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*Full Length Research Paper*

# Microalgae culture for the mosquito pre-adults feeding used as food in the aquaculture

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**Microalgae are used as food in aquaculture because they have essential amino acids, polysaccharides, enzymes, and proteins, being a food that provides great benefits to cultures. Some mosquito pre-adults (*Culex quinquefasciatus*) are microalgae consumers and they are used as living food for fish. Mosquito pre-adults support various fish characteristics, for instance they retain the behaviour of hunters, fish develop more intense coloration, and increase their growth. We described the microalgae culture required for live food growth (mosquito pre-adults) in aquaculture, and recognized the amount of carotenoids encapsulate in these organisms that support the colour and immune system of fish consumers. We founded that *Chlorella* sp. is the most abundant microalgae in living food culture and this specie had a high carotenoid pigment concentration. Additionally, we founded that mosquito pre-adult keeps a high carotenoid pigment concentration (at initial and final culture cycle) which support that this living food is an excellent food to fish production.**

**Keywords:** Microalgae abundance, living food, carotenoids, fertilization.

## INTRODUCTION

Microalgae are microscopic organisms that usually lives suspended in the water column (Spolaore et al., 2006). In aquaculture, microalgae are used in nutrition and represent an important group because they have essential amino acids, polysaccharides, enzymes, and proteins, being a food that provides great benefits to cultures (Muller-Feuga, 2000). Microalgae are used as fish food in the early development stages and as food for other groups that will later be supplied to the fish (Reitan et al., 1997). For instance, the microalgae, *Chlorella vulgaris*, it has been used to improve pigmentation in rainbow trout (Gouveia et al., 2002). Others organisms used as fish food are insect

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larvae (Barroso et al., 2014; Luna-Figueroa et al., 2019). In several studies it has been found that the fish fed with mosquito larvae are more colourful and they are bigger in less time (Mares and Arce, 2017; Arce et al., 2018).

The characteristic that most positive influence has in the fish production is the nutritional quality food (Puello-Cruz et al., 2010). Quality food determines essential aspects for fish development such as the immune system, growth, coloration, and survival rate (Torrisen, 1984; Yong and Lee, 1991; Clotfelter et al., 2007; Sefc et al., 2014). Commercial foods commonly in flakes or pellets presentation have consistently sought to improve each of these requirements; however, nowadays they are not always considered the best option for aquaculture (Sampaio et al., 2009). In view of this situation, live foods with high nutritional quality have been used (Woods, 2003). Mosquito pre-adults support various fish characteristics, among the most relevant are that the organisms fed with mosquito pre-adults retain the hunters behaviour, fish develop more intense coloration, and increase their growth (Katya et al., 2017). We described the microalgae culture required for live food growth (mosquito pre-adults)in aquaculture,and recognized the amount of present in these organisms that support the colour and immune system of fish consumers.

## MATERIALS AND METHODS

### Live food culture

The live food culture was prepared in 590 L tanks, the tanks were fertilized with one kilogram of chicken manure (8 tanks with a replica, total= 16 tanks, Nieves et al., 1996; Votolina et al., 1999). Once the was done, the tanks remained under observation and microalgae counts were carried out in order to know the abundance and the time collection of mosquito pre-adults, *Culex quinquefasciatus*. The temperature was registered daily during all culture cycle (HANNA HI98129 sensor).

### Pigments measurement

In order to know the microalgae carotenoid pigments concentration, 100 ml of water was filtered from each culture live food tank with a Millipore filter (membrane pore opening 0.45 µm). Once the filtration was finished, the membrane was placed in a centrifuge tube with 10 ml of methanol and stirred until disintegrated. Subsequently, the sample was centrifuged for 10 minutes at 1500 rpm. The readings were made in a spectrophotometer (HACH DR/2010; De la Lanzaand Hernández, 1998; Minh et al., 2014).The carotenoids concentration (mg/m$^3$) was recorded for each culture tank (16 tanks, at initial and final culture).

To obtain mosquito pre-adults pigments, mosquito pre-adults samples were collected from the culture tank.The samples were rinsed with water and macerated, which was dissolved with methanol (10 ml). Subsequently, the sample was centrifuged for 10 min at 1500 rpm; the supernatant fluid was placed in cells in order to be read in the spectrophotometer (HACH DR/2010; De la Lanza and Hernández, 1998 Minh et al., 2014).

The carotenoid pigments extraction was carried out twice per culture tank during the experiment period. The first extraction was performed on 7 day after fertilization because at thistime the coloration of the tank denotes microalgae presence. The second extraction was established considering that decreased and that the mosquito pre-adults presence was low.

### Statistics

Carotenoids pigments concentration in a culture (microalgae and mosquito pre-adults) at initial and final culture cycle was evaluated using *t* test. All Statistical analyses were conducted using Statistics® 10 Software (Zar, 2010).

## RESULTS

The most abundant microalgae species in the living food culture was*Chlorella* sp., (100 % at initial collection and 95 % at the final culture cycle). The microalgae abundance suitable for the living food culture and mosquito pre-adult collection was at 7 day next to the fertilization and it was 630431 ± 23922 cell/ml (Fig. 1-2). The final living food final cycle was when microalgae abundance was 226844 ± 10003 cel/ml (14 days next the fertilization, Fig. 1-2). Thetemperature culture was 27 ± 1.2 °C.

Mean microalgae carotenoids of the living food culture tank in all cycle were 1179 ± 200 mg/m$^3$. Carotenoid concentration in microalgae was higher at initial living food collection (1750 ± 250 mg/m$^3$) than at the final collection (630 ± 150 mg/m$^3$; $t_{(2,8)}$= 2.89, $P$< 0.05; table 1). As to mosquito pre-adults mean carotenoids of the living food culture tank in all cycle were 587 ± 69 mg/m$^3$. Carotenoid concentration in mosquito pre-adults was the same at initial living food collection (610 ± 100 mg/m$^3$) than at the final collection (570 ± 100 mg/m$^3$; $t_{(2,8)}$= 0.006, $P$> 0.05; table 1).

At the initial living food collection, carotenoids concentration was higher in microalgae (1750 ± 250 mg/m$^3$) than in the mosquito pre-adults(610 ± 100 mg/m$^3$; $t_{(2,8)}$= 4.33, $P$< 0.01; table 1) while at the final living food collection, carotenoids concentration was the same in microalgae (630 ± 150 mg/m$^3$) than in mosquito pre-adults (570 ± 100 mg/m$^3$; $t_{(2,8)}$= 0.45, $P$> 0.05; table 1).

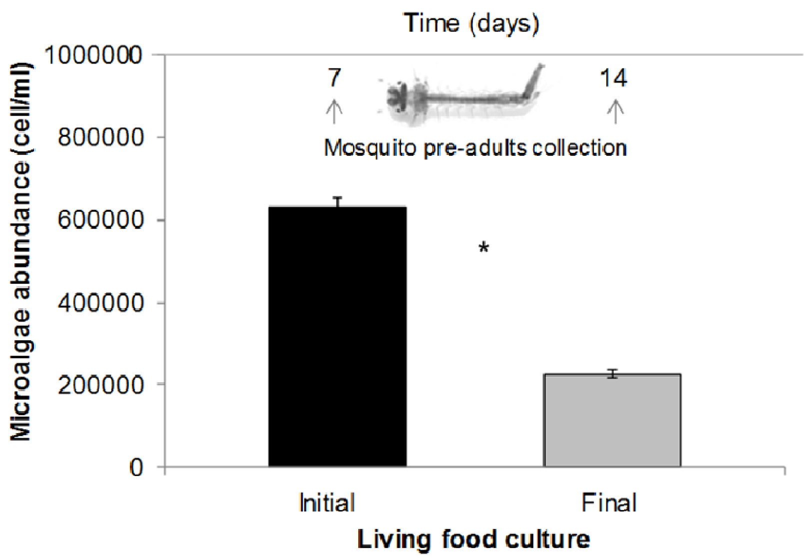


**Figure 1.** Microalgae abundance at the initial and final living food culture. Asterisks indicate significant differences ($P< 0.05$). Mosquito pre-adults collection period are shown.

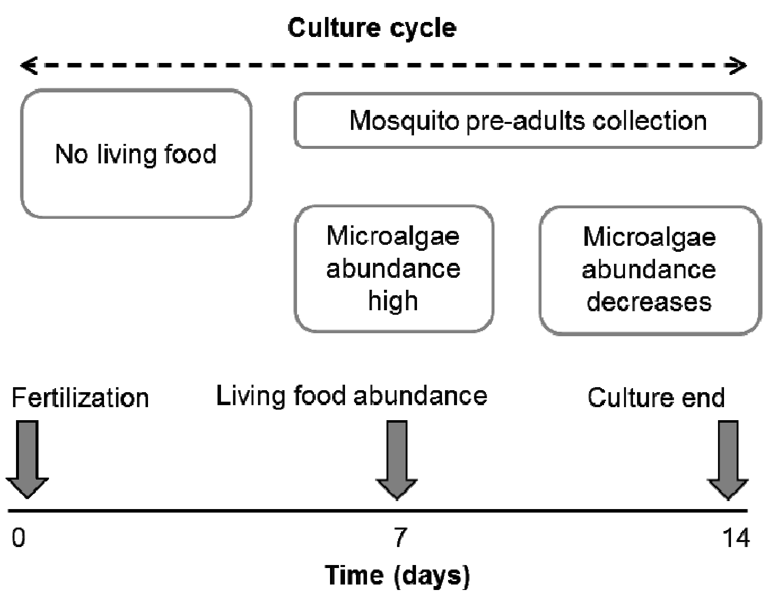


**Figure 2.** Microalgae living food culture cycle for feed mosquito pre-adults used as food in the aquaculture.

**Table 1.** Microalgae carotenoids pigments and mosquito pre-adults carotenoids pigments at the initial and final living food culture cycle. Mean values and SE are shown. Letters indicate significant differences (*P*< 0.05).

| Organism | Carotenoids (mg/m$^3$) | |
|---|---|---|
| | Initial | Final |
| **Microalgae** | 1750 ± 250 (a) | 630 ± 150 (b) |
| **Mosquito pre-adults** | 610 ± 100 (b) | 570 ± 100(b) |

## DISCUSSION

*Chlorella* sp. is a common species in the microalgae culture (Gouveia et al., 2002).We founded that *Chlorella* is the microalgae that produces carotenoids pigment in our living food culture.Microalgae have high carotenoid concentrations, which are synthesized in the cells ribosomes and transported to chloroplasts (Yong and Lee, 1991). *Chlorella vulgaris* contains carotenoid pigments in concentrations of up to 0.4%, of which 80% are carotene (canthaxanthin and astaxanthin, Gouveia et al., 1996). The microalgae abundance determines the success of a live food culture since the zooplankton species (living food to fish) and other invertebrate larvae are feed with microalgae (Sipaúba-Tavares et al., 2016). The optimal microalgae abundance was presented 7 days after fertilization, at this time the carotenoids concentration is higher in microalgae than in mosquito pre-adults. Microalgae consumers are not able to produce carotenoids in a natural way; instead, they need to consume them from the primary producers as *Chlorella* sp. (Khatoon et al., 2010). Carotenoids expression in fish is considered an honest signal of the immune resistance (Houde and Torio, 1992). Additionally, carotenoids are responsible for the nutritional properties of fish muscle for human consumption (Rajasingh et al., 2006); while in ornamental fish the carotenoids determined the animal coloration and the price of this fish (Marañón et al., 1999).We founded that microalgae in culture has high carotenoids concentration. Gouveia and Rema (2005) observed the effect of microalgae biomass and the temperature in *Carassiusauratus* on skin pigmentation. They founded that the best results were in fish fed with high levels of *C. vulgaris* in 26 to 30°C temperature range, and this range is adequate for the microalgae culture (Paripatanamont et al., 1999).Additionally, *Clorella* sp. increases the coloration of *Acipenserruthenus* as complementary food (Sergejevova and Masojìdek, 2013). We demonstrated that mosquito pre-adults are a food with a high concentration of carotenoids pigments that are made available to fish in production. Mosquito pre-adults does not undergo drying processes, freezing, or packaging, which decreases its original value, on the contrary, this food retains its nutritional value until ingestion and encourages consumers to hunt (Luna-Figueroa et al., 2009). Further, mosquito pre-adults obtain sources of carotenoids from microalgae present in cultured and even when the carotenoids pigments concentration of microalgae decreases, mosquito pre-adults are able to maintain the carotenoids pigment concentration until the end of the culture.Food fish has been enriched with microalgae, for instance, it has been used *Nostocellipsosporum* and *Navicula minima* to feed *Daphnia* sp. as food to Goldfish, *Carassiusauratus* (Khatoon et al., 2010).

In conclusion, we founded that *Chlorella* sp. had a high carotenoid pigment concentration and we founded that mosquito pre-adult keeps a high carotenoid pigment concentration (at initial and final culture cycle) which support that this living food is an excellent food to fish production.

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