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Article

The Effects of Salinity on the Growth and Survival, Feeding of *Sanderia malayensis* (Cnidaria: Scyphozoa) Ephyrae

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Abstract: *Sanderia malayensis* is a species from the phylum Cnidaria, class Scyphozoa, and order Semaestomeae, found in tropical waters across the Indian Ocean, Pacific Ocean, Red Sea, and Malaysia, with its range extending to Australian and Japanese waters. This study aimed to evaluate the effects of salinity on the growth and survival of *S. malayensis* ephyrae and to identify its optimal salinity range. The experimental design involved two temperature conditions (20°C and 24°C) and three salinity levels (21 PSU, 24 PSU, 27 PSU). The results indicated that growth and feeding abilities of *S. malayensis* ephyrae were significantly higher in 24 PSU and 27 PSU environments compared to 21 PSU at both temperatures. Survival rates were also higher in 24 PSU (20°C: 90%, 24°C: 79%) and 27 PSU (20°C: 87%, 24°C: 86%) compared to 21 PSU (20°C: 70%, 24°C: 55%). Despite lower survival in 21 PSU, the species exhibited notable environmental adaptability. These findings suggest that *S. malayensis* ephyrae are highly adaptable to varying salinity conditions, indicating the potential for expansion into South Korean waters affected by climate change, with possible implications for local marine ecosystems.

KEYWORDS: *Sanderia malayensis*; salinity; growth; survival rate; feeding rate; ephyrae; Koreans waters

1. Introduction

Jellyfish outbreaks have significant impacts on marine ecosystems and human activities [1-4]. In particular, they are known to have highly negative social and economic effects on human activities, especially in sectors such as fisheries and tourism [5-6]. These impacts include damage to aquaculture species, loss of fishing nets, a decrease in fishery resources due to food competition, disruptions to coastal power plant systems, and human sting incidents at beaches [7-11].

Jellyfish outbreaks occurring worldwide are primarily attributed to Scyphozoan species from the phylum Cnidaria [12-13], and species such as *Aurelia coerulea* and *Nemopilema nomurai* frequently experience annual outbreaks in East Asian waters, including those of Korea, Japan, and China [14-17].

The life cycle of most Scyphozoans is primarily carried out through sexual reproduction [18]. Eggs fertilized through mating develop into planula larvae, which then attach to the substrate and transform into polyps. These polyps undergo asexual reproduction to form numerous colonies. Subsequently, polyps release ephyrae through strobilation, and the ephyrae grow and develop into adult medusae.

Research on Scyphozoans primarily focuses on various ecological studies of *Aurelia* spp. (*A. coerulea*, *Aurelia solida*, *Aurelia labiata*) [19-24]. *Aurelia* spp. have the widest distribution range globally and are not only easy to sample for experimental research but also exhibit exceptional survival in

diverse environmental conditions. As a result, they have long been used as standardized model organisms for experimental studies on Scyphozoan ecology. [23,25]. However, outside of *Aurelia spp.* ecological research on Scyphozoans is highly limited, and there is an increasing need for broader ecological studies on Scyphozoans. [3,26]. This is particularly important as recent phenomena have shown a rise in the abundance of various Scyphozoan populations [27], and climate change-driven alterations in marine environments may lead to the expansion of their distribution ranges [28]. Such changes could potentially destabilize marine ecosystems, posing a risk of significant ecological impacts [4].

Sanderia malayensis (Hereafter *S. malayensis*) is a species belonging to the phylum Cnidaria, class Scyphozoa [29], and is reported to inhabit tropical waters including the Indian Ocean, Pacific Ocean, Red Sea, and Malaysian waters [30-31]. However, the distribution of this species has expanded to include the northern Australian waters and East Asian waters, including those of Japan [32,33]. The first discovery of *S. malayensis* in Japan was made in 1938 in the Amakusa region of Kyushu [34]. Later, in 2004, the discovery of natural polyps of *S. malayensis* forming colonies and inhabiting deep-sea areas of the Kagoshima region was reported [35].

Ecological studies on *S. malayensis* have primarily focused on various asexual reproductive strategies and habitat conditions during the polyp stage [29-30,36,37]. However, ecological research on the ephyra stage, the free-swimming phase produced through the strobilation process, remains highly limited [38].

S. malayensis has continued to be observed in the East Asian waters of Japan since 2010, with reports indicating that its distribution range is gradually expanding in the Japanese waters due to the influence of marine environmental changes [39-41]. This suggests that *S. malayensis* could potentially become an invasive species with the possibility of expanding its distribution into South Korean waters, which are geographically the closest to Japan. Shin and Choi [38] conducted an experiment that simulated the summer temperature conditions of the southern coast of Korea and confirmed the stable growth and high survival rates of *S. malayensis* ephyrae, suggesting that the environmental factors affecting the growth and survival of this species are more strongly influenced by temperature than by salinity. Additionally, they reported that no significant effects on growth and survival were observed when exposed to salinity levels close to those found in natural environments [38]. The ephyra stage of Scyphozoans is known to be significantly influenced by salinity conditions, which are one of the key factors affecting growth and survival [42-43], suggesting the need for further research to specifically evaluate the impact of salinity on the growth and survival of *S. malayensis* ephyrae.

Therefore, this study aimed to predict the potential changes in growth and survival of *S. malayensis* ephyrae when exposed to coastal marine environments with frequent salinity fluctuations, by creating salinity conditions lower than the natural salinity environment used in the experiment by Shin and Choi [38], and to identify the optimal salinity range for this species. Through this, the study aims to understand the impact of salinity environmental factors on the growth and survival of this species, providing fundamental insights, and contributing to the use of this data as an ecological indicator to assess the potential for the expansion of its distribution into South Korean waters due to changes in marine environments caused by future climate change.

2. Materials and Methods

2.1. Acquisition of *S. malayensis* Polyps and Ephyrae

For the acquisition of *S. malayensis* ephyrae in this experiment, *S. malayensis* polyps were provided by researchers at the Shinagawa Aquarium, located in Minato, Tokyo, Japan (provided in May 2015), and the original source of the polyps could not be verified. *S. malayensis* polyps were cultured and maintained in an incubator at the Aqua Planet Gwangyo Jellyfish Laboratory in Suwon, South Korea. The method for inducing strobilation to obtain ephyrae was based on the temperature stimulation method used by Fuchs et al. [44] and the temperature conditions under which ephyrae

were released from *S. malayensis* polyps as reported by Avian et al. [29]. The ephyrae, released in large quantities from the polyps, were stored following the method of Shin and Choi [38]. They were maintained in a transparent acrylic cylindrical tank (Diameter 35 cm, Height 40 cm, Thickness 5 mm), where water flow was generated through an air stick to prevent the ephyrae from sinking to the bottom, until the experiment commenced. The seawater used for storage and cultivation was prepared using Red Sea Salt (Public Aquarium Part A) artificial seawater product from Red Sea, which underwent three stages of filtration: stage 1: sand filter, stage 2: biological filter, and stage 3: housing filter. No additional feeding was provided to the ephyrae to limit their growth until the commencement of the experiment.

2.2. Evaluation of Growth, Survival, and Feeding of *S. malayensis* Ephyrae According to the Effect of Salinity

The setup of the experimental treatments (Temperature and Salinity conditions) and the rearing information are shown in Figure 1. The tanks used in the experiment were Breeding Air Kreisel tanks designed and manufactured by Schuran Seawater Equipment BV (Netherlands). The temperature settings for the experimental treatments were designed based on the natural temperature range of 20–22°C reported by Uchida and Sugiura [45–46] and the temperature condition of 24°C, which showed the highest survival rate (98%) in the study by Shin and Choi [38]. The two temperature conditions, 20°C and 24°C, were chosen as the optimal temperature environments for evaluating the effects of salinity. The salinity settings for the experimental treatments were designed to evaluate the effects of low salinity conditions, with three low salinity treatments at 21 PSU, 24 PSU, and 27 PSU, which were established to be lower than the salinity range found in natural environments, and these treatments were applied equally in the two temperature environments of 20°C and 24°C. All experimental treatments contained 50 ephyrae, which were cultured as described in section 2-1, with each treatment set up in replicates. The seawater used in this experiment was filtered artificial seawater, the same product as used in section 2-1. The experiment lasted for a total of 20 days. During the experimental period, feeding was provided according to the method proposed by Purcell et al. [2] and the feeding method used in the study by Riisgård [47], where a sufficient amount of *Artemia* sp. was supplied once a day to ensure that all ephyrae in each treatment received enough food. This was to ensure that the feeding behavior of the cultured ephyrae was not restricted by providing a sufficient amount of *Artemia* sp. to each treatment. The water quality maintenance for ephyrae health was managed through 100% water exchange every other day with pre-filtered clean artificial seawater following the feeding of *Artemia* sp.

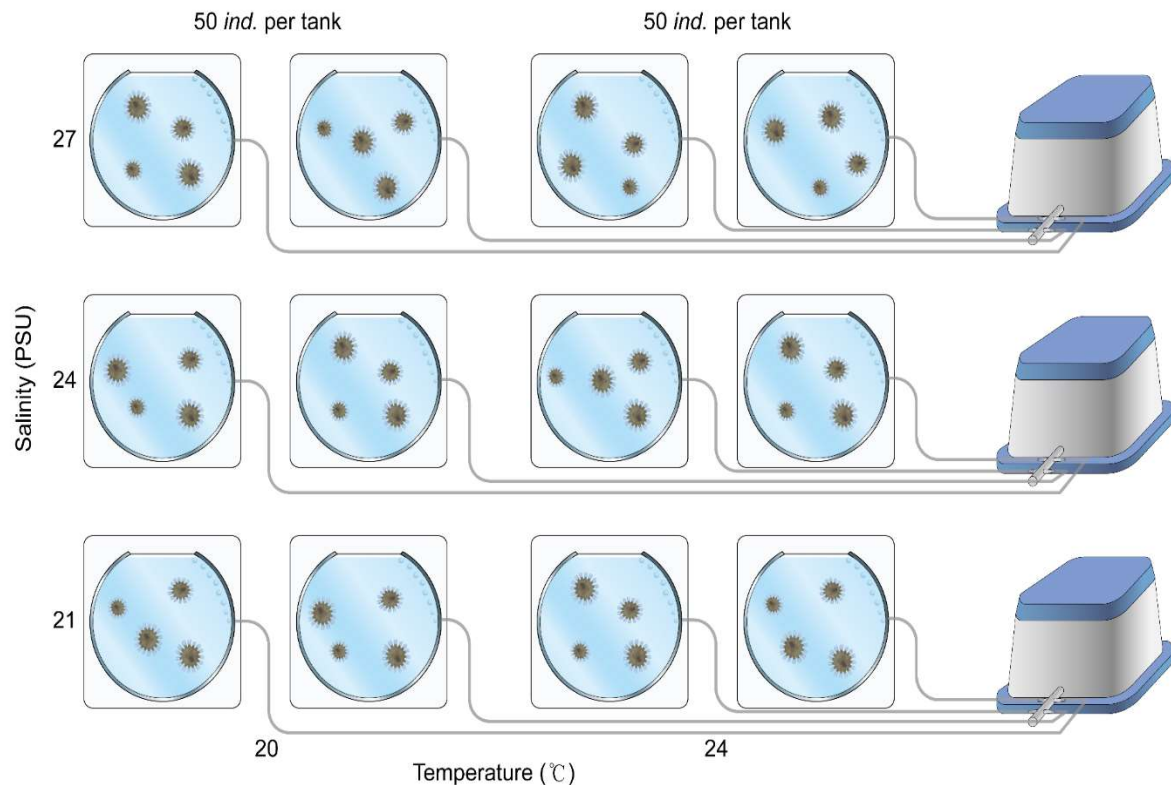


Figure 1. The experimental design to assess the effects of low salinity on the survival and growth of *S. malayensis* ephyrae is as follows. The experiment was conducted under two temperature conditions, 20°C and 24°C, with three different salinity levels. Each experimental group was replicated twice, with 50 ephyrae cultured in each Breeding Air Kreisel tank. As a result, 100 ephyrae were cultured per salinity level under each temperature condition, totaling 600 ephyrae across both temperature conditions.

The growth, feeding, and survival records of the ephyrae were measured a total of 10 times at 2-day intervals throughout the 20-day experimental period. Growth and feeding were recorded by randomly extracting 10 ephyrae from each treatment, which were set up in replicates, and observing them under an optical microscope (Olympus model SZX2-ILLK, Tokyo, Japan). The growth size increase (mm) was measured according to the method devised by Straehler-Pohl et al. [48], using the total diameter of the ephyrae (Central disk diameter + Total marginal lappet length) as the measurement standard (Figure 2). On day 1, 10, and 20 of the experiment, the largest individual in terms of diameter was visually selected from each treatment to observe the growth changes of the ephyrae, and images were captured using an optical microscope.

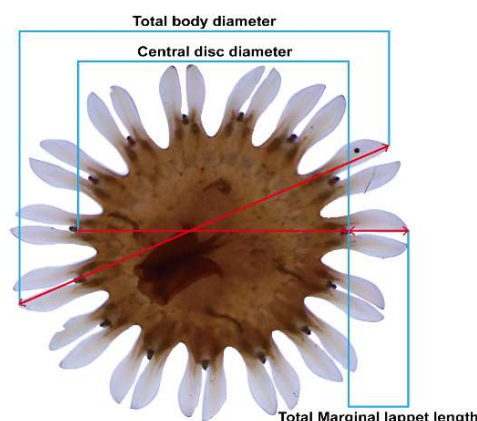


Figure 2. In this experiment, the measurement points and range criteria for assessing the growth changes of *S. malayensis* ephyrae were adapted from Straehler-Pohl et al. (2010). Measurements were taken based on the central disc diameter (mm) and total marginal lappet length (mm) of the ephyrae.

The growth rate of the ephyrae (% d⁻¹) was calculated according to the method for measuring ephyra growth rate devised by Widmer [43], as follows. This method was developed through Båmstedt et al. [49].

$$\% \text{ Growth day}^{-1} = \ln [(D2 / D1)^3] / (t2 - t1) \times 100$$

Here, D1 represents the average diameter of the ephyrae on the first measurement day (Day 1), and D2 represents the average diameter two days later (Day 2). Since measurements were recorded at 2-day intervals for a total of 10 times during the 20-day experimental period, the average diameter at each measurement point was represented as D1 and D2 and calculated accordingly. The feeding records of the ephyrae were measured by randomly extracting ephyrae from each treatment 1 hour after feeding *Artemia sp.* and visually confirming the gut content under an optical microscope. The number of *Artemia sp.* consumed, which was considered completed feeding, was counted and recorded. The survival records of the ephyrae were measured by counting all surviving ephyrae from each treatment and selecting individuals that showed good swimming and feeding behavior, which were then recorded.

3. Statistical Analysis

To evaluate the effects of salinity environmental factors on the growth and feeding of *S. malayensis* ephyrae, we applied the Aligned Rank Transform ANOVA (ART-ANOVA) method. The dependent variables were growth (mm) and feeding (number of individuals), while the independent variables were the three salinity levels (21 PSU, 24 PSU, 27 PSU) and the number of days measured during the experimental period (a total of 10 measurements: 2-day intervals over the 20-day experimental period). All analyses were performed independently under the experimental temperature conditions of 20°C and 24°C. To assess the effects of salinity over the course of the experimental period, the interaction effect of salinity × experimental days was included for each temperature condition.

As a result of preliminary testing for homogeneity of variance using Levene's test, it was found that the assumption of homogeneity was not met for the growth changes measured at the three salinity levels at 20°C and the feeding changes measured at the three salinity levels at 24°C. Therefore, to avoid the potential occurrence of statistical errors when applying traditional ANOVA, we employed a non-parametric approach based on ART-ANOVA analysis. ART-ANOVA is a validated non-parametric alternative known to effectively adjust for errors due to violations of homogeneity of variance and can also assess interaction effects between factors [50-51].

Since the assumption of homogeneity of variance was met for the feeding changes measured at the three salinity levels at 20°C according to Levene's test, ANOVA analysis was applied. This was

done to ensure high statistical power through the parametric method of ANOVA and to estimate clear statistical results and reliability (ART-ANOVA analysis results for the three salinity levels of feeding changes measured at 20°C, Supplementary Table S1, S2). The Holm adjustment was applied as a post-hoc test (For the growth changes at three salinity levels at 20°C and 24°C, and for the feeding changes at three salinity levels at 24°C). For the feeding changes measured at three salinity levels at 20°C, Tukey's Honest Significant Difference (HSD) test was used to compare the differences between groups.

To evaluate the effect of salinity environmental factors on the survival rate of *S. malayensis* ephyrae, we independently compared survival curves for each of the three salinity conditions at 20°C and 24°C using Kaplan-Meier survival analysis. The hazard ratio based on the survival analysis was assessed using the Cox Proportional Hazards Model to evaluate the impact of salinity differences. All statistical analyses were performed using R version 4.4.2 (R Core Team, 2024). The ARTool package [52] was applied for growth and feeding changes analysis (ART-ANOVA), and the Survival package [51] was used for survival analysis.

4. Results

4.1. Effect of Salinity Environmental Factors on the Growth of *S. malayensis* Ephyrae

During the 20-day experimental period, the growth changes of *S. malayensis* ephyrae cultured under three different salinity conditions (21 PSU, 24 PSU, 27 PSU) at 20°C and 24°C were captured on Day 1, Day 10, and Day 20. The images of these growth changes for each treatment are shown in Figure 3 and Figure 4. At the end of the 20-day experimental period, the growth changes (size increase) of *S. malayensis* ephyrae were measured as follows: at 20°C, the size increased to 6.34 ± 0.14 mm (Mean $\pm$ SD) at 21 PSU, 8.47 ± 0.15 mm (Mean $\pm$ SD) at 24 PSU, and 8.51 ± 0.13 mm (Mean $\pm$ SD) at 27 PSU (Figure 5, Table 1). At 24°C, the size increased to 6.35 ± 0.14 mm (Mean $\pm$ SD) at 21 PSU and 8.50 ± 0.13 mm (Mean $\pm$ SD) at both 24 PSU and 27 PSU (Figure 5, Table 1). The growth rate (% d⁻¹) of *S. malayensis* ephyrae during the 20-day experimental period was as follows: at 20°C, the growth rate was 10.31 % d⁻¹ at 21 PSU, 14.88 % d⁻¹ at 24 PSU, and 14.86 % d⁻¹ at 27 PSU. At 24°C, the growth rate was 10.24 % d⁻¹ at 21 PSU, 14.80 % d⁻¹ at 24 PSU, and 14.94 % d⁻¹ at 27 PSU (Table 2). The results of the ART-ANOVA analysis for growth changes under three different salinity conditions at 20°C and 24°C showed statistically significant main effects for time (20°C: $F = 633.97$, 24°C: $F = 633.98$), salinity (20°C: $F = 6023.03$, 24°C: $F = 6284.18$), and the interaction effect between time and salinity (20°C: $F = 234.34$, 24°C: $F = 207.09$). These results are presented in Table 3 ($p < 0.001$). To further examine the interaction effect between time and the three different salinity environments, the Holm post-hoc test results showed that the growth at 21 PSU was significantly lower than at 24 PSU and 27 PSU at both 20°C and 24°C (Table 1, $p < 0.001$). However, the difference between 24 PSU and 27 PSU was not statistically significant (Table 1, 20°C: $p = 0.461$, 24°C: $p = 0.840$).

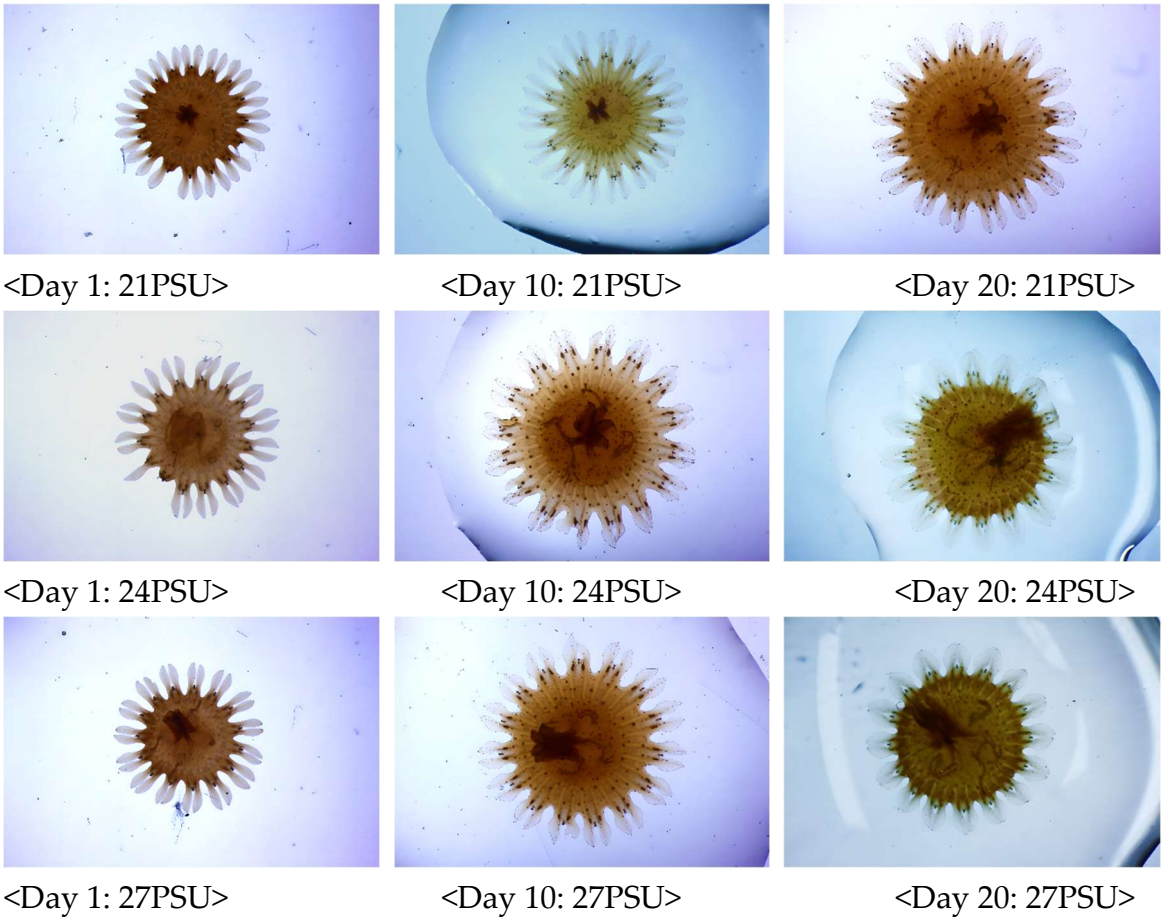
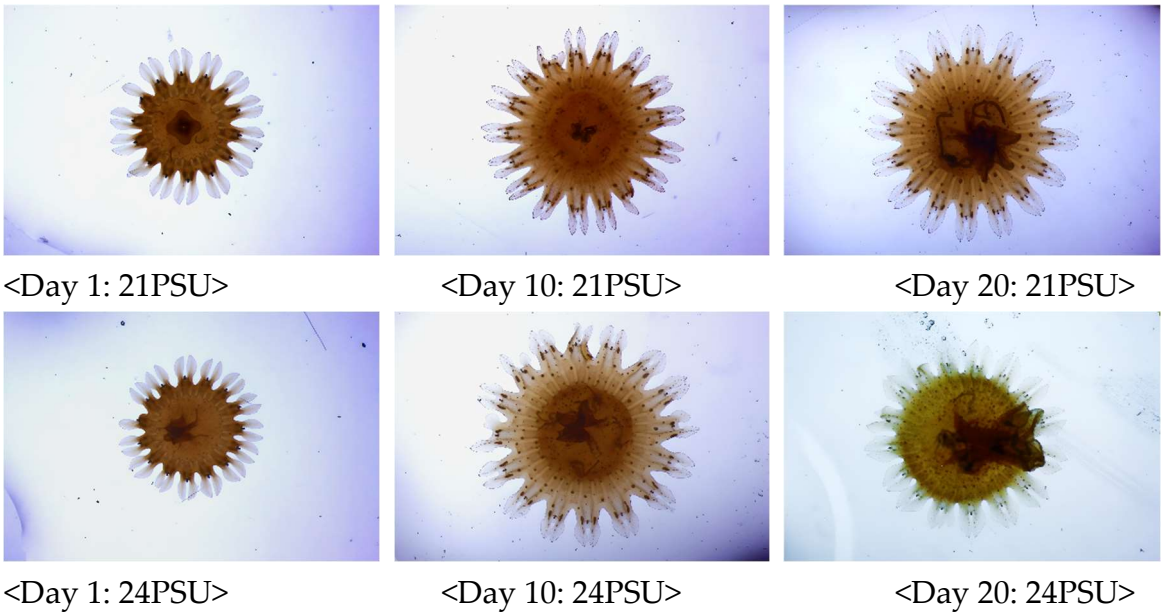


Figure 3. The growth changes of *S. malayensis* ephyrae were recorded three times during the 20-day experimental period (On days 1, 10, and 20). The experiment was conducted at a temperature of 20°C with three different salinity conditions: 21PSU, 24PSU, and 27PSU.



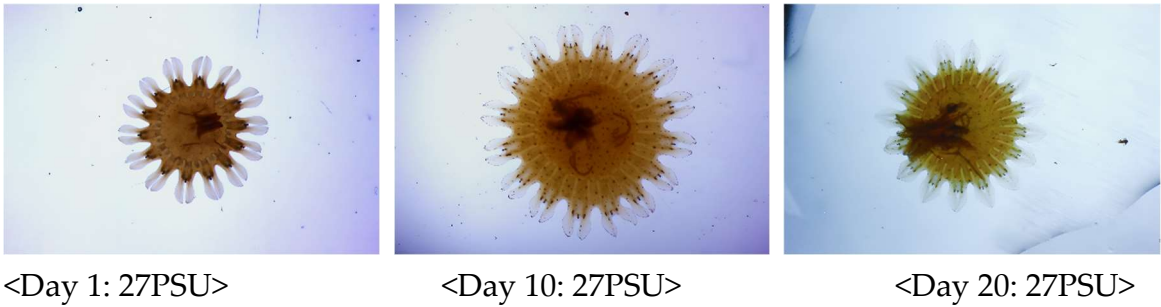
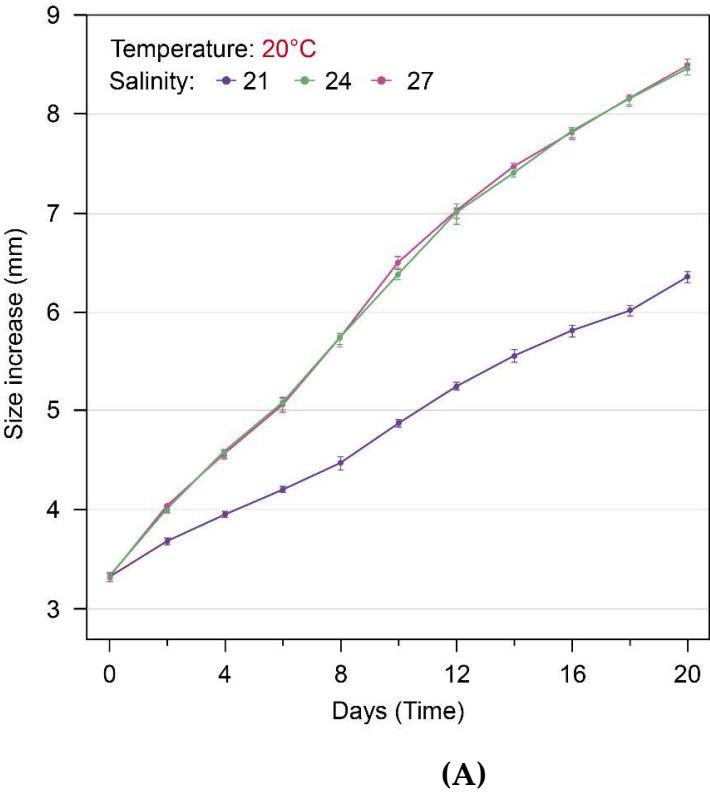


Figure 4. The growth changes of *S. malayensis* ephyrae were recorded three times during the 20-day experimental period (On days 1, 10, and 20). The experiment was conducted at a temperature of 24°C with three different salinity conditions: 21PSU, 24PSU, and 27PSU.



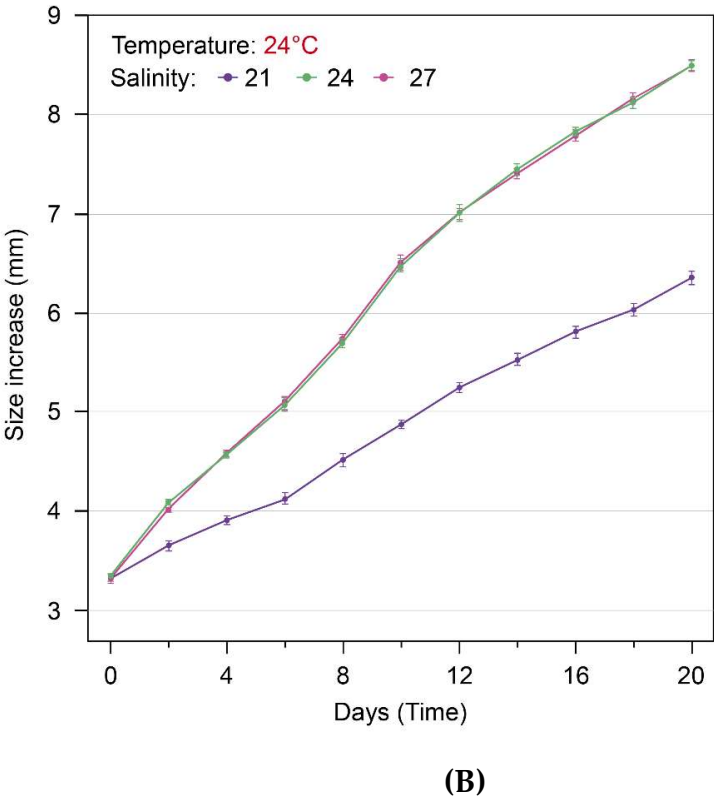


Figure 5. The growth changes (size increase: mm) of *S. malayensis* ephyrae over 20 days: At temperatures of 20°C and 24°C, the growth in the 21PSU salinity environment was significantly lower than in the 24PSU and 27PSU environments (A, B: $p < 0.001$). However, no significant differences were observed between the 24PSU and 27PSU salinity environments (A: $p = 0.461$, B: $p = 0.840$).

Table 1. The growth changes (size increase) of *S. malayensis* ephyrae were measured under two temperature conditions (20°C, 24°C) across three salinity levels (21PSU, 24PSU, 27PSU). Statistical significance was determined using the Holm post-hoc test. Growth at 21PSU was significantly lower than at 24PSU and 27PSU ($p < 0.001$), while no significant difference in growth was observed between 24PSU and 27PSU ($p > 0.05$).

Temperature (°C)	Salinity (PSU)	Size Increase (mm, n = 10)		95% Confidence interval	
		Mean	SD	Lower Limit (CI)	Upper Limit (CI)
20	21	6.34 ^a	0.14	6.10	6.70
	24	8.47 ^b	0.15	8.20	8.70
	27	8.51 ^b	0.13	8.30	8.70
24	21	6.35 ^a	0.14	6.10	6.60
	24	8.50 ^b	0.13	8.30	8.70
	27	8.50 ^b	0.13	8.30	8.70

Table 2. The growth rates (% d-1) of *S. malayensis* ephyrae at three salinity levels (21PSU, 24PSU, 27PSU) under two temperature conditions (20°C, 24°C) were measured following the method outlined by Bamstedt et al. (1997). The sample size for each treatment group was n = 10.

Temperature (°C)	Salinity (PSU)	Growth rate (% d ⁻¹ , n = 10)	95% Confidence interval	
			Lower Limit (CI)	Upper Limit (CI)
20	21	10.31	10.19	10.71
	24	14.88	14.83	14.86
	27	14.86	14.83	15.05
24	21	10.24	10.19	10.47
	24	14.80	14.38	15.05
	27	14.94	14.38	15.05

Table 3. The statistical significance of growth changes (size increase) in *S. malayensis* ephyrae under two temperature conditions (20°C, 24°C) and three salinity levels (21PSU, 24PSU, 27PSU) was assessed using ART-ANOVA. A significant interaction effect between time and salinity was observed ($p < 0.001$), and the results of the Holm post-hoc analysis are provided in the main text.

Temperature(°C) Source	Size Increase (mm, n = 10)					
	Degrees of freedom	Sum of Squares <i>F-value</i>	<i>p-</i> Value			
20 Salinity	Time	2	15988153.54 633.97	<0.001		
	Salinity	10	23703043.10 6023.03	<0.001		
		20	20791713.35 234.03	<0.001	9	<0.001
24 Salinity	Time	2	15974650.91 633.98	<0.001	2	<0.001
	Salinity	10	23715381.33 6284.18	<0.001	18	<0.001
		20	20437093.72 207.09	<0.001		

4.2. Effect of Salinity Environmental Factors on the Feeding of *S. malayensis* Ephyrae

During the 20-day experimental period, the average feeding rate (Preys ind·hr⁻¹) of *S. malayensis* ephyrae was as follows: at 20°C, 2.10 ± 0.908 Preys ind·hr⁻¹ (Mean ± SD) at 21 PSU, 7.58 ± 1.138 Preys ind·hr⁻¹ (Mean ± SD) at 24 PSU, and 7.69 ± 1.165 Preys ind·hr⁻¹ (Mean ± SD) at 27 PSU (Figure 6, Table

4). At 24°C, the average feeding rate was 2.16 ± 0.869 Preys ind·hr⁻¹ (Mean $\pm$ SD) at 21 PSU, 7.75 ± 1.118 Preys ind·hr⁻¹ (Mean $\pm$ SD) at 24 PSU, and 7.64 ± 1.136 Preys ind·hr⁻¹ (Mean $\pm$ SD) at 27 PSU (Figure 6, Table 4). Levene’s test was used for preliminary testing of homogeneity of variance, and based on the results, the analysis method was chosen. The average feeding rate at 20°C was analyzed using ANOVA, while the average feeding rate at 24°C was analyzed using ART-ANOVA (Section 3. Statistical Analysis). The results of the ANOVA analysis for the average feeding rate (Preys ind·hr⁻¹) under three different salinity conditions (21 PSU, 24 PSU, 27 PSU) at 20°C showed statistically significant main effects for time ($F = 2029.04$) and salinity ($F = 3.16$; Table 5, $p < 0.001$). However, the interaction effect between time and salinity ($F = 1.47$) was not statistically significant (Table 5, $p = 0.085$). To further examine the differences in the average feeding rate (Preys ind·hr⁻¹) between the three salinity conditions, Tukey post-hoc analysis revealed significant differences between 21 PSU and 24 PSU, as well as between 21 PSU and 27 PSU (Table 4, $p < 0.001$). However, no significant difference was found between 24 PSU and 27 PSU, indicating that the average feeding rate was similar in the relatively higher salinity environments of 24 PSU and 27 PSU compared to 21 PSU (Table 4, $p = 0.522$).

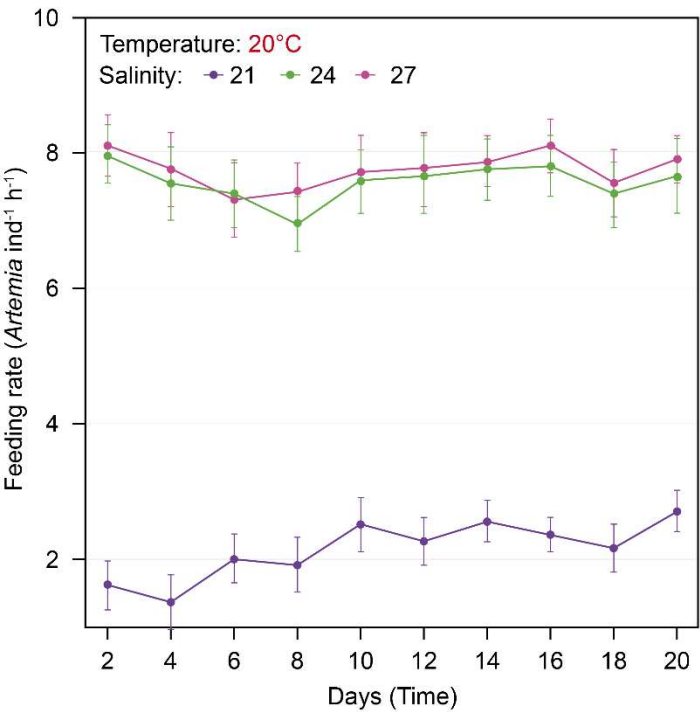
Table 4. The feeding rate (*Preys ind⁻¹ hr⁻¹*) results of *S. malayensis* ephyrae under two temperature conditions (20°C, 24°C) and three salinity levels (21PSU, 24PSU, 27PSU) are presented. Statistical significance was determined using the Tukey post-hoc test at 20°C and the Holm post-hoc test at 24°C. Growth at 21PSU was significantly lower compared to 24PSU and 27PSU ($p < 0.001$), while no significant difference in growth was observed between 24PSU and 27PSU ($p > 0.05$).

Temperature (°C)	Salinity (PSU)	Feeding rate (<i>Artemia ind⁻¹ h⁻¹</i> , n = 10)		95% Confidence interval	
		Mean	SD	Lower	Upper
				Limit (CI)	Limit (CI)
20	21	2.10 ^a	0.908	0.00	4.00
	24	7.58 ^b	1.138	5.00	10.00
	27	7.69 ^b	1.165	4.00	10.00
24	21	2.16 ^a	0.869	0.00	5.00
	24	7.75 ^b	1.118	5.00	10.00
	27	7.64 ^b	1.136	5.00	11.00

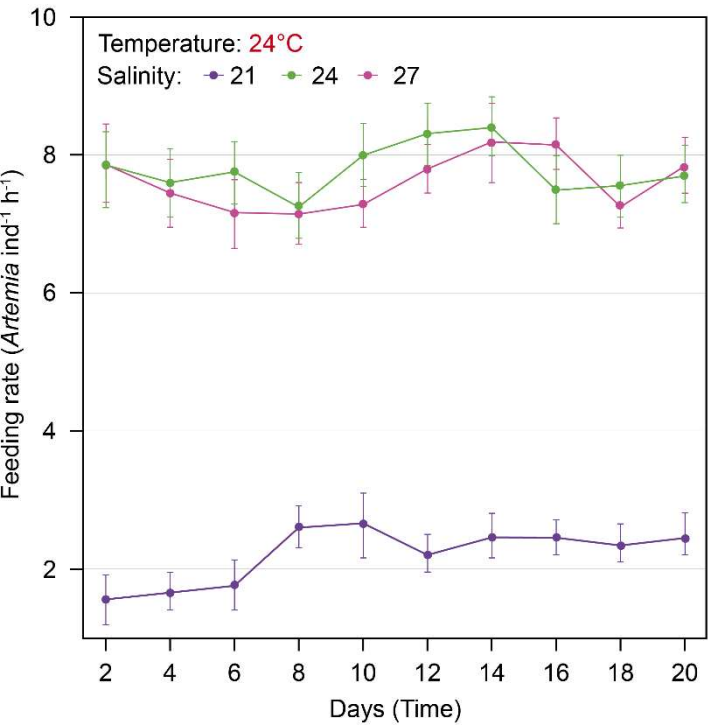
Table 5. The statistical significance of feeding rates of *S. malayensis* ephyrae at three salinity levels (21PSU, 24PSU, 27PSU) under a temperature condition of 20°C was assessed using ANOVA. No significant interaction effect between time and salinity was observed ($p = 0.085$), and the results of the Tukey post-hoc analysis are provided in the main text.

Source	Feeding rate (<i>Artemia ind⁻¹ h⁻¹</i> , n = 10)				
	Sum of squares	Degrees of freedom	Mean of squares	F- value	p- Value
Time	4489	2	2245	2029.04	<0.001

Salinity	35	10	3	3.16	<0.001		
Salinity × Days	33	20	2	1.47	0.085	9	<0.001



(A)



(B)

Figure 6. The feeding rate changes (preys ind⁻¹ h⁻¹) of *S. malayensis* ephyrae over 20 days: At temperatures of 20°C (Tukey post-hoc test) and 24°C (Holm post-hoc test), the feeding rate in the 21PSU salinity environment

was significantly lower compared to the 24PSU and 27PSU environments (A, B: $p < 0.001$). However, no significant differences were observed between the 24PSU and 27PSU salinity environments (A: $p = 0.522$, B: $p = 0.100$).

The results of the ART-ANOVA analysis for the average feeding rate (Preys ind⁻¹·hr⁻¹) under three different salinity conditions at 24°C showed statistically significant main effects for time ($F = 633.18$), salinity ($F = 3.27$), and the interaction effect between time and salinity ($F = 2.75$) (Table 6, $p < 0.001$). To further examine the interaction effects between time and the three salinity conditions, the Holm post-hoc test revealed significant differences between 21 PSU and 24 PSU, as well as between 21 PSU and 27 PSU (Table 4, $p < 0.05$). However, no significant difference was found between 24 PSU and 27 PSU, indicating that the average feeding rate was similar in the relatively higher salinity environments of 24 PSU and 27 PSU compared to 21 PSU (Table 4, $p = 0.100$).

Table 6. The statistical significance of feeding rates of *S. malayensis* ephyrae at three salinity levels (21PSU, 24PSU, 27PSU) under a temperature condition of 24°C was assessed using ART-ANOVA. A significant interaction effect between time and salinity was observed ($p = 0.001$), and the results of the Holm post-hoc analysis are provided in the main text.

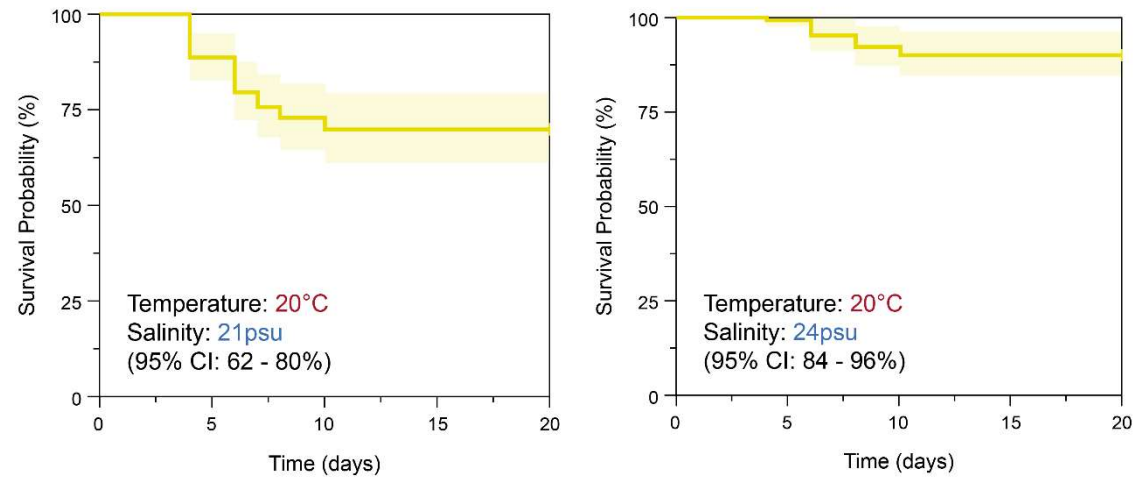
Source	Feeding rate (<i>Artemia</i> ind ⁻¹ h ⁻¹ , n = 10)					
	Degress of freedom	Sum of squares	F-value	p-Value		
Time	2	15983505.68	633.18	<0.001		
Salinity	10	1186144.58	3.27	<0.001		
Salinity × Days	20	1927731.32	2.75	<0.001	9	<0.001

4.3. Effect of Salinity Environmental Factors on the Survival of *S. malayensis* Ephyrae

The Kaplan–Meier survival analysis results for *S. malayensis* ephyrae cultured under three different salinity conditions (21 PSU, 24 PSU, 27 PSU) at 20°C and 24°C during the 20-day experimental period are shown in Figure 7. The survival rates at 20°C were 70% (95% CI: 62–80%) at 21 PSU, 90% (95% CI: 84–96%) at 24 PSU, and 87% (95% CI: 81–94%) at 27 PSU (Figure 7, Table 7). The results of the Log-rank test showed a significant difference in the survival curves between the three different salinity conditions ($\chi^2 = 17.746$, $df = 2$, Figure 7, $p < 0.001$). The survival rates at 24°C were 55% (95% CI: 46–66%) at 21 PSU, 79% (95% CI: 69–86%) at 24 PSU, and 86% (95% CI: 79–93%) at 27 PSU (Figure 7, Table 7). The Log-rank test results also showed a significant difference in the survival curves between the three salinity conditions ($\chi^2 = 28.008$, $df = 2$, Figure 7, $p < 0.001$). In the Cox Hazard Ratio analysis to assess the effects of the three different salinity conditions (21 PSU, 24 PSU, 27 PSU), with 21 PSU set as the reference, the survival rates at both 20°C and 24°C were found to significantly increase at 24 PSU and 27 PSU ($p < 0.001$, Supplementary Figure S1, S2). At 20°C, the hazard ratios for 24 PSU and 27 PSU were 0.29 (95% CI: 0.14–0.59, $p < 0.001$) and 0.38 (95% CI: 0.20–0.72, $p = 0.003$), respectively. At 24°C, the hazard ratios for 24 PSU and 27 PSU were 0.42 (95% CI: 0.25–0.69, $p < 0.001$) and 0.25 (95% CI: 0.14–0.46, $p < 0.001$), respectively. The p-value for the Global Log-Rank Test was calculated to be <0.001, indicating that there was a statistically significant difference in survival rates between the three different salinity conditions ($p < 0.001$).

Table 7. The Kaplan-Meier survival analysis results of *S. malayensis* ephyrae under two temperature conditions (20°C, 24°C) and three salinity levels (21PSU, 24PSU, 27PSU) are presented. The Log-rank test revealed a significant difference in survival curves among the three salinity levels ($p < 0.001$).

Temperature (°C)	Salinity (PSU)	Survival rate (%)	Observed Event	95% Confidence interval	
				Lower CI	Upper CI
20	21 ^a	70	30	0.62	0.80
	24 ^b	90		0.84	0.96
	27 ^c	87		0.81	0.94
24	21 ^a	55			
	24 ^b	45		0.46	0.66
	27 ^c	79		0.69	0.86
		21			
		86		0.79	0.93
		14			



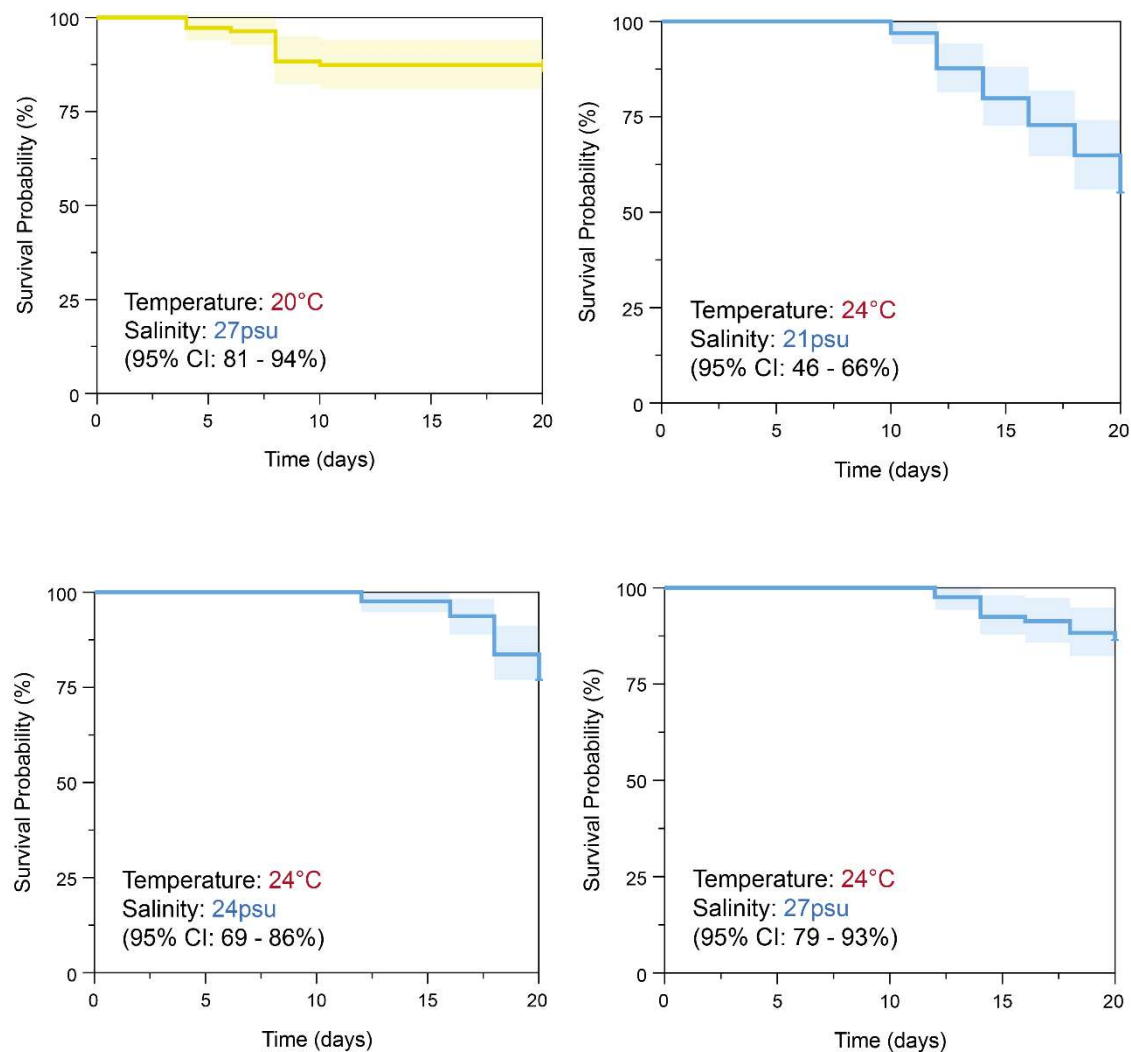


Figure 7. Kaplan-Meier survival analysis of *S. malayensis* ephyrae over 20 days: Significant differences in survival curves were observed between the salinity conditions (21PSU, 24PSU, 27PSU) at 20°C and 24°C. (20°C: $\chi^2 = 17.746$, DF = 2; 24°C: $\chi^2 = 28.008$, DF = 2, $p < 0.001$). The effects of the three different salinity conditions were further evaluated using the Cox Hazard Ratio analysis, as shown in Figure S1 and S2.

5. Discussion

This study aimed to evaluate the effects of salinity on the growth and survival of *S. malayensis* ephyrae and to determine its optimal salinity range. *S. malayensis* ephyrae showed stable growth changes in environments with salinities of 24 PSU and 27 PSU (Figure 5, Table 1), which was also confirmed by the survival rate results (Figure 7, Table 7). The environment with 21 PSU showed significantly lower growth changes (Figure 5, Table 1, $p < 0.001$) and survival rates (Figure 7, Table 7, $p < 0.001$) compared to 24 PSU and 27 PSU. However, a certain level of environmental adaptability was observed. These findings demonstrate that this species has the ability to adapt very well to a wide range of salinity conditions. Previous studies on *S. malayensis* ephyrae have primarily focused on morphological characteristics [36,48,53], as well as ecological studies during the polyp stage [29-30,37,46]. However, biological and ecological research on the free-swimming phase, which follows the polyp stage, remains largely unexplored [38]. This may be due to the technical challenges involved in studying the free-swimming stage, including the need for advanced techniques and the establishment of life-support systems [54]. *S. malayensis* is known to be a representative species of Scyphozoans commonly exhibited in aquariums worldwide [29], and aquariums possess the

necessary technologies and manuals for year-round display of various Scyphozoans [55]. This aspect may present one of the significant challenges faced by researchers in studying the free-swimming phase of Scyphozoan species, including *S. malayensis*. The effects of salinity environmental factors on the survival and growth of Scyphozoan ephyrae during the free-swimming stage have been reported in various studies [23,42-43,56-58]. Båmstedt et al. [42] found no significant impact on growth rate and growth efficiency between 17.5 PSU and 35 PSU in their experiment on *A. aurita*, but they reported very high feeding rates when exposed to 35 PSU, suggesting that the stable feeding rate of *A. aurita* is attributed to higher salinity environments. Purcell et al. [56] suggested that *C. quinquecirrha* prefers survival in environments with salinities below 20 PSU, and Windmer [43] confirmed a high growth rate of *C. capillata* in the low salinity environment of 21 PSU, while *C. hyoscella* showed maximum growth rate at 34 PSU. Additionally, in studies on *A. coerulea*, Fu et al. [57] reported that *A. coerulea* increased its growth rate when exposed to lower salinity, and research by Schäfer et al. [23] and Yu et al. [58] also suggested that salinity levels of 20 PSU and 25 PSU resulted in higher growth rates of *A. coerulea* compared to environments with salinities above 30 PSU, indicating that lower salinity environments had a significant effect on growth. In this study, *S. malayensis* ephyrae showed a tendency for both growth (Figure 5, Table 1) and survival (Figure 7, Table 7) to increase with higher salinity, while feeding rates were found to be lower in the 21 PSU environment compared to 24 PSU and 27 PSU (Figure 6, Table 4, $p < 0.001$). These results suggest that this species has the ability to adapt to a wide range of salinity conditions, but it prefers higher salinity environments for stable growth and survival. In the study by Shin and Choi [38], which simulated the summer temperature conditions of the southern coast of Korea, no significant differences were observed in the growth and survival of *S. malayensis* ephyrae under salinity conditions of 30–33 PSU at 24°C, with a very high survival rate of 98%. In this study, the survival rates at 24 PSU and 27 PSU were 90% and 87% (Figure 7, Table 7) at 20°C, and 79% and 86% (Figure 7, Table 7) at 24°C. However, the survival rate at 21 PSU was significantly lower than at 24 PSU and 27 PSU (20°C: 70%, 24°C: 55%, $P < 0.001$). Therefore, based on the results of both the Shin and Choi [38] study and this study, the ideal salinity range for the growth and survival of *S. malayensis* ephyrae is estimated to be between 24 PSU and 33 PSU. In the study by Shin and Choi [38], the salinity range of 30 PSU to 33 PSU may represent the stable optimal salinity range for this species. *Chrysaora* species such as *C. capillata* and *C. lamarckii* have a medusivores ecology, feeding on gelatinous plankton [43], and Avian et al. [29] reported in a laboratory study that *S. malayensis* ephyrae were observed to unilaterally prey on *A. solida* ephyrae. In natural environments, the abundance of zooplankton is a contributing factor to Scyphozoan outbreaks [1] and feeding on prey with appropriate nutrients has a significant impact on the growth and survival of Scyphozoans [59]. The most commonly used zooplankton in global Scyphozoan research are *Artemia* sp. and *Brachiounus* spp. [8,60]. In this study, *Artemia* sp. was provided as the prey for *S. malayensis* ephyrae, with no additional nutritional supplementation. Considering that *S. malayensis* has a medusivores ecology feeding on gelatinous plankton [29], it is possible that rapid growth could be induced when *S. malayensis* ephyrae prey on *Aurelia* spp. (*A. solida*, *A. labiata*, *A. coerulea*). Additionally, if *S. malayensis* expands its distribution into South Korean waters in the future, it may compete for food in the marine ecosystem with *A. coerulea*, which is known for its outbreaks during the summer and may prey on *A. coerulea* [4]. This possibility is supported by the findings of this study, which demonstrate that *S. malayensis* is highly adaptable to a wide range of salinity conditions, similar to the results found in studies on *Aurelia* spp. [23,42,57-58,61]. Shin and Choi [38] suggested that the optimal temperature range for the growth and survival of *S. malayensis* is between 20°C and 24°C, while Avian et al. [29] reported that the polyps of this species have the ability to reproduce even at 20°C. Furthermore, this species has a wide distribution range, not only in the Indian Ocean, Pacific Ocean, and Suez Canal but also in East Asian waters, including Japan [31]. Notably, the species has been reported to appear from spring to summer May to August in the waters of Japan, which are geographically close to the waters of South Korea [39-41]. This study confirmed that *S. malayensis* ephyrae possess a high level of adaptability, allowing for stable growth and survival across a range of salinity environments. These findings suggest that *S. malayensis* could potentially

become an invasive species capable of expanding its distribution into South Korean waters, which are affected by climate change. Furthermore, there is a pressing need for additional biological and ecological research to understand the potential impacts of this species' presence on marine ecosystems.

6. Conclusions

This study confirmed that *S. malayensis* ephyrae exhibit stable growth (Figure 5, Table 1) and high survival rates (Figure 7, Table 7) across a range of salinity environments, demonstrating their high adaptability to varying salinity conditions. Considering that *S. malayensis* is distributed in the East Asian waters, including Japan, these findings suggest the potential for this species to expand its distribution into South Korean waters, which are influenced by climate change. If the distribution of this species expands, there is a pressing need for additional research to understand the potential impacts on marine ecosystems. Future studies should focus on thoroughly analyzing the potential ecological consequences of this species' distribution expansion on local ecosystems.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1, S2: ART-ANOVA analysis results for feeding rate changes at three salinity levels (21 PSU, 24 PSU, 27 PSU) measured at 20°C. Figure S1, S2: Cox Hazard Ratio analysis results for survival rates at salinities of 21 PSU, 24 PSU, and 27 PSU under two different temperatures (20°C and 24°C) for evaluation.

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