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Effect of different experimental diets and salinity on growth performance of *Penaeus monodon*

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Abstract. This study evaluated the effect of different experimental diets on the growth of *Penaeus monodon* cultured at different salinities. We used four diets in this experiment, such as diet-1 (F_1), diet-2 (F_2), diet-3 (F_3) and diet-4 (F_4). All the diets were fed to *P. monodon* juveniles cultured at salinities of 5 ppt (S_5), 10 ppt (S_{10}), 15 ppt (S_{15}), and 20 ppt (S_{20}) for a 45-day tank trial. In this study, the feed's fishmeal protein was replaced with concentrated protein as an alternative approach to aquaculture sustainability. There was no interaction effect and no main effects of feed and salinity on the growth of *P. monodon*. Feed and salinity had an interaction effect, and salinity had a main effect on the survival of *P. monodon*. Overall, survival was higher in the tanks with higher salinity, such as 15 ppt and 20 ppt. There was also a higher survival at salinity 10 ppt where shrimps were fed with F_1 ($83.33\pm3.33\%$) and F_2 ($86.67\pm8.82\%$). Estimated marginal survival also indicated that survival tends to be raised with increased salinity. There was an environmental fluctuation during this study. Hence, this data might not indicate the general features of growth and survival of *P. monodon*.

Keywords: Salinity, Diets, *Penaeus monodon*

Introduction

The tiger shrimp, *Penaeus monodon* is one of the primary species which are widely cultured in the southern part of Bangladesh (AftabUddin *et al.* 2018). It has high demand to the customers due to its taste, nutritious and economic value, hence popularly known as 'white gold' (Paul and Vogl 2011). About 1.2 million people are directly or indirectly involved in shrimp farming in Bangladesh (Paul and Vogl 2012). Bangladesh ranked the 5th position among the aquaculture producing countries (FAO 2018). The total production of cultured prawns in Bangladesh was 359,887 MT in 2015-2016 (DoF 2017), which increased to 368,882 MT in 2019–20 (DoF 2019). Because of its export potential, high demands, and high profits in European Union (EU), USA, Japan, Russia, China, freshwater prawn farming is now a significant economic activity in rural Bangladesh (Hasan *et al.* 2020).

Dietary supplements are now widely used in shrimp aquaculture to enhance growth, immune response, digestive enzyme activity and nutrient absorption in shrimp. Commercial shrimp feed constitutes 50–60% operational cost of intensive shrimp farming (Wasielesky *et al.* 2006). So, formulation of nutritionally balanced feed with quality dietary supplements is an imperative area of research to meet the nutritional demand and maximize the growth of shrimp. The costs of formulated feed and labor associated with feeding are a major component of cost of cultured shrimp production. It is well established that the nutrient contents of the feed influence growth, survival and the amount of metabolic and excreted waste products entering the system. However, processing of feed also plays a critical role as it influences the stability of the feed and hence the availability of the feed over time (Smith *et al.* 2002).

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Although filter feeding animals like mollusks or herbivorous silver carp and bighead carp do not require feeding (FAO 2013); however, fed aquaculture represents 81 percent of global fish and crustacean aquaculture production. Fed aquaculture contributes about 60 percent of global aquaculture (Tacon and Metian 2013). Although, in developing countries, fewer supplementary feeds and fishmeal are used for lower trophic level species culture, higher trophic level species, like shrimp, salmon, trout are still dependent on fish from the wild for fishmeal and oil in formulated feeds (HLPE 2014). The use of fish from the wild in fishmeal and fish oil to produce fish has been the cause of major public controversy. In 2012, about 10 percent of total catch fish (16 million tons) was reduced to fishmeal and fish oil (Tacon *et al.* 2011).

The aquaculture research community and the aquafeeds industry have long recognized and anticipated issues impacting the sustainability of fishmeal in aquafeeds (Barrows and Hardy 2001) and have been researching and developing aquafeeds by using alternative protein ingredients, particularly plant-derived proteins (Gatlin *et al.* 2007). Blue economy challenge project tried to find an alternative approach of protein source in collaboration with Wageningen University and Research, WorldFish Bangladesh, Vietnam and Khulna University and developed different types of feeds supplemented with concentrated microbial proteins into shrimp feed. Moreover, most of the shrimp culture farms at different coastal districts in Bangladesh have been developed at different salinities. There are a few literatures available on protein supplementation in shrimp feed with microbial proteins and its suitability in application at different salinities, and its performance in growth of *P. monodon*.

Materials and Methods

***Experimental tank set up and salinity adjustment*:** Newly purchased transparent plastic tanks of 30 L water capacity were used for this experiment. The tank water volume was maintained up to 25 L throughout the experimental period. All the tanks under different treatments were distributed randomly in the laboratory. Different salinity levels were adjusted following the formula, $S_1V_1 = S_2V_2$. Brine water of 120 ppt was used to adjust the salinity.

***Collection of juvenile and experimental design*:** Juvenile shrimps were collected from a farmer's nursery pond from Bagerhat District and taken to the wet lab of the Fisheries and Marine Resource Technology discipline. The juveniles were acclimatized in brackish water tanks for three days and transferred to the experimental tanks. The animals were cultured in four different salinity levels (5, 10, 15, and 20 ppt) with four different experimental diets (F_1, F_2, F_3, and F_4) (Table I and II). We also stocked juvenile at 0 ppt, but after 3 days of stocking, all the animals died. The tanks were well set up with continuous aeration and covered with plastic lids to avoid unwanted contamination. Each of the treatments was repeated in three tanks with equal facilities.

Table I. Layout of the experiment: F_1, F_2, F_3, and F_4 indicating four different experimental feeds, and S_5, S_{10}, S_{15}, S_{20} indicating different salinity levels at salinity 5, 10, 15, and 20 ppt

Salinity	F_1	F_2	F_3	F_4
S_5	F_1S_5	F_2S_5	F_3S_5	F_4S_5
S_{10}	F_1S_{10}	F_2S_{10}	F_3S_{10}	F_4S_{10}
S_{15}	F_1S_{15}	F_2S_{15}	F_3S_{15}	F_4S_{15}
S_{20}	F_1S_{20}	F_2S_{20}	F_3S_{20}	F_4S_{20}

Feeding management: During the experiment, the shrimps were fed to satiation twice daily for the entire experiment. The feed ration allocation was determined by feeding to slight excess (110-120% of satiation). Initial feeding rate was 6% of tank biomass daily (initial 1.2g a day).Uneaten feed pellet was counted from each tank in the morning before feeding, with the scoring used to estimate the amount of uneaten feed and used to adjust the afternoon ration (as per the table below). A smaller amount of feed was given to each tank in the morning after counting (feed 0.3g in AM), the remaining individually adjusted ration was offered at 4 pm (Table III). Bulk feed was kept in the freezer except when weighing feed.

Table II. Basic proximate composition of four different diets

Diet	% Moisture	% Crude Lipid	% Crude Protein	% Ash	% Crude Fiber	% Carbohydrate
F1	15.61	7.17	38.09	10.57	4.20	24.36
F2	14.91	6.70	37.00	12.43	5.20	23.76
F3	13.67	6.77	39.70	9.44	4.30	26.12
F4	13.97	7.33	40.46	7.29	5.66	25.29

Table III. Feeding adjustment table

Pellet Count in AM	Adjustment
30 + Pellets	Decrease PM ration by ~0.3g
1 to ~30 Pellets	No adjustment
No pellets	Increase PM ration by ~0.3g

***Sampling and growth* measurement:** The weight of shrimp was measured every seven days interval by collecting all the individuals from all the tanks by using a scoop net. The weight of the shrimp was taken by an electronic weight measuring balance. The growth parameters were calculated by the following equations:

Weight gain (WG) = Final weight (W_t) – Initial weight (W_0)

$$RGR = \frac{Wt - W0}{W0} \times 100$$

Where, RGR, relative growth rate or weight gain (%); W_t, final weight; W_0, initial weight.

$$SGR(\%) = \frac{Ln(Wt) - Ln(W0)}{t} \times 100$$

Where, SGR, specific growth rate (in % Body weight per day); W_t, final weight; W_0, initial weight; t, the duration of the experiment in days.

$$SR(\%) = \frac{Nt}{N0} \times 100$$

Where, SR, survival rate; N_t, number of live prawns harvested at the end of experiment; N_0, number of prawns stocked.

Data analysis: The data obtained during the experiment were calculated using Microsoft Excel 10.0, and all the statistical analysis was done using RStudio. The effects of feed and salinity, and/or their interactions on the growth of *P. monodon* were tested by two-way analysis of variance (ANOVA) using general linear model. Survival was considered as a binomial distribution and survival data was analyzed by a generalized linear model with 'logit' link function. All the graphs/charts were generated by 'ggplot' function.

Results

Growth of P. monodon: The %SGR was found to be maximum in the treatment F_4S_{10} (0.94±0.56), and the minimum (0.22±0.09) in treatment F_1S_{10}. There was no interaction or main effects of the treatments on %SGR. Similarly, effects of feed, salinity, or their interactions were not significant on other growth parameters, such as final weight (W_t), weight gain (WG), and percent relative growth rate (%RGR). The final individual body weight varied from 1.34±0.18 to 2.38±0.54g with the highest value in F_2S_5 and the lowest in F_1S_{10}. In case of individual weight gain, it was higher in F_4S_{10} (0.80±0.59g) and minimum in F_2S_{10} (0.12±0.05g). The relative growth rate was higher in F_4S_{10} compared to other combinations, which was lowest in F_1S_{10} (9.30±3.91%) (Table IV). We experienced a sudden environmental temperature fall due to a weather depression during this study, which might impact on the growth of the *P. monodon*.

Table IV. Growth of *P. monodon* with their statistical parameters

Feed	Salinity	W_t(g)	%SGR	WG (g)	%RGR
F_1	S_5	1.78±0.36	0.73±0.44	0.50±0.33	38.33±24.63
F_2	S_5	2.38±0.54	0.57±0.47	0.55±0.49	30.06±26.00
F_3	S_5	1.80±0.18	0.90±0.31	0.56±0.21	45.74±18.55
F_4	S_5	1.83±0.52	0.84±0.33	0.59±0.33	42.52±18.82
F_1	S_{10}	1.34±0.18	0.22±0.09	0.12±0.05	9.30±3.91
F_2	S_{10}	1.61±0.13	0.33±0.13	0.21±0.09	14.38±5.87
F_3	S_{10}	1.55±0.06	0.36±0.04	0.21±0.03	15.37±1.84
F_4	S_{10}	2.17±0.72	0.94±0.56	0.80±0.59	53.35±34.57
F_1	S_{15}	1.56±0.10	0.66±0.06	0.36±0.04	30.37±3.20
F_2	S_{15}	1.81±0.10	0.85±0.06	0.52±0.02	40.69±3.61
F_3	S_{15}	1.81±0.11	0.50±0.14	0.32±0.08	22.78±6.91
F_4	S_{15}	1.35±0.22	0.65±0.36	0.33±0.20	32.33±20.24
F_1	S_{20}	1.42±0.20	0.65±0.20	0.30±0.07	30.29±10.40
F_2	S_{20}	1.94±0.20	0.56±0.20	0.37±0.12	26.15±9.98
F_3	S_{20}	1.88±0.40	0.62±0.36	0.47±0.32	31.19±19.52
F_4	S_{20}	1.44±0.06	0.46±0.10	0.24±0.04	20.36±4.92
Interaction effect, Feed:Salinity					
$F_{9,32}$		0.735	0.537	0.513	0.556
P value		0.675	0.836	0.854	0.822
Effect of Feed					
$F_{3,41}$		1.191	0.280	0.331	0.359
P value		0.325	0.840	0.803	0.783
Effect of salinity					
$F_{3,41}$		0.890	0.868	0.705	0.781
P value		0.454	0.466	0.555	0.511

Survival of P. monodon: We found an interaction effect (LRT, $G_9 = 19.76$, $P = 0.019$), and salinity as the main effect (LRT, $G_3 = 53.50$, $p < 0.001$) on the survival of *P. monodon*. Feed did not have any impact on the survival of shrimp (LRT, $G_3 = 4.73$, $P = 0.193$). Overall, higher survival was recorded in the treatments with salinity 15 ppt and 20 ppt. Survival rate varied from 80.00±11.55 to 96.67±3.33% in the treatments which had all the feeds and salinity 15 ppt. Similarly, it was 83.33±16.67 to 96.67±3.33% in all treatments with salinity 20 ppt. In addition, above 80% survival was also recorded from the S_{10} treatments with diets F_1 (83.33±3.33%) and F_2 (86.67±8.82%). Osmotic stress might be the reason for higher mortality in the treatments with salinity 5 ppt (50.00±15.28 to 66.67±8.82%), and two other feed treatments (F_3 (53.33±17.64%) and F_4 (60.00±10.00%)) with salinity 10 ppt (Fig. 1). Highest survival (96.67±3.33%) was found in F_1S_{15}, F_1S_{20} and F_3S_{20}, and the lowest was in F_1S_5 (50.00±15.28%).

Estimated survival interaction revealed that salinity level above 15 ppt increase the survival rate. It stayed stable at 20 ppt which may start to decline after reaching a certain salinity level. On the other hand, higher protein level in the diet F_3 and F_4 might cause an impact on the water quality of culture tanks, which may cause higher mortality at salinity 10 ppt (Fig. 4).

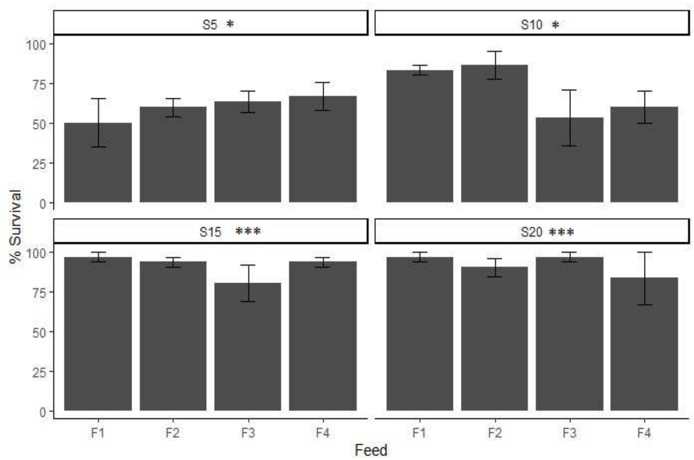

Fig. 1. Survival rate of *P. monodon* treated with different diets and salinity. Error bar represents the standard error of three replicates. Asterisk indicates significant differences between the treatments (Generalized linear model with 'logit' link function, $p < 0.05$).

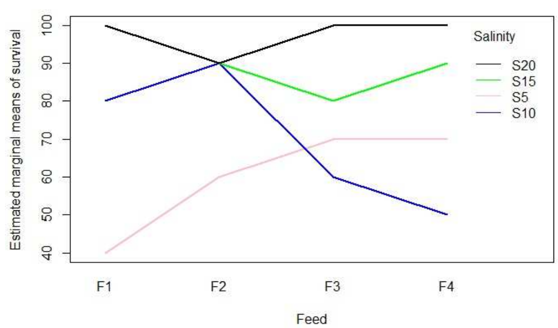

Fig. 2. Estimated survival of *P. monodon* under different feed and salinity treatments.

Discussion

Most penaeid shrimps are euryhaline growing in a wide range of salinities. With the salinity fluctuations, *P. monodon* exhibits different osmotic responses. They perform hyper-osmotic ionic regulation at lower level of salinity and hypo-osmotic at higher levels (Cheng and Liao 1986). However, the low survival rate at 5 ppt found in this study, which was similar to the result obtained with post larvae by Cawthorne *et al.* (1983) (Fig. 1). On the other hand, we experienced a complete mortality of juveniles at 0 ppt in this study. Hence, lower salinity is stressful for tiger shrimps and their adaptability is relatively weak when salinity goes down (Chen *et al.* 2016).

Abnormal molting may also increase the opportunities of cannibalism due to weakness during molting, which may be a risk for higher mortality. We scored an enhanced molting at lower salinity, which finally disappeared from the cultured tank and recorded as dead. That might be one of the reasons of higher mortality in the treatments with lower salinity. In fact, similar observations have been reported in other crustaceans (Allan and Maguire 1992, Romano and Zeng 2006, Vijayan and Diwan 1995). Lower salinity levels might also impact on the psychological responses of shrimps, such as increasing or decreasing the dissolve oxygen consumption. In addition to salinity stress, higher mortality might be happened due to lower adaptability of shrimp with newly formulated feeds. The other major causes might be the environmental fluctuations and seasonal transition during study period. On the other hand, at higher salinities ($\geq$15 ppt), they may have optimum ionic balance with strong physiological support. Thus, they could cope with environmental and/or feed associated stresses and resulting a higher survival (>80%) in this study. Complete survival of *P. monodon* was also reported at 15°C in a previous study (Chen *et al.* 2016).

In this study, the growth data was not found statistically significant. There were considerable differences in the growth parameters between the treatments. However, it was insignificant, which might be due to the higher standard deviations which overlapped each other. Although not significant, we observed higher %SGR in treatments with high protein enriched feed and lower salinity (F_3S_5, F_4S_5, F_3S_{10}, F_4S_{10}), which then reduced at same diets with higher salinities (F_3S_{15}, F_4S_{15}, F_3S_{20}, F_4S_{20}) (Table 4). This might be happened due to the chance of the animals survived in low saline treatments, where they had fewer living animals, less competition, and higher change to get feeds. Hence, they had higher possibility to increase their growth. Variation of %SGR in different tanks might be varied due to variations in salinities which might influence the respiratory metabolism of *Penaeus monodon* (Ye *et al.* 2009). Similarly, individual weight gain was higher in the treatment with salinity S_5, except F_4S_{10}; which then felt rapidly in S_{10}, and slowly increasing to S_{15} and S_{20}. Highest weight gain in F_4S_{10}, might be due to a smaller number of shrimps alive in this treatment, and they got feed with highest protein level (F_4) (Fig. 1, Table 1). Similarly, higher weight gain was found in *P. vannamei* at lower salinity (<15 ppt) compared to higher salinity ($\geq$15 ppt) (Bray *et al.* 1994). DGR and %RGR followed the similar trend like %SGR and weight gain.

This study indicates that salinity has an immediate and significant effect on survival, and growth of *P. monodon*. A previous study recommended a salinity range between 20-30 ppt for juvenile culture of *P. monodon* (Ye *et al.* 2009). The study also suggests that larvae and juveniles should pass a weaning or adaptability stage before applying a newly formulated feed in shrimp culture. Hence, these experimental diets might be effective in shrimp culture when a comprehensive study will go through closer observation.

In this study, we identified that either experimental diets or salinity levels, and their interactions have no effect on the growth of *P. monodon*. We further identified that interaction of feed and salinity, and salinity itself controlled the survival of tiger shrimp. Hence, it might not be a general feature of *P. monodon* growth, and further study will be required to make this feed commercialize for shrimp farming.

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