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# BIOENCAPSULATION OF *Bifidobacterium animalis* AND *Lactobacillus johnsonii* IN *Artemia franciscana* AS FEED FOR CHARAL (*Chirostoma jordani*) LARVAE

# BIOENCAPSULACIÓN DE *Bifidobacterium animalis* Y *Lactobacillus johnsonii* EN *Artemia franciscana* COMO ALIMENTO DE LARVAS DE CHARAL (*Chirostoma jordani*)

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**Abstract**

An experiment was designed to evaluate the filling and effect of probiotic bacteria in metanauplii of *Artemia* as a vehicle and feed source for *Chirostoma jordani*, an endemic fish species with socio-economic importance and as a protein source for the population from the central region of México. The optimum filling of metanauplii of *Artemia* with *Lactobacillus johnsonii* and *Bifidobacterium animalis* was achieved in 40 min with a concentration of $2.0 \times 10^3$ CFU mL$^{-1}$ of viable cells of *L. johnsonii* and *B. animalis*, respectively, being enough to fill up in 40 min the metanauplii of *Artemia* with 628 cells of *B. animalis* and 688 cells of *L. johnsonii*. During the experiment, 20 metanauplii with or without probiotic bacteria were consumed per fish per day. The encapsulated probiotics promoted the growth and increased the weight and specific growth rate of the larvae of *C. jordani* compared with the control ($P < 0.05$). *L. johnsonii* had a better probiotic effect on metanauplii of *C. jordani* ($P < 0.05$).

*Keywords*: bacterial supplements, enriched live food, *Artemia*, charal, *Chirostoma jordani*, growth indicators.

**Resumen**

Se diseñó un experimento para evaluar la capacidad de ingestión de *Artemia* y el efecto de dos bacterias probióticas encapsuladas en metanauplios de *Artemia* como vehículo y fuente de alimento para larvas de charal *Chirostoma jordani*, especie endémica de importancia socioeconómica y, fuente de proteínas y de recursos económicos de los pobladores de la región central de México. La capacidad de llenado en metanauplios de *Artemia* se evaluó en 40 min, en agua enriquecida con células viables de *Lactobacillus johnsonii* y *Bifidobacterium animalis* en una concentración de $2.0 \times 10^3$ CFU ml$^{-1}$. Durante el experimento de alimentación de larvas, 20 metanauplii de *Artemia* con o sin bacterias fueron consumidos por pez por día. Los probióticos bioencapsulados en *Artemia* favorecieron el incremento en peso y talla así como tasa específica de crecimiento en larvas de *C. jordani*, en comparación al grupo control ($P$ <0,05). *Artemia* enriquecida con *L. johnsonii*, además mejoró significativamente la supervivencia larvas de *C. jordani* ($P$ <0,05).

*Palabras clave*: bacterias benéficas, alimento vivo enriquecido, *Artemia*, charal, *Chirostoma jordani*, indicadores de crecimiento.

## 1 Introduction

In the production of fish, metanauplii of *Artemia* are considered the best food for the diet of aquatic organisms in their early larval stages. *Artemia* is considered the most widely used food as feed in the larval stages or larval production due to its nutritional value and easy digestion (Tonheim *et al*., 2000). Regarding its nutritional composition, *Artemia* is considered a feed of high quality due to its protein content (57%) and fatty acids, which are essential for the nutrition of fish and crustaceans (Malpica *et al*., 2004; Luna *et al*., 2009).

Other attributes is the adaptability of *Artemia* to several culture media, easy handling and ability to incorporate materials such as nutrients, pigments, emulsions (Kyungmin *et al*., 2000), antibiotics (Rodríguez *et al*., 2011), microalgae, and bacteria

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(Subhadra *et al*., 2010) into its body. In aquaculture, bioencapsulation is a technique of enrichment of live prey widely used, which includes improvement living feed through the incorporation of microorganisms or substances inside rotifers, Cladocerans or *Artemia*, which function at the same time as vectors or carriers of such components facilitating the administration of its content to host (Saarela *et al*., 2000; Dey *et al*., 2015). *Artemia* can undergo encapsulation, which facilitates the administration of different products or microorganisms because it acts as a carrier or live capsule, allowing products to reach consumers directly while avoiding deterioration by water (Verschuere *et al*., 2000; Gelabert, 2003).

Probiotics have been widely used at the larval stages to control bacterial infections and the presence of potential pathogens instead of antibiotics in subtherapeutic doses (Monroy-Dosta *et al*., 2010); however, the use of antibiotics has increased the tolerance and virulence of the bacteria and generated significant economic losses (Verschuere *et al*., 2000) as well as making practices more environmentally friendly (Taoka *et al*., 2006). This fact has favored the use of probiotics, which in adequate concentrations, confer health benefits to the host (Irianto & Austin, 2002), whilst addressing global demands to reduce the use of antibiotics in animal production. According to Ziaei-Nejad *et al*., (2006) metanauplii of *Artemia* have been used as vehicles for the transport of probiotics in aquatic animals, to modify the properties of the intestinal microbiota of fish. Alterations in the intestinal microbiota are related to interactions between probiotics and pathogens mediated by production of hydrogen peroxide, organic acids and antimicrobial substances known as bacteriocins (Ringø *et al*., 2010).

The use of beneficial microorganisms or probiotics for the control of pathogens through several mechanisms is considered an alternative in animal production and as a way to decrease the dose of antibiotic treatment to prevent infectious diseases. The use of probiotics in human and animal nutrition is well documented (Fuller, 1992; Verschuere *et al*., 2000; Wang *et al*., 2008; Bidhan *et al*., 2014). Strains such as *L. johnsonni* and *B. animalis* subsp. *lactis* have been chosen for studies in animals as they are known to be safe for human use, which is of major importance because fish are meant for human consumption (Nikoskelainen *et al*., 2001; Rodríguez-Miranda *et al*., 2014).

In fish, these beneficial microorganisms or probiotics produce vitamins, amino acids and enzymes that improve digestibility, feed efficiency, performance and growth (Wang *et al*., 2008). The addition of probiotics in diets has been suggested as an alternative to improve the survival, welfare and growth of fish, including larval stages (Ringø and Gatesoupe 1998; Gómez-Gil *et al*., 1998; Verschuere *et al*., 2000; Wang *et al*., 2008). Furthermore, produce organic acids as metabolic products that inhibit the growth of pathogenic bacteria, and also antibacterial substances such as bacteriocins. Lactic acid bacteria are a common bacterial probiotic for animal and fish production. In the case of *Bifidobacteria*, there is not enough information about its effect on fish production; there has only been found information regarding trout (Kopečný *et al*., 2010). Lactic acid bacteria and *Bifidobacteria* have $\alpha$-and $\beta$-glucosidase, $\beta$-galactosidase, $\beta$-fructofuranosidase and phytase activities, among others, that help with good intestinal colonization (Hayek *et al*., 2013, Pokusaeva *et al*., 2011). For good intestinal colonization, these bacteria also show good tolerance to acidic conditions and bile acids. Some of these properties of probiotics have been shown in a few species of fish, mainly species such as trout (Araújo *et al*., 2015), but not in small animals by its reduced size. All of these properties are critical for conferring health benefits on the host (Ren *et al*., 2014). The addition of probiotics in feed has shown a significant increase in the yield of freshwater species such as goldfish (Ahilan *et al*., 2004) and swordfish green swordtail with the use of probiotic products commercially available compounds of *Saccharomyces cerevisiae* and/or *Lactobacillus sporogens*, *L. acidophilus*, *Bacillus subtilis*, *B. licheniformis*, *Streptococcus faecium* (Abraham *et al*., 2007). In angelfish *Pterophyllum scalare* improvements in growth they were obtained with the addition of bacilli *B. licheniformis*, *B. subtilis*, *B. polymyxa*, *B.* and *B. circulans laterosporas* (Farahi *et al*., 2011).

Among the wild species that require an enrichment of live feed during growth are the endemic fish well known as "charal" (*Chirostoma jordani*), which represents a fishery resource because it forms the basis of artisanal fisheries from the central region of Mexico (Martínez *et al*., 2006). Although this species has not been adequately studied, they have an economic impact on fishing communities living of this resource. Although there have been several efforts to cultivate this species, unfavorable results have been obtained, mainly due to its high mortality during the early stages of growth, caused by the immaturity of the digestive tract and immune system,

and gastrointestinal disorders related to environmental microbiota (Hernández-Rubio *et al*., 2006). The aim of the present study was to encapsulate probiotic bacteria inside of metanauplii of *Artemia* and to test the effect of these probiotics on the survival and larval growth of *C. jordani*i.

# 2 Material and methods

## 2.1 Microorganisms

The probiotic strains used were *Bifidobacterium animalis* sp. lactis ATCC27536 and *Lactobacillus johnsonii* generously donated by D. Roy and P. Ward from Food Research and Development Centre, Saint-Hyacinthe, Québec, Canada. The growth medium for the inocula of *B. animalis* and *L. johnsonii* was prepared in 50 mL vials with 38 mL TPYG culture medium, which consisted of the following formulation (in $gL^{-1}$): trypticase peptone, 10; phytone peptone, 5; yeast extract, 2.5; glucose, 5; cysteine HCl, 0.5; $K_2HPO_4$, 2; $MgCl_2.6H_2O$, 0.5; $ZnSO_4.7H_2O$, 0.25; $CaCl_2$, 0.15; $FeCl_3$, 0.03; and 1 mL Tween 80. The pH was adjusted to 6.9-7.0. The oxygen was displaced from the culture medium by gassing with $CO_2$ for 1 min. The vials used were then sealed with a rubber stopper to be sterilized. The strains were incubated at 37 °C at 200 rpm for 24 h in an orbital incubator (Gellemkamp, UK). Cell concentrations were determined in all cases by decimal dilutions in MRS agar (Difco, Becton Dickinson and Company, USA) supplemented with 0.05% L-cysteine, 0.02% $NaCO_3$ and 0.01% $CaCl_2$. Plates were incubated in an anaerobic chamber (Thermo Scientific, USA) with a gas mixture of $CO_2$ (10%) hydrogen (5%) and nitrogen (85%) at 37 °C for 72 h.

## 2.2 Metanauplii of Artemia

For the experiment, cysts of *Artemia franciscana* (INVE Aquaculture NV, Great Salt Lake Utah, USA), which were hatched in containers with 3 L of water at 30 g $L^{-1}$ salinity, room temperature (28 ºC), light (2,000 lux) and constant aeration (Castro *et al*., 2003), were used. After 48 h of hatching, the metanauplii stage was used for bioencapsulation.

## 2.3 Artemia enrichment procedure - bioencapsulation assay

The *Artemia* nauplii were stocked for enrichment at a density of 3 nauplii per 1 mL of fresh water disinfected with sodium hypoclorite (NaClO), filtered with activated carbon and ionized (Magna Power Ionizer, México). Total nauplii were enriched with $2.3\times10^3$ CFU $mL^{-1}$ of *L. johnsonii* or $2.06\times10^3$ CFU $mL^{-1}$ of *B. animalis* and one control for each probiotic without *Artemia*. The bioencapsulation media were homogenized gently with continuous agitation to prevent settling of the cells. Encapsulation of bacteria by *Artemia* was evaluated indirectly by quantifying the amount of remaining microorganisms in CFU $mL^{-1}$ after 0, 10, 20, and 40 min by taking 100 $\mu$L of the enrichment medium using the pour-plate method with MRS agar (Difco, USA). The plates were incubated at 37 °C in an anaerobic chamber (Thermo Forma USA). In each sample, the metanauplii were observed under the microscope (Olympus ZX12) to verify the filling of the digestive tract at the three times tested. All experiments were conducted in triplicate.

## 2.4 Feeding trials - bioencapsulated probiotic bacteria in Artemia as feed for fish larvae

The growth experiment with *C. jordani* larvae and probiotic bacteria lasted 90 days. Treatments were carried out in triplicate: *Artemia* with cells of *L. johnsonii*, *Artemia* with cells of *B. animalis*, and *Artemia* without bacteria. Each treatment consisted of 30 fish larvae, 45 days old, randomly distributed into three plastic containers, which contained 8 L of saline water. Fish were fed *ad libitum* three times per day (9:00, 13:00, and 17:00 h) with the rotifer *Brachiojus plicatilis*, supplementing the diet with the bacterial strains bioencapsulated in *Artemia* (20 metanauplii per fish per day); 40 min after feeding, feces and uneaten live feed were removed. The larval culture was maintained with continuous light and aeration at a temperature of 22 ± 1 °C. The mean values determined for the water were as follows: pH 7.9 ± 0.2, dissolved oxygen 4.8 ± 0.3 mg $L^{-1}$, nitrates 0.4 ± 0.2 mg $L^{-1}$, and ammonium 0.05 ± 0.4 mg $L^{-1}$ (Multiparameter Photometer, Hanna Instruments Hi83203). Indicators of fish growth as wet weight and total length were recorded monthly with an analytical balance (precision ± 0.0001 g) and a digital Vernier caliper (accuracy ± 0.01 mm). The total daily length ($L_t$), daily weight ($W_t$), specific growth rate ($SGR$) and larval survival was determined as follows: $L_t$ increase = [(final $L_t$− initial $L_t$)/no. days], $W_t$ increase = [(final $W_t$− initial $W_t$)/no. days], specific growth rate $(SGR)_L$ = [(Ln $L_t$ final − Ln $L_t$ initial)/no. days × 100], $SGR_W$ = [ (Ln $W$ final − Ln $W$ initial)/no. days × 100]. The

survival percentage was calculated as survival (%) = $[(N_f - N_i) \times 100]$, where Ni is the number of larvae at the beginning of the experiment and $N_f$ is the number of larvae at a subsequent particular time.

### 2.5 *Statistical analysis*

The results of bacterial concentrations (CFU $mL^{-1}$) consumed by *Artemia* and growth parameters of fish larvae were analyzed by one-way analysis of variance ($P < 0.05$). The comparison of means was performed with the Tukey test using SYSTAT 10.2, Software Inc. (Chicago USA). Survival results were analyzed with the non-parametric Kruskal-Wallis test ($\alpha$ = 0.05), a comparison of means was performed using the Nemenyi test (Zar, 1999).

# 3 Results

### 3.1 *Bioencapsulation of bacteria in metanauplii of Artemia*

During the intake assay, Figure 1 shows that in the first 10 min, the remaining *B. animalis* in the bioencapsulation assay was 212 CFU $ml^{-1}$ for fill up the metanauplii of *Artemia*. For *L. johnsonii* 10 additional minutes were necessary to fill up each metanauplii of *Artemia*. In both experiments, from minute 20 to the end, there were no significant changes in the concentration of bacteria in the enrichment water. At the end of the experiments, the remaining microorganisms were 176 ± 11 CFU $mL^{-1}$ of *B. animalis* and 286 ± 20 CFU $mL^{-1}$ of *L. johnsonii*. These means that each metanauplii was filled up with 628 cells of *B. animalis* and 688 cells of *L. johnsonii* in 40 min. Furthermore, significant differences between encapsulated *B. animalis* ($P < 0.05$) with respect to *L. johnsonii* (Fig. 1) were observed. The loading of metanauplii with probiotic bacteria resulted in a lack of transparency in the body (Fig. 2). It was also observed that the alimentary canal was initially empty and began to fill after initiating encapsulation. The metanauplii with or without probiotic bacteria were used as feed for the larvae of *C. jordani* for a period of 90 days. No changes in bacterial survival were found in the control without *Artemia*.

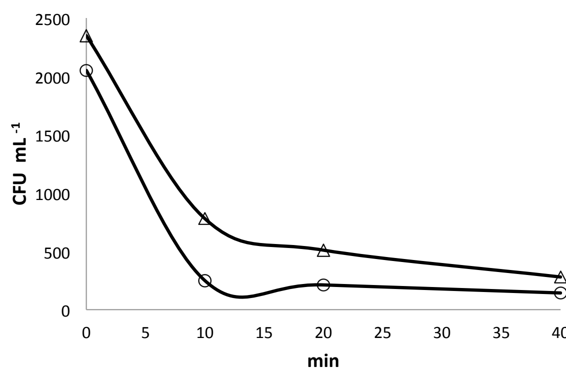


Fig. 1. Kinetics of the disappearance of probiotic bacteria by filtration of the metanauplii of *Artemia*. *Bifidobacterium animalis* (o) and *Lactobacillus johnsonii* (△).

### 3.2 *Effect of encapsulated probiotics in the larvae of C. jordani*

The probiotic *B. animalis* and *L. johnsonii* encapsulated in *Artemia* promoted the growth of larvae of *C. jordani* in the first 30 days of cultivation (Table 1). *L. johnsonii* showed higher growth ($P <$ 0.05) regarding the total larval length (1.61 ± 0.03 cm), weight increase (0.0024 ± 0.0002 g $day^{-1}$), and TCE (0.06% ± 1.71 $day^{-1}$) than the group of larvae supplemented with *B. animalis* and that consisting of *Artemia* without probiotics. The presence of both probiotics significantly improved the response of all growth parameters ($P < 0.05$) after 60 and 90 days of administration compared with the group that was only administrated with *Artemia*, indicating that probiotic supplementation greatly improves the utilization of nutrients for the development of larvae compared with the control diet.

The administration of encapsulated probiotic in metanauplii of *Artemia* during the first 30 days did not significantly increase the survival of *C. jordani* larvae ($P > 0.05$). However, significant differences in larvae supplemented with *L. johnsonii* and *B. animalis* were observed after 60 and 90 days, compared with larvae without probiotic supplementation (Fig. 3).

# 4 Discussion

The use of encapsulated probiotics in *Artemia* has shown positive effects in aquaculture, due to the active inhibition of the colonization of the digestive tract by pathogenic bacteria through mechanisms of antagonism and competition for nutrients and space

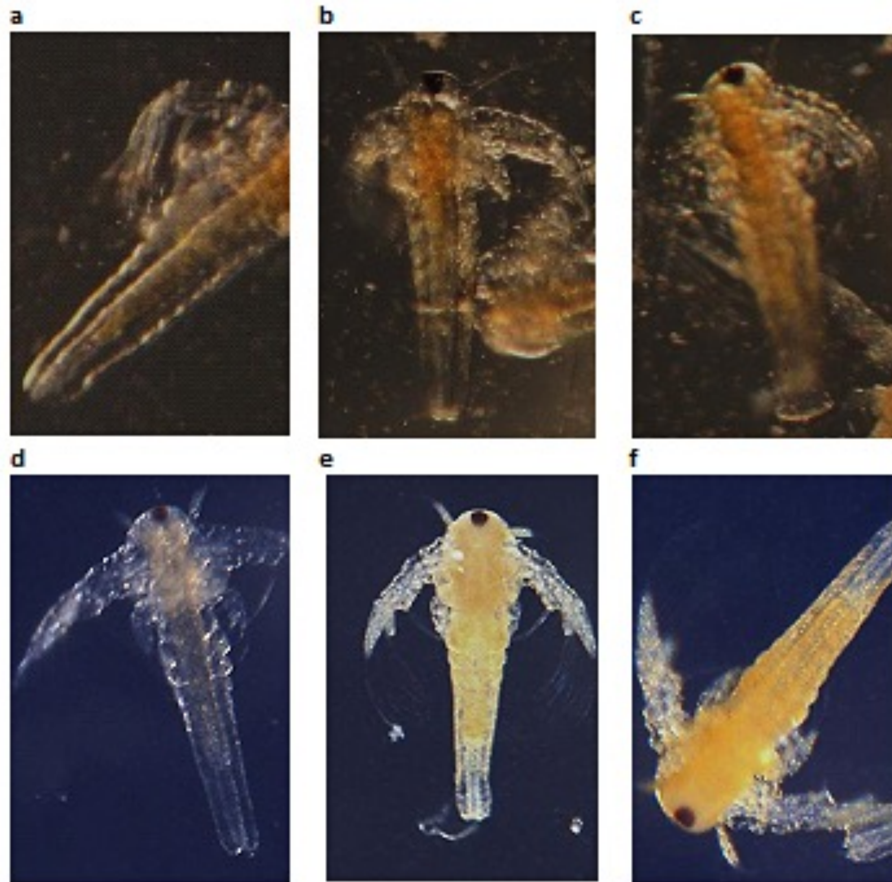


Fig. 2: Metanauplii of *Artemia* in liquid medium with cells of *Lactobacillus johnsonii* at a concentration of $2.3\times10^3$ CFU $mL^{-1}$ (a) at the beginning of bioencapsulation, (b) after 20 min and (c) after 40 min. Metanauplii with cells of *Bifidobacterium animalis* at a concentration of $2.06\times10^3$ CFU $mL^{-1}$, (d) at the beginning of bioencapsulation, (e) after 20 min, (f) after 40 min.

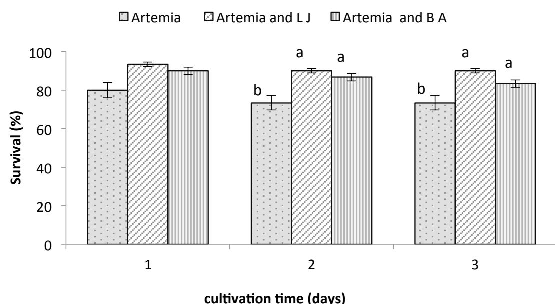


Fig. 3: Survival of *Chirostoma jordani* fed with bioencapsulated bacteria in *Artemia* in the metanauplii phase. *Artemia* without probiotic. *Artemia* and *Lactobacillus johnsonii* (LJ) *Artemia* and *Bifidobacterium animalis* (BA). Different letters above each group of bars indicate significant differences ($P < 0.05$).

(Garcés *et al*., 2015) and by the production of inhibitory compounds such as bacteriocins, thereby increasing the immune system and growth and survival of the host (Nikoskelainen *et al*., 2001; Martínez-Cruz *et al*., 2012). In fish production systems, the use of bioencapsulated probiotic bacteria in *Artemia* and rotifers has increased to protect cod larvae (Lauzon *et al*., 2008) and trout larvae (Ramos *et al*., 2013) from opportunistic and pathogenic infections and the use of molecules encapsulated to control infections in shrimp larvae (Subhadra *et al*., 2010). According to Ziaei-Nejad *et al*. (2006) metanauplii of *Artemia* have been used as vehicles for the transport of probiotics in aquatic animals to modify the properties of the intestinal microbiota of fish. Castro *et al*. (2005) incorporated *L. casei* into metanauplii of *Artemia* to feed to the ornamental fish *Astronotus ocellatus* and *Pterophyllum scalare* for 9 weeks.

Table 1. Effect of encapsulated probiotic bacteria in *Artemia* on the growth of *C. jordani*

| Variables | Treatments (n = 30) | | | P |
|---|---|---|---|---|
| | *Artemia* SP | *Artemia* and LJ | *Artemia* and BA | |
| Initial length (cm) | 1.43 ± 0.12 | 1.39 ± 0.15 | 1.40 ± 0.10 | |
| Initial weight (g) | 0.024 ± 0.004 | 0.020 ± 0.007 | 0.021 ± 0.001 | |
| | | 30 days | | |
| Length (cm) | 1.48b ± 0.03 | 1.61 a ± 0.03 | 1.54ab ± 0.06 | 0.008 |
| Increase in length (cm $day^{-1}$) | 0.0018c ± 0.00 | 0.0071a ± 0.01 | 0.0047b ± 0.002 | 0.002 |
| TCE in length (% $day^{-1}$) | 0.04b ± 0.02 | 0.13a ± 0.03 | 0.08ab ± 0.05 | 0.006 |
| Weight (g) | 0.08 ± 0.003 | 0.09 ± 0.005 | 0.06 ± 0.03 | 0.052 |
| Increase in weight (g $day^{-1}$) | 0.0018b ± 0.0002 | 0.0024a ± 0.0002 | 0.0019ab ± 0.00 | 0.026 |
| TCE in weight (% $day^{-1}$) | 1.29b ± 0.07 | 1.71a ± 0.06 | 1.46b ± 0.1 | 0.003 |
| | | 60 days | | |
| Length (cm) | 2.28b ± 0.05 | 2.67a ± 0.06 | 2.53a ± 0.08 | 0.003 |
| Increase in length (cm $day^{-1}$) | 0.014b ± 0.001 | 0.021a ± 0.001 | 0.020a ± 0.002 | 0.001 |
| TCE in length (% $day^{-1}$) | 0.52b ± 0.04 | 0.69a ± 0.02 | 0.63a ± 0.04 | 0.001 |
| Weight (g) | 0.12b ± 0.01 | 0.15a ± 0.004 | 0.14a ± 0.01 | 0.0002 |
| Increase in weight (g $day^{-1}$) | 0.0016b ± 0.0002 | 0.0021a ± 0.0001 | 0.0020a ± 0.0002 | <0.0001 |
| TCE in weight (% $day^{-1}$) | 1.77c ± 0.11 | 2.21a ± 0.001 | 2.11b ± 0.001 | <0.0001 |
| | | 90 days | | |
| Length (cm) | 3.14b ± 0.07 | 3.71a ± 0.02 | 3.37b ± 0.04 | 0.001 |
| Increase in length (cm $day^{-1}$) | 0.019c ± 0.002 | 0.026a ± 0.001 | 0.022b ± 0.001 | 0.001 |
| TCE in length (% $day^{-1}$) | 0.87c ± 0.05 | 1.06a ± 0.02 | 0.95b ± 0.03 | 0.001 |
| Weight (g) | 0.20b ± 0.01 | 0.25a ± 0.01 | 0.22b ± 0.02 | 0.002 |
| Increase in weight (g $day^{-1}$) | 0.0019b ± 0.0003 | 0.0025a ± 0.0001 | 0.0022b ± 0.0002 | 0.001 |
| TCE in weight (% $day^{-1}$) | 2.33b ± 0.14 | 2.60a ± 0.03 | 2.43b ± 0.08 | 0.001 |

*Artemia* SP = *Artemia* without probiotic, *Artemia* LJ = *Artemia* and *L. johnsonii*, *Artemia* BA = *Artemia* and *B. animalis*.

TCE = Specific growth rate (%/day).

abDifferent letters in a row indicate significant differences ($P < 0.05$)

The filling of the digestive tract of metanauplii of *Artemia* of approximately 0.9 to 1.5 mm in size was performed in 40 min with a concentration of $2.3 \times 10^3$ CFU $mL^{-1}$ of *L. johnsonii* and $2.06 \times 10^3$ CFU $mL^{-1}$ of *B. animalis* in a liquid medium (Fig. 2). Both probiotics showed a similar kinetic of encapsulation (Fig. 1). The rapid decrease in probiotics in the system of bioencapsulation might be due to high filtration efficiencies and the exoskeleton of the metanauplii of *Artemia* could also accumulate microorganisms by simple adsorption (Rodríguez *et al*., 2011). Van-Hai *et al*. (2010) reported that 48 h was the optimum period of encapsulation for *Pseudomonas aeruginosa* and *P. synxantha* in *Artemia* using an inoculum of $10^5$ CFU $mL^{-1}$ of each probiotic. Gomez-Gil *et al*. (1998) and Makridis *et al*. (2000) reported studies of encapsulation of some bacteria in *Artemia* at a density of $10^3$ to $10^5$ CFU $mL^{-1}$, and Subhadra *et al*. (2010) reported the bioencapsulation of *E. coli* with a recombinant fluorescent protein in *Artemia* and found the highest expression at 10 h. These results are probably related to the size of the digestive tract. Gelabert (2003), reported that metanauplii of *Artemia* of small size (0.8-3.2 mm) exhibited high filtration efficiencies, despite the lower level of development in the filtering system at the early stages: this behavior appears to be associated with the structures and strategies of filtration in the early stages of metanauplii. Therefore, from the obtained results, it can be noted that the size of the microorganism and the concentrations of the particles, which allow an appropriate level of saturation of the digestive system, should be taken into account in bioencapsulation. In this case, both bacteria are approximately 5 to 10 $\mu$m long and 0.5 to 1.5 $\mu$m wide. Gomez-Gil *et al*. (1998) demonstrated that the encapsulation of bacteria in nauplii of *Artemia* strongly depends on the type of bacteria used, the exposure time and their status, dead or alive. Rodríguez *et al*. (2011) suggest that *Artemia* can incorporate substances in three ways: particle

absorption to incorporate them into their exoskeleton, particulate filtration into the digestive tract, or via both mechanisms.

The administration of probiotics encapsulated within metanauplii of *Artemia* significantly improved the survival of larvae of *C. jordani* at later stages of 60 and 90 days (Fig. 3). This larval survival might be due to the establishment of *L. johnsonii* and *B. animalis* in the gut of larvae or because the probiotics that colonize the gut might be dominant over harmful bacteria and trigger a saturation of the adhesion receptors (Venkat *et al*., 2004). Furthermore, the administration of the probiotic might significantly change the proportion of probiotic bacteria in the intestinal microbiota, thereby improving the immune response of fish and the water quality (Thompson *et al*., 1999; Verschuere *et al*., 2000), or by improving the nutrition of *C. jordani* larvae via the production of digestive enzymes from *Lactobacillus* (Hayek *et al*., 2013) and *Bifidobacterium* (Janer *et al*., 2005) and increasing the activity of the digestive tract of *C. jordani*.

In the present study, the encapsulated probiotic bacteria in *Artemia* significantly increased the overall length, weight, specific growth rate (Table 1), and survival (Fig. 3), indicating that the addition of *L. johnsonii* and *B. animalis* was effective compared with *C. jordani* larvae fed with *Artemia* without probiotics. Similar results have been reported in *M. rosenbergii* fed with bioencapsulated *L. cremoris* (Suralikar and Sahu, 2001). Gatesoupe (1994) also reported a weight increase in *Scophthalmus maximus* with lactic acid bacteria and encapsulated *Bacillus toyoi*. The significant increase in weight, length, and survival could be attributed to the synthesis of vitamin B and to the enzymatic contribution of probiotics in the gut of aquatic animals (Mondal *et al*., 2003; Abraham *et al*., 2007).

## Conclusion

In the present study, the use of encapsulated probiotics in the metanauplii of *Artemia* to feed larvae of *C. jordani* showed the potential of *L. johnsonii* and *B. animalis* for use as a feed additive in the manufacture of formulated diets because they can improve the length, weight and growth rate of aquatic animals. This is the one of the efforts that has been made to develop diets for *C. jordani* larvae in order to increase nutritional efficacy, weight gain, and survival, to establish commercial aquaculture of this endemic species and thereby try to reduce the pressure fisheries wild populations.

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