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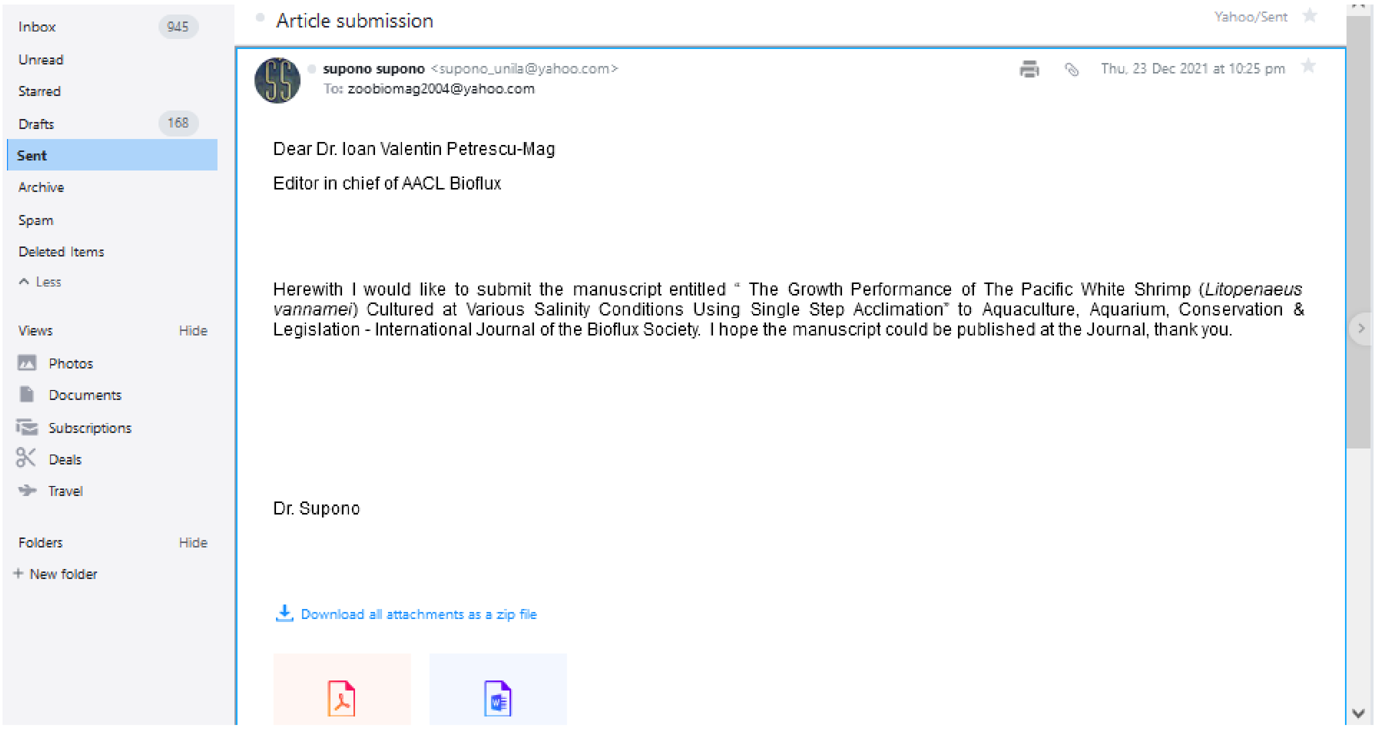
The growth performance of the Pacific white shrimp (*Litopenaeus vannamei*) cultured at various salinity conditions using single step acclimation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To: zoobiomag2004@yahoo.com

Thu, 23 Dec 2021 at 10:25 pm

Dear Dr. Ioan Valentin Petrescu-Mag

Editor in chief of AACL Bioflux

Herewith I would like to submit the manuscript entitled " The Growth Performance of The Pacific White Shrimp (*Litopenaeus vannamei*) Cultured at Various Salinity Conditions Using Single Step Acclimation" to Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society. I hope the manuscript could be published at the Journal, thank you.

Dr. Supono

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THE GROWTH PERFORMANCE OF THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) CULTURED AT VARIOUS SALINITY CONDITIONS USING SINGLE STEP ACCLIMATION

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Abstract. The Pacific white shrimp (*L. vannamei*) is a euryhaline species, which can be reared in aquatic environments with a wide range of salinity from 0.5 to 40 ppt. The ability of Pacific white shrimps to adapt to extreme saline conditions allows for the expansion of shrimp farming in inland aquatic environments away from the coast. Therefore, this study aimed to analyze specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) of Pacific white shrimp reared at various salinity levels using a single-step acclimation method. The study employed a completely randomized design with five treatments and three replications including T1, T2, T3, T4, and T5 at 5 ppt, 10 ppt, 15 ppt, 20 ppt, 25 ppt, respectively. Each treatment unit was incorporated with Pacific white shrimp PL 15 at a density of 2000 PL's/m3. Subsequently, the shrimps were fed commercial pellets three times daily through a blind feeding program during the 40 days of rearing. The results showed that different salinities of culture media had an effect on SGR, SR, and FCR of Pacific white shrimp. The most desirable specific growth rate and feed conversion ratio were observed in 15 ppt culture media at 12.3% and 1.5, respectively, while the best survival rate was observed in 20 ppt salinity at 79.3%. Generally, Pacific white shrimp performed best in the culture media with a salinity of 15 ppt.
Keywords: *Litopenaeus vannamei*, salinity euryhaline, growth, survival rate

Introduction. The Pacific white shrimp (*L. vannamei*) is a euryhaline species that can tolerate a wide range of salinity between 0.5 to 45 ppt (Wyban and Sweeny 1991), allowing for high-yield rearing away from the coast. The rearing of Pacific white shrimp at low salinity is one of the best options to optimize the use of low-salinity ponds located far from seawater sources due to the high salinity tolerance range of this shrimp (Chong-Robles 2014). Furthermore, this is important since there are numerous brackish water aquaculture centers located inland that can be used for Pacific white shrimp farming (Supono 2021).

Although the Pacific white shrimp can adapt to a wide range of salinities, it grows best in iso-osmotic media at a salinity equal to the osmotic tolerance range of common shrimps (Anggoro et al 2018). Therefore, the rate of osmosis is a measure of the processes that occur in aquatic animals during times of environmental stress (Cheng et al 2006). Differences in media salinity and fluid osmolarity in shrimp (hemolymph) can lead to increased energy requirements for adaptation, retardation of shrimp growth, and metabolism (Gao et al 2016). Meanwhile, shrimps can only survive by utilizing available energy during conditions of low salinity, where an extremely high osmotic load reduces shrimp survival (Susilowati et al 2014; Jayasankar et al 2009). The salinity of the media can be adjusted based on the osmotic pressure of the media to increase the growth of cultured species (Lukas et al 2017). Acclimatization to temperature and salinity is an important factor that affects the success of low-salinity shrimp farming. Acclimatization systems for stocking shrimp seeds are classified into two types, which include one-step acclimation and gradual acclimation (Jayasankar et al 2009), where each technique has its advantages and disadvantages. The single-step acclimation is easy to implement with a low survival rate sometimes, while gradual acclimation is difficult to implement in the field. Therefore, this study aimed to evaluate the growth rate, survival rate, and feed conversion ratio of Pacific white shrimp reared under various salinity conditions.

Material and Method

Experimental Design and Setup. This study used a completely randomized design (CRD), which consisted of 5 treatments and 3 replications. The treatments include the following:

T1: culture media at salinity of 5 ppt
T2: culture media at salinity of 10 ppt
T3: culture media at salinity of 15 ppt
T4: culture media at salinity of 20 ppt
T5: culture media at salinity of 25 ppt

The study made use of 15 plastic containers filled with 70 liters of water, which was sterilized with chlorine at a concentration of 30 mg/L. Each container was filled with 70 liters of water at varying salinity based on the treatment. Furthermore, the aeration settings were performed in each maintenance container, with up to four aeration stones per container serving as an oxygen supply to the culture container. Each treatment unit was stocked with 140 shrimp (PL 15), or the equivalent of 2000 PL's/m^3. Acclimatization was conducted using a single-step acclimation system, while feeding using a blind feeding program three times daily at 6.30, 12.00, and 19.00, throughout the 45-day culture period. The feed used was commercial feed containing protein fat, fiber, ash, and moisture at 41%, 7%, 3%, 13%, and 10%, respectively.

Statistical analysis Pacific white shrimp was evaluated based on specific growth rate (SGR), survival rate (SR), biomass, and feed conversion ratio (FCR). The data were analyzed using ANOVA at a 95% confidence level (P<0.05) using the SPSS 23.0 program. Further testing was conducted using the Duncan test when there is a significant difference between variables.

Results. Table 1 showed the data from observations made during the study on SGR, SR, biomass, and FCR of Pacific white shrimp. The Anova statistical test results showed that the rearing of white shrimps in various saline media has a significant effect on its SGR, SR, biomass, and FCR (P<0.05).

Tabel 1.

Growth Performance of Pacific White Shrimp

Parameter	P1 (5 ppt)	P2 (10 ppt)	P3 (15 ppt)	P4 (20 ppt)	P5 (25 ppt)
Growth rate (g)	0.6 ± 0.1^{a}	1.4 ± 0.2^{c}	2.8 ± 0.1^{d}	0.8 ± 0.2^{ab}	1.2 ± 0.1^{bc}
SGR (%)	$8.,4 \pm 0.7^{a}$	$10,4. \pm 0.3^{c}$	$12.3 \pm 0,1^{d}$	9.4 ± 0.8^{ab}	$10.,3 \pm 0.2^{bc}$
Biomass (g)	$40.6 \pm 17,5^{a}$	114.0 ± 19.4^{b}	251.3 ± 33.3^{c}	98.3 ± 29.3^{b}	$106,0 \pm 11.5^{b}$
SR (%)	47 ± 6^{a}	56 ± 2^{ab}	63 ± 11^{b}	79 ± 6^{c}	61 ± 2^{b}
FCR	1.9 ± 0.2^{b}	1.8 ± 0.1^{b}	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}	1.8 ± 0.2^{b}

Note: Different lowercase letters indicate significantly different treatment effects (P<0.05)

The highest absolute weight growth and SGR were obtained in the 15 ppt salinity at 2.8±0.1 g and 12.3±0.1% per day, respectively. Additionally, this was followed by 10 ppt salinity at 1.4±0.2 g and 10.4±0,3% per day, 25 ppt salinity at 1.2±0.1 g and 10.3±0.2% per day, as well as 20 ppt salinity at 0.8±0.2 g and 9.4±0.8% per day, respectively. The lowest value was obtained in the 5 ppt salinity at 0.6±0.1 g and 8.4±0.7% per day. According to Duncan's further test, there was a significant difference between the salinity conditions at 15 ppt and the 5 ppt, 10 ppt, 20 ppt, and 25 ppt.

The highest SR of Pacific white shrimp occurred in 20 ppt salinity at 79±6%, which was followed by 15 ppt, 25 ppt, 10 ppt, and 5 ppt salinity at 63±11%, 61±2%, 56±2%, and 47±6%, respectively. According to Duncan's further test, there was a significant difference in the salinity of 20 ppt compared to the 5 ppt, 10 ppt, 15 ppt, and 25 ppt.

The highest FCR occurred in the 5 ppt media salinity at 1.9 ± 0.2, which was followed by 10, 25, 20, and 15 ppt at salinities of 1.8±0.1, 1.8±0.2, 1.6±0.1, and 1.5±0.1, respectively. Duncan's further test showed that there was a significant difference in the salinity conditions of 5 ppt and 10 ppt compared to the 15 ppt, 20 ppt, and 25 ppt.

Table 2 shows the water quality data collected during the study, which included, sodium, magnesium, calcium, potassium, pH, temperature, and dissolved oxygen. The water quality in the culture media is still in accordance with the standards for Pacific white shrimp culture.

Table 2.

Water Quality Parameters during Culture

No	Parameters	Unit	5 ppt	10 ppt	15 ppt	20 ppt	25 ppt
1	Sodium (Na)	mg/L	1195	2384.5	3585	4774.6	5964.1
2	Magnesium (Mg)	mg/L	130.6	258.5	387.5	515.3	643.2
3	Calsium (Ca)	mg/L	73	142.2	212	281.2	350.4
4	Potassium (K)	mg/L	65.1	128.8	193	256.6	320.3
5	pH		7.2-7.9	7.2-7.9	7.3-7.9	7.3-7.9	7.2-7.9
6	Suhu	° C	28-29	28-29	28-29	28-29	28-29
7	Dissolved oxygen	mg/L	4.6-6.2	4.6-6.2	4.9-6.4	4.9-6.4	4.8-6.5

Discussion. The use of single-step acclimation reduced SR of Pacific white shrimp. The results show that the SR of Pacific white shrimp ranges between 47 to 79%. According to Jayasankar et al (2009), the single-step acclimation on Pacific white shrimp with a media salinity of 5 ppt led to SR of 53%. A sudden drop in salinity over a wide range makes osmoregulation difficult for aquatic animals, which can lead to death (Laramore 2001).

The highest SR was observed in the culture media with a salinity of 20ppt at 79±6% when compared to the other treatments. The shrimp had little difficulty in adapting to a salinity of 20 ppt, which was similar to the salinity of the previous culture media. Salinity differences have a significant impact on the SR of Pacific white shrimp. Meanwhile, shrimp mortality during rearing could be caused by a large difference in the osmotic pressure of the shrimp's body and its environment. Physiological processes can be disrupted when the osmotic value between the body fluids and the environment is too high, leading to stress and cell damage (Brocker et al 2012). Additionally, the SR of shrimps can be affected by the frequency of shrimp molting, cannibalism, and water quality. Low salinity treatment led to the incomplete molting of vannamei shrimps since the exoskeleton was not hard enough to trigger the release of the carapace. Therefore, this reduces SR of Pacific white shrimp, as well as their gradual death.

The Pacific white shrimp in a culture media with a salinity of 15 ppt produced the best growth (SGR = 12.3 ±0.1%), which was accompanied by a drop in the value of FCR. According to Wyban and Sweeny (1991) and Davis et al (2002), Pacific white shrimp grow very well at low salinity around 10-15 ppt where there is an iso-osmotic equilibrium between the environment and the hemolymph of the shrimp, while according to Jaffer et al (2020), the isosmotic point occurs at a salinity of 21.1 ppt. A previous study by Rahman et al (2016) is consistent with this study, which discovered that a salinity treatment of 15 ppt is the most effective condition for Pacific white shrimp growth. According to Bückle et al (2006), *L. vannamei* hyperregulates between at salinities of 10 and 20 ppt and hyporegulates between 20 and 40 ppt.

The osmotic pressure of the culture medium osmotic pressure has an effect on the osmoregulation of aquatic organisms, such as shrimp. According to Anggoro (2018), shrimps lose much water through the diffusion process at high salinity (hypertonic). Furthermore, they adapt to this condition by drinking much water and avoiding excess salt through various mechanisms, such as the hardening of the exoskeleton, which inhibits the molting process. The regulation of ion concentration in the blood of crustaceans is very important since the osmotic pressure of the blood is higher than the external pressure of the environment at low salinity, and vice versa at high salinity. Additionally, the differences in osmotic pressure affect the bioenergetics of aquatic organisms (Ingraham and Lignot 2017). According to Anggoro et al (2018), the salinity of isoosmotic media has an effect on the level of osmotic workload required to maintain osmolarity pressure balance (media and blood) or electrolyte content balance, such that the higher the osmotic work rate, the more energy is wasted on osmotic performance, which leads to a reduction in shrimp growth. According to Rachmawati et al (2012), the growth of white vaname shrimps can also be inhibited when more energy is consumed during osmoregulation since this reduces the amount of energy available for growth. Therefore, aquatic organisms will grow optimally if the culture medium is isosmotic with their body fluids (Ferraris et al 1987).

Minerals are important for the growth and survival of cultured shrimps, especially potassium (Kaligis 2010), calcium (Kaligis, 2015), and magnesium (Roy et al 2010). The salinity of the water can give a measure of its mineral content, which has more influence on the growth performance of shrimps, even though salinity plays a role in SGR and SR (Boyd, 2018). Shrimp cultures often experience mineral deficiencies in low salinity, such as calcium and potassium deficiency. Table 3 shows that the calcium and potassium concentrations in the culture media are still within the standards for Pacific white shrimp farming.

Table 3.
Calcium and potassium concentrations in culture media and standards

Salinitas (ppt)	Calcium (mg/L)	Potassium (mg/L)	Standards (Boyd, 2018)	
			Calsium (mg/L)	Potassium (mg/L)
5	73	65	58	55
10	142.2	129	116	110
15	212	193	174	165
20	281.2	257	232	220
25	350.4	320	290	275

Conclusions. The salinity of the culture medium had an effect on SGR, SR, and FCR of Pacific white shrimp. The optimal SGR, biomass, and FCR occurred at 15 ppt salinity, while the optimal SR occurred at 20 ppt salinity. Therefore, it can be concluded that a media salinity of 15 ppt provided the best performance for Pacific white shrimp rearing.

Acknowledgment. This study was funded by Lampung University through the Postgraduate Research program in 2021.

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AACL Bioflux - manuscript reception confirmation 6 — Yahoo/Inbox

Tudor Papuc <ptudor2008@yahoo.com> — Tue, 28 Dec 2021 at 9:44 pm
To: supono_unila@yahoo.com

Dear Dr. Supono,

This is a message of confirmation regarding the reception of your manuscript THE GROWTH PERFORMANCE OF THE PACIFIC WHITE SHRIMP (Litopenaeus vannamei) CULTURED AT VARIOUS SALINITY CONDITIONS USING SINGLE STEP ACCLIMATION , submitted to our journal AACL Bioflux. I will soon contact you with further information regarding your manuscript.

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2. Dr. G. Nugroho Susanto (scopus ID: 7801438504 ; E-mail: gnugrohos@gmail.com)

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supono supono Thanks a lot — Fri, 21 Jan at 10:28 am

supono supono Dear Tudor Papuc I'm sorry, I'd like to ask about the progress of the article with the title: The Growth Performance of The Pacific White Shrimp (Litopenaeus vannamei) Cultured at Various S. — Tue, 22 Feb at 9:19 am

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Sat, 26 Feb at 3:40 pm

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After the evaluation arrives (it will most likely be positive), I will need about 1-2 weeks to correct the English and the formatting, and then to send you the manuscript with comments from reviewers.

So, I think that sometime between 25-30 April I will be able to send you the paper for revisions (or sooner). Afterwards, the publication will go much faster, because it depends only on us.

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Tudor Păpuc
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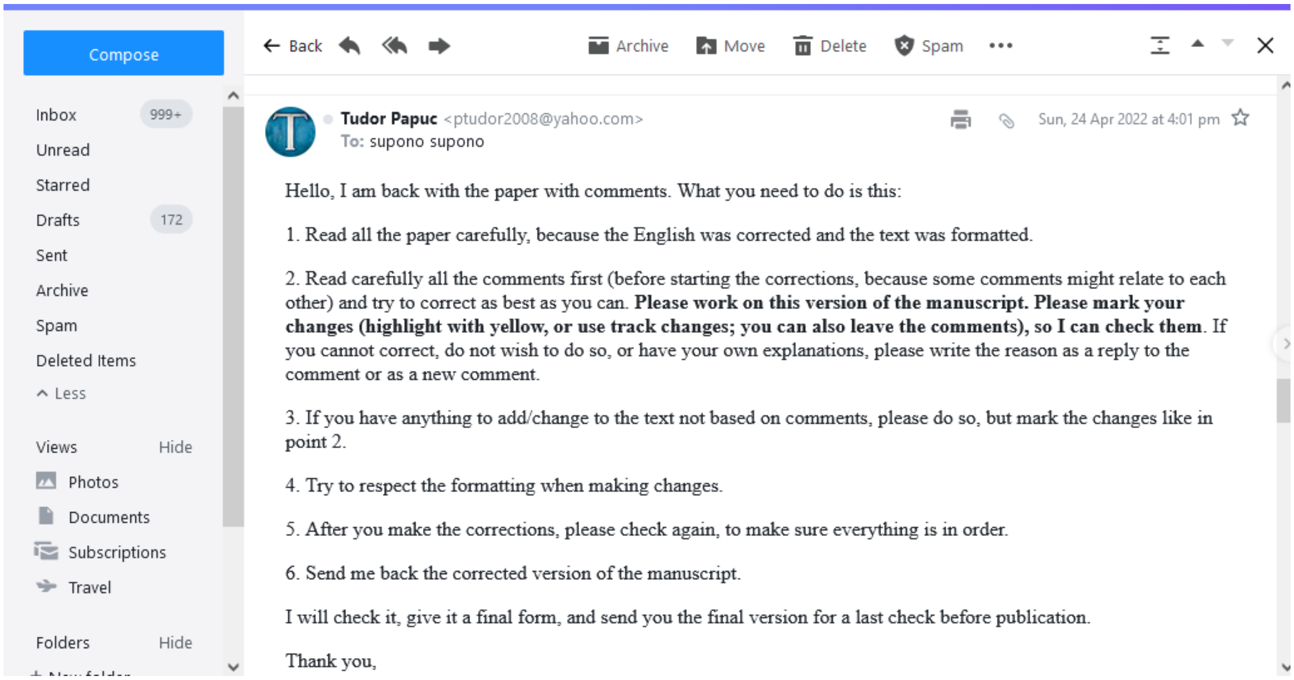

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2. Read carefully all the comments first (before starting the corrections, because some comments might relate to each other) and try to correct as best as you can. **Please work on this version of the manuscript. Please mark your changes (highlight with yellow, or use track changes; you can also leave the comments), so I can check them**. If you cannot correct, do not wish to do so, or have your own explanations, please write the reason as a reply to the comment or as a new comment.

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Thank you,

The growth performance of the Pacific white shrimp (*Litopenaeus vannamei*) cultured at various salinity conditions using single step acclimation

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Abstract. The Pacific white shrimp (*Litopenaeus vannamei*) is a euryhaline species that can be reared in aquatic environments with a wide range of salinity, from 0.5 to 40 ppt. The ability of Pacific white shrimps to adapt to extreme saline conditions allows the expansion of shrimp farming in inland aquatic environments, away from the coast. Therefore, this study aimed to analyze the specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) of Pacific white shrimp reared at various salinity levels using a single-step acclimation method. The study employed a completely randomized design with five treatments and three replications, namely T1, T2, T3, T4, and T5 at 5 ppt, 10 ppt, 15 ppt, 20 ppt, and 25 ppt, respectively. Each treatment unit was incorporated with Pacific white shrimp PL 15 at a density of 2000 PL m^{-3}. Subsequently, the shrimps were fed commercial pellets three times daily through a blind feeding program during the 40 days of rearing. The results showed that different salinities of culture media had an effect on SGR, SR, and FCR of Pacific white shrimp. The most desirable SGR and FCR were observed in the 15 ppt culture media at 12.3% and 1.5, respectively, while the best survival rate was observed in the 20 ppt salinity at 79.3%. Generally, Pacific white shrimp performed best in the culture media with a salinity of 15 ppt.

Key Words: euryhaline, growth, *L. vannamei*, survival rate.

Comment [u1]: at first mentioning, please use the full words, and only afterwards the abbreviations

Introduction. The Pacific white shrimp (*Litopenaeus vannamei*) is a euryhaline species that can tolerate a wide range of salinity between 0.5 to 45 ppt (Wyban & Sweeny 1991), allowing for high-yield rearing away from the coast. The rearing of Pacific white shrimp at low salinity is one of the best options to optimize the use of low-salinity ponds located far from seawater sources, due to the high salinity tolerance range of this shrimp (Chong-Robles et al 2014). Furthermore, this is important since there are numerous brackish water aquaculture centers located inland that can be used for Pacific white shrimp farming (Supono 2021).

Comment [u2]: inland where?

Although the Pacific white shrimp can adapt to a wide range of salinities, it grows best in iso-osmotic media at a salinity equal to the osmotic tolerance range of common shrimps (Anggoro et al 2018). The rate of osmosis is a measure of the processes that occur in aquatic animals during times of environmental stress (Cheng et al 2006). Differences in media salinity and fluid osmolarity in shrimp (hemolymph) can lead to increased energy requirements for adaptation, retardation of shrimp growth and metabolism (Gao et al 2016). Meanwhile, shrimps can only survive by utilizing available energy during conditions of low salinity, where an extremely high osmotic load reduces shrimp survival (Jayasankar et al 2009; Susilowati et al 2014). The salinity of the media can be adjusted based on the osmotic pressure of the media to increase the growth of cultured species (Lukas et al 2017). Acclimatization to temperature and salinity is an important factor that affects the success of low-salinity shrimp farming. Acclimatization systems for stocking shrimp juveniles are classified into one-step acclimation and gradual acclimation (Jayasankar et al 2009). Each technique has its advantages and disadvantages. The single-step acclimation is easy to implement, but sometimes has a

low survival rate. Gradual acclimation is difficult to implement in the field. Therefore, this study aimed to evaluate the growth rate, survival rate, and feed conversion ratio of Pacific white shrimp reared under various salinity conditions.

Material and Method

Experimental design and setup. This study used a completely randomized design (CRD), which consisted of 5 treatments and 3 replications. The treatments include the following: T1 - culture media at salinity of 5 ppt; T2 - culture media at salinity of 10 ppt; T3 - culture media at salinity of 15 ppt; T4 - culture media at salinity of 20 ppt; T5 - culture media at salinity of 25 ppt.

Comment [u3]: please mention when and where the study was conducted

The study made use of 15 plastic containers filled with 70 L of water, which was sterilized with chlorine at a concentration of 30 mg L^{-1}. Each container was filled with 70 L of water at varying salinity based on the treatment. Furthermore, the aeration settings were performed in each maintenance container, with up to four aeration stones per container serving as an oxygen supply to the culture container. Each treatment unit was stocked with 140 Pacific white shrimp (PL 15), or the equivalent of 2000 PL m^{-3}. Acclimatization was conducted using a single-step acclimation system, while feeding was carried out using a blind feeding program three times daily at 6.30, 12.00, and 19.00, throughout the 45-day culture period. The feed used was commercial feed containing protein fat, fiber, ash, and moisture at 41%, 7%, 3%, 13%, and 10%, respectively.

Comment [u4]: where were the shrimp obtained from?

Statistical analysis. Pacific white shrimp was evaluated based on specific growth rate (SGR), survival rate (SR), biomass, and feed conversion ratio (FCR). The data were analyzed using ANOVA at a 95% confidence level ($p<0.05$) using the SPSS 23.0 software. Further testing was conducted using the Duncan test when there were significant differences between variables.

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Results and Discussion. Table 1 shows the data from observations made during the study on SGR, SR, biomass, and FCR of Pacific white shrimp. The ANOVA statistical test results showed that the rearing of white shrimps in various saline media has a significant effect on their SGR, SR, biomass, and FCR ($p<0.05$).

Table 1

Growth performance of Pacific white shrimp

Parameter	*P1 (5 ppt)*	*P2 (10 ppt)*	*P3 (15 ppt)*	*P4 (20 ppt)*	*P5 (25 ppt)*
Growth rate (g)	0.6±0.1^{a}	1.4±0.2^{c}	2.8±0.1^{d}	0.8±0.2^{ab}	1.2±0.1^{bc}
SGR (%)	8.4±0.7^{a}	10.4±0.3^{c}	12.3±0.1^{d}	9.4±0.8^{ab}	10.3±0.2^{bc}
Biomass (g)	40.6±17.5^{a}	114±19.4^{b}	251.3±33.3^{c}	98.3±29.3^{b}	106±11.5^{b}
SR (%)	47±6^{a}	56±2^{ab}	63±11^{b}	79±6^{c}	61±2^{b}
FCR	1.9±0.2^{b}	1.8±0.1^{b}	1.5±0.1^{a}	1.6±0.1^{a}	1.8±0.2^{b}

Note: SGR - specific growth rate; SR - survival rate; FCR - feed conversion ratio; different superscripts indicate significant differences ($p<0.05$).

Comment [u6]: please use the same notations as presented in Material and Method

The highest absolute weight growth and SGR were obtained in the 15 ppt salinity, 2.8±0.1 g and 12.3±0.1% per day, respectively. Additionally, this was followed by 10 ppt salinity, with 1.4±0.2 g and 10.4±0.3% per day, 25 ppt salinity at 1.2±0.1 g and 10.3±0.2% per day, as well as 20 ppt salinity at 0.8±0.2 g and 9.4±0.8% per day, respectively. The lowest value was obtained in the 5 ppt salinity at 0.6±0.1 g and 8.4±0.7% per day. According to Duncan's test, there were significant differences among the salinity conditions at 15 ppt and the 5 ppt, 10 ppt, 20 ppt, and 25 ppt.

The highest SR of Pacific white shrimp occurred in 20 ppt salinity at 79±6%, which was followed by 15 ppt, 25 ppt, 10 ppt, and 5 ppt salinity at 63±11%, 61±2%, 56±2%, and 47±6%, respectively. According to Duncan's further test, there was a significant difference in the salinity of 20 ppt compared to the 5 ppt, 10 ppt, 15 ppt, and 25 ppt.

The highest FCR occurred in the 5 ppt media salinity at 1.9 ± 0.2, which was followed by 10, 25, 20, and 15 ppt at salinities of 1.8±0.1, 1.8±0.2, 1.6±0.1, and 1.5±0.1, respectively. Duncan's further test showed that there was a significant difference in the salinity conditions of 5 ppt and 10 ppt compared to the 15 ppt, 20 ppt, and 25 ppt.

Table 2 shows the water quality data collected during the study, which included sodium, magnesium, calcium, potassium, pH, temperature, and dissolved oxygen. The water quality in the culture media is still in accordance with the standards for Pacific white shrimp culture.

Table 2

Water quality parameters during culture

No	*Parameters*	*Unit*	*5 ppt*	*10 ppt*	*15 ppt*	*20 ppt*	*25 ppt*
1	Sodium (Na)	mg L^{-1}	1195	2384.5	3585	4774.6	5964.1
2	Magnesium (Mg)	mg L^{-1}	130.6	258.5	387.5	515.3	643.2
3	Calcium (Ca)	mg L^{-1}	73	142.2	212	281.2	350.4
4	Potassium (K)	mg L^{-1}	65.1	128.8	193	256.6	320.3
5	pH		7.2-7.9	7.2-7.9	7.3-7.9	7.3-7.9	7.2-7.9
6	Suhu	°C	28-29	28-29	28-29	28-29	28-29
7	Dissolved oxygen	mg L^{-1}	4.6-6.2	4.6-6.2	4.9-6.4	4.9-6.4	4.8-6.5

The use of single-step acclimation reduced SR of Pacific white shrimp. The results show that the SR of Pacific white shrimp ranged between 47 and 79%. According to Jayasankar et al (2009), the single-step acclimation of Pacific white shrimp in a media of 5 ppt salinity led to a SR of 53%. A sudden drop in salinity over a wide range makes osmoregulation difficult for aquatic animals, which can lead to death (Laramore et al 2001).

The highest SR was observed in the culture media with a salinity of 20 ppt, 79±6%. The shrimp had little difficulty in adapting to a salinity of 20 ppt, which was similar to the salinity of the previous culture media. Salinity differences have a significant impact on the SR of Pacific white shrimp. Meanwhile, shrimp mortality during rearing could be caused by a large difference in the osmotic pressure between the shrimp's body and its environment. Physiological processes can be disrupted when the osmotic value between the body fluids and the environment is too high, leading to stress and cell damage (Brocker et al 2012). Additionally, the SR of shrimps can be affected by the frequency of shrimp molting, cannibalism, and water quality. The low salinity treatment led to the incomplete molting of vannamei shrimps since the exoskeleton was not hard enough to trigger the release of the carapace. Therefore, this reduced the SR of Pacific white shrimp, provoking their gradual death.

The Pacific white shrimp in the culture media with a salinity of 15 ppt produced the best growth (SGR=12.3±0.1%), which was accompanied by a drop in the value of FCR. According to Wyban & Sweeny (1991) and Davis et al (2002), Pacific white shrimp grow very well at low salinities around 10-15 ppt, where there is an iso-osmotic equilibrium between the environment and the hemolymph of the shrimp. According to Jaffer et al (2020), the isosmotic point occurs at a salinity of 21.1 ppt. A previous study by Rahman et al (2016) obtained results consistent with those of this study, discovering that a salinity treatment of 15 ppt is the most effective for Pacific white shrimp growth. According to Bückle et al (2006), *L. vannamei* hyperregulates between salinities of 10 to 20 ppt and hyporegulates between 20 to 40 ppt.

The osmotic pressure of the culture medium has an effect on the osmoregulation of aquatic organisms, such as shrimp. According to Anggoro et al (2018), shrimps lose much water through the diffusion process at high salinity (hypertonic). Furthermore, they adapt to this condition by drinking much water and avoiding excess salt through various mechanisms, such as the hardening of the exoskeleton, which inhibits the molting

Comment [u7]: please delete; the info has been already presented in the table, and the text does not bring anything new to what was already presented

Comment [u8]: please mention what parameters you determined, how, and with what instruments in Material and Method

Comment [u9]: please add a reference for the standard values

Comment [u10]: please add another column in the table with the standard values, for a clear comparison

Comment [u11]: temperature? please use English process. The regulation of ion concentration in the blood of crustaceans is very important, since the osmotic pressure of the blood is higher than the external pressure of the environment at low salinity, and vice versa at high salinity. Additionally, the differences in osmotic pressure affect the bioenergetics of aquatic organisms (Rivera-Ingraham & Lignot 2017). According to Anggoro et al (2018), the salinity of iso-osmotic media has an effect on the level of osmotic workload required to maintain osmolarity pressure balance (media and blood) or electrolyte content balance; a higher osmotic work rate means more energy wasted on osmotic performance, which leads to a reduction in shrimp growth. According to Rachmawati et al (2012), the growth of white vannamei shrimps can also be inhibited when more energy is consumed during osmoregulation, since this reduces the amount of energy available for growth. Therefore, aquatic organisms will grow optimally if the culture medium is iso-osmotic with their body fluids (Ferraris et al 1987).

Minerals are important for the growth and survival of cultured shrimps, especially potassium (Kaligis 2010), calcium (Kaligis 2015), and magnesium (Roy et al 2010). The salinity of the water can give a measure of its mineral content, which has an influence on the growth performance of shrimps, even though salinity itself plays a role in SGR and SR (Boyd 2018). Shrimp cultures often experience mineral deficiencies in low salinity, such as calcium and potassium deficiency. Table 3 shows the calcium and potassium concentrations in the culture media, which were still within the standards for Pacific white shrimp farming.

Table 3

Calcium and potassium concentrations in culture media and standards

Salinity (ppt)	*Current study*		*Standards (Boyd 2018)*	
	Calcium (mg L-1)	*Potassium (mg L^{-1})*	*Calcium (mg L^{-1})*	*Potassium (mg L^{-1})*
5	73	65	58	55
10	142.2	129	116	110
15	212	193	174	165
20	281.2	257	232	220
25	350.4	320	290	275

Conclusions. The salinity of the culture medium had an effect on SGR, SR, and FCR of Pacific white shrimp. The optimal SGR, biomass, and FCR occurred at 15 ppt salinity, while the optimal SR occurred at 20 ppt salinity. Therefore, it can be concluded that a media salinity of 15 ppt provided the best performance for Pacific white shrimp rearing.

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

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The growth performance of the Pacific white shrimp (*Litopenaeus vannamei*) cultured at various salinity conditions using single step acclimation

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Statistical analysis. Pacific white shrimp was evaluated based on specific growth rate (SGR), survival rate (SR), biomass, and feed conversion ratio (FCR). The data were analyzed using ANOVA at a 95% confidence level ($p<0.05$) using the SPSS 23.0 software. Further testing was conducted using the Duncan test when there were significant differences between treatments.

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SGR (%)	8.4 ± 0.7^{a}	10.4 ± 0.3^{c}	12.3 ± 0.1^{d}	9.4 ± 0.8^{ab}	10.3 ± 0.2^{bc}
Biomass (g)	40.6 ± 17.5^{a}	114 ± 19.4^{b}	251.3 ± 33.3^{c}	98.3 ± 29.3^{b}	106 ± 11.5^{b}
SR (%)	47 ± 6^{a}	56 ± 2^{ab}	63 ± 11^{b}	79 ± 6^{c}	61 ± 2^{b}
FCR	1.9 ± 0.2^{b}	1.8 ± 0.1^{b}	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}	1.8 ± 0.2^{b}

Note: SGR - specific growth rate; SR - survival rate; FCR - feed conversion ratio; different superscripts indicate significant differences ($p<0.05$).

Table 2 shows the water quality data collected during the study, which included sodium, magnesium, calcium, potassium, pH, temperature, and dissolved oxygen. The water quality in the culture media is still in accordance with the standards for Pacific white shrimp culture.

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5	pH		7.2-7.9	7.2-7.9	7.3-7.9	7.3-7.9	7.2-7.9	7.4-8.9 (wyban and Sweeney 1991)
6	Temperature	°C	28-29	28-29	28-29	28-29	28-29	23-30 (briggs et al 2004)
7	Dissolved oxygen	mg L^{-1}	4.6-6.2	4.6-6.2	4.9-6.4	4.9-6.4	4.8-6.5	4-5 (Wyban and Sweeney 1991)

The use of single-step acclimation reduced SR of Pacific white shrimp. The results show that the SR of Pacific white shrimp ranged between 47 and 79%. According to Jayasankar et al (2009), the single-step acclimation of Pacific white shrimp in a media of 5 ppt salinity led to a SR of 53%. A sudden drop in salinity over a wide range makes osmoregulation difficult for aquatic animals, which can lead to death (Laramore et al 2001).

The highest SR was observed in the culture media with a salinity of 20 ppt, 79±6%. The shrimp had little difficulty in adapting to a salinity of 20 ppt, which was similar to the salinity of the previous culture media. Salinity differences have a significant impact on the SR of Pacific white shrimp. Meanwhile, shrimp mortality during rearing could be caused by a large difference in the osmotic pressure between the shrimp's body and its environment. Physiological processes can be disrupted when the osmotic value between the body fluids and the environment is too high, leading to stress and cell damage (Brocker et al 2012). Additionally, the SR of shrimps can be affected by the frequency of shrimp molting, cannibalism, and water quality. The low salinity treatment led to the incomplete molting of vannamei shrimps since the exoskeleton was not hard enough to trigger the release of the carapace. Therefore, this reduced the SR of Pacific white shrimp, provoking their gradual death.

The Pacific white shrimp in the culture media with a salinity of 15 ppt produced the best growth (SGR=12.3±0.1%), which was accompanied by a drop in the value of FCR. According to Wyban & Sweeny (1991) and Davis et al (2002), Pacific white shrimp grow very well at low salinities around 10-15 ppt, where there is an iso-osmotic equilibrium between the environment and the hemolymph of the shrimp. According to Jaffer et al (2020), the isosmotic point occurs at a salinity of 21.1 ppt. A previous study by Rahman et al (2016) obtained results consistent with those of this study, discovering that a salinity treatment of 15 ppt is the most effective for Pacific white shrimp growth. According to Bückle et al (2006), *L. vannamei* hyperregulates between salinities of 10 to 20 ppt and hyporegulates between 20 to 40 ppt.

The osmotic pressure of the culture medium has an effect on the osmoregulation of aquatic organisms, such as shrimp. According to Anggoro et al (2018), shrimps lose much water through the diffusion process at high salinity (hypertonic). Furthermore, they adapt to this condition by drinking much water and avoiding excess salt through various mechanisms, such as the hardening of the exoskeleton, which inhibits the molting process. The regulation of ion concentration in the blood of crustaceans is very important, since the osmotic pressure of the blood is higher than the external pressure of the environment at low salinity, and vice versa at high salinity. Additionally, the differences in osmotic pressure affect the bioenergetics of aquatic organisms (Rivera-Ingraham & Lignot 2017). According to Anggoro et al (2018), the salinity of iso-osmotic media has an effect on the level of osmotic workload required to maintain osmolarity pressure balance (media and blood) or electrolyte content balance; a higher osmotic work rate means more energy wasted on osmotic performance, which leads to a reduction in shrimp growth. According to Rachmawati et al (2012), the growth of white vannamei shrimps can also be inhibited when more energy is consumed during osmoregulation, since this reduces the amount of energy available for growth. Therefore, aquatic organisms will grow optimally if the culture medium is iso-osmotic with their body fluids (Ferraris et al 1987).

Minerals are important for the growth and survival of cultured shrimps, especially potassium (Kaligis 2010), calcium (Kaligis 2015), and magnesium (Roy et al 2010). The salinity of the water can give a measure of its mineral content, which has an influence on the growth performance of shrimps, even though salinity itself plays a role in SGR and SR (Boyd 2018). Shrimp cultures often experience mineral deficiencies in low salinity, such as calcium and potassium deficiency. Table 3 shows the calcium and potassium concentrations in the culture media, which were still within the standards for Pacific white shrimp farming.

Table 3

Calcium and potassium concentrations in culture media and standards

Salinity (ppt)	*Current study*		*Standards (Boyd 2018)*	
	Calcium (mg L-1)	*Potassium (mg L^{-1})*	*Calcium (mg L^{-1})*	*Potassium (mg L^{-1})*
5	73	65	58	55
10	142.2	129	116	110
15	212	193	174	165
20	281.2	257	232	220
25	350.4	320	290	275

Conclusions. The salinity of the culture medium had an effect on SGR, SR, and FCR of Pacific white shrimp. The optimal SGR, biomass, and FCR occurred at 15 ppt salinity, while the optimal SR occurred at 20 ppt salinity. Therefore, it can be concluded that a media salinity of 15 ppt provided the best performance for Pacific white shrimp rearing.

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The growth performance of the Pacific white shrimp (*Litopenaeus vannamei*) cultured at various salinity conditions using single step acclimation

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Abstract. The Pacific white shrimp (*Litopenaeus vannamei*) is a euryhaline species that can be reared in aquatic environments with a wide range of salinity, from 0.5 to 40 ppt. The ability of Pacific white shrimps to adapt to extreme saline conditions allows the expansion of shrimp farming in inland aquatic environments, away from the coast. Therefore, this study aimed to analyze the specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) of Pacific white shrimp reared at various salinity levels using a single-step acclimation method. The study employed a completely randomized design with five treatments and three replications, namely T1, T2, T3, T4, and T5 at 5 ppt, 10 ppt, 15 ppt, 20 ppt, and 25 ppt, respectively. Each treatment unit was incorporated with Pacific white shrimp postlarvae (PL) 15 at a density of 2000 PL m^{-3}. Subsequently, the shrimps were fed commercial pellets three times daily through a blind feeding program during the 40 days of rearing. The results showed that different salinities of culture media had an effect on SGR, SR, and FCR of Pacific white shrimp. The most desirable SGR and FCR were observed in the 15 ppt culture media at 12.3% and 1.5, respectively, while the best survival rate was observed in the 20 ppt salinity at 79.3%. Generally, Pacific white shrimp performed best in the culture media with a salinity of 15 ppt.
Key Words: euryhaline, growth, *L. vannamei*, survival rate.

Introduction. The Pacific white shrimp (*Litopenaeus vannamei*) is a euryhaline species that can tolerate a wide range of salinity between 0.5 to 45 ppt (Wyban & Sweeny 1991), allowing for high-yield rearing away from the coast. The rearing of Pacific white shrimp at low salinity is one of the best options to optimize the use of low-salinity ponds located far from seawater sources, due to the high salinity tolerance range of this shrimp (Chong-Robles et al 2014). Furthermore, this is important since there are numerous brackish water aquaculture centers located inland in Lampung Province, Indonesia that can be used for Pacific white shrimp farming (Supono 2021).

Although the Pacific white shrimp can adapt to a wide range of salinities, it grows best in iso-osmotic media at a salinity equal to the osmotic tolerance range of common shrimps (Anggoro et al 2018). The rate of osmosis is a measure of the processes that occur in aquatic animals during times of environmental stress (Cheng et al 2006). Differences in media salinity and fluid osmolarity in shrimp (hemolymph) can lead to increased energy requirements for adaptation, retardation of shrimp growth and metabolism (Gao et al 2016). Meanwhile, shrimps can only survive by utilizing available energy during conditions of low salinity, where an extremely high osmotic load reduces shrimp survival (Jayasankar et al 2009; Susilowati et al 2014). The salinity of the media can be adjusted based on the osmotic pressure of the media to increase the growth of cultured species (Lukas et al 2017). Acclimatization to temperature and salinity is an important factor that affects the success of low-salinity shrimp farming. Acclimatization systems for stocking shrimp juveniles are classified into one-step acclimation and gradual acclimation (Jayasankar et al 2009). Each technique has its advantages and disadvantages. The single-step acclimation is easy to implement, but sometimes has a

low survival rate. Gradual acclimation is difficult to implement in the field. Therefore, this study aimed to evaluate the growth rate, survival rate, and feed conversion ratio of Pacific white shrimp reared under various salinity conditions.

Material and Method

Experimental design and setup. The study was conducted from July to September 2021 at the Laboratory of Aquaculture, Lampung University, Indonesia. This study used a completely randomized design (CRD), which consisted of 5 treatments and 3 replications. The treatments include the following: T1 - culture media at salinity of 5 ppt; T2 - culture media at salinity of 10 ppt; T3 - culture media at salinity of 15 ppt; T4 - culture media at salinity of 20 ppt; T5 - culture media at salinity of 25 ppt.

The study made use of 15 plastic containers filled with 70 L of water, which was sterilized with chlorine at a concentration of 30 mg L^{-1}. Each container was filled with 70 L of water at varying salinity based on the treatment. Furthermore, the aeration settings were performed in each maintenance container, with up to four aeration stones per container serving as an oxygen supply to the culture container. Each treatment unit was stocked with 140 Pacific white shrimp (PL 15), or the equivalent of 2000 PL m^{-3}. The shrimp were obtained from a hatchery in Kalianda City, South Lampung. Acclimatization was conducted using a single-step acclimation system, while feeding was carried out using a blind feeding program three times daily at 6.30, 12.00, and 19.00, throughout the 45-day culture period. The feed used was commercial feed containing protein fat, fiber, ash, and moisture at 41%, 7%, 3%, 13%, and 10%, respectively. Sodium (Na), magnesium (Mg), calcium (Ca), and potassium (K) concentrations were analyzed using the U.S. EPA 200.7 method (U.S. EPA 1994), while pH, temperature, and dissolved oxygen were measured using a water quality checker.

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Statistical analysis. Pacific white shrimp was evaluated based on specific growth rate (SGR), survival rate (SR), biomass, and feed conversion ratio (FCR). The data were analyzed using ANOVA at a 95% confidence level ($p<0.05$) using the SPSS 23.0 software. Further testing was conducted using the Duncan test when there were significant differences between treatments.

Results and Discussion. Table 1 shows the data from observations made during the study on SGR, SR, biomass, and FCR of Pacific white shrimp. The ANOVA statistical test results showed that the rearing of white shrimps in various saline media has a significant effect on their SGR, SR, biomass, and FCR ($p<0.05$).

Table 1

Growth performance of Pacific white shrimp

Parameter	*T1 (5 ppt)*	*T2 (10 ppt)*	*T3 (15 ppt)*	*T4 (20 ppt)*	*T5 (25 ppt)*
Growth rate (g)	$0.6±0.1^{a}$	$1.4±0.2^{c}$	$2.8±0.1^{d}$	$0.8±0.2^{ab}$	$1.2±0.1^{bc}$
SGR (%)	$8.4±0.7^{a}$	$10.4±0.3^{c}$	$12.3±0.1^{d}$	$9.4±0.8^{ab}$	$10.3±0.2^{bc}$
Biomass (g)	$40.6±17.5^{a}$	$114±19.4^{b}$	$251.3±33.3^{c}$	$98.3±29.3^{b}$	$106±11.5^{b}$
SR (%)	$47±6^{a}$	$56±2^{ab}$	$63±11^{b}$	$79±6^{c}$	$61±2^{b}$
FCR	$1.9±0.2^{b}$	$1.8±0.1^{b}$	$1.5±0.1^{a}$	$1.6±0.1^{a}$	$1.8±0.2^{b}$

Note: SGR - specific growth rate; SR - survival rate; FCR - feed conversion ratio; different superscripts indicate significant differences ($p<0.05$).

Table 2 shows the water quality data collected during the study, which included sodium, magnesium, calcium, potassium, pH, temperature, and dissolved oxygen. The water quality in the culture media is still in accordance with the standards for Pacific white shrimp culture.

Table 2

Water quality parameters during culture

No	*Parameters*	*Unit*	*5 ppt*	*10 ppt*	*15 ppt*	*20 ppt*	*25 ppt*	*Standards*
1	Sodium	mg L^{-1}	1195	2384.5	3585	4774.6	5964.1	1522-7609 (Boyd 2018)
2	Magnesium	mg L^{-1}	130.6	258.5	387.5	515.3	643.2	196-976 (Boyd 2018)
3	Calcium	mg L^{-1}	73	142.2	212	281.2	350.4	60-290 (Boyd2018)
4	Potassium	mg L^{-1}	65.1	128.8	193	256.6	320.3	55-275 (Boyd 2018)
5	pH		7.2-7.9	7.2-7.9	7.3-7.9	7.3-7.9	7.2-7.9	7.4-8.9 (Wyban & Sweeney 1991)
6	Temperature	°C	28-29	28-29	28-29	28-29	28-29	23-30 (Briggs et al 2004)
7	Dissolved oxygen	mg L^{-1}	4.6-6.2	4.6-6.2	4.9-6.4	4.9-6.4	4.8-6.5	4-5 (Wyban & Sweeney 1991)

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The highest SR was observed in the culture media with a salinity of 20 ppt, 79±6%. The shrimp had little difficulty in adapting to a salinity of 20 ppt, which was similar to the salinity of the previous culture media. Salinity differences have a significant impact on the SR of Pacific white shrimp. Meanwhile, shrimp mortality during rearing could be caused by a large difference in the osmotic pressure between the shrimp's body and its environment. Physiological processes can be disrupted when the osmotic value between the body fluids and the environment is too high, leading to stress and cell damage (Brocker et al 2012). Additionally, the SR of shrimps can be affected by the frequency of shrimp molting, cannibalism, and water quality. The low salinity treatment led to the incomplete molting of vannamei shrimps since the exoskeleton was not hard enough to trigger the release of the carapace. Therefore, this reduced the SR of Pacific white shrimp, provoking their gradual death.

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Calcium and potassium concentrations in culture media and standards

Salinity (ppt)	*Current study*		*Standards (Boyd 2018)*	
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