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Research article

# Acute toxicity assessment and real-time metabolic rate responses of early life stage *Macrobrachium rosenbergii* to ammonia exposures at different salinities

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ABSTRACT

*Macrobrachium rosenbergii*, the giant freshwater prawn, is an important aquaculture species cultivated worldwide. As a catadromous species, it requires brackish water for early development (larval stages) and grows optimally under low-salinity conditions. This tolerance enables production using brackish groundwater or desalination concentrate, helping reduce disposal costs. However, aquaculture systems often accumulate nitrogenous waste such as ammonia, which can negatively affect growth, survival, and health. The interactive effects of ammonia and salinity on *M. rosenbergii* remained understudied, particularly during juvenile stages that coincide with the transition to brackish water. Therefore, we first determined the 3, 6, 24, and 48 h median lethal concentrations ($LC_{50}$) of total ammonia nitrogen (TAN) across three salinities (1, 5, and 10 ppt) at pH 8.2. Toxicity increased with both salinity and exposure time, with $LC_{50}$ values ranging from 5.6 mg/L (95 % CI: 4.9–6.3) to 42 mg/L (95 % CI: 37–48) TAN. Based on these $LC_{50}$ values, we tested how increasing waterborne ammonia concentrations affect the routine metabolic rate (RMR) of juvenile *M. rosenbergii* using static intermittent respirometry. Analysis by a linear mixed-effects model revealed a significant salinity × ammonia interaction where the positive relationship between ammonia concentration and RMR became steeper at higher salinities. The model also identified a significant main effect of ammonia, with RMR increasing as ammonia concentration rose, but no significant main effect of salinity. These findings inform aquaculture management of *M. rosenbergii* and demonstrate the potential for sentinel respirometry systems to detect real-time water quality changes by monitoring metabolic rates.

## 1. Introduction

Aquaculture is the world's fastest-growing food production sector and now surpasses capture fisheries in global aquatic animal production (Anderson et al., 2017; FAO, 2024). This continued expansion, driven by advances in genetics, animal health, and system design, is essential to meet rising food demand (Bjørndal et al., 2024). However, intensive production systems remain vulnerable to fluctuations in water quality, which can compromise animal health and growth before obvious signs of mortality occur (Badiola et al., 2012; Raza et al., 2025). Addressing these challenges requires not only improved water quality treatment strategies, but also early indicators of physiological stress that can serve as warning signals for producers.

Technological innovations, such as recirculating aquaculture systems (RAS) aim to address many of these concerns by reducing space usage, water consumption, and feed requirements, while also facilitating improved disease control (Badiola et al., 2018; Ed-Idoko, 2021; Engle, 2023). RAS recycles water continuously within the system rather than relying on open or flow-through systems, thereby allowing environmental conditions to be highly controlled. This ability to optimize water quality parameters reduces physiological stress in cultured animals and promotes the allocation of metabolic functions to growth (Bregnballe, 2022; Martins et al., 2010; Tirsgaard et al., 2015). However, the limited water renewal and high biomass loads typical of RAS can compound the

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effects of even minor water quality disturbances. Integrating physiological monitoring tools, such as real-time respirometry, could serve as an early warning system by detecting sublethal stress before declines in performance occur.

*Macrobrachium rosenbergii*, the giant freshwater prawn, is a commercially important aquaculture species valued for its large size, rapid growth, and relative disease tolerance (Banu and Christianus, 2016; Hooper et al., 2023). As a catadromous species, it requires brackish water for early life stage development before migrating to freshwater. This makes it especially well-suited for production in systems that recycle otherwise unusable brackish groundwater or desalination waste concentrate, an approach that can lower input costs and improve the economic viability of aquaculture operations (Bowles et al., 2000; Chand et al., 2015). However, as in most aquaculture systems, high density rearing can lead to the accumulation of nitrogenous waste, particularly in the form of ammonia, especially under suboptimal water quality conditions (Tan and Wang, 2022). While both ammonia and salinity are known stressors, their interactive effects on *M. rosenbergii*, particularly during late juvenile life stages, are not well defined (Naqvi et al., 2007). Sublethal stress can also increase aggressive behaviors such as cannibalism, reducing survival and production efficiency (Reyes-Avalos, 2020). This highlights the need for species-specific tools capable of detecting early physiological or behavioral changes, enabling timely intervention before losses occur.

One of the most common and challenging stressors in intensive aquaculture systems, including RAS, is ammonia (Badiola et al., 2012; Zhao et al., 2020). Ammonia accumulates from the decomposition of organic waste and protein metabolism, producing total ammonia nitrogen (ammonia-N; TAN), which includes both ammonia ($NH_3$) and ammonium ions ($NH_4^+$). The relative proportion of each form is strongly influenced by environmental pH (Zhao et al., 2020). With a pK for $NH_3$/$NH_4^+$ of ~9.5, speciation plays a significant role in determining toxicity (Ip et al., 2001). Although temperature, pressure, and ionic strength can also affect speciation, their effects are generally minor compared to the effects of pH (Molins-Legua et al., 2006). While nitrifying microbes can aid in converting ammonia to less harmful compounds, ammonia production can exceed removal capacity, especially during biofilter disruptions or system overload (Camargo et al., 2005; Romano and Zeng, 2013).

Physiologically, elevated ammonia acts as a metabolic stressor, disrupting homeostasis and forcing organisms to divert energy from growth and reproduction toward maintenance and survival (Barton, 2002; Sandblom et al., 2016). Environmental factors such as temperature, dissolved oxygen (DO), salinity, and ammonia, can shift this energetic balance, driving metabolic adjustments that prioritize stress mitigation over performance (Fry, 1971). Given its relevance as a common contaminant and physiological stressor, ammonia was selected in this study as a model stressor to evaluate its effects on both survival and real-time metabolic responses in *M. rosenbergii*.

Routine metabolic rate (RMR), measured as oxygen consumption, serves as an integrated physiological indicator of these shifts (McBryan et al., 2013). Intermittent-flow respirometry offers a non-invasive method for monitoring real-time changes in RMR, providing a sensitive approach to detect sublethal stress before it impacts overall performance. While this study was conducted under controlled laboratory conditions, such physiological monitoring holds potential for application in intensive systems, such as RAS, where early detection of stress could inform timely management responses to optimize water quality parameters and improve animal welfare.

This study aimed to evaluate the interactive effects of ammonia and salinity on juvenile *M. rosenbergii* by first determining the 3, 6, 24, and 48 h median lethal concentrations ($LC_{50}$) across three different salinities (1, 5, 10 ppt). The selected salinities were chosen to represent the range most relevant to juvenile *M. rosenbergii*, which transition from brackish water to freshwater as they grow from postlarvae to juveniles and exhibit optimal growth and survival during these stages at salinities below 10 ppt (Chand et al., 2015; Nair and Salin, 2012; New, 2002). Informed by these values, we then examined real-time changes in RMR in response to stepwise increases in sublethal ammonia concentrations using intermittent-flow respirometry. This approach allowed us to identify early physiological responses to water quality stress and evaluate the potential for metabolic rate monitoring to serve as an early warning system for deteriorating conditions in aquaculture.

## 2. Materials and methods

### 2.1. Experimental animals

*M. rosenbergii* were sourced from Aquaculture of Texas, Inc. (Willow Park, TX, USA) and transported to the University of North Texas for acclimation and maintenance. Biometric data (mean ± SEM) for the mass and total length of all prawns used in experiments are provided in Tables 1 and 2. Prawns used for acute toxicity testing were randomly distributed among three Rubbermaid tanks (~380 L each), while those used for respirometry experiments were collected separately and randomly distributed among three living stream tanks (~500 L each; Frigid Units, Inc., Toledo, OH, USA). All tanks were filled with dechlorinated City of Denton tap water, maintained at 28 °C with constant aeration, and adjusted to one of three salinities (1, 5, or 10 ppt). All tanks operated as static systems and were not connected to a RAS. Plastic mesh and PVC tubing were added to each tank to provide refuge and reduce stress.

To achieve experimental salinities of 1, 5, or 10 ppt, Instant Ocean Sea Salt (Instant Ocean, Blacksburg, VA, USA) was gradually added in daily increments not exceeding 2.5 ppt. Prawns were acclimated to their respective salinities for at least two weeks prior to initiating toxicity and respirometry experiments. Animals were fed TetraMin flakes (47 % protein, 10 % fat, 3 % fiber) ad libitum and maintained under a 14:10 light:dark cycle. Water quality was monitored daily during acclimation and toxicity trials, and weekly thereafter during respirometry experiments (see Supplementary Table 1). Temperature and DO were measured using a YSI ProODO meter (YSI Incorporated, Yellow Springs, OH, USA); pH was measured with an Orion Star A121 portable pH meter (Thermo Fisher Scientific, Waltham, MA, USA); conductivity with an Oakton CON 6+ conductivity meter (Thermo Fisher Scientific, Waltham, MA, USA); and ammonia with an aquarium test kit (Mars, Inc., Chalfont, PA, USA).

### 2.2. Acute toxicity tests

Bioassays were conducted following standard acute toxicity test procedures in accordance with USEPA guidelines (EPA, 2002). Three separate bioassays were performed simultaneously at a salinity of 1, 5, and 10 ppt. For each salinity treatment, seven concentrations of total ammonia nitrogen (TAN) were tested with three replicates per concentration: 0, 2.5, 5, 10, 20, 40, and 80 mg/L. These concentrations were selected based on preliminary range-finding trials and previously published studies on *M. rosenbergii*, which documented both lethal and sublethal effects across a broad concentration range (Armstrong et al.,

**Table 1**
Biometric data (mean ± SEM) of the mass and total length of *M. rosenbergii* across salinity treatments in toxicity bioassays.

| Salinity (ppt) | Mass (g) | Length (cm) |
|---|---|---|
| 1 ppt (10) | 0.089 ± 0.014 | 2.32 ± 0.14 |
| 5 ppt (10) | 0.094 ± 0.012 | 2.44 ± 0.13 |
| 10 ppt (10) | 0.070 ± 0.007 | 2.17 ± 0.09 |

No significant differences were observed between treatments for either mass or length ($P > 0.05$). Statistical tests were conducted using one-way ANOVA (mass: $F_{2,27} = 1.297$, $P = 0.290$, $\eta^2 = 0.087$; length: $F_{2,27} = 1.230$, $P = 0.308$, $\eta^2 = 0.083$). Sample sizes are indicated in parentheses.

**Table 2**
Biometric data (mean ± SEM) of the mass and total length of *M. rosenbergii* at each dosing rate and salinity treatment used in respirometry tests.

| Dosing rate | Salinity (ppt) | Mass (g) | Length (cm) |
|---|---|---|---|
| Control | 1 ppt (9) | 2.32 ± 0.24 | 6.52 ± 0.18 |
| | 5 ppt (8) | 2.36 ± 0.16 | 6.76 ± 0.13 |
| | 10 ppt (8) | 1.85 ± 0.11 | 6.27 ± 0.15 |
| Low rate | 1 ppt (7) | 2.63 ± 0.35 | 6.88 ± 0.30 |
| | 5 ppt (8) | 2.15 ± 0.17 | 6.44 ± 0.18 |
| | 10 ppt (8) | 2.40 ± 0.11 | 6.77 ± 0.09 |
| High rate | 1 ppt (6) | 2.56 ± 0.23 | 6.90 ± 0.20 |
| | 5 ppt (8) | 2.43 ± 0.15 | 6.79 ± 0.17 |
| | 10 ppt (8) | 2.21 ± 0.23 | 6.52 ± 0.22 |

No significant differences were observed between treatments for either mass or length ($P > 0.05$). One-way ANOVA results for mass and length at both high and low doses showed no significant effects mass (high: $F_{2,19} = 0.986$, $P = 0.391$, $\eta^2 = 0.072$), length (high: $F_{2,19} = 0.986$, $P = 0.740$, $\eta^2 = 0.094$), mass (low: $F_{2,20} = 0.931$, $P = 0.411$, $\eta^2 = 0.085$), length (low: $F_{2,20} = 1.365$, $P = 0.278$, $\eta^2 = 0.120$) Sample sizes are indicated in parentheses.

1978; Naqvi et al., 2007; Zhang et al., 2015). The selected range was intended to span the full response curve and to ensure accurate $LC_{50}$ estimation. Ammonia solutions were prepared from a 50 g/L stock of $NH_4Cl$ (Fisher Scientific, Pittsburgh, PA, USA) and adjusted to a pH of 8.2 using $NaHCO_3$ (Sigma-Aldrich, St. Louis, MO, USA). A pH of 8.2 was chosen because it falls within the optimal range for *M. rosenbergii* (Chen and Chen, 2003; New, 1995). Target salinities were achieved using Instant Ocean Sea Salt (Instant Ocean, Blacksburg, VA, USA).

Each replicate comprised 10 randomly selected prawns housed in individual 1 L plastic beakers. Animals were fasted for 24 h prior to experimentation and were not fed during the trial. Preliminary experiments revealed significant declines in DO at this density; therefore, aquarium air pumps were used to gently aerate each beaker via PE60 tubing (Braintree Scientific, Inc., MA, USA). Beakers were covered with foil to minimize evaporative loss and prevent escape. All bioassays were conducted within an environmental chamber maintained at a constant temperature of 28 °C and subject to a 14:10 light:dark cycle. Mortality rates and water quality parameters were monitored at 3, 6, 24, and 48 h. The mean mass and length of *M. rosenbergii* were measured from the remaining individuals in the respective tanks following the conclusion of the bioassay. Length was measured from the tip of the rostrum to the end of the telson using ImageJ version 1.53 (National Institutes of Health, Bethesda, MD, USA).

Water samples were collected at time 0 (initial) and after 24 and 48 h. In replicates where complete mortality occurred before the 48 h mark, final water samples were collected upon confirmation of 100 % mortality. Across trials, the percent difference between the initial and final TAN concentrations was approximately 5 %, with slightly higher variation at the lowest concentrations. Ammonia concentrations were measured using a Multiparameter Photometer (Hanna Instruments model HI83399, Woonsocket, RI, USA). Samples were diluted as needed to ensure compatibility with the photometer's operational range. $LC_{50}$ estimates were limited to 48 h to align with the study's goal of evaluating early responses that would coincide with rapid changes within an aquaculture system.

### 2.3. Respirometry

Respirometry tests were conducted using glass intermittent-flow static respirometers (Loligo Systems, Viborg, Denmark), allowing for up to four prawns to be measured simultaneously. DO and temperature were monitored using mini fiber optic oxygen probes, each paired with an internal sensor spot and Pt1000 temperature probe (Loligo Systems). DO probes were calibrated using a two-point calibration at the appropriate acclimation salinity and temperature. The 100 % air saturation point was achieved by aerating the water with an air stone, while the 0 % saturation was established using a 10 g/L sodium sulfite solution (Avantor, Allentown, PA, USA). Experimental data were collected using AutoResp version 2.1.2 (Loligo Systems, Viborg, Denmark).

Preliminary control trials were conducted to determine the recovery period required for prawns to overcome handling stress, habituate to the chambers, and reach a stable RMR prior to initiating ammonia exposure (Mager et al., 2014). Up to four prawns per trial were placed in static respirometers and monitored until metabolic rates ($\dot{M}O_2$) stabilized to RMR, defined by at least three consecutive measurements <10 % variation. Initially, individuals exhibited elevated $\dot{M}O_2$ due to handling stress, but values typically stabilized within approximately 3 h, after which prawns entered a quiescent state consistent with what was described by Ern et al. (2013). Standard metabolic rate (SMR) was calculated as the average of the lowest 10th percentile of $\dot{M}O_2$ values following habituation. Initial trials showed no diurnal variation in $\dot{M}O_2$ over a 24 h period; therefore, subsequent measurements were conducted over a 12 h duration.

All prawns were fasted for 24 h prior to testing to minimize potential confounding effects of specific dynamic action. Individual masses were measured by weighing each prawn in a beaker of water before placement in the chamber. Final masses were obtained at the end of each trial by directly weighing the animals. A correction factor, calculated as the ratio of initial to final mass, was applied to adjust $\dot{M}O_2$ values accordingly. After each trial, the chambers were resealed and run without animals to quantify background microbial respiration. Total lengths were measured using the same protocol described for the bioassay subjects.

A 10-gallon (38 L) cylindrical white plastic tank served as the water reservoir for the respirometry system. Temperature was controlled using a submerged heating coil connected to a LAUDA Alpha 8 heating/cooling circulating water bath (LAUDA-Brinkman, LP, Delran, NJ, USA). The tank was continuously aerated. Water was pumped from the reservoir to the respirometry system using a pump (Eheim universal 300), and then returned via overflow back into the reservoir, forming a closed-loop system with a total of 30 L. To reduce visual disturbance, the entire system (excluding the reservoir) was concealed behind a curtain. Ammonia stock solution was added directly to the reservoir without disturbing the animals, ensuring uniform exposure and allowing consistent water quality monitoring.

Respirometry loops were initially set to 10 min and shortened to 5 min to increase data resolution. Each loop included a flush, wait, and measurement period. The wait period was held constant at 15 s while the durations of the flush and measurement phases were optimized to ensure high-quality oxygen traces ($r^2$) and allow sufficient time to replenish the chamber with nearly 100 % air-saturated water. During the ammonia exposure trials, ammonia was added near the end of the measurement phase of the cycle (when the chambers were sealed) preceding each new dose. At this point, flush pumps to the chambers were turned off, allowing the ammonia to disperse and equilibrate between the reservoir and the water bath surrounding the chambers. During the next loop's flush phase, the pumps were reactivated, delivering the target exposure concentration to the animals. Depending on the loop duration (i.e., 10 or 5 min), two or four $\dot{M}O_2$ values were collected per concentration and averaged.

A dose-response gradient experimental design was used to evaluate the effects of ammonia on $\dot{M}O_2$. Initial exposure concentrations were informed by $LC_{50}$ data, with a starting maximum TAN concentration of 24 mg/L. These concentrations were selected to assess sublethal physiological responses and to identify thresholds at which $\dot{M}O_2$ begins to change. Due to minimal responses at these initial levels, the concentration range was extended up to 48 mg/L TAN. Following the initial habituation period, ammonia concentrations were increased every 20 min in 3 or 6 mg/L increments, depending on the concentration range being tested. The exposure concentration gradient tested in the experiment was: 0, 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, and 48 mg/L (Fig. 1). After each addition, pH was immediately adjusted to 8.2 using NaOH (Thermo Fisher Scientific, Waltham, MA, USA). Water samples were

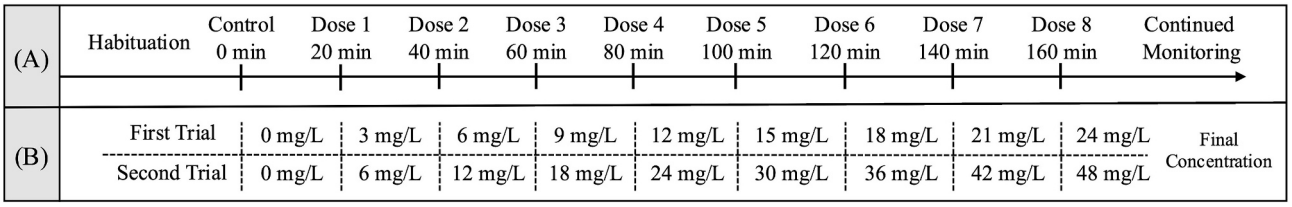


**Fig. 1.** (A) Experimental timeline showing the control period and timing of eight sequential ammonia doses administered every 20 min. (B) Total ammonia nitrogen concentrations for the 24 mg/L and 48 mg/L trials, corresponding to cumulative doses delivered over time. Dosing increments (3 or 6 mg/L TAN) were selected to assess sublethal physiological responses based on $LC_{50}$ data.

collected 10 min after post-addition and processed using the same methods described for the toxicity trials.

### 2.4. Statistical analysis

Acute toxicity data for *M. rosenbergii* were analyzed by the Trimmed Spearman-Kärber Method (USEPA, 2002) to estimate the $LC_{50}$ based on initial measured TAN concentrations. Differences in biometric data, as well as in SMR and RMR control salinity treatments were tested for statistical significance using SigmaPlot version 12.3 (Systat Software, Inc., San Jose, CA, USA). Shapiro-Wilk normality equal variance tests were run before proceeding with one-way ANOVA. Data were log-transformed or subjected to a reciprocal transformation as necessary to meet model assumptions. In all cases, differences were deemed significant at $P < 0.05$.

Respirometry tests were analyzed by averaging the $\dot{M}O_2$ value across loops for each ammonia concentration. The 20 min period preceding the first dose served as the control condition. Two days of testing were required for testing each salinity at a given dosing level to achieve the desired sample size of $N = 8$ (i.e., 4 per day). All respirometry data were analyzed using linear mixed-effect regression models (LMM) to assess the continuous fixed effects of ammonia concentration, salinity, and their interaction on the $\dot{M}O_2$ of *M. rosenbergii*. The terms 'Day' and 'Individual' were included as random effects in the model to account for variability between testing days and to address the non-independence of repeated measures from individual subjects, respectively. $\dot{M}O_2$ was log-transformed to meet model assumptions. Mixed model analyses were conducted in R (version 4.4.0) using the lme4 v1.1-37 package (Bates et al., 2015). Model diagnostics, including checks for normality and homoscedasticity, were conducted using the performance v0.15.0 package (Lüdecke et al., 2021). The significance of fixed effects was tested using Type II ANOVA. Statistical significance was set at $P < 0.05$, and all visualizations of predicted interaction effects were produced using ggplot2 and ggeffects (Lüdecke, 2018; Wickham, 2016).

## 3. Results

### 3.1. Biometric data

No significant size differences were observed among the salinity treatments in the toxicity bioassays ($N = 30$; $n = 10$ per treatment; Table 1). Mean mass ranged from 0.070 ± 0.007 g (10 ppt) to 0.094 ± 0.012 g (5 ppt), and mean total length ranged from 2.17 ± 0.09 cm to 2.44 ± 0.13 cm. One-way ANOVA confirmed that neither mass nor length differed significantly among treatments. Similarly, no significant differences in mass or length were observed among prawns used in the respirometry experiments across salinity treatments (Table 2). Mean mass across all respirometry trials ranged from 1.85 ± 0.11 g to 2.63 ± 0.35 g, and total length ranged from 6.27 ± 0.15 cm to 6.90 ± 0.20 cm.

### 3.2. Influences of salinity on ammonia toxicity

Ammonia toxicity increased significantly with exposure duration across all salinity treatments, as reflected in the decreasing TAN $LC_{50}$ values from 3 h to 48 h (Fig. 2; Supplementary Table 2). For example, in the 1 ppt treatment, the $LC_{50}$ declined from 42 mg/L (95 % CI: 37–48) at 3 h to 9.5 mg/L (95 % CI: 8.1–11) at 48 h. Similar patterns were observed at 5 ppt (33 to 7.2 mg/L) and 10 ppt (22 to 5.6 mg/L). Differences between 3 h and 6 h $LC_{50}$s were not significant within any salinity, but values declined significantly at 24 h and again at 48 h.

When comparing salinity treatments at the same time point, $LC_{50}$ values at 3 h and 6 h were significantly higher at 1 and 5 ppt than at 10 ppt, although 1 and 5 ppt did not differ from each other. At 24 h, the 1 ppt treatment had a significantly higher $LC_{50}$ than the 10 ppt treatment, while the 5 ppt group did not differ statistically from either. By 48 h, a significant salinity-dependent effect emerged, with ammonia toxicity increasing as salinity increased. $LC_{50}$ values were 9.5 mg/L (95 % CI: 8.1–11) at 1 ppt, 7.2 mg/L (95 % CI: 6.9–7.6) at 5 ppt, and 5.6 mg/L (95 % CI: 4.9–6.3) at 10 ppt. These findings demonstrate both time- and salinity-dependent increases in ammonia sensitivity in juvenile *M. rosenbergii*.

### 3.3. Respirometry

A significant interaction between ammonia concentration and salinity was observed ($P = 0.046$), where the positive relationship between ammonia concentration and RMR became steeper at higher salinities. In the 10 ppt salinity treatment, $\dot{M}O_2$ increased more steeply with each ammonia dose compared to the 1 and 5 ppt treatments, suggesting that juvenile *M. rosenbergii* exhibit real-time metabolic responses to ammonia exposure and that the magnitude of this response varies

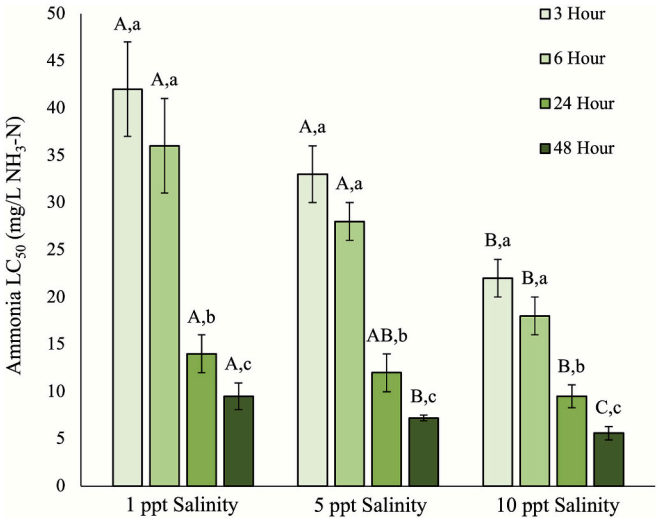


**Fig. 2.** Acute $LC_{50}$ values (mg/L TAN) for juvenile *M. rosenbergii* exposed to ammonia at three salinities. Uppercase letters indicate significant differences among salinities at each exposure duration, and lowercase letters denote significant differences among time points within a salinity. Significance was determined by non-overlapping 95 % confidence intervals.

with salinity.

Ammonia concentration also had a significant positive effect on $\dot{M}O_2$ across all salinities ($P = 0.006$), indicating that metabolic rate increased in response to rising ammonia levels (Fig. 3). However, no significant main effect of salinity on $\dot{M}O_2$ was detected ($P = 0.523$). Among control prawns, there were no significant differences in SMR ($P = 0.194$) or RMR ($P = 0.159$) across salinity treatments (Fig. 4A, B).

## 4. Discussion

This study examined the interactive effects of ammonia and salinity on juvenile *M. rosenbergii* using acute toxicity bioassays and real-time metabolic monitoring. The aim was to determine how salinity stress influences ammonia sensitivity and to evaluate whether metabolic rate responses can serve as early indicators of physiological stress. Understanding these relationships is critical for improving stress detection and management in intensive aquaculture systems such as RAS.

$LC_{50}$ values decreased over time at all salinities, indicating that ammonia toxicity increased with longer exposure. The highest ammonia toxicity was observed at 10 ppt across all time points, where the 48 h $LC_{50}$ was 5.6 mg/L TAN (95 % CI: 4.9–6.3 mg/L). This pattern contrasts with findings from other shrimp species, where ammonia toxicity tends to decrease with increasing salinity. For instance, Kir and Kumlu (2006) and Lin and Chen (2001), as summarized in Lin et al. (2022), reported progressively higher $LC_{50}$ values in *Penaeus semisulcatus* and *Litopenaeus vannamei* at elevated salinities, likely due to reduced ammonia uptake under more saline conditions. Comparable $LC_{50}$ values for other shrimp and crab species across life stage and salinities are summarized in Lin et al. (2022; Tables 1–2), which show that many shrimp and crab species exhibit higher ammonia tolerance at elevated salinities. The difference may reflect contrasting life history strategies. As a catadromous species, *M. rosenbergii* transitions from brackish to freshwater environments during development, whereas penaeid shrimp typically migrate from estuarine to fully marine habitats. As a result, *M. rosenbergii* may experience greater osmoregulatory challenges at elevated salinities, reducing its ability to excrete or detoxify ammonia and thereby increasing sensitivity under these conditions.

The elevated toxicity at 10 ppt aligns with known challenges in ammonia management in intensive aquaculture systems, particularly in RAS, where waste can accumulate rapidly under high biomass loading (Badiola et al., 2012). Excess ammonia can impair physiological function, reduce growth, and ultimately lead to mortality. When faced with high environmental ammonia, hemolymph ammonia is primarily eliminated through active excretion across the gills in crustaceans, a process closely tied to ion and acid-base regulation (Romano and Zeng, 2011; Weihrauch et al., 2009, 2004). This excretion is driven by ion transport mechanisms, such as the $Na^+/K^+$-ATPase and potentially a $Na/NH_4^+$ exchanger (Romano and Zeng, 2012). At sufficiently high concentrations, this gradient can reverse, impairing excretion and increasing internal ammonia load. Understanding how these processes interact across salinities is critical for managing toxicity risk.

Salinity is a key determinant of physiological performance in *M. rosenbergii* (Wang et al., 2023; Yen and Bart, 2008). Adults can maintain hemolymph osmolality near 400–450 mOsm at low salinities (<12 ppt), but their osmoregulatory capacity diminishes in full strength seawater, with difficulty maintaining $Na^+$ levels (Wang et al., 2023; Wilder et al., 1998). Although hemolymph osmolarity was not measured, the absence of salinity related increases in RMR suggests that prawns maintained ionic balance without measurable metabolic costs across 1–10 ppt. However, it remains unclear whether concurrent ammonia exposure increases the energetic cost of osmoregulation at these salinities, warranting further investigation.

Ammonia excretion, osmoregulation, and acid-base balance are all tightly integrated through gill function in crustaceans (Romano and Zeng, 2013; Weihrauch et al., 2009). In *M. rosenbergii*, elevated external ammonia impairs sodium uptake and promotes internal ammonia accumulation (Armstrong et al., 1978). High $NH_4^+$ concentrations disrupt $Na^+/NH_4^+$ exchange, leading to sodium loss, osmoregulatory stress, and acid-base disturbance (Lin et al., 2022). Hemolymph pH, which tends to decline with salinity (Henry et al., 2012), further influences ammonia speciation: lower pH favors $NH_4^+$ formation over $NH_3$, potentially hindering excretion and increasing toxicity (Ip et al., 2001).

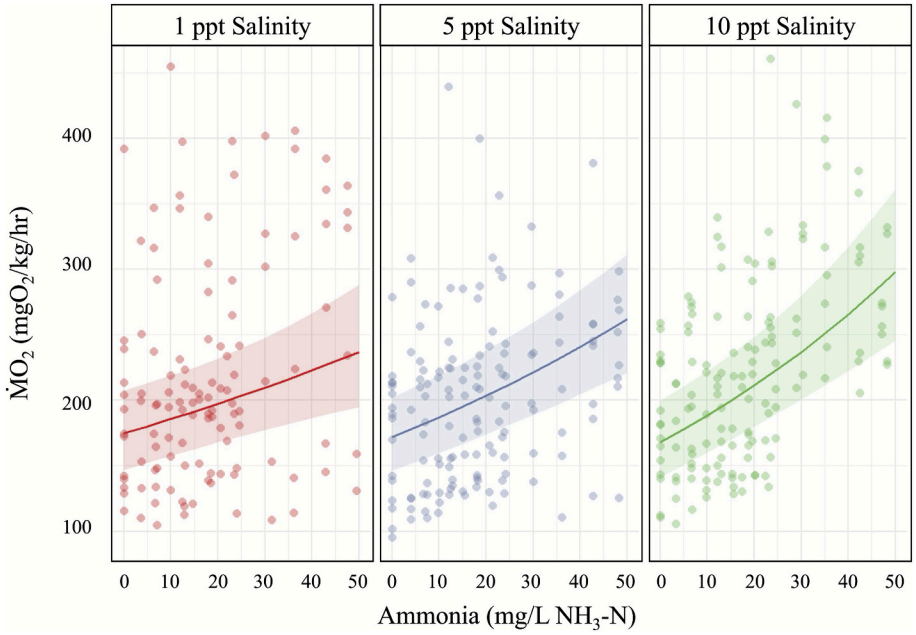


**Fig. 3.** Mean metabolic rates of juvenile *Macrobrachium rosenbergii* exposed to increasing ammonia concentrations (mg/L $NH_3$-N) across three salinity levels (1, 5, and 10 ppt). Model predictions are shown with 95 % confidence intervals for each salinity level. A significant interaction between ammonia and salinity was observed, ($F_{1,397.08} = 4.020$, $P = 0.046$, $\eta^2 = 0.01$), where the positive relationship between ammonia concentration and RMR became steeper at higher salinities. The analysis also revealed a significant main effect of ammonia across all salinities ($F_{1,397.12} = 7.613$, $P = 0.006$, $\eta^2 = 0.02$), indicated by the overall increasing trend. No significant main effect of salinity was detected ($F_{1,397.17} = 0.409$, $P = 0.523$, $\eta^2 = 0.001$).

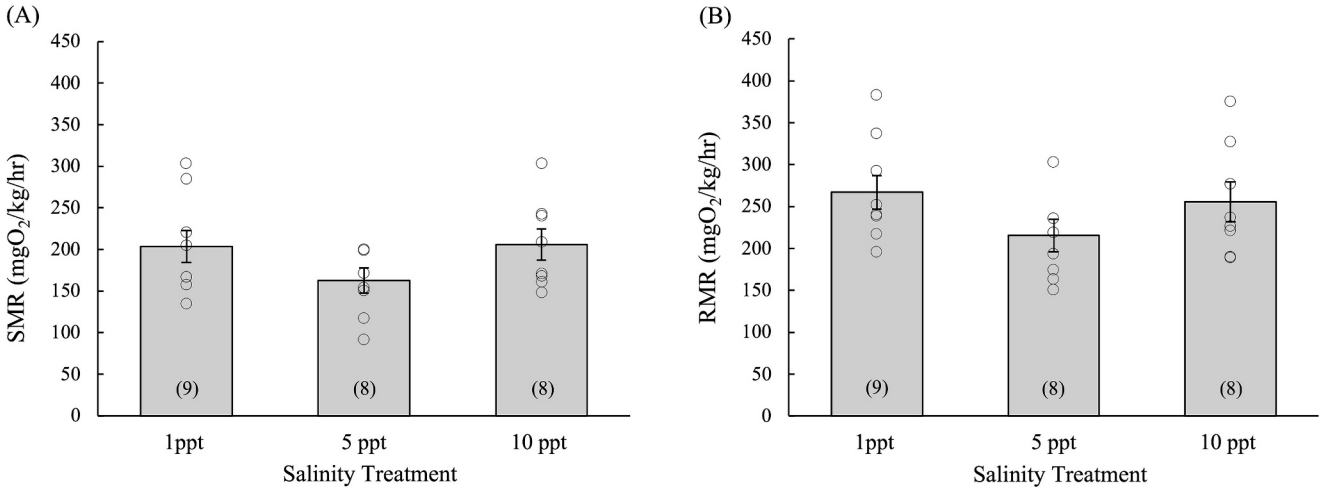


**Fig. 4.** (A) Standard metabolic rate (SMR) and (B) routine metabolic rate (RMR) of juvenile *M. rosenbergii* under control conditions across salinity treatments. One-way ANOVA showed no significant effect of salinity on SMR ($F_{2,22} = 1.770$, $P = 0.194$, $\eta^2 = 0.139$) or RMR ($F_{2,22} = 2.004$, $P = 0.159$, $\eta^2 = 0.154$). Sample sizes are indicated in parentheses. Open circles represent individual data points.

These coupled ionoregulatory and acid-base disturbances likely underlie the metabolic responses observed. Ammonia exposure significantly increased oxygen consumption, whereas salinity alone had no effect on SMR or RMR. This result is consistent with previous findings supporting the species' viability in brackish water aquaculture and aligns with our dosing experiments, which showed no significant main effect of salinity across trials (Ern et al., 2013). Although prawns used for respirometry were larger than those used in the bioassays, the latter served primarily to establish a suitable sublethal exposure range, after which the respirometry experiments quantified metabolic responses within that range. The ammonia doses selected represent rare but plausible conditions in aquaculture systems, such as those that might occur during biofiltration failures.

The observed increase in $\dot{M}O_2$ most likely reflects elevated ATP demand associated with ammonia detoxification and ionoregulatory stress, potentially including partial conversion of ammonia to urea through energetically costly pathways described in some decapods (Lee and Chen, 2003; Romano and Zeng, 2013). Prolonged exposure can also damage gill architecture, including lamellar fusion that impairs gas exchange (Bridges, 2001; Lin et al., 2022; Romano and Zeng, 2013). Recent work on *Macrobrachium acanthurus* confirms that ammonia exposure causes gill damage and osmoregulatory disruption, with reversible lesions at moderate concentrations and irreversible damage at higher levels (Miranda et al., 2025). Some crustaceans may additionally modulate boundary-layer pH using an ATP-dependent proton pump at the gill surface to facilitate outward $NH_3$ diffusion (Romano and Zeng, 2013). Together, these mechanisms impose substantial energetic costs on ionic and acid-base regulation that is expressed as elevated oxygen consumption. Although some contribution from increased activity cannot be excluded, the consistent and graded rise in $\dot{M}O_2$ across ammonia doses supports a primarily physiological explanation. Future work integrating behavioral observations and hemolymph chemistry would help clarify the relative contributions of these mechanisms.

The inter-individual variation in $\dot{M}O_2$ observed in this study is consistent with previous work on *M. rosenbergii*, where resting oxygen consumption differed by approximately fourfold among individuals of similar size and molt status (Taylor et al., 2002). This inherent variability likely reflects physiological and developmental differences within populations, and while typical for some species, may complicate the use of metabolic rate as a sensitive indicator in monitoring systems.

Overall, these results suggest that ammonia toxicity in *M. rosenbergii* arises not only from direct nitrogenous stress but also from the compounded energetic cost of maintaining ionic and acid-base homeostasis under saline conditions. For RAS operations, where ammonia accumulation and variable salinity can co-occur, real-time metabolic monitoring provides a promising tool for early detection of physiological stress. Integrating respirometry with continuous water-quality monitoring could enable proactive management before irreversible harm occurs. While the concept of integrating respirometry into RAS management is promising, several technical and practical challenges must be addressed before large-scale implementation. Besides the considerable inter-individual variation in $\dot{M}O_2$ for this species corroborated in this study, continuous respirometry systems require regular calibration, are prone to sensors biofouling, and the interpretation of metabolic signals in multi-stressor environments can be complex, as fluctuations in oxygen consumption may reflect interacting effects such as water quality, feeding activity, or stress responses rather than a single stressor. However, it should be noted that this approach of integrating real-time respirometry into aquaculture has been previously developed using finfish species (Stiller et al., 2013). Although ammonia was used here as a model stressor, this framework can be extended to other environmental challenges, offering a pathway toward improved welfare and performance in intensive aquaculture systems.

## 5. Conclusions

This study demonstrates that real-time respirometry can detect ammonia-induced metabolic stress in juvenile *Macrobrachium rosenbergii*, and that this response is modulated by salinity. Ammonia exposure significantly increased oxygen consumption, while salinity alone had no measurable effect on metabolic rate. These findings indicate that *M. rosenbergii* maintains osmoregulatory balance across low salinities but experiences clear physiological cost when exposed to elevated ammonia and that this response increases with salinity up to 10 ppt. The use of intermittent-flow respirometry offers a sensitive means of detecting sublethal stress, highlighting its potential as an early warning tool in intensive aquaculture systems such as RAS. However, translating this approach from the laboratory to production-scale settings will require addressing challenges related to high intrinsic variation in metabolic rates, sensor maintenance, calibration stability, costs, and data interpretation and modeling in complex rearing environments. Future work should focus on integrating respirometry with continuous water quality monitoring and evaluating its performance under commercial conditions and additional stressors. Together, these efforts could advance physiology-based monitoring frameworks that improve animal welfare and production efficiency in intensive aquaculture systems.

## CRediT authorship contribution statement

**Cameron M. Emadi:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fabio Dos Santos Neto:** Writing – review & editing, Conceptualization. **Jason R. Bohenek:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Data curation. **Breana Smithers:** Writing – review & editing, Project administration, Funding acquisition. **Miguel F. Acevedo:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Edward M. Mager:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpc.2025.110401.

## Data availability

Data will be made available on request.

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