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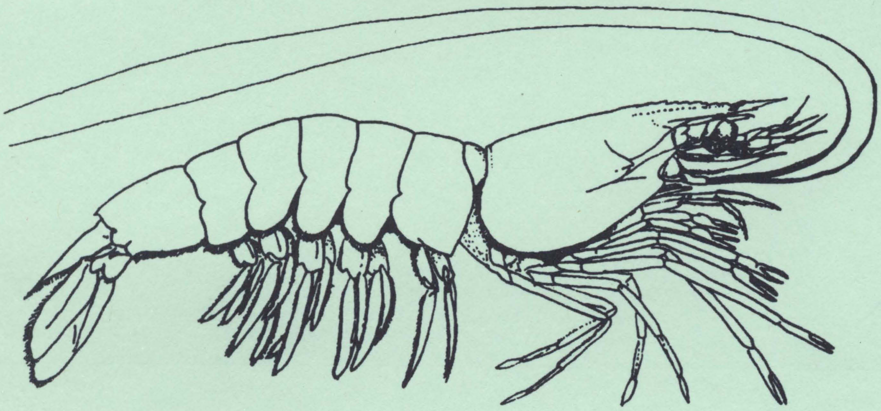

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# Shrimp Culture Research at Texas A&M University: 1989 to 1991

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## Abstract

The following accomplishments in shrimp mariculture research were completed in 1989, 1990, and 1991. Growth trials conducted on *Penaeus vannamei* in outdoor tanks indicated that (1) a substrate was not necessary for optimal growth; (2) requirements for some minerals could be satisfied from the substrate and seawater, the substrate being more important than seawater; and (3) production was greater at low than at high salinities. However, the growth at high salinities could be increased by raising the protein level of the feed.

Growth studies of *Penaeus vannamei* in pens in earthen ponds demonstrated that (1) growth (2.31 to 2.22 g/week) and production (6,608 to 6,636 kg/ha) were similar for feed rates of 4 to 8 percent body weight per day but greater than a feed rate of 2 percent body weight per day (1.70 g/week and 5,912 kg/ha); (2) growth was either equal to or greater in shrimp fed during the day than in shrimp fed at night; (3) growth increased as feeding frequency increased from 1 to 4 times per day, (4) shrimp fed a 25 percent protein feed had a similar growth and harvest biomass (2.09 g/week and 6,723 kg/ha) to those fed a 45 percent protein feed (2.31 g/week and 6,606 kg/ha); (5) shrimp fed a commercial feed containing 35 percent protein and 2.5 percent squid meal had similar growth and production rates (2.29 g/week and 6,989 kg/ha) to those presented a feed containing 45 percent protein and 15 percent squid meal (2.30 g/week and 6,578 kg/ha); and (6) growth and production were 2.19 g/week and 6,232 kg/ha using 13 mg vitamin C per kg of feed and 2.22 g/week and 6,288 kg/ha using 246 mg vitamin C per kg of feed. Growth trials conducted on native species in pens in earthen ponds demonstrated that a harvest biomass of 4,003 to 4,288 and 2,443 to 2,578 kg/m² and a growth rate of 0.93 to 1.04 and 0.69 to 0.76 g/week was obtained with *P. setiferus* and *P. aztecus* in pens in earthen ponds and that similar growth and survival were obtained for *P. setiferus* and *P. vannamei* fed 28 percent protein feeds. With *P. vannamei*, growth of more than 2 g/week was obtained for at least one treatment in six different experiments conducted in either outdoor tanks or pens within earthen ponds.

Intensive round pond technology was transferred from Oceanic Institute in Waimanolo, Hawaii, to Texas. Its use demonstrated that intensive production (growth rates of 1.82 to 1.90 g/week and harvest levels from 7,229 to 9,107 kg/ha) of *Penaeus vannamei* in square ponds was similar to that in round ponds having a bottom area of about 0.07 to 0.1 ha.

New technology was developed for removing waste and for water aeration and circulation for nursery raceway production of *Penaeus vannamei*. More than 7,000 juvenile *Penaeus vannamei* per m² with a minimum of 92 percent survival and a food conversion ratio (FCR) of about 0.6 in raceways were successfully produced using this system. In addition, raceway technology was developed for production of 6.5 to 8.1 g *P. vannamei* with survival and production of 78 to 83 percent and 13,600 to 15,600 kg/ha, respectively, at an initial stocking density of 233 or 299 PL/m².

A method for evaluating *Penaeus vannamei* postlarvae (PL) quality was developed. It indicated that lower quality postlarvae have a lower growth rate in the nursery phase.

Research on shrimp diseases indicated that Necrotizing Hepatopancreatitis (NHP) and the Texas Pond Mortality Syndrome (TPMS), synonym Granulomatous Hepatopancreatitis (GH), does not appear to be correlated to the presence of IHHNV inclusion bodies, and hence IHHNV is not a factor in

**Keywords:** shrimp, pond and raceway production, nutrition, disease.

this disease. Use of oxytetracycline (OTC)-medicated feed markedly increased the harvest biomass in 1990 (6,569 kg/ha) versus that of 1989 (881 kg/ha) and 1988 (1,025 kg/ha) on a commercial shrimp farm in Texas. Husbandry practices oriented toward production of specific pathogen-free (SPF) postlarvae for grow-out to marketable size were developed to maintain SPF broodstock.

Although a tremendous amount of new technology has been developed during the last 3 years, commercial shrimp culture in Texas is still marginal. Additional technology is needed in terms of intensification of raceway and pond culture, feeds and feeding strategies, disease prevention, and development of high-quality shrimp populations.

## Introduction

The shrimp culture research effort during the last 3 years has been in four areas: (1) intensification of raceway and pond culture; (2) feeds and feeding strategies; (3) disease prevention; and (4) development of high-quality shrimp populations. In this review, an attempt will be made to give an overview of the research conducted at the Shrimp Mariculture Project, Texas Agricultural Experiment Station, Texas A&M University System.

## Intensification of Raceway and Pond Culture

**Demonstrated that *Penaeus vannamei* production in tanks without substrate and in lined ponds is equal to or better than production in tanks containing either sand or clay substrate and unlined ponds.**

Conceptually, as shrimp culture in earthen ponds intensifies, maintaining desired quality of the pond bottom substrate will become increasingly difficult. Three experiments were conducted in outdoor tanks using different culture conditions in the presence of substrate (either sand and clay) and in the absence of substrate (bare bottoms). In the first experiment using either seawater from a power plant cooling lake or from a natural bay (i.e., Laguna Madre), *Penaeus vannamei* cultured at 45 ppt without substrate had greater growth than in the presence of a sand substrate. At 25 ppt, *P. vannamei* growth was the same in the presence and absence of a substrate. In the second experiment, using natural seawater at 27 and 45 ppt, growth of *P. vannamei* without substrate was greater than growth with either sand or clay substrates (Bray et al. 1990). The third experiment showed there were no significant differences in growth of *P. vannamei* either in the presence or absence of substrate using either 10 ppt or 35 ppt seawater (Davis et al. 1990). Thus, in all experiments, growth of juvenile *P. vannamei* in outdoor tanks without substrate was either greater than or the same as growth in the presence of either sand or clay substrates.

Two trials were conducted by stocking *Penaeus vannamei* into a single lined round pond and a single round earthen pond (unlined) of approximately 0.08- to 0.1-ha size (Bray et al. 1992). Table 1 gives data from these two trials. In both trials, production of harvest biomass from lined ponds was greater than from unlined ponds in each trial. Growth rate, survival, and harvest size values were similar for the shrimp cultured in lined versus unlined ponds.

**Table 1. Data on *Penaeus vannamei* cultured in lined and unlined, small round ponds. Each value represents a single observation.**

| Parameter | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| | Lined pond | Unlined pond | Lined pond | Unlined pond |
| Stocking weight (g/shrimp) | 1.33 | 1.31 | 1.32 | 1.32 |
| Stocking density (shrimp m$^2$) | 81.9 | 77.1 | 71.4 | 72.7 |
| Trial duration (days) | 70 | 70 | 92 | 92 |
| Growth rate (g/week) | 1.82 | 1.86 | 1.25 | 1.23 |
| Survival (%) | 57.1 | 55.6 | 67.2 | 56.6 |
| Harvest size (g/shrimp) | 19.5 | 19.9 | 18.1 | 17.4 |
| Harvest biomass (kg/ha) | 9,107 | 8,530 | 8,688 | 7,151 |

These data from experiments conducted in outdoor tanks and from production trials in small round ponds suggest that production of *Penaeus vannamei* in lined ponds would be either equal to or greater than production in unlined ponds. Other advantages of lined ponds include shorter time between crops, reduced probability for development of substrate toxicity, and significantly greater ability to disinfect the pond. Whether it is economically advantageous to use lined ponds cannot be determined from available data at this time.

**Demonstrated that *Penaeus vannamei* production in outdoor tanks increases as salinity decreases from 49 ppt to 5 ppt.**

Most people assume that high salinities decrease growth of *Penaeus vannamei*. Thirty 1.2-m diameter outdoor tanks with no substrate were stocked with 15 1.6-g juvenile *P. vannamei* per m$^2$ bottom area for 37 days. Five salinities (with six replicates each) of 5, 15, 25, 35, and 49 ppt were evaluated. Survival

Table 2. Data on *Penaeus vannamei* cultured in either outdoor tanks or pens in earthen ponds. Each value represents the mean of 6 and 12 observations if the experiment is conducted in outdoor tanks or in pens in ponds, respectively. Experiment number is in parenthesis.

| Parameter | Outdoor tanks | | Pens in ponds | | | |
|---|---|---|---|---|---|---|
| | (1) | (2) | (1) | (2) | (3) | (4) |
| Stocking density (shrimp/m²) | 30 | 30 | 40 | 40 | 40 | 40 |
| Salinity (ppt) | 12 | 46 | 33 | 33 | 33 | 33 |
| Feed quality (% protein) | 35 | 45 | 25/45 | 45 | 35 | 45 |
| Trial duration (days) | 42 | 42 | 42 | 42 | 42 | 42 |
| Growth rate (g/week) | 2.54 | 2.20 | 2.32 | 2.17 | 2.22 | 2.45 |
| Survival (%) | 89.6 | 91.6 | 88.4 | 90.9 | 82.3 | 83.9 |
| Harvest size (g/shrimp) | 20.5 | 18.5 | 19.7 | 18.8 | 19.1 | 20.5 |
| Harvest biomass (kg/ha) | 5,512 | 5,082 | 6,983 | 7,180 | 6,288 | 6,876 |

rates did not differ significantly, but the final weight of shrimp grown in 5 or 15 ppt seawater (12.2 and 11.9 g, respectively) were greater than those grown in 25 and 35 ppt seawater (11.0 and 10.9 g, respectively). Final weights of shrimp in 25 and 35 ppt seawater were greater than the final weight of shrimp in 49 ppt (10 g) seawater (Bray et al. 1990). These data definitely indicate that the growth of *P. vannamei* is greater at lower salinities of 5 to 15 ppt than at higher salinities above 35 ppt.

**Obtained growth better than 2 g/week of *Penaeus vannamei* for at least one treatment in six experiments conducted in either outdoor tanks or pens in earthen ponds.**

At initial stocking densities under various conditions (e.g., salinities, feed quality, stocking densities, etc.), growth rates from 2.17 to 2.54 g/week with extrapolated harvest biomass of 5,512 to 7,180 kg/ha were obtained for at least one treatment in each of six experiments in outdoor tanks and pens in earthen ponds (Table 2) (Robertson et al., 1992a, b, c, d). The significance of these data is that the potential exists for achieving *Penaeus vannamei* growth of more than 2 g/week in production ponds that yield more than 5,000 kg/ha and harvest sizes greater than 18 g. Thus, a long-term goal would be to develop production technology with the necessary supporting physical system (raceways and/or ponds) to achieve this potential high growth rate with larger production levels. With growth rates above 2 g/week and "head starting" the nursery phase in raceways under greenhouses, two crops per year of *P. vannamei* in south Texas would be more economically feasible.

**Transferred intensive round pond technology developed at Oceanic Institute to Texas and demonstrated that intensive production (growth rates of 1.29 to 1.32 g/week and harvest levels from 7,151 to 8,530 kg/ha) of *Penaeus vannamei* in square ponds was similar to round ponds having a bottom area of about 0.07 to 0.1 ha.**

Intensive round pond trials were initiated in 1989 and 1990 to transfer technology developed at the Oceanic Institute for production of shrimp in small round ponds. Initial results obtained at the Oceanic Institute showed extremely promising results: 7,400 to 16,000 kg/ha/crop in 1988 and 1989. The production achieved in the Texas trials varied from 7,151 to 8,530 kg/ha/crop (Table 3), corresponding to results at Oceanic Institute in 3 trials in 1989 to 1990 that produced 7,700 to 11,100 kg/ha/crop (Bray et al. 1992).

Table 3. Data on *Penaeus vannamei* cultured in square and round small ponds. Each value represents a single observation.

| Parameter | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| | Square pond | Round pond | Square pond | Round pond |
| Stocking weight (g/shrimp) | 1.29 | 1.31 | 1.32 | 1.32 |
| Stocking density (shrimp/m²) | 82.0 | 77.1 | 75.0 | 72.7 |
| Trial duration (days) | 70 | 70 | 92 | 92 |
| Growth rate (g/week) | 1.90 | 1.86 | 1.01 | 1.23 |
| Survival (%) | 43.4 | 55.6 | 67.7 | 56.6 |
| Harvest size (g/shrimp) | 20.3 | 19.9 | 14.6 | 17.4 |
| Harvest biomass (kg/ha) | 7,229 | 8,530 | 7,446 | 7,151 |

Particularly noteworthy is the lack of difference in harvest biomass of *Penaeus vannamei* in square versus round earthen ponds using the intensive round pond technology. The growth rate, survival rate, and harvest size are similar for *P. vannamei* cultured in square compared with round ponds. Because the capital cost for construction of square ponds is less than that for round ponds, this observation indicates that small square ponds would be economically justified compared with that of small round ponds.

**Developed new technology for removing waste and improving aeration and water circulation for nursery raceway production of *Penaeus vannamei*.**

A new method of water circulation and waste removal for raceway systems resulted in the production of 14,200 and 18,900 kg/ha of juvenile *Penaeus vannamei* (final weight was 0.48 and 0.77 g, respectively) and a survival rate above 90 percent (Samocha et al. 1993). Requiring less labor than others, the new system provided better water circulation and more efficient removal of the waste produced by shrimp. A second trial of this new system successfully produced more than 3,500 and 7,000 juvenile *P. vannamei* per m$^2$ in 33 days with a minimum of 92 percent survival at initial stocking densities of 4,000 and 7,800/m$^2$, respectively (Table 4). The small harvest size was due to low mean seawater morning and evening temperatures of 22.9 and 24.0 °C. Survival and harvest size did not differ among the different stocking densities. Notably, these shrimp had normal growth in commercial production ponds even though low seawater temperature caused slow growth in the 33-day nursery raceway period.

**Developed technology for production of 6.5 to 8.1 g *Penaeus vannamei* with survival of 78 to 83 percent and production of 13,600 to 15,600 kg/ha at an initial stocking density of 223 to 299 PL/m$^2$ in raceways.**

A 49-day intensive growth trial in 68.4-m$^2$ raceways under greenhouse conditions was conducted with 80-mg *Penaeus vannamei* at a density of 223 to 229 PL/m$^2$. Shrimp grew at an average rate of 0.94 to 1.19 g/week. They were harvested at a mean size of 6.5 and 8.1 g. The harvest biomass reached 12,600 and 15,600 kg/ha, and 78 to 83 percent survived. Although this trial was done during the spring in Texas when ambient morning seawater temperatures ranged from 18.3 to 28.3 °C, temperatures in the raceways were maintained at a mean daily low temperature of 26.8 °C and a mean daily high of 29.8 °C (Robertson et al. 1992e).

## Feed and Feeding Strategies

**Demonstrated that the growth of *Penaeus vannamei* at high salinities of 46 ppt can be improved by increasing the protein level of the feed.**

An experiment was conducted in outdoor tanks with *Penaeus vannamei* (average stocking weight of 5.3 g and density of 30 shrimp/m$^2$) using three feeds (25, 35, and 45 percent protein levels) at two salinities (12 ppt and 46 ppt) for 6 weeks (Robertson et al. 1992a). Salinity and protein level of the feed interacted significantly. At 46 ppt, shrimp fed 45 percent protein grew faster and had a higher harvest biomass (2.2 g/week and 5,073 kg/ha) than did shrimp fed 35 and 25 percent protein (2.02 and 1.89 g/week and 4,463 and 4,073 kg/ha, respectively). At 12 ppt, there was no significant difference in growth and harvest biomass of shrimp fed either 35 or 45 percent protein feeds. However, the shrimp fed 35 percent protein feed had a higher growth rate and harvest biomass (2.54 g/week and 5,513 kg/ha) than did shrimp fed 25 percent protein feed (2.3 g/week and 4,888 kg/ha). Results indicate that nutritional requirements vary with culture salinity and suggest

**Table 4. Data on 5- to 9-day-old postlarvae *Penaeus vannamei* (mean initial weight is 2.16 mg/postlarva) cultured for 33 days in raceways. Each value represents a single observation.**

| Parameter | Raceway | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Stocking density (shrimp/m$^2$) | 7,830 | 4,040 | 7,830 | 4,290 | 7,830 | 4,290 |
| Survival (%) | 92.5 | 108.5 | 96.9 | 107.8 | 95.2 | 99.0 |
| Harvest size (g/shrimp) | 0.070 | 0.064 | 0.060 | 0.062 | 0.064 | 0.080 |
| Harvest density (shrimp/m$^2$) | 7,240 | 4,380 | 7,580 | 4,630 | 7,830 | 4,290 |
| Harvest biomass (kg/ha) | 5,080 | 2,810 | 4,610 | 2,900 | 4,770 | 3,420 |
| FCR (feed conversion ratio) | 0.66 | 0.57 | 0.72 | 0.64 | 0.65 | 0.66 |

that use of higher protein feeds under hypersaline culture conditions may produce higher yields.

**Collected data indicating that *Penaeus vannamei* obtain some of their requirements for minerals from the substrate and seawater, the substrate being more important than seawater.**

An 8-week experiment was conducted with juvenile *Penaeus vannamei* using 42 1.2-$m^2$ outdoor tanks (Davis et al. 1990). Seven treatments with replicates of six each evaluated growth and survival of shrimp fed semipurified diets with and without mineral supplementation at two salinities and in the presence and absence of substrate. The deletion of dietary mineral supplementation at 10 and 35 ppt in the absence of substrate resulted in significant depression of growth with no effect on survival. The depression of growth was the highest at 10 ppt, indicating that some minerals were obtained from seawater and/or its associated biota. The data suggest (1) that the greater the stocking density, the greater the necessary mineral supplementation and (2) that culture in lined ponds may require a higher mineral supplementation in the diet than in unlined earthen ponds.

**Demonstrated that the growth (2.31 to 2.22 g/week) and harvest biomass (6,608 to 6,636 kg/ha) was similar for constant feed rates of 4, 6, and 8 percent/day but greater than a constant feed rate of 2 percent/day (1.70 g/week and 5,912 kg/ha) for *Penaeus vannamei* in earthen ponds.**

*Penaeus vannamei* (6.67 g mean weight) were stocked at a density of 40 shrimp/$m^2$ into 1-$m^2$ pens in an earthen pond for 42 days. The shrimp were fed a 45 percent protein feed at 2, 4, 6, and 8 percent of body weight, four times per 24-hour period. The data in terms of growth rate, harvest size, and harvest biomass indicate that a feed rate of 4 percent/day is adequate for the conditions of this experiment (Table 5). It should be emphasized that if the growth rate had been 1.0 g/week instead of the 1.7 to 2.3 g/week, the required feed rate would probably be less than 4 percent.

**Obtained data suggesting that *Penaeus vannamei* fed during the day grew faster than those fed at night and that growth increased as feeding frequency increased from one to four times per day.**

*Penaeus vannamei* were stocked at a density of 40 shrimp/$m^2$ into 1-$m^2$ pens in an earthen pond and fed 40 percent protein commercial feed one, two, or four times during either the night or day (Robertson et al. 1991b). The mean minimum and maximum temperatures were 22.2 and 26.7 °C, respectively, with a minimum oxygen tension of 5.3 mg/L. Growth increased as the feeding frequency increased from one

**Table 5. Data on *Penaeus vannamei* fed at various percentages of body weight per day. Each value represents a mean of 12 observations.**

| Parameter | Feed rate | | | |
|---|---|---|---|---|
| | 2% | 4% | 6% | 8% |
| Growth rate (g/week) | 1.70 | 2.31 | 2.22 | 2.17 |
| Survival (%) | 92.2 | 88.4 | 86.6 | 90.9 |
| Harvest size (g/shrimp) | 16.0b* | 19.8a | 19.2a | 18.8a |
| Harvest biomass (kg/ha) | 5,912 | 6,983 | 6,636 | 7,180 |

* SNK (Student Newman Keul) groupings are indicated with lowercase letters.

to four times per day. Growth of shrimp fed during the day was marginally significantly greater ($P = 0.0633$) than growth of shrimp fed during the night. Results indicate that feeding *P. vannamei* during the day is at least as good as and may be preferable to feeding at night.

**Demonstrated that *Penaeus vannamei* fed a 25 percent protein feed had a similar growth and harvest biomass (2.09 g/week and 6,723 kg/ha) to those fed a 45 percent protein feed (2.31 g/week and 6,606 kg/ha) in pens in an earthen pond.**

Two experiments stocked *Penaeus vannamei* at densities of 30 and 40 shrimp/$m^2$ into pens in an earthen pond and fed them either 25, 35, or 45 percent protein feeds at a 4 percent feed rate. One additional treatment consisted of shrimp fed 25 percent protein feed for 14 days, 35 percent protein feed for the next 14 days, and 45 percent protein for the last 14 days. Surprisingly, none of the treatments in both experiments differed significantly. Growth rates were 2.09 to 2.32 and 1.85 to 1.97 g/week, and harvest biomass levels were 6,677 to 7,180 kg/ha and 5,101 to 5,255 kg/ha in shrimp stocked at 40 and 30 shrimp/$m^2$, respectively. These data indicate that a much lower feed quality can be considered for shrimp production levels ranging from 5,000 to 6,000 kg/ha.

**Demonstrated that *Penaeus vannamei* fed a commercial feed containing 35 percent protein and 2.5 percent squid meal had a similar growth and production rates (2.29 g/week and 6,989 kg/ha) to those presented a feed containing 45 percent protein and 15 percent squid meal (2.30 g/week and 6,578 kg/ha) in pens in earthen ponds.**

An experiment was conducted by stocking *Penaeus vannamei* at 40 shrimp/$m^2$ into pens in an earthen pond and feeding either a 35 percent protein containing 2.5 percent squid meal, 40 percent protein containing 5 percent squid meal, 45 percent protein containing 10 percent squid meal, or 45 percent protein containing 15 percent squid meal feeds at a 4 percent feed rate (Table 6). Similar to the previous data, none of the treatments differed significantly. Growth rates were 2.29 to 2.45 g/week, and harvest biomass levels were 6,296 to 6,989 kg/ha. These data indicate that a much lower feed quality can be considered for shrimp production levels of as much as 5,000 to 6,000 kg/m$^2$. Of significance is the fact that the treatment containing unfed shrimp in pens in the same pond had a harvest biomass production level of more than 50 percent of the production level in the fed treatments.

**Table 6. Data on *Penaeus vannamei* fed practical feeds containing 35/2.5, 40/5, 45/10, and 45/15 protein squid levels. Each value represents a mean of 12 observations.**

| Parameter | Unfed | Protein/squid level in feed | | | |
|---|---|---|---|---|---|
| | shrimp | 35/2.5 | 40/ 5 | 45/10 | 45/15 |
| Growth rate (g/week) | 0.79 | 2.29 | 2.42 | 2.45 | 2.30 |
| Survival (%) | 88.0 | 89.4 | 77.5 | 83.9 | 83.8 |
| Harvest size (g/shrimp) | 10.50 | 19.6 | 20.3 | 20.5 | 19.6 |
| Harvest biomass (kg/ha) | 3,696 | 6,989 | 6,296 | 6,876 | 6,578 |

**Demonstrated that growth and production of *Penaeus vannamei* in earthen ponds were 2.19 g/week and 6,232 kg/ha with 13 mg vitamin C per kg of the feed and 2.22 g/week and 6,288 kg/ha with 246 mg vitamin C per kg of feed.**

Three experiments were conducted using *Penaeus vannamei*. The first two experiments were feeding trials conducted in the laboratory under highly controlled conditions and in the absence of natural productivity. The third experiment was a feeding trial conducted in pens in an earthen pond in the presence of two potential food sources: presented feed and natural productivity.

In the first laboratory experiment, small shrimp having an initial body weight of 226 mg were fed semipurified diets containing 0, 25, 50, 75, 100, and 1,000 ppm of a protected form of vitamin C, L-ascorbyl-2-polyphosphate (APP). After 14 days, the survivals were 23, 36, 50, 52, 63, and 91 percent for shrimp fed semipurified diets containing 0, 25, 50, 75, 100, and 1,000 ppm vitamin C, respectively. These data indicate that the vitamin C requirement was more than 100 ppm but probably less than 1,000 ppm in the feed. In the second laboratory experiment, larger shrimp having an initial body weight of 900 mg were fed the same semipurified diets containing 50, 75, 100, and 1,000 ppm vitamin C levels. After 2 weeks, the survival of shrimp fed diets containing 50 and 75 ppm vitamin C (39 and 57 percent, respectively) were significantly less than survival of shrimp fed diets contain 100 and 1,000 ppm vitamin C (89 and 96 percent, respectively). These data indicate that shrimp are highly sensitive to vitamin C deficiencies, that the protected form of vitamin C L-ascorbyl-2-polyphosphate (APP) is available to shrimp, and that vitamin C dietary requirements change with age (He and Lawrence 1993).

Forty *Penaeus vannamei*/m$^2$ having an initial weight of 5.81 g were stocked into 1-m$^2$ pens in an earthen pond for 42 days. Practical feeds were formulated to contain 0, 75, 150, and 250 ppm vitamin C (APP) and were analyzed to contain 13, 100, 153, and 246 ppm vitamin C. At the end of the experiment, no treatment differed with respect to growth (1.88 to 1.98 g/week), final body weight (18.9 to 19.7 g/shrimp), survival (80.2 to 83.1 percent), and harvest biomass (6,120 to 6,370 kg/ha) (Table 7). There was no difference in vitamin C level (50 to 54 ppm) in tissues of shrimp fed practical feeds containing 13 to 153 ppm vitamin C, although the level of vitamin C in tissue of shrimp fed the practical feed containing 246 ppm was significantly higher (66 ppm). The nutritional contribution to the shrimp from natural productivity was estimated to be about 33.8 to 35.1 percent. These data indicate that for these conditions and for harvest biomass levels of as much as 6,000 kg/ha, no vitamin C is required in the prepared feed (Robertson et al. 1992d). Initial biomass of shrimp stocked into pens was equivalent to 2,324 kg/ha.

**Table 7. Data on *Penaeus vannamei* fed practical feeds containing 13, 100, 153, and 246 ppm vitamin C levels. Each value represents a mean of 12 observations.**

| Parameter | Unfed | Analyzed vitamin C level in ppm | | | |
|---|---|---|---|---|---|
| | shrimp | 13 | 100 | 153 | 246 |
| Growth rate (g/week) | 0.79 | 2.19 | 2.32 | 2.24 | 2.22 |
| Survival (%) | 88.0 | 82.3 | 80.2 | 83.1 | 82.3 |
| Harvest size (g/shrimp) | 10.50 | 18.9 | 19.7 | 19.2 | 19.1 |
| Harvest biomass (kg/ha) | 3,696 | 6,232 | 6,320 | 6,382 | 6,288 |
| *Pond biomass increase (%) | 1,372 | 3,908 | 3,996 | 4,058 | 3,964 |
| **Contribution (%) | | 35.1 | 34.3 | 33.8 | 34.6 |

* Harvest biomass minus initial biomass (2,324 kg/ha) in kg/ha.

** Increase in pond biomass of unfed treatment divided by increase in pond biomass of a fed treatment times 100.

## Disease

**Demonstrated that Necrotizing Hepatopancreatitis (NHP) and Texas Pond Mortality Syndrome (TPMS), synonym Granulomatous Hepatopancreatitis (GH), do not appear to be correlated to the presence of inclusions, and hence IHHNV does not cause this syndrome.**

Six or more shrimp were collected from various commercial ponds and at different selected times during the culture period and were processed. Four sections were prepared from each shrimp: a sagittal section of the cephalothorax, a section of the gills, a cross section of the third abdominal segment, and a sagittal section of the sixth abdominal segment. Analysis of these tissues indicated that hepatopancreatic granuloma formation did not appear to be correlated to the presence of inclusions; hence IHHNV does not cause NHP.

**Determined that use of oxytetracycline (OTC)-medicated feed markedly increased the harvest biomass in 1990 (6,569 kg/ha) compared with that of 1989 (881 kg/ha) and 1988 (1,025 kg/ha) on a commercial shrimp farm in Texas.**

Necrotizing Hepatopancreantitis (NHP) has reduced production of shrimp in ponds in Texas since 1985. Recently, a rickettsia-like organism was proposed as the etiologic agent of NHP (Frelier et al. 1992). Previous diagnostic reports from the Texas Veterinary Medical Diagnostic Laboratory in 1988 and 1989 verified that TPMS was present in the ponds of a commercial farm at the time mortalities were observed. In 1990, when TPMS was initially observed in ponds of the same commercial farm, a field trial using oxytetracycline (OTC)-medicated feed was initiated. This research was conducted in cooperation with Tom Bell from the University of Arizona. The USDA (via the Center for Tropical and Subtropical Aquaculture) sponsored the FDA-approved experimental testing of OTC-medicated feeds. The medicated feed was fed for 10 to 14 days. A minimum withdrawal period of 15 days before harvest was included in the protocol. For the 30 treated ponds in 1990, the average harvest biomass was 6,569 kg/ha with a survival of 44.5 percent. In 1989, 15 of these same ponds had an average harvest biomass of 881 kg/ha with a survival of 11.4 percent. For 1988, 20 of these same ponds averaged a harvest biomass of 1,025 kg/ha with a survival of 24.5 percent. These data indicate that the harvest biomass and survival of ponds containing shrimp with TPMS may be increased with the use of OTC-medicated feed (Bell et al. 1992, Bell and Frelier 1991).

## Development of High-Quality Shrimp Populations

**Demonstrated that a harvest biomass of 4,003 to 4,288 kg/m² and 2,443 to 2,578 kg/m² with a growth rate of 0.93 to 1.04 and 0.69 to 0.76 g/week was obtained with *Penaeus setiferus* and *Penaeus aztecus* in earthen ponds.**

*Penaeus setiferus* (3.4 g mean weight), *P. aztecus* (7.4 g mean weight), and *P. vannamei* (6.9 g mean weight) were stocked at a density of 40 shrimp/m² into 1-m² pens in an earthen pond for 56 days (Robertson et al. 1992c). The shrimp were fed either a 30, 40, or 50 percent protein feed. The mean salinity and minimum and maximum temperatures were 32.4 ppt and 26.8 and 31.4 °C, respectively. The *P. setiferus* postlarvae, produced at Waddell Mariculture Center in South Carolina in captivity, were shipped to Texas A&M University. The *P. vannamei* PL produced by a commercial hatchery in Texas were shipped to Texas A&M University. Both of these species then grew to stocking size at Texas A&M University. *P. aztecus* was obtained from Corpus Christi Bay and acclimatized to pond conditions for a minimum of 1 week before stocking the pens. Thus, the *P. setiferus* and *P. vannamei* were from a laboratory source, whereas *P. aztecus* were from a natural source.

As seen in Table 8, *Penaeus aztecus* has a lower growth rate, survival, weight gain, and harvest biomass than do *P. setiferus* and *P. vannamei* (Robertson et al. 1992c). The growth rate and weight gain of *P. setiferus* and *P. vannamei* are similar, but the survival rate of *P. setiferus* is greater than that of *P. vannamei*. The greater harvest biomass of *P. vannamei* than *P. setiferus* is due to the much larger initial size of *P. vannamei* (6.9 g/shrimp) than that of *P. setiferus* (3.6 g/shrimp). The *P. vannamei* used in this experiment were of low quality.

**These data indicate that of the native species *Penaeus setiferus* is much more productive than *P. aztecus* under pond conditions. For the conditions of this experiment, the data indicate that *P. setiferus* can be considered for pond culture in Texas because growth was similar and survival was better than for a low-quality population of *P. vannamei*. The data obtained in other experiments using higher quality *P. vannamei* yielded much greater growth and production values for *P. vannamei* than the growth and production values of *P. setiferus* obtained in this experiment.**

Table 8. Data on *Penaeus setiferus*, *P. aztecus*, and *P. vannamei* fed 30, 40, and 50 percent protein feeds. Each value represents a mean of 12 observations.

| Parameter | *P. setiferus* | | | *P. aztecus* | | | *P. vannamei* | | |
|---|---|---|---|---|---|---|---|---|---|
| | 30% | 40% | 50% | 30% | 40% | 50% | 30% | 40% | 50% |
| Stocking size (g/shrimp) | 3.6 | 3.6 | 3.6 | 7.4 | 7.4 | 7.4 | 6.9 | 6.9 | 6.9 |
| Growth rate (g/week) | 0.93 | 1.01 | 1.04 | 0.69 | 0.69 | 0.76 | 0.97 | 1.04 | 1.07 |
| Survival (%) | 90.4 | 90.8 | 90.0 | 47.3 | 49.8 | 47.3 | 78.5 | 82.7 | 80.6 |
| Weight gain (g/shrimp) | 7.5 | 8.1 | 8.3 | 5.5 | 5.5 | 6.1 | 7.7 | 8.3 | 8.5 |
| Harvest biomass (kg/ha) | 4,003 | 4,224 | 4,288 | 2,443 | 2,578 | 2,554 | 4,603 | 5,031 | 4,984 |

**Demonstrated that growth and survival rates were similar for *Penaeus setiferus* and *Penaeus vannamei* fed 28 percent protein feeds in earthen pond culture.**

*Penaeus setiferus* (1.5 g mean weight and initial densities of 15 and 30 shrimp/m$^2$) and *P. vannamei* (0.7 g mean weight and initial density of 15 shrimp/m$^2$) were stocked into 1-m$^2$ pens in an earthen pond for 28 days. The shrimp were fed one of three different 28 percent protein feeds of three different protein qualities as represented by 100 percent animal protein, 50 percent animal and 50 percent plant protein, and 100 percent plant protein. The data in Table 9 indicate that all three protein-quality feeds are adequate when the shrimp are stocked at 15 shrimp/m$^2$. However, when the density for *P. setiferus* is increased to 30 shrimp/m$^2$, the growth of shrimp fed feed containing 100 percent plant protein is significantly less than of shrimp fed feeds containing 100 percent animal protein or 50 percent animal protein and 50 percent plant protein. Growth and weight gain is slightly less but survival is slightly greater in *P. setiferus* than *P. vannamei*. Harvest biomass of *P. setiferus* is larger compared with *P. vannamei* because the initial stocking weight of *P. setiferus* was larger than of *P. vannamei*. The growth rate, survival, and weight gain of *P. setiferus* is lower at the initial stocking density of 30 shrimp/m$^2$ versus 15 shrimp/m$^2$. Again, the data indicate that *P. setiferus* can be considered for pond culture in Texas because its growth rate and survival are similar to *P. vannamei* for the conditions of this experiment. It must be noted that data obtained in other experiments using higher quality *P. vannamei* yielded much greater growth and production values for *P. vannamei* than growth and production values for *P. setiferus* obtained in this experiment (Lawrence and Houston, 1992).

**Developed a method for evaluating *Penaeus vannamei* postlarvae (PL) quality and obtained data indicating that the lower the postlarvae quality, the lower the growth rate in the nursery phase.**

Four- to eight-day-old PL from two different populations of *Penaeus vannamei* broodstock were obtained from a commercial hatchery in Texas. One population, designated lower quality (LQ), was produced from a commercial broodstock cultured through several generations at the hatchery and was identified as having the presence of IHHNV (Infectious Hypodermal and Hematopoietic Necrosis Virus). The

Table 9. Data on *Penaeus setiferus* and *P. vannamei* fed 28 percent protein feed consisting of either 100 percent animal protein (ani), 50 percent animal protein, and 50 percent plant protein (a/p) or 100 percent plant (plt). Each value represents a mean of eight observations.

| Parameter | *P. vannamei* 15/m$^2$ | | | *P. setiferus* 15 m$^2$ | | | *P. setiferus* 30 m$^2$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | ani | a/p | plt | ani | a/p | plt | ani | a/p | plt |
| Stocking size (g/shrimp) | 0.7 | 0.7 | 0.7 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Growth rate (g/week) | 1.30 | 1.30 | 1.25 | 1.15 | 1.10 | 1.08 | 1.00 | 1.00 | 0.83* |
| Survival (%) | 80.4 | 75.3 | 77.0 | 88.1 | 93.7 | 88.3 | 79.2 | 72.8 | 71.3 |
| Weight gain (g/shrimp) | 5.2 | 5.2 | 5.0 | 4.6 | 4.4 | 4.3 | 4.0 | 4.0 | 3.3 |
| Harvest biomass (kg/ha) | 690 | 666 | 655 | 830 | 850 | 799 | 1,306 | 1,205 | 1,029 |

*Value is significantly less ($P < 0.05$) than for respective values of shrimp fed either all animal protein or 50 percent animal protein and 50 percent plant protein.

other population, designated higher quality (HQ), was produced from broodstock from the Oceanic Institute in Waimanolo, Hawaii. Repeated histologic examination of the HQ animals failed to identify evidence of Hepatopancreatic Parvo-like Virus (IHHNV), HPV, Baculovirus Penaei (BP)-type baculovirus, microsporidians, gregarines, and nematodes or cestodes. All postlarvae were produced simultaneously using identical culture conditions and methods. Four batches were obtained from each of the LQ and HQ populations. Each batch was stocked into one 2.4-m-diameter tank (5,000 PL/tank) and eight 28-cm-diameter tanks (20 PL/tank). No survival differences were noted, but the PL from the HQ population grew faster than the PL from the LQ population. An additional growth trial on juvenile shrimp indicated that the differences observed in PL growth between the two populations continued to be expressed during juvenile growth. Correlations of PL growth to larval duration and survival suggest that larval survival was a good indicator of PL quality but that time required for larval development (from nauplii to PL) was inadequate to predict growth.

These data indicate that a 14-day growth trial using 4- to 8-day-old PL can be used to predict the quality of PL produced in the hatchery and that the quality of PL is important in determining the growth rate of shrimp (Castille et al. 1993).

**Developed husbandry practices for maintaining specific pathogen-free (SPF) broodstock to produce SPF postlarvae for marketable-size shrimp in ponds.**

The following husbandry practices for maintaining SPF broodstock to produce SPF postlarvae (PL) for marketable-size shrimp in ponds were developed and applied. SPF is defined as free of IHHNV, HPV, BP-type, microsporidians, gregarina, nematodes, and cestodes.

## Prespawning

1. On the day of arrival and for the following 2 days (first 72 hours), fix all but the first moribund animal in Davidson's fixative for 36 to 48 hours and then transfer them to 50 percent ethanol solution. Each bottle contains only one animal and is labeled with the time and date each animal was killed. Fixation includes injecting each animal with fixative at multiple sites and then making two longitudinal cuts along the side of the cuticle before the animal is immersed into a bottle of fixative. The ratio of tissue to fixative should be kept to 1 part shrimp to 10 parts fixative. Proper fixation is imperative to demonstrate the intranuclear inclusions of IHHNV.
2. Dry the first moribund animal with paper towels and freeze it. This animal should be double-bagged and if possible, placed into a freezer that has never contained shrimp tissue.
3. At the end of the initial 72-hour period, take samples from 27 additional animals (not counting the frozen animals). This represents a total sample of 27 animals in addition to the moribund animals collected. It is recommended that six expendable animals be chosen to test the amputation and cauterization technique. The sample size of 27 is used because 27 corresponds to the number of samples needed to detect at least 1 infected animal in a population of 500 with an assumed pathogen prevalence of 10 percent. The pereiopods may be fixed and stored in one or two bottles as long as each pereiopod is from a single animal.
4. Kill all moribund animals found before they spawn and fix and process them as in step 1.
5. The week before spawning commences, sacrifice three animals; fix and process them as in step 1. This sample size was determined because it represents the number of shrimp that must be sampled to detect at least 1 affected animal in a population of 500 when 50 percent of the population is affected. It is assumed that the population was originally SPF and naive to IHHNV, and if infected, then at least 50 percent of the population should become infected.
6. Individually fix and label with the date killed all moribund animals found during the spawning period.

## Postspawning

7. At the end of the spawning period, fix 60 broodstock in Davidson's fixative. Included in this number will be all the animals that had undergone amputation of the pereiopod during the first 3 days of arrival.
8. From each larval tank, fix approximately 600 to 700 5- to 7-day-old PL in Davidson's fixative.
9. At the end of each nursery period, kill and fix 30 animals from each lot.
10. At the end of the grow-out period, kill and fix 30 animals from each pond stocked with SPF PL's.

## Specific Husbandry Procedures

11. Receive the broodstock as late as possible in the year before they spawn, but allow for acclimation.

12. Use a "clean" vehicle, which has not been used for any farm work the preceding week, for transporting the shipment.

13. Individuals handling the shipment should not have been on the farm that day. If they have, they must return home, shower, and change clothes before receiving the shipment.

14. Wipe off the boxes containing broodstock with alcohol or an iodophor (e.g., Betadine) before loading into the vehicle and again before entering the hatchery room.

15. Use automatic feeders for night and day feeding, thus eliminating a night worker and reducing labor during the day.

16. Change foot baths (200 ppm minimum chlorinated water) in the SPF areas at least once every third day.

17. Make specific individuals responsible for the SPF broodstock.

18. Perform as many duties as possible concerning the broodstock first in the morning, before people contact infected areas.

19. Prepare food for the SPF broodstock before that of other broodstock, and keep the feed separate. These facilities should be as clean as possible and wiped down with a suitable disinfectant after use. Keep a segregated final feed storage area for the broodstock.

20. Use only pasteurized shrimp products in the feed unless experimental data indicate otherwise.

21. Wall off the broodstock tanks with a plastic barrier about 1 foot below the overhead lights. Weigh down the bottom and seal with silicone.

22. Allow only one entrance to the broodstock tanks and a short hall serving as a dressing and washing room. Follow this sequence when entering the broodstock area:
    A. Wear a specific pair of boots, assigned to no one else, for work in the SPF broodstock area.
    B. Walk through a foot bath.
    C. Disinfect hands and arms with alcohol or an iodophor.
    D. Put on a long surgery/laboratory gown having elastic cuffs.
    E. Put on gloves and wash hands.

23. Wash the floors of the SPF area weekly with disinfectant after the adjacent contaminated areas are cleaned.

24. Spawn the SPF animals (if possible) before other stocks.

25. Give the SPF stock a separate spawning room and/or a movable partition that can be used to isolate spawning tanks.

26. Provide SPF stock an isolated "hatching room" with a foot bath at the entrance.

27. House SPF larvae in a semi-isolated area of the building, preferably as far from the contaminated area as possible. Plastic "drapes" can be hung to isolate these tanks. Provide at the entrance to the tanks a cleaning station.

28. Perform maintenance of the larval tanks before coming in contact with contaminated tanks.

29. Before each use, disinfect and wash the final tanks used to count and acclimate PL's and the truck tanks for transport to the pond.

30. When possible, designate equipment used on the SPF stock for that stock.

## Results

1. Examinations to date indicate that the husbandry recommendations instituted were sufficient to maintain SPF broodstock, including those free of IHHNV infection, for the production of SPF PL's.
2. Examinations to date suggest that the three commercial production ponds initially stocked with SPF PL's are either free of IHHNV infection or that the prevalence of infection is below the level detected by histological methods.

## Reviews

Four published reviews are also noteworthy. *Learning to Determine Quality* (Bray and Lawrence 1992) reviews new concepts in seedstock production. The second discusses penaeid larviculture using the "Galveston Method" (Smith et al. 1992a). The third is about the use of 1-L Imhoff cones to optimize larviculture production (Smith et al. 1992b). The fourth reports on reproduction of penaeid shrimp in captivity (Bray and Lawrence 1992).

## Summary

Major accomplishments reported in this review are as follows:

1. *Penaeus vannamei* production in tanks without substrate and lined ponds is equal to or better than production in tanks containing either sand or clay substrate and unlined ponds.

2. *P. vannamei* production in outdoor tanks increases as salinity decreases from 49 ppt to 5 ppt.

3. Growth better than 2 g/week was obtained in *P. vannamei* for at least one treatment in six experiments conducted either in outdoor tanks or pens in earthen ponds.

4. Intensive round pond technology developed by Oceanic Institute was transferred to Texas, and intensive production (growth rates of 1.82 to 1.90 g/week with harvest levels from 7,229 to 9,107 kg/ha) of *P. vannamei* in square ponds was similar to round ponds having a bottom area of about 0.07 to 0.1 ha.

5. Technology was developed for production of 0.5- to 0.8-g juvenile *P. vannamei* in raceways with survival rates of 91 to 99 percent and production of 14,200 and 18,900 kg/ha, at initial stocking densities of 3,100 to 3,300 PL/m$^2$.

6. New technology was developed for removing waste and for aerating and circulating water for nursery raceway production of *P. vannamei*; more than 7,000 juvenile *P. vannamei* PL/m$^2$ were successfully produced with a minimum survival rate of 92 percent and a food conversion ratio (FCR) of about 0.6 in raceways.

7. Technology was developed for producing 6.5- to 8.1-g *P. vannamei* in raceways with survival of 78 to 83 percent and production of 13,600 to 15,600 kg/ha, at an initial stocking density of 233 or 299 PL/m$^2$.

8. The lower growth of *P. vannamei* at high salinities of 46 ppt can be improved by increasing the protein level of the feed.

9. *P. vannamei* obtained some of their requirements for minerals from the substrate and seawater, the substrate being more important than seawater.

10. Growth (2.31 to 2.22 g/week) and production (6,608 to 6,636 kg/ha) were similar for constant feed rates of 4, 6, and 8 percent body weight per day but were greater than a constant feed rate of 2 percent body weight per day (1.70 g/week and 5,912 kg/ha) for *P. vannamei* in pens in earthen ponds.

11. Experiments conducted in pens in earthen ponds suggest that *P. vannamei* fed during the day grew as fast as shrimp fed at night.

12. Experiments conducted in pens in earthen ponds indicate that growth of *P. vannamei* increased as feeding frequency increased from one to four times per day.

13. Growth (2.09 g/week) and production (6,723 kg/ha) when *P. vannamei* in pens in earthen ponds were presented a 25 percent protein quality feed was not significantly different when *P. vannamei* were presented a 45 percent protein quality feed (2.31 g/week and 6,606 kg/ha).

14. *P. vannamei* fed a commercial feed containing 35 percent protein and 2.5 percent squid meal had similar growth (2.29 gm/week) and production (6,989 kg/ha) rates to those presented a feed containing 45 percent protein and 15 percent squid meal (2.30 g/week and 6,578 kg/ha) in pens in earthen ponds.

15. Growth and production of *P. vannamei* in pens in earthen ponds were 2.19 g/week and 6,232 kg/ha with 13 mg vitamin C per kg of feed and 2.22 g/week and 6,288 kg/ha with 246 mg vitamin C per kg of feed.

16. Necrotizing Hepatopancreatitis (NHP) and Texas Pond Mortality Syndrome (TPMS), synonyms Granulomatous Hepatopancreatitis (GH), do not appear to be correlated to the presence of inclusions, and hence IHHNV is not the etiology of this syndrome.

17. The use of oxytetracycline (OTC)-medicated feed markedly increased the harvest biomass in 1990 (6,569 kg/ha) compared with those of 1989 (881 kg/ha) and 1988 (1,025 kg/ha) on a commercial shrimp farm in Texas.

18. A harvest biomass of 4,003 to 4,288 and 2,443 to 2,578 kg/m$^2$ with a growth rate of 0.93 to 1.04 and 0.69 to 0.76 g/week was obtained in *P. setiferus* and *P. aztecus* in pens in earthen ponds.

19. Similar growth and survival was obtained in *P. setiferus* and a low-quality population of *P. vannamei* fed 28 percent protein feeds and cultured in pens in an earthen pond.

20. A method was developed for evaluating *P. vannamei* postlarvae quality, and data indicate that the lower the quality of the postlarvae, the lower the growth rate in the nursery phase.

21. Husbandry practices were developed to maintain SPF broodstock for SPF and subsequently for production of marketable-size shrimp in ponds.

22. Seedstock quality and evaluation methods, penaeid shrimp hatchery production, raceway production, and an experimental system for optimizing shrimp hatchery systems using Imhoff cones have been reviewed.

A tremendous amount of new technology has been developed during the last 3 years. However, commercial shrimp culture in Texas is still marginal. Additional technology is needed for intensification of raceway and pond culture, feeds and feeding strategies, disease prevention, and development of high-quality shrimp populations.

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