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Artemia sp. biomass production using three different microalgae (*Pinnularia* sp., *Porphyridium* sp., and *Dunaliella* sp.) with yeast supply

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Abstract
Artemia plays an important role for fish and crustacean fed. The artemia culture system was made in 160 L culture medium at 60 gL^{-1} salinity. Three experimental diets were made: a) 6 L *Pinnularia* sp; b) 6 L *Porphyridium* sp.; and c) 6 L *Dunaliella* sp. Growth curves were obtained from each experiment. A Life Table was made from each one to obtain the reproductive potential. The highest density value was found in the *Dunaliella* sp. diet with 85928±233 org 160 L-1, Ro value with 42.25. The lowest density value was obtained with *Porphyridium* sp. diet con cero value until 39 culture days. Their Ro value was 8.62. The Tc values were similar to the *Dunaliella* sp. and *Pinnularia* sp. diet in comparison with the *Porphyridium* sp. diet. This experiment shows that is necessary to make mixed diets between *Pinnularia* and *Dunaliella* to obtain better results of organism densities. The *Porphyridium* sp. diet cannot be used as the only diet source for Artemia. We suggest that is necessary for *Porphyridium* sp. diet supply with brown or green microalgae from 21 culture days to complement the nutritional value of the food diet to obtain the biggest rates of survival.

Keywords: *Artemia, Pinnularia, Dunaliella, Porphyridium*, Bio-mass

1. Introduction
Zooplankton culture was a bottleneck for all aquaculture projects, principally for good management of crustacean larval and fry fish stages. One of these zooplankton groups was the resource Artemia sp. which is widely distribution in many hypersaline waters reservoirs or was introduced, in most cases successfully, allowing the establishment of new populations that were commercially exploited [1]. Actually, not only the nauplius stages were used, but also juvenile, preadult, and adult stages were used as biomass resources to feed fishes and crustaceans. This organism is important because it can supply the highest energetic value through the supplied polyunsaturated fatty acids which itself contain or can be incorporated by the bioencapsulation process during all their life cycle stages [2, 3, 4, 5, 6].
In another way, *Artemia* can be cultured easily because of their high salinity tolerance until 300 g L^{-1}. It is a wide-range oxygen-tolerant organism because it can resist concentrations below 1 mgL^{-1} of dissolved oxygen until saturation range. It is a non-selective filter organism because it can feed organic matter with proper size (< 50µm) and microalgae like *Dunaliella* sp. (green microalgae) which contributes with protein and pigments content, as same as *Pinnularia* sp. (brown microalgae) which contributes with carbohydrates and fatty acids content, and *Porphyridium* sp. (red microalgae), which contributes with minerals like iron. These microalgae were easy to produce in laboratory conditions, but *Porphyridium* sp. microalgae did not have as many research studies as *Artemia* sp. monoculture.
For the above, the present study was made to know about the use of the microalgae *Porphyridium* sp. in *Artemia* sp. culture and make a survival, growth and final biomass comparison with other two microalgae that have already been used like *Dunaliella* sp. and *Pinnularia* sp. [1, 2, 7, 8] and yeast supply which contributes with B12 vitamin.

2. Materials and Methods

2.1 Microalgae culture

The three microalgae used in this experiment were from the Life Food Production Laboratory at Universidad Autónoma Metropolitana-Xochimilco, México cultured in a Petri dish with bacteriological agar. They were inoculated in a 500 mL container for growth and then, inoculated in a 10 L container with fertilizer. The microalgae culture media was maintained in continuous light and aeration [9, 10, 11].

2.2 *Artemia* cysts

Six grams of *Artemia* sp. cysts were decapsulated and in three 10 L containers and hatched nauplii were fed with rice bran for three days. All meta nauplii stages were collected and introduced in a 200 L plastic container.

2.3 Experimental design

The *Artemia* culture was made in 200 L plastic containers, one for each experimental treatment. The plastic containers were filled with 160 L of saline water (60 gL^{-1}), with continuous light and aeration, at 24±2°C, and a pH value between 7 to 8, for 60 days. Six liters of microalgae were added to plastic containers. The three experimental diets were: a) *Pinnularia* sp; b) *Porphyridium* sp.; and c) *Dunaliella* sp. Also, was added to each treatment 40 mL of yeast (2 g 1000 mL^{-1} of salty water) once a week (Fig. 1).
(Insert Fig.1)

2.4 Density sampling

Each container was inoculated with 20 g of *Artemia* sp. adult biomass and every third day a sample of 1 L was obtained to take 10 subsamples to make a total density counting to obtain a mean value and extrapolate to 160 L.

2.5 Processing data

All data were introduced in an Excel 2019 database to obtain descriptive statistical processes to obtain de growth curves. A Life Table was made to obtain the reproductive potential with the following formulas.

Reproduction rate (Ro)

$$Ro = \sum_{i=0}^{n} lx * mx$$

Where
lx = Survival proportion of organisms produced in each sampling phase
mx = Organisms produced pear each survival female in each sampling phase

Cohort generation time (Tc)

$$Tc = \sum \frac{x * lx * mx}{Ro}$$

Where
x = sampling phase
lx = Survival proportion of organisms produced in each sampling phase
mx = Organisms produced pear each survival female in each sampling phase
Ro = Reproduction rate
Instantaneous growth rate = r

$$r = \frac{Log_e Ro}{Tc}$$

Where
Ro = Reproduction rate
Tc = Cohort generation time

3. Results

Table 1 shows density organisms per each experimental treatment and Fig.2 shows growth curves with each formula.
(Insert Table 1)
Table 1 shows that the highest density was found in *Dunaliella* sp. treatment with 85928±233 org 160 L^{-1}, meanwhile, the lowest density was found in *Porphyridium* sp. treatment because of the organisms' dye at 39 culture days. Between *Dunaliella* sp. and *Pinnularia* sp. treatment showed significant differences (p<0.05) in density value.
Table 2 shows the potential reproductive values obtained per each treatment. It can be observed that *Dunaliella* sp. treatment obtained the highest values with respect Ro with 42.25 org $female^{-1}$, and the lowest value was found with *Porphyridium* sp. treatment with only 8.62 org $female^{-1}$. Tc and r values with Dunaliella sp. and *Pinnularia* sp. treatments were close concerning *Porphyridium* sp. treatment. (Insert table 2)
Concerning produced biomass per each experimental treatment, *Dunaliella* sp. and *Pinnularia* sp. diets show values of 859.28 g and 796.7 g respectively, *Porphyridium* sp. diets only reach 72.087 g. *Porphyridium* sp. biomass value was obtained with 24 culture day value.

4. Discussion

At *Artemia* sp. culture system was used as diet live microalgae, dry microalgae, yeast, industries food sub-products like rice bran, wheat bran, and soy pellets [12,13,14,15], but live microalgae were found as better fed source because they support better survival and growth rates as was mentioned in Dhont and van Stappen [13] research, according with our results with *Pinnularia* sp. with a production of 2,347 org $160L^{-1}$, and *Dunaliella* sp. diet with 2,031 org 160 L^{-1}.
According to [7] mentioned that survival rates in *Artemia* sp. culture with *Dunaliella* sp. and *Chaetoceros* sp. diets were above 66±1%. According to [16] mentioned that *Artemia* sp. cultured with *Dunaliella* sp. reach the adult stage early with respect *Chaetoceros* sp. and *Spirulina* sp. monoculture diets. According to [17] evaluated the culture system of *Artemia* sp. using different live microalgae and concluded that the use of *Dunaliella bardwill* obtain better results. These authors mentioned that *Porphyridium cruentum* microalgae can be used to feed *Artemia* sp. but, our results showed better results only until 24 culture days were reached with only 202 org $160L^{-1}$ until they dye at 39 culture days.
Although this research wanted to observe *Artemia* sp. growth at different mono-cultured live microalgae like *Pinnularia*, *Dunaliella*, and *Porphyridium*, and supply with yeast which was rich with vitamin B12 complex, it is important to mention that [18], which work with two different fed types of mixed diets *Tetraselmis suecica* and *Dunaliella salina* diets, both with wheat bran. Better results were shown in *Tetraselmis suecica* and wheat bran. Also, according to [19,20] suggest the use of *Pinnularia* sp. mixed with *Tetraselmis* sp. for biomass production in the laboratory with *Artemia franciscana*.

According to [21] mentioned when a mix of green and brown microalgae was used it can obtain better results, because green microalgae supply proteins and pigments, and brown microalgae supply carbohydrates and fatty acids. According to [19,20] who worked with a mixed diet of *Isochrysis galbana* and *Tetraselmis suecica*, mentioned better results when they used it at the same proportion (1:1).

According to [22, 23] mentioned that wall cells of microalgae were different and the juvenile stages of *Artemia* sp., in many cases, cannot ingest rigid cell walls and the growth process can be affected not only by the microalgae size, but for his compositions and structural cell walls too. Perhaps, this condition occurred with *Porphyridium* sp. diet.

Another variable that many authors according were about *Artemia* sp. survival and culture density growth was the supply of microalgae culture growth phase as food for this crustacean. This important variable is according to [24] work, which use seven different marine microalgae species at three different growth phase stage, and the study according to [9] which evaluate the growth of different larval stages of *Artemia franciscana* fed with two different proportion of microalgae. They concluded that using the microalgae stage in their exponential phase influences *Artemia* sp. growth and survival because, in different stages of the culture microalgae phase, they produced different concentrations of proteins, fatty acids, carbohydrates, and lipids. This condition was maintained in this experiment. The microalgae culture was harvested every third day to maintain the microalgae cultures in this exponential phase.

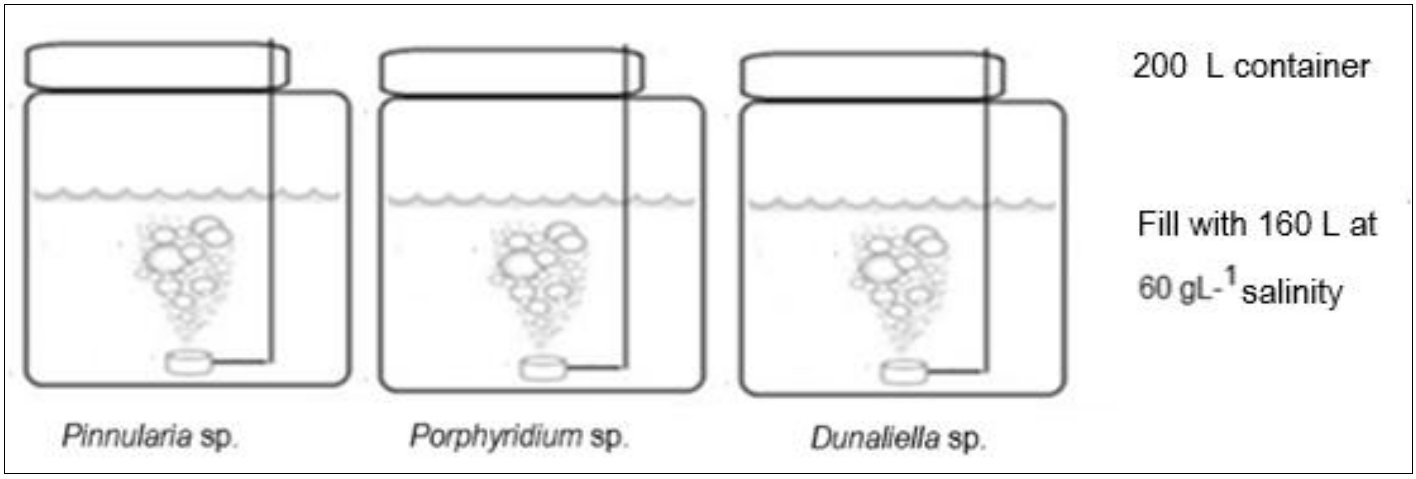

Fig 1: Experimental design of *Artemia* sp. culture at three experimental treatments.

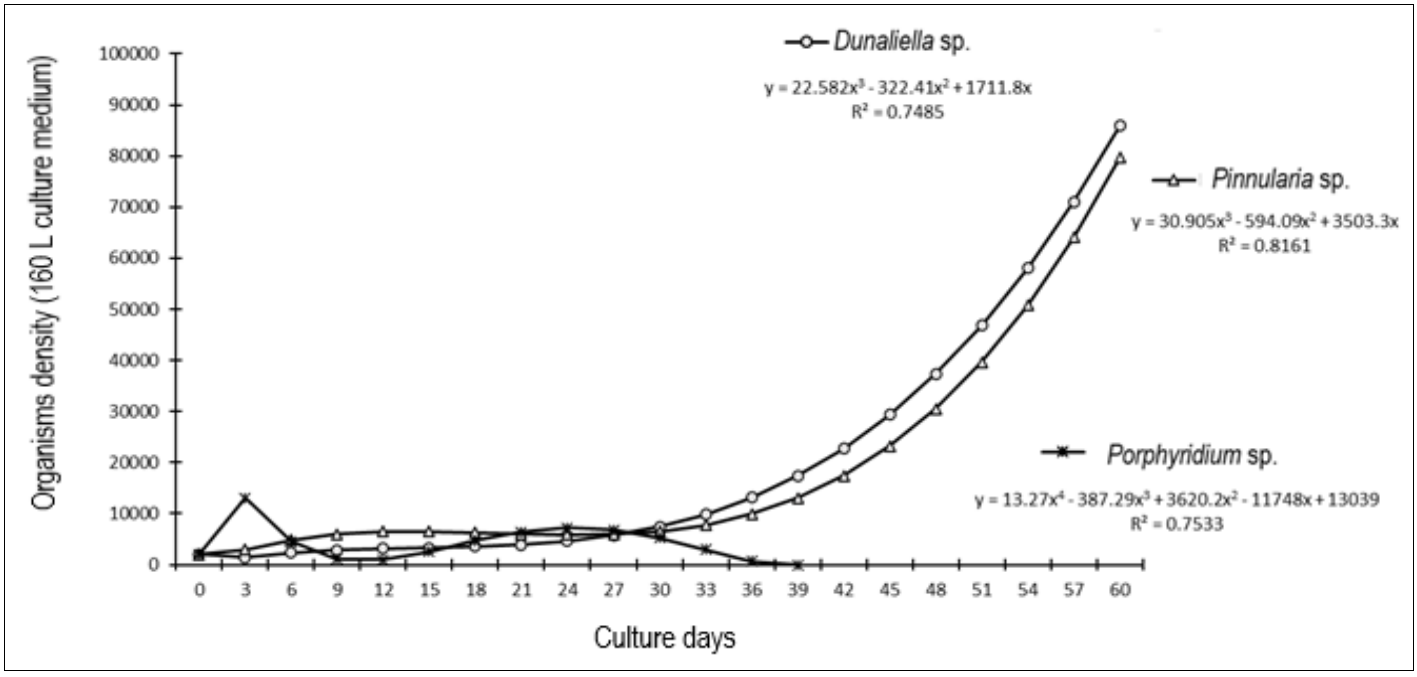

Fig 2: Growth density curves of cultured *Artemia* sp. experimental treatment.

Table 1: Organisms density of *Artemia* sp. produced per each experimental treatment.

Culture days	*Artemia* sp. experimental treatment		
	Dunaliella	***Pinnularia***	***Porphyridium***
0	2000±31	2000±83	2000±67
3	1412±68	2940±31	13039±60
6	2315±79	4877±80	4537±89
9	2843±40	5998±75	1137±100
12	3134±74	6486±42	994±95
15	3322±19	6527±25	2579±29
18	3542±49	6308±51	4684±56
21	3930±20	6013±22	6418±40
24	4622±91	5828±57	7209±126
27	5753±119	5938±107	6803±80
30	7459±227	6529±132	5265±92
33	9875±145	7786±102	2979±150
36	13136±166	9894±112	646±158
39	17379±166	13040±112	0
42	22738±168	17408±113	
45	29349±173	23184±169	
48	37348±174	30553±188	
51	46869±181	39700±201	
54	58050±205	50812±203	
57	71024±207	64074±209	
60	85928±233	79670±211	

Table 2: Organisms production values of *Artemia* sp. per each experimental treatment.

Experimental treatment	Reproduction rate (Ro)	Cohort generational time (Tc)	Instantaneous growth rate (r)
	$\sum l_x m_x$	$\sum x l_x m_x / R_o$	$\log_e R_o / T_c$
Dunaliella sp.	42.25	15.24	0.24
Pinnularia sp.	39.18	15.36	0.23
Porphyridium sp.	8.62	21.90	0.13

5. Conclusions

Although *Artemia* sp. culture density growth was obtained with microalgae *Pinnularia* and *Dunaliella*, it is necessary to make mixed diets between them to obtain better results at final density and biomass. *Porphyridium* sp. microalgae cannot be used as a mono-culture food source in the culture system after 21 culture days. It is necessary to apply other microalgae like green or brown. To supply nutritional value.

6. References

1. Castro BT, De Lara AR, Castro MG, Castro MJ, Malpica SA. Alimento vivo en la acuicultura. Contactos. 2003;48:27-33.
2. Cisneros-Burga RE. Producción semi-intensiva de biomasa de *Artemia franciscana* kellogg 1906 (cepa Virrilá, Perú) utilizando diferentes dietas. Thesis. Universidad Nacional Mayor de San Marcos, Lima, Perú; c2002.
3. Cisneros R, Vinatea E. Producción de biomasa de *Artemia franciscana* Kellogg 1906 utilizando diferentes dietas. Ecología aplicada. 2009;8(1-2):9-14.
4. Sanabria-Jiménez S, Cárdenas-Hincapié JS, Fonseca AN, Seincobio S. Producción de alimento vivo como complemento nutricional para la conservación y el mantenimiento de peces ornamentales, Cultivando conocimiento: estrategia de acercamiento a la Investigación. 2015;97-106.
5. Garrido-Weber ER. Producción y Evaluación del Uso de la *Artemia salina* como Alimento Vivo en Alevinos de Tilapia Roja (*Oreochromis* spp). Limitaciones y oportunidades para el desarrollo del Caribe Colombiano. Centro Agroempresarial y Acuícola, SENA, Regional Guajira; c2018. p. 73-83.
6. Gaspar-Reyes W, Niño-Velásquez A, Alejos-Cabrera R, Ynga-Huamán G. Manual para la producción de *Artemia franciscana* como alimento para larvas y juveniles de peces. Inf. Inst. Mar Perú. 2021;48(1):35-49. https://hdl.handle.net/20.500.12958/3531.
7. Band-Schmidt CJ. Efecto de la composición bioquímica de microalgas sobre el valor nutritivo de dos cepas de *Artemia*. Doctoral Thesis dissertation. Instituto Politécnico Nacional. Centro Interdisciplinario de Ciencias Marinas; c1999. http://tesis.ipn.mx/handle/123456789/213.
8. Romero-López ME, Peeters J. Utilización del afrechillo de arroz en la producción de biomasa de *Artemia*. [Thesis]. Escuela Superior Politécnica del Litoral; 1999, 88. http://www.dspace.espol.edu.ec/handle/123456789/4601.
9. Godínez D, Gallo M, Del C, Gelabert R, Díaz A, Gamboa J, *et al*. Crecimiento larvario de *Artemia franciscana* (Kellog, 1906) alimentada con dos especies de microalgas vivas. Zootecnia Tropical. 2004;22(3):265-276.
10. Hernández-Pérez A, Labbé JI. Microalgas, cultivo y beneficios. Revista de biología marina y oceanografía. 2014;49(2):157-173.
11. Browne RA, Wanigasekera G. Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. Journal of Experimental Marine Biology and Ecology. 2000;244(1):29-44.
12. Balachandar S, Rajaram R. Influence of different diets on the growth, survival, fecundity, and proximate composition of brine shrimp *Artemia franciscana*

(Kellog, 1906). Aquaculture Research. 2019;50(2):376-389. https://doi.org/10.1111/are.13882.
13. Dhont J, Van Stappen G. Biology, tank production and nutrition value of *Artemia*. In J. Støttrup & L. McEvoy (Eds.), Live feeds in marine aquaculture; c2003. p. 65-121.
14. Seixas P, Coutinho P, Ferreira M, Otero A. Nutritional value of the cryptophyte *Rhodomonas lens* for *Artemia* sp. Journal of Experimental Marine Biology and Ecology. 2009;381:1-9. https://doi.org/10.1016/j.jembe.2009.09.007.
15. Dhont J, Dierckens K, Støttrup J, Van Stappen G, Wille M, Sorgeloos P. Rotifers, *Artemia*, and copepods as live feeds for fish larvae in aquaculture. In G. Allan & G. Burnell (Eds.), Advances in aquaculture hatchery technology; c2013. p. 157-202.
16. Arriaga Haro V, Re Araujo A. Uso de microalgas vivas e Inertes como alimento para *Artemia franciscana* (Anostraca: Artemiidae). Revista de Biología Tropical. 2015;45(2):803-811. https://revistas.ucr.ac.cr/index.php/rbt/article/view/20872. Consultado [13 de abril 2022].
17. Cunzhen S, Qing L, Xiuye L, Liangchen W. Studies on feeding brine shrimp with several kinds of algae. Nan kai da xue xue bao. Zi ran ke xue ban= Acta Scientiarum Naturalium Universitatis Nankaiensis. 1995;28(1):102-104.
18. Ribeiro VM, Da N. Contribuição para o conhecimento da biologia de *Artemia* sp. em aquacultura e na dinâmica daquele ecossistema. [Thesis] Assistente da Faculdade de Ciências da Universidade do Porto; c1989.
19. Castro-Mejia J, Castro-Mejia G, Castro-Barrera T, Monroy-Dosta MC, de Lara-Andrade R. Cultivo semiintensivo de *Artemia franciscana* proveniente de la salinera del Istmo, Juchitan, Oaxaca, bajo condiciones controladas en laboratorio. ContactoS. 2006;60:49-54.
20. Castro MG, Malpica SA, De Lara R, Castro MJ, Castro B. Técnicas de cultivo de especies planctónicas e invertebrados útiles para la acuicultura. Serie Académicos CBS Num. 37. Universidad Autónoma Metropolitana Unidad Xochimilco; c2001.
21. Nimura NK, Idris MM. Food utilization in Artemia for growth, reproduction, and maintenance. Fisheries Science. 1994;60;493-503.
22. Bernaerts TM, Gheysen L, Kyomugasho C, Kermani ZJ, Vandionent S, Foubert I, *et al*. Comparison of microalgal biomasses as functional food ingredients: Focus on the composition of cell wall-related polysaccharides. Algal Research. 2018;32:150-161. https://doi.org/10.1016/j.algal.2018.03.017.
23. Dan S, Yamashita K, Matsunari H, Nagakura K, Hamasaki K. The capability of *Artemia* to digest *Chlorella vulgaris* and *Nannochloropsis oculata* under stagnant culture conditions. Aquaculture Research. 2022;53:4316-4326. https://doi.org/10.1111/are.15929.
24. Fernández-Reiriz MJ, Pérez-Camacho A, Ferreiro MJ, Blanco J, Planas M, Campos MJ, *et al*. Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids, and fatty acids) of seven species of marine microalgae. Aquaculture. 1989;83(1-2):17-37.