The Effect of Biomass Density on Transport Survival of Juvenile Freshwater Prawn, *Macrobrachium rosenbergii*

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ABSTRACT. In temperate regions freshwater prawn, *Macrobrachium rosenbergii*, juveniles are nursed to more advanced sizes ($\geq$0.3 g) in indoor tanks, then transported to ponds for growout. Stress during transport can produce immediate mortality and undetected mortality after pond stocking. This study was designed to evaluate the effect of biomass density during transport in sealed containers on pre-stocking prawn survival. Nine replicate styrofoam transport containers were prepared. Each contained one double bagged plastic bag with 10 L of oxygen-saturated 22EC water with an atmosphere of 10 L pure oxygen. Juvenile prawn weighing 0.26±0.02 g (average weight ±S.D.) were randomly stocked into transport containers at either 10, 25 or 50 g of prawn per liter of water, then sealed for eight hours (maximum regional transport period). There were three replicate transport containers per density. At eight hours post-stocking, bags were opened, water quality determinations were conducted, and live and dead animals were separated and counted. Total ammonia-nitrogen and un-ionized ammonia-nitrogen concentrations were significantly higher ($P \leq 0.05$) in containers stocked at 50 g/L than in containers stocked at either 10 or 25 g/L, which were also significantly different ($P \leq 0.05$) from each other. Nitrite-nitrogen concentrations were significantly higher ($P \leq 0.05$) for containers stocked at 50 g/L than in those stocked at 10 g/L. However, nitrite concentrations in containers stocked at 25 g/L were not significantly different ($P > 0.05$) from containers stocked at other densities. Dissolved oxygen was significantly lower ($P \leq 0.05$) in transport containers stocked at 50 g/L (1.3

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mg/L) than those stocked at 25 g/L or 10 g/L (1.6 mg/L and 3.2 mg/L, respectively), which were also significantly different ($P \leq 0.05$). Survival was significantly lower ($P \leq 0.05$) in transport containers stocked at 50 g/L (86.6%) than in containers stocked at 25 g/L (93.0%) and 10 g/L (97.2%), which were also significantly different ($P \leq 0.05$). These data indicate that transport densities greater than 10 g/L should be avoided for transport $\geq$ 8 hours in sealed containers.

KEYWORDS. Freshwater prawn, *Macrobrachium*, survival, transport

INTRODUCTION

In temperate climates the production of freshwater prawn, *Macrobrachium rosenbergii*, includes three phases: hatchery, nursery and pond growout. During nursery production, freshwater prawn attain an average weight of 0.3-0.6 g in 40-60 days (D'Abramo et al. 1989) prior to transport to ponds for growout. Recently, prawn production in the south-central United States has been increasing; however, the number of nurseries (suppliers of juvenile prawns) supplying several states is currently very limited. This means that prawn juveniles may need to be transported long distances from nursery facilities to growout ponds.

Poor survival during transport has been attributed to deteriorated water quality and predation in transport containers (Smith and Wannamaker 1983; Alias and Siraj 1988). Stress during transport may also cause unseen mortalities after prawn are stocked into ponds. Since individual pond stocking allocations are usually determined before prawn are transported to growout ponds, mortality during transport, or undetected mortality after stocking, can result in reduced pond stocking density and reduced pond production (Tidwell et al. 1996).

Various methods of transporting live prawn have been used including: open containers (hauling trucks), sealed inflated plastic bags, and transport in non-water environments using wet packing materials (Joshi and Raje 1993). Transporting prawn in plastic bags has the advantage of enabling producers who do not have specialized hauling equipment to pickup juveniles at nursery facilities and transport them, inside temperature controlled vehicles, to their ponds. Styrofoam transport containers are widely used in the ornamental fish industry. Within these containers, fish are placed in partially-filled plastic bags for shipments up to 24 hours. These containers are the method of choice in the ornamental fish industry because they are lightweight, insulating, watertight, durable, and inexpensive (Froese 1998). However, for producers to efficiently utilize this mode of transport, allowable densities and transport times must be established.

Relatively few studies have focused on the survival of freshwater prawn juveniles during transportation to growout ponds. Stocking densities and associated water quality in transport containers are important factors affecting immediate and subsequent prawn survival. Smith and Wannamaker (1983) successfully shipped 6 g juveniles in aerated plastic bags at 18 g/L for 24 hours and reported that neither increased salinity nor increased substrate benefitted survival. Harrison and Lutz (1980) reported survival of juvenile prawns was increased when water temperature was reduced during transport (17-23EC). Smaller post-larvae (0.01-0.1g) prawn are generally shipped in plastic bags at densities of 1.5-4.5 g/L of shipping water (Smith and Wannamaker 1983). Other published reports used 7-28 day juveniles which are typically stocked into growout ponds in tropical areas, and found that densities of approximately 15 g/L resulted in survivals >90% after 6 and up to 12 hrs (Alias and Siraj 1988).

Major differences have been reported regarding acceptable biomass densities and water quality tolerances of different size prawns (Smith and Wannamaker 1991; Strauss et al. 1991). It is generally accepted that aquatic animals are more sensitive to decreases in dissolved oxygen and less sensitive to the toxicity of nitrogenous waste products (particularly NH_3) as size and age increase. This is due primarily to the development of physiological detoxification mechanisms and a decrease in weight specific metabolic rate (Gasca-Leyva et al. 1991; Strauss et al. 1991).

No published studies have specifically addressed the effect of biomass density on the 40-60 day nursed juveniles (0.3-0.5 g), which are typically stocked in growout ponds in temperate regions of the United States. This study was designed to evaluate the effect of biomass density within sealed shipping containers on water quality and survival of 60 day nursed prawn juveniles during an eight hour transport period.

MATERIALS AND METHODS

Nine replicate styrofoam transport containers (39 cm $\times$ 39 cm $\times$ 25 cm) were prepared as they would be for prawn transport from nursery to growout ponds. Each contained a plastic bag filled with 10 L of reservoir water under an atmosphere of 10 L pure oxygen. This bag was then sealed inside a second plastic bag. Baseline water quality values were: temperature, 21.8EC; dissolved oxygen, 5.2 mg/L; nitrite-nitrogen, 0.02 mg/L; total ammonia-nitrogen 0.78 mg/L; un-ionized ammonia-nitrogen 0.08 mg/L and pH 8.4.

Prawn were fasted for 24 hours prior to stocking. Juvenile prawn (60 day post-larvae) weighing 0.26±0.02 g were then randomly stocked at regular 15 minute intervals into each of nine transport containers at either 10, 25 or 50 grams of prawn biomass per liter of water. The water was saturated (8.6 mg/L at 22EC) with pure oxygen prior to sealing the bags. There were three replicate containers per treatment (biomass density). At eight hours post-stocking (maximum regional transport period), bags were opened at corresponding 15 minute intervals, water quality determinations were conducted, and live and dead animals were separated visually and counted.

Water temperature and dissolved oxygen were measured using a YSI Model 55 oxygen meter (YSI Industries, Yellow Springs, Ohio[1]). Total ammonia-nitrogen and nitrite-nitrogen were measured using a DREL 2000 spectrophotometer (Hach Company, Loveland, Colorado); pH was measured with a electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio). Un-ionized ammonia was calculated as a percentage of total ammonia according to Boyd (1979).

Survival and water quality data were analyzed by analysis of variance (ANOVA) using Statistix version 4.1 (Analytical Software, Tallahassee, Florida). If ANOVA indicated significant treatment effects, Fisher's Least Significant Difference test (LSD) was used to determine differences among means ($P \leq 0.05$). All percentage and ratio data were transformed to arcsin values prior to analysis (Zar 1984). Data are presented untransformed to facilitate interpretation.

RESULTS AND DISCUSSION

Total ammonia-nitrogen and un-ionized ammonia-nitrogen concentrations were significantly higher ($P \leq 0.05$) in containers stocked at 50 g/L than in containers stocked at either 10 or 25 g/L, which were also significantly different ($P \leq 0.05$) (Table 1). Measured pH values were not significantly different between treatments ($P > 0.05$) and averaged 8.1 overall. Nitrite-nitrogen was significantly higher ($P \leq 0.05$) in containers stocked at 50 g/L than in containers stocked at 10 g/L. Nitrite concentrations in containers stocked at 25 g/L were not significantly different ($P > 0.05$) from containers stocked at other densities (Table 1). Dissolved oxygen concentrations decreased with increasing prawn density and were significantly lower ($P \leq 0.05$) in transport containers stocked at 50 g/L (1.3 mg/L) than in those stocked at 25 g/L or 10 g/L (1.6 mg/L and 3.2 mg/L, respectively) which were also significantly different

1. Use of trade or manufacturer's name does not imply endorsement.

TABLE 1. Prawn survival (%), dissolved oxygen (mg/L), total ammonia (mg/L), un-ionized ammonia (mg/L), nitrite (mg/L), and pH in transport containers stocked at 10, 25, and 50 g/L for eight hours. Values are averages±S.D. of three replicate containers. Values in the same row followed by different were significantly different ($P \leq 0.05$).

	Biomass Densities During Transport		
	10 g/L	25 g/L	50 g/L
Survival (%)	97.2±0.2a	93.0±0.1b	86.6±2.1c
Dissolved oxygen (mg/L)	3.1±0.1a	1.5±0.2b	1.3±0.1c
Total ammonia-nitrogen (mg/L)	3.7±0.3a	4.9±0.2b	6.7±0.9c
Un-ionized ammonia-nitrogen (mg/L)	0.23±0.05a	0.31±0.05b	0.40±0.09c
Nitrite-nitrogen (mg/L)	0.04±0.00a	0.12±0.11ab	0.23±0.07b
pH	8.1±0.1a	8.0±0.0a	8.0±0.1a

($P \leq 0.05$) (Table 1). Survival was significantly lower ($P \leq 0.05$) in transport containers stocked at 50 g/L (86.6%) than in those stocked at 25 g/L or 10 g/L (93% and 97.2%, respectively) which were also significantly different ($P \leq 0.05$) (Table 1).

After eight hours of simulated shipping conditions dissolved oxygen concentrations decreased; where total ammonia, un-ionized ammonia, and nitrite concentrations increased. This has been consistently observed in other transport studies (Smith and Wannamaker et al. 1983; Alias and Siraj 1988; Vadhyar et al. 1992). Strauss et al. (1991) determined that juvenile freshwater prawn could tolerate exposure to >2 mg/L un-ionized ammonia at a pH of 8.5 for up to 72 hours. Armstrong et al. (1976) indicated that post-larvae prawn could tolerate nitrite concentrations up to 1.8 mg/L. This indicates that decreased survivals at the higher stocking density in this study were not likely a result of the toxicity of nitrogenous compounds as measured NH_3 concentrations were not considered lethal at the pH and temperature values in this study.

New (1990) indicated that survival in transport of *M. rosenbergii* is more closely related to decreased dissolved oxygen level than any other water quality variable. Although, adult *M. rosenbergii* can tolerate dissolved oxygen levels as low as 1 mg/L for short time periods (Avault 1987), the dissolved oxygen concentrations measured in transport containers stocked at 50 and 25 g/L (<2 mg/L) may represent stressful conditions for juvenile freshwater prawn (D'Abramo et al. 1989). Also, when applying these data to practice, it is essential to understand that increases in water temperatures during transport could increase oxygen consumption and nitrogen excretion rates (Chen and

Kou 1996), further reducing acceptable biomass densities and/or transport times.

Vadhyar et al. (1992) reported 100% survival at biomass densities similar to the low stocking density in this study (10 g/L) and 87% survival for those stocked at approximately 20 g/L when transporting smaller, 10-15 day old, postlarvae for 6 hours. Smith and Wannamaker (1983) reported 100% survival for 6 g juveniles stocked at 18 g/L for 8 hours with no mention of adverse affects on water quality. However, the smaller prawn used in this study have higher oxygen consumption rates.

To determine the most economical transport density, "real costs" for individual juveniles stocked live into growout ponds were computed and compared by considering the cost of stocker prawn, transport containers, labor, and survival. Real cost calculations were based on a stocking target of 10,000 total prawn, a cost of $0.10 per juvenile prawn, a cost of $10.00 for each shipping container (which includes packing labor), and the 97%, 93% and 87% survivals obtained under the 10 g/L, 25 g/L, and 50 g/L transport densities, respectively. Real costs were calculated to be $0.114/individual for prawn transported at 25 g/L, $0.117/individual for prawn transported at 10 g/L, and $0.118/individual for prawn transported at 50 g/L.

While these economic data indicate that transporting at 25 g/L has the lowest cost per individual prawn, water quality and survival data indicate that stocking densities greater than 10 g/L, for durations in excess of 8 hours in sealed containers, may result in deteriorated water quality and stressful conditions for transported prawn. This study did not quantify delayed mortality after pond stocking, which could be significant. Future studies should determine delayed mortality 5-7 days following the simulated transport conditions to more accurately determine transport success and profitability. Additional research should also be conducted to determine methods to increase transport densities and times including: further reducing water temperatures, increased volumes of pure oxygen atmospheres, utilization of substrate materials, and the use of ammonia absorbent materials, and anesthetics.

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