Combined effects of ocean acidification and temperature on planula larvae of the moon jellyfish *Aurelia coerulea*  
Zhijun Dong*, Tingting Sun  
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**ABSTRACT**  
Rapidly rising levels of atmospheric CO₂ have caused two environmental stressors, ocean acidification and seawater temperature increases, which represent major abiotic threats to marine organisms. Here, we investigated for the first time the combined effects of ocean acidification and seawater temperature increases on the behavior, survival, and settlement of the planula larvae of *Aurelia coerulea*, which is considered a nuisance species around the world. Three pH levels (8.1, 7.7 and 7.3) and two temperature levels (24 °C and 27 °C) were used in the present study. There were no interactive effects of temperature and pH on the behavior, survival, and settlement of planula larvae of *A. coerulea*. We found that the swimming speed and mortality of the planulae of *A. coerulea* were significantly affected by temperature, and low pH significantly affected settlement. Planulae of *A. coerulea* from the elevated temperature treatment moved faster and showed higher mortality than those at the control temperature. The settlement rate of *A. coerulea* planulae was significantly higher at the pH level of 7.3 than at other pH levels. These results suggest that seawater temperature increase, rather than reduced pH, was the main stress factor affecting the survival of *A. coerulea* planulae. Overall, the planulae larvae of the common jellyfish *A. coerulea* appeared to be resistant to ocean acidification, but may be negatively affected by future seawater temperature increases.

**1. Introduction**  
Rapidly rising atmospheric carbon dioxide (CO₂) concentrations are causing ocean warming and decreasing seawater pH, which represent two important abiotic threats to marine ecosystems (Hoegh-Guldberg et al., 2007; Fabricius et al., 2011; McCulloch et al., 2012). The global ocean temperature has increased by 0.5 °C since the 1970s and an additional increase of 2.6–3.1 °C has been projected to occur by 2100 (IPCC, 2013; Rogelj et al., 2016). The average surface ocean pH has declined by approximately 0.1 units since the industrial revolution and is predicted to decrease by another 0.4 units by 2100 under a “business-as-usual” CO₂ emission scenario (IPCC, 2013; Gattuso et al., 2015).

The effects of ocean acidification and ocean warming on marine organisms, such as corals, sea urchins and mussels, have been studied extensively to predict the future population trends of these organisms (e.g., Crain et al., 2008; Byrne and Przeslawski, 2013; Kroeker et al., 2013; Wangenstein et al., 2013; Duarte et al., 2014; Garcia et al., 2015; Hu et al., 2015; Li et al., 2015; Wang et al., 2015a,b; Przeslawski et al., 2015; Wu et al., 2016). These studies indicate that the biological responses of marine organisms to the combined stressors of ocean acidification and seawater temperature increases vary across taxonomic groups, life-history stages and trophic levels (Crain et al., 2008; Byrne and Przeslawski, 2013; Harvey et al., 2013; Kroeker et al., 2013; Przeslawski et al., 2015). For example, Nguyen et al. (2012) showed that warming, not acidification, was the dominant stressor affecting development of the sea star *Meridiastra calcar*. However, results from Wangenstein et al. (2013) showed that high temperature and low pH had a positive effect on the reproduction of the sea urchin *Arbacia lixula* (Wangensteen et al., 2013). Chua et al. (2013) found that temperature and pH had negligible or no effects on the larval development of scleractinian corals *Acropora millepora* and *A. tenuis* (Chua et al., 2013).

The moon jellyfish *Aurelia coerulea* is a common scyphozoan jellyfish, found in the major warm temperate regions (e.g., East Asian Marginal Seas, the Mediterranean Sea and the Atlantic Coast of the USA) (Dawson et al., 2005; Ki et al., 2008; Dong et al., 2015; Scorrano et al., 2016). 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; therefore, it has been suggested that *A. coerulea* is categorised as a nuisance species (Dong et al., 2010; Uye, 2011; Purcell et al., 2013). On the other hand, mounting evidence indicates that this jellyfish species is preyed upon by other...
organisms and might also play an important role in the marine pelagic food web (Cardona et al., 2012; Jarman et al., 2013; Hamilton, 2016).

The early developmental stages (i.e., planula, polyp and ephyra) of *A. coerulea* are sensitive to environmental changes and are crucial to the abundance of the adult medusa population (Lucas et al., 2012). Many research efforts have been conducted to address the impacts of seawater temperature (Liu et al., 2009; Schiariti et al., 2014; Pascual et al., 2015; Wang et al., 2015a,b) or pH conditions (Winans and Purcell, 2010; Tills et al., 2016) on the polyp and ephyra stages of the moon jellyfish *Aurelia* spp.

The pelagic larvae of marine invertebrates are thought to be vulnerable to predators, physical and chemical stress (Pechenik, 1999). Additionally, there has been growing evidence that ocean acidification and elevated seawater temperatures influence the survival and settlement of pelagic larvae of other marine invertebrate species (reviewed in Gibson et al., 2011).

*Aurelia* spp. planulae, released by mature female medusae, usually settle on suitable substrate within one week (Brewer, 1978; Conley and Uye, 2015). Hence, the pre-settlement survival and settlement of planula larvae is crucial for the establishment of new polyp populations (Webster and Lucas, 2012; Gambill et al., 2016). During this stage, environmental factors (i.e., temperature, salinity, light and dissolved oxygen), substrate properties (physical properties and bacterial biofilms), and biological factors (conspecifics, competitors and predators) may affect the survival, settlement and metamorphosis of *Aurelia* spp. planulae (Lucas et al., 2012). Several recent studies have reported how water temperature and salinity influence the survival and settlement of *Aurelia* spp. planulae (Webster and Lucas, 2012; Conley and Uye, 2015). However, previous work has not determined the impact of ocean warming and reduced pH conditions on the planula larvae of scyphozoan jellyfish.

Ocean acidification and ocean warming can act in a combined manner, affecting the physiological progress of marine organisms. Studies have shown that elevated seawater temperature can either exacerbate the negative effects of ocean acidification on marine organisms (Rodolfo-Metalpa et al., 2011; Wu et al., 2016) or mitigate the negative effects (García et al., 2015). The aim of this study was to evaluate for the first time the combined effects of ocean acidification and seawater temperature increases on the behavior, survival, and settlement of planula larvae of the moon jellyfish *A. coerulea*. *A. coerulea* is recognized as a highly tolerant species to environmental stressors, including temperature, salinity and oxygen conditions (Lucas et al., 2012). Therefore, we hypothesized that the elevated seawater temperature and reduced pH levels would not influence the behavior, survival, or settlement of planula larvae of the moon jellyfish *A. coerulea*.

2. Materials and methods

2.1. *Aurelia coerulea* collection and planulae cultivation

The planulae of *A. coerulea* were obtained following the methods of Conley and Uye (2015). Five mature *A. coerulea* medusae with visible planulae were collected with a hand net at Sishili Bay, northern Yellow Sea, China (37°29.40′ N; 121°2.89′ E) in August. Medusae were maintained in a 30 L plastic container filled with 160-μm-filtered seawater (salinity 31 psu) and transported to a controlled temperature laboratory (24 °C). On the second day, medusa incubation seawater was filtered through a 500-μm mesh to remove mucus and gelatinous tissue, and the planulae were concentrated with the use of a 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.

2.2. Experimental design and procedures

A factorial experimental design was conducted with two main effects: pH (8.1, 7.7 and 7.3) and temperature (24 and 27 °C), for a total of six treatments. The pH of 8.1 was chosen to represent the current pH level in the Yantai Sishili Bay, northern Yellow Sea, China. It is predicted that the pH of surface seawater could fall by up to 0.4 U by 2100 and 0.8 U by 2300 (Caldeira and Wickett, 2003; Orr et al., 2005). Therefore, 7.7 and 7.3 were chosen as intermediate and extreme low pH levels. 24 °C is the average seawater temperature during the month of August, when *A. coerulea* are most sexually active in the Yantai Sishili Bay. The elevated temperature used (+3 °C, 27 °C) represents the predicted future sea surface temperature during the month of August at this site by 2100 (IPCC, 2013). The temperatures were controlled using incubators (BSG-800, Boxun, Shanghai). Target pH levels were achieved by adding exactly equal amounts of ultra-pure HCl and NaHCO₃, which is chemically equivalent to adding CO₂ (Shi et al., 2009; Gattuso et al., 2010).

To determine the effects of different treatments on the swimming behavior of *A. coerulea* planulae, video motion analysis was performed following the protocol of Lonnstedt and Eklov (2016), with minor revisions. Briefly, 5 planulae were randomly selected and kept in a 10 ml plastic tube containing 0.45-μm-filtered seawater equilibrated to the respective pH levels. Three replicate tubes were used in each of the six treatments. Then, the tubes with planulae were maintained overnight in two different incubators that were set to 24 and 27 °C. After 24 h, the swimming activity of the planulae was analyzed in a temperature-controlled room that was set to 24 or 27 °C, according to the treatment. One planula was randomly selected and kept in a glass aquarium (20 × 20 cm; 5 mm × 5 mm grid drawn on the bottom), under the same pH conditions as the seawater in the plastic tubes. Videos were recorded using an Olympus SZX10 stereo microscope fitted with an Optec TP510 digital camera. The total number of lines crossed on a grid at the bottom of the aquarium (5 × 5 mm) was recorded for each 3-min sampling period and served as an indicator of larval swimming behavior. This procedure was then repeated for the remaining 4 planulae from each treatment. The entire test was conducted on a single day.

The settlement assays were conducted using 24 culture plates (24-well plates; Canvic, Shanghai). Each of the wells was filled with 10 ml of 0.45-μm-filtered seawater equilibrated to the respective pH levels. *A. coerulea* planulae were individually transferred into each well. A 3-cm-diameter polyethylene terephthalate (PET) disc was placed on the water surface as a settlement substrate. Four replicates, each containing one tray of 24 planulae, were used for each temperature and pH combination. The culture plates with planulae were then maintained in two different incubators, which were set to 24 and 27 °C with a light regime of 12 h light/12 h dark. Examinations of the number of dead and settled planulae were conducted every 24 h for a period of 7 days. To determine the effects of different treatments on the morphology of *A. coerulea* planulae, two planulae were sampled randomly from each replicate and measured at both the beginning and end of the experiment. The length and width of the sampled planulae larvae were measured using an Olympus SZX10 stereo microscope fitted with an Optec TP510 digital camera. The planulae larval sizes were estimated using the formula for an ellipsoid \( [4/3 \times \pi \times (\text{length}/2 \times (\text{width}/2)^2] \).

2.3. Seawater chemistry

The seawater was changed every two days by gently pipetting old seawater out of the rearing containers and pipetting in 0.45-μm-filtered seawater equilibrated to the respective pH levels under the microscope. The seawater salinity, pH and total alkalinity were measured in the filtered seawater samples at the beginning of the experiment and at each subsequent water exchange. The seawater pH was measured using a calibrated PHS-3C pH meter (INESA, Shanghai). Total alkalinity (TA) was measured by Gran titration with 0.1 N HCl using an automated
The temperature and salinity were measured with a YSI-600 multiparameter water quality sonde (YSI, Yellow Springs, OH). All other related carbonate system parameters (pCO₂, HCO₃⁻, and CO₃²⁻) were calculated using the software CO₂ SYS (Pierrot et al., 2006).

2.4. Data analysis

The effects of the two factors (temperature and pH) and their interactions on the swimming speed, settlement, mortality and size of A. coerulea planulae were analyzed with two-way ANOVA. For all analyses, the homogeneity of variances and normality of residuals were assessed with Levene's and Shapiro-Wilk tests, respectively. Data on number of lines crossed per three minutes was square root transformed to improve the homogeneity. Multiple comparisons were carried out using Tukey's a posteriori HSD test on each factor that showed significant differences (Underwood, 1997). All statistical analyses were performed using SPSS statistics software, version 19 (IBM, Armonk, NY, USA).

3. Results

3.1. Seawater chemistry

Mean (± SD) values of carbonate system parameters in various pH treatments are shown in Table 1. The pH values measured in the filtered seawater samples were close to the target pH (within 0.03 units, Table 1). Total alkalinity remained relatively constant across different pH treatments.

3.2. Behavior, survival and settlement of A. coerulea planula larvae

The effect of the temperature-pH interaction on the swimming speed of A. coerulea planulae is shown in Fig. 1. The change in the larval size of A. coerulea planulae is shown in Fig. 2. The day-to-day settlement and mortality of A. coerulea planulae under different temperature-pH combinations are illustrated in Fig. 3 and Fig. 4, respectively. No statistically significant interactive effects between temperature and pH were detected on the swimming speed, settlement, mortality, and size of A. coerulea planulae (P = 0.218, P = 0.293, P = 0.594, P = 0.299, respectively; Table 2).

The results from the two-way ANOVA analysis indicate that temperature had a significant effect on the swimming speed of the A. coerulea planulae (P = 0.024; Table 2; Fig. 1), while pH had no significant effect (P = 0.207; Table 2). A. coerulea planulae moved more rapidly at 27 °C than at 24 °C (Fig. 1). The mean number of lines crossed by the planulae was 15 ± 7 (± SD) at 24 °C and 23 ± 3 (± SD) at 27 °C.

Results from the two-way ANOVA revealed that pH significantly affected the larval size of A. coerulea over the duration of 7 days (P = 0.047; Table 2; Fig. 2). Larval sizes were smaller at low pH conditions (7.3) than at ambient pH conditions (8.1) (Fig. 2). On day 7, the larval size of A. coerulea planulae was 0.0023 ± 0.0007 mm³ (± SD), 0.0019 ± 0.0005 mm³ (± SD) at pH 7.7, and 0.0017 ± 0.0006 mm³ (± SD) at pH 7.3.

The results from the two-way ANOVA showed that temperature had a significant effect on the mortality of A. coerulea planulae (P = 0.009; Table 2), while pH had no significant effect (P = 0.158; Table 2). On day 7, the mortality of A. coerulea planulae was higher at the elevated temperature of 27 °C than at the ambient temperature (24 °C) (Fig. 3).

The settlement of A. coerulea planulae was significantly influenced by the pH level (P = 0.001; Table 2), while temperature had no significant effect (P = 0.413; Table 2). On day 7, the settlement rate of A. coerulea planulae was significantly higher at the pH level of 7.3 than at other pH levels (Fig. 4). On day 7, the settlement rate was 68% ± 14% (± SD) at pH 7.3, 41% ± 17% (± SD) at pH 7.7, and 39% ± 12% (± SD) at pH 8.1.

4. Discussion

Previous studies suggest that larvae that calcify are sensitive to both warming and acidification, whereas those that do not calcify are more sensitive to warming (Byrne and Przeslawski, 2013; Kroeker et al., 2013). Our results are consistent with the hypothesis that seawater temperature increase, rather than reduced pH, is the main stress factor affecting the survival of noncalcifying A. coerulea planulae. Elevated seawater temperatures have been confirmed to reduce the survival of the pelagic larvae of corals, sea urchins and oysters (e.g., Negri et al., 2007; Randall and Sz mant, 2009; Gibson et al., 2011; Nguyen et al., 2012; Putnam et al., 2013). Negri et al. (2007) hypothesized that the high mortality of pelagic larvae at elevated temperatures was due to the impairment of functional enzymes and proteins. Results from Putnam et al. (2013) showed that respiration and the expression of a protein critical to photosynthesis and carbon fixation were both significantly depressed in Pocillopora damicornis larvae exposed to elevated temperatures. Similar molecular mechanisms may be at work in A. coerulea planulae experiencing stress due to high temperatures, although further studies are needed to confirm this hypothesis.

The pelagic larvae of marine invertebrates are triggered to settle by external cues, typically including chemical cues from the water column, appropriate substrata, prey species or conspecific individuals (Hadfield, 2000). Other environmental factors, such as temperature, salinity, pH and dissolved oxygen, can also influence the settlement of pelagic larvae (e.g., Cigliano et al., 2010; Holst and Jarms, 2010; Doropoulos et al., 2012; Webster and Lucas, 2012; Doropoulos and Diaz-Pulido, 2013; Conley and Uye, 2015). Several recent studies have examined the impacts of environmental factors (e.g., temperature and salinity) on the planula larvae of scyphozoans (Holst and Jarms, 2010; Webster and Lucas, 2012; Conley and Uye, 2015). Webster and Lucas (2012) studied the effects of three temperatures (6 °C, 12 °C and 18 °C) on the settlement of Aurelia aurita planulae in southern England and found that significantly fewer A. aurita planulae settled when exposed to the

Table 1

Setting seawater pH, measured pH, salinity, total alkalinity (TA, μmol kg⁻¹) and the concentration of pCO₂ (μatm), CO₃²⁻ (μmol kg⁻¹), HCO₃⁻ (μmol kg⁻¹) in seawater in each group (n = 4; Mean ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental treatments: temperature and pH</th>
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<tbody>
<tr>
<td></td>
<td>pH 8.1</td>
</tr>
<tr>
<td></td>
<td>24 °C</td>
</tr>
<tr>
<td>pH (Measured)</td>
<td>8.10 ± 0.02</td>
</tr>
<tr>
<td>Salinity</td>
<td>33.55 ± 0.24</td>
</tr>
<tr>
<td>TA (μmol kg⁻¹)</td>
<td>2486 ± 38</td>
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<tr>
<td>pCO₂ (μatm)</td>
<td>397.5 ± 11.8</td>
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<tr>
<td>CO₃²⁻ (μmol kg⁻¹)</td>
<td>241.8 ± 7.6</td>
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<tr>
<td>HCO₃⁻ (μmol kg⁻¹)</td>
<td>1905.8 ± 59.8</td>
</tr>
</tbody>
</table>
highest temperature (Webster and Lucas, 2012). However, the settlement of *A. coerulea* planulae appeared not to be affected by moderate (+3 °C) increases in temperature.

Our results show that the settlement of *A. coerulea* planulae was significantly higher at the pH level of 7.3 than at higher pH levels. This suggests that *A. coerulea* planula larvae will be able to cope with future severe acidic conditions through rapid settlement. The planula larvae of *A. coerulea* are small, lecithotropic and sensitive to environmental stress (Webster and Lucas, 2012; Conley and Uye, 2015). The smaller size of *A. coerulea* planulae at low pH conditions suggested that they might spend more energy against the acid conditions. Thus, the rapid transition from pelagic to benthic stages may allow for ecological adaptation under environmental stress. Previous studies have shown that the response of marine invertebrate larvae to ocean acidification was species-specific (Talmage and Gobler, 2009; Cigliano et al., 2010). Most marine invertebrate larvae showed decreased settlement success when exposed to acidic conditions (Talmage and Gobler, 2009; Cigliano et al., 2010). For example, results from Cigliano et al. (2010) showed calcareous foraminifers, serpulid polychaetes, gastropods and bivalves showed highly significant reductions in recruitment when exposed to severely acidified conditions (pH 7.08–7.79). The settlement of the *Acropora selago* declined as pCO2 increased (705 and 1214 μatm pCO2) with three crustose coralline algae (Doropoulos and Diaz-Pulido, 2013). Delayed metamorphosis due to exposure to acidic waters (pH 7.50) was also found in 3 species of marine bivalves: *Crassostrea virginica*, *Argopecten irradians*, and *Mercenaria mercenaria* (Talmage and Gobler, 2009). However, some species of marine invertebrate larvae showed high settlement success under acidic conditions (Cigliano et al., 2010). For example, the syllid polychaete *Syllis prolifera* was more abundant in the most acidified conditions tested (pH 7.08–7.79) (Cigliano et al., 2010). Our study confirmed that the planula larvae of the moon jellyfish *A. coerulea* may be better adapted to the threats of ocean acidification than many other invertebrates. In addition, Albright et al. (2010) indicated that the acidification of seawater might alter the community composition of the substrata during the conditioning phase, thereby altering the biological and chemical cues responsible for
settlement. Therefore, we cannot exclude the possibility that exposure to low pH levels may also alter the bacterial communities associated with the substrate, which are important for the settlement of invertebrate pelagic larvae.

The recruitment success during the early life stages (i.e., planula, polyp and ephyra) of Aurelia spp. can have a major effect on the abundance of the adult medusa population and can contribute to jellyfish blooms (Lucas et al., 2012). No studies have been conducted to investigate the combined effects of ocean acidification and elevated seawater temperature on the other life stages of Aurelia spp. The responses of different life stages of Aurelia spp. to each of these factors individually (e.g. ocean acidification or elevated seawater temperature) were varied across life stages (Winans and Purcell, 2010; Schiariti et al., 2014; Pascual et al., 2015; Wang et al., 2015a,b; Tills et al., 2016). For example, the polyps of Aurelia spp. showed high tolerance to low pH conditions, surviving and reproducing asexually at the lowest tested pH (Winans and Purcell, 2010). However, the swimming activities of Aurelia spp. ephyrae were inhibited under elevated pCO2 or reduced pH levels (Tills et al., 2016). In addition, warm seawater temperatures increased the asexual production of Aurelia spp. polyps in France, Germany, Spain, Japan, and China (Schiariti et al., 2014; Pascual et al., 2015; Wang et al., 2015a,b). Our results showed that elevated seawater temperatures reduced the survival of the A. coerulea planula larvae. These different responses among the life stages suggest that sensitivity to environmental stress is related to life stages. Thus, a single study integrating all life stages is needed to determine the effects of ocean acidification and ocean warming on the scyphozoan jellyfish, in order to predict the population size trends of this blooming scyphozoan in the context of future climate change.

In summary, our study was the first to address the impact of ocean acidification and seawater temperature increases on the planula larvae of scyphozoans. No interactive effects of ocean warming and decreased pH levels on the survival and settlement of planula larvae in A. coerulea were found in our study. Our study suggests that A. coerulea planula larvae can cope well with the decreased pH conditions through rapid settlement, but cope poorly with elevated seawater temperature. Elevated seawater temperature appears to be a crucial stress factor for the planula larvae of A. coerulea. Future studies are needed to understand the mechanisms behind the influence of ocean acidification and elevated seawater temperature on the planula larvae of A. coerulea.

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