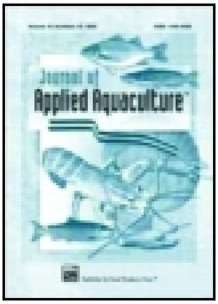

Journal of Applied Aquaculture
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/wjaa20

The Effect of Biomass Density, Temperature, and Substrate on Transport Survival of Market-Size Freshwater Prawn, Macrobrachium rosenbergii

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Version of record first published: 04 Oct 2008.

To cite this article: Shawn D. Coyle, James H. Tidwell, David K. Yasharian, Angela Caporelli & Nicholas A. Skudlarek (2005): The Effect of Biomass Density, Temperature, and Substrate on Transport Survival of Market-Size Freshwater Prawn, Macrobrachium rosenbergii , Journal of Applied Aquaculture, 17:4, 61-71

To link to this article: http://dx.doi.org/10.1300/J028v17n04_04

The Effect of Biomass Density, Temperature, and Substrate on Transport Survival of Market-Size Freshwater Prawn, *Macrobrachium rosenbergii*

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ABSTRACT. After harvest, adult prawn are often transported for sales to live markets, such as ethnic Asian outlets, in major urban centers. Poor survival during transportation has hindered development and expansion of these markets. Methodologies to increase survival during transport could contribute to industry viability. Three independent trials were conducted. In the first trial, three biomass densities (25, 50 and 100 g/L) were evaluated in 100-L, open plastic containers aerated with pure oxygen and compressed air. Water quality analyses were performed prior to stocking. After 24 hours in the model transport containers, water quality analysis was conducted and all prawn were removed, determined to be alive or dead, and each group weighed and counted. There was no significant difference ($P > 0.05$) for prawn survival (overall mean 98%) among the three densities. Concentrations of dissolved oxygen and nitrite were not effected by hauling density ($P < 0.05$). Total ammonia-nitrogen and un-ionized ammonia-nitrogen concentrations increased ($P < 0.05$) as biomass densities increased, though values remained within

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Journal of Applied Aquaculture, Vol. 17(4) 2005
Available online at http://www.haworthpress.com/web/JAA

doi:10.1300/J028v17n04_04

what are considered tolerable ranges. In the second trial, the effect of added substrate and temperature on transport survival was evaluated with prawns stocked at the high density (100 g/L). Two water temperatures (21°C and 26°C) with and without substrate were evaluated in a 2 × 2 factorial with three replicate, 100-L model transport containers per treatment combination (12 containers total). Factorial analysis indicated no significant statistical interaction ($P > 0.05$) between the presence of substrate and water temperature on any measured variable. The main effects of substrate and temperature were then analyzed separately. The presence or absence of substrate had no significant impact ($P > 0.05$) on prawn survival. However, temperature had a highly significantly impact ($P < 0.01$) on survival; prawn survival at 21°C averaged 97% compared to 24% at 26°C. The third trial was a commercial verification trial in which 500 kg of live prawn were transported to New York from Kentucky. These data indicate prawn can be successfully transported at 100 g/L for 24 hours when temperatures are maintained near 21°C. Adding substrate to the transport tank appears to provide no benefit.

KEYWORDS. Transport, substrate, temperature, freshwater prawn, *Macrobrachium rosenbergii*

INTRODUCTION

According to the FAO, annual global expansion of freshwater prawn farming over the decade 1992-2001 was 27.4% per year compared to only 3.9% per year for marine shrimp (New 2005). Farmed production in the United States was reported to be 44 mt in 2001 (FAO 2003). Production in the US offers the opportunity for prawns to be produced in close proximity to large inland urban markets and for producers to supply these markets with unique product forms, such as fresh or live product (Tidwell and D'Abramo 2000). In general, marketing freshwater prawns alive will generate a higher price than other product forms (Phillips and Lacroix 2000). Live products are especially important for fulfilling the demand of special niche markets, such as the large ethnic communities, found in many urban areas of the United States. Preliminary market research has identified a market potential in excess of 50,000 kg per year for live freshwater prawn in ethnic Asian communities located in urban areas of the

US and Canada (personal communication: Michael Lamb, Big Land Farm, Toronto, Canada). However, supplying these markets has been difficult due to poor survival during transport. If techniques could be developed that would enable market size prawns to be successfully transported live at relatively high densities, there appears to be significant market opportunity (Tidwell et al. 2005).

Stocking density is one of the most important factors affecting survival and economics during transportation of freshwater prawn. Previous research reported acceptable transport densities for juvenile prawn to be 10-25 g/L (Coyle et al. 2001). Harrison and Lutz (1980) reported survival of juvenile prawn increased when transported at reduced water temperature (20°C). However, relatively little research has focused on the survival of adult freshwater prawn during transport. Smith and Wannamaker (1983) successfully shipped small (17 g) adults in aerated plastic bags at 12-15 g/L for up to 48 hours with good success, but reported poor survival at 19 g/L. They reported that neither increased salinity or added substrate increased survival and concluded that survival rates were more dependant on dissolved oxygen concentrations than to other water quality parameters. However, these densities are very low compared to those reported for finfish 100-200 g/L (Jensen 1990), and are not economically feasible for long distance transport of market-size prawn.

When live market-size finfish are transported, it is normally in trucks equipped with larger (300-1,000 L) transport tanks and a constant source of oxygen. However, most prawn work has used sealed bags and little, if any, research has evaluated transport of prawns in these larger vented containers. D'Abramo et al. (1995), referring to unpublished data, indicated that adult prawns could be successfully live-hauled in open containers for at least 24 hours at a density of 60 g/L with little mortality. This suggests that much higher densities may be possible in open containers than those reported for sealed containers (15 g/L).

This research was designed to determine appropriate technologies for the transport of market size prawns in open model transport containers for up to 24 hours. Trial 1 was designed to evaluate acceptable biomass densities for transport. Trial 2 was designed to evaluate the impact of water temperature and added substrate on transport survival. Trial 3 was a commercial verification trial in which 500 kg of prawn were transported live to New York from Kentucky.

MATERIALS AND METHODS

Water Quality

The water used to fill transport containers in both experiments was spring water (15°C) which was heated to the appropriate treatment temperature. Baseline water quality values were determined for each replicate container prior to stocking. The same analyses were conducted at the termination of each experiment (24-hours post-stocking). In both trials, water temperature and dissolved oxygen were measured using a YSI Model 58 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 an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio). Un-ionized ammonia was calculated as a percentage of total ammonia based on temperature and pH according to Boyd (1979). Baseline water quality characteristics of the spring water used in the studies were: dissolved oxygen, 7.1-8.3 mg/L; nitrite-nitrogen, 0.0-0.1 mg/L; total ammonia-nitrogen, 0.2-0.3 mg/L; un-ionized ammonia nitrogen, 0.0 mg/L; total alkalinity, 97-106 mg/L; total hardness, 102-104 mg/L, and pH 7.3.

Trial 1

This research was conducted at Kentucky State University's, Aquaculture Research Center, Frankfort, Kentucky. Un-graded, mixed-sex, adult prawn (47.5±3.2 g) were obtained at harvest from research ponds. Approximately 50 kg were stocked into each of two 3,700-L fiberglass rectangular tanks. Prawn were not fed and held at 20°C with a flow rate of 60 L/minute for one week prior to conducting experiments to ensure that all prawn were in a post-molt or "hardened" state. Three stocking densities (25, 50, and 100 g/L) were evaluated with three replications per treatment. Insulated 100-L rectangular plastic coolers (H × W × L: 57 cm × 57 cm × 144 cm, respectively) were used as model transport tanks. Each container was supplied with both pure oxygen from an oxygen tank and compressed air from a regenerative blower to provide circulation. This combination is the commercial standard when transporting marine shrimp (personal communication, Steven Guise, Todays Fresh

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

Catch, Inc., Spring Hill, Florida). These gases were delivered through separate 5.0 cm × 2.5 cm air stones in each container to maintain dissolved oxygen concentrations near saturation. After 24 hours, water quality analysis was performed, all prawn were removed, determined to be alive or dead, weighed and counted.

Trial 2

This study evaluated the effect of added substrate and water temperature on transport survival. The experiment was designed as a 2×2 factorial evaluating two temperatures (21°C and 26°C) and the presence of substrate (with and without). Each treatment combination was replicated three times (12 experimental units). The experimental system was the same as described for Trial 1. Each container was stocked with 10 kg of adult market size prawn (100 g/L; average individual weight 46.5±6.2 g). The substrate consisted of 2.0-mm plastic mesh supported by a PVC frame with a 5-cm spacing between two layers, and was included at a rate sufficient to increase bottom surface area by 200% (1.6 m^2). Water quality analysis was performed prior to stocking and after 24 hours. All prawn were then removed, determined to be alive or dead, and each group was weighed and counted.

Trial 3

Based on the results of these experiments a commercial scale trial was conducted. A commercial live hauler (Steven Guise, Todays Fresh Catch, Inc., Spring Hill, Florida) was contracted to haul 500 kg of market-size prawns from Kentucky to New York using technologies adapted from Trials 1 and 2. Each of five 950-L insulated containers were stocked with 100 kg of prawns (average weight approximately 45 g). Five different un-replicated water temperature and salinity combinations were evaluated: 14°C with 10 ppt salinity, 15°C with 9 ppt salinity, 15.5°C with 5.5 ppt salinity, 16°C with 0 ppt salinity, and 16.5°C with 5.5 ppt salinity. As suggested by D'Abramo et al. (1995), approximately 2.7 kg of live prawn were confined in perforated Plastic containers (LWH: 43.5 cm, 30 cm, 9 cm, respectively; Thunderbird Plastics Inc., Canada) stacked within transport tanks (40 containers/950-L tank; 108 g/L). After 18-20 hours of transport, prawns were offloaded at 2 different grocery stores and a distribution "warehouse" which supplies Asian supermarkets and fish markets in New York City.

Statistical Analysis

In Trial 1, 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 ($P \leq 0.05$). For Trial 2, data were analyzed by the two-way analysis of variance to determine the effects of water temperature, presence or absence of substrate, and their interactions on water quality and prawn survival. Since factorial analysis indicated no significant statistical interaction ($P > 0.05$) between the presence of substrate and water temperature on any measured variable, the main effects of substrate and temperature were then compared separately using Student's T-test. All percentage and ratio data were transformed to arc sin values prior to analysis (Zar 1984). Data are presented in the untransformed form to facilitate interpretation.

RESULTS

For Trial 1, which evaluated prawn stocked at biomass densities of 25 g/L, 50 g/L and 100 g/L, there was no significant difference ($P > 0.05$) in prawn survival (mean 98%) after 24 hours. There was also no significant difference ($P > 0.05$) in final concentrations of dissolved oxygen, nitrite-nitrogen or pH between treatments which averaged 6.6±0.6 mg/L, 0.3±0.1 mg/L, and 7.7±0.1, overall (Table 1). Total ammonia-nitrogen levels increased ($P < 0.05$) as biomass densities in transport containers increased. Un-ionized ammonia nitrogen levels also increased ($P < 0.05$) as biomass densities increased. However, ammonia concentrations did not reach lethal concentrations, primarily due to a relatively low pH and water temperature.

Trial 2 evaluated the effect of added substrate and temperature on transport survival of prawns stocked at the highest biomass evaluated in Trial 1 (100 g/L). There was no significant interaction ($P > 0.05$) between the presence of substrate and water temperature on any measured variable. This allows substrate and temperature to be analyzed separately for their effect on water quality and prawn survival. Substrate had no significant impact ($P > 0.05$) on any measured water quality variable or prawn survival after 24 hours of simulated transport (Table 2).

TABLE 1. Prawn survival, dissolved oxygen, total ammonia, un-ionized ammonia, nitrite-nitrogen, and pH in transport containers stocked with market size freshwater prawn at 25 g/L, 50 g/L, and 100 g/L for twenty-four hours under simulated transport conditions. Values in the same row followed by different letters were significantly different ($P < 0.05$).

		Biomass densities during transport		
	Initial	25 g/L	50 g/L	100 g/L
Survival (%)	100	100±0.0 a	97.5±2.2 a	100±0.0 a
Dissolved oxygen (mg/L)	8.3	6.7±0.5 a	6.8±0.9 a	6.1±0.2 a
Total ammonia (mg/L)	0.32	2.1±0.9 c	4.6±1.6 b	15.7±6.3 a
Un-ionized ammonia (mg/L)	0.00	0.0±0.0 c	0.1±0.0 b	0.3±0.1 a
Nitrite-nitrogen (mg/L)	0.05	0.2±0.0 a	0.3±0.2 a	0.4±0.1 a
pH	7.3	7.7±0.1 a	7.8±0.1 a	7.6±0.1 a

In the temperature comparison, water temperatures in transport containers were maintained within 1°C of target temperatures through the duration of the experiment. Water temperature had a highly significant effect ($P < 0.01$) on prawn survival; averaging 24% in the 26°C transport containers compared to 97% in those maintained at 21°C. There was no significant difference ($P > 0.05$) in total ammonia-nitrogen, un-ionized ammonia-nitrogen, or pH between temperature treatments, which averaged 28.6 mg/L, 0.6 mg/L, and 7.6 mg/L, overall (Table 2). There was a significant difference ($P < 0.05$) in final dissolved oxygen concentrations due to the different transport temperatures; which averaged 3.7 mg/L for the 21°C containers and 2.4 mg/L for the 26°C containers. Although some of this difference is directly related to the different solubilities of oxygen at the different water temperatures (Boyd 1979); the percent saturation of oxygen was also statistically different ($P < 0.05$) for the two temperatures and averaged 43% for the 21°C treatment and 30% for the 26°C treatment (Table 2). Nitrite-nitrogen was significantly higher ($P < 0.05$) in 26°C containers (0.9 mg/L) compared to 21°C containers (0.6 mg/L).

In Trial 3 survival in the containers ranged from 95-98.7% with no obvious difference in survival related to the different environmental conditions tested; although, it appeared that 16°C with 0 ppt salinity was the best based on visual assessment of liveliness (activity within transport containers) and exterior quality of the product (bright coloration). Total ammonia-nitrogen, un-ionized ammonia-nitrogen, and nitrite-nitrogen

TABLE 2. Main effect means* of prawn survival, dissolved oxygen, total ammonia, un-ionized ammonia, nitrite-nitrogen and pH in transport containers either with or without plastic mesh substrate after twenty-four hours stocked with market size prawn at 100 g/L under simulated transport conditions. *Means (±SE) of six replicate containers. For the main effects of substrate and temperature, means within a row followed by different letters were significantly different ($P < 0.05$).

		Substrate		Temperature	
	Initial	With	Without	21°C	26°C
Survival (%)	100	58.8±41.8 a	62.0±41.5 a	96.5±2.1 a	24.3±18.7 b
Dissolved oxygen (mg/L)	7.1	3.2±1.2 a	2.9±1.2 a	3.7±1.3 a	2.4±0.5 b
Dissolved oxygen (% sat)	85.2	37.3±13.8 a	36.8±15.6 a	42.8±14.9 a	30.2±2.8 a
Total ammonia (mg/L)	0.2	27.1±6.8 a	30.1±6.2 a	25.2±1.6 a	32.0±2.8 a
Un-ionized ammonia (mg/L)	0.0	0.6±0.3 a	0.7±0.3 a	0.4±0.1 a	0.8±0.2 a
Nitrite-nitrogen (mg/L)	0.0	0.8±0.2 a	0.8±0.2 a	0.6±0.1 b	0.9±0.2 a
pH	7.3	7.6±0.1 a	7.6±0.1 a	7.6±0.1 a	7.6±0.1 a

concentrations increased over the duration of the transport trial with no large differences between treatments and averaged 23 mg/L, 0.5 mg/L, and 0.2 mg/L, respectively.

DISCUSSION

In Study 1, total and un-ionized ammonia concentrations increased with increased stocking density; however, the measured values were within the acceptable range for the species. 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 and that the toxicity of NH_3 decreases as *M. rosenbergii* juveniles increase in size and weight. Therefore, measured NH_3 concentrations were not considered stressful and likely did not effect survival at the pH and temperature values measured in this study. However, if stocking densities, water temperatures or pH values are increased, NH_3 concentrations could become limiting.

In Study 2, inclusion of substrate in transport containers did not improve survival. These data are in agreement with Smith and Wannamaker

(1983), who reported that increased substrate did not benefit survival of 6 g juveniles in aerated plastic bags at 18 g/L for 24 hours. The authors also evaluated immobilization of adult prawns by individually wrapping each animal in plastic mesh and found that this procedure actually decreased survival compared to the unconfined treatment. D'Abramo et al. (1995) suggested that prawns be distributed on "shelves" stacked vertically in the water column to avoid mortality caused by crowding and localized deterioration of water quality.

In Study 2, decreased water temperature greatly improved survival. This is in agreement with Harrison and Lutz (1980) who reported survival of juvenile prawn increased when transported at reduced water temperature (20°C). In the transport studies conducted by Smith and Wannamaker (1983), water temperatures of 18-23°C were used. These studies indicate that water temperature is a dominate factor affecting prawn survival during transport. Within the optimal temperature range for most species, increases in transport temperatures significantly increases metabolism and, therefore, oxygen consumption and nitrogenous excretion (Chen and Kou 1996). This reduces acceptable biomass densities and/or transport times. The optimum temperature range for growth of *M. rosenbergii* is between 26°C and 32°C (Boyd and Zimmerman 2002). Rogers and Fast (1988) reported that *M. rosenbergii* are stressed at water temperatures below 22°C. However, it appears that water temperatures of 20-21°C may produce an anesthesia-like state in prawn; which appears to be beneficial for transport. The effect of water temperatures < 20°C are not known and should be evaluated.

The dissolved oxygen concentrations in Study 2 were lower than in Study 1 and may represent stress conditions for freshwater prawn. This difference may be due to increased oxygen demand of the spring water used in the second trial because of recent rains. Baseline dissolved oxygen concentrations in Trial 2 were approximately 1 mg/L less than in Trial 1. In both trials, each container was equipped with a flow meter set to deliver 1.5 L/min of pure oxygen. Also, the depth of the model transport containers were relatively shallow (57 cm); which likely resulted in poor oxygen transfer due to a relatively short contact time. *M. rosenbergii* are reportedly able to tolerate dissolved oxygen levels as low as 1 ppm for short time periods (Avault 1987), and become stressed at dissolved oxygen levels < 2 mg/L (Boyd and Zimmerman 2002). While oxygen concentrations were maintained > 2 mg/L at all times, the percent of saturation is a more biologically important value. Usually values < 50% are considered stressful. At 21°C saturation values averaged 43%, but only 30% in containers maintained at 26°C. Furthermore, the combined effects of marginally high nitrite, with relatively low oxygen levels, is not known, and may represent a stressful condition for prawns.

In the commercial verification trial, it appeared that further reductions in water temperature (< 20°C) may be advantageous. Although there were no obvious difference in survival; based on the appearance and activity of the prawns, 16°C with 0 ppt salinity appeared to be optimal. As in Trials 1 and 2, measured ammonia concentrations in transport containers following transport were very high (> 20 mg/L); therefore, evaluation of methodologies to reduce the buildup of nitrogenous metabolites would be advantageous. The use of perforated plastic containers stacked within transport tanks appears to be favorable for transport and beneficial for distribution and should be evaluated further. In this market, the majority of the product is sold within 24 hours of arrival and post-transport survival is not a major consideration. However, in some live markets it is necessary to maintain product for several days.

In Trial 1, 100% survival was obtained at a biomass density of 100 g/L using open containers provided with both pure oxygen and compressed air for circulation. Prior to this, the highest transport densities that could be found in the literature was 60 mg/L by D'Abramo et al. (1995), but was based on unpublished data. Trial 2 indicated that reduced water temperature improved survival, while addition of substrate provided no benefit. The results of Trial 3 indicate that further reductions in water temperature (16°C) and the confinement of prawns in perforated containers stacked within transport tanks may be advantageous. Future studies should evaluate methodologies to further increase hauling densities (i.e., anesthesia, biofiltration, colder temperatures), as well as, technologies for post-transport holding of live product.

ACKNOWLEDGMENTS

Special thanks to Jason Danaher and Russell Neal for technical support throughout the study. This research was supported by a USDA/CREES grant to Kentucky State University under agreement KYX-80-91-04A and funding was provided by Kentucky's Regional University Trust Fund to the Aquaculture Program as KSU's Program of Distinction.

REFERENCES

Avault, J.W. 1987. Species profile–freshwater prawn and marine shrimp. Aquaculture Magazine 13(3):53-56.

Boyd, C. E. 1979. Water quality in warmwater fish ponds. Auburn University, Agriculture Experiment Station, Auburn, Alabama.

Boyd, C., and S. Zimmerman. 2000. Grow-out systems-water quality and soil management. Pages 234-236 *in* M.B. New and W.C. Valenti, eds. Freshwater Prawn Culture–The Farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford, United Kingdom.

Chen, J.C., and T.T. Kou. 1996. Effects of temperature on oxygen consumption and nitrogenous excretion of juvenile *Macrobrachium rosenbergii*. Aquaculture 145: 295-303.

Coyle, S.D., J.H. Tidwell, and A. VanArnum. 2001. The effect of biomass density on transport survival of juvenile freshwater prawn, *Macrobrachium rosenbergii*. Journal of Applied Aquaculture 11(3):57-63.

D'Abramo, L.R., W.H. Daniels, M.W. Fondren, and M.W. Brunson. 1995. Management practices for the culture of freshwater prawns in temperate climates. Bulletin 1030. Mississippi Agricultural Forestry Experimental Station, Mississippi Sate University, Mississippi.

FAO. 2003. Fishstat Plus (V. 2.30), Issued 15.03.2003. FIDI, Fisheries Department, FAO, Rome, Italy.

Harrison, K.E., and P.L. Lutz. 1980. Studies on the ontogenesis of osmoregulation in *Macrobrachium rosenbergii* with application to shipping post-larvae. Proceedings of the World Mariculture Society 11:181-182.

Jensen, G.L. 1990. Transportation of warmwater fish: Loading rates and tips by species. Southern Regional Aquaculture Publication No. 393.

New, M.B. 2005. Freshwater prawn farming: Global status, recent research and a glance at the future. Aquaculture Research 36: 210-230.

Phillips, H., and D. Lacroix. 2000. Marketing and preparation for consumption. Pages 345-368 *in* M.B. New and W.C. Valenti, eds. Freshwater Prawn Culture–The Farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford, United Kingdom.

Rogers, G.L., and A.W. Fast. 1988. Potential benefits of low energy water circulation in Hawaiian prawn ponds. Aquacultural Engineering 7:155-165.

Smith, T.I.J., and A.J. Wannamaker. 1983. Shipping studies with juvenile and adult Malaysian prawn *Macrobrachium rosenbergii*. Aquaculture Engineering 2: 287-300.

Strauss, D.L., H.R. Robinette, and J.M. Heinen. 1991. Toxicity of un-ionized ammonia and high pH to post-larval and juvenile freshwater shrimp, *Macrobrachium rosenbergii*. Journal of the World Aquaculture Society 22(2):128-133.

Tidwell, J.H., and L.R. D'Abramo. 2000. Grow-out systems–culture in temperate zones. Pages 177-186 *in* M.B. New and W.C. Valenti, eds. Freshwater Prawn Culture–The Farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford, United Kingdom.

Tidwell, J.H., L.R. D'Abramo, S.D. Coyle, and D. Yasharian. 2005. Overview of recent research and development in temperate culture of the freshwater prawn *Macrobrachium rosenbergii* de Man) in the South Central United States. Aquaculture Research 36: 264-277.

Zar, J.H. 1984. Biostatistical Analysis. Prentice Hall, Engelwood Cliffs, New Jersey.