Variation in the growth and toxin production of Gymnodinium catenatum under different laboratory conditions

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Abstract

The chain-forming dinoflagellate Gymnodinium catenatum is the only known gymnodinioid dinoflagellate that produces paralytic shellfish toxins (PST). Dense blooms caused by the dinoflagellate have been frequently reported in coastal waters of Fujian, China since 2017. While there is still limited understanding of the major physiological characteristics of G. catenatum isolated from Fujian coastal waters, the growth and toxin production of the G. catenatum strain were examined in batch cultures with different levels of irradiance, temperature, salinity, nitrate, and phosphate conditions. The results indicated that the highest maximum cell density of the strain was achieved at 70 µmol m$^{-2}$ s$^{-1}$, with the highest growth rate at 120 µmol m$^{-2}$ s$^{-1}$. The strain grew well within the temperature range of 15–30°C, with maximum growth rate and cell density achieved at 20°C. The dinoflagellate also showed higher tolerance to salinity variation (20–40), with the highest growth rate at salinity 25. Meanwhile, G. catenatum showed higher demand for nitrogen and phosphorus as indicated by its higher half saturation constant. Decrease in nitrate and phosphate greatly inhibited the growth of G. catenatum. The toxin profile of the G. catenatum strain was conservative and dominated mainly by the N-sulfcarbamoyl C-toxins (> 95%), indicating its hypotoxicity. The cellular toxicity increased with the algal growth, with the highest cellular toxicity observed at the stationary growth phase. The cellular toxicity of G. catenatum also responded to environmental variations including lower temperature (15°C), lower salinity (20), nitrate-repletion, and phosphate-depletion conditions which enhanced the cellular toxicity, while irradiance exerted non-significant influence. The present study depicted the physiological characteristics of the particular G. catenatum strain and provided valuable insight on the ecophysiology of G. catenatum in natural coastal waters.

1. Introduction

Over the last several decades, coastal regions throughout the world have experienced frequent occurrence of harmful algal blooms (HAB). The most common effects of HAB are poisoning events, resulting in mass mortality or illness of marine organisms. The accumulated toxins in seafood may lead to eventual human poisoning (Anderson et al. 2002; Green et al. 2004; Shumway et al. 2018; Usup et al. 1994). Among the algal toxins, paralytic shellfish toxins (PST) are among the most hazardous biotoxins due to their severe toxicity and wide distribution. They are mainly produced by eukaryotic dinoflagellates and prokaryotic cyanobacteria (Hummert et al. 1997; Lefebvre et al. 2008; Usup et al. 2012). Recently six cases of human poisoning occurred in Qinhuangdao, China, due to consumption of seafood containing PST during blooms in 2019. Monitoring by local government indicated PST levels reached 2220 MU 100g$^{-1}$, which was four times over the limit allowed in seafood products (People's Republic of China Agricultural Industry standard, NY5073-2006).

Paralytic shellfish toxins (PST) contain non-sulfated, mono-sulfated, di-sulfated, decarbamoylated and deoxy-decarbamoylated analogues, with each moiety indicating different levels of toxicity (Llewellyn 2006; Oshima 2008; Wiese et al. 2010). The PST can block Na$^+$ conductance by binding with voltage-
gated sodium channels and resulting the interference of Na\(^+\) temporary permeability (Stevens et al. 2011), thereby cause extremities numbness, breathing difficulties and even complete paralysis or death (Wang et al. 2016). The chain-forming dinoflagellate *Gymnodinium catenatum*, known as the only gymnodinioid dinoflagellate capable of producing PST (Bolch et al. 2002; Cembella et al. 2018), produces 38 forms of saxitoxin and its analogs, including saxitoxin (STX), neosaxitoxin (neoSTX), the N-sulfocarbamoyl gonyautoxins (GTX1-6), the N-sulfocarbamoyl-11-hydroxysulfate C-toxins (C1-4), the decarbamoylated analogs (dcSTX, dcneoSTX, dcGTX1-4), the deoxy-decarbomol toxins (doSTX, doGTX1-2), and benzoate analogs (GC1-3) (Negri et al. 2003; Oshima et al. 1993; Vale 2008). Its toxin profile and cellular toxicity, however, varied among different geographic regions. In most regions, such as Mexico, Japan, Australia, Spain, and China, the toxin profile has been characterized by the dominance of N-sulfocarbamoyl analogs (C1-4, B1/2) (Band-Schmidt et al. 2014; Negri et al. 2007; Oshima et al. 1993). While, the specific content and other trace toxins were rather diverse. For example, more than 80% of the toxins detected in the strains from Japan and Australia were C toxins, while less than 40% were C toxins in the species from Spain (Oshima et al. 1993). In addition, differences in the toxin profile of different algal strains within the same region were also observed (Band-Schmidt et al. 2014; Ordas et al. 2004).

Major environmental factors exert varying degrees of impact on the growth and cellular toxicity of *G. catenatum*. The algal strains from different geographic regions showed distinct toxin profiles as well. In particular, the optimum temperature for growth of *G. catenatum* strains from Tasmania was 14.5–20°C, and was 22–25°C in the strains isolated from coastal waters of southern China (Blackburn et al. 1989; Ye et al. 2018; Zhang 2009). Higher cellular toxicity was detected in the Japanese strain cultured in 18°C was twice as toxic as that cultured at 25°C (Oh et al. 2010), while there was no such variation in the strain from Mexico (Band-Schmidt et al. 2014). The growth and toxicity characters of *G. catenatum* showed higher intraspecific variability (strain differences). Intraspecific variability in key characteristics such as life-history traits, behavior, nutrition, genetics and toxicity, has been experimentally documented for many toxigenic microalgae, including species of cyanobacteria, dinoflagellates, haptophytes, raphidophytes and diatoms ((Burkholder et al. 2009; Sinclair et al. 2009; Thessen et al. 2009). Although often overlooked, it is likely of fundamental importance to species survival and evolution. Maintenance of an array of co-existing genotypes within a population might be especially important for commonly haploid dinoflagellates (Tillmann et al. 2009). The cause of intraspecific variability is unknown and may arise from environmental variability and epi-genetic factors.

In the coastal waters of China, *G. catenatum* cysts were reported ubiquitously (Gu et al. 2013; Qi et al. 1996), and massive *G. catenatum* blooms have occurred in the coastal waters of the East China Sea (e.g. Jiangsu and Fujian coasts) (Cheng et al. 2009; Zhang et al. 2020), while its vegetative cells have rarely been observed in the South China Sea and the Yellow Sea (Jiao et al. 2010; Lu et al. 2004). In 2017, a large-scale bloom of *G. catenatum* occurred in Fujian coastal waters and resulted in huge economic losses to local aquaculture ventures and severe PSP events (Chen 2018). The PST produced by this strain were dominated by C1/2 and dcGTX2/3 without C3/4 (Liu et al. 2020). The toxin profile was unique compared to those strains from Japanese, Australia, South Korea, Mexico, Spain, Portugal, Uruguay, Malaysia, Hong Kong and Yellow Sea of China (Liu et al. 2020), suggesting it may represent a separate
strain. Limited studies have been conducted to explore physiological responses of this strain to major environmental factors. In the present study, growth, cellular toxicity, and toxin composition of *G. catenatum* in batch cultures under different environmental conditions were investigated to provide better understanding of the toxin production of the species.

2. Materials And Methods

2.1 Algal isolation and identification

The dinoflagellate *Gymnodinium catenatum* (isolate ID# TIO523) was originally isolated from Xiamen Bay, Fujian Province, China by Prof. Haifeng Gu’s laboratory at the Third Institute of Oceanography, SOA. The monoculture of the strain was further established by pipetting single cells into 24-well polystyrene cell culture plates for laboratory experiments in the Institute of Oceanology, CAS. The stock cultures were maintained in the exponential growth phase by transferring the cells to fresh medium biweekly and were gently shaken once daily. The cultures were maintained routinely in f/2 medium supplemented with selenium (10⁻⁹ M H₂SeO₃, (Doblin et al. 2000) at 20°C, 80 µmol photons m⁻² s⁻¹ under a 12-h light/12-h dark cycle.

Genomic DNA was extracted from the vegetative cultures of *G. catenatum* harvested at the exponential growth phase, using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories Inc., USA). Partial D1-D2 of LSU rDNA and ITS regions were amplified using primers D1R and D2C (Scholin et al. 1994) and primers ITS-A and ITS-B (Adachi et al. 1994). The PCR reaction procedure was 5 min at 94°C, followed by 40 cycles of 1 min at 94°C, 2 min at 55°C, 1 min at 72°C, and final extension of 7 min at 72°C. Amplification products were purified using the E.Z.N.A® Gel Extraction Kit (OMEGA, USA) and sequenced using ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences of each gene were aligned using default setting of ClustalW 2.0 (Larkin et al. 2007) and then concatenated for tree construction. Additional sequences of *G. catanetum* were acquired from the National Center for Biotechnology Information (NCBI) database. *Gymnodinium nolleri* (GenBank number: FN649409), *Gymnodinium microreticulatum* (GenBank number: AY036078), *Lepidodinium viride* (GenBank number: AY464689), *Gymnodinium nolleri* (GenBank number: AM998535), and *Gymnodinium inusitatum* (GenBank number: KF234061) were selected as outgroups. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) based on the maximum composite likelihood model (Tamura K. 2004) with 1000 bootstrap replicates, using MEGA 7.0 software (Kumar S. et al. 2016).

2.2 Characterization of algal growth and toxin profile

Triplicate batch cultures (180 mL) were grown in f/2 + Se medium and maintained under the conditions described above, with an initial cell density of 220 ± 30 cells mL⁻¹ in individual Erlenmeyer flasks (250 mL in volume). Every second day, 1mL of subsample was fixed in Lugol’s solution (final concentration 0.5%), loaded on a plankton counting chamber (0.1 mL), and then counted with an inverted microscope (Olympus, IX71, Japan). To assess cellular density, algal cells were collected at the lag phase (Day 2), the
exponential phase (Day 8) and the stationary phase (Day 14) post transfer of *G. catenatum* cells into the batch cultures. The acquired cell densities were used to generate growth curves in which the growth rates (μ, day⁻¹) were calculated with the following equation:

\[
\mu = \frac{lnC_n - lnC_0}{t_n - t_0} #(1)
\]

where \(C_0\) and \(C_t\) are the cell density at time \(t_0\) and \(t_n\), respectively.

In addition, aliquots of 50–100 ml algal cultures were pipetted according to the cell density, filtered onto GF/C membranes (nominal pore size 1.2 µm, Whatman, Kent, U) packaged in tinfoil and stored at -20°C until further toxin analysis.

### 2.3 The effects of major environmental factors on algal growth

Time-course experiments were carried out to determine the effects of major environmental factors on growth of *G. catenatum* in batch cultures. The set-up of culture conditions was detailed in Table 1. All experiments were conducted in triplicate in culture flasks, with an initial cell density of 300 ± 30 cells mL⁻¹. To monitor the growth of *G. catenatum* in the experimental cultures, a 1-ml sub-sample was pipetted from each flask and fixed immediately with Lugol’s solution (final concentration 0.5%). The cell densities and grow rates of *G. catenatum* in the fixed samples were evaluated as described previously. The relationship between growth rate and environmental conditions were fitted to a Monod growth kinetic model using the following equation (Monod 1942):

\[
\mu = \frac{\mu_m \cdot S}{K_{\mu} + S} #(2)
\]

where \(\mu\) was the calculated growth rate at the exponential growth phase (Eq. 1)), \(\mu_m\) was the maximum specific growth rate (day⁻¹), \(S\) was the irradiance level (µmol m⁻² s⁻¹) or nutrient concentration (µmol L⁻¹).
1), and $K_p$ was the irradiance level or substrate concentration which supports half-maximum specific growth rate.

### 2.4 The effects of major environmental factors on algal toxin production

Additional time-course experiments were conducted to study further the effects of major environmental factors on toxin production of *G. catenatum*, based on the preliminary results of algal growth experiments. The experimental set up is summarized in Table 2. All treatments were conducted independently in triplicate cultures, with an initial cell density of 300 ± 30 cells mL$^{-1}$. During the stationary phase of experimental cultures, 50 mL of algal culture was harvested by filtration. The filtered membranes (GF/C, nominal pore size 1.2 µm, Whatman, Kent, UK) were frozen immediately and stored (-20°C) until further processed for toxin analysis.

| Environmental factors     | Levels     | Unit                   |
|---------------------------|------------|------------------------|
| Irradiance                | 0, 10, 30, 70, 120, 190 | µmol photons m$^{-2}$ s$^{-1}$ |
| Temperature               | 10, 15, 20, 25, 30 | °C                     |
| Salinity                  | 20, 25, 30, 35, 40 | /                      |
| Nitrate concentration     | 0, 25, 50, 100, 200, 800 | µmol L$^{-1}$        |
| Phosphate concentration   | 0, 2, 4, 8, 16, 36 | µmol L$^{-1}$        |

### 2.5 LC-MS/MS analysis of algal toxin

The membrane filters were minced and treated with 5-mL acetic acid (1%), then sonicated in an ice bath using a sonication probe (JY92-DN, Scientz Biotechnology Co., Ltd., Ningbo City, China) for 6 min. Aliquots (2 mL) of extract was centrifuged at 10,000 r min$^{-1}$ for 10 min at 4°C. Then part of the above extract (1 mL) was filtered through a syringe membrane filter (0.22 µm) and then processed through liquid chromatography coupled with quadrupole/linear ion trap tandem mass spectrometry (LC-MS/MS) (5500 QTRAP MS system; AB Sciex, Framingham, MA, USA) as described in Wu et al. (2017), to study the toxin profile of *G. catenatum* in the experimental cultures. Briefly, TSK-Amide-80 (3 µm, 2 mm×150 mm) was used to separate toxins at 40°C in the speed of 0.4 mL min$^{-1}$. The sample injection temperature and volume were 4°C and 5 µL, respectively. Ammonium formate (2 mmol L$^{-1}$) and formic acid (50 mmol L$^{-1}$) were used for mobile phase solvent A (water) and B (95% acetonitrile). Gradient elution: 0.01-3.00 min,
80% B; 3.01-5.00 min, 80%-40% B; 5.01-10.00 min, 40% B; 10.01-11.00 min, 40%-80% B; 11.01-13.00 min, 80% B. Multiple reactions monitoring (MRM) was used to monitor saxitoxin and its analogs. Data were acquired and processed with Analyst software version 1.5.2 (AB Sciex).

Total cellular toxicity was calculated from the individual toxin concentrations based on the specific toxicities of each toxin according to Turner et al. (2015) and expressed in picogram STX equivalent per cell (pg STX eq cell⁻¹).

2.6 Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the differences in growth rates, cellular toxin concentrations, and toxin composition of the various cultures treated with different levels of irradiance, temperature, salinity, nitrogen, and phosphate concentrations, as well as the toxin concentration and composition at different growth phases. All the data were tested for normality and homogeneity of variance before applying Duncan's post hoc multiple comparison test using the SPSS Statistics software (version 21.0, SPSS, USA) with the significance level of \( p < 0.05 \). Growth curves, Monod and Michaelis-Menten curves were fitted with Origin software (OriginPro 9, USA).

3. Results

3.1 Molecular identification G. catenatum isolate

The G. catenatum strain (TIO523) shared identical LSU sequence (GenBank sequence code ON183163) with the other strains, including GCCW991 (AY036125, Korea), DC88A44 (AY036121, Singapore); CCMP414 (DQ779990, Spain), GCCV-11 (JQ616825, Mexico), GCCC21 (AY036072, Australia), VG0743 (FN647677, Algeria) (Fig. 1A). The strain shared similar ITS sequence (GenBank sequence code ON183162) with the strains isolated from Japan (CS-397), Mexico (GCCV-11), Korea (GCCW991) and Algeria (CSIC744) (Fig. 1B). Further, the strain TIO523, as well as other Chinese strains, shared identical LSU sequence and ITS sequence (Fig. 1A, 1B). The 5th base in the 5.8S rDNA gene of the G. catenatum strain was cytosine, indicating it belonged to the C-gene ribotype (Bolch et al. 2007).

3.2 Growth and characteristics of G. catenatum

After inoculation into the f/2 + Se medium, G. catenatum entered a lag growth phase until day 4. Then the algae grew exponentially and reached the highest cell density of 12,898 ± 790 cells mL⁻¹ on day 14, with a growth rate of 0.19 ± 0.10 d⁻¹ (Fig. 2A). Toxin analysis showed that N-sulfocarbamoyl toxins (C1/2) dominated the toxin profile of the algal isolate (>99%), with trace amounts of GTX3-5, dcneoSTX contributing less than 1%, thus these trace derivatives were referred collectively as “Others” in the following report of toxin profile (Fig. 2B). The equivalent cellular toxicity of G. catenatum increased along with the growth of experimental cultures, varying from 8.72 ± 0.47 on day 2 to 12.26 ± 0.10 pg STX eq cell⁻¹ on day 14 (\( p < 0.05 \)). As for toxin profile per cell on a molar basis, the relative contents of C1 toxin increased significantly from 8.96% (day 2) to 16.96% (day 14) (\( p < 0.05 \)), whereas the percentage of C2
decreased significantly from 91.03–82.39% ($p < 0.05$), the proportion of other trace toxins also increased significantly ($p < 0.05$) during the sampling period, from 0 (day 2) to 0.65% (day 14) (Fig. 2C).

### 3.3 Effects of environmental factors on growth of G. catenatum

Irradiance significantly affected the growth of *G. catenatum*. Increments in the cell densities of experimental *G. catenatum* cultures were observed in all treatments except at 0 µmol m\(^{-2}\) s\(^{-1}\). The maximum cell densities increased with irradiance in the range of 0–70 µmol m\(^{-2}\) s\(^{-1}\), reaching the highest value of 12,120 ± 275 cells mL\(^{-1}\) by day 20 in the 70 µmol m\(^{-2}\) s\(^{-1}\) treatment (Fig. 3A). The maximum growth rate (0.26 ± 0.02 d\(^{-1}\)) was observed in 120 µmol m\(^{-2}\) s\(^{-1}\). Additionally, the growth of *G. catenatum* in experimental cultures exhibited standard Monod-type kinetics within the range of 0–190 µmol m\(^{-2}\) s\(^{-1}\), with the maximum specific growth rate of 0.28 ± 0.02 d\(^{-1}\) and the half saturation constant ($K_{\mu}$) of 24.54 ± 10.32 µmol m\(^{-2}\) s\(^{-1}\) (Fig. 3B).

Temperature also had a significant effect on the growth of *G. catenatum* under laboratory conditions. Growth occurred in all treatments and showed an exponential increase except in 10°C, with the maximum cell density (12,898 ± 790 cells mL\(^{-1}\)) was obtained in the 20°C treatment by day 14 (Fig. 3C). The growth rates of experimental cultures in the temperature treatments varied between 0.05 ± 0.004 and 0.25 ± 0.01 d\(^{-1}\), with the maximum growth rate obtained in the 20°C treatment (Fig. 3D). The dinoagellate exhibited constant growth in the salinity range of 20 ~ 40, and the growth rates varied from 0.13 ± 0.013 to 0.24 ± 0.012 d\(^{-1}\), with the maximum growth rate in the salinity 25 treatment (Fig. 3F). The maximum cell density increased within the salinity range of 20–30 and reached the highest of 5,703 ± 232 cells mL\(^{-1}\), while there was no significant difference ($p > 0.05$) in the maximum cell densities within the salinity range of 25–35 (Fig. 3E).

The *G. catenatum* strain was able to grow in different concentrations of nitrate, but showed different growth patterns. Limited growth was observed in the cultures at the initial nitrate concentration of 0 µmol L\(^{-1}\) (cell density < 2,000 cells mL\(^{-1}\)). The algal growth increased with nitrate concentration, with the maximum cell density of 11,638 ± 722 cells mL\(^{-1}\) obtained in 800 µmol L\(^{-1}\) nitrate treatment (Fig. 4A). Growth rates also increased with nitrogen concentrations, within the range of 0.04 ± 0.004–0.26 ± 0.02 d\(^{-1}\). Additionally, the growth fit standard Monod type kinetics, with the maximum specific growth rate ($\mu_m$) of 0.29 ± 0.08 d\(^{-1}\) and the half saturation constant ($K_{\mu}$) of 53.91 ± 50.49 µmol L\(^{-1}\) (Fig. 4B). Similarly, the algal growth increased with increased phosphate concentrations. Limited growth was observed in the treatment without supplement of phosphate (cell density < 2,000 cells mL\(^{-1}\)), and both the highest maximum cell density (12,898 ± 790 cells mL\(^{-1}\)) and growth rate (0.28 ± 0.02 d\(^{-1}\)) were obtained in the 36 µmol L\(^{-1}\) phosphate treatment (Fig. 4C, 4D). Growth also exhibited standard Monod-type kinetics, with the maximum specific growth rate ($\mu_m$) of 0.38 ± 0.08 d\(^{-1}\) and the half saturation constant ($K_{\mu}$) of 9.51 ± 4.36 µmol L\(^{-1}\) (Fig. 4D).
3.4 Effects of environmental factors on toxin production of G. catenatum

Irradiance exerted no overt influence on toxin production of *G. catenatum* in the laboratory treatments. The cellular toxicity varied from 11.43 ± 0.68 to 13.62 ± 1.73 pg STX eq cell⁻¹ in the algal cells cultured under different irradiance levels, but with no significant difference among treatments (*p* > 0.05). The toxin compositions of the laboratory cultures were all dominated by C2, with no significant difference (*p* > 0.05) in the proportions of major toxins among treatments (Fig. 5A).

Temperature affected both the cellular toxicity and toxin composition of *G. catenatum* in the laboratory conditions. The cellular toxicity was 14.5 ± 1.29 pg STX eq cell⁻¹ at 15°C, but was 12.03 ± 0.08 pg STX eq cell⁻¹ at 20°C and 11.30 ± 1.17 pg STX eq cell⁻¹ at 30°C, and the percentage of C1 analog was higher (13.79%) in the 20°C treatment than that of 30°C and 15°C (4.3% and 1%, respectively), with other toxins accounting for 0.59% at 15°C and 2.33% at 30°C (Fig. 5B). The cellular toxicity was significantly different among salinity treatments (*p* < 0.05), which was 15.15 ± 1.02 pg STX eq cell⁻¹ at 20, 11.29 ± 0.81 pg STX eq·cell⁻¹ at 30, 9.14 ± 1.03 pg STX eq·cell⁻¹ at 40, respectively. In addition, C2 contributed to the major proportion (93.5% ~ 95.7% in molar percentage, *p* > 0.05). The percentage of C1 toxin in *G. catenatum* cultured at salinity 30 was significantly higher than the other two salinities (*p* < 0.01), with the other toxins (GTX3-5, dcneoSTX) accounting for the lowest percentage (Fig. 5C).

The toxin production by *G. catenatum* in laboratory cultures changed with nitrate concentration in culture media. There was significant difference in the cellular toxicities of different nitrate treatments (*p* < 0.01), with 7.77 ± 1.31 pg STX eq·cell⁻¹ at 25 µmol L⁻¹, 10.53 ± 0.67 pg STX eq cell⁻¹ at 100 µmol L⁻¹, 12.97 ± 1.18 pg STX eq·cell⁻¹ at 800 µmol L⁻¹, respectively. While no significant difference was observed in the toxin profiles among different nitrate concentrations, which were consistently dominated by C-toxins (> 95%) (Fig. 6A). The cellular toxicity was significantly different among phosphate concentrations (*p* < 0.01), with 15.26 ± 0.54 pg STX eq·cell⁻¹ at 2 µmol L⁻¹, 13.45 ± 1.27 pg STX eq cell⁻¹ at 8 µmol L⁻¹, 11.68 ± 0.83 pg STX eq cell⁻¹ at 36 µmol L⁻¹. The proportion of toxins was significantly different among treatments (*p* < 0.01). The percentage of C2 was 82.36% at 36 µmol L⁻¹, but was 76.56% at 2 µmol L⁻¹ and 78.54% at 8 µmol L⁻¹ while the percentage of C1 was 16.95% at 36 µmol L⁻¹, 21.38% at 8 µmol L⁻¹ and 23.32% at 2 µmol L⁻¹, with the range of the other trace toxins 0.08% ~ 0.65% (Fig. 6B).

4. Discussion

The toxic dinoflagellate *Gymnodinium catenatum* is a cosmopolitan species distributed widely in temperate (12–18°C) and tropical waters (21–30°C), and blooms have been associated with outbreaks of paralytic shellfish poisoning (PSP) events in some regions (Band-Schmidt et al. 2018; Oh et al. 2010; Ordas et al. 2004; Proena et al. 2001). Nutrients, together with light and temperature, are among the major enviromental factors impacting the development of dinoflagellate blooms. In the present study, *G. catenatum* was very sensitive to variation of environmental factors in laboratory treatments, and the
fluctuations in irradiance, temperature, salinity, nitrate, and phosphate concentrations affected not only the growth of *G. catenatum*, but also its toxin production. The eurythermal and euryhaline species was able to tolerate a wide range of temperature (10–30°C) and salinity (20–40). Its growth responded positively to irradiance levels, while deplete nutrients limited the growth of *G. catenatum*. In addition, lower levels of temperature and salinity, as well as limitation of phosphate, exhibited beneficial effects on the toxin production of *G. catenatum*. Moreover, increases in nitrate concentrations enhanced the cellular toxicity of *G. catenatum* significantly, suggesting nitrogen might be a key element for its toxin production.

### 4.1 The effects of major environmental factors on growth of *G. catenatum*

The *G. catenatum* strain isolated from Fujian, China, grew well in f/2 + Se medium, and the exponential growth phase lasts for 10–15 days, which was similar to the Korean strains (Han et al. 2019). The maximum cell density reached 12,000 cells mL\(^{-1}\), which was two times higher than those from Hongkong and Guangxi of China (Hu et al. 2012; Ye et al. 2018). The highest growth rate was 0.28 d\(^{-1}\), which was comparable to the algal strains from Mexico and Tasmania, Australia, but was slightly lower than that of the Japanese strain (0.31 d\(^{-1}\)) and the strain from Derwent, Australia (0.34 d\(^{-1}\)) (Band-Schmidt et al. 2014; Doblin et al. 2000; Yamamoto et al. 2010). The differences in the maximum cell densities and growth rates may be related to their geographic origin, as well as the differences of culture conditions. For example, *G. catenatum* exhibited much higher growth rate in GSe medium (0.82 d\(^{-1}\)) attributing to chelation of trace metals and provision of nutrients (Band-Schmidt et al. 2006; Usup et al. 1989). The trace element selenium was also an important factor for growth of *G. catenatum*, and depressed maximum cell density and growth rate were observed in the dinoflagellate cultured in Se-deplete medium (Doblin et al. 2000). The strain TIO523 grew well in the temperature range of 10–30°C, attaining maximum growth rate at 20°C. Its optimal salinity range (25–35) was similar to the Australian (23–34) and Japanese (20–32) strains (Blackburn et al. 1989; Yamamoto et al. 2010), but was slightly higher than the Korean strains which could tolerate salinity as low as 15 (Han et al. 2019). Such tolerance of salinity changes reflects the natural habitat favorable for the algal bloom. For example, a Venezuelan strain grew better in the salinity range of 33.2 and 39, and the algal blooms were observed in the northeast coastal waters with salinity of 36.5–38 (BarberaSánchez et al. 2001).

The growth of *G. catenatum* was greatly enhanced in nitrate sufficient cultures, which was consistent with several other dinoflagellates (Lei et al. 2011; Liu et al. 2015; Wang et al. 2002). Nitrogen is a component of numerous cellular structures and thought to be the primary limiting macronutrients for algae growth. The maximum cell density and growth rate decreased with nitrate concentrations, probably due to the retarded cell cycle resulting from nitrate stress (Lei et al. 2011). In the present study, the calculated half saturation constant (\(K_{\mu}\)) of *G. catenatum* was 53.91 ± 50.49 µmol L\(^{-1}\), indicating its higher demand for nitrate. The algae evolved physiological functions to adapt nitrate stress, including efficient uptake subsystems (Lei et al. 2011), and the capability to utilize multiple forms of dissolved nitrogen (unpublished data). As observed in previous studies (Hu et al. 2012; Zhang 2009), phosphate
also promoted the growth of *G. catenatum*. The calculated half saturation constant (K_µ) of *G. catenatum* was 9.51 ± 4.36 µmol L⁻¹, while the phosphate concentration in most marine environments was below 1 µmol L⁻¹ (Xu et al. 2010). Thus, to survive the phosphate-deplete conditions, microalgae have evolved mechanisms in utilization of phosphate and counteraction of phosphate stress (Chai et al. 2006; Cheng et al. 2009; Chung et al. 2003; Zhao et al. 2009). Similarly, *G. catenatum* could also utilize various forms of phosphate substrates (e.g. sodium-β-glycerophosphate, unpublished data). Moreover, algae could store phosphorus intracellularly in phosphorus-replete environments, then utilize it in phosphate-deplete environment (John et al. 2000).

### 4.2 Effects of environmental factors on toxic characteristic of *G. catenatum*

The cellular toxicity and toxin profile of *G. catenatum* are extraordinarily variable among different populations or strains around the world. The cellular toxicity (7.77 ± 1.31 to 15.26 ± 0.54 pg STX eq cell⁻¹) of the present strain was at levels comparable to the strains isolated from Japan, Korea, Brazil and Mexican Pacific (Band-Schmidt et al. 2014; Oh et al. 2010; Park et al. 2010; Proena et al. 2001), but was much lower than the Mexican strain reported in Bahía de Mazatlan (> 101 pg STX eq cell⁻¹) (Band-Schmidt et al. 2006). The observed difference in cellular toxicity among strains might be attributed to the difference of toxin composition, particularly the low-toxic strains contained extreme higher proportions of N-sulfocarbamoyl C-toxins (Band-Schmidt et al. 2014; Lee et al. 2012; Park et al. 2010). Among the *G. catenatum* strains isolated in different areas of the East China Sea, C1/C2 toxins were the dominant components (47% ~ 70%) in the toxin profiles, with trace or even no C3/4 toxins (Lin et al. 2022; Liu et al. 2020). Similar toxin profiles were also reported in the Singapore and Japanese strains (Holmes et al. 2010; Oh et al. 2010), indicating their closer relationship with those isolates from East China Sea. Even though the toxin profile was a conservative characteristic within a strain or natural population (Oshima et al. 1993), there was still noticeable difference in the detailed toxin components of particular dinoflagellates. The present *G. catenatum* strain (TIO523) produced trace GTX toxins, while the strain (MEL11) isolated from the same region had a high content of GTX5/6 (Lin et al. 2022). Such discrepancies may be attributed to the difference of initial isolation methods as the present strain was separated from cysts and the other strain was isolated directly from vegetative cells(Oshima et al. 1992).

Among the environmental factors tested in the present study, temperature appeared to be the prominent factor affecting the variation of toxin production in *G. catenatum*. The cellular toxicity of *G. catenatum* was higher in the lower temperature treatments, which was also observed in previous studies (Band-Schmidt et al. 2014; Ye et al. 2018). Similar trends have been found in other PST-producing species, for example *Alexandrium fundyense* produced four times higher toxin levels at 5°C than that at 20°C (Etheridge et al. 2005). Low temperature (< 20°C) suppressed the cell division process leading to the accumulation of arginine (one precursor of PST) (Wang et al. 2016), thereby accelerated the synthesis of toxins. Yoshida et al. (2002) found the activity of N-sulfotransferase in *G. catenatum* was affected by temperature. The decreasing percentage of C2, but increasing percentage of C1, in *G. catenatum* cultured at 30°C might also result from stimulated enzyme activity (e.g. keto-enol epimerization). The response of
toxin synthesis to salinity changes was complicated (Etheridge et al. 2005; Flynn et al. 1996; Lim et al. 2005). One of the well accepted explanations is that rapid growth resulted in the reduced cellular toxicity in the daughter cells (Koji et al. 2001), which may explain the observed higher toxicity at 20 than that of 30. To adapt higher salinity (40), more nutrients and energy were used to enhance osmoregulation, leading to the decrease of toxin production (Lim et al. 2005). In general, effects of salinity on toxicity were species-dependent. Decline of toxicity with elevated salinities has been reported in *Alexandrium minutum*, which was consistent with our results (Lim et al. 2005). In addition, a tight correlation with cell volume and PST quota (Pearson correlation, r = 0.63, p < 0.05) was also observed in *Alexandrium ostenfeldii* at different salinities (Martens et al. 2016).

Variation in the toxin production of harmful algae cultured in different nutrient conditions has been observed in multiple marine dinoflagellates (Flynn et al. 1996; Lee et al. 2012; Li et al. 2016; Lin et al. 2022; Wang et al. 2002; Zhang 2009). In general, it is well documented that sufficient N and depleted P increase toxin production in toxic dinoflagellates (Lee et al. 2012; Macintyre et al. 1997; Touzet et al. 2007). The current results present a similar pattern in that the cellular toxicity was enhanced with the increasing nitrate or decreasing phosphate concentrations. It is not surprising that sufficient supply of N appeared to be important for high toxin production in *G. catenatum*. This is because PSP toxins compose high molecular contents of N, which accounted 5–10% of the total N in the cell of *Alexandrium tamarense* (Macintyre et al. 1997). As a result, insufficient N supply will influence the toxin synthesis directly. In contrast to N, P was not contained in PST molecules. Phosphorus stress could cause an increase in the availability of intracellular arginine, a presumed precursor in PST biosynthesis (Anderson et al. 1990; John et al. 2000; Taroncher-Oldenburg et al. 1997), thereby the cellular toxicity was promoted. Compared with the cellular toxicity, the toxin composition remained constant with nitrate variations rather than with phosphate. In the present study, the ratio of C2:C1 toxins decreased in lower phosphate concentration. Other strains of *G. catenatum* indicated that the toxin composition was not affected by nutrient status (Flynn et al. 1996; Oshima et al. 1993). In *Alexandrium tamarense*, however, the ratio increased slightly at low phosphate conditions (Lee et al. 2012). This reaffirms that the stability of toxin composition is greatly species/strain dependent.

In summary, the *G. catenatum* strain in the present study presented special growth and toxic characteristics, although it shared similar ribosomal sequences with strains from other regions. Variations in environmental conditions could also affect its growth and toxin production, and should be taken into consideration when assessing the harmful effects of *G. catenatum* blooms in the field. In addition to the environmental factors in the present study, various other factors, such as biotic factors and nutrient types, have also been shown to affect both the growth and cellular toxicity of other dinoflagellates (Band-Schmidt et al. 2018; Xu et al. 2012), which need to be further studied to reveal the comprehensive physiological characteristic of *G. catenatum*.

**Declarations**

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**Data Availability:** The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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**References**

1. Adachi M, Sako Y, Ishida Y (1994). Restriction fragment length polymorphism of ribosomal DNA internal transcribed spacer and 5.8s regions in Japanese *Alexandrium* species (Dinophyceae). Journal of Phycology 30: 857-863.

2. Anderson DM, Glibert PM, Burkholder JM (2002). Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25: 704-726. https://doi.org/10.1007/BF02804901

3. Anderson DM, Kulis DM, Sullivan JJ, Hall S, Lee C (1990). Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. Marine Biology 104: 511-524. https://doi.org/10.1007/BF01314358

4. Band-Schmidt C, José BG, Morquecho L, Gárate-Lizárraga I, Alonso-Rodríguez R, Reyes-Salinas A, Erler K, Luckas B (2006). Variations of psp toxin profiles during different growth phases in *Gymnodinium catenatum* (Dinophyceae) strains isolated from three locations in the Gulf of California, Mexico. Journal of Phycology 42: 757-768. https://doi.org/10.1111/j.1529-8817.2006.00234.x

5. Band-Schmidt CJ, Bustillos-Guzman JJ, Hernandez-Sandoval FE, Nunez-Vazquez EJ, Lopez-Cortes DJ (2014). Effect of temperature on growth and paralytic toxin profiles in isolates of *Gymnodinium catenatum* (Dinophyceae) from the Pacific coast of Mexico. Toxicon 90: 199-212. https://doi.org/10.1016/j.toxicon.2014.08.002

6. Band-Schmidt CJ, Fernandez-Herrera LJ, Ramirea-Rodriguez DV, Zumaya-Higuera MG, Hernández-Sandoval FE, Núñez-Vázquez EJ, Bustillos-Guzmán JJ, López-Cortés DJ, Leyva-Valencia I (2018). Effect of different taxonomic groups on the growth and toxin content in *Gymnodinium catenatum* cultures from the Pacific coast of Mexico. Marine and Fresh-water Harmful Algae 54-57.

7. BarberaSánchez A, Gamboamaruez JF (2001). Distribution of *Gymnodinium catenatum* Graham and shellfish toxicity on the coast of Sucre State, Venezuela, from 1989 to 1998. Journal of Shellfish
Research 20: 1257-1261.
8. Blackburn SI, Hallegraeff GM, Bolch CJ (1989). Vegetative reproduction and sexual life cycle of the toxic dinoflagellate Gymnodinium catenatum from Tasmania, Australia. Journal of Phycology 25: 577-590. https://doi.org/10.1111/j.1529-8817.1989.tb00264.x

9. Bolch CJS, De Salas MF (2007). A review of the molecular evidence for ballast water introduction of the toxic dinoflagellates Gymnodinium catenatum and the Alexandrium "tamarensis complex" to Australasia. Harmful Algae 6: 465-485. https://doi.org/10.1016/j.hal.2006.12.008

10. Bolch CJS, Reynolds MJ (2002). Species resolution and global distribution of microreticulate dinoflagellate cysts. Journal of Plankton Research 24: 565-578. https://doi.org/10.1093/plankt/24.6.565

11. Burkholder JM, Glibert PM (2009). The importance of intraspecific variability in harmful algae—Preface to a collection of topical papers. Harmful Algae 8: 744-745. https://doi.org/10.1016/j.hal.2009.03.006

12. Cembella AD, Band-Schmidt CJ (2018). Harmful Algal Species Fact Sheet: Gymnodinium catenatum 605-612. In: Harmful Algal Blooms, Wiley-Blackwell.

13. Chai C, Yu Z, Song X, Cao X (2006). The Status and Characteristics of Eutrophication in the Yangtze River (Changjiang) Estuary and the Adjacent East China Sea, China. Hydrobiologia 563: 313-328. https://doi.org/10.1007/s10750-006-0021-7

14. Chen H (2018). Emergency treatment and reflection of red tide event of Gymnodinium catenatum in Fujian sea area in 2017. Journal of Fisheries Research 40: 308-314.

15. Cheng J, Zhang Y, Zhang D, Liu J, Chen S (2009). Analysis of ecological environment elements during the red tide occurring in Haizhou Bay. Advances in Marine Science 27: 217-223. https://doi.org/10.3969/j.issn.1671-6647.2009.02.012

16. Chung CC, Hwang SPL, Chang J (2003). Identification of a High-Affinity Phosphate Transporter Gene in a Prasinophyte Alga, Tetraselmis chui, and Its Expression under Nutrient Limitation. Applied & Environmental Microbiology 69: 754. https://doi.org/10.1128/AEM.69.2.754-759.2003

17. Doblin MA, Blackburn SI, Hallegraeff GM (2000). Intraspecific variation in the selenium requirement of different geographic strains of the toxic dinoflagellate Gymnodinium catenatum. Journal of Plankton Research: 421-432. https://doi.org/10.1093/plankt/22.3.421

18. Etheridge SM, Roesler CS (2005). Effects of temperature, irradiance, and salinity on photosynthesis, growth rates, total toxicity, and toxin composition for Alexandrium fundyense isolates from the Gulf of Maine and Bay of Fundy. Deep-Sea Research Part II 52: 2491-2500. https://doi.org/10.1016/j.dsr2.2005.06.026

19. Flynn KJ, Flynn K, John EH, Reguera B, Reyero MI, Franco JM (1996). Changes in toxins, intracellular and dissolved free amino acids of the toxic dinoflagellate Gymnodinium catenatum in response to changes in inorganic nutrients and salinity. Journal of Plankton Research 18: 2093-2111. https://doi.org/10.1093/plankt/18.11.2093
20. Green DH, Llewellyn LE, Negri AP, Blackburn SI, Bolch CJS (2004). Phylogenetic and functional diversity of the cultivable bacterial community associated with the paralytic shellfish poisoning dinoflagellate Gymnodinium catenatum. FEMS Microbiology Ecology 47: 345-357. https://doi.org/10.1016/s0168-6496(03)00298-8

21. Gu H, Liu T, Vale P, Luo Z (2013). Morphology, phylogeny and toxin profiles of Gymnodinium inusitatum sp. nov., Gymnodinium catenatum and Gymnodinium microreticulatum (Dinophyceae) from the Yellow Sea, China. Harmful Algae 28: 97-107. https://doi.org/10.1016/j.hal.2013.06.001

22. Han, Kyong HL, Zhun K, Byeong JY, Joo YS, Hyeon H (2019). Toxic dinoflagellate Gymnodinium catenatum Graham (Dinophyceae) from the southern coast of Korea: morphology, phylogeny and effects of temperature and salinity on growth. Environmental Biology Research 37: 31-41. https://doi.org/10.11626/kjeb.2019.37.1.031

23. Holmes MJ, Bolch CJS, Green DH, Cembella AD, Teo SLM (2010). Singapore isolates of the dinoflagellate Gymnodinium catenatum (Dinophyceae) produce a unique profile of paralytic shellfish poisoning toxins. Journal of Phycology 38: 96-106. https://doi.org/10.1046/j.1529-8817.2002.01153.x

24. Hu R, Xu Y, Zhang W, Wu N, Jiang T, Jiang T (2012). Effect of different concentration of N, P, Mn and Fe on the growth and toxin production of Gymnodinium catenatum Graham. Marine Environmental Science 31: 167-172.

25. Hummert C, Ritscher M, Reinhardt R, Luckas B (1997). Analysis of the characteristic PSP profiles of Pyrodinium bahamense and several strains of Alexandrium by HPLC based on ion-pair chromatographic separation, post-column oxidation, and fluorescence detection. Chromatographia 45: 312-316. https://doi.org/10.1007/BF02505576

26. Jiao X, Xu H, Hua W, Sheng J (2010). The red tide organisms in Haizhou Bay. Journal of Aquaculture 31: 25-29.

27. John EH, Flynn KJ (2000). Growth dynamics and toxicity of Alexandrium fundyense (Dinophyceae): the effect of changing N:P supply ratios on internal toxin and nutrient levels. European Journal of Phycology 35: 11-23. https://doi.org/10.1080/09670260010001735581

28. Koji H, Michiko H, Sanae T, Tatsuki T, Satoru T (2001). Variability in toxicity of the dinoflagellate Alexandrium tamarense isolated from Hiroshima Bay, western Japan, as a reflection of changing environmental conditions. Journal of Plankton Research: 271-278. https://doi.org/10.1093/plankt/23.3.271

29. Kumar S., Stecher G., K. T (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33. https://doi.org/10.1093/molbev/msw054

30. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H (2007). Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948. https://doi.org/10.1093/bioinformatics/btm404

31. Lee TCH, Kwok OT, Ho KC, Lee FWF (2012). Effects of different nitrate and phosphate concentrations on the growth and toxin production of an Alexandrium tamarense strain collected from Drake
Passage. Marine Environmental Research 81: 62-69. https://doi.org/10.1016/j.marenvres.2012.08.009

32. Lefebvre KA, Bill BD, Erickson A, Baugh KA, Trainer VL (2008). Characterization of intracellular and extracellular saxitoxin levels in both field and cultured Alexandrium spp. samples from Sequim Bay, Washington. Marine Drugs 6: 103-116. https://doi.org/10.3390/md20080006

33. Lei Q, Lu S (2011). Molecular ecological responses of dinoflagellate, Karenia mikimotoi to environmental nitrate stress. Marine Pollution Bulletin 62: 2692-2699. https://doi.org/10.1016/j.marpolbul.2011.09.021

34. Li A, Jiang B, Chen H, Gu H (2016). Growth and toxin production of Azadinium poporum strains in batch cultures under different nutrient conditions. Ecotoxicology and Environmental Safety 127: 117-126. https://doi.org/10.1016/j.ecoenv.2016.01.017

35. Lim PT, Ogata T (2005). Salinity effect on growth and toxin production of four tropical Alexandrium species (Dinophyceae). Toxicon 45: 699-710. https://doi.org/10.1016/j.toxicon.2005.01.007

36. Lin Z, Geng H, Zhang Q, Chen Z, Dai L, Yu R (2022). Toxin production of dinoflagellate Gymnodinium catenatum isolated from the East China Sea. Harmful Algae 113: 102188. https://doi.org/10.1016/j.hal.2022.102188

37. Liu M, Gu H, Krock B, Luo Z, Zhang Y (2020). Toxic dinoflagellate blooms of Gymnodinium catenatum and their cysts in Taiwan Strait and their relationship to global populations. Harmful Algae 97: 101868. https://doi.org/10.1016/j.hal.2020.101868

38. Liu Y, Chen T, Song S, Li C (2015). Effects of nitrogenous nutrition on growth and nitrogen assimilation enzymes of dinoflagellate Akashiwo sanguinea. Harmful Algae 50: 99-106. https://doi.org/10.1016/j.hal.2015.10.005

39. Llewellyn LE (2006). Saxitoxin, a toxic marine natural product that targets a multitude of receptors. Natural Product Reports 23: 200-222. https://doi.org/10.1039/b501296c

40. Lu S, Hodgkiss IJ (2004). Harmful algal bloom causative collected from Hong Kong waters. Hydrobiologia 512: 231-238. https://doi.org/10.1023/b:hydr.0000020331.75003.18

41. Macintyre J, Cullen J, Cembella A (1997). Vertical migration, nutrition and toxicity in the dinoflagellate Alexandrium tamarense. Marine Ecology Progress Series 148: 201-216.

42. Martens H, Van de Waal DB, Brandenburg KM, Krock B, Tillmann U (2016). Salinity effects on growth and toxin production in an Alexandrium ostenfeldii (Dinophyceae) isolate from The Netherlands. Journal of Plankton Research 38: 1302-1316. https://doi.org/10.1093/plankt/fbw053

43. Monod J (1942). Recherches sur la croissance des cultures bacteriennes, second ed. Hermann, Paris: 211.

44. Negri A, Stirling D, Quilliam M, Blackburn S, Bolch C, Burtonl, Eaglesham G, Thomas K, Walter J, Willis R (2003). Three novel hydroxybenzoate saxitoxin analogues isolated from the dinoflagellate Gymnodinium catenatum. Chemical Research in Toxicology 16: 1029–1033. https://doi.org/10.1021/tx034037j
45. Negri AP, Bolch CJS, Geier S, Green DH, Park TG, Blackburn SI (2007). Widespread presence of hydrophobic paralytic shellfish toxins in *Gymnodinium catenatum*. Harmful Algae 6: 774-780. https://doi.org/10.1016/j.hal.2007.04.001

46. Oh SJ, Matsuyama Y, Yoon YH, Miyamura K, Choi CG, Yang HS, Kang IJ (2010). Comparative analysis of paralytic shellfish toxin content and profile produced by dinoflagellate *Gymnodinium catenatum* isolated from Inokushi Bay, Japan. Journal of the Faculty of Agriculture Kyushu University 55: 47-54. https://doi.org/10.1017/S002185960999027X

47. Ordas MC, Fraga S, Franco JM, Ordas A, Figueras A (2004). Toxin and molecular analysis of *Gymnodinium catenatum* (Dinophyceae) strains from Galicia (NW Spain) and Andalucia (S Spain). Journal of Plankton Research 26: 341-349. https://doi.org/10.1093/plankt/fbh037

48. Oshima Y (2008). Chemical and biochemical studies on paralytic shellfish toxins. Nippon Suisan Gakkaishi 74: 767-771. https://doi.org/10.2331/suisan.74.767

49. Oshima Y, Blackburn SI, Hallegraeff GM (1993). Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodinium catenatum* from three different countries. Marine Biology 116: 471-476. https://doi.org/10.1007/BF00350064

50. Oshima Y, Bolch CJ, Hallegraeff GM (1992). Toxin composition of resisting cysts of *Alexandrium tamarense* (Dinophyceae). Toxicon 30: 1539-1544.

51. Park TG, Kim CH, Oshima Y (2010). Paralytic shellfish toxin profiles of different geographic populations of *Gymnodinium catenatum* (Dinophyceae) in Korean coastal waters. Phycological Research 52: 300-305. https://doi.org/10.1111/j.1440-183.2004.00351.x

52. Proena LAO, Tamanaha MS, Souza NPD (2001). The toxic dinoflagellate *Gymnodinium catenatum* Graham in southern Brazilian waters: occurrence, pigments and toxins. Atlantica (Rio Grande) 23: 59-65.

53. Qi Y, Hong Y, Zheng L, Kulis D, Anderson DM (1996). Dinoflagellate cysts from recent marine sediments of the South and East China Seas. Asian Marine Biol. 13: 87-103.

54. Scholin CA, Herzog M, Sogin M, Anderson DM (1994). Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (dinophyceae). II. sequence analysis of the LSU rRNA gene. Journal of Phycology 30: 999-1011.

55. Shumway SE, Burkholder JM, Morton SL (2018). Harmful algal blooms: A compendium desk reference. In: Harmful Algal Blooms.

56. Sinclair G, Kamykowski D, Glibert PM (2009). Growth, uptake, and assimilation of ammonium, nitrate, and urea, by three strains of *Karenia brevis* grown under low light. Harmful Algae 8: 770-780. https://doi.org/10.1016/j.hal.2009.02.006

57. Stevens M, Peigneur S, Tytgat J (2011). Neurotoxins and their binding areas on voltage-gated sodium channels. Frontiers in Pharmacol 2: 71. https://doi.org/10.3389/fphar.2011.00071

58. Tamura K. NM, Kumar S., (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA 101: 11030-11035. https://doi.org/10.1073/pnas.0404206101
59. Taroncher-Oldenburg G, Kulis DM, Anderson DM (1997). Toxin variability during the cell cycle of the dinoflagellate *Alexandrium fundyense*. Limnology and Oceanography 42: 1178-1188.

60. Thessen AE, Bowers HA, Stoecker DK (2009). Intra- and interspecies differences in growth and toxicity of *Pseudo-nitzschia* while using different nitrogen sources. Harmful Algae 8: 792-810. https://doi.org/10.1016/j.hal.2009.01.003

61. Tillmann U, Hansen PJ (2009). Allelopathic effects of *Alexandrium tamarense* on other algae: evidence from mixed growth experiments. Aquatic Microbial Ecology 57: 101-112. https://doi.org/10.3354/ame01329

62. Touzet N, Franco JM, Raine R (2007). Influence of inorganic nutrition on growth and PSP toxin production of *Alexandrium minutum* (Dinophyceae) from Cork Harbour, Ireland. Toxicon 50: 106-119. https://doi.org/10.1016/j.toxicon.2007.03.001

63. Turner AD, McNabb PS, Harwood DT, Selwood AI, Boundy MJ (2015). Single-Laboratory Validation of a Multitoxin Ultra-Performance LC-Hydrophilic Interaction LC-MS/MS Method for Quantitation of Paralytic Shellfish Toxins in Bivalve Shellfish. Journal of AOAC International 98: 609-621. https://doi.org/10.5740/jaoacint.14-275

64. Usup G, Ahmad A, Ismail N (1989). *Pyrodinium bahamense* var. *compressum* red tide studies in Sabah, Malaysia. In: Hallegraeff, G.M., Maclean, J.L. (Eds.), Biology, Epidemiology and Management of Pyrodinium Red Tides. ICLARM, Manila: 97-110.

65. Usup G, Ahmad A, Matsuoka K, Lim PT, Leaw CP (2012). Biology, ecology and bloom dynamics of the toxic marine dinoflagellate *Pyrodinium bahamense*. Harmful Algae 14: 301-312. https://doi.org/10.1016/j.hal.2011.10.026

66. Usup G, Kulis DM, Anderson DM (1994). Growth and toxin production of the toxic dinoflagellate *Pyrodinium bahamense* var. compressum in laboratory cultures. Natural toxins 2: 254-262.

67. Vale P (2008). Complex profiles of hydrophobic paralytic shellfish poisoning compounds in *Gymnodinium catenatum* identified by liquid chromatography with fluorescence detection and mass spectrometry. Journal of Chromatogr A 1195: 85-93. https://doi.org/10.1016/j.chroma.2008.04.073

68. Wang DZ, Hsieh DPH (2002). Effects of nitrate and phosphate on growth and C2 toxin productivity of *Alexandrium tamarense* C101 in culture. Marine Pollution Bulletin 45: 286-289. https://doi.org/10.1016/S0025-326X(02)00183-2

69. Wang DZ, Zhang SF, Zhang Y, Lin L (2016). Paralytic shellfish toxin biosynthesis in cyanobacteria and dinoflagellates: A molecular overview. Journal of Proteomics 135: 132-140. https://doi.org/10.1016/j.jprot.2015.08.008

70. Wiese M, D'Agostino PM, Mihali TK, Moffitt MC, Neilan BA (2010). Neurotoxic alkaloids: saxitoxin and its analogs. Mar Drugs 8: 2185-2211. https://doi.org/10.3390/md8072185

71. Wu H, Guo M, Bing X, Zheng G, Peng J, Tan Z, Zhai Y (2017). Simultaneous identification and detection of paralytic shellfish toxin in bivalve mollusks by liquid chromatography coupled with quadrupole/linear ion trap tandem mass spectrometry. Oceanologia et limnologia sinica 48: 508-515. https://doi.org/10.11693/hyhz20161000229
72. Xu J, Ho A, He L, Yin K, Hung C, Choi N, Lam P, Wu R, Anderson D, Harrison P (2012). Effects of inorganic and organic nitrogen and phosphorus on the growth and toxicity of two *Alexandrium* species from Hong Kong. Harmful Algae 16: 89-97. https://doi.org/10.1016/j.hal.2012.02.006

73. Xu S, Song J, Li X, Yuan H, Li N, Duan L, Sun P (2010). Changes in nitrogen and phosphorus and their effects on phytoplankton in the Bohai Sea. Chinese Journal of Oceanology and Limnology 28: 945-952.

74. Yamamoto T, Seok OH, Kataoka Y (2010). Effects of temperature, salinity and irradiance on the growth of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae) isolated from Hiroshima Bay, Japan. Fisheries Science 68: 356-363. https://doi.org/10.1046/j.1444-2906.2002.00433.x

75. Ye Z, Cao J, Wu N, Jiang T (2018). Effects of temperature, light intensity and salinity on growth and toxin production of paralytic shellfish poisoning toxins-producing dinoflagellates. Marine Environmental Science 37: 321-348. https://doi.org/10.13634/j.cnki.mes.2018.03.001

76. Yoshida T, Sako Y, Uchida A, Kakutani T, Arakawa O, Noguchi T, Ishida Y (2002). Purification and characterization of sulfotransferase specific to O-22 of 11-hydroxy saxitoxin from the toxic dinoflagellate *Gymnodinium catenatum* (dinophyceae). Fisheries Science 68: 634-642. https://doi.org/10.1046/j.1444-2906.2002.00471.x

77. Zhang C, Lim PT, Li X, Gu H, Anderson DM (2020). Wind-driven development and transport of *Gymnodinium catenatum* blooms along the coast of Fujian, China. Regional Studies in Marine Science 39: 101397. https://doi.org/10.1016/j.rsma.2020.101397

78. Zhang W (2009). Effect of different environment factors on the growth and toxin production of toxic dinoflagellate *Gymnodinium catenatum* Graham. Dissertation, Jinan university.

79. Zhao Y, Yu Z, Song X, Cao X (2009). Effects of different phosphorus substrates on the growth and phosphatase activity of *Skeletonema costatum* and *Prorocentrum donghaiense*. Environmental Science 30: 693-699.

**Figures**

**Figure 1**

Molecular phylogenetic analysis of *G. catenatum* inferred from NJ analyses based on partial large subunit (LSU) rDNA (A) and internal transcribed spacer region (ITS) sequences (B). The numbers near each node are bootstrap support values in NJ tree. The scale bar represents the clade length and the red pentacle indicate the algal strain of the present study.

**Figure 2**
A: growth curve of the cultured *G. catenatum*; B: the percentage of major toxin components detected at the late exponential phase of laboratory cultures; C: cellular toxicity and toxin composition of *G. catenatum* collected from the different growth stages of experimental cultures.

**Figure 3**

Growth curves (A, C, E) and growth rates (B, D, F) of cultured *G. catenatum* in response to different irradiance levels, temperatures and salinities.

**Figure 4**

Growth curves (A, C) and kinetic curves (B, D) of cultured *G. catenatum* in response to different nitrogen and phosphate concentrations.

**Figure 5**

Cellular toxicity and toxin composition of cultured *G. catenatum* in response to different irradiance levels (A), temperatures (B) and salinities (C).

**Figure 6**

Cellular toxicity and toxin composition of cultured *G. catenatum* in response to different nitrogen (A) and phosphate concentrations (B).