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Effects of temperature and salinity on *Ruppia sinensis* seed germination, seedling establishment, and seedling growth

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ABSTRACT

As typical submerged aquatic vegetation, *Ruppia* species are facing population reductions due to anthropogenic impacts. In this study, we investigated the effects of temperature and salinity on seed germination and seedling establishment of *Ruppia sinensis* seeds collected from northern China. The effects of seven salinities (0–50) and six water temperatures (0–30 °C) on seed germination were investigated to identify the environmental conditions that could potentially limit survival and growth. We found that: 1) optimum seed germination was salinity 5 at 30 °C; 2) high salinity (salinity 40–50) and low temperature (0 °C) significantly inhibited seed germination; 3) seed germination with increasing temperature showed a bimodal pattern at suitable salinities (5–10); 4) storing seeds at high salinities (40–50) or low temperature (0 °C) promoted germination after transferal to optimal germination conditions. These findings may serve as useful information for *R. sinensis* habitat establishment and restoration programs.

1. Introduction

Seagrass meadows are extremely valuable coastal marine ecosystems, comprising prominent marine foundation species widely acknowledged for their ecological and economic importance (Costanza et al., 1997; Barbier et al., 2011; Liu et al., 2013; Tol et al., 2016). The latest research shows that seagrass meadows not only provide a penaeid nursery habitat in wave dominated estuaries (Taylor et al., 2017), but also reduce human, fish, and invertebrate bacterial pathogen exposure (Lamb et al., 2017). However, seagrass meadows are degenerating at an alarming rate globally due to anthropogenic influences (Orth et al., 2006; Waycott et al., 2009; Short et al., 2011). Effective management and active restoration programs are therefore becoming increasingly important (Orth et al., 2006; Lefcheck et al., 2017).

Ruppia is a globally distributed submerged aquatic vegetation genus (Bigley and Harrison, 1986; Ito et al., 2015; Jacobs and Brock, 1982; Mannino et al., 2015; Murcia et al., 2015; Rodrigues et al., 2009). In addition to representing a food source for water fowl (Charalambidou et al., 2003; Triest and Sierens, 2014) and providing a spawning and nursery habitat for fish (Whitfield, 2017), it also improves the quality of the sediment environment (Strazisar et al., 2015). With increases in the disturbance of coastal ecosystems, the biotic environment of coastal communities has been greatly affected (Waycott et al., 2009); changing environmental conditions are leading to instability in *Ruppia* population size (Menendez, 2009; Strazisar et al., 2016). In temperate China, farmers often occupy the *Ruppia* habitats to culture shrimps, and the *Ruppia* meadows have been facing degeneration. The restoration effort should be paid attention to. Although numerous studies on restoring *Zostera* seagrasses have been conducted over the past several years in China (e.g. Liu et al., 2013; Zhang et al., 2014, 2015a; Zhou et al., 2014, 2015; Lin et al., 2016; Xu et al., 2017), relatively little research has been performed on *Ruppia*.

As a result of the large phenotypic differences among populations in different habitats, the classification and identification of *Ruppia* solely based on morphology have long been controversial (Verhoeven, 1979; Brock, 1982a, 1982b). According to Short et al. (2011) the genus *Ruppia* comprises six species, i.e. *R. maritima, R. cirrhosa, R. tuberosa, R. megacarpa*, *R. polycarpa,* and *R. filifolia*. With the development of molecular classification methods, taxonomists have constantly improved and developed the classification system of *Ruppia*, involving the

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addition of new species (Den Hartog et al., 2016; Martinez-Garrido et al., 2016). *Ruppia* has been documented across China after a nationwide survey (Yu and den Hartog, 2014). In this survey, both the molecular genetics and morphology of *Ruppia* in China were found to be completely different from the species found elsewhere globally. This led to the discovery of two new species, which are widely distributed in China, including *R. brevipedunculata* distributed in southern China and *R. sinensis* distributed in northern China (Yu and den Hartog, 2014). However, the physiological differences between these new species and common global species have not been reported.

Ruppia populations can be characterized by two different life strategies, i.e. annual or perennial growth cycles (Mannino and Graziano, 2014). Nevertheless, in variable and disturbed environments, the regeneration of *Ruppia* meadows can highly depend on sexual reproduction (Strazisar et al., 2016). A persistent seed bank is important to maintain the plant population (Bossuyt and Honnay, 2008). Moreover, in consideration that transplanting of adult plants is highly labor- and cost-intensive, and harmful to the donor meadows (Paling et al., 2009), the seeds of *Ruppia* are considered to be excellent material for large-scale SAV restoration projects (Harwell and Orth, 1999). It is important to understand the effects of environmental factors such as salinity and temperature on *Ruppia* seed germination.

While Strazisar et al. (2016) reported a large annual production of *Ruppia* seeds, 85% germinated too early, resulting in low seedling survival percentages. Meanwhile, the salinity and temperature are the two main factors affecting *Ruppia* seed germination, while light conditions have no obvious effects, as suggested by past studies (Koch and Dawes, 1991a, 1991b). So, we hypothesized: temperature and salinity can significantly influence *R. sinensis* seed germination. This study aimed to determine the optimal conditions for seed germination and seedling growth of this species, and may provide fundamental information for *R. sinensis* habitat establishment and restoration.

2. Materials and methods

2.1. Study site and species

The seagrass *Ruppia sinensis*, named by Shuo Yu (Yu and den Hartog, 2014), is a widely distributed species in the coastal water bodies including lagoons, estuaries, abandoned salt pans, and aquaculture ponds along the coastline of northern China. Our sample collection site was located in Diaokou village, Dongying City, (37°59′52″N, 118°36′33″W), which is near the Yellow River Estuary, northern China. The *R. sinensis* population at this site is perennial, and in 2016 the surrounding temperature and salinity ranged from 0 to 29 °C and salinity 8.6 to 32.2, respectively.

2.2. Seed collection

On September 30th 2016, seeds for the germination experiment were collected from reproductive shoots to ensure that all seeds were current-year-grown (Kahn and Durako, 2005). Seeds bearing shoots were directly hand-picked and returned to the laboratory on the same day. After removal of epiphyte cover, the shoots were transferred to large containers with aerated seawater (natural seawater mixed with deionized water at salinity 12 at room temperature (~20 °C) in darkness (Koch and Seeliger, 1988). After 28 days, the seeds that had fallen to the bottom, whose exocarps had decayed naturally, were collected. The mature seeds with black and hard endocarps were then selected.

2.3. Germination experiments

2.3.1. Germination-experiment 1

Seed germination and growth status were measured in six temperature treatments (0, 5, 10, 15, 22, and 30 °C) and seven salinity treatments (salinity 0, 5, 10, 20, 30, 40, and 50). There were three replicates for each treatment, and each replicate contained 30 seeds placed in a 150 mL glass beaker. The seeds were kept in artificial seawater (dissolving sea salt in deionized water) at the salinity levels mentioned above, and placed in a light incubator at the different temperatures with a light irradiance of 70 $\mu E \cdot m^{-2} \cdot s^{-1}$ in a 12-h photoperiod. Artificial seawater was changed every 4 days and the numbers of the germinated seeds in each treatment were recorded. Seeds with an emerging cotyledon were considered germinated seeds (Koch and Seeliger, 1988). During the experimental period, treatments with the maximum germinated seeds were determined to be the optimum conditions for *R. sinensis* seed germination. The germination process of the optimum treatment was recorded with an industrial digital camera (precision of 0.01 mm).

2.3.2. Germination-experiment 2

Since none of the seeds germinated at 0 °C after 58 days (Germination-experiment 1), the second seed germination experiment (Germination-experiment 2) was conducted to determine the vitality of the seeds. The ungerminated seeds were transferred from 0 °C to 22 °C, and all the treatment groups continued using the original salinity to culture the corresponding seeds. The numbers of germinated seeds were recorded every four days, and the artificial seawater was changed at the same time.

2.3.3. Germination-experiment 3

Less seeds germinated in the relative high salinity treatment groups (salinity 20–50) than those in relative low salinity conditions (salinity 0–10) in Germination-experiment 1. To test the vitality of the remaining seeds which did not germinate in the relative high salinity conditions, the third germination experiment (Germination-experiment 3) was conducted. At the end of Germination-experiment 1, eight treatments (three replicates for each treatment) at two experimental temperatures (5 and 10 °C) and four relative high salinities (salinity 20–50) were selected. The remaining ungerminated seeds of these treatment groups were then transferred to the optimal germination condition (salinity 5 at 30 °C). The new seed germination percentage was calculated as the ratio of the new germinated seeds in the new germination condition for 10 days to the remaining seeds at end of Germination-experiment 1.

2.4. Seedling establishment experiment

The germinated seeds in Germination-experiment 1 were transferred to 500 mL glass bottles containing artificial seawater at the corresponding salinity for seedling establishment, and the artificial seawater was changed every 4 days. According to the preliminary experiment, the germinated seeds with cotyledons exceeding 1 cm in length and differentiated roots had the greatest chance for growing into adult plants. Therefore, the numbers of germinated seeds with cotyledons longer than 1 cm and adventitious roots were recorded, which represented the numbers of seedlings establishing successfully. Also, the length of the seedling from the end of cotyledon to the longest fresh leaf was measured every 2 days.

2.5. Statistical analyses

A two-way ANOVA was applied to compare the effects of temperature and salinity on seed germination in Germination-experiment 1. Also, the number of the germinated seeds after transferal from 0 to 22 °C was compared with that at the original temperature of 5, 22, and 30 °C by the two-way ANOVA. When the effects of salinity and temperature on seed germination were both significant ($P < 0.05$), a one-way ANOVA was used to compare the salinity effect at each temperature, and the temperature effect at each salinity, respectively (Zar, 1999). The length of seedlings cultured in the suitable conditions (salinity 5–10 at 22 and 30 °C) at different times was analyzed by a one-