North American Journal of Aquaculture 68:271–275, 2006

DOI: 10.1577/A05-049.1

[Article]

The Effect of Biomass Density, Salinity, and Substrate on Transport Survival of Juvenile Freshwater Prawns *Macrobrachium rosenbergii* in Continuously Oxygenated, Vented Containers

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Abstract.—During the stocking of grow-out ponds, juvenile prawns are usually transported from regional nurseries by truck in vented tanks. Hauling stress and associated delayed mortality have been implicated as potential causes of low pond survival. An experiment was conducted to evaluate the effects of biomass density, substrate, and salinity on water quality and transport survival of juvenile freshwater prawns *Macrobrachium rosenbergii*. The trial was designed as a 3 × 2 × 2 factorial and evaluated three biomass densities (10, 20, and 30 g/L), the presence or absence of added substrate (plastic mesh to provide a 200% increase in surface area), and the presence or absence of added salt (0‰ or 6‰). Model transport tanks were 15-L, open styrofoam containers aerated with pure oxygen and compressed air. Water quality analyses were performed prior to stocking. After 24 h, water quality analyses were again conducted and all prawns were removed, designated as alive or dead, weighed, and counted. After 24 h of simulated transport, three-way analysis of variance indicated no significant interactions ($P > 0.05$) between the main effects of density, substrate, or salinity for prawn survival or water quality. When the main effects were analyzed separately, survival was significantly influenced by density. Survival was significantly lower ($P < 0.05$) in the high-density treatment (30 g/L: 89% survival) than in the medium- (20-g/L) and low-density (10-g/L) treatments, which were not significantly different ($P > 0.05$) and averaged 95% survival overall. Total ammonia nitrogen and un-ionized ammonia nitrogen concentrations significantly increased ($P < 0.05$) as biomass density increased. The addition of salt to the transport water did not significantly affect survival ($P > 0.05$) but did result in a significant decrease ($P < 0.05$) in both pH and un-ionized ammonia nitrogen. Added substrate had no significant ($P > 0.05$) impact on any measured variable. These data indicate that good survival can be achieved at a biomass density of 20 g/L and that increased salinity appears to reduce the buildup of toxic metabolites. The addition of substrate to the transport tank appears to provide no benefit.

Juvenile freshwater prawns *Macrobrachium rosenbergii* are typically transported in vented tanks by truck from nurseries to pond grow-out facilities. Of the published studies related to the transport of prawn juveniles, most have used sealed, oxygenated polyethylene bags (Smith and Wannamaker 1983; Alias and Siraj 1988; Coyle et al. 2001). The efficacy of such procedures is constrained by the inevitable reduction of dissolved oxygen levels and increases in dissolved ammonia concentrations in the shipping water due to the metabolism of the animals (Schmitt and Uglow 1996). Furthermore, transport of juveniles in plastic bags is not economically feasible for transporting large quantities of juveniles. Consequently, the transport of juveniles in trucks equipped with larger (300–1,000-L) transport tanks and with a constant source of oxygen is more commonly practiced. However, relatively little, if any, research has focused on the transport of freshwater prawn juveniles in these larger vented containers. Since oxygen is continuously supplied and does not become limiting, the densities and technologies that are optimal for transport in vented containers may not be the same as in sealed plastic bags.

Stocking density is one of the most important factors affecting survival and economics during transportation of aquatic animals. Previous research reported acceptable transport densities for juvenile prawns as 10–25 g/L when transported in sealed, oxygenated plastic bags for up to 24 h (Coyle et al. 2001). Smith and Wannamaker (1983) successfully shipped 6-g juveniles in plastic bags at 18 g/L for 24 h and reported that survival in transport was more closely related to dissolved oxygen concentrations than any other water quality variable. Another study found that densities of approximately 15 g/L resulted in survivals above 90% after 6 h and up to 12 h (Alias and Siraj 1988). Coyle et al. (in press) indicated that adult prawns (48 g) could be successfully live-hauled (i.e., with little mortality) at a density of 100 g/L for up to 24 h in open containers continually supplied with oxygen. This suggests that

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Received April 29, 2005; accepted August 10, 2005

increased stocking densities may be possible in open containers and that such systems should be evaluated to reduce the cost of transporting juveniles to grow-out ponds. However, studies to determine the appropriate biomass densities of juvenile prawns in open, continuously oxygenated containers are lacking.

Artificial substrates are used as a method to increase survival and production in prawn nursery tanks (Smith and Sandifer 1975; Alston and Sampaio 2000; Coyle et al. 2003) and to increase stocking densities and production in ponds (Cohen et al. 1983; Ra'anan et al. 1984; Tidwell et al. 2000) by reducing prawn-to-prawn interaction. Smith and Wannamaker (1983) reported that substrate did not increase the survival of juvenile prawns transported in oxygenated polyethylene bags. However, the effect of substrates has not been evaluated in larger vented containers.

Increasing salinity is a method that is often utilized when transporting finfish to alleviate stress by providing a more isotonic environment (Jensen 1990) and is used during the transport of prawn juveniles by some hatcheries (New 1990). However, Smith and Wannamaker (1983) reported that increased salinity did not benefit the survival of prawn juveniles transported in sealed polyethylene bags. Increasing salinity lowers the solubility of oxygen in water (Boyd 1979) but may decrease oxygen demand and nitrogenous excretion by reducing the energy needed for osmoregulation. The effect of salinity on prawn survival and associated water quality variables in transport containers should be evaluated.

The objective of this study was to evaluate the effects of biomass density, salinity, substrate presence, and their potential interactions on the survival of juvenile freshwater prawns and subsequent water quality variables in open, "model" transport containers supplied with a continual source of oxygen for up to 24 h.

Methods

Water quality.—Springwater was used to fill transport containers. Water quality analysis was performed on each replicate container prior to stocking and at the conclusion of the experiment. Water temperature and dissolved oxygen were measured by use of a YSI Model 58 oxygen meter (YSI Industries, Yellow Springs, Ohio). Total ammonia nitrogen and nitrite-nitrogen were measured with a DREL 2000 spectrophotometer (Hach Company, Loveland, Colorado); pH was measured with an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio). Un-ionized ammonia was calculated as a percentage of total ammonia according to Boyd (1979). Baseline water quality values were as follows: temperature was 21.2°C; dissolved oxygen was 6.2 mg/L; nitrite-nitrogen was 0.08 mg/L; total ammonia nitrogen was 0.37 mg/L; un-ionized ammonia nitrogen was 0.03 mg/L; and pH was 8.3.

System.—This research was conducted at Kentucky State University's Aquaculture Research Center (ARC), Frankfort, Kentucky. Postlarval (PL) prawns were shipped by air from a commercial hatchery (Aquaculture of Texas, Weatherford, Texas) and nursed in a greenhouse at ARC for 60 d. Approximately 50,000 ungraded 60-d-old PL freshwater prawns (0.75 ± 0.2 g) were removed from nursery tanks and stocked into each of two 3,700-L fiberglass rectangular tanks. Prawns were fasted and held at 20°C with a flow rate of 60 L/min for 2 d prior to experiments to ensure that all prawns were in a postmolt or "hardened" state. The trial was designed as a 3 × 2 × 2 factorial and evaluated three biomass densities (10, 20, and 30 g/L), the presence or absence of added substrate (plastic mesh to provide a 200% increase in surface area), and the presence or absence of added salt (0‰ or 6‰).

Substrate consisted of 2.0-mm plastic mesh supported by a polyvinyl chloride frame included at a rate sufficient to increase the bottom surface area by 200%. The salt used to increase salinity was high-grade evaporated salt (Cargill, Inc., Minneapolis, Minnesota). Twenty-seven styrofoam transport containers (39 × 39 × 25 cm) were used as model transport tanks. There were three replicates of each of the nine treatment combinations. The system was indoors and remained static throughout the experiment. Each container was supplied with both pure oxygen from an oxygen tank and compressed air from a regenerative blower (to provide circulation). These were delivered through separate 5.0 × 2.5-cm air stones in each container to maintain dissolved oxygen concentrations near saturation. After 24 h, water quality analysis was again performed and all prawns were removed, designated as alive or dead, weighed, and counted.

Statistical analysis.—Data were analyzed by three-way analysis of variance (ANOVA) in Statistix version 7.0 (Analytical Software, Tallahassee, Florida) to determine the effects of density, substrate, salinity, and their interactions on water quality and prawn survival (Steel and Torrie 1980). If no statistical interactions were identified ($P > 0.05$), the main effects of density, substrate, and salinity were subjected to separate ANOVAs to evaluate density effects. If ANOVA indicated significant treatment effects, the least-significant-difference test was used to determine differences among means ($P < 0.05$). Student's t-test was used to evaluate the effects of substrate and salinity. All percentage and ratio data were transformed to arcsine values prior to analysis (Zar 1984). Data are presented as untransformed values to facilitate interpretation.

Results

After 24 h of simulated transport, three-way ANOVA indicated no significant interactions ($P > 0.05$) between the main effects of density, substrate, and salinity for prawn survival (Table 1). There were also no significant interactions ($P > 0.05$) between the main effects on any measured water quality variable. This allowed density, substrate, and salinity to be analyzed separately for their effects on water quality and prawn survival (Table 2).

Survival was significantly lower ($P < 0.05$) in the high-density treatment (30 g/L: 89% survival) than in the medium- (20-g/L) and low-density (10-g/L) treatments, which were not significantly different ($P > 0.05$) and averaged 95% survival overall. Total ammonia nitrogen and un-ionized ammonia nitrogen concentrations increased ($P < 0.05$) as biomass density increased. The nitrite-nitrogen concentration was significantly higher ($P < 0.05$) in the high-density treatment (0.27 mg/L) than in the medium- and low-density treatments, which were not significantly different ($P > 0.05$; combined mean = 0.12 mg/L) (Table 2).

Increasing the salinity of the transport water had no significant ($P > 0.05$) impact on prawn survival. However, the addition of salt did result in a significant decrease ($P < 0.05$) in both pH and un-ionized ammonia nitrogen of the transport water relative to those of treatments without added salt (Table 2). The presence or absence of substrate had no significant impact ($P > 0.05$) on any measured water quality variable or on prawn survival after 24 h of simulated transport.

Discussion

In the high-density treatment, the un-ionized ammonia concentration averaged 2.2 mg/L. Strauss et al. (1991) determined that juvenile freshwater prawns could tolerate exposure to 2.0-mg/L un-ionized ammonia at a pH of 8.5 for up to 72 h. Therefore, measured un-ionized ammonia concentrations in the high-density treatment were near the lethal concentration at the pH and temperature values measured in this study and probably contributed to the decrease in survival. Smith and Wannamaker (1983) indicated that survival during the transport of freshwater prawns is more closely related to dissolved oxygen level than to any other water quality variable when prawns are transported in sealed plastic bags. However, these data suggest that when vented containers supplied with a continual source of oxygen are used, the first limiting factor is likely to be un-ionized ammonia.

Previous research has reported that acceptable transport densities for juvenile prawns are 10–25 g/L in sealed, oxygenated plastic bags for up to 24 h (Smith and Wannamaker 1983; Alias and Siraj 1988; Coyle et al. 2001). The results of this study indicate that allowable transport densities for prawn juveniles are similar when transported in vented containers (20 g/L). However, Coyle et al. (in press) reported 100% survival after 24 h of simulated transport of adult prawns (48 g) at a biomass density of 100 g/L in vented containers, indicating that much higher densities are possible with older, larger animals. Additional research should characterize allowable transport densities for different sizes and age-groups of freshwater prawns in continuously oxygenated containers.

In this study, increasing salinity reduced the buildup of toxic metabolites but did not improve survival. This is in agreement with the results of Smith and Wannamaker (1983), who also found that the use of brackish water (8‰) did not result in any significant improvement in survival, but differs from the results of Venkataswamy et al. (1992), who reported increased juvenile survival of the prawn *M. malcolmsonii* during transport in brackish water (10‰).

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TABLE 1.—Analysis of variance results testing the validity of the relationship between freshwater prawn survival and the indicated values during simulated transport (SS = sum of squares; MS = mean square).

Source	SS	df	MS	*F*
Main effects				
Prawn (g/L) (*A*)	278.9	2	139.5	17.5*
Salt (0‰ or 6‰)	16.1	1	16.1	2.0
Substrate (presence or absence) (*C*)	5.1	1	5.1	0.6
Interaction				
$A \times B$	2.2	1	2.2	0.3
$A \times C$	5.6	1	5.6	0.7
$B \times C$	9.9	2	5.0	0.6
$A \times B \times C$	22.4	2	11.2	1.4
Error	190.9	24	8.0	
Total	531.3	35	15.2	

TABLE 2.—Comparison (mean ± SE) of the main effects of density, salt, and substrate on the indicated values after 24 h of simulated transport of juvenile freshwater prawns (DO = dissolved oxygen; TAN = total ammonia nitrogen; UAN = un-ionized ammonia nitrogen.) Values followed by different letters within columns and main effects are significantly different ($P = 0.05$).

Main effects	Treatment	Survival (%)	Temperature (°C)	DO (mg/L)	TAN (mg/L)	UAN (mg/L)	Nitrite (mg/L)	pH
Density	10 g/L	94.6 ± 0.7 z	23.8 ± 0.0 z	7.3 ± 0.3 z	8.4 ± 1.2 x	0.8 ± 0.1 y	0.1 ± 0.0 y	8.3 ± 0.0 z
	20 g/L	94.8 ± 0.8 z	23.9 ± 0.0 z	7.3 ± 0.4 z	13.9 ± 0.8 y	1.0 ± 0.1 y	0.1 ± 0.0 y	8.2 ± 0.0 y
	30 g/L	88.8 ± 0.9 y	23.6 ± 0.1 z	7.2 ± 0.4 z	23.8 ± 1.3 z	2.2 ± 0.3 z	0.3 ± 0.0 z	8.3 ± 0.1 zy
Salt	Present	92.1 ± 1.0 z	23.5 ± 0.1 z	7.3 ± 0.3 z	15.7 ± 1.8 z	1.1 ± 0.1 y	0.2 ± 0.0 z	8.2 ± 0.0 y
	Absent	93.4 ± 0.9 z	23.5 ± 0.2 z	7.9 ± 0.3 z	15.1 ± 1.8 z	1.6 ± 0.2 z	0.2 ± 0.0 z	8.3 ± 0.0 z
Substrate	Present	93.1 ± 0.9 z	23.5 ± 0.1 z	7.6 ± 0.3 z	15.4 ± 1.8 z	1.3 ± 0.2 z	0.2 ± 0.0 z	8.2 ± 0.0 z
	Absent	92.3 ± 1.0 z	23.5 ± 0.2 z	7.6 ± 0.3 z	15.4 ± 1.8 z	1.4 ± 0.2 z	0.2 ± 0.0 z	8.3 ± 0.0 z

animals is a common practice for providing a more isotonic environment, which reduces the metabolic demand for osmoregulation and generally reduces oxygen consumption and nitrogenous waste production and stress. New and Singholka (1985) reported that some hatcheries add seawater to transport water, claiming that survival rates are better in brackish water than in freshwater. The isosmotic point for freshwater prawns is reported to be 17‰ (Sandifer et al. 1975), which could potentially further reduce ammonia efflux rates and oxygen consumption (Singh 1980). In this study, 6‰ did decrease un-ionized ammonia concentrations and therefore should be considered beneficial. Future studies should evaluate salinity levels closer to the isosmotic concentration (17‰) in transport to determine the effect on ammonia efflux, oxygen uptake, and survival.

The presence or absence of substrate had no significant effect ($P > 0.05$) on any measured water quality variable or prawn survival after 24 h of simulated transport. These data are in agreement with the findings of Smith and Wannamaker (1983), who reported that increased substrate did not benefit survival when 6-g juveniles were shipped in aerated plastic bags at 18 g/L for 24 h. However, Alias and Siraj (1988) reported increased survival of PL prawns by providing polypropylene netting as habitat during transport. Vadhyar et al. (1992) evaluated different substrate materials (plastic straws, plastic ribbon, and plastic netting) at different densities (100, 200, 250, 300, 400, and 800 PL prawns/L) in model transport containers to determine the effect on the safe duration (time) and found that only plastic straws at the lowest densities (100 and 200 PL prawns/L) resulted in a significant increase in safe transport duration relative to that of controls. Additional research is required to evaluate the effect of different types of substrate that provide cavernous refuge areas on the transport survival of juvenile prawns.

Increases in transport temperatures significantly increase oxygen consumption and nitrogenous excretion (Chen and Kou 1996), thereby reducing acceptable biomass densities or transport times. Harrison and Lutz (1980) reported that the survival of juvenile prawns increased when transported at a reduced water temperature (20°C). The water temperature in this study increased from 21°C to 24°C over the duration of the experiment. A direct relationship between temperature increase and nitrogen efflux rates was reported for freshwater prawns by Schmitt and Uglow (1996). The authors concluded that temperature changes during the shipment of freshwater prawns should be avoided or made gradually, thus indicating the importance of insulated transport containers. Further temperature reductions and better maintenance of transport temperature may allow higher biomass densities and reduce the accumulation of nitrogenous by-products.

Previous research has reported acceptable transport densities of 10–25 g/L for juvenile prawns placed in sealed, oxygenated plastic bags for up to 24 h (Coyle et al. 2001). The results of this study indicate that (1) allowable transport densities in vented containers are similar (20 g/L) and (2) during the use of vented containers supplied with a continual source of oxygen, un-ionized ammonia will probably become the first limiting factor. Due to the decrease in un-ionized ammonia concentrations of transport water with increased salinity, the addition of salt to transport water should be considered beneficial and higher rates should be evaluated. The addition of substrate to the transport tank appears to provide no benefit, but only one type of substrate was evaluated. Additional research should evaluate reductions in transport temperature (<20°C), the use of different types of substrate, and the incorporation of biofiltration as techniques to increase acceptable biomass densities.

Acknowledgments

Special thanks to Russell Neal and Angela Caporelli for technical support throughout the study. This research was funded by the U.S. Department of Agriculture Federal and State Market Improvement Program and by Kentucky's Regional University

Excellence Trust Fund to the Aquaculture Program as Kentucky State University's Program of Distinction.

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