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*Research Article*

# Effect of light and feed density on ingestion rate, protein and lipid content of *Artemia franciscana* juveniles

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**ABSTRACT.** *Artemia franciscana* is one of the most used organisms as live food to larvae of different marine species, so it is important to determine the appropriate food ration to improve its biochemical composition and maintain the nutritional stability of the *Artemia*. Furthermore, even though light is important in newly hatched nauplii, its effect in the late stages of *Artemia* have been poorly studied. This study aimed to evaluate the effect of food density (*Chaetoceros muelleri* microalgae) on the ingestion rate, protein, and lipid content of *A. franciscana* juveniles exposed and unexposed to light during 6 h. The experiment evaluated six treatments, resulting from combining three densities of *C. muelleri* as a food: 600,000, 900,000, and 1,200,000 cell $mL^{-1}$ and two illumination conditions: exposed and unexposed to the light. The ingestion rate was evaluated at 3 and 6 h, while protein and lipid content at the end of the experiment. The highest ingestion rates were observed in the treatment of 900,000 cell $mL^{-1}$ exposed to light at 3 h (253,118 cell $ind^{-1}$ $h^{-1}$) and unexposed to light at 6 h (164,712 cell $ind^{-1}$ $h^{-1}$). At the same time, the highest content of lipid (13.52 µg $ind^{-1}$) and proteins (38.28 µg $ind^{-1}$) per organism was obtained with the treatment of 900,000 cell $mL^{-1}$ unexposed to light. There was an interaction between cell density and light since both factors influenced the results of the ingestion rate and lipid content of the juveniles of *A. franciscana*.

**Keywords:** *Artemia franciscana*; *Chaetoceros muelleri*; ingestion rate; lipid; protein; aquaculture

## INTRODUCTION

The use of live food in aquaculture remains essential, especially during the larval and juvenile stages of many species of fish and crustaceans. It is frequently observed that commercial balanced foods do not satisfy the species nutritional requirements due to essential nutrients commonly having low availability and assimilation (Sales & Janssens 2003). Live food has several advantages over balanced food. Besides its high nutritional value, it can be nutritionally improved using compounds or ingredients (e.g. fatty acids, vitamins, minerals, antioxidants, probiotics, among others), which is incorporated by the prey and later consumed by the cultivated organisms, resulting in high assimilation rates, greater tolerance to diseases, and higher growth and survival rates (Ocampo et al. 2010, Sánchez-Estudillo 2011). Among the species of zooplankton, rotifers and *Artemia* are the most used organisms as live prey in aquaculture (Martínez-Córdova et al. 2014).

*Artemia* is widely used as live food for more than 85% of marine species, from nauplii to the adult stage (Lakshmana-Senthil et al. 2011). Its importance as food is because it tolerates various culture conditions is easily digested and also can be used as a carrier of interest compounds for the species in culture. Another attribute of *Artemia* is that its eggs can encyst and hatch whenever be necessary, and as a result, obtain large biomass in any season of the year (Viciano-Delibano 2015).

According to previous studies, the hatching of *Artemia* cysts is affected by lighting conditions (Lavens et al. 1986, Lavens & Sorgeloos 1987), such as light

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intensity, illumination periods, and wavelength of light (Browne & Wanigasekera 2000). Therefore, it is recommended to maintain a constant illumination of around 2000 lux during the hydration phase or the incubation of the cysts to maximize the efficiency of hatching (Sorgeloos 1973, Sharahi & Zarei 2016).

The effect of light throughout the life cycle of *Artemia* has been poorly studied, especially in late stages, so it is not clear how light affects the proximal composition or the ingestion rate of juveniles of *Artemia*. However, it has been observed that newly hatched organisms are attracted to the light during the first days, while from the fifth day, they move away from the light and concentrate in the non-illuminated area (Gallego-Galiano 2011).

On the other hand, the nutritional quality of *Artemia* can vary considerably due to geographic origin, differences between batches of cysts, and the analysis methods used (Leger et al. 1986). In addition, the food ration has been shown to affect the reproductive rate of *Artemia* and its protein, lipid, and carbohydrate content (Ronsón-Paulín 2010). Regarding the proximal composition of the juveniles of *Artemia*, in general, it has been observed that various strains fed with diets based on *Chaetoceros* sp. have a higher content of proteins (50%), lipids (15%), and carbohydrates (5%) compared to other microalgae (Lora-Vilchis et al. 2004, Shanmugam & Rajendran 2018). Also, higher than 90% survival percentages were obtained when *Artemia* was feeding with this microalgae genera (Arriaga & Re 1997, Lora-Vilchis 2004).

For all the above, the objective of this study was to evaluate the effect of food density of *Chaetoceros muelleri* microalgae on the ingestion rate and protein and lipid content in juveniles of *Artemia franciscana* exposed and unexposed to light.

## MATERIALS AND METHODS

### Culture of the juveniles of *Artemia franciscana*

The standard procedure of hydration-decapsulation-incubation of the cysts was followed to obtain the nauplii (Great Salt Lake *Artemia*, INVE Aquaculture, lot 7116496181), applying some of the recommendations proposed by Sorgeloos et al. (1986). In a transparent plastic container of 3 L for 1 h in fresh water and constant aeration were weighed and hydrated 1.5 g of cysts. Afterward, cysts were harvested with a 105 µm mesh and transferred to a 1 L container for decapsulation. Five percent commercial sodium hypochlorite at a rate of 125 mL of chlorine for every 10 g of cysts was used as a decapsulation solution.

Once the sodium hypochlorite was added, intense stirring was maintained for approximately 3.5 min, until the color of the cysts turned orange, thus indicating the degradation of the chorion. Immediately afterward, the cysts were flushed with fresh water to remove residual chlorine and avoid any damage. The decapsulated cysts were incubated for 24 h in a conical transparent plastic container, with a capacity of 1.5 L of seawater at 35 of salinity, constant aeration, and lighting (2000 lux) at 28°C. Then, the newly hatched *A. franciscana* was harvested and moved to a 14 L circular container at a density of 2 ind $mL^{-1}$.

According to the feeding rate obtained from previous studies, the *A. franciscana* was fed with *Chaetoceros muelleri* microalgae for eight days for its growth (Table 1). Likewise, a 30% daily exchange was carried out with seawater to eliminate the organic matter residues in the container. In addition, samples of the *A. franciscana* were taken at zero and nine days to determine the initial and final length of the individuals.

### Ingestion rate (IR) of *Artemia franciscana* juveniles

Juveniles of *A. franciscana* were transferred to 18 experimental 1 L units of seawater at a rate of 0.5 ind $mL^{-1}$ to carry out the IR experiment. The experiment evaluated six treatments, resulting from combining three densities of *C. muelleri* as a food: 600,000, 900,000, and 1,200,000 cell $mL^{-1}$ and two illumination conditions: exposed (EL) and unexposed (UL) to the light. For the EL treatments, the lighting was continuous and supplied by two white light fluorescent lamps of 2000 lux of intensity. The containers did not have any lighting in the UL treatments and were protected from light using a black curtain. In addition, there were six control treatments, which consisted of the combination of the mentioned factors without the juveniles of *A. franciscana*, to evaluate the reproduction of the microalgae in the experimental units and thus be able to correct the IR readings of *A. franciscana* juveniles.

**Table 1.** Feeding for *Artemia franciscana* fed with the microalgae *Chaetoceros muelleri* (CH-M-1).

| Day | CH-M-1(cell $mL^{-1}$) |
|---|---|
| 0 | 100,000 |
| 1 | 150,000 |
| 2 | 200,000 |
| 3 | 250,000 |
| 4 | 300,000 |
| 5 | 400,000 |
| 6 | 550,000 |
| 7 | 650,000 |
| 8 | 750,000 |

The treatments were carried out in triplicate. Therefore, there were 36 units: 18 units (9 EL and 9 UL) corresponding to the treatments with *A. franciscana* and 18 (9 EL and 9 UL) control units. It is important to mention that, before starting the experiment, it was verified that the intestine of the organisms was empty.

The experiment lasted 6 h; water samples were taken at 3 and 6 h from the treatments to perform microalgae counts to determine the ingestion rate. Furthermore, at the end of the experiment, samples were taken to determine the protein and lipid concentration of the juveniles of *A. franciscana*.

The ingestion rate was determined using the formula proposed by Paffenhofer (1971):

$$IR = \frac{V\ (Co - Ct)}{nt}$$

where IR: ingestion rate (cell ind$^{-1}$ h$^{-1}$), V: volume per experimental unit (mL), Co: cell density of the control treatment (cell mL$^{-1}$), Ct: cell density of the sample (cell mL$^{-1}$), n: number of individuals per experimental unit, t: time (h).

### Proximal composition analysis

The protein content was determined with the method of Lowry et al. (1951) using 1 N sodium hydroxide for 30 min to the extraction, and the extraction of total lipids was carried out according to the methodology proposed by Bligh & Dyer (1959) and its quantification was made with the method of Pande et al. (1963).

### Statistical analysis

To the data obtained from the ingestion rate and the proximal composition (proteins and lipids), the Lilliefors normality test and Bartlett's homoscedasticity test (Zar 2010) were applied to define the application of parametric statistical analysis methods or non-parametric. As the data met the statistical assumptions, two-factor analysis of variance was applied to determine statistical differences between the treatments. When the analysis revealed significant differences, the Student-Newman-Keuls (SNK) multiple comparison test was applied to determine these differences. The effect per factor (density of food and light) is presented as the averages of the data. All statistical analyzes were made in the SigmaStat 3.5 program, with a significance level of 5%.

## RESULTS

The newly hatched nauplii of *Artemia franciscana* registered an average length of 0.23 ± 0.01 mm, while, at the end of the culture, the juveniles reached an average length of 3.53 ± 0.12 mm with 90% survival on day 9.

### Ingestion rate (IR)

The IR was influenced by the food density as well as the light exposition since the interaction between the two factors (cell density and light) was observed in both samplings: at 3 h ($P$ = 0.013) and 6 h ($P$ = 0.029).

The highest IR of the *A. franciscana* juveniles at 3 h was recorded on the 900,000 cell mL$^{-1}$ treatment exposed to the light (EL), reaching an ingestion rate of 253,118 ± 12,123 cell ind$^{-1}$ h$^{-1}$, which showed significant differences with the other treatments (Table 2). In contrast, the lowest ingestion rates were recorded in the 600,000 cell mL$^{-1}$ of both treatments EL and UL, and the 1,200,000 cell mL$^{-1}$ unexposed to light treatment, whose values were significantly lower than those registered by the other treatments.

When the effect of light was evaluated, it was found that the highest ingestion rates were obtained on treatments EL, which were significantly higher than those obtained on treatments UL. On the other hand, the effect of cell density revealed that the highest IR was obtained when the juveniles of *A. franciscana* were fed with 900,000 cell mL$^{-1}$, with significant differences ($P$ < 0.001) concerning the other cell densities.

Regarding 6 h, the highest ingestion rate for juveniles of *A. franciscana* was obtained with the treatment of 900,000 cell mL$^{-1}$ and UL, achieving a consumption of 164,712 ± 3,593 cell ind$^{-1}$ h$^{-1}$, which was significantly higher than the other treatments (Table 2).

On the contrary, the lowest ingestion rate was in the 1,200,000 cell mL$^{-1}$ and EL treatments. This value was significantly lower ($P$ < 0.001) than those registered by the other treatments. When the effect of lighting was evaluated, it was observed that the highest IR was obtained on treatments UL, being significantly higher than those obtained on EL. In addition, the density of 900,000 cell mL$^{-1}$ registered the highest values of IR in the juveniles, showing significant differences compared to the other cell densities.

### Lipid and protein content of the juveniles of *A. franciscana*

Both cell density and illumination influenced the results obtained from lipid content since an interaction ($P$ = 0.038) was found between the two factors. The highest content of lipids per organism was obtained with the treatment of 900,000 cell mL$^{-1}$ and UL, reaching a concentration of 13.52 ± 0.10 µg ind$^{-1}$, which showed significant differences with the other treatments (Table 3). In contrast, the lowest lipid content was registered

**Table 2.** Ingestion rate (cell ind$^{-1}$ h$^{-1}$) of juveniles of *Artemia franciscana*, fed with three cell densities of *Chaetoceros muelleri* and exposed (EL) and unexposed (UL) to light, after 3 and 6 h. Results are expressed as the mean ± standard error (n = 3). Different superscript letters between the two factors (cell density and light) indicate significant differences ($P < 0.05$). Two-way ANOVA and SNK tests.

| Time | | Cell density (cell mL$^{-1}$) | | | Average |
|---|---|---|---|---|---|
| | | 600,000 | 900,000 | 1,200,000 | |
| 3 h | EL | 129,833 ± 10,191$^{a}$ | 253,118 ± 12,123$^{c}$ | 221,799 ± 17,150$^{b}$ | 201,583 ± 19,688$^{b}$ |
| | UL | 124,993 ± 1,993$^{a}$ | 194,181 ± 2,482$^{b}$ | 148,910 ± 7,419$^{a}$ | 156,028 ± 10,408$^{a}$ |
| | Average | 127,413 ± 4,768$^{a}$ | 223,649 ± 14,294$^{c}$ | 185,354 ± 18,316$^{b}$ | |
| *P*-value | | | | | |
| cell mL$^{-1}$ effect | | <0.001 | | | |
| Light effect | | <0.001 | | | |
| cell mL$^{-1}$ *vs.* light | | 0.013 | | | |
| 6 h | EL | 66,767 ± 6,052$^{b}$ | 134,389 ± 10,239$^{c}$ | 45,413 ± 2,433$^{a}$ | 82,190 ± 13,859$^{a}$ |
| | UL | 121,292 ± 2,502$^{c}$ | 164,712 ± 3,593$^{d}$ | 65,201 ± 5,482$^{b}$ | 117,068 ± 14,544$^{b}$ |
| | Average | 94,030 ± 12,539$^{b}$ | 149,550 ± 8,338$^{c}$ | 55,307 ± 5,174$^{a}$ | |
| *P*-value | | | | | |
| cell mL$^{-1}$ effect | | <0.001 | | | |
| Light effect | | <0.001 | | | |
| cell mL$^{-1}$ *vs.* light | | 0.029 | | | |

**Table 3.** Lipid and protein content (µg ind$^{-1}$) of juvenile *Artemia franciscana* fed with three cell densities of *Chaetoceros muelleri* exposed (EL) and unexposed (UL) to light, after 6 h. Equal letters indicate that there are no significant differences. Results are expressed as the mean ± standard error (n = 3). Different letters between the two factors (cell density and light) indicate significant differences ($P < 0.05$). Two-way ANOVA and SNK tests.

| | | Cell density (cell mL$^{-1}$) | | | Average |
|---|---|---|---|---|---|
| | | 600,000 | 900,000 | 1,200,000 | |
| Lipid | EL | 9.57 ± 0.15$^{b}$ | 12.10 ± 0.08$^{d}$ | 8.73 ± 0.11$^{a}$ | 10.13 ± 0.51$^{a}$ |
| | UL | 11.30 ± 0.13$^{c}$ | 13.52 ± 0.10$^{e}$ | 9.72 ± 0.17$^{b}$ | 11.52 ± 0.55$^{b}$ |
| | Average | 10.43 ± 0.40$^{b}$ | 12.81 ± 0.32$^{c}$ | 9.23 ± 0.24$^{a}$ | |
| *P*-value | | | | | |
| cell mL$^{-1}$ effect | | <0.001 | | | |
| Light effect | | <0.001 | | | |
| cell mL$^{-1}$ *vs.* light | | 0.038 | | | |
| Protein | EL | 33.55 ± 0.36$^{a}$ | 36.41 ± 0.50$^{a}$ | 31.37 ± 0.37$^{a}$ | 33.78 ± 0.76$^{a}$ |
| | UL | 35.89 ± 0.32$^{a}$ | 38.28 ± 0.36$^{a}$ | 33.19 ± 0.36$^{a}$ | 35.79 ± 0.76$^{b}$ |
| | Average | 34.72 ± 0.57$^{b}$ | 37.34 ± 0.50$^{c}$ | 32.28 ± 0.47$^{a}$ | |
| *P*-value | | | | | |
| cell mL$^{-1}$ effect | | <0.001 | | | |
| Light effect | | <0.001 | | | |
| cell mL$^{-1}$ *vs.* light | | 0.761 | | | |

in the treatment of 1,200,000 cell mL$^{-1}$ and EL, whose value was significantly lower than those registered by the other treatments. An effect per factor was also observed; the lipid content was significantly higher on treatments UL. Likewise, it was registered that the highest lipid content was obtained when the juveniles were fed with the density of 900,000 cell mL$^{-1}$. In the case of proteins, there was no interaction ($P = 0.761$) between cell density and illumination (Table 3).

However, the main protein content was observed on the treatment of 900,000 cell mL$^{-1}$, in both condition EL and UL, compared with the low and highest density of the food. On the other hand, an effect per factor was recorded; the protein content on the 900,000 cell mL$^{-1}$ treatment was significantly higher than the other treatments, and when the illumination factor was evaluated, it was observed that the highest protein content was obtained on the treatments UL.

## DISCUSSION

The results of survival (90%) and growth of *Artemia franciscana* (3.53 ± 0.12 mm) fed with *Chaetoceros muelleri* in this study were superior to those found by Rivera-Reyes (2013). They obtained a survival of 78.9% and an average length of 2.80 mm at day 11 of culture with the same species but fed with a cell density of 120,000 cell $mL^{-1}$ of *C. muelleri*, which could affect the growth and the length of the *Artemia*. Likewise, in this work, the length recorded at day 9 was greater than that reported by Lora-Vilchis (2004) for *A. franciscana* fed with 900,000 cell $mL^{-1}$ of *C. muelleri* the day 7, who obtained a growth of 2.81 mm of total length; as well as a survival superior to 89%, which was similar in both studies.

It was observed, in both illumination conditions, that the IR of *A. franciscana* juveniles was higher at the first sampling (3 h), and as time passed, it tended to decrease, obtaining lower IR values during the second sampling (6 h). At 6 h, the IR on EL treatments decreased around 51% in the density of 600,000 cell $mL^{-1}$, 53% at 900,000 cell $mL^{-1}$, and 80% for 1,200,000 cell $mL^{-1}$ compared to the IR at 3 h. Nevertheless, on the UL treatments, the IR at 6 h decreased 3, 15, and 56% for the cell densities of 600,000, 900,000, and 1,200,000 cell $mL^{-1}$, respectively, compared to the value at 3 h. The consumption decreased according to the time could be because the organisms consumed more food initially because they had an empty intestine and required food intake to reach their needs, which was not the case in the second sampling.

On the other hand, at 3 h, the IR was higher on the EL treatments, while at 6 h, the UL treatments registered the highest IR. However, during both samplings (3 and 6 h), the highest ingestion rate was obtained with the treatment of 900,000 cell $mL^{-1}$, so this cell density could be optimal to feed juveniles of *A. franciscana* regardless of the light condition. In this sense, determining the ingestion rate is very useful since it is an index that contributes to developing a good eating strategy. If the IR increases, this means that the amount of feed supplied must also increase. Contrarily, if the IR decreases, the amount of feed supplied should be reduced. An increment in IR may be a reflection of an improvement in the food supply (You et al. 2008).

At the highest cell density (1,200,000 cell $mL^{-1}$), the consumption was lower than the other densities, probably because concentration exceeded the consumption limit of the juveniles of *A. franciscana*. From the point of view of the consumer's functional response, the ingestion rate is directly proportional to the food concentration, up to the incipient limiting concentration, from which the ingestion rate becomes independent of the food concentration (Ramos-Jiliberto 1995).

The results of this study do not agree with the recorded by Lora-Vilchis (2004), who observed the ingestion rates of *A. franciscana* varied directly proportionally with the supplied ration, although the said author did not reach the consumption limit for *Artemia*. On the other hand, this behavior occurs only until reaching a critical concentration, above which ingestion remains constant or low, as observed in *Daphnia magna* (McMahon & Rigler 1963) and the copepod *Calanus pacificus* (Frost 1972).

It is worth mentioning that *A. franciscana* tended to be attracted to light during the first four days of cultivation, which changed from the fifth day as the organisms moved away. Although studies on the light effect on *Artemia* behavior are scarce, the results of this research are similar to those found by Gallego-Galiano (2011). He maintained a culture of *A. franciscana* for 15 days. He observed that these organisms presented a variable behavior against light throughout their development until they reached the adult stage, initially showing positive phototropism, followed by a negative phototropism phase, and finally positive phototropism. The observed behavior of *Artemia* according to age may be linked to the needs of the first larval stages, which look for surface waters with a greater presence of light and favorable temperatures to facilitate their development and allow them to feed on primary production (Cebrián & Valiela 1999). In addition to this, the development of *Artemia* requires the performance of several molts that allow the shedding of the chitinous exoskeleton and the increase of muscle mass and the development of secondary sexual characteristics until reaching the adult stage (Castrejón et al. 1993). During these molting phases, the organisms are particularly vulnerable, so the juveniles may show a negative phototropism in response to the search for deeper waters or refuge, which reduces such vulnerability against possible predators. Once the *Artemia* has reached adult size, it would show positive phototropic behavior, searching for the most illuminated layer where the phytoplankton that serves as food is concentrated (Isenmann 1975).

Regarding the proximal composition of *Artemia* sp. and *A. franciscana* starving, Guevara & Lodeiros (2003) analyzed the protein and lipid content of nauplii (24 h after hatched) and metanauplii (72 h after hatched) from cysts obtained from two different sites: Araya saltworks and San Francisco Bay. The authors found a higher protein proportion than lipids for both populations, which agrees with the results obtained in this study since proteins were more abundant than lipids. However, they also recorded a higher protein

concentration in metanauplii, while lipids were more abundant in nauplii. Studies support the hypothesis of the accumulation of proteins for structural and energetic effects in advanced stages, rather than lipids, which are used in the earliest phases such as naupliar. Nauplii present higher lipid content than metanauplii since they represent the form of energy available for the first hours of life since the digestive system is not functional (Katavic et al. 1985, Leger et al. 1986).

As mentioned above, in this study, the major components found in the *A. franciscana* juveniles were proteins and, to a lesser extent, lipids, which is in agreement with some authors (Lora-Vilchis et al. 2004, Sánchez-Saavedra & Paniagua-Chávez 2017), who found a similar proportion for these compounds. On the other hand, it was observed that both proteins and lipids, concentrations, coincided with the data of the ingestion rate since the organisms that presented the highest IR also presented the highest concentration of these compounds. The organic constitution is shown to be similar among microalgae, with proteins being the predominant components (Parsons et al. 1984, Lavens & Sorgeloos 1996). Such is the case of the microalgae *C. muelleri*, which has been characterized by having a higher concentration of proteins than lipids, registering values >20 and >10 pg cell$^{-1}$ for these compounds (Lemus et al. 2006, Piña et al. 2007), which agrees with the results obtained in this study for *A. franciscana* juveniles fed with this microalgae. Furthermore, *C. muelleri* is considered one of the most appropriate live diets for the culture of *A. franciscana* due to its optimal development and high survival rates when feeding it with this microalga (De Micco & Hubbard 2001). In turn, the *Artemia* fed with *C. muelleri* can improve its nutritional quality (Palma et al. 2011) and that of the organisms fed with this *Artemia*, resulting in good growth and a high survival rate (Widiastuti et al. 2012, Herawati et al. 2014).

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