



Ammonia toxicity in Southern King Crab (*Lithodes santolla*, Molina 1742) larvae

Soledad L. Diodato · Oscar A. Amin · Laura I. Comoglio

Received: 8 January 2019 / Accepted: 9 July 2019
© The Author(s) 2019

Abstract Effects of ammonia on zoea I of the Southern King Crab, *Lithodes santolla* (Decapoda, Lithodidae) were analyzed through acute (96 h) and chronic (29 days in total) assays (seven total ammonia nitrogen (TAN) concentrations: 9, 15, 25, 41, 67, 110, and 182 mg L⁻¹, plus control). The estimated LC₅₀-96 h was 107.97 mg TAN L⁻¹ (1.93 mg NH₃-N L⁻¹), while the safe level of ammonia was 10.79 mg TAN L⁻¹ (0.19 mg NH₃-N L⁻¹). Survival was highest in the three lowest ammonia concentrations throughout 96 h (93.3%, 90% and 93.3% in 9, 15 and 25 mg TAN L⁻¹, respectively). In chronic assays, the percentage of survival decreased along with the exposure time and the ammonia concentration. Zoeae's mean life time tended to increase almost gradually with the increment of ammonia concentration. Mean molting time from zoea I to II was 4.06 days, while it increased from zoea II to III, and zoea III to the post-larval stage (6.00 and 8.39 more days, respectively) with ammonia concentration. The percentage of individuals that have molted in every molt stage tended to decrease while ammonia concentration increased. Therefore, the results obtained in the present study bring new information about ammonia toxicity in early stages of development of crab *Lithodes santolla*, an important commercial species of the Beagle Channel.

Keywords Acute toxicity · Ammonia · Chronic toxicity · Commercial crustacean species · Molting · Zoea

Introduction

Among the environmental stressors found in natural waters, ammonia is one of the most common toxic substances. It enters aquatic ecosystems through several sources including industrial wastes, municipal sewage effluents, agricultural run-off and decomposition of biological wastes (Camargo and Alonso 2006; Randall and Tsui 2002; USEPA 2009). It is also a waste product of protein metabolism of fish and crustaceans and is not a persistent pollutant, such as metals or some organic compounds (Borgmann and Borgmann 1997). The accumulation of ammonia in the aquatic environment in intensive aquacultural systems as well as in the wild

S. L. Diodato (✉)
Centro Austral de Investigaciones Científicas (CADIC-CONICET), B. Houssay 200, Ushuaia, Tierra del Fuego, Argentina
e-mail: sole22diodato@gmail.com

S. L. Diodato
Instituto de Ciencias Polares, Ambiente y Recursos Naturales (ICPA), Universidad Nacional de Tierra del Fuego (UNTDF),
Fuegia Basket 251, Ushuaia, Tierra del Fuego, Argentina

O. A. Amin · L. I. Comoglio
Ushuaia, Tierra del Fuego, Argentina



has been identified as one of the common causes of death in aquatic organisms (Cobo et al. 2014; Dutra et al. 2016; Ip et al. 2001; Lin et al. 1993; Xu et al. 2004). Thus, it is important to know the tolerance to ammonia of aquatic species in order to assess its sensitivity and to improve the efficiency of aquacultural systems.

Ammonia occurs in natural waters in two forms: un-ionized (NH_3) and ionized ammonia (NH_4^+); the sum of the two is the total ammonia (TAN). The toxicity of ammonia to aquatic organisms has been attributed to the NH_3 species because it is lipid-soluble and it diffuses across branchial membranes easily. The NH_4^+ species is considered nontoxic or significantly less toxic (Armstrong et al. 1978; Camargo and Alonso 2006; Evans and Cameron 1986; Thurston et al. 1981) although there is evidence that the ammonium ion also contributes to toxicity (USEPA 2009). The proportion of NH_3 to NH_4^+ depends on pH and temperature and, to a lesser extent, salinity of the medium. The percentage of NH_3 in aqueous ammonia solutions generally increases at higher pH and temperature and at lower salinities (Emerson et al. 1975; Spotte and Adams 1983).

Several studies have demonstrated that the tolerance to ammonia increases with the ontogenetic developmental progress of the animals and decreases with exposure time (Dutra et al. 2016; Liao et al. 2011; Neil et al. 2005; Ostrensky and Wasielesky 1995; Zhao et al. 1997). The toxicity of waterborne ammonia to crustaceans has been studied by several authors in larval stages as well as juveniles and adults (Harris et al. 2001; Hutchinson et al. 1998; Liao et al. 2011; Lin et al. 1993; Marazza et al. 1996; Moore et al. 1997; Racotta and Hernández-Herrera 2000; Romano and Zeng 2010). As larval stages support the population recruitment, it is ecotoxicologically relevant to know what effects ammonia has on crustacean larvae. The exposure of crustaceans to high levels of environmental ammonia is strongly linked to the physiological processes that are involved in osmoregulation. In this sense, gills play a significant role in ammonia excretion, osmoregulation, gas exchange and pH balance; for instance, damages at branchial level is a possible cause of death (Camargo and Alonso 2006; Henry et al. 2012; Romano and Zeng 2012). The susceptibility to ammonia is a consequence of gill damage since they are in direct contact with the external environment. Additionally, elevated environmental ammonia has been reported to reduce growth, to affect the immune system, and could enhance or delay molting in crustaceans (Chen and Kou 1992; Forward et al. 2001; Henry et al. 2012). Molting is a very important physiological process for crustaceans because it not only allows growth and development bearing a rigid and confining exoskeleton, but it is also tied with metamorphosis during the early stages of the life cycle and with reproduction during the adult stage. The post-molt stage is characterized by a rapid increase in body size during the short soft-skinned period caused by water uptake from the environment (Hartnoll 2001). This is why before and immediately after molt, some crustaceans are more sensitive to ammonia and consequently it is relevant to evaluate ammonia toxicity at different molt stages (Wajsbrodt et al. 1990).

The Southern King Crab *Lithodes santolla* is a decapod crustacean of the family Lithodidae. It is a cold and cold-temperate water species and is the most important shellfish commercially exploited in the Beagle Channel, Argentina. Although its population has not shown a trend of recovery of its abundance after ca. 19 years of fishing restrictions, since 2013 its fishery is not restricted (Provincial Law No. 931). Stock enhancement program has been proposed as an alternative strategy to revert local depletion of the Southern King Crab population (Sotelano et al. 2016, 2018; Tapella et al. 2012). Shallow waters like bays have been mentioned as a possible recruitment area (Lovrich 1997; Vinuesa and Lovrich 1992). External fertilization takes place during late November and early December. Females carry the eggs in the abdomen during 9–10 months until they hatch between mid-September and October (Vinuesa 1991). Larval development involves three zoeae stages (zoea I, II and III) during a period of about 21 days in experimental conditions and a post-larval stage (megalopa) (Anger et al. 2004; Calcagno et al. 2004; Campodónico 1971). Larval stages of the Southern King Crab have been studied in several biological aspects, including their exposure to pollutants like heavy metals and hydrocarbons (Amin and Comoglio 2002, 2010; Amin et al. 2003); however, ammonia toxicity has not been evaluated yet.

Within this context, the aim of this work was to assess the effect of ammonia on larval development of *L. santolla*, in terms of survival and molting. Results of the present study will be a useful contribution to the biology of the species, and will help to optimize the rearing of larvae and juveniles for future attempts to repopulate natural lithodid stocks.



Materials and methods

Larvae collection and maintenance

Larvae were obtained from ovigerous females collected in the Beagle Channel during September 2010 using commercial fishery traps. Females, kept in the laboratory until zoeae hatched, were maintained in glass aquaria each containing 100 L of filtered seawater with continuous aeration (dissolved oxygen = 10.5 mg L^{-1}), water temperature of $7.5 \pm 0.5 \text{ }^\circ\text{C}$, salinity of 23–24, pH 8.15 and photoperiod of 12L:12D (fluorescent light). Filtration and partial renewal of seawater were done every day, along with the discard of new hatched larvae in order to assure that the new ones had less than 24 h from hatch. Natural seawater used in the experiments was collected from a pristine zone in the Beagle Channel; it was filtered through a 10-mm polypropylene cartridge filter and then through UV light to minimize the activity of microorganisms. It was kept with constant aeration in 250-L dark tanks until use.

Acute and chronic toxicity experiments were carried out starting from newly hatched larvae (day 0) up to complete metamorphosis to post-larval stage. For the assays, zoeae of less than 24 h were selected from the general stock. Only actively swimming larvae were selected and no food was given to them during the experiments, since no effects of starvation on their survival were previously noted owing to their lecithotrophic development (Calcagno et al. 2004; Comoglio and Vinuesa 1991; Kattner et al. 2003).

Acute toxicity

To evaluate acute toxicity, a 96 h survival assay was carried out in laboratory at $7.5 \pm 0.5 \text{ }^\circ\text{C}$ using seven different total ammonia concentrations (9, 15, 25, 41, 67, 110, and $182 \text{ mg TAN L}^{-1}$) and a control (no ammonia added). Each treatment was run in triplicate. A stock solution (1000 mg L^{-1}) of ammonia chloride was prepared by mixing reagent-grade NH_4Cl (Mallinckrodt Inc; 99.5%) in filtered seawater, from which small aliquots were added to dilution water. Concentrations of $\text{NH}_3\text{-N}$ were calculated from TAN according to Bower and Bidwell (1978) based on salinity of 23–24, pH of 8.15 and temperature of $7.5 \text{ }^\circ\text{C}$. The percentage of $\text{NH}_3\text{-N}$ was determined to be 1.79%. Groups of 10 newborn zoeae were placed in 150 mL of test solution with no aeration. Dead larvae were recorded daily and surviving animals were transferred to the corresponding fresh test solutions, according to the static renewal method described by USEPA (2002). Cessation of zoea heartbeat, watching through a stereomicroscope, was considered as death criterion (Rodríguez and Amin 1991).

Daily survival was compared among experimental groups by means of one-way ANOVA, followed by Fisher's post hoc comparisons (Sokal and Rohlf 1981). The 96 h lethal concentration 50 (LC_{50}) with its 95% confidence limits and Pearson's correlation coefficient were estimated using probit analysis including Abbott's correction for the mortality of the control (Finney 1971). Additionally, it was defined the safe level of the LC_{50} of ammonia as the concentration of ammonia which does not have an adverse sublethal or chronic effect on *L. santolla* larvae, and it is equal to $\text{LC}_{50}\text{-96 h} \times 0.1$ (Sprague 1971).

Chronic toxicity

To evaluate chronic toxicity, the experimental assay was carried out at $7.5 \pm 0.5 \text{ }^\circ\text{C}$ using seven different total ammonia concentrations (9, 15, 25, 41, 67, 110, and $182 \text{ mg TAN L}^{-1}$), run in triplicate and including a control. Test solutions were made as in the acute assay and groups of 10 newborn zoeae were placed in 150 mL of test solution with no aeration. Dates of molting and death were daily recorded in every treatment, and surviving organisms were transferred to corresponding fresh test solutions, grouping those that molted simultaneously and that belonged to identical treatment. The experiment finished when the post-larval stage was reached or when all the assayed larvae died.

Several indexes were calculated from chronic survival and molting according to ammonia exposure. The time at which 50% of the assayed larvae had died was defined as "mean life time" (LT_{50}) and was estimated for each ammonia treatment. The time at which 50% of the assayed larvae molt from one stage to the next was defined as "mean molting time" (MT_{50}) and was estimated for each molt (zoea I to II, II to III and III to post-larva) and ammonia treatment by probit analysis (Finney 1971). In addition, molting percentage (MP) was



estimated as the proportion of molted larvae to the total number of larvae in each ammonia treatment. Significant differences between ammonia treatments were evaluated by means of one-way ANOVA followed by Fisher's post hoc comparisons (Sokal and Rohlf 1981) in daily survival, LT_{50} , MT_{50} and MP.

Results

Acute toxicity

The percentage of survival of zoea I larvae of *L. santolla* exposed 96 h to different concentrations of ammonia is shown in Fig. 1. Control treatment and the three lowest ammonia concentrations tested (9, 15, and 25 mg TAN L⁻¹) presented the highest percentage of survival (higher than 86.7%) throughout 96 h, whereas in the highest ammonia concentrations the mortality increased. At 48 and 72 h of exposure, statistical differences were only found in the highest concentration (182 mg TAN L⁻¹). At 96 h of exposure, treatments of 67, 110, and 182 mg TAN L⁻¹ differ significantly from the lowest ones (9, 15, and 25 mg TAN L⁻¹).

The estimated LC_{50-96} h value reported in terms of both total and un-ionized ammonia nitrogen, was 107.97 mg TAN L⁻¹ and 1.93 mg NH₃-N L⁻¹, respectively (Table 1). Therefore, the observed safe level of ammonia was estimated at 10.79 mg TAN L⁻¹ and 0.19 mg NH₃-N L⁻¹. No molting was observed neither in controls nor ammonia treatments during the 96 h of exposure.

Chronic toxicity

The chronic bioassay concluded when the last larva reached the post-larval stage, that is, 29 days after hatching. The percentage of survival decreased in all treatments along with the exposure time and the ammonia concentration (Fig. 2). At the end of the assay, the highest survival value registered was 50% (in 15 mg TAN L⁻¹). Nevertheless, after 29 days of exposure, the survival in the control was slightly lower than in 9 and 15 mg TAN L⁻¹ (40, 43 and 50% of survival, respectively). Significant differences between ammonia treatments were only found in the first 15 days of exposure (Fig. 2).

The mean life time (LT_{50}) tended to increase almost gradually with the increment of ammonia concentration, although this increment was only significant in 41 mg TAN L⁻¹ ($F = 8.867$; $p = 0.0048$). In other

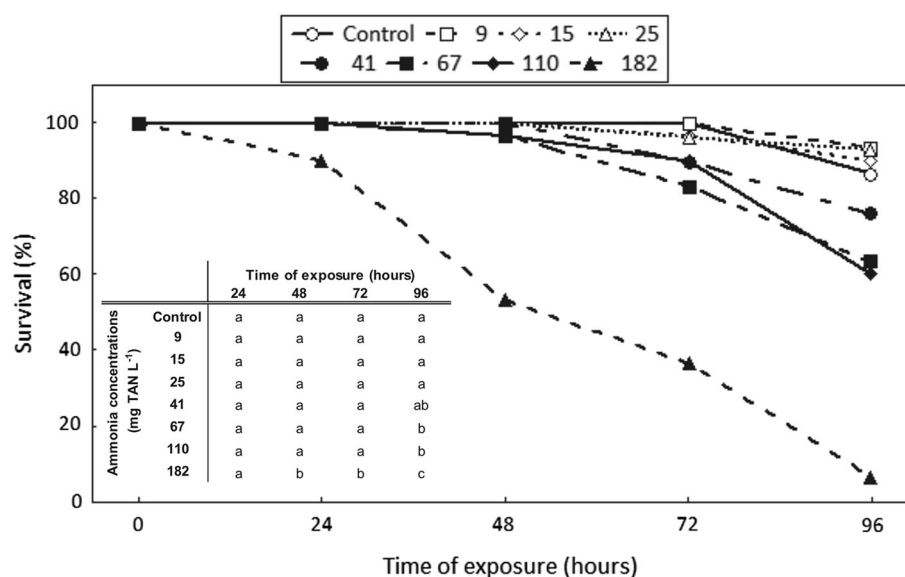
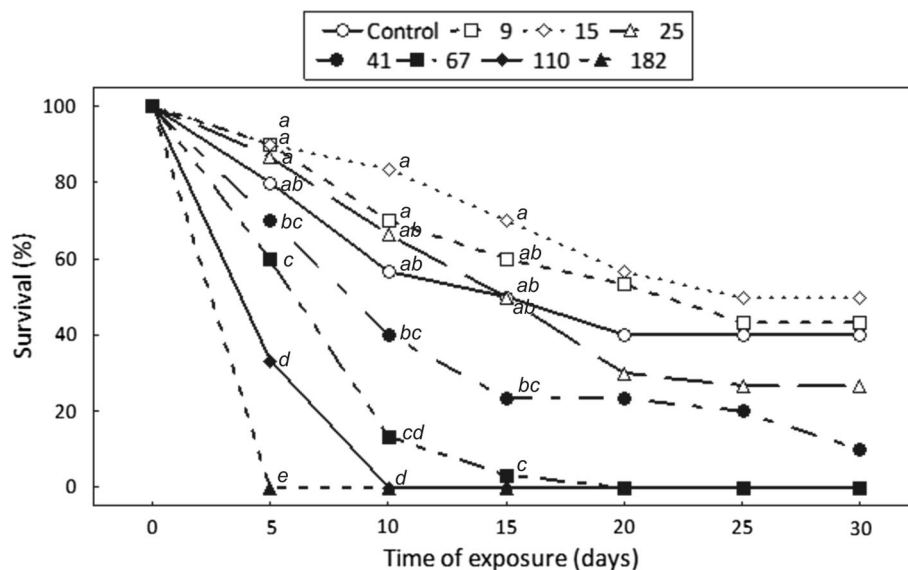


Fig. 1 Percentage of survival (%) of zoea I larvae of *Lithodes santolla* exposed to a total of seven ammonia concentrations (9, 15, 25, 41, 67, 110, and 182 mg TAN L⁻¹, plus control) throughout 96 h. The table indicates statistical differences ($p < 0.05$) between ammonia treatments for each time of exposure (24, 48, 72 and 96 h). Ammonia treatments sharing the same letter for each time of exposure do not differ significantly ($p > 0.05$)

Table 1 Parameters of acute lethal toxicity for zoea I larvae of *Lithodes santolla* exposed to increasing concentrations of ammonia during 96 h: lethal concentration₅₀ for 96 h values (LC₅₀-96 h expressed in mg TAN L⁻¹ and in mg NH₃-N L⁻¹), their 95% confidence limits and the correlation coefficient (*R*²) from probit analysis

	mg TAN L ⁻¹	mg NH ₃ -N L ⁻¹
LC ₅₀ -96 h	107.97	1.93
Confidence limits (95%)	87.37–130.87	1.56–2.34
<i>R</i> ²	0.67	

**Fig. 2** Percentage of survival (%) of *Lithodes santolla* larvae exposed to a total of seven ammonia concentrations (9, 15, 25, 41, 67, 110, and 182 mg TAN L⁻¹, plus control) until post-larval stage was reached (day 29). Ammonia treatments sharing the same letter for each time of exposure (5, 10, 15, 20, 25 and 30 days) do not differ significantly (*p* > 0.05)

words, a zoea I of the control treatment took, on average, 19.51 days to reach the post-larval stage, whereas a zoea I exposed to 41 mg TAN L⁻¹ took, on average, 23.25 days in doing it (Table 2). The mean molting time (MT₅₀) for every stage and every ammonia treatment is shown in Table 2. For the 50% of the larvae tested in control treatment, molting from zoea I to II took 4.06 days whereas molting from zoea II to III took almost 6 more days and finally, from zoea III to the post-larval stage took 8.39 more days (Table 2). Comparing MT₅₀ values of the different ammonia treatments it was observed that this parameter did not follow a defined trend in ammonia treatments from zoea I to II as well as from zoea II to III. However, the MT₅₀ from zoea III to post-larval stage increased with ammonia concentration; it was 8.39 days in the control and 12.04 days in the highest ammonia concentration where post-larvae were found (41 mg TAN L⁻¹).

Except for the highest ammonia treatments in which no survival zoeae I were registered (110 and 182 mg TAN L⁻¹), all survival larvae had molted from zoea I to II and from zoea II to III. However, molting from zoea III to post-larval stage was successful in the lowest concentrations (control, 9, 15, 25 and 41 mg TAN L⁻¹). The percentage of molting in every molt tended to decrease while ammonia concentration increased (Fig. 3), although significant differences were only found from zoea I to II (*F* = 9.38; *p* = 0.0008). From all zoeae I tested in control treatment, only 40% reached the post-larval stage. This percentage tended to decrease in ammonia concentrations until no zoeae III were registered that could molt to post-larval stage (in 67, 110 and 182 mg TAN L⁻¹ treatments).

Time course for molting is shown in Fig. 4. The molting percentage of each curve was calculated from the proportion obtained between the number of molted larvae in the stage and the number of larvae that had molted from the previous stage. In all ammonia treatments, molting from zoea I to zoea II began the 4th day after hatching (Fig. 4). Nevertheless, in treatments 9, 15 and 41 mg TAN L⁻¹ molting from zoea II to III



Table 2 Mean life time (LT₅₀) and mean molting time (MT₅₀) values for zoea I, II and III of *Lithodes santolla* to the next stages (zoea II, III, and post-larva (PL), respectively), exposed to a total of seven ammonia concentrations (9, 15, 25, 41, 67, 110, and 182 mg TAN L⁻¹, plus control)

Ammonia concentration (mg TAN L ⁻¹)	LT ₅₀ ± SD (days)	MT ₅₀ [95% confidence limits] (days)		
		Zoea I–II	Zoea II–III	Zoea III–PL
Control	19.51 ± 1.38 (12)	4.06 [3.42–4.44] (21)	5.93 [5.42–6.48] (16)	8.39 [7.95–8.75] (12)
9	19.25 ± 0.25 (13)	4.58 [3.99–5.02] (25)	5.41 [4.92–6.01] (19)	8.69 [8.30–9.05] (13)
15	19.75 ± 0.29 (15)	4.21 [3.79–4.48] (26)	5.69 [5.41–5.97] (25)	9.27 [8.81–9.59] (15)
25	19.75 ± 1.06 (6)	4.32 [4.08–4.56] (21)	6.04 [5.20–6.44] (16)	nd (6)
41	23.25 ± 0.35 (3)**	4.09 [2.65–4.89] (15)	5.44 [4.75–6.20] (11)	12.04 [nd–nd] (3)
67	–	4.14 [3.47–4.94] (5)	6.99 [nd–nd] (2)	–
110	–	–	–	–
182	–	–	–	–

Number of live larvae for LT₅₀ and MT₅₀ is indicated between brackets

nd not determined, SD standard deviation, – no results

**Significant differences ($p < 0.05$) between treatments

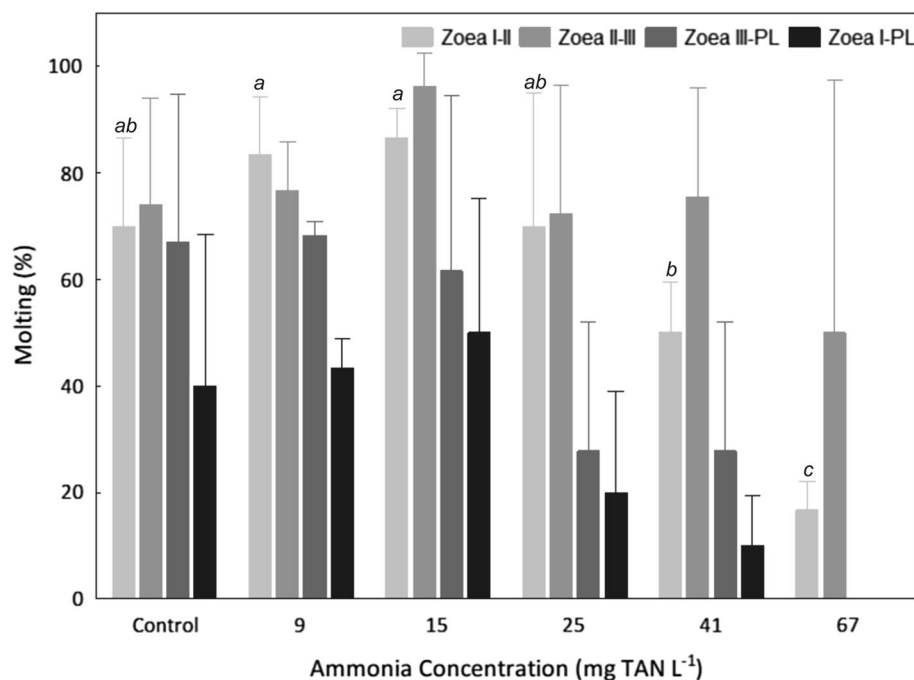


Fig. 3 Molting percentages (%) for zoea I, II and III of *Lithodes santolla* to the respective next stages (zoea II, III, and post-larva (PL), respectively), and for zoea I to post-larval stage, exposed to a total of seven ammonia concentrations (9, 15, 25, 41, 67, 110, and 182 mg TAN L⁻¹, plus control). Only ammonia treatments with molting events are shown. Ammonia treatments sharing the same letter for each molting event (zoea I–II; zoea II–III; zoea III–PL; and zoea I–PL) do not differ significantly ($p > 0.05$)

began between the 6th and the 8th day after hatching, whereas in 67 mg TAN L⁻¹ it was delayed until the 11th day; taking into account that in control treatment it began in the 10th day, as well as in 15 mg TAN L⁻¹. Between the 6th and the 11th day, there was an overlapping between molting of zoea I–II and II–III in different treatments. Finally, molting to post-larval stage came early in 9 and 15 mg TAN L⁻¹ (started on the



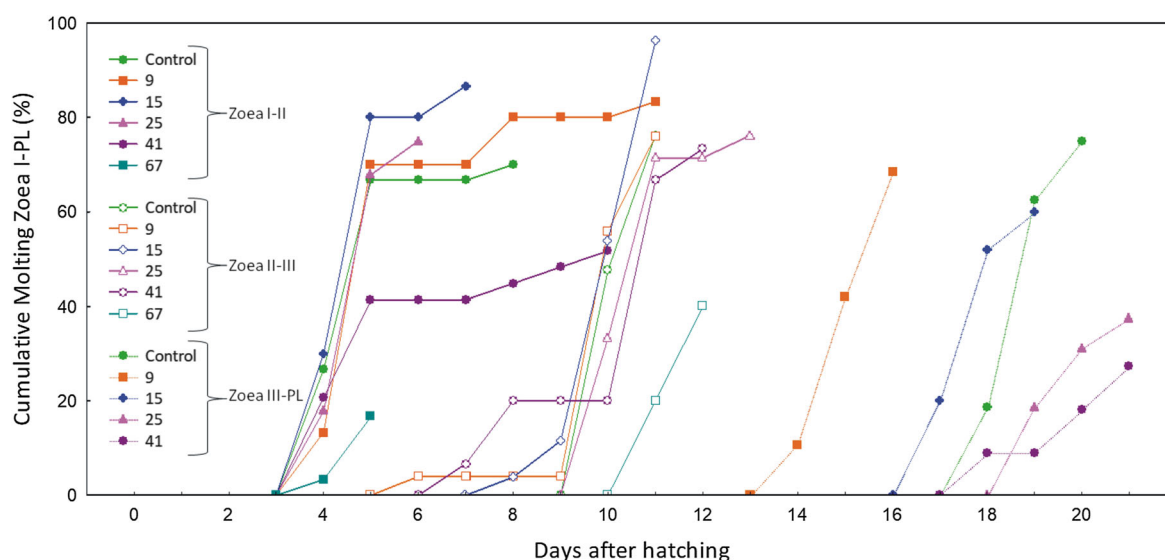


Fig. 4 Cumulative percentage of molting (%) from zoea I, II and III of *Lithodes santolla* to the respective next stages (zoea II, III, and post-larva (PL), respectively), exposed to a total of seven ammonia concentrations (9, 15, 25, 41, 67, 110, and 182 mg TAN L⁻¹, plus control). Only ammonia treatments with molting events are shown

14th and 17th day, respectively), considering that in control, 25 and 41 mg TAN L⁻¹ it occurred since the 18th day after hatching (Fig. 4).

Discussion

This study provides the first evidence about ammonia toxicity on early life stages of *Lithodes santolla*. In comparison to other crustaceans, early stages of this species have a substantially higher tolerance to ammonia (Table 3). In this sense, the LC_{50-96 h} obtained in the present study (107.97 mg TAN L⁻¹ and 1.93 mg NH₃-N L⁻¹) was almost 10 times higher than the value reported for zoeae I of the crab *Portunus pelagicus* (11.16 mg TAN L⁻¹; no data reported in mg NH₃-N L⁻¹) (Liao et al. 2011). Even more, the seventh stage of juvenile of *P. pelagicus*, that was considered highly tolerant with regard to other species (Romano and Zeng

Table 3 The lethal concentration₅₀ for 96 h (LC_{50-96 h}) values of total ammonia (TAN) and un-ionized ammonia (NH₃-N) concentrations for several aquatic crustacean species

Species	Life stage	LC _{50-96 h}		References
		(mg TAN L ⁻¹)	(mg NH ₃ -N L ⁻¹)	
<i>Portunus pelagicus</i>	Zoea I	11.16	–	Liao et al. (2011)
<i>Portunus pelagicus</i>	7th crab	50.65	3.62	Romano and Zeng (2007)
<i>Eriocheir sinensis</i>	Zoea I	5.7 (72 h)	0.20 (72 h)	Zhao et al. (1997)
<i>Eriocheir sinensis</i>	Juvenile	31.6	0.90	Zhao et al. (1997)
<i>Eriocheir sinensis</i>	Juvenile	119.97	1.31	Hong et al. (2007)
<i>Scylla serrata</i>	Zoea I	61.70 (24 h)	4.05 (24 h)	Neil et al. (2005)
<i>Penaeus paulensis</i>	Zoea	9.39	0.73	Ostrensky and Wasielesky (1995)
<i>Macrobrachium amazonicum</i>	Post-larva	21.14	0.67	Dutra et al. (2016)
<i>Lithodes santolla</i>	Zoea I	107.97	1.93	This study



2007), presented a $LC_{50-96\text{ h}}$ of $50.65\text{ mg TAN L}^{-1}$ ($3.62\text{ mg NH}_3\text{-N L}^{-1}$), which is the half of the value found in the present study in terms of TAN L^{-1} . These and other comparisons shown in Table 3 indicate that the first larval stage of *L. santolla* presents high resistance to waterborne ammonia. There are two main factors that could explain that *L. santolla* larvae are more resistant to ammonia exposure: one factor is associated with its significant size compared with other decapods' larvae, and the other factor is that they do not depend on exogenous food sources (Amin et al. 2003). Concerning the first factor, Campodónico (1971) reported that zoeae I of *L. santolla* have a carapace length of $3.75 \pm 0.14\text{ mm}$ (total length of 6 mm; Boschi et al. 1984), while this parameter in the same stage of related commercial species like *Paralomis granulosa* and *Paralithodes camtschaticus* is between 2.75 and 3.25 mm for the former, and $1.39 \pm 0.03\text{ mm}$ for the latter (Epelbaum et al. 2006; McLaughlin et al. 2003). Furthermore, zoeae I of *P. pelagicus*, a more sensitive species to ammonia, presented values between 0.44 and 0.54 mm in carapace length (Josileen and Menon 2004). Regarding the second factor, *L. santolla* larvae are lecithotrophic, which means that they have evolved a non-feeding mode of larval development (Calcagno et al. 2004; Kattner et al. 2003; Oyarzún 1992). In this sense, as they do not have to incorporate food in these first developmental stages, the entry of pollutants as well as nitrogenous compounds to the organism through the digestive tract is avoided (Amin et al. 2003). However, the entry of ammonia through the body surface could be possible owing to many causes: during molting event, water uptake from the environment is necessary as a result of which other compounds could be also absorb; also because gill surface ratio to body weight is bigger, and because the physiological detoxifying mechanisms are still immature (Figueroa Lucero et al. 2012; Rand and Petrocelli 1985). New physiological studies must be done to understand the causes of toxicity in the larvae of this decapod species.

On the other hand, the safe level found in this study ($10.79\text{ mg TAN L}^{-1}$; $0.19\text{ mg NH}_3\text{-N L}^{-1}$) is higher than other values reported previously for other commercial species. For example, the safe level for zoea I of *Eriocheir sinensis* was $0.57\text{ mg TAN L}^{-1}$ ($0.02\text{ mg NH}_3\text{ L}^{-1}$) for 72 h (Zhao et al. 1997); while this value for zoea I of *Portunus pelagicus* was 1.12 for 96 h (Liao et al. 2011). To know the value of this parameter is relevant because it could be applied to general water quality management in intensive larval rearing.

Ammonia toxicity can also presents long-term effects on the exposed organisms, principally in the molting process (Chen and Kou 1992; Koo et al. 2005; Miranda-Filho et al. 2009). In the present study, chronic survival of *L. santolla* larvae diminished with increasing time and ammonia concentration. It is noticeable that from all zoeae I tested in control treatment, only 40% reached the post-larval stage. However, other studies concerning *L. santolla* larval culture carried out with ovigerous females also from the Beagle Channel have reported up to 50% of survival to reach the megalopa stage (Tapella et al. 2009). In this case, massive culture was performed in recirculation water systems with seawater parameters (including pH, salinity, nitrogen wastes, ammonium and temperature) strictly controlled. Moreover, an extreme vulnerability of the first larval stage was observed when newborn zoeae were released in natural waters of the Beagle Channel, as no live larvae were recovered after 55 days (Sotelano et al. 2018).

From the analysis of molting percentages and mean life time (LT_{50}) between ammonia treatments, several conclusions can be stated. On the one hand, it was observed that molting percentage diminished when ammonia concentration was increased. Thus, high levels of ammonia exert a toxic effect on the life cycle of *L. santolla* larvae. Only zoeae exposed to the lowest ammonia concentrations (9, 15, 25 and 41 mg TAN L^{-1}) could reach the post-larval stage. Moreover, they took more days to reach it in the highest ammonia concentrations which they survived (25 and 41 mg TAN L^{-1}). The most critical passage from one molting stage to the next one was from zoea III to post-larval stage since it was notable the reduction in the number of individuals, mainly in 25 mg TAN L^{-1} . On the other hand, differences in the initial time of molting between treatments were also found. Comparing to the control treatment, molting events came early in larvae exposed to the lower ammonia concentrations (9 and 15 mg TAN L^{-1}), whereas it were delayed in those exposed to the higher ammonia concentrations. The overtaking in molting events could be explained as a defense strategy for detoxification (Amin et al. 2003; du Preez et al. 1993), a fact that has been also registered in other crustacean species as *Litopenaeus stylirostris* (Mugnier et al. 2008) and *Penaeus monodon* (Chen and Kou 1993). Similar results have been found when other crabs were exposed to other pollutants. For example, cadmium has inhibited the molt in *Chasmagnathus granulata* (Rodríguez Moreno et al. 2003), as well as lindane and acetone provoked delay and strong inhibition in molt, respectively, in larvae of *Lithodes antarcticus* (Lombardo et al. 1991). Furthermore, Amin et al. (2003) found in *L. santolla* larvae that heavy metals like Cd, Pb and Zn cause significant effects delaying molting events.



Conclusions

The present study provides new and valuable information about the tolerance to ammonia toxicity in a highly commercial crustacean species. The implementation of a restocking program of *L. santolla* larvae (that is provisioning the natural habitats with a massive number of early juvenile stages) would be a feasible initiative in order to improve the population numbers of overfished populations of this crustacean of commercial interest. Unfortunately, until now it has not been possible to establish in a productive way the breeding of king crab larvae. These results are an important supply because knowing the tolerance of newborn zoeae to culture parameters like ammonia concentration will help to achieve a successful aquaculture production in the near future.

Acknowledgements We want to specially thank Lic. Natalia Y. Nohra for her assistance in the laboratory. This study was funded by the Agencia Nacional de Promoción Científica y Tecnológica (PICT 06-1261). This paper is based on work done by S.L. Diodato in partial fulfillment on the requirements for the Doctoral degree at the Universidad Nacional del Sur, Argentina, through a doctoral fellowship from CONICET.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Amin O, Comoglio L (2002) Toxicidad del petróleo diesel en el primer estadio larval de la centolla (*Lithodes santolla*) y del centollón (*Paralomis granulosa*). Rev Biol Mar Oceanogr 37:139–144
- Amin O, Comoglio L (2010) Effects of copper on the physiological responses of the commercial crab *Lithodes santolla* (Decapoda: Anomura) larvae. Sci Mar 74(2):215–221
- Amin O, Comoglio L, Rodríguez E (2003) Toxicity of cadmium, lead, and zinc to larval stages of *Lithodes santolla* (Decapoda, Anomura). Bull Environ Contam Toxicol 71:527–534
- Anger K, Lovrich G, Thatje S, Calcagno J (2004) Larval and early juvenile development of *Lithodes santolla* (Molina, 1782) (Decapoda: Anomura: Lithodidae) reared at different temperatures in the laboratory. J Exp Mar Biol Ecol 306:217–230
- Armstrong D, Chippendale D, Knight A, Colt J (1978) Interaction of ionized and un-ionized ammonia on short-term survival and growth of prawn larvae, *Macrobrachium rosenbergii*. Biol Bull 154:15–31
- Borgmann U, Borgmann A (1997) Control of ammonia toxicity to *Hyalella azteca* by sodium, potassium and pH. Environ Pollut 95(3):325–331
- Boschi E, Bertuche D, Wyngaard J (1984) Estudio biológico pesquero de la centolla (*Lithodes antarcticus*) del Canal Beagle, Tierra del Fuego, Argentina. Contrib. No. 441 INIDEP, Mar del Plata, p 72
- Bower C, Bidwell J (1978) Ionization of ammonia in seawater: effects of temperature, pH, and salinity. J Fish Res Board Can 35(7):1012–1016
- Calcagno J, Anger K, Lovrich G, Thatje S, Kaffenberger A (2004) Larval development of the subantarctic king crabs *Lithodes santolla* and *Paralomis granulosa* reared in the laboratory. Helgol Mar Res 58:11–14
- Camargo J, Alonso A (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ Int 32:831–849
- Campodónico I (1971) Desarrollo larval de la centolla *Lithodes antarctica* Jaquinot en condiciones de laboratorio (Crustacea Decapoda Anomura: Lithodidae). An Inst Patagon 2(1–2):181–190
- Chen J, Kou Y (1992) Effects of ammonia on growth and molting of *Penaeus japonicus* juveniles. Aquaculture 104:249–260
- Chen J, Kou Y (1993) Accumulation of ammonia in the haemolymph of *Penaeus monodon* exposed to ambient ammonia. Aquaculture 109:177–185
- Cobo M, Sonnenholzner S, Wille M, Sorgeloos P (2014) Ammonia tolerance of *Litopenaeus vannamei* (Boone) larvae. Aquac Res 45(3):470–475
- Comoglio L, Vinuesa J (1991) Larval culture under laboratory conditions of southern king crab *Lithodes santolla* and false king crab *Paralomis granulosa*. European Aquaculture Society, Special Publication No. 15, pp 349–351
- du Preez H, Steenkamp V, Schoonbee H (1993) Bioaccumulation of zinc and lead in selected tissues and organs of the freshwater crab, *Potamonautes warreni*. Sci Total Environ 134(1):469–478
- Dutra F, Forneck S, Caramelo Brazão C, Arruda Freire C, Ballester E (2016) Acute toxicity of ammonia to various life stages of the Amazon river prawn, *Macrobrachium amazonicum*, Heller. Aquaculture 1862(453):104–109



- Emerson K, Russo R, Lund R, Thurston R (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J Fish Res Board Can* 32(12):2379–2383
- Epelbaum A, Borisov R, Kovatcheva N (2006) Early development of the red king crab *Paralithodes camtschaticus* from the Barents Sea reared under laboratory conditions: morphology and behaviour. *J Mar Biol Assoc UK* 86:317–333
- Evans D, Cameron J (1986) Gill ammonia transport. *J Exp Zool* 239:17–23
- Figuerola Lucero G, Hernández-Rubio M, Guevara M (2012) Acute toxicity of ammonia on *Macrobrachium tenellum* (Smith) larvae. *Rev Int Contam Ambient* 28:145–150
- Finney D (1971) Probit analysis, 3rd edn. Cambridge University Press, Cambridge, p 333
- Forward R, Tankersley R, Rittschof D (2001) Cues for metamorphosis of brachyuran crabs: an overview. *Am Zool* 41:1108–1122
- Harris R, Coley S, Collins S, Mc Cabe R (2001) Ammonia uptake and its effects on ionoregulation in the freshwater crayfish *Pacifastacus leniusculus* (Dana). *J Comp Physiol B* 171:681–693
- Hartnoll R (2001) Growth in Crustacea—twenty years on. *Hydrobiologia* 449:111–122
- Henry R, Lucu C, Onken H, Weihrach D (2012) Multiple functions of the crustacean gill: osmotic/ionic regulation, acid-base balance, ammonia excretion, and bioaccumulation of toxic metals. *Front Physiol* 3(431):1–33
- Hong M, Chen L, Sun X, Gu S, Zhang L, Chen Y (2007) Metabolic and immune responses in Chinese mitten-handed crab (*Eriocheir sinensis*) juveniles exposed to elevated ambient ammonia. *Comp Biochem Phys C* 145:363–369
- Hutchinson T, Solbé J, Kloepper-Sams P (1998) Analysis of the ecetoc aquatic toxicity (EAT) database III—comparative toxicity of chemical substances to different life stages of aquatic organisms. *Chemosphere* 36(1):129–142
- Ip Y, Chew S, Randall D (2001) Ammonia toxicity, tolerance, and excretion. In: Wright P, Anderson P (eds) *Fish physiology* 20: Nitrogen excretion. Academic, New York, pp 109–148
- Josileen J, Menon N (2004) Larval stages of the blue swimmer crab, *Portunus pelagicus* (Linnaeus, 1758) (Decapoda, Brachyura). *Crustaceana* 77(7):785–803
- Kattner G, Graeve M, Calcagno J (2003) Lipid, fatty acid and protein utilization during lecithotrophic larval development of *Lithodes santolla* (Molina) and *Paralomis granulosa* (Jacquinot). *J Exp Mar Biol Ecol* 292:61–74
- Koo J, Kim S, Jee J, Kim J, Bai S, Kang J (2005) Effects of ammonia and nitrite on survival, growth and moulting in juvenile tiger crab, *Orithyia sinica* (Linnaeus). *Aquac Res* 36:79–85
- Liao Y, Wang H, Lin Z (2011) Effect of ammonia and nitrite on vigour, survival rate, moulting rate of the blue swimming crab *Portunus pelagicus* zoea. *Aquac Int* 19(2):339–350
- Lin H, Thuet P, Mounet-Guillaume R, Charmantier G (1993) Effects of ammonia on survival and osmoregulation of juvenile and subadult *Penaeus stylirostris*. *Aquaculture* 209:307–317
- Lombardo R, Ferrari L, Vinuesa J (1991) Effects of lindane and acetone on the development of larvae of the southern king crab (*Lithodes antarcticus* Jaquinot). *Bull Environ Contam Toxicol* 46:185–192
- Lovrich G (1997) La pesquería mixta de las centollas *Lithodes santolla* y *Paralomis granulosa* (Anomura: Lithodidae) en Tierra del Fuego, Argentina. *Invest Mar* 25:41–57
- Marazza D, Bornens P, Le Gal Y (1996) Effect of ammonia on survival and adenylate energy charge in the shrimp *Palaemonetes varians*. *Ecotoxicol Environ Safe* 34(2):103–108
- McLaughlin P, Anger K, Kaffenberger A, Lovrich G (2003) Larval and early juvenile development in *Paralomis granulosa* (Jacquinot) (Decapoda: Anomura: Paguroidea: Lithodidae), with emphasis on abdominal changes in megalopal and crab stages. *J Nat Hist* 37:1433–1452
- Miranda-Filho K, Lopes Leães Pinho G, Wasielesky W Jr, Bianchini A (2009) Long-term ammonia toxicity to the pink-shrimp *Farfantepenaeus paulensis*. *Comp Biochem Phys C* 150:377–382
- Moore D, Bridges T, Gray B, Duke M (1997) Risk of ammonia toxicity during sediment bioassays with the estuarine amphipod *Leptocheirus plumulosus*. *Environ Toxicol Chem* 16(5):1020–1027
- Mugnier C, Zipper E, Goarant C, Lemonnier H (2008) Combined effect of exposure to ammonia and hypoxia on the blue shrimp *Litopenaeus stylirostris* survival and physiological response in relation to molt stage. *Aquaculture* 274:398–407
- Neil L, Fotedar R, Shelley C (2005) Effects of acute and chronic toxicity of unionized ammonia on mud crab, *Scylla serrata* (Forsskal, 1755) larvae. *Aquac Res* 36:927–932
- Ostrensky A, Wasielesky W Jr (1995) Acute toxicity of ammonia to various life stages of the Sao Paulo shrimp, *Penaeus paulensis* Pérez-Farfante, 1967. *Aquaculture* 132:339–347
- Oyarzún S (1992) Cultivo de centolla (*Lithodes antarcticus*) con fines de repoblación. I. Metamorfosis de postlarvas a juvenil y crianza de larvas y postlarvas a escala intermedia. *Inf Inst Pat, Punta Arenas, Chile*, p 38
- Racotta I, Hernández-Herrera R (2000) Metabolic responses of the white shrimp, *Penaeus vannamei*, to ambient ammonia. *Comp Biochem Physiol A* 125:437–443
- Rand G, Petrocelli S (1985) *Fundamentals of aquatic toxicology*. Hemisphere Publ., Washington, D.C.
- Randall D, Tsui T (2002) Ammonia toxicity in fish. *Mar Pollut Bull* 45(1–12):17–23
- Rodríguez E, Amin O (1991) Acute toxicity of parathion and 2, 4-D to larval and juvenile stages of *Chasmagnathus granulata* (Decapoda, Brachyura). *Bull Environ Contam Toxicol* 47:634–640
- Rodríguez Moreno P, Medesani D, Rodríguez E (2003) Inhibition of molting by cadmium in the crab *Chasmagnathus granulata* (Decapoda Brachyura). *Aquat Toxicol* 64(2):155–164
- Romano N, Zeng C (2007) Ontogenetic changes in tolerance to acute ammonia exposure and associated histological gill alterations during early juvenile development of the blue swimmer crab, *Portunus pelagicus*. *Aquaculture* 266:246–254
- Romano N, Zeng C (2010) Survival, osmoregulation and ammonia-N excretion of blue swimmer crab, *Portunus pelagicus*, juveniles exposed to different ammonia-N and salinity combinations. *Comp Biochem Phys C* 151(2):222–228
- Romano N, Zeng C (2012) Osmoregulation in decapod crustaceans: implications to aquaculture productivity, methods for potential improvement and interactions with elevated ammonia exposure. *Aquaculture* 334–337:12–23
- Sokal R, Rohlf R (1981) *Biometry*. W.H. Freeman and Company, New York, p 859



- Sotelano M, Lovrich G, Tapella F (2016) Cannibalism among *Lithodes santolla* (Molina 1782) juveniles: effect of stocking density, stage and molt condition. *Aquac Int* 24(4):1025–1034
- Sotelano M, Lovrich G, Di Salvatore P, Florentín O, Giamportone A, Tapella F (2018) Suspended mesh-bags enclosures for Southern King Crab *Lithodes santolla* (Molina 1782) larvae and juvenile culture in the sea. *Aquaculture* 495:575–581
- Spotte S, Adams G (1983) Estimation of the allowable upper limit of ammonia in saline waters. *Mar Ecol Prog Ser* 10:207–210
- Sprague J (1971) Measurement of pollutant toxicity to fish-III: sublethal effects and “safe” concentrations. *Water Res* 5:245–266
- Tapella F, Sotelano M, Romero M, Lovrich G (2009). Survival and condition of southern king crab *Lithodes santolla* larvae in cultivation: tools for stock enhancement. In: International symposium on aquaculture, biology and management of commercially important crabs (ISABMC). Shanghai Ocean University, Shanghai
- Tapella F, Sotelano M, Romero M, Lovrich G (2012) Experimental natural substrate preference of southern king crab *Lithodes santolla* larvae. *J Exp Mar Biol Ecol* 411:70–77
- Thurston R, Russo R, Vinogradov G (1981) Ammonia toxicity to fish. Effect of the pH on the toxicity of the un-ionized ammonia species. *Environ Sci Technol* 15:837–840
- USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th edn. EPA-821-R-02-012, p 266
- USEPA (2009) Draft 2009 update aquatic life ambient water quality criteria for ammonia–freshwater. EPA-822-D-09-001, p 184
- Vinuesa J (1991) Biología y pesquería de la centolla (*Lithodes santolla*). *Atlántica* 13:233–244
- Vinuesa J, Lovrich G (1992) Biología y pesca de la centolla en el Canal Beagle, Tierra del Fuego, Argentina. Recomendaciones para su manejo. *Contr Cient* No 15, CADIC, Ushuaia, p 27
- Wajsbrodt N, Gasith A, Krom M, Somocha T (1990) Effect of dissolved and the molt stage on the acute toxicity of ammonia to juvenile green tiger prawn *Penaeus semisulcatus*. *Environ Toxicol Chem* 9:497–504
- Xu H, Song W, Warren A (2004) An investigation of the tolerance to ammonia of the marine ciliate *Euplotes vannus* (Protozoa, Ciliophora). *Hydrobiologia* 519:189–195
- Zhao J, Lam T, Guo Y (1997) Acute toxicity of ammonia to the early stage-larvae and juveniles of *Eriocheir sinensis* H. Milne-Edwards, 1853 (Decapoda: Grapsidae) reared in the laboratory. *Aquac Res* 28:517–525

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.