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Effects and Interactions of Stocking Density and Added Substrate on Production and Population Structure of Freshwater Prawns *Macrobrachium rosenbergii*

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Abstract

The effects and interactions of added artificial substrate with different stocking densities of prawns were evaluated. Juvenile prawns (0.2 ± 0.1 g) were stocked into 12 0.04-ha ponds at 60,000/ha and 120,000/ha with and without substrate. Added substrate consisted of horizontal plastic mesh and strips of "oyster netting" sufficient to increase available surface area 80%. There were no statistically significant ($P > 0.05$) interactions between stocking density and presence of added substrate, allowing these main effects to be compared separately. Increasing stocking density produced a significant increase ($P < 0.01$) in total production but a significant decrease ($P < 0.01$) in average prawn size and production ($P < 0.05$) of market size prawns (>20 g, −27%; > 30 g, −56%). Added substrate also produced a significant increase ($P < 0.05$) in total production (18%). However, average prawn size was not decreased and production of marketable shrimp was increased (>20 g, 25%; >30 g, 19%). Feed conversion ratios were significantly decreased ($P < 0.01$) by the presence of substrate. Increased stocking density significantly increased ($P < 0.05$) the percentage of males which were small males (SM) and decreased ($P < 0.01$) the percentage of orange claw males (OC), but had no impact ($P > 0.05$) on numbers of reproductive (RF) and virgin (VF) female morphotypes. Increased stocking density also produced a significant decrease ($P < 0.05$) in average size of OC, RF, and VF morphotypes while the addition of substrate had no statistically significant impact ($P > 0.05$) on the number or size of different morphotypes. The mathematical relationship between available surface area and average prawn size should be determined to produce recommended inclusion rates for added substrate based on desired levels of total production, stocking rates, and optimum market sizes.

In temperate regions, production of the freshwater prawn *Macrobrachium rosenbergii* is limited to a single seasonal crop. To achieve commercial viability, high production rates must be attained. Total pond production can be increased by increasing stocking densities; however, this decreases average individual prawn size (Karplus et al. 1986; D'Abramo et al. 1989). This can negatively impact production economics as the proportion of the crop that reaches marketable sizes is decreased (Smith et al. 1978). Cohen et al. (1983) reported that when artificial substrate was added to ponds, total production was increased 14% while average size increased 13%. Tidwell et al. (1998) examined added substrate under temperate conditions and reported that in prawns stocked at relatively low densities (59,280/ha), production and average size were increased 20 and 23%, respectively. Ra'anan et al. (1984) reported that added substrate was more effective in intensively-stocked systems. This research was designed to evaluate the effects and interactions of stocking density and added substrate on growth, survival, and population structure of freshwater prawns.

Materials and Methods

Pond Preparation and Stocking

Two weeks prior to the anticipated stocking date, 12 0.04-ha ponds located at the Aquaculture Research Center (ARC), Kentucky State University, Frankfort, Kentucky, USA were drained and allowed to dry. Less than one week prior to stocking, ponds were filled with water from a reservoir filled by runoff from the surrounding watershed. The water-surface area of all experimental ponds was 0.04 ha and average water depth was approximately 1.1 m. A ½-hp aerator operated continuously at the surface of the deepest area of each pond to aerate and prevent thermal stratification. Two applications of liquid fertilizer (NPK, 10:34:0) were added to each pond one week apart, at a rate of 9.0-kg phosphorous/ha, to achieve an algal bloom. Water to replace evaporative losses of the ponds was obtained from the reservoir.

Post-larval prawns were shipped by air from a commercial hatchery (Aquaculture of Texas, Weatherford, Texas, USA), nursed in a greenhouse at ARC for 45 d, and stocked 11 June 1997. On the stocking date the mean stocking weight was determined from a sample of 50 prawns that were blotted free of surface water and individually weighed (0.24 ± 0.13 g; $\bar{x}$ ± SD). Prawns were hand-counted and stocked into six ponds at 60,000/ha and six ponds at 120,000/ha. Three replicate ponds per density were randomly assigned to receive added substrate, consisting of two multi-level habitat structures constructed of a PVC pipe frame with three separate horizontal levels, each 30 cm apart, composed of 12.7-mm plastic mesh sheeting. The substrate unit floated approximately 30 cm above the pond bottom so that four levels (including the pond bottom) were actually available. Within each pond, 60-cm wide strips of polyethylene "oyster netting" with 2.5-cm mesh were also suspended horizontally across the pond. Based on dimensions of mesh (length × width), these additions were calculated to have increased available surface area in each pond approximately 80%. Open area within the mesh itself was not subtracted from surface area calculations.

Samples

A 3.2-mm seine was used to collect a sample of prawns from each pond every 3 wk. Structures were not removed and only open areas were seined. Prawns in the sample were group-weighed (drained weight) to the nearest 0.1 g, counted, and returned to the pond. Prawns in the last sample obtained prior to harvest were also individually weighed and classified into either one of three female morphotypes—berried (egg carrying; BE), open (previously egg carrying; OP), and virgin (VF)—or one of three male morphotypes—blue claw (BC), orange-claw (OC), and small (<20 g; SM) as described by D'Abramo et al. (1989) and modified from Cohen et al. (1981). For data presented here BE and OP females were combined into a composite group of mature females termed reproductive females (RF).

Feeds and Feeding

Prawns were fed a 32% protein prawn diet processed into 5-mm sinking pellets. The formulation is described in Tidwell et al. (1997). One-half of the daily ration was distributed over the entire surface of each pond twice daily between 0900 and 1000 h and between 1500 and 1600 h. Prawns were initially fed at a set rate of 25 kg/ha per day until an average individual weight of 5 g was achieved. For weights greater than 5 g, prawns were fed a percentage of body weight based on a feeding schedule reported by D'Abramo et al. (1995). Feeding rates were adjusted weekly based on a feed conversion ratio of 2.5 and survival was assumed to be 100%.

Water Quality Management

Dissolved oxygen (DO) and temperature of all ponds were monitored twice daily (0900 h and 1530 h) using a YSI Model 57 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Levels of total ammonia-nitrogen (TAN) and nitrite-nitrogen in water samples collected from each pond at approximately 1300 h were determined weekly, according to outlined procedures for a HACH DR/2000 spectrophotometer (Hach Co., Loveland, Colorado, USA). The pH of each pond was determined daily at 1300 h using an electronic pH meter (Hanna Instruments, Ltd., Mauritius).

TABLE 1. *Main effect means for prawns raised at two stocking densities in ponds with and without added substrate. Means (± SE) of six replicate ponds; means within a column within a main effect followed by different single letters are significantly different at* P < *0.05, double letters indicate* P < *0.01.*

Main effect	Final weight (g)	Total yield (kg/ha)	Yield ≥20 g (kg/ha)	Yield ≥30 g (kg/ha)	Survival (%)	FCR
Density (prawns/ha)						
60,000	25.0 ± 3.9^{aa}	$1,156 \pm 80^{bb}$	687 ± 157^{a}	339 ± 119^{a}	78.3 ± 10.6^{a}	2.6 ± 0.4^{a}
120,000	18.2 ± 3.5^{bb}	$1,557 \pm 242^{aa}$	504 ± 145^{b}	150 ± 99^{b}	73.2 ± 15.6^{a}	2.7 ± 0.4^{a}
Substrate						
Without	20.3 ± 4.0^{a}	$1,243 \pm 155^{b}$	531 ± 148^{a}	222 ± 115^{a}	74.5 ± 14.7^{a}	2.9 ± 0.3^{aa}
Added	22.9 ± 5.9^{a}	$1,469 \pm 334^{a}$	661 ± 183^{a}	267 ± 175^{a}	77.0 ± 12.3^{a}	2.4 ± 0.2^{bb}

Harvest

Prawns were cultured for 95 d. Beginning one day prior to harvest, 14 September 1997, the water levels in each pond were lowered to approximately 0.9 m at the drain end. On the following day, substrates were removed and each pond was seined three times, with a 1.3-cm square mesh seine, and then completely drained. Remaining prawns were manually harvested from the pond bottom and purged in clean water. Total bulk weight and number of prawns from each pond were recorded. All prawns in each pond were then individually classified into one of the six previously described sexual morphotypes. Prawns, in each morphotype within each pond, were bulk weighed and counted.

Statistical Analyses

Water quality data, interim sample weights, and harvest data were analyzed as a 2 × 2 factorial and tested for significant interactions ($P \leq 0.05$) between main effects (stocking density and presence of substrate) and between levels within main effects (Steel and Torrie 1980) using Statistix Version 4.1 (Statistix Analytical Software 1994). Percentage and ratio data were converted to arc sin values prior to analysis (Zar 1984).

Results and Discussion

There were no significant interactions or differences ($P > 0.05$) in measured water quality variables. Overall means for water quality variables were: afternoon temperature, 26.9 C; morning dissolved oxygen, 7.5 mg/L; afternoon pH, 8.8; total ammonia-nitrogen, 0.81 mg/L; and nitrite-nitrogen, 0.04 mg/L.

Analyses indicated no significant interactions ($P > 0.05$) between stocking density and presence of added substrate on any of the measured production variables. These data differ from R'anan et al. (1984) who reported that the effects of added substrate were increased at higher stocking rates. Based on this lack of significant interactions, main effects (stocking density and added substrate) may now be considered separately (Dowdy and Wearden 1983).

Density

There was no significant difference in survival or feed conversion ratio ($P > 0.05$) in prawns stocked at the two densities, averaging 76% and 2.7 overall (Table 1). In prawns stocked at high density, production was significantly greater ($P < 0.01$) than in those stocked at low density (1,587 and 1,156 kg/ha, respectively). However, average individual prawn weight was significantly decreased ($P < 0.01$) in prawns stocked at high density. These data agree with previous studies indicating increased production, but reduced average sizes, at higher stocking rates (Karplus et al. 1986). Not only is total production important, the

TABLE 2. *Percent distribution (% of sex) according to number of each male and female morphotype for prawns raised at two stocking densities in ponds with and without added substrate. Means (± SE) of six replicate ponds; means within a column within a main effect followed by different letters are significantly different at* P *< 0.05, double letters indicate* P *< 0.01.*

	Male			Female	
Main effect	Blue claw (BC)	Orange claw (OC)	Small (SM)	Reproductive (RF)	Virgin (VF)
Density (prawns/ha)					
60,000	3.1 ± 1.8[a]	49.4 ± 3.9[aa]	47.4 ± 3.9[b]	11.0 ± 5.2[a]	89.0 ± 5.2[a]
120,000	2.2 ± 1.3[a]	32.8 ± 10.0[bb]	65.0 ± 11.1[a]	11.5 ± 4.9[a]	88.5 ± 4.9[a]
Substrate					
Without	1.9 ± 1.4[a]	40.5 ± 12.9[a]	57.5 ± 13.8[a]	9.3 ± 4.3[a]	90.7 ± 4.3[a]
Added	3.4 ± 1.4[a]	41.8 ± 10.6[a]	54.9 ± 11.4[a]	13.2 ± 4.8[a]	86.8 ± 4.8[a]

proportion of a crop that attains harvestable weight also has a strong effect on commercial viability (Smith et al. 1978). If 20 g is used as the minimum harvestable weight (Maclean et al. 1989), prawns stocked at high density actually produced significantly less ($P < 0.05$) harvestable prawns than ponds stocked at low density (504 and 687 kg/ha, respectively). If 30 g is used as a minimum harvestable weight (D'Abramo et al. 1989), there was again a statistically significant difference ($P < 0.05$) with ponds stocked at high density actually producing 56% less harvestable prawns (Table 1).

Population structures were significantly affected by stocking density. The proportion of OC males in high density ponds (Table 2) was significantly lower ($P < 0.01$) than in low density ponds (33 and 49%, respectively). Average weight of OC was also significantly lower ($P < 0.05$) at high density (Table 3). The number of SM as a percentage of total males was significantly higher ($P < 0.05$) in high density ponds. Average weights of RF and VF were significantly higher ($P < 0.05$) in ponds stocked at the lower density. These results are in agreement with those reported by Karplus et al. (1986). Data also agree closely with those reported previously by Tidwell et al. (1998) for ponds with added substrate reacting as if stocked at reduced density.

Substrate

Survival and average weight of prawns were not significantly affected ($P > 0.05$) by the presence of added substrate (Table 1). Addition of added substrate had a highly significant effect ($P < 0.01$) on feed conversion ratios (FCR). In ponds with added substrate FCR's were 2.4, while in ponds without substrate they averaged 2.9, an 18% difference. It may be that the increased surface area in substrate ponds allows increased periphyton development. Periphyton communities have a high photosynthetic yield, and increased surface area could increase the production of natural forage items (Milstein 1997). The organisms commonly composing periphytic communities (Klots 1966) closely match organisms found to be preferred prey items for prawns in ponds (Tidwell et al. 1997). This concept has been utilized in marine shrimp production by anchoring mesh balls in ponds to provide additional substrate for prey organisms (Jory 1995).

Total prawn production was significantly increased ($P < 0.05$) by the presence of added substrate (Table 1). In ponds without substrate, total production averaged 1,244 kg/ha compared to 1,469 kg/ha in ponds with added substrate, an 18% increase. Production of marketable prawns ≥20 g and ≥30 g was 25% and 20% greater, respec-

TABLE 3. *Average weight (g) of each male and female morphotype for prawns raised at two stocking densities in ponds with and without added substrate. Means (± SE) of six replicate ponds; means within a column within a main effect followed by different letters are significantly different at* P < *0.05.*

	Male			Female	
Main effect	Blue claw (BC)	Orange claw (OC)	Small (SM)	Reproductive (RF)	Virgin (VF)
Density (prawns/ha)					
60,000	55.5 ± 6.3^a	36.3 ± 5.2^a	13.0 ± 2.7^a	35.5 ± 3.8^a	22.9 ± 4.3^a
120,000	45.3 ± 8.2^a	30.1 ± 2.9^b	11.9 ± 3.9^a	28.3 ± 5.1^b	16.9 ± 1.8^b
Substrate					
Without	50.2 ± 9.1^a	32.3 ± 2.7^a	11.9 ± 4.1^a	31.3 ± 5.7^a	18.6 ± 3.0^a
Added	50.7 ± 9.3^a	41.8 ± 10.6^a	12.9 ± 2.6^a	32.5 ± 6.2^a	21.2 ± 5.4^a

tively, in ponds with added substrate, though differences were not statistically significant ($P > 0.05$) due to high within treatment variation.

The population structure of prawns (number and average size of sexual morphotypes) was not significantly impacted ($P > 0.05$) by the presence of added substrate (Tables 2, 3). These results differ from Tidwell et al. (1998) who found that added substrate significantly decreased ($P < 0.05$) the number of SM and significantly increased ($P < 0.05$) the number of OC males and average sizes of BC, OC, RF, and VF. This may be due to the different stocking rates used.

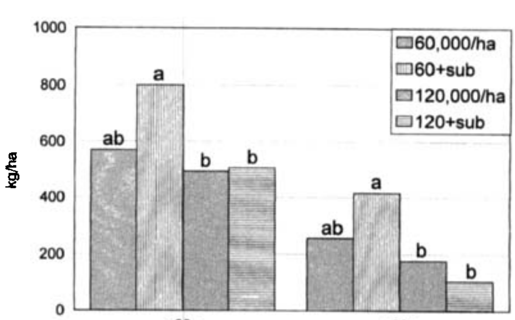

FIGURE 1. *The percentage of prawns reaching minimum marketable size of 20 g or 30 g at harvest from ponds stocked at 60,000 or 120,000/ha, with or without added substrate. Each bar represents a mean of three replicate ponds per treatment combination. Bars with different letters were significantly different (*P < *0.05) as analyzed by ANOVA.*

In summary, increasing stocking density increased total production, but actually decreased marketable production by decreasing average weights. Added substrate increased total production, increased marketable production, and improved feed efficiency. Although there was no statistically significant interaction, the highest yields of marketable product were obtained in the treatment combination of 60,000/ha stocking density with added substrate (Figure 1). Future research should examine the relationship between average prawn size and surface area available per animal, to allow recommendations to be developed as to the amount of added substrate needed to produce target size animals under different conditions of pond size and stocking rate.

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