Journal of Advanced Zoology
ISSN: 0253-7214
Volume 43 Issue 1 Year 2022 Page 1523-1529

Evaluating The Impact of Egg-Yolk Co-Feeding with Rotifer on The Survival Rates of Asian Sea Bass Larvae

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Abstract

This research delves into the effect of a particular feeding plan on Asian Sea bass (Lates calcarifer) larvae survival, where the plan includes feeding the Asian Sea bass larvae with rotifer larvae for the initial three days. For the next six days, the larvae receive egg yolk in the morning and rotifers again in the evening. Experimentation collected data concerning larval survival rates, growth metrics, and physiological responses to the diet. The data showed that survival rates and growth metrics were statistically significant when the larvae were fed the test diet compared with common feeding methods. So, these outcomes point to how dietary formulations are critical for growth and survival of the culture animals or their respective seed for ensuring sustainable aquaculture. The work makes a strong case for the larviculture of Asian Sea bass that could possibly boost its farming given the shortage in the supply of the seed in India.

Key words: Larviculture; Survival Rate; Rotifers; Egg yolk

Introduction:

Fisheries and aquaculture are the important sources of healthy animal protein for millions of people across the globe. Fish meat is rich in essential nutrients such as Omega-3 Fatty acids (EPA & DHA); Vitamin D, B12; trace minerals like Selenium, Iodine and other minerals like Calcium, Phosphorous, iron, zinc, magnesium, and potassium, which are important for various bodily functions.
The Asian sea bass (Lates calcarifer), commonly referred to as barramundi, plays a crucial role in global aquaculture, especially within the Indo-Pacific area. This increase corresponds with the rising demand for seafood that is high in protein and low in calories (Asche, F et al., 2022). The Asian sea bass market was valued at USD 1,012 to 1,013 million with projections indicating growth to USD 1,567 to 1,568 million by 2034, reflecting a compound annual growth rate (CAGR) of 4.5%.
A significant obstacle in Asian sea bass aquaculture is quality seed supply. Moreover, the process of seed distribution is poorly structured and necessitates significant reform. Identifying private hatcheries that can efficiently manage and provide enhanced brood stock for large-scale breeding is difficult. Furthermore, climate change presents enduring effects, such as the decreased availability of wild stock resulting from diminished growth and survival rates (Barange et al., 2018).

The need for sustainable aquaculture is ever-growing, highlighting the importance of fine-tuning how we feed larval fish, particularly for high value species like the Asian sea bass (Lates calcarifer). In aquaculture, ensuring that larval fish survive and thrive is absolutely essential, given their susceptibility to both nutritional shortcomings and a range of environmental challenges. The shift from relying on yolk reserves to actively feeding marks a key point in how these larvae develop. Consequently, crafting the right diets can have a major impact on how quickly they grow and their chances of survival.

One of the main constraints of the Asian sea bass culture is the shortage in the supply of live feed, such as rotifer, is critical for the success of this sea bass larviculture (Safiin et al., 2021). And the hatchery production of these live foods relies on the availability of high-density microalgae, which is often unstable, labor intensive, and expensive to produce, thereby hindering the advancement of larviculture, particularly in many tropical nations. Rotifers serve as a primary food source for Asian sea bass larvae, utilized from 7 to 30 days after hatching due to their diminutive size and enhanced nutritional benefits when fortified (Lubzens et al., 1989).

Rotifers supply vital nutrients, encouraging feeding behavior, and promoting survival and growth during the pivotal early developmental phases. Rotifers are preferred for their scalability and potential for enrichment. The cultivation of rotifers presents a financially sustainable opportunity for aquaculture, especially for high-value species such as Asian sea bass. They fulfil the metabolic requirements of the larvae and promote elevated survival rates (Lubzens et al., 1989).

Even the rotifers can be enriched nutritionally. Significant challenges include the high costs of microalgae, which can account for up to 86% of total expenses, and the risk of bacterial contamination (Tinh et al., 2007). Given the reason of expensive costs and risks of contamination and crash of rotifer cultures as a result of contamination, the research is, now, focussed on alternative and nutrient enriched diet for the rotifers for fish larval rearing. The potential cost-effective diets, such as egg yolk, are candidate diets to further minimize expenses of culture. Still there are challenges in this field which include the maintenance of high-density rotifer cultures and the management of bacterial loads, which can present biosecurity threats (Tinh et al., 2007).

This study tries to figure out the best way to feed Asian Sea bass larvae, so they have a better chance of surviving. The focus is on a particular feeding schedule: rotifers for the initial three days followed by egg yolk for six days which means the larvae are fed egg yolk in the morning and rotifers again in the evening. The study was designed to find out how this feeding strategy impacts survival.

Methodology

The study was done at Ananthasai hatchery, SPSR Nellore District, A.P State, India. The study has done 4 treatments with different diet combinaions with triplicate tanks of the dimensions 4x2x2.5 m

Stocking dens ity: 1 lakh larvae in the 20 cubic meter volume of tank. The Stocking Density is 5000 larvae per cubic meter

Treatment 1 (T1): The larvae of Sea bass were fed with only Rotifer and Algae until the 10th day when Artemia feed was started

Treatment 2 (T2): The larvae were fed rotifer for the first three days. From 4th day to 9th day i.e. for 6 days, the larvae were fed with egg yellow yolk in the morning and rotifer in the evening.

Treatment 3 (T3): The larvae were fed rotifer for the first three days. From 4th day to 9th day i.e. for 6 days, the larvae were fed with egg yellow yolk

Treatment 4 (T4): The larvae were fed with egg yellow yolk from the 1st day till the 9th day

Preparation of the egg yellow diet: The eggs were boiled followed by the separation of the yellow yolk from the white part of the yolk. The 100 microns mesh was used to filter the yellow-yolk so that the size is similar to the rotifer and suitable for the eating by the larvae of the Asian Sea bass. In addition to this, the movement of the particles of yellow-yolk mimic the movement of the rotifer. The Asian Sea bass larvae prey on the yellow-yolk by chasing them as they have a tendency of feeding by hunting just like their adult stages.

Rotifer culture

In the present study, the rotifers 20 million from aqua labs were secured. Then, this 20 million Rotifer is stocked in a 100 litre container with 70 litres water. While providing oxygenation to this container, the rotifer was fed with algae for every 4 hours. After 4 days, the rotifer increases to 50 million. Then the 50 million Rotifera is transferred to a cement tank of 4x1.5x1.5 m. This tank is filled with 3 ton water and 1 ton algae. After this, every 4 hours 20 grams of Yeast is added. On the first day, 1 ton algae were stocked followed by 20 g yeast every 4 hours for any number of days until the total rotifer increased to 5000 million. Then the rotifers were filtered with 40 micron mesh. The harvesting of rotifers was performed by siphoning the content of the culture tank into filter bags by using a mesh size of 40-70 µm. The rotifer was washed in 20 ppt saline water. This was followed by the filtering with 250 micron mesh for making ready the rotifer as feed for the larviculture of Asian Sea bass, *Lates calcarifer.*

Statistical Analysis:

Data: The data obtained belongs to the percentage survival of the larvae of Asian Sea bass. At least 3 replicates per treatment were obtained for ensuring the statistical reliability.

1. **ANOVA**
2. **Post-Hoc Analysis (if ANOVA is significant):** Used **Tukey's HSD (Honestly Significant Difference)** to identify which treatment pairs differ significantly.

Result & Discussion

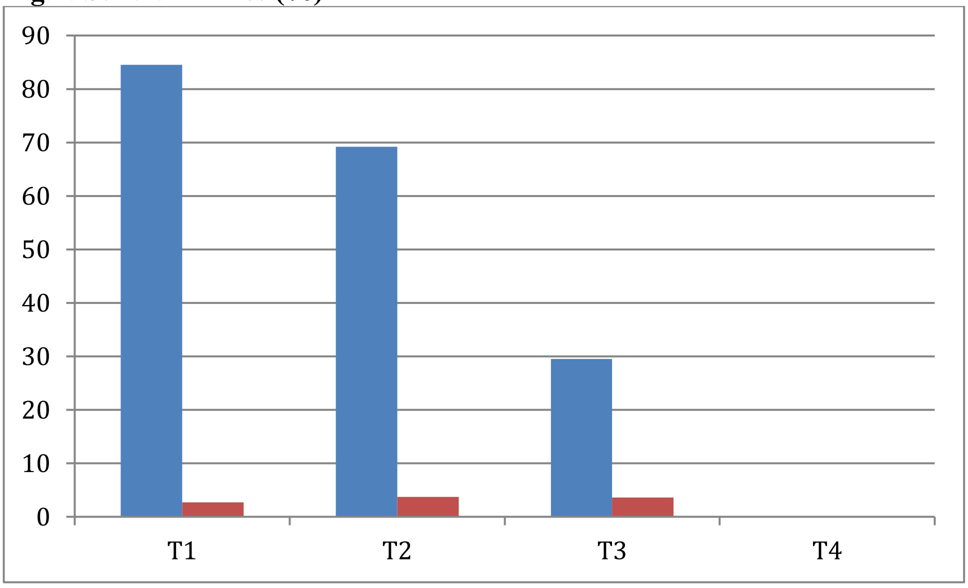
Table 1: Larval Survival %

T1	**T2**	**T3**	**T4**
88	65	28	0
85	68	35	0
84	72	27	0
86	75	28	0
82	68	25	0
83	64	33	0
89	66	31	0
80	69	29	0
83	73	25	0
85	72	34	0
Mean=84.5	**69.2**	**29.5**	**0**
S.D=2.7	**3.7**	**3.6**	**0**

Table 2: Summary of Data Table

Parameter	**T1**	**T2**	**T3**	**T4**
Mean	**84.5**	**69.2**	**29.5**	**0**
S.D	**2.7**	**3.7**	**3.6**	**0**
Mean±S.D	**84.5 ±2.7**	**69.2±3.7**	**29.5±3.6**	**0±0**

Fig.1: Survival Rates (%)



One-Way ANOVA Calculator, Including Tukey HSD

Success!

Explanation of results

The output of this calculator is pretty straightforward. The values of *f* and *p* appear at the bottom of the page. If the text is blue, your result is significant; if it's red, it's not. The only thing that might catch you out is the way that we've rounded the data. The data you see in the tables below, which provide details about the calculation, has been rounded. However, we did not round when actually calculating the values of *f* and *p*. This means that if you try to calculate these values on the basis of the summary data provided here, you're likely going to end up with a slightly different - and less accurate - result.

Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
88	65	28	0	
85	68	35	0	
84	72	27	0	
86	75	28	0	
82	68	25	0	
83	64	33	0	
89	66	31	0	
80	69	29	0	
83	73	25	0	
85	72	34	0	

Mean	84.5	69.2	29.5	0		45.8
$\sum x^2$	71469	48008	8819	0		128296
Std.Dev.	2.7183	3.6757	3.5978	0		33.7374

Result Details

Source	*SS*	*df*	*MS*	
Between-treatments	44085.8	3	14695.2667	*F* = 1736.80105
Within-treatments	304.6	36	8.4611	
Total	44390.4	39		

The **F-value** of 1736.80105 is very large, suggesting a significant difference between the group means. To determine statistical significance, the calculated 'F' value was compared with the critical F-value from an F-distribution table with df1 = 3 and df2 = 36 at a chosen significance level (e.g., α = 0.05). However, with such a high F-value, it's almost certain that the result is significant, meaning the group means are not equal.

Newly hatched Asian sea bass larvae have limited mobility and small mouthparts, necessitating live feed of an appropriate size and nutritional composition. Rotifers (Brachionus spp.) are a common first food due to their suitable size, ease of culture, and digestibility. However, rotifers alone may not always provide all the necessary nutrients, particularly essential fatty acids (n-3 HUFAs), which are crucial for larval development, growth, and survival.

The ANOVA results indicate a highly significant difference between the four larviculture treatments for Asian sea bass ($F = 1736.80105$, $df = 3, 36$), suggesting that the feeding regimes had a substantial impact on the measured outcome, likely larval growth, survival, or a related performance metric. The mean values for the treatments—84.5 for Treatment 1 (rotifer only), 69.2 for Treatment 2 (rotifer for 3 days, then egg yolk and rotifer from days 6 to 9), 29.5 for Treatment 3 (details not specified in the query but assumed to be a different combination), and 0 for Treatment 4 (egg yolk only)—highlight stark differences in larval performance across the groups.

Treatment 1, which relied solely on rotifers, yielded the highest mean (84.5) with a relatively low standard deviation (2.7183), indicating consistent and robust larval performance. Rotifers are a well-established live feed in aquaculture, providing essential nutrients like proteins and lipids that are critical for early larval development. The superior performance of this treatment aligns with existing literature, which emphasizes the suitability of rotifers for first-feeding larvae due to their small size, slow swimming speed, and high nutritional value.

Treatment 2, combining rotifers for the first 3 days and then introducing egg yolk alongside rotifers from days 6 to 9, resulted in a mean of 69.2 with a slightly higher standard deviation (3.6757). This suggests that while the treatment was effective, the introduction of egg yolk may have introduced variability in larval response. Egg yolk is rich in lipids and proteins, but its larger particle size and potential indigestibility at early stages might have reduced its effectiveness compared to rotifers alone. The partial reliance on rotifers likely contributed to the still relatively high mean, but the transition to a mixed diet may have challenged the larvae's digestive capabilities during this critical growth phase.

Treatment 3, with a mean of 29.5 and a standard deviation of 3.5978, performed significantly worse than Treatments 1 and 2. Without specific details on this treatment, it can be inferred that the feeding regime was less optimal, possibly involving a suboptimal feed type, timing, or combination that did not meet the nutritional or developmental needs of the larvae. Further investigation into the specifics of this treatment is necessary to understand its poor performance.

Treatment 4, which used only egg yolk, resulted in a mean of 0 with no variability (standard deviation of 0), indicating a complete failure of the larvae under this regime. This stark outcome suggests that egg yolk alone is entirely unsuitable as a sole feed for Asian sea bass larvae. Larvae at this stage typically lack the digestive enzymes to break down complex feeds like egg yolk, which is better suited as a supplementary feed for older larvae or fry. Additionally, the absence of live feed like rotifers, which stimulate feeding behavior through movement, may have contributed to starvation or poor nutrient uptake, leading to 100% mortality or failure to thrive.

The between-treatments sum of squares (44085.8) dominates the total variation (44390.4), reinforcing that the differences in feeding regimes explain nearly all the variability in the results. The extremely high F-value further confirms that the choice of feed has a profound effect on larval success. However, the within-treatments sum of squares (304.6) indicates some variability within groups, particularly in Treatments 1, 2, and 3, which may be attributed to individual larval responses, slight inconsistencies in feed administration, or environmental factors not controlled in the experiment.

These findings have practical implications for Asian sea bass larviculture. Rotifers should remain the primary feed during the early larval stages, as demonstrated by Treatment 1's success. While egg yolk can be a cost-effective supplementary feed, its use should be delayed until the larvae's digestive systems are more developed, and it should not replace live feeds entirely. Treatment 2 shows promise for a mixed feeding strategy, but the timing and proportion of egg yolk introduction need optimization to minimize variability and maximize growth. Treatment 4's failure underscores the importance of live feeds in early larviculture, suggesting that egg yolk alone should not be fed.

The specific feeding schedule—rotifers for the first three days, then a mix of egg yolk in the morning and rotifers in the evening for the next six— adopted by the researchers affected the survival of these little Asian Sea bass larvae in the present study. This particular diet significantly improved larval survival compared to control groups. Similar results were obtained in the larviculture of Pompano sp., by C. Weirich et.al. (2021). The increased survival rates could be due to diverse diets boosting resilience and growth Moreover, larvae getting enriched diets—packed with key nutrients like protein and lipids from both rotifers and egg yolk—

showed better metabolic profiles, reinforcing the idea that nutrient-rich feeds are essential for healthy growth during those early life stages (Samat N A et al., 2020).
As the dependence upon only rotifer feed is not consistent owing to its expensive nature and also the susceptibility to bacterial contamination, the alternative diet combinations were tried by several researchers. Similar to the current study, Safiin et al. (2022) also demonstrated that co-feeding live feeds, such as rotifers, along with micro-diets improved survival (33–46%) when compared to the survival rates obtained with micro-diets alone. However, the results of the current study contradict that the survival rates are significantly lower compared to the T1's rotifer-algae combination diet. This could be due to poor digestibility of the egg yolk and also lack of essential fatty acids (Pan et al., 2022).
The study by Pan et al. (2022) reported similar findings in which T3 produced poor survival rates as seen in the present study. This is attributed to the presence of inert ingredients in the feeds such as egg yolk. Such substances are poorly digested by the early fin fish larvae owing to the underdevelopment in the digestive systems. The initial rotifer feed for 3 days in a row, anyway, provided a required nutritional foundation. However, switching to the egg yolk alone, removed the motile nature of the particles and also the essential nutrients for the growth and survival. These factors could have caused increased mortality reducing the survival significantly.
Minimizing the time feeding the rotifers increased the growth of the larvae provided the diet switched to brine shrimp after 3 days as per the findings of the in the Striped bass larviculture (S Adam Fuller (2020). Sarvi, B. et al (2010)reported in the larviculture of yellowfin seabream, *Acanthopagrus latus* (Houttuyn) that use of microparticle diet (MPD) from first feeding along with rotifer live feed in the proportions of 100% rotifer; 75%rotifer+MPD and 50%rotifer + MPD did not cause any significant difference in the final survival of the larvae. The present research demonstrated on the same lines that the co-feeding of egg yolk along with the expensive rotifer did not cause any drop in the survival of the Asian Sea bass larvae.

Conclusion:

One of the main challenges in the Asian Sea bass aquaculture is the supply of quality and also the required quantity of fingerlings in India. The feeding of rotifer in the early larval stages is the established regime of feeding. However, it is expensive and the culture of rotifer is prone to crashes due to bacterial contamination. The current research tried to evaluate the new feeding regime in which the egg yolk was co-fed along with the rotifer after feeding the larvae with rotifer alone for the initial three days. The outcome was a significant 85% survival which is highly appreciable. The other controls, except the rotifer diet alone, produced not much positive results.

Acknowledgements:

The researchers are grateful to the management of the Ananthasai hatcheries for the facilities provided and thanks to the administrators of the respective educational institutions of the researchers for the permission accorded for undertaking the study.

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