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Composition of Zooplanktonic communities associated with the culture of *Heros severus* (Heckel, 1840) in a biofloc system

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Abstract
This investigation aimed to identify the composition of zooplanktonic communities associated with flocs in a Biofloc culture of *Heros severus* with different carbon sources and diets rich in carotenoid pigments. To form microbial flocs, a culture was carried out in 80 L tanks, where no water changes were made. The used carbon sources were moringa flour for the organisms fed with trout feed and TetraColor®, beetroot, and carrot flours for organisms fed with formulated diets with beetroot and carrot respectively, in all cases a C:N ratio of 15:1 was maintained. Regarding the zooplanktonic communities observed, the carrot and beetroot meal cultures allowed a better system since they presented nematodes and a large zooplanktonic community that helped to maintain water quality and high survival levels.

Keywords: Zooplankton, Biofloc, Ornamental fish, *Heros severus*

1. Introduction
Biofloc technology (BFT) has proven to be a good technique for fish and crustacean culture, which helps to increase culture density along with decreasing water and space use [1]. Likewise, it allows the development of microbial flocs formed from a carbon-nitrogen ratio in the water, with little or no water change (0.5 to 1% per day) [2] and high oxygenation [3, 4], in which diets with low crude protein content [5] and external carbon sources such as molasses (sugar cane), rice bran, wheat bran [4], and Yucca, Moringa, and Macroalgae meals are used [6]. These carbon sources allow the growth of a microbial community, mostly heterotrophic bacteria that metabolize carbohydrates and take up inorganic nitrogen solving the problems of nutrient saturation from its transformation to less harmful substances [7, 8], decreasing the levels of ammonium, and nitrites, and consequently the need for water changes [9, 10].
This also allows the microbial biomass to form the flocs that serve as natural food for the species in culture and increases the efficiency of food use [11, 12]. In addition to the bacteria associated with the Biofloc, another important part of this culture system is that it allows planktonic organisms attached to the flocs to play a fundamental role as producers and consumers of dissolved oxygen, recycling nutrients and producing food for larger organisms [13], such as microalgae, protozoa (ciliates and flagellates), rotifers and nematodes, which are going to serve as a 24-hour available food source for different aquatic organisms in culture [6].
Protozoa are considered a rich natural food for fish and shrimp [14]. In this group, we can find the genus: *Paramecium*, *Stylonychia*, *Vorticella*, *Colpidium*, *Epistylis*, *Halteria*, *Unorema*, *Litonotus*, and *Euplotes* [15, 1]. Rotifers are frequently associated with Biofloc, because they can fragment the flocs and consume the attached bacteria, and the mucilage produced by their excretions also helps the formation of new flocs [16]. *Lecane*, *Keratella*, and *Philodina* genus have been identified in this system [1]. Finally, nematodes represent an important group within the Biofloc as they have high contents of crude protein and essential fatty acids in their composition [17], their abundance is determined by the presence of various ciliates that serve as food [18].
Therefore, the objective of the present work was to identify the communities associated with the Biofloc and how they helped to maintain water quality in a Biofloc system culture of *Heros severus*, with different carbon sources.

2. Materials and Methods

The experimental work was carried out in the facilities of the Biofloc and Live Food Production Laboratory at the Universidad Autónoma Metropolitana, Unidad Xochimilco.

2.1 Experimental Design and culture conditions

For the culture of *H. severus*, fish were acclimatized for four weeks in 25 L aquariums with a 150 W thermostat to maintain the temperature at 27±2 °C. Also, a mechanical filter based on rocks and a sponge was placed to maintain the cleanliness of the aquarium, and an aeration stone to maintain oxygenation in the aquarium.

Once the acclimatization period passed, the organisms were transferred to an 80 L tank to start the Biofloc, which was filled to 60 L, and 20 *H. severus* juveniles were introduced. In each culture vessel, an aeration system was placed with a 25 cm long aeration stone, with sufficient intensity to move the entire water column. In the same way, a 150 W thermostat was placed to maintain the temperature of the medium at 27±2 °C. Each treatment was done per triplicate.

2.2 Feeding of the organisms

The experimental diets were a) Trout feed, El Pedregal®; b) TetraColor®; c) Carrot-based meal and; d) Beetrootroot-based meal. The carrot and beetrootroot -based diets contained approximately 30% protein and 10% lipids provided by chicken gizzards, and 3.5% fiber with apple, banana, and 250 g of oats; two mineral and multivitamin supplement tablets were also added. The carrot and beetrootroot cubes were agglomerated with 50 g of liquid gelatin.

The diets were provided at 5% of the total biomass of the organisms present in the culture vessel. The feed was supplied twice a day (2.5% in the morning and 2.5% in the afternoon).

2.3 Biofloc Production

For the formation of microbial flocs, the source of carbon incorporated into the cultures was moringa flour for the trout and Tetracolor feeds; for the diets with carrot and beetroot, carrot and beetroot flours were used, respectively, made with the waste from the diets. In all cases a C:N ratio of 15:1 was maintained, by using the next formulas [12]

g of C in food = ((g of food) (0.9) (0.7)) / 2
g of N in food = ((g of food) (0.9) (0.7) (0.32)) / 6.25
g of necessary C = (g of N in food) (15)
g of external C = g of necessary C – g of C in food

Where
0.9 = 90% of dry matter in food
0.7 = 70% of waste that is maintained in the system
2 = carbon content in food is ~ 50% based in dry matter
0.32 = 30% content of raw protein in food
6.25 = constant
15 = C/N relation of 15:1

2.4 Water quality monitoring

Every seven days a sample of 100 mL of water was taken from the culture medium of each tank and the concentration of nitrite (NO_2^- mgL^{-1}), nitrate (NO_3^- mgL^{-1}), ammonium (NH_4^- mgL^{-1}), phosphate (PO_4^{-3}) was determined using a HANNA® Model No. HI83325 multiparameter.

2.5 Composition of the Biofloc

After the conditioning period, a sample of 100 mL was taken every 15 days and allowed to settle for 30 minutes. Subsequently, a 1 mL sample was taken and observed under a Leica ICC50 HD optical microscope (20X and 40X), connected to the imaging, and counting program Image® Pro Plus 7.0. The taxonomic identification of the observed groups was carried out at the genus level with the help of specialized literature [19].

3. Results

3.1 Water physicochemical parameters

The evaluation of the physicochemical parameters did not present significant variations since the parameters of ammonium (NH_3), nitrites (NO_2), nitrates (NO_3), phosphates (PO_4^-), and pH remained constant during the experimental period. Table 1 shows the averages of each parameter in the four experimental diets.

Table 1: Average values of the physicochemical variables in the experimental diets.

Experimental Diet	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	PO_4^- (mg L^{-1})	pH
Control Diet	0.2	1.89	52.92	5.34	8.3
TetraColor Diet	0.35	0	84.86	9.76	8.3
Carrot Diet	0.11	1.33	107.62	7.56	8.2
Beetroot Diet	0.36	2.06	28.34	10.49	7.9

3.2 Biofloc Composition

All treatments began to show floc formation after eight weeks of culture. The pictures of the main groups are presented in figure1.

3.2.1 Control treatment

By the 12th week of culture, microalgae, and ciliates of the genus *Colpidium* sp. and *Tokophryrav* sp. were present, as well as flagellates of the genus *Peranema* sp. and rotifers of the genus *Lecane* sp. and protozoa of the genus *Vorticella* sp. By the 16th week of culture, annelids of the genus *Aeolosoma* sp. were present, reaching the last week of culture without nematodes.

3.2.2. Tetra Color treatment: Like the previous treatment, in the 12th week of culture, rotifers of the genus *Aeolosoma* sp were present. By the 16th week, protozoa of the genus *Arcella* sp, *Centropyxis* sp, and ciliates of the genus *Acineta* sp, *Tokophyra* sp, and *Paramecium* sp were present. In addition, rotifers of the genus *Lecane* sp and *Lepadella* sp, protozoa of the genus *Vorticella* sp, and a greater number of gastrotrichs and annelids were present. At the end of the culture period, there were floccules with large numbers of rotifers of the genera *Lecane* sp, *Philodina* sp, and *Lepadella* sp, as well as protozoa of the genus *Vorticella* sp. and annelids of the genus *Aeolosoma* sp. In this treatment, nematodes were also absent.

3.2.3 Carrot and beetroot treatment

The carrot and beetroot diets were the ones that presented the best Biofloc, since they reached the presence of nematodes at the end of the culture for the carrot diet and in the 16th week for the beetroot diet. The carrot treatment at the 12th week

already had a Biofloc with ciliates and flagellates of the genus *Litonotus* sp. and *Peranema* sp, and protozoa such as *Centropyxis* sp, and *Amoeba* sp. Similarly, it already had a large presence of rotifers of the genera *Lecane* sp. and *Lepadella* sp, and protozoa of the genus *Vorticella* sp, and annelids of the genus *Aelos* sp, and annelids of the genus *Aeolosoma* sp. For the treatment with beetroots, in the 16th week, rotifers of the genera *Philodina* sp, and *Lecane* sp were present, and annelids of the genus *Aeolosoma* sp. For the 18th week, the predominant groups in the Biofloc were nematodes and annelids together with four rotifer genera (*Lecane* sp, *Lepadella* sp, and *Philodina* sp) predominant in the Biofloc were nematodes and annelids together with four rotifer genera (*Lecane* sp, *Lepadella* sp, and *Philodina* sp).

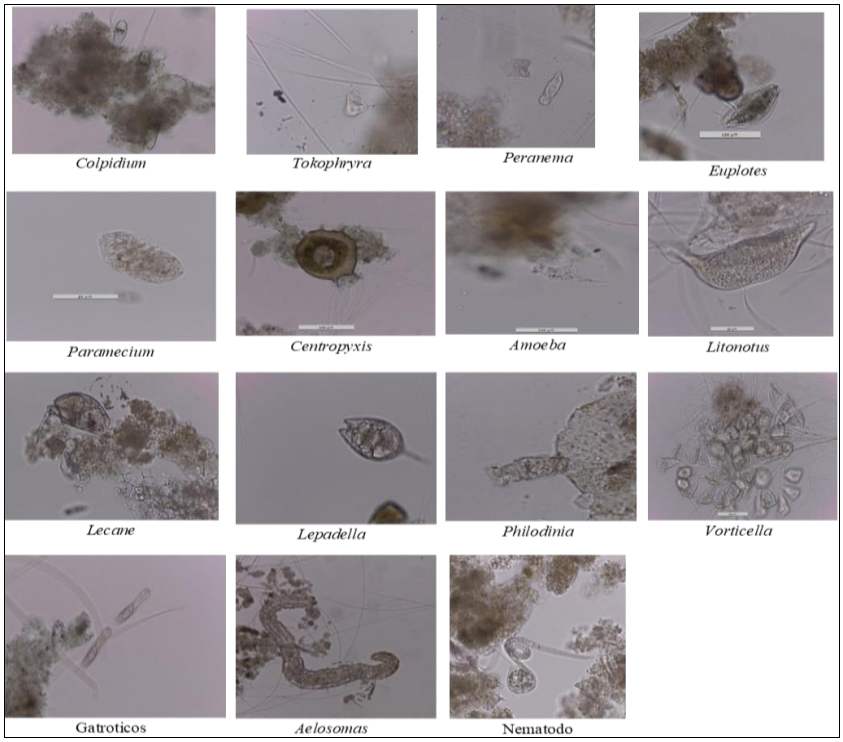

Fig 1: Microorganisms present in the different Biofloc treatments in a culture of *Heros severus*.

3.3 Survival of *H. severus*

The diet with the highest survival was the beetroot diet with 95%, followed by the TetraColor diet at 90%, the carrot diet at 70%, and finally the control diet with 30% survival (Figure 2).

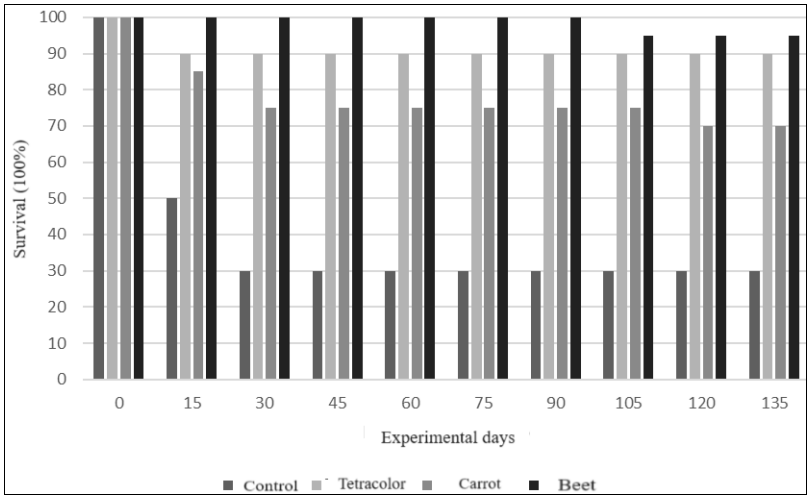

Fig 2: Percent survival of *H. severus* with the four experimental diets.

4. Discussions

Regarding the culture in Biofloc, the system allowed maintaining the organisms for six weeks (after the four weeks of acclimatization), without water changes and managing to maintain the physicochemical parameters within the optimal range for the species, especially ammonium, since Veras *et al.*,[20] mentions that the optimum is 1.02±0.46 mg L^{-1}, in this experiment the levels were maintained at an average of 0.25±0.12 mg L^{-1}, this was reflected in the survival of the organisms since from the fifth week onwards no major mortalities were observed. In this regard, the organisms fed with the carrot and beetroot diet maintained above 90% survival, having mortalities between 5 to 10%, while the organisms in the control diet had mortalities of 70%. There is

experimental work with the incorporation of three different pigment substrates (*Rhodococcus* sp, *Tagetes erecta*, and *Capsicum annuum*) with another ornamental fish, *Pterophyllum scalare* [21], although it was not placed in a Biofloc system. The main food was Wardley flakes, and they obtained survivals of 75, 72.22, and 77.8% respectively, unlike the experiment conducted in which 20% more survival was obtained than the experiment with the angelfish without Biofloc treatment.

According to the obtained results from the composition of the Biofloc in the different diets, it can be said that the carrot and beetroot treatments presented a better Biofloc than the control and Tetracolor, because they presented large rotifer communities in earlier stages and even presented nematodes. According to Pérez [16], rotifers can fragment the flocs and consume the attached bacteria, and the mucilage produced by their excretions also helps the formation of new flocs. For their part, nematodes are an important group within the Biofloc since they have high crude protein and essential fatty acid content in their composition, being a rich source of live food in situ and available 24 hr [1]. These authors found that in addition to zooplankton, microalgae, colloids, organic polymers, cations, and dead cells also serve as a source of protein, reducing feeding costs by more than 25% [22, 23, 24].

Ray *et al*., [18] and Newall *et al*., [25] point out that a good technique to characterize zooplanktonic organisms in the Biofloc is visual microscopy, which allows for determining the main groups of microorganisms in the flocs, which is why this technique was used in this experiment [25]. In the work of Monroy-Dosta *et al*., [1], the appearance of ciliates in the third week is mentioned. The difference in appearance concerning this research is because tilapia is a much larger organism and can shed a greater amount of waste that can be transformed by bacterial microorganisms and used by microalgae, increasing their density and therefore the presence of food for the different zooplanktonic groups. As in the work of Monroy-Dosta *et al*., [1] and Eilious *et al*., [26] rotifers are present throughout the culture, although not the same groups and this could be mainly due to the carbon source used. These authors point out that the elements that produce the flocs such as the carbon source, the balanced feed used for feeding, as well as the fish conditioned for the system, can have a direct influence on the groups of organisms that develop, and this can be observed in the difference of groups found in this experimentation. It should be noted, as mentioned by Monroy-Dosta *et al*., [1], that the presence of rotifers that fragment the Biofloc flocs and produce mucilage in their excretions is an essential component for the presence of nematodes in crops. Nematodes are a source of crude protein and essential fatty acids [26]. Ray *et al*., [18] mentioned that nematodes are one of the most important groups in Biofloc systems and that their abundance is determined by the presence of various ciliates, which were present in the carrot and beetroot treatments.

Becerril *et al*., [27] and Wang *et al*., [28], mention that an aquatic organism culture system is more stable when it is based on a zooplanktonic microorganism production culture and not only on phytoplankton-based cultures since it can more easily control the water quality system, since they actively participate, not only in the metabolism of feces but in the production of waste products and therefore the utilization and transformation of ammonium into non-toxic nitrogenous compounds. This means, that the water expenditure is diminished, as well as the application of a greater amount of feed, decreasing the costs in the cultivation of organisms, for human food use, as well as for ornamental species [6].

5. Conclusion

According to the obtained results in this research, it can be concluded that *H. severus* fish can be cultured in Biofloc using beetroot and carrot residues as a carbon source, since it was shown that these help the establishment of the Biofloc system, managing to maintain good water quality and floc formation with a good community of microorganisms, using a smaller amount of feed and therefore a more sustainable and profitable aquaculture.

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