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Ammonia tolerance of *Corbicula japonica* (brackish water clam) at various growth stages in rearing water

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Abstract: *Corbicula japonica*, a brackish water bivalve, is an important inland fishery resource in Japan. However, in recent years, the commercial catch of *C. japonica* in Japan has declined to approximately one-fifth of its peak. The reduction in catch is believed to be caused by basin-derived substances, such as ammonia, as well as habitat degradation, including the deterioration of water quality and sediment composition. The purpose of the present study was to clarify the mechanism of ammonia toxicity and ammonia tolerance according to the growth stages of the brackish water clam, *C. japonica*. The ammonia median lethal concentration (LC50) value for *C. japonica* larvae was the lowest during the growth stages. The LC50 values for ammonia decreased as shell length increased at 0.5 mm, 3 mm, and 25 mm. The histological appearance of ctenidium in *C. japonica* changed with an increase in ammonia concentration. According to the results of the present study, it is considered that the ctenidium's destructive effect was the underlying mechanism of ammonia toxicity in *C. japonica*.

Key words: *Corbicula japonica*; Ammonia tolerance; Histological appearance of ctenidium

Corbicula japonica, the brackish water clam, inhabits a wide geographic area from Hokkaido to Kyushu and is an important inland fishery resource in Japan. However, in recent years, the commercial catch of *C. japonica* in Japan has declined to approximately one-fifth of its peak (Ministry of Agriculture, Forestry and Fisheries 2020). This reduction in *C. japonica* catch is believed to be caused by basin-derived substances, such as pollutants, as well as habitat degradation, including deterioration of water quality and sediment composition (Nakamura 2000). Extensive research has been conducted on the suitable environments for *C. japonica* (Nakamura et al. 1996a, 1996b, 1997a, 1997b; Nakamura 2000; Sugahara et al. 2017). In addition, the effects of basin-derived substances on brackish water organisms have been reported, such as neonicotinoide-based agrochemicals on zooplankton (Yamamuro et al. 2019), herbicides dioxins in paddy fields (Masunaga et al. 2001) and organophosphorus-based agrochemicals on zooplankton (Kashiwada et al. 1995, 1998). The concentration of ammonia with effects on the larvae of *C. japonica* have been reported to be 0.43 mg/*l* for a decrease in feeding activity and 2.15 mg/*l* for mortality (Aomori Prefectural Industrial Research Center 2019). Matsuda and Sonoda (2021a, 2021b) reported the tolerance of nitrite, nitrate, and heavy metal of *C. japonica*. However, the effects of ammonia of juvenile and adult *C. japonica* clams remain unclear.

Lake Abashiri, the coastal lagoons on the Sea of Okhotsk in Hokkaido, is Hokkaido's largest fishing ground for *C. japonica*. The Lake Abashiri basin is approximately 80% forest and approximately 19% farmland

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(Ministry of Land, Infrastructure, Transport and Tourism 2006). The maximum ammonia concentrations at difference water depths at the center of Lake Abashiri from 1994 to 2017 were 1.10 mg/*l*, 1.2 mg/*l*, 3.10 mg/*l*, and 5.37 mg/*l* at depths of 1, 2, 3, and 4 m, respectively, and the mean concentrations were 0.07 mg/*l*, 0.08 mg/*l*, 0.10 mg/*l*, and 0.15 mg/*l*, respectively (Ministry of Land, Infrastructure, Transport and Tourism 2020). The ammonia concentration at the center of Lake Abashiri was the highest at a depth of 4 m. Lake Furen is located in Nemuro, Hokkaido, Japan. Basin-derived substances have had an impact on the *C. japonica* population in Lake Furen (Mikami et al. 2008; Mikami and Igarashi 2014; Tsuji and Montani 2016; Nagasaka 2017). From 1971 to 1988, the average commercial catch of *C. japonica* in the lake was approximately more than 100 t/year (Mikami and Igarashi 2014). However, the catch of *C. japonica* declined after 1985, and the fishery was closed in 2000. On the other hand, dairy farming has developed in the Lake Furen basin, and it is speculated that the decrease in the *C. japonica* population in Lake Furen is related to nitrogen compound discharge from the surrounding area (Mikami et al. 2008; Mikami and Igarashi 2014; Tsuji and Montani 2016; Nagasaka 2017). Notably, in September 1999, the highest and averaged ammonia concentrations in the Nokoribetu River flowing into Lake Furen were 5.01 mg/*l* and 0.10 ± 0.45 mg/*l* (Mikami et al. 2008). The ammonia concentrations in the rivers flowing into Lake Furen was showed high value. The concentrations of ammonia in the lakes could affect *C. japonica*.

Ammonia is a nitrogen compound that exists in water as both unionized (NH_3) and ionized (NH_4^+) forms. NH_3 is extremely toxic to aquatic organisms (Nishimura 2007), and several studies have explored its effects (Hickey and Vickers 1994; Boardman et al. 2004; Phillips et al. 2005; Cong et al. 2017; Zhang et al. 2023). Water temperature, salinity, and pH all influence NH_3 concentration in total ammonia nitrogen (TAN). The amount of NH_3 in seawater is generally lower than that in freshwater, however, it can increase significantly under high temperature and alkaline conditions (Franklin and Edward 2019; Japan Fisheries Resource Conservation Association 2022). The concentration of NH_3 in brackish water where *C. japonica* inhabits may be higher than that in seawater.

The ammonia toxicity mechanism in *Ruditapes philippinarum* has been examined (Cong et al. 2017, 2019). Ammonia promotes lysosome membrane destabilization, gill cells apoptosis, and muscle element decomposition in the gill tissue (Cong et al. 2017). Furthermore, it alters branched chain amino acids, neurotransmitters, and causes metabolic disturbances in the gills (Cong et al. 2017). Cong et al. (2019) reported that ammonia modifies the structure and function of mitochondria, inhibits of Ca^{2+}-ATPase activity, and alters H^+/K^+-ATPase activity significantly. However, the effect of ammonia on *C. japonica* gills remains unclear.

Ammonia toxicity is considered to vary based on ammonia uptake because of effect the adverse effects of ammonia are largely manifested via gills. Nakamura et al. (1988) reported that the filtered water content of *C. japonica* with shell heights of 15.4, 19.1, and 21.1 mm was not different. However, filtered water content could differ between juvenile and adult clam; juvenile is defined as individual before maturity in Lake Abashiri, and its size is under 10 mm (Maru 1981). Therefore, ammonia tolerance between juvenile and adult clam could differ. However, the amounts of water filtered by juveniles of *C. japonica* are unclear. The purpose of the present study was to clarify the mechanism of ammonia toxicity and ammonia tolerance according to the growth stages of the brackish water clam, *Corbicula japonica*. The present study carried out ammonia tolerance experiments at difference growth stages, ctenidium histological appearance, and measured of filtered water amount.

Materials and Methods

Testing individuals of C. japonica and seed production

C. japonica was tested for ammonia tolerance

at various growth stages (larva, shell lengths of 0.5, 3, and 25 mm). In October 2021, *C. japonica* with a shell length of 25 mm (mean ± standard deviation: 25.0 ± 2.3 mm) were collected from Lake Abashiri. They were cultured for 2 weeks in water with a water temperature of approximately 18℃ and a salinity of 5 psu. Afterward, the water temperature was increased by 1℃ every 5 days until it became to 22℃. This was performed to prevent the death by the sudden increase of water temperature. *Chaetoceros gracilis* (YANMAR, 1.0×10^8 cells/m*l*) feed (100 m*l*) was given to approximately 250 individuals once every 2 or 3 days. Seed production was carried out using adult *C. japonica* collected in Lake Abashiri from July to September 2018–2020. *C. japonica* larvae were cultured until their shell lengths reached 0.5 mm (approximately 1 to 2 months) and 3 mm (approximately 1 year) on the Abashiri Fisheries Science Center. Feeding of *C. japonica* larvae was fed concentrate *Chaetoceros* (2500 cells/m*l*) once a day. Feeding after settlement of *C. japonica* larvae was fed concentrate *Chaetoceros* or the sufficiently cultured *Nannochloropsis oculate* once every 1 or 2 days. Feeding amount was decided based on the amount of feed remaining in a tank.

Preparation of the experimental water and water quality analysis

The experimental water of larva was prepared using a circular water tank (styrol; diameter: 29.5 cm; height: 15 cm (Fig. 1(a)). The experimental water of 0.5 and 3 mm

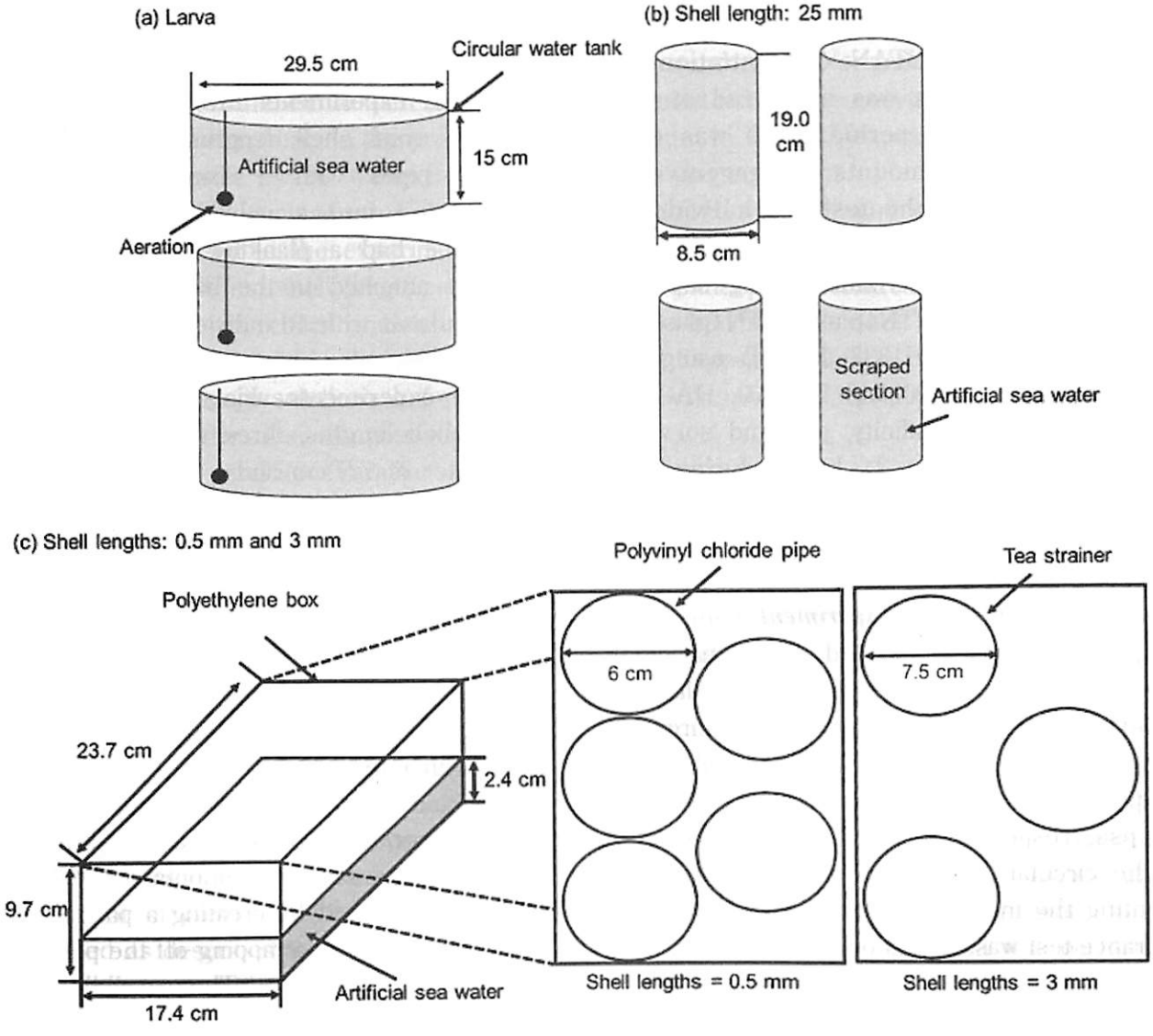

Fig. 1. Experiment model. (a) Larva. (b) 25 mm shell length. (c) 0.5 and 3 mm shell length.

shell lengths was prepared in a polyethylene container (length: 23.7 cm; width: 17.4 cm; height: 9.7 cm) (Fig. 1(c)). The experimental water of larva, 0.5 and 3 mm shell lengths were prepared using a mixture of ammonium chloride (NH_4Cl, KANTO CHEMICAL Co., Inc.), artificial seawater (SEAwater, GEX) and sodium hypochlorite in tap water was removed by aeration over one day. The experimental water of 25 mm shell length was prepared using a mixture of an ammonia standard solution to either 100 mg/*l* or 1000 mg/*l* and artificial seawater (MARINE ART SF-1, Tomita). The ammonia standard solution was prepared using ammonium chloride (Ammonium Chloride, FUJIFILM Wako Pure Chemical Corporation).

TAN concentration was determined during the experimental period as follows. TAN concentration of larva, 3 and 25 mm shell lengths was measured at the start and end of the toxicity period. TAN concentration of 0.5 mm shell length was measured at the end of the toxicity period. This was due to the equivalent amounts of reagent and water employed in the tests of individuals with shell lengths of 3 mm. The TAN concentration was determined using salicylic acid ammonia reagent (TNTplus831, TNTplus832, HACH1336, and HACH1337, HACH) using an absorptiometer (DR900 and DR3900, HACH). Water temperature, salinity, pH, and survival were measured every 24 hours during the experiment. The pH was adjusted to 8.00 ± 0.05 using hydrochloric acid and sodium hydroxide.

Ammonia tolerance larva experiment

The larvae were tested for ammonia tolerance after 96 hours, and 27500 larvae (D-shaped larva) were placed in a circular water tank (Fig. 1 (a)). The experimental water temperature and salinity were set at 20℃ and 5.5 psu, respectively. The number of larvae in the circular water tank was calculated by counting the individuals in a 10 m*l* sample. A tolerance test was carried out while feeding the individuals with *Nannochloropsis oculata* (5000 ~ 10000 cells/m*l*) under moderate aeration. The purpose of the condition was to exclude factors other than ammonia that could cause larval mortality. Tolerance tests were performed with TAN concentrations of 0, 5, 10, and 20 mg/*l*. Each concentration was tested in triplicate.

Ammonia tolerance experiment for individuals with shell lengths 0.5, 3, and 25 mm

Ammonia tolerance tests were conducted with shell lengths of 0.5, 3 and 25 mm at a water temperature of 22℃, salinity of 5 psu, and photoperiod of 12 L:12 D., without sediment referring previous studies (e.g., Nakamura et al. 1996a). The test lasted 144 hours, consisting of a 96-hour toxicity period and a 48-hour recovery period. The reason for application of a recovery period was to confirm death after exposure to ammonia. During the toxicity period, the exposure involved ammonia exposure, stagnant water conditions, and no feeding. However, the recovery period included aeration, feeding, and no exposure to ammonia.

For the experiments involving individuals with 0.5 mm shell lengths, five polyvinyl chloride pipes with a diameter of 6 cm were placed into a polyethylene container. Each pipe had a plankton net (mesh size: 530 μm) attached to the bottom. Each pipe was populated with 10 individuals, resulting in a total of 50 individuals employed in the test. For the experiments using individuals with 3 mm shell lengths, three tea strainers with a diameter of 7.5 cm and a height of 6.3 cm were placed into a polyethylene container. Five individuals were placed in each tea strainer, yielding a total of 15 individuals. For the 25 mm shell length, the experiments were performed using a reagent bottle with three individuals, for a total of 12 individuals. *C. japonica* exhibit an escape behavior by closing the shell when there is change in water quality (e.g., salinity) (Ishida and Ishii 1971). To counteract this escape behavior effect above, and to soak the soft part of the clam in ammonia solution, the test was performed by creating a partial hole of three individuals, scrapping off the posterior shell margin by a rasp. The possibility that damages to the mantle or leakage of pallial cavity due to scraping may affect survival

rates was examined based on mortality rates of scraped individuals in the control. The TAN concentration applied of individuals in each shell length class were as follows: 0, 20, 30, 40, 60, 80, 100, 150, 200 and 300 mg/*l* for 0.5 mm, and 0, 10, 20, 30, 40, 80, 100, 120, 160, 200 and 400 mg/*l* for 3 mm, and 0, 20, 40, 60, 80, 100, 200, 300 and 400 mg/*l* for 25 mm. The concentrations were set based on previous studies (e.g., Mummert et al. 2003; Boardman et al. 2004). *C. japonica* with shell lengths of 3 and 25 mm were considered dead if their valves were opened and they did not move when their foot was touched. *C. japonica* with a shell length of 0.5 mm that did not move for 10 minutes were considered deceased and removed immediately from the experimental water.

Ctenidium histological appearance assessment under ammonia toxicity

The histological appearance of ctenidium in *C. japonica* was examined to determine the effect of ammonia exposure. In the preliminary experiment, ctenidia were collected from deceased 4 to 6 individuals at a toxicity period. The individuals used in the experiment were approximately 22.8 mm shell length, and were exposed to ammonia concentrations of 200, 300 and 400 mg/*l*. Ctenidium was dehydrated in a series of ethanol solutions before being replaced with xylene. The samples were then soaked in soft paraffin (Paraffin (48–50), Hayashi Pure Chemical Ind., Ltd.): xylene (1:1), soft paraffin I, soft paraffin II, hard paraffin (Tissue-Tec® Paraffin wax II, Sakura Finetec Japan Co., Ltd.) I, hard paraffin II. Finally, the sections were embedded in hard paraffin. The embedded ctenidium were sectioned to a continuous 6–8 μm thickness, using a rotation-type microtome. Double staining was performed using Mayer's hematoxylin and eosin. Ctenidium histological appearance in *C. japonica* was observed under a microscope. Assessment of ctenidium of *C. japonica* was performed as described by Cong et al. (2017) and Yamamoto et al. (2017).

Measurement of filtered water content in C. japonica

Filtered water amount in *C. japonica* was measured using phytoplankton. This was done to clarify difference in ammonia uptake quantity according to the growth stages. The water temperature was maintained at 22℃, salinity was set at 5 psu, and the lighting conditions were controlled to be dark. The experimental water was prepared by combining 100 m*l* of *Nannochloropsis oculata* (approximately 1.0×10^7 cells/m*l*) and 900 m*l* of artificial seawater (salinity 5 psu) in a hard PVC bottle (diameter: 10.0 cm; height: 16.0 cm). The test was repeated every 2 hours, and the experimental water sampled every half-hour. Measurement of *C. japonica* filtered water content was conducted for the control (no individual), and the 0.5 mm ($n = 10$), 3 mm ($n = 5$) and 22 mm ($n = 3$) shell length groups. Three bottles were used for each experimental group. The cell number of *Nannochloropsis oculata* were counted using a hemocytometer. Filtered water amount in *C. japonica* was calculated using the following equation:

$$\text{Filtered water amount} = w \times \ln(i/f)/t/N \quad (1)$$

w = Water amount.
i = Initial phytoplankton count.
f = Final phytoplankton count.
t = Experimental time.
N = Number of individuals of each bottle.

Statistical analysis

Difference in the time of death between individuals with scraped-off shells and those with normal 25 mm shell lengths was performed using the Mann–Whitney U test. The lethal concentration for 50% (LC50 value) of *C. japonica* was calculated using the ammonia tolerance test. TAN and NH_3 LC50 values were also calculated. Water temperature, salinity, and pH were used to determine NH_3 concentration (Japan Fisheries Resource Conservation Association 2022). LC50 values were computed using R (version 4.0.2). Logarithmic logistic regression was performed using the drc package. Regression significance was confirmed using the χ^2 goodness-of-fit test (Ritz and Streibig 2005; Ritz et al. 2015).

Results

Larvae ammonia tolerance experiments

The temperature, salinity, and pH of the water during the larval experimental period were recorded as 19.6 ± 0.63℃, 5.60 ± 0.07 psu, and 8.28 ± 0.13, respectively. The control group had a larval survival rate of 51.6 ± 7.6%. The larva survival rates in the 5, 10 and 20 mg/*l* experiments were 37.0 ± 4.3%, 24.7 ± 10.3%, and 8.0 ± 1.2%, respectively. The larval survival rate decreased as the level of ammonia exposure increased. The LC50 value for larval NH_3 was 0.31 mg/*l* (95% confidence interval:0.31–0.32) (Fig. 2(a)). The LC50 value for larval TAN was 3.51 mg/*l* (95% confidence interval:3.46–3.56) (Fig. 2 (b)).

Ammonia tolerance experiment for 0.5, 3 and 25 mm shell lengths

The water quality for the *C. japonica* experiment, covering a range of shell lengths from 0.5 to 25 mm, was characterized by a water temperature of 22.1 ± 0.5℃ and salinity of 5.1 ± 0.2 psu. The TAN concentrations at which deceased individuals appeared were 20, 80 and 20 mg/*l* for shell lengths of 0.5 mm, 3 mm, and 25 mm, respectively. The time of death between individuals with scraped-off shells and those with normal shells of shell length 25 mm showed similar extent (Fig. 3 (a) and (b); $p > 0.05$; Mann–Whitney U test). In addition, the mortality rate of scraped individuals in the control was zero. The LC50 values for NH_3 in *C. japonica* at each growth stage were 18.62 mg/*l* (95% confidence interval: 8.07–29.16), 5.65 mg/*l* (95% confidence interval: 4.85–6.45), and 2.85 mg/*l* (95% confidence interval: 2.42–3.29) for the 0.5, 3 and 25 mm shell length groups, respectively (Fig. 2 (a)). The LC50 values for TAN in *C. japonica* at each growth stage were 415.9 mg/*l* (95% confidence interval: 180.3–651.5), 126.2 mg/*l* (the 95% confidence interval: 108.4–144.0), and 63.8 mg/*l* (95% confidence interval: 54.1–73.5) for the 0.5, 3 and 25 mm shell length groups, respectively (Fig. 2 (b)). The LC50 values decreased as shell length

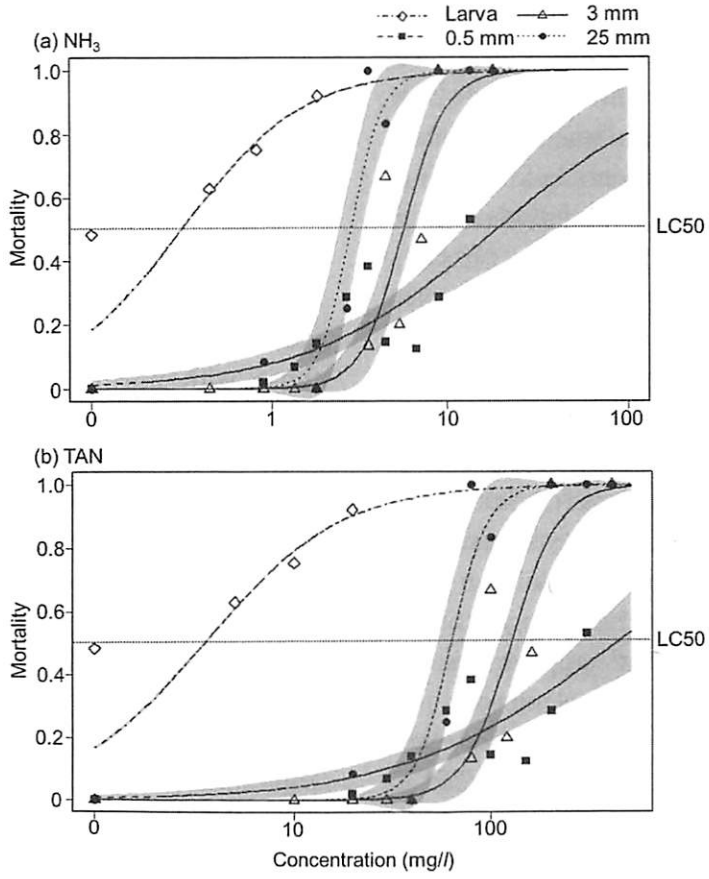

Fig. 2. Ammonia tolerance of *Corbicula japonica* at each life stages. (a) NH_3 (b) TAN.

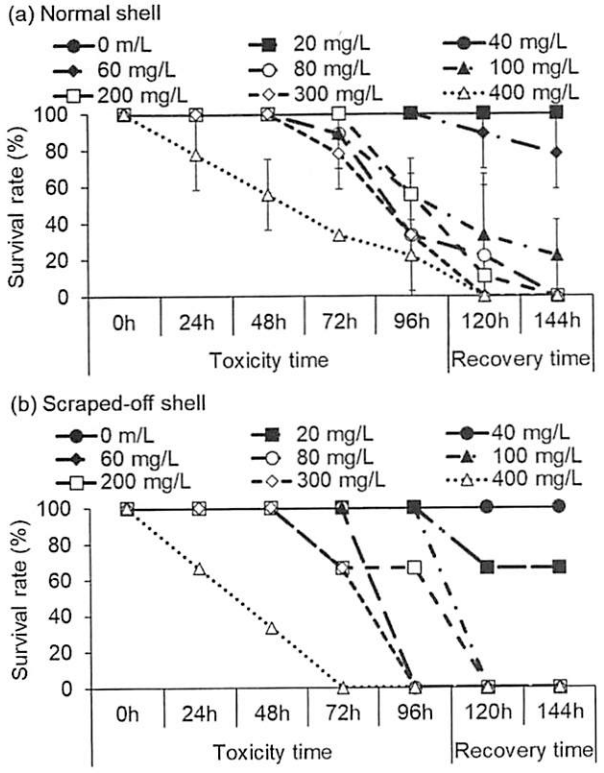

Fig. 3. Comparison of survival rate of individuals with normal shells and individuals with shells with partial holes. (a) Normal shell (b) Scraped-off shell.

increased (Pearson's correlation coefficient, $r = -0.71$).

Ctenidium histological appearance assessment

Ctenidium histological appearance in *C. japonica* changed as ammonia concentration increased (Fig. 4). *C. japonica* ordinary filament was extended at 200 mg/*l* (Fig. 4 (b)). It was unclear at 300 and 400 mg/*l* (Fig. 4 (c and d)). However, branchial cavity was expanded at 200 and 300 mg/*l*. At 300 mg/*l*, the interfilamentar junction of the form of intra-plical band of *C. japonica* was damaged (Fig. 4 (c)). After being exposed to 400 mg/*l*, the ctenidium collapsed (Fig. 4 (d)).

C. japonica filtered water content

Under light conditions, *C. japonica* individuals with 0.5, 3 and 22 mm shell lengths filtered 0.003 *l*/h, 0.010 *l*/h, and 0.073 *l*/h (Fig. 5). Under dark conditions, *C. japonica* individuals with 0.5, 3 and 22 mm shell lengths filtered 0.005 *l*/h,

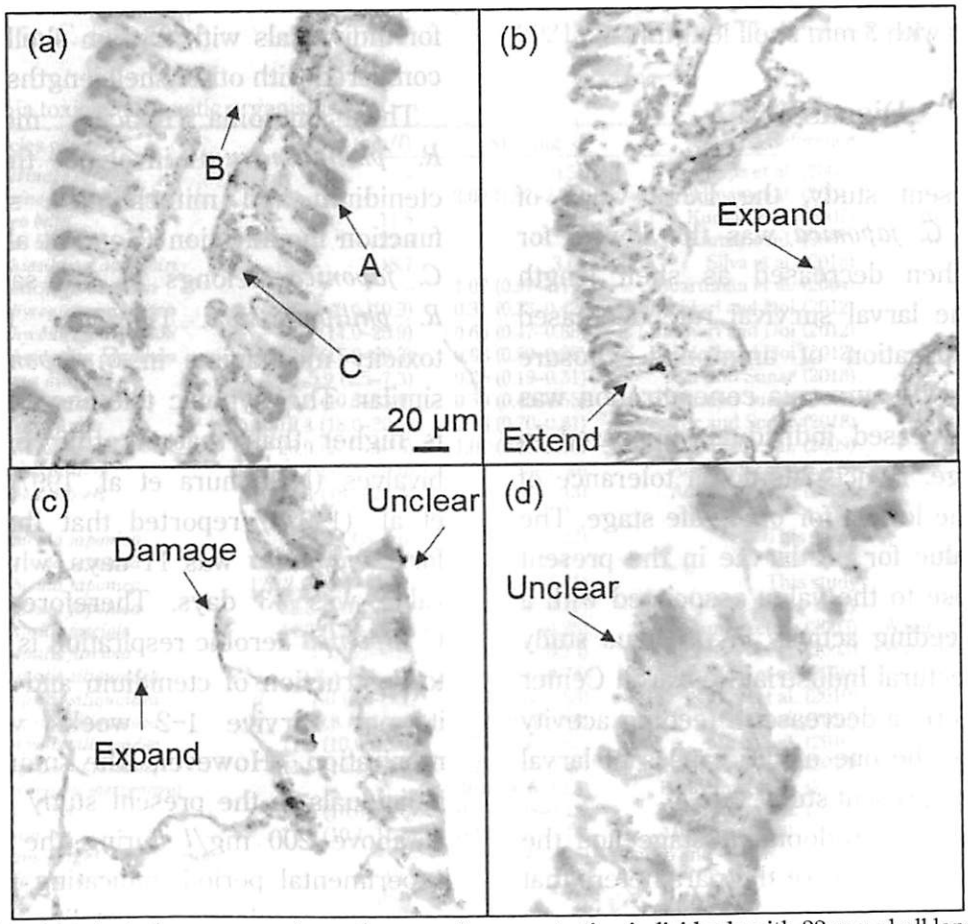

Fig. 4. Ctenidium histological appearance for each ammonia concentration individuals with 22 mm shell length. (a) 0 mg/*l*, (b) 200 mg/*l*, (c) 300 mg/*l*, (d) 400 mg/*l*. A–C indicates gill tissue structures; A: Ordinary filament, B: Branchial cavity and C: Interfilamentar junction of the form of intra-plical band. Scalebars indicate 20 μm in all pictures.

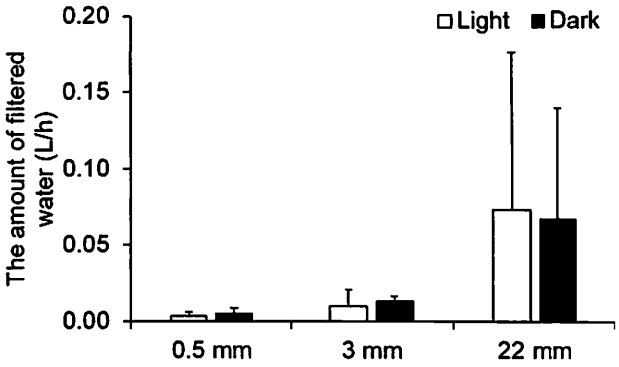

Fig. 5. The amount of water filtered by *Corbicula japonica* individuals at each life stage.

0.013 *l*/h, and 0.067 *l*/h (Fig. 5). A comparison of filtered water volume between the dark and light conditions with 0.5 and 3 mm shell lengths revealed slightly higher volume under dark conditions, however, no statistically significant differences were observed among the shell length groups. The filtered water amount for individuals with 3 mm shell length was approximately thrice that for individuals with 0.5 mm shell length, and the filtered water amount for individuals with 22 mm shell length was five-fold to seven-fold that for individuals with 3 mm shell length.

Discussion

In the present study, the LC50 value of ammonia for *C. japonica* was the lowest for larvae and then decreased as shell length increased. The larval survival rate decreased as the concentration of ammonia exposure increased, and its ammonia concentration was lower than deceased individuals appeared in other life stage. Hence, ammonia tolerance of the larva is the lowest for other life stage. The TAN LC50 value for the larvae in the present study was close to the value associated with a decrease in feeding activity in previous study (Aomori Prefectural Industrial Research Center 2019). Therefore, a decrease in feeding activity was thought to be one of the causes of larval mortality in the present study.

Gill (ctenidium) development stage and the filtered water amount were the parameters that contributed to change in ammonia tolerance for shell lengths of 0.5, 3, and 25 mm in the present study. After the juvenile stage, bivalve ctenidia exhibit rapid development (Amio et al. 1999). Unlike aquatic mollusks without gills, bivalves exchange gas through their gills (Nishiwaki 1999). Based on these findings, it is possible that the 0.5 mm shell length individuals used in the present study during the early stages had not developed gills fully and utilized both body surface and infant gills for respiration. Hence, individuals of 0.5 mm in shell length exhibited high ammonia tolerance because they exposed to smaller amount of ammonia. Filtering rate of an adult clam *C. japonica* was reported to be 0.097 *l*/h by Suemitsu et al. (2001). This value is a little higher than that of adult clam in the present study. This may be derived from the difference of water temperature and/or experimental method. The amount of filtered water in present study rose as shell length increased. This is presumably related to the development stage of the gill with its growth. The difference of the amount of filtered water suggests that ammonia uptake increases as it grows. This may have resulted in lower LC50 for individuals with 25 mm shell length when compared with other shell lengths.

The ammonia toxicity mechanism in *R. philippinarum* involved the effect on ctenidium and mitochondria structure and function modification (Cong et al. 2017, 2019). *C. japonica* belongs to the same order as *R. philippinarum*. Therefore, the ammonia toxicity mechanism in *C. japonica* could be similar. The hypoxic tolerance of *C. japonica* is higher than that of other brackish water bivalves (Nakamura et al. 1997c). Nakamura et al. (1997a) reported that the LT50 value for *C. japonica* was 11 days, while the LT100 value was 13 days. Therefore, even if the *C. japonica* aerobic respiration is hindered due to destruction of ctenidium and mitochondria, it may survive 1–2 weeks via anaerobic respiration. However, the mortality of all individuals in the present study was observed at above 200 mg/*l* during the 144 h (6 d) experimental period, indicating that ammonia exposure accelerates mortality in *C. japonica* when compared with anoxic conditions. Sugahara et al. (2017), who investigated

hydrogen sulfide tolerance in *C. japonica*, also noted that high concentrations of hydrogen sulfide produced by sulfate-reducing bacteria that exploit anaerobic metabolites inside the shells of closed individuals may have influenced the mortality of individuals. However, there was no discernible difference in mortality time between individuals with and without partial holes in shells in the present study. In addition, the mortality rate of individuals with partial holes in the control was zero, suggesting that scraping itself had no effect of survival rates. Therefore, shell-closed behavior was considered to not affect the ammonia tolerance of *C. japonica*. The finding indicates that the *C. japonica* ammonia toxicity mechanism may have resulted in death due to ctenidium tissue destruction and metabolic disturbances in the gills by changes of branched chain amino acids and neurotransmitters, similar to that observed in *R. philippinarum*. Further study will clarify the relationship among ctenidium tissue destruction, filtered water amount, and the metabolite in *C. japonica*.

Table 1 shows the TAN and NH_3 LC50 values for numerous aquatic organisms, including *C. japonica*. The LC50 values of the larvae were lower than those of other aquatic organisms. However, individuals with a shell length greater than 0.5 mm exhibited higher tolerance than other aquatic organisms. Quahog, *Mercenaria mercenaria*, exhibited higher tolerance than *C. japonica* with shell lengths greater than 0.5 mm. A factor influencing the high tolerance observed in quahog is that when exposed to high concentrations of NH_3, the closed shell minimizes exposure to high concentrations of NH_3 (Boardman et al. 2004). However, since there was no clear difference in mortality time between individuals with and without partial holes in shells in the present study, closed shells are unlikely to have increased tolerance. *C. japonica* has been shown to be highly tolerant to environmental factors, including water temperature, salinity, hypoxia, nitrite, nitrate, and heavy metal (Nakamura et al. 1996a, 1996b, 1997a, 1997b; Matsuda and Sonoda 2021a, 2021b). The result of present study

Table 1. Ammonia toxicity in aquatic organisms

Taxon	Species name	NH_4-N (mg/*l*)	NH_3 (mg/*l*)	Reference	Note
Fish	*Atherinops affinis*	–	0.56	Phillips et al. (2005)	
	Cyprinodon variegatus	–	2.09 (1.97–2.23)	Boardman et al. (2004)	
	Labeo bata	11.5	–	Kumar et al. (2007)	Weight = 200.0 ± 5.0 mg
	Labeo bata	22.5	–	Kumar et al. (2007)	Weight = 500.0 ± 4.0 mg
	Lophiosilurus alexandri	18.7	3.66	Silva et al. (2018)	
	Paralichthys dentatus	–	1.07 (0.97–1.17)	Boardman et al. (2004)	
	Rachycentron canadum	8.1 (6.0–10.9)	0.31 (0.23–0.42)	Barbieri and Doi (2012)	Salinity = 5 ‰
	Rachycentron canadum	19.1 (14.0–25.9)	0.65 (0.47–0.88)	Barbieri and Doi (2012)	Salinity = 20 ‰
	Rachycentron canadum	22.7 (17.6–29.3)	0.68 (0.50–0.88)	Barbieri and Doi (2012)	Salinity = 35 ‰
	Sparus aurata	5.9 (4.5–7.3)	0.25 (0.19–0.31)	Kir and Sunar (2018)	Salinity = 10 ppt
	Sparus aurata	11.7 (10.5–13.0)	0.50 (0.45–0.55)	Kir and Sunar (2018)	Salinity = 20 ppt
	Sparus aurata	19.4 (18.0–20.7)	0.76 (0.70–0.81)	Kir and Sunar (2018)	Salinity = 30 ppt
	Topeka shiners	28.9 (27.3–30.5)	1.01 (0.97–1.06)	Adelman et al. (2009)	Juvenile (15 month)
	Topeka shiners	18.7 (17.1–20.6)	1.37 (1.24–1.50)	Adelman et al. (2009)	Juvenile (16 month)
	Topeka shiners	21.4 (18.8–24.5)	0.99 (0.86–1.13)	Adelman et al. (2009)	Adult
Mollusca					
Bivalvia	*Corbicula japonica*	3.5 (3.5–3.6)	0.31 (0.31–0.32)	This study	Larva
	Corbicula japonica	415.9 (180.3–651.5)	18.62 (8.07–29.16)	This study	Shell length = 0.5 mm
	Corbicula japonica	126.2 (108.4–144.0)	5.65 (4.85–6.45)	This study	Shell length = 3 mm
	Corbicula japonica	63.8 (54.1–73.5)	2.85 (2.42–3.29)	This study	Shell length = 25 mm
	Lampsilis fasciola	14.9 (13.4–16.6)	0.23 (0.21–0.25)	Mummert et al. (2003)	Water temperature = 12 ± 1
	Lampsilis fasciola	7.7 (6.5–9.3)	0.28 (0.23–0.33)	Mummert et al. (2003)	Water temperature = 20 ± 1
	Lampsilis siliquoidea	8.8 (7.4–10)	0.64 (0.56–0.74)	Miao et al. (2010)	
	Lampsilis siliquoidea	7.0 (5.3–9.1)	0.55 (0.44–0.68)	Miao et al. (2010)	
	Lampsilis siliquoidea	9.8 (8.4–11)	0.70 (0.52–0.8)	Miao et al. (2010)	
	Lampsilis siliquoidea	11.0 (10.0–12.0)	0.75 (0.66–0.85)	Miao et al. (2010)	
	Limnoperna fortunei	11.5 (10.3–12.9)	0.25 (0.24–0.27)	Montresor et al. (2013)	
	Mercenaria mercenaria	–	36.6 (30.5–42.8)	Boardman et al. (2004)	
	Villosa iris	20.6 (16.6–25.6)	0.10 (0.08–0.13)	Mummert et al. (2003)	Water temperature = 12 ± 1
	Villosa iris	11.4 (10.1–12.9)	0.12 (0.11–0.14)	Mummert et al. (2003)	Water temperature = 20 ± 1
Gastropoda	*Potamopyrgus antipodarum*	–	2.02 (1.56–2.45)	Alonso and Camargo (2003)	
Cephalopoda	*Sepia pharaonis*	18.3	0.96	Peng et al. (2017)	
Arthropoda					
Crustacean	*Eulimnogammarus toletanus*	–	0.65 (0.59–0.72)	Alonso and Camargo (2004)	
	Holmesimysis costata	–	0.84	Phillips et al. (2005)	
	Mysidopsis bahia	–	0.76 (0.69–0.83)	Boardman et al. (2004)	
	Palaemonetes pugio	–	1.67 (1.60–1.75)	Boardman et al. (2004)	

provides high tolerance to ammonia adding to them, thus *C. japonica* possess significant physiological tolerance to multiple water quality and basin-derived substances. However, it has been shown that ammonia tolerance of aquatic invertebrates varies with factors such as water temperature, salinity, pH, and the amount of food available (Zhang et al. 2023). Salinity fluctuations are an important factor for brackish invertebrates because they alter NH_3 concentration by causing changed in pH and also cause physiological changes through osmoregulation. For example, the survival rate of white-leg shrimp, *Litopenaeus vannamei* juveniles, to TAN was significantly lower at low salinity (Tu et al. 2022). Therefore, it is likely that ammonia tolerance in *C. japonica* may also be altered by environmental factors such as salinity.

The LC50 values obtained in the present study were compared with those of coastal lagoons on the Sea of Okhotsk and Nemuro in Hokkaido. Maximum TAN concentrations at each water depth at the center of Lake Abashiri and the Nokoribetu River flowing into Lake Furen were 5.37 mg/*l* (water depth of 4 m) and 5.01 mg/*l* (September 1999), respectively (Mikami et al. 2008; Ministry of Land, Infrastructure, Transport and Tourism 2020). These maximum concentration values of Lake Abashiri at a depth of 4 m and the inflowing river of Lake Furen were higher than the larvae LC50 values, suggesting a potential risk if similar concentrations occur during the spawning season. The coastal lagoons on the Sea of Okhotsk were confirmed to have high TAN concentrations for the spawning season. *C. japonica* fisheries have been undertaken in the Lakes Shibunotsunai and Mokoto. In addition, agricultural and livestock industry activities have been undertaken in the basins. TAN concentration in the inflow rivers of Lake Shibunotsunai was high from July to September, the spawning season for *C. japonica* (Matsuda et al. in prep.). TAN concentration in Lake Mokoto was high in the lake mouths and in the lake from July to September (Matsuda et al. in prep.). Therefore, TAN levels tend to be higher during the spawning season of *C. japonica* fisheries in the coastal lagoons on the Sea of Okhotsk in Hokkaido, posing a potential risk. Furthermore, Mikami and Igarashi (2019) reported that the water quality environment is different substantially between the waters upper and lower haloclines, with the lower of the halocline being anaerobic and containing high TAN concentrations. Hence, upwelling of water masses below the halocline could be the ecological risk to *C. japonica* in Lake Abashiri. From the above, the potential ammonia risk is a concern in the Sea of Okhotsk and Nemuro regions in Hokkaido, where large scale agriculture and livestock industries are conducted in the river basins.

Conclusion

The ammonia LC50 value of *Corbicula japonica* was lowest in planktonic larvae, highest in settled juveniles, and then decreased with growth. The mechanism of ammonia toxicity is proposed to be due to its destructive influence on the ctenidium, leading to death. Ammonia tolerance in *Corbicula japonica* was higher than in other aquatic organisms. However, a comparison of ammonia concentrations in *Corbicula japonica* fishing grounds in Hokkaido and the LC50 value revealed that it may be one of the potential ecological risk factors for *Corbicula japonica*, since values exceeding the LC50 of planktonic larvae were observed. Conservation of *Corbicula japonica* is essential for managing water quality risks, and this require continuous monitoring of ammonia levels in the fishing ground environment.

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飼育水中における汽水性二枚貝ヤマトシジミの成長段階に応じたアンモニア耐性

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汽水性二枚貝ヤマトシジミは日本の水産有用種であるが，近年の漁獲量は最盛期の約1/5まで減少している。ヤマトシジミ漁獲量の減少要因としては，好適な水質や底質を有する生息地の減少やアンモニアなどの流域由来物質の影響が考えられる。そこで本研究は，ヤマトシジミの成長段階によるアンモニア耐性の違いとアンモニア毒性メカニズムについて明らかにすることを目的とした。ヤマトシジミの浮遊幼生の半数致死濃度は成長段階の中で最も低く，着底後は殻長の増大に伴って半数致死濃度が低下した。また，アンモニア濃度の増加に伴ってヤマトシジミの鰓組織に変化が起こっていた。以上のことから，ヤマトシジミのアンモニア毒性メカニズムは，鰓組織の破壊による影響であることが考えられた。