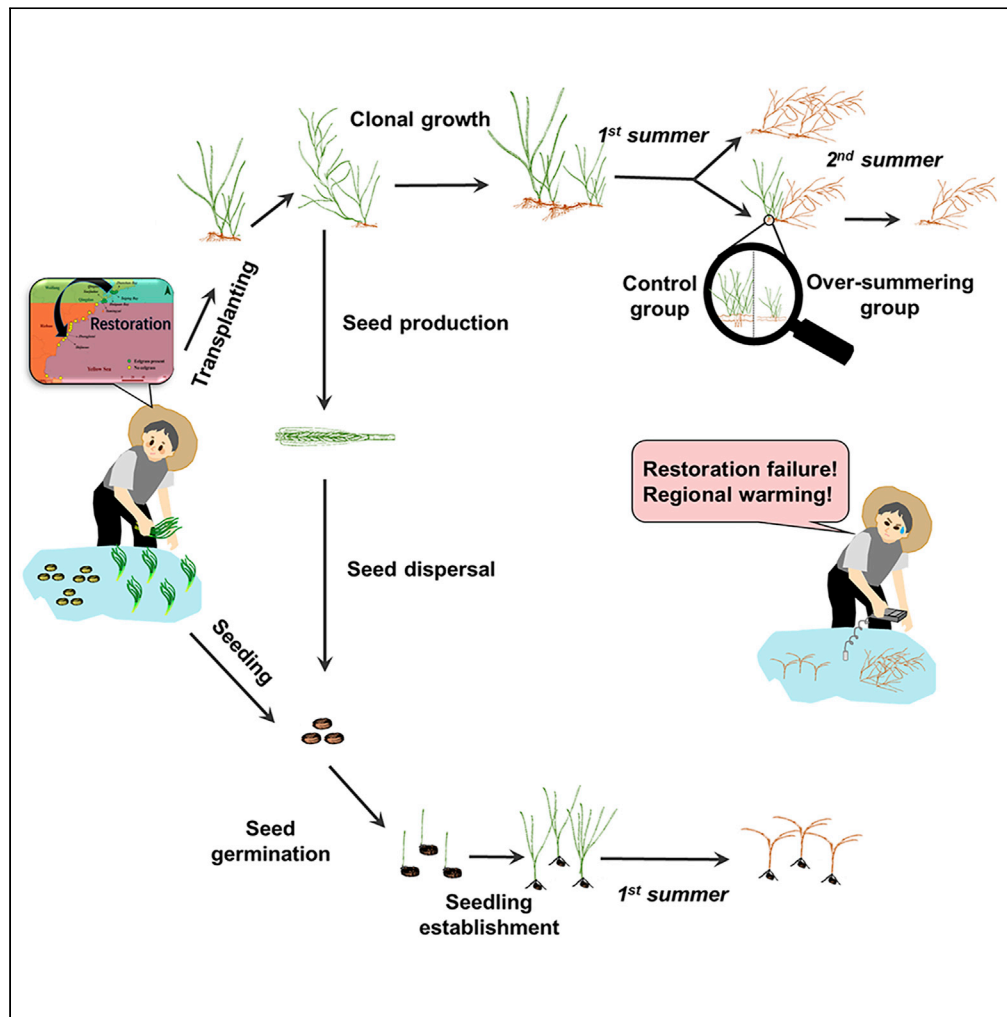


Article

Warming northward shifting southern limits of the iconic temperate seagrass (*Zostera marina*)



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Highlights

High temperatures trigger seagrass (*Zostera marina* L.) restoration failure

None seedlings and adult shoots survived the first or second summer

Over-summering shoots with lower density, height, and rhizome diameter

Warming northward shifting eelgrass habitat range along the eastern coast of China



Article

Warming northward shifting southern limits of the iconic temperate seagrass (*Zostera marina*)

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SUMMARY

Global warming can shift the range edges of numerous species poleward. Here, eelgrass distribution was reinvestigated at its southern limits on the eastern coast of China, which indicated that there has been a northward shift in the southern limit of *Z. marina*. To determine if regional warming resulted in a northward shift in suitable eelgrass habitats, sixteen transplantations of adult eelgrass shoots and seeds at the historical southern distribution limit of eelgrass were conducted between 2016 and 2021. The results showed that high water temperatures in summer had negative effects on eelgrass growth, and directly triggered shoot mortality during 2016–2018. Under heat stress, antioxidant enzyme activity was initially increased, but then decreased under more stressful heat conditions; and the HSP70 protein and its molecular chaperone protein were highly expressed under heat stress. These results demonstrated that suitable eelgrass habitat was now located further north along the eastern coast of China.

INTRODUCTION

Seagrasses are marine flowering plants that have evolved a combined suite of adaptations multiple times to survive, and thrive, in the sea (Olsen et al., 2016). As important ecosystem engineers and habitat formers, seagrasses form extensive and highly productive ecosystems, providing habitat, food, and nursery areas for numerous vertebrate and invertebrate species (Costanza et al., 1997; Duffy, 2006; Unsworth et al., 2018). In addition, seagrass ecosystems can act as carbon sinks by capturing and storing carbon (Fourqurean et al., 2012), and they can reduce exposure to bacterial pathogens in fishes, invertebrates, and humans (Lamb et al., 2017). However, seagrasses have been declining at a rate of 7% per annum since 1990 owing to natural threats (e.g., global warming, ocean acidification) and human activities (e.g., coastal urban development, fishing activities, aquaculture) (Orth et al., 2006; Waycott et al., 2009).

Global warming has resulted in an increase in the average upper ocean temperature by 0.07°C per decade (Burrrows et al., 2011). These temperature increases affect marine species and ecosystems in many ways, including enhanced mortality of key habitat-forming species such as seagrasses (Coma et al., 2009; Marbà and Duarte, 2010), changes in species distributions (Cavanaugh et al., 2019; Hyndes et al., 2016; Precht and Aronson, 2004; Saintilan et al., 2014; Wernberg et al., 2016), and a greater incidence of disease (Hoegh-Guldberg and Bruno, 2010). Future global warming will likely result in the continued northward migration of animals and plants to escape increasing temperatures. Although the impacts of global warming on seagrass reproduction (Qin et al., 2020) and mortality (Marbà and Duarte, 2010) have been reported, the literature does not currently adequately reflect the shift of seagrass species distributions as a result of global warming.

The seagrass *Zostera marina* L. (eelgrass) is a marine foundation species and is the most widespread seagrass species throughout the Atlantic and Pacific coasts of the temperate northern hemisphere (Green and Short, 2003). Owing to its widespread distribution, eelgrass is exposed to a wide range of temperatures ranging from below 0°C to over 30°C (Orth and Moore, 1986; Phillips and Backman, 1983); however, the optimum temperature for eelgrass growth is typically between 15 and 20°C (Lee et al., 2007). Greve et al. (2003) previously reported that *Z. marina* respiration exceeds photosynthesis at temperatures above 30°C, leading to reduced growth or shoot mortality of the plants. Future global warming will likely result in a decrease in eelgrass populations at the southern distribution limit, where eelgrass is expected to have reached its maximal adaptation

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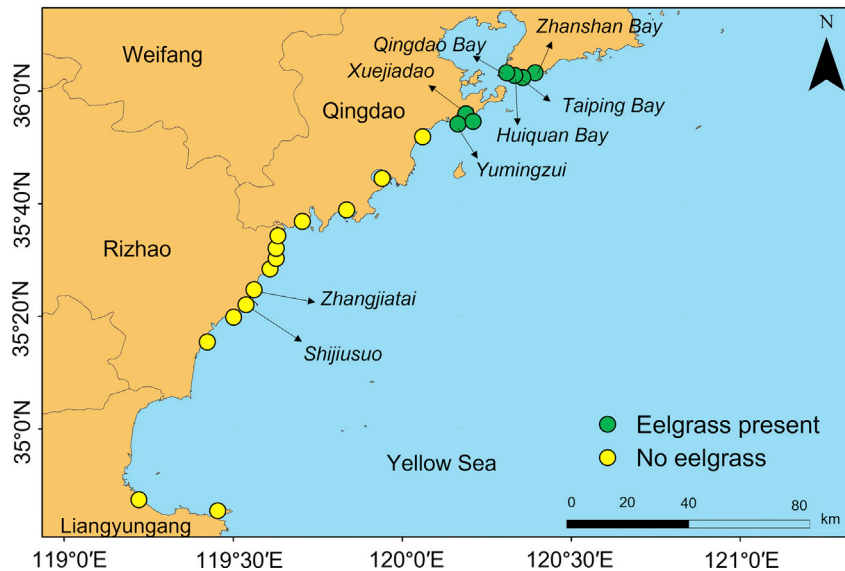


Figure 1. Eelgrass distribution

Distribution of eelgrass (*Zostera marina*) along the coast from Lianyungang, Jiangsu Province to Qingdao, Shandong Province according to an eelgrass distribution survey in December 2015.

to thermal stress (Moore et al., 2012). In China, eelgrass is common along the coast of the northern temperate provinces of Liaoning, Hebei, and Shandong (Xu et al., 2020a, 2020b, 2021a, 2021b; Zheng et al., 2013). In the 1950s the most southerly distribution of eelgrass was recorded at Shijiusuo (35°20'N, 119°30'E), Rizhao City, Shandong Province on the eastern coast of China (den Hartog and Yang, 1990), although ecological data for the species in this area are limited. However, eelgrass was not found at this locality in 2011 (Liu, 2013), indicating that there may have been a northward shift of the southern distribution limits of this species over the last 70 years, potentially related to increasing temperatures associated with regional warming. In Rizhao, a gradual warming trend (0.248°C/10 a) has been observed over the past 50 years (Lu et al., 2012).

Global climate change is imposing increasing thermal stress on seagrass (Koch et al., 2013; Short and Neckles, 1999). High temperatures are likely stressing and damaging seagrass shoots resulting in a decrease in shoot abundance and even sudden die-backs (Nejrup and Pedersen, 2008; Zimmerman et al., 1989). To assess the effects of heat stress, biochemical and transcriptomic analysis have recently been carried out on seagrasses (Franssen et al., 2014; Hammer et al., 2018; Marín-Guirao et al., 2017). Biochemical and transcriptomic analysis can be used to identify changes in antioxidant enzymes and gene expression associated with heat shock proteins (HSP), respectively, offering a tool to evaluate the phenotypic responses of organisms to stressful heat conditions (Bergmann et al., 2010; Bush et al., 1997; Elias et al., 1999; Franssen et al., 2011; Winters et al., 2011).

In the current study, we provide evidence that high temperatures associated with regional warming have resulted in a northward shift of suitable eelgrass habitats, through eelgrass restoration at the historical southern distribution limit of eelgrass (Rizhao, China) between 2016 and 2021. This was achieved by transplanting eelgrass shoots and seeds from Qingdao Bay (seagrass donor site, DS) to Rizhao (seagrass restoration site, RS), monitoring seawater temperature and seagrass metrics at RS and DS, and conducting biochemical and transcriptomic analysis. It was hypothesized that high temperatures associated with regional warming could decrease shoot growth and survival, and eventually lead to restoration failure, indicating that regional warming is resulting in a northward shift of the southern limits of *Z. marina* along the eastern coast of China.

RESULTS

Eelgrass distribution

Eelgrass was recorded at seven of the 21 survey sites, including six previously observed meadows and one newly recorded meadow (Figure 1). All seven sites were located in Qingdao, with no eelgrass recorded north of Lianyungang, Rizhao, and south of Qingdao. No eelgrass was recorded in Shijiansuo and Zhangjiatai in

Rizhao, where eelgrass meadows had been observed in the 1950s, indicating that eelgrass meadows have disappeared at this location, which is consistent with the results of an investigation conducted in 2011 (Liu, 2013).

Eelgrass restoration

Environmental parameters

Daily SSTs at both DS and RS exhibited clear seasonal trends, and they were generally highest in August (Figure 2A). Some of daily SSTs at RS were above 30°C in August during the study period with the exception of 2019, while no daily SSTs at DS were above 30°C (Figure 2A). The $D_{>30}$ and $H_{>30}$ at RS were generally higher than those at DS, and the highest $D_{>30}$ (60 days) was recorded at RS in 2018 (Figure 2B). Even though $D_{>30}$ at RS between 2017 and 2019 were similar, $H_{>30}$ was generally higher in 2017, indicating that in 2017 eelgrass was likely exposed to increased heat stress (Figures 2B and 2C).

Air temperatures have gradually increased at study sites since 1961 (Figure 3). The yearly numbers of days at RS when daily air temperature exceeded 20 and 25°C were approximately 991 and 352 days during the 1960s, respectively, whereas the numbers of days were approximately 1,248 and 496 days during the 2010s (Figures 3A and 3B). The yearly numbers of days at RS when daily air temperature exceeded 30°C were approximately 4 days during the 1960s and 23 days during the 2010s (Figure 3C).

The salinity ranged from 30.1 to 31.5 at RS, and 30.4 to 31.5 at DS.

Seagrass metric responses

In the transplants of shoots or seeds between 2016 and 2021, most shoots did not survive the first summer and none survived the second.

Adult eelgrass shoots transplanted over the period 2016–2017 completely died in the first summer (Figure 4). However, before the eelgrass disappearance in summer, no significant difference in plant height was found between RS and DS ($p > 0.05$; Figure 5), and the two sites exhibited very similar trends in plant height (Figure 5). In addition, reproductive shoots with blossoming spathes were observed in April, and a large number of seeds within reproductive shoots were observed in June. Meanwhile, the leaves of the eelgrass vegetative shoots turned black. It should be noted that a seedling, with five clone shoots, was observed in April 2018, indicating that one of the seeds produced by transplants in 2017 successfully germinated.

Between August 2018 and August 2019, adult shoot transplantation and seed planting was conducted. Most transplants disappeared at the hottest time of year, but in two cases (Dec 2018 seeds, Feb 2019 shoots) died earlier in summer. The early disappearance of February transplants may be because most shoots transformed into reproductive plants and then died (Figure 6). Results from the seed transplant suggest that seedlings may be less tolerant of high temperature than vegetative shoots. Germination occurred by 10 Feb 2019, and shoot density peaked (about 22 shoots per group) in May 2019 (Figure 7A). Seedling shoot height rapidly increased from about 12 cm in February to about 35 cm in June 2019 (Figure 7B). Finally, the seedlings completely disappeared on July 21, 2019 (Figure 7A).

Eelgrass transplanted in October and December 2018 reached the highest shoot density (327 shoots m^{-2} and 218 shoots m^{-2} , respectively) in June 2019, and then died (Figure 6). Although some of the plants transplanted in October and December 2018 survived through August 2019, the shoot density (Figure 6) and height (Figure 5) decreased significantly ($p < 0.05$). Moreover, in June 2020 the shoot density, height, and rhizome diameter of those shoots that survived through the summer (over-summering group) were significantly lower than the newly transplanted seagrass in August 2019 (control group, Figure 6; Table 1), indicating that the eelgrass in the control group, with more energy storage belowground, was stronger and healthier than the over-summering group.

For seagrass transplanted in the middle of August and November 2019, there was no significant difference in plant height between RS and DS ($p > 0.05$; Figure 5) before August, and the density of seagrass at RS reached the level of DS (about 366 shoots m^{-2} ; Figure 6). However, the density of shoots decreased rapidly and reached <10 shoots m^{-2} in August, before completely disappearing in December 2020 (Figure 6).

In terms of reproduction, there was no significant inter-annual and location variation in the number of spathes per flowering shoot (Table 2; $p > 0.05$), whereas the number of seeds per spathe at DS was

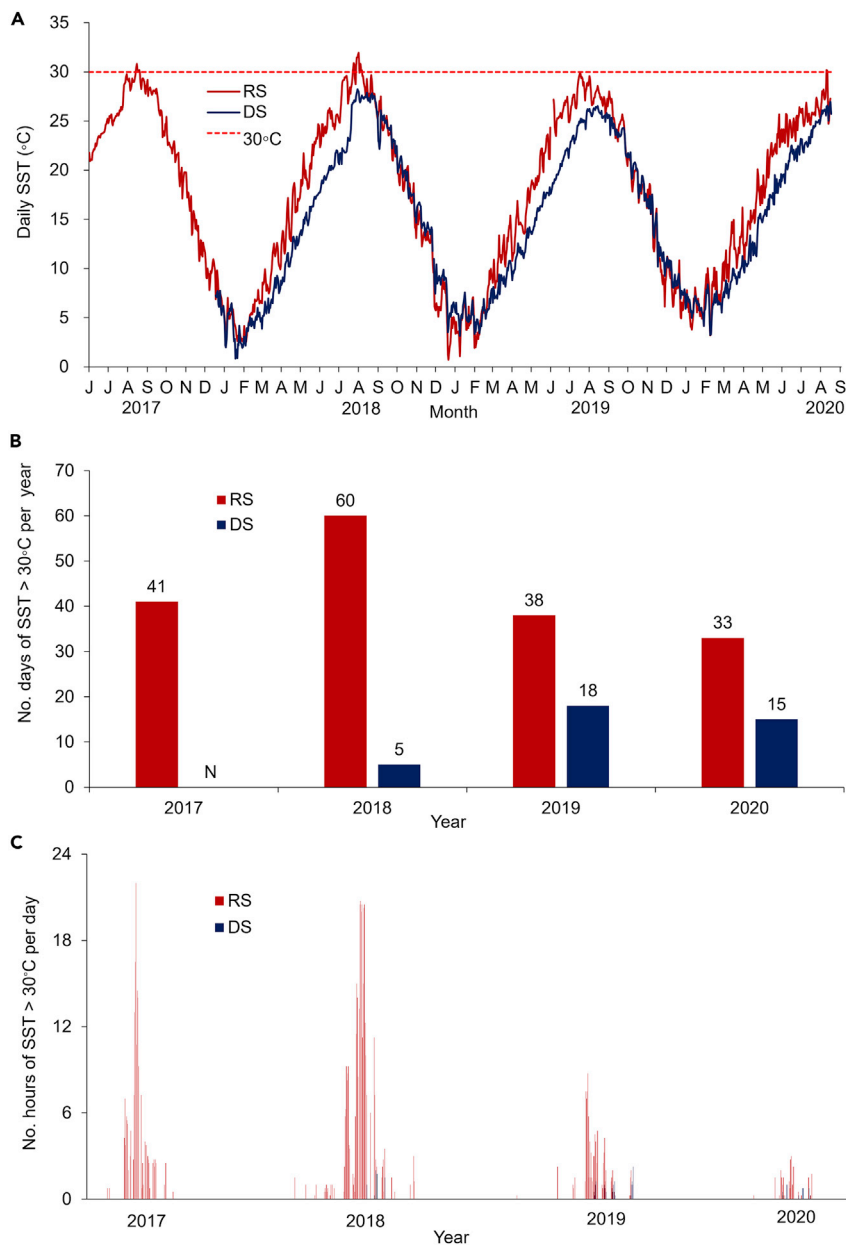


Figure 2. Mean daily sea surface temperature

Mean daily sea surface temperature (SST; A), number of days with SST exceeding 30°C per year ($D_{>30}$; B), and number of hours with SST exceeding 30°C per day ($H_{>30}$; C) at the seagrass restoration site (RS; Rizhao) and donor site (DS; Qingdao) on the northern coast of China from 2017 to 2020. The red dotted line in panel A represents an SST of 30°C. The “N” in panel B shows that there was no record at DS in 2017. As temperatures were not recorded after August 2020, $D_{>30}$ and $H_{>30}$ were calculated based on recorded data.

significantly higher than RS in 2019 and 2020 ($p < 0.05$). There was no significant inter-annual variation in the number of seeds per spathe at DS ($p > 0.05$), whereas that value at RS in 2020 was significantly higher than that in 2019 ($p < 0.05$). The number of seeds per flowering shoot at DS was 1.5–5 times those at RS (Table 2).

Antioxidant enzyme responses

In general, the antioxidant enzyme activity of eelgrass leaf blades at RS in May and June 2019 was higher than in July 2019, with daily SSTs increasing from 18.79°C to 29.05°C. On the contrary, antioxidant enzyme

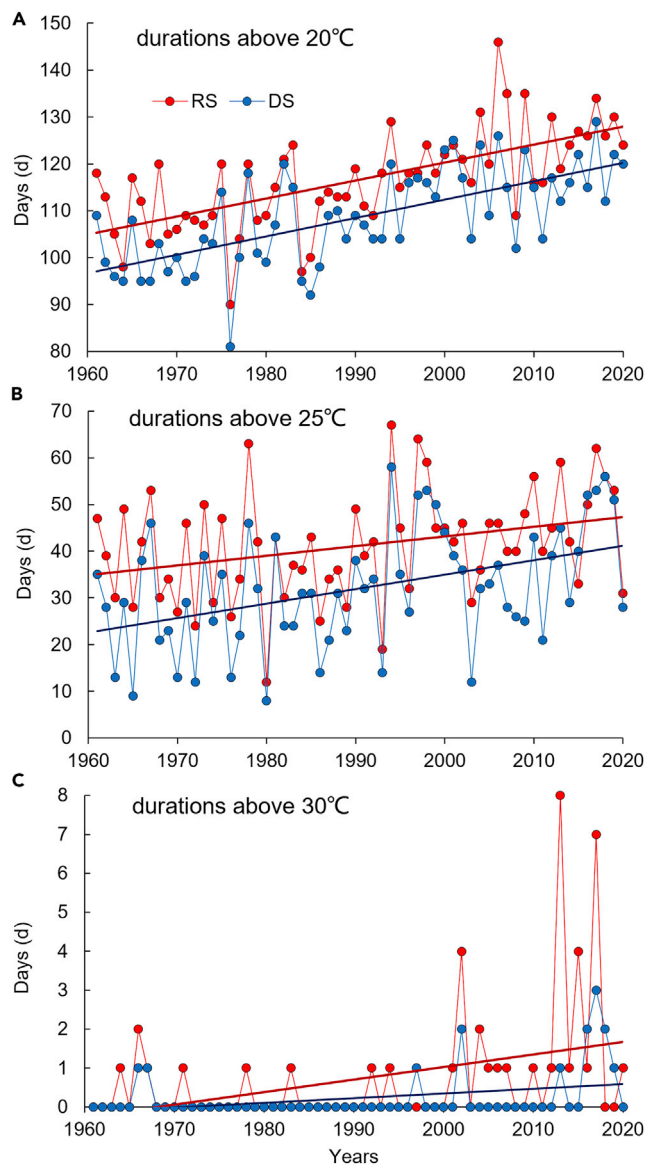


Figure 3. Long term air temperature changes

The yearly number of days with air temperatures exceeding 20°C (A), 25°C (B), and 30°C (C) at the seagrass restoration site (RS; Rizhao) and donor site (DS; Qingdao) since 1961.

activity at DS in May and June was generally lower than in July, with daily SSTs increasing from 14.93°C to 24.54°C (Figure 8).

By comparing the antioxidant enzyme activity in *Z. marina* leaf blades at RS and DS, we found that with the exception of MDA at RS, antioxidant enzyme activity was generally higher at RS compared with DS in May and June, while antioxidant enzyme activity at RS was generally lower than DS in July (Figure 8 and Table 3). For example, SOD and GPX activities at RS were significantly higher than those at DS in May and June ($p < 0.05$), while SOD and GPX activities at RS were significantly lower than those at DS in July ($p < 0.05$).

Gene expression response to heat stress

A total of 320,861,002 raw read pairs were obtained using the Illumina sequencing platform with the PE150 sequencing strategy. After quality control, 313,391,247 clean read pairs were obtained, and 22,553 genes were annotated. Of the clean reads, 96.37–97.43% were total mapped, 5.41–10.89% were multiple

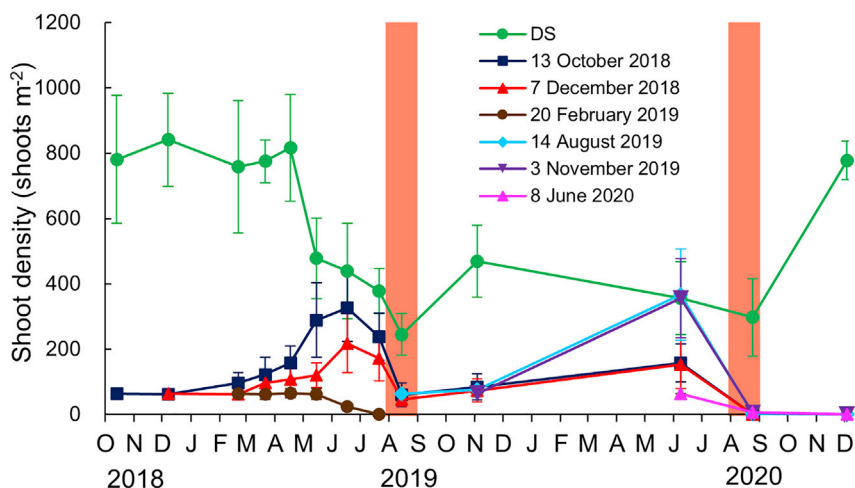


Figure 6. Adult eelgrass shoot density

Variations in shoot density (shoots m^{-2}) of *Zostera marina* at the seagrass restoration site (RS; Rizhao) and the donor site (DS; Qingdao) in six transplantations from October 2018 to June 2020. Orange bars represent the hottest month (August). Values are presented as means \pm SD.

northward shift of the southern limits of *Z. marina*, and spikes in water temperature that occurred in summer at the seagrass restoration site led to declines in seagrass growth and increased mortality, which resulted in restoration failure. Therefore, we suggest that high temperatures associated with regional warming resulted in a northward shift of suitable eelgrass habitats.

Seagrass metrics

In this study, the high water temperature at RS was presumed to be the most critical factor leading to restoration failure in the current study, for the following reasons. Firstly, the sediment at DS was mainly composed of sand, while the sediment at RS was mainly composed of sands and gravels (Xu et al., 2022). However, eelgrass can survive in substrates ranging from mud to gravel, and thus the variations of sediments in the present study likely have minimal effects on seagrass survival. Secondly, RS and DS are both sheltered sites (Xu et al., 2022), suggesting minimal effects on the restoration project from waves or currents. Thirdly, the marine water of the two sites (RS and DS) achieved Grade I water quality status (good water quality) during the study period, according to the Bulletin of Marine Ecology and Environment Status of China (<https://www.mee.gov.cn/hjzl/sthjzk/jagb/>). In addition, RS is a remote site with little human disturbance that would affect transplanted eelgrass. Overall, high water temperatures provide a logical explanation for restoration failure in the current study.

Many studies have reported that seagrasses are affected by high water temperature stress, and sharp reductions in photosynthetic efficiency occur after temperatures exceed optimum thresholds (Bulthuis, 1983; Campbell et al., 2006; Pérez and Romero, 1992; Ralph, 1998), breaking the balance between carbon uptake (photosynthesis) and carbon consumption (respiration) (Bulthuis, 1987; Pérez and Romero, 1992). With variations of seagrass species in sensitivity to elevated temperature, tropical and subtropical seagrass species can tolerate higher temperatures than temperate species (Campbell et al., 2006; Collier et al., 2011). *Z. marina* is a temperate species and water temperatures exceeding 30°C will lead to higher plant respiration than photosynthesis (Greve et al., 2003), and negative daily carbon balances of leaves, which could contribute to mortality or reduced plant growth (Marsh et al., 1986). Even though temperatures exceeded 30°C at both RS and DS, the negative effects of high water temperature (>30°C) on seagrass at RS were more persistent and continuous than at DS, indicating that the eelgrass at RS was likely exposed to higher heat stress. This may explain the declines in seagrass growth (lower shoot height and energy storage in the rhizome) and the increased mortality recorded at RS. Seagrass rhizomes are recognized as storage and redistribution organs for carbohydrate reserves (Stapel et al., 1997). Therefore, lower rhizome development (lower rhizome diameters), resulting from high water temperature in summer, may make eelgrass less resilient, although these eelgrass plants could survive the first summer. Additionally, eelgrass died in the summer of 2017 and survived in the summer of 2019, which could be

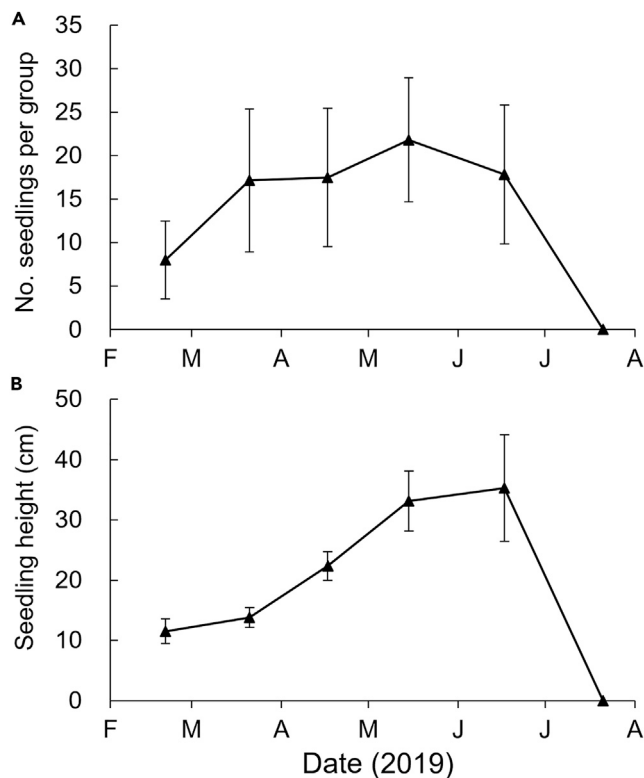


Figure 7. Eelgrass seedling density and height

Variations in seedling shoot density (shoots m^{-2}) and height (cm) of *Zostera marina* at the seagrass restoration site (RS; Rizhao) transplanted in December 2018. Values are presented as means \pm SD.

explained by the generally higher $H_{>30}$ in 2017, indicating more persistent and continuous heat stress in 2017. Finally, in the current study, seedlings vanished before August, supporting the idea that eelgrass seedlings have higher mortality than adult shoots with increasing water temperature (Salo and Pedersen, 2014).

Most studies indicate that temperature is critical for eelgrass reproduction, including flower appearance and seed production (Larkum et al., 2006; Walker et al., 2001). However, the reproductive eelgrass shoots at RS with higher temperature exhibited lower seed production, as a result of the lower number of seeds per spathe and number of seeds per flowering shoot, which may be owing to pollen limitation resulting from insufficient pollination in the small-scale restoration (Van Tussenbroek et al., 2010). The implication of these findings is that seagrass restoration needs to be carried out on a large scale to decrease pollen limitation (Van Tussenbroek et al., 2010). In addition, eelgrass shoots transplanted on February 20, 2019 completely disappeared before hot summer, mainly because most of the transplanted eelgrass shoots transformed into reproductive plants and then died before summer. Generally, it is difficult to distinguish reproductive shoots at early stages (Park and Lee, 2007) and it is likely that these shoots in February 2019 transplants were already cued to flower before being transplanted. Additionally, seagrasses also show increased reproduction under disturbance (Cabaco and Santos, 2012), and transplantation may have served as a type of disturbance that caused shoots to differentiate to flowering.

Antioxidant enzyme activity

Heat stress from increased temperature can induce excessive production of reactive oxygen species (ROS) in cells, causing progressive oxidative damage and ultimately cell death (Potters et al., 2007; Suzuki and Mittler, 2006), and scavenging of excess ROS is achieved by an efficient antioxidative system mainly comprising of antioxidant enzymes, such as SOD, GPX, CAT, POD, and MDA. The results of the current study

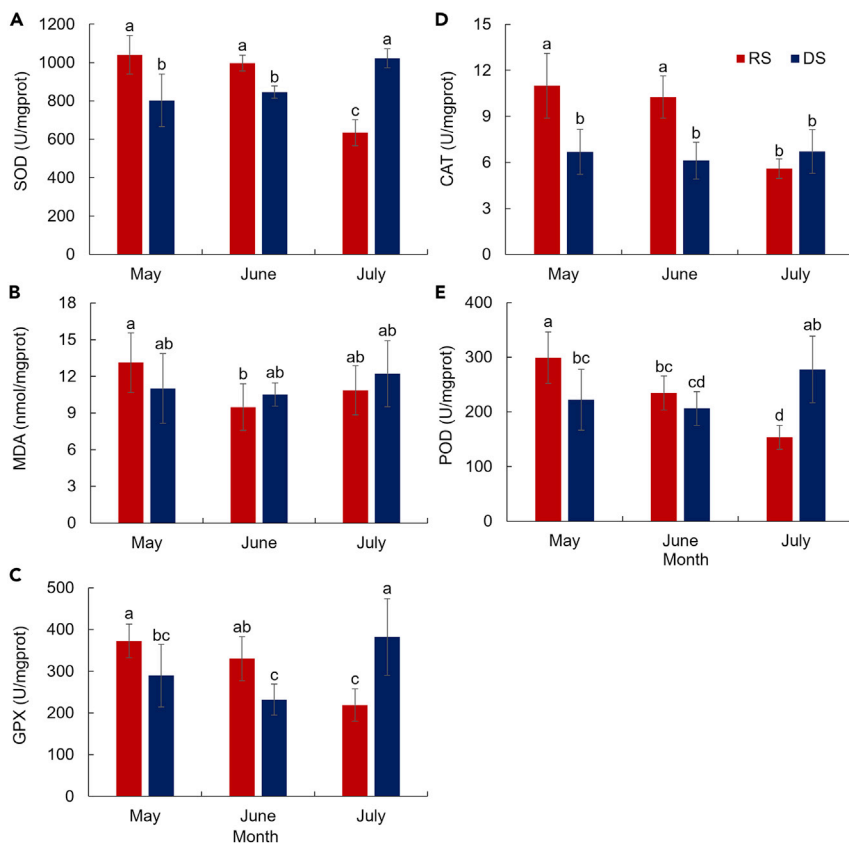


Figure 8. Antioxidant enzyme responses

Temporal variations of SOD (A), MDA (B), GPX (C), CAT (D), and POD (E) activities in *Zostera marina* at the seagrass restoration site (RS; Rizhao) and the donor site (DS; Qingdao) in the middle of May, June, and July 2019. Values are presented as means \pm SD. Different letters indicate a significant difference ($p < 0.05$).

clearly showed that antioxidant enzyme activity at RS with higher temperatures was significantly higher than that of DS with lower temperatures in May and June, indicating that there was a more intense up-regulation of antioxidant enzyme activity in response to heat stress at RS. Tutar et al. (2017) reported that antioxidant enzyme activity was significantly up-regulated in response to heat stress in Mediterranean seagrasses (*Posidonia oceanica* and *Cymodocea nodosa*). Similarly, the seagrasses *Zostera noltii* and *Z. marina* also showed an increasing antioxidant enzyme activity response to heat stress (Franssen et al., 2014; Hammer et al., 2018). However, if ROS reach very high levels, which can't be effectively removed by the antioxidative system, they will cause significant damage, leading to a collapse of the antioxidative system and triggering apoptosis and cell necrosis in marine organisms (Lesser, 2006). In the present study, there was an obvious decrease in antioxidant enzyme activity with increasing temperatures, with the exception of MDA at RS, suggesting a collapse of the antioxidative system, with concomitant eelgrass damage, eventually causing death (Huang et al., 2019; Mittler, 2002). Therefore, the antioxidant activity at RS with higher temperatures

Table 1. Shoot height, root length, rhizome node length, and rhizome diameter of *Zostera marina* transplanted in October and December 2018 (over-summering group) and late August of 2019 (control group) at the seagrass restoration site (RS; Rizhao) in the Yellow Sea on June 8, 2020

	Shoot height (cm)	Root length (cm)	Rhizome node length (cm)	Rhizome diameter (mm)
Over-summering group	39.29 \pm 14.68 ^a	9.62 \pm 3.38	1.44 \pm 0.33	2.90 \pm 0.14 ^a
Control group	91.43 \pm 22.68 ^b	9.14 \pm 1.86	1.27 \pm 0.10	4.74 \pm 0.36 ^b

Data with different superscript letters (a, b) in the same column indicate significant differences. Values are presented as means \pm SD.

Table 2. Seed production of *Zostera marina* at the seagrass restoration site (RS; Rizhao) and the donor site (DS; Qingdao) in the Yellow Sea on the north coast of China in June 2019 and June 2020

Seed production	RS		DS	
	17 June 2019	8 June 2020	16 June 2019	5 June 2020
No. spathes per flowering shoot	11.57 ± 3.82	9.83 ± 2.71	11.43 ± 4.08	9.71 ± 1.38
No. seeds per spathe	0.95 ± 1.44 ^c	2.71 ± 2.98 ^b	4.26 ± 3.06 ^a	4.69 ± 2.42 ^a
No. seeds per flowering shoot	10.00 ± 8.23 ^b	26.67 ± 11.6 ^{ab}	48.71 ± 22.26 ^a	39.57 ± 6.40 ^a

Data with different superscript letters (a, b, c) in same column indicate significant differences.
Values are presented as means ± SD.

was significantly lower than that at DS with lower temperatures in July. Similar results were also found for antioxidant enzyme activity in aquatic plants (e.g. *Zostera japonica*) exposed to heavy metals (Pb and Cd), with a decline in antioxidant enzyme activity at higher metal concentrations (Lin et al., 2016).

Transcriptome

Of the DEGs enriched in the KEGG pathway of protein processing in the endoplasmic reticulum, the genes related to HSP70 were the most numerous, including HSP70, HSC70, and molecular chaperones. In terrestrial plants, the important role of HSP in tolerance to heat stress has been clearly studied in model species (e.g., *Arabidopsis*, Dong et al., 2019; Queitsch et al., 2000) as well as in some common plants (e.g., potato, Ahn et al., 2004; spinach, Guy and Li, 1998; soybean, Khan et al., 2020; tobacco, Park and Hong, 2002; wheat, Rampino et al., 2009; pea, Srikanthbabu et al., 2002; tomato, Zhuang et al., 2020). Wang et al. (2004) have reviewed the role of plant heat-shock proteins and molecular chaperones, the overexpression of the HSP70 gene was positively correlated with the acquisition of thermotolerance, and members of the HSP70 chaperone protein were significantly expressed under heat stress conditions. HSP response to heat stress has been studied rarely in seagrasses. Bergmann et al. (2010) have found that the expression of the HSP70 gene in *Z. marina* was significantly up-regulated under the simulation of global warming conditions, which provided a good validation of our results.

The accumulation of misfolded proteins in the cytoplasm leads to increased expression of heat shock proteins, and the accumulation of misfolded proteins in the endoplasmic reticulum stimulates the expression of many endoplasmic reticulum proteins, most of which can function as molecular chaperones (Bush et al., 1997). The results of the current study also revealed that Luminal-binding protein (BiP), an HSP70 chaperone located in the inner endoplasmic reticulum, was significantly up-regulated. BiP can bind to newly synthesized proteins, making the proteins more efficient in a state of subsequent folding and oligomerization. Therefore, BiP can be used to facilitate the folding and assembly of newly synthesized proteins (Gething, 1999). In addition, BiP is essential for the transport back across the membrane of aberrant polypeptides destined for degradation by the proteasome (McClellan et al., 1998; Plemper et al., 1997). Bergmann et al. (2010) reported that the expression levels of BiP gene during simulated heat waves were also significantly up-regulated (13-fold change), which were second to that of HSP80 (34-fold change), and slightly higher than that of HSP70 (4-fold change).

Table 3. Two-way ANOVA analysis of antioxidant enzyme response in leaf blades of *Zostera marina* at the seagrass restoration site (RS; Rizhao) and the donor site (DS; Qingdao) in the middle of May, June, and July 2019

Enzymes	SOD	MDA	GPX	CAT	POD
Location	0.000128	0.014	0.142	21.773***	0.155
Time	4.455*	2.306	1.877	9.576***	3.201
Location*time	44.4***	1.843	15.279***	11.66***	14.385***

Because the assumption of homogeneity of variance for GPX was not met by Levene's test, log-transformation was used for the GPX data.

Values are presented as means ± SD.

*p < 0.05.

***p < 0.001.

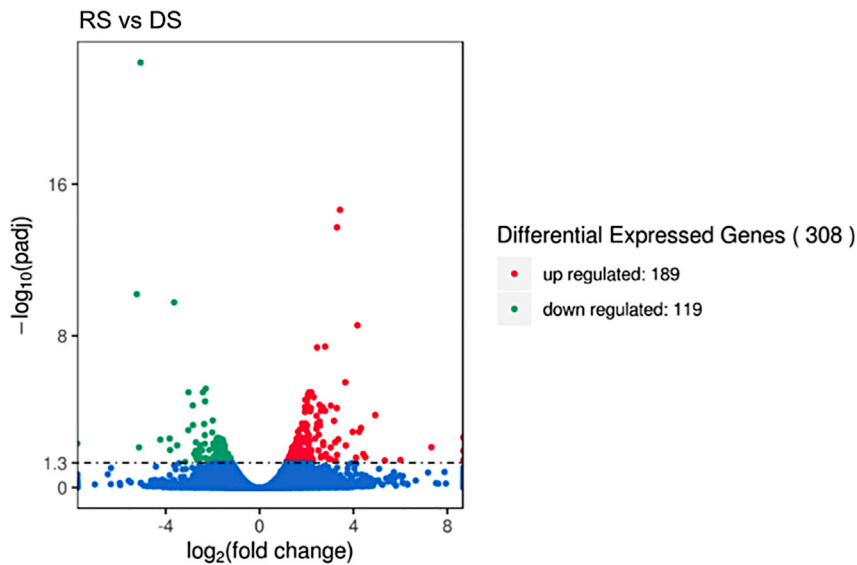


Figure 9. Volcano plot

Volcano plot of DEGs from the seagrass restoration site (RS; Rizhao) and the donor site (DS; Qingdao).

In the present study, under a long period of heat stress, eelgrass responded to the adverse effects of increasing temperatures by up-regulating the expression of the HSP70 protein family gene and its molecular chaperone BiP. However, because prolonged heat stress exceeds the self-regulation of eelgrass, it is less likely to be able to resist heat stress, leading to plant death in summer.

Conclusion

There is a northward shift of the southern distribution limit of *Z. marina* on the eastern coast of China. High water temperatures resulted in the restoration failure of *Z. marina* at the edge of its biogeographical range in Rizhao, China. Transplanted eelgrass was negatively affected by high water temperatures. The transplanted eelgrass that experienced thermal stress exhibited declines in seagrass growth, lower shoot height, reduced energy storage ability (rhizome diameters), and an increased mortality rate. Antioxidant enzyme activity changed with increasing water temperature, and HSP70 and BiP were significantly up-regulated under heat stress conditions. The results of the current study demonstrated that high water temperature, which has been proven to be one of the direct causes of seagrass shoot mortality (Marbà and Duarte, 2010), poses a significant threat to seagrass restoration, especially at the edge of its biogeographical range. This study also indicated that increasing temperatures associated with global warming are likely to continue to shift the range edges of eelgrass in a northerly direction.

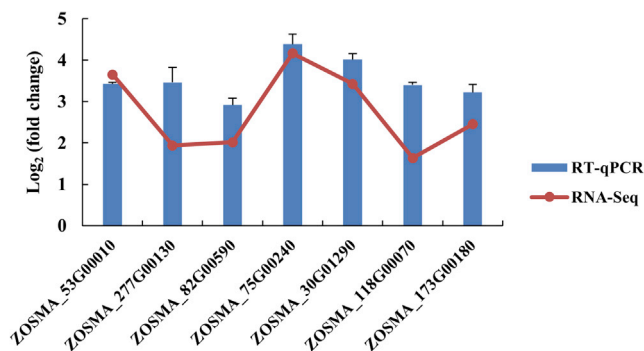


Figure 10. Gene expression comparison

Comparison of gene expression obtained by RNA-Seq and RT-qPCR. RT-qPCR values are presented as means \pm SE.

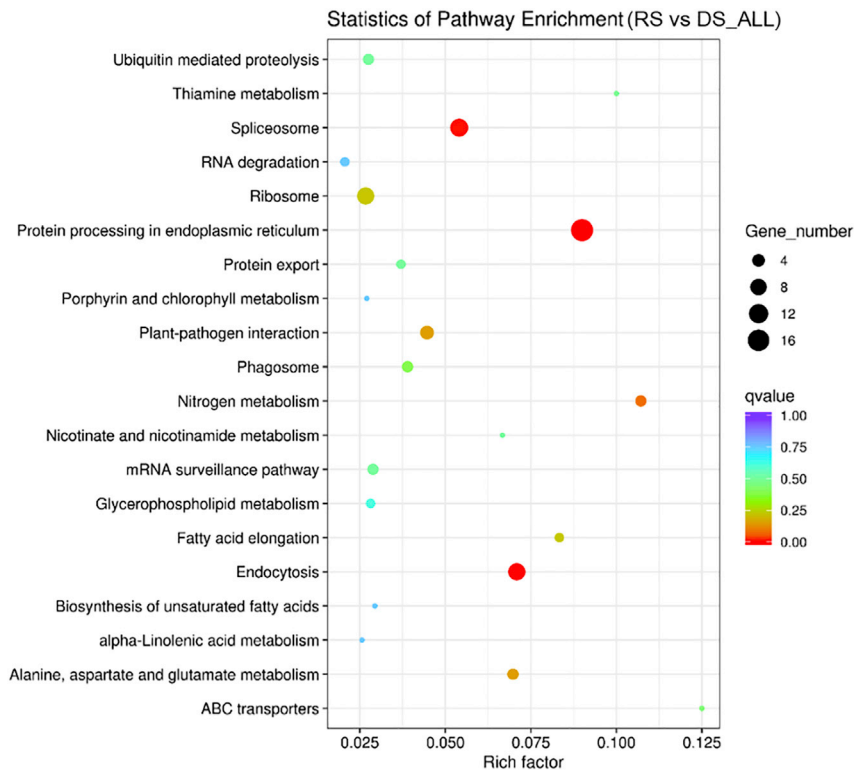


Figure 11. KEGG pathway enrichment

Statistical analysis of KEGG pathway enrichment of DEGs from the seagrass restoration site (RS; Rizhao) and the donor site (DS; Qingdao). Each circle represents a KEGG pathway, the ordinate represents the pathway name, and the abscissa is the enrichment factor. The larger the enrichment factor is, the greater the degree of enrichment is. The circle color represents q-value, the smaller the q-value is, the more reliable the enrichment significance is. The size of circle indicates the number of genes enriched in the pathway, the larger the circle, the more abundant the genes.

Limitations of the study

The current data provide a strong set of evidence that high water temperatures resulted in the restoration failure of *Z. marina*, and the southern limit of *Z. marina* in China has shifted north, which indicates that warming is northward shifting the southern limits of the iconic temperate seagrass (*Zostera marina*) on the western Pacific coast (China). Yet, the disappearance of natural eelgrass meadows at the edge of its biogeographical range might be explained by many factors including warming, water pollution, or disturbance. In the follow-up research, it is expected to determine the cause of the disappearance of seagrass through remote sensing.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.104755>.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.-C. XU and Y. ZHANG; methodology, S.-C. XU and Y. ZHOU; investigation, S.-C. XU, Y. ZHANG, Y. ZHOU, S. XU, S.-D. YUE, M.-J. LIU, and X. ZHANG; writing – original draft, S.-C. XU; writing –review & editing, Y. ZHANG and Y. ZHOU; funding acquisition, Y. ZHOU; supervision, Y. ZHOU.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

- Ahn, Y.-J., Claussen, K., and Lynn Zimmerman, J. (2004). Genotypic differences in the heat-shock response and thermotolerance in four potato cultivars. *Plant Sci.* 166, 901–911.
- Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biol.* 11. <https://doi.org/10.1186/gb-2010-11-10-r106>.
- Bergmann, N., Winters, G., Rauch, G., Eizaguirre, C., Gu, J., Nelle, P., Fricke, B., and Reusch, T.B.H. (2010). Population-specificity of heat stress gene induction in northern and southern eelgrass *Zostera marina* populations under simulated global warming. *Mol. Ecol.* 19, 2870–2883.
- Bulthuis, D. (1983). Effects of temperature on the photosynthesis-irradiance curve of the Australian seagrass, *Heterozostera tasmanica*. *Mar. Biol. Lett.* 4, 47–57.
- Bulthuis, D.A. (1987). Effects of temperature on photosynthesis and growth of seagrasses. *Aquat. Bot.* 27, 27–40.
- Burrows, M.T., Schoeman, D.S., Buckley, L.B., Moore, P., Poloczanska, E.S., Brander, K.M., Brown, C., Bruno, J.F., Duarte, C.M., Halpern, B.S., et al. (2011). The pace of shifting climate in marine and terrestrial ecosystems. *Science* 334, 652–655.
- Bush, K.T., Goldberg, A.L., and Nigam, S.K. (1997). Proteasome inhibition leads to a heat-shock response, induction of endoplasmic reticulum chaperones, and thermotolerance. *J. Biol. Chem.* 272, 9086–9092.
- Cabaço, S., and Santos, R. (2012). Seagrass reproductive effort as an ecological indicator of disturbance. *Ecol. Indic.* 23, 116–122. <https://doi.org/10.1016/j.ecolind.2012.03.022>.
- Campbell, S.J., McKenzie, L.J., and Kerville, S.P. (2006). Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature. *J. Exp. Mar. Biol. Ecol.* 330, 455–468.
- Cavanaugh, K.C., Dangremond, E.M., Doughty, C.L., Williams, A.P., Parker, J.D., Hayes, M.A., Rodriguez, W., and Feller, I.C. (2019). Climate-driven regime shifts in a mangrove–salt marsh ecotone over the past 250 years. *Proc. Natl. Acad. Sci. USA* 116, 21602–21608.
- Collier, C.J., Uthicke, S., and Waycott, M. (2011). Thermal tolerance of two seagrass species at contrasting light levels: implications for future distribution in the Great Barrier Reef. *Limnol. Oceanogr.* 56, 2200–2210.
- Coma, R., Ribes, M., Serrano, E., Jiménez, E., Salat, J., and Pascual, J. (2009). Global warming-enhanced stratification and mass mortality events in the Mediterranean. *Proc. Natl. Acad. Sci. USA* 106, 6176–6181.
- Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., et al. (1997). The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260.
- Dazy, M., Masfaraud, J.-F., and Féraud, J.F. (2009). Induction of oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica* Hedw. *Chemosphere* 75, 297–302.
- den Hartog, C., and Zongdai, Y. (1990). A catalogue of the seagrasses of China. *Chin. J. Ocean. Limnol.* 8, 74–91.
- Dong, Z.-Y., Narsing Rao, M.P., Wang, H.-F., Fang, B.-Z., Liu, Y.-H., Li, L., Xiao, M., and Li, W.-J. (2019). Transcriptomic analysis of two endophytes involved in enhancing salt stress ability of *Arabidopsis thaliana*. *Sci. Total Environ.* 686, 107–117.
- Duffy, J.E. (2006). Biodiversity and the functioning of seagrass ecosystems. *Mar. Ecol. Prog. Ser.* 311, 233–250.
- Elias, S., Arner, E., Zhong, L., and Holmgren, A. (1999). Methods in enzymatic analysis. In *Oxidants and antioxidants*, L. Packer, ed. (Academic press), pp. 226–239.
- Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., and Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nat. Geosci.* 5, 505–509.
- Franssen, S.U., Gu, J., Bergmann, N., Winters, G., Klostermeier, U.C., Rosenstiel, P., Bornberg-Bauer, E., and Reusch, T.B.H. (2011). Transcriptomic resilience to global warming in the seagrass *Zostera marina*, a marine foundation species. *Proc. Natl. Acad. Sci. USA* 108, 19276–19281.

- Franssen, S.U., Gu, J., Winters, G., Huylmans, A.-K., Wienpahl, I., Sparwel, M., Coyer, J.A., Olsen, J.L., Reusch, T.B.H., and Bornberg-Bauer, E. (2014). Genome-wide transcriptomic responses of the seagrasses *Zostera marina* and *Nanozostera noltii* under a simulated heatwave confirm functional types. *Mar. Genomics* **15**, 65–73.
- Galbraith, P.S., Larouche, P., Chassé, J., and Petrie, B. (2012). Sea-surface temperature in relation to air temperature in the Gulf of St. Lawrence: interdecadal variability and long term trends. *Deep Sea Res II Top. Stud. Oceanogr.* **77–80**, 10–20.
- Gething, M.-J. (1999). Role and regulation of the ER chaperone BiP. *Semin. Cell Dev. Biol.* **10**, 465–472.
- Green, E., and Short, F. (2003). *World Atlas of Seagrasses* (University of California Press).
- Greve, T.M., Borum, J., and Pedersen, O. (2003). Meristematic oxygen variability in eelgrass (*Zostera marina*). *Limnol. Oceanogr.* **48**, 210–216.
- Guy, C.L., and Li, Q.-B. (1998). The organization and evolution of the spinach stress 70 molecular chaperone gene family. *Plant Cell* **10**, 539–556.
- Hammer, K.J., Borum, J., Hasler-Sheetal, H., Shields, E.C., Sand-Jensen, K., and Moore, K.A. (2018). High temperatures cause reduced growth, plant death and metabolic changes in eelgrass *Zostera marina*. *Mar. Ecol. Prog. Ser.* **604**, 121–132.
- Hodges, D.M., DeLong, J.M., Forney, C.F., and Prange, R.K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* **207**, 604–611.
- Hoegh-Guldberg, O., and Bruno, J.F. (2010). The impact of climate change on the world's marine ecosystems. *Science* **328**, 1523–1528.
- Huang, H., Ullah, F., Zhou, D.-X., Yi, M., and Zhao, Y. (2019). Mechanisms of ROS regulation of plant development and stress responses. *Front. Plant Sci.* **10**, 800.
- Hyndes, G.A., Heck, K.L., Jr., Vergés, A., Harvey, E.S., Kendrick, G.A., Lavery, P.S., McMahon, K., Orth, R.J., Pearce, A., Vanderklift, M., et al. (2016). Accelerating tropicalization and the transformation of temperate seagrass meadows. *Bioscience* **66**, 938–948.
- Khan, M.A., Asaf, S., Khan, A.L., Jan, R., Kang, S.-M., Kim, K.-M., and Lee, I.-J. (2020). Thermotolerance effect of plant growth-promoting *Bacillus cereus* SA1 on soybean during heat stress. *BMC Microbiol.* **20**, 175–214.
- Koch, M., Bowes, G., Ross, C., and Zhang, X.H. (2013). Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Glob. Chang. Biol.* **19**, 103–132.
- Lamb, J.B., van de Water, J.A.J.M., Bourne, D.G., Altier, C., Hein, M.Y., Fiorenza, E.A., Abu, N., Jompa, J., and Harvell, C.D. (2017). Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. *Science* **355**, 731–733.
- Larkum, A., Orth, R., and Duarte, C. (2006). *Seagrasses: Biology, Ecology and Conservation* (Springer).
- Lee, K.-S., Park, S.R., and Kim, Y.K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *J. Exp. Mar. Biol. Ecol.* **350**, 144–175.
- Lesser, M.P. (2006). Oxidative stress in marine environments: biochemistry and physiological ecology. *Annu. Rev. Physiol.* **68**, 253–278.
- Lin, H., Sun, T., Zhou, Y., and Zhang, X. (2016). Anti-oxidative feedback and biomarkers in the intertidal seagrass *Zostera japonica* induced by exposure to copper, lead and cadmium. *Mar. Pollut. Bull.* **109**, 325–333.
- Liu, K. (2013). *Preliminary Studies on Conservation Genetics and Breeding Biology of Zostera marina along Shandong Province*. Master (Shanghai Ocean University).
- Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods* **25**, 402–408.
- Lu, G., Hu, F., HZ, Y., YJ, Z., and YZ, Z. (2012). Temperature trend in Rizhao City in recent 55 years. *Agric. Technol.* **32**, 135–137.
- Marbà, N., and Duarte, C.M. (2010). Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality. *Glob. Chang. Biol.* **16**, 2366–2375.
- Marín-Guirao, L., Entrambasaguas, L., Dattolo, E., Ruiz, J.M., and Procaccini, G. (2017). Molecular mechanisms behind the physiological resistance to intense transient warming in an iconic marine plant. *Front. Plant Sci.* **8**, 1142.
- Marsh, J.A., Jr., Dennison, W.C., and Alberte, R.S. (1986). Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). *J. Exp. Mar. Biol. Ecol.* **101**, 257–267.
- McClellan, A.J., Endres, J.B., Vogel, J.P., Palazzi, D., Rose, M.D., and Brodsky, J.L. (1998). Specific molecular chaperone interactions and an ATP-dependent conformational change are required during posttranslational protein translocation into the yeast ER. *Mol. Biol. Cell* **9**, 3533–3545.
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* **7**, 405–410.
- Moore, K.A., Orth, R.J., and Nowak, J.F. (1993). Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: effects of light, oxygen and sediment burial. *Aquat. Bot.* **45**, 79–91.
- Moore, K.A., Shields, E.C., Parrish, D.B., and Orth, R.J. (2012). Eelgrass survival in two contrasting systems: role of turbidity and summer water temperatures. *Mar. Ecol. Prog. Ser.* **448**, 247–258.
- Nejrup, L.B., and Pedersen, M.F. (2008). Effects of salinity and water temperature on the ecological performance of *Zostera marina*. *Aquat. Bot.* **88**, 239–246.
- Olsen, J.L., Rouzé, P., Verhelst, B., Lin, Y.-C., Bayer, T., Collen, J., Dattolo, E., De Paoli, E., Dittami, S., Maumus, F., et al. (2016). The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature* **530**, 331–335.
- Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., et al. (2006). A global crisis for seagrass ecosystems. *Bioscience* **56**, 987–996.
- Orth, R.J., and Moore, K.A. (1986). Seasonal and year-to-year variations in the growth of *Zostera marina* L. (eelgrass) in the lower Chesapeake Bay. *Aquat. Bot.* **24**, 335–341.
- Park, J.-I., and Lee, K.-S. (2007). Site-specific success of three transplanting methods and the effect of planting time on the establishment of *Zostera marina* transplants. *Mar. Pollut. Bull.* **54**, 1238–1248.
- Park, S.M., and Hong, C.B. (2002). Class I small heat-shock protein gives thermotolerance in tobacco. *J. Plant Physiol.* **159**, 25–30.
- Pérez, M., and Romero, J. (1992). Photosynthetic response to light and temperature of the seagrass *Cymodocea nodosa* and the prediction of its seasonality. *Aquat. Bot.* **43**, 51–62.
- Phillips, R.C., and Backman, T.W. (1983). Phenology and reproductive biology of eelgrass (*Zostera marina* L.) at Bahia Kino, sea of Cortez, Mexico. *Aquat. Bot.* **17**, 85–90.
- Plempner, R.K., Böhmeler, S., Bordallo, J., Sommer, T., and Wolf, D.H. (1997). Mutant analysis links the translocan and BiP to retrograde protein transport for ER degradation. *Nature* **388**, 891–895.
- Potters, G., Pasternak, T.P., Guisez, Y., Palme, K.J., and Jansen, M.A.K. (2007). Stress-induced morphogenic responses: growing out of trouble? *Trends Plant Sci.* **12**, 98–105.
- Precht, W.F., and Aronson, R.B. (2004). Climate flickers and range shifts of reef corals. *Front. Ecol. Environ.* **2**, 307–314.
- Qin, L.-Z., Kim, S.H., Song, H.-J., Kim, H.G., Suonan, Z., Kwon, O., Kim, Y.K., Park, S.R., Park, J.-I., and Lee, K.-S. (2020). Long-term variability in the flowering phenology and intensity of the temperate seagrass *Zostera marina* in response to regional sea warming. *Ecol. Indic.* **119**, 106821.
- Queitsch, C., Hong, S.-W., Vierling, E., and Lindquist, S. (2000). Heat shock protein 101 plays a crucial role in thermotolerance in Arabidopsis. *Plant Cell* **12**, 479–492.
- Ralph, P.J. (1998). Photosynthetic response of laboratory-cultured *Halophila ovalis* to thermal stress. *Mar. Ecol. Prog. Ser.* **171**, 123–130.
- Rampino, P., Mita, G., Pataleo, S., De Pascali, M., Di Fonzo, N., and Perrotta, C. (2009). Acquisition of thermotolerance and HSP gene expression in durum wheat (*Triticum durum* Desf.) cultivars. *Environ. Exp. Bot.* **66**, 257–264.
- Saintilan, N., Wilson, N.C., Rogers, K., Rajkaran, A., and Krauss, K.W. (2014). Mangrove expansion and salt marsh decline at mangrove poleward limits. *Glob. Chang. Biol.* **20**, 147–157.
- Salo, T., and Pedersen, M.F. (2014). Synergistic effects of altered salinity and temperature on estuarine eelgrass (*Zostera marina*) seedlings and

- clonal shoots. *J. Exp. Mar. Biol. Ecol.* 457, 143–150.
- Short, F.T., and Neckles, H.A. (1999). The effects of global climate change on seagrasses. *Aquat. Bot.* 63, 169–196.
- Srikanthbabu, V., Krishnaprasad, B.T., Krishnaprasad, B.T., Gopalakrishna, R., Savitha, M., and Udayakumar, M. (2002). Identification of pea genotypes with enhanced thermotolerance using temperature induction response technique (TIR). *J. Plant Physiol.* 159, 535–545.
- Stapel, J., Manuntun, R., and Hemminga, M.A. (1997). Biomass loss and nutrient redistribution in an Indonesian *Thalassia hemprichii* seagrass bed following seasonal low tide exposure during daylight. *Mar. Ecol. Prog. Ser.* 148, 251–262.
- Suzuki, N., and Mittler, R. (2006). Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. *Physiol. Plant.* 126, 45–51.
- Tutar, O., Marín-Guirao, L., Ruiz, J.M., and Procaccini, G. (2017). Antioxidant response to heat stress in seagrasses. A gene expression study. *Mar. Environ. Res.* 132, 94–102.
- Unsworth, R.K., Nordlund, L.M., and Cullen-Unsworth, L.C. (2018). Seagrass meadows support global fisheries production. *Conserv. Lett.* 12, e12566.
- Van Tussenbroek, B.I., Muhlia Montero, M., Wong, R., Barba Santos, M., and Márquez Guzmán, J. (2010). Pollen limitation in a dioecious seagrass: evidence from a field experiment. *Mar. Ecol. Prog. Ser.* 419, 283–288.
- Walker, D., Hillman, K., Kendrick, G., and Lavery, P. (2001). Ecological significance of seagrasses: assessment for management of environmental impact in Western Australia. *Ecol. Eng.* 16, 323–330.
- Wang, W., Vinocur, B., Shoseyov, O., and Altman, A. (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci.* 9, 244–252.
- Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., et al. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proc. Natl. Acad. Sci. USA* 106, 12377–12381.
- Wernberg, T., Bennett, S., Babcock, R.C., De Bettignies, T., Cure, K., Depczynski, M., Dufois, F., Fromont, J., Fulton, C.J., Hovey, R.K., et al. (2016). Climate-driven regime shift of a temperate marine ecosystem. *Science* 353, 169–172.
- Winters, G., Nelle, P., Fricke, B., Rauch, G., and Reusch, T. (2011). Effects of a simulated heat wave on photophysiology and gene expression of high- and low-latitude populations of *Zostera marina*. *Mar. Ecol. Prog. Ser.* 435, 83–95.
- Xu, S., Qiao, Y., Xu, S., Yue, S., Zhang, Y., Liu, M., Zhang, X., and Zhou, Y. (2021a). Diversity, distribution and conservation of seagrass in coastal waters of the Liaodong Peninsula, North Yellow Sea, northern China: implications for seagrass conservation. *Mar. Pollut. Bull.* 167, 112261.
- Xu, S., Wang, P., Zhou, Y., Zhang, X., Gu, R., Liu, X., Liu, B., Song, X., Xu, S., and Yue, S. (2018). New insights into different reproductive effort and sexual recruitment contribution between two geographic *Zostera marina* L. populations in temperate China. *Front. Plant Sci.* 9, 15.
- Xu, S., Xu, S., Zhou, Y., Gu, R., Zhang, X., and Yue, S. (2020a). Long-term seed storage for desiccation sensitive seeds in the marine foundation species *Zostera marina* L. (eelgrass). *Glob. Ecol. Conserv.* 24, e01401.
- Xu, S., Xu, S., Zhou, Y., Yue, S., Qiao, Y., Liu, M., Gu, R., Song, X., Zhang, Y., and Zhang, X. (2020b). Sonar and *in situ* surveys of eelgrass distribution, reproductive effort, and sexual recruitment contribution in a eutrophic bay with intensive human activities: implication for seagrass conservation. *Mar. Pollut. Bull.* 167, 111706. <https://doi.org/10.1016/j.marpolbul.2020.111706>.
- Xu, S., Zhou, Y., Qiao, Y., Yue, S., Zhang, X., Zhang, Y., Liu, M., Zhang, Y., and Zhang, Z. (2022). Seagrass restoration using seed ball burial in northern China. *Restor. Ecol.* e13691.
- Xu, S., Zhou, Y., Wang, P., Wang, F., Zhang, X., Yue, S., Zhang, Y., Qiao, Y., and Liu, M. (2021b). Temporal-spatial variations in the elemental and stable isotope contents of eelgrass (*Zostera marina* L.) in the Bohai Sea and Yellow Sea, northern China: sheath as a novel ecological indicator for geochemical research. *Ecol. Indic.* 121, 107181. <https://doi.org/10.1016/j.ecolind.2020.107181>.
- Young, M.D., Wakefield, M.J., Smyth, G.K., and Oshlack, A. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biol.* 11, R14.
- Fengying, Z., Guanglong, Q., Hangqing, F., and Wei, Z. (2013). Diversity, distribution and conservation of Chinese seagrass species. *Biodivers. Sci.* 21, 517–526.
- Zhou, Y., Liu, P., Liu, B., Liu, X., Zhang, X., Wang, F., and Yang, H. (2014). Restoring eelgrass (*Zostera marina* L.) habitats using a simple and effective transplanting technique. *PLoS One* 9, e92982.
- Zhuang, K., Gao, Y., Liu, Z., Diao, P., Sui, N., Meng, Q., Meng, C., and Kong, F. (2020). WHIRLY1 regulates HSP21.5A expression to promote thermotolerance in tomato. *Plant Cell Physiol.* 61, 169–177.
- Zimmerman, R.C., Smith, R.D., and Alberte, R.S. (1989). Thermal acclimation and whole-plant carbon balance in *Zostera marina* L. (eelgrass). *J. Exp. Mar. Biol. Ecol.* 130, 93–109.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Eelgrass morphological and biological performances	This paper	https://doi.org/10.12157/IOCAS.20220526.001
RNA-sequencing data	This paper	PRJNA841441
Software and algorithms		
IBM SPSS Statistics		RRID:SCR_016479
DE-Seq		RRID:SCR_000154
Goseq		RRID:SCR_017052
KEGG		RRID:SCR_012773

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yi Zhou (yizhou@qdio.ac.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Raw data on eelgrass morphological and biochemical performances can be found online at <https://doi.org/10.12157/IOCAS.20220526.001>. RNA-sequencing data are available through the NCBI Sequence Read Archive database (SRA:PRJNA841441).

METHOD DETAILS

Distribution investigation

A total of 21 sites along the coast from Lianyungang, Jiangsu Province to Qingdao, Shandong Province were selected for an eelgrass distribution survey in December 2015 (Figure S1). Eight of the 21 sites, two sites in Rizhao and six sites in Qingdao, were meadows with historical distribution information (1950–2013) of eelgrass specimens from the Biological Museum, Chinese Academy of Sciences, Qingdao, China and previous records in the literature (Zheng et al., 2013). Additionally, two sites in Lianyungang Jiangsu Province were also selected for reinvestigation, and eelgrass was found by Yang (den Hartog and Yang, 1990), but there is no herbarium material from Lianyungang in the Qingdao herbarium. Therefore, in the present study, Shijiusuo, Rizhao City, Shandong Province was identified as the southernmost locality of eelgrass on the eastern coast of China. The surveys were conducted through site visits during low tides.

Restoration site (RS) and donor site (DS)

The restoration area, within intertidal zone, was located in the coastal waters of Shijiusuo, Rizhao City, northern China (35°20'11"N, 119°30'05"E; Figure S1). The average water depth for Shijiusuo is about 3.5 m, and the tides at the restoration area are semidiurnal, with an average tidal range of about 3.00 m. The sediments were mainly composed of sands (51.19 ± 5.15%) and gravels (43.21 ± 5.23%) at RS (Xu et al., 2022). Historical distribution information (1950–2013) of eelgrass specimens from the Biological Museum, Chinese Academy of Sciences, Qingdao, China and the literature (den Hartog and Yang, 1990) revealed that there were *Z. marina* beds in the coastal waters of Shijiusuo in the 1950s. At that time, this location represented the southern geographical distribution limit of eelgrass on the west coast of the Yellow Sea. However, no seagrass was found at this location during the National Seagrass Resource Survey (2015–2020).

The donor population, within intertidal zone, selected for the transplant operation was located in Qingdao Bay, Qingdao City, northern China (36°03'39"N, 120°18'57"E; Figure S1). Qingdao Bay is located ca. 110 km north of the transplant area. The average water depth for Qingdao Bay is about 3.5 m, and the tides at this location are semidiurnal with an average tidal range of about 2.78 m (Xu et al., 2021a; 2021b). The sediments were mainly composed of sands ($89.50 \pm 7.48\%$) at DS (Xu et al., 2022). The eelgrass in Qingdao is a perennial form of *Z. marina*, which can colonize both asexually, through clonal growth, and sexually, through the production of flowers and seeds (Xu et al., 2018). Asexual recruitment of seagrass plays a critical role in population recruitment. The seed germination of eelgrass mainly occurs in autumn.

Environmental parameters at RS and DS

Water temperature (°C) at the plant canopy level was measured every 15 min using HOBO data loggers (USA) from June 2017 to August 2020 at RS, and from January 2018 to August 2020 at DS. Daily mean sea surface temperatures (SSTs) were calculated for the study period. The number of days per year with SST exceeding 30°C ($D_{>30}$) were counted, and the number of hours per day with SST exceeding 30°C ($H_{>30}$) were calculated by multiplying the number of recorded values exceeding 30°C by 0.25 (hours).

Air temperature is considered a good proxy for sea surface temperature (SST) at shallow study sites, certainly including our study sites (Galbraith et al., 2012). To detect the warming trend of temperatures at study sites over the last 60 years, air temperature data were obtained from the China Meteorological Data Service Center (<http://data.cma.cn>). The yearly number of days with air temperatures exceeding 20°C, 25°C, and 30°C were counted from the 1961 to 2020.

YSI Pro 30 (USA) was used to measure the salinity at RS and DS during the study period.

Transplant method

As eelgrass can reproduce both asexually and sexually (Xu et al., 2018), two different transplant methods based on adult shoots and seeds were used (Table S1). A total of 15 transplantations were conducted using adult shoots, and one transplantation was conducted using seeds.

Adult shoots of *Z. marina* that had at least 1–2 cm of rhizome with roots were collected carefully with a shovel from DS at low tide. The shoots were transplanted using a stone anchoring method (Zhou et al., 2014). A transplant unit (PU) consisting of four shoots with rhizomes and roots was anchored to a small elongate stone 50–150 g in weight using biodegradable hemp string. These small stones were collected from the seashore of RS. At RS, PUs were buried at a depth of 2–4 cm with a shovel. A $5 \times 5 \text{ m}^2$ plot was planted using this method. Specimens were transplanted 25 cm apart with a row interval of 25 cm, providing a total of ~400 PUs (equal to 1600 adult shoots) for each transplantation. Fifteen transplantations were performed between 2016 and 2021, in spring, summer, autumn, and winter (Table S1).

Reproductive shoots of *Z. marina* containing seeds were collected in July 2018 from DS. The shoots were cultured in a circular, aerated flow-through tank (1.2 m \times 1.2 m \times 1.2 m) in the laboratory until the seeds were released. The seeds were stored at a salinity of 50psu at 0°C (Xu et al., 2020a; 2020b) until the seed transplant was conducted. On December 7, 2018 at RS, 50 eelgrass seeds were wrapped in wet clay balls (diameter ~1.5 cm), to form a transplant unit, and then buried at a depth of 2–3 cm with a shovel, to minimize seed loss induced by waves and predation (Moore et al., 1993). PUs were planted 20 cm apart with a row interval of 20 cm, and a total of 25 PUs (equal to 1250 seeds) were transplanted in a $1 \times 1 \text{ m}^2$ plot (Table S1).

Transplant monitoring method

At RS, the main monitoring parameters included adult/seedling shoot density and height (Table S1). Monitoring was done every 30–218 days from 2016 to 2021, until no biomass was observed in the summer. Synchronously, adult shoot density and height were also monitored at DS. To compare seed productions between RS and DS, number of spathes per flowering shoot, number of seeds per spathe, and number of seeds per flowering shoot were also monitored in June 2019 and 2020. Most adult plants transplanted before 2019 died in the summer, however some transplanted plants survived the summer of 2019 (over-summering group). Therefore, a comparison of additional parameters (root length, rhizome node length, and rhizome diameter) of surviving shoots in the over-summering group and the newly transplanted shoots in August 2019 (control group) were also conducted in June 2020, and shoots with belowground parts were

randomly sampled from each group. In the present study, adult shoot density (shoots m^{-2}) was calculated by counting the number of shoots from a quadrat (30 × 30 cm), with 4–5 replicates randomly selected in the 5 × 5 m^2 plot, and seedling shoot density was calculated by counting the number of shoots from the clay balls, with six replicates randomly selected in the 1 × 1 m^2 plot. To determine shoot height (cm), 20 shoots were randomly selected for measurement.

Biochemical analysis

Eelgrass leaf blades were sampled in the middle of May, June, and July 2019 at both RS and DS. Five replicates were sampled each time. Superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and peroxidase (POD) were measured following the methods described by Dazy et al. (2009). Malondialdehyde (MDA) was measured using the method described by Hodges et al. (1999). The biochemical analysis work was finished by Jiancheng Laboratories (Nanjing, China).

Transcriptomics analysis

Eelgrass leaf blades, were sampled at about 3:00 pm on July 17 and July 20, 2019 at RS and DS, respectively. Five replicates were sampled on each occasion. The sequencing work was carried out by Allwegene Technology (Beijing, China).

The total RNA of each sample was extracted using a TRIzol RNA kit following the manufacturers protocol. RNA quality was detected after extraction and 2 μ g RNA was taken for library sequencing. Illumina Hi-seq4000 and PE 150 were used for sequencing. Raw data were filtered to remove the reads with joint contamination and low quality to get clean data. The genome of *Z. marina* was sequenced in 2016 (Olsen et al., 2016), and its annotation was updated in 2017. Therefore, in the present study, sequence alignment and splicing were performed with the reference genome of *Z. marina*, and subsequent annotation was performed with the reference genomes of *Z. marina* and *Oryza sativa*.

After checking RNA-Seq correlation, DESeq R package(v1.10.1) was used for differential expression analysis (Anders and Huber, 2010). Goseq (v1.22) software was used for GO enrichment analysis (Young et al., 2010). DEG pathways were analyzed based on KEGG (Kyoto Encyclopedia of Genes and Genomes, <http://www.kegg.jp>).

Real Time (RT)-qPCR was used to evaluate the reliability of RNA-Seq. In total, seven genes (three replicates) were selected for RT-qPCR validation using cDNA as a template. The relative expression level was calculated by $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001).

Data analysis

Differences in seagrass biological characteristics (shoot height, root length, rhizome node length, and rhizome diameter) and seed production (number of spathes per flowering shoot, number of seeds per spathe, and number of seeds per flowering shoot) were tested using a one-way ANOVA or independent t test. Variations in seagrass antioxidant enzyme activities (SOD, MDA, GPX, CAT, and POD) between RS and DS, and among various sampling times were tested using a two-way ANOVA. GPX data were transformed into log data to achieve homogeneity of variance. When the interaction was significant, one-way ANOVA was used to test temporal variations at each study site, and spatial variations at each sampling time. The homogeneity of variance was tested using Levene's test. Differences were considered significant at a probability level of $p < 0.05$. SPSS 20.0 was used for all data analyses.