JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

doi: 10.1111/jwas.12402

# Acute and Chronic Effects of Ammonia on Juvenile Cuttlefish, *Sepia pharaonis*

Rui-Bing Peng, Peng-Shuai Wang, Ke-Xin Le, Yuan Wang, Qing-Xi Han, and Xia-Min Jiang[1]

*School of Marine Sciences, Ningbo University, Ningbo 315211, China*

## Abstract

**The aim of this study was to provide a reference value for the safe regulation and control of ammonia nitrogen in the aquaculture of *Sepia pharaonis*. The effects of acute and chronic toxicity of ammonia on the cuttlefish, *S. pharaonis*, were tested experimentally using juvenile *S. pharaonis*. The results showed that the half-lethal concentration ($LC_{50}$) values of ammonia nitrogen in juvenile *S. pharaonis* with a body weight of 6.52 ± 0. 23 g at 24, 48, 72, and 96 h were 31.72, 25.77, 23.33, and 18.33 mg/L, respectively, and the corresponding un-ionized ammonia nitrogen (UIA-N) concentrations were 1.66, 1.35, 1.22, and 0.96 mg/L, respectively. Compared with the control, the survival rate, specific growth rate, and feed intake of juvenile *S. pharaonis* declined significantly, and the feed conversion ratio and hepatosomatic index increased significantly at 56 d after exposure to >1 mg/L ammonia nitrogen. Juvenile *S. pharaonis* should be maintained at a concentration of ammonia nitrogen of no more than 1 mg/L (UIA-N is 0.056 mg/L) in culture, and removing harmful nitrogenous wastes from the seawater is critical in maintaining cuttlefish culture.**

KEYWORDS

**ammonia nitrogen, chronic toxicity, growth, half-lethal concentrations, *Sepia pharaonis***

The cuttlefish, *Sepia pharaonis*, is an important warm-ocean demersal cephalopod that inhabits offshore waters at a depth of approximately 15–100 m and is mainly distributed from the Indian Ocean to the western Pacific (Chen et al. 2009). Wild populations of *S. pharaonis* have declined dramatically in the last 10 yr due to overfishing and environmental deterioration. Due to its rapid growth, high nutritional profile, and high market value, *S. pharaonis* is recognized as a potential species for commercial aquaculture (Gao et al. 2014). However, the development of the breeding and culturing of this species has resulted in several problems, such as deteriorating water conditions in ponds, reducing the cuttlefish growth rate and causing a high incidence of disease; decreased cuttlefish production; and low economic return (Jiang et al. 2014).

Ammonia nitrogen can contribute to pollution of water bodies, causing water eutrophication, and a subsequent decrease of dissolved oxygen (DO) that can lead to deterioration of water quality (Foss et al. 2004; Ip and Chew 2010). The concentration of ammonia nitrogen ideally should not exceed 0.6 mg/L in aquaculture (Cheng et al. 2013). Jiang et al. (2014) indicated that the level of ammonia nitrogen can reach as high as 1.03 mg/L in *S. pharaonis* ponds. The ammonia nitrogen in water is mainly composed of un-ionized ammonia ($NH_3$) and ionic ammonia ($NH_4^+$) (Wajsbrot et al. 1993). Considerable research results confirm that $NH_3$ is chemically toxic due to its ability to diffuse through the cell membranes, causing damage to gill epithelial cells and, consequently, interfering with osmotic adjustment mechanisms (Smart 1976; Thurston et al., 1981a; Benli et al. 2008. Ip and Chew 2010). The growth performance, food conversion efficiency, nonspecific immunity, and physiological functions of tissues and

[1] Correspondence to: jiangxiamin@nbu.edu.cn

© Copyright by the World Aquaculture Society 2017

organs of fish will be affected if the fish are constantly exposed to long-term low levels of ambient ammonia nitrogen (Dosdat et al. 2003; Foss et al. 2004; Spencer et al. 2008). In aquaculture, *S. pharaonis* are fed fresh-frozen fishes (Jiang et al. 2014); the ammonification of the unconsumed fresh fishes and excretion of the cuttlefish lead to an increase of ammonia nitrogen content in seawater. Several deaths of cuttlefish have been reported because of the high content of ammonia nitrogen in water during the breeding process. Therefore, the time frame for effects of chronic and acute toxicities of ammonia on cuttlefish must be taken into consideration to optimize rearing conditions and prevent seawater quality from becoming a limiting factor for the optimal growth and welfare of cuttlefish.

Like most marine invertebrates, cephalopods release their nitrogen waste products mainly as ammonia (Katsanevakis et al. 2005); cephalopods are also extremely sensitive to the level of ambient ammonia (Lee et al. 1998). Vidal and Boletzky (2014) reported the importance of maintaining the level of un-ionized ammonia nitrogen (UIA-N) at <0.1 mg/L in cephalopod cultures. The first toxicological evaluation of a certain combination "toxicant + organism" in general involves the determination of the average half-lethal concentration ($LC_{50}$) and safe concentration (SC) based on an acute toxicity experiment of short duration (96 h) (Cheng et al. 2013). Surprisingly, ammonia toxicity has been extensively studied for teleosts and crustaceans, but data on the chronic and acute toxicities of ammonia for cephalopods are relatively scarce. Further, the chronic and acute effects of exposure to ammonia in cephalopods have rarely been examined. With the development of cuttlefish aquaculture, research on the effects of exposure to ammonia on cephalopods has become urgent. The purpose of this study is to provide information about (1) the $LC_{50}$ of ammonia nitrogen on *S. pharaonis* juveniles at 24, 48, 72, and 96 h and the SC of ammonia nitrogen; (2) the effect of chronic ammonia exposure on the survival and growth of *S. pharaonis* juveniles; (3) and the reference value for safe regulation and control of ammonia nitrogen in the aquaculture of *S. pharaonis*.

## Materials and Methods

### *Aquarium Tank System and Water Quality Management*

The aquarium tank system consisted of 30 individual plastic tanks (350 L). All plastic tanks had identical, smooth inner surfaces. The temperature in each plastic container was controlled using submersible titanium heaters. The container tank had an outlet and an inlet with the use of air stones for continuous aeration.

The ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen were measured twice per day according to the methods of the Hach reagent kit (HACH®, Loveland, CO, USA) using a spectrophotometer (Hach DR-2000, HACH). To assess the water quality, the salinity, pH, and DO were measured using a YSI Pro Plus instrument (YSI, Yellow Springs, OH, USA).

### *Experimental Cuttlefish and Acclimation in an Aquarium Tank System*

Juvenile cuttlefish, *S. pharaonis*, were obtained from the Marine Fisheries Research Institute of Zhoushan (Zhejiang Province, China), where the ammonia exposure trial experiment was conducted. Prior to the ammonia toxicity trial, all cuttlefish juveniles were reared for 7 d to get acclimated to the new environment. One thousand cuttlefish of similar size (initial weight: $5.25 \pm 0.05$ g for the acute ammonia toxicity experiment) and 900 cuttlefish of similar size (initial weight: $4.35 \pm 0.03$ g for the chronic ammonia toxicity experiment) were evenly distributed into 30 tanks housed indoors. The juvenile cuttlefish were reared under a natural photoperiod in sand-filtered, aerated, and UV-irradiated seawater (temperature, $24.5 \pm 0.5$ C; salinity, $29.5 \pm 0.5$; pH, $8.01 \pm 0.09$; DO, $7.36 \pm 0.54$ mg/L; ammonia nitrogen, $0.03 \pm 0.01$ mg/L; nitrate nitrogen, $0.03 \pm 0.01$ mg/L; nitrite nitrogen, $0.01 \pm 0.01$ mg/L). The juvenile cuttlefish were fed fresh shrimp three times per day to visual satiety (0730, 1130, and 1730 h). The tanks were thoroughly cleaned once every 2 d. Every 12 h, 80% of the seawater in each tank was refreshed (0830 and 1830 h) with seawater at the same

salinity and temperature, and water samples were taken at 0900 and 1800 h.

### *Experimental Design of the Acute Ammonia Toxic Experiment*

The design for the acute ammonia toxicity experiment depends on the maximum ammonia nitrogen concentration that results in the death of all affected animals in 24 h ($LC_{100,\,24\,h}$) and the minimum ammonia nitrogen concentration that results in no deaths in 96 h ($LC_{0,\,96\,h}$); this concentration range (with its upper and lower limits being $LC_{0,\,96\,h}$ and $LC_{100,\,24\,h}$, respectively) was divided into six different experimental concentrations according to the logarithmic interval (Cheng et al. 2013). In this study, we established a control group (0 mg/L) and six different ammonia nitrogen concentrations of 11.45, 15.00, 19.63, 25.70, 33.57, and 44.00 mg/L for the acute ammonia toxicity experiment according to preliminary experimental results. The six different ammonia concentrations were adjusted by diluting a high-purity $NH_4Cl$ (10 g/L) stock solution.

After acclimation, a total of 900 *S. pharaonis* juveniles of similar size (with an average body weight of $6.52 \pm 0.23$ g) were randomly allocated into 18 tanks (350 L) at a breeding density of 50 cuttlefish per tank. The 21 individual test tanks were divided into seven groups (seven ammonia concentrations × three tanks). The cuttlefish juveniles were reared under a natural photoperiod in sand-filtered, aerated, and UV-irradiated seawater. The seawater quality was measured at 0, 6, 12, 24, 36, 48, 60, 72, 84, and 96 h of the experimental period (Table 1). Every 12 h, 80% of the seawater in each tank was refreshed (at 0830 and 1830 h) with seawater at the same salinity, temperature, pH, and ammonia nitrogen concentration. The acute ammonia toxicity trial lasted for 96 h; the number of deaths was recorded at 6, 12, 24, 48, 72, and 96 h; and the dead cuttlefish were promptly removed. The cuttlefish juveniles were not fed diets during the experimental period. The $LC_{50}$ and SC were calculated at the end of the experiment.

### *Experimental Design of Chronic Ammonia Toxic Experiment*

In this study, the design for the chronic ammonia toxic experimental regimes of 0, 1, 1.8, 3, and 5 mg/L was chosen based on some studies that reported the importance of maintaining the level of un-ionized ammonia at <0.1 mg/L in cephalopod cultures (Boletzky and Hanlon 1983; Vidal and Boletzky 2014), whereas Jiang et al. (2014) indicated that the level of ammonia nitrogen can reach as high as 1.03 mg/L in *S. pharaonis* ponds, and the SC of ammonia nitrogen for juvenile *S. pharaonis* was 1.8 mg/L according to the above experimental results of the acute ammonia toxicity experiment. The four different ammonia nitrogen concentrations were adjusted by diluting a high-purity $NH_4Cl$ (10 g/L) stock solution.

After acclimation, a total of 300 *S. pharaonis* juveniles of similar size (with an average body weight of $5.68 \pm 0.13$ g) were randomly allocated into 15 tanks (350 L) at a breeding density of 20 cuttlefish per tank. The 15 individual test tanks were divided into five groups (five ammonia nitrogen concentrations × three tanks). The cuttlefish juveniles were reared under natural photoperiod in sand-filtered, aerated, and UV-irradiated seawater. Every 12 h, 80% of the

TABLE 1. *Water quality parameters of acute ammonia toxic experiment during experimental period.*[a]

| Variables | 0 mg/L | 11.45 mg/L | 15.00 mg/L | 19.63 mg/L | 25.70 mg/L | 33.57 mg/L | 44.00 mg/L |
|---|---|---|---|---|---|---|---|
| Ammonia nitrogen (mg/L) | $0.02 \pm 0.01$ | $11.62 \pm 0.21$ | $15.22 \pm 0.11$ | $19.89 \pm 0.09$ | $25.92 \pm 0.13$ | $33.67 \pm 0.21$ | $44.21 \pm 0.17$ |
| Temperature (C) | $24.5 \pm 0.3$ | $24.5 \pm 0.3$ | $24.5 \pm 0.3$ | $24.5 \pm 0.3$ | $24.5 \pm 0.3$ | $24.5 \pm 0.3$ | $24.5 \pm 0.4$ |
| Dissolved oxygen (mg/L) | $7.23 \pm 0.45$ | $7.31 \pm 0.51$ | $7.16 \pm 0.42$ | $7.12 \pm 0.22$ | $7.42 \pm 0.36$ | $7.21 \pm 0.25$ | $7.28 \pm 0.32$ |
| Salinity | $29.4 \pm 0.2$ | $29.4 \pm 0.2$ | $29.4 \pm 0.2$ | $29.4 \pm 0.2$ | $29.5 \pm 0.2$ | $29.5 \pm 0.2$ | $29.4 \pm 0.2$ |
| pH | $8.02 \pm 0.03$ | $8.01 \pm 0.04$ | $8.02 \pm 0.05$ | $8.01 \pm 0.05$ | $8.01 \pm 0.04$ | $8.03 \pm 0.05$ | $8.01 \pm 0.03$ |
| Nitrate nitrogen (mg/L) | $0.03 \pm 0.01$ | $0.02 \pm 0.01$ | $0.02 \pm 0.01$ | $0.03 \pm 0.01$ | $0.03 \pm 0.01$ | $0.03 \pm 0.02$ | $0.03 \pm 0.01$ |
| Nitrite nitrogen (mg/L) | $0.01 \pm 0.01$ | $0.01 \pm 0.01$ | $0.02 \pm 0.01$ | $0.02 \pm 0.01$ | $0.01 \pm 0.01$ | $0.02 \pm 0.01$ | $0.01 \pm 0.01$ |

[a]Values are presented as the mean ± SE.

TABLE 2. *Water quality parameters of chronic ammonia toxic experiment during experimental period.*[a]

| Variables | 0 mg/L | 1 mg/L | 1.8 mg/L | 3 mg/L | 5 mg/L |
|---|---|---|---|---|---|
| Ammonia nitrogen (mg/L) | 0.10 ± 0.02 | 1.06 ± 0.07 | 1.76 ± 0.11 | 3.11 ± 0.09 | 5.09 ± 0.09 |
| Temperature (C) | 25.5 ± 0.6 | 25.5 ± 0.6 | 25.5 ± 0.6 | 25.5 ± 0.6 | 25.5 ± 0.5 |
| Dissolved oxygen (mg/L) | 7.33 ± 0.41 | 7.47 ± 0.25 | 7.16 ± 0.46 | 7.16 ± 0.21 | 7.16 ± 0.25 |
| Salinity | 29.5 ± 0.2 | 29.5 ± 0.2 | 29.5 ± 0.2 | 29.5 ± 0.2 | 29.5 ± 0.2 |
| pH | 8.02 ± 0.03 | 8.01 ± 0.04 | 8.02 ± 0.05 | 8.01 ± 0.05 | 8.01 ± 0.04 |
| Nitrate nitrogen (mg/L) | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 |
| Nitrite nitrogen (mg/L) | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 |

[a]Values are presented as the mean ± SE.

seawater in each tank was refreshed (0830 and 1830 h) with seawater at the same salinity, temperature, pH, and ammonia nitrogen level. The seawater quality was measured twice per day (at 0900 and 1800 h) during the experimental period (Table 2). The cuttlefish juveniles were fed fresh shrimp by hand three times per day to visual satiety (at 0730, 1130, and 1730 h), and the residual feed was promptly removed after feeding. The chronic ammonia toxicity trial lasted for 56 d, and diet consumption was recorded daily. The tanks were thoroughly cleaned once every 2 d, and mortality was recorded daily. The survival rate (SR), specific growth rate (SGR), feed conversion ratio (FCR), and hepatosomatic index (HSI) were calculated at the end of the experiment.

### *Calculations and Statistical Analysis*

The parameters were calculated as follows:

The SC was calculated according to the Sprague (1971) study.

$$SC = 0.1 \times LC_{50,\ 96\ h}$$

The UIA-N fraction was calculated according to Emerson et al. (1975).

$$\text{UIA-N} = \left[NH_4^+ + NH_3\right] / \left[1 + 10\ (\text{pKa} - \text{pH})\right]$$

where $NH_4{}^+ + NH_3$ is total ammonia and $\text{pKa} = 0.09018 + 2729.92/\text{T}$ ($\text{T} = 273 + t\,\text{C}$).

$$\text{SR}\ (\%) = 100 \times (\text{final number of cuttlefish}) / (\text{initial number of cuttlefish})$$

$$\text{SGR}\ (\%/\text{d}) = 100 \times \left(\text{Ln}W_t - \text{Ln}W_i\right) / t$$

where $W_t$ is the final body weight (g), $W_i$ is the initial body weight (g), and $t$ is the experimental duration in days.

$$\text{FCR} = \text{feed consumption}/\text{weight gain}$$

$$\text{Feed intake (FI, \%/d)} : \text{feed consumption} / \left(\text{d} \times \left(W_t + W_i\right)/2\right)$$

$$\text{HSI} = \text{liver weight (g, weight)} / \text{body weight (g, weight)}$$

The results were presented as the means ± SD. Group normality was initially evaluated using the Shapiro-Wilk test, and one-way ANOVA for significant differences was then performed using the software package SPSS 18.0 (SPSS, Chicago, IL, USA). The homogeneity of variances was checked using Bartlett's test. When necessary, post hoc analyses using the LSD test were applied.

## Results

### *Acute Ammonia Toxicity Experiment*

As shown in Figure 1, as the ammonia nitrogen concentration increased from 0 to 44.00 mg/L, the mortality rate of the juvenile *S. pharaonis* significantly increased. The mortality rate was not significantly different ($P > 0.05$) at 24, 48, 72, and 96 h after exposure to 11.45 mg/L ammonia nitrogen compared with the control (0 mg/L). The mortality rate of the other experimental group was significantly different from the control ($P < 0.05$). The mortality rate showed a notable dose-dependent relationship with the

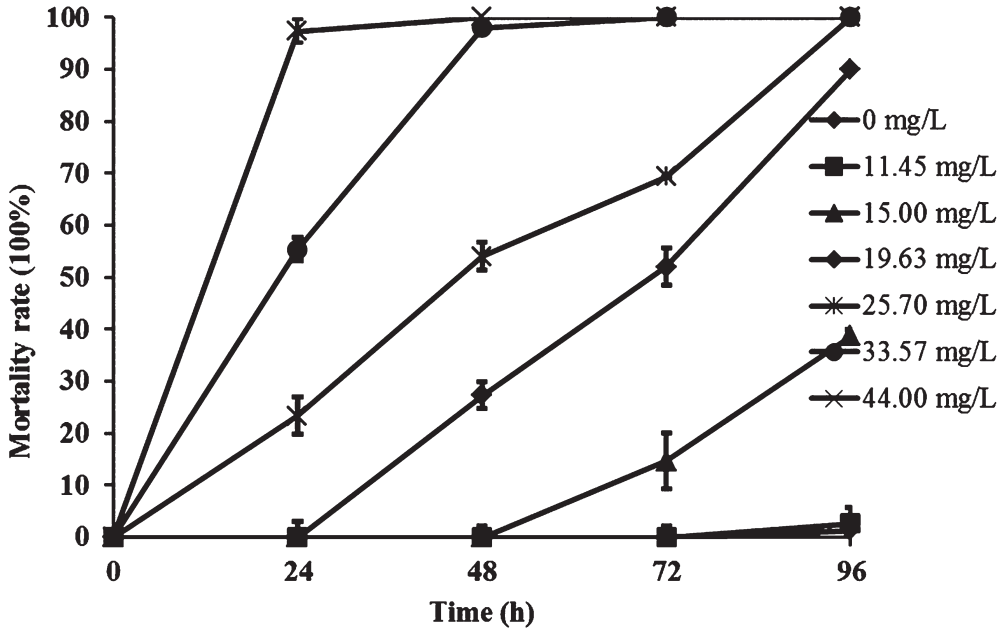


FIGURE 1. *Mortality rate of juvenile cuttlefish,* Sepia pharaonis*, exposed to different concentrations of ammonia nitrogen for 96 h. Values are presented as the mean ± SE.*

TABLE 3. *The half-lethal concentration ($LC_{50}$) and safe concentration (SC) values of juvenile* Sepia pharaonis *exposed to different concentrations of ammonium nitrogen and un-ionized ammonia nitrogen (UIA-N).*

| Time (h) | Ammonium nitrogen concentration (mg/L) | | UIA-N concentration (mg/L) | |
|---|---|---|---|---|
| | $LC_{50}$ | SC | $LC_{50}$ | SC |
| 24 | 31.72 | 1.833 | 1.66 | 0.096 |
| 48 | 25.77 | | 1.35 | |
| 72 | 23.33 | | 1.22 | |
| 96 | 18.33 | | 0.96 | |

ammonia nitrogen concentration and time of ammonia exposure.

The $LC_{50}$ values for juvenile *S. pharaonis* with body weight of 6.52 ± 0. 23 g at 24, 48, 72, and 96 h were 31.72, 25.77, 23.33, and 18.33 mg/L, respectively, and the corresponding UIA-N concentrations were 1.66, 1.35, 1.22, and 0.96 mg/L, respectively (Table 3). The SC of ammonia nitrogen of juvenile *S. pharaonis* with a body weight of 6.52 ± 0. 23 g was 1.83 mg/L, and the corresponding UIA-N concentration was 0.096 mg/L (Table 3).

### *Chronic Ammonia Toxicity Experiment*

As shown in Figure 2(A), in all groups of juvenile *S. pharaonis* exposed to ammonia, except for those exposed to 1 mg/L, significant effects on the SR were observed. The SRs of juvenile *S. pharaonis* exposed to 0, 1, 1.8, 3, and 5 mg/L (UIA-N of 0, 0.056, 0.101, 0.168, and 0.280, respectively) were 77.3, 74.7, 69.3, 55.3, and 33.7%, respectively. The SR decreased by 3.5, 9.3, 28.5, and 60.4 at 56 d after exposure to 1, 1.8, 3, and 5 mg/L ammonia nitrogen, respectively, in comparison with the controls.

As shown in Figure 2(B), in all groups of juvenile *S. pharaonis* exposed to ammonia nitrogen, except for 1 mg/L, significant effects on the SGR were observed. The SGRs of juvenile *S. pharaonis* exposed to 0, 1, 1.8, 3, and 5 mg/L were 4.6, 4.6, 4.3, 3.5, and 3.1%, respectively. The SGRs decreased by 0.9, 6.5, 23.9, and 32.6% at 56 d after exposure to 1, 1.8, 3, and 5 mg/L ammonia nitrogen, respectively, compared with the controls.

As shown in Figure 2(C), the FCRs of juvenile *S. pharaonis* exposed to 0, 1, 1.8, 3, and 5 mg/L were 3.1, 3.2, 3.4, 3.6, and 3.7, respectively,

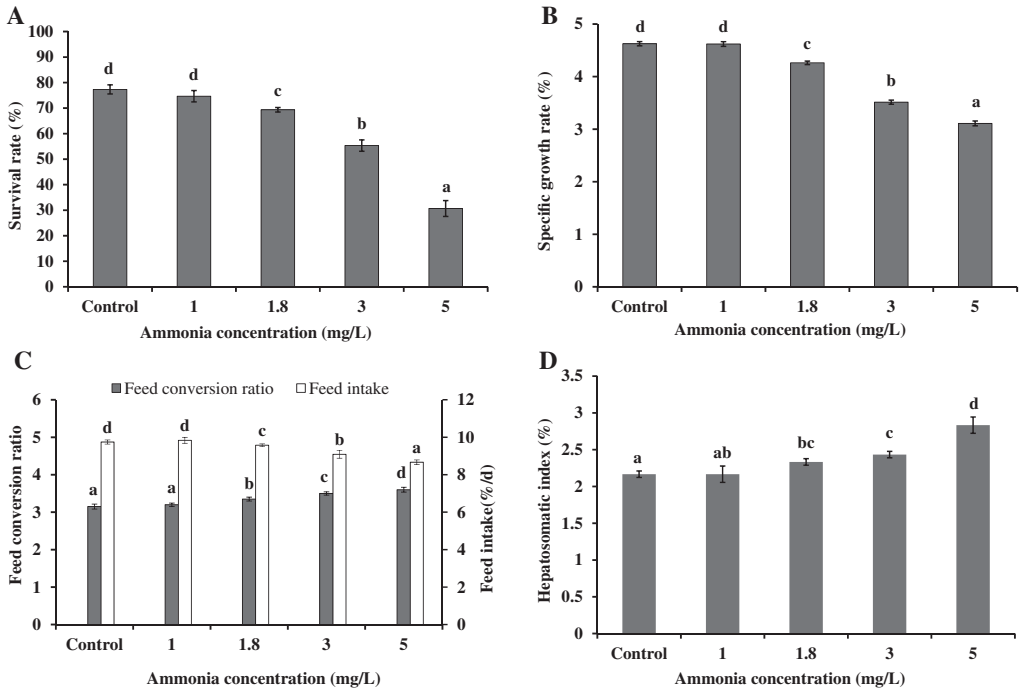


FIGURE 2. *Effect of chronic ammonia exposure on the survival rate, specific growth rate, feed conversion ratio, and hepatosomatic index of juvenile cuttlefish,* Sepia pharaonis. *Values are presented as the mean ± SE. Values with different superscripts in the same row differ significantly (*$P < 0.05$*).*

and had a positive correlation with the ammonia exposure concentration. The FI of juvenile *S. pharaonis* exposed to 0, 1, 1.8, 3, and 5 mg/L were 9.8, 9.8, 9.5, 9.0, and 8.7 %/d, respectively, and had a negative correlation with the ammonia exposure concentration.

As shown in Figure 2(D), the HSI was significantly affected by the ammonia exposure concentration. The HSIs of the juvenile *S. pharaonis* exposed to 0, 1, 1.8, 3, and 5 mg/L of ammonia nitrogen were 2.2, 2.2, 2.3, 2.4, and 2.8, respectively, and had a positive correlation with the ammonia exposure concentration.

## Discussion

The $LC_{50}$ is a common indicator used for pollutants as well as for toxicants in toxicology (Seneriches-Abiera et al. 2007; Liao et al. 2011). The main purposes of the acute ammonia toxicity experiment are to study the relationship between dose dependence and ammonia exposure concentration, to determine the specific levels for acute toxicity, to provide information on patterns of acute toxicity, and to inform the chronic toxicity experiment (Liao et al. 2011). The $LC_{50}$ and SC provide important information about acute ammonia toxicity. The $LC_{50}$ of ammonia nitrogen for juvenile *S. pharaonis* (6.52 g) at 96 h was 18.33 mg/L, and the corresponding UIA-N concentration was 0.962 mg/L. The SC of ammonia nitrogen was 1.83 mg/L, and the corresponding UIA-N concentration was 0.096 mg/L. Some studies have shown that the level of tolerance for ammonia was related to the species and has a positive relationship with individual size (Qu et al. 2007; Seneriches-Abiera et al. 2007; Cheng et al. 2013). Zheng (2012) found that the $LC_{50}$ of ammonia nitrogen for juvenile *Epinephelus coioides* (9.96 g) at 96 h was 51.40 mg/L and the SC of ammonia nitrogen was 5.13 mg/L. Cheng et al. (2013) showed that the $LC_{50}$ of ammonia nitrogen for juvenile sea cucumber, *Apostichopus japonicas* (1.48 g), at 96 h was 212.1 mg/L and the SC of

ammonia nitrogen was 21.2 mg/L. The $LC_{50}$ of UIA-N concentration in turbot, *Scophthalmus maximus* (length: 2.0–2.4 cm), was 1.73 mg/L (Qu et al. 2007). This might have been related to the species and individual size. The level of tolerance of ammonia of juvenile *S. pharaonis* is significantly lower than that of other aquatic animals. This finding shows that the cuttlefish, *S. pharaonis*, is sensitive to ammonia nitrogen. In this study, the toxic effect of ammonia nitrogen on juvenile *S. pharaonis* was closely correlated with exposure time and dose. Therefore, it is important to remove harmful ammonia nitrogen wastes from seawater in maintaining cuttlefish culture and to periodically detect the concentration of ammonia nitrogen in seawater during the rearing period. Investigating the effects of chronic ammonia exposure on the survival, growth, health, and FI of juvenile *S. pharaonis* provides a more comprehensive understanding of the effects of ammonia exposure on this species.

The SR and SGR in treatment groups as the test progressed indicate an acclimation response to water ammonia in aquatic animals (Foss et al. 2004). Some studies had indicated that long-term exposure to ammonia nitrogen in water could delay the growth and development and decreased survival of the tiger crab, *Orithyia sinica* (Koo et al. 2005); the abalone, juvenile European sea bass, *Dicentrarchus labrax* (Lemarie et al. 2004); juvenile greenlip abalone, *Haliotis laevigata* (Harris et al. 1998; Kasturi et al. 2006); and slimy sculpin, *Cottus cognatus* (Spencer et al. 2008). Our study also showed that SGR and the SR declined sharply at 56 d after exposure to more than 1 mg/L ammonia nitrogen. This might be because $NH_3$ can diffuse through the cell membranes, causing damage to organs and tissues and results in growth inhibition and SR decline. Our study showed that long-term exposure of aquatic animals to ammonia nitrogen in water could increase the FCR. With the increase of ammonia nitrogen, FI decreased dramatically. Some studies also presented evidence that the stress of ammonia nitrogen can affect the food utilization of fish, which leads to decline in growth performance (Foss et al. 2003, 2009; Paust et al. 2011; Pinto et al. 2007). As ammonia nitrogen causes an increased cost of repair and maintenance, fewer calories would be left for growth, which leads to a decreased FCR. However, Li et al. (2015) presented evidence that no differences were found in fish final weight, weight gain, and feed efficiency between ammonia group (5.65–5.80 mg/L) and control group. This may be related to species and size of experimental aquatic animals, stress degrees, and stress times. Because ammonia nitrogen causes an increased cost of repair and maintenance, fewer calories are left for growth, which leads to a decreased FCR. The liver is central to metabolism and detoxification. This study showed that the HSI was increased by long-term ammonia exposure concentrations of 1.8, 3, and 5 mg/L compared with the control. This finding suggests the proliferation of liver tissue of the cuttlefish, *S. pharaonis*, as a result of long-term exposure to ammonia nitrogen in water. Li et al. (2015) reported that the HSI of juvenile yellow catfish, *Pelteobagrus fulvidraco,* in the ammonia group was significantly higher than that of the fish in the control group; the livers were swollen and functioned abnormally in the juvenile *P. fulvidraco* in the ammonia group. The results of this study provide evidence that the high concentration of ammonia nitrogen was not appropriate for juvenile *S. pharaonis*; thus, a concentration of ammonia nitrogen no more than 1 mg/L should be maintained in cephalopod cultures based on the effects of chronic ammonia nitrogen on the SR, SGR, FCR, and HSI. The SC of ammonia nitrogen for juvenile *S. pharaonis* is 1.83 mg/L according to the Sprague model ($SC = 0.1 \times LC_{50,\,96\,h}$). However, in the chronic ammonia toxic experiment, compared with the control, the SR and SGR of juvenile *S. pharaonis* declined significantly, and the FCR and HSI increased significantly at 56 d after exposure to 1.8 mg/L ammonia nitrogen. The final results show that the SC of ammonia nitrogen for juvenile *S. pharaonis* is 1 mg/L and the Sprague model was inapplicable to juvenile *S. pharaonis*. Our results also indicated the limitations in the SC of ammonia nitrogen for some species by using the Sprague model calculation.

The study found that the concentration of ammonia nitrogen with no observable effect

(1 mg/L, UIA-N of 0.056 mg/L) is relatively low compared with that reported in similar studies on other commonly cultured aquatic animals. Wajsbrot et al. (1993) reported that the concentration of UIA-N with no observable effect was 0.27 mg/L for juvenile gilthead sea bream, *Sparus aurata*. Foss et al. (2003) reported that the concentration of UIA-N with no observable effect was 0.13 mg/L for juvenile spotted wolf fish. Similar results have also been reported for juvenile sea bass, at 0.26 mg/L (Lemarie et al. 2004); juvenile yellow catfish, *Pelteobagrus fulvidraco*, at 0.32 mg/L (Li et al. 2015); and juvenile sea cucumber, *A. japonicas*, at 0.8 mg/L (Cheng et al. 2013). This might be due to the fact that *S. pharaonis* is sensitive to ammonia nitrogen. Therefore, in practice, more attention should be paid to the concentration of ammonia nitrogen in water changes in aquaculture production to reduce its toxicity and side effects on *S. pharaonis.* Regular monitoring of ammonia nitrogen concentration in the water of aquaculture systems for *S. pharaonis* is particularly important. Some studies have demonstrated that the toxicity of ammonia nitrogen in water is related to DO (Wajsbrot et al. 1991; Thurston et al. 2011), salinity (Alabaster et al. 1979), pH (Thurston et al. 1981b), and carbon dioxide (Randall and Wright 1989). Therefore, it is necessary to take effective measures to ensure the range of suitable pH and abundance of oxygen in the water, and cuttlefish breeders should clear, transport, and dispose of the egesta and waste produced in raising cuttlefish.

## Conclusion

The results of this study indicate that the $LC_{50}$ values of ammonia nitrogen in juvenile *S. pharaonis* with a body weight of $6.52 \pm 0.23$ g at 24, 48, 72, and 96 h are 31.72, 25.77, 23.33, and 18.33 mg/L, respectively, and the corresponding UIA-N concentrations are 1.66, 1.35, 1.22, and 0.96 mg/L, respectively. The high concentration of ammonia nitrogen was not appropriate for juvenile *S. pharaonis*; their weight gain, SGR, and FI decreased significantly and the FCR and HSI of juvenile *S. pharaonis* increased significantly at 56 d after exposure to more than 1 mg/L ammonia nitrogen (UIA-N of 0.056 mg/L). Juvenile *S. pharaonis* are highly sensitive to ammonia. Removing harmful nitrogenous wastes from the seawater is critical in maintaining cuttlefish culture.

## Acknowledgments

We would like to thank Ming Li, Zheng Tao, Jingjing Liang, and Peng Ruan for their assistance with caring for cuttlefish and sampling. This research was funded by the Science and Technology Department of Ningbo (2014C11001) and also partly sponsored by K.C. Wong Magna Fund, Ningbo University.

## Literature Cited

**Alabaster, J. S., D. G. Shurben, and G. Knowles.** 1979. The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, *Salmo salar* L. Journal of Fish Biology 15:705–712.

**Benli, A. C., K. G. Kösal, and A. Özkul.** 2008. Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus* L.): effects on gill, liver and kidney histology. Chemosphere 72(9):1355–1358.

**Boletzky, S. V. and R. T. Hanlon.** 1983. A review of the laboratory maintenance, rearing and culture of cephalopod molluscs. Cephalopods 44:147–187.

**Chen, X. J., B. L. Lin, and X. G. Wang.** 2009. Cephalopods of the world. Ocean Press, Beijing, China.

**Cheng, L., J. Song, Z. He, and J. H. Song.** 2013. Acute toxicity of ammonia nitrogen on juvenile sea cucumber (*Apostichopus japonicas*). Journal of Anhui Agricultural Science 41(33):12933–12934 (in Chinese).

**Dosdat, A., P. L. Ruyet, D. Covès, G. Dutto, E. Gasset, R. A. Le, and G. Lemarie.** 2003. Effect of chronic exposure to ammonia on growth, food utilization and metabolism of the European sea bass (*Dicentrarchus labrax*). Aquatic Living Resources 16:509–520.

**Emerson, K., R. C. Russo, R. E. Lund, and R. V. Thurston.** 1975. Aqueous ammonia equilibrium calculations: effects of pH and temperature. Journal of Fisheries Research Board of Canada 32:2379–2383.

**Foss, A., T. H. Evensen, T. Vollen, and V. Øiestad.** 2003. Effects of chronic ammonia exposure on growth and food conversion efficiency in juvenile spotted wolfish. Aquaculture 228(1–4):215–224.

**Foss, A., S. I. Siikavuopio, B. R. Sæther, and T. H. Evensen.** 2004. Effect of chronic ammonia exposure on growth in juvenile *Atlantic cod*. Aquaculture 237:179–189.

**Foss, A., A. K. Imsland, B. Roth, E. Schram, and S. O. Stefansson.** 2009. Effects of chronic and periodic exposure to ammonia on growth and blood physiology in juvenile turbot (*Scophthalmus maximus*). Aquaculture 296(1–2):45–50.

**Gao, X. L., X. M. Jiang, and K. X. Le.** 2014. Analysis and evaluation of nutritional components in different tissues of wild *Sepia pharaonis*. Chinese Journal of Animal Nutrition 26:3858–3867 (in Chinese).

**Harris, J. O., G. B. Maguire, S. Edwards, and S. M. Hindrum.** 1998. Effect of ammonia on the growth rate and oxygen consumption of juvenile greenlip abalone, *Haliotis laevigata* Donovan. Aquaculture 160:259–272.

**Ip, Y. K. and S. F. Chew.** 2010. Ammonia production, excretion, toxicity, and defense in fish: a review. Frontiers in Physiology 134(1):1–20.

**Jiang, X. M., J. Luo, R. B. Peng, F. Tang, X. L. Gao, K. X. Le, and Z. P. Sun.** 2014. Growth characteristics of *Sepia pharaonis* under pond-culture. Journal of Ningbo University (NSEE) 27:1–6 (in Chinese).

**Kasturi, R., L. Auerswald, and P. Cook.** 2006. Ammonia toxicity and its effect on the growth of the South Africana balone *Haliotis midae* Linnaeus. Aquaculture 261:678–687.

**Katsanevakis, S., S. Stephanopoulou, H. Miliou, M. Moraitou-Apostolopoulou, and G. Verriopoulos.** 2005. Oxygen consumption and ammonia excretion of *Octopus vulgaris* (Cephalopoda) in relation to body mass and temperature. Marine Biology 146: 725–732.

**Koo, J. G., S. G. Kim, J. H. Jee, J. M. Kim, S. C. Bai, and J. C. Kang.** 2005. Effects of ammonia and nitrite on survival, growth and moulting in juvenile tiger crab, *Orithyia sinica* (Linnaeus). Aquaculture Research 36:79–85.

**Lee, P. G., P. E. Turk, J. W. Forsythe, and F. P. Dimarco.** 1998. Cephalopod culture: physiological, behavioral and environmental requirements. Aquaculture Science 46:417–422.

**Lemarie, G., A. Dosdat, D. Coves, G. Dutto, E. Gasset, and J. P. Ruyet.** 2004. Effeet of chronic ammonia exposure on growth of European seabass (*Dicentrarchus labrax*) juveniles. Aquaculture 229:479–491.

**Li, Q., S. Y. Gong, and M. Li.** 2015. Chronic ammonia toxicity induces glutamine accumulation, oxidative damage and immunosuppression of juvenile yellow catfish *Pelteobagrus fulvidraco*. Journal of Fisheries of China 39:728–735 (in Chinese).

**Liao, Y. Y., H. H. Wang, and Z. G. Lin.** 2011. Effect of ammonia and nitrite on vigour, survival rate, moulting rate of the blue swimming crab *Portunus pelagicus* zoea. Aquaculture International 19:339–350.

**Paust, L. O., A. Foss, and A. K. Imsland.** 2011. Effects of chronic and periodic exposure to ammonia on growth, food conversion efficiency and blood physiology in juvenile Atlantic halibut (*Hippoglossus hippoglossus L.*). Aquaculture 315(3–4):400–406.

**Pinto, W., C. Aragao, F. Soares, M. T. Dinis, and L. E. C. Conceicao.** 2007. Growth, stress response and free ammonia acid levels in Senegalese sole (*Solea senegalensis* Kaup 1858) chronically exposed to exogenous ammonia. Aquaculture Research 38:1198–1204.

**Qu, K. M., Y. Xu, S. S. Ma, and J. Li.** 2007. Acute toxic effects of nitrite and non-ion ammonia on turbot (*Scophthalmus maximus*) at different DO levels. Marine Fisheries Research 4:83–89 (in Chinese).

**Randall, D. J. and P. A. Wright.** 1989. The interaction between carbon dioxide and ammonia excretion and water pH in fish. Canadian Journal of Zoology 67:2936–2942.

**Seneriches-Abiera, M. L., F. Parado-Estepa, and G. A. Gonzales.** 2007. Acute toxicity of nitrite to mud crab *Scylla serrata* (Forsskal) larvae. Aquaculture Research 38(14):1495–1499.

**Smart, G.** 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). Journal of Fish Biology 8:471–475.

**Spencer, P., R. Pollock, and M. Dube.** 2008. Effects of un-ionized ammonia on histological, endocrine, and whole organism endpoints in slimy sculpin (*Cottus cognatus*). Aquatic Toxicology 90:300–309.

**Sprague, J. B.** 1971. Measurement of pollutant toxicity to fish. III. Sublethal effect and SC. Water Research 5:245–266.

**Thurston, R. V., C. Chakoumakos, and R. C. Russo.** 1981a. Effect of fluctuating exposures on the acute toxicity of ammonia to rainbow trout (*Salmo gairdneri*) and cutthroat trout (*S. clarki*). Water Research 15(7):911–917.

**Thurston, R. V., R. C. Russo, and G. A. Vinogradov.** 1981b. Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionized ammonia species. Environmental Science and Technology 15:837–840.

**Thurston, R. V., G. R. Philipps, and R. C. Russo.** 2011. Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen. Canadian Journal of Fisheries and Aquatic Sciences 38(8):983–988.

**Vidal, E. A. G. and S. V. Boletzky.** 2014. *Loligo vulgaris* and *Doryteuthis opalescens*. Pages 271–313 *in* J. Iglesias, L. Fuentes, and R. Villanueva, editors. Cephalopod culture. Springer Netherlands, London, UK.

**Wajsbrot, N., A. Gasith, M. D. Krom, and D. M. Poppe.** 1991. Acute toxicity of ammonia to juvenile gilthead seabream *Sparus aurata* under reduced oxygen levels. Aquaculture 92:277–288.

**Wajsbrot, N., A. Gasith, A. Diamant, and D. M. Popper.** 1993. Chronic toxicity of ammonia to juvenile gilthead seabream *Sparus aurata* and related histopathological effects. Journal of Fish Biology 42:321–328.

**Zheng, L. Y.** 2012. Acute toxic effects of ammonia and nitrite on *Epinephelus coioides* fry. Marine Sciences 5:81–86 (in Chinese).