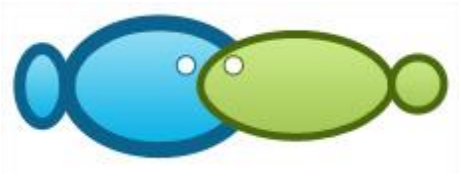

Study on different salinities and diets for the survival and growth of newly introduced *Penaeus merguiensis* larvae culture

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Abstract. Salinity is one of the leading environmental determinants affecting the survival, growth, and distribution of aquatic animals. Although various crustaceans have general euryhalinity standards, optimal salinity for certain species is unequivocal. In tropical areas with periodical changes in the rainy and dry seasons, salinity fluctuations are obvious. This study aims to analyze the effect of variations in salinity (28 ppt; 32 ppt; 36 ppt) and the feeding types (Diet A: 100% live feed; Diet B: 100% FRIPPAK; Diet C: a combination of FRIPPAK and live feed 50% each) on the survival and growth of the newly introduced *Penaeus merguiensis* larvae. The results showed that artificial feed significantly succeeded in replacing natural feed for the survival and growth of Zoea-1 to Zoea-3 at a salinity of 28 ppt. In the Mysis stage (Mysis-1 to Mysis-3), Diet A is preferred in the 32 and 36 ppt salinities. The relationships between larva length and days of life at a salinity of 28 ppt were: (Diet A) YA=0.5623x+0.632, with R^2=0.921; (Diet B) YB=0.4389x+0.8807, with R^2=0.9484; (Diet C) YC=0.5694x+0.6287, with R^2=0.9469. The relationship between larva length and day of life at a salinity of 32 ppt were: (Diet A) YA=0.4017x+0.9573, with R^2=0.8343; (Diet B) YB=0.3646x+0.884, with R^2=0.9242; (Diet C) YC=0.38x+0.77, where R^2=0.9403. Finally, The relationship between larva length and day of life at a salinity of 36 ppt: (Diet A) YA=0.3914x+0.9, with R^2=0.9081; (Diet B) YB=0.2566x+0.1187, with R^2=0.7624; (Diet C) YC=0.3343x+1.0233, with R^2=0.7833. The larval length significance to the variation of days was positive, the larva length increasing with increasing day number.
Key Words: euryhalinity, feed, larvae length, *Penaeus merguiensis*.

Introduction. Salinity is one of the most important abiotic factors affecting the survival and growth of banana prawn (*Fenneropenaeus merguiensis*) farming (Anand et al 2014) and profoundly influences many aquatic organisms. Although various crustaceans have different degrees of euryhalinity, salinity for growth, survival, and production for specific species has certain optimal levels (Kumlu & Jones 1995). Therefore, determining the optimal salinity level to meet the viability and performance of each commercial shrimp species necessary (Labrador et al 2016).

In tropical areas characterized by rainy and dry seasons, salinity fluctuations are apparent. However, sea-level rise, coastal flooding, and tropical cyclones in recent years have caused salinity imbalances in freshwater fisheries worldwide and are threatening the sector of aquaculture. As salinity intrusion happens in freshwater aquaculture, many freshwater species experience severe salinity stress; some species even become extinct due to their inability to cope with these extreme conditions. Therefore, it is essential to determine the salinity tolerance for freshwater-cultured species; this information can determine some freshwater species that can be cultivated in saline water areas.

Shrimp in low salinities tend to have low immunity and are susceptible to pathogens such as yellow head virus, white spot syndrome virus, or *Vibrio alginolyticus* infection. Low salinity stress can increase the oxygen consumption of brine shrimp. Consequently, shrimp needs more food to compensate for the extra energy used in the osmoregulation system to improve the growth performance (Li et al 2015).

Several studies on the *Penaeus merguiensis* diet have been conducted. Based on a study of gastric content from 35 specimens with a carapace length between 17-33 mm, it was found that *P. merguiensis* ate other crustaceans, vegetables, phytoplankton, and benthic foraminifera. However, studies of penaeid shrimp diets show that they eat a wide variety of foods (Nehru et al 2018). In the offshore of the Madras Coast of India, the morphospecies of *Penaeus indicus* were fed with vegetables and crustaceans, mollusks, foraminifera, polychaetes, hydroids, trematodes, and echinoderm larvae (Liao et al 2020).

In the farming practice of *P. merguiensis* larvae, the community has applied several artificial and natural feeds. However, no detailed information regarding optimal diet, bowel evacuation time, trypsin analysis, and the diet's particle size was found (Bojórquez-Mascareño & Soto-Jiménez 2013). In shrimp farming, various environmental stimuli such as pH (Han et al 2018), salinity (Wang et al 2016), dissolved oxygen (Han et al 2018), temperature (Madeira al 2015), and pollutants such as nitrates, ammonia, and sulfides (Duan et al 2018) affect growth (Bu et al 2017; Valenzuela-Madrigal et al 2017), physiological performance (Xu et al 2012; Khanjani et al 2016), and shrimp survival (Ayaz et al 2015). In addition, it is also known that temperature changes with more than 13°C can lead to cessation of feeding, swimming ability, and shrimp mortality (Xu et al 2012; Zhang et al 2017).

Generally, penaeid shrimp are categorized as heterosexual (dioecious) so that the male and female genitals can be distinguished morphologically (sexual dimorphism) (Sakas 2016). At the same age, the overall size of the female shrimp is larger than the male one, especially their abdomens. The male reproductive organs consist of a pair of testes, vas deferens, petasma, and masculine appendixes. Petasma is found in the first pair of pleopods, while the genital canal hole is located between the bases of the fifth walking legs (Sentosa et al 2017).

Salinity is defined as the total concentration of dissolved ions in seawater expressed in units per million (‰) or ppt (units per thousand) or gr L^{-1}. Salinity is composed of seven significant ions: sodium, potassium, calcium, magnesium, chloride, sulfate, bicarbonate. Although other water substances do not directly influence salinity (Marlina & Panjaitan 2020), they are also crucial for ecological functions. If the salinity is too high, the conversion ratio will be higher for continuous air circulation. In marine animals, glycine betaine is one of the main osmolytes that expands under osmotic stress conditions; however, little information is known about the course involved in glycine betaine's biosynthesis (Mizanur & Bai 2014; Valle et al 2015).

In animal cells, glycine betaine is synthesized by the enzyme Betaine Aldehyde Dehydrogenase (BADH) (Gao et al 2016). The effect of salinity and glycine concentration of betaine on white shrimp *Litopenaeus vannamei* has been studied, the shrimp being treated with salinities of 10, 20, 35, 40, 50, and 60 ppt for seven days. Another result found that increased BADH activity occurred in the hepatopancreas and gills of shrimp in salinity above 35 ppt (Muhammad et al 2016). The BADH activity in muscle decreased at 35 ppt salinity, but in the hepatopancreas, it increased by 1.1 and 1.7 at 50 and 60 ppt, respectively. Then, at a salinity of 60 ppt, the gills' BADH activity increased 1.5 times compared to the salinity of 35 ppt. Increased glycine betaine concentrations in the hepatopancreas, gills, and shrimp muscle occurred at salinity above 35 ppt. However, the glycine concentration of betaine also increased at a salinity of 20 ppt (Yang et al 2020) but was not detected at a salinity of 10 ppt. Betaine glycine most likely occurs to avoid the denaturalization of protein. Ammonia concentration in the air only increased at a salinity of 20 ppt and 10 ppt (1.1-fold compared to 35 ppt). Salinity regulates BADH and glycine content, specifically in *L. vannamei* tissues (Delgado-Gaytán et al 2020).

Live food used for some larval production can increase cannibal behavior in the cultured shrimp species (Chand et al 2015). Live feed in cultured larvae is considered necessary because of its high nutritional value; later, they can balance their diet according to their natural habitat (de Lourdes Cobo et al 2014). However, when cultivation is carried out in captivity, live food must be chosen to meet the best dietary pattern (Liu et al 2020). Once the temperature and salinity of farming are established, they can be used to predict larval survival and growth rates for future planning (Crisp et

al 2017). Food and salinity are believed to cause stress in the cultured system, impacting gut morphology and nutrient digestion (Tran-Ngoc et al 2017).

By analyzing the effect of variations in salinity (28, 32, and 36 ppt) and the feeding types (100% live feed, 100% FRIPPAK, and a combination of FRIPPAK and live feed 50% each), the survival and growth of the newly introduced *P. merguiensis* larvae can be objectively assessed for better farming configuration. This study's authenticity lies in the research object, *P. merguiensis*, as a native species from Indonesia that can be introduced to various parts of the world. Research novelty consists of three aspects: material, location, and methodology. The materials used in this study were *P. merguiensis* larvae from the Jepara Government Marine Research Institute's hatcheries. The research was conducted in the Maritime Research and Development Center for the Government of Jepara, Indonesia, and this research is new to this location. The research on salinity and feed on *P. merguiensis* larvae is new, and *P. merguiensis* is a native Indonesian giant prawn.

Material and Method

Salinity experiment. Nine beaker glasses were used, being placed in aquariums containing 20 L of saline water. Each beaker glass was placed in an aquarium with a water heater to adjust the water temperature to 31°C. The beaker glasses were filled with water with different salinities (28, 32, and 36 ppt up to 2.5 L). Later, filtered seawater with salinities of 28, 32, and 36 ppt was added. For this experiment, 250 nauplii of *P. merguiensis* were allocated per beaker glass before it was placed into the specified salinity.

Diet experiment. The diet experiment was carried out for 10 days, by giving live microalgae and FRIPPAK (consisting of fishmeal, vitamins, and minerals) in the form of microencapsulated diet (MED). Considering zoea was the successor of the nauplius stage where its digestive system was not yet fully developed, we treated Zoea 1 to Zoea 3 with Diet A containing 2000 cells mL^{-1} of *Skeletonema costatum*. However, we increased the number of *Skeletonema costatum* to 7500 cells mL^{-1} for the mysis stage. For Diet B containing FRIPPAK MED, we served 4 mg L^{-1} for Z1 to Z3 and 6 mg L^{-1} for M1 to M3. Lastly, Diet C containing 50% of Diet A and 50% of Diet B was administered for Z1 to Z3 and M1-M3 with the same feeding pattern (4 mg L^{-1} for Z1 to Z3 and 6 mg L^{-1} for M1 to M3). Zoea stage was fed once a day at 8 am to avoid overeating that can cause death. The Mysis stage was administered feed 5 times a day at 8.00, 11.00, 16.00, 23.00, and 04.00.

Stages of shrimp. The used stages included zoea and mysis phases. Larvae were stocked in beaker glasses at a density of 100 individuals L^{-1} (250 individuals in each 2.5 L beaker glass). Each treatment consisted of 3 repetitions. The assessments were carried out at different salinity levels (28, 32, and 36 ppt).

Water quality standard. Temperature, oxygen, ammonia, acidity, and alkalinity were measured once per day in the morning with a DO meter (YSI550A-25). Temperature, oxygen, ammonia, acidity, and pH were measured once a day from 6.00-16.00.

Length and survival rate. The calculation was carried out at the beginning and end of the experiment. The survival rate and length were measured daily for 10 days. Three samples were extracted daily for inspection and measurement. After ten days, the survival rate and daily development were calculated.

The daily development calculation (length) was done by measuring the difference in length:

$L_Z=L_x-L_y$

Where: L_y is the length before the treatment; L_x is the length where the particular treatment was done. The product difference between the two lengths was the development (L_Z).

The survival rate was calculated using the following formula:

Survival Rate = (total number of living larvae at the end of the experiment/the initial number of larvae) x 100%

Source of larvae. The material used in this research was shrimp larvae (*P. merguiensis*). Larvae, as raw material, were produced by the government at the Hatchery Marine Research Center. Larvae at the zoea stage were brought from the Marine Cultivation Research Center. The stages for shrimp include zoea to mysis. Larvae were stored in beaker glasses. With 250 samples per glass (2.5 L), each treatment consisted of 750 animals (three repetitions).

Statistical analysis. The data obtained were analyzed using the analysis of variance by looking at the interaction of each factor. If the analysis of variance results showed a significant difference, then further examination using the Duncan's Multiple Range Test (DMRT) was conducted to determine the significance and interaction between treatments with a 95% confidence level ($p<0.05$). Experimental data were analyzed by two-way analysis of variance using SPSS v.16.

Results and Discussion

Stages of cultured larva from zoea to mysis. The stages from zoea to mysis are presented in Figure 1.

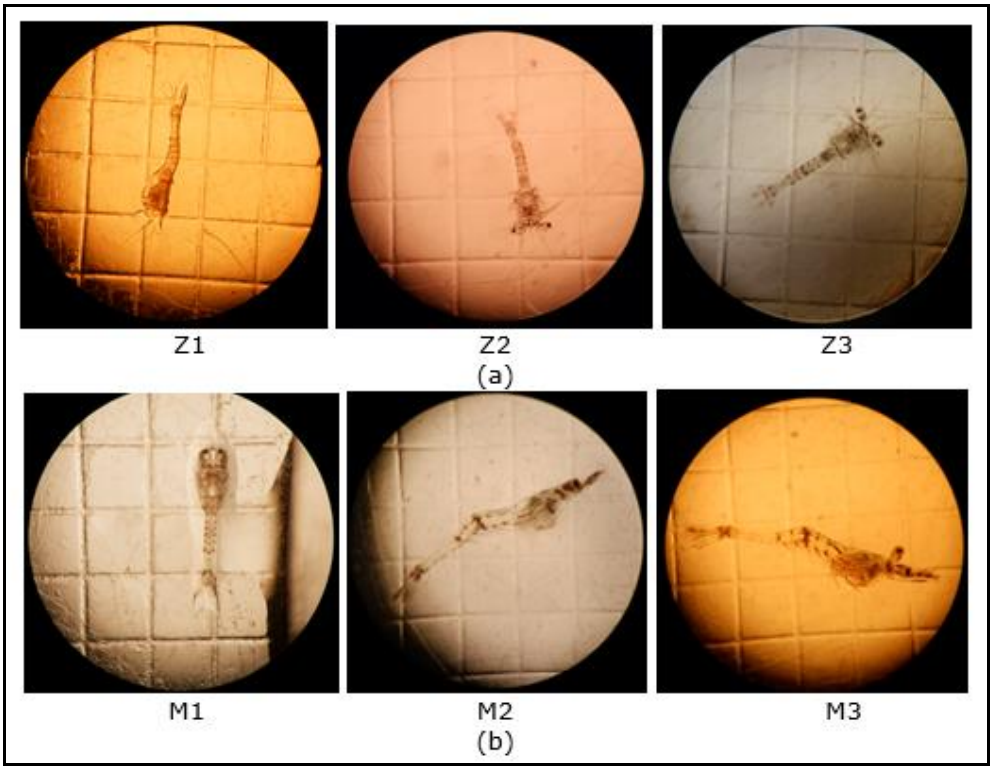

Figure 1. The development observation of *Penaeus merguiensis* larvae: a - zoea; b - mysis. Images were captured using a stereo microscope with high-magnification objectives (100x).

From Figure 1, it can be seen that the larvae in the zoea stage were fully developed, and reach the shape of shrimp. At the mysis stage, the shrimp development continued. The

phases of Z1 to Z3 took one day each (day 1 to day 3) and the phases of M1-M3 also took one day each (day 4 to day 6), as presented in the following figures.

Correlation between larvae lengths and days in salinity of 28 ppt. Figure 2 presents the correlation between larvae length and days of life for Zoea and Mysis.

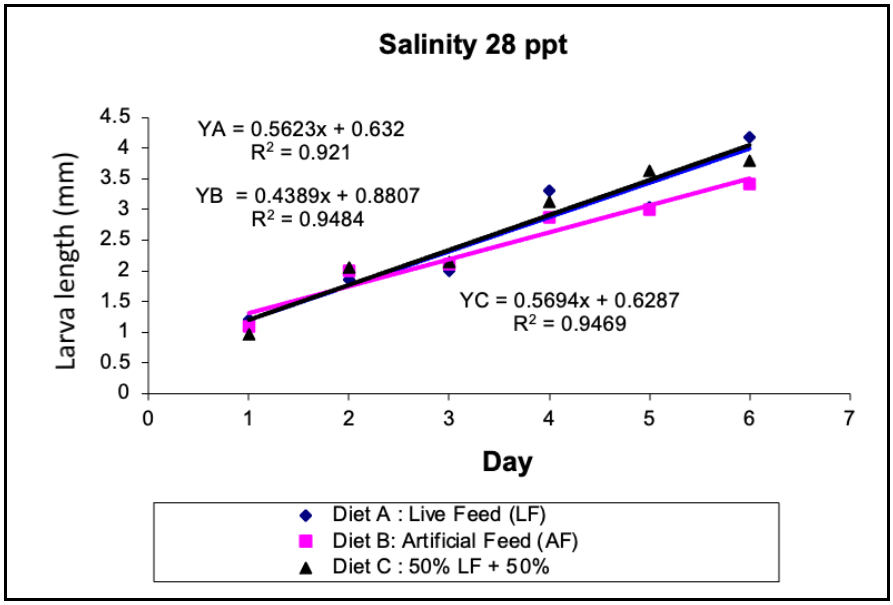

Figure 2. Correlation between *Penaeus merguiensis* larvae lengths and days (days 1-3: Z1-Z3; days 4-6: M1-M3) at a salinity of 28 ppt.

As presented in Figure 2, the equations obtained are: YA=0.5623x+0.632, with R^2=0.921, for Diet A; YB=0.4389x+0.8807, with R^2=0.9484, for Diet B; YC=0.5694x+0.6287, with R^2=0.9469, for Diet C.

Correlation between larvae lengths and days in salinity of 32 ppt. Figure 3 presents the correlation between larvae length and days for zoea and mysis.

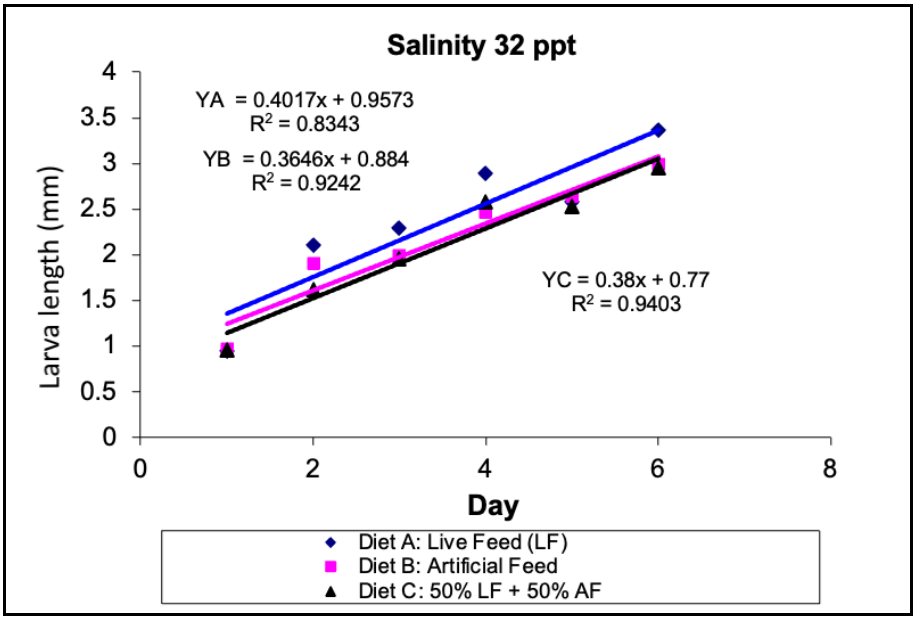

Figure 3. Correlation between *Penaeus merguiensis* larvae lengths and days (days 1-3: Z1-Z3; days 4-6: M1-M3) at a salinity of 32 ppt.

As presented in Figure 3, the equations obtained were: YA=0.4017x+0.9573, with R^2=0.8343, for Diet A; YB=0.3646x+0.884, with R^2=0.9242, for Diet B; YC=0.38x+0.77, with R^2=0.9403, for Diet C.

Correlation between larvae lengths and days in salinity of 36 ppt. Figure 4 presents the correlation between larvae length and days for Zoea and Mysis.

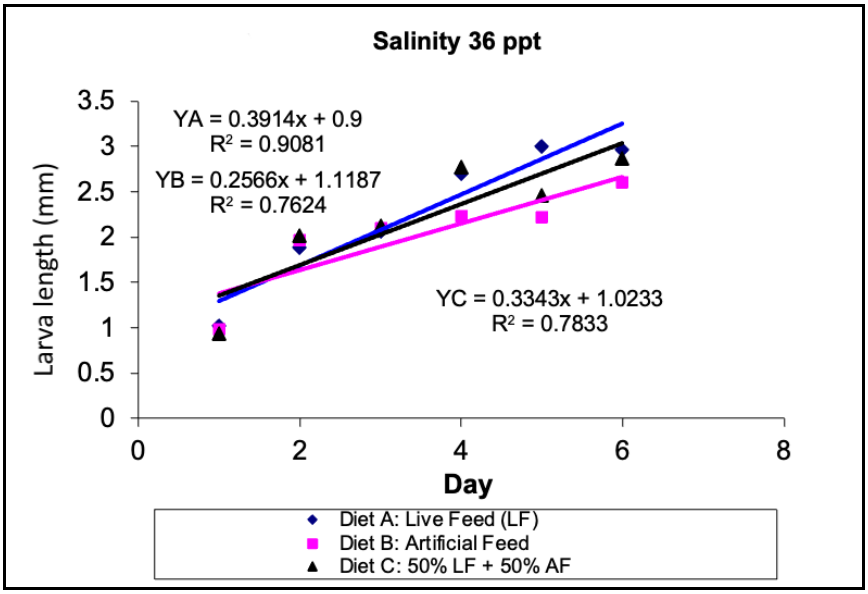

Figure 4. Correlation between *Penaeus merguiensis* larvae lengths and days (days 1-3: Z1-Z3; days 4-6: M1-M3) at a salinity of 36 ppt.

As presented in Figure 4, the equations obtained were: YA=0.3914x+0.9, with R^2=0.9081, for Diet A; YB=0.2566x+1.1187, with R^2=0.7624, for Diet B; YC=0.3343x+1.0233, with R^2=0.7833, for Diet C.

Tests of between-subjects effects for Zoea-1. The test results of the average length of Zoea-1 in different salinities and feed can be seen in Table 1.

Table 1

Tests of between-subjects effects for *Penaeus merguiensis* larvae of Zoea-1

Source	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Corrected model	0.204[a]	8	0.026	1512	0.221
Intercept	27462	1	27462	1624000	0.000
Salinity	0.095	2	0.047	2799	0.087
Feed	0.055	2	0.028	1633	0.223
Salinity x Feed	0.055	4	0.014	0.807	0.537
Error	0.304	18	0.017		
Total	27971	27			
Corrected Total	0.509	26			

Note: a - R Squared=0.402 (Adjusted R Squared=0.136); df - degrees of freedom.

From Table 1, it can be concluded that there is no significant connection between the length of Zoea-1 with variations in the salinity and composition of the diets. Also, the interaction between salinity and feed composition that affected the mean length of Zoea-1 was not significant ($p>0.05$). With the adjusted R Square value of 0.402, the independent variable affects the dependent variable by 40.2%, while other variables influence the remaining 59.8%.

Tests of between-subjects effects for Zoea-2. The test results of the average length of Zoea-2 in different salinities and feed can be seen in Table 2.

Table 2

Tests of between-subjects effects for *Penaeus merguiensis* larvae of Zoea-2

Source	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Corrected model	0.501[a]	8	0.063	2582	0.045
Intercept	101121	1	101121	4171000	0.000
Salinity	0.043	2	0.022	0.891	0.427
Feed	0.021	2	0.011	0.434	0.655
Salinity x Feed	0.436	4	0.109	4501	0.011
Error	0.436	18	0.024		
Total	102058	27			
Corrected Total	0.937	26			

Note: a - R Squared=0.534 (Adjusted R Squared=0.327); df - degrees of freedom.

From Table 2, it can be concluded that there is no significant correlation between Zoea-2 length and variations in salinity and feed composition. However, the interaction between salinity and feed composition in influencing the length of Zoea-2 was significant ($p<0.05$). The adjusted R Square value of 0.534 means that the independent variable affects the dependent variable by 53.4%, while other variables influence the remaining 46.6%.

Tests of between-subjects effects for Zoea-3. The test results of the average length of Zoea-3 in different salinities and feed are presented in Table 3.

Table 3

Tests of between-subjects effects for *Penaeus merguiensis* larvae of Zoea-3

Source	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Corrected model	0.226[a]	8	0.028	0.940	0.509
Intercept	117509	1	117509	3912000	0.000
Salinity	0.001	2	0.000	0.010	0.990
Feed	0.014	2	0.007	0.234	0.794
Salinity x Feed	0.211	4	0.053	1759	0.181
Error	0.541	18	0.030		
Total	118275	27			
Corrected Total	0.767	26			

Note: a - R Squared=0.295 (adjusted R Squared=-0.019); df - degrees of freedom.

From Table 3, it can be concluded that there is no significant correlation between Zoea-3 length on variations in salinity and feed composition. Also, the interaction between salinity and feed composition in influencing Zoea-3 length was not significant ($p>0.05$). With the adjusted R Square value of 0.295, the independent variable affects the dependent variable by 29.5%, while other variables influence the remaining 70.5%.

Tests of between-subjects effects for Mysis-1. The test results of the average length of Mysis-1 in different salinities and feed are presented in Table 4.

From Table 4, it can be concluded that there is a significant association between the length of Mysis-1 on variations in salinity and the diet composition ($p<0.05$). However, the interaction between salinity and feed composition that affects the length of Mysis-1 was not significant ($p>0.05$). With an adjusted R Square value of 0.656, the independent variable affects the dependent variable by 65.6%, while other variables influence the remaining 34.4%.

Table 4

Tests of between-subjects effects for *Penaeus merguiensis* larvae of Mysis-1

Source	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Corrected model	2.549[a]	8	0.319	4289	0.005
Intercept	206112	1	206112	2774000	0.000
Salinity	1537	2	0.769	10346	0.001
Feed	0.862	2	0.431	5799	0.011
Salinity x Feed	0.150	4	0.038	0.505	0.732
Error	1337	18	0.074		
Total	209998	27			
Corrected Total	3886	26			

Note: a - R Squared=0.656 (adjusted R Squared=0.503); df - degrees of freedom.

Tests of between-subjects effects for Mysis-2. The test results of the average length of Mysis-2 in different salinities and feed can be seen in Table 5.

Table 5

Tests of between-subjects effects for *Penaeus merguiensis* larvae of Mysis-2

Source	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Corrected model	4.312[a]	8	0.539	7318	0.000
Intercept	210148	1	210148	2853000	0.000
Salinity	2579	2	1290	17511	0.000
Feed	0.377	2	0.188	2559	0.105
Salinity * Feed	1356	4	0.339	4601	0.010
Error	1326	18	0.074		
Total	215786	27			
Corrected Total	5638	26			

Note: a - R Squared=0.765 (adjusted R Squared=0.660); df - degrees of freedom.

From Table 5, it can be concluded that there was a significant association between the length of Mysis-2 with salinity variations ($p<0.05$) but not with the composition of the feed. The test results also found that the interaction between salinity and feed composition affecting the length of Mysis-2 was significant ($p<0.05$). With an adjusted R Square value of 0.756, the independent variable affects the dependent variable by 75.6%, while other variables influence the remaining 24.4%.

Tests of between-subjects effects for Mysis-3. The test results of the average length of Mysis-3 in different salinities and feed can be seen in Table 6.

Table 6

Tests of between-subjects effects for *Penaeus merguiensis* larvae of Mysis-3

Source	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Corrected model	6.013[a]	8	0.752	4099	0.006
Intercept	282684	1	282684	1541000	0.000
Salinity	4631	2	2315	12626	0.000
Feed	1127	2	0.564	3074	0.071
Salinity x Feed	0.255	4	0.064	0.348	0.842
Error	3301	18	0.183		
Total	291998	27			
Corrected Total	9314	26			

Note: a - R Squared=0.646 (adjusted R Squared=0.488); df - degrees of freedom.

From Table 6, it can be concluded that there is a significant correlation between the length of Mysis-3 with salinity variations ($p<0.05$) but not with the composition of the feed. The test results also found that the interaction between salinity and feed composition affecting the length of Mysis-3 was not significant ($p>0.05$). With an adjusted R Square value of 0.646, the independent variable affects the dependent variable by 64.6%, while other variables influence the remaining 35.4%.

The polynomial regression of time development in different salinity and diet for zoea. Figure 5 presents the polynomial graph of the development period from Zoea-1 to Zoea-3 in different salinities and diets.

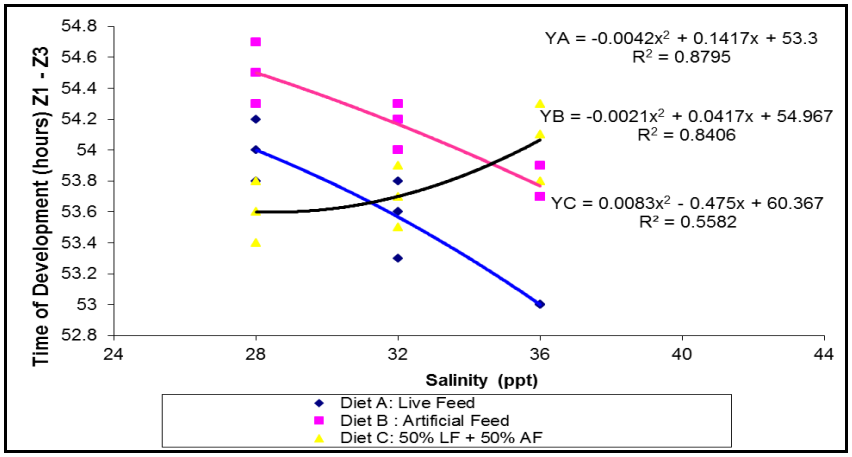

Figure 5. The polynomial regression of time development of *Penaeus merguiensis* larvae from Zoea-1 to Zoea-3 in different salinity and diet.

Figure 5 shows the variation of the polynomial graph: time of development in Diet A with a salinity of 28 ppt fell from 54 to 53 hours at a salinity of 36 ppt; time of development in Diet B with a salinity of 28 ppt decreased from 54.5 to 53.8 hours at a salinity of 36 ppt; time of development of Diet C with a salinity of 28 ppt increased from 53.6 to 54.1 hours at a salinity of 36 ppt.

The linear regression of time development in different salinities and diets for zoea. Figure 6 shows the polynomial graph of time development from Zoea-1 to Zoea-3 in different salinities and diets.

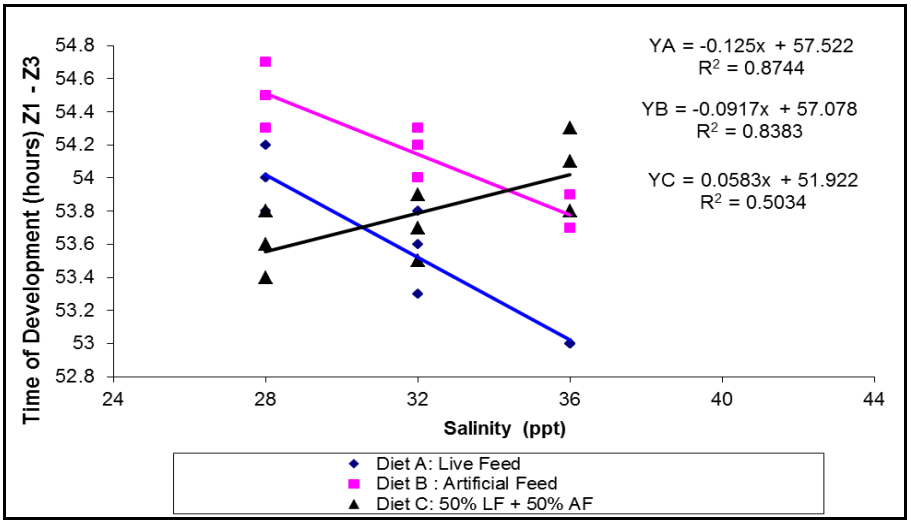

Figure 6. The linear regression of time of development of *Penaeus merguiensis* larvae from Zoea-1 to Zoea-3 in different salinity and diet.

Based on Figure 6, the equations obtained were: YA=-0.125x+57.522, with R^2=0.8744, for Diet A; YB=-0.0917x+57.078, with R^2=0.8383, for Diet B; YC=0.0583x+51.922, with R^2=0.5034, for Diet C. Thus, Diet C is the best feed for Zoea, being marked with a positive line.

The polynomial regression of time development in different salinities and diets for mysis. Figure 7 shows the polynomial graph of time development from Mysis-1 to Mysis-3 in different salinities and diets.

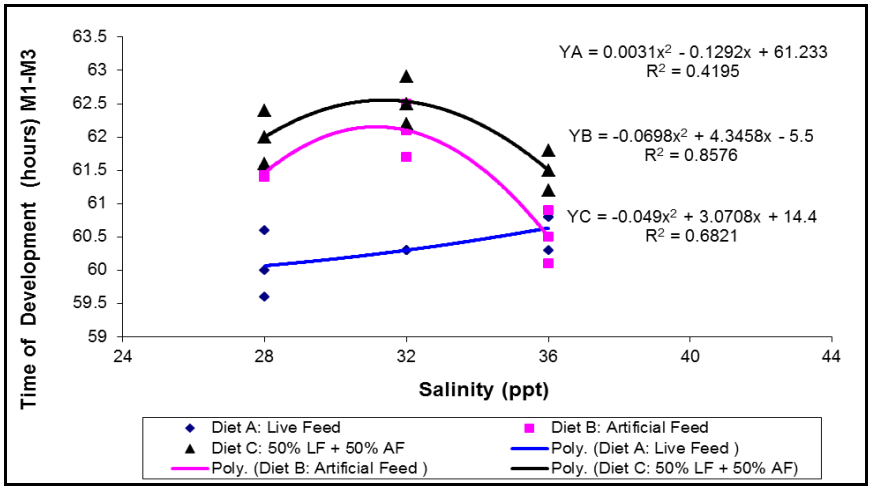

Figure 7. The polynomial regression of time development of *Penaeus merguiensis* larvae from Mysis-1 to Mysis-3 in different salinities and diets.

Figure 7 shows the variation of the polynomial graph: the time of development of *P. merguiensis* larvae for Diet A at a salinity of 28 ppt increased from 60 to 60.5 hours at a salinity of 36 ppt; the time of development for Diet B at a salinity of 28 ppt decreased from 61.5 to 60.5 hours at a salinity of 36 ppt; the time of development for Diet C at a salinity of 28 ppt decreased from 62 to 61.5 hours at a salinity of 36 ppt.

The linear regression of time development in different salinities and diets for mysis. Figure 8 shows the polynomial graph of time development from Mysis-1 to Mysis-3 in different salinities and diets.

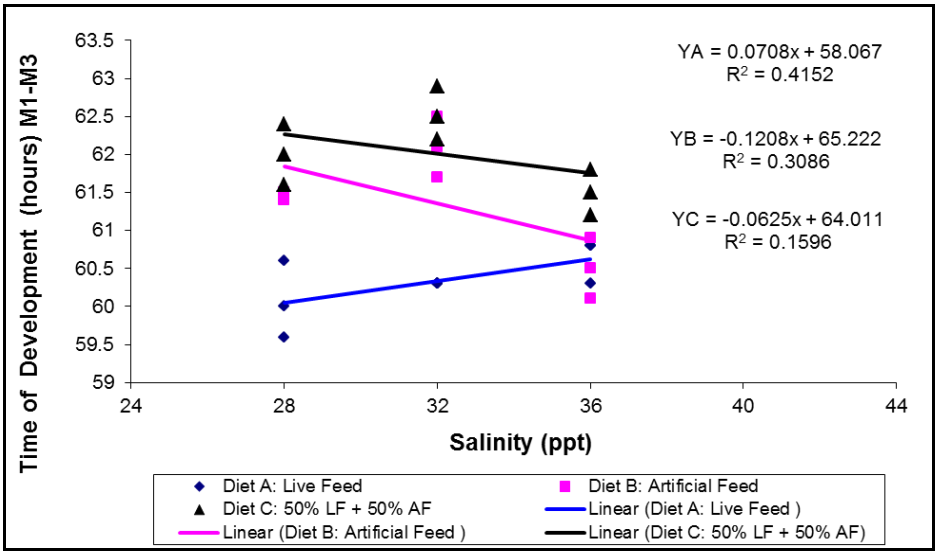

Figure 8. The linear regression of time of development of *Penaeus merguiensis* larvae from Mysis-1 to Mysis-3 in different salinities and diets.

From Figure 8, the equations obtained were: YA=0.0708x+58.067, with R^2=0.4152, for Diet A; YB=-0.1208x+65.222, with R^2=0.3086, for Diet B; YC=-0.0625x+64.011, with R^2=0.1596, for Diet C. Thus, Diet A is the best feed for Mysis, being is marked with a positive line.

Figure 9 shows the survival rate of *P. merguiensis* larvae, showing that the best survival rates were found in the salinity of 28 ppt, with diet A.

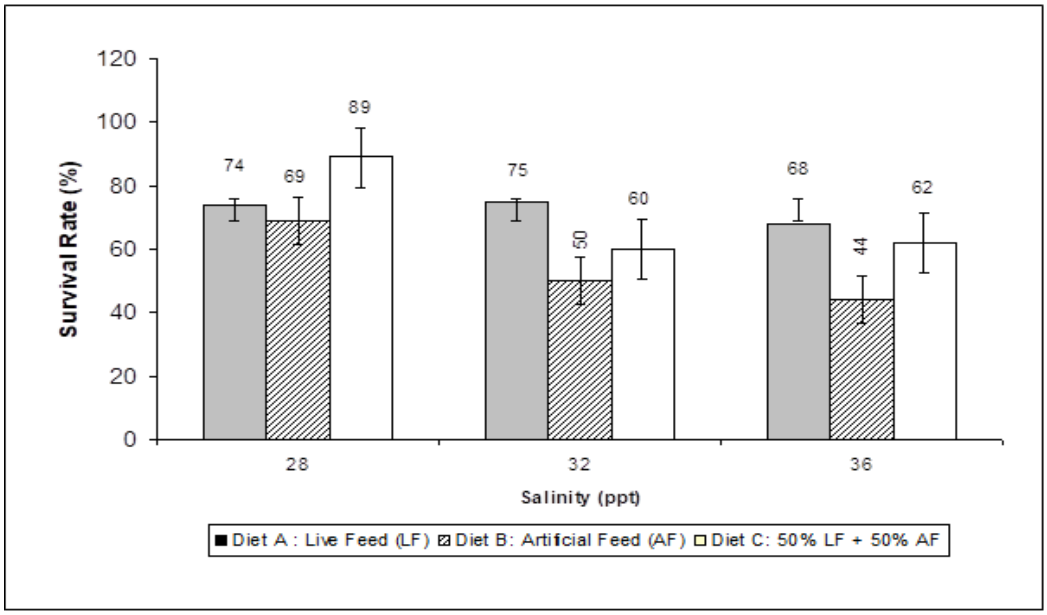

Figure 9. Survival rate based on different salinities and diets.

This study observed that different salinities and types of diets do not significantly alter the length of Zoea 1-3. However, the interaction of salinity and feed was significant for Zoea-2 but not for Zoea-1 and Zoea-3. However, there were significant differences in the length of Mysis 1-3, with different salinities ($p<0.05$), the feed composition influenced Mysis-1 and Mysis-3 only. Also, the interaction between salinity and feed combination only affected the mean length of Mysis-2. Figures 2 to 4 showed a growth trend for zoea and mysis in different salinities and diets, which is supported by Susanto et al (2020), who stated that differences in time development based on salinities and diets for juvenile larvae of *P. merguiensis* varied.

The majority of shrimp larvae were not accustomed to receiving the artificial feed, even though this aspect is important in increasing hatchery intensity. However, based on the polynomial graphs, this study found that artificial diets were positive for Zoea-1 to Zoea-3, and live feed was best for Mysis-1 to Mysis-3. These findings are in line with a study of de Lourdes Cobo et al (2014) on *L. vannamei* larvae, who stated that nutritious-manufactured feed for early cultured larva was considered necessary because of its high nutritional value; later, larvae can balance their diet according to their natural habitat.

Salinity in the waters affects the osmoregulatory balance of the body, and the energetic processes further affect growth (Thi Thu et al 2019). Thus, aquatic organisms have to expend large amounts of energy to adjust to the salinity of their environment. Salinity is a significant environmental parameter that influences crustaceans' survival, distribution, abundance, and physiology (Zhang et al 2009; Antony et al 2019). Although variations in salinity and different dietary patterns affect the growth and survival of cultured larvae (Praveen & Krishna 2015), the unsuitable feed can lead to higher mortality due to inappropriate larval phase duration (Qian et al 2015). Hyposalinity can reduce salt diffusion, and water absorbed from the environment can lead to swollen cells (Chen et al 2015). Accordingly, the present study shows that larvae (zoea to mysis) grew better at lower salinity (28 ppt) than at higher salinity (32 ppt).

Generally, juvenile growth tends to be escalating at low (5-10 ppt) and moderate (15-20 ppt) salinity, and decreased growth with high salinity also characterizes the life history of shrimp (Gao et al 2016). Based on Figure 9, the best survival rates were found in the salinity of 28 ppt. This finding is in line with the study of Yang et al (2010), where the growth differences of shrimp in salinities from 2 to 20 ppt were not significant. Therefore, shrimp are believed to grow optimally in salinity over 20 ppt, but the upper limit should still be considered.

The osmoregulation of a species depends on its distribution patterns and ecological conditions and is an exciting area to understand ecological distribution patterns in order to increase aquaculture production (Maicá et al 2014). Penaeid shrimp have different osmoregulatory abilities during ontogeny (development stage), related to their ecological models. Changes in freshwater flow and salinity can affect the growth and development of penaeid shrimp, and the relationship between salinity and shrimp abundance is complicated because other environmental variables are also very influential. Therefore, laboratory experiments can provide insight into salinity selection by examining shrimp's salinity preferences (Doerr et al 2016).

From the regression line on salinity-based and survival rate results, it is found that artificial feed (Diet C) significantly succeeded in replacing natural feed in accelerating the survival and growth of the newly introduced *P. merguiensis* larvae from Zoea-1 to Zoea-3 at a salinity of 28 ppt. In the mysis stage, Diet A is preferred in salinities of 32 and 36 ppt.

In the last phase, larvae migrate from the ocean to estuarine nurseries with low salinities for more stable growth and development. However, *P. semisulcatus* larvae in the Mediterranean Sea favor higher salinities during the nursery stage. Hence, the newly introduced *P. merguiensis* larvae in the Indo-Pacific region grew and survived better at a salinity between 28-32 ppt (Diet C for zoea and Diet A for mysis).

Conclusions. In the zoea phase, Zoea 1-3 lengths did not change significantly at different salinities. Different feed composition was not significant for lengths of Zoea 1-3, and the interaction between salinity and the combination of feed on the mean length of Zoea-1 and Zoea-3 was not found. Although there were significant differences in the length of Mysis 1-3 in different salinities ($p<0.05$), the feed composition did not have a considerable influence (only for Mysis-1 and Mysis-3), and the interaction between salinity and feed combination affected the mean length of Mysis-1-3 was not significant ($p>0.05$).

This research has empirically proven that an artificial diet (Diet C) was positive for Zoea-1 to Zoea-3 growth. Diet C was the best combination (artificial feed and live feed, 50% each) for the first stage of the cultured larva, and it provided a higher survival rate in 28 ppt salinity. Mysis-1 to Mysis-3 survived better at a salinity of 32 ppt with live feed (Diet A). Therefore, it is better to treat Zoea with Diet C at 28 ppt salinity and treat mysis with Diet A at the salinity of 28 and 32 ppt afterward. Thus, the newly introduced *P. merguiensis* larvae in the Indo-Pacific region grew and survived better at a salinity between 28 and 32 ppt (Diet C for zoea and Diet A for mysis).

Due to several limitations, this study suggests further research to satisfy several questions concerning *P. merguiensis*: optimal farming systems, potential alternatives related to its biodiversity, general description of opportunities and limitations of its biodiversity, restoration of its biodiversity, its state on biophysical changes (soil, water, flora, and fauna) resulting from socio-economic changes, recognition of effects on ecosystem connectivity and possible cumulative effects, and information on conditions for its potential farming. The improvement of services to support sustainable ecology principles must be highlighted to prevent and reduce damage to shrimp biodiversity.

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Conflict of interest. The authors declare that there is no conflict of interest.

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