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Acute and Sub-chronic Ammonia Toxicity in *Artemia franciscana*: Effects of Developmental Stage and Environmental Conditions

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Abstract

Ammonia is a widespread aquatic contaminant that can adversely affect aquatic organisms, and its toxicity is strongly influenced by environmental conditions and developmental stage. However, ammonia toxicity remains poorly understood in many marine and euryhaline crustaceans, particularly under varying environmental conditions. This study evaluated the acute toxicity (24-h LC_{50}) of total ammonia in brine shrimp (*Artemia franciscana*) nauplii and assessed the effects of a 5-day sublethal ammonia exposure on larval growth. In addition, the influence of temperature (20 and 25°C), salinity (20, 35, and 50 ppt), pH (6.5, 8.0, and 9.0), and photoperiod on ammonia toxicity was investigated. Results revealed a clear concentration-dependent increase in mortality under all experimental conditions ($P<0.05$). The 24-h LC_{50} values were 338 mg/L for Instar I and 665 mg/L for Instar II nauplii, while survival rates were consistently higher in Instar II, indicating greater sensitivity of Instar I nauplii. Ammonia toxicity was strongly affected by environmental conditions, with lower mortality observed at higher salinity and pH, lower temperature, and continuous darkness. Sublethal ammonia exposure significantly affected larval growth, resulting in reduced body length after 5 days of exposure ($P<0.05$). These findings contribute to a better understanding of how developmental stage and environmental conditions influence ammonia toxicity in marine and euryhaline crustaceans and provide ecologically relevant information for assessing ammonia risk in saline aquatic environments.

Introduction

Ammonia (NH_3) is a ubiquitous environmental contaminant that can impair physiological processes in aquatic organisms and alter ecosystem functioning (Yun et al., 2026). It originates from natural nitrogen cycling, including excretion and microbial decomposition processes, as well as from anthropogenic inputs such as agricultural runoff, sewage discharge, industrial effluents, and organic matter degradation (Constable et al., 2003). In aquatic environments, ammonia exists in a pH and temperature-dependent equilibrium between un-ionized (NH_3) and ionized (NH_4^+) forms, with NH_3 being the more toxic species due to its ability to diffuse across biological membranes (Ip et al., 2001). This leads to disruption of ion regulation, inhibition of enzymatic activity, and oxidative stress (Xu et al., 2021; Lin et al., 2023). Acute and chronic exposure to ammonia has been associated with reduced growth, impaired reproduction, immunosuppression, and histopathological alterations in aquatic organisms (Neil et al., 2005; Zhang et al., 2023; Yun et al., 2026). Such effects can ultimately influence survival, growth, and reproductive performance, potentially affecting population persistence and ecosystem processes in contaminated aquatic environments.

In recent decades, ammonia levels in aquatic environments have increased due to intensified

anthropogenic activities associated with agricultural expansion, urbanization, aquaculture development, and industrial discharge (Edwards et al., 2024). Consequently, ammonia contamination has become a major environmental concern (Edwards et al., 2024), with implications for organismal performance and ecosystem stability (Constable et al., 2003). Ammonia toxicity is strongly influenced by environmental conditions that regulate its chemical speciation and organismal physiological responses (Ip et al., 2001; Romano & Zeng, 2013). In parallel, climate change alters key environmental variables, including temperature, salinity, pH, and hydrological regimes (IPCC, 2023; Jeppesen et al., 2023), thereby increasing physiological stress on aquatic organisms and modifying contaminant toxicity through effects on metabolism, osmoregulation, and energy allocation (Allan and Maguire, 1992; Fabbri and Dinelli, 2014). Photoperiod may also affect physiological performance by regulating circadian rhythms, feeding activity, and energy metabolism, potentially influencing organismal responses to environmental stressors (Fanjul-Moles & Prieto-Sagredo, 2003; Cheng et al., 2026). Nevertheless, the role of environmental conditions in influencing ammonia toxicity and its potential ecological consequences remains poorly understood in marine and euryhaline invertebrates.

Ammonia toxicity has been extensively studied in aquatic organisms, with a strong emphasis on freshwater systems and fish species (Hickey and Vickers, 1994; Colombo et al., 2008; Fan et al., 2021; Xu et al., 2021; Parvathy et al., 2023; Edwards et al., 2024; Jayasree et al., 2025; Yu and Liu, 2026). In contrast, marine and euryhaline invertebrates have received considerably less attention (Zhang et al., 2023). Among crustaceans, research has mainly focused on commercially important aquaculture species (Lin et al., 2023), particularly shrimps (Lin et al., 2024), prawns (Emadi et al., 2026), and crabs (Zhang et al., 2023), while information on other ecologically and taxonomically diverse crustacean groups remains limited. Given the central role of crustaceans in aquatic food webs, nutrient cycling, and ecosystem functioning (Thiel and Gutow, 2025), this taxonomic bias represents an important knowledge gap. Consequently, the ecological implications of ammonia exposure for a broader range of crustacean taxa remain insufficiently understood. Furthermore, developmental stage-specific sensitivity and the sub-chronic effects of ammonia exposure on growth remain poorly understood in *Artemia* species.

A. franciscana is a brine shrimp widely distributed in hypersaline ecosystems worldwide, where it plays an important role in saline aquatic food webs (Sorgeloos et al., 1998; Dhont and Sorgeloos, 2002). It is also extensively used as live feed in aquaculture (Dhont et al., 2013), highlighting its ecological and economic importance. Because Artemia occupies a key position in saline food webs, factors that affect its survival and growth may have broader ecological consequences for hypersaline ecosystems. Owing to its short life cycle, high fecundity, ease of culture, and sensitivity to environmental stressors, it has become a widely used model organism in aquatic ecotoxicology (Libralato et al., 2016; Khandan Barani et al., 2025; Lee et al., 2025; Gayathri et al., 2026). However, despite its extensive application in toxicity testing, information on ammonia toxicity in *A. franciscana* remains scarce. This is particularly relevant because Artemia populations are naturally exposed to substantial fluctuations in environmental conditions, which can influence both ammonia toxicity and organismal tolerance.

Therefore, the present study aimed to evaluate the acute and sub-chronic toxicity of ammonia in *A. franciscana*. Acute toxicity was assessed by determining 24-h LC_{50} values in 1- and 2-day-old nauplii, and comparative sensitivity between these developmental stages was evaluated to identify the most susceptible stage. In addition, the effects of key environmental factors, including temperature, pH, salinity, and photoperiod, were investigated in 1-day-old nauplii to evaluate their influence on ammonia toxicity. A 5-day sub-chronic exposure experiment was conducted to evaluate the effects of prolonged ammonia exposure on growth. Collectively, these findings provide new insights into ammonia toxicity and its modulation by environmental factors in a widely used halophilic crustacean model and provide ecologically relevant information on ammonia toxicity in saline aquatic environments

Materials and Methods

Test Organism

Cysts of *A. franciscana* were purchased from a commercial supplier in Tehran, Iran, and stored at -20°C in the dark prior to experimentation. For hatching, artificial seawater (ASW; salinity 35 g/L; pH 8.1±0.05) was prepared by dissolving commercial sea salt in deionized water. Approximately 1 g of dry cysts was incubated in 1 L of ASW in a conical hatching vessel with continuous aeration under a 16 h light:8 h dark photoperiod (~2000 lux) at 25±1°C for 24 h (Van Stappen et al., 1996; Sorgeloos et al., 2001). The newly hatched nauplii (Instar I) were collected and transferred to fresh ASW. A portion of newly hatched nauplii was used immediately for acute toxicity tests, whereas the remaining individuals were maintained for an additional 24 h to obtain Instar II nauplii.

Chemical Preparation

Ammonia stock solutions were prepared by dissolving ammonium chloride (NH_4Cl, Merck reagent grade) in sterile artificial seawater (ASW) to a concentration of 1000 mg/L total ammonia. Working solutions were prepared by diluting the stock solution with ASW to achieve the target nominal ammonia

concentrations. Stock solutions were renewed daily to maintain target exposure concentrations. Water quality parameters, including pH, temperature, salinity, and photoperiod, were monitored throughout the experiment and maintained at the designated levels for each treatment.

Acute Toxicity Test

The effects of ammonia on *A. franciscana* nauplii were evaluated under different experimental conditions, including variations in life stage, pH, salinity, temperature, and photoperiod (Table 1). Initially, a range of ammonia concentrations (0, 150, 300, 450, 600, 800, and 1000 mg/L total ammonia) was used to determine the 24-h median lethal concentration (LC_{50}) for Instar I and Instar II nauplii under reference conditions (35 ppt salinity, pH 8.0, 25±1°C, and a 16 h:8 h light: dark photoperiod) (Sorgeloos et al., 2001).

Additional 24-h experiments were conducted using a reduced concentration range (0, 75, 150, 300, and 700 mg/L total ammonia) to evaluate the effects of environmental factors (pH, salinity, temperature, and photoperiod) on ammonia toxicity in Instar I nauplii. Because Instar I nauplii were more sensitive to ammonia, they were selected for the environmental-factor experiments.

Each treatment consisted of a 24-h exposure and was conducted in triplicate using 30-mL glass beakers containing 20 mL of test solution and 10 nauplii per replicate. No food was provided during the exposure period. Individuals that did not exhibit movement for at least 10 s were considered immobile. Mortality data were analyzed using Probit regression to estimate LC_{50} values and their 95% confidence intervals.

Sub-chronic Toxicity Test

A five-day exposure experiment was conducted using newly hatched Instar I nauplii of *A. franciscana*. A total of 100 nauplii were transferred into 150-mL Erlenmeyer flasks containing 100 mL of ammonia solutions at nominal concentrations of 0, 15, 50, 75 and 150 mg/L total ammonia, selected based on the results of the acute toxicity tests. Control flasks contained artificial seawater (ASW) without ammonia. Each treatment, including the control, was performed in triplicate. Flasks were maintained under reference conditions (25±0.5°C, pH 8.0, salinity 35 ppt, and a 16 h:8 h light: dark photoperiod) with gentle aeration. The exposure medium was renewed every 2 days to maintain stable ammonia concentrations and water quality. Nauplii were fed throughout the experiment with fresh *Dunaliella tertiolecta* at a density of 1.2×10^5 cells m/L.

A subsample of 20-30 nauplii per replicate was collected, fixed in 4% formaldehyde, and stored at 8°C until analysis. Body length was measured from digital images captured using a Euromex CMEX DC.5000 camera mounted on a Euromex GE 3035 microscope and analyzed using Digimizer image analysis software (version 6.4.4; MedCalc Software Ltd., Ostend, Belgium).

Statistical Analysis

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Data were first evaluated for normality and homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively. Differences among treatment groups were determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Comparisons between the two groups were conducted using an independent-samples t-test. The 24-h median lethal concentration (LC_{50}) values and their 95% confidence intervals were estimated using Probit analysis. All results are presented as mean±standard deviation (SD), and statistical significance was accepted at $P<0.05$.

Results

Acute Toxicity (LC_{50})

The 24-h acute toxicity of total ammonia to *A. franciscana* nauplii is summarized in Table 2. The estimated 24-h LC_{50} values were 338 mg/L for Instar I

Table 1. Experimental design and conditions for total ammonia toxicity tests on *A. franciscana* nauplii

Purpose of the test	Life stage	Photoperiod	Temperature (°C)	Salinity (ppt)	pH
Effect of developmental stage on ammonia toxicity	a) Instar I, b) II	16 h:8 h light: dark	25	35	8
Effect of pH on ammonia toxicity	Instar I	16 h:8 h light: dark	25	35	a) 6.5 b) 8 c) 9
Effect of salinity on ammonia toxicity	Instar I	16 h:8 h light: dark	25	a) 20 b) 35 c) 50	8
Effect of temperature on ammonia toxicity	Instar I	16 h:8 h light: dark	a) 25 b) 20	35	8
Effect of photoperiod on ammonia toxicity	Instar I	a) 16 h:8 h light: dark b) Continuous light (24 h light) c) Full darkness (no light)	25	35	8

*Instar I and Instar II refer to the first and second naupliar developmental stages of *A. franciscana*.

and 665 mg/L for Instar II nauplii. The LC_{50} values obtained in this study were higher than those reported for most crustacean species (Table 3).

Survival Response to Ammonia Exposure

Ammonia exposure significantly affected the survival of *A. franciscana* nauplii after 24 h of exposure (Figure 1). Survival remained above 90% in the control treatment for both instar stages but declined progressively with increasing total ammonia concentration. Significant reductions in survival were observed at concentrations ≥300 mg/L compared with the control ($P<0.05$). Instar I nauplii consistently exhibited lower survival than instar II nauplii throughout the exposure range. At the highest ammonia-N concentration (1000 mg/L), survival decreased to 0% in instar I and approximately 7% in instar II. Significant differences between developmental stages were detected at concentrations ≥450 mg/L ($P<0.05$).

Table 2. Percent mortality (%) of *A. franciscana* nauplii (Instar I and II) exposed to different total ammonia concentrations for 24 h and estimated LC50

Salinity	Total ammonia (mg/L)							LC50 of total ammonia (mg/L)
	0.0	150	300	450	600	800	1000	
Instar 1	7	27	37	56	68	83	100	338
Instar 2	0	10	26	28	43	67	93	665

*LC_{50}= median lethal concentration causing 50% mortality after 24 h of exposure.

Table 3. Acute 24-h LC_{50} values of total ammonia in selected crustacean species

Species	Stage	Duration (h)	pH	T (°C)	Salinity (ppt)	LC50 (mg/L)	Reference
Penaeus semisulcatus	Juvenile	24	8.2	26	39	25.89	Kır et al., 2004
Penaeus japonicus	Nauplius	24	-	25	36	28.4	Lin et al., 1993
Litopenaeus schmitti	Juvenile	24	8.0	20	35	54.32	Barbieri, 2010
Litopenaeus vannamei	postlarvae	24	8.2	26.1	3	48.4	Valencia-Castañeda et al., 2018
Litopenaeus vannamei	Juvenile	24	8.2	26	3	116	Valencia-Castañeda et al., 2019
Macrobrachium carcinus	Zoea VIII	24	8.0	28.9	20	63.06	Gomes et al., 2016
Scylla paramamosain	-	24	8.2	28.3	21	325	Wang et al., 2025
Ampelisca abdita	adult	96	8.0	15	30	49·8	Kohn et al., 1994
Rhepoxynius abronius	adult	96	8.0	15	30	78·7	Kohn et al., 1994
Eohaustorius estuarius	adult	96	8.0	15	30	125·5	Kohn et al., 1994
Grandidierella japonica	adult	96	8.0	15	30	148·3	Kohn et al., 1994
Artemia sp.	Nauplius	24	-	-	-	650	Landau and Sanchez, 1991
Artemia sp.	Instar I	24	7.5	27	30	839.5	Chen et al., 1989

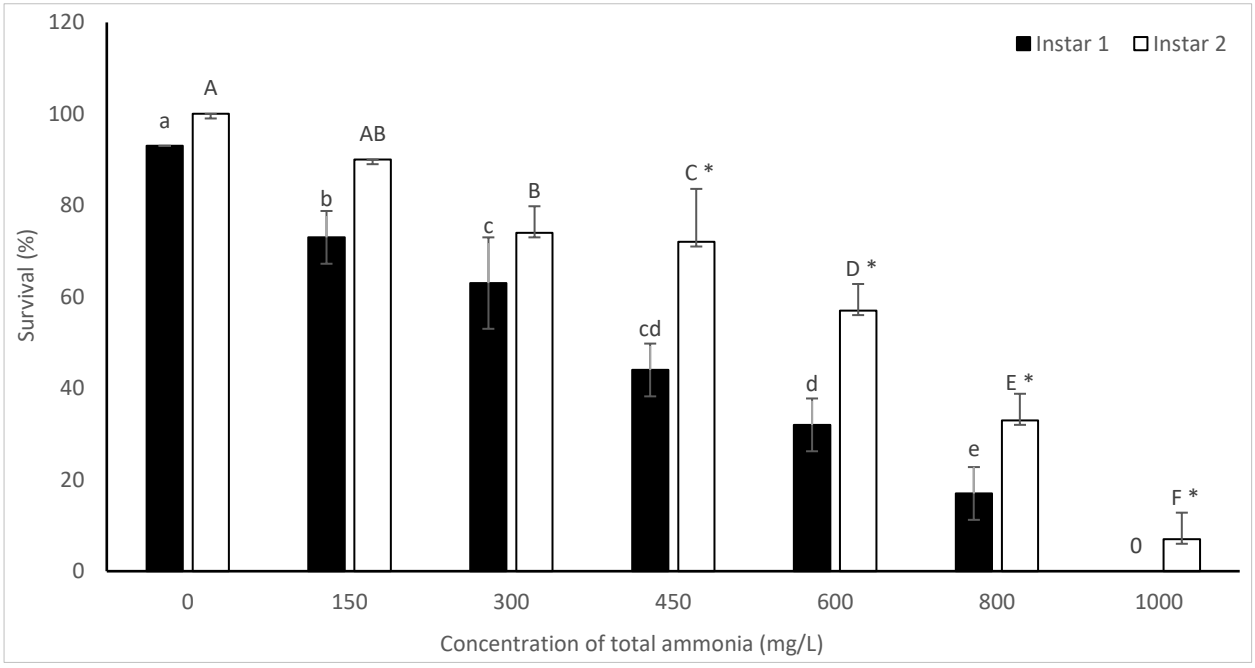

Figure 1. Survival (%) of *A. franciscana* instar I and instar II nauplii exposed to different total ammonia concentrations for 24 h. Data are presented as mean ± SD. Bars sharing different lowercase letters (instar I) or uppercase letters (instar II) differ significantly among total ammonia treatments ($P<0.05$). Asterisks (*) indicate significant differences between developmental stages at the same total ammonia concentration ($P<0.05$).

Effect of pH on Mortality Under Ammonia Exposure

Mortality of *A. franciscana* exposed to total ammonia was significantly influenced by pH (Figure 2). The highest mortality rates were consistently observed at pH 6.5, followed by pH 8, whereas the lowest mortality rates occurred at pH 9. Significant differences among pH treatments were detected in several exposure groups (P<0.05), demonstrating a clear pH-dependent variation in mortality.

Effect of Temperature on Mortality Under Ammonia Exposure

Temperature significantly influenced mortality associated with total ammonia exposure, which was consistently higher at 25°C than at 20°C across all exposure levels (P<0.05; Figure 3). This pattern indicates that elevated temperature intensifies the toxic effects of total ammonia, particularly under higher exposure concentrations.

Effect of Salinity on Mortality Under Ammonia Exposure

Mortality was significantly affected by salinity (P<0.05; Figure 4), showing a clear inverse relationship between salinity level and ammonia toxicity. Across all total ammonia concentrations, the highest mortality was observed at 20 ppt, followed by 35 ppt, whereas the lowest mortality occurred at 50 ppt. At 700 mg/L ammonia, mortality reached 96% at 20 ppt, 73.3% at 35 ppt, and decreased considerably to 40% at 50 ppt, indicating a dose-dependent reduction in ammonia

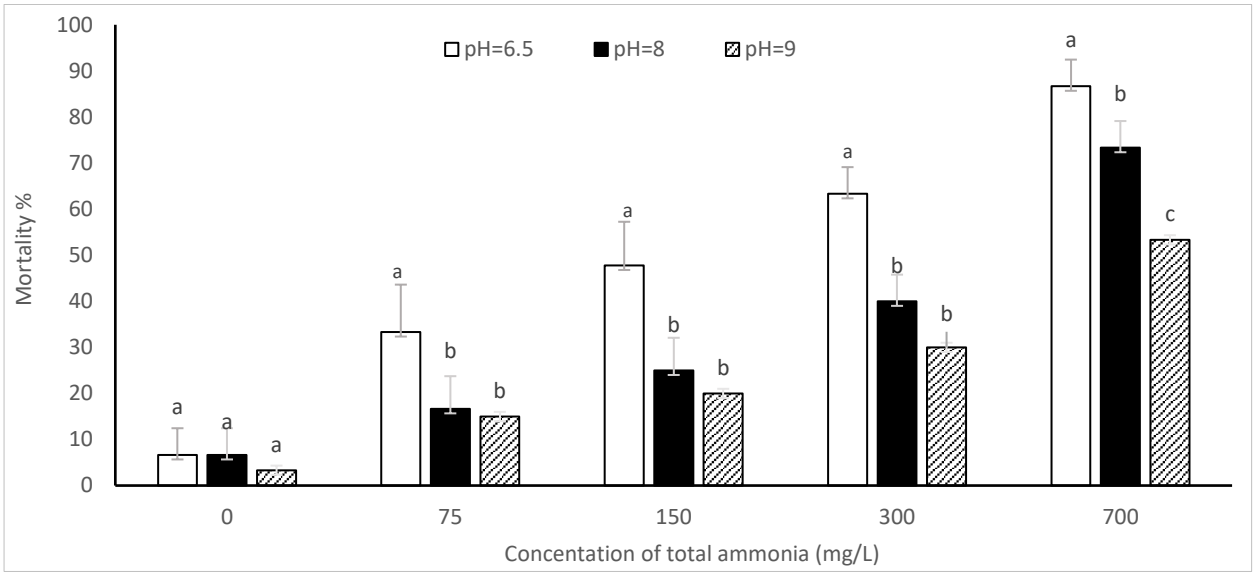

Figure 2. Effect of pH on mortality (%) of *A. franciscana* nauplii exposed to total ammonia after 24 h. Values are expressed as mean±SD. Within each total ammonia concentration, bars sharing different letters are significantly different (P<0.05).

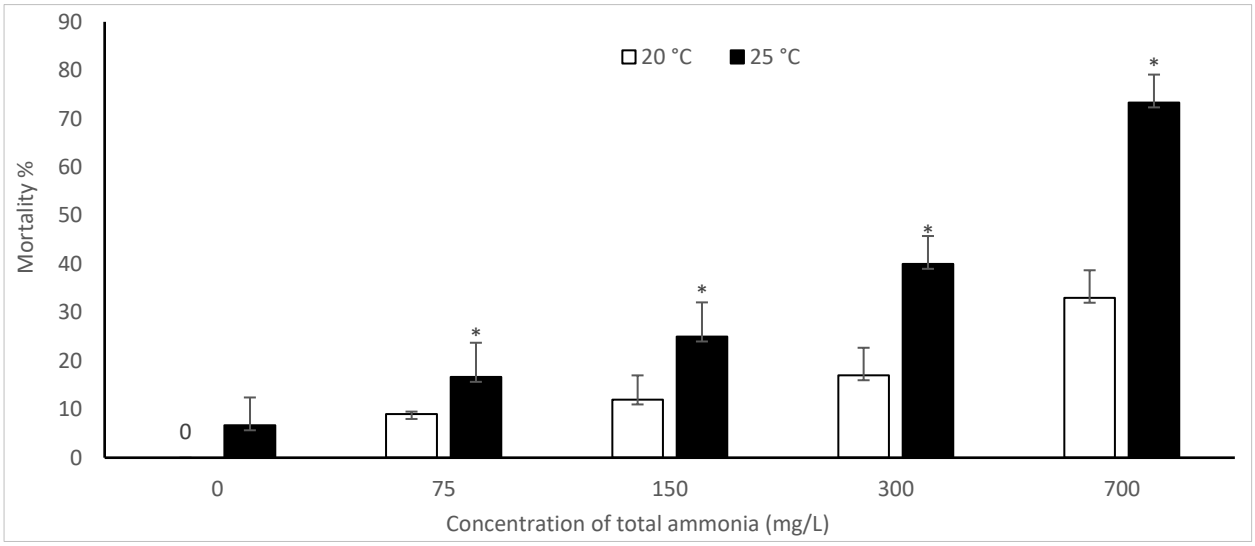

Figure 3. Effect of temperature on mortality (%) of *A. franciscana* nauplii exposed to total ammonia after 24 h. Asterisks indicate significant differences between temperature treatments at each concentration.

toxicity with increasing salinity. This pattern became more evident at higher ammonia concentrations, suggesting that the effect of salinity on mortality was dependent on exposure level.

Effect of Photoperiod on Mortality Under Ammonia Exposure

Photoperiod significantly affected mortality under total ammonia exposure in *A. franciscana* (P<0.05; Figure 5). Under complete darkness, mortality remained relatively lower across concentrations, reaching 66.6% at 700 mg/L. Under a 16 h:8 h light-dark cycle, mortality increased progressively, reaching 40% and 73.3% at 300 and 700 mg/L, respectively. Continuous light (24 h illumination) resulted in the highest mortality, reaching 96.6% at 700 mg/L. Overall, extended photoperiod exposure, particularly continuous illumination, intensified ammonia toxicity, whereas darkness reduced lethal effects.

Effect of Ammonia on Body Length

Ammonia exposure significantly affected the body length of *A. franciscana* (P<0.05; Figure 6). No significant difference was observed between the control and 15 mg/L treatments. However, body length decreased significantly at ammonia concentrations of 50 mg/L and above, with the lowest mean body length recorded in the 150 mg/L treatment.

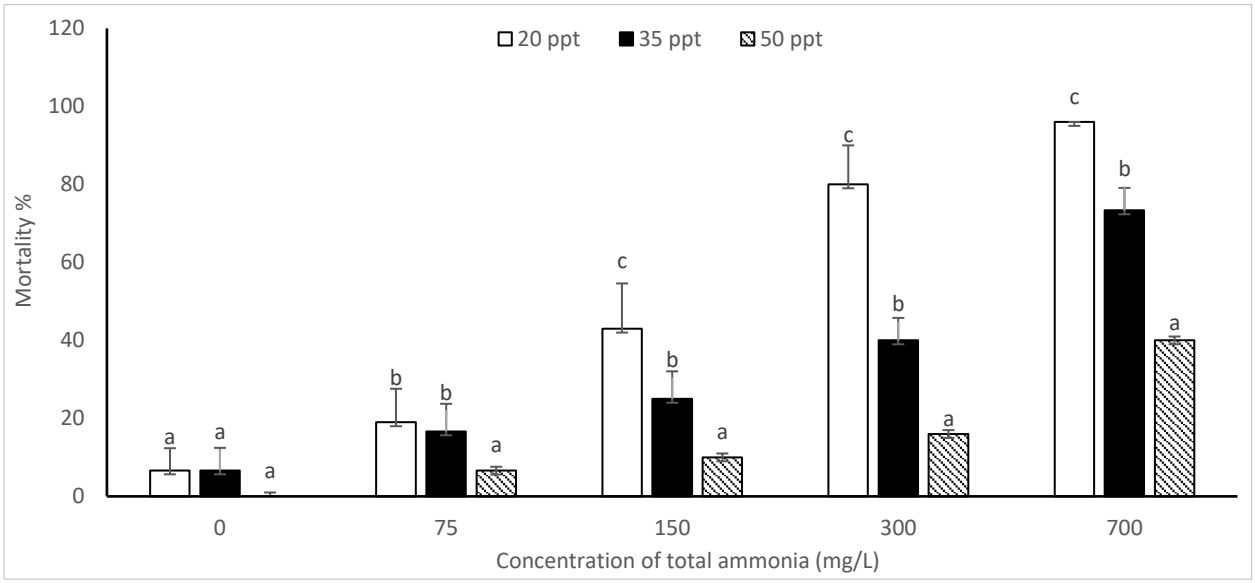

Figure 4. Effect of salinity on mortality (%) of *A. franciscana* nauplii exposed to total ammonia after 24 h. Values are expressed as mean±SD. Within each total ammonia concentration, bars sharing different letters are significantly different (P<0.05).

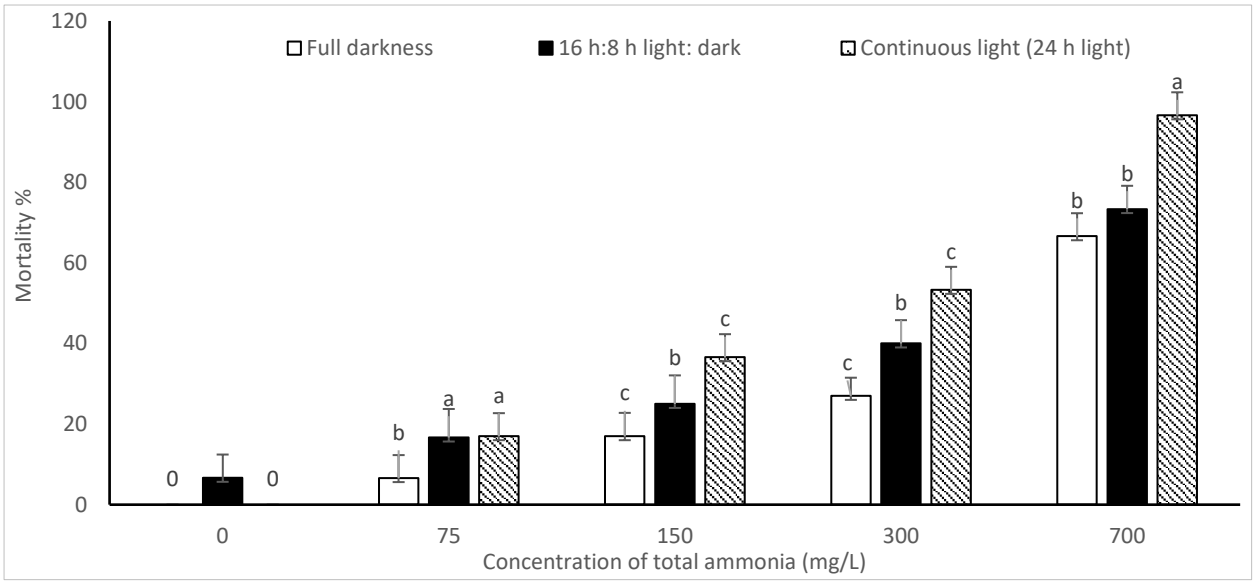

Figure 5. Effect of Photoperiod on mortality (%) of *A. franciscana* nauplii exposed to total ammonia after 24 h. Values are expressed as mean±SD. Within each total ammonia concentration, bars sharing different letters are significantly different (P<0.05).

Discussion

The acute toxicity of ammonia has been investigated in a wide range of crustacean species (Miranda-Filho et al., 2009; Barbieri et al., 2010; Lin et al., 2024; Miranda et al., 2025; Liang et al., 2026; Wu et al., 2026). However, information on *Artemia* species remains scarce. In the present study, toxicity experiments conducted on *A. franciscana* nauplii yielded 24 h LC_{50} values of 338 and 665 mg/L (total ammonia) for instar I and instar II, respectively. As summarized in Table 3, these values are considerably higher than those reported for most other crustaceans. Penaeid shrimps, including *Litopenaeus vannamei*, *Litopenaeus schmitti*, *Penaeus japonicus*, and *Penaeus semisulcatus*, exhibit LC_{50} values ranging from ~25 to 116 mg/L depending on the life stage and environmental conditions (Kır et al., 2004; Lin et al., 1993; Barbieri, 2010; Valencia-Castañeda et al., 2018, 2019). The freshwater prawn *Macrobrachium carcinus* shows LC_{50}≈ 63 mg/L (Gomes et al., 2016), the mud crab *Scylla paramamosain* exhibits LC_{50}= 325 mg/L (24 h), and marine amphipods, including *Ampelisca abdita*, *Rhepoxynius abronius*, *Eohaustorius estuarius*, and *Grandidierella japonica*, display LC_{50} values between 49.8 and 148.3 mg/L under 96 h exposure (Kohn et al., 1994). In contrast, *A. franciscana* exhibited substantially greater tolerance to ammonia, with LC_{50} values higher than those reported for most crustacean species summarized in Table 3.

Comparable patterns have previously been reported for *Artemia* nauplii (Chen et al., 1989; Landau and Sanchez, 1991), suggesting that elevated ammonia tolerance may be a characteristic feature of the genus. This resilience is likely associated with species-specific physiological adaptations. As a halophilic crustacean inhabiting hypersaline and environmentally variable habitats, *Artemia* possesses highly efficient osmoregulatory mechanisms that are closely linked to nitrogen excretion processes (Weihrauch and O'Donnell, 2015). As an ammoniotelic organism, it can rapidly eliminate ammonia, thereby limit intracellular accumulation and maintain physiological homeostasis (Criel and MacRae, 2002). Moreover, ammonia excretion in *Artemia* is strongly associated with metabolic activity and physiological status, reflecting considerable metabolic flexibility and an enhanced capacity to cope with environmental stressors. Collectively, these physiological attributes may promote efficient nitrogen turnover, reduce internal ammonia burden, and contribute to the exceptionally high ammonia tolerance observed in *A. franciscana* relative to other aquatic crustaceans.

Previous studies have demonstrated that *Artemia* species exhibit stage-dependent sensitivity to pollutants (Toğulga, 1998; Zhu et al., 2017; Wang et al., 2017; Lish et al., 2019; Khandan Barani et al., 2025). This study showed that instar I nauplii were more sensitive to ammonia exposure than instar II nauplii. This pattern is consistent with observations in other crustaceans, including shrimps and crabs, where younger individuals are generally more sensitive to ammonia than later developmental stages (Lin et al., 2023), a pattern commonly reported among crustacean species.

The higher sensitivity observed in Instar I nauplii is likely related to developmental and physiological limitations. At this stage, osmoregulatory and metabolic systems are still developing, resulting in reduced capacity for ion regulation and stress tolerance (Anger, 2001; Charmantier and Charmantier-Daures, 2001; Romano and Zeng, 2013). Instar I nauplii primarily rely on yolk reserves, whereas Instar II individuals begin active feeding, which may increase metabolic flexibility and stress tolerance. Although size differences are

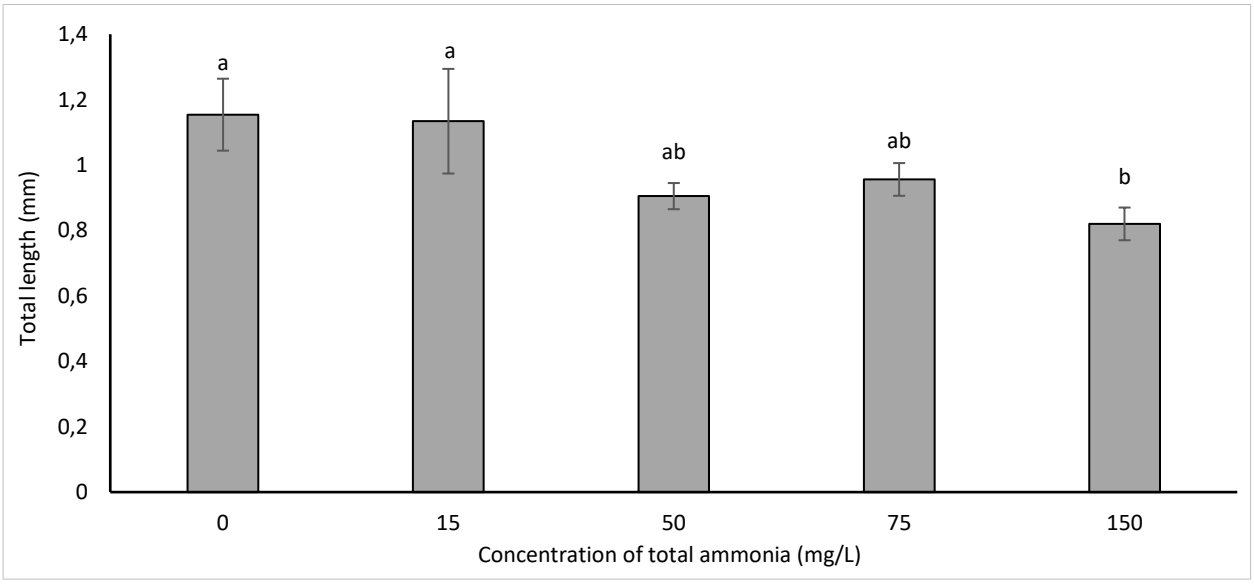

Figure 6. Total length (mean±SD) of *A. franciscana* larvae after 5 days of exposure to different total ammonia concentrations. Different letters above the bars indicate significant differences among treatments (P<0.05).

relatively small, they may still influence passive ammonia diffusion across epithelial surfaces (Péqueux, 1995). Collectively, these factors may contribute to higher ammonia susceptibility in Instar I compared with Instar II nauplii.

Ammonia toxicity in aquatic organisms is strongly modulated by environmental factors such as pH, salinity, temperature, and photoperiod through their effects on ammonia speciation and organismal physiology (Lin & Chen, 2001). Although these factors have been widely studied in relation to the physiology and performance of *Artemia* (Browne and Wanigasekera, 2000; Nambu et al., 2004; Sui et al., 2014; Xue et al., 2024), their roles in influencing ammonia toxicity remain poorly understood. This study demonstrated that ammonia toxicity in *A. franciscana* was significantly affected by all tested environmental factors, highlighting the importance of environmental context.

Mortality of *A. franciscana* nauplii was significantly higher at pH 6.5 than at pH 8 and 9, which is contrary to the commonly reported pattern of increased ammonia toxicity at higher pH due to higher NH_3 availability (Mallasen & Valenti, 2005; Romano & Zeng, 2013; Gündoğdu et al., 2021). However, *Artemia* appears to exhibit a distinct response pattern. Landau and Sanchez (1991), for example, also reported higher mortality at lower pH values in *Artemia*. These observations indicate that ammonia toxicity in *Artemia* is not solely determined by ammonia speciation in the external medium. Instead, acidic conditions are likely to impose additional physiological constraints that impair epithelial ion transport and acid-base regulation (Henry and Wheatly, 1992). In this context, NH_4^+-Na^+ competitive interactions have been proposed as a potential contributing mechanism in *A. franciscana* (Landau and Sanchez, 1991; Young-Lai et al., 1991). Such disturbances may interfere with ammonia excretion efficiency and promote the accumulation of nitrogenous waste, thereby increasing susceptibility to ammonia stress.

Temperature also significantly influenced ammonia toxicity in *A. franciscana*, with mortality increasing at 25°C compared with 20°C. These results are consistent with previous studies demonstrating that elevated temperature can increase ammonia toxicity in crustaceans (Kır et al., 2004; Dehedin et al., 2013). This response may reflect the combined effects of changes in ammonia chemistry and physiological stress. Elevated temperature shifts the NH_4^+/NH_3 equilibrium toward the more toxic un-ionized form (Romano and Zeng, 2013) while simultaneously increasing metabolic rate, oxygen demand, and ventilation activity (Salomon and Buchholz, 2000; Yang et al., 2022). These changes may enhance ammonia uptake and increase the energetic costs associated with ion regulation and acid-base balance. Consequently, the capacity to maintain physiological homeostasis may be reduced, leading to increased susceptibility to ammonia toxicity. Given ongoing climate warming, these interactions may increase the ecological risks associated with ammonia pollution in aquatic ecosystems.

Salinity reduced ammonia toxicity, with higher survival observed at elevated salinity levels. This result is consistent with previous findings in crustaceans (Lin and Chen, 2001; Kır and Kumlu, 2006; Barbieri, 2010), supporting an inverse relationship between salinity and ammonia toxicity. The observed reduction in toxicity may be explained by reduced NH_3 bioavailability at higher salinities, which can limit passive diffusion across biological membranes (Tang et al., 2024). In addition, elevated salinity may improve osmoregulatory performance by stabilizing ionic gradients and enhancing physiological resilience under toxic stress. Collectively, these mechanisms may increase the tolerance of *A. franciscana* to ammonia exposure and highlight salinity as an important modulating factor in aquaculture systems.

Photoperiod also influenced ammonia toxicity, with the highest mortality observed under continuous light (24 h illumination), followed by a 16 h:8 h light–dark cycle, whereas the lowest mortality occurred under continuous darkness. Although studies directly addressing photoperiod–ammonia interactions are limited, light regimes regulate circadian rhythms, metabolic activity, and stress physiology in crustaceans (Fanjul-Moles et al., 1998; Simčič and Brancelj, 2007). Continuous illumination may increase metabolic demand and stress-related energy expenditure, which could reduce the capacity of organisms to cope with ammonia stress. In contrast, continuous darkness may provide more stable metabolic conditions, potentially enhancing tolerance to toxic exposure (Farhadi et al., 2021; Cheng et al., 2023). However, the underlying physiological mechanisms remain unclear and require further investigation.

Collectively, the present findings indicate that ammonia toxicity in *A. franciscana* is influenced by ammonia concentration and environmental conditions. These factors may interact to shape physiological responses and determine stress tolerance. The results emphasize the importance of considering multiple environmental variables when assessing ammonia toxicity in aquatic invertebrates.

Growth reduction following chronic ammonia exposure has been reported in several crustacean species, including *Penaeus japonicus* (Chen and Kou, 1992), *Orithyia sinica* (Koo et al., 2004) and *Litopenaeus vannamei* (Han et al., 2017). Consistent with these observations, chronic exposure to total ammonia significantly reduced the body length of *A. franciscana* after 5 days of exposure. This growth inhibition is likely attributable to the physiological stress associated with ammonia exposure. Elevated ammonia concentrations can disrupt normal cellular and metabolic processes, resulting in increased energetic demands for detoxification, ion regulation, and maintenance of internal homeostasis (Lin et al., 2023; Zhang et al., 2023). Consequently, energy that would otherwise be

allocated to growth may be redirected toward coping with physiological stress. In addition, ammonia exposure may reduce feeding activity, increase metabolic costs, and interfere with nutrient assimilation and energy metabolism (Romano and Zeng, 2013; Miranda-Filho et al., 2009). Collectively, these effects may limit the resources available for somatic growth, ultimately leading to reduced body size in ammonia-exposed individuals. The reduction in growth observed in the present study suggests that sublethal total ammonia exposure may impair individual performance even in the absence of acute mortality, highlighting the potential consequences of chronic ammonia stress for individual performance in *Artemia*.

Conclusion

The present study demonstrates that total ammonia causes both acute and chronic adverse effects in *A. franciscana*, with responses strongly influenced by developmental stage and environmental conditions. Instar I nauplii were more sensitive to ammonia exposure than Instar II nauplii, indicating stage-dependent variation in ammonia tolerance. Environmental factors, including pH, temperature, salinity, and photoperiod, significantly affected ammonia toxicity, emphasizing the importance of environmental conditions in shaping toxic responses. In addition, prolonged exposure to sublethal ammonia concentrations reduced larval growth, demonstrating that ammonia can impair developmental performance even in the absence of acute mortality. Overall, these findings improve our understanding of ammonia toxicity in *A. franciscana* and highlight the combined role of developmental stage and environmental conditions in determining toxic effects.

Ethical Statement

The experiments were performed using Artemia franciscana nauplii. As this species is an invertebrate, no ethical approval was required under the applicable institutional and national regulations.

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Author Contribution

HKB; Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Supervision, Visualization, Writing - Review & Editing.

MM; Investigation, Data Curation, Formal Analysis, Writing - Original Draft Preparation.

NS; Investigation, Validation, Writing - Review & Editing. All authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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