E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(5): 372-377

www.fisheriesjournal.com
Received: 20-07-2017
Accepted: 21-08-2017

Comparison of weight gain of *Astronotus ocellatus* and *Danio rerio* cultured directly in Biofloc system and live food diet enriched with heterotrophic bacteria

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Abstract
One of the aims of aquaculture is the increase of productivity of aquatic resources through deliberated manipulation of its processes. The nutrition of organisms is the highlight aspect in aquaculture, that is why the effect of different diets (live food enriched with heterotrophic bacteria and Biofloc) in *A. ocellatus* and *D. rerio* were evaluated in this investigation. The organisms of each specie were divided in six plastic containers of 80L, and every 15 days, total population weight of each specie was taken to obtain absolute growth rate (AGR) and instantaneous growth rate (IGR) values. In general, the highest gain of weight in both species was obtained with the diet of live food enriched with bacteria produced in a Biofloc system, with an AGR of 0.037g and an IGR of 2.023g in *D. rerio* culture, and an AGR of 0.089g and IGR of 2.702g in *A. ocellatus* culture. It was concluded that this type of diets is a better option than commercial food, which is the normal food that was applied to in ornamental fish, pointing out that live food enriched with bacteria diet produced in a Biofloc system, obtained better results than only Biofloc diet.

Keywords: growth rates, Biofloc, *Danio rerio* and *Astronotus ocellatus*

1. Introduction

In Mexico, ornamental aquaculture is an important activity and it is commercialized around 43 million of ornamental fishes, mainly fresh water fish [1]. Among the most produced ornamental fish species in Mexico, are Oscar fish (*Astronotus ocellatus*) and zebrafish (*Danio rerio*) [2].

A. ocellatus, better known as "Oscar fish", is a fresh water fish with benthopelagic behavior, the mean length was 24 cm (maximum of 45.7 cm) and maximum recorded weight of 1.6 kg; usually feeds of little fish, crabs, worms, and insect larvae. It is mainly found in South America: Peru, Colombia, Brazil, and reports in Argentina [3]. It is very popular among aquarist but little used by aqua culturists, because it has slow growth

D. rerio fish, better known as "zebra fish", is a cyprinid from tropical regions of Asia and is mainly found in shallow calm waters. It usually occupies entire water column, feeding from substrate to surface. It has an omnivorous diet, but mainly feeds on insects and microcrustaceans. This species has a mean length value of 2.5 cm, with a maximum weight registered of 3.8 g [3].

A technology to improve the production in a sustainable way in aquaculture was Biofloc system (BFT), which consists in the development of microbial flocs formed from a relation carbon-nitrogen in cultured water, with little or non-water change (0.5 to 1% per day) [4] and high oxygenation [5, 6], in which diets of low raw protein content was used [7] and external carbon sources such as molasses (sugar cane), rice bran, wheat bran, among others [8]. These carbon sources allow the development of a microbial community, mostly of heterotrophic bacteria that metabolize carbohydrates and use inorganic nitrogen solving the problems of nutrient saturation, because it was recycling [9, 10].

The phyto and zoo planktonic composition of flocs, change through maturity time of Biofloc, so a recent one will be constituted mainly by heterotrophic bacteria while an old one will be constituted of fungus [11]. Besides of the recycling of nutrients, other derivatives are provided by Biofloc consumption [4].

In Biofloc cultures, the systems for the incorporation of oxygen into water (aerators) are fundamental, because they must meet three main needs: first one are the needs of respiration by the cultured specie; second are the respiration and nitrification reactions in nitrogenous compounds metabolism contained in the system (by action of heterotrophic bacteria); and third is that Biofloc should be kept in constant suspension to avoid decantation and accumulation of solids that lead to anaerobic reactions that can affect culture medium [12].

There are two ways in which Biofloc is use as food source for the cultured organisms. The first one is when the development of Biofloc is stimulated in cultures (*in situ*) by maintaining a high proportion of C:N through addition of carbon sources. This can include the use of food with low nitrogen content. The second involves the separated production of microbial biomass. The water of the fish or crustaceans culture that contain inorganic nitrogen is used as additional carbon source, and bioflocs are harvested and incorporated to culture of fish and crustaceans as food (*ex situ*). A third alternative is the use of separated reactors where *ex-situ* bioflocs are harvested and incorporated to food formula [13]. Biofloc systems function better with species that can obtain a nutritional benefit from direct consumption of floc as well as tolerate a poor water quality [4].

Because of all above, the aim of this study is to maintain an ornamental fish culture such as *A. ocellatus* and *D. rerio*, in an *in situ* Biofloc system and a system where the organisms where fed with live food enriched with Biofloc bacteria, in laboratory conditions and compare, which diet, can obtain a higher weight gain that allows to consider it as a viable alternative as food to ornamental aquaculture.

2. Material and methods

2.1 Experimental design and culture conditions of live food enriched with Biofloc bacteria diet

One hundred and twenty organisms of each specie (*A. ocellatus* and *D. rerio*) were used, and were divided in equal number (40 organisms) in three 80L plastic beakers with mechanical and biological filter (PVC tube with stones, sponge, bio-balls and a 1mm mesh sieve), which filter water culture medium 24 hours day^{-1} (Fig. 1). This study was made for 120 days.

For each species, food was supplied twice per day (11.7g) of cladocerans of genus *Daphnia* sp. (water flea), which were enriched with phytoplankton (50 mL of mixed culture of unicellular microalgae) and 200 mL of heterotrophic bacteria extracted from a Tilapia Biofloc system, for 15 minutes before applying to fishes' beakers. Each week, six randomly selected organisms per each experimental beaker were registered, using an analytic balance OHAUS (0.01g precision).

2.2 Experimental design and culture conditions for Biofloc diet

For this experimental test, six containers of 80L were used with Biofloc treatment. Three of them were used for zebra fish with 40 organisms each, and other three for Oscar fish, with six organisms' each (Fig. 1).

The containers were kept with continuous aeration and supplied with commercial food "El Pedregal S.A. de C.V." for tilapia twice per day with a protein content of 45%. To guarantee floc formation, it was supplied ground dried coffee as external carbon source once per day. The food and the coffee were supplied at 3% and 1% respectively of the total fish population biomass from each beaker. Quantity was adjusted every 15 days (at 28 cultured days food was adjusted to 5% of total biomass).

Before starting the experiment (one week), Biofloc culture system was inoculated with 125 mL of culture medium of four cladocerans species: *Simocephalus vetulus*, *Daphnia pulex*, *Ceriodaphnia dubia* and *Ceriodaphnia laticaudata*; and one species of rotifer: *Branchionus angularis*.

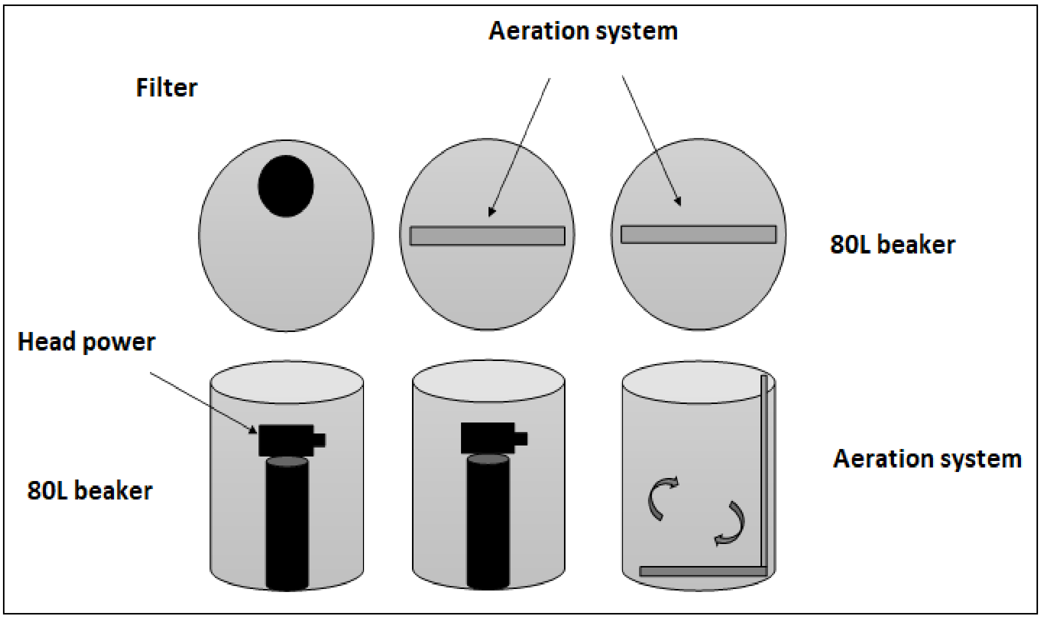

Fig 1: Culture system for both experiments

2.3 Data processing

The measurement was made every 15 days during the experiment period that last 120 days. Total population biomass was weight from each culture beaker. All organisms were placed in 1000 mL glass beaker with 500 mL of freshwater and their total weight was determined with an electronic balance OHAUS, model Scout PRO SP402.

Weight values and number of organisms per sample were incorporated to a data base in Excel 2013, to determine averages and tendency curves.

Also, absolute growth rate (AGR) and the instantaneous growth rate (IGR) were determined with the next formulas:

$$AGR = \frac{\text{Final weight} - \text{Inital weight}}{\text{Total time of experiment}}$$

$$IGR = \frac{(\text{LN(Final Weight)} - \text{LN(Initial weight)})}{\text{Total time of experiment}} \times 100$$

Gain weight was obtained with the next formula:
Gain = Final weight – Initial weight

2.4 Statistical analysis

To obtain significant differences ($P<0.05$) between experimental diets, it was made a one-way variance analysis (ANOVA) with obtained data values.

3. Results

Table 1 show the comparison between weight mean values per organism of *A. ocellatus*, in different used diets. In only Biofloc, the organisms gain 5.443g, and the AGR and IGR gain 5.443g day^{-1} and 1.477g day^{-1} respectively. The diet of live food enriched with bacteria of Biofloc obtained a higher gain with 10.752g, also with a higher AGR and IGR with 0.089g day^{-1} and 2.702g day^{-1} respectively. In the two experimental diets, the organisms grew in a similar way the first 60 days, after the experimental diet of live food enriched with Biofloc bacteria, the weight of the animals increased with respect to the other diet.

Table 1: Weight average (g) per organism of *A. ocellatus* according to experimental diet

Sample	Experimental diets	
	Live food enriched with Biofloc bacteria	Biofloc
0	0.437	1.1141
15	0.946	1.4956
30	1.6936	1.9625
45	2.6798	2.5148
60	3.9046	3.1525
75	5.368	3.8756
90	7.07	4.6841
105	9.0106	5.578
120	11.1898	6.5573
Gain	10.7528	5.4432
AGR	0.0896	0.0453
IGR	2.7023	1.4771

The diets showed a second-grade polynomic growth tendency curve, both diets obtained a correlation of R=1 (Fig. 2).

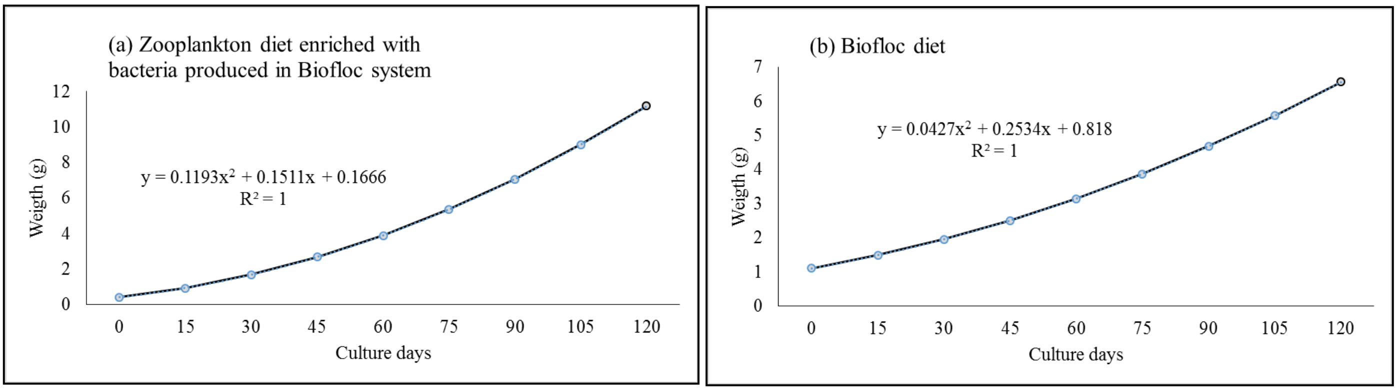

Fig 2: Growth tendency curves in experimental diets with *A. ocellatus*.

Regarding to *D. rerio*, the live food enriched with Biofloc bacteria diet obtained a higher gain with 4.517g, also a higher AGR and IGR with 0.037g day^{-1} and 2.023g day^{-1} respectively (Table 2). while organisms cultivated with only Biofloc, gained only 0.308g, and their AGR and IGR obtained 0.0025g day^{-1} and 0.571g day^{-1} respectively.

Table 2: Weight average (g) per organism of *D. rerio* according to experimental diet

Sample	Experimental diets	
	Live food enriched with Biofloc bacteria	Biofloc
0	0.437	0.3123
15	0.1666	0.3242
30	0.1348	0.3437
45	0.3416	0.3708
60	0.787	0.4055
75	1.471	0.4478
90	2.3936	0.4977
105	3.5548	0.5552
120	4.9546	0.6203
Gain	4.5176	0.308
AGR	0.0376	0.0025
IGR	2.0234	0.5718

It was obtained a second-grade polynomic growth tendency curve for both diets, with a R=1 for Biofloc diet and live food enriched with Biofloc bacteria diet (Fig. 3).

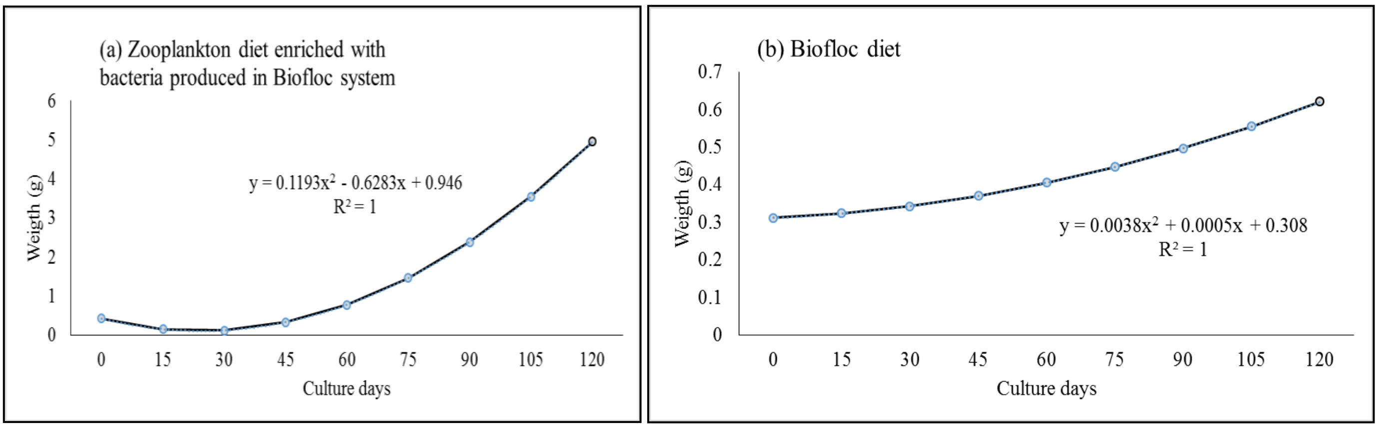

Fig 3: Growth tendency curves in experimental diets with *D. rerio*.

The ANOVA analysis showed significant differences ($P<0.05$) between diets for both experimental organisms.

4. Discussion

The obtained results show that live food enriched with Biofloc bacteria diet was more efficient and provide a higher gain in weight, followed by Biofloc diet. Similar obtained by other authors [14], that compared growth of *A. ocellatus* in five different diets (live, commercial, elaborated, live+commercial and live+elaborated food), reaching the conclusion that, diets elaborated and commercial food, can present nutritional limitations affecting the growth of organisms, and by adding more quantity of live food in diet, the organisms present a higher weight gain, specially *A. ocellatus* because food habit of this fish consumes and assimilates more efficiently this type of food.

These can be related [4] with other study, that mentions that Biofloc is good vitamin and mineral source, especially phosphorus, and it is estimated that it has probiotic effects. Likewise, it mentions that Biofloc has a wide nutritional quality and can be mentioned that range of raw protein and lipids vary from 12 to 49% and from 13 to 46% respectively [15].

Other authors [16, 17], mentioned that TCA values were affected by food type proportionated to organisms and it is an indicator of protein quality of diets. Therefore, the TCA increases with high protein content in diet that is why, it can be said that based on the obtained results the diet of live food enriched with Biofloc bacteria provided higher quantity of protein to organisms and therefore these obtained a higher AGR and IGR rates.

These authors [17] mentioned that commercial food produce low growth rates and high mortality when are supplied as only food source and, the excess of protein in diet increases additional energy costs for deamination and therefore reduction of energy for growth [18, 11]. This can be observed in other study [19], in which they cultured *D. rerio* during nine weeks with seven experimental diets (five commercial and two experimental) and their results showed that in experimental diets it was obtained a weight gain significantly higher than in all other diets. This can be related to results obtained with *D. rerio* in this study, because AGR rate was of 0.037g per day in live food enriched with Biofloc bacteria diet, which means that organisms increase their commercial weight (0.13g) every three days.

Regarding to *A. ocellatus*, its weight gain was higher with live food enriched with Biofloc bacteria diet by obtaining an AGR of 0.089g which means that they increase their commercial weight (2.235g) every 25 days. Similar result obtained [14] with *A. ocellatus* cultured with five different diets (live food, commercial, elaborated, live + commercial and live + elaborated) in which the live food diet, organisms increased their weight until reaching commercial weight in 24 days, while in commercial diet they reached commercial weight in 69 days. These author mentions that even though the content of protein in live food diet was lower than all other diets. The growth was higher because this protein is highly digestible and assimilable, while commercial food does not present an optimum protein quality for the specie.

Therefore, the use of live food as well as Biofloc are a better alternative for ornamental fish culture than commercial food that is normally used, because commercial food cannot provide a 100% nutrition to aquatic organisms, and some vitamins are highly unstable in water [20], which makes a reduction in the food nutritional value, which directly influences the use of nutritional components, manifesting in a lower growth and malformations due to deficient nutrition [21]. Another problematic with commercial food apply was not considered the organism development stage and principally storage conditions, because food flakes suffer alteration due to chemical reactions (oxidation and slow processes of protein denaturalization) causing that nutritive quality of flakes decrease [14].

Biofloc technology is economically profitable [22], because it is easy to operate and has lower feed and water expenditure. Also, it decreases the risk of input or output of pathogens and diseases [23], it increases the survival and growth of organisms as shown in this study, because for eight cultured weeks, no water exchange was made. This contributes to survival values remaining above 90%, reaching optimum increases in biomass, AGR, and IGR values.

Likewise, it has been observed in different investigation that Biofloc is a viable alternative for culture of fish like *Oreochromis mossambicus* [24], *Carassius auratus* [25], *Chirostoma jordani* [11], *Puntius conchonius* [1], crustaceans like *Macrobrachium rosenbergii* [26], and with *Litopenaeus vannamei* [27-29].

Cultured ornamental fishes, in this case *A. ocellatus* and *D.*

rerio, with live food enriched with bacteria produced by external Biofloc system or directly in Biofloc system was a viable option to growth and produce this organisms in cities aquarium industry, which need to improve better their production units and do not spend in artificial foods (poor in nutrients) and spend the water resource with minimum water exchanges.

5. Conclusions

By mentioned above, it can be concluded that use of live food enriched with Biofloc bacteria and Biofloc system are a better alternative than use of commercial diets in culture of *A. ocellatus* and *D. rerio*, in which the use of first diet, represents a better option. The use of Biofloc is a new alternative that can be used in the culture of different ornamental species produced in Mexico, if the nutritional requirements for each species are considered.

6. Reference

1. Ramón LA, Germán CM, María CMD, Jorge CM, José AOC, Fernando DS. Crecimiento y supervivencia de *Puntius conchonius* (Hamilton, 1822) cultivado en un sistema Biofloc. Revista Digital del Departamento El Hombre y su Ambiente. E-BIOS. 2017; 1(13):43-53.
2. Carlos RM, Roberto MA, Carlos AG. Estado actual y perspectivas de la producción y comercialización de peces de ornato en México. Universidad Autónoma de Nuevo León, Monterrey, México. 2010, 112.
3. Froese R, Pauly ED. FishBase. World Wide Web electronic publication. Disponible en: www.fishbase.org, (02/2017), 2017.
4. Jhon AJ. Biofloc Production Systems for Aquaculture. Southern Regional Aquaculture Center (SRAC) from the United States Department of Agriculture, National Institute of Food and Agriculture. SRAC Publication. 2013; 4503:12.
5. Yoram A. Biofloc technology -a practical guide book. The World Aquaculture Society, Baton Rouge. 2012, 272.
6. Mauricio E, Gerard C, Miguel A, Gabriela G. Biofloc technology in intensive broodstock farming of the pink shrimp *Farfantepenaeus duorarum*: spawning performance, biochemical composition and fatty acid profile of eggs. Aquaculture. Research. 2013; 45:1713-1726.
7. Azim ME, Little DC. The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture. 2008; 283:29-35.
8. Mauricio E, Eduardo LCB, Ronaldo OC, Wilson W. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). Aquaculture Research. 2012; 43:447-457.
9. Yoram A. Biofloc technology a practical guide book. The World Aquaculture Society, Baton Rouge. 2009, 181.
10. Roselien C, Malik K, Willy V, Yoram A. Bio-flocs technology application in over-wintering of tilapia. Aquaculture Engineering. 2009; 40:105-112.
11. Jorge CM, Ramón LA, María CMD, Stephanie MG, Germán CM, Fernando PJ. Presencia y abundancia de fitoplancton y zooplancton en un sistema de producción de Biofloc utilizando dos aportes de carbono: 1) Melaza y 2) Melaza + pulido de arroz cultivando al pez *Oreochromis niloticus*. Revista Digital E-BIOS. Departamento El Hombre y su Ambiente. 2016; 1(13):33-42.
12. Luis FCL, José AAC. Fundamentos de la tecnología biofloc (BFT). Una alternativa para la piscicultura en Colombia. Una revisión. Orinoquia. 2015; 19(1):77-86.
13. Luis RMCV, Marcel MP, Mauricio GCE, Teresa G. From microbes to fish the next revolution in food production. Critical Reviews in Biotechnology. 2016, 9.
14. Domínguez TA, Soto PE. Evaluación experimental del crecimiento de *Astronotus ocellatus* (pez oscar) alimentado con diferentes dietas, así como el análisis económico en su producción. Tesis de Licenciatura. Facultad de Estudios Superiores Zaragoza. Universidad Nacional Autónoma de México. 2001, 116.
15. María CMD, Ramón LA, Jorge CM, Germán CM, Mauricio EGC. Composición y abundancia de comunidades microbianas asociados al biofloc en un cultivo de tilapia. Revista de Biología Marina y Oceanografía. 2013; 48(3):511-520.
16. Erland A, Terje R. Effect of varying dietary protein level in different families of rainbow trout. Aquaculture. 1979; 18:145-156.
17. Elsah AU, Jorge LF. Efecto de dietas con diferente contenido proteico en las tasas de crecimiento de crías de Bagre de Balsas en condiciones de cautiverio. AquTIC. 2003; 18:39-47.
18. Carlos AMP, Janett CS, Santoy GOV, Antonio CM, Carlos CMC, Lindsay GR. The effect of photoperiod on the reproduction of *Chirostoma estor estor* Jordan 1879 from Lago de Pátzcuaro, Mexico. Journal of Applied Ichthyology. 2007; 23:621-623.
19. Anthony JS, Heath WG, Warren TJ, Dorothy BM, Louis RD, Stephen AW. Growth and survival of zebrafish (*Danio rerio*) fed different commercial and laboratory diets. PubMed doi:10.1089/zeb.2008.0553. 2009; 6(3):275-280.
20. Mark PD. Fish diseases. T.F.H. Publications, Inc. USA. 1998, 89.
21. Stephen S. Marine Aquarium Keeping. The Science, Animals and Art. John Wiley Sons. New York-London-Sydney-Toronto. Awiley-Interscience Publication. 1973, 93-112.
22. Newman S. Understanding biofloc in aquaculture production systems. Aquaculture Asia Pacific Magazine. 2011; 7(2):25-26.
23. Irshad A, Babitha AM, Verma AK, Maqsood M. Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. Aquaculture International. 2017; 25(3):1215-1226.
24. Yoram A. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture. 2007; 264(1-4):140-147.
25. Guangjun W, Yu EM, Jun X, Deguang Y, Zhifei L, Wen L *et al*. Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. Aquaculture. 2015; 443:98-104.
26. Asaduzzaman M, Abdul W, Marc CJV, Haque S, Abdus S, Ekram A. C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. Aquaculture. 2008; 280(1):117-123.
27. Michele AB, Peter JT, Robins PM, Robert HB, Doug CP.

The contribution of locculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero exchange system. Aquaculture. 2004; 232:525-537.

28. Wu JX, Lu QP, Da HZ, Jie H. Preliminary investigation into the contribution of bioflocs on protein nutrition of *Litopenaeus vannamei* fed with different dietary protein levels in zero-water exchange culture tanks. Aquaculture. 2012a; 350-353:147-153.
29. Wu JX, WJ, Lu QP, Xiao HS, Jie H. Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. Aquaculture Research. 2012b; 44:1093-1102.

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