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Impact of Substrate Physical Characteristics on Grow Out of Freshwater Prawn, *Macrobrachium rosenbergii*, in Ponds and Pond Microcosm Tanks

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Abstract

Two independent trials were conducted to evaluate the effects of different physical characteristics of substrate materials on growth and survival of freshwater prawn. In Trial 1, juvenile prawns (0.4 ± 0.2 g) were stocked at 123 prawns/m² of tank bottom into twenty-one 18,000-L fiberglass tanks managed as pond microcosms. Six substrate materials were chosen to allow comparisons of surface area, mesh size, color, and texture. A control treatment received no added substrate. There were three replicate tanks per treatment. Substrates were positioned vertically at a rate sufficient to increase the bottom surface area by 100%. Prawns were fed a 32% protein sinking diet according to a feed chart. After 110 d, there was no significant difference ($P > 0.05$) in survival among treatments, averaging 72.6% overall. Prawns in the control treatment (no substrate) had significantly lower ($P \leq 0.05$) average weights (9.5 g), lower production (1342 kg/ha), and higher feed conversion ratio (FCR; 2.5) than those in substrate treatments, which were not significantly different ($P > 0.05$) and averaged 13.4 g, 2404 kg/ha, and 1.3, respectively. For Trial 2, the least expensive substrate material from Trial 1 (lightweight polyethylene bird netting) was compared with the substrate most commonly used in commercial production (heavyweight orange polyethylene safety fencing) under practical pond conditions. Juvenile prawns (0.8 ± 0.3 g) were stocked at 61,600/ha into six 0.04-ha earthen ponds. Each was randomly assigned one of the two substrate materials, and there were three replications per treatment. After 101 d, there were no significant differences ($P > 0.05$) between treatments in terms of survival (91%), average weight (34 g), total production (2150 kg/ha), or FCR (3.1). In these studies, physical characteristics of the substrate materials had little impact. The lightweight netting represents a 68% cost savings compared to the currently recommended substrate material.

In temperate climates, production of freshwater prawn is temperature limited to a single seasonal crop, with approximately a 100- to 180-d growing season (Tidwell and D'Abramo 2000). Under the constraints of temperate zone culture, pond production rates must be maximized to achieve commercial viability. Many technologies have been evaluated to increase per unit production (kg/ha) of prawns including stocking larger juveniles (Eble et al. 1977; D'Abramo et al. 1989), increasing stocking densities (D'Abramo et al. 1989), size grading animals prior to stocking (Daniels et al. 1995), and selective harvest of large animals periodically throughout the growing season (D'Abramo et al. 1995). Despite these efforts, expected mean yields for prawns under practical conditions (1120 kg/ha; D'Abramo et al. 1995) remains far below the yields of other commercially grown species such as white shrimp, *Penaeus vannamei* (2500 kg/ha; Lim and Persyn 1989), and channel catfish, *Ictalurus punctatus* (5000 kg/ha; Busch 1985).

Prawns are primarily benthic animals and as such are constrained to a two-dimensional area rather than a three-dimensional volume (as with many finfish species). This is further exacerbated by the fact that they are territorial and cannibalistic (Cohen et al. 1981). Several studies have documented the benefits of adding artificial substrate to production units (e.g., tanks and ponds) to increase available surface area. Sandifer and Smith (1977) reported that the addition of substrate in nursery tanks allowed prawns to use the entire water column and

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reduced mortality. Cohen et al. (1983) reported that added substrate in ponds increased prawn production by 14% and average size by 13%. Tidwell et al. (2000) found that prawns provided with an 80% increase in pond bottom surface area by inclusion of orange plastic mesh (‘‘safety fence’’) were 33% larger, with a 24% increase in total yield, compared to those without substrate.

However, the mechanisms of action for substrate have not been clearly defined or identified. Potential functions include physical separation of animals, increased production of natural foods, and even improved water quality through the actions of attached periphyton (Tidwell and Bratvold 2005). Depending on which of these functions is most important, the physical characteristics of the materials used could have a major impact on substrate effectiveness. Potential variables include mesh size, surface area, texture, and color (Huchette et al. 2000; Keshavanath et al. 2001; Mariappan and Balasundaram 2004; Yasharian et al. 2005). To evaluate these variables, Trial 1 was designed to compare substrate materials with different physical attributes in outdoor tanks and Trial 2 was designed to evaluate the most promising substrate material identified in the first experiment, under actual pond culture conditions.

Materials and Methods

Trial 1

The experiment was conducted in 21 outdoor, 18,000-L round fiberglass tanks (Dolphin Fiberglass Products, Inc., Homestead, FL, USA) at Kentucky State University’s Aquaculture Research Center (ARC), Frankfort, Kentucky. The tanks were designed and managed as ‘‘pond microcosms.’’ Tank bottoms provided 16 m² of available surface area. In substrate treatments, 16 m² of substrate materials were added. The substrate was divided into four ‘‘curtains’’ suspended vertically in the water column. Amounts were based on total length × width of the substrate materials, with no adjustment for mesh openings. After all substrates were in place, the tanks were filled with filtered reservoir water and aerated for 1 wk prior to prawn stocking.

Each tank contained one 3.75- × 3.75- × 30-cm medium-pore diffuser (Aquatic Ecosystems, Inc., Apopka, FL, USA) provided with air from a regenerative blower (Aquatic Ecosystem, Inc.). Tanks were supplied with filtered (1000 μm) reservoir water at a rate of 3.2 L/min for the first 16 d. This flow rate proved insufficient for maintenance of good water quality, and the flow rate was increased to 7.6 L/min for the remainder of the study. Flow rate was measured and adjusted accordingly every 2 wk. Tanks were equipped with an external standpipe and an internal 1000 μm discharge screen.

Six substrate materials were evaluated (Fig. 1). Treatment 1 was a lightweight polyethylene bird netting (2.5 × 3.8 cm; InterNet, Inc., Minneapolis, MN, USA). Treatment 2 was a heavyweight polyethylene mesh (2.5 × 3.4 cm; InterNet,

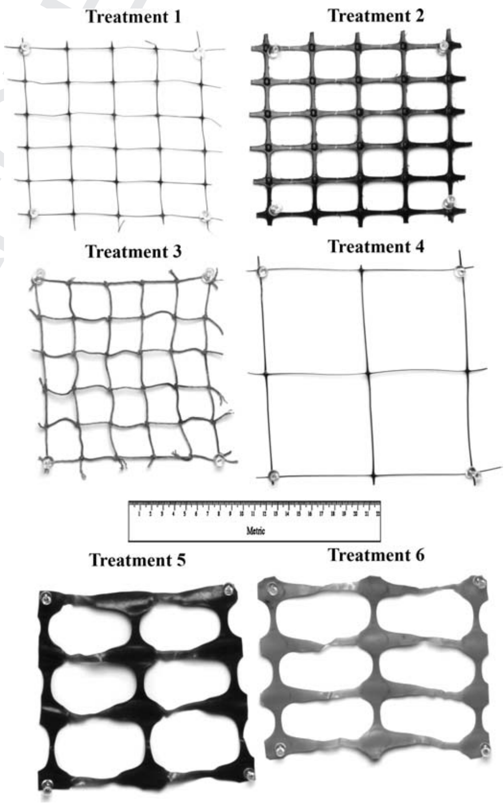

FIGURE 1. *Six substrate materials used in Trial 1 to evaluate the impacts of substrate color, texture, surface area, and mesh size on prawn survival and growth.*

Inc.). Treatment 3 was a dyed nylon seine material (3 × 3 cm; Delta Net and Twine, Inc., Greenville, MS, USA). Treatment 4 was a lightweight polyethylene bird netting (10.2 × 10.2 cm; InterNet, Inc.). Treatment 5 was a green heavyweight polyethylene barrier fence (2.5 × 3.4 cm; US Fence, Inc., Erie, PA, USA). Treatment 6 was the same heavyweight polyethylene barrier fence as in Treatment 5 but in orange; this treatment is the most commonly used substrate in the region and served as the commercial control (2.5 × 3.4 cm; US Fence, Inc.). Treatment 7 received no added substrate.

Substrate materials were chosen to allow comparison of specific attributes or variables such as the effect of mesh size (Treatment 1 vs. Treatment 4), surface area of the mesh (Treatment 1 vs. Treatment 2), material texture (Treatment 1 vs. Treatment 3), and color (Treatment 5 vs. Treatment 6). The costs in US dollars for each of the substrate types were as follows: Treatment 1, \$0.18/m^2; Treatment 2, \$2.33/m^2; Treatment 3, \$8.22/m^2; Treatment 4, \$0.23/m^2; Treatment 5, \$1.11/m^2; Treatment 6, \$0.56/m^2; and Treatment 7, \$0.00/m^2.

Postlarval prawns were shipped by air from a commercial hatchery (Aquaculture of Texas, Weatherford, TX, USA) and nursed in a greenhouse at ARC for 60 d prior to stocking. The mean stocking weight was determined from a sample of 100 prawns that were blotted free of surface water and individually weighed. Individual mean stocking weight ($\overline{X} \pm$ SD) was 0.4 ± 0.2 g. Prawns were hand-counted and stocked into 21 tanks at 123 prawns/m^2 of tank bottom, the equivalent of approximately 123,000/ha. There were three replicate tanks per treatment.

Feeds and Feeding. Prawns were fed a commercial sinking-extruded prawn grow-out diet containing 32% protein and 10% lipid (Aquafare, US Energy Partners LLC, Russellville, KA, USA). Feed rates were based on a standardized feeding chart (D'Abramo et al. 1995). The daily ration was divided between AM and PM feedings.

Sampling. Every 3 wk, tanks were sampled with dip nets to obtain a sample of >30 individuals per tank. Sampled prawns were bulk weighed and counted to determine average weight and then returned to the culture tank.

Water Quality. Dissolved oxygen and temperature were monitored twice daily (0800 and 1600 h) using a Model 85 oxygen meter (YSI Industries, Yellow Springs, OH, USA). The pH of the water was also monitored twice daily (0800 and 1600 h) using a model 340i pH meter (Wissenschaftlich Technische Werkstätten, Weilheim, Germany). Total ammonia–nitrogen (TAN) and nitrite–nitrogen were monitored thrice a week using a Hach Odyssey digital spectrophotometer (Hach Company, Loveland, CO, USA). Alkalinity and hardness were monitored once a week using a Hach digital titrator (Hach Company).

Harvest. Prawns were harvested after 110 d (Tidwell and D'Abramo 2000). Tanks were drained and prawns harvested by hand with dip nets. All harvested prawns were bulk weighed and counted to determine percent survival and average weight. Prawns from individual tanks were placed into separate aerated 900-L fiberglass tanks supplied with a continuous flow of water. All prawns were individually weighed and classified into either one of three female morphotypes: berried (egg carrying; BE), open (previously egg carrying; OP), and virgin female, or one of three male morphotypes: blue claw (BC), orange claw, and small male (<20 g) as described by Cohen et al. (1981) and modified by D'Abramo et al. (1989). For data presented here, BE and OP females were combined into a composite group of mature females termed reproductive females (RFs).

Statistical Analysis. Effects of substrate treatments on water quality, prawn growth, total productivity, and survival were evaluated by ANOVA (Zar 1999) using Statistix version 7.0 (Analytical Software, Tallahassee, FL, USA). If significant differences were indicated by ANOVA ($P < 0.05$), means were separated using the least significant difference test (Zar 1999). Student's *t*-test was used to make individual comparisons in terms of mesh size, material texture, surface area, and color.

Trial 2

The following year, six ponds located at the ARC, Kentucky State University, Frankfort, Kentucky, were prepared as described previously in Tidwell et al. 2000. A 0.5-hp vertical pump surface aerator (Airolator, Kansas City, MO, USA), modified with a "deep-draw" tube, operated nightly at the surface of the deepest area of each pond to aerate and prevent thermal stratification.

Postlarval prawns were shipped by air from a commercial hatchery (Aquaculture of Texas, Weatherford, TX, USA) and then nursed in a greenhouse at ARC for 60 d prior to stocking. The mean stocking weight was determined from a sample of 100 prawns that were blotted free of surface water and individually weighed. Individual mean stocking weight ($\overline{X} \pm$ SD) was 0.8 ± 0.3 g. Prawns were hand-counted and stocked into six 0.04-ha ponds at 61,600/ha. There were three replicate ponds per treatment. Feeding and water quality monitoring were the same as previously described for Trial 1. Sampling was conducted every 3 wk (Tidwell et al. 2000).

Two types of substrate from Trial 1 were compared under practical pond conditions. For Treatment 1, the substrate consisted of 120-cm-wide panels of orange polyethylene "safety fence" with a mesh opening (length × width) of 7.0 × 3.4 cm (same as the commercial control, Substrate 6 in Trial 1). In Treatment 2, substrate was a 2.5- × 3.8-cm lightweight polyethylene bird netting (Substrate 1 in Trial 1) and was chosen based on its low cost and lack of production differences in Trial 1. Substrates were hung in vertical orientation and stretched the length of the pond between metal fence posts. The substrate was positioned approximately 30 cm above the pond bottom, with a 30-cm separation between layers. Surface area of the substrate was calculated based on dimensions of one side of the mesh (length × width) and was added at a rate to increase the pond bottom surface area 100%.

Harvest. Prawns were cultured for 101 d. One day prior to harvest, the water levels in each pond were lowered to approximately 0.5 m at the drain end. On the following day, substrates were removed and each pond was seined thrice with a 1.3-cm square mesh seine and then drained. Remaining prawns were manually harvested from the pond bottom, and all prawns were purged of mud by holding in tanks with flowing water. Total bulk weight and number of prawns from each pond were recorded. A random sample of $500 prawns from each pond were then individually weighed and classified into one of the six previously described sexual morphotypes.

Statistical Analyses. Effects of substrate type on water quality and prawn growth in ponds were compared by Student's *t*-test using Statistix version 4.1 (Analytical Software). Means were considered different at $P < 0.05$. Growth performance and feed conversion were measured in terms of final individual weight (g), percent survival, total yield (kg/ha), and feed conversion ratio (FCR). Percentage and ratio data were converted to arc sin values prior to analysis. These data are presented in the

TABLE 1. *Trial 1: Means (±SE) of final individual weight (g), SGR, total production (kg/ha), percent survival, and FCR of freshwater prawn reared in outdoor tanks containing different types of artificial substrate.*[1]

Treatment	Final weight (g)	SGR (%/d)	Production (kg/ha)	Survival (%)	FCR
1	13.3 ± 1.2^{a}	3.4 ± 0.0^{a}	2252.4 ± 174.7^{a}	70.9 ± 5.4^{a}	1.3 ± 0.1^{b}
2	13.0 ± 0.0^{a}	3.4 ± 0.0^{a}	2418.7 ± 75.9^{a}	77.5 ± 4.0^{a}	1.2 ± 0.0^{b}
3	13.2 ± 1.8^{a}	3.4 ± 0.1^{a}	2535.1 ± 277.8^{a}	80.5 ± 3.6^{a}	1.2 ± 0.1^{b}
4	12.9 ± 1.8^{a}	3.3 ± 0.1^{a}	2252.0 ± 31.4^{a}	72.5 ± 3.8^{a}	1.3 ± 0.2^{b}
5	14.5 ± 0.4^{a}	3.5 ± 0.0^{a}	2476.9 ± 133.3^{a}	71.3 ± 11.6^{a}	1.2 ± 0.2^{b}
6	13.5 ± 0.5^{a}	3.4 ± 0.0^{a}	2489.4 ± 133.3^{a}	76.9 ± 7.1^{a}	1.2 ± 0.1^{b}
7	9.5 ± 0.3^{b}	3.1 ± 0.0^{b}	1342.2 ± 489.7^{b}	58.5 ± 21.1^{a}	2.5 ± 1.0^{a}

FCR = feed conversion ratio; SGR = specific growth rate.

[1] Values within columns followed by different letters are significantly different ($P < 0.05$).

untransformed form to facilitate comparison and interpretation.

Results

Trial 1

There were no significant differences ($P >$ 0.05) between treatments for monthly or overall means of measured water quality variables. Overall means for dissolved oxygen, temperature, pH, alkalinity, hardness, unionized ammonia, and nitrite–nitrogen for the study period were 8.1 mg/L, 25.9 C, 8.6, 149.8 mg/L, 115.6 mg/L, 0.08 mg/L, and 0.01 mg/L, respectively. These values represent suitable conditions for prawn culture.

Survival did not differ significantly between treatments and averaged 73% overall (Table 2). Prawns in Treatment 7 (no substrate) had significantly lower ($P < 0.05$) average weights (9.5 g), lower production (1342 kg/ha), and higher FCR (2.5) than all substrate treatments. There was no significant difference ($P > 0.05$) between substrate treatments (Treatments 1–6) for any production variable, and overall means were average weight 13.4 g, total production 2404 kg/ha, and FCR 1.2.

Population structure of prawns was only slightly affected by substrate treatments. There were no significant differences ($P > 0.05$) in the percent distribution of the different morphotypes for either sex between substrate treatments and nonsubstrate controls. Few sexually mature animals (BCs and RFs) were produced in this study compared to previous experiments in ponds (Tidwell et al. 1996, 1999, 2000). The lack of sexually mature animals in this study was largely because of low average weights, resulting from high stocking rates, and may also indicate the nutritional importance of benthic invertebrates in production ponds (Tidwell et al. 1997).

Two sample *t*-tests were then used to evaluate the potential impact of specific physical characteristics of the different substrate materials including mesh size (Treatment 1 vs. Treatment 4), material texture (Treatment 1 vs. Treatment 3), surface area (Treatment 1 vs. Treatment 2), and color (Treatment 5 vs. Treatment 6). No significant differences ($P > 0.05$) were found in these comparisons.

Trial 2

There was no significant difference between treatments in terms of measured water quality variables, either monthly or overall. Overall means for water quality variables were the following: morning temperature, 25.2 C; afternoon temperature, 27.7 C; morning dissolved oxygen, 7.0 mg/L; afternoon dissolved oxygen, 11.7 mg/L; pH, 8.9; TAN, 0.6 mg/L; unionized ammonia–nitrogen, 0.14 mg/L; and total nitrite–nitrogen, 0.02 mg/L. Over the duration of the study, all water quality samples for the above parameters represented suitable conditions for prawn culture (Boyd and Zimmerman 2000).

At harvest, there were no significant differences ($P > 0.05$) between the two substrate treatments in terms of survival, average weight, total production, or FCR, which averaged 91%, 34 g, 2150 kg/ha, and 3.1 overall, respectively (Table 2), or in the population structure in terms of numbers or average weight of morphotypes (Table 3).

Discussion

Trial 1

In this study, average weights of prawns in substrate treatments were 41% greater than those of nonsubstrate controls, and total production (kg/ha) in substrate treatments were 80% greater than nonsubstrate controls. Feed conversion efficiency was also improved more

TABLE 2. *Trial 2: Means (±SE) of final individual weight (g), SGR, total production (kg/ha), percent survival, and FCR of freshwater prawn reared in ponds containing two different types of artificial substrate.*[1]

Treatment	Final weight (g)	SGR (%/d)	Production (kg/ha)	Survival (%)	FCR
Orange mesh	28.8 ± 3.6[a]	3.5 ± 0.1[a]	1830.7 ± 324.6[a]	89.8 ± 5.4[a]	3.7 ± 0.6[a]
Bird netting	38.4 ± 1.3[a]	3.8 ± 0.0[a]	2469.7 ± 71.5[a]	92.0 ± 2.5[a]	2.6 ± 0.1[a]

FCR = feed conversion ratio; SGR = specific growth rate.

[1] Values within columns followed by different letters are significantly different ($P < 0.05$).

TABLE 3. *Trial 2: Means (±SE) of percent distribution of sex (%) and average weights (g) of each male (BC, OC, and SM) and female (RF and VF) morphotype at harvest for freshwater prawns reared in ponds containing two different types of artificial substrate.*[1]

Treatment	BC	OC	SM	RF	VF
			Sex (%)		
Orange mesh	6.0 ± 2.7[a]	76.0 ± 3.2[a]	12.0 ± 5.0[a]	39.6 ± 9.4[a]	60.4 ± 9.4[a]
Bird netting	11.0 ± 2.3[a]	69.4 ± 4.2[a]	19.6 ± 4.9[a]	60.8 ± 6.0[a]	39.2 ± 6.0[a]
			% Average weight (g)		
Orange mesh	53.1 ± 3.6[a]	39.8 ± 0.8[a]	11.1 ± 0.9[a]	33.9 ± 1.6[a]	27.9 ± 1.5[a]
Bird netting	48.4 ± 1.8[a]	38.3 ± 1.6[a]	11.2 ± 1.6[a]	32.8 ± 1.1[a]	26.0 ± 0.4[a]

BC = Blue claw; OC = orange claw; RF = reproductive female; SM = small male; VF = virgin female.
[1] Values within columns within data type followed by different superscripts are significantly different ($P \leq 0.05$).

than 100% in prawns in substrate treatments compared to controls.

A relatively high stocking density (123,000/ha) was intentionally used in this study and likely explains the relatively low average weights of prawns at harvest. This protocol was intended to amplify the potential effects of the different substrate materials. Tidwell et al. (1999) reported that added substrate improved feed conversion efficiency and suggested that this may be as a result of increased surface area for periphyton production and increased availability of natural food. Data in this study do not appear to support this hypothesis. There was no improvement in prawn production or feed conversion efficiencies, as surface area was increased (Treatment 2 had the same mesh size but a threefold increase in surface area compared to Treatment 1). Reductions in antagonistic interactions between prawns have been shown to reduce stress, improve growth, and thereby improve feed conversion efficiency (Karplus et al. 1992). From these data, it appears that the primary benefit of substrate is to provide the prawns the ability to physically separate themselves from each other, thus reducing prawn–prawn interaction and stress.

Because these data indicate that physical characteristics of the substrate materials have little impact, cost then becomes a primary consideration. The costs (in US dollars) associated with supplying a prawn production pond with 100% inclusion of artificial substrate can be significant. For the commonly used orange construction fence (Trial 1, Treatment 6), costs would be approximately \$5600/ha, whereas the least expensive material (Trail 1, Treatment 1) would cost only \$1800/ha. This would represent a 68% reduction in substrate costs. Trial 2 then compared these substrate materials under practical pond conditions.

Trial 2

Production differences between the two substrate types were not statistically significant ($P > 0.05$). However, the ponds using the less expensive monofilament material actually produced numerically superior results for all production parameters (Table 2). The lack of statistical significance appears to be because of high within-treatment variation in the control treatment, the reason for which is unknown.

There also appeared to be a shift toward sexually mature female morphotypes in the monofilament treatment (Table 3). Tidwell et al. (2000) reported that the number of females achieving reproductive status increased as the amount of available substrate increased, also suggesting that the monofilament material may actually perform slightly better. Overall, the distribution in population structure and average weights of individual morphotypes compare similarly with previous experiments at this laboratory (Tidwell et al. 1996, 1999, 2004).

The results of Trial 2 appear to confirm the results of Trial 1, which indicate that substrates differing in material texture, mesh size, and color perform similarly. Because of the dramatic cost reduction of using the lightweight bird netting, its use would appear economically advantageous. However, the functional lifespan of these substrate materials has not yet been evaluated. While the type of substrate did not have a strong impact, the presence of substrate of any type did dramatically improve total production and average weight. Further research should focus on the application, practicality, and durability of different substrate materials under commercial pond production conditions.

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2	Please provide the department name (if any) for the authors' affiliation in the affiliation field.	
3	Please note that the year "2006" in the reference citation "Tidwell and Bratfold (2006)" has been changed to "2005" and also note that the spelling of the author name "Bratfold" has been changed to "Bratvold" as per the reference list. Please check if these are correct.	
4	Please spell out "CREES" in the sentence "This research was . . ."	
5	Please note that the reference "Coyle et al. (2003)" is not cited in the text. Please provide a citation for the same in text or delete it from the list.	
6	Please provide the city name for reference "D'Abramo et al. (1995)."	
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