Enhancing growth performance, survival rates and stress resistance in larval and early post larval rearing of *Litopenaeus vannamei*

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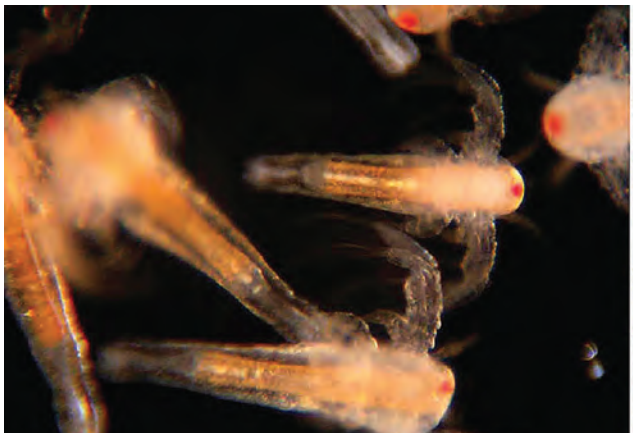
Unraveling the beneficial impact of Artemia franciscana feeding regimes in a field trial.

Summary

In a field trial, L. vannamei larval and early post-larval stages (Zoea I through PL5) were fed different levels of Artemia to replace all or a portion of an artificial feed regime (the "Non-Artemia Diet"). Results showed improved survival, growth, and stress resistance of shrimp fed increasingly higher levels of Artemia.



Introduction

Previous research consistently demonstrated that increased stocking densities generally result in higher mortality and reduced feeding efficiency among Penaeid shrimp (Guillermo *et al.*, 2021). Therefore, the primary aim of the hatchery industry is to optimize production capabilities by implementing feeding regimes that promote larval metamorphosis and enhance survival, growth, and stress resistance of the post larvae (PL). This approach allows hatchery operators to improve economic margins, even when dealing with higher stocking densities, without subjecting the larvae to excessive mortalities and stress. Traditionally, *Artemia* has been widely used as a co-feed for Mysis (M3) to post-larval stages in the aquaculture industry (Bengtson, 1991; Sorgeloos, 2001; Dirk Halet, 2007; Azra, 2022; Sahandi, 2022). *L. vannamei* larvae have shown a strong feeding response to live *Artemia nauplii,* resulting in increased consumption and improved feeding efficiency (Sheen *et al.*, 1994). During the last two decades, attempts to eliminate *Artemia* from a traditional co-feeding regime and replace *Artemia* entirely with artificial feeds in *L. vannamei* have yielded mostly inferior results.

In general, live feeds have always provided consistent positive results in terms of growth and survival in *L. vannamei* larvae (Puello-Cruz *et al*., 2002) and recent studies continue to demonstrate this improvement with increased feeding levels of live *Artemia* throughout the late larval and post-larval stages (Gamboa-Delgado & Le Vay, 2009; Sommer, 2019; Yathish *et al*., 2022). More recent field and research studies have also demonstrated that incorporating live or attenuated forms of *Artemia* during the early larval stages of *L. vannamei* promotes better health, particularly in terms of stress resistance, enabling them to better cope with environmental challenges and leading to improved survival rates (Liqing, 2022).

Artemia continues to play a vital role in supporting the larval and post-larval diets of *L. vannamei*, providing high levels of protein (62.7%) and lipids (21.7%) on a dry weight basis. Artemia also contains high levels of the most limiting amino acids such as methionine, threonine, and lysine, essential for early larval stages (Niu *et al*., 2012) which contribute significantly to protein synthesis, enzyme production, energy metabolism, osmoregulation, and immune function. Additionally, Artemia provides essential fatty acids and cholesterol necessary to maintain cell membranes, hormone production, absorption and utilization of lipids, energy metabolism, and antioxidant protection (Hernández, 2004).

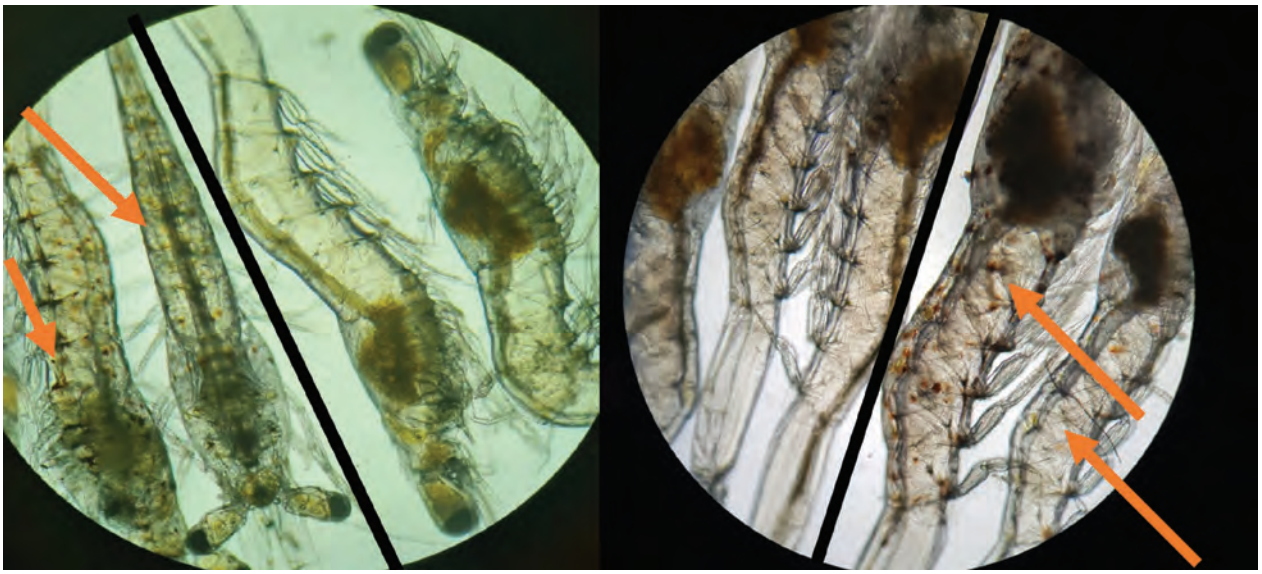
In general, the high digestibility and essential nutrients provided by Artemia nauplii enable efficient nutrient absorption and utilization, supporting the growth and survival of *L. vannamei* larvae (Jones *et al*., 1997a, 1997b). This field trial aimed to assess the potential effects of increased feeding levels of *Artemia* instar 1 nauplii during the early rearing of *L. vannamei* larvae (Mysis I to PL5) compared to a non-*Artemia* feed regime.

The field trial, performed in a commercial production environment, showed that the replacement of artificial feeds with increased levels of Artemia in larval and early post-larval (Zoea I to PL5) rearing significantly improved survival, growth, and stress resistance of *L. vannamei* post larvae.

Methods

The trial implemented four different dietary treatments replacing a fixed percentage of the microparticulate feed with *Artemia* instar 1 nauplii ranging from 25%, 50%, 75% to 100%. Eight randomized replicated tanks for each treatment were employed with 5,800,000 Zoea 1 in 30,000 L-1 U-shaped rectangular concrete tanks, equivalent to a density of 200 Zoea/L-1. The water salinity was consistently maintained at 26ppt, while the temperature was 28°C.

The *Artemia* hatching quality used in the study was 200,000 nauplii per gram (GSL Artemia cysts, GSLA brand, USA). All cysts were hatched using 2g/L-1



Picture 1. Density patterns of higher/sharper chromatophores observed in Artemia-fed PL5 *L. vannamei* larvae across the dorsal body are indicated with orange arrows. Non-Artemia-fed post-larvae lack high development.

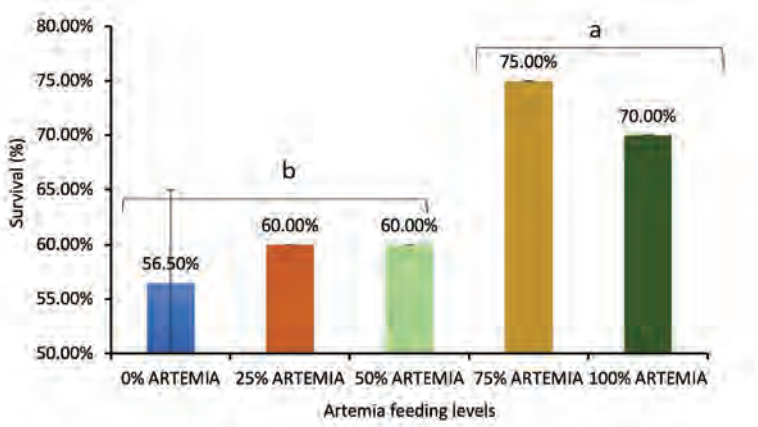


Figure 1. Survival rates of *L. vannamei* post-larvae (PL5) fed with different dietary levels of *Artemia franciscana* nauplii during the larval and post-larval rearing periods.

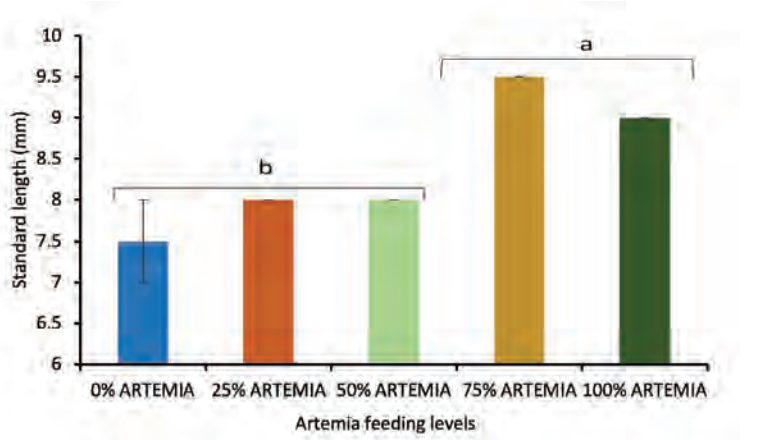


Figure 2. Length (mm) of *L. vannamei* post-larvae (PL5) fed with different dietary levels of *Artemia franciscana* nauplii during the larval and post-larval rearing periods.

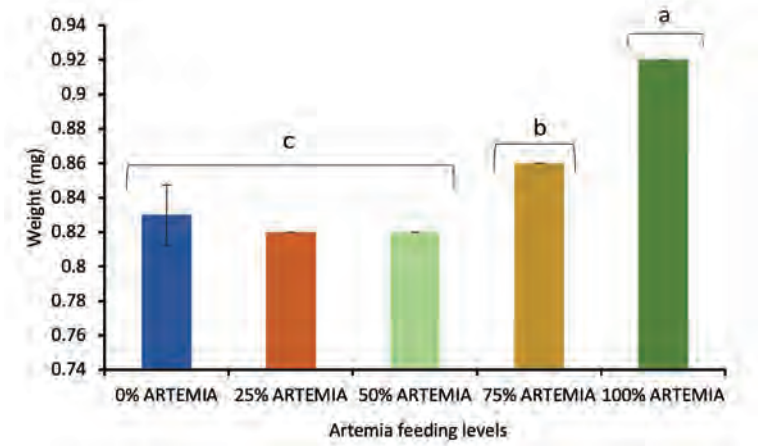


Figure 3. Whole-body wet weight (mg) of *L. vannamei* post-larvae (PL5) fed with different dietary levels of *Artemia franciscana* nauplii during the larval and post-larval rearing periods.

stocking density, 28°C, strong aeration, 2,000 lux, in cylindro-conical 60L clear plastic tanks. All Artemia instar 1 nauplii were harvested, rinsed, and re-suspended for hatch calculations and then frozen in plastic feeding bags ready for the shrimp larvae from Zoea 3 onwards.

In those tanks fed *Artemia*, initially 250 grams of cysts were hatched and used to feed each dietary treatment tank from Zoea 3 to the Mysis I stage. The amount of dry cysts was subsequently increased to 400 grams, 545 grams, 750 grams, 800 grams, and 875 grams for each dietary treatment tank during the rearing period until reaching PL5. A total of 7,250g of dry cysts were used for 5.8 million Zoea stocking density, or 1,250g for one million PL5.

A proprietary feeding regime of live algae and commercial shrimp microparticulate feeds made up the non-*Artemia* diet. The *Artemia* 75%, 50%, and 25% feeding regimes (the *Artemia* diets) included a proportionate decrease in *Artemia* as compared to the 100% *Artemia* feeding and thus a proportionate increase in the artificial diets.

For all treatments, trials were terminated at PL5. All larvae were harvested into fine nylon meshes from each tank (as they will be re-stocked in raceways thereafter) and gently compressed to remove excess water. The whole biomass was then weighed (Ohaus) and the subsequent 3 sub-samples were removed, each weighing 1g. From these sub-samples, all 3 parameters were calculated. Survival (%) was determined by counting surviving larvae. The total length (mm) of 10 randomly selected larvae from all the replicates of each treatment was measured from the tip of the rostrum to the tip of the telson for PL5, viewed under a binocular microscope with a graticule calibrated against a stage micrometer. Finally, wet weight (mg larvae-1) from every replicate and treatment was

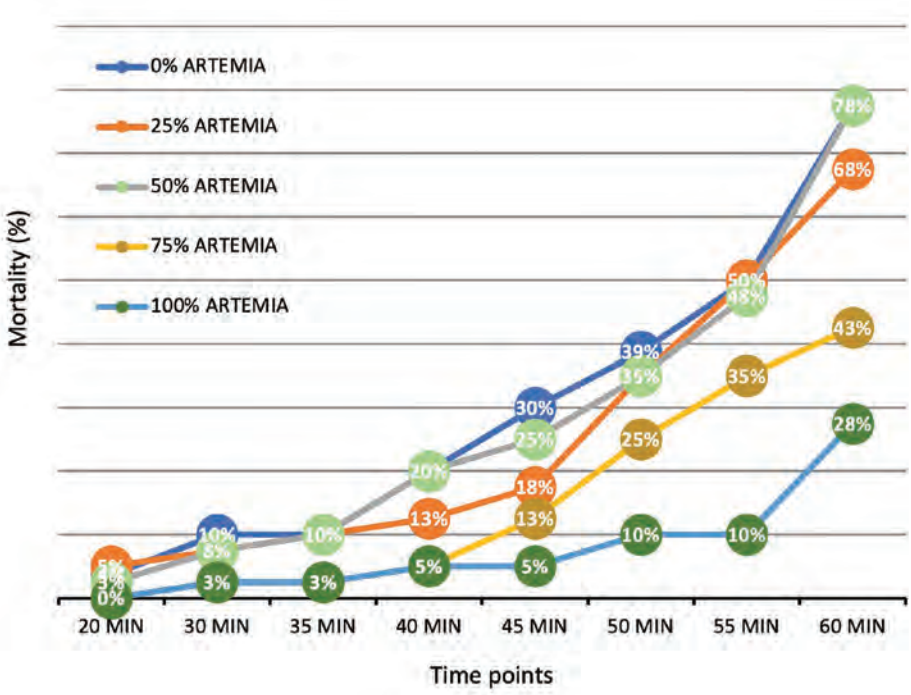


Figure 4. Freshwater stress test: Mortality rates of *L. vannamei* post-larvae fed with different dietary levels of *Artemia franciscana* nauplii during the larval and post-larval rearing periods.

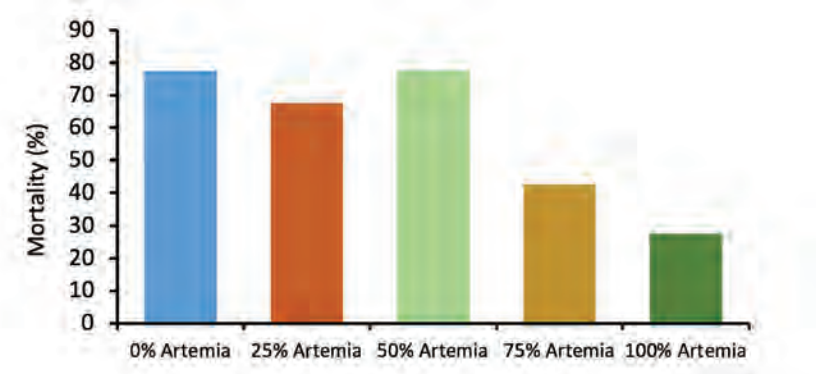


Figure 5. Mortality (%) at 60 minutes of *L. vannamei* post-larvae fed with different dietary levels of Artemia franciscana nauplii during the larval and post-larval rearing periods.

assessed by removing 20 animals and subsequently weighed on an electronic balance (Ohaus).

Stress test

A controlled experimental setup was used to induce salinity stress through a sudden decline in salinity from 26 to 0 ppt using fresh water at 33.6°C (shrimp hatchery condition). In triplicate 4L buckets per treatment, 100 PL5 animals were directly immersed in 2L of freshwater without undergoing any gradual acclimatization procedure. Time was noted as T0 and subsequently, total mortalities in each bucket were carefully recorded at regular five-minute intervals up to 60 minutes.

Results

During the culture period, a post-larval health analysis was conducted, which revealed high chromatophore development throughout the bodies of the shrimps (as shown in Picture 1) that were fed 75% to 100% *Artemia*, in comparison to the other dietary treatments.

Generally, survival, growth, and stress resistance improved with increasing dietary inclusion of *Artemia* (Fig. 1-5), verifying earlier research that demonstrated the importance of feeding adequate levels of *Artemia* in the larval and early post-larval rearing of *L. vannamei*. Statistical analysis indicates a strong effect of the dietary treatments on survival rates (p=0.0001) between the *Artemia* diets (Fig. 1). Tanks that received 75% and 100% *Artemia* inclusion levels showed notably higher survival rates of 75% to 70%, respectively, which were significantly higher than the survival rates of 56% to 60% observed in tanks fed lower levels of *Artemia* or the non-*Artemia* diet.

A similar trend was observed in terms of the length. PLs in tanks that received 75% (9.5mm) and 100%

(9.00mm) *Artemia* showed significantly ($p>0.0001$) greater lengths (Fig. 2) compared to those in the remaining dietary treatments.

Weight gain data also revealed statistically significant differences ($p<0.0001$) among the different dietary treatments. PL5 that were fed a diet consisting of 100% *Artemia* exhibited the highest weight gain, with a mean increase of 0.92 mg (Fig. 3). In comparison, those fed a diet containing 75% *Artemia* had a slightly lower mean weight gain of 0.86 mg, but still significantly higher than the tanks fed 0% (0.83mg), 25% (0.82mg), and 50% (0.82mg) *Artemia*.

In the stress resistance experiment, different dietary treatments of *Artemia* resulted in varying mortality rates, demonstrating higher stress resistance of the PLs fed higher levels of *Artemia*. Within the first 20 minutes of exposure to freshwater, there were no mortalities in any of the treatments. However, as time progressed, the mortality rate gradually increased. The mortality increased more rapidly in the 0%, 25% and 50% *Artemia* treatments, reaching a mortality rate at or above 68% after 60 minutes. In the 75% and 100% Artemia treatments, the PL mortality remained lower (<5%) during the first 40 to 45 minutes after which it gradually increased reaching final mortality of 43% and 28%, respectively, after 60 minutes of salinity stress. The total mortality rates after 60 minutes of exposure to freshwater were significantly lower for the tanks that received higher inclusion rates of *Artemia* at or above 75% (Fig. 5).

Overall, feeding higher levels of *Artemia* (75% and 100% *Artemia* diets) significantly improved survival, growth, and stress resistance during the early hatchery cycle (up to PL5) when compared to the non-*Artemia* diet and the 25% and 50% *Artemia* diets. These results demonstrate the positive impact of feeding higher levels of *Artemia* during the larval and early post-larval development stages. The results of the field study support the view that higher feeding levels of *Artemia* during larval and early post-larval development (up to PL5) optimize hatchery performance by substantially improving the survival, growth, and stress resistance of the *L. vannamei* PLs.

Conclusion

This one-of-a-kind field study presented a comprehensive assessment of the nutritional suitability of *Artemia* as a crucial dietary source for larvae and early post-larvae stages of *L. vannamei* and demonstrated the value of Artemia inclusion for these developmental stages. These results again emphasize the significance of the highly digestible *Artemia* as a feed to improve overall hatchery production during the larval and early post-larval stages of *L. vannamei* and establish a solid foundation for the subsequent rearing of advanced PL stages.

Further refinement of optimal *Artemia* feeding levels and investigations into the underlying mechanisms and physiological responses associated with these results would provide valuable insights for optimizing larval-rearing practices and promoting the successful cultivation of *L. vannamei*.

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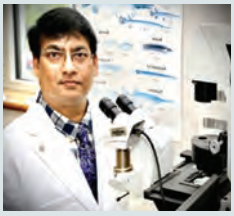

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