



AQUACULTURE CRSP 22ND ANNUAL TECHNICAL REPORT

EFFECT OF STOCKING DENSITY OF RED HYBRID TILAPIA (*Oreochromis Sp.*) ON GROWTH AND SURVIVAL OF JUVENILE TILAPIA AND SHRIMP (*Litopenaeus Vannamei*) IN POLYCULTURE

*Tenth Work Plan, New Aquaculture Systems/New Species Research 3D (10NSR3D)
Final Report*

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ABSTRACT

A single earth pond (3/4 hectare) was prepared and divided into nine (7 x 30 m) enclosures that were considered to be three blocks of three enclosures. During water exchange, the water passed first through block A (first use water), then block B (second use) and finally block C (third use). Each block was randomly stocked with shrimp (*Litopenaeus vannamei*) and three different densities of red tilapia hybrids (with an *Oreochromis mossambicus* genetic component) to give three different treatments: T0: zero tilapia per m² and 30 shrimp m²; T25: 0.25 tilapia per m² and 30 shrimp m²; T50: 0.50 tilapia per m² and 30 shrimp m². Tilapia and shrimp populations in each enclosure were sampled and growth was estimated approximately every two weeks. During the two month experiment temperature ranged from 16.2 to 25.6 °C and salinity from 28 to 35ppt. Growth rates of the tilapia and shrimp across the different treatments were similar and no significant differences were observed in mean sample weights for any sample date. Growth of the tilapia was also similar across the experimental blocks and no significant differences were observed. The tilapia grew from 4.6 ± 0.9g to mean final weights from the replicates that ranged from 32.8 ± 8.1g to 38.8 ± 10.0g.

However, the experimental blocks had a significant ($P < 0.05$) affect on mean sample weight of shrimp and when the experiment finished shrimp in blocks B and C were significantly ($P < 0.05$) bigger than shrimp from block A. The shrimp grew from 0.004 g to mean weights from the replicates that ranged from 1.82 ± 0.40 g to 2.07 ± 0.45 g in block A and from 2.08 ± 0.48 g to 2.29 ± 0.47 g in blocks B and C. There was no significant difference in survival of tilapia among the different treatments and blocks, survivals ranged from 86 to 92%. A larger variation was observed in the survival of the shrimp, the replicates exhibited a range from 46.6 to 73.9%. Survivals from block C that ranged from 66.4 to 73.9% were significantly ($P < 0.05$) higher than in blocks A and B that exhibited survivals that ranged from 46.6 to 57.0%. The three stocking densities of tilapia (0, 0.25, and 0.50 tilapia per m²) did not affect survival or growth of the tilapia or shrimp. Shrimp survival and growth was affected by position of the enclosure in the pond, survival and growth was significantly higher with water that had previously passed through other enclosures.

INTRODUCTION

Disease outbreaks and price fluctuations continue to affect the stability of the shrimp industry in many parts of the world. The results from the survey of shrimp and tilapia farming (Aquaculture CRSP project polyculture) in Thailand, Philippines and Mexico showed that shrimp ponds are not being fully utilized in some areas probably

as a result of production problems. The project's surveys and experiments also support the suggestion that tilapia production, supplemented with low densities of shrimp, in shrimp ponds can provide an opportunity to develop a sustainable aquaculture system (Fitzsimmons, 2001). Tilapia production in former shrimp ponds (with and without shrimp) has increased rapidly in many locations including Thailand (Yi and Fitzsimmons, 2002), the

Philippines (Bolívar et al, 2002), Ecuador, Peru and the inland desert of Arizona.

The majority of the Mexican shrimp farming industry is situated in the North West of Mexico (SAGARPA CONAPESCA, 2002). Aquaculture production of shrimp in Mexico has developed rapidly from 8,326 tons in 1992 to 45,853 tons in 2002 (SAGARPA CONAPESCA, 2002). The industry directly employs 23,505 and many more indirectly in secondary industries that supply feeds and equipment and process and export the final product. A large number of these employees are local people. The development of the industry has been affected through a mixture of Government intervention and complications of coastal land tenure that has restricted the speed of growth compared to other shrimp producing countries. Although cases exist from other countries of salination of agricultural land and destruction of mangrove and artisanal fisheries, the development of shrimp aquaculture in Mexico has not caused wide spread environmental problems (Hernández-Cornejo and Ruiz-Luna 2000).

Production procedures are changing rapidly to deal with these disease outbreaks and low yields (Roque and Gómez-Gil, 2000). Many farms have reduced stocking densities and the number of cycles of shrimp production in a year. These changes have allowed farms to avoid environmental conditions conducive to diseases and provide the possibility of producing larger shrimp, which command better prices in the shrimp markets.

In a polyculture setting, tilapia and shrimp can utilize different niches in the culture setting. In an extensive farm, tilapia can filter feed on phytoplankton and zooplankton in the upper water column. Shrimp spend most of the time in the pond bottom grazing on bacterial films on the bottom substrate and on the detritus settling from above. This detrital matter consists of dying algae cells and fecal matter from the tilapia. In a more intensive farm receiving pelleted feeds, the tilapia monopolize the feed, especially if it is a floating feed. However, some feed particles will probably be available to the shrimp at the bottom of the pond. More importantly, the fecal matter from the tilapia contributes to the detrital rain that supports the shrimp. *Macrobrachium*-tilapia polyculture reduces the yield of prawns compared to monoculture, but increases total yield of fish and prawns (García-Pérez et al., 2000). A similar effect occurs with brackish water polyculture of tilapia and shrimp (Yap, 2001). Anggawa (1999) reported that yields of shrimp increased when tilapia were stocked into existing shrimp ponds. The suggested stocking rate was 20-25 g fish/m² and the fish size at stocking of 50-100 g/fish. The use of all-male fish was needed to control reproduction. Fish were stocked when the shrimp biomass was at least 80 g/m² (for 3-4 g shrimp) or 150 g/m² (for 5-6 g shrimp). Tilapia harvest biomass was 40-50 g/m² and shrimp

survival was 70%.

From the disease aspect, tilapia seem to provide advantages in several ways. Growers in Ecuador have reported that tilapia will consume dead or moribund shrimp in polycultured ponds. Cannibalism is one of the primary vectors for transmission of shrimp diseases. Tilapia, which do not appear to be susceptible or carriers of these viruses, disrupt cannibalism as a mode of transmission. Tilapia also consume small crustaceans in shrimp ponds. These crustaceans are of concern as potential vectors. Having tilapia directly in the ponds or alternating with shrimp in a crop rotation can be effective for reducing crustacean populations. Bacterial infections also may be impacted by polyculture. *Vibrio* and most other bacterial pathogens common in shrimp culture are gram negative while waters which have been used for fish culture tend to be predominated by gram positive bacteria. Using water from a fish culture pond seems to reduce the prevalence of luminous *Vibrio* bacterial infections in shrimp ponds (Yap, 2001). Growers in Asia and South America have provided anecdotal reports that shrimp production increases due to higher survival in some of these polyculture systems, however, carefully controlled and replicated trials are needed to better study these systems and confirm the results.

METHODS AND MATERIALS

Experimental Animals

The tilapia used were sex reversed male red hybrids with a genetic component of *Oreochromis mossambicus*. The tilapias were brought from Jala, Colima, Mexico to the installations of CIAD Mazatlan Unit. To acclimatize the tilapias to full strength sea water (35ppt) the salinity of the holding water was raised daily by 5ppt over a 7 day period. After acclimatization, the tilapias were maintained, until the starting date of the experiment, in six 400 litre tanks with 24 hour aeration, 50% water (35ppt) exchange every 3 days and were fed daily to satiation with Silver Cup tilapia feed.

The shrimp (*Litopenaeus vannamei*) used were at developmental stage PL15 and were air freighted from a hatchery in Yucatan, Mexico for the start of the experiment. The supplier tested and certified that the shrimp were free from white spot syndrome virus (WSSV).

Experimental Pond and Preparation

The pond was a conventional earth pond with a light loam soil with low permeability that was situated on the edge of an estuary lagoon. Water was supplied to the pond by gravity from a reservoir canal that was filled from the estuary lagoon with a large bore axial pump run by a diesel engine, the outlet from the pond led back to the lagoon. The pond was dried over a 2

month period and then the earth bottom was tilled using a conventional tractor and plough. The divisions were constructed in the pond (see Experimental design below) and the bottom was limed. The pond was then filled and left for 1-2 weeks to develop an algal bloom.

Experimental Design

The experimental pond measured approximately 150m x 30m, with the water inlet at one end and the outlet at the other end (figure 1). An area of the pond with maximum depth in the middle of approximately 1 m was set up with the experimental enclosures. Each enclosure measured 7m x 30m, an area of 210 m². To form the enclosures, divisions of 1mm mosquito mesh supported with a wooden stack every 3m were set up across the pond. The enclosures were numbered from 1 to 9 from the inlet of the pond to the outlet. The enclosures were considered to be in 3 blocks, enclosures 1 to 3 formed block A, with first use water, 4 to 6 block B with second use water and 7 to 9 block C with third use water.

Three experimental treatments were used, T0: zero tilapia per m² and 30 shrimp m²; T25: 0.25 tilapia per m² and 30 shrimp m²; T50: 0.50 tilapia per m² and 30 shrimp m². Each treatment was randomly placed into one of the 3 enclosures in each block to give 3 replicates of each treatment, T0 in enclosure 1, 5 and 8, T25 in enclosure 3, 4 and 9 and T50 in enclosure 2, 6 and 7 (Figure 1).

Stocking

A total of 500 tilapia of a similar size were selected. A random sample of 30 tilapia was individually weighed to the nearest 0.1g using an electronic balance (Scout, Ohaus) and the fork length was measured to the nearest 1mm. On the 5th of December, the tilapia were transported to the farm from CIAD (1.5 hour journey time) in oxygenated plastic bags and randomly distributed and counted into the 6 enclosures to give the appropriate stocking densities 0.25 tilapia per m² or 0.50 tilapia per m² (figure 1.)

On the 5th of December, the shrimp were transported to the farm from the Mazatlan airport (1.5 hour journey time) in oxygenated plastic bags. An average weight per shrimp was calculated from counted batch weights. The shrimp were then randomly stocked into each enclosure by weight to give a stocking density of 30 shrimp per m² in each enclosure.

Operation and Sampling

No aeration was available for the pond. At regular intervals before sun-set the pump was operated to fill the reservoir. The pond was then left with a flow-through of water to renew the water during the night. Temperature, oxygen and salinity were monitored, with dissolved

oxygen level determined before dawn. The tilapia were fed Silver Cup (45% protein) tilapia feed daily at 10% body weight and the shrimp were fed a Camaronina Malta Cleyton (45% protein) shrimp feed daily at 10% body weight.

Approximately every two weeks, samples were caught using a fine mesh cast net and measured. To catch the tilapia, the cast net was thrown over the area where the tilapias were observed. To catch the shrimp, the cast net was thrown in the same way and in the same area of each enclosure. A total of 30 shrimp and 20 tilapia were weighed to the nearest 0.1g. All organisms were lightly dried before they were weighed. The fork lengths of the tilapia were also measured to the nearest 1mm. After 8 weeks on the 2nd of February the pond was drained and all shrimp and tilapia were caught and counted. Thirty shrimp and 20 tilapia from each enclosure were sampled as described above.

Statistics

Statistical analyses were conducted using Minitab release 13.1. Normality was determined with the Shapiro-Wilk W test, homogeneity of variance was determined with the Bartlett test. Percentage survival of tilapia and shrimp were compared with a two way ANOVA. Some distributions of shrimp weights were skewed towards a few heavy shrimp and could not be transformed to a normal distribution. Some tilapia weight distributions appeared to be bimodal. Shrimp and tilapia weight distributions were compared with the 2 way Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf 1995), with a multiple comparison made with the Dunn test (Zar 1999). All means are expressed as \pm one standard deviation.

RESULTS

Environmental Conditions

During the experiment, water temperature ranged from 16.2 to 25.6 °C, salinity from 28 to 35 ppt and dissolved oxygen from 2.7 to 9.6 mg/L.

Growth

The growth of the tilapia was similar (Figure 2), no significant differences were observed among the different treatment groups on any sample date. On Dec. 5th the mean weight at stocking was 4.6 ± 0.9 g. On the 22nd of December the mean weights from the replicates of the two treatments ranged from 14.8 ± 2.3 g for T50 in block B to 16.6 ± 3.2 g for T25 in block B. On the 5th of Jan. the mean weights from the replicates ranged from 22.1 ± 3.5 g for T50 in block A to 23.0 ± 3.7 g for T25 in block A. On the 19th of Jan. the mean weights from the replicates ranged from 27.4 ± 2.1 g for T25 in block C to 28.2 ± 2.7 g

for T50 in block A. On the 2nd of Feb. the mean weights from the replicates ranged from $32.8 \pm 8.1\text{g}$ for T50 in block B to $38.8 \pm 10.0\text{g}$ for T25 in block C. Overall, during a 59 day period, the tilapias grew approximately 30.1g from $4.6 \pm 0.9\text{g}$ to mean final weights from the replicates that ranged from $32.8 \pm 8.1\text{g}$ to $38.8 \pm 10.0\text{g}$. Sample distributions of the tilapia were not normal, exhibiting an apparently bimodal distribution. A group of tilapia exhibited slower growth and formed a smaller mode. At the end of the experiment, gonadal development of the tilapia was examined, the percentage of mature female tilapia in the enclosures ranged from 23 to 42%.

The growth of the shrimp in the different tilapia treatments was similar (Figure 3), no significant differences were observed between the treatments T0, T25 and T50. On the 5th of Dec. the mean weight at stocking was 0.004g. On the 22nd of Dec. the mean weights from the replicates of the three treatments ranged from $0.18 \pm 0.09\text{g}$ for T50 in block C to $0.22 \pm 0.13\text{g}$ for T50 in block B. On the 5th of Jan. the mean weights from the replicates ranged from $0.51 \pm 0.2\text{g}$ for T50 in block A to $1.0 \pm 0.25\text{g}$ for T50 in block C. On the 19th of Jan. the mean weights from the replicates ranged from $1.11 \pm 0.4\text{g}$ for T25 in block A to $1.51 \pm 0.5\text{g}$ for T0 in block A. On the 2nd of Feb. the mean weights from the replicates ranged from $1.82 \pm 0.4\text{g}$ for T25 in block A to $2.29 \pm 0.5\text{g}$ for T0 in block C.

However, significant differences ($P < 0.05$) were observed among shrimp from the different blocks (Figure 4). On the 22nd of Dec there were no significant differences among blocks. On the 5th of Jan. the shrimp in block C ($0.88 \pm 0.34\text{g}$) were significantly ($P < 0.05$) bigger than shrimp in blocks A ($0.58 \pm 0.26\text{g}$) and B ($0.65 \pm 0.29\text{g}$). On the 19th Jan. the shrimp in block C ($1.36 \pm 0.50\text{g}$) were significantly ($P < 0.05$) bigger than shrimp in block A ($1.14 \pm 0.53\text{g}$), blocks A and C were not significantly different from B ($1.29 \pm 0.53\text{g}$). On the 2nd of Feb. the shrimp in blocks C ($2.27 \pm 0.52\text{g}$) and B ($2.17 \pm 0.52\text{g}$) were significantly ($P < 0.05$) bigger than shrimp in block A ($1.90 \pm 0.43\text{g}$). Overall, during a 59 day period, the shrimp grew approximately 2.17g from 0.004g to mean weights from the replicates that ranged from $1.82 \pm 0.40\text{g}$ to $2.07 \pm 0.45\text{g}$ in block A and from $2.08 \pm 0.48\text{g}$ to $2.29 \pm 0.47\text{g}$ in blocks B and C. A number of sample distributions of the shrimp were not normal and exhibited a skewed distribution, with a small number of shrimp that exhibited higher weights compared to the weights of the majority of the shrimp in the sample.

Survival

Survival of the tilapia in the different treatments were similar, there were no significant differences among the different treatments (Figure 5). Survivals ranged from 86% for T25 in block A to 92% in groups T50 block A and T25 block B.

Survival of the shrimp in the different tilapia treatments were similar, there were no significant differences among the different treatments T0, T25 and T50 (Figure 5). However, there were significant ($P < 0.05$) differences in the survivals among blocks, block C exhibited significantly ($P < 0.05$) higher survival than blocks A and B. Survivals ranged from 66.4% for T50 to 73.9% for T0 in block C and for blocks A and B survivals ranged from 46.6% for T0 in block B to 57.0% for T50 in block A.

DISCUSSION

The three stocking densities of tilapia (0, 0.25 and 0.50 tilapia per m^2) did not affect survival or growth of juvenile tilapia or shrimp. This suggests that tilapia can be stocked with shrimp into earth ponds with out affecting shrimp growth and the tilapia will be an added product from the pond system. Saelee et al (2002) obtained similar results using the same stocking densities to culture shrimp (*Penaeus monodon*) and Nile tilapia (*Oreochromis niloticus*) in fresh water ponds in Thailand. However, Anggawa (1999) reported that yields of shrimp increased when tilapia were stocked into existing shrimp ponds and Garcia-Perez et al. (2000) found that *Macrobrachium*-tilapia polyculture reduced the yield of prawns compared to monoculture, but increased total yield of fish and prawns, while Yap (2001) had a similar result with tilapia and shrimp polyculture in brackish water. From the results in this study, poly culture clearly offers an opportunity to increase production of shrimp pond systems without affecting shrimp production. It is interesting that the tilapia density of 0.5 tilapia per m^2 did not affect either shrimp or tilapia growth and survival. This suggests that higher densities of tilapia could be used to further increase possible gains from polyculture. Stocking densities have been reported of 25 to 100 fish per m^2 for tilapia pre-ongrowing monoculture (Watanabe et al. 1997, Green and Engle, 2000) and of 1 to 3 tilapia per m^2 for tilapia ongrowing monoculture (Lovshin, 2000, Popma and Rodriquez, 2000).

Shrimp survival and growth was affected by position of the enclosure in the pond. Survival and growth were significantly higher with water that had previously passed through other enclosures. It is interesting that shrimp growth and survival was significantly better under the poorer conditions that would be expected from water that had previously passed through other enclosures containing shrimp and tilapia. It is possible that these conditions provided some nutritional advantage as the used water may carry sediments and provide a better environment for bacterial growth that the shrimp could use as a nutritional source.

CONCLUSION

This study demonstrated that under these culture condi-

tions juvenile shrimp growth and survival was not affected by juvenile tilapia and the production from the pond system was increased with the added tilapia production. Tilapia stocking density did not affect production and it is possible that higher stocking densities of tilapia could be considered. However, this study only considered juveniles of tilapia and shrimp. Studies are being completed to look at the polyculture system in Mexico to harvest size.

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Figure 1. Experimental set up in the pond. Arrows indicate water inlet and outlet, dashed lines represent the mosquito mesh divisions that formed the enclosures, enclosures were numbered from 1 to 9, the number below the enclosure number indicates the stocking density of tilapia per m^2 , all enclosures were stocked with 30 shrimp per m^2 . Enclosure 1 to 3 formed block A, enclosure 4 to 6 block B and enclosure 7 to 9 block C.

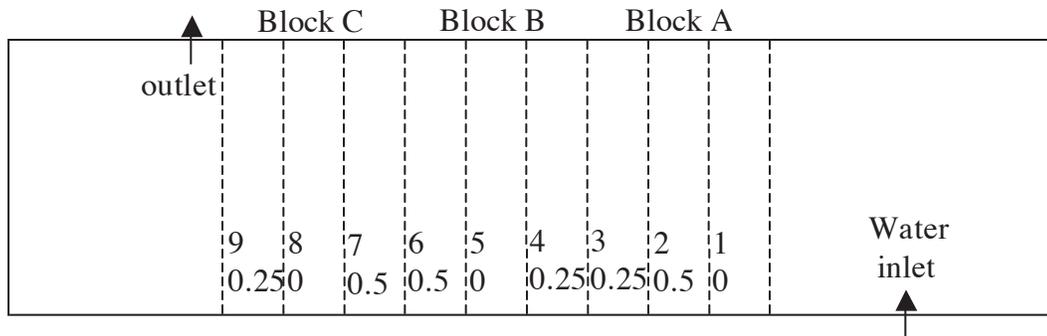


Figure 2. Growth of tilapia, weight in grams for 5 dates, the stocking date (5th Dec.) of the experiment and 4 sampling dates. Light shading represents replicates of the T25 treatments stocked at 0.25 tilapias per m^2 , dark shading represents the T50 treatments stocked at 0.50 tilapias per m^2 . The replicates are arranged from lowest numbered enclosure on the left to highest on the right (block A to block C). All means are presented with standard deviation (n=20).

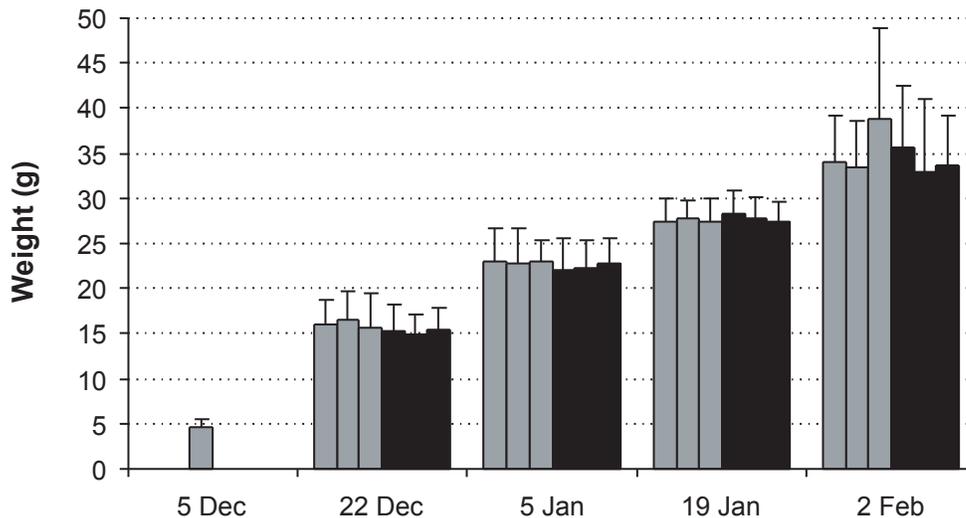


Figure 3. Growth of shrimp, weight in grams for 5 dates, the stocking date (5th Dec.) of the experiment and 4 sampling dates. All treatments were stocked with 30 shrimp per m² and 3 stocking densities of tilapia: No shading represents replicates of the T0 treatment stocked at 0 tilapias per m²; light shading represents replicates of the T25 treatment stocked at 0.25 tilapias per m²; dark shading represents the T50 treatment stocked at 0.50 tilapias per m². The replicates are arranged from the lowest numbered enclosure on the left to highest on the right (block A to block C). All means are presented with standard deviation (n=30).

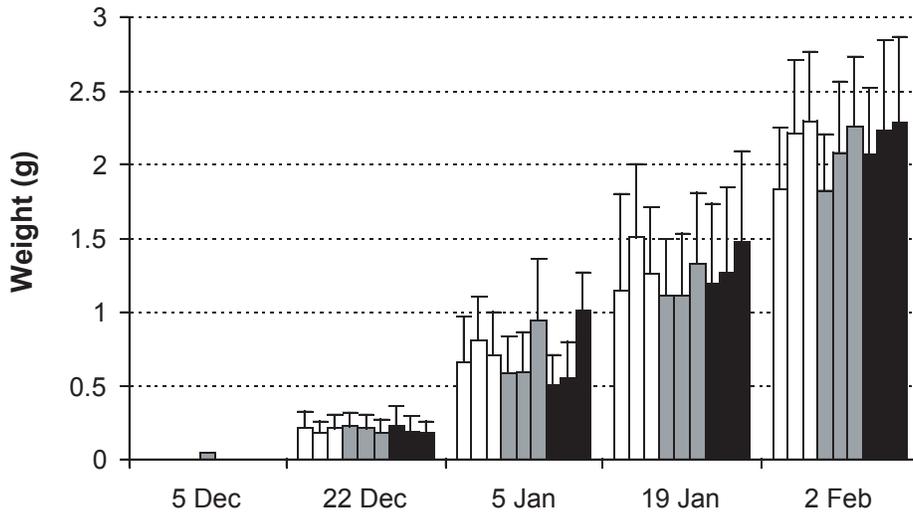


Figure 4. Growth of shrimp, weight in grams for 4 dates for each block, block A spotted, block B diagonal strip and block C horizontal strip. Different letters on a particular sample date indicate significant differences (P<0.05). All means are presented with standard deviation (n=90).

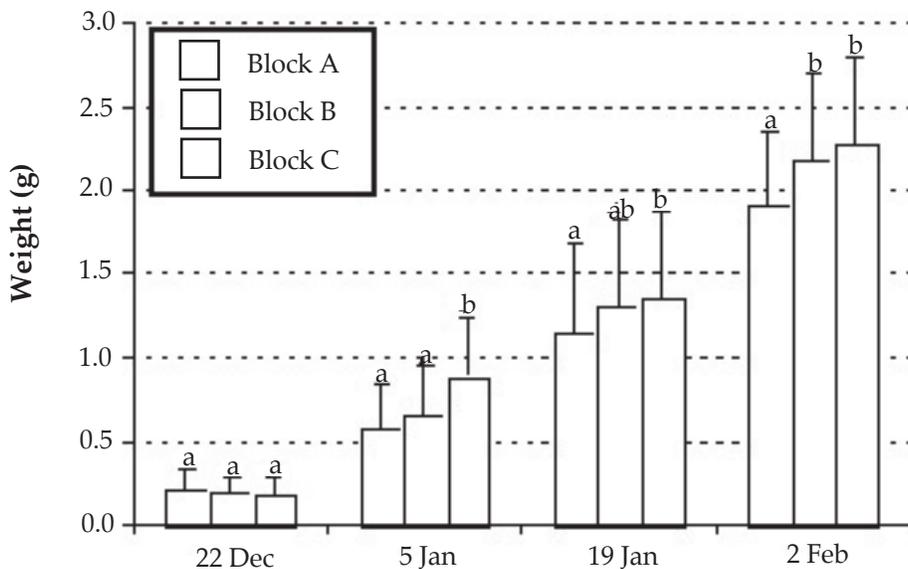


Figure 5. Percentage survival for Shrimp and Tilapia grouped in tilapia treatments, T0, T25 and T50 and in blocks A, B and C. Different letters within blocks or treatments for shrimp and tilapia indicate significant differences ($P < 0.05$). All means are presented with standard deviation ($n=3$).

