

# Effects of main ecological factors on the growth of marine green alga *Caulerpa sertularioides* using the response surface methodology

Bingxin Huang<sup>1,2</sup>, Yue Chu<sup>1,2</sup>, Rongjuan Wang<sup>1,2</sup>, Yixiao Wang<sup>1,2</sup>, Lanping Ding<sup>1,2\*</sup>

<sup>1</sup> College of Life Sciences, Tianjin Normal University, Tianjin 300387, China

<sup>2</sup> Tianjin Key Laboratory of Animal and Plant Resistance, Tianjin 300387, China

Received 25 October 2022; accepted 11 January 2023

© Chinese Society for Oceanography and Springer-Verlag GmbH Germany, part of Springer Nature 2023

## Abstract

*Caulerpa sertularioides* is an invasive potential blooming green alga in China but it remains poorly studied. We studied the effects of ecological factors on its growth. Optimum conditions of ecological factors, i.e., irradiance, temperature, and salinity, for the growth of its fragments were determined in the response surface methodology (RSM). The specific growth rates (SGR) of the fragments were determined in single-factor experiment. The results show that the SGR of *C. sertularioides* peaked under the conditions of irradiance 37.5  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , temperature 25°C, and salinity 30. Meanwhile, using the Box-Behnken design, the conditions were further optimized and verified to be: irradiance 39.03  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , temperature 25.29°C, and salinity 30.06, under which the SGR reached 4.66%. The results provide new theoretical data and solutions for the cultivation, invasion prediction, and monitoring of *Caulerpa* species in China and the world. The RSM method may have great potential applications in the environmental adaptation characteristics of new macroalgal cultivars, intensive orientation cultured germplasm, and environmental hazard analysis of cultivated species in the field.

**Key words:** *Caulerpa sertularioides*, fragment, response surface methodology (RSM), ecological factor

**Citation:** Huang Bingxin, Chu Yue, Wang Rongjuan, Wang Yixiao, Ding Lanping. 2023. Effects of main ecological factors on the growth of marine green alga *Caulerpa sertularioides* using the response surface methodology. Acta Oceanologica Sinica, 42(11): 90–97, doi: 10.1007/s13131-023-2171-0

## 1 Introduction

*Caulerpa* is a genus of marine green algae distributed mainly in tropical and subtropical waters and has more than 90 species. The thalli contain caulerpin, an alkaloid extracted from *Caulerpa*. The compound has anti-inflammatory activity, is rich in minerals and vitamins (Jiang et al., 2011), and has great value in application in the industries of food (de Gaillande et al., 2017), medicine (Gao, 2014; Chaves Filho et al., 2022), high-grade fertilizer (Wang, 2010), energy (Huang, 2012), and bioremediation of marine waters (Landi et al., 2022). Physiological and ecological studies have mostly focused on some environmental factors such as temperature (Li et al., 2022; Shi et al., 2022), salinity (Guo et al., 2015; Cai, 2021), irradiances (Stuthmann et al., 2021; Zhong et al., 2021), nutrient (Liu et al., 2016; Zhang et al., 2020) and heavy metal salt stress (Pang et al., 2021) for cultivated *Caulerpa lentilifera* in Southeast Asian countries. However, due to invasive nature, some species caused ecological problems in the Mediterranean, America, Australia, and other waters in the world (Davis et al., 1997; Meinesz et al., 2001; Verlaque et al., 2004; Anderson, 2005; Lapointe et al., 2005; Klein and Verlaque, 2008; Kang et al., 2021). In a eutrophic sea where some species boom excessively, vast and thick algal mats would form on the seafloor, which hamper sunlight transmission and seawater exchange, posing a large threat to local mariculture activities or coral reef growth (Williams and Schroeder, 2004). In environments with poor water quality, these blooming species have a strong ability to ab-

sorb nutrients from water and sediments (Williams, 1984; Kolar and Lodge, 2001). Therefore, they have the potential for sewage treatment (Landi et al., 2022).

Some of the *Caulerpa* species are highly invasive such as *Caulerpa taxifolia* and *Caulerpa racemosa* (Anderson, 2005; Fernández and Cortés, 2005; Liu et al., 2019). *Caulerpa sertularioides* (Anderson, 2005; Fernández and Cortés, 2005), also known as green feather alga, is similar in appearance and has the same mode of reproduction as *C. taxifolia*, including well-developed stolons and rhizoids for easy attachment to substrates (Fig. 1). It is highly invasive because its fragment of stolons can grow continuously from its apices (Smith and Walters, 1999), and spread quickly in the tropical northeast Pacific (Withgott, 2002). Its bloom damaged the coral reefs seriously (Smith et al., 2010). Some scientists believed that the high invasion ability might be related to a favorite temperature and the quick proliferation of thallus fragments (Fernández and Cortés, 2005). Some affected countries (such as Spain and France) are very concerned about the invasion and have tried to eradicate or control it by banning the trade of aquatic species (Klein and Verlaque, 2008).

In China, *C. sertularioides* was distributed mainly along the coast of the South China Sea in Taiwan, Hainan, and other islands (Ding et al., 2015; Liu et al., 2019). During the growing season, its blooms and the biomass abounds along the coast of the South China Sea (Fig. 2). However, few previous studies focused on its antimicrobial activities (Kumar et al., 2011), and polysac-

Foundation item: The National Natural Science Foundation of China under contract Nos 31970216, 32270219 and 31670199.

\*Corresponding author, E-mail: skydpl@tjnu.edu.cn



Fig. 1. The external morphology of *Caulerpa sertularioides*.



Fig. 2. The field population of *Caulerpa sertularioides* in the intertidal zone of Yinggehai, Hainan.

charides and sterols (Shevchenko et al., 2009; Chaves Filho et al., 2022). More studies are required to elucidate its invasion characteristics and potential.

The response surface methodology (RSM) is a new statistical method for solving multivariable problems and exploring optimal process parameters by analyzing the regression equation (Mee, 2009; Mäkelä, 2017). It has been widely used in the research of life science in recent years because of its advantage of direct display selection of optimal operating conditions in experiment design (Kim et al., 2019; Srinivas et al., 2019; Faramarzi et al., 2019; da Silva et al., 2019; Vishwakarma and Banerjee, 2019; Ebadi et al., 2019; Nur et al., 2019). In China, the RSM method has recently been used to analyze the algal polysaccharides of *Caulerpa lentillifera*, demonstrating its advantages in the analysis of the active ingredients of algae (Tong et al., 2022).

In this study, effects of irradiance, temperature, and salinity on the growth of *C. sertularioides* are studied by using RSM. By determining and analyzing the optimum conditions of multiple ecological factors, it will provide reasonable data for cultivation, prevention and control of biological invasion of *C. sertularioides* in the natural waters.

## 2 Materials and methods

### 2.1 Materials

The samples of *C. sertularioides* were bought from Tianjin's

aquariums and pre-cultured for one week in a seawater tank at room temperature, the thalli were washed and cleaned with disinfected seawater to remove debris, and then cultured under aeration for 30 days under the conditions of irradiance ( $37.5 \pm 6.25$ )  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , temperature ( $25 \pm 1$ )  $^{\circ}\text{C}$ , and salinity ( $30 \pm 1$ ) to obtain adequate biomass for the following experiment.

### 2.2 Methods

The thalli with long stolons were picked out and cut with sterilized scissors into at least 3 cm-long fragments carefully, making sure each fragment containing some blades and rhizoids (Smith and Walters, 1999), and placed in the air for 15 s for better wound healing. The fresh weight of each fragment was between 0.31–0.38 g (the mean standard deviation was  $(0.35 \pm 0.02)$  g).

Referring to the cultivation experience and data, three ecological single-factor experiments, in which three main factors, e.g., irradiance, salinity, and temperature are tested and each is assigned a variable value, while the other two are constants. The conditions were set as: (1) irradiance at 18.75  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , 25.00  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , 31.25  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , 37.50  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , 43.75  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$  and 50.00  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , salinity at 30 and temperature at 25  $^{\circ}\text{C}$ ; (2) temperature at 19  $^{\circ}\text{C}$ , 22  $^{\circ}\text{C}$ , 25  $^{\circ}\text{C}$ , 28  $^{\circ}\text{C}$ , 31  $^{\circ}\text{C}$  and 34  $^{\circ}\text{C}$ , irradiance at 37.5  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$  and salinity at 30; and (3) salinity at 15, 20, 25, 30, 35 and 40, irradiance at 37.5  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$  and temperature at 25  $^{\circ}\text{C}$ .

Each sample (including three fragments) was placed in a 250 mL conical flask containing 200 mL seawater that was renewed daily and cultured in the thermostatic illumination incubator (Jiangnan Instrument Co., Ltd., Ningbo, type GXZ) referring to the different treatments above. The total cultivation cycles last a week. Each treatment group was replicated three times. All samples were taken out and weighted wet at the end of the week, and then the SGR (specific growth rate) was calculated.

A central combination design experiment and the verification experiment on the results of the RSM of the three factors were performed using multi-factor experimental method. The results of the single-factor experiment were used to confirm the horizontal range of the ecological factor. The Box-Behnken central combination design, or the Box-Behnken design for simplicity—an RSM method was used to determine the SGR of the thalli fragments, and the optimum conditions of irradiance, temperature, and salinity.

The experimental data were statistically analyzed using Design-Expert 10.

SGR can be expressed as:

$$\text{SGR} = \frac{W_t - W_0}{t} \times 100\%, \quad (1)$$

where  $W_0$  is the fresh mass (g) of fragments at the initial stage;  $W_t$  is the fresh mass (g) of fragments at the end of the experiment; and  $t$  is the number of days of the experiment.

## 3 Results

### 3.1 The single-factor experiment

SGR of the fragments increased at first and then decreased with the increase of the variable factor. Peaks appeared at irradiance 37.5  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , temperature 25  $^{\circ}\text{C}$ , and salinity 30, under which the SGR was 4.35%, 4.22%, and 4.33%, respectively (Figs 3–5).

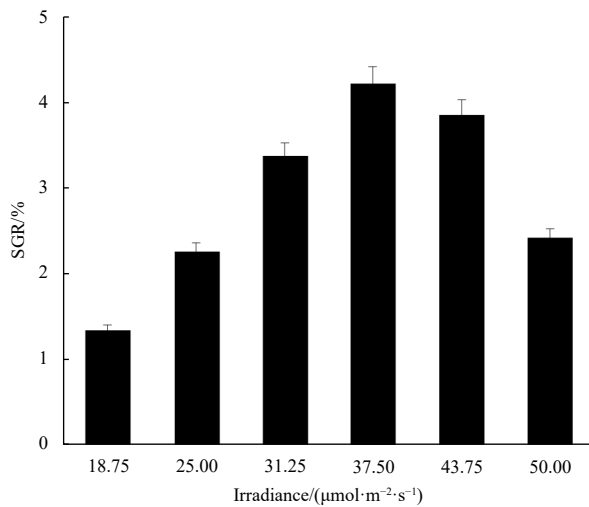


Fig. 3. The SGR of *Caulerpa sertularioides* in different irradiances.

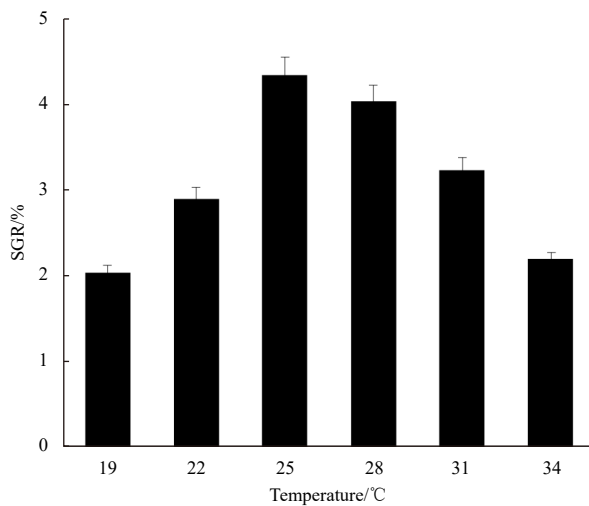


Fig. 4. The SGR of *Caulerpa sertularioides* in different temperatures.

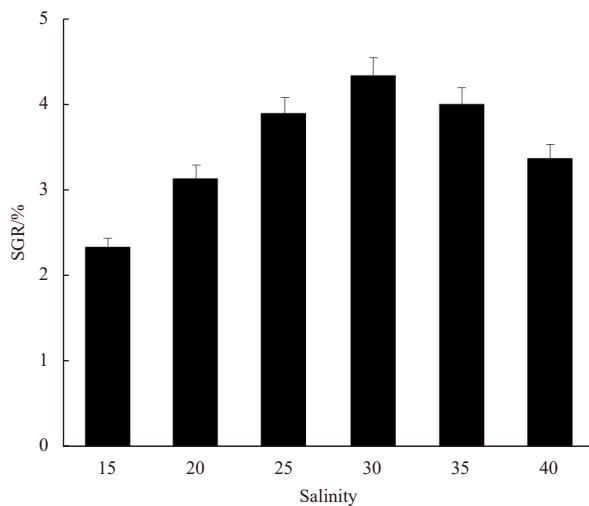


Fig. 5. The SGR of *Caulerpa sertularioides* in different salinities.

### 3.2 Ecological multi-factor experiment

Irradiance, temperature, and salinity are considered the three key factors affecting the growth of the species, and SGR is the response value. According to the results of the single-factor experiments, the horizontal ranges of the three factors were set and analyzed in the Box-Behnken design (Table 1), in which 17 three-factor combinations were determined and the SGRs of thalli fragments of the 17 combinations were measured (Table 2).

Design-Expert 10 was used for the quadratic multiple regression of the data shown in Table 2. The regression equation was established as below:

$$\text{SGR}(\%) = 4.60 + 0.46A + 0.22B + 0.049C - 0.17AB - 0.074AC - 0.14BC - 0.90A^2 - 0.94B^2 - 0.75C^2, \quad (2)$$

where  $A$  is irradiance,  $B$  is temperature, and  $C$  is salinity. The influences of factors  $A$  and  $B$  were highly significant ( $P < 0.0001$ ), and those of factors  $AB$  and  $BC$  were extremely significant ( $P < 0.01$ ).

In addition, as ANOVA results show, the model of regression equation was highly significant ( $P < 0.0001$ ), while the lack-of-fit was not significant ( $P > 0.05$ ), thus the model could better describe the real relationship between various factors and the response value (SGR) (Table 3). In our response surface analysis (Figs 6–11), the factors involved in multiple interactions were irradiance (in  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ ), temperature ( $^{\circ}\text{C}$ ), and SGR (%). The results show that within the set range, the SGR of the fragments increased first and then decreased with the increases in irradiance, temperature, and salinity, and the interactions of irradiance-temperature and temperature-salinity were extremely significant. According to the analysis of the model, the best combination condition for the growth of fragments was irradiance  $39.03 \mu\text{mol}/(\text{m}^2\cdot\text{s})$ , temperature  $25.29^{\circ}\text{C}$ , and salinity  $30.06$ , un-

Table 1. Levels of variables in the Box-Behnken experimental design

Factor	Irradiance / ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Temperature/ $^{\circ}\text{C}$	Salinity
Low	31.25	22	25
High	43.75	28	35

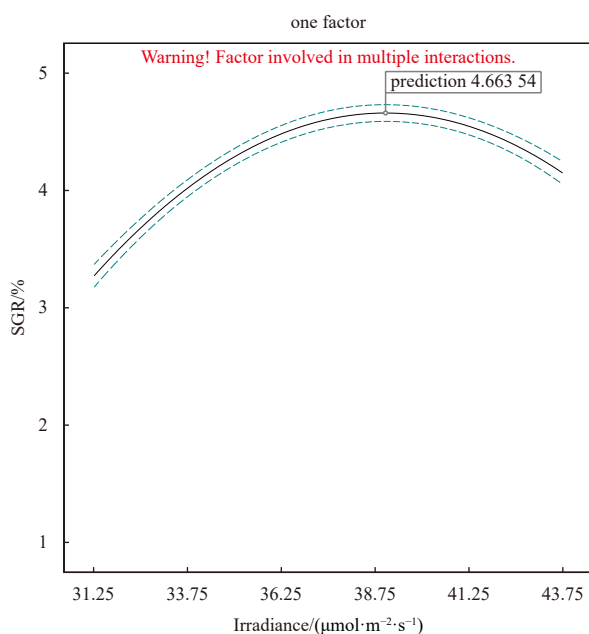
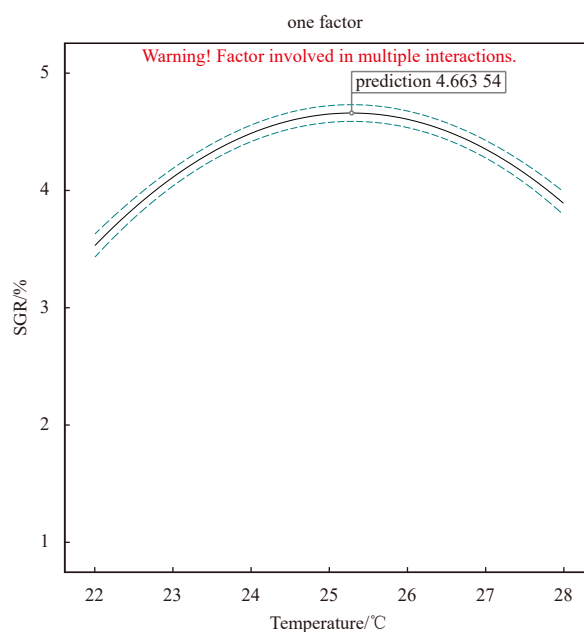
Table 2. Combinations of the Box-Behnken experimental design

Serial number	Irradiance/ ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Temperature/ $^{\circ}\text{C}$	Salinity	SGR/%
1	37.5	22	35	2.84
2	37.5	25	30	4.62
3	37.5	28	35	3.06
4	37.5	25	30	4.52
5	37.5	22	25	2.46
6	43.75	28	30	3.25
7	31.25	22	30	1.93
8	37.5	25	30	4.52
9	43.75	22	30	3.20
10	31.25	28	30	2.65
11	43.75	25	25	3.41
12	37.5	25	30	4.70
13	31.25	25	35	2.63
14	37.5	28	25	3.26
15	31.25	25	25	2.38
16	43.75	25	35	3.38
17	37.5	25	30	4.62

**Table 3.** Analysis of variance

Source	Sum of squares	df	Mean square	F value	P value	Significance
Model	12.89	9	1.43	304.92	<0.000 1	Significant
A–irradiance	1.67	1	1.67	355.29	<0.000 1	–
B–temperature	0.4	1	0.4	85.16	<0.000 1	–
C–salinity	0.02	1	0.019	4.08	0.083	–
AB	0.11	1	0.11	23.76	0.001 8	–
AC	0.02	1	0.022	4.64	0.068	–
BC	0.08	1	0.083	17.67	0.004	–
A <sup>2</sup>	3.4	1	3.4	724.44	<0.000 1	–
B <sup>2</sup>	3.74	1	3.74	796.58	<0.000 1	–
C <sup>2</sup>	2.34	1	2.34	498.31	<0.000 1	–
Residual	0.033	7	4.70 × 10 <sup>-3</sup>	–	–	–
Lack of fit	9.07 × 10 <sup>-3</sup>	3	3.02 × 10 <sup>-3</sup>	0.51	0.80	Insignificant
Pure error	0.024	4	5.95 × 10 <sup>-3</sup>	–	–	–
Cor total	12.93	16	–	–	–	–

Note: df: Degrees of Freedom. Cor total: This row shows the amount of variation around the mean of the observations. The model explains part of it, the residual explains the rest.

**Fig. 6.** SGR of *Caulerpa sertularioides* affected by irradiance.**Fig. 7.** SGR of *Caulerpa sertularioides* affected by temperature.

der which the SGR was the best, reaching 4.66%.

To verify the optimum combination conditions, another experiment was conducted under the above-stated optimum condition. Within the scope of the precision of experimental instruments, the SGR of the thalli fragments was measured to be 4.66%, which is consistent overall with the predicted results of response surface method.

#### 4 Discussion

Some species of genus *Caulerpa* are common invasive green algae. Their growth and development are affected by local coastal environmental conditions. Taking *C. racemosa* as an example, its growth varied in temperature in terms of season, region, or water depth. As reported previously, its blade length reached 6 cm in October at 0–3 m depth in Leghorn, Italy (Piazzi and Cinelli, 1999), and at a deeper depth of 17 m in Marseille, France; its blade height in summer was on average 2 cm only and no such a summer peak was observed (Ruitton et al., 2005). Moreover, *C.*

*racemosa* had fewer blades in winter at a depth of 2 m in coastal waters of northern Italy (Piazzi et al., 2001). In Japanese waters, *Caulerpa* species begin to develop in spring and become mature in summer (Wang, 2015). In salinity and irradiance, *C. racemosa* grew fastest in salinity 30–40 and light intensity 20–60  $\mu\text{E}/(\text{m}^2\cdot\text{s})$  as reported in the intertidal zone/subtidal reef of southwestern coastal Australia (Carruthers et al., 1993). In China, a study showed that the optimum conditions for *C. sertularioides* growth were 26°C, salinity 27.5, and irradiance 25  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$  (Zhong et al., 2021). Similarly, *C. racemosa* on Taiwan Island in China grew best in seawater temperatures ranging from 24–28°C, while the biomass reduced dramatically below 22°C or above 31°C (Shi, 2008).

All these data provide references for the monitoring and control of the invasion of *Caulerpa* species. However, the above-mentioned cases are complicated and imprecise among the three parameters. Therefore, RSM was introduced and applied to this study.

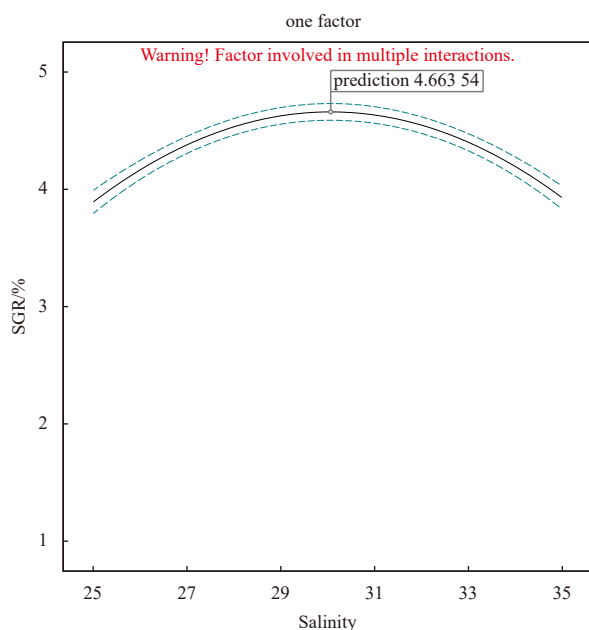


Fig. 8. SGR of *Caulerpa sertularioides* affected by salinity.

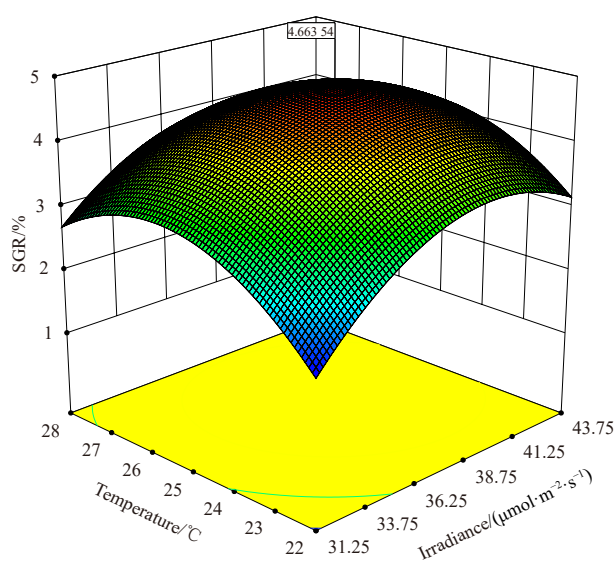


Fig. 9. SGR of *Caulerpa sertularioides* affected by irradiance and temperature.

RSM is a commonly used method for experimental design, which is applicable for multi-factor and multi-level experimental designs and is convenient, and has good predictability (Stensrud et al., 2000; Nazzal et al., 2002; Kramar et al., 2003; Hadiyat et al., 2022). Currently, it is widely used for biological enzyme medium configuration and in food processing (Zhao et al., 2013; Gong et al., 2022; Pinheiro et al., 2022). In this study, we first determined preliminarily growth conditions of *C. sertularioides* fragments in a single-factor manner: 25°C in temperature, 30 in salinity, and 37.5  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$  in irradiance, under which the SGR was the best. Subsequently, the interactions among irradiance, temperature, and salinity, and an optimum ecological multi-factor combination condition were established and analyzed in RSM.

The results of RSM show that the interactions between irradiance and temperature, and temperature and salinity were ex-

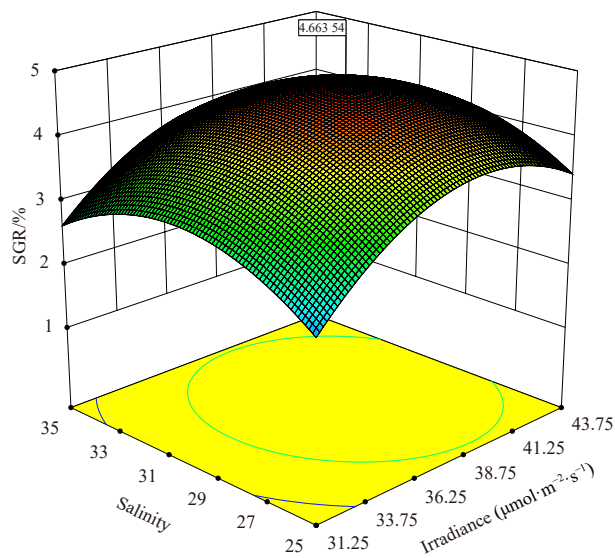


Fig. 10. SGR of *Caulerpa sertularioides* affected by irradiance and salinity.

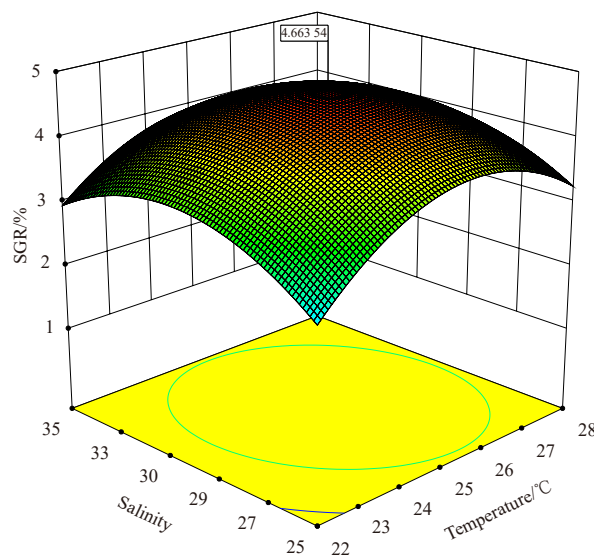


Fig. 11. SGR of *Caulerpa sertularioides* affected by temperature and salinity.

tremely significant. Temperature regulates algal growth by affecting enzyme activity (Wang et al., 2014; Feng et al., 2021). Salinity regulates ion exchange by affecting osmotic pressure (Flexas et al., 2004). The enzymes require the activation of specific ions (Wells and Di Cera, 1992); too high or too low salinity could affect the activity of enzymes or their carriers (Okur et al., 2002). The irradiance mainly affects the photosynthesis of algae (Denison, 1987), in which certain enzymes are involved (Bischof et al., 2000). The interactions between temperature and salinity, and between temperature and irradiance have been observed to be significant in other algae *Prorocentrum donghaiense* (Xu et al., 2010) and *Skeletonema costatum* (Yu, 2005).

The optimum combination condition indicated by RSM was: irradiance 39.03  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , temperature 25.29°C, and salinity 30.06. The  $R^2_{\text{Adj}}$  (adjusted coefficient of determination) of the multiple correlation coefficient  $R$  after the analysis of variance was 0.99, indicating that 99% of the change in the response value

is derived from the selected variable, which means that the error of this experiment is very small. By analyzing the response surface of the interaction terms in the regression equation (Figs 9–11), we found that the interaction among temperature, salinity, and irradiance is significant in the selected range, which is consistent with the result of the model analysis (Table 3), indicating that the model could be used to optimize the growth conditions of the fragments of *C. sertularioides*, and to predict its SGR. The SGR value determined by the verification experiment was higher than the maximum SGR of the single-factor experiment, and also higher than the SGR of *C. sertularioides* measured under 16 of the total 17 combined conditions determined in the Box-Behnken design (see Table 2), but slightly lower than one of the conditions, which is speculated that it was caused by an experimental error. The result indicates that the optimum combination conditions of ecological factors for the growth of *C. sertularioides* optimized by RSM (irradiance 39.03  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , temperature 25.29°C, and salinity 30.06) are suitable for the growth of *C. sertularioides*. In addition, it also indicates that the optimum combination conditions (the irradiance, temperature, and salinity) of ecological factors for the growth of *C. sertularioides* optimized in this study can be taken as the center and be appropriately extended to combine in the range of appropriate growth conditions, which provided new theoretical data and solutions for the cultivation, invasion prediction, and monitoring of *Caulerpa* species in China and around the world, and offer some new scientific data for future in-depth researches in this regard. Based on the literatures and our result, with further research and data mining, we predict that the RSM method will be better applied in the following aspects of macroalgae: (1) species or taxa (new cultivars) that have not been studied because an optimal set of culture conditions need to be obtained; (2) germplasms that require intensive orientation cultured, where changes in the microenvironment often cause them to undergo qualitative changes, such as the transition from the growth to the reproductive stage; (3) the analysis of environmental hazards of cultivated species in the field, which facilitates the acquisition of new insights.

As known from the current works of literature, those blooming macroalgae generally adapt to their environment very quickly through multiple pathways, which means they are extremely viable. *C. sertularioides* is also extremely adaptable to its environment which is similar to other *Caulerpa* species. In addition to sexual reproduction (which had few been seen in the literature), it can grow and spread on the seafloor through its stolons and fragments or branches. In them, the rate of speed by the fragments is much faster. In the previous research and cultivations of *Caulerpa* species, asexual materials were generally used, mainly fragments or branches. They are much more economical and conveniently available than sexual ones. Therefore, our experiment was implemented using fragments rather than whole individuals.

## 5 Conclusions

The effects of ecological factors on growth of *C. sertularioides*, an invasive potential blooming green alga, were studied. Its optimum conditions of irradiance, temperature and salinity for the growth of its fragments were determined in the response surface methodology (RSM). Using the Box-Behnken design, the conditions were optimized and verified to be irradiance 39.03  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , temperature 25.29°C, and salinity 30.06, under which the SGR reached 4.66%. As the research progresses and the data are fully explored, the RSM method may have great potential application in the environmental adaptation characteristics of new mac-

roalgal cultivars, intensive orientation cultured germplasms, and environmental hazard analysis of cultivated species in the field.

## Acknowledgements

We thank Roger Z. YU, a Canadian english editor for help in modifying the language.

## References

- Anderson L W J. 2005. California's reaction to *Caulerpa taxifolia*: a model for invasive species rapid response. *Biological Invasions*, 7(6): 1003–1016, doi: [10.1007/s10530-004-3123-z](https://doi.org/10.1007/s10530-004-3123-z)
- Bischof K, Hanelt D, Wiencke C. 2000. Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae. *Planta*, 211(4): 555–562, doi: [10.1007/s00425000313](https://doi.org/10.1007/s00425000313)
- Cai Yixun. 2021. Effect of environmental factors on growth, physiological and biochemical characteristics of *Caulerpa lentillifera* (in Chinese)[dissertation]. Guangzhou: South China University of Technology
- Carruthers T J B, Walker D I, Huisman J M. 1993. Culture studies on two morphological types of *Caulerpa* (Chlorophyta) from Perth, Western Australia, with a description of a new species. *Botanica Marina*, 36(6): 589–596
- Chaves Filho G P, Batista L A N C, de Medeiros S R B, et al. 2022. Sulfated glucan from the green seaweed *Caulerpa sertularioides* inhibits adipogenesis through suppression of adipogenic and lipogenic key factors. *Marine Drugs*, 20(8): 470, doi: [10.3390/md20080470](https://doi.org/10.3390/md20080470)
- da Silva L P, Pereira E, Prieto M A, et al. 2019. *Rubus ulmifolius* Schott as a novel source of food colorant: extraction optimization of coloring pigments and incorporation in a bakery product. *Molecules*, 24(11): 2181, doi: [10.3390/molecules24112181](https://doi.org/10.3390/molecules24112181)
- Davis A R, Roberts D E, Cummins S P. 1997. Rapid invasion of a sponge-dominated deep-reef by *Caulerpa scalpelliformis* (Chlorophyta) in Botany Bay, New South Wales. *Austral Ecology*, 22(2): 146–150, doi: [10.1111/j.1442-9993.1997.tb00653.x](https://doi.org/10.1111/j.1442-9993.1997.tb00653.x)
- de Gaillande C, Payri C, Remoissenet G, et al. 2017. *Caulerpa* consumption, nutritional value and farming in the Indo-Pacific region. *Journal of Applied Phycology*, 29(5): 2249–2266, doi: [10.1007/s10811-016-0912-6](https://doi.org/10.1007/s10811-016-0912-6)
- Dennison W C. 1987. Effects of light on seagrass photosynthesis, growth and depth distribution. *Aquatic Botany*, 27(1): 15–26, doi: [10.1016/0304-3770\(87\)90083-0](https://doi.org/10.1016/0304-3770(87)90083-0)
- Ding Lanping, Huang Bingxin, Luan Rixiao. 2015. New classification system of marine green algae of China. *Guangxi Sciences* (in Chinese), 22(2): 201–210
- Ebadi S, Ashaari Z, Late Masoumi H, et al. 2019. Evaluation of bending strength of hydrothermally treated oil palm wood in various buffered media using response surface methodology. *Journal of Tropical Forest Science*, 31(2): 151–161, doi: [10.26525/jtfs2019.31.2.151161](https://doi.org/10.26525/jtfs2019.31.2.151161)
- Faramarzi S, Anzabi Y, Jafarizadeh-Malmiri H. 2019. Selenium supplementation during fermentation with sugar beet molasses and *Saccharomyces cerevisiae* to increase bioethanol production. *Green Processing and Synthesis*, 8(1): 622–628, doi: [10.1515/gps-2019-0032](https://doi.org/10.1515/gps-2019-0032)
- Feng Lina, Shi Xiaoyong, Chen Yuehong, et al. 2021. Effects of temperature on the nitrate reductase activity and growth of *Ulva prolifera*. *Journal of Phycology*, 57(3): 955–966, doi: [10.1111/jpy.13141](https://doi.org/10.1111/jpy.13141)
- Fernández C, Cortés J. 2005. *Caulerpa sertularioides*, a green alga spreading aggressively over coral reef communities in Culebra Bay, North Pacific of Costa Rica. *Coral Reefs*, 24(1): 10, doi: [10.1007/s00338-004-0440-8](https://doi.org/10.1007/s00338-004-0440-8)
- Flexas J, Bota J, Loreto F, et al. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in  $C_3$  plants. *Plant Biology*, 6(3): 269–279, doi: [10.1055/s-2004-820867](https://doi.org/10.1055/s-2004-820867)
- Gao Kunshan. 2014. *Algal Carbon Fixation: Basis, Advances and Methods* (in Chinese). Beijing: Science Press
- Gong Shusen, Yang Fei, Wang Qingfen, et al. 2022. Processing of Noni

- Liquor based on response surface methodology. *PeerJ*, 10: e13817, doi: [10.7717/peerj.13817](https://doi.org/10.7717/peerj.13817)
- Guo Hui, Yao Jianting, Sun Zhongmin, et al. 2015. Effects of salinity and nutrients on the growth and chlorophyll fluorescence of *Caulerpa lentillifera*. *Chinese Journal of Oceanology and Limnology*, 33(2): 410–418, doi: [10.1007/s00343-015-4105-y](https://doi.org/10.1007/s00343-015-4105-y)
- Hadiyat M A, Sopha B M, Wibowo B S. 2022. Response surface methodology using observational data: a systematic literature review. *Applied Sciences*, 12(20): 10663, doi: [10.3390/app122010663](https://doi.org/10.3390/app122010663)
- Huang Jianhui. 2012. Effects of concentrations of nitrogen and phosphorus and different culture methods on the growth of *Caulerpa lentillifera*. *Journal of Fujian Fisheries (in Chinese)*, 34(5): 416–419
- Jiang Haiyan, Wu Sichao, Cen Yingzhou. 2011. Nutrient component analysis of six kinds of economic algae in Zhanjiang sea area. *Guangzhou Chemical Industry (in Chinese)*, 39(7): 105–108
- Kang E J, Lee S, Kang J, et al. 2021. Performance of a potentially invasive species of ornamental seaweed *Caulerpa sertularioides* in acidifying and warming oceans. *Journal of Marine Science and Engineering*, 9(12): 1368, doi: [10.3390/jmse9121368](https://doi.org/10.3390/jmse9121368)
- Kim M, Lee B, Kim H S, et al. 2019. Increased biomass and lipid production of *Ettlia* sp. YC001 by optimized C and N sources in heterotrophic culture. *Scientific Reports*, 9(1): 6830, doi: [10.1038/s41598-019-43366-5](https://doi.org/10.1038/s41598-019-43366-5)
- Klein J C, Verlaque M. 2008. The *Caulerpa racemosa* invasion: a critical review. *Marine Pollution Bulletin*, 56(2): 205–225, doi: [10.1016/j.marpolbul.2007.09.043](https://doi.org/10.1016/j.marpolbul.2007.09.043)
- Kolar C S, Lodge D M. 2001. Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, 16(4): 199–204
- Kramar A, Turk S, Vrečer F. 2003. Statistical optimisation of diclofenac sustained release pellets coated with polymethacrylic films. *International Journal of Pharmaceutics*, 256(1–2): 43–52, doi: [10.1016/S0378-5173\(03\)00061-9](https://doi.org/10.1016/S0378-5173(03)00061-9)
- Kumar M, Gupta V, Kumari P, et al. 2011. Assessment of nutrient composition and antioxidant potential of Caulerpaceae seaweeds. *Journal of Food Composition and Analysis*, 24(2): 270–278, doi: [10.1016/j.jfca.2010.07.007](https://doi.org/10.1016/j.jfca.2010.07.007)
- Landi S, Santini G, Vitale E, et al. 2022. Photosynthetic, molecular and ultrastructural characterization of toxic effects of zinc in *Caulerpa racemosa* indicate promising bioremediation potentiality. *Plants*, 11(21): 2868, doi: [10.3390/plants11212868](https://doi.org/10.3390/plants11212868)
- Lapointe B E, Barile P J, Wynne M J, et al. 2005. Reciprocal *Caulerpa* invasion: Mediterranean native *Caulerpa ollivieri* in the Bahamas supported by human nitrogen enrichment. *Aquatic Invasions*, 16(2): 2–5
- Li Tianjingwei, Ma Bin, Zou Xiaoxiao, et al. 2022. Physiological mechanism of response to temperature stress in seaweed *Caulerpa lentillifera*. *Chinese Journal of Fisheries (in Chinese)*, 35(4): 67–73
- Liu Rui, Li Yongmei, Wang Yixiao, et al. 2019. Biogeographical distribution, biological characteristics and biological invasion of *Caulerpa* (Chlorophyta) seaweeds. *Journal of Anhui Agricultural Sciences (in Chinese)*, 47(4): 95–98, 104
- Liu Hengtong, Wang Fang, Wang Qiaohan, et al. 2016. A comparative study of the nutrient uptake and growth capacities of seaweeds *Caulerpa lentillifera* and *Gracilaria lichenoides*. *Journal of Applied Phycology*, 28(5): 3083–3089, doi: [10.1007/s10811-016-0858-8](https://doi.org/10.1007/s10811-016-0858-8)
- Mäkelä M. 2017. Experimental design and response surface methodology in energy applications: a tutorial review. *Energy Conversion and Management*, 151: 630–640, doi: [10.1016/j.enconman.2017.09.021](https://doi.org/10.1016/j.enconman.2017.09.021)
- Mee R W. 2009. *A Comprehensive Guide to Factorial Two-Level Experimentation*. New York: Springer
- Meinesz A, Belsheer T, Thibaut T, et al. 2001. The introduced green alga *Caulerpa Taxifolia* continues to spread in the mediterranean. *Biological Invasions*, 3(2): 201–210, doi: [10.1023/A:1014549500678](https://doi.org/10.1023/A:1014549500678)
- Nazzal S, Nutan M, Palamakula A, et al. 2002. Optimization of a self-nanoemulsified tablet dosage form of ubiquinone using response surface methodology: effect of formulation ingredients. *International Journal of Pharmaceutics*, 240(1–2): 103–114, doi: [10.1016/S0378-5173\(02\)00130-8](https://doi.org/10.1016/S0378-5173(02)00130-8)
- Nur M M A, Garcia G M, Boelen P, et al. 2019. Enhancement of C-phycocyanin productivity by *Arthrospira platensis* when growing on palm oil mill effluent in a two-stage semi-continuous cultivation mode. *Journal of Applied Phycology*, 31(5): 2855–2867, doi: [10.1007/s10811-019-01806-9](https://doi.org/10.1007/s10811-019-01806-9)
- Okur N, Çengel M, Göçmez S. 2002. Influence of salinity on microbial respiration and enzyme activity of soils. *Acta Horticulturae*, (573): 189–194
- Pang Meixia, Huang Zhili, Liu Xiaolong, et al. 2021. Effects of heavy metal cadmium on *Caulerpa lentillifera* based on transcriptome analysis. *Chinese Journal of Applied Ecology (in Chinese)*, 32(12): 4447–4456
- Piazzi L, Ceccherelli G, Cinelli F. 2001. Threat to macroalgal diversity: effects of the introduced green alga *Caulerpa racemosa* in the Mediterranean. *Marine Ecology Progress Series*, 210: 149–159, doi: [10.3354/meps210149](https://doi.org/10.3354/meps210149)
- Piazzi L, Cinelli F. 1999. Développement et dynamique saisonnière d'un peuplement méditerranéen de l'algue tropicale *Caulerpa racemosa* (Forsskl) J. Agardh. *Cryptogamie Algologie*, 20(4): 295–300, doi: [10.1016/S0181-1568\(00\)88146-5](https://doi.org/10.1016/S0181-1568(00)88146-5)
- Pinheiro A M, Maia G A, Figueiredo R W, et al. 2022. Response surface methodology optimization of blended fruit nectar: cashew apple and açai. *International Journal of Fruit Science*, 22(1): 275–286, doi: [10.1080/15538362.2022.2037037](https://doi.org/10.1080/15538362.2022.2037037)
- Ruitton S, Verlaque M, Boudouresque C F. 2005. Seasonal changes of the introduced *Caulerpa racemosa* var. *cylindracea* (Caulerpaceae, Chlorophyta) at the northwest limit of its Mediterranean range. *Aquatic Botany*, 82(1): 55–70, doi: [10.1016/j.aquabot.2005.02.008](https://doi.org/10.1016/j.aquabot.2005.02.008)
- Shevchenko N M, Burtseva Y V, Zvyagintseva T N, et al. 2009. Polysaccharides and sterols from green algae *Caulerpa lentillifera* and *C. sertularioides*. *Chemistry of Natural Compounds*, 45(1): 1–5, doi: [10.1007/s10600-009-9223-3](https://doi.org/10.1007/s10600-009-9223-3)
- Shi Jianhong. 2008. Field survey and culture studies of *Caulerpa* in Taiwan (in Chinese)[dissertation]. Taipei: National Sun Yat-Sen University
- Shi Xiaohan, Zou Dinghui, He Quan, et al. 2022. Photophysiological characteristics of the branch and stolon of macroalga *Caulerpa lentillifera* (Caulerpaceae, Caulerpa) under different growth light conditions, and their responses to temperature rise. *Journal of Tropical Oceanography (in Chinese)*, 41(5): 150–160
- Smith T B, Fong P, Kennison R, et al. 2010. Spatial refuges and associational defenses promote harmful blooms of the alga *Caulerpa sertularioides* onto coral reefs. *Oecologia*, 164(4): 1039–1048, doi: [10.1007/s00442-010-1698-x](https://doi.org/10.1007/s00442-010-1698-x)
- Smith C M, Walters L J. 1999. Fragmentation as a strategy for *Caulerpa* species: fates of fragments and implications for management of an invasive weed. *Marine Ecology*, 20(3–4): 307–319, doi: [10.1046/j.1439-0485.1999.2034079.x](https://doi.org/10.1046/j.1439-0485.1999.2034079.x)
- Srinivas G, Nidoni U, Ramachandra C T, et al. 2019. Supercritical fluid extraction of pupae oil from mulberry silkworm (*Bombyx mori* L.). *Journal of Pharmacognosy and Phytochemistry*, 8(3): 4507–4513
- Stensrud G, Sande S A, Kristensen S, et al. 2000. Formulation and characterisation of primaquine loaded liposomes prepared by a pH gradient using experimental design. *International Journal of Pharmaceutics*, 198(2): 213–228, doi: [10.1016/S0378-5173\(00\)00338-0](https://doi.org/10.1016/S0378-5173(00)00338-0)
- Stuthmann L E, Springer K, Kunzmann A. 2021. Cultured and packed sea grapes (*Caulerpa lentillifera*): effect of different irradiances on photosynthesis. *Journal of Applied Phycology*, 33(2): 1125–1136, doi: [10.1007/s10811-020-02322-x](https://doi.org/10.1007/s10811-020-02322-x)
- Tong Yanmei, Chen Xiuli, Hu Tingjun, et al. 2022. Optimization of extraction process and antioxidant activity of polysaccharides from *Caulerpa lentillifera* by response surface methodology. *Journal of Fisheries of China (in Chinese)*. <https://kns.cnki.net/kcms/detail/31.1283.S.20220518.1019.004.html>[2022-05-18/2022-12-20]

- Verlaque M, Afonso-Carrillo J, Gil-Rodríguez M C, et al. 2004. Blitzkrieg in a marine invasion: *Caulerpa racemosa* var. *cylindracea* (Bryopsidales, Chlorophyta) reaches the Canary Islands (north-east Atlantic). *Biological Invasions*, 6(3): 269–281, doi: [10.1023/B:BINV.0000034589.18347.d3](https://doi.org/10.1023/B:BINV.0000034589.18347.d3)
- Vishwakarma R, Banerjee R. 2019. Process optimization for enhancement of fermentable sugar from *Cyperus* sp. through enzymatic saccharification. *Journal of Biofuels*, 10(1): 1–11
- Wang Zewen. 2010. Study on the determination of seaweed growth regulator and fertilizer efficiency (in Chinese)[dissertation]. Qingdao: Ocean University of China
- Wang Zhan. 2015. Preliminary studies on environmental adaptation and paling mechanism of *Caulerpa* (in Chinese)[dissertation]. Shantou: Shantou University
- Wang Zonghua, Sun Na, He Yao, et al. 2014. DNA assembled gold nanoparticles polymeric network blocks modular highly sensitive electrochemical biosensors for protein kinase activity analysis and inhibition. *Analytical Chemistry*, 86(12): 6153–6159, doi: [10.1021/ac501375s](https://doi.org/10.1021/ac501375s)
- Wells C M, Di Cera E. 1992. Thrombin is a sodium ion activated enzyme. *Biochemistry*, 31(47): 11721–11730, doi: [10.1021/bi00162a008](https://doi.org/10.1021/bi00162a008)
- Williams S L. 1984. Uptake of sediment ammonium and translocation in a marine green macroalga *Caulerpa cupressoides*. *Limnology and Oceanography*, 29(2): 374–379, doi: [10.4319/lo.1984.29.2.0374](https://doi.org/10.4319/lo.1984.29.2.0374)
- Williams S L, Schroeder S L. 2004. Eradication of the invasive seaweed *Caulerpa taxifolia* by chlorine bleach. *Marine Ecology Progress Series*, 272: 69–76, doi: [10.3354/meps272069](https://doi.org/10.3354/meps272069)
- Withgott J. 2002. California tries to rub out the monster of the lagoon. *Science*, 295(5563): 2201–2202, doi: [10.1126/science.295.5563.2201](https://doi.org/10.1126/science.295.5563.2201)
- Xu Ning, Duan Shunshan, Li Aifen, et al. 2010. Effects of temperature, salinity and irradiance on the growth of the harmful dinoflagellate *Prorocentrum donghaiense* Lu. *Harmful Algae*, 9(1): 13–17, doi: [10.1016/j.hal.2009.06.002](https://doi.org/10.1016/j.hal.2009.06.002)
- Yu Ping. 2005. Effect of temperature, irradiance and population interaction on the growth of phytoplankton of East China Sea (in Chinese)[dissertation]. Qingdao: Ocean University of China
- Zhang Meijian, Ma Yurong, Che Xinyi, et al. 2020. Comparative analysis of nutrient composition of *Caulerpa lentillifera* from different regions. *Journal of Ocean University of China*, 19(2): 439–445, doi: [10.1007/s11802-020-4222-x](https://doi.org/10.1007/s11802-020-4222-x)
- Zhao Yuping, Chen Xiaowang, Shen Pengwei. 2013. Optimization of culture medium to produce manganese peroxidase by using Box-Behnken experimental design and response surface methodology. *Science and Technology of Food Industry* (in Chinese), 34(4): 207–211
- Zhong Yiyun, Yang Yunqi, Gao Xiaofeng, et al. 2021. Effects of salinity, temperature and light intensity on growth and photosynthetic activity of *Caulerpa sertularioides*. *Journal of Tropical and Subtropical Botany* (in Chinese), 29(6): 626–633