JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 33, No. 1
March, 2002

Effects of Substrate Amount and Orientation on Production and Population Structure of Freshwater Prawns *Macrobrachium rosenbergii* in Ponds

JAMES H. TIDWELL, SHAWN D. COYLE, AARON VANARNUM,
AND CHARLES WEIBEL

Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky 40601 USA

Abstract.—Previous studies have demonstrated a direct linear relationship between freshwater prawn *Macrobrachium rosenbergii* production intensity (kg/ha) and amount of added substrate. However, increases greater than 80% have not been practicable with multiple layers of substrate installed in horizontal orientation. Vertical orientation could allow higher inclusion rates, but might not be useable by prawns which had recently molted. This study compared the effectiveness of substrate (in the form of polyethylene fencing) oriented horizontally or vertically, and evaluated the impact of increasing amounts of vertical substrate (0, 50, and 100%) on prawn production. Juvenile prawns (0.2 ± 0.1 g) were stocked into 12 0.04-ha ponds at 65,000/ha. Ponds were randomly assigned one of four treatments with three replicate ponds per treatment: 1) controls containing no substrate, 2) 50% increase in horizontal orientation, 3) 50% increase in vertical orientation, or 4) 100% increase in vertical orientation. After 106 d, orientation of substrate (horizontal or vertical) had no significant ($P > 0.05$) impact on prawn survival, production, or population structure. In ponds with different amounts of vertical substrate, average weight (42 g), survival (89%), and feed conversion ratio (2.7) were not significantly different ($P > 0.05$) among treatments. However, total yield (kg/ha) was significantly greater ($P \leq 0.05$) in ponds with 100% increase in surface area (2,653 kg/ha) than in control ponds (2,140 kg/ha). Ponds with a 50% increase in surface area had yields (2,452 kg/ha) that were not significantly different ($P > 0.05$) from control ponds or ponds with a 100% increase. These data indicate that orientation does not impact substrate effectiveness in ponds and that increasing amounts of added substrate continue to increase prawn production up to at least an increase of 100% in available surface area.

Production of freshwater prawns *Macrobrachium rosenbergii* in temperate regions has become increasingly popular based on an ability to produce fresh, and even live, prawns of large average sizes (> 30 g) in close proximity to urban inland markets (Tidwell and D'Abramo 2000). However, in these temperate regions production is limited to one seasonal crop, increasing the need to intensify production during that limited period. While production increases can be achieved by simply increasing stocking rates, average individual weights and thereby marketable percentages are usually decreased (Karplus et al. 1986). In the 1980s researchers in Israel reported that pond production rates could be intensified, without decreasing average weights, by adding artificial substrate to ponds to increase available surface area (Cohen et al. 1983; Ra'anan et al. 1984). These production increases are based on the determination that prawns, as aggressive non-burrowing benthic invertebrates, are primarily constrained by the amount of surface area provided by the pond bottom.

To evaluate and develop methods to intensify production of prawns under temperate conditions, a series of studies has been conducted on the use of artificial substrate in ponds. Tidwell et al. (1998) reported that in prawns stocked at 60,000/ha, total production and average weight were increased 20 and 23%, respectively, when substrate was added to ponds. Research the following year found that substrate effects were not necessarily increased at higher stocking densities (Tidwell et al. 1999). Tidwell et al. (2000) found that in prawns stocked at one density (74,000/ha), production was increased in a direct linear relationship with the amount of substrate added. However, that concept could not be pursued further than an increase of 80% in surface area, under practical pond conditions, due to an inability to keep substrate layers physically separated when in a horizontal orientation.

It was proposed that vertical orientation would allow greater inclusion rates, but hypothesized that the horizontal orientation might be essential for utilization by recently molted animals (Tidwell et al. 2000).

To pursue these results further, the present study was designed to evaluate whether added substrate could function as effectively if added in a vertical orientation and if so, would higher inclusion rates (> 80%) further increase production.

Materials and Methods

Pond Preparation and Stocking

Two wk prior to the anticipated stocking date, 12 ponds located at the Aquaculture Research Center (ARC), Kentucky State University, Frankfort, Kentucky, USA, were drained and allowed to dry. Less than 1 wk prior to stocking, ponds were filled with water from a reservoir filled by runoff from the surrounding watershed. The water-surface area of each experimental pond was 0.04 ha, and average water depth was approximately 1.1 m. A ½-hp vertical pump surface aerator (Airolator, Kansas City, Missouri, USA) modified with a "deep-draw" tube operated continuously at the surface of the deepest area of each pond to aerate and prevent stratification. Liquid fertilizer (NPK, 10:34:0) was added to each pond at filling, at a rate of 9.0-kg phosphorous/ha, to achieve an initial algal bloom. Water to replace evaporative losses was obtained from the reservoir as needed.

Post-larval prawns were shipped by air from a commercial hatchery (Aquaculture of Texas, Weatherford, Texas, USA), nursed in a greenhouse at ARC for 60 d, and stocked on 1 June 1999. The mean stocking weight (0.24 ± 0.13 g; $\bar{x} \pm$ SD) was determined from five samples of 50 prawns that were blotted free of surface water and group weighed. Prawns were hand-counted and stocked into 12 ponds at 65,000/ha. Ponds were randomly assigned to receive one of four treatments: 1) no substrate (controls); 2) 50% increase in surface area—horizontal orientation; 3) 50% increase—vertical orientation; 4) 100% increase—vertical orientation.

Substrate consisted of 120-cm wide panels of polyethylene "construction/safety fence" with a mesh opening (length × width) of 7.0 cm × 3.5 cm, suspended either horizontally or vertically across the pond. The substrate was positioned approximately 30 cm above the pond bottom with a 30-cm separation between layers. Horizontal substrate was held in position with stakes made from PVC pipe driven into the pond bottom. Vertical substrate was spaced similarly and stretched the length of the pond between metal fence posts. Surface area increases were based on dimensions of mesh (length × width) with open area within the mesh subtracted from surface area calculations.

Samples

A 3.2-mm mesh seine was used to collect a sample of prawns (≥ 50) 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

For the first 6 wk prawns were fed unpelleted distillers grains with solubles (DDGS; 27% protein) (Tidwell et al. 1997), for weeks 7–12 a 32% prawn diet as described in Tidwell et al. (1997) was fed, and for weeks 13–16 prawns were fed a 40%

protein penaeid diet (Rangen Inc., Buhl, Idaho, USA). 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 d of DDGS until an average individual weight of 5 g was achieved in samples. For weights greater than 5 g, prawns were fed a percentage of body weight based on a feeding schedule modified from D'Abramo et al. (1995) by increasing daily allotments 20% above table values. Feeding rates were adjusted weekly based on an assumed feed conversion ratio of 2.5 and an assumed survival of 100%. Rates for all ponds within a treatment were based on the treatment average, not on individual pond sample weights.

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 were determined weekly from water samples collected from each pond at approximately 1300 h 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). Sample data were compiled into monthly pond means for statistical analysis.

Harvest

Prawns were cultured for 106 d. One day prior to harvest, 15 September 1999, 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 from 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. As in sample data, open (OP) and berried (BE) morphotypes were later combined into a composite group of sexually mature reproductive females (RF).

Statistical Analyses

The potential effects of orientation on survival, production, and population structure were evaluated using Student's *t*-test ($P \leq 0.05$) to compare ponds containing the same amount of substrate (50% increase) in horizontal or vertical orientation. Effects of increasing amounts of vertical substrate (0, 50, and 100%) were evaluated using ANOVA (Steele and Torrie 1980). If significant differences were indicated by ANOVA ($P \leq 0.05$) means were separated using the least significant difference (LSD) test. Production/Size Index (PSI) was calculated as PSI = production (kg/ha) x average weight (g) ÷ 1,000 (Tidwell et al. 2000). All percentage and ratio data were arc sin transformed prior to analysis. Data are presented as untransformed values to facilitate comparisons and interpretation. Statistical analyses were conducted using Satistix, version 4.1 (Statistix Analytical Software 1994).

Results and Discussion

Neither the orientation or amount of substrate had significant impact ($P > 0.05$) on monthly water quality means. Overall values for the study were: combined AM/PM water temperature, 26.6 C; combined AM/ PM DO, 7.5 mg/L; total ammonia-N, 0.06 mg/L; nitrite-N, 0.03 mg/L; and pH, 8.7, respectively.

Addition of increasing amounts of vertical substrate had no statistically significant impact ($P > 0.05$) on final average weight (42 g), survival (89%), or feed conversion ratio (FCR) (2.7) (Table 1). These results differ from Tidwell et al. (1999) and Tidwell et al.

TABLE 1. *Mean harvest weight, total yield, survival, feed conversion ratio (FCR), production/size index (PSI), and daily yield of prawns cultured in ponds with different amounts (0, 50, and 100% increases in surface area) of added substrate and two orientations (50% increase, horizontal and vertical). Values are means ± SEM of three replicate ponds. Treatment means within a row, for different amounts of vertical substrate, followed by a different letter are significantly different* ($P \leq 0.05$) *by ANOVA. Ponds with 50% increases in surface area in different orientations (vertical and horizontal) were compared using Students* t-*test and were not significantly different* ($P > 0.05$).

	Substrate amount and orientation			
Variable	0% Control	50% Horz.	50% Vert.	100% Vert.
Harvest weight (g)	39.0 ± 1.5 a	41.8 ± 1.3	44.2 ± 1.7 a	42.6 ± 2.4 a
Total yield (kg/ha)	2139.5 ± 32.0 b	2452.8 ± 115.8	2452.6 ± 60.8 ab	2653.0 ± 213.8 a
Survival (%)	84.5 ± 2.0 a	90.1 ± 2.0	85.5 ± 4.4 a	95.8 ± 6.7 a
FCR	3.0 ± 0.1 a	2.7 ± 0.1	2.6 ± 0.1 a	2.5 ± 0.2 a
PSI	83.5 ± 4.5 b	102.8 ± 8.0	103.0 ± 4.4 ab	113.4 ± 13.2 a
Daily yield (kg/ha per d)	19.6 ± 0.3 b	22.5 ± 1.1	22.5 ± 0.6 ab	24.3 ± 2.0 a

(2000) who reported that added substrate significantly decreased FCR. In the current study, FCR demonstrated the same consistent relationship (i.e., decreasing as the amount of substrate was increased); however, differences were not statistically significant ($P > 0.05$). Total yield, daily yield, and PSI were significantly greater ($P \leq 0.05$) in ponds with a 100% increase in available surface area than in control ponds. Ponds with a 50% increase in surface area had yields and average PSIs that were not significantly different ($P > 0.05$) from control ponds or ponds with a 100% increase in surface area. Increasing availability of substrate had no significant impact ($P > 0.05$) on the numbers of animals

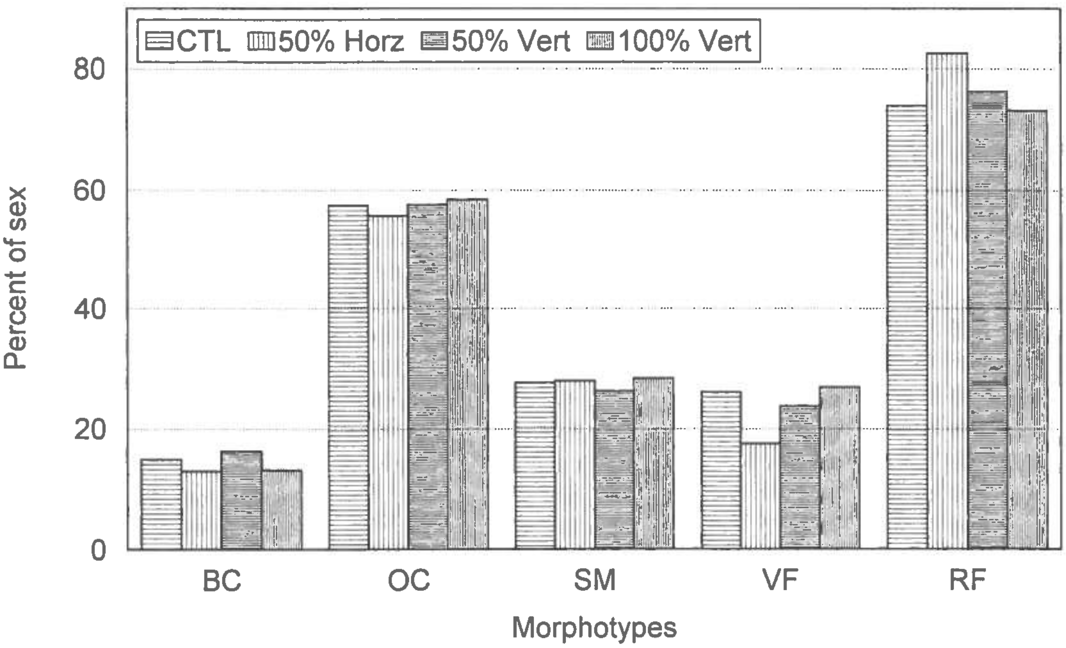

FIGURE 1. *The number of prawns classified into five morphotypes (BC, blue claw males; OC, orange claw males; SM, small males; VF, virgin females; RF, reproductive females) as a percentage of the total number within the respective sex. There were no statistically significant differences* ($P > 0.05$) *among treatments by amount of added substrate or orientation.*

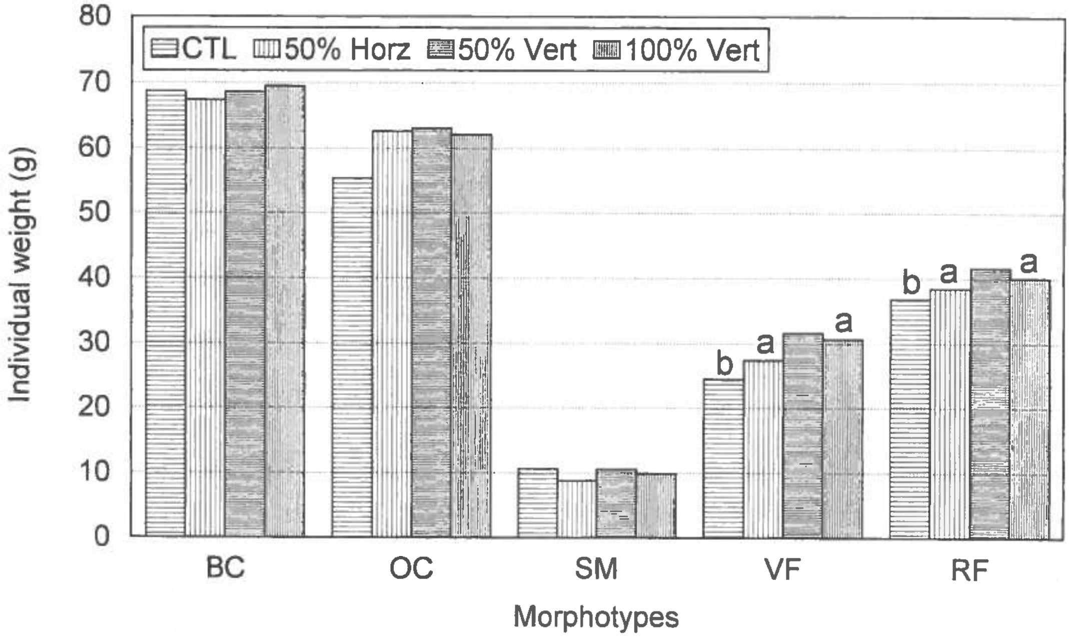

FIGURE 2. *Average individual weights (g) of prawns classified into five morphotypes (BC, blue claw males; OC, orange claw males; SM, small males; VF, virgin females; RF, reproductive females) at final harvest in ponds supplied with 0%, 50%, or 100% increases in available surface area. Different letters indicate significant differences (*$P \leq 0.05$*) by ANOVA. The 50% horizontal and 50% vertical were compared using Student's* t*-test and were not significantly different (*$P > 0.05$*).*

classified into the five morphotypes (as a percentage of their respective sex) (Fig. 1). This differs from Tidwell et al. (2000) who reported that the number of females achieving reproductive status increased as the amount of available substrate increased. In the present study, average individual weights of females (both reproductive and virgin) were significantly higher ($P \leq 0.05$) in ponds with added substrate, while weights of male morphotypes were not significantly affected ($P > 0.05$) (Fig. 2).

Comparison of ponds with the same amount of substrate, in either horizontal or vertical orientation, indicates that orientation had no significant impact ($P > 0.05$) on prawn survival, production (Table 1), or population structure (Figs. 1, 2). Previous studies at this laboratory had evaluated substrate only in a horizontal orientation based on the theory that the positive effects of substrate were at least partially manifested during the critical period after molting (before the new exoskeleton has hardened). During this period "soft-shell" prawns are extremely susceptible to cannibalism and predation (Peebles 1978), but might not have the structural integrity and strength needed to utilize substrate installed in a vertical orientation. These data indicate that in ponds, orientation does not appear to influence the effectiveness of added substrate. This should allow greater amounts of substrate to be installed more easily in production ponds.

Production results in this study were extremely high for seasonal production of prawns in temperate ponds. Even in control ponds (with no substrate), production was as much as 100% greater than results reported for similar ponds in earlier studies (D'Abramo et al. 1989; Tidwell et al. 1999) as were PSI indices (Tidwell et al. 2000). These large production increases compared to previous work may be due to the changes in the feed types and rates used in the current study. Feed rates were increased approximately 20% above feed table recommendations to insure that feed availability did not limit potential substrate effectiveness. The switch to phase feeding of different feed stuffs was based on data presented in Tidwell et al. (1997) which indicated that small prawns utilize meal feeds and natural foods efficiently while prawns of large average sizes (> 40 g) may require more nutrient dense feedstuffs. Studies should be conducted to evaluate the relative impact of increased feed rates and phase feeding of different feed types.

In summary, production results in this study indicate that substrate functionality is not decreased in vertical orientation and that production continues to increase up to at least, and possibly beyond, an increase of 100% in available surface area. The positive effects of increasing substrate amounts appear to be additive with other management improvements. Future research should quantify and refine the relative contributions and efficiencies of substrate inclusion, feed type, and feeding rates. These data indicate that under these conditions prawn production rates in excess of 2,000 kg/ha, with average weights of > 40 g, are consistently achievable within the approximately 120-d pond growing period of temperate regions.

Acknowledgments

We thank Daniel Yancey and James Johnson for daily management of culture ponds and Karla Hochstrasser for typing this manuscript. This research was funded by a grant from the Southern Regional Sustainable Agriculture Research and Education Program (SARE) funded jointly by the United States Department of Agriculture and the Environmental Protection Agency under SARE Grant Number L597-89 and a USDA-CSREES grant to Kentucky State University under agreement KYX-80-91-04A.

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