ECOSISTEMAS Y RECURSOS AGROPECUARIOS

Changes in mortality rates during the larval stage of the Pacific white shrimp (*Litopenaeus vannamei*) on the basis of algal (*Chaetoceros calcitrans* or *Tetraselmis suecica*) food density

Cambios en las tasas de mortalidad durante el estadio larval del camarón blanco del Pacífico (*Litopenaeus vannamei*) en base a la densidad de alimento algal (*Chaetoceros calcitrans* o *Tetraselmis suecica*)

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ABSTRACT. In the shrimp culture, larval stages have high mortality rates, particularly in the zoea stage, this because to the star of phytoplankton feeding and an inadequate microalga species and cell density used in commercial hatcheries. Several microalgae species are used as food in shrimp larviculture, and the most common are *Chaetoceros calcitrans* and *Tetraselmis suecica*. Therefore, in this study, we quantified changes in the mortality rates of zoea larvae of *L. vannamei* fed either *C. calcitrans* or *T. suecica* at different cell densities. Results showed higher mortality rates when fed *L. vannamei* larvae with *T. suecica* than *C. calcitrans*. This study demonstrates that when zoea begin to feed on phytoplankton, they are highly sensitive to the microalga diet and cell densities supplied, which significantly affect the survival of *L. vannamei* larvae.
Key words: Aquaculture, microalgae, shrimp larvae, zoea stage, zooplankton

RESUMEN. En el cultivo de camarón, los estadios larvales presentan altas mortalidades, particularmente en el estadio de zoea, debido al inicio de la alimentación fitoplanctófaga y principalmente por una inadecuada selección de la especie de microalga y de densidad celular suministrada como alimento en laboratorios de producción larvaria. Diversas microalgas son usadas como alimento en la larvicultura del camarón; las más comunes son *Chaetoceros calcitrans* y *Tetraselmis suecica*. En el presente trabajo se cuantificaron los cambios en las tasas de mortalidad de larvas en estadio de zoea de *L. vannamei* alimentadas con *C. calcitrans* o *T. suecica* a diferentes densidades celulares. Los resultados mostraron mayores mortalidades en larvas de *L. vannamei* cuando se alimentaron con *T. suecica* en comparación con *C. calcitrans*. Este estudio demostró que cuando las larvas en zoea comienzan a alimentarse de fitoplancton, son altamente sensibles al tipo de microalga y a la densidad celular suministrada, lo cual afecta significativamente la sobrevivencia de larvas de *L. vannamei*.
Palabras clave: Acuacultura, estadio de zoea, larvas de camarón, microalgas, zooplancton

INTRODUCTION

In Mexico, shrimp aquaculture is a highly profitable industry widely developed in the states of Sinaloa, Sonora, and Nayarit. The main farm-raised species in these regions is the Pacific white shrimp *Litopenaeus vannamei* (Boone 1931), for which have been developed intensive breeding and rearing techniques from the post-larval to adult stages. However, the larval stages of zoea have the highest mortalities in the shrimp life cycle, predominantly because to an inadequate selection of

microalgae species and cell densities used as food in commercial hatcheries (Godínez *et al*. 2005, Piña *et al*. 2006). Microalgae are widely used as the main source of food for larvae of farmed crustaceans, although several characteristics need to be considered, such as biochemical composition (fatty acids, amino acids, vitamins, and enzymes), digestibility, size, and shape, examples of suitable microalgae are *Isochrysis, Chaetoceros, Thalassiosira, Tetraselmis, Skeletonema, Chlorella*, and *Dunaliella* (Volkman *et al*. 1989, Brown *et al*. 1999, Núñez *et al*. 2002, Hemaiswarya *et al*. 2011).

Microalgae commonly used in hatcheries of Mexico for routine feeding protocols are *Chaetoceros calcitrans* and *Tetraselmis suecica* (Pérez-Morales *et al*. 2015); these microalgae are supplied without quantifying the cell density to feed shrimp larvae, which may cause high mortalities in the zoea stage (Godínez *et al*. 2005, Piña *et al*. 2006). Therefore, in this study, we quantified changes in the mortality rates of zoea of *L. vannamei* fed either *C. calcitrans* or *T. suecica* at different cell densities.

MATERIAL AND METHODS

Strains of microalgae used as the food source, *C. calcitrans* (8-12 μm) and *T. suecica* (30-35 μm) were grown at 23 $\pm$ 1 °C, 37 $\pm$ 1 ups, pH 8.0 $\pm$ 0.1, photoperiod of 12:12 h light:dark cycle, and artificial illumination of 150 μmol m^{-2} s^{-1} in 2-L bottles with gentle air influx and semi-continuous cultures using the f/2 medium; silicates were added for *C. calcitrans* and not added for *T. suecica* (Pérez-Morales *et al*. 2015). Both strains were cultured for several weeks prior to the bioassays. Cell density was estimated by direct counting with a Neubauer hemocytometer (Hausser Scientific); seawater previously autoclaved and filtered under a low vacuum with Whatman GF/F filters was used for dilutions.

Three batches of *L. vannamei* nauplii were donated by a local hatchery. In the laboratory, the three batches were mixed to eliminate the effect caused by progeny. Stage III nauplii (N III) were maintained in darkness at room temperature (26 °C). Nauplii vitality was verified by observing attraction to light. All nauplii that swam vigorously towards the light source were considered good candidates for the bioassay. These nauplii were incubated by adding one nauplius per well to 48 polystyrene microdilution well-plates with sterile Pasteur pipettes. In each well, 1 mL of different cell densities of *C. calcitrans* (0.045, 0.09, 0.25, 0.75, 2, and 4 $\times$ 10^6 cells mL^{-1}) or *T. suecica* (60, 90, 125, 180, and 250 $\times$ 10^3 cells mL^{-1}) was added. Each set of nauplii was incubated in triplicate at 23 °C and 37 ups of salinity in an acrylic incubator. Our experimental design was as follows: for *C. calcitrans*, we used 864 nauplii (6 cell densities $\times$ 3 replicates with 48 nauplii in each plate), and for *T. suecica*, we used 720 nauplii (5 cell densities $\times$ 3 replicates with 48 nauplii in each plate). In total, 33 polystyrene microdilution well-plates were used.

The plates were observed every day, and the survival of *L. vannamei* was quantified. The bioassay ended when nauplii reached the mysis stage (M I). Identification of the early development stages of *L. vannamei* was based on the descriptions reported by Kitani (1986). To find differences among treatments, mortality data for each microalga species used as food were statistically tested using two-way statistical analyses of variance (ANOVA, $p < 0.05$); for multiple comparisons, Tukey's post-hoc tests were performed (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

At the beginning of day three, the stage I zoea was observed, while stage I mysis was found at the end of day six in both microalgal bioassays. These results showed different mortality rates for zoea stages depending on the microalga supplied as feed. The *C. calcitrans* bioassay showed the highest mortality ($\sim$60 %) for the zoea stage at a cell density of 4 $\times$ 10^6 cells mL^{-1} (Figure 1 A); cell densities of 2, 0.09, and 0.045 $\times$ 10^6 cells mL^{-1} showed mortalities close to 40 %. A lower mortality rate ($\sim$15 %) was observed at cell densities of 0.25 and 0.75 $\times$ 10^6 cells mL^{-1}. In the *T. suecica* bioassay, mortality rate of zoea increased from 4 % to 92 % after day two until day four at a cell density of 250 $\times$ 10^3

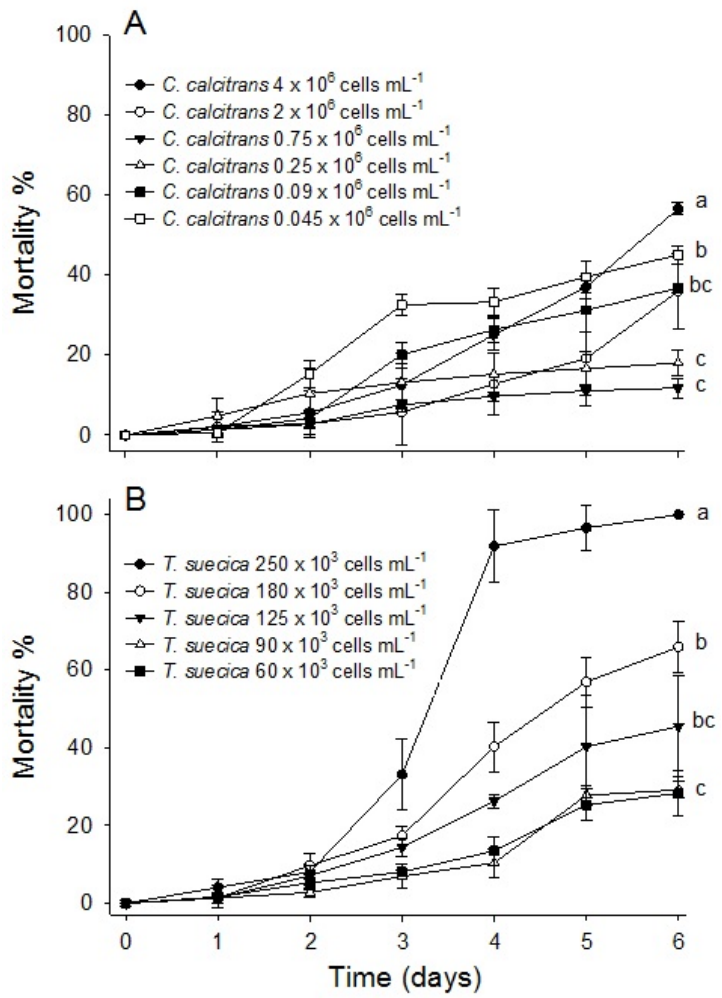

Figure 1. Mortality (%) of *Litopenaeus vannamei* from nauplii (N III) to mysis (M I) exposed to (A) *Chaetoceros calcitrans* (0.045, 0.09, 0.25, 0.75, 2, and 4 $\times$ 10^6 cells mL^{-1}) and (B) *Tetraselmis suecica* (60, 90, 125, 180, and 250 $\times$ 10^3 cells mL^{-1}); lowercase letters beside the lines indicate statistically significant differences.

cells mL^{-1} (Figure 1 B), and then a slight increase was observed until the end of the bioassay (100 % mortality). A lower mortality rate ($\sim$25 %) was observed at cell densities of 60 and 90 $\times$ 10^3 cells mL^{-1}. These results showed statistically significant differences ($p < 0.05$).

Mortality rates were higher when larvae of *L. vannamei* were fed with *T. suecica* than with *C. calcitrans*. Similar results have been observed in other shrimp species such as *Penaeus japonicus, Penaeus semisulcatus*, and *Penaeus monodon*, in which a lower mortality rate was observed when the larvae were fed *Chaetoceros muelleri* than *T. suecica* (D'Souza and Loneragan 1999). Piña *et al.* (2006) reported higher survival and growth rates, and longer organisms when *C. muelleri* was used as food in comparison with *T. suecica* or *Isochrysis galbana* at densities from 0.1 to 0.2 $\times$ 10^6 cells mL^{-1}; they observed 100 % mortality when stage II zoea of *L. vannamei* were fed only *T. suecica*. In contrast, Loya-Javellana (1989) reported a survival rate between 85 % and 100 % in *P. monodon* fed

Tetraselmis sp. at densities from 2.5 to 17.5 $\times$ 10^3 cells mL^{-1}. Our results for *C. calcitrans* differ from those reported by Godínez *et al*. (2005) for *Litopenaeus stylirostris*. They tested the effects of several cell densities of *C. calcitrans* on the survival of zoea stages and found that 0.09 $\times$ 10^6 cells mL^{-1} was the best cell density for reducing shrimp mortality. For *L. vannamei* in this study, the optimum range was from 0.25 to 0.75 $\times$ 10^6 cells mL^{-1}.

Microalgae are the main source of proteins, lipids, and carbohydrates for shrimp larvae in the zoea stage (Pérez-Morales *et al*. 2015); the most important nutrients that affect the performance of shrimp larvae are polyunsaturated fatty acids such as eicosapentaenoic acid (20:5ω3 [EPA]) and arachidonic acid (20:4ω6 [ARA]) (D'Souza and Loneragan 1999, Palacios *et al*. 2002). Nutritional differences among diatoms and chlorophytes have been reported: in *C. calcitrans*, protein content is 43.1 %; lipids, 11.7 %; and carbohydrates, 6.6 %; in *T. suecica*, protein content is $\sim$42.6 %; lipids, 19 %; and carbohydrates, 21.7 % (% of dry weight) (Banerjee *et al*. 2011, Abiusi *et al*. 2014). Differences in the fatty acid composition have also been found: *C. calcitrans* has higher quantities of EPA and ARA ($\sim$11 and 5.7 %, respectively) than *T. suecica* ($\sim$4.8 and 1.8 %, respectively) (Volkman *et al*. 1989, Abiusi *et al*. 2014). With respect to vitamin content, Brown *et al*. (1999) have reported that *Chaetoceros muelleri* has a higher proportion ($\sim$125 μg g^{-1}) of thiamine (B1) than *Tetraselmis* sp. ($\sim$109 μg g^{-1}). Nevertheless, *Tetraselmis* sp. exhibits a higher content of other essential vitamins (retinol (A), cobalamin (B12), and ascorbic acid (C); 2.2, 1.95, and 3 000 μg g^{-1}, respectively) than other microalgae commonly used in shrimp aquaculture. EPA and ARA are commonly detected in higher quantities in *C. calcitrans* than in *T. suecica* (see above), which may explain, in part, the higher survival of *C. calcitrans* observed in this study.

The structure of the digestive tract in decapod crustacean species is similar; however, digestive responses to specific nutrients differ widely. Particularly, the digestive system in penaeid shrimp does not have a gastric mill during early larval development; thus, nutrients are assimilated using enzymes released mainly from the anterior midgut diverticulum and, to a lesser extent, by the hepatopancreas (Ceccaldi 1989, Kumlu 1999). Hence, activities of digestive enzymes in *L. vannamei* larvae could result in the assimilation of more nutrients from diatoms than chlorophytes, mainly because of intrinsic differences among microalgal groups (Pérez-Morales *et al*. 2015). This may explain some of the differences in the mortality rates with respect to *C. calcitrans* and *T. suecica* in this study; such has those observed in other penaeid shrimps (D'Souza and Loneragan 1999, Piña *et al*. 2006).

The digestive physiology of crustacean filter feeders is regulated by a direct relationship among suspended particles available as food, feeding and filtration rates, and retention time in the digestive tract (Kumlu 1999, Pérez-Morales *et al*. 2014). Our results indicate that cell densities less than the optimal value may be insufficient for the energy required for normal metabolism and adequate growth rate. Besides, cell densities higher than the optimal value may induce higher particle ingestion and diminish the retention time in the digestive tract, releasing intact microalgal cells without assimilation of nutrients and causing death by starvation in zoea larvae; this has been reported in the early development stages of different decapod crustaceans (Ceccaldi 1989, Loya-Javellana 1989, Kumlu 1999).

In commercial hatcheries of Mexico, a monoalgal diet as food is a very common practice for shrimp larviculture, so the appropriate microalga species and cell density are fundamental for reducing the mortality rate in the zoea stage. In conclusion, this study demonstrates that when zoea begin to feed on phytoplankton, they are highly sensitive to the microalgal diet and cell densities supplied, which significantly affect the survival of *L. vannamei* larvae.

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