



ACUTE TOXICITY OF AMMONIA FOR SOFT CORAL *Xenia umbellata* (Lamarck, 1916)

Toxicidade aguda de amônia para o coral mole *Xenia umbellata* (Lamarck, 1916)

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ABSTRACT

*Coral reefs are important ecosystems on the planet and provide a wide variety of ecosystem services that are extremely important for the development and survival of several species, including humans. The lack of information and knowledge about the influence of abiotic parameters on the physiology of marine cnidarians with ornamental potential has hampered the cultivation of coral species on a large scale in aquaculture. The objective of this work was to determine the median lethal concentration after 96 h (LC_{50}^{96h}) of nonionized ammonia (NH_3-N) for the octocoral white pulse soft coral (*Xenia umbellata*) over a period of 96 h. Eighty colonies of *X. umbellata* coral were exposed to five different ranges of $N-NH_3$ (0.0, 0.92, 3.87, 5.95, 8.46 or 10.18 $mg L^{-1}$, all in triplicate. Tests were performed on a standard semistatic system with 100% daily water renewal. The LC_{50}^{96h} was estimated to be 0.28 $mg NH_3-N L^{-1}$ (0.22~0.36 $mg L^{-1}$). The lethal concentration of ammonia decreased with increasing time. Furthermore, concentrations below 0.96 $mg L^{-1}$ total ammonia (0.08 $N-NH_3$) are not lethal for this organism in an exposure period of 96 h.*

Keywords: Aquaculture; Octocoral; Regenerative; White pulse soft coral.

RESUMO

Os recifes de corais são ecossistemas importantes do planeta, fornecendo uma grande variedade de serviços ecossistêmicos que são extremamente importantes para o desenvolvimento e sobrevivência de várias espécies, incluindo humanos. A falta de informação e conhecimento sobre a influência dos parâmetros abióticos na fisiologia dos cnidários marinhos com potencial ornamental tem dificultado o cultivo de espécies de corais em larga escala na aquicultura. O objetivo deste trabalho foi determinar a concentração letal mediana após 96 h (CL_{50}^{96h}) de amônia não ionizada (NH_3-N) para o octocoral *Xênia pulsante* (*Xenia umbellata*) em um período de 96 h. Para isso, 80 colônias de coral *X. umbellata* foram expostas a cinco diferentes concentrações de $N-NH_3$ (0,0; 0,92; 3,87; 5,95; 8,46; ou 10,18), todas em triplicata. Os testes foram realizados em sistema semiestático padrão com 100% de renovação diária de água nas unidades experimentais. A CL_{50}^{96h} foi estimada em 0,28 mg $NH_3-N L^{-1}$ (0,22~0,36 mg L^{-1}). Concluiu-se que a concentração letal de amônia é menor com o aumento do tempo. Além disso, concentrações abaixo de 0,96 mg L^{-1} de amônia total (0,08 $N-NH_3$) não foram letais para este organismo em um período de exposição de 96 h.

Palavras-chave: Aquicultura; Octocoral; Regenerativo; *Xênia pulsante*.

INTRODUCTION

Coral reefs are important ecosystems on the planet and provide a wide variety of ecosystem services that are extremely important for the development and survival of several species, including humans (Connell, 1978; Hughes *et al.*, 2003). The class Octocorallia consists exclusively of polypoid organisms belonging to the class Anthozoa, the largest class in the phylum Cnidaria (Haddad, 2006; Pérez *et al.*, 2016). It is estimated that the subclass Octocorallia includes approximately 3000 known species, which are distributed in 46 families belonging to three major orders: Helioporacea, Pennatulacea, and Alcyonacea (Daly *et al.*, 2007), in which the genus *Xenia* is found.

Xenia umbellata (Lamarck, 1816) is a soft coral species that resembles a mushroom and is characterized as an autotrophic species that has a reduced gastrovascular cavity and greater photosynthetic activity than other corals without pulsating movement (Schlichter *et al.*, 1983; Reinicke, 1997; Kremien *et al.*, 2013). Furthermore, it is a coral that multiplies very quickly (Benayahu, 1991; Daly *et al.*, 2007) and is easy to manage, making it highly desirable to beginner aquarists (Kim *et al.*, 2022).

In aquaculture, coral farming is a growing activity that can act as a regenerative tool and control the degradation and exploitation of coral reefs around the world (Pomeroy *et al.*, 2006; Leal *et al.*, 2016). However, coral aquaculture is still underdeveloped (Leal *et al.*, 2016). Aquaculture can offer a sustainable alternative to produce these animals and supply the ornamental trade (Barton *et al.*, 2017). Nevertheless, to make commercial cultivation of a given species viable, it is necessary to establish safe levels of some abiotic factors, such as nitrogenous compounds, which potentially limit the survival of aquaculture species (Boyd and Watten, 1989; Tomasso, 1994).

Ammonia reaches aquatic systems through complex biogeochemical interactions and is released into the environment by various anthropogenic activities. Despite the toxic properties of ammonia in water, it also plays a pivotal role in the biogeochemical cycle of nitrogen (N) and regulates the mechanisms of normal and abnormal physiology in aquatic

organisms (Constable *et al.* 2003; Edwards *et al.*, 2023). Ammonia cycling between a cnidarian animal host and intracellular symbiotic algae is a key metabolic pathway for corals; for example, recycled nitrogen accounts for 90% of the nitrogen demand of zooxanthellae in the coral *Stylophora pistillata*, and nitrogen cycles occur at a rate of 0.13% per day in algae and 0.013% per day in animal tissue (Rahav *et al.*, 1989). External ammonium concentrations equal to 0.6 mM can sustain the growth of zooxanthellae populations in the coral *Stylophora pistillata* (Grover *et al.*, 2002). However, a concentration of 0.001 mmol L⁻¹ of ammonia or nitrate can significantly increase the expulsion of symbiotic algae from *Acropora nobilis*, *Palythoa* sp. and *Alveopora verrilliana* (Zhu *et al.*, 2004).

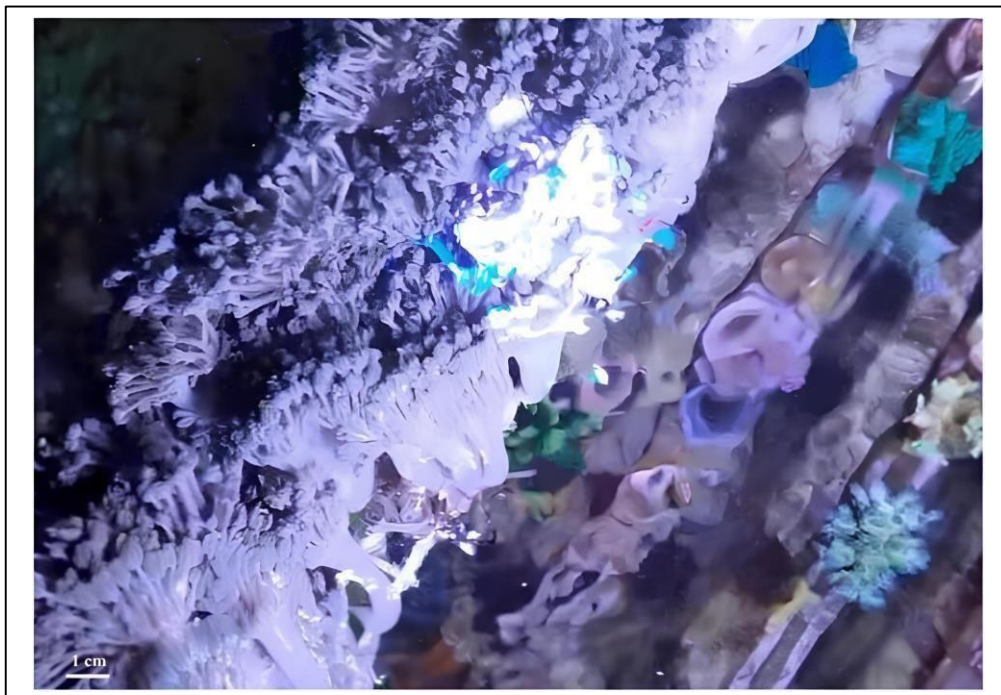
The threshold concentration of ammonia in aquaculture is still unknown for several potentially produced coral species. Interactions between aquaculture research and the environment have emerged as promising technologies to contribute to ecosystem sustainability. Therefore, to enable large-scale cultivation of *X. umbellata*, the present study aimed to estimate the lethal concentration (LC₅₀^{96 h}) of nonionized ammonia for this soft coral.

MATERIAL AND METHODS

Experimental design

The experiment was carried out at the Laboratory of Marine Fish Culture (LAPMAR) of the Federal University of Santa Catarina (UFSC). For this purpose, 80 specimens of *Xenia umbellata* were obtained from a small local producer in the city of Florianópolis, Brazil (Figure 1). The animals were acclimatized for 10 days in a 20 L tank with water from the ocean collected at Mozambique beach Florianópolis, Brazil (27°34'02''S, 48°25'44''W). The samples were subsequently transferred to experimental units filled with artificial marine water obtained by mixing fresh water purified via reverse osmosis with the addition of the natural marine salt Coral Pro Salt (Red Sea, USA), which was equipped with constant aeration and a thermostat to maintain a constant temperature at 24 °C.

Figure 1 – Colonies of the octocoral *Xenia umbellata* acquired for the experiment



Water preparation

In a 200 L tank, 120 L of freshwater sterilized with ultraviolet light and deionized water by reverse osmosis and an electrical conductivity of 0.714 $\mu\text{S m}^{-1}$ were prepared. Afterward, 4.453 kg of Coral Pro Salt (Red Sea, USA) was added and dissolved via a submersible pump to homogenize the water for two hours until the salts were completely dissolved, following the manufacturer's recommendations. The salt used was chosen because it comes from the Red Sea, a place where *X. umbellata*, the species used in the experiment, naturally occurs, and, according to the manufacturer, meets the basic requirements of the chemical parameters of the water necessary for the maintenance of the species.

The saltwater was divided into six 20 L reservoir tanks. One of the tanks did not receive ammonium chloride (NH_4Cl) addition, whereas the other tanks received NH_4Cl concentrations calculated on the basis of the molecular weight of this salt. Water samples from each of the reservoir tanks were subsequently analysed. Water prepared and stored without ammonia content was used to carry out the necessary dilution of the samples for analysis and for the control group. The analyses were carried out at the Water Quality Laboratory of the Mariculture Station Elpídio Beltrame/UFSC via irradiance spectrophotometry, and the NH_4Cl weights and concentrations are shown in Table 1.

Table 1 - Weight of ammonium chloride used, and concentrations obtained in treatments

Treatments	Weight (g)	Analyzed (mg L^{-1})
0	-	0,001
1	0,0595	0,92
2	0,1788	3,87
3	0,2969	5,95
4	0,4158	8,46
5	0,5346	10,18

Determination of the median lethal concentration (LC_{50})

Sixteen experimental units with a capacity of 2 L (useful 1.5 L) were used, with LED lighting from an Al@ Hydra 52 HD luminaire (Aquaillumination, USA), a light density (PAR peak) of 225 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and an average PAR over an area of 107 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, which is specific for coral development. The temperature was maintained at 24 °C, and the photoperiod was 12 h. The colonies of five animals were randomly distributed in the experimental units with five different concentrations (mg L^{-1}) of N- NH_3 [0.92], [3.87], [5.95], [8.46], [10.18], and a control group [0].

The experiment was conducted for 96 h. In the first 12 h, observations were made every 30 min to monitor animal movement and mortality. After this period, these observations were performed every 24 h at the same time daily. Temperature and pH analyses of the experimental units were also carried out during this period. Twenty-four hours after the beginning of the experiment, the first water changes were made through siphoning, and 100% of the water in each experimental unit was renewed so that the ammonia concentration was maintained at the predetermined levels. During daily maintenance, in addition to water changes, dead animals in each treatment were removed and counted. Data collection and physical-chemical parameters of the water occurred throughout the experimental period and were carried out in the morning, always at 10 am.

Accumulated mortality data were used to calculate the median lethal concentration over 96 h ($LC_{50}^{96\text{ h}}$) and their respective confidence intervals via the mathematical method Trimmed Spearman-Kärber (Hamilton *et al.*, 1977). Organisms that did not show pulsation and that shrivelled during daily maintenance, lost their physical integrity (i.e., lost parts of the body) and caused turbidity in the water were considered dead.

RESULTS

The physical–chemical parameters of the water did not significantly differ among the groups at 96 h (Table 2). The median lethal concentrations of total ammonia and unionized ammonia were estimated for *Xenia umbellata* (Table 3).

The species *Xenia umbellata* presented a mortality curve within expectations, and almost all the treatments resulted in 100% lethality within a period of up to 96 h, except for the treatment with the lowest concentration of ammonia [0.92] and the control group, which did not experience mortality (Figure 2).

Table 2 – Water quality variables during assay of lethal concentration (LC_{50}) of non-ionized ammonia for soft coral *Xenia umbellata*. (*) Manufacturer's reference values for Coral Pro Salt (Red Sea – USA)

Variables	Control	[0,92]	[3,87]	[5,95]	[8,46]	[10,18]
Dissolved oxygen (mg L^{-1})	7.12±0.51	6.22±0.85	6.02±0.55	6.92±0.31	6.26±0.7	6.92±0.47
temperature °C	23.9±0.21	24.1±0.1	23.8±0.25	23.9±0.17	23.9±0.32	24.0±0.2
Salinity (‰)	34	34	34	34	34	34
pH	8.3±0.4	8.7±0.2	8.2±0.1	8.7±0.3	8.2±0.1	8.3±0.2
Total alkalinity ($\text{mg CaCO}_3\text{L}^{-1}$) *	250	250	250	250	250	250
Calcium (mg L^{-1}) *	435 - 465	435 - 465	435 - 465	435 - 465	435 - 465	435 - 465
Magnesium (mg L^{-1}) *	1,310 – 1,390	1,310 – 1,390	1,310 – 1,390	1,310 – 1,390	1,310 – 1,390	1,310 – 1,390
Potassium (mg L^{-1}) *	375 - 405	375 - 405	375 - 405	375 - 405	375 - 405	375 - 405
Phosphate (mg L^{-1}) *	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03

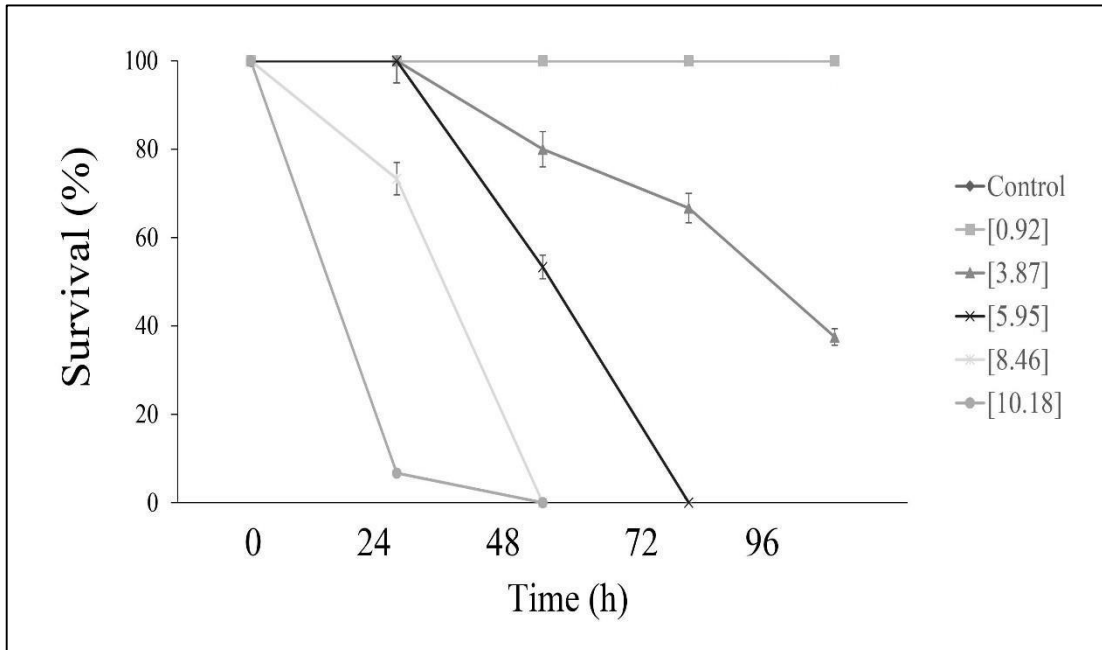
DISCUSSION

Ammonia is a chemical compound that originates mainly from the decomposition of biological residues and is toxic or lethal to animals, depending on the concentrations of N-NH_3 and the metabolic conditions of the animals (Randall and Tsui, 2002). Excess ammonia in the natural environment can harm coral reefs through eutrophication and degradation of water quality, with a consequent reduction in the photic zone that impairs the photosynthesis of symbiotic algae (zooxanthellae), which are essential for their survival (Constable *et al.*, 2003; Hughes *et al.*, 2003; Haas *et al.*, 2009; Edwards *et al.*, 2023). Currently, domestic and industrial effluents containing ammonia, originating from untreated or inadequately treated sewage, are a significant source of ammonia for marine environments (Edwards *et al.*, 2023).

A concentration of 0.001 mmol L^{-1} ammonia or nitrate significantly increased the expulsion of zooxanthellae in *Acropora nobilis*, *Palythoa* sp. and *Alveopora verrilliana* (Zhu *et al.*, 2004), whereas for the coral *Stylophora pistillata*, severe oxidative stress and a reduction in the aerobic

scope were observed when exposed to 3 μM NH_4^+ seawater enrichment combined with thermal stress (Marangoni *et al.*, 2020).

Figure 2 – Survival of *Xenia umbellata* during acute exposure to ammonia for 96 h



According to Bassim and Sammarco (2003), increasing the seawater temperature in the presence of ammonia caused a significant decrease in the ciliary activity (motility) and larval settlement rate in a scleractinian coral (*Diploria strigosa*). On the other hand, Haas *et al.* (2009) described the negative impact of increased ammonia together with other inorganic compounds on the health of corals in the Red Sea, specifically on the deterioration of animal tissue, which was very similar to the findings of the present study; however, the direct impact of ammonia in coral cells was not detailed. A hypothesis for corals of the genus *Xenia*, as in other classes of Anthozoans, is that at high levels of inorganic nitrogen in water, zooxanthellae can retain more carbon for their own metabolism and growth, and consequently, less of this carbon is translocated to their host, causing coral mortality (Dubinsky and Jokiel, 1994).

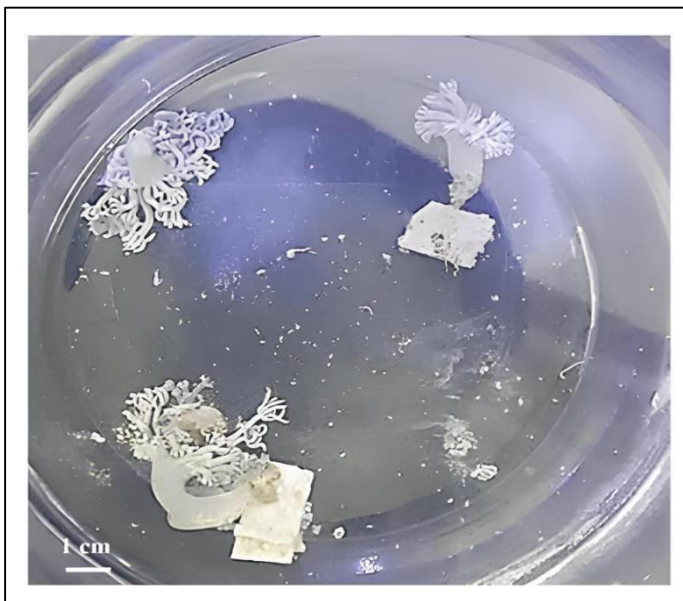


Figure 3 – Dead specimens of the coral *Xenia umbellata* after 72h in Treatment 3

The study of Octocorallia corals is incipient worldwide and became more popular in Brazil in the 1980s, with diving expeditions identifying native species of this group (Castro *et al.*, 2010). The species of the present study is an Octocorallia nonnative to Brazil but is commonly found in the Brazilian marine ornamental market due to its relative rusticity, fast growth, and ease of management. The possibility of the proliferation of this species in Brazilian waters must be considered in the case of inadequate disposal of parts of colonies from domestic reef aquariums. This practice possibly led to the appearance of a similar species, popularly known as Xênia-azul (*Sansibia* sp.), described by Alderslade (2000) on the coast of Bahia; this species is also found on a tropical rocky reef in the southwest Atlantic, Brazil (Mantelatto *et al.*, 2018).

Table 3 - Mean lethal concentrations of ammonia obtained by the Trimmed Spearman-Kärber method according to Hamilton *et al.* (1977)

Time (h)	Total ammonia N-NH ₄ ⁺	Confidence interval	Non-ionized ammonia	Confidence interval
			N-NH ₃	
24	8.81	8.18 - 9.50	0.77	0.71 - 0.82
48	4.9	3.95 - 6.10	0.42	0.34 - 0.53
72	3.74	3.02 - 4.63	0.32	0.26 - 0.40
96	3.3	2.61 - 4.18	0.28	0.22 - 0.36

Corals are examples of the richness and complexity of marine life, and the science developed in support of coral aquaculture can contribute to reducing the degradation of coral reefs around the world, supplying the ornamental, pharmaceutical and reef restoration industries (Leal *et al.*, 2016).

On the basis of the present study, we conclude that the lethal concentration of ammonia decreases with increasing time and that the longer the exposure of the coral *X. umbellata* to ammonia is, the smaller the amount of ammonia necessary to intoxicate the animal. Furthermore, it was possible to conclude that concentrations below 0.96 mg L⁻¹ total ammonia (0.08 N-NH₃) are not lethal for this organism in an exposure period of 96 h, since no mortality was observed in this group. Thus, the mean lethal concentration (LC₅₀) after 96 h of exposure to N-NH₃ was estimated to be 0.28 mg L⁻¹. These findings provide fundamental and important data on the toxic effects of ammonia on the coral *X. umbellata*. Some knowledge gaps were identified to support future research involving the cultivation of this species in aquaculture.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

G.M.B. – Conceptualization, Conducting the experiment, Investigation, Data curation. **M.S.O.** – Data analysis, Manuscript review, Final writing. **R.V.R.**: Data analysis, manuscript review, final writing. **C.M.** – Methodology, Validation, Supervision, manuscript review.

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.

DATA AVAILABILITY

The data will be made available upon reasonable request.

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