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Responses of *Sargassum thunbergii* Germlings to Acute Environmental Stress

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Abstract: The responses of *Sargassum thunbergii* germlings to high temperature, low salinity, desiccation, combined thermal and osmotic stress (35 ºC combined with 12 psu), anthracene, and eutrophication were examined. Probit regression analysis results showed that the median lethal time (LT50) values of high temperature decreased with the increase in temperature. The 24 h median lethal temperature was 36.9 ºC. For salinity treatment, the LT50 value of fresh water was 47.6 h. Survival rates of germlings were over 60% when germlings were exposed to salinities ranging from 27 psu to 7 psu at a time interval of 108 h post-treatment. The LT50 values of desiccation and combined thermal and osmotic stress (35 ºC combined with 12 psu) were 7.0 h and 9.8 h, respectively. Anovas showed that germlings were inhibited by high concentrations of anthracene (5 mg L^{-1} and 10 mg L^{-1}) with low survival rates of below 50% and low relative growth rates of below 1% after 25 days of culture; however, low concentrations (0.01-1 mg L^{-1}) had no significant effects. In addition, neither severe eutrophication nor disproportionality of N/P showed any significant effect on the survival and growth of germlings. Of the environmental stresses tested, possible occurrence of high temperature of 40 ºC and combined thermal and osmotic stress directly impacted the survival of germlings, suggesting that the deterioration of S. *thunbergii* bed may be related more to increasing extreme climatic events.

Keywords: Environmental stress, germling, median lethal value, *Sargassum thunbergii.*

1. INTRODUCTION

As a member of *Sargassum* beds, *Sargassum thunbergii* is ecologically important in the maintenance of a healthy coastal ecosystem [1], such as serving as a primary producer; in spawning, nursery and feeding ground for marine organisms; and as nutrient cycling controller [2]. However, the natural populations of *S. thunbergii* along the coast of China have evidently deteriorated in recent years [3]. It is necessary to explore the reason for this phenomenon. Mostly, deterioration of seaweed beds is caused by various anthropogenic interferences and climate changes which cause increasing extreme events and physical stresses [4]. For example, increased temperature is generally thought to have negative effects on spore production, germination, sporophyte growth and recruitment of seaweeds [5]. Particularly, when they experience periods of temperature change, which are sufficiently high to result in disruptive stress, such damage and any reallocation of resources for protection and repair can cause slow growth, delay development and also lead to mortality [6]. In addition, seaweed growths have declined due to water pollution such as polycyclic aromatic hydrocarbons (PAHs) and eutrophication. Anthracene is a PAH with higher solubility than most other PAHs due to its low molecular capacity and may prove a threat to the environment if widely distributed [7]. It acts as a photosensitizer causing an oxidative damage of algal cells [8]. The eutrophication of coastal zones resulted from land run-off, river inflow and sewage discharges with an imbalanced N/P ratio can cause harmful algal blooms [9] and deterioration of macroalgal communities [10]. Deeper in water, total algal abundance and abundance of perennial algae decrease along a eutrophication gradient [11]. In brief, both physical and chemical stresses may have adverse effects on the survival of seaweed.

The role of recruitment from germlings in the *S. thunbergii* populations has not been investigated. In other Sargassum species, the role of recruitment from propagules in local persistence and stabilising densities of populations remains controversial [12]. In any case, early stage is a bottleneck for algae [9]. Therefore, it is necessary to test the tolerance of *S. thunbergii* germlings to environmental stresses to search for the reasons of deterioration of *S. thunbergii* bed.

Our previous studies investigated the responses of *S. thunbergii* germlings to combined physical stresses [13]. However, the effects of individual physical and chemical stress are still unknown. Therefore, the present study focuses on the chemical stress and the median lethal values of germlings responding to the individual physical stress. As a common experimental measure of stress tolerance, the acute LD50 (median lethal dose) is used for evaluating tolerance to physical stress due to its high accuracy and flexibility [14]. The lower the value of LD50 , the higher is the damage of the stress. The median lethal time was also estimated for the combined thermal and osmotic stress (35 ºC combined with 12 psu) which previously resulted as extreme for germlings [13]. The objective of this study was to investigate if the

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physical and chemical parameters emerge as environmental stresses for *S. thunbergii* germlings; and if the environmental stresses caused by local (pollution) and overall (climate change) anthropogenic disturbances can explain the deterioration of *S. thunbergii* beds.

2. MATERIAL AND METHODS

2.1. Collection of Germlings

Fertile female and male specimens of *S. thunbergii* were collected on June 25, 2011, in the intertidal zone of Zhanqiao (37°31'N, 121°26'E), Yantai. Selected thalli (about 25 cm) were healthy and yellowish-brown in appearance with intact and inflated receptacles which had no obvious shedding. Once transported to the laboratory, they were placed in two 15L plastic tanks (30cm ×20cm×25cm) filled with filtered seawater which was continuously aerated. Tanks were kept at 25 ºC, 60 μmol photons $m^{-2}s^{-1}$ and with a 10L:14D (light: dark cycle) photoperiod. The irradiance was provided with fluorescent illumination and measured on the water surface.

Released germlings sank to the bottom of tanks within 24 h after fertilization. Germlings were then collected by filtering the remaining seawater with an 80-mesh nylon sieve, followed by filtration with a 200-mesh nylon sieve. A total of about 3.0×10^5 germlings were obtained. They were subsequently transferred to a 3 L glass tank and even stirred to produce a homogeneous suspension.They were then immediately poured into each Petri dish (60mm × 15mm). A total of 109 Petri dishes with about 700 germlings in each were used for the experiment.

2.2. Stress Treatments

After 24 h, germlings attached to the Petri dishes were cultured in seven light and temperature-controlled incubators on the basis of the following experimental design. Three independent replicates were used for each treatment. For desiccation treatment, samples were cultured in a total of five durations of desiccation: 3 h, 6 h, 9 h, 10 h and 12 h. The desiccation treatment was conducted at relative humidity of about 80%. The chosen level of relative humidity was in accordance with that in the sample plot. The relative humidity was measured with a hygrometer and adjusted by placing soaked filter paper or calcium sulfate in three incubators. After desiccation, germlings were fully immersed for 12 h prior to the record of survival rate.

For hyposalinity treatment, samples were cultured at five levels of salinity: 27, 20, 13, 7 and 0. Solutions of different salinities were prepared from seawater diluted with distilled water, measured with a salinity hydrometer (GM Manufacturing Co.) and changed on a daily basis.

For anthracene treatment, samples were cultured at seven concentrations of anthracene: 0.01 mg L^{-1}, 0.05 mg L^{-1}, 0.1 mg L^{-1}, 0.5 mg L^{-1}, 1 mg L^{-1}, 5 mg L^{-1} and 10 mg L^{-1}, respectively. The tests of anthracene were performed on the basis of two controls (germlings in culture with and without 0.5% DMSO).

A two-way factorial experimental design was used to test the effects of severe eutrophication and disproportionality of N/P on germlings, along-with the concentrations of N and P as fixed factors [15]. The concentrations of N and P sources were designed as 0.072 mg L^{-1}, 0.36 mg L^{-1} and 2 mg L^{-1} for N; and 0.011 mg L^{-1}, 0.03 mg L^{-1} and 0.3 mg L^{-1} for P. $NaNO_3$ and KH_2PO_4 were added to the seawater as respective N and P sources for a final concentration. Therefore, high N/P ratios (0.36/0.011, 0.36/0.03, 2/0.011 and 2/0.03 mg L^{-1}) and low N/P ratios (0.072/0.03, 0.072/0.3 and 0.36/0.3 mg L^{-1}) were obtained. In addition, severe eutrophication with normal N/P ratio (2/0.3 mg L^{-1}) was also included.

The treatments of desiccation, reduced salinity, anthracene and eutrophication listed above were conducted at 25 ºC. For high temperature treatment, samples were cultured at six temperature conditions: 35 ºC, 36 ºC, 37 ºC, 38 ºC, 39 ºC and 40 ºC. The extreme combined condition (35 ºC combined with salinity of 12) was also conducted to estimate the median lethal time. All treatments were cultured at irradiance of 60 μmol photons $m^{-2}s^{-1}$ with a 10 L: 14 D light: dark cycle. The irradiance within the wavelength range 400-700 nm was measured using a Li-Cor LI-250 light meter equipped with a LI-190SA quantum sensor.

2.3. Measurements of Survival and Growth

Prior to the stress treatment, the mean survival rate of germlings in four dishes was measured as the initial survival rate. The survival rate used in the present study was calculated as a ratio of the actual survival rate in the treatment to the initial survival rate. In each dish, germlings were counted in five areas arranged in a cross pattern (the upper, lower, left and right peripheries and the center) under stereoscopy microscope. A total of about 200 germlings were used to calculate the survival rate of each dish. Germlings were classified as dead if they collapsed or were structurally fragmented.

Considering that anthracene and eutrophication may have no evident effects on the survival of germlings, for the treatments of anthracene and eutrophication, growth of germlings was measured in terms of changes in lengths excluding rhizoids at the end of the experiment. Lengths were measured using a microscope with an ocular micrometer in five areas in each Petri dish as mentioned above. The initial mean length of germlings in four dishes was also measured before the stress treatment. A total of 20 germlings in each dish were used to estimate the relative growth rate (RGR, % d^{-1}). RGR was calculated as 100 (ln (L_1) – ln (L_0))/t, from initial versus final values, where L_0 and L_1 are germlings lengths at the start and at the end of treatment, respectively, and t is the length of treatment period calculation in days [16].

2.4. Statistical Analysis

All the data were analyzed using SPSS 13.0 for Windows. Median lethal values of temperature, salinity, desiccation and extreme combined condition with 95% confidence limits (95% CL) were determined using the probit regression analysis [17]. Probit regression equations were estimated as Y = a + b X; where Y is the percent of mortality in probit units, a and b are the intercept and slope constants, and X is the log time or log dose of stress. The effects of DMSO and anthracene on the survival of

germlings were tested by repeated measures ANOVA. One-way ANOVAs were used to test the effect of DMSO and anthracene on the growth of germlings. Main effects and interactions of N and P on the survival of germlings were analyzed by two-way repeated measures ANOVA. A two-way univariate analysis of variance was performed to test the significance of the main effects and interactions on the growth of germlings, with N and P as fixed factors. For repeated measured ANOVA, the Huynh-Feldt correction was used to adjust the degrees of freedom when the sphericity assumption was violated (*i.e.* if Mauchly's test of sphericity was statistically significant at $p < 0.05$). Tukey's tests were used for post-hoc comparisons. The differences were considered to be statistically significant if the probability value was less than 5% ($p < 0.05$).

3. RESULTS

3.1. Acute Physical Stress for Germlings

For high temperature treatment, the survival rates of germlings decreased with time (Fig. **1**). In comparison to the gradual decline of survival rates at 35 ºC and 36 ºC, the survival rates decreased sharply to 0% within 40 h when exposed to 37 ºC to 40 ºC. Especially at 40 ºC, the survival rate of 0% was even recorded at about 10 h after treatment (Fig. **1**). The results of acute thermal stress tests are listed in Table **1**. Variation in LT_{50} values at different temperatures was evident.50% mortalities occurred after exposure to 35 ºC and 36 ºC at over 50 h and 70 h, respectively (Table **1**). However, LT_{50} values decreased sharply from 37 ºC to 40 ºC (Table **1**). When exposed to 40 ºC, the LT_{50} value was even lower than 5 h (Table **1**). By the end of 24 h thermal tolerance experiment, the median lethal temperature was estimated as 36.9 ºC ($\chi^2 = 73.8$, df = 13, $p < 0.001$, 95% CL, 36.6 – 37.1 ºC).

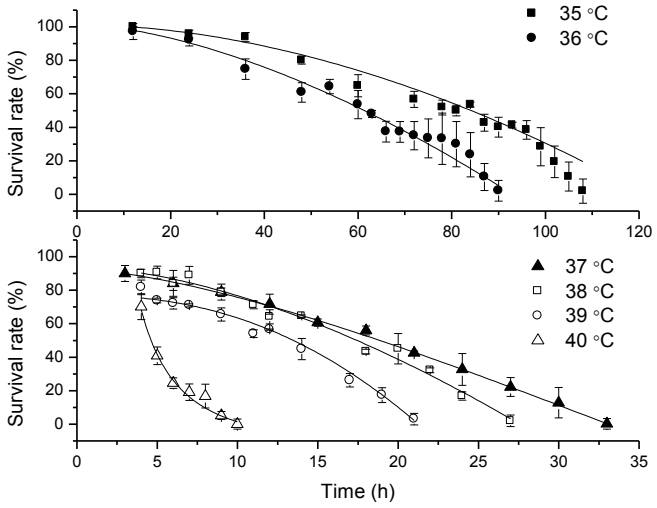

Fig. (1). Survival rates of germlings exposed to various temperatures (35 ºC, 36 ºC, 37 ºC, 38 ºC, 39 ºC and 40 ºC). Solid lines are logistic fits (all p values < 0.01 and all R^2 values > 0.97). Values are means ± SE (n = 3).

For hyposalinity treatment, germlings maintained high survival rates at over 90% when exposed to 27 psu, 20 psu and 13 psu for 108 h (Fig. **2**). Over 60% survival rate was obtained after 108 h exposure to 7 psu (Fig. **2**). However, in fresh water, survival rate decreased sharply to about 0% at a time period of 60 h (Fig. **2**). Probit analysis estimated an LT_{50} value of 47.6 h in fresh water (Table **1**).

For desiccation treatment, the survival rates decreased quickly in a short time span and all germlings died at 12 h (Fig. **3**). The LT_{50} value of desiccation was estimated as 7.0 h (Table **1**).

For combined thermal and osmotic stress treatment, the survival rates decreased to about 0% at 21 h (Fig. **3**). The LT_{50} value of combined thermal and osmotic stress was estimated as 9.8 h (Table **1**).

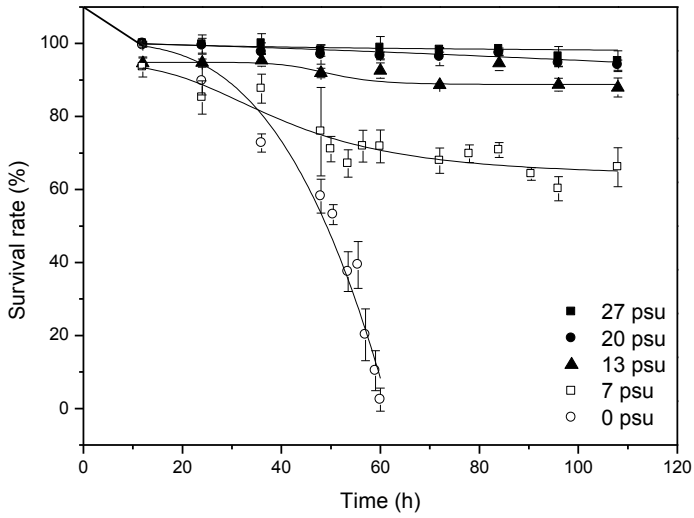

Fig. (2). Survival rates of germlings exposed to various salinities (27 psu, 20 psu, 13 psu, 7 psu and 0 psu). Solid lines are logistic fits (all p values < 0.01 and all R^2 values > 0.70). Values are means ± SE (n = 3).

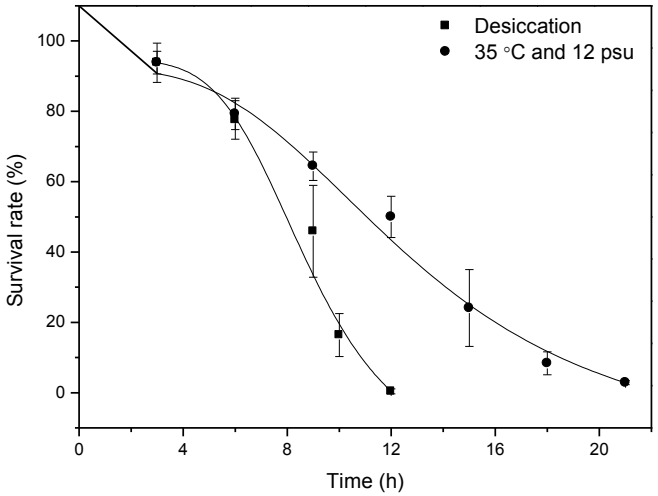

Fig. (3). Survival rates of germlings exposed to desiccation and 35 ºC combined with 12 psu. Solid lines are logistic fits (all p values < 0.01 and all R^2 values > 0.99). Values are means ± SE (n = 3).

3.2. Effect of Anthracene on Germlings

Results of one-way ANOVA indicated that the difference between seawater and DMSO (0.5% v/v) for the growth of germlings was not significant ($F = 0.053$, $p = 0.830$). DMSO also had no significant effect on the survival of germlings

Table 1. Median lethal time of germlings exposed to various stresses (n = 3).

Variable	*df*	χ^2	Regression Equation	LT_{50} (h)	95% Confidence Limit (h)	
					Lower	Upper
35 ºC	49	123.7*	Y = - 7.9 + 4.2 X	77.2	74.6	12.0
36 ºC	40	189.0*	Y = - 6.1 + 3.5 X	57.9	80.0	3.5
37 ºC	28	155.1*	Y = - 3.0 + 2.5 X	16.4	54.3	4.8
38 ºC	34	98.5*	Y = - 3.3 + 2.8 X	15.8	61.5	45.0
39 ºC	28	70.5*	Y = - 2.5 + 2.4 X	11.0	14.6	50.4
40 ºC	13	63.0*	Y = - 2.5 + 4.0 X	4.3	18.4	6.2
Fresh water	22	76.6*	Y = - 7.9 + 4.7 X	47.6	14.7	8.3
Desiccation	13	169.3*	Y = - 4.9 + 5.6 X	7.0	17.1	8.7
35 ºC + 12 psu	19	137.6*	Y = - 4.0 + 4.0 X	9.8	10.2	10.9

* Since Goodness-of-Fit test is significant ($p < 0.05$), a heterogeneity factor is used in the calculation of confidence limits.

(Table **2**). Therefore, the interference of 0.5% DMSO on the growth and survival of *S. thunbergii* germlings can be excluded. Although anthracene significantly affected the survival (Table **2**), low concentrations (0.01 ~ 1 mg L^{-1}) had no significant effects (Tukey's tests: p= 0.433, p = 0.996, p = 0.830 and p = 0.340, respectively). Survival rates of over 70% were obtained after 25 days of exposure to concentrations rangeing from 0.01 mg L^{-1} to 1 mg L^{-1} (Fig. **4**). Similar to the survival effect, although a significant effect of anthracene on the growth of germlings was found (F = 49.360, $p < 0.001$), low concentrations of anthracene (0.01 mg L^{-1} – 1 mg L^{-1}) had no significant effects on growth (Tukey's tests, p = 0.510, p = 0.982, p = 0.163, p = 0.676 and p = 0.234, respectively). Germlings were severely inhibited by high concentrations of anthracene (5 mg L^{-1} and 10 mg L^{-1}) with low survival rates and RGRs of below 50% and 1%, respectively (Tukey's tests, $p < 0.001$) (Fig. **4**). The initial mean length of germlings was measured as 127.6 μm. At the end of anthracene treatment, final lengths ranged from 129.3 to 461 μm.

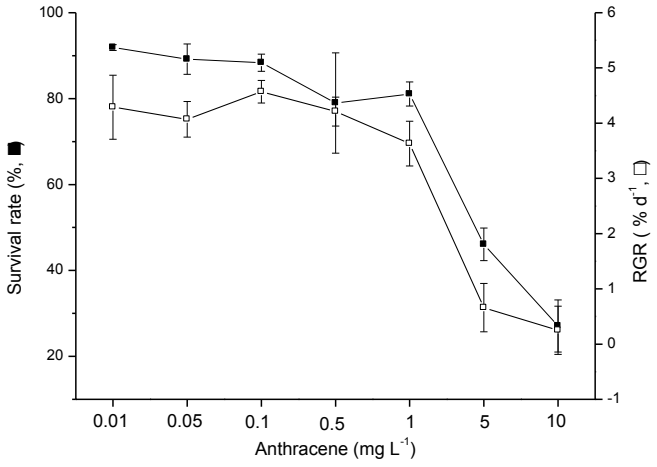

Fig. (4). Relative growth rates (RGR; % day^{-1}) and survival rates of germlings exposed to various concentrations of anthracene (0.01mg/L, 0.05 mg/L, 0.1 mg/L, 0.5 mg/L, 1 mg/L, 5mg/L and 10 mg/L) after 25 days of culture. Values are means ± SE (n = 3).

3.3. Effect of Eutrophication on Germlings

The results of repeated measures ANOVA showed that neither N nor P source had significant effect on the survival of germlings (Table **3**). No significant effects of N or P on the growth were found (Table **4**). Therefore, severe eutrophication had little effect on germlings. Tables **3** and **4** also showed that the interaction between N and P source had no significant effects on the survival and growth, indicating that various imbalanced N/P ratios also had no effect on germlings. By the end of eutrophication treatment, final lengths of germlings ranged from 227.115 to 344.93 μm.

4. DISCUSSION

Of the environmental stresses tested in the present study, high temperature of 40 ºC and combined thermal and osmotic stress (35 ºC combined with 12 psu) may directly impact the survival of germlings.

Although the LT_{50} value of 39 ºC was evaluated as 11h, its duration in the mid-tidal zone occupied by *S. thunbergii* cannot exceed the LT_{50} value even with this temperature , because the temperature decreases due to immersion in seawater during the high tide. This suggests that the high temperature not over 39 ºC causes no mass mortality of *S. thunbergii* germlings in the field. However, the LT_{50} value of 40 ºC was only about 4 h. Although *S. thunbergii* germlings are rarely exposed to 40 ºC for 4 h during low tide, this extreme thermal condition possibly occurs under extreme hot weather.

The LT_{50} value of combined thermal and osmotic stress (35 ºC combined with 12 psu) was much lower than the separate LT_{50} values of 35 ºC or 13 psu indicating that there is a synergistic effect between thermal and osmotic stress, that is, germlings living near the limit of one tolerance were more sensitive to additional stress, which is in accordance with the results reported for *Fucus vesiculosus* [18]. Low salinity usually results from strong rainfall which also causes a decrease in temperature. Hence, high temperature and low

Table 2. Repeated measures ANOVA for effects of DMSO and anthracene on survival of germlings.

	Variable	*df*	Mean Square	*F*	*p*
DMSO	Within-subjects				
	Time	24	25.785	5.555	< 0.001
	Time × DMSO	24	7.264	1.565	0.066
	Error (Time)	96	4.642		
	Between-subjects				
	DMSO	1	29.695	0.343	0.59
	Error	4	86.532		
Anthracene	Within-subjects				
	Time	19.174	302.948	23.310	< 0.001
	Time × Anthracene	115.047	292.546	22.509	< 0.001

Table 3. Repeated measure ANOVA for effects of $NaNO_3$ and KH_2PO_4 on survival of germlings. N: $NaNO_3$, P: KH_2PO_4.

Variable	*df*	Mean Square	*F*	*p*
Within-Subjects				
Time	4	91.923	14.428	< 0.001
Time × N	8	5.178	0.813	0.594
Time × P	8	15.936	2.501	0.019
Time × N × P	16	3.847	0.604	0.871
Error (Time)	72	6.371		
Between-Subjects				
N	2	12.68	0.394	0.680
P	2	27.278	0.849	0.444
N × P	4	10.557	0.328	0.855
Error	18	32.145		

Dependent variable (survival) was untransformed and the assumption of homogeneity met Levene's test ($F = 4.367$, $p = 0.212$).

salinity can generally not be concurrent with each other in the field. However, extreme climatic events, such as El Niño which can cause high temperatures and storms, may severely destroy *S. thunbergii* germlings. For example, on the Pacific Coast of California and Baja California, a reduction of the brown algae *Macrocystis pyrifera* bed size and biomass with up to 100% in some areas resulted from the influence of El Niño [4].

Table 4. Univariate analysis of variance for effects of $NaNO_3$ and KH_2PO_4 on relative growth rate (RGR, % d^{-1}) of germlings. N: $NaNO_3$, P: KH_2PO_4.

Variable	*df*	Mean Square	*F*	*p*
N	2	< 0.001	1.779	0.197
P	2	< 0.001	2.824	0.086
N × P	4	< 0.001	1.887	0.156
Error	18	< 0.001		

Dependent variable (RGR) was untransformed and the assumption of homogeneity met Levene's test ($F = 0.843$, $p = 0.547$).

The capacity to tolerate desiccation is thought to be a major factor in determining the upper limits of distribution for intertidal seaweed [19]. In comparison to other stresses, germlings were more vulnerable to desiccation at a relative humidity of 80%, which is consistent with our previous study [13]. In contrast, small thallus pieces of *Codium fragile* (green alga) can survive long periods of emersion (90 days) when kept under high relative air humidity of 90% [20]. We suggest that desiccation tolerance is related to relative humidity. Therefore, lower relative humidity may have greater adverse effects on *S. thunbergii* germlings in a short span of time. During the low tide, adult canopy can act as a nurse thalli by buffering germlings beneath them from desiccation stress. It is inferred that in the upper tidal zone which is characterized by its long periods of emergence, germlings beneath the adult canopies are the main sources of *S. thunbergii* recruitments.

Intertidal seaweeds are subjected to low salinity stress when exposed to low tide or trapped in tide pools, where fresh water from rain may cause a decrease in salinity [21]. In seaweeds, hypo-osmotic stress causes increases in cell

volume and turgor, resulting in the loss of ions and organic solutes as well as in damage to membranes and organelles, culminating in cell rupture [22]. However, in this study, *S. thunbergii* germlings exhibited high tolerance to reduced salinity, even in fresh water. Similar phenomena were reported in *Fucus*. Simulated rainfalls during low tides caused photosynthetic activity of *Fucus spiralis* to drop to 50% of initial Fv/Fm, independent of the length of the rain period. Treated thalli also fully recovered after 6 min re-submersion in seawater [23]. It suggested that even heavy rain during the low tide cannot result in mass deaths of *S. thunbergii* germlings. Furthermore, extremely low salinity cannot be maintained for a long time due to tidal motions. Therefore, the deterioration of *S. thunbergii* beds may not be related to hyposalinity resulting from heavy rain.

It has been reported that algae are more tolerant to PAHs than other aquatic organisms [24]. In the present study, *S. thunbergii* germlings also showed high tolerance to anthracene. Although the survival and growth of germlings were significantly affected by the concentration of over 5 mg L^{-1}, the solubility of anthracene in water (0.073 mg L^{-1}) wasmuch lower than its concentration [25]. Although anthracene at 0.25 mg L^{-1} reduced the growth of three *Scenedesmus* species [26], growth of *S. thungergii* germlings was not significantly affected at this concentration. It appears that direct anthracene toxicity is not important in assessing the environmental hazard posed to algae by anthracene and PAH contamination [27]. However, there was a significant interaction between anthracene and UV-A radiation, which, in combination, caused significant toxic effects on *Selenastrum capricornutum* [27]. Anthracene at nominal concentrations exceeding 0.05 mg L^{-1} inhibited the growth of the algae in a concentration- and irradiance-dependent manner [8]. Therefore, response of *S. thunbergii* germlings to the interaction between anthracene and irradiance needs further investigation.

Eutrophication can result in accelerated development of the early stages of some algal species [9]. However, in the present study, neither positive nor negative effects of eutrophication on *S. thunbergii* germlings were found. Effect of N/P ratio on marine environment has received great deal of attention. The appearance of different red tides was related to the N/P ratio [28]. Our results showed that N/P ratio had no significant effect on *S. thunbergii* germlings. However, indirect effects including increased sediment cover of substrata, scouring caused by wind-induced resuspension of sediments, and grazing, were also expected to be negative [9]. A deterioration of the light climate due to increased phytoplankton biomass, suspended matter and overgrowing (shading) by epiphytes are likely causes for the decline of Fucus spp. in Kiel Bay [29], and for a decrease in macrophyte numbers in general [30]. Therefore, indirect effects of eutrophication may be partially responsible for the deterioration of *S. thunbergii* beds.

Since the young stages of seaweeds are a sensitive link in species life cycle [18], recovery of populations from anthropogenic stress is likely to depend upon recruitment of these early stages [9]. Pollution of PAHs and eutrophication seems to have little effect on *S. thunbergii* germlings. Although high temperature of 40 ºC and combined thermal and osmotic stress (35 ºC combined with 12 psu) directly impacted the survival of germlings, such extreme conditions rarely exist in the middle of the latitude region unless extreme events occur. It is suggested that increasing extreme climatic events caused by various anthropogenic interferences are more responsible for the deterioration of *S. thunbergii* beds and other seaweed beds. These results will be useful in the search for evidence regarding the reasons of deterioration of *S. thunbergii* beds.

CONCLUSION

As an ecologically important member of *Sargassum* beds, the natural populations of *S. thunbergii* along the coast of China have evidently deteriorated in recent years. This paper represents the responses of *S. thunbergii* germlings to acute environmental stress. The median lethal time (LT50) values of high temperature, low salinity and combined thermal and osmotic stress (35 ºC combined with 12 psu) by Probit regression analysis, and the effects on the survival and growth of germlings of high concentrations of anthracene (5 mg L^{-1} and 10 mg L^{-1}), low concentrations (0.01-1 mg L^{-1}), severe eutrophication and disproportionality of N/P by anovas, were examined. Results indicated that the deterioration of *S. thunbergii* beds may be related more to the increasing extreme climatic events.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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