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Effect of Stocking Density on the Survival of Litopenaeus vannamei Larvae through the PL5 Stage

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ABSTRACT

The whiteleg shrimp (Litopenaeus vannamei) is a leading Indonesian aquaculture commodity, and the success of postlarval production in hatcheries is a key determinant of downstream productivity. Initial nauplius stocking density is one of the main hatchery variables affecting water quality and larval survival. This study analyzed the effect of two stocking densities, 90 individuals/L (P90) and 120 individuals/L (P120), on the survival rate (SR) of L. vannamei larvae from the nauplius stage (N5–N6) through the PL5 stage. The study was conducted at the hatchery of PT. Central Proteina Prima, Kalianda, South Lampung, using a field-experiment approach with a Completely Randomized Design (CRD) comprising two treatments and three replicate tanks per treatment (six independent experimental units in total). Each 45-ton tank constituted one experimental unit. SR was the primary response variable, while temperature, salinity, pH, ammonia, and nitrite were monitored as supporting water-quality variables. Data were analyzed with an independent-samples t-test at $\alpha = 0.05$, preceded by Shapiro–Wilk normality and Levene homogeneity checks. P90 produced a mean SR of 83.70 ± 7.77%, significantly higher than P120 at 65.23 ± 6.51% ($t = 3.156 > t\text{-critical} = 2.776$, $df = 4$; mean difference = 18.47 percentage points, 95% CI 2.22–34.72; Cohen's $d \approx 2.58$). The lower SR in P120 coincided with higher ammonia (0.340–0.357 mg/L) and nitrite (0.085–0.194 mg/L) concentrations, consistent with — though not proof of — greater metabolic-waste loading at the higher density; dissolved oxygen, feed competition, and cannibalism were not measured directly in this study and are discussed as plausible contributing mechanisms rather than confirmed causes. In terms of production efficiency, P90 achieved a postlarval output nearly equal to P120 while using approximately 25% fewer nauplii. Among the two densities tested, 90 individuals/L is the more suitable choice for improving both survival and input efficiency in commercial-scale hatchery operations; whether an even better density exists outside the tested range remains an open question for further research.

INTRODUCTION

The whiteleg shrimp (Litopenaeus vannamei) is a high-value aquaculture commodity and a key Indonesian export. Its advantages — rapid growth, adaptability to environmental change, and efficient feed utilization — make it the most widely cultivated penaeid shrimp species (Ghufron *et al.,* 2017; Mustafa *et al.,* 2023). Successful L. vannamei farming depends heavily on the quality of the postlarvae produced in the hatchery. The larval rearing phase, spanning the nauplius to postlarva stages, is the most critical period: larvae undergo rapid morphological and physiological change and require stable environmental conditions. Failure during this phase can compromise seed quality and negatively affect subsequent pond productivity (de Lorenzo *et al.,* 2016; Hasniar, 2014).

Stocking density is a crucial factor influencing the success of larval rearing. Increasing stocking density can improve space-use efficiency and production capacity, but it also risks degrading water quality through elevated metabolic waste, greater oxygen demand, and organic-matter accumulation, all of which can reduce larval survival rate (SR) (Krummenauer *et al.,* 2011; Mugwanya *et al.,* 2022). Nauplius stocking densities applied in Indonesian commercial hatcheries commonly range from about 80 to 120 individuals/L depending on tank design and management capacity (Hidayat *et al.,* 2017). Whether raising density from 90 to 120 individuals/L remains within the carrying capacity of large commercial tanks (45-ton scale) has not been well documented.

This study therefore compares the survival of L. vannamei larvae reared at 90 individuals/L and 120 individuals/L, and evaluates the accompanying changes in water-quality parameters, to identify the more suitable stocking density of the two for commercial-scale hatcheries. This study aims to (1) compare SR of L. vannamei larvae at stocking densities of 90 and 120 individuals/L, (2) describe changes in temperature, pH, salinity, and ammonia/nitrite during rearing as supporting variables, and (3) examine the relationship between stocking density, water quality, and larval survival.

The study is limited to rearing from the nauplius stage (N5–N6) through PL5 in 45-ton tanks under two stocking densities, 90 and 120 individuals/L. SR is the primary response variable; larval length and weight are outside the scope of this study.

H_0: stocking densities of 90 and 120 individuals/L do not produce different SR in L. vannamei larvae from the nauplius stage through PL5. H_1: the two densities produce a significantly different SR.

METHOD

Study site, duration, and experimental unit

The study was conducted in 2024 at the hatchery facility of PT. Central Proteina Prima, Kalianda, South Lampung Regency, Lampung Province. Harvest of the six experimental units (three P90 tanks and three P120 tanks) took place on three consecutive dates, in pairs of two tanks per day: 23, 24, and 25 October 2024. Given a nominal rearing duration of 14 days from nauplius stocking (N5–N6) to PL5 harvest (Table 1), the corresponding stocking dates are estimated at approximately 10, 11, and 12 October 2024, respectively. This 3-day harvest window (and the corresponding ~3-day stocking window) indicates that all six units were run concurrently within the same short production cycle rather than in temporally separated batches, supporting comparability across replicates. All six tanks received nauplius originating from the same production source, were operated under identical standard operating procedures (water source, sterilization protocol, aeration configuration, feeding schedule, and prophylaxis program; Section 3.3.2 of the underlying thesis), and were housed within the same hatchery complex, so that ambient conditions external to the treatment (light, air temperature, personnel handling) were shared across units. Reported rearing duration ranged 14–20 days per replicate due to minor between-tank variation in micro-environmental conditions (Table 1); the dates above correspond to the nominal 14-day schedule and should be cross-checked by the author against the original hatchery production log if replicate-level deviations from this nominal schedule need to be reported.

Each 45-ton concrete tank was treated as one independent experimental unit. The Completely Randomized Design (CRD) comprised a single factor (initial nauplius stocking density) with two levels: P90 (90 individuals/L; N_0 = 3,600,000 per tank) and P120 (120 individuals/L; N_0 = 4,800,000 per tank), each replicated three times (n = 3), for six experimental units in total.

Equipment and materials

Primary equipment included 45-ton concrete rearing tanks, a blower-and-diffuser aeration system, a binocular microscope (40–400×), a refractometer (0.1 ppt), a digital pH meter (0.01), mercury/digital thermometers, and a spectrophotometer with ammonia/nitrite

reagent kits. Test organisms were L. vannamei nauplii at stage N5–N6 (25,200,000 individuals in total across all replicates). Feed and management materials comprised Chaetoceros sp. culture, Artemia salina nauplii, artificial crumble feed, probiotics, EDTA-2Na, vitamin C, and standard biosecurity/disinfection agents (chlorine, formalin, iodine).

Feeding schedule and density adjustment

Feed was not dosed at a fixed tank-level quantity but scaled to the estimated larval population of each unit, following the hatchery's standard operating dosage per million larvae: artificial feed at 5–9 kg per million individuals (nauplius–PL10) and 2–4 kg per million (PL4–PL10); Artemia nauplii at 2.5–4 kg per million (nauplius–PL10), 1.5–2.5 kg per million (nauplius–PL5), and 1–2 kg per million (PL4–PL10); and Chaetoceros sp. at a cell density of 3,000–5,000 cells/mL at the nauplius–early mysis stages rising to 10,000–20,000 cells/mL at the mysis stage, with a stage-wise increase from 40,000 cells/mL (Z1) to 80,000 cells/mL (Z3). Because dosage was scaled per estimated million larvae rather than fixed per tank, the P120 units received proportionally more total feed than the P90 units at every stage; feeding frequency followed the same schedule across all six units. This design was intended to keep feed availability per larva comparable between treatments; nevertheless, because uneaten feed and its decomposition products scale with both stocking density and the larger absolute feed mass required at P120, a residual density-linked difference in effective per-capita food access, and in organic loading, cannot be excluded and is discussed as a contributing mechanism below.

Prophylaxis and health management

Prophylactic agents applied uniformly across all units comprised: probiotics (2–25 ppm, dose adjusted to stage and water condition), EDTA-2Na (10–20 ppm, to chelate potentially toxic heavy-metal ions), molasses/Supermedia (6–12 ppm, as a substrate for beneficial bacterial growth), and, conditionally, vitamin C (2 ppm), micro-minerals (1–3 ppm), or Ecoguard (1–5 ppm). Tank and water sterilization prior to stocking used iodine (300 ppm), formalin (1,000 ppm for 24 h), and chlorine/virkon per the biosecurity protocol described in the underlying thesis (Section 3.6). Because the same agents, doses, and schedule were applied to both treatments, differential prophylaxis is not considered a likely explanation for the SR difference between P90 and P120; nevertheless, mortality attributable to residual pathogen load cannot be fully excluded without pathogen-monitoring data, which were not collected in this study.

Water-quality monitoring

Temperature, salinity, and pH were monitored daily. Ammonia and nitrite were quantified spectrophotometrically using colorimetric reagent kits; the spectrophotometric method measures total ammonia nitrogen (TAN = NH_3 + NH_4^+). The un-ionized ammonia fraction (NH_3-N), which is the toxicologically relevant species, was subsequently derived from TAN using the temperature- and pH-dependent equilibrium of Emerson et al. (1975): pKa = 0.09018 + 2729.92/(273.15 + T), with the NH_3 fraction (%) = 100 / (1 + 10^(pKa − pH)) and NH_3-N = TAN × fraction. Applying this conversion to the replicate-level TAN, pH, and temperature data yielded NH_3-N concentrations of 0.0285–0.0329 mg/L (mean 0.0304 mg/L) for P90 and 0.0307–0.0325 mg/L (mean 0.0314 mg/L) for P120 — both well below the 0.2 mg/L NH_3-N safety threshold (Fendjalang *et al.,* 2016), even though total ammonia nitrogen exceeded that threshold in both treatments. This indicates that, once expressed as the toxic un-ionized species rather than TAN, ammonia was unlikely to be acutely lethal in either treatment, and the small but consistent difference between treatments (P120 marginally higher) should be interpreted as a modest, not severe, contributing stressor. Dissolved oxygen was maintained above 5 ppm as a standard-operating control criterion via continuous aeration but was not logged as quantitative time-series data in this study; consequently, DO is reported here as a controlled condition rather than a measured outcome, and its potential role in the observed SR difference is discussed as inferred rather than demonstrated.

Sampling and survival rate determination

Harvest was conducted once larvae reached PL5, confirmed by microscopic verification of stage-specific morphology (a bifid telson, functional pleopods, and a juvenile-shrimp body form). The total number of postlarvae harvested from each tank was estimated using volumetric sampling: a minimum of five sub-samples were drawn from different horizontal locations and depths within each tank to ensure a representative estimate, and the resulting larval density was extrapolated to the full tank volume to obtain N_t. Survival rate (SR) was calculated as:

$$SR\ (\%) = (N_t / N_0) \times 100$$

where N_t is the number of larvae alive at the end of rearing (PL5) and N_0 is the number of nauplii stocked at the start.

Stage-transition monitoring (descriptive)

In addition to terminal SR, the approximate population at the zoea and mysis stages was estimated from sequential stage-transition survival percentages (nauplius→zoea, zoea→mysis), calculated the same way as terminal SR but applied between consecutive stages. This stage-transition efficiency was recorded as a descriptive, exploratory metric to help interpret when during rearing the density effect emerged; it was not defined as a primary outcome of this study and was not subjected to formal inferential statistical testing (no replicate-level variance or hypothesis test is reported for it). Only terminal SR at PL5 was analyzed inferentially.

Statistical analysis

Terminal SR data from the six experimental units were analyzed using an independent-samples t-test (pooled-variance) at $\alpha = 0.05$, because the design compares two independent groups. Prior to the t-test, normality within each treatment group was assessed with the Shapiro–Wilk test (P90: $W = 0.999$, $p = 0.936$; P120: $W = 0.869$, $p = 0.293$) and variance homogeneity with Levene's test ($F = 0.010$, $p = 0.924$); both assumptions were satisfied ($p > 0.05$ in all cases), justifying the pooled-variance t-test. In addition to the hypothesis-test decision, the mean difference between treatments, its 95% confidence interval, and Cohen's d effect size are reported to characterize the magnitude and precision of the treatment effect, consistent with current recommendations for reporting beyond a binary significance decision. With only three replicates per treatment, the Shapiro–Wilk test has limited power to detect non-normality, so these results should be read as not contradicting the normality assumption rather than as strong confirmation of it.

RESULT AND DISCUSSION

Larval development

Rearing spanned 14–20 days across four developmental stages (nauplius, zoea, mysis, postlarva; Table 1). Based on descriptive observation, the chronological progression through these stages appeared similar between P90 and P120, suggesting stocking density did not detectably alter the pace of metamorphosis. This observation is qualitative: stage timing was not quantified with replicate-level precision or compared statistically, so it should be read as a descriptive note rather than a tested finding.

Stage-transition efficiency (descriptive)

The estimated nauplius-to-zoea transition efficiency averaged 91.67% for P90 versus 82.67% for P120, and the zoea-to-mysis transition averaged 87.67% for P90 versus 79.67% for P120. As these are descriptive estimates rather than a formally tested outcome (see Method), they are presented only to indicate that the density-associated difference in survival was already apparent early in rearing and appeared to widen with time, a pattern consistent with the cumulative water-quality and competition mechanisms discussed below, and with the general pattern reported by Renitasari et al. (2023).

Terminal survival rate (SR) at PL5

P90 produced a mean SR of 83.70 ± 7.77% (estimated 3,013,200 postlarvae per tank from 3,600,000 nauplii stocked), while P120 produced a mean SR of 65.23 ± 6.51% (estimated 3,131,200 postlarvae per tank from 4,800,000 nauplii stocked). Although the absolute postlarval output of P120 was marginally higher (~3.9% more), this required 33% more nauplius input, so input-to-output efficiency clearly favored P90. The P90 SR exceeded the commonly cited commercial-hatchery benchmark of ≥70% (Widiastuti *et al.,* 2021), whereas P120 fell below it.

Water quality

Temperature (31°C), salinity (29–30 ppt), and pH (8.03–8.11) remained within optimal ranges in both treatments and are not considered limiting factors. Total ammonia nitrogen was higher in P120 (0.340–0.357 mg/L) than in P90 (0.292–0.353 mg/L), and nitrite was likewise higher in P120 (0.085–0.194 mg/L, approaching the 0.2 mg/L threshold) than in P90 (0.059–0.122 mg/L). These differences are consistent with greater metabolic-waste loading at the higher stocking density (Fendjalang *et al.,* 2016; Duan *et al.,* 2025) and provide a plausible chemical mechanism for the lower SR in P120; because dissolved oxygen was not measured as a time series and larval-level physiological stress was not assayed directly, this remains a correlational, not a directly demonstrated, causal link.

Statistical test

The independent-samples t-test yielded t = 3.156, exceeding the two-tailed critical value $t_{0.05,4}$ = 2.776 (df = 4); H_0 was therefore rejected. The mean difference in SR between P90 and P120 was 18.47 percentage points (SE = 5.853), with a 95% confidence interval of approximately 2.22 to 34.72 percentage points, and a standardized effect size of Cohen's d ≈ 2.58 — a very large effect by conventional benchmarks. Reporting the interval alongside the test decision indicates that, while the direction and general magnitude of the effect are well supported, the precision of the point estimate is limited by the small number of replicates (n = 3 per treatment), and the interval is wide.

Plausible mechanisms (not directly measured)

Three mechanisms are proposed to explain the lower SR at P120, each treated as a plausible contributor inferred from the water-quality data and prior literature rather than as directly measured in this study: (1) elevated ammonia/nitrite loading, supported by the water-quality measurements above; (2) intensified competition for feed, space, and oxygen at higher density — plausible given the larger absolute population, but not directly evidenced here since feed intake per larva, crowding behavior, and dissolved-oxygen time series were not measured; and (3) an increased likelihood of cannibalism during molting when larvae are in a vulnerable soft-shell state — a mechanism reported elsewhere in penaeid literature (Kharisma & Manan, 2012) but not directly observed, filmed, or quantified in this study. These three factors are discussed as a plausible, literature-consistent explanation for the observed SR gap, not as confirmed causal findings; direct measurement of DO, per-capita feed intake, and cannibalism incidence is recommended in future work.

Practical implications

Of the two densities tested, 90 individuals/L is the more suitable choice for 45-ton commercial hatchery tanks: it achieved higher SR, exceeded the commercial benchmark, and required about 25% fewer nauplii to reach a comparable postlarval output. This is a comparative statement limited to the two densities examined here; because no density below 90 or between 90 and 120 individuals/L was tested, the true optimum could lie outside this range, and this study does not establish 90 individuals/L as a global optimum.

CONCLUSION

Stocking density had a statistically significant effect on the SR of L. vannamei larvae from the nauplius (N5–N6) stage through PL5 (t = 3.156 > t-critical = 2.776; df = 4; H_0 rejected).

Of the two densities tested, P90 (90 individuals/L) produced a significantly higher SR (83.70 ± 7.77%) than P120 (120 individuals/L; 65.23 ± 6.51%), a mean difference of 18.47 percentage points (95% CI 2.22–34.72; Cohen's d ≈ 2.58). This difference coincided with higher ammonia and nitrite concentrations at P120, and is discussed — though not proven — alongside possible contributions from feed/space/oxygen competition and cannibalism during molting, none of which were measured directly. Temperature, salinity, and pH remained within optimal ranges in both treatments and are not considered limiting factors. In production terms, P90 is the more suitable of the two tested densities, yielding a nearly equivalent postlarval output while using approximately 25% fewer nauplii.

RECOMMENDATIONS

A stocking density of 90 individuals/L is recommended as an operational reference for 45-ton commercial hatchery tanks pending confirmation with a wider density gradient. Follow-up studies should (1) test a broader and finer density gradient (e.g., 60–150 individuals/L) to locate the true optimum, since only two points were tested here; (2) directly measure dissolved oxygen as a time series and quantify per-capita feed intake; (3) directly document and quantify cannibalism incidence, e.g., via video monitoring or gut-content analysis, during molting windows; and (4) increase the number of replicates to narrow the confidence interval around the treatment effect.

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