Note

The biogenic reefs formed by the alien polychaete Hydroides dianthus (Serpulidae, Annelida) favor the polyp stage of Aurelia coerulea (Cnidaria, Scyphozoa) in a coastal artificial lake

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A B S T R A C T

Blooms of the moon jellyfish Aurelia coerulea frequently occur in coastal waters. The increased availability of substrates for the settlement and proliferation of polyps due to the expansion of artificial structures in coastal areas has been proposed as a possible contributing factor in jellyfish blooms. This paper investigates whether a marine artificial lake (Fenghuang Lake) provides additional substrates for A. coerulea polyps and contributes to jellyfish blooms. High densities of A. coerulea ephyrae were discovered in this lake, with a mean density of 41 individuals/m² and a maximum measured density of 128 individuals/m². Meanwhile, A. coerulea ephyrae were also found in the two emptying channels outside the lake, with a mean density of 13 individuals/m². Underwater surveys revealed that dense colonies of A. coerulea polyps occurred mainly on biogenic reefs formed by a polychaete, which was identified as an invasive serpulid species Hydroides dianthus, based on the phylogenetic analysis of mitochondrial COI gene sequences. Our study highlights the potential modification of habitats by the alien polychaete H. dianthus, which might provide complex benthic habitats suitable for the settlement and proliferation of A. coerulea polyps and may contribute to jellyfish blooms in the marine artificial lake and nearby coastal waters.

1. Introduction

The moon jellyfish (Aurelia spp.) is the most common scyphozoan jellyfish, with a wide geographic distribution (Lucas, 2001). Blooms of Aurelia spp. occur frequently in the harbors, marine lakes and coastal waters, negatively affecting coastal power plant operations, local fisheries and aquaculture (Dong et al., 2010; Uye, 2011; Dong et al., 2014; Purcell et al., 2013). Previous case studies have indicated that coastal eutrophication, increased artificial structures for jellyfish larval settlement, increased seawater temperature, and decreased predation of jellyfish due to overfishing could be important contributors to the Aurelia spp. blooms (Araki, 2001; Lo et al., 2008; Richardson et al., 2009; Purcell, 2012; Duarte et al., 2012).

Aurelia spp. has a metagenetic life cycle, alternating between a sexual reproduction phase and an asexual reproduction phase (Lucas, 2001). Sexually mature medusae of Aurelia spp. produce pelagic planulae, which settle on hard substrates and develop into sessile polyps. After settlement, polyps of Aurelia spp. can reproduce asexually, rapidly increasing their population size. For example, one polyp of A. aurita (Linnaeus, 1758) can produce approximately 100 polyps during a 201-day culturing period (Pascual et al., 2015). During a strobilation event, one polyp of A. aurita can release as many as 40 ephyrae (Lucas, 2001). Therefore, the recruitment success during the asexual reproduction stage affects the population size of medusae and is a key stage in the formation of jellyfish blooms (Lucas et al., 2012).

The population dynamics of A. coerulea (von Lendenfeld, 1884) medusae in Chinese coastal waters have been well studied (Dong et al., 2012; Wan and Zhang, 2012; Dong et al., 2014; Wang and Sun, 2015). In Chinese temperate coastal waters, A. coerulea medusae are mainly found between April and September (Dong et al., 2014), while ephyrae of A. coerulea are mainly found in coastal waters during the early spring (Wan and Zhang, 2012). However, information on the population dynamics of in-situ polyps in Chinese coastal waters is relatively scarce. Therefore, it is difficult to reliably predict A. coerulea blooms, due to the challenges in locating the natural habitat of the polyps (Boero et al., 2008; Willcox et al., 2008; Purcell et al., 2009; Ceb and Riscos, 2017).

Previous studies have revealed that polyps of Aurelia spp. mainly settle on the undersides of artificial structures, including floating docks, buoys and piers, because artificial structures provide suitably shaded substrates for the settlement and proliferation of polyps (Duarte et al.,...
Increased human activities have had a large impact on coastal areas of China. Harbor construction and aquaculture in coastal areas use numerous artificial structures that may provide substrates for A. coerulea polyps. Fenghuang Lake is an artificial marine lake with a concrete dam located on the coast of the Northern Yellow Sea, in which we found high densities of A. coerulea medusae in summer. Thus, we hypothesized that the increased artificial structures in this artificial lake would provide additional suitable substrates for the settlement of the moon jellyfish A. coerulea and contribute to the blooms in the marine artificial lake and nearby coastal waters. Therefore, the possible occurrence and distribution of A. coerulea ephyrae and polyps in this coastal artificial lake were investigated in our present study.

2. Materials and methods

2.1. Study area

This study was conducted in Fenghuang Lake, a coastal marine lake located in Shidao Bay on the Northern Yellow Sea, China (36°55′ N 122°24′ E; Fig. 1). It covers an area of 1.39 km² and has an average depth of about five meters (Zhang et al., 2007). The dam of Fenghuang Lake is made of concrete. One intake valve and two emptying valves are used to exchange seawater with Shidao Bay (Fig. 1). Fenghuang Lake was used for sea cucumber aquaculture (Apostichopus japonicas) from 2007 until 2010, and since 2010 has been used for landscape tourism and recreational fishing. Dense blooms of A. coerulea medusae often occur in the lake in summer, averaging approximately 10.5 individuals/m³.

2.2. Distribution of ephyrae

Five stations (E1-E5) in Fenghuang Lake and six stations (O1-O6) in the two emptying channels outside the lake were selected for surveys of ephyrae (Fig. 1). In May 2015, the ephyrae and zooplankton samples were collected using a plankton net (31.6 cm mouth diameter and 160 um mesh size) equipped with a flow meter (Hydrobio, Germany). At each station, all samples were hauled vertically from near the bottom to the surface of the seawater and preserved in 4% formalin. In the laboratory, zooplankton samples were counted and sorted into species. The ephyrae were identified according to the morphological characteristics described in previous studies (Straehler-Pohl and Jarms, 2010; Dong et al., 2017). The ephyrae were counted using an Olympus SZX10 stereo microscope fitted with an Optec TP510 digital camera. The densities of A. coerulea ephyrae and polyps in this coastal artificial lake were investigated in our present study.
polyps was the target for all of the identifications of polychaete colonies. The habitat of polyp colonies was determined by eye for the whole substrate using a scuba diver swam approximately 10 cm above the transect lines with the camera lens pointing directly downward and approximately 0.2 m in width was surveyed along each transect line. The software program A. coerulea polyps. The number of A. coerulea polyp colonies in each habitat were recorded. The percent coverage of polyp colonies was determined by eye for the whole substrate using intervals of 5% (Toyokawa et al., 2011). The habitat of A. coerulea polyps was the target for all of the identified frames. In our study, four main habitat categories were identified: (1) polychaete reefs; (2) metals; (3) plastic nets; (4) soft muds.

### 2.4. Identification of the reef-forming polychaete

Three serpulid specimens were randomly collected, preserved in 95% ethanol and stored at −20 °C until DNA extraction. Total genomic DNA was extracted from the specimens using the TIANamp Marine Animals DNA Kit (TIANGEN, Beijing, China), following the manufacturer’s protocol. The mitochondrial COI fragments were amplified using the primers Hydro-COIF (CWRTWRTKACDGTKCATGCTA) and Hydro-COIR (CMRYAGGWTSAAAARACCTAGTA) under the PCR conditions previously described in Sun et al. (2012). PCR-amplified DNA fragments were purified and sequenced with an ABI 3730 automatic DNA sequencer at Sangon Biotech Co., Ltd. (Shanghai, China) using the primers described above. All PCR products were sequenced in both directions to ensure acquisition of accurate sequences. The DNA sequence fragments were verified, edited and assembled with BioEdit 7.0 (Hall, 2005). The sequences were blasted in NCBI to confirm their identities. Additionally, related sequences were obtained from GenBank for phylogenetic analyses. Neighbor joining analysis of COI data was performed using the K80 model with 1000 bootstrap replicates. Phylogenetic analyses were conducted with MEGA 5.0 (Ballard and Melvin, 2010).

### 3. Results

#### 3.1. Distribution of ephyrae

Based on morphological characteristics, all the sampled ephyrae were identified as A. coerulea (Strachler-Pohl and Jarms, 2010; Dong et al., 2017). The mean density of ephyrae in Fenghuang Lake was 41 ± 49 individuals/m² (mean ± SD; N = 5). The highest density of ephyrae occurred at station E1 with a density of 128 individuals/m². A. coerulea ephyrae were also found in the two emptying channels outside the lake. The mean density of ephyrae in the two emptying channels was 13 ± 16 individuals/m³ (mean ± SD; N = 6).

At the time of sampling, the seawater temperature was 16.7 °C and the salinity was 33.0. The mean abundance of zooplankton was 93,068 ± 60,614 individuals/m³ (mean ± SD; N = 5). Polychaete larvae was the dominant organism in the zooplankton samples, with a mean density of 57,800 ± 40,531 individuals/m³ (mean ± SD; N = 5). The mean Chl a concentration was 13.23 ± 5.65 μg/L (mean ± SD; N = 5). The dissolved inorganic nitrogen, dissolved inorganic phosphate and dissolved silicate concentrations were 7.0 ± 8.59 μM, 1.05 ± 0.49 μM, and 4.48 ± 1.10 μM respectively (mean ± SD; N = 5). No significant relationships between the density of A. coerulea ephyrae and zooplankton density, Chl a concentration or nutrient concentration were detected in this study.

#### 3.2. Distribution of polyps

In order to identify the scyphozoan polyps, colonies of polyps were sampled and maintained in the laboratory until the release of ephyrae, all of which were identified as A. coerulea. Therefore, we treated all scyphozoan polyps in this study as A. coerulea polyps. The number of polyp colonies, percent coverage, and type of substrate for each transect site can be found in Table 1. Fig. 2 shows the various substrates and colonies of A. coerulea polyps found in Fenghuang Lake. Colonies of A. coerulea polyps were only found on the biogenic reefs formed by a polychaete; no colonies were found in the transects near the soft muds, plastic nets and metal dams (Table 1). The coverage of A. coerulea polyps was highest in the transect P1 (5%–80%, median 25%).

#### 3.3. Identification of polychaete

The three partial sequences of mitochondrial COI genes were identical. The sequence was deposited in GenBank under accession number KY605381. A BLAST search of the GenBank database revealed that the mtDNA COI sequences determined in this study were identical to COI sequences of Hydroides dianthus from the east coast of the USA (KU051460) and the Mediterranean (KY386656). Phylogenetic analysis of aligned 380-bp mtDNA COI sequences also indicated that the polychaetes sampled in this study were closely related to Hydroides dianthus; this was supported by a 100% bootstrap value in neighbor joining trees (Fig. 3).

### 4. Discussion

The calcareous tubeworm Hydroides dianthus is one of the most well-documented invasive foulers (Sun and Yang, 2000; Link et al., 2009; Sun et al., 2017). H. dianthus was originally described from individuals on the coast of Massachusetts, USA (Verrill, 1873). However, Sun et al. (2017) argue that it might be a native Mediterranean species. This species was then introduced to West Africa, Europe and East Asia (Sun et al., 2017).
A recent study, based on the molecular genetic analysis of mtDNA COI sequences from 17 locations around the world, revealed two cryptic species (Sun et al., 2017). The polychaetes sampled in our study were identical to the *H. dianthus* collected in the coast of USA, Italy, Brazil, and Japan. In Chinese coastal waters, *H. dianthus* was first reported on scallop aquaculture farms in Dalian Bay (Sun and Yang, 2000). The arrival of *H. dianthus* in Rongcheng may be the result of a spread of individuals from scallop aquaculture farms to natural habits (Sun et al., 2017).

Tube dwelling polychaetes, such as *Serpula vermicularis*, *Ficopomatus enigmaticus* and *H. dianthus* can produce dense aggregations of calcareous tubes, forming substantial structures that are considered to be
biogenic reefs (Bianchi and Morri, 2001; Moore et al., 2003; Poloczanska et al., 2004; Chapman et al., 2012). Our study found that dense aggregations of H. dianthus formed patch reefs above the concrete dam. This is the first report of biogenic reefs formed by the invasive polychaete H. dianthus in Chinese coastal waters. H. dianthus has also been reported to build small reefs in the lagoon of Orbetello, Italy (Bianchi and Morri, 2001). Biogenic reefs formed by polychaetes have been reported to support a rich and diverse community, which utilizes the habitat for a substrate, a food source and for refuge (Haines and Maurer, 1980; Rabaut et al., 2009; Haanes and Gulliksen, 2011; Chapman et al., 2012). Our results indicated that the biogenic reefs formed by the alien polychaete H. dianthus also provided complex benthic habitats suitable for the settlement and proliferation of A. coerulea polys.

Tubeworms have also previously been reported as suitable substrates for the settlement of Aurelia spp. especially in soft-sediment environments (Miyake et al., 2002; Miyake et al., 2004). For example, Aurelia spp. polys were observed on polychaete tubes in Kagoshima Bay (Miyake et al., 2002) and polyps of Sanderia malayensis and Aurelia aurita were found attached to the siboglinid polychaete Lamellibrachia satsuma at depths of 80–105 m in Kagoshima Bay, Japan (Miyake et al., 2004). The bottom sediment of the Yellow Sea is mainly composed of sand, mud and mixed sediment and is unsuitable for the settlement of A. coerulea planulae larvae (Chen and Zhu, 2012; Duarte et al., 2012). Therefore, biogenic reefs formed by H. dianthus may provide additional suitable substrates for the settlement of A. coerulea polys.

The habitat modifications of the alien polychaete H. dianthus result in increased habitat complexity and heterogeneity (Haines and Maurer, 1980; Link et al., 2009). Biogenic reefs formed by H. dianthus form complex three-dimensional structures, providing abundant hidden and shaded areas for the settlement of A. coerulea planulae. Previous studies have suggested that Aurelia spp. planulae prefer to settle on the undersides of artificial structures with low light intensity (Miyake et al., 2002; Purcell et al., 2009; Duarte et al., 2012; Malej et al., 2012). Miyake et al. (2002) found that no polyps of Aurelia spp. were attached to the upper or vertical sides of artificial structures. In comparison to the vertical concrete dam walls, habitat modifications by H. dianthus decrease the light intensity and provide more shaded areas of substrate for the settlement of A. coerulea planulae.

Artificial structures including plastic nets and metals have been suggested as suitable substrates for the settlement of Aurelia spp. planulae (Holst and Jarms, 2007; Duarte et al., 2012; Marques et al., 2015). However, no polyps were found on the plastic nets and metal dams near the intake and emptying valves of Fenghuang Lake, which suggests that Aurelia spp. planulae cannot settle on the substrate in areas with strongly flowing water. Biogenic reefs can stabilize underlying substrates against erosion and influence the flow of water (Reise, 2002), and may therefore provide a stable environment for the settlement and proliferation of A. coerulea polys.

Diverse assemblages of vertebrate and invertebrate fauna associated with biogenic reefs frequently serve as a food source for predators and grazers (Witman et al., 2003; De Smet et al., 2015). In addition, our study highlighted the potential modification of habitats by the alien polychaete H. dianthus, which might provide a suitable substrate for A. coerulea polys (Rabaut et al., 2009; Chapman et al., 2012). The high density of A. coerulea ephyrae found in this artificial lake during the spring may contribute to the blooms of A. coerulea in nearby coastal waters due to the outflow of ephyrae into Shidao Bay.

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