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Original Article

Replacing *Artemia salina* with bigeye scad (*Selar crumenophthalmus*) roe for larval rearing of giant freshwater prawn (*Macrobrachium rosenbergii*, De Man 1879)

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Abstract: Bigeye scad, *Selar crumenophthalmus*, roe was studied as an alternative to costly Artemia during larval rearing (20 days) of *Macrobrachium rosenbergii*, followed by nursing (35 days). Four treatments with Artemia replacement at 0, 50, 75, and 100% were compared in 12 round-shaped plastic buckets (30 L), each stocking 2,400 larvae. All the larvae fed with 100% fish roe died after one week. Average survival of larvae fed with 0, 50, and 75% fish roe was 38.5±6.7, 36.5±1.6, and 33.6±0.9%, respectively, which were not significantly different. However, specific growth rate and gains in weight and length were similar in the group fed 50% fish roe compared with the control (0%). Therefore, a 50% replacement is recommended, although polynomial regression suggests that a 30.4% replacement might result in the highest survival rate (46.2%). Further research should be conducted using varying levels of replacement, ranging from 0 to 80%, to determine the economically optimal replacement level more precisely.

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Introduction

The giant freshwater or river prawn (*Macrobrachium rosenbergii*) has been introduced to many countries for aquaculture due to success in hatchery technology. Its culture has been thriving and is expanding in many countries, with total production of approximately 0.6 million metric tons in 2022, representing about 4.7% of the total crustacean catch (FAO, 2024). More than 50% of global production comes from China, followed by Thailand, Vietnam, India, and Bangladesh, which collectively account for a value exceeding US$2 billion (Keawthong et al., 2023). Over 98% of production occurs in Asia. The giant freshwater prawn is highly popular in Asia, and demand in both domestic and international markets is high (New et al., 2010; Farook et al., 2019).

In Thailand, among freshwater aquaculture species, the giant freshwater prawn ranks second in value after tilapia. Its production increased from 22.4 thousand metric tons in 2017 to over 41.9 thousand metric tons in 2020 (AST, 2022). Recently, demand for prawn larvae (hatched but still with an underdeveloped body) or post-larvae (well-developed body parts with fully formed appendages) has increased rapidly, whereas supply remains limited. Unpredictably low and variable survival (5-50%) during larval and post-larval rearing is critical (Tansakul 1983; Miglio et al., 2021; Wei et al., 2021). It is mainly due to cannibalism and a lack of quality and the right food. At the initial larval stages, sensory systems are not yet developed. They capture food through random encounters and non-selectively (New et al., 2010). During the first feeding period, larvae prefer live or freely moving food in the water column, which increases the likelihood of more encounters. Prawn larvae require exogenous food immediately, one or two days after hatching (Jones and Holland, 1997; Lavens and Sorgeloos, 2000). However, their digestive systems and enzymes are not well-developed. The simplest food, i.e., egg custard, was suggested; however, survival of larvae was still very low, e.g., 15-20% (Ali, 2005; New et al., 2010), or even nil in some cases. The custard egg alone is

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insufficient, particularly after 10 days (Ali, 2005), as it releases nutrients into the environment and pollutes the water. As a result, water quality deteriorates, especially with high ammonia and nitrite, which can be lethal to prawn larvae (Dong et al., 2020; Brazão et al., 2022).

Artemia salina is commonly used as an alternative food source due to its high protein content (Nik-Sin and Shapawi, 2016). The hatcheries feed prawn larvae with newly hatched *Artemia* nauplii, typically prepared fresh (Kutty and Valenti, 2010). To date, it remains the primary food for the commercial rearing of giant freshwater prawn larvae, supporting relatively high survival and growth (New et al., 2010). For the commercial culture of *M. rosenbergii,* an optimal *Artemia* density of approximately five nauplii mL^{-1} day^{-1} has been recommended (New et al., 2010; Aviz et al., 2018). However, Artemia nauplii intake varies with larval developmental stage and nauplii density and must be adjusted throughout the larval cycle. Coelho-Filho et al. (2018) reported that prawn larvae need more live food in the initial developmental stages. At each stage, low nauplii density may result in reduced growth and increased cannibalism (Barros and Valenti 2003). On the other hand, at high density (>20/mL), excess nauplii may generate waste and increase the concentration of toxic nitrogenous compounds, especially ammonia and nitrite (Barros and Valenti, 2003; Brazão et al., 2022).

Artemia has been the predominant live food so far, but it is mainly imported and costly. Some researchers have also argued that *Artemia* alone is insufficient and that supplementation is necessary (Barros and Valenti, 2003). Therefore, many hatchery and nursery operators are seeking alternative live food and/or supplementary diets. Rotifers (*Brachionus plicatilis*) were initially tested but proved ineffective (Emmerson, 1984; Lovett and Felder, 1988). The prawn larvae that were fed a diet consisting of 100% rotifers perished on day 14 (Thomaz et al., 2004). Murthy et al. (2012) found that the maximum level of *Artemia* that rotifers can replace is 30%, and a 50% replacement significantly lowers survival. They also found that higher levels of rotifers could not support prawn larval growth, likely because rotifers have a lower protein content (29.2%) than Artemia (54%; Jeeja et al., 2011).

The other options could be small and light pelagic fish eggs or fish roe, which are highly nutritious (Mouritsen, 2023). Those fish eggs are soft and light, and they can float, move freely, or remain suspended in the water column. If not overfed, fish eggs do not pollute the water, as their nutrients are encapsulated in cell walls, similar to those of live food organisms. Eggs from marine species (e.g., tuna, king mackerel, mullet fish, bigeye scad fish, seabass, etc.) are available in large volumes as byproducts of fish processing factories. Usually, they are cheap and abundant. For the feeding of larvae, the size of eggs matters, as their size needs to be smaller than the size of the mouth of the larvae. One potential source of fish roe is the bigeye scad (Selar crumenophthalmus), a member of the family Carangidae. It is a coastal pelagic fish commonly found in the Indo-Pacific, including the Andaman Sea and the Gulf of Thailand. Thai fishermen catch them using purse seines, luring nets, and sonar-equipped purse seines. When preparing dried and salted fish, they typically discard the eggs and entrails. Therefore, if their roe/eggs could be used as food for prawn larvae, fishermen could also have additional income. This fish breeds throughout the year, with two peaks occurring in March-June and October-November each year. Therefore, it could be an alternative food source for prawn larvae due to its high nutritional value, suitable size, buoyancy in the water column, and low price. However, no research has been done to utilize fish roe as a potential feed for prawn larvae. Therefore, the present study was carried out to see the effects of fish roe inclusions on growth and survival during larval as well as post-larval stages, and to determine the optimum level (percent) of the bigeye scad roe to replace *Artemia*.

Materials and Methods

Study site and prawn larvae procurement: The larval stage experiment (Day 1-20) was conducted at a commercial prawn hatchery located in Chachoengsao Province, Central Thailand. The newly

hatched prawn larvae were obtained from the company's brood farm and packed in plastic bags (approx. 4,000/bag) with water and oxygen. Upon arrival, the larvae were counted and transferred to culture tanks (2,400 larvae/tank) for a 20-day trial at the farm. On the 20th day, the post-larvae were counted and weighed. On the 21st day, all surviving post-larvae were transferred to the university facility and packed in plastic bags containing 1 L of water each from the trial buckets to assess effects on growth and survival during the post-larval stage (Days 21-56).

Fish roe collection and preparation: Bigeye scad eggs were purchased from the fisheries village of Trang Province. They were in 0.5-kg or 1.0-kg packets. The price was 180-200 baht (approximately US$5-6) per kilogram, which is about 10 times cheaper than *Artemia* cysts found in the local market. Because the bigeye scad is popular in its salted form, its roe is separated for sale as a byproduct. The fish roe was collected fresh in plastic bags and kept in a deep freezer (-15°C). The eggs in plastic bags were stored in foam boxes and transported to the farm in Chachoengsao by a cold-chain vehicle. The frozen fish roe was thawed gradually at room temperature before use. Whole fish roe was placed in two hand nets, with mesh sizes of 450 microns on top and 300 microns below. Fish roe was washed with clean tap water and filtered to collect single cells from the remaining eggs using a 300-mesh net. The fish roe were then placed in a cup with a cover and stored in a standard refrigerator (4-7°C) for approximately 2-3 days before being fed to prawn larvae during the trial.

Egg custard preparation: The egg custard was made locally (New et al., 2010). Ten chicken eggs were broken and poured into a mixer; 10 g milk powder, DHA, and Bio-asta (activate pigment) were added, 10 mL spirulina extract, and 500 mL of freshwater were also added; and then they were mixed well. After mixing, it was steamed in a stainless-steel bowl for about 30 minutes. After steaming, the egg custard was screened for smaller particles that passed through a net (2 mm). The fine particles of the egg custard were collected and kept in a plastic bowl. Approximately 5 g of DHA, 5 mL of Biomega, and 5 mL of fish oil were added and mixed well by hand, then stored in an icebox.

Water preparation and water exchange

***Larvae* (*Days 1-20*):** Brine water (around 60-70 ppt salinity) was purchased from Samut Prakan province. Freshwater was mixed with brine to obtain a 15 ppt solution, which was disinfected with 65% chlorine at 16 g per 1 ton of water, as described by New et al. (2010). Brackish water was stored in a 2-ton tank, and shell lime (for calcium carbonate) and 32 g/ton of sodium bicarbonate were added. A mineral mixture and alkaline, plus 8 g, were added per ton of water. Magnesium (320 g/ton) was added. All those items were thoroughly mixed in a 20-liter bucket and then filled into a tank that passed through the net. Before use, the brackish water was checked for chlorine using the chlorine test kit. The water for the nursery system was maintained at a salinity of 15 ppt, a pH of 7.5-8.5, a temperature of 32-34°C, with magnesium at 475 ppm, calcium at 200 ppm, and alkalinity at 170 ppm. The photoperiod was maintained at 12:12 hours dark: light. The first water exchange was conducted on the seventh day of the experiment, and subsequent exchanges were performed every 2 days thereafter at 07:30 am. The initial water exchange was 30%, and it remained at 50% throughout the experiment.

***Post-larvae* (*Days 21-56*):** Following the guidelines by New et al. (2010), approximately 300 L of freshwater was stored in nine plastic tanks, each measuring 89 x 118 x 65 cm. The water in the tanks was disinfected with chlorine at 5 g per ton (5 ppm). Before use, the water was checked for chlorine using a chlorine test kit. Three pieces of black polyvinyl (PVC) nets (70 x 30 cm) were provided as shelters in each tank. Aeration was provided with a blower (2 HP) using two air stones per tank, connected through PVC pipes and plastic tubes. Water was exchanged weekly at 07.30 am. The feces and uneaten food were siphoned out, and 50% of the water was replaced with fresh water weekly to replenish 300 L per tank.

Experimental design

***Larvae* (*Days 1-20*):** Four combinations of feeding with *Artemia* and fish roe were tested as treatments with three replications: larvae fed with *A. salina*

nauplii only (i.e., 100% *Artemia*) as the control, and other treatments consisted of *Artemia* replaced at 50, 75, and 100% by bigeye scad roe. The experiment was conducted using 12 black round-shaped plastic buckets (40 cm height and 37 cm in diameter) with a capacity of 40 L. Stocking density in each bucket was 2,400 larvae in 30 L water, i.e., 80 larvae L^{-1} (New et al., 2010). The experiment was continued for 20 days, until 80% of the larvae (or more) had achieved metamorphosis to stage 12 or postlarval stage. On day 20, all the replicate larvae were counted, and 100 larvae were randomly sampled from each replicate to estimate the average weight. For length measurement, 20 larvae were sampled from each replicate group. The larvae were weighed on both the first and final days using a digital balance (0.0001 g precision) (OHAUS Corporation, USA). Larvae length on the first and final days was measured using a plastic standard vernier caliper to a precision of 0.05 mm.

***Post-larvae* (*Days 21-56*):** After larval rearing at the farm, all the larvae and post-larvae were brought to the university hatchery on the 21st day of the experiment to monitor the growth performance during the post-larval stage to see whether the type of food (treatments) fed during the larval stage had any effects on their survival and growth at later stages. The larvae or post-larvae were packed in a plastic bag containing approximately 1 L of water, one bag per replicate group. They were kept in nine plastic tanks (300 L of water). Out of the remaining post-larvae, 150 were randomly sampled and kept in each replicate tank for the post-larvae nursing experiment. This experiment with post-larvae was conducted for 35 days. The remaining post-larvae were counted for survival and batch weighed by using the same digital balance (0.0001 g precision). For length, 10 post-larvae were randomly sampled from each replicate group. Lengths were measured with a plastic standard vernier caliper with a precision of 0.05 mm.

Feeding

***Larvae* (*Days 1-20*):** Initially, larvae were fed three times, i.e., at 07:00, 12:00, and 17:00 hrs. At the time, 5 g of feed was prepared and kept separately in a refrigerator. For example, to achieve 5 g in the case of a 50% replacement, 2.5 g of fish roe and the same amount of *Artemia* were mixed to create 5 g. Similarly, for a 75% replacement, 3.75 g of fish roe with 1.25 g of *Artemia* were mixed. Live *Artemia* and fish roe were weighed using a balance with a precision of up to three decimals in gram units (RIA Digital Scale; China, 0.001 g) while feeding the larvae each time. Egg custard was added twice daily at 10:30 and 14:30 hrs. when the larvae reached the 5^{th} stage in all the treatment replicates. Details of the feeding schedule (total feed in grams per meal per day) are shown in Table 1.

***Post-larvae* (*Days 21-56*):** The post-larvae of all the treatments were fed the same commercial feed (INVE THAILAND, Ltd.) twice a day at 08:00 and 17:00 hrs. The particle size of the feed was 200-400. The feed composition included 40% crude protein, 6% crude lipid, less than 3% crude fiber, and less than 10% moisture. During the first week of the experiment, the post-larvae were fed 3 g per meal in each tank; thereafter, the feed rate was increased by 1 g per week.

Water quality analysis

***Larvae* (*Days 1-20*):** Water temperature and pH were monitored using a EUTECH pH multiparameter probe (Thermo Fisher Scientific Eutech Industries Pte Ltd, Singapore), and DO was measured with a DO meter (LAQUA, Horiba, Model 220; HORIBA Advanced Techno Co. Ltd, Kyoto, Japan) three times a day, i.e., at 07:30, 14:30, and 17:30 hrs. Collection of water samples (500 mL/replicate) for Ammonia-N and Nitrite-N started by the seventh day of the larval experiment and every two days afterwards (based on water exchange) at 07:30 hrs. Water samples were stored in a deep freezer at -15°C until transport to the university, where they were analyzed by titration (Boyd and Tucker, 1992). At the end of the experiment, water from each bucket (replicate) was collected in a one-liter bottle to analyze total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), total volatile solids (TVS), and fixed solids (FS) (Boyd and Tucker, 1992).

***Post-larvae (Days 21-56*):** DO was measured by using a DO meter (LAQUA, Horiba, Model 220; HORIBA Advanced Techno Co. Ltd, Kyoto, Japan), and water

Table 1. Feeding table for the rearing of *Macrobrachium rosenbergii* larvae (1-20 days) (adapted from Carvalho and Mathias, 1998; Valenti et al., 1998; New, 2002; Aviz et al., 2018)

Time➔ Day	Quantity of feed or daily ration (g)				
	07:00 (*Artemia* and/or Fish roe)	**10:30 (Only egg custard)**	**12:00 (*Artemia* and/or Fish roe)**	**14:30 (Only egg custard)**	**17:00 (*Artemia* and/or Fish roe)**
1	-	-	-	-	-
2	0.3	0	0.6	0	0.6
3	0.6	0	0.6	0	1
4	1	0	1	0	1.5
5	1	0	1	0	1
6	1	0.3	0	0	1
7	1	0.3	1	0.6	1
8	1	0	0	0.6	1
9	1.5	0.6	1	0.6	1
10	1	0.6	1	0.6	1.5
11	1	0.6	1	0.6	1.5
12	1.5	0.3	0	0	1.5
13	1	0.6	1	0.6	1
14	0.6	1	0.6	1	0.6
15	0.6	1.5	0.6	1.5	0.6
16	0.6	1.5	0.6	1.5	0.6
17	0.6	1.5	0.6	1.5	0.6
18	0.6	1.5	0.6	1.5	0.6
19	0.6	1.5	0.6	1.5	0.6
20	0.6	1.5	0.6	1.5	0.6

temperature and pH were monitored using a EUTECH pH multiparameter probe (Thermo Fisher Scientific Eutech Industries Pte Ltd, Singapore) daily at 07:30 hrs. Approximately 500 mL of water from each tank was collected weekly at 07:30 hours for analysis of Ammonia-N and Nitrite-N using the method described by Boyd and Tucker (1992).

Performance indicators: Survival, specific growth rate (SGR, %/day), daily weight gain (DWG), and daily length gain (DLG) were compared between the treatments, which were calculated based on the following equations (Maciel et al., 2012):

Survival rate = $(N_t/N_0) \times 100$

Specific growth rate (SGR, %) = $(\ln(w_t)-\ln(w_0)/t) \times 100$

Daily weight gain (DWG, mg/day) = $(W_t-W_0) / t$

Daily length gain (DLG, mm/day) = $(L_t-L_0)/t$

Where N_t = number of larvae and post-larvae taken at the final harvest, N_0 = number of larvae stocked, W_t = final weight, W_0 = initial weight, Lt = final length, L_0 = initial length, and t = time of day.

Proximate analysis of the feed: *Artemia*, bigeye scad roe, and egg custard were collected in three replications each for proximate analysis (AOAC, 1990). Crude protein was determined by the micro-Kjeldahl method. The FOSS Kjeltec 8100 apparatus was used for analysis (FOSS Analytical AB, Hoganas, Sweden). Crude lipid was analyzed applying the Soxhlet Method using the FOSS Soxtec 2043 apparatus (FOSS Scino Co. Ltd, Suzhou, China). Crude fiber was estimated by the Weende method using the FOSS Fibertec 1020 apparatus (FOSS Scino Co. Ltd., Suzhou, China). Moisture was assessed using an air oven (Model LDO-100E; Lab Tech, Daihan Labtech Co., Ltd., Namyangju City, Korea). Ash was estimated using the muffle furnace method.

Statistical analysis: One-way analysis of variance (ANOVA) was used to test the effects of the factor (feed replacement) on growth (weight and length gains) and survival of larvae and post-larvae. Tukey's HSD test was used to compare the means of the treatment groups. The statistical significance level was considered 5%. In addition, polynomial regression analysis was performed to examine the

Table 2. Survival rate (%), specific growth rate (%/day), Daily weight gain (mg/day) and Daily length gain (mm/day) of the larvae after 20 days of nursing which were fed with the 100% *Artemia* nauplii (control) and replaced at 50%, 75% and 100% by the fish roe (Average ±SE).

Performance	Treatments			
	Artemia	50% Roe	75% Roe	100% Roe
Initial weight (mg)	0.20±0.0	0.20±0.0	0.20±0.0	0.20±0.0
Initial length (mm)	2±0	2±0	2±0	2±0
Final weight (mg)	4.11±0.31[a]	3.7±0.25[a]	2.59±0.16[b]	-
Final length (mm)	7.95±0.13 [a]	8.03±0.13 [a]	6.53±0.27[b]	-
Survival (%)	38.5±6.7[a]	36.5±1.6[a]	33.6±10.9[a]	-
Specific growth rate (SGR%/day)	15.1±0.4[a]	14.6±0.3[a]	12.8±0.3[b]	-
Daily weight gain (mg/day)	0.20±0.02[a]	0.18±0.01[a]	0.12±0.01[b]	-
Daily length gain (mm/day)	0.30±0.01[a]	0.30±0.01[a]	0.23±0.01[b]	-

relationships between larval survival and size (length gain) and the replacement rate, to determine optimal levels. All statistical analyses were performed using the IBM SPSS Statistics (Version 23).

Results

Survival and growth of larvae: Survival and growth performance of the larvae are presented in Table 2. Larvae fed 100% fish roe survived for only 9 days. The survival rates of the groups fed 100% *Artemia* nauplii (38.5%), 50% (36.5%), and 75% (33.6%) replacement treatments were not statistically different ($P>0.05$). SGR was significantly lower in the group fed 75% fish roe compared to the 50 and 100% *Artemia* groups. The 75% fish roe-fed group had an SGR of 12.8±0.3%, approximately 12-15% lower than the SGRs of the other treatments (i.e., 50% replacement and no replacement) at 100% Artemia. On the other hand, the SGRs for 100% Artemia (15.1±0.4%) and 50% replacement (14.6±0.3%) were not significantly different ($P>0.05$). Considerable differences in daily weight gains (DWG) were observed during the larval rearing. The average daily weight gains were 0.20±0.02 and 0.18±0.01 mg/day for the treatments fed 100% *Artemia* and 50% replacement, respectively, and were not significantly different. The average daily weight gain was lowest (0.12±0.01 mg/day) in the treatment with 75% *Artemia* replacement by fish roe, which was 33% lower than that of the 50% replacement and 40% lower than that of the group with *Artemia* ($P<0.01$). DLG was the lowest (0.23±0.01 mm/day) with 75% *Artemia* replaced with fish roe, but no difference was observed between 50% and 100% Artemia.

Polynomial regression analysis was performed to investigate the relationship between the *Artemia* replacement percentage by fish roe and the survival of prawn larvae and their size (length gain). The results showed the relationship between the replacement percent (x) and larvae survival (y) is $y = -0.0091x^2 + 0.5531x + 37.768$ ($R^2 = 70\%$, n = 11, Xmax = 30.4%, Ymax = 46.2%, Fig. 1) which means highest survival of larvae 46.2% can be achieved at 30.4% replacement of *Artemia* by fish roe from which survival starts declining. Similarly, the relationship between the replacement rate (x) with the size of the prawn indicated by length gain (y) is $y = -0.0008x^2 + 0.0428x + 5.95$ ($R^2 = 0.8707$, n = 9, Xmax = 26.8%, Ymax = 6.5 mm; Fig. 2) which means largest size of prawn larvae can be obtained when fish roe replaces the 26.8% *Artemia*. If replaced at higher levels, the length gain or size of prawn larvae starts to decline.

Post-larvae performance: Average survival rate of post-larvae ranged from 79.3 to 83.3%, and there were no differences due to the feed type during the larval stage (Table 3). SGR was the highest, 11.74±0.15%/day, with a 75% replacement of *Artemia* with fish roe. Two groups: 100% *Artemia* (10.79±0.18%/day) and replacing 50% of fish roe (10.66±0.23%/ day), were similar in terms of SGR during the post-larval stage. The DWG of the post-larvae showed significantly higher levels ($P<0.01$), i.e., 7.54±0.0.11 mg/day, with the 100% Artemia treatment compared with the 50% (6.48±0.13 mg) and 75% (6.65±0.11 mg) fish roe treatments. Daily length gain (DLG) did not differ significantly among treatment means.

Proximate composition and size of the feed: In the larval stage, fish roe, *Artemia*, and egg custards were

Table 3. Survival rate (%), specific growth rate (%/day), Daily weight gain (mg/day), and Daily length gain (mm/day) of post-larvae after 56 days fed with a commercial feed, after the experiment with 100% *Artemia* nauplii and replaced at 50%, 75% and 100% by fish roe (Average±SE).

Post larval performance	Treatments			
	Artemia	50% Roe	75% Roe	100% Roe
Initial weight (mg)	4.1±0.3[a]	3.7±0.3[a]	2.6±0.2[b]	-
Initial length (mm)	7.95±0.13[a]	8.03±0.13[a]	6.53±0.27[b]	-
Final weight (mg)	268±4[a]	231±4[b]	235±4[b]	-
Final length (mm)	32.77±2.34[a]	32.0±0.87[a]	28.70±0.95[b]	
Survival (%)	83.3±1.9[a]	79.3±2.0[a]	80.4±2.0[a]	-
Specific growth rate (SGR%/day)	11.95±0.18[a]	11.82±0.23[a]	12.90±0.15[b]	-
Daily weight gain (mg/day)	7.54±0.12[a]	6.48±0.13[b]	6.65±0.11[b]	-
Daily length gain (mm/day)	0.71±0.0.7[a]	0.72±0.03[a]	0.63±0.03[a]	-

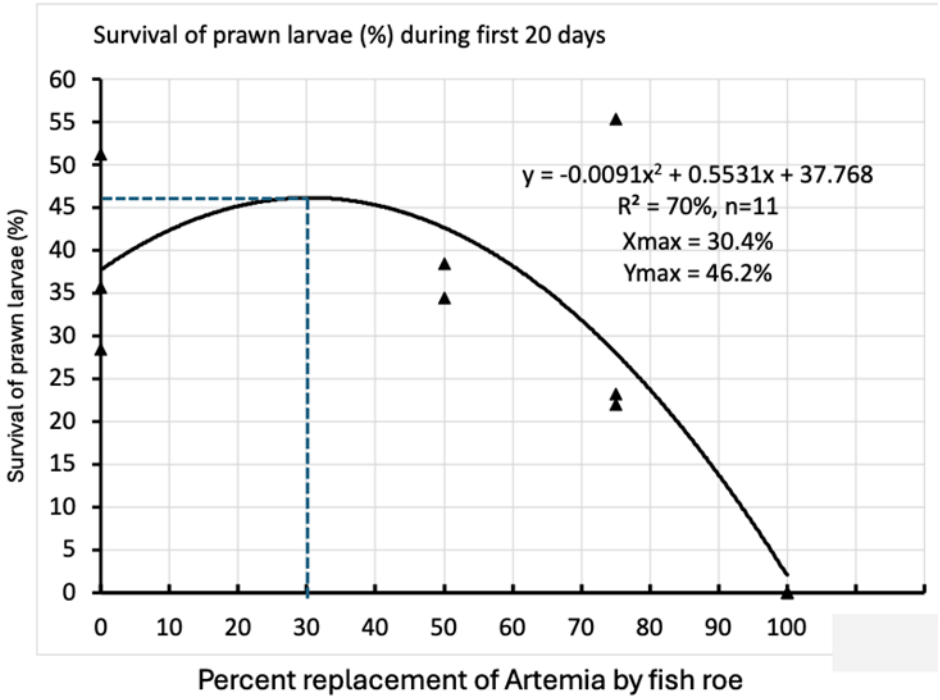

Figure 1. Relationship between the survival and the replacement of *Artemia* by fish roe.

fed at different ratios according to the planned treatments. Crude protein content ranged from 50 to 54%, but was not significantly different among them (P>0.05). However, the crude lipid contents differed among them. Egg custard had the highest crude lipid content, followed by fish roe, whereas *Artemia* had the lowest (Table 4).

Water quality in the larvae rearing system: During larvae rearing, the average water pH ranged from 6.8 to 8.9, and the average DO ranged from 6.27 to 8.8 mg/L. During the post-larvae experiment, average water temperature was 28.8±0.16°C, pH 8.69±0.09, and DO 7.69±0.1 mg/L. Water temperature range was 28-29°C, pH 8.56-8.75, and DO 7.56-7.78 mg/L. Average Ammonia-N levels were 0.58±0.33, 0.58±0.23, and 0.66±0.2 mg/L in treatments with 100% *Artemia*, and replacing 50%, and 75% with fish roe, respectively, and were not significantly different among them. Nitrite-N levels ranged from 0.17 to 0.18 mg/L and were not significantly different among the treatments.

Average total solids in 100% *Artemia* and replacing 75% of fish roe treatment were 14,770±2,608 mg/L, 16,923±1,004 mg/L, and 8,160±1,311 mg/L. Total suspended solids (TSS) in each treatment were not significantly different (P>0.05). The average total suspended solids were 28, 22, and 32 mg/L in the 100% *Artemia* treatment and in treatments replacing

Table 4. Proximate composition of Fish roe, Artemia, and egg custard (Average±SE)

Proximate composition	Treatments		
	Artemia	Eggs custard	Fish roe
Moisture (%)	83.6±0.07[b]	78.3±1.22[c]	86.2±0.48[a]
Crude protein (%)	54.1±0.13[a]	50.0±1.44[a]	53.7±0.12[a]
Crude lipid (%)	15.0±0.47[c]	34.6±3.52[a]	20.5±0.48[b]
Ash (%)	10.5±0.28[a]	2.67±0.05[b]	2.7±0.17[b]
Fiber (%)	0.57±0.01[a]	0.59±0.13[a]	0.55±0.01[a]
NFE (%)	23.8±0.31[a]	12.1±3.49[b]	22.5±0.37[ab]
Size (μ)	479.6±59 (L)	300-500	340.7±25.6

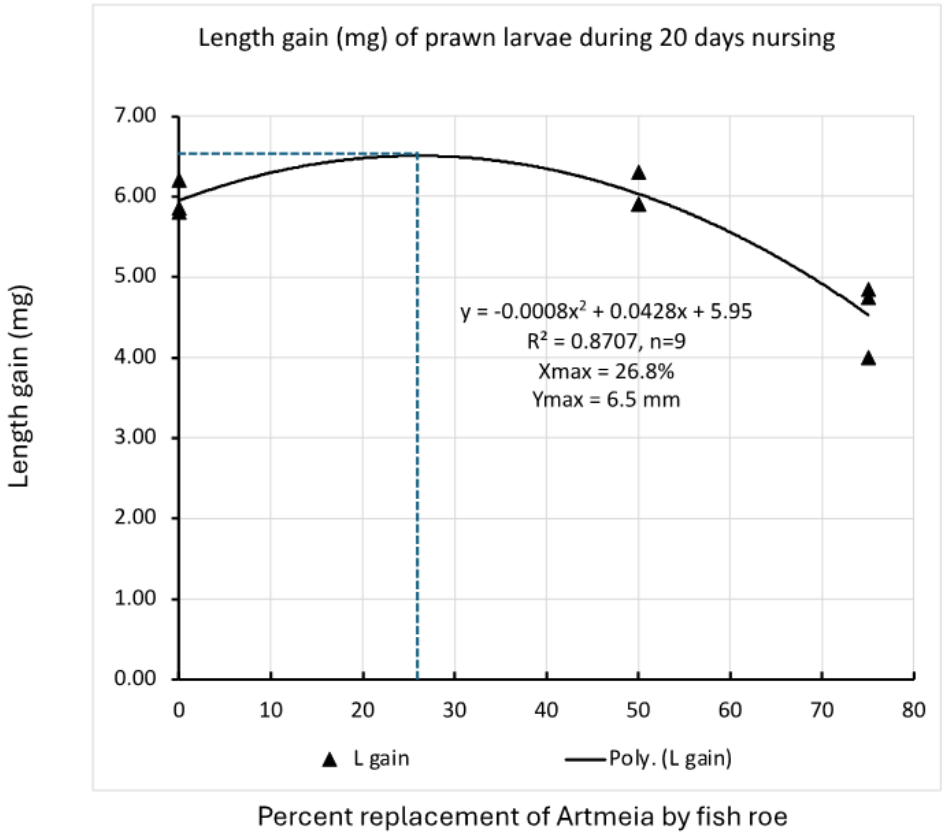

Figure 2. Relationship between the length gain and the replacement of *Artemia* by fish roe.

50% and 75% of fish roe, respectively. Total dissolved solids (TDS), in 100% *Artemia*, and replacing 75% of fish roe treatment were significantly higher (P<0.05) than in 50% replacement. The average total dissolved solid in the 50% replacement of fish roe treatment was 8,138 mg/L, higher than in other treatments. The average total volatile solid was 1,932 mg/L, which was the lowest in the 50% replacement scenario (Table 5).

Discussions

Efficient incubation of eggs followed by larval and post-larval rearing in controlled environments ensures mass-scale production of a predictable quantity and quality of larvae, which is required for the development of the prawn industry. In the present study, the survival rate of larvae ranged from 33.6 to 38.5% and post-larvae from 79.3 to 83.3%, which are reasonably good as compared to many other reports (Tansakul, 1983; Ali, 2005; New et al., 2010; Miglio et al., 2021; Wei et al., 2021). Membranes or cell walls well protect fish and crustacean eggs, but when they hatch into larvae and emerge into the external environment, they are highly vulnerable and delicate. Their survival can vary widely and depends on physical, chemical, and microbial conditions. The

Table 5. The average of total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), total volatile solids (TVS), and fixed solids (FS).

Treatment	Total solids (TS;mg/L)	Total suspended solids (TSS; mg/L)	Total dissolved solids (TDS; mg/L)	Total volatile solids (TVS; mg/L)	Fixed Solids (FS; mg/L)
Artemia	14,770±1,506[b]	28±3[a]	14,742±1,503[b]	3,701±348[b]	13,097±498[b]
50% Roe	8,160±757[a]	22±0[a]	8,138±757[a]	1,932±83[a]	6,756±409[a]
75% Roe	16,923±1004[b]	32±5[a]	16,879±999[b]	4,275±333[b]	12,649±672[b]
100% Roe	-	-	-	-	-

death of all larvae across all replicates when fed only fish roe, without *Artemia*, indicated that appropriate natural food or commercial feeds are crucial for these transitional life forms to ensure high survival and proper physiological development. As the larvae of fish and crustaceans are microscopic, fine fishmeal, powder, and paste, or custard made from chicken eggs, blood, and liver have been tried as the basic food for rearing larvae (New, 2002; New et al., 2010). However, they degrade water quality over time and may sink to the bottom, reducing their availability to free-swimming larvae. Feed has to be suspended in the water column, buoyant or freely swimming, so that prawn larvae can encounter it more often to grab and eat *(*Kovalenko et al., 2002). Nauplii of *Artemia salina* are among the most widely used live foods and are commercially available, particularly for the crustacean industry. Various product forms, such as powders, frozen, and others, are available in cans, plastic bags, and bottles. However, a very high price is a problem for farmers. Therefore, various alternatives have been tried. One of them is a group of fish eggs, also known as roe.

In the present study, it was hypothesized that small, floating pelagic fish eggs could partially or fully replace *Artemia*. Therefore, a larval rearing trial was conducted using 50, 75, and 100% replacements of *Artemia* by bigeye scad roe, which is about 10 times cheaper. The results were very promising. Although a 100% replacement of Artemia by fish roe resulted in the total death of prawn larvae within a week, replacing up to 75% yielded a reasonable level of survival. The polynomial regression indicated that the highest survival (46.2%) can be achieved when only 30.4% *Artemia* is replaced by fish roe. If we increase the inclusion of fish roe, their survival and growth decline. The relationship has been mainly influenced by 100% mortality when all *Artemia* were replaced. Further research using replacement levels between 30% and 80% would help determine the replacement rate more precisely. The present study showed that up to a 50% replacement, larval survival and growth were reasonably good and comparable to the control (only Artemia). However, at a replacement rate of 75%, survival was highly variable, and growth, in terms of weight and length gains, was hampered. Typically, nursing farmers prefer to purchase larger larvae because they believe they are healthier, stronger, and of higher quality. Therefore, a 50% replacement of *Artemia* with fish roe is recommended, given the survival, growth, and cost-reduction benefits, although the highest survival rate occurs at approximately 30% replacement.

Present research indicates that up to 75% can be replaced if larval growth or size is not important at this stage, since larvae can compensate for growth later, as occurred in this study during the second phase of the trial. However, alternatives such as rotifers and fish roe are not complete alternatives; they are only supplementary (Barros and Valenti, 2003; Murty et al., 2012). The bigeye scad roe has a protein content comparable to that of Artemia (53.7 vs. 54.1%), higher lipid content (20.5 vs. 15.0%), but lower ash content (2.7 vs. 10.5%). These indicate that higher lipid content may have contributed to low digestibility, as most young animals' digestive systems are not yet fully developed. At the same time, lower ash content may result in deficiencies of essential minerals, such as calcium, phosphorus, magnesium, and zinc, which are crucial for growth, development, and survival, particularly during molting, which occurs more frequently in the early stages. Another difference is that *Artemia* is live and can swim freely in the water column, which may lead to encountering more, while

fish eggs only remain in suspension without their own movement. Live animals still require a high protein content; for example, rotifers are live but may contain as little as 29.2% protein (Jeeja et al., 2011), which is significantly lower than that of *Artemia*.

The second phase of the trial was conducted to assess the effects of larval rearing under different treatments and to generate additional knowledge. Interestingly, the larvae which were smaller or shorter at the end of larval stage (20th day) as a result of feeding 75% fish roe with 25% *Artemia* had higher SGR during the post-larval period, or they grew at faster rate to catch up the lost growth earlier which means prawn may have compensatory growth advantages (Pyet al., 2022; Jesus et al., 2023). However, final weights and lengths were still lower in the 75% replacement group compared to the groups fed 50% and 100% *Artemia*. It indicates that poor feeding during the larval stage affects the post-larval stage, despite some compensatory growth.

While exploring alternatives, food or feed particle size, nutritional composition, color, and movement are crucial factors to consider, especially during the larval stage of crustaceans or other aquaculture species. In the first week, the larvae are small, capable of taking newly hatched Artemia nauplii, which are approximately 300 µm in length and 174 µm in width. The complete death of larvae in this study may have occurred in the case of 100% fish roe, due to the larger size of the fish eggs, which the larvae could not ingest. The size of the fish eggs was 340.7±25.6 µm. It clearly indicates that *Artemia* nauplii is required for the early stages, at least for up to a week. Alternative or supplementary feeds can help after the first week. Therefore, the strategy could involve a low replacement rate in the first week, then gradually increasing it to higher levels in the second and third weeks. As they grow bigger, they need bigger prey, for which fish roe can fit in. The benefit of live feed is that it grows alongside the larvae and is available within the same culture system. Alternatively, it can be grown separately and offered according to its needs. They are likely to accept larger prey, such as fish eggs, if they can crush them to sizes compatible with their ingestive capacity, which ranges from 250 to 1,190 (Valent et al., 1998; Barros and Valenti, 2003). They also do not pollute the water by releasing nutrients, which occurs when powder feed is used. Therefore, fish roe appears to be a better option than egg custard, as it contains higher nutrient levels.

More interestingly, fish roe contains 20.5% crude lipid, whereas Artemia contains only about 15%. It is a well-established fact that lipids play important roles in the reproduction and development of embryos and larvae (Rainuzzo et al., 1997; Cavalliet al., 1999; Izquierdo et al., 2000; Bhujel, 2002; Mohantyet al., 2013). However, high lipid levels could also cause indigestion, as young larvae have an underdeveloped digestive system. However, low ash content (i.e., low in minerals such as Ca, P, Mg, and Zn) in fish roe may contribute to mortality, as molting in larvae occurs more frequently and requires a high level of minerals for shell formation each time. In cases of mineral deficiencies, shell formation may take longer, increasing the risk of cannibalism and potentially leading to mortality (New et al., 2010) when larvae are fed 100% fish roe.

It is also important to note that the bigeye scad eggs contain high protein (53.7±0.12%), which is similar to the level in Artemia and egg custard, and as required by the shrimps and prawns, which is in the range of 52-57% (Deshimaru and Yone, 1978). Nitrogen-free extract, i.e., carbohydrates, is a source of energy, as the fish roe contains high levels of unsaturated fatty acids that make it a very healthy food item for humans (Vilgis, 2020; Bunga et al., 2022; Mouritsen, 2023). The only disadvantage of fish roe is that most single cells of fish eggs are round-shaped, which can make it difficult for the larvae to catch them.

The present study highlights the scope for further research, as many fish species produce large numbers of single-cell eggs that vary with species and female weight. Non-sticky, smallest-size eggs that remain in the water column are more suitable. Providing a variety of food sources, such as fish roe combined with Artemia and other items, is an effective strategy, as it increases the likelihood that larvae encounter prey in the rearing system. Reports have shown that higher

capture rates occurred due to an increase in the number of prey (Anger, 2001; Maciel et al., 2012). On the other hand, when exploring additional food options, matching color preferences may be important. Kawamura et al. (2016) reported that they prefer dark blue, light blue, and white. The color of the fish roe is usually yellow-creamy, and the color of the *Artemia* is red; larvae prefer neither of these. Therefore, more types of food/feed of their favored color should be explored. If not, they may still take them if the preferred color is not available. They can learn and still consume as they are opportunistic in nature.

In this study, water quality was maintained within the standard range considered appropriate for aquaculture (New, 2002). The total solid (TS) content in the water differed between the groups. TS includes dead *Artemia*, prawn larvae, and uneaten fish roe, including the molt of the larvae. Most of them may remain suspended as total suspended solids (TSS); some of them are actually food or sources of nutrients for larvae. However, these can cause excessive turbidity in the water and may also provide shelter for pathogens. Therefore, it should be at an optimal level and not persist for long. Water exchange plays a key role. During larval rearing, water exchange began after 7 days and was performed every 2 days thereafter. Therefore, TSS in this study remained low, i.e., 28-32 mg/L, and it may not have any effects on survival and growth performance, as it becomes a problem if it exceeds 300 mg/L *(*Gaona et al., 2015; Liu et al., 2022). Lima et al. (2021) reported that the highest TSS in a biofloc system was associated with the lowest survival of *M. carcinus* larvae. Therefore, 50% replacement of *Artemia* with the fish roe shows the lowest TSS and TVS, which supports the recommendations not to go for higher replacements prawn larvae are reared under high prey density conditions adding more *Artemia* or fish roe, weight or productivity may not be increased, but create a superfluous situation that can be highly risky (Maciel et al., 2012).

Conclusion

Based on the results of the present study, we concluded that *Artemia* live feed can be replaced by bigeye scad fish roe up to 50%. However, the highest larval survival rate (46.2%) is achieved at a 30.4% replacement rate. As the price of fish roe is about 10 times lower, prawn hatchery operators can take advantage of reducing the cost of larval rearing considerably. However, complete replacement is detrimental. Therefore, further research should be conducted using replacement levels from 0 to 80% to determine the economically optimal level. Further efforts should be made to explore the potential of using the roe of other fish, which may be more suitable with respect to color, size, shape, and other attractiveness factors, including palatability.

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