

Specific Growth Rate (SGR) was calculated by the following formula:

$$SGR=100\cdot(\ln DW2-\ln DW1)\cdot T^{-1}$$

where SGR is the specific growth rate (%·d^{-1}), DW1 and DW2 are the worms' body weight (g) in the beginning and in the final stage of experiment, respectively. T is the experimental time(d).

The production and biomass of worms in tanks were calculated by the methods in Gray J S,(1987) [9].

Statistical analyses were performed using SPSS (version 13.0). One-way ANOVA was applied to determine the difference of worms' growth in each tank.

3. RESULTS

3.1. Worms' Growth

The growth in the worms with different diet ratio under the temperature of 20℃ is shown in Fig. (**2-5**). From Figs. (**2-5**), it is obvious that the mean-weight of worms of different groups increased following the experimental time. The M4 group was the most significant with a mean weight of 1.117±0.221g/ind in the beginning of the experiment. After 40 days of aquaculture, the weight of worms was 1.570±0.352g/ind, while the mean-weight of worms in the other groups was 1.374±0.208g/ind, 1.215±0.332g/ind, 0.986±0.211g/ind, 1.448±0.361g/ind. Through statistical analysis, the effect of different diets ratio on the worms' growth was significantly analyzed ($F=23.05$, $P<0.01$).

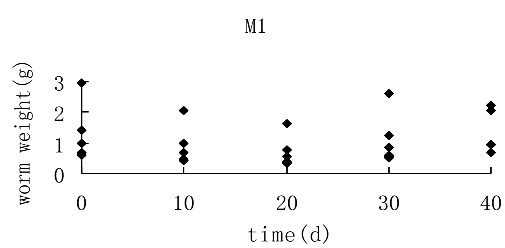


Fig. (2). the weight change of worms in M1.

Table 1. The nutritional composition of food.

Index	Dry Matter (%)	Organic Matter (%)	Crude Protein (%)	Crude Fat (%)	P (%)	C (%)
Food	90	71.22	35.60	17.79	0.09	35.11

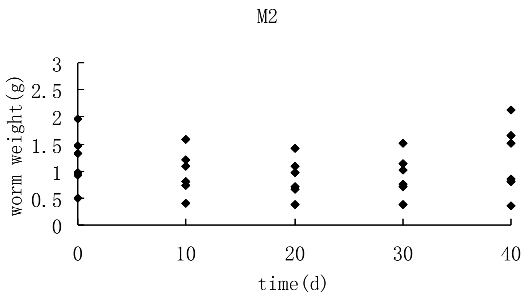


Fig. (3). The weight change of worms in M2.

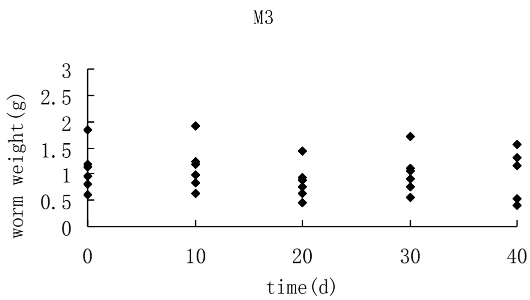


Fig. (4). The weight change of worms in M3.

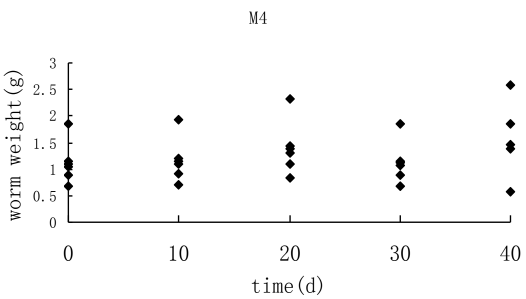


Fig. (5). The weight change of worms in M4.

The SGR of different groups is shown in Fig. (**7**). It is obvious that the growth and SGR of worms with the different food levels changed differently. In the ARAS, the aquaculture period was 10-20 days, the SGR of M2, M4 and M5 increased, but it was decreased in groups M1 and M3. When aquaculture period was 20-30 days, the SGR of M5 decreased, while the SGR of the rest of the groups increased. Throughout the experiment period, the SGR of M2 increased with special growth rate of M4 being 0.97. The values of SGR of the rest of the groups were 0.56, 0.32 and 0.20. The SGR of the group M3 was negative, being -0.37.

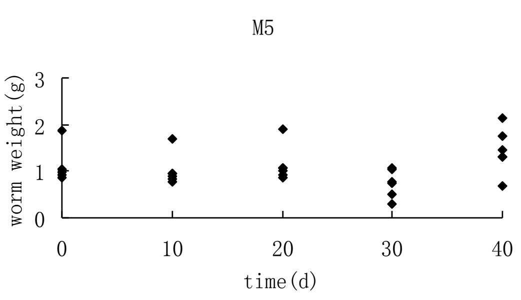


Fig. (6). The weight change of worms in M5.

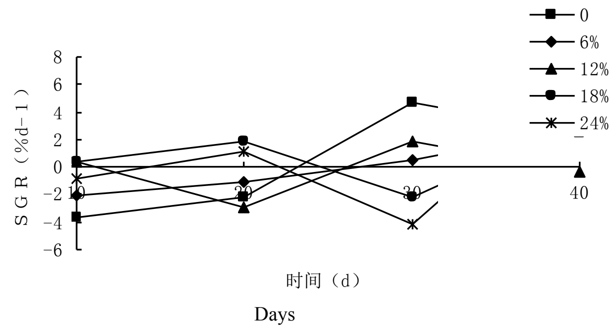


Fig. (7). The SGR of worms in each group.

In Table **2**, it is shown that the production of M1, M2, M3, M4 and M5 decreased at the beginning of the experiment, which was -82.56 g/m^2, -62.73 g/m^2, 2.63 g/m^2, -40.92 g/m^2 and -33.77 g/m^2, respectively. The production of each group increased markedly after 30 days, with the highest production of M3 as 2.36 g/m^2, while the production of M1 was the lowest as -82.56, and the production of M4 was close to M5.

3.2. The Change in Water Chemistry Parameters in Each Group

Figs. (**8-11**) show change in the water chemistry parameters in each group. It could be seen from Figures 7-10 that the change in Total Nitrogen (TN) was similar in each group, which increased and decreased smoothly throughout the experiment. However, the overall trend inclined towards its increment. In the early 20 days of experiment, the ammonia nitrogen (NH_4^+-N) concentration significantly changed. It first reached the peak in the experiment of the larger group M3 and subsequently the groups M5, M2, M4 and M1 reached the peak. In 20-40 days, ammonia nitrogen concentration in all the groups declined to the lowest point. The concentration of nitrite nitrogen (NO_2^--N) increased rapidly at first, then decreased to maintain a stable level, and again increased at the end of the experiment. The nitrate nitrogen (NO_3^--N) concentration overall showed an increasing trend.

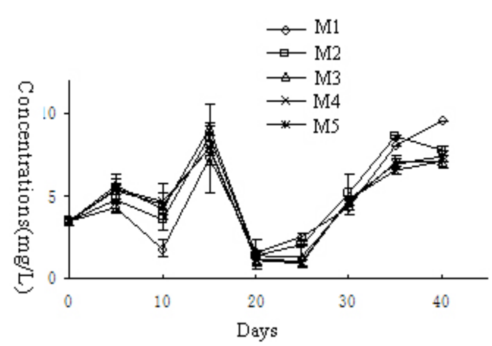


Fig. (8). The total nitrogen change in each group.

4. DISCUSSION

Polychaetes can reduce the pollution from aquaculture and wastewater, therefore, they can promote sustainable aquaculture development [10]. Different polychaete was used to reuse the aquaculture solid waste [11, 12]. In this experiment, *P.aibuhitensis* not only acted well (the worms average

Table 2. The biomass and production of worms in the experiment.

Index	Time (d)	Mean Weight (g)	Density (ind/m^2)	Biomass (g/m^2)	Production (g/m^2)
M1	1	1.594	100	159.43	
	10	1.152	100	115.16	-44.27
	20	0.832	100	83.18	-31.98
	30	1.153	66.67	76.87	-6.31
Total				434.64	-82.56
M2	1	1.468	100	146.82	
	10	1.017	100	101.73	-45.09
	20	1.129	100	112.95	11.22
	30	1.261	66.67	84.08	-28.86
Total				445.58	-62.73
M3	1	1.616	100	161.55	
	10	1.409	100	140.92	-20.63
	20	0.928	100	92.81	-48.80
	30	1.907	83.33	158.92	71.46
Total				591.10	2.63
M4	1	1.221	100	122.12	
	10	1.185	100	118.50	-3.62
	20	0.779	100	77.91	-40.59
	30	1.218	66.67	81.20	3.29
Total				399.73	-40.92
M5	1	1.441	100	144.08	
	10	0.979	100	97.88	-46.21
	20	0.641	100	64.09	-33.79
	30	1.324	83.33	110.32	46.23
Total				416.37	-33.77

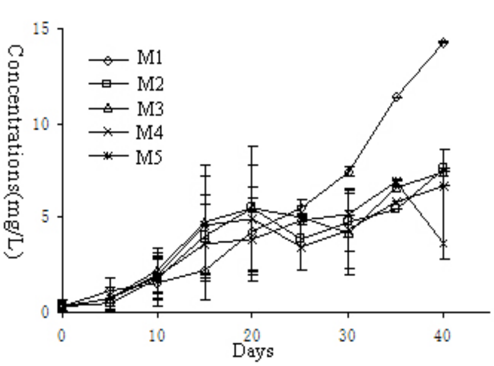


Fig. (9). The change of nitrate in each group.

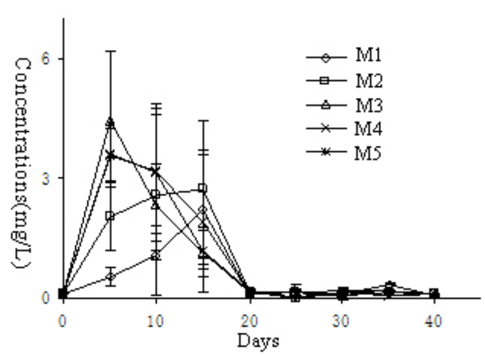


Fig. (10). The change of ammonia in each group.

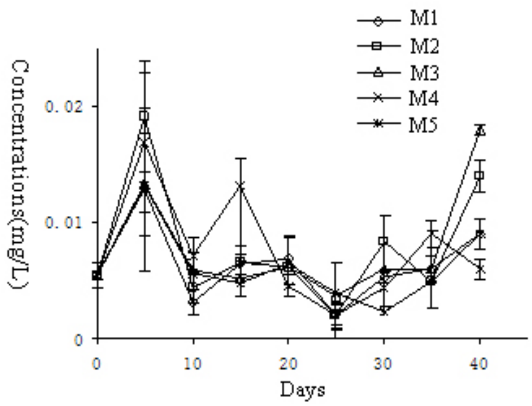


Fig. (11). The change of nitrite in each group.

survival rate was more than 70%), but it also showed growth, depending on the feces and residual food of the fish in the ARAS. In spite of the SGR, under different feeding levels, the growth and production were low. This phenomenon can be explained by several reasons. Firstly, the nutrition of food was very low, and the crude protein was 35.60%, which resultantly made the growth limited. At the same time, the density of worms in the tank became high, therefore, the food was not enough in most of the tanks. The food involved feces and residual food with inadequate viscosity, which were easily dispersed in water after feeding, therefore the worms did not get sufficient food. The particularity of the distribution food also affected the absorbing efficiency of worms. Secondly, there was a consanguineous relation between the creature and the space, where it lived. Most of the studies on aquatic-organism indicated that space is a limiting factor to the growth of the creature. Ali (2003) studied fish in the containers of different sizes in the same feeding condition. It was shown that the fishes which were fed in bigger containers grew rapidly than other fishes [13]. The feeding to shrimp, polliwog and carp gave the same results [14]. The tank used in this experiment was small with a capacity of 5L, therefore, the growth of worms was restricted due to limited space. Thirdly, the substrate was the important habitation environment of polychaete. In the natural ecosystem, the surface of sediment can accumulate a large amount of organic matter by sedimentation. It is the major food resource of polychaete. In the natural sediments, the content of organic matter is 4-5% [15]. Compared to this, the density of worms was not low in the experiment [16], but in the ASRS, the organic matter was very low at the beginning of the experiment, being lower than 2%. As a result, the serious shortage of surface organic matter deposition also affected the growth and survival of worms. Our results show that in the ARAS, under the condition, in which, the feces and residual feeds of fish farm were the only food for *P.aibuhitensis* and without water exchange, most of the worms could survive. The ratio of food/total worms' weight was 12% (M3 group), and the biomass and production of worms were the highest. From the water chemistry results, we also observed that the ARAS could maintain the water quality, especially the concentration of ammonia-N could be kept at lower level in spite of substantial wasted food given to the worms. The nitrite was not stable in water and would be transferred to the nitrate by microorganisms living in the sediments. The concentration of nitrite was high during the experiment, but the worms tolerated and showed growth in high concentration nitrate [17]. The experimental results provided us a confident trial to reuse the waste of aquaculture by the polychaete airlift recirculating aquaculture system.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was funded by the National Marine Public Welfare Research Project (No. 201305002, 201305043), and the project of marine ecological restoration technology research to the Penglai 19-3 Oil Spill Accident and Dalian Natural Foundation (No. 2012J21DW014).

REFERENCES

[1] Chen J, Xu H, Ni Q, Liu H. The study report on the development of China industrial recirculating aquaculture. Fishery Modernization 2009;36:1-7.

[2] Bunting SW. Principles of Sustainable Aquaculture, Promoting Social, Economic and Environmental Resilience. Routledge, Abingdon, UK., 2013 ; pp.301.

[3] Carvalho AN, Vaz ASL, Sérgio TIB, Santos PJ. Sustainability of bait fishing harvesting in estuarine ecosystems–Case study in the Local Natural Reserve of Douro Estuary, Portugal. Revista da Gestão Costeira Integrada, 2013;13:157-168

[4] Roscia G, Falciani C, Bracci L, Pini A. The development of antimicrobial peptides as new antibacterial drugs. Curr Protein Pept Sci 2013;14:641-649.

[5] McArthur M, Brooke B, Przeslawski R, Ryan D, Lucieer V, Nichol S, McCallum A, Mellin C, Cresswell I, Radke LC. On the use of abiotic surrogates to describe marine benthic biodiversity. Estuar Coast Shelf S 2010;88:21-32.

[6] Gerlach SA. Food-chain relationships in subtidal silty sand marine sediments and the role of meiofauna in stimulating bacterial productivity. Oecologia 1978; 33:55-69.

[7] Zhao H, Zhou Y, Li Y, Li S, Yang D. Molecular cloning and expression of the gene for G protein alpha subunit induced by bisphenol A in marine polychaete *Perinereis aibuhitensis*. Environ Toxicol Phar 2014;37:521-528.

[8] The Specification for Marine Monitoring-Part 4: Seawater Analysis, General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of the People's Republic of China, 2008.

[9] Gray, JS. In: Gee, JHR and Giller, PS, Ed.Organization of Communities Past and Present.Species-abundance patterns,Blackwell Science, Oxford 1987;53-68.

[10] Aguado-Giménez F, Piedecausa M, Carrasco C, Gutiérrez J, Aliaga V, García-García B. Do benthic biofilters contribute to sustainability and restoration of the benthic environment impacted by offshore cage finfish aquaculture? Mar Pollut Bull 2011; 62:1714-1724.

[11] Honda H, Kikuchi K.Nitrogen budget of polychaete *Perinereis nuntia* vallata fed on the feces of Japanese flounder. Fisheries SCI 2002;68:1304-1308.

[12] Palmer PJ. Polychaete-assisted sand filters. Aquaculture 2010;306:369-377.

[13] Ali M, Nicieza A, Wootton RJ. Compensatory growth in fishes: a response to growth depression. Fish Fish 2003; 4:147-190.

[14] Mascaró M, Rodríguez-Pestaña L, Chiappa-Carrara X, Simões N. Host selection by the cleaner shrimp *Ancylomenes pedersoni*: Do anemone host species, prior experience or the presence of conspecific shrimp matter? J Exp Mar Biol Ecol 2012;413:87-93.

[15] Heiri O, Lotter AF, Lemcke G. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. J Paleolimnol 2001; 25:101-110.

[16] Woodin SA, Wethey DS, Dubois SF. Population structure and spread of the polychaete *Diopatra biscayensis* along the French Atlantic coast: Human-assisted transport by-passes larval dispersal. Mar Environ Res 2014;102:110-121.

[17] Gutierrez-Wing MT, Malone RF. Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquacult Eng 2006;34:163-171.

Received: June 05, 2015 Revised: August 20, 2015 Accepted: September 11, 2015