

Safe Usage of Antibiotic (Oxytetracycline) in Larval Rearing of Mud Crab, *Scylla serrata* (Forsskål, 1775) in Fiji

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Abstract: The mud crab aquaculture industry has found much success by the use of antibiotics such as oxytetracycline (OTC) to increase yield from larval rearing, but there needs to be a control in its usage to avoid the bacterial resistance to the drug. Therefore this research was carried out to assess the safe usage of oxytetracycline in mud crab larvae rearing process in Fiji. The experiment was done to assess the optimal concentration and duration of the use of oxytetracycline. Four treatments were tested in two runs (10ppm and 25ppm); OTC used from zoea 1 to zoea 2, OTC from zoea 1 to zoea 3, OTC from zoea 1 to zoea 4 and a control (without OTC). Results concluded that the use of OTC made significant differences ($P \leq 0.05$) in the survival rate (%) of larvae in comparison to control. OTC usage till zoea 2 and at 25ppm indicated better growth rate while the extended use of OTC till zoea 4 gave a lower percentage yield of megalopae. The bacteria leading to mass mortality from zoea 1-zoea 2 coincided with rotifer feeding to the larvae, while the slow growth rate from the extended OTC use may have been due to the emergence of antibiotic resistant bacteria which slowed the larval growth.

Key words: Mud Crab Oxytetracycline • Larval Stages • Growth Rate • Fiji

INTRODUCTION

The aquaculture industry is fast developing and intensifying in most regions of the world, having grown at an annual rate of 10 % from 1984 to 1988 compared to 3% for livestock and meat and 1.6 % for capture fisheries production [1]. This makes aquaculture one of the fastest growing food producing sectors [2] and now many Pacific Island Countries, including Fiji have ventured into this industry by farming prawns, tilapia, milk-fish, seaweed, pearls, etc., with increasing production rates [2]. To date the freshwater prawns have been the major commodity that has been farmed in Fiji [2] while mud crabs have recently been introduced as a commodity in aquaculture to Fiji.

Farming of mud crabs (*Scylla serrata*) tends to be a very lucrative business as it fetches a higher price due to its juicy flesh and bigger size [3, 4] as compared to prawns. Currently, mud crab farming in Fiji is still very young and it has not been able to intensify due to constraints in being able to provide crablets (seed-stock) for nurseries and grow-out ponds. Practice has it, that

seed-stock is usually collected from the wild and then supplied to nurseries and grow-out ponds. This practice tends to threaten the wild stock [5] leads to an inconsistency of seed-stock supply and could also be the cause of disease introduction as larvae would not come from a controlled environment [1]; hence the need for hatchery reared crablets to supply seed-stock for mud crab farming.

While many Pacific Island Countries such as Cayman Islands, French Polynesia, Palau and Papua New Guinea have already been successfully farming mud crab, *Scylla serrata*, Fiji has yet to establish a standard protocol for larval rearing to be able to potentially commercialize this product. The major constraint faced is the high mortality in the larval rearing process. This issue has successfully been countered in many countries with the use of antibiotics [6] and also probiotics [1]. The import of probiotics has yet to be permitted into Fiji, whereas the use of antibiotics need to be controlled and minimized as it could bring about antibiotic resistance in bacteria [7]. Antibiotic resistant bacteria become immune to that specific kind of drug thus reversing its effects and the

transmission of drug resistant micro-organism from animals to humans have also been detected through the food chain, that could cause the spreading of these micro-organisms in hospitals and communities [8].

Hence, this research report assessed the minimum concentration and minimum extent of the usage of the antibiotic *Oxytetracycline* to provide optimal larval survival of mud crab *Scylla serrata*. Along with survival, larval health and growth rate were also assessed.

MATERIALS AND METHODS

Maintenance of Berried Crabs: The brood stock was bought from the fish market area in Suva. These crabs were then disinfected with 150ppm of formalin for about 30 minutes before being stocked into tanks. Once a crab had spawned, it was disinfected again with formalin for 30 minutes at the same concentration. Crabs were then transferred into the hatching tanks. Protocol was similar to procedures carried out by Quinito and Parado-Estepa [5] and Shelley and Lovatelli [6]. The hatching tank contained about 500 liters of aerated seawater. Temperature was controlled at 28-30°C using a heater while the salinity was maintained at 28-29 ppt. Hygiene and water quality was optimized through 50-80% water exchange done at a three day interval. Any detached eggs were also siphoned out. Hygiene was maintained throughout the experiment to ensure that infections did not stress the animals [9]. The egg development of each spawned crab was monitored so that only the hatching from a properly developed egg mass was used for each run.

If the hatching was delayed for more than 2 days, it was not used in the experiment.

Collection and Stocking of Zoea: After the zoea had hatched, the aeration was turned off to allow dead eggs to settle. The larvae were then siphoned out by concentrating the healthy larva in one area of the tank. This was done by concentrating light in only that area of the tank. The larvae were siphoned into a smaller tank of about 60 liters, which had a temperature of 29°C and salinity of 29 ppt [10].

Once the larvae were stocked, they were immediately fed with live rotifers at a density of 40 rotifers per milliliter. A high density of rotifers was stocked as the larvae (zoeae 1) were not active predators. Hence increasing density of rotifers increased feeding opportunities [6]. Rotifers were continued to be fed until zoea 2, which was day 3. Microalgae *Nannochloropsis* (algal paste) was

also added, as they were essential in providing fatty acids for mud crab larval development [6]. Artemia was fed from zoeae 3-zoeae 5 at 3 per milliliter. Larger 5-7 day old artemia was fed from zoeae 5- to early megalopae.

Water Management: All the seawater that was used for the experiment was bought from Galoa, Navua. This seawater was filtered with a carbon filter and it was also treated by running it under ultraviolet light. This was done to minimize bacteria content in the water.

Experimental Design: 1.5 liter containers were used to hold one liter of seawater at 29ppt salinity and 29°C water temperature. The containers were stocked with 50 zoeae each. There were four treatments i.e. T1- OTC (oxytetracycline) from zoea1-zoea 2; T2 - OTC from zoea 1-zoea 3; T3 - OTC from zoea 1- zoea 4; T4 - without OTC (control). Each treatment had four replicates and two runs were done. First run was with 10ppm concentration of OTC while the second run was with 25ppm concentration. For each treatment, OTC was applied at 0700 hours each day, then discontinuing usage for T1 from day 3, T2 from day 6 and T3 from day 9. Only for the control, there was no OTC addition. Also, only the healthy and active larval were stocked for the experiment. This was done to withdraw bad egg quality and unhealthy larvae, which could affect the experiment and give inaccurate results.

Data Collection: The following parameters were measured on a daily basis to ensure that the larvae are kept in optimal conditions [11]:

- Temperature, salinity and dissolved oxygen were measured every morning at 0800 hours with an YSI meter.
- Larval counts were done every 4 days; this was done as it normally took 3 days for the zoea stages to change.
- Feed consumption was also monitored daily by residual feed counts. This information was used to maintain adequate diet for the larvae.
- Gross observation of the container to monitor slime and fungi.

RESULTS AND DISCUSSION

Temperature of the experimental units measured at 0800 hours remained between 28.7°C and 29.6°C and salinity ranged from 29 ppt at stocking, up to 30.1 ppt. at the end of the experiment. Dissolved oxygen measured from 5 mg/l to 6.25 mg/l.

There was a highly significant difference ($P = 0.05$) in survival rate (%) between treatments and control. Control had significantly poorer survival rate [5] in comparison to other treatments. However, there were no significant differences in survival rate between any of the treatments receiving OTC ($P > 0.05$). 95% of all the mortality for the treatment with control occurred within the first 3 days (zoea 1- zoea 2) when rotifers were fed. After the molting of the larvae from zoea 1 to zoea 2, the larvae mortality slowed down.

There was a high significant difference in survival rate (%) between treatments with control and treatments with OTC, ($P = 0.05$). While there were no significant difference between the treatments with OTC ($P > 0.05$). It was observed again that majority of the mortality for the control (no OTC) occurred within the first 3 days. Run 2 showed similar results as Run 1, implying same reasons for the low larvae survival from zoea 1-zoea 2.

The growth rate was also determined by counting the number of megalopae on the day of first megalopa sighting (Tables 3 and 4).

For both of the runs (10 ppm and 25 ppm), the megalopa on the 15th day (Table 3 and 4) were counted while for the control, only 1 megalopa was counted. For OTC Treatment 1, it was 7 and 16 for 10 ppm and 25 ppm respectively, for OTC Treatment 2 it was 7 and 18 for 10 ppm and 25 ppm and for the OTC Treatment 3, the count was 5 and 10 for 10 ppm and 25 ppm respectively. The percentage growth remained the same for the control for both runs, while for the OTC treatments the percentage growth increased markedly. For T1 (treatment 1), the percentage growth increased by 128.57% while for T2 and T3, this increased by 157.14% and 100% from Run1 to Run 2.

Results from Run 1 (10 ppm) and Run 2 (25 ppm) indicated similar pattern with respect to larvae survival rate (%). For both the Runs, it was seen that there was a significant difference between T2, T3 and T4 and control. The admission of OTC distinctly increased survival of the zoea, but there was no significant difference in the larvae survival from usage of OTC among T2, T3 and T4. It could be inferred that high mortality in the larval rearing may be primarily associated with bacteria present in the rearing system and these bacteria were highly vulnerable to OTC [12].

From figure 1 and 2, it was observed that there was a high number of mortality from zoea 1 to zoea 2, which is the period when rotifers are fed to the larvae. It was clearly found that once rotifers were stopped from feeding, mortality rate decreased. In control treatment, almost 70% of the larval deaths were from zoea1 to zoea 2 while 25% of mortality between zoea 2 to megalopa.

Table 1: Mean survival (%) (\pm SE) Z1 to megalopa for the various treatments for Run 1 at 10 ppm

Treatment	Control	Z1-Z2	Z1-Z3	Z1-Z4
Final Survival (%)	5.00 \pm 6.16	20.48 \pm 1.09	22.21 \pm 0.66	25 \pm 2.41

Table 2: Mean survival (%) (\pm SE) Z1 to megalopa for the various treatments for Run 2 at 25 ppm

Treatment	Control	Z1-Z2	Z1-Z3	Z1-Z4
Final Survival (%)	7.00 \pm 2.17	45.30 \pm 0.74	44.21 \pm 6.01	53.09 \pm 4.09

Table 3: Number of megalopa harvested on the 15th day since post hatch for Run 1, 10 ppm OTC concentration

Treatments	Control	Z1-Z2	Z1-Z3	Z1-Z4
Number of larvae stocked (mean of replicates)	50	50	50	50
Mean % growth	2	14	14	10

Table 4: Number of megalopa harvested on the 15th day since post hatch for Run 2, 25 ppm OTC concentration

Treatments	Control	Z1-Z2	Z1-Z3	Z1-Z4
Number of larvae stocked (mean of replicates)	50	50	50	50
Mean % growth	2	32	36	20

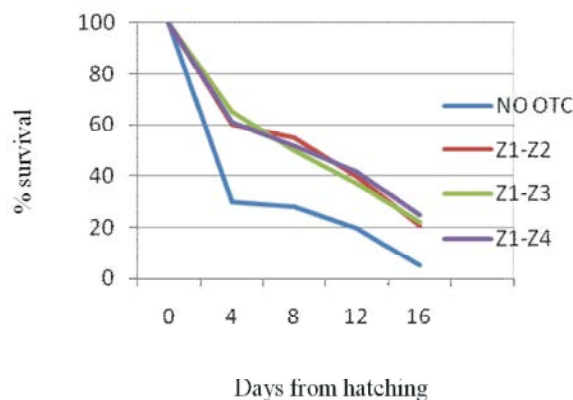


Fig. 1: Survival of larvae from zoea 1 to 1st megalopa.

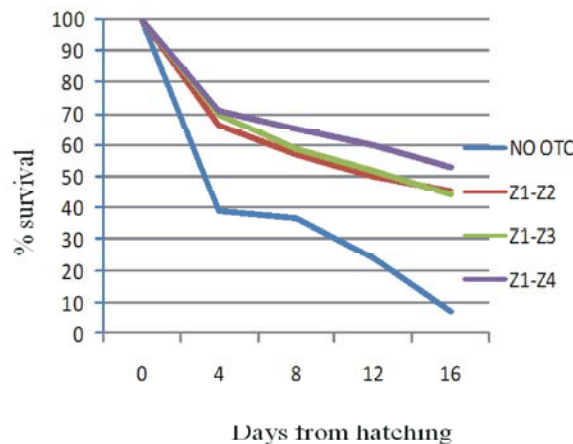


Fig. 2: Survival of larvae from zoeae 1 to 1st megalopa.

These results suggested that bacteria responsible for majority of the deaths were linked to rotifer feeding [7]. Results observed in Table 1 and Table 2 further supported these implications. It was found that there was no significant difference in the survival rate of larvae in T where OTC was only used till zoea 2 in comparison to T3 and T4 where OTC usage was carried on till zoea 3 and zoea 4 respectively. Therefore, the application of OTC could be restricted to zoea 1 and zoea 2 and from zoea 2 or from when rotifer feeding ceased.

The growth rate of the larvae was determined by calculating the percentage of the larvae that had molted into megalopae on the first day of megalopae sighting. For the run at 10 ppm and 25 ppm, the treatment without OTC showed that 2% of the originally stocked zoea turned into megalopae. The percentage growth from 10 ppm to 25 ppm seemed to double for the treatments with OTC. It could also be noted that for both runs, T3 had lesser number of megalopae compared to T1 and T2. It would be expected that since T3 had received OTC for a longer period of time, the percentage growth would also be similar to the survival but this was not the case. Although treatment with OTC had increased the larval survival, it may have slowed down the growth rate and development of larvae. Prolonged exposure of the larvae to OTC would have led to bacteria becoming less susceptible or resistant to the effects of OTC due to oxytetracycline residue accumulation [13].

From assessing the results for larval survival and growth rate, there is indication that the OTC usage could be restricted to zoea 2 and that even at 25 ppm growth rate is optimal when usage is restricted to zoea 2 as seen in T2.

CONCLUSION

At the end of the experiment, the safe usage of antibiotic Oxytetracycline was successfully determined. At both concentration levels of 10 ppm and 25 ppm where the use of OTC was stopped at Zoea 2, good survival and growth rate was achieved. Therefore the use of the antibiotic oxytetracycline should be restricted to zoea 2 and 25 ppm concentration could be preferred as it gave a better growth rate. When the use of OTC continued till zoea 4, the growth rate slowed down. This may be due to the emergence of some antibiotic resistant bacteria. On the other hand, the lower concentration of 10 ppm could be also used. Once the first megalopae were sighted, it should be screened so that there would be no preying on zoea 5 by the molted megalopae. Hence, either of the 2

concentrations could be used to improve larval survival and growth while the duration should not exceed from zoea 2. This particular protocol may be implemented for hatchery operations in Fiji as it minimizes the concentration of antibiotic and at the same time optimizes seed-stock for nursery and grow-out ponds. This protocol therefore could help commercialize mud crab aquaculture in Fiji. Alternately, the use of probiotics could be used instead of antibiotics if allowed to be imported. Since probiotics are micro-organisms, they would not give rise to resistant bacteria which could be extended usage to increase larval survival and growth rate [14, 15].

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