

Original Article

The synergistic influence of water salinity and temperature on the routine oxygen consumption and apparent heat increment of *Penaeus vannamei* (Boone, 1931) juveniles

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Abstract: The Pacific white shrimp, *Penaeus vannamei* (Boone, 1931), is a precious species in aquaculture due to its increasing market demand. This study evaluated the routine oxygen consumption rate (OCR) and apparent heat increment (AHI) of *P. vannamei* juveniles at four salinities of 5, 15, 25, and 35 ppt and two temperatures of 28 and 32°C levels to understand their respiratory adaptations to salinity fluctuations, which is essential for optimizing their culture conditions. The results indicated that white shrimp juveniles are well-adjusted to withstand salinity changes up to 35 ppt, as evidenced by a 100% survival rate following exposure to various salinity levels (5, 15, 25, and 35 ppt) at 28 and 32°C, respectively. However, the findings show that salinity significantly influenced the metabolic rate of *P. vannamei* juveniles. *Penaeus vannamei* juveniles exposed to various salinity levels exhibited higher oxygen consumption and AHI at 32°C compared to 28°C. Oxygen consumption (fasting and feeding) elevated as salinity reduced, with the maximum values at 5 ppt and the minimum at 25 ppt at both temperatures. When salinity was lowered from 25 to 5 ppt, the OCR of shrimp after feeding increased. At low salinity (5 ppt), oxygen consumption increased by 160.78% and 197.48% at 28°C and 32°C, respectively. However, OCR increased with increasing salinity from 25 to 35 ppt at both temperatures. A surge in metabolic rate at low salinity (5 ppt) was likely due to a decreased ability to maintain ionic and osmotic balance as temperature increased. The study also demonstrated that the oxygen consumption of unfed *P. vannamei* juveniles significantly depends on salinity, with no significant influence from temperature and the interaction between the two factors (temperature x salinity). However, the OCR of *P. vannamei* juveniles after feeding was significantly influenced by salinity, temperature, and their interaction. These findings suggest that to optimize the production of *P. vannamei* in controlled conditions, the shrimp should be cultivated at a salinity range of 15 to 25 ppt at 28 and 32°C, respectively.

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Introduction

For more than a decade, there has been a remarkable surge in the worldwide production of marine shrimp due to rising market demand. Marine shrimp remains one of the largest commodities in aquaculture, and the Pacific white shrimp, *Penaeus vannamei* (Boone, 1931), is the most widely farmed crustacean species worldwide (FAO, 2016, 2018). In recent years, *P. vannamei* has drawn considerable attention as one of the topmost major penaeid shrimp species commercially reared in the Philippines. This penaeid species is rapidly replacing the black tiger shrimp (*Penaeus monodon*) as the primary cultured species due to its relatively higher growth rate, higher

stocking rate, and yield, and lower production costs than *P. monodon* (Limhang et al., 2010). This euryhaline white shrimp thrives in a wide range of salinities, from brackish (1-2 ppt) to hypersaline (45 ppt) waters (Bray et al., 1994; Parnes et al., 2004; Re et al., 2012). It also has a broad temperature tolerance (~15-30°C) and high resistance to certain diseases and viral pathogens, making it a promising species for brackish water and low salinity inland aquaculture in many parts of the world (Pan et al., 2007; Re et al., 2012; Gao et al., 2016). The optimal salinity for best growth of *P. vannamei* has been reported at 33-40 ppt for post larvae (Ponce-Palafox et al., 1997) and 5 ppt for juveniles (mean weight=1.6 g; Bray et al., 1994).

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However, 15-25 ppt salinities are ideal for *P. vannamei* culture (Boyd, 1989). Huang (1983) indicated that the fastest growth of *P. vannamei* juveniles occurs at 25 ppt and the poorest growth at 5 and 45 ppt, respectively. Conversely, Bray et al. (1994) found better growth of *P. vannamei* juveniles at 5-15 ppt and decreased growth at 49 ppt. Yan et al. (2007) also reported that elevating salinity from 11 to 21 to 31 ppt led to a gradual increase in the specific growth rate of shrimp. Despite these studies, the optimal salinity for the growth of *P. vannamei* remains inconclusive and controversial (Ponce-Palafox et al., 1997; Jiang et al., 2000; Li et al., 2007). While many studies have explored the adaptive physiology of *P. vannamei*, conflicting results on the effects of salinity have been reported (Rosas et al., 2001a). The optimal temperature for better growth of *P. vannamei* appears to be size-specific, with recommendations of 28-30°C for post larvae (Ponce-Palafox et al., 1997), above 30°C for small juveniles (<5 g), and around 27°C for sub-adults (Wyban et al., 1995). The ability of *P. vannamei* to regulate daily and seasonal variations in salinity and temperature is a crucial factor, making it an attractive species for aquaculture (Ponce-Palafox et al., 1997; Díaz et al., 2001).

Salinity and temperature are among the most important abiotic factors in aquaculture that influence not only the physiology but also the growth and survival of aquatic organisms (Jiang et al., 2000; Kumlu et al., 2000, 2010; Ye et al., 2009; Chong-Robles et al., 2014). Salinity fluctuation is directly linked to osmoregulation (Chong-Robles et al., 2014) and poses challenges to organisms' biological processes and physiological homeostasis (Kültz, 2015). The organisms modify the osmotic concentration of their hemolymph in response to changes in environmental salinity, which disrupts osmotic equilibrium (Díaz et al., 2001). The penaeid shrimp have an osmoregulatory capacity that allows them to adjust hemolymph osmolytes to control hemolymph osmotic concentration, which is crucial for their survival (Parado-Esteva et al., 1987; Díaz et al., 2001; Chong-Robles et al., 2014). The energy allocated to metabolic processes appears to be

influenced by salinity and temperature (Chen and Lai, 1993; Chen and Nan, 1993, 1994; Rosas et al., 1999a, b). Acclimating shrimp to wide salinity fluctuations may involve more energy to regulate iono-osmotic equilibrium, which could affect their metabolic requirements for survival. Since it is associated with the metabolic activity and energy flow that an organism needs for its homeostatic control mechanisms, the oxygen consumption rate (OCR) is one of the physiological responses that can be linked with changes in environmental conditions (Salvato et al., 2001).

The metabolic rate, indirectly determined by oxygen consumption, represents the overall energy-demanding aerobic metabolism of food consumed and the regular metabolism to sustain biological processes (Jobling, 1993; De Boeck et al., 2000). Numerous studies have extensively documented the combined effects of salinity and temperature on the metabolism of penaeid shrimps, quantified by oxygen consumption (Chen and Lai, 1993; Chen and Nan, 1993, 1994; Villarreal and Rivera, 1993; Villarreal et al., 1994; Salvato et al., 2001). However, the interaction between low water salinities and temperatures on the mechanisms involved in respiratory metabolism adjustments and the calorogenic effect of feeding in *P. vannamei* has not been extensively studied. Quantifying oxygen consumption has been a valuable index for evaluating the energetic cost of osmoregulation and the physiological state of shrimp, including active, routine, and postprandial metabolism. This approach allows for determining ingested energy pathways related to different dietary components or environmental parameters (Villarreal and Rivera, 1993; Rosas et al., 1996, 1999a, b, 2001; Salvato et al., 2001). The energy allocated to respiratory and energetic metabolism was evaluated as the sum of metabolic activity (routine rate of oxygen consumption) and the calorogenic effect of food or apparent heat increment (AHI) (Beamish and Trippel, 1990; Lemos et al., 1996; Burggren et al., 1993; Rosas et al., 1996). Post-prandial stimulation in metabolism is called apparent heat increment (AHI) or specific

dynamic action (SDA), and calorogenic or thermic effect of feeding, which measures the metabolic rate (energy expenditure) of the post-absorptive processes following diet ingestion (Beamish and Trippel, 1990; Burggren et al., 1993; Jobling, 1993; Lemos et al., 1996). However, there is limited information on the metabolic and respiratory adjustments in *P. vannamei* juveniles to withstand variations in salinity and temperature. Information about the interaction of salinity and temperature on white shrimp's physiological and respiratory adaptations is crucial for determining the adaptive capacities of this penaeid shrimp species, optimizing rearing procedures, and improving production conditions. Thus, to determine the tolerance limit towards optimizing the rearing techniques of the white shrimp, this study was conducted to assess the synergistic influence of water salinity and temperature on the routine metabolic rates of *P. vannamei* juveniles as measured by oxygen consumption and AHI.

Materials and Methods

Experimental animals and acclimatization: The *P. vannamei* juveniles (mean body weight \pm standard deviation (SD): 1.34 ± 0.37 g) used in this study were sourced from a 70 m³ rectangular outdoor tank in the high-density polyethylene (HDPE) concrete pond system of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miag-ao, Iloilo, Philippines. The *P. vannamei* juveniles were transported to the laboratory in oxygen-saturated brackish water (25 ppt) at $27.67 \pm 0.58^\circ\text{C}$.

Upon arrival, the shrimp were randomly distributed into four rectangular plastic tanks containing 50-L of previously aerated rearing water at the original salinity of 25 ppt. The rearing water had been aerated for at least 24 hours, and each tank housed a maximum of 10 shrimps. Each tank was covered with a black mesh cover to prevent the animal from escaping. During acclimatization and experimentation, a static water system with constant aeration was provided in each tank using a single air-stone to keep dissolved oxygen (DO) levels above 5 ppm. Salinity acclimatization

involved a stepwise increase/ decrease at a rate of 5 ppt d⁻¹ from the original salinity (25 ppt) by diluting natural seawater (33 ppt) with freshwater using a portable refractometer (ATC S-100 model). Aerated freshwater aged 24 h was diluted in the seawater until the desired salinities were reached. Natural sea salt (Díaz et al., 2001) was dissolved into filtered natural seawater to achieve the required salinity of 35 ppt.

After adjusting to the desired experimental salinity, the shrimp were acclimatized for at least 4 days (Rosas et al., 2001b). The shrimps were kept under constant 12-h light and 12-h dark photoperiod and hand-fed to satiation twice daily (0800 and 1700) with a commercially available diet (14.99 KJ g⁻¹) for *P. vannamei* (Table 1). No mortality was observed during or after the salinity reduction. In each tank, 75% of the rearing water was changed daily to ensure optimal water quality. Unconsumed feed, feces, and molted exuviae were siphoned out daily before water management. Optimum water quality parameters were monitored throughout the experiment, with ammonia at 0.06 ± 0.1 ppm, dissolved oxygen (DO) above 5 ppm, and pH at 8.2 ± 0.1 .

Experimental design and set-up: Metabolic oxygen consumption was determined using a respirometric assay. The routine oxygen consumption and the AHI were quantified in homogenous-sized *P. vannamei* juveniles (1.34 ± 0.37 g) acclimatized for 4 d at 35, 25, 15, and 5 ppt salinities in rectangular plastic tanks (10 shrimps 50 L⁻¹). Salinity adjustments were made at a rate of 5 ppt d⁻¹ (Rosas et al., 2001b), and the 4-day acclimatization period commenced after the desired experimental salinity was reached. Previous results indicated that a 4-day acclimatization period was sufficient for shrimp acclimatization (Rosas et al., 2001b). Temperature adjustments were made at a rate of 1°C d⁻¹, achieving different temperatures (28 and 32°C) using a temperature-controlled water bath. During acclimatization, the *P. vannamei* juveniles were hand-fed twice daily with a commercial feed (37% protein and 4% lipid). After acclimatization, intermolt shrimp, regardless of sex, were individually placed in 250 ml Pyrex Erlenmeyer flasks (respirometric chambers) filled with 200 ml of

Table 1. Proximate composition of commercial *Penaeus vannamei* starter pellet (Oversea Feeds Corp., Balud, San Fernando, Cebu, Philippines).

Proximate analysis	
Crude protein (%)	37.0
Crude lipid (%)	4.0
Crude fiber (%)	4.0
Crude ash (%)	16.0
NFE (%) *	39.0
Gross energy (KJ g ⁻¹) **	14.99

* Nitrogen-free extract, computed by difference. ** Gross energy was calculated using the following factors: protein: 24 KJ g⁻¹; lipid: 38 KJ g⁻¹; carbohydrates: 17 KJ g⁻¹ (Jobling, 1993).

dechlorinated filtered water for each experimental combination of salinity and temperature (Chen and Lai, 1993; Villarreal and Rivera, 1993; Chen and Nan, 1994). The shrimp were kept in sealed respirometric chambers, with oxygen-saturated water maintained using air diffusers (Burggren et al., 1993; Villarreal and Rivera, 1993). The oxygen consumption and the AHI of shrimp juveniles were evaluated in triplicated combinations of four salinities (35, 25, 15, and 5 ppt) and two temperatures (28 and 32°C). Each chamber was sealed to prevent shrimp escape and minimize water evaporation (Jiang et al., 2000). All respirometric chambers were placed in a temperature-controlled water bath using a submersible 150W heater.

The shrimp were starved for 24 hours (Rosas et al., 1996, 1999; Díaz et al., 2001; Re and Diaz, 2011) to ensure maximum gut evacuation. Before any measurements, the unfed shrimp were acclimatized to the chamber for one hour to minimize the handling effect (Rosas et al., 1996). After 24 h of feed deprivation, the first oxygen consumption measurement was conducted on unfed shrimp with a static renewal of experimental water after one hour to maintain constant chamber volume (Chen and Nan, 1994; Rosas et al., 1996, 1999b). Measurement of DO consumption commenced with the cessation of airflow in the respirometer chamber (Burggren et al., 1993). During this time, oxygen consumption readings for the unfed *P. vannamei* juveniles were collected. The oxygen consumption of unfed shrimp, measured in triplicates, served as an index of oxygen consumption for fully acclimatized shrimp and was used as reference values (Dal and Smith, 2001). Water in each

test chamber was sampled through a plastic cannula connected to volume-calibrated glass syringes (Lemos et al., 2001). Oxygen concentration was measured using Winkler's method after introducing appropriate reagents into sample-filled syringes (Fox and Wingfield, 1938). Variations in oxygen consumption were detected by comparing experimental and control (no animals) units (Lemos et al., 2001).

Following this first metabolic measurement in unfed shrimps, individuals in each chamber, including the control chamber (without shrimp), were hand-fed with 0.07 g dry weight (dw) *P. vannamei* starter pellet (14.99 KJ g⁻¹ dw). The introduced food in the control chamber without shrimp served as blanks, which was used to correct the error due to the oxygen consumption by the microorganisms that fed on unconsumed food, as well as to detect if the presence of food altered the concentration of oxygen in the respirometric chamber (Beamish and Trippel, 1990; Rosas et al., 1999a, b, 2001b). In most cases, the oxygen consumption in the control chambers was negligible. After feeding, oxygen consumption was measured again hour for one hour. All measurements were conducted during the daytime between 0800 and 1000 h. The AHI was determined from the difference between the oxygen consumption of the feeding and unfed shrimp (Beamish and Trippel, 1990; Rosas et al., 1996, 2001b). A 14.3 joules mg⁻¹ oxycaloric equivalent was used to convert the routine oxygen consumption into energy utilization (AHI) in joules h⁻¹ g⁻¹ dw (Lucas, 1996). At the end of the experiments, the shrimp were weighed, euthanized, and dried overnight to 60°C, placed on a desiccator for two hours, and weighed to determine the dry weight (Díaz

Table 2. Factorial analysis on the oxygen consumption (fasting and feeding) and AHI of white shrimp juveniles subjected to various salinity and temperature combinations.

Salinity (ppt)	Temperature (°C)	Oxygen consumption (mgO ₂ h ⁻¹ g ⁻¹ dw)		AHI* (joules h ⁻¹ g ⁻¹ dw)
		Unfed	Feeding	
Salinity effect (<i>P</i> -value)		0.021	0.000	0.000
5		1.56±0.20 ^a	4.36±0.51 ^a	40.04±6.46 ^a
15		1.40±0.19 ^{ab}	2.66±0.16 ^b	18.09±4.29 ^b
25		1.21±0.10 ^b	1.84±0.10 ^c	9.06±2.17 ^c
35		1.42±0.12 ^{ab}	2.67±0.13 ^c	12.75±6.35 ^{bc}
Temperature effect (<i>P</i> -value)		0.456	0.016	0.004
	28	1.37±0.05 ^a	2.77±0.06 ^b	17.42±1.07 ^b
	32	1.42±0.05 ^a	3.00±0.06 ^a	22.55±1.07 ^a
Salinity x Temperature (<i>P</i> -value)		1.000	0.029	0.038

Note: Values (mean±SD) appearing in each column with unlike superscripts are significantly different ($P<0.05$).

*AHI=Apparent heat increment.

et al., 2001; Rosas et al., 2001b). Oxygen consumption results were related to the dry weight of the experimental animals and expressed as mg O₂ h⁻¹ g⁻¹ dw (Díaz et al., 2001; Rosas et al., 2001b).

Statistical analysis: The normality of the data in each parameter was evaluated using the Shapiro-Wilk test. A two-way analysis of variance (ANOVA) was utilized to assess the synergistic effects of water salinity, temperature, and their interaction on the oxygen consumption and AHI of the experimental animals. One-way ANOVA was employed to evaluate the mean values after a significant interaction was found. If significant differences were detected, Tukey's HSD post-hoc test ($P<0.05$) was used to determine which groups exhibited significant differences. Statistical analyses were performed among various parameters using IBM SPSS Version 20.0 and presented as mean ± SD.

Results

Oxygen consumption: The fasting and feeding oxygen consumptions of *P. vannamei* juveniles subjected to various salinity fluctuations (5, 15, 25, and 35 ppt) and temperature (28 and 32°C) levels are presented in Tables 2 and 3. The results showed that temperatures ($P=0.456$) and the interaction of salinity and temperature ($P=1.000$) had no significant influence on the oxygen consumption of unfed *P. vannamei* juveniles (Table 2). However, the effect

of different salinity fluctuation regimes ($P=0.021$) on the oxygen consumption of unfed *P. vannamei* juveniles was significant ($P=1.000$; Table 2). At both temperatures (28 and 32°C), oxygen consumption of unfed *P. vannamei* juveniles exposed to fluctuating salinity levels was highest at 5 ppt and lowest at 25 ppt. The fasting oxygen consumption (mgO₂ h⁻¹ g⁻¹ dw) of *P. vannamei* juveniles exposed to fluctuating salinity levels (5, 15, 25, and 35 ppt) was higher at 32°C than at 28°C. However, the findings indicated no significant influence on the oxygen consumption of unfed *P. vannamei* juveniles exposed to different fluctuating salinity conditions, regardless of temperature levels ($P>0.05$; Table 3). The OCR increased when the unfed animals were subjected to descending salinities of 25 to 5 ppt at both temperatures, but the variations were insignificant ($P>0.05$). However, the OCR of the unfed shrimps decreased when exposed to descending salinities of 25 to 15 ppt at 28° and 32°C (Table 3). Moreover, the results showed that the unfed shrimp juveniles exposed to 25 ppt had the lowest oxygen consumption (mgO₂ h⁻¹ g⁻¹ dw) than other groups ($P=0.131$; Table 3).

After feeding, the results showed that the various salinity levels ($P=0.000$) and temperatures ($P=0.016$) significantly influenced the oxygen consumption of shrimp juveniles (Table 2). In addition, the interaction between water salinity and temperature significantly

Table 3. Comparison of the mean oxygen consumption (fasting and feeding) and AHI of white shrimp juveniles subjected to various salinity and temperature combinations.

Salinity (ppt)	Temperature (°C)	Oxygen consumption (mgO ₂ h ⁻¹ g ⁻¹ dw)		AHI*	
		Unfed	Feeding	A	B
5	28	1.53±0.20 ^a	3.99±0.46 ^b	2.46±0.36 ^a	35.18±5.10 ^a
15	28	1.37±0.23 ^a	2.63±0.16 ^c	1.26±0.36 ^b	18.02±5.10 ^b
25	28	1.18±0.10 ^a	1.81±0.10 ^d	0.63±0.12 ^b	8.96±1.66 ^b
35	28	1.39±0.10 ^a	2.64±0.16 ^c	1.25±0.24 ^b	17.92±3.40 ^b
5	32	1.59±0.24 ^a	4.73±0.18 ^a	3.14±0.19 ^a	44.90±2.73 ^a
15	32	1.42±0.20 ^a	2.69±0.18 ^c	1.27±0.31 ^b	18.16±4.46 ^b
25	32	1.23±0.12 ^a	1.87±0.10 ^d	0.64±0.21 ^b	9.15±3.00 ^b
35	32	1.44±0.15 ^a	2.70±0.10 ^c	1.26±0.25 ^b	17.97±3.54 ^b
One-way ANOVA (<i>P</i> -value)		0.131	0.000	0.000	0.000

Note: Values (mean±SD) appearing in each column with unlike superscripts are significantly different ($P<0.05$). *AHI=Apparent heat increment; A = mgO₂ h⁻¹ g⁻¹ dw; B = joules h⁻¹ g⁻¹ dw.

influenced the oxygen consumption of shrimp juveniles after feeding ($P=0.029$; Table 2). The results indicated that the oxygen consumption of shrimp juveniles was significantly influenced ($P<0.05$) by various experimental conditions after feeding ($P=0.000$; Table 3). The newly fed *P. vannamei* juveniles exposed to 5 ppt at 32°C significantly resulted in higher OCR (4.73±0.18 mg O₂ h⁻¹ g⁻¹ dw) than those exposed to 15, 25, and 35 ppt at both temperature (28 and 32°C) levels ($P=0.000$; Table 3). In contrast, the newly fed *P. vannamei* juveniles exposed to 25 ppt of both temperature levels (28 and 32°C) significantly resulted in lower oxygen consumption than the other groups. However, no significant differences were noticed in the oxygen consumption of the *P. vannamei* juveniles exposed to 15 and 35 ppt at both temperature (28 and 32°C) levels after feeding ($P>0.05$; Table 3).

The OCR of the newly fed *P. vannamei* juveniles (0.5 h) increased markedly with that of the 24-h-fasting ones. During this time, the oxygen consumption of *P. vannamei* juveniles acclimatized to 5 ppt rapidly elevated significantly ($P<0.05$) at 28°C (160.78%; 3.99.01±0.30 mg O₂ h⁻¹ g⁻¹ dw) and 32°C (197.48%; 4.73±0.18 mg O₂ h⁻¹ g⁻¹ dw), respectively (Table 3). The shrimps acclimatized to 15 ppt at 28°C; the oxygen consumption reached its maximum of 0.5 h following food ingestion (2.63 mg O₂ h⁻¹ g⁻¹ dw) (Table 3), about 92 % higher than that of the unfed shrimps (1.37 mg O₂ h⁻¹ g⁻¹ dw). However, the

shrimps acclimatized to 15 ppt at 32°C, the oxygen consumption after 0.5 h feeding was about 89% higher (2.69 mg O₂ h⁻¹ g⁻¹ dw) than that of the unfed shrimps (1.42 mg O₂ h⁻¹ g⁻¹ dw). The oxygen consumption of the shrimp acclimatized to 25 ppt at 28°C after 0.5 h was 53% greater (1.81 mg O₂ h⁻¹ g⁻¹ dw) than that of the unfed shrimps (1.18 mg O₂ h⁻¹ g⁻¹ dw). However, the oxygen consumption of the shrimp acclimatized to 25 ppt at 32°C after 0.5 h was 52% higher (1.87 mg O₂ h⁻¹ g⁻¹ dw) than that of the unfed shrimps (1.23 mg O₂ h⁻¹ g⁻¹ dw). While the oxygen consumption of the newly fed shrimp (0.5 h) acclimatized to 35 ppt also increased significantly ($P<0.05$) at 28°C (89.93%; 2.64 mg O₂ h⁻¹ g⁻¹ dw) and 32°C (87.50%; 2.70 mg O₂ h⁻¹ g⁻¹ dw), respectively (Table 3).

Apparent heat increment: The results showed that the salinity ($P=0.000$), temperature ($P=0.004$), and the interaction of salinity and temperature ($P=0.038$) had a significant influence on the AHI (joules h⁻¹ g⁻¹ dw) of *P. vannamei* juveniles (Table 2). The AHI of shrimp juveniles were significantly highest in animals acclimatized at 5 ppt in 28°C (2.46±0.36 mg O₂ h⁻¹ g⁻¹ dw; 35.18±5.10 joules h⁻¹ g⁻¹ dw) and 32°C (3.14±0.19 mg O₂ h⁻¹ g⁻¹ dw; 44.90±2.73 joules h⁻¹ g⁻¹ dw), respectively ($P=0.000$; Table 3). The AHI of *P. vannamei* juveniles exposed to fluctuating salinity levels (5, 15, 25, and 35 ppt) was higher at 32 than at 28°C. However, the AHI was significantly highest at 5 ppt and lowest at 35 ppt at 28°C and 32°C ($P=0.000$; Table 3). The juveniles exposed to 5 ppt at both

temperatures (28 and 32°C) had significantly higher AHI than those exposed to 35 ppt ($P=0.000$; Table 3). The shrimp juveniles exposed to 25 ppt at both temperatures had significantly lower AHI than other groups (Table 3). However, no significant differences were noticed in the AHI of the *P. vannamei* juveniles exposed to 15, 25, and 35 ppt at both temperatures (28 and 32°C) after feeding (Table 3). The AHI represented the energy allocated to the metabolic process, ranging from 44.90 ± 2.73 to 8.96 ± 1.66 joules $\text{h}^{-1} \text{g}^{-1} \text{dw}$ across the different experimental conditions (Table 3). Various experimental conditions significantly affected the AHI of *P. vannamei* juveniles ($P < 0.05$).

Discussions

The respiratory rate in crustaceans is regulated by external factors such as water temperature, salinity, dissolved oxygen (DO), and light intensity (Dalla-Via, 1986; Kurmaly et al., 1989; Martínez-Palacios et al., 1996). The ability of penaeids to effectively acquire and utilize energy for growth and maintenance depends on these factors. According to Rosas et al. (2001b), the respiratory rate behavior, especially in juvenile *P. vannamei*, is influenced by acclimatization time, linked to various adjustment mechanisms that shrimp and other crustaceans employ to cope with salinity changes. The osmoregulatory capacity of penaeid shrimp to regulate changes in salinity and temperature is crucial for their survival. Any variations in these environmental conditions disrupt their osmotic equilibrium, necessitating readjustments in the osmotic concentration of their hemolymph (Parado-Esteva et al., 1987; Díaz et al., 2001; Chong-Robles et al., 2014). When comparing the internal osmotic concentration to various environmental salinity exposures, oxygen consumption is frequently utilized as a metabolic indicator to show an energetic demand (Ern et al., 2014). The energetic cost of osmoregulation generally accounts for 25-50% of metabolic activity (Ahmadi et al., 2016). Numerous studies have reported minimal OCR in aquatic animals at their natural habitat salinity (Boeck et al., 2000; McKenzie et al., 2001; Kidder et al., 2006; Scott et al.,

2007), corroborated by the current study. The OCR of unfed *P. vannamei* juveniles is comparable and independent of DO across all treatments, demonstrating the shrimp's ability to modulate oxygen intake (Villarreal et al., 1994).

In 4-d acclimatized *P. vannamei* juveniles, a reduction of salinity from 25 to 5 ppt increased the OCR of the shrimps. This result aligns with Rosas et al. (2001b) findings, indicating that four days is sufficient for *P. vannamei* acclimatization. Earlier studies also reported that energetic costs associated with osmotic stress would cause physiological and metabolic responses, such as elevating DO consumption (Chen and Chia, 1996; Spanopoulos-Hernández et al., 2005). Similarly, when fishes were exposed to salinities beyond their natural environment, metabolic oxygen consumption increased (Rahmah et al., 2020). Since these mechanisms involve metabolic energy, the OCR of the shrimp increases to meet the energetic requirements needed to maintain essential physiological function and for the mechanisms of homeostatic control (Chen and Nan, 1995; Rosas et al., 1998; Salvato et al., 2011), this suggests that energy allocated for metabolic activity increases under osmotic stress conditions, as validated in the current study. Therefore, the reduction of DO in the rearing water is likely linked to the rising OCR demand as a metabolic response to various salinity fluctuations in cultured shrimp.

When the salinity changed from 25 to 15 ppt and from 15 to 5 ppt, the OCR of the shrimps significantly increased after feeding. There was a noticeable decrease in animal motor activity in respirometric chambers due to this salinity reduction. Given that the salinity range of 25 to 15 ppt is within the normal range of salinity for the white shrimp juveniles, it is anticipated that the animal will initially conserve energy to meet physiological adaptations before utilizing that energy for motor activity. The decrease in oxygen intake coupled with the motor activity could be considered an energy-saving strategy in which the shrimp used its energy to compensate for these salinity variations rather than increase muscular activity

(Rosas et al., 2001b). The influence of salinity on the respiratory metabolism of penaeid shrimp is usually thought to be an energy consumption modification caused by an osmotic pressure difference between the external environment and body fluid (Ye et al., 2009). Panikkar (1968) reported that euryhaline shrimp species can adjust to external salinity change by hemolymph osmoregulation and ionic regulation. This type of osmotic pressure-driven regulatory mechanism requires the shrimp to expend energy to adjust to the external salinity change, which elevates its metabolic rate at the expense of slower growth. Generally, shrimps are hypo-osmotic for their natural environment, and their metabolic energy requirements are not generally adapted to low salinities (Gaudy and Sloane, 1981). In particular, *P. vannamei* is an euryhaline shrimp species that can be reared in a wide range of salinities and has a relatively low metabolic rate compared to that observed in *Litopenaeus setiferus* (Rosas et al., 2001a).

The OCR of fasting *P. vannamei* juveniles exposed to salinity fluctuations was not significantly different. In other osmoregulator decapods, salinity did not significantly influence oxygen consumption when the organisms were acclimatized to experimental salinities, provided these were not extreme (Gaudy and Sloane, 1981; Díaz-Herrera et al., 1992; Villarreal and Rivera, 1993; Villarreal et al., 1994; Salvato et al., 2001). The 4-day acclimatization period for *P. vannamei* juveniles to each salinity condition was sufficient to stabilize their internal environment (Díaz et al., 2001). Numerous studies have reported similar behaviors in decapod crustaceans. For instance, Rosas et al. (1997) noted a 158% increase in the OCR of *L. setiferus* and *L. schmitti* Burkenroad postlarvae acclimatized to salinities below 5 ppt compared to shrimp acclimatized to 37 ppt. Chen and Chia (1996) observed a similar trend in juvenile *Scylla serrata* acclimatized to combinations of 15, 20, 25, and 30 ppt and temperatures of 16, 22, 27, and 32°C. Rosas et al. (1999a) reported a 259% increase in oxygen consumption in *L. setiferus* postlarvae when salinity was reduced from 40 to 10 ppt. Puig and Sanz (1987) found that oxygen consumption in *Palaemonetes*

zariquieyi Sollaud was 300% higher in shrimp acclimatized to 1 ppt than those acclimatized to 30 ppt. Several authors have linked this difference in oxygen consumption to increased metabolic energy expenditure required to maintain routine metabolism and homeostasis (Gérald and Gilles, 1972; Haberfield et al., 1975; Savage and Robinson, 1983; Almeida, 1985; Rasmussen and Bjerregard, 1995).

In the shrimp juveniles subjected to fluctuating salinity, the AHI value significantly increased as salinity lowered from 25 to 5 ppt, with the highest values noted in shrimp acclimatized to 5 ppt at 28 and 32°C. However, no significant differences were observed in AHI as the shrimps were fed the same diet (Rosas et al., 2002; Re et al., 2010; Re and Diaz, 2011). Previous studies have shown that AHI increases with dietary protein content (Rosas et al., 1996; Taboada et al., 1998) and decreases in salinity (Rosas et al., 1999a). Although the metabolism of proteins, lipids, and carbohydrates contribute to AHI, amino acid deamination and protein synthesis are likely the primary factors affecting AHI increase (Beamish and Trippel, 1990). Based on earlier findings, a reduction in salinity is expected to increase AHI, presumably due to increased amino acid catabolism to maintain the shrimp's hemolymph osmotic concentration (Claybrook, 1983). This is because, at lower salinities, shrimp use dietary proteins as the primary energy source (Chen, 1998). Further studies are needed to verify if energetic parameters are influenced by nutritional factors (e.g., food quantity and quality), showing the effect on the growth and survival of juveniles.

Most penaeid shrimps are known as euryhaline species that can thrive in various salinities (Ye et al., 2009). This study demonstrates that *P. vannamei* juveniles are effective at regulating environmental changes; when acclimatized, they can adjust their osmoregulatory patterns with their hemolymph osmotic concentration (Díaz et al., 2001; Rosas et al., 2001b). While the isosmotic point has been associated with optimal growth conditions for penaeid shrimp, Rosas et al. (2001b) reported no direct relation between the isosmotic point and the maximum growth

rate. The findings suggest that an osmotic gradient below the isosmotic point enhances the assimilation mechanisms, thereby reducing energy expenditure and motor activity, which supports the maintenance of metabolic processes and allows more energy for growth. This hypothesis may explain why 5 and 15 ppt are considered optimal salinities for the growth of *P. vannamei* juveniles under laboratory conditions (Bray et al., 1994).

The lowest OCR of *P. vannamei* juveniles exposed to fluctuating salinity levels was recorded at 25 ppt at 28 and 32°C. This indicates that the shrimp utilized less energy to maintain its routine metabolic process (Re et al., 2010). This finding was anticipated since this salinity is known as an isosmotic environment for *P. vannamei* juveniles (Díaz et al., 2001), where the animals do not need active processes to compensate for the internal environmental equilibrium in response to external environment changes (Re et al., 2010). Castile and Lawrence (1981) reported that the isosmotic point for *P. vannamei* is about 24.7 ppt salinity. Similarly, Díaz et al. (2001) reported the isosmotic point for *P. vannamei* juveniles exposed to descending and ascending salinities and temperatures, ranging from 712 to 777 mmolKg⁻¹ (24.5-25 ppt). The shrimp do not need to expend energy hypo- or hyper-osmoregulation at this salinity level to maintain hemolymph osmolality (Ray and Lotz, 2017). As the temperature increased, these metabolic peaks appeared at the lower salinity level, potentially indicating a reduced capacity to maintain the ionic and osmotic balances of the animals (Villarreal et al., 1994). Salinity levels outside the 15-25 ppt range will elevate the energy expenditure required to maintain homeostasis, leading to increased DO consumption and poor growth and feed conversion efficiency.

Conclusion

In conclusion, the findings showed that *P. vannamei* juveniles can withstand a broad gamut of salinity up to 35 ppt. However, the optimal routine metabolic demand by the white shrimp was found within a salinity range of 15 to 25 ppt at both 28 and 32°C water temperatures. This salinity range was

considered ideal for the optimal growth requirement of *P. vannamei* juveniles (Bray et al., 1994). Thus, a salinity range between 15 and 25 ppt of both temperatures (28 and 32°C) is recommended to optimize the culture of *P. vannamei* juveniles. This range corresponds with the optimal salinity closer to the isosmotic point (Castile and Lawrence, 1981; Rodriguez, 1981; Díaz et al., 2001) and ideal temperature (Wyban et al., 1995; Ponce-Palafox et al., 1997) determined for this species. In this salinity range, the white shrimp juveniles invest less energy in metabolic demands, channeling more energy towards growth in a stress-free and favorable environmental condition (Valdez et al., 2008). Given this favorable and stress-free environment, the white shrimp are expected to optimize their physiological adjustments without excessive metabolic energy for osmoregulation, permitting more available energy for growth enhancement. Moreover, this salinity range is within the typical range found in their native environment. Rearing white shrimp juveniles at optimal fluctuating salinity could result in elevated assimilation, favoring the growth of the animal. To attain the highest shrimp growth, aquaculturists and farm operators should prudently consider the timing of freshwater additions to modulate the salinity levels closer to the isosmotic point to meet the optimal salinity requirements of *P. vannamei*. These findings provide valuable insights about the sustainable management practices in shrimp aquaculture. In addition, this result will provide valuable information to maximize output in abandoned brackish shrimp ponds to promote nursery and grow-out production of *P. vannamei*.

Ethics statement

Ethical review and approval were not required for the study involving animals, as it complies with local legislation and institutional requirements. This study adheres to the Philippine National Standard (PNS) on the Code of Good Aquaculture Practices (GAqP) for Shrimp and Crab (PNS/BAFS 197:2017; BAFES, 2017). Rearing, handling, and animal welfare protocols were strictly followed according to the

guidelines stipulated in the Philippine Republic Act Number 8485, otherwise known as the Animal Welfare Act 1998.

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