

To the Graduate Council:

I am submitting herewith a thesis written by Holly Alford Marlowe entitled "The Effects of Nursery Length and Pond Substrate Use in Production of Freshwater Prawn *Macrobrachium rosenbergii*." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

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The Effects of Nursery Length and
Pond Substrate Use in
Production of Freshwater Prawn
Macrobrachium rosenbergii

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Holly Alford Marlowe
May 2006

ACKNOWLEDGEMENTS

I am extremely grateful to Dr. J. Larry Wilson for providing the opportunity for me to pursue an advanced degree at the University of Tennessee, Knoxville, and for his guidance throughout graduate school. I would also like to thank the other members of my committee, Dr. Thomas Hill and Dr. Barry Sims, for invaluable support and guidance throughout this study.

Special thanks to Misty Huddleston for her willingness to help in all aspects of this study. Thanks also to my fellow graduate students, Mike Gaughler, Brac Salyers, Melinda Wilson, Alison Ashby, and Joyce Coombs for help with counting, stocking, and harvesting. Thanks also to the undergraduate Forestry, Wildlife and Fisheries students at the University of Tennessee in Knoxville and Martin for help in counting prawn.

I would like to thank the staff at the Joseph E. Johnson Animal Research and Teaching Unit for their technical assistance, as well as the staff at Highland Rim Research and Education Center (HRREC), formerly called the Highland Rim Experiment Station, at Springfield, Tennessee, and the University of Tennessee, Martin, for managing the ponds during this experiment.

I extend thanks to the Department of Forestry, Wildlife and Fisheries for funding through a tuition waiver and stipend for a teaching assistantship. Additional thanks to J. Fred Heitman and Pete Wyatt for guidance throughout my college career. Finally, I would like to thank my husband and family for their unwavering support throughout my years in college.

ABSTRACT

Pond production of freshwater prawn (*Macrobrachium rosenbergii*) is limited in temperate regions by the length of the growing season. The indoor nursery phase is intended to increase the growing season, but can be costly to maintain. This study compared the total weight and survival (%) of prawn grown indoors for 30 days to prawn grown indoors for 60 days. Artificial substrate was used in the nursery tanks to increase yield and survival of the post-larvae. The prawn nursed for 60 days were stocked at 4.6 prawn/L of water and the prawn nursed for 30 days were stocked at 4.75 prawn/L; the mean weight of all post-larvae at the beginning of the study was 0.01 g. The 60-day group was significantly larger at the end of the nursery experiment than the 30-day group (average individual weight was 0.12 g, compared to 0.07 g).

Prawn were stocked at two sites in Tennessee with six 0.04-ha outdoor ponds at each site. The ponds at one site used artificial substrate and were stocked at 27,375 prawn/ha (10,950 prawn/acre), while ponds at the other site did not have artificial substrate and were stocked at 30,000 prawn/ha (12,000 prawn/acre). After approximately 120 days, the prawn were harvested, weighed, and counted. The ponds with artificial substrate had significantly increased survival between the 30-day group and the 60-day group (82% and 88%, respectively). The total weight between the treatments was significantly different with 76.4 kg (168.5 lbs) for the 30-day group and 108.2 kg (238.5 lbs) for the 60-day group. The survival in the ponds without substrate was 81% for the 30-day

group and 76% for the 60-day group and was not statistically significant. The total weight between the treatments was not significantly different with 99.7 kg (219.7 lbs) for the 30-day group and 100.7 kg (222.1 lbs) for the 60-day group. Stocking post-larvae nursed for 60 days results in a larger yield and higher survival than those nursed for 30 days if artificial substrate is used in the ponds. There is little difference in total yield between stocking post-larvae reared for 30 versus 60 days if artificial substrate is not used. The reduced survival in the 60-day group without substrate is likely due to increased cannibalism as a result of the increased size compared to available substrate surface area.

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CHAPTER I

INTRODUCTION

Freshwater prawn (*Macrobrachium rosenbergii*) are indigenous to the tropical Indo-Pacific region. Culture began in Malaysia and Thailand in the 1950s, although this consisted of growing wild-caught prawn in ponds (Wickins 1976). After Ling and Merican (1961) discovered that larvae require brackishwater to survive, broodstock were introduced to Hawaii in the 1970s, and subsequent research led to the development of culture management practices (New 2002).

Further research found that even though prawn culture was limited by temperature, culture could be economically feasible in temperate regions. In order to extend the growing season in these areas, temperate prawn culture has three distinct phases: 1) rearing larvae in brackishwater for approximately 30 days (hatchery), 2) growing post-larvae indoors for 30-60 days to improve growout (nursery), and 3) stocking juveniles in outdoor warmwater ponds for approximately 120 days to allow prawn to reach maximum adult size (growout) (D'Abramo and Brunson 1996).

In the mid-1990s, the freshwater prawn industry began to develop in Mississippi and Kentucky, and in the last few years, in Tennessee, Georgia, and southern Illinois. The estimated total acreage devoted to freshwater prawn production in Tennessee was over 200 acres in 2002 (T. K. Hill, personal

communication). This increase in production was due in part to more cost-efficient culture practices. While recent experiments have improved the total harvest yield through studies on stocking densities (Kneale and Wang 1979; Smith and Sandifer 1979a; Smith et al. 1981, 1983; Karplus et al. 1986; D'Abramo et al. 1989; Daniels et al. 1995; Marques et al. 2000; Marcus 2002), feed options (Heinen and Mensi 1991; Ashby 2003), artificial substrate in the nursery and ponds (Sandifer and Smith 1977; Smith and Sandifer 1975, 1979b; Cohen 1983; Ra'anani et al. 1984; Sandifer et al. 1983; Karplus et al. 1992; Tidwell et al. 1998, 1999; Alston and Sampaio 2000; Ashby 2003), substrate color and orientation (Smith and Sandifer 1979a, 1979b; Molina 1990; Tidwell et al. 2000, 2002; Marcus 2002; Posadas et al. 2003), production at a commercial level tends to be approximately 50% of that reported in research trials (Tidwell et al. 1999). Tidwell et al. (2005) reported that this inconsistency appears to be related to poor survival (50-60%) in commercial operations when compared with that in research ponds (85-90%).

According to S. Coyle (personal communication) with Thoroughbred Shrimp Company, an estimated 90% of prawn producers purchase juvenile prawn from nurseries as opposed to raising their own. Dasgupta and Tidwell (2003) reported that most farmers purchase prawn that have been nursed for 60 days, which cost approximately \$0.06 each (Coyle, personal communication). Post-larvae nursed for 30 days, however, cost approximately \$0.04-0.05 each (Coyle, personal communication). It may be economically beneficial to spend

more money to purchase larger prawn if the total yield at harvest is significantly higher.

This study was designed to compare survival and growth of two groups of post-larvae in grow-out ponds, one group that had been nursed for 30 days and the other which had been nursed for 60 days. Comparisons of the total production weight and percent survival of both groups may help the producer decide which group may offer the best economical advantage in terms of end-of-year profit.

CHAPTER II

LITERATURE REVIEW

Biology

Freshwater prawn, *Macrobrachium rosenbergii*, in the family Palaemonidae, are indigenous to the Indian sub-continent and Malaysia (Wickins 1976). Over 200 species of *Macrobrachium* have been described worldwide; these organisms can be found in most inland freshwater areas such as lakes, rivers, swamps, ditches, canals, and ponds, as well as in estuarine waters for at least part of their life (New 2002). *M. rosenbergii* are the largest species of *Macrobrachium*, and are therefore the most used for commercial aquaculture.

New (2002) described *M. rosenbergii* as easily distinguishable from other species of *Macrobrachium* by the following characteristics: 1) it has a very long rostrum with 11-14 dorsal teeth and 8-10 ventral teeth, 2) adult males have long second chelipeds in which all segments are elongate with blunt spines, and 3) adult males have a movable finger covered with dense velvet-like fur at the end of the second cheliped. New (2002) also stated that *M. rosenbergii* is the largest species in the genus with a total length of up to 33 cm in adult males and up to 29 cm in adult females.

New (2002) further provided a physical description of freshwater prawn. Like other crustaceans, prawn have a hard exoskeleton that must be regularly shed. The shedding, called molting, is followed by an incremental increase in

size and weight. Prawn are vulnerable for a period of time after each molt until the new exoskeleton hardens.

Ismael and New (2000) gave a complete description of the morphology of *M. rosenbergii*, including information on mating and spawning. Mating can only occur between hard-shelled males and soft-bodied females. The male deposits semen in a gelatinous mass on the ventral thoracic region of the female. Eggs are laid after a few hours, fertilized by the semen on the exterior of the female, and attached to her underside. The fertilized eggs are aerated by the movement of the abdominal legs. The number of eggs produced by the female ranges from 5,000 to 20,000 for the first spawn, and 80,000 to 100,000 eggs in a fully mature female. Eggs development takes from two to three weeks, depending on environmental conditions. Egg color changes from orange to gray just before hatching.

Although adults live in fresh water, larvae require a salinity of 10-14 parts per thousand to survive. Females move to brackish water and hatch free-swimming larvae. Larvae go through 11 distinct stages of development before becoming post-larvae (PL), or juveniles. Immediately after hatching, larvae are planktonic and swim upside down and tail-first. They are aggressive sight-feeders, constantly feeding on zooplankton, small worms, or the larvae of other organisms. Metamorphosis is complete after approximately 16 days, although development varies according to feeding and temperature. In captivity, most larvae metamorphose to PLs within 25-28 days. Behavior and appearance mark the transition to post-larvae, where the PL resembles adult prawn, swims dorsal-

side up and forward, and becomes benthic. Post-larvae can tolerate freshwater and move into areas of lower salinity. Post-larvae are omnivorous, feeding on insects, algae, nuts, grain, seeds, fruit, mollusks, crustaceans, fish, and animal waste.

Culture

Culture of prawn began in Malaysia and Thailand in the 1950s where adults were captured and raised in captivity, although attempts to hatch and rear larvae were unsuccessful (Wickins 1976). In 1961, Ling and Merican discovered that larvae require brackish water to survive, leading to the introduction of broodstock to Hawaii by Fujimura and Okamoto (1972). The availability of post-larvae for subsequent research led to the development of a substantial worldwide market, although temperature requirements of prawn prevented successful culture in temperate regions (New 2000).

Perhaps the most limiting environmental factor affecting prawn culture is sensitivity to cold temperatures. Prawn can survive in 14-35°C, although the optimum range for growth is 28-31°C (New 2002). According to New (2002), the growing season in temperate regions ranges from 100 to 150 days. To extend the growing season, production in temperate regions requires three phases of prawn culture: 1) the hatchery phase, 2) nursery phase, and 3) pond grow-out (D'Abramo and Brunson 1996). The hatchery phase involves the brackishwater larval period; the nursery phase consists of indoor temperature-controlled freshwater tanks to advance stocking size of juveniles prior to stocking in outdoor

ponds for grow-out. Stocking growout ponds with nursery-reared juveniles, as opposed to newly metamorphosed post-larvae, results in larger individuals and higher survival, which leads to larger individuals at harvest (Alston and Sampaio 2000), and an increased total production (D'Abramo et al. 1989).

Nursery

A number of studies have been conducted to maximize growth and survival in the nursery phase. Studies have been conducted to determine the most productive stocking density in the nursery to offset the cost of rearing post-larvae indoors. Kneale and Wang (1979), Smith and Sandifer (1979a), and D'Abramo et al. (1989) found that increased stocking densities resulted in lower survival, reduced individual average weight, and reduced total production. The optimal stocking density was found to be less than or equal to 600 PL/m² (Kneale and Wang 1979). Prawn are cannibalistic when in higher densities, whether from lack of adequate food or from incidents of territoriality.

Ling (1969) recommended placing vegetation in ponds to increase surface area for the prawn to reduce the incidence of cannibalism. Experiments have further proven that total production can be greatly increased by added artificial substrate in both the nursery tanks and grow-out ponds. Adding artificial substrate to nursery tanks increases survival and total production by increasing two-dimensional space and allowing the prawn to utilize the entire water column and not just the sides and bottom of the tank, thereby reducing mortality (Sandifer and Smith 1977; Smith and Sandifer 1975, 1979b; Sandifer et al. 1983,

Alston and Sampaio 2000). Ashby (2003) reported that increasing the surface area in the nursery tanks by 100% yielded larger prawn than a 50% increase, although survival was not significantly different.

Alston and Sampaio (2000) reported that aside from increasing stocking size of post-larvae and lengthening the growing season in temperate regions, the nursery phase increases the average individual size of prawn at harvest when stocked in ponds. Nursed juveniles were also reported to be more resistant to predation, cannibalism, and changes in environmental conditions in growout ponds than post-larvae stocked directly into ponds.

Nursery substrate orientation and the types of materials used as substrate have been topics of research studies. Smith and Sandifer (1979a, 1979b) found that post-larvae preferred layered substrate and had a significant response to black plastic mesh when compared to fiberglass strips. Molina (1990) compared the effects of various sizes of black substrate to white substrate, as well as no substrate. Prawn in tanks without substrate had significantly lower survival. Optimum stocking density associated with substrate in this study was 7 PL/L. Marcus (2002) found that there were no significant differences in survival or individual weight between horizontal and vertically oriented substrate after 65 days, although survival of prawn was significantly higher over dark brown substrate (46%) than tan substrate (40%).

With the nursery phase usually taking place indoors in temperate areas, research on the effects of photoperiod and artificial light has been conducted. Tidwell et al. (2001) described several studies conducted with other species of

crustaceans on the effect of photoperiod. Photoperiod has been shown to affect food consumption in the red frog crab (*Ranina ranina*) (Minagawa and Murano 1993; Minagawa 1994) and cannibalism in the Australian giant crab (*Psuedocarcinus gigas*) (Gardner and Maguire 1998). Photoperiod was found to affect molting frequency in crayfish (*Procambarus clarkia*) (Constanon-Cervantes et al. 1995) and growth performance in American lobsters (*Homarus americanus*) (Aiken et al. 1983), although the effects are highly variable among different genera. Withyachumnarnkul et al. (1990) found that post-larvae grown in total darkness had increased growth rates and increased survival when compared to other light regimens; however, Lin (1991) reported that growth rate and survival increased with increased photoperiod. Tidwell et al. (2001) compared different light regimens and concluded that while growth rates were not significantly impacted by photoperiod, post-larvae exposed to continual light had a significantly higher rate of survival than those exposed to other regimens: i.e., 12 hours of light/12 hours of dark, and 24 hours darkness.

Size-grading nursery populations before stocking into production ponds have been found to increase individual mean harvest weight and total yield. Tidwell et al. (2005) stated that as the duration of the nursery phase increased, size variation increased because of differential growth rates and may have contributed to poor survival because of cannibalism by larger individuals. Therefore, size-grading juveniles at some time during the nursery period may decrease size variation and increase survival.

Tidwell and colleagues (2005) reported that post-larvae that were size-graded during the nursery phase had significantly higher survival (89%) than ungraded prawn (81%) although average weight was higher in ungraded prawn populations than in populations that were graded. The same study by Tidwell and colleagues reported that survival at 30 days was significantly higher than survival at 60 days (92% versus 82%, respectively), indicating that nursery duration had a significant effect on survival.

Growout

The impact of stocking densities with and without the use of artificial substrate has also been studied in production ponds. Karplus et al. (1986) found that while prawn survival was independent of stocking density, yield increased and mean prawn size decreased with stocking density. D'Abramo et al. (1989) also reported that average harvest weight decreased 23% when stocking densities were increased from 39,536 to 79,072 prawn/ha. The addition of artificial substrate in ponds has been found to improve total production yield, for the same reasons it is used in nurseries. Cohen et al. (1983) reported that the addition of artificial substrate increased total production and average prawn size. Ra'anani et al. (1984) reported that artificial substrate was more effective in intensively-stocked production ponds, although research by Tidwell et al. (1999) found that stocking ponds at 60,000 prawn/ha resulted in higher yield of marketable prawn (>30 g each) than ponds stocked at 120,000 prawn/ha.

Karplus et al. (1992) found that while artificial substrate reduced antagonistic interactions between prawn, it also improved the feed conversion rate. Tidwell et al. (1999) found that substrate increased feeding efficiency by providing more surface area for periphyton to grow; the organisms that generally make up periphytic communities closely match preferred prey for prawn (Tidwell et al. 1997).

Tidwell et al. (2002) reported that substrate orientation (horizontal versus vertical) in production ponds did not affect the functionality of substrate with regard to increased survival and total production. Total production continued to increase with up to at least, and perhaps beyond, an increase of 100% in available surface area in ponds. Ashby (2003) compared survival, mean individual weight, and feed conversion in ponds with 1.22-m high vertical substrate to 0.61-m high vertical substrate and reported no significant differences.

Nutrition

Feed costs constitute 40-60% of operational costs in production of prawn (D'Abramo and Sheen 1991). In an effort to be more cost-effective, numerous experiments have been conducted to determine the dietary requirements of prawn. Weindenbach (1980) found that prawn will consume natural pond biota regardless of the presence of formulated feed pellets, although natural productivity is neither optimally employed nor promoted in prawn culture (MacLean et al. 1989). Early research studies have shown that commercially

available pelleted feed will usually dissolve before direct consumption by prawn (Stahl 1979). Feed that was not directly consumed by prawn was thought to contribute organic and inorganic material to the pond; this addition of material would then contribute to the pond food chain, i.e., phytoplankton, detritus, and microorganisms (Goodwin and Hanson 1974). Tidwell et al. (1995) also suggested that the addition of pelleted feed may have positively impacted benthic macroinvertebrate fauna by the addition of macro- and micro-nutrients to the pond, although the addition of organic fertilizer likely increased the numbers of benthic macroinvertebrates used as forage by prawn.

Corbin et al. (1983), however, reported that protein needs to be supplied to prawn by prepared feeds, although other nutritional requirements could be attained by the consumption of naturally-occurring organisms in production ponds. Research has been conducted to reduce this cost by replacing certain ingredients in prawn feed with less expensive ingredients. Tidwell et al. (1995) and Hill et al. (1997) found that prawn increase predation on natural pond biota to supplement their nutritional intake when expensive nutrients are removed from the feed.

CHAPTER III

METHODS

This study was conducted to determine if a period of 60 days versus 30 days in an indoor nursery facility impacts growth and survivorship of prawn during the pond grow-out period. The research was performed from March 2004 to September 2004. The nursery facility was located at the Joseph E. Johnson Animal Research and Teaching Unit, University of Tennessee, Knoxville, and the grow-out ponds were located at the Highland Rim Research and Education Center in Springfield, Tennessee, and at the University of Tennessee, Martin.

Nursery Design

The nursery design for this experiment consisted of twelve fiberglass tanks; six were circular and six were rectangular. The circular tanks were 121.9 cm in diameter and 49.5 cm in height and the rectangular tanks were 208.3 cm long, 7.2 cm wide, and 53.3 cm deep. The system design was a flow-through aquaculture system using city water dechlorinated by a charcoal filter and two carbon filters. The dechlorinated water flowed into an 1135-L rubberized head tank on a metal rack approximately 3 m off the floor. The twelve fiberglass tanks were situated 0.5 m lower than the head tank for gravity-assisted flow. This design was identical to the one described by Ashby (2003) (Figure 1).



Figure 1. Nursery layout at Joseph Johnson Animal and Research Teaching Unit.

Water temperature was maintained by two 1800-watt digitally controlled immersion heaters (Process Technology) in the head tank. Oxygen concentrations were supplemented by air bubbles pumped to each culture tank by a linear magnetic piston air pump (#MAPXL-MANAIR XL, Ultralife Reef Products). Water and air were distributed to each tank through polyvinyl chloride pipes (PVC) and each culture tank had an air stone connected by plastic tubing to the air line. The rate of air entering the tank could be adjusted by a twist valve at the join of the pipe and the tubing. Water inflow to each tank was distributed through PVC caps with holes drilled in the center. Water inflow to each tank was approximately 0.2 L/minute resulting in a 100% water exchange every 33 hours.

Each culture tank contained horizontal artificial substrate. Each circular tank had four substrate frames that were 46 cm tall, 50 cm long, and 33 cm wide. The substrate consisted of 6x6-mm brown polyester mesh horizontally stretched and attached to 1.3-cm PVC pipes in four layers, providing approximately 6 cm of space between each layer (Figure 2). Measuring the horizontal surfaces only, the substrate increased the overall substrate surface area of each circular tank to 46,454 cm² (87%). Each rectangular tank had one substrate frame that was 27 cm tall, 165 cm long, and 28 cm wide. The same type of 6x6 mm brown polyester mesh was stretched horizontally and attached to the PVC pipes in three layers, providing approximately 9 cm of space between each layer (Figure 3). Measuring the horizontal surfaces only, the substrate in the rectangular tanks increased the overall usable substrate surface area of the tank to 46,454 cm² (45%). The substrate was submerged in each tank and the top layer rested just below the water surface. The substrate used is identical to the substrate described by Marcus (2002) and Ashby (2003).

Nursery Experiment

A total of 11,100 prawn were obtained from Shawn Coyle at Thoroughbred Shrimp Company (Frankfort, Kentucky) and transported in four polyethylene bags at a density of 5g/L with each bag containing approximately 3,500 prawn. Bags were filled with brackish water (5ppt) to reduce ammonia-related stress and water temperatures were lowered to 22.2°C to reduce



Figure 2. Horizontal substrate used in circular tanks in the nursery experiment.



Figure 3. Horizontal substrate used in the rectangular tanks in the nursery experiment.

metabolic activity. The bags were filled with oxygen, sealed, and packed into styrofoam shipping boxes. Prawn in bags were shipped via ground transportation to the nursery facility; the transport time was approximately four hours.

The first group of post-larvae (the 60-day group) was stocked in the nursery on 29 March 2004. The prawn were hand-counted and weighed in groups of 300, then stocked into three circular tanks (C1-C3) and three rectangular tanks (R1-R3). Each tank was stocked at a density of 4.625/L (N=1850), and the average individual weight was 0.01 g. The second group of post-larvae (the 30-day group) was stocked on 30 April 2004. The prawn were also hand-counted and weighed in groups of 300 and stocked into the remaining three circular tanks (C4-C6) and three rectangular tanks (R4-R6). These prawn were stocked at a density of 4.75/L (N=1900), and the average individual weight was 0.01 g.

The prawn were fed a commercially available granular feed formulated for penaeid shrimp (Rangen, Inc., #1) consisting of 41% protein. Prawn were fed once daily between 1300 and 1700 hours and the amount was recalculated every week assuming a feed conversion rate of 2.0 (Ashby 2003). Prawn were fed 10% of their average body weight for days 1-30, 8% of their estimated average body weight for days 31-45, and 6% of their estimated average body weight for days 46-60. Survivorship was assumed to be 100% when calculating the feed amount.

Water quality measurements were taken once weekly to monitor any changes in water quality. Dissolved oxygen (%) and pH were measured using a handheld YSI Model 60 meter. Ammonia and alkalinity were tested with LaMotte testing kits (LaMotte Company, Chestertown, Maryland). Water temperature was recorded daily with a hand-held thermometer, as well as air temperature of the room. There was no natural light available in the nursery; six fluorescent lights on the ceiling above the tanks were on 24 hours per day.

At the end of the nursery segment of the experiment, the tanks were drained and the prawn were scooped out using dip nets. Prawn from each tank were hand-counted into groups of 100 and weighed. Three nursery tanks were randomly selected from each treatment group and were combined in the transport tank, with each treatment having a separate transport tank. Half of the juveniles were counted, weighed, and transported on 1 June 2004 and the other half were counted, weighed, and transported 2 June 2004.

The 400-L insulated haul tanks were filled with dechlorinated water from the nursery head tank. Pure oxygen in cylinders was bubbled into the tanks through plastic tubing and through an air stone. Temperature and oxygen levels were checked periodically during transport.

Pond Growout

The six ponds at Highland Rim Research and Education Center (HRREC), formerly called Highland Rim Experiment Station, were constructed in 2000. Each 0.04-ha pond was 24.8 m long and 16.5 m wide and had an average depth

of 1.37 m. Each pond had 0.6 m of freeboard with a 2:1 inside slope and a 1% bottom slope (Figure 4). Each pond had a 6x6-m catch basin on the lower end with a 20.3-cm diameter PVC standpipe. The standpipe was 1.82 m high and was connected to a PVC elbow to direct water out of the pond during harvest. The PVC ran downslope from each pond to an outlet where the water was diverted away from the pond area.

The production ponds at HRREC were earthen cut-and-fill type ponds. The production ponds were oriented on a slope with three sets of two ponds. Ponds 1 and 2 were at the highest elevation, with Ponds 3 and 4 at mid-level and Ponds 5 and 6 at the lowest elevation. The production ponds were filled by water from a 0.62-ha reservoir pond at a higher elevation for gravity-assisted flow. Water was routed to each pond through a 10-cm diameter PVC pipe. A 4.95-ha wastewater pond was at a lower point in the watershed to trap pond effluent during harvest. Figure 5 illustrates the schematic of the pond layout.

Prior to stocking, ponds were drained and allowed to dry completely. The reservoir pond was treated with rotenone to ensure that no fish were present to invade the production ponds through the water supply. Four weeks before stocking, each pond was filled and fertilized using one-half bale of good-quality grass hay, along with 4,536 g of cottonseed meal, and 363 g of inorganic fertilizer (19-19-19). A similar amount of inorganic fertilizer was reapplied to each pond two weeks after stocking.

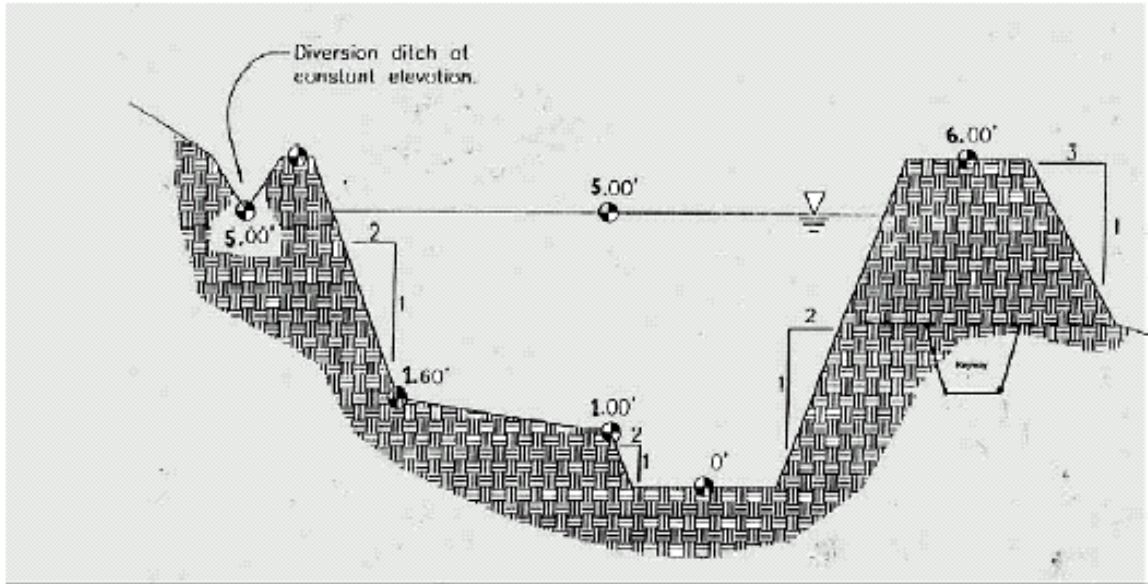


Figure 4. Schematic representation of pond layout.

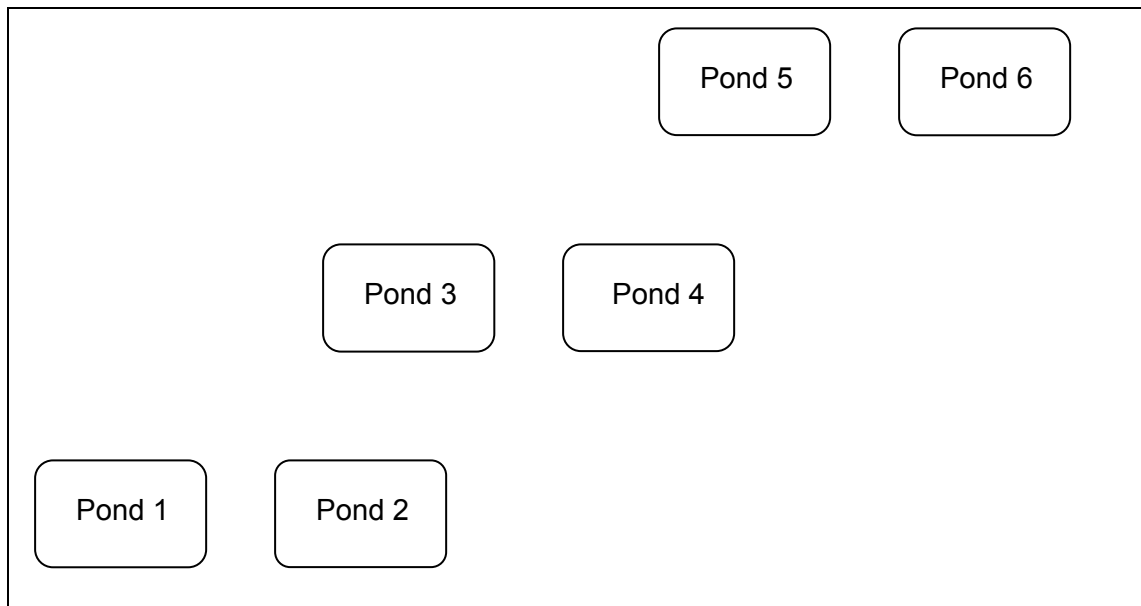


Figure 5. Schematic of pond orientation at Highland Rim Research and Education Center.

Vertical substrate was installed in each pond (Figure 6). Nine 15-m long rows of 1.22-m orange polyethylene construction fencing were stretched across each pond. The fencing was attached to metal stakes and reached the pond bottom. Propeller aerators (Kasco 110 volt) were used twenty-four hours per day to prevent thermal stratification in the ponds and to ensure a level of dissolved oxygen sufficient to sustain the prawn.

The six UT Martin (UTM) ponds were constructed for this research in 2004 with similar pond dimensions as those at the Highland Rim site. These ponds were filled by an underground well and allowed to reach ambient temperature before prawn were stocked. Each pond was equipped with a 25-cm diameter PVC pipe to drain water to a 15.2-cm diameter pipe which drained into a diversion ditch. The six ponds were arranged in two sets of three ponds (Figure 7).

The ponds at the UTM site did not contain any type of artificial substrate. Propeller aerators were used continuously in Ponds 1, 2, and 3, while wind-powered aerators were used for Ponds 4, 5, and 6.

Juvenile prawn were counted and stocked into each pond in groups of 300. Ponds were randomly assigned and stocked with juveniles from either the 30-day group or the 60-day group; each treatment had three replications. These data were used to determine survival rates and total yield for each pond.

Prawn were fed a commercial pelleted sinking catfish feed (32% protein). Prawn were fed half of the amount of feed twice daily, except for weekends when they were fed once per day.



Figure 6. Vertical substrate used at ponds at Highland Rim Research and Education Center.

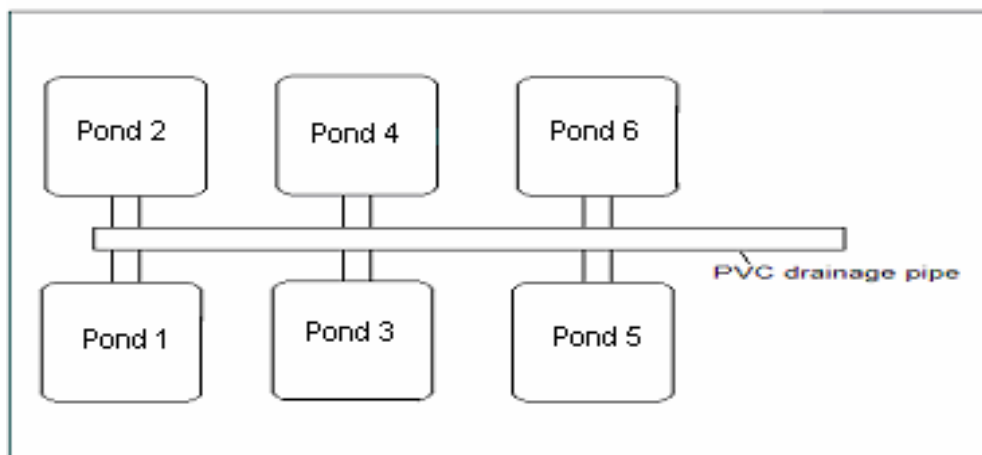


Figure 7. Schematic of pond orientation at UT Martin.

Harvest

The HRREC ponds were harvested after 110 days. The standpipe was lowered and water and prawn were diverted out through the drain pipe. The water was directed through a wooden purge box with holes drilled in the bottom (Figure 8). This allowed any prawn that went through the pipe to be collected. Remaining prawn were hand-picked from the pond and added to the harvest. Prawn in the purge box were rinsed in fresh water and then purged in clean water for 30 minutes (Figure 9). Prawn were then submerged in ice water for 15 minutes to quickly kill them and preserve the quality of the flesh. Prawn from each pond were separately counted and weighed in batches of 100. Prawn were immediately put in coolers on ice.

The ponds at UT Martin were harvested after 126 days. The standpipes were lowered, but due to inefficient draining, the remaining water



Figure 8. Water and prawn diverted through the drain to a wooden purge box.



Figure 9. Prawn in an ice bath after harvesting from ponds.

was ultimately pumped out of each pond (Figure 10). All prawn were hand-picked from each pond (Figure 11), rinsed in freshwater, purged, and submerged in ice water, then counted and weighed as previously described.

Statistical Analyses

Analysis of the total survival per treatment and total weight per treatment for the nursery experiment and the growout experiment was performed using the latest version of SPSS software. Survival data were analyzed using a Chi-Square test for independence of the variables. The variances of the weights were tested for equality using the Levene's test statistic, and an analysis of variance (ANOVA) was run to determine dependence between the treatments in each experiment.



Figure 10. Water was pumped out of ponds at the UT Martin site due to inefficient draining.



Figure 11. All prawn were hand-picked at the UT Martin site for harvest.

CHAPTER IV

RESULTS

Nursery Experiment

The data for this experiment are presented in Table 1. After 60 days in the nursery, Tank C1 had a total weight of 228.3 g with an average individual weight of 0.16 g and 74% survival. Tank C2 had a total weight of 201.4 g with an average individual weight of 0.14 g and 75% survival. Tank C3 had a total weight of 205.6 g with an average individual weight of 0.15 g and 74% survival. Tank R1 had a total weight of 166.7 g with an average individual weight of 0.13 g and 66% survival. Tank R2 had a total weight of 181.2 g with an average individual weight of 0.14 g and 69% survival. Tank R3 had a total weight of 175.5 g with an average individual weight of 0.15 g and 63% survival. In this treatment, the average individual weight of the post-larvae was 0.15 g with an average survival of 70.2%.

After 30 days in the nursery, Tank C4 had a total weight of 70.9 g with an average individual weight of 0.06 g and 67% survival. Tank C5 had a total weight of 61.6 g with an average individual weight of 0.06 g and 54% survival. Tank C6 had a total weight of 88.9 g with an average individual weight of 0.06 g and 75% survival. Tank R4 had a total weight of 77.3 g with an average individual weight of 0.07 g and 75% survival. Tank R5 had a total weight of 64.8 g with an average

Table 1. Data collected from each tank in the nursery experiment.

<u>60-day Treatment</u>				
<u>Tank</u>	<u>Number Harvested</u>	<u>Survival (%)</u>	<u>Total Weight (g)</u>	<u>Avg. Ind. Weight (g)</u>
C1	1408	74	228.3	0.16
C2	1426	75	201.4	0.14
C3	1397	74	205.6	0.15
R1	1260	66	166.7	0.13
R2	1304	69	181.2	0.14
R3	1202	63	175.5	0.15
Average		70		0.15
<u>30-day Treatment</u>				
<u>Tank</u>	<u>Number Harvested</u>	<u>Survival (%)</u>	<u>Total Weight (g)</u>	<u>Avg. Ind. Weight</u>
C4	1232	67	70.9	0.06
C5	1002	54	61.6	0.06
C6	1385	75	88.9	0.06
R4	1073	58	77.3	0.07
R5	1009	55	64.8	0.06
R6	1284	70	85.7	0.07
Average		63		0.06

individual weight of 0.06 g and 75% survival. Tank R6 had a total weight of 85.7 g with an average individual weight of 0.07 g and 70% survival. In this treatment, the average individual weight of the post-larvae was 0.06 g with an average survival of 63%.

Using the Fisher's Exact Test statistic in the Chi-Square test, the higher survival in the 60-day treatment was statistically significant ($\alpha = 0.05$). A one-way ANOVA was run to compare total weight between treatments (Table 2). The 60-day treatment resulted in a significantly higher total weight from each tank than the 30-day treatment ($p = 0.001$).

There were unexpected differences between the circular and rectangular tanks within each treatment. Within the 60-day treatment, total weight and survival was higher for prawn nursed in the circular tanks than in the rectangular tanks. The surface area of the circular tanks (24,807 cm²) was less than that of the rectangular tanks (32,044 cm²), although both tank types had the same total surface area with added artificial substrate (46,454 cm²). There were minimal differences within the 30-day treatment with regard to tank type.

Table 2. One-way ANOVA comparing total weight and length of nursery phase.

Weight					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7830.893	1	7830.893	186.575	.000
Within Groups	2182.537	52	41.972		
Total	10013.430	53			

Pond Growout

The data for this experiment are presented in Table 3. At the Highland Rim Research and Education Center (HRREC), Ponds 1, 4, and 5 were stocked with 1095 post-larvae from the 60-day treatment. A total of 1049 prawn were harvested from Pond 1 (95.8% survival) for a total of 38.5 kg (84.8 lbs). A total of 874 prawn were harvested from Pond 4 (79.8% survival) for a total of 36.0 kg (79.5 lbs). A total of 969 prawn were harvested from Pond 5 (88.5% survival) for a total of 33.7 kg (74.2 lbs).

Ponds 2, 3, and 6 were stocked with 1096 post-larvae from the 30-day treatment. A total of 847 prawn were harvested from Pond 2 (77.3% survival) for a total of 25.6 kg (56.5 lbs). A total of 944 prawn were harvested from Pond 3 (86.1% survival) for a total of 23.2 kg (51.1 lbs). A total of 892 prawn were harvested from Pond 6 (81.4% survival) for a total of 27.6 (60.9 lbs).

The ponds at HRREC stocked with the 60-day treatment prawn had an average survival of 88%. There were 2892 prawn harvested of the 3285 stocked, and the total weight of the prawn harvested from these three ponds was 108.2 kg (238.5lbs). The three ponds at HRREC that had the 30-day treatment prawn had an average survival of 81.6%. Of the 3288 total prawn stocked into the three ponds, 2683 survived, and the total weight of the prawn harvested was 76.4 kg (168.5lbs).

At UT Martin, the six ponds were stocked with 1200 post-larvae. Ponds 1, 4, and 6 were stocked with post-larvae from the 60-day treatment. A total of 787 prawn were harvested from Pond 1 (65.6% survival) for a total of 32.8 kg

Table 3. Data collected from the growout ponds at each site.

Highland Rim Research and Education Center ^a				
<u>60-day Treatment</u>				
<u>Pond</u>	<u>Stocked</u>	<u>Total harvested</u>	<u>Total weight (kg)</u>	<u>% Survival</u>
1	1095	1049	38.5	95.8
4	1095	874	36.0	79.8
5	1095	969	33.7	88.5
Total	3285	2892	108.2*	
Average			36.0	88.0*
<u>30-day Treatment</u>				
<u>Pond</u>	<u>Stocked</u>	<u>Total harvested</u>	<u>Total weight (kg)</u>	<u>% Survival</u>
2	1096	847	25.6	77.3
3	1096	944	23.2	86.1
6	1096	892	27.6	81.4
Total	3288	2683	76.4	
Average			25.5	81.6
UT Martin ^b				
<u>60-day Treatment</u>				
<u>Pond</u>	<u>Stocked</u>	<u>Total harvested</u>	<u>Total weight (kg)</u>	<u>% Survival</u>
1	1200	787	32.8	65.6
4	1200	1052	36.0	87.7
6	1200	912	32.0	76.0
Total	3600	2751	100.8	
Average			33.6	76.4
<u>30-day Treatment</u>				
<u>Pond</u>	<u>Stocked</u>	<u>Total harvested</u>	<u>Total weight (kg)</u>	<u>% Survival</u>
2	1200	1062	36.1	88.5
3	1200	925	30.5	77.1
5	1200	944	33.0	78.7
Total	3600	2931	99.6	
Average		977	33.0	81.4

^a Ponds with artificial substrate

^b Ponds without artificial substrate

* indicates significance

(72.3 lbs). A total of 1052 prawn were harvested from Pond 4 (87.7% survival) for a total of 35.96 kg (79.3 lbs). A total of 912 prawn were harvested from Pond 6 (76% survival) for a total of 32.0 kg (70.5 lbs).

Ponds 2, 3, and 5 were stocked with post-larvae from the 30-day treatment. A total of 1062 prawn were harvested from Pond 2 (88.5% survival) for a total of 36.13 kg (79.7 lbs). A total of 925 prawn were harvested from Pond 3 (77.1% survival) for a total of 30.5 kg (67.2 lbs). A total of 944 prawn were harvested from Pond 5 (78.7% survival) for a total of 33.1 kg (72.8 lbs).

The ponds at UT Martin stocked with 60-day treatment prawn had an average survival of 76.4%. There were 3600 prawn stocked in the three ponds and 2751 were harvested, with a total of 100.7 kg (222.1lbs) harvested. The three ponds stocked with 30-day treatment prawn had an average survival of 81.4%. There were 2931 of the 3600 stocked prawn harvested, with a total of 99.7 kg (219.7lbs).

There was a significant difference in survival between the two treatments at HRREC ($p < 0.001$, $\alpha = 0.05$), although the survival at UT Martin was not significantly different between the two treatments. The total weight at HRREC was significantly higher in the 60-day treatment than in the 30-day treatment ($p < 0.001$, $\alpha = 0.05$) (Table 4). The total weight at UT Martin was not significantly different between treatments.

Table 4. One-way ANOVA comparing total weight and length of nursery phase in growout ponds at Highland Rim Research and Education Center.

weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	166.260	1	166.260	11.128	.002
Within Groups	567.751	38	14.941		
Total	734.011	39			

CHAPTER V

DISCUSSION

Successful commercial production of *Macrobrachium rosenbergii* in temperate regions requires special considerations at stocking and harvest (Tidwell et al. 2005). The relatively short growing season, limited by temperature, has been overcome by the practice of growing post-larvae in an indoor nursery facility (D'Abramo et al. 1996). The nursery phase is primarily used to increase the post-larval size prior to pond stocking.

Nursery Experiment

Numerous studies have been conducted to maximize growth and survival in the nursery phase. Kneale and Wang (1979), Smith and Sandifer (1979a), and D'Abramo et al. (1989) found that increased stocking densities resulted in lower survival, reduced individual average weight, and reduced total production. The optimal stocking density was found to be less than or equal to 600 PL/m² (Kneale and Wang 1979).

After Ling (1969) found that placing natural substrate in ponds could reduce cannibalism, various experiments have indicated that total production can be increased by the addition of artificial substrate in both the nursery and in the growout ponds. Artificial substrate allows prawn to better utilize the entire water column and, also, reduces aggressive encounters (Sandifer and Smith 1977;

Smith and Sandifer 1975, 1979b; Sandifer et al. 1983, Alston and Sampaio 2000). Increasing the substrate surface area in the nursery tanks has also been found to yield larger prawn (Ashby 2003).

Marcus (2002) found that there were no significant differences in survival or individual weight between horizontal and vertically oriented substrate after 65 days, although survival of prawn was significantly higher over dark brown substrate (46%) than tan substrate (40%). Smith and Sandifer (1979a, 1979b) found that post-larvae preferred layered substrate and had a significant response to black plastic mesh when compared to fiberglass strips. In a study designed to compare the color of substrate versus no substrate, Molina (1990) reported that while there was significantly less survival in tanks without substrate, prawn had no preference between the two colors.

The results from the present study indicated that keeping prawn in the nursery for 60 days resulted in a significantly higher average individual weight than prawn kept in the nursery for 30 days (0.15 g and 0.06 g, respectively). The average individual weight was expected to be higher in the post-larvae held for 60 days (S. Coyle, personal communication). The average individual weights of the post-larvae were underestimated due to the range of sizes; grading the population would have resulted in a more uniform size distribution and a higher average individual weight.

Survival was significantly higher after 60 days in the nursery versus 30 days (70.2% and 63%, respectively); although previous research stated that survival was significantly higher in 30-day nursed prawn compared to 60-day-old

prawn (Tidwell et al. 2005). The lack of size grading throughout the nursery experiment likely changed the population structure and led to unexpected results.

According to New (2002), the shape of the nursery tank has no effect on post-larvae growth and survival. Tidwell et al. (2005) reported that survival was significantly lower in 60-day-old nursed post-larvae (82%) compared to that of 30-day-old nursed post-larvae (92%); the difference in weight and survival within the 60-day group could be a factor of increased vigor or aggression within the larger post-larvae.

Pond Growout

Pond growout in temperate regions typically lasts 120-130 days. Karplus et al. (1986) and D'Abramo et al. (1989) found that while prawn survival was independent of stocking density, yield increased and mean prawn size decreased with stocking density. To maximize growth rate and survival, artificial substrate has been recommended in production ponds as a means of increasing total production and average weight of prawn (Cohen et al. 1983; Tidwell et al. 1999).

Cohen et al. (1983) reported that the addition of artificial substrate to growout ponds led to increased total production and average prawn size. Research by Ra'anani and colleagues (1984) found that artificial substrate was more effective in intensively-stocked production ponds; Tidwell et al. (1999), however, found that stocking ponds at 60,000 prawn/ha resulted in higher yield of marketable prawn (>30 g each) than ponds stocked at 120,000 prawn/ha. In addition, artificial substrate has been found to contribute to natural productivity in

ponds (Tidwell et al. 1999), and Karplus et al. (1992) reported that artificial substrate not only reduced antagonistic interactions between prawn, but it also led to an improvement in the feed conversion rate of prawn.

In the present study, stocking 60-day nursery-reared prawn in growout ponds with artificial substrate resulted in a significantly higher survival than that of 30-day nursed prawn (88% versus 81.6%). The use of substrate also resulted in a significant increase in total weight, from 108.2 kg with 60-day PLs, compared to 76.4 kg with 30-day PLs (an increase of 29%).

Post-larvae from the 30-day treatment were not significantly different in total weight or survival from 60-day nursery-reared prawn when stocked in ponds without artificial substrate. The survival of prawn from the 30-day treatment was actually slightly higher than that of prawn from the 60-day treatment (81.4% versus 76.4%, respectively). Without substrate, the total weight at harvest for the 60-day treatment was nearly identical to that of the 30-day treatment (100.7 kg versus 99.7 kg, respectively). Growth of prawn is density-dependent (Tidwell et al. 2005); therefore, without artificial substrate, the larger 60-day prawn exhibited reduced growth rate as the population reached a critical biomass.

Culture Implications

According to an overview of recent research and development in temperate prawn culture by Tidwell and colleagues (2005), culture of freshwater prawn in the South Central United States offers positive opportunities. Furthermore, there are several crucial management practices that must be

implemented for success of prawn farming in this region, such as stocking of advanced juveniles, size grading of juveniles, and the use of artificial substrate in growout ponds. When these management practices are combined, production of 2000 kg/ha, with a mean harvest size of >30 g, has been consistently achieved in commercial operations in less than 140 days in growout ponds. Based on research by Dasgupta and Tidwell (2003), the average prawn producer in Kentucky pays \$0.10 each for 60-day-old post-larvae, although many farmers do not use artificial substrate in the growout ponds. The research conducted at UT Martin suggests that without artificial substrate, smaller juveniles can be stocked without differences among total production yield, average individual weight, and survival. However, research conducted at HRREC suggests that stocking 60-day-old prawn with artificial substrate would be more profitable than stocking without artificial substrate.

CHAPTER VI

SUMMARY

This research was conducted to compare survival and total weight of two groups of post-larval prawn reared in production ponds, one group having a 60-day nursery phase and the other a 30-day nursery phase. Prawn were weighed and counted after each treatment and then stocked into growout ponds at two sites. The treatments were also compared with regard to growout in ponds with and without artificial substrate. The major findings were:

1. In the nursery experiment, post-larvae held for 60 days were significantly larger and had significantly higher survival than those held for 30 days.
2. When stocked in ponds with artificial substrate (HRREC), stocking post-larvae that had been in the nursery for 60 days resulted in significantly higher survival and a significantly higher total yield at harvest than 30-day-old post-larvae.
3. In growout ponds without substrate (UTM), there was no significant difference in survival or total yield between stocking 30-day-old and 60-day-old post-larvae.

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