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## The reproductive potential of *Artemia franciscana* fed with different concentrations mixed diet with *Porphyridium cruentum* and *Pinnularia* sp. in laboratory

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### Abstract

This experiment was made with two microalgae that produce HUFA (20:5w3, 22:6w3) used in different metabolisms functions, not only for this crustacean but also all the aquatic species that eat them. Five grams of cysts from *Artemia franciscana* were hatching and the nauplii were introduced in 180 L plastic containers with 160 L of saline water (60 gL<sup>-1</sup>). Five experimental treatments were made: A) 100% of *Porphyridium*; 100% of *Pinnularia*; c) 50:50% of both microalgae; d) 25% *Porphyridium* and 75% *Pinnularia*; e) 75% *Porphyridium* and 25% *Pinnularia*. Better production of biomass was obtained in 50:50% of both microalgae with 417.83 g, with Ro value of 16.57, Tc of 16.52, and r value of 0.19. The lowest value was with 100% *Porphyridium* sp. because all organisms were dyed before 45 days. In the other treatments, the population density decreased during all experiments. The conclusion of this experiment was that is better to use a mixed diet with these two microalgae.

**Keywords:** *Artemia franciscana*, microalgae, microalgae

### 1. Introduction

The aquaculture industry allows the environmentally controlled culture media, whether it's in small or big size habitats to produce aquatic species with commercial importance to human consumption, which can be profitable to those people who sell it or for their own family-fed source [1]. However, is not easy to obtain a food source with all nutrient requirements and palatability to obtain crustacean larval or fish fry to obtain a successful culture system to maintain better growth (total length and biomass) and survival of those organisms [2, 3, 4].

One of the most live foods used in those first crustacea and fish stages was the crustacean *Artemia franciscana*, from Class Branchiopoda which lives in hypersaline waters and was considered an obligate, continuous, and non-selective filter organism [5]. The principal stages that were used in the aquaculture of this crustacean were the unhatched embryos, called cysts. These embryos were dehydrated and stored in a vacuum until they hydrated again and decapsulated to carry on to the hatching process for 24 hours [6]. Nauplius were used as live food from the first stages of different fishes and crustaceans [7, 8, 9, 10, 11, 12], because their high protein and high unsaturated fatty acids (HUFA) content (20.5w3: 22:6w3) which play an important role in different metabolic functions of cultured organisms [13, 14], but it is necessary that all stages of *A. franciscana* was cultured in an optimal diet to acquire better nutritional components to those species which fed them [15].

In the last few years, *A. franciscana* culture technique was modified to reach high biomass volumes with low water content and costs. The advantage of this production type was the pre-established quantities of good-quality feed at the time it was required [16]. Those culture techniques use different microalgae species which can be selected by their size, digestibility of their wall cells, and nutritional value that impact this crustacean's survival, growth, and metamorphosis [17]. It has been documented that the use of mixed microalgae species as a diet for many invertebrates, like *Artemia*, provided a nutritional balance and improved their growth compared with mono microalgae diet [18], and above all, with those microalgae produce

essential fatty acids necessary for those crustaceans and fish's species which were cultured in different aquaculture systems and consequently, these can be transferred through trophic chain [19, 20, 21]. One of the most important microalgae group were the diatoms for their highest lipid content, reaching in polyunsaturated fatty acids, specifically in eicosatetraenoic acid (20:5w3) which can reach 7 to 34% of microalgae cell dry weight [22]. Also, *Porphyridium* sp. Microalgae can synthesize different bioactive substances like phycoerythrin, extracellular polysaccharides, and polyunsaturated fatty acids during their growth process [23]. Also, this microalga can storage arachidonic acid (20:4w6) and eicosatetraenoic acid (20:5w3) [24]. These characteristics of these two microalgae (*Pinnularia* and *Porphyridium*) can be used in *Artemia* biomass production per volume and time [25].

For all above, the goal of this research was to evaluate the population growth of *A. franciscana* fed with these two microalgae (*Pinnularia* sp. and *Porphyridium* sp.) at different percentage concentration levels to obtain better biomass productions and ensure its nutritional quality with unsaturated fatty acids necessary for nutrition of other aquatic species.

## 2. Material and Methods

### 2.1 Artemia cysts

Cyst of *A. franciscana* was obtained by Biogrow® company and stored in the Live Food Production and Biofloc laboratory from Universidad Autónoma Metropolitana Xochimilco.

### 2.2 Experimental design

Two grams of the cyst were hydrated for one hour and then placed in a 100 mL sodium hypochlorite and brine water (120 gL<sup>-1</sup>) solution (1:1) until they were decapsulated and washed with sodium thiosulphate solution (1 gL<sup>-1</sup>) and placed in a 5 L conic container with 40 gL<sup>-1</sup> of salt water and continuous light and air supply. Hatched nauplii were transferred in a plastic container (200 L) with 160 L of water at 60gL<sup>-1</sup> salinity. Nauplii were fed with 25 mL of rice bran solution (300g rice bran in 4 L of brine water) for three days. Five experimental diets were applied: *Pinnularia* 100%; 2) *Porphyridium* 100%; 3) *Porphyridium* 25% and *Pinnularia* 75%; 4) *Porphyridium* 50% and *Pinnularia* 50%, and y 5) *Porphyridium* 75% and *Pinnularia* 25%. Every third day, were take three samples of 10 mL and all organisms were counted to obtain a mean value of population density. Culture systems have a duration of 60 days.

### 2.3 Data analysis

All data values were placed in an Excel database to obtain their descriptive analysis. All mean values were extrapolated to 160 L containers. Also, growth curves were obtained for each experimental treatment. Population density values were placed in an Excel database to obtain Life Table data to obtain

the following productive parameters:

$$\text{Reproduction rate} = \sum lx * mx$$

Where:

$\sum$  = summation

lx = Organisms survival in each phase

mx = Organisms produced for each sample phase

$$\text{Intrinsic Growth Rate (r)} = \frac{\text{Log}_e \text{Ro}}{\text{Tc}}$$

Where:

$\text{Log}_e \text{Ro}$  = logarithm of natural reproduction rate

Tc = Cohort generational time

$$\text{Cohort generational time (Tc)} = \frac{\sum \frac{x * lm * mx}{\text{Ro}}}{\text{Ro}}$$

Where

$\sum$  =summary

lx = survival in each phase

mx = Organisms produced in each phase

Ro = Reproduction rate

## 3. Result ADOS

Table 1 shows *A. franciscana* mean values of population density per sampling day and Fig.1 shows growth curves per experimental treatment.

The organisms fed with *Porphyridium* (50%) + *Pinnularia* (50%) reach the highest density value with 41,783 org. at 60 culture day, meanwhile lowest values were obtained with *Porphyridium* 100% experimental diet with 88 org at 45 culture days.

(Insert Table 1 and Fig.1)

The reproductive values are shown in Table 2. The highest Ro value was obtained in *Porphyridium* (50%) + *Pinnularia* (50%) experimental diet with 16.57 org, Tc = 16.52 days, and r = 0.17. The lowest value was obtained in *Porphyridium* 100% experimental diet with 0.58 org female-1, a Tc value of 2.54, and an r value of -0.21.

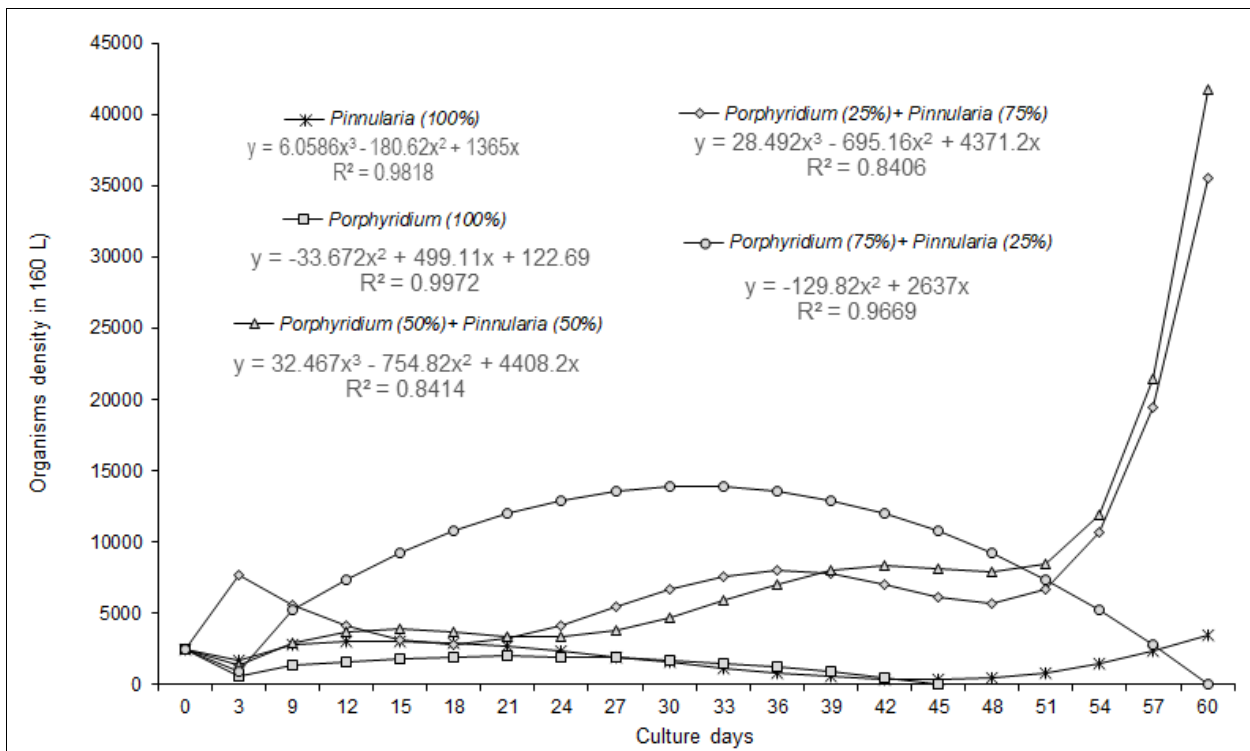
(Insert Table 2)

If highest density production value was considered and if each mean adult organism weight was 0.01 g, it was obtained a better biomass production when these two microalgae were mixed in a proportion 50:50% (417.83 g). When the concentration of *Pinnularia* was decreased until 25%, biomass content only reaches 138.52 g. (Fig. 2). *Pinnularia* 100% and *Porphyridium* 100% diets obtained values below 40 g and 20 g respectively. (Insert Fig. 2)

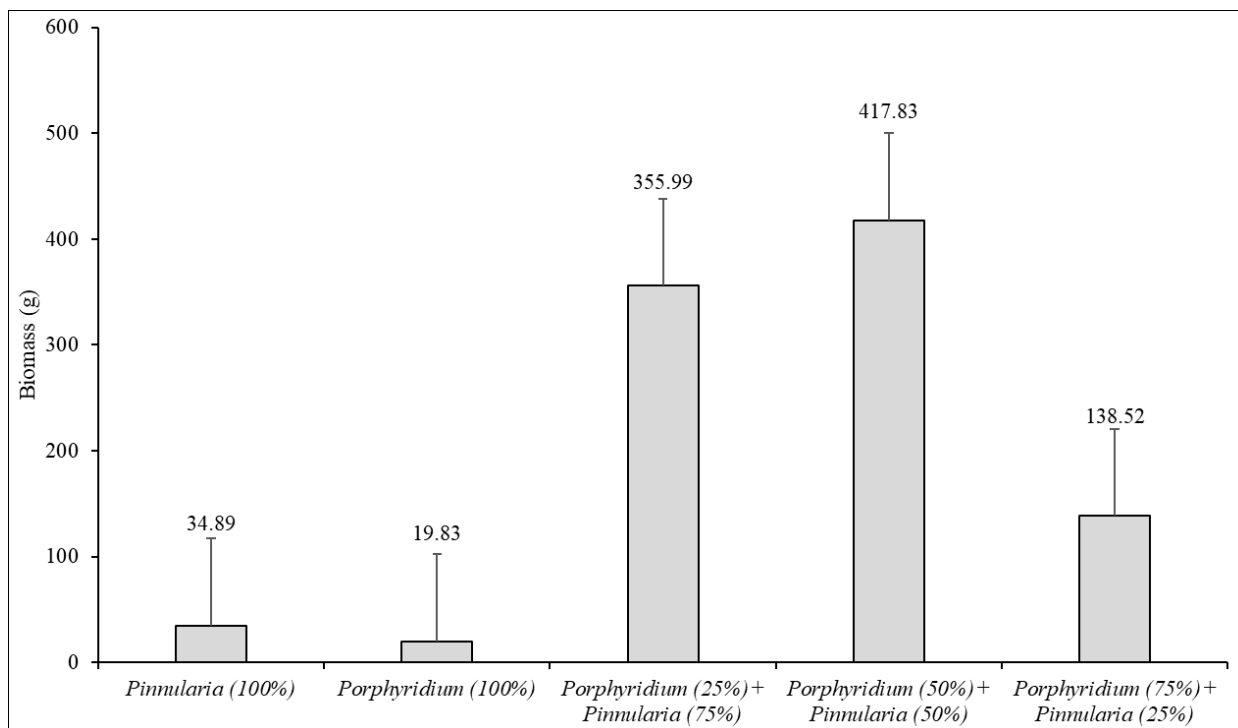
**Table 1:** Mean values of *A. franciscana* population density from each five experimental treatments.

Culture days	<i>Pinnularia</i> (100%)	<i>Porphyridium</i> (100%)	<i>Porphyridium</i> (25%) <i>Pinnularia</i> (75%)	<i>Porphyridium</i> (50%) <i>Pinnularia</i> (50%)	<i>Porphyridium</i> (25%) <i>Pinnularia</i> (75%)
0	2500	2500	2500	2500	2500
3	1717	533	7665	1333	960
6	2385	978	7080	1592	2770
9	2806	1338	5624	2972	5233
12	3013	1615	4139	3725	7388
15	3037	1813	3131	3865	9234
18	2911	1935	2827	3644	10774
21	2667	1983	3231	3390	12005
24	2338	1961	4188	3394	12928

27	1956	1871	5435	3827	13544
30	1553	1717	6667	4701	13852
33	1162	1502	7592	5870	13852
36	814	1228	7987	7066	13545
39	543	899	7762	7976	12929
42	380	518	7015	8355	12006
45	358	88	6091	8186	10775
48	508	0	5642	7867	9236
51	865		6682	8447	7389
54	1459		10650	11896	5235
57	2323		19466	21415	2772
60	3489		35590	41783	2



**Fig 1:** Growth curves of *A. franciscana* population density fed with the five experimental treatments diets.



**Fig 2:** Adult biomass obtained at the five experimental diets.

**Table 2:** Mean reproductive values of *A. franciscana*, fed with the five experimental treatments diets.

Experimental diets	Reproductive rate per female (Ro)	Cohort Generation time (Tc)	Population Growth Rate (r)
<i>Pinnularia</i> (100%)	1.78	13.03	0.044
<i>Porphyridium</i> (100%)	0.58	2.542	-0.21
<i>Porphyridium</i> (25%) + <i>Pinnularia</i> (75%)	16.11	14.72	0.19
<i>Porphyridium</i> (50%) + <i>Pinnularia</i> (50%)	16.57	16.52	0.17
<i>Porphyridium</i> (75%) + <i>Pinnularia</i> (25%)	5.16	3.87	0.42

#### 4. Discussion

One of the principal variables to make good management of *Artemia* culture to obtain biomass and cysts was not only the food concentration, but also the nutritional quality of that food, that's why it is necessary to consider the use of mixed diets with microalgae and bacteria to allow better growth and female maturity rates to obtain better production of nauplii and cysts [26]. Also, was important to consider the food concentration, because of this, the nauplii survival was a successful consequence in the obtaining of adult biomass and production of cysts per female. According to [27], mentioned that is necessary to consider not only the food supply but also, climatic conditions, low inoculation nauplii concentration in the culture system, bad selection of microalgae and it is important to consider a dry diet as a carbohydrate source to produce heterotrophic bacteria with the capacity to eliminate nitrogen compounds of culture medium and their income as bacteria biomass as fed for this crustacean.

According to [28], mentioned that is important the apply carbohydrate sources in *Artemia* sp. diet to obtain the highest total length value and biomass quantity. In this aspect, not only carbohydrates are an important source as fed to this crustacean, but also, as fed to bacteria biomass which can be an important source to *Artemia* culture. The presence of carbohydrate sources as fed to *Artemia* and bacteria helps the digestion process of microalgae nutrient content because of their enzyme content, that's why important to consider carbohydrate sources like rice or wheat bran [29]. That's why is important to consider the *Artemia* Biofloc culture system to obtain better adult biomass.

According to [30], mentioned when *Artemia* sp. culture system was made with high salinity content it's important to supply a carbohydrate source to maintain C/N relation to increase growth, organism maturity, and female fecundity. These authors used pig and tapioca compost to increase C/N relation and observed in their culture system an increase of 9.96 kg (wet weight ha<sup>-1</sup>) with respect to control with only 2.84 kg (wet weight ha<sup>-1</sup>). Adequate fertilizer supply in this *Artemia* culture system to increase C/N relation not only produces better microalgae but also, heterotrophic bacteria production in column water, which can be consumed for this organism to obtain better energy content to make their metabolic functions affected by high salinity content. According to [31], mentioned that C/N relation in 20:1 relation increases cyst production per female in the 24 to 90 range and therefore obtained 28 to 38 kg in 120 m<sup>2</sup> system ponds. In our experiment, perhaps the supply of carbohydrate sources can be improved for better biomass production. According to [32], also mentioned that carbohydrate supply in *Artemia* culture systems can produce a bacteria substratum to allow the nitrogen content to decrease and also a biomass source that this crustacean can be fed with microalgae source too.

According to [32], mentioned that in natural *Artemia* culture systems is important to make partial harvesting activities and not only final harvesting because intraspecific competition

between different stages of this organism can be eliminated. These authors mentioned that is better to make adult biomass harvest every third day [33], but it is important to make continuous nauplii inoculations in the culture system because this harvesting method did not allow that adult biomass can be reproduced, and contributed the nauplii supply to culture system naturally form. These authors mentioned that in natural biomass production in ponds only biomass harvest is possible in 3 to 4 weeks because adult biomass decreases considerably if nauplii inoculation is not made. These authors obtained in 300 m<sup>2</sup> pond culture systems a 1,323 kg ha<sup>-1</sup> biomass in the first week, but nine weeks later they obtained only 975 kg ha<sup>-1</sup>.

According to [34], which made an experiment in a 648 L pond with 120 gL<sup>-1</sup> salinity found better productions at 21-22 culture days with a biomass production of 3.7 kg per pond, meanwhile, at 25-26 culture days, the population began to produce cysts. In comparison with this experiment in a 160 L container, was obtained 417 g of adult biomass, and converted to a 648 L culture system we can obtain 1.668 kg of biomass. The difference with [35], is that they added a mix of egg yolk, rice bran, cattle manure, and oil in addition to microalgae. According to [35], did the same experiment but 180 L obtained 63.06 g per container in 15 culture days, the lowest value with our results because we obtained 150 g in 15 culture days.

According to [36], mentioned that carbohydrate source needs to be supplied in the highest proportion to microalgae and this carbohydrate source needs a maximum diameter of 50 µm, but microalgae is an important nutrient source not only for growth but also to improve better nutritional quality to adult *Artemia* biomass [37]. These authors and [38, 39, 40], mentioned also that carbohydrates source in *Artemia* culture systems allows the production of heterotrophic bacteria which can eliminate nitrogen compounds and it was an important biomass source as food. According to [36], mentioned that adequate salinity in culture systems to maintain the *Artemia* organisms during the pre-reproductive period was between 38 to 50 gL<sup>-1</sup>, meanwhile during the reproductive period is important to maintain salinity culture medium between 60 to 80 gL<sup>-1</sup>. According to [41], confirm that culture salinity needs to be up 60 gL<sup>-1</sup> when females reach the reproductive stage because female fertility increases. These authors mentioned that salinity concentration needs to be between 70 to 150 gL<sup>-1</sup> of salinity to produce biomass, but, between 110 and 200 gL<sup>-1</sup> salinity to produce cysts. This fertility increase in *Artemia* populations at those salinities was observed when salinity change concentration was made gradually at a 15% increase because this gradual increment allows the females to synchronize nauplii and cysts production in culture systems.

For all above, is important to consider not only a mixed diet of microalgae because of their nutritional content, but also, a carbohydrate source not only to use as food for *Artemia*, but also as food and energy source to produce heterotrophic bacteria that can be used to eliminate nitrogen compounds in culture systems, but as biomass source as food to *Artemia*

from their different stages.

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