Effects of copper and reduced salinity on the early life stages of the moon jellyfish *Aurelia coerulea*

Zhijun Dong\(^a,b,c,\) Fuhao Wang\(^c\), Saijun Peng\(^a\), Guofu Chen\(^c\), Shan Sun\(^d\)

\(^a\) Muping Coastal Environment Research Station, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, Shandong 264003, PR China
\(^b\) Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, Shandong 266071, PR China
\(^c\) Harbin Institute of Technology, Harbin, Heilongjiang 150001, PR China
\(^d\) Shandong Marine Resources and Environment Research Institute, Yantai, Shandong 264006, PR China

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**ABSTRACT**

The moon jellyfish *Aurelia coerulea* is a nuisance species around the world, and is considered highly tolerant of a wide range of environmental conditions. The recruitment success during the early life stages of scyphomedusae can have a major effect on the abundance of the adult medusa population and can contribute to jellyfish blooms. The environmental stress factors of elevated copper concentrations and reduced salinity often act simultaneously during the summer time. In this study, we investigated the combined effects of three concentrations of copper (0, 10 and 25 μg L\(^{-1}\)) and reduced salinity (from ambient 31 psu to 22 psu) on the early life stages of *A. coerulea*. We found that the swimming speed of the planula larvae of *A. coerulea* was significantly affected by the copper concentration. Planula larvae of *A. coerulea* from the highest copper concentration (25 μg L\(^{-1}\)) moved slower at than at lower copper concentrations. The results showed significant interactive effects between copper concentrations and salinity on the settlement of *A. coerulea* planulae. High copper concentration (25 μg L\(^{-1}\)) and reduced salinity (22 psu) significantly increased the settlement of *A. coerulea* planulae. Additionally, copper concentration had a significant effect on the asexual reproduction rate of *A. coerulea* polyps, which was significantly higher at the moderate copper concentration (10 μg L\(^{-1}\)). These results suggest that the current concentration of copper was not a stress factor for the early life stages of *A. coerulea* and may potentially stimulate population increases of *A. coerulea* in Chinese coastal waters.

1. **Introduction**

The moon jellyfish *Aurelia* spp. is the most common scyphozoan jellyfish in global coastal waters (Lucas, 2001). Recently, blooms of *A. coerulea* medusae have been reported in the East Asian margin seas, including the coastal waters of China, Japan, and Korea, and these blooms can negatively impact coastal power plant operations, local fisheries, and aquaculture (Dong et al., 2010; Uye, 2011; Purcell et al., 2012). Previous studies have shown that many environmental factors (e.g. temperature, pH, dissolved oxygen and food availability) affect the population dynamics of *Aurelia* spp. in various stages of their early life cycle (Lucas et al., 2012). Therefore, understanding the ways in which environmental factors affect the early life stages, including the survival, settlement and metamorphosis of planulae and the survival and asexual reproduction of polyps, is key to understanding the population dynamics of *A. coerulea* (Lucas et al., 2012; Schariri et al., 2014).

Copper is a renowned marine toxicant that is widely used in the shipping industries. Previous studies have revealed that different marine organisms have different copper tolerance, ranging from < 10 to > 10,000 μg L\(^{-1}\) (Grosell et al., 2007). Elevated concentrations of copper in coastal waters are mostly due to anthropogenic inputs from coastal runoffs and antifouling paint (Alsterberg et al., 2007; Srinivasan...
and Swain, 2007), and the highest recorded concentration of copper in the Bohai Sea was approximately 25 μg L⁻¹ (Gao et al., 2014). Heavy rainfall frequently occurs during the summer, which could cause extremely low sea surface salinity in the coastal waters. Seawater salinity could be as low as 22 psu or below during the summer in coastal sea cucumber culture ponds, where high densities of A. coerulea medusae have been reported (Yuan et al., 2010; Dong et al., 2014). Thus, the environmental stress factors of elevated copper concentrations and reduced salinity often act simultaneously during the summer time.

Sexual recruitment of A. coerulea in Chinese coastal waters mainly occurs during the summer monsoon time, when the coexisting stress factors of copper and reduced salinity are likely to occur (Gao et al., 2014; Yuan et al., 2010). Therefore, A. coerulea planulae are likely to encounter low salinity conditions and high copper concentration during the planktonic and settling periods. Several studies have investigated the effects of salinity or copper on the early life-history stages of Aurelia spp. (Lucas and Horton, 2014; Conley and Uye, 2015; Sokolowski et al., 2016; Dong et al., 2018). However, the interactive effects of copper and reduced salinity on the early life-history stages of A. coerulea have never been studied. The moon jellyfish A. coerulea is reported to be highly tolerant of a wide range of environmental conditions, including large variations in temperature, salinity and dissolved oxygen (Lucas, 2001; Thein et al., 2012). Therefore, we hypothesized that the moon jellyfish A. coerulea would also have a high tolerance to the combination of copper and salinity stress. The aim of our study was to determine how the combined effects of copper and reduced salinity affect swimming behavior and settlement of A. coerulea planulae, and asexual reproduction of A. coerulea polyps.

2. Materials and methods

2.1. Experimental organisms

Five mature A. coerulea medusae with visible planulae larvae for use in the swimming and behavior experiments were collected using a hand net at Yantai Sishili Bay, in the northern Yellow Sea, China (37°29.40′ N, 121°2.89′ E) in August 2016. Medusae in a 30 L plastic container filled with 160-μm filtered seawater (salinity 31 psu) were transported to a controlled temperature laboratory (24 °C). On the second day, medusa incubation seawater was filtered through 500-μm mesh to remove mucus and gelatinous tissue, and then the planulae were concentrated using 38-μm mesh. Thereafter, the concentrated planulae were rapidly washed with 0.45-μm filtered natural seawater and transferred to a graduated cylinder (1000 mL volume). The planulae were pipetted into a beaker with 0.45-μm filtered natural seawater (salinity 31 psu) and used in the following experiments.

For the asexual reproduction experiment, colonies of A. coerulea polyps attached to tubeworms were collected from a coastal lake in Rongcheng in April 1, 2016 and transported to the laboratory. Polyps were placed in plastic tanks filled with filtered seawater in a low-temperature incubator that provided a constant temperature of 6 °C (Yiheng, Shanghai), and they were fed newly hatched Artemia salina nauplii, twice weekly. After the polyps were fed for four hours, the seawater was replaced with filtered seawater. The polyps were maintained in the laboratory until the beginning of the experiment. The polyps of similar size were separated from the main cultures and placed in the wells of 24-well culture plates (Canvic, Shanghai) for re-attachment.

2.2. Experimental design

A factorial experimental design was conducted with two main effects: copper (Cu) doses (0, 10 and 25 μg L⁻¹) and salinity (31 psu and 22 psu). The Cu concentrations were selected because the highest recorded concentration of Cu in the Bohai Sea was approximately 25 μg L⁻¹ (Gao et al., 2014). The salinity of 31 psu represents the ambient salinity level at Sishili Bay, while the reduced salinity of 22 psu represents the lowest salinity due to high precipitation and river flow during the summer (Yuan et al., 2010).

Cu stock solutions were prepared in the laboratory by dissolving CuCl₂ (Alfa, 99.995%, metals basis, CAS: 7447-39-4, USA) in Milli-Q water. The test solutions of Cu were prepared using the Cu stock solutions and an appropriate amount of artificial seawater. The artificial seawater was prepared by mixing sea salt (Sigma, USA) and Milli-Q water, adjusted to a salinity of 31 psu. Low-salinity artificial seawater was prepared by diluting artificial seawater with a volume of Milli-Q water. The salinity was measured with a YSI-600 multiparameter water quality sonde (YSI, Yellow Springs, OH).

The equipment used in the experiments, including the graduated cylinder, 24-well plates, plastic and automated pipettes, plastic tube and Artemia tanks, were first washed in detergent and then acid washed in 1 M HCl, rinsing with Milli-Q water before each step to prevent any contamination.

2.3. Experimental procedures

The swimming behavior of A. coerulea planulae in different Cu and salinity treatments was determined using video motion analysis. 5 planulae were kept in a 10 mL plastic tube containing artificial seawater equilibrated to the respective Cu and salinity levels and maintained at 24 °C in the incubators (Shanghai Boxun incubator BSG-800) overnight. Three replicate tubes were used in each of the six treatments. After 24 h, the swimming activity of the planulae was analyzed in a temperature-controlled room at 24 °C. Five planulae from each replicate of each treatment were selected for recording. For each recording session, one planula was selected and kept in a glass aquarium (5 cm × 2 cm × 0.5 cm, with a 5 mm × 5 mm grid on the bottom) containing artificial seawater with the applicable salinity and Cu concentration. The videos were recorded using an Olympus SZX10 stereo microscope fitted with an Optec TP510 digital camera. The total number of lines crossed on the grid at the bottom of the aquarium was recorded for each 3-min sampling period as an indicator of larval swimming behavior. This procedure was repeated with each of the remaining 4 planulae from each treatment. The entire test was conducted on a single day.

The settlement assays were conducted using 24-well culture plates (Canvic, Shanghai), which were filled with 10 mL artificial seawater equilibrated to the relevant Cu and salinity levels. A 3-cm diameter polyethylene terephthalate (PET) disc was added on the water surface as a settlement substrate. A. coerulea planulae were individually transferred into each well. Four replicates, each consisting of one tray of 12 planulae, were used for each Cu concentration and salinity combination. The culture plates with planulae were then maintained in an incubator set to 24 °C with a light regime of 12-h light/12-h dark. Examinations of the number of settled planulae were conducted every two days by gently pipetting old seawater out of the rearing containers and pipetting in new artificial seawater in under the microscope.

24-well culture plates (Canvic, Shanghai) with re-attached polyps were maintained at 19 °C for one week prior to the asexual reproduction experiment. Then, the plates were filled with 10 mL artificial seawater equilibrated to the respective Cu and salinity levels. Four replicates, each containing one tray of 12 polyps, were used for each Cu and salinity combination. The culture plates with polyps were maintained at an incubator (Shanghai Boxun incubator BSG-800) that was set to 19 °C with a light regime of 12-h light/12-h dark. Polyps were fed with excess newly-hatched Artemia once a week and the artificial seawater was changed 2-4 h after feeding. Once a week during four weeks of the assay, the total number of polyps in each well was observed under the microscope.
Results from a two-way ANOVA on differences in the mean number of lines crossed and settlement (%) of *A. coerulea* planula larvae, and asexual reproduction of *A. coerulea* polyps under different copper concentrations (0, 10 and 25 μg L⁻¹) and salinity (31 psu and 22 psu) treatments.

| Source                | df  | MS     | F     | P     |
|-----------------------|-----|--------|-------|-------|
| Number of lines crossed |     |        |       |       |
| Salinity              | 1   | 90.676 | 2.660 | 0.129 |
| Cu                    | 1   | 212.302 | 6.229 | 0.014*|
| Error                 | 18  | 34.084 |       |       |
| Settlement            |     |        |       |       |
| Salinity              | 1   | 0.182  | 15.408| 0.001**|
| Cu                    | 1   | 0.194  | 16.864| 0.000**|
| Salinity * Cu         | 2   | 0.234  | 19.777| 0.000**|
| Error                 | 18  | 0.012  |       |       |
| Asexual reproduction  |     |        |       |       |
| Salinity              | 1   | 2.994  | 1.721 | 0.206 |
| Cu                    | 1   | 15.293 | 8.787 | 0.002**|
| Salinity * Cu         | 2   | 0.860  | 0.494 | 0.618 |
| Error                 | 18  | 1.740  |       |       |

df: degree of freedom; MS: mean square; F: F-value; P: P-value. Asterisks denote statistically significant differences (*: P < .05; **: P < .01).

### 2.4. Data analysis

The effects of the two factors (copper concentration and salinity) and their interactions on the swimming speed and settlement of *A. coerulea* planulae, and the asexual reproduction of *A. coerulea* polyps were examined using two-way analysis of variance (ANOVA). For all analyses, homogeneity of variances and normality of residuals were tested using the Levene and Shapiro-Wilk tests, respectively. If required, variances were homogenized using a SQRT transformation. When the analysis showed significant interactions, a one-way ANOVA was performed for each factor separately in each level from the other factor, followed by Tukey's a posteriori HSD test. All statistical analyses were performed using SPSS Statistics version 19 (IBM, Armonk, NY, USA).

### 3. Results

There were no interactive effects of copper and salinity on the swimming speed of *A. coerulea* planulae (P > .05; Table 1). Results of two-way ANOVA showed that copper concentration had a significant effect on the swimming speed of the *A. coerulea* planulae (P = .014; Table 1; Fig. 1), while salinity had no significant effect (P > .05; Table 1). *A. coerulea* planulae moved slower at the highest copper concentration (25 μg L⁻¹) than at lower copper concentrations (Fig. 1).

Significant interactive effects between copper concentrations and salinity were detected on the settlement of *A. coerulea* planulae (P < .001; Table 1). The settlement of *A. coerulea* planulae was influenced by salinity (P = .001; Table 1) and copper concentrations (P < .001; Table 1). The highest settlement rate was 63.6% ± 14.8% at the salinity of 22 psu and copper concentration of 25 μg L⁻¹ (Fig. 2).

There were no interactive effects of copper concentration and salinity on the asexual reproduction rate of *A. coerulea* planulae (P = .002; Table 1), while salinity had no significant effect on the asexual reproduction rate of *A. coerulea* polyps (P > .05; Table 1). The asexual reproduction rate of *A. coerulea* polyps was higher at the copper concentration of 10 μg L⁻¹ than at other Cu concentrations (Fig. 3).

### 4. Discussion

We investigated the combined effects of elevated copper concentrations and reduced salinity on the early life stages of the moon jellyfish *Aurelia coerulea*, which is considered a nuisance species around the world. The results presented here suggest that high copper concentration (25 μg L⁻¹) and reduced salinity (22 psu) significantly increased the settlement of *A. coerulea* planulae. Meanwhile, asexual reproduction of *A. coerulea* polyps was increased at the moderate copper concentration (10 μg L⁻¹).

Several studies have investigated the effects of seawater salinity on the survival and settlement of *Aurelia* spp. planulae (Conley and Uye, 2015; Dong et al., 2018). Conley and Uye (2015) reported that salinity was a principal factor that affected planulae dispersion and distribution of *A. coerulea* in temperate monsoon regions. Dong et al. (2018) showed that reduced salinity had a significant negative effect on the swimming behaviour and settlement of *A. coerulea* planulae. However, no studies have previously been conducted to investigate the effects of copper on *Aurelia* spp. planulae. Previous studies have shown that low or moderate copper concentration might not be a threat to the settlement of other invertebrate larvae and could have positive effects on invertebrate cells and larvae, and that this concentration range varies between species. (Watling, 1983; Reichelt-Brushett and Harrison, 2000; Ng and Keough, 2003). Results from Reichelt-Brushett and Harrison (2000) showed that copper concentrations of 2, 10 and 20 μg L⁻¹ did...
not inhibit larval settlement of the reef-building coral *Acropora tenuis*. Similarly, copper concentration of 100 μg L$^{-1}$ accelerated larval attachment of bryozoan *Watersipora subtorquata* (Ng and Keough, 2003) and 20–40 μg L$^{-1}$ of copper stimulated larval settlement of the oyster *Crasostrea gigas* (Watling, 1983). Copper concentration of 30 μg L$^{-1}$ improved cell aggregation and sped up larval settlement of the sponge *Scopalina lophyropoda* (Cebrian and Uriz, 2007). In Bohai Sea, the highest reported Cu concentration was 25 μg L$^{-1}$ (Gao et al., 2014), so it is currently a stimulating factor for settlement of *A. coerulea*, especially with reduced salinity during the summer time.

The settlement of hard-substrate invertebrates pelagic larval can be induced or inhibited by chemical or physical cues (Hadfield and Paul, 2001). Changes in water chemistry or surface-bound biotic signals released by conspecific individuals or microbial biofilms are important chemical settlement cues (McKenzie et al., 2012). Low levels of heavy metals are known to stimulate reproductive growth in colonial hydrozoans, a phenomenon termed hormesis (Lucas and Horton, 2014). Based on previous research results, we speculate that there are several possible mechanisms underlying the physiological response of *A. coerulea* planulae to copper. Previous studies have shown that copper might affect the settlement of other invertebrate larvae in different ways. For example, Cu$^{2+}$ could enter the cells via Ca$^{2+}$ channels, promoting the release of cytosolic calcium, which may be responsible for enhancement of cell aggregation and speeding up larval settlement of *Scopalina lophyropoda* (Cebrian and Uriz, 2007). Meanwhile, results from Bao et al. (2010) showed that Copper affected larval settlement of *Hydroids elegans* most readily by affecting the biofilm development process.

*A. coerulea* planulae usually settle on suitable substrate within one week, during which time they will be subject to risks of predation, offshore transport away from suitable substrates and exposure to extreme environmental conditions (Brewer, 1976; Lucas et al., 2012; Conley and Uye, 2015). Therefore, reduction of the time planulae spend seeking suitable substrata may increase the survival rate of *A. coerulea* planulae. Our results showed that the combination of the current highest concentration of copper and low salinity significantly increased the settlement rate of *A. coerulea* planulae. In addition, copper tolerance would be advantageous for *A. coerulea*. Copper-intolerant species can no longer persist in copper-polluted estuaries, allowing copper-tolerant *A. coerulea* to form colonies in a relatively low-competition habitat.

After settlement, *A. coerulea* polyps can reproduce asexually, rapidly increasing their population. Several studies have investigated the effects of salinity on the asexual reproduction of *Aurelia* spp. polyps (Purrell, 2007; Willcox et al., 2007). Results from Purrell (2007) showed that the effects of salinity on asexual reproduction by *Aurelia labiata* polyps were significant. However, asexual reproduction of *Aurelia* sp. scyphistomae in Australian waters was not found to be influenced by salinity (Willcox et al., 2007). Our results showed that asexual reproduction of *A. coerulea* polyps was not influenced by salinity. The differences in the responses of *Aurelia* sp. polyps to salinity may be attributed to the differing salinity ranges used in various studies or the different strains of *Aurelia* sp. polyps.

Only one previous study investigated the effects of copper on the asexual reproduction of *Aurelia* sp. polyps (Lucas and Horton, 2014). Lucas and Horton (2014) determined the effects of three concentrations of copper (ambient, 20 and 200 μg L$^{-1}$) on *Aurelia aurita* polyps and found increased budding at low copper concentration (20 μg L$^{-1}$) compared to the control. Our results also showed that low concentrations of copper (10 μg L$^{-1}$) increased asexual production of *A. coerulea* polyps. Similarly, results from Stebbing (2002) showed that low levels of copper (1–10 μg L$^{-1}$) stimulate reproductive growth in the colonial hydroid *Laomedea flexuosa*. Copper tolerance may be important for the proliferation of *A. coerulea* polyps because many other species have reduced reproductive success and survival in such concentrations (Lucas and Horton, 2014).

In summary, we examined the combined effects of reduced salinity and increased concentrations of copper on the early life stages of *A. coerulea*. Our results showed that significant interactive effects between copper concentrations and salinity were detected on the settlement of *A. coerulea* planulae. High copper concentration (25 μg L$^{-1}$) and reduced salinity (22 psu) significantly increased the settlement of *A. coerulea* planulae. Meanwhile, *A. coerulea* polyps asexually produced more new polyps at a copper concentration of 10 μg L$^{-1}$. Therefore, our study suggests that the current concentration of copper may be beneficial to the early life-history stages of *A. coerulea* and may potentially stimulate an increase in the population of *A. coerulea* in Chinese coastal waters.

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