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# DEVELOPMENT OF MYSIS SHRIMP LITOPENAEUS VANNAMEI (DECAPODA:PENAEIDAE) FED WITH THE ROTIFER BRACHIONUS ROTUNDIFORMIS (PLIONA: BRACHIONIDAE)

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## ABSTRACT

As the demand increases shrimp production, *Artemia* cysts is also increased, their availability and prices. In our case Mysis larvae *Litopenaeus vannamei* (Boone) was fed five different portions of the rotifer *Brachionus rotundiformis* (Tschugunoff), with the aim of determining ration that produces more survival, larval development, growth and weight. The rotifers were cultured in 20 containers using the semi-continuos technique with a working volume of 15 L. The strains were kept in containers of 3.5 L with 2 L of filtered seawater, were fed the microalga *Thalassiosira weissflogii* (Grunow). Daily volume of 3 L of rotifers plus 2 L through a sieve of 55µm, which equivalent to 30% of the volume harvested, it is added to food for the purpose of completing 5 L. Experiment 1 was completed in 74 hours and 93 hours in Experiment 2. The best results were obtained with surviving larvae fed 240-400 rotlarvae$^{-1}$; survival ranged from 40 to 88%. The development index 3.60 to 3.88 was achieved with the highest density of rotifers, which indicates that 60-88% of the larvae reached the PL1 stage in a given time. The growth in both size and weight, no significant differences between treatments were found, except for the increase in size experiment 2, where the three treatments with the greatest portion of rotifers were larvae had larger sizes, from 3.63 to 3.76 mm. In the case of experiment 1 larger count were taken with the 240-420 rot·larvae$^{-1}$ treatments with a size of 3.44 mm. The organic growth in dry weight, no significant differences were found, most larvae dry weight were fed treatment 1: 320-420 rotlarvae$^{-1}$, with a weight of 71.78 mglarvae$^{-1}$ and the treatment 2: 240-400 rotlarvae-$^{1}$ weighing 91.67 mglarvae$^{-1}$; the highest values in the organic weight had larvae fed treatment 1: 240-420 rotlarvae$^{-1}$ with values 77.72, and 55.02 mglarvae $^{-1}$, for treatment 2.

**KEYWORDS:** *Brachionus rotundiformis,* Development index, *Litopenaeus vannamei,* Mysis, Rotifer, Surviving.

## INTRODUCTION

In the last larval stage (Mysis), change its eating habits becoming omnivores, but are primarily carnivores and their main food is zooplankton organisms, this phase lasts only 3 days until metamorphosis to postlarvae (PL1). For these reasons there have been researches proposing replacing *Artemia* nauplii with other prey such as rotifers and copepods, there are also studies with other food microencapsulated (Pedroza-Islas et al. 2004); (De Lima & Souza-Santos 2007); (Campaign-Torres et al. 2009). The use of rotifers as natural food has been implemented in the cultivation of various aquatic species, including shrimp in their larval stages (Wilcox et al. 2006). The rotifer *B. rotundiformis* has characteristics that make it a good alternative for live food for larval rearing, which can be grown at high densities under controlled conditions (Hagiwara et al. 2001); (Tanaka et al., 2009). In addition, these organisms possess characteristics such as small size, average size of 72.5-235.0 µm lorica length and 52.5-162.5 µm and lorica width in size are ideal for the mandible of larval shrimp, being able to eat in early larval stages from the stage of zoea I (Cabrera. 2008); (Bermudez-Lizárraga, 2009).

Tovar-Guillén (2003) conducted a job feeding the three stages of *L. vannamei* Mysis larvae with rotifer *B. plicatilis* and concluded that the most appropriate and efficient both in development and growth and survival of larval diets concentrations were 60, 330 and 600 rotiferslarvae$^{-1}$day$^{-1}$ to Mysis I, II and III, respectively. Medina-Jasso (2004) reported that larvae of *L. vannamei* in zoea III stage, when fed with rotifers and microalgae have an assimilation efficiency of 71% and 90% when they are fed only rotifers. While, for the three Mysis stages, the greater efficiency of food absorption obtained when the rotifers were supplied as the sole diet (81-86%). Piña, et al. (2004) conducted a feeding study with larvae of *L. vannamei* using *B. plicatilis* rotifer as alternative to replace the traditional diet of *Artemia* nauplii during the final stage of three zoea and Mysis, this rotifer feeding not modify survival but favored the growth and development of the larvae. Campaign-Torres et al. (2009) conducted a study testing the effect of rotifer *B. rotundiformis* on water quality and production in super-intensive shrimp culture *L. vannamei*, finding that higher concentrations of this rotifer improving production parameters shrimp without being significantly impair water quality.

Rotifers feeding improve nutritional content; microalgae offer a high nutritional value due to its content of vitamins and essential fatty acids. The genera most frequently used with aquacultural purposes are *Chaetoceros, Thalassiosira, Isochrysis, Nannochloropsis* and *Tetraselmis* (Duerr et al., 1998). Bermudez-Lizárraga (2009) fed shrimp larvae of *L. vannamei* with the microalga *T. weissflogii*, the rotifer *B. rotundiformis* and *Artemia* nauplii, zoea from phase I to PL1, finding index values of development and growth higher in the treatments fed rotifers with respect to those fed *Artemia* nauplii in the survival values and organic dry weight were the same in both rotifer diet to those fed *Artemia* nauplii. Indicating that the use of rotifers in larviculture shrimp from zoea I stage does not change much the results to those obtained with microalgae, suggesting that the rotifer *B. rotundiformis* can completely replace *Artemia* nauplii.

This study's main objective is to evaluate survival, larval development and growth of the three stages of *L. vannamei* Mysis larvae fed with the rotifer *B. rotundiformis*.

## MATERIAL AND METHODS

Larvae shrimp *L. vannamei* that were used in this study were obtained by donating a commercial laboratory in the development stage zoea II and III. In the laboratory Ecophysiology of Aquatic Organisms and Crop support, larvae were acclimated and placed in a container of 400 L seawater with salinity of 35‰ at a temperature of 30° C, which was maintained constant with a heater 500 W connected to a thermoregulatory. Once the larvae reached 100%, zoea phase III, the experiment proceeded to assemble. Two experiments were carried out: experiment 1 was completed in 74 hours and 93 hours the experiment 2.

Daily volume of 3 L of rotifers were harvested to feed shrimp larvae plus 2 L water over through a sieve of 55µm, both volumes is equivalent to 30% of the volume of each container. This procedure was performed with the aim of completing 5 L offered food crops. This was enough to feed three Mysis stages of larvae, having an average harvest 131 rot·ml$^{-1}$ per day.

***Survival:*** was calculated using the daily count of larvae of sample of each aquarium, before taking the sample the entire water column is homogenized, the volume was measured, approximately 0.5 L to then scale the volume number of larvae total aquarium. Knowing the latter number, the volume was adjusted to maintain a constant density of larvae per liter.

***Development Index:*** The cultures were monitored at intervals of 6 hours, to check the temperature of the culture. Before collecting samples for larval development throughout the water column aquarium homogenized, proceeded to concentrate a sample of that aquarium calculating that had at least 20 larvae were observed in a watch glass to be checked under a stereoscope Leica brand, model 2000 Zoom. These data were used to calculate incidence rates of each stage, plus the rate of larval development (DI) of larvae present was determined. Which was calculated as Villegas and Kanazawa (1979), by the equation: $DI = \Sigma in_i / n$ where i is the value attributed to each larval stage (0 absolute value zoea III, 1: Mysis I, 2: Mysis II; 3: Mysis III and 4: PL I). $n_i$ is the total number of larvae in the stage i and n is the total number of organisms in the sample. This index development or metamorphoses of the larvae were evaluated during their growth.

***Growth:*** The growth in length was calculated with samples of at least 10 to 15 organisms per tank at intervals of 24 hours, same as they were preserved in the fixative solution described by Correa-Sandoval and Bückle-Ramírez (1993). Were

measured on a compound microscope OLYMPUS brand CH3ORF100 model, equipped with an eyepiece with a rule graduated to the target pre-calibrated using a slide micrometer.

The experiments were terminated when 50% or more of the larvae reached PL stage I, at the end of each test samples from each of the experimental units were taken to determine total organic individual and dry weight of the larvae.

***Dry and organic weight:*** At the beginning of each experiment larvae filtered in phase zoea III in a pre-calibrated fiberglass mark Whatman type GF / C of 25 mm diameter, filter for pre-calibration of the filters, were deposited in a vessel capacity of 1 L with distilled water for disposal, leaving on blotting paper for 24 hours and then are introduced into a muffle mark Termolyne model 48000 which were given a time of 4 hours at a temperature of 250° C, in order to remove any organic residue in the filters. End of this time were placed in an oven Barnstrad Lab-Line Model 3513, leaving for 24 hours at a temperature of 60° C to start weighing the filters daily on an analytical balance brand Denver Instrument model M-220D, giving at least 4 heavy to determine the average weight of the filter .

The dry weight (DWU) and the organic weight ( OWU ) per larvae was performed with a number of four replicates for each experiment, they were placed in the oven for at least 72 hours at 60° C; after this time reweighed filters on analytical balance described, to determine the dry weight of the larvae being repeated at least four weighing filter with uniform weights, we proceeded to place these filters in muffle for incineration hence leaving at least 12 hours at a temperature of 450° C, and then return them to the oven at the same temperature of 60° C and bringing them again to a constant weight. The dry weight was calculated by difference between the weight of the filter + sample filter weight less the weight of ash is performed in the same manner and the body-weight, the difference between the dry weight and ash weight per filter.

The experiments were analyzed by analysis of variance parametric or non-parametric way, depending on the outcome of the Lilliefors test for normality and homogeneity of variance test of Bartlett. Testing a posteriori multiple comparisons were performed when the ANOVA shows significant differences (Zar 1996).

***Experimental design*:** The experiments started in the phase III and zoea finished when a minimum of 50% of organisms PL1 phase four replicates of each treatment was recorded.

Tests were made in plastic containers with a volume of 12 L with a density of 150 larvae·$L^{-1}$, 5 treatments were established at each stage taking care provided there overlap between the 3 Mysis stages dose. The feeding table that was used in this work was based on the number of *Artemia* nauplii used in commercial laboratories, making a number of rotifers based on organic weight and considering the maximum and minimum for each stage of Mysis, this is shown in (Table 1).

Samples every 6 hours to observe larval development and at 24 hours the survival rate was checked and collected at least 15 organisms for growth were taken.

All treatments were done in quadruplicate, based on daily diaries mortality, concentration of larvae in each container was kept constant, in order to avoid density-dependent responses.

**RESULTS**

**Survival:** Fig. 1, the first day of the experiment, four of the five treatments had a 90% survival; the end of the experiment, treatments which had the lower concentration of rotifers had the poorest survival with 48 and 51%, respectively. Upon completion of the experiment the five treatments were statistically equal, since no significant differences were found between treatments with percentages ranging 48-66% survival.

***Development Index*:** Experiment 1 (Fig. 2) was terminated at 74 h was found in four of the five treatments percentages above 50% of their larval phase PL1 values ranging between 55 and 61%. At the end of the experiment, the percentage of larvae PL1 phase were similar to those that were fed rations with rotifers older, also observed Fig. 2 a line, was obtained by averaging the values of five treatments for each sampling time during the experiment, with a coefficient of determination of $R^2 = 0.984$, indicating that 98% of the data is located on the line. The values of DI: the experiment showed no significant differences between treatments, but is to be noted that there is a parallel to increase the number of rotifers per larvae trend.

**Experiment 2 (Fig. 2):** the end of the experiment at 93 hours, all treatments showed percentages from 72 to 87% of PL1. At the end of the experiment no significant differences between treatments were found.

The value of DI, the experiment showed no significant differences between treatments, but is to be noted that there is a parallel to increase the number of rotifers per larvae trend.

***Growth in size:*** Fig. 3. Generally these larvae had a growth rate of 0.34 mm per day in average length. At the end of the experiments, no significant differences among the five treatments were detected, noticing that the three major treatments have a higher growth compared to larvae under both treatments, with an increasing trend.

***Dry and organic weight:*** At the beginning of the experiment a known number of larvae were filtered to determine their dry weight per unit (DWU) and organic weight per unit (OWU). Values DWU, OWU and OW / DW end shown in Table 2, had a coefficient of variation (CV) ranging from 3.41 to 7.60 mglarvae$^{-1}$ in the DWU market, whereas it had a OWU (CV) values of 5.40 and 6.16 mglarva$^{-1}$ as minimum and maximum and the OW / DW a CV of 0.84 was obtained 8.53 mglarvae$^{-1}$.

The values of (DWU) do not show significant differences between treatments, with values that are ranging from 61.00 to 71.78 mglarvae$^{-1}$ having a CV between the five treatments, values ranging from 3.41 to 7.60 mglarvae$^{-1}$

For values of (OWU) in the same way that the (DWU) no significant differences between treatments were found showing fluctuating values 49.22 to 55.02 mglarvae$^{-1}$, showing a variation coefficient of 5.40 to 6.16 mglarvae$^{-1}$. The percentage of organic matter present in the dry weight of the sample, no significant differences were found between treatments, with values ranging from 77 to 81% of organic matter, these data had a CV ranging from 1-7%.

**DISCUSSION**

Bermudez-Lizárraga (2009) with the end of 26 to 30% survival, this low survival may be because they use a higher density of the larvae than used in this study and handling of the animals had to do, and the start of the experiment from nauplii stage V and completed in phase PL1. Similar values to mine the (D' Abramo et al. 2006; Piña et al. (2004) where the first authors mentioned, they fed larvae of *L. vannamei Artemia* nauplii, copepod (*Tisbe monozota*) and microencapsulated microbound, obtaining survivals of 48 to 69%, having the best results with the microencapsulated, and the second author mentioned getting survivals of 36 to 58%, with the lowest survival diet of *Artemia* nauplii superior results to those obtained in this work were Focken et al. (2006 ) fed them shrimp *L. vannamei* larvae with *Artemia* nauplii, and microalgae mass *Panagrellus redivivus* nematode, obtaining survivals of 70 and 86%, the best results were obtained with larvae fed nematodes mass and Pedroza-Islas et al. (2004) and Garcia et al. (2004 ) with a percentage of 96 to 98 % and from 72 to 85 % survival.

Very similar results to those in this work were obtained Bermudez-Lizárraga (2009) who fed larvae of *L. vannamei* with *T. weissflogii* microalgae, *Artemia* nauplii and the rotifer *B. rotundiformis*, obtaining percentages of late development 71 and 86% of larvae PL1 phase after 96 hours, and those obtained by Piña et al. (2004) where they fed Mysis *L. vannamei* larvae with *Artemia* nauplii and rotifers *B. plicatilis* with which development had values of 40 and 53% of larvae PL1 phase, this result is obtained larvae fed rotifer, while larvae were fed *Artemia* nauplii only 18% were in stage PL1, obtaining this result to 144 hours, they began experiments when 100% of the larvae was in the zoea II stage, maybe this longer period of which was obtained in this work due time.

Tovar-Guillen (2003) who fed to Mysis larvae of *L. vannamei* with different rotifer *B. plicatilis* rations which had rates of development of the 19% maximum of larvae PL1 phase lasting 72 hours, both papers ended when at least 80% was in the Mysis stage III. As these values lower than those obtained in this work as in the first experiment was obtained 31 to 61% of larvae at the stage of PL1 and this was at 74 hours of the experiment. López-Prado (2003) which feed on larvae of *L. vannamei* with rotifer *B. plicatilis* which was fed with microalgae *Tetraselmis suecica* and I*sochrysis* sp. producing a development index 45 and 50% in stage larvae PL1, achieving these results at 120 hours of experiment. But it is different in the degree of development of the larvae which in this work was 74 and 93 hours in both experiments more than 50% of PL1 were obtained.

Similar results to those obtained by Bermudez-Lizárraga (2009); Piña et al. (2004) having their larvae a final size of 3.53 to 3.81 and 3.50 to 3.57 mm. Focken et al. (2006) and D'Abramo et al. (2006) had larger sizes than those obtained in this work, since they had their PL1 size from 4.4 to 5.7 mm and 4.02 to 5.46 mm in length, respectively. The work of López-Prado (2003) ended their postlarvae with a larger size than the postlarvae of this work with carvings of 3.66 to 3.98 mm. Similar to the work of Bermudez-Lizarraga (2009) and Piña et al. (2004) and my work is that of Tovar-Guillén (2003) & Ulloa-Moreno (2003) having larvae with final size of 3.62 to 3.72 mm and 3.4 to 3.6 mm in length, respectively.

Similar results to those obtained by Bermudez-Lizárraga (2009) with 84.35 to 89.42 PL1 uglarvae$^{-1}$ OWU DWU and 58.37 to 64.95 mglarvae$^{-1}$. Also the work of D'Abramo et al. (2006); Focken et al. (2006); López-Prado (2003) larvae were 89.4 PSU 106 mglarvae$^{-1}$, 80.2 to 82.8 mglarvae$^{-1}$ and 80-90 mglarva$^{-1}$, respectively, similar to the results obtained in this work. Piña et al. (2004) obtained values of 57.89 to 76.88 OWU uglarvae$^{-1}$, these values are also similar to those obtained in this work.

**Table 1. Program larval feeding of Mysis of *L. vannamei* with rotifer *B. rotundiformis* (values rot·larvae$^{-1}$).**

| | TREATMENTS | | | | |
|---|---|---|---|---|---|
| **LARVAL STAGE** | **A** | **B** | **C** | **D** | **E** |
| Mysis I | 80 | 160 | 240 | 320 | 400 |
| Mysis II | 160 | 240 | 320 | 400 | 460 |
| Mysis III | 240 | 320 | 400 | 480 | 560 |

**Table 2. Average values and standard deviation initial and final dry weights of the unit (DWU, in uglarvae$^{-1}$), organic weight unit (OWU in uglarvae$^{-1}$) and organic content in the dry weight of the larvae (OW / DW in %) of *L. vannamei* Mysis larvae fed with different concentrations of the rotifer *B. rotundiformis*. Similar letters indicate lack of significant differences between treatments.**

| | TREATMENTS | | | | |
|---|---|---|---|---|---|
| **INITIAL** | **A** | **B** | **C** | **D** | **E** |
| DWU | 26.12 ± 1.85 | 26.12 ± 1.85 | 26.12 ± 1.85 | 26.12 ± 1.85 | 26.12 ± 1.85 |
| OWU | 22.91 ± 1.54 | 22.91 ± 1.54 | 22.91 ± 1.54 | 22.91 ± 1.54 | 22.91 ± 1.54 |
| OW/DW | 87.76 ± 0.84 | 87.76 ± 0.84 | 87.76 ± 0.84 | 87.76 ± 0.84 | 87.76 ± 0.84 |
| **FINAL** | **A** | **B** | **C** | **D** | **E** |
| DWU | 65.76$^{a}$ ± 5.00 | 61.00$^{a}$ ± 4.39 | 68.83$^{a}$ ± 2.35 | 71.78$^{a}$ ± 4.68 | 65.33$^{a}$ ± 4.27 |
| OWU | 52.25$^{a}$ ± 3.00 | 49.22$^{a}$ ± 2.74 | 51.74$^{a}$ ± 2.80 | 55.02$^{a}$ ± 3.39 | 52.25$^{a}$ ± 3.17 |
| OW/DW | 79.63$^{a}$ ± 4.90 | 81.32$^{a}$ ± 1.86 | 76.83$^{a}$ ± 3.62 | 78.61$^{a}$ ± 6.71 | 80.06$^{a}$ ± 0.67 |

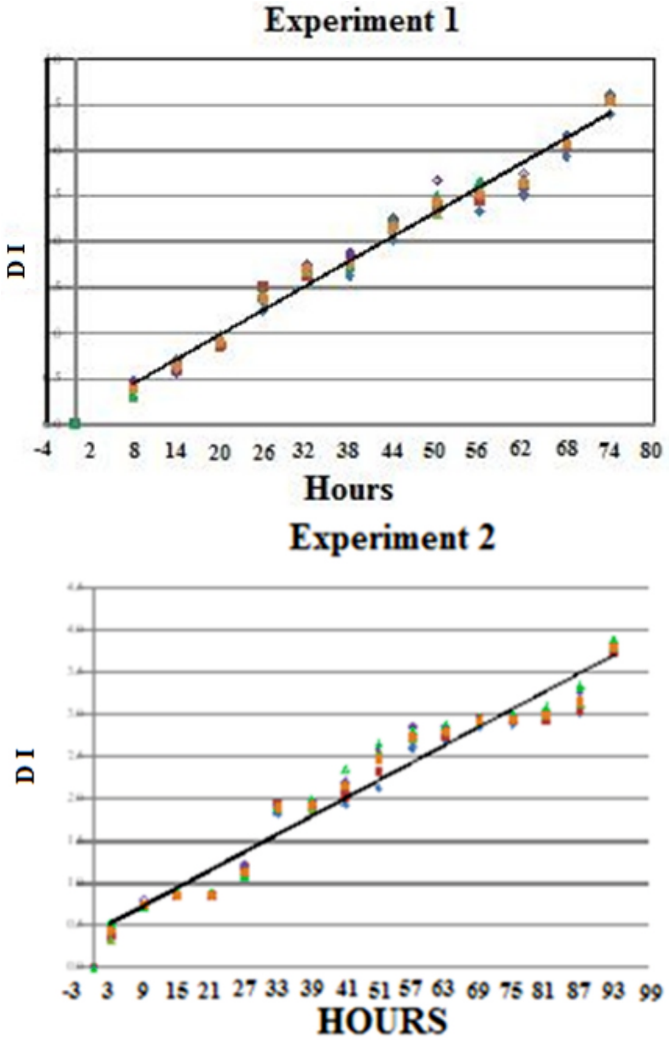


**Figure 1. Survival percentages of the three stages of L. vannamei Mysis larvae fed five concentrations of rotifer *B. rotundiformis*. ♦ A, ■ B, ▲ C D, Δ E.**

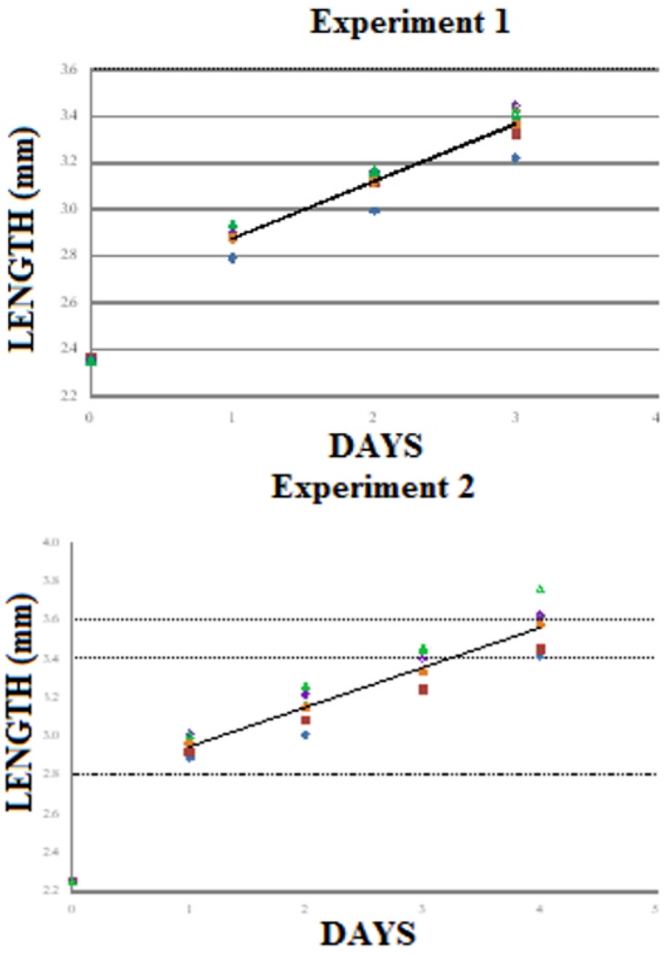


**Figure 2. Index larval development (DI) of the three phases of *L. vannamei* Mysis larvae fed five concentrations *B. rotundiformis* rotifer. ♦ A, ■ B, ▲ C D, Δ E.**

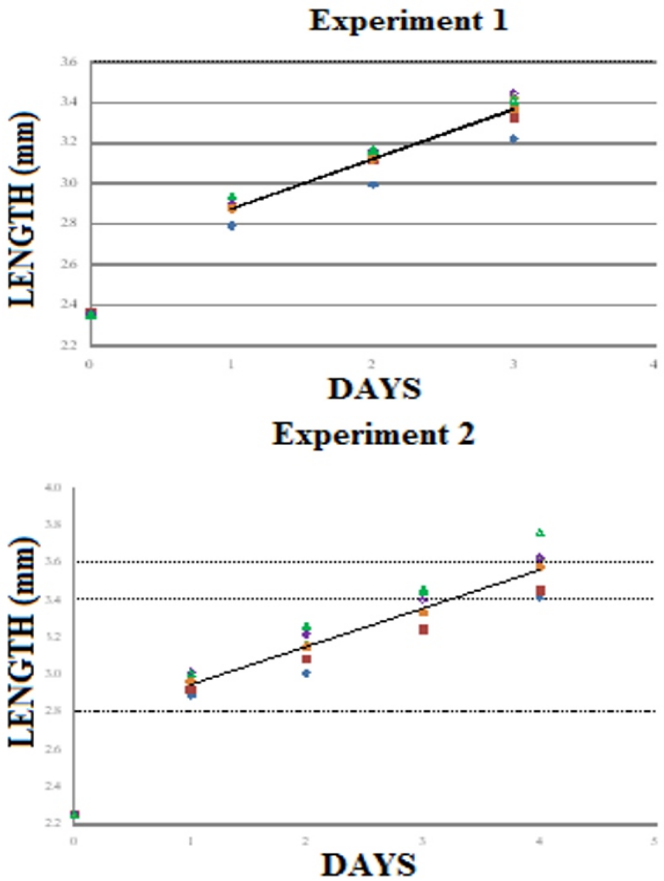


**Figure 3. Growth in mm of the three phases of L. vannamei Mysis larvae fed five concentrations *B. rotundiformis* rotifer. ♦ A, ■ B, ▲ C D, Δ E.**

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