

## Toxic dinoflagellate *Centrodinium punctatum* (Cleve) F.J.R. Taylor: an examination on the responses in growth and toxin contents to drastic changes of temperature and salinity

Shin Hyeon Ho <sup>1,\*</sup>, Li Zhun <sup>2</sup>, Réveillon Damien <sup>3</sup>, Savar Veronique <sup>3</sup>, Hess Philipp <sup>3</sup>, Mertens Kenneth Neil <sup>4</sup>, Yoon Joo Youn <sup>1</sup>, Shin Kyoungsoon <sup>5</sup>, Lee Jihoon <sup>6</sup>, Shin A-Young <sup>6</sup>, Byun Eunjung <sup>6</sup>, Yoo Yeong Du <sup>7</sup>, Son Moon Ho <sup>8</sup>, Lee Minji <sup>9</sup>, Seo Min Ho <sup>10</sup>

<sup>1</sup> Library of Marine Samples, Korea Institute of Ocean Science & Technology, Geoje 53201, Republic of Korea

<sup>2</sup> Biological Resource Center/Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology, Jeongeup, 56212, Republic of Korea

<sup>3</sup> Ifremer, PHYTOX unit, F-44000 Nantes, France

<sup>4</sup> Ifremer, LITTORAL unit, Place de la Croix, BP40537, 29900 Concarneau CEDEX, France

<sup>5</sup> Ballast Water Research Center, Korea Institute of Ocean Science & Technology, Geoje 53201, Republic of Korea

<sup>6</sup> Marine Biotechnology Research Center, Korea Institute of Ocean Science & Technology, Busan 49111, Republic of Korea

<sup>7</sup> Department of Oceanography, Kunsan National University, Gunsan 54150, Republic of Korea

<sup>8</sup> National Institute of Fisheries Science, Busan, 619-705, Republic of Korea

<sup>9</sup> National Institute of Fisheries Science, Yeosu, 59780, Republic of Korea

<sup>10</sup> Marine Ecology Research Center, Yeosu 59697, Republic of Korea

\* Corresponding author : Hyeon Ho Shin, email address : [shh961121@kiost.ac.kr](mailto:shh961121@kiost.ac.kr)

### Abstract :

To understand environmental effects affecting paralytic shellfish toxin production of *Centrodinium punctatum*, this study examined the growth responses, and toxin contents and profiles of a *C. punctatum* culture exposed to drastic changes of temperature (from 5–30°C) and salinity (from 35 to 15–40). *C. punctatum* grew over a temperature range of 15–25°C, with an optimum of 20°C., and over a salinity range of 25–40, with optimum salinities of 30–35. This suggests that *C. punctatum* prefers relatively warm waters and an oceanic habitat for its growth and can adapt to significant changes of salinity levels. When *C. punctatum* was cultivated at different temperature and salinity levels, the PST profile included four major analogs (STX, neoSTX, GTX1 and GTX4, constituted >80% of the profile), while low amounts of doSTX and traces of dc-STX and dc-GTX2 were also observed. Interestingly, though overall toxin contents did not change significantly with temperature, increases in the proportion of STX, and decreases in proportions in GTX1 and GTX4 were observed with higher temperatures. Salinity did not affect either toxin contents or profile from 25 to 35. However, the total toxin content dropped to approximately half at salinity 40, suggesting this salinity may induce metabolic changes in *C. punctatum*.

---

## Highlights

► *Centrodinium punctatum* prefers warm waters and an oceanic habitat for its growth. ► Toxin contents of *Centrodinium punctatum* do not change significantly with temperature. ► A salinity of 40 can decrease the toxin contents of *Centrodinium punctatum*.

**Keywords** : East China Sea, PST, STX, neoSTX, Gonyautoxin

# 1. Introduction

Over the last few decades, paralytic shellfish poisoning (PSP) has been reported from many coastal areas (e.g. Sommer et al., 1937; Anderson et al., 2012; Yñiguez et al., 2020), and several PSP outbreaks have caused human intoxication and death, serious economic losses in fisheries industries and negative impact on marine ecosystem (e.g. Hallegraeff, 1993; Azanza and Taylor, 2001; Anderson et al., 2012). Because of such outbreaks, the relationships between toxin production of the causative

organisms and environmental factors have been intensively studied, and numerous reports indicated that toxin contents and profiles are affected by variations in salinity (White, 1978; Cembella, 1998; Hamasaki et al., 2001; Etheridge and Roesler, 2005; Lim and Ogata, 2005; Laabir et al., 2013; Shi et al., 2022) and temperature (Cembella, 1998; Hamasaki et al., 2001; Etheridge and Roesler, 2005; Lim et al., 2006; Navarro et al., 2006; Laabir et al., 2013; Band-Schmidt et al., 2014; Kim et al., 2021; Lin et al., 2022; Shi et al., 2022).

Many species of *Alexandrium*, *Gymnodinium catenatum* and *Pyrodinium bahamense* have been reported as the main toxic dinoflagellates responsible for the PSP outbreaks in marine waters (Harada et al., 1982; Oshima, 1987; Anderson et al., 1994; Taylor et al., 2003; Lundholm et al., 2009; Lassus et al., 2016; Band-Schmidt et al., 2019; Mertens et al., 2020), while some freshwater cyanobacteria have also been shown to produce paralytic shellfish toxins (PSTs) (Wiese et al., 2010). Recently, the marine dinoflagellate, *Centrodinium punctatum* (Cleve) F.J.R. Taylor, was reported to produce saxitoxins (STXs) (Shin et al., 2020). According to Shin et al. (2020), *C. punctatum* maintained at a fixed temperature and salinity (20°C and a salinity of 35) produced high cellular toxicity compared to other STX-producing dinoflagellates; the estimated cellular toxicity was between 91 and 212 pg cell<sup>-1</sup> (or 259 and 601 fmol cell<sup>-1</sup>), and the toxin profile was dominated by STX, GTX1, neoSTX, GTX2, GTX4 and GTX3. However, in that study the effects of change in temperature and salinity levels on the toxin contents and profiles of *C. punctatum* had not been assessed.

*Centrodinium* species are widely distributed in oceanic waters (Hernández-Becerril et al., 2010 and references therein). Nevertheless, information on morphology, phylogeny and ecological niche of *Centrodinium* species is very limited or lacking. In the *Centrodinium* species, the phylogenetic position and detailed morphological features of *C. punctatum* were reported, recently; *C. punctatum* has a close genetic relationship with the genus *Alexandrium*, despite the significant morphological differences between *C. punctatum* and *Alexandrium* species (Li et al., 2019). The previously studied *C. punctatum* strain was established by isolating single cell from the East China Sea (ECS) (Fig. 1). The ECS is a highly dynamic sea that is strongly influenced by several different hydrographically distinct

water bodies, including the Changjiang River diluted water (CDW), the Tsushima Warm Current (TWC) that is a branch of Kuroshio water, and the Taiwan Warm Current water (Hu and Yang, 2001; Yuan et al., 2008). Several particular properties of these waters are known to promote blooms of harmful or toxic species in the ECS (Zhou et al., 2008; Li et al., 2009; Li et al., 2012; Zhou et al., 2015; Wang et al., 2018; Zhou et al., 2019; Sun et al., 2022).

The effects of environmental factors on growth and toxin contents of toxic dinoflagellates such as *Alexandrium* species and *G. catenatum* have been well studied (e.g. Anderson et al., 2012; Band-Schmidt et al., 2019), leading to major advances in understanding their physiology, ecology and toxicology. Water temperature and salinity have been commonly regarded as the main environmental factors that affect the toxin production and growth of harmful or toxic dinoflagellates (White, 1978; Anderson et al., 1990a; Usup et al., 1994; Cembella, 1998; Parkhill and Cembella, 1999; Hamasaki et al., 2001; Lim and Ogata, 2005; Wang and Hsieh, 2005; Laabir et al., 2013; Band-Schmidt et al., 2014; Brandenburg et al., 2020; Shi et al., 2022). However, the growth and toxin production of *C. punctatum* in relation to changes in water temperature and salinity remain largely unknown, as cultured isolates are rare. Hence, this study examines for the first time the growth responses and changes in the toxin contents and profiles of *C. punctatum* exposed to drastic changes of temperature and salinity.

## 2. Materials and methods

### 2.1. Culture of *Centrodinium punctatum*

A strain of *Centrodinium punctatum* (LIMS-PS-2525; previously studied by Li et al., 2019, Shin et al., 2020), which was established from a surface seawater sample that was collected from the ECS (31°59'53"N, 127°41'59"E) (24°C and salinity of 33.7) (Fig. 1), was obtained from the Library of Marine Samples, Korea Institute of Ocean Science and Technology (KIOST). As preliminary examinations indicated that cell growth of *C. punctatum* under a 12L:12D photo-cycle is lower than that under a 24L:0D photo-cycle and that *C. punctatum* did not maintain growth without a roller

apparatus (Shin et al., 2020), all experimental cultures in this study were incubated using a roller apparatus (2 rpm) (Wheaton, USA) under a 24L:0D photo-cycle, with an irradiance of ca. 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  cool-white illumination. The strain in KIOST has been maintained in 2L culture bottles (SPL, Korea) containing f/2 culture medium (Marine Water Enrichment Solution, Sigma Aldrich, USA) without silicate, prepared with sterile sea water (salinity of 35) (filtered through a 47 mm GF/F filter (Hyundai micro, Korea) with a pore size of 0.7 and autoclaved) at 20 °C and ca. 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  cool-white illumination. In this study, experimental cultures were established from exponentially growing cells.

## **2.2. Culturing of *Centrodinium punctatum* at different temperatures and salinities**

To estimate the growth responses of *Centrodinium punctatum* culture exposed to drastic changes of temperature and salinity, experiments were performed under six different water temperatures (5, 10, 15, 20, 25, and 30°C) and eight levels of salinity (15, 20, 25, 27.5, 30, 32.5, 35 and 40) without acclimation of the culture, but the conditions of the combined temperature and salinity levels reflect the range of naturally occurring conditions (e.g. Sun et al., 2022). The target temperatures were established in temperature-controlled incubators, and salinity levels were obtained by evaporating or diluting seawater with ultra-distilled water. The culture experiments to investigate the effect of temperature were performed at a salinity of 35, while the effect of salinity was examined at 20°C. For the culture experiments, aliquots of *C. punctatum* with concentrations ranging from 200 to 300 cells  $\text{mL}^{-1}$  were inoculated into 1L culture bottles (SPL, Korea) filled with f/2-Si culture medium using seawater (salinity of 35) from the ECS (32°00'N, 127°00'E), and placed in the incubators for 26 days. All experiments on growth were conducted in triplicate. Duplicate samples were harvested at the end of the incubation period for toxin analysis.

## **2.3. Calculation of growth rate**

For growth experiments, 1 mL subsamples were collected from each of the three replicate cultures and fixed with Lugol's solution (final concentration 1%). The cells were counted with a

Sedgewick-Rafter counting slide on an upright microscope (ECLIPSE Ni; Nikon, Japan) every two days. Mean cell concentration (from three replicates) was used to calculate the specific growth rates ( $\mu$ ,  $d^{-1}$ ) of *Centrodinium punctatum*. Calculation for the specific growth rate was made the following equation (Guillard, 1973):

$$\text{Specific growth rate } (\mu) = \ln(N_t/N_0) / \Delta t$$

where  $N_0$  and  $N_t$  are the cell concentrations (cells  $mL^{-1}$ ) at the initial and final time during the incubation experiments, respectively, and  $\Delta t$  is the length of exponential phase (day) (Supplementary data).

#### 2.4. Toxin analysis

In this study, toxin analysis was performed as in Shin et al. (2020), on the cultures of *Centrodinium punctatum* exposed to different temperature and salinity levels only, and two samples were used for PST analysis (Table S1). Briefly, freeze-dried cell pellets were extracted with acetic acid 1% in an ultrasonic bath, followed by Solid Phase Extraction purification on ENVI-carb cartridges (250 mg, Supelco). Analysis was performed by HILIC-MS/MS (hydrophilic interaction liquid chromatography) and samples were injected randomly. Toxins (C1–4, GTX1–6, dc-GTX2–3, dc-NEO, dc-STX also used to quantify do-STX, neoSTX, STX) were quantified using certified calibration solutions (NRCC, Halifax, NS, Canada) and matrix-matched calibration curves. The final concentrations were obtained after correction by the solid-phase extraction (SPE) recovery (SPE-recoveries are given per batch and analog in Table S2).

#### 2.5. Statistical analysis

Data for growth rates and maximum cell densities of *C. punctatum* cultivated at different temperatures and salinity levels were presented as mean and standard deviation, and values for toxin contents were expressed as mean. One-way analysis of variance (ANOVA) followed by Tukey's test was performed using MS Excel Professional Plus 2016 Analysis ToolPAK to compare the difference

in cell densities between treatments for temperature and salinity.. Differences were considered significant when  $p < 0.05$ , and  $p$  value was given to indicate the level of difference in the text.

### 3. Results

#### 3.1. Growth responses of *Centrodinium punctatum* exposed to drastic changes of temperature and salinity

Growth of *Centrodinium punctatum* exposed to drastic changes of temperature was observed between 15 and 25°C, whereas at 5 and 10°C the cell densities gradually decreased after inoculation (Fig. 2A; Table 1). No survival was observed at 30°C. At 15°C, *C. punctatum* slowly grew until day 16 of incubation (i.e. exponential growth phase) and then entered the stationary phase. Cells at 20°C grew consistently until day 18 of incubation, and the senescence phase was observed from day 24 of incubation. The maximum cell density (1,087 cells mL<sup>-1</sup>) during the incubation period was observed at 20°C, which was the optimal temperature here (Fig. 2A; Table 1; Supplementary data). At 25°C, *C. punctatum* grew until day 12 and then entered the stationary phase, and cell density rapidly decreased at day 24 of incubation. Within the temperature range that the growth of *C. punctatum* was observed (15, 20 and 25°C), the changes in cell densities showed statistically significant differences (one-way ANOVA,  $p < 0.05$ ), and growth rate in the exponential growth phase ranged from 0.06 to 0.08 day<sup>-1</sup> (Table 1).

When *Centrodinium punctatum* was exposed to drastic changes of salinity, it grew at salinities from 25 to 40, while no growth and eventually cell death were observed at salinities of 15 and 20 (Fig. 2B; Table 1). Similar growth patterns with relatively high cell densities were observed at salinities from 30 to 35 (optimal salinity) (one-way ANOVA,  $p > 0.05$ ); *C. punctatum* grew exponentially until 22 days of culture, which was followed by a senescence phase. With decreasing salinities, the duration of the exponential growth was reduced (i.e. up to day 14 for a salinity of 25, day 18 for a salinity of 27.5 and day 24 for a salinity of 40) (Supplementary data). Maximum cell densities during the incubation period were higher at salinities from 27.5 to 40 (> 1000 cells mL<sup>-1</sup>) and highest at



salinities of 32.5 (1271 cells mL<sup>-1</sup>) (Table 1). Within the salinity ranges that growth of *C. punctatum* was observed, no significant difference (one-way ANOVA,  $p > 0.05$ ) in cell density and specific growth rate (0.07 to 0.08 day<sup>-1</sup>) at the exponential phase were observed among the cultures with different salinities (Table 1).

### ***3.2. Toxin profiles and contents of *Centrodinium punctatum* exposed to drastic changes of temperature and salinity***

Toxin profiles in cultures of *Centrodinium punctatum* exposed to drastic changes of temperature and salinity were dominated by 6 analogs (STX, neoSTX, GTX1, GTX2, GTX3 and GTX4), while low amounts of doSTX (quantified in 16/20 samples,  $< \text{LOQ}$  for 2/20), or traces of dc-STX and dc-GTX2 were sometimes observed (quantified in only 3/20 samples) (Fig. 3; Table 2). Surprisingly, no toxin was detected at 5°C in both the duplicated samples.

When toxins were detected (10-25°C), STX, GTX1, GTX4 and neoSTX were the four major analogs (>80% of the total toxin content), followed by GTX2, GTX3 and doSTX. The concentration of STX was detected to be the lowest at 10°C (55 fmol cell<sup>-1</sup>) and the highest at 25°C (118 fmol cell<sup>-1</sup>), respectively. At 15°C, 20°C and 25°C, GTX1 and GTX4 decreased with increase in temperature (Fig. 3; Table 2). Deoxy-saxitoxin (doSTX) was undetectable at 10°C, and was the highest at 25°C. The mean total toxin content ranged from 210 to 288 fmol cell<sup>-1</sup> (from 227 to 297 fmol STXeq cell<sup>-1</sup>) when toxicity equivalent factors based on Botana et al. (2017) were considered (i.e. traces of doSTX were excluded). The highest value of toxin contents was obtained at 15°C (Table 2). No clear tendency was observed for the toxin contents in relation to different temperature levels.

For cultures of *C. punctatum* harvested at different salinity levels, the toxin profile was characterized by the dominance of STX, GTX4, GTX1 and neoSTX (> 85% of the total toxin content), followed by GTX3 and GTX2 (Fig. 3; Table 2). Two additional analogs (dc-STX and dc-GTX2) were

also detected, albeit at very low levels ( $<0.29$  fmol cell<sup>-1</sup>, Table 2). The analogs dc-STX and dc-GTX2 were not observed when *C. punctatum* cultivated at different temperatures, and were both detected in cultures with a salinity of 30, while only dc-GTX2 was detected at a salinity of 40. The proportion of each analog (STX, GTX4, GTX1, neoSTX, GTX3 and GTX2) was quite similar to those at different temperatures and remained constant in all samples. The highest proportion of STX (42.9%) was found at a salinity of 40, whereas STX had the lowest proportion at a salinity of 30 (34.5%) (Fig. 3). The total toxin content ranged from 111 to 276 fmol cell<sup>-1</sup> (from 113 to 286 fmol STXeq cell<sup>-1</sup>), with the highest value at salinity 27.5 and the lowest at salinity 40.

## 4. Discussion

### 4.1. Temperature and salinity preferences of *Centrodinium punctatum* for growth

According to Li et al. (2019), *Centrodinium punctatum* was isolated from the ECS when the water temperature was 24°C and salinity of 33.7, and then immediately cultured at room temperature of 22°C and salinity of 35. In addition, the isolate was transported to laboratory and incubated at 20°C, and then a strain was successfully established. This indicates that *C. punctatum* may have the ability to adapt quickly to sudden changes of temperature and salinity. Consequently, we consider that the growth responses of the *C. punctatum* culture exposed to significant changes of temperature and salinity in this study reflect its temperature and salinity preference for growth, although the culture was not acclimated to the respective experimental conditions.

The strain of *Centrodinium punctatum* had a narrow temperature range for growth (15-25°C), with an optimal temperature of 20°C, indicating that this species prefers warm waters. Okolodkov and Dodge (1997) recorded that *C. punctatum* is found in the warm waters of the Atlantic including North of the Canary Islands and Pacific Ocean. Similarly, according to Hernández-Becerril et al. (2010), *Centrodinium* species are mainly distributed in warm-water regions, including tropical and subtropical areas.

Several studies have reported that the growth of dinoflagellates under different temperature conditions can vary among strains of same species, and this may be indicative of local adaptation to different temperatures in specific geographic areas (Ichimi et al., 2001; Navarro et al., 2006; Kibler et al., 2012; Tawong et al., 2015; Sparrow et al., 2017; Gémin et al., 2021; Ramilo et al., 2021). The ECS, from which a single cell of *C. punctatum* was isolated to incubate the strain studied here, is strongly affected by both the TWC, that is the branch of the Kuroshio Current characterized by warm temperature and high salinity, and the intrusion of the CDW characterized by relatively high temperature and low salinity (Fig. 1) (Ichikawa and Beardsley, 2002; Teague et al., 2003; Yasuda, 2003). According to Zhou et al. (2019), Sun et al. (2022) and Wang et al. (2022), the warm water currents in the ECS are known to play an important role in affecting the abundance and distribution of dinoflagellates. It is thus possible that the temperature preference of *C. punctatum* can be a successful adaptation to the warm water currents in the ECS. However, more growth experiments on other isolates of *C. punctatum* are needed to determine whether the temperature preference is stable for all isolates collected from the ECS.

Among the PST-producing dinoflagellates, *Alexandrium* species are phylogenetically related to *C. punctatum*, and in particular *Alexandrium affine* displayed the closest genetic resemblance to *C. punctatum* (Li et al., 2019). Growth of *A. affine* collected from Asian coastal waters has been recorded in temperature ranges between 15 and 30°C, and its maximum growth rates were in the range of 0.37–0.70 day<sup>-1</sup> (Nguyen-Ngoc, 2004; Lim et al., 2007; Jeong et al., 2010; Kim et al., 2019; Lim et al., 2019). Compared to the growth responses of *C. punctatum*, *A. affine* seems to be able to tolerate broader temperature ranges and have higher growth rates (Nguyen-Ngoc, 2004; Lim et al., 2007). In addition, maximum growth rates of more than 0.3 day<sup>-1</sup> have been reported for other *Alexandrium* species (*Alexandrium catenella*, *A. fraterculus*, *A. insuetum*, *A. minutum*, *A. pacificum*, *A. peruvianum*, *A. tamarense* and *A. tamiyavanichii*) from Asian coastal waters (Fukuyo, 1982; Yamamoto and Tarutani, 1997; Hamasaki et al., 2001; Ichimi et al., 2001; Lim and Ogata, 2005; Wang and Hsieh, 2005; Matsuda et al., 2006; Oh et al., 2012; Shin et al., 2014; Lee et al., 2019; Li et al. 2021; Han et al. 2022), indicating that the growth rate of *C. punctatum* is quite low compared to those of *Alexandrium*

species.

*C. punctatum* grew at a wide salinity range of 25–40, however lower salinities (15 and 20) inhibited its growth, indicating that *C. punctatum* exhibits a preference to higher salinity and should thus be considered to prefer an oceanic habitat. Hernández-Becerril et al. (2010) also documented that *Centrodinium* species including *C. punctatum* mainly occurred in oceanic areas (see table 2 in their publication). In contrast to *C. punctatum*, *A. affine* tolerated the salinity of 10 (Nguyen-Ngoc, 2004), and many *Alexandrium* species exhibited strong tolerance to low salinity (< 15) (Nhu Bui et al., 2021 and references therein). The majority of *Alexandrium* species are known to be rather euryhaline and have salinity adaptation ranges reflecting the natural habitats from which the species or strains originated (Lim and Ogata, 2005; Lim et al., 2011; Nhu Bui et al., 2021). In the ECS, the seasonal variability of surface salinity for three years (2018–2020) is in the range of 29.8–34.1 (Sun et al., 2022), which are within the recorded optimal salinity (salinities of 30 and 35) of *C. punctatum*. This species is therefore expected to be well adapted to the salinity range typically occurring in the ECS.

The ECS is connected with the East Sea of Korea (Sea of Japan) through the Korea Strait, and along the continental slope of the ECS, the TWC that branch from the Kuroshio Current, enter into the Korean and Japanese coastal areas (Fig. 1) (Lie and Cho, 1994; Hu and Yang, 2001). These currents have been suggested to transport dinoflagellates including harmful species such as *Margalefidinium polykrikoides* and *Prorocentrum obtusidens* into Korean and Japanese coastal bays (Lee, 2006; Shin et al., 2019). However, occurrences of *C. punctatum* have not been recorded in Chinese, Japanese and Korean coastal bays so far. Salinity in Korean and Japanese coastal bays can dramatically change and lower salinity waters (<25) may also occur, because of the input of fresh water from the many streams and rivers during the summer monsoon (Jang et al., 2010; Lee et al., 2014; Yoshimatsu et al., 2014 and references therein). In addition, the ocean currents around the Changjiang river estuary of China, which is located at western part of the ECS (Fig. 1), are very complicated and salinity can vary dramatically (Zhou et al., 2003). Thus, such a low salinity effects in Asian coastal areas may be able to restrict the distribution of *C. punctatum* within the ECS.

In the present study, it is interesting to note that the growth experiments of *C. punctatum* could not be carried out without a roller apparatus, albeit at a low movement rate (2 rpm) (e.g. Li et al., 2019; Shin et al., 2020). Similarly, stock cultures of some dinoflagellates cultivated on a rotating wheel have been used for the growth experiments (e.g. Jeong et al., 1999; Gribble et al., 2009; Yoo et al., 2009). The roller apparatus ensures that cells stay in suspension as caused by turbulent mixing (Arnott et al., 2021), indicating that cells of *C. punctatum* in stagnant water may not highly control their buoyancy or swimming, which is possibly related to the comparatively large cell size. Unless water masses are highly stratified such as in estuarine embayments, phytoplankton in the ocean experiences an active and variable fluid mixing caused by wind, waves and currents, and the turbulent water motion may have both positive and negative effects on cell physiology (e.g. Kiorboe, 1993; Tomas et al., 1995, 1997; Karp-Boss et al., 2000; Sullivan and Swift, 2003). Although in the present study the relationship between growth of *C. punctatum* and quantified turbulent mixing was not examined, small-scale turbulence seems to have a positive effect on *C. punctatum* growth. Probably, the experimental conditions in the present study indicate the habitat preference of *C. punctatum* for mixed water column conditions in the natural environment.

#### **4.2. Toxin profiles and contents of *Centrodinium punctatum***

Interestingly, there were no significant compositional changes in the toxin profiles of *Centrodinium punctatum* cultures exposed to drastic changes of temperature and salinity. In *Alexandrium* species and *Gymnodinium catenatum*, the toxin profile can be related to a phenotypic trait, although amounts are variable among strains (Anderson et al., 1990a; Oshima et al., 1993; Franco et al., 1994; Parkhill and Cembella, 1999; Yoshida et al., 2001). Shin et al. (2020) concluded that the toxin profile of *C. punctatum*, which has been maintained under the fixed culture conditions (20°C and a salinity of 35), is distinct from *Alexandrium* species, excluding *Alexandrium minutum* (e.g., Yoshida et al., 2001; Wang and Hsieh, 2005; Lim et al., 2006; Wang et al., 2006; Orr et al., 2011;

Laabir et al., 2013; Band-Schmidt et al., 2019). The conclusion is supported by the results in the present study as toxin analogs did not change significantly even at drastic changes of temperature and salinity. However, more strains of *C. punctatum* from the ECS and also from different geographical regions should be investigated to clarify this phenotypic trait.

It is noteworthy that at 15°C, 20°C and 25°C, where significant growth of *C. punctatum* was observed, lower proportions of GTX1 and GTX4 were observed with higher temperatures (Fig. 3; Table 2). The relationship between toxin proportion and temperature is not well understood. However, as several studies documented the responses of GTX1 and GTX4 of *Alexandrium* species to changes in concentrations of nutritional factors, salinity, and growth phase (e.g. Anderson et al., 1990b; Hwang and Lu, 2000; Lim et al., 2006; Xu et al., 2012; Laabir et al., 2013; Perini et al., 2014), more studies are needed to examine the production of these toxin analogs in response to environmental factors including temperature.

Some publications documented that high growth of *Alexandrium* species may result in reduced toxicity, because the toxin concentrations can get quickly diluted in the daughter cells during the frequent divisions, establishing an inverse relationship between growth rates and toxicity (Ogata et al., 1987; Sakamoto and Kotani, 1998; Lim and Ogata, 2005). Several publications agree with this conclusion (e.g. Anderson et al., 1990a; Hamasaki et al., 2001; Ichimi et al., 2002; Navarro et al., 2006; Kim et al., 2021). Similarly, in the present study, a small decrease in total toxin content was observed at 20°C where the cell densities of *C. punctatum* slightly increased, compared with 15 and 25°C (Table 1 and 2). In addition, the toxin content at 10°C with no growth of *C. punctatum* was slightly higher than that at 20°C, and at salinity of 25 where the highest growth rate of *C. punctatum* was recorded the toxin content was low (Table 1 and 2). Kodama (1990) found no clear correlation between growth rate and cellular toxin content in different isolates of *A. tamarense*. Similarly, toxin production of *C. punctatum* here does not appear strongly affected by growth, other than dilution by higher cellular division rates (e.g. Lim et al., 2006).

In previous studies, there was no determined optimal salinity for toxin production in PST-

producing dinoflagellates, and the effects of salinity on toxicity were species-dependent (e.g. White, 1978; Anderson et al., 1990a; Usup et al., 1994; Parkhill and Cembella, 1999; Lim and Ogata, 2005; Wang and Hsieh, 2005; Laabir et al., 2013; Shi et al., 2022). In the present study, we found no clear tendency for the toxin contents of *C. punctatum* in relation to different salinity levels. The toxin content of *C. punctatum* was nearly two times lower at a salinity of 40 than at other salinities (Table 2), which may be related to leakage of toxins from lysing cells as growth rapidly declined in this condition. Similarly, the lowest toxicity and growth rate in *G. catenatum* was also obtained at salinity of 40 (Shi et al., 2022). Shi et al. (2022) speculated that to adapt to higher salinity (salinity of 40), more nutrients and energy are used for osmoregulation, leading to a decrease in toxin production. Grzebyk et al. (2003) and Hamasaki et al. (2001) also found that toxicities of *A. minutum* and *A. tamarense* were low at higher salinity, and Hamasaki et al. (2001) concluded that the stress caused by osmotic change can cause the suppression of toxin production or the leakage of toxin from cells. In addition, Stefels (2000) reported that both lower and higher salinities may reduce the growth due to enhanced energy loss by osmoregulation. In the ECS, the highest salinity was 34.1 (Sun et al., 2022), indicating that *C. punctatum* did not experience the salinity of 40 *in situ*, and at salinity of 35 and 40 the growth rates of *C. punctatum* were low in the experiments. Presumably, under high salinity-induced osmotic stress *C. punctatum* is likely to prioritize the growth, rather than toxin biosynthesis.

Previous studies documented that sufficient nitrogen (N) and depleted phosphorus (P) generally increase toxin production of dinoflagellates such as *Alexandrium* species and *G. catenatum* (Anderson et al., 1990b; MacIntyre et al., 1997; Murata et al., 2006; Touzet et al., 2007; Brandenburg et al., 2020; Lee et al., 2012; Shi, 2022). It is not surprising that sufficient supply of N can be important for toxin production, because PSTs have a high molecular content of N (MacIntyre et al., 1997). However, several studies reported that P limitation alone did not result in an increase of toxin production (e.g. Cembella, 1998; John and Flynn, 2000; Lim et al., 2006). As in the present study the growth responses of *C. punctatum* to different temperatures and salinities were conducted with culture medium sufficiently enriched in N and P, the effects of different concentrations of N and P on toxin production of *C. punctatum* could not be assessed. In future work, the effects of nutritional sources

and its different concentrations on toxin production and profile of *C. punctatum* should be examined.

### **Declaration and Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### **Acknowledgements and funding**

This work was supported by grants funded by Korean culture collection of microalgae and collaboration center (NRF-2022M3H9A1083416), the Marine Biotics project (20210469) funded by the Ministry of Ocean and Fisheries, techniques development for management and evaluation of biofouling on ship hulls (20210651) and KIOST project (PEA0121). The authors acknowledge the European H2020 program for funding the EMERTOXX project (grant number 778069).



## References

- Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012. The globally distributed genus *Alexandrium*: Multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae* 14, 10-35. <http://doi.org/10.1016/j.hal.2011.10.012>.
- Anderson, D.M., Kulis, D.M., Doucette, G.J., Gallagher, J.C., Balech, E., 1994. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. *Mar. Biol.* 120(3), 467–478. <http://doi.org/10.1007/bf00680222>.
- Anderson, D.M., Kulis, D.M., Sullivan, J.J., Hall, S., Lee, C., 1990a. Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. *Marine Biology* 104(3), 511–524. <http://doi.org/10.1007/bf01314358>.
- Anderson, D.M., Kulis, D.M., Sullivan, J.J., Hall, S., 1990b. Toxin composition variations in one isolate of the dinoflagellate *Alexandrium fundyense*. *Toxicon* 28(8), 885–893. [http://doi.org/10.1016/0041-0101\(90\)90018-3](http://doi.org/10.1016/0041-0101(90)90018-3).
- Arnott, R.N., Cherif, M., Bryant, L.D., Wain, D.J., 2021. Artificially generated turbulence: a review of phycological nanocosm, microcosm, and mesocosm experiments. *Hydrobiologia* 848(5), 961–991. <http://doi.org/10.1007/s10750-020-04487-5>.
- Azanza, R.V., Taylor, F.J., 2001. Are *Pyrodinium* blooms in the Southeast Asian region recurring and spreading? A review at the end of the millennium. *Ambio* 39(6), 356–364. <http://doi.org/10.1579/0044-7447-30.6.356>.
- Band-Schmidt, C.J., Durán-Riveroll, L.M., Bustillos-Guzmán, J.J., Leyva-Valencia, I., López-Cortés, D.J., Núñez-Vázquez, E.J., Hernández-Sandoval, F.E., Ramirez-Rodriguez, D.V., 2019. Paralytic toxin producing dinoflagellates in Latin America: Ecology and physiology. *Front. Mar. Sci.* 6, 42. <http://doi.org/10.3389/fmars.2019.00042>.
- Band-Schmidt, C.J., Bustillos-Guzman, J.J., Hernandez-Sandoval, F.E., Nunez-Vazquez, E.J., Lopez-Cortes, D.J., 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. <http://doi.org/10.1016/j.toxicon.2014.08.002>.
- Botana, L.M., Hess, P., Munday, R., Nathalie, A., DeGrasse, S. L., Feeley, M., Suzuki, T., Berg, M., Fattori V., Gamarro, E.G., Tritscher, A., Nakagawa R., Karunasagar, I., 2017. Derivation of toxicity equivalency factors for marine biotoxins associated with Bivalve Molluscs. *Trends Food Sci. Technol.* 59, 15–24. <http://doi.org/10.1016/j.tifs.2016.09.015>.
- Brandenburg, K., Siebers, L., Keuskamp, J., Jephcott, T.G., Van de Waal, D.B., 2020. Effects of nutrient limitation on the synthesis of N-rich phytoplankton toxins: A meta-analysis. *Toxins* 12, 221. <http://doi.org/10.3390/toxins12040221>.

- Cembella, A.D., 1998. Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae. In Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), *Physiological Ecology of Harmful Algal Blooms*, Springer Verlag, Heidelberg, pp. 381–403.
- Chen, B., Wang, K., Guo, H., Lin, H., 2021. *Karenia mikimotoi* blooms in coastal waters of China from 1998 to 2017. *Estuar. Coast. Shelf Sci.* 249, 107034. <http://doi.org/10.1016/j.ecss.2020.107034>.
- Etheridge, S.M., Roesler, C.S., 2005. Effects of temperature, irradiance, and salinity on photosynthesis, growth rates, total toxicity, and toxin composition for *Alexandrium fundyense* isolates from the Gulf of Marine and Bay of Fundy. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 52(19–21), 2491–2500. <http://doi.org/10.1016/j.dsr2.2005.06.026>.
- Franco, J.M., Fernandez, P., Reguera, B., 1994. Toxin profiles of natural populations and cultures of *Alexandrium minutum* Halim from Galician (Spain) coastal waters. *J. Appl. Phycol.* 6(3), 275–279. <http://doi.org/10.1007/bf02181938>.
- Fukuyo, Y., 1982. Taxonomical and ecological studies on *Protogonyaulax* occurring in Japanese coastal waters. PhD thesis, Tokyo University, Japan [in Japanese].
- Gémin, M.P., Bertrand, S., Séchet, V., Amzil, Z., Réveillon, D., 2021. Combined effects of temperature and light intensity on growth, metabolome and ovatoxin content of a Mediterranean *Ostreopsis cf. ovata* strain. *Harmful Algae* 106, 102060. <http://doi.org/10.1016/j.hal.2021.102060>.
- Gribble, K.F., Anderson, D.M., Coats, W., 2009. Sexual and asexual process in *Protoperidinium steidingerae* Balech (Dinophyceae), with observation on life-history stages of *Protoperidinium depressum* (Bailey) Balech (Dinophyceae). *J. Eukaryot. Microbiol.* 56, 88–103. <http://doi.org/10.1111/j.1550-7408.00378.x>
- Grzebyk, D., Béchemin, C., Ward, C.J., Vérité, C., Codd, G.A., Maestrini, S.Y., 2003. Effects of salinity and two coastal waters on the growth and toxin content of the dinoflagellate *Alexandrium minutum*. *J. Plank. Res.* 25, 1185–1199. <http://doi.org/10.1093/plankt/fbg088>.
- Guillard R.R.L., 1973. Division rates. In: Stein, J.R. (Ed.), *Handbook of phycolgical methods: culture methods and growth measurements*. Cambridge University Press, Cambridge. 289–311.
- Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32(2), 79–99. <http://doi.org/10.2216/i0031-8884-32-2-79.1>.
- Hamasaki, K., Horie, M., Tokimitsu, S., Toda, T., Taguchi, S., 2001. Variability in toxicity of the dinoflagellate *Alexandrium tamarense* isolated from Hiroshima Bay, Western Japan, as a reflection of changing environmental conditions. *J. Plankton. Res.* 23(3), 271–278. <http://doi.org/10.1093/plankt/23.3.271>.
- Han, K.H., Youn, J.Y., Kwak, K.Y., Li, Z., Lee, W., Shin, H.H., 2022. *Alexandrium pacificum* (Group IV) isolated from Jangmok Bay, Korea: Morphology, phylogeny, and effects of temperature, salinity, and nutrient levels on growth. *Korean J. Environ. Biol.* 40(3), 290–300. <http://doi.org/10.11626/kjeb.2022.40.3.290>.

- Harada, T., Oshima, Y., Kamiya, H., Yasumoto, T., 1982. Confirmation of paralytic shellfish toxins in the dinoflagellate *Pyrodinium bahamense* var. *compressa* and bivalves in Palau. *Bull. Japan Soc. Sci. Fish* 48(6), 821–825. <http://doi.org/10.2331/suisan.48.821>.
- Hernández-Becerril, D.U., Bravo-Sierra, E., Ceballos-Corona, J.G.A., Esqueda-Lara, K., Escobar-Morales, S., Parra-Toriz, D., 2010. Morphology and taxonomy of the marine planktonic dinoflagellate *Centrodinium pulchrum* Böhm (Dinophyta) from the tropical Mexican Pacific, with comments on the taxonomy and distribution of the genus *Centrodinium*. *Phycologia* 49(5), 461–470. <http://doi.org/10.2216/09-80.1>.
- Hu, D.X., Yang, Z.S., 2001. The key process of marine fluxes in East China Sea. Ocean Press, Beijing [in Chinese].
- Hwang, D.F., Lu, Y.H., 2000. Influence of environmental and nutritional factor on growth, toxicity, and toxin profile of dinoflagellate *Alexandrium minutum*. *Toxicon* 38(11), 1491–1503. [http://doi.org/10.1016/s0041-0101\(00\)00080-5](http://doi.org/10.1016/s0041-0101(00)00080-5).
- Ichikawa, H., Beardsley, R.C., 2002. The current system in the Yellow and East China Seas. *Journal of Oceanography* 58, 77–92. <http://doi.org/10.1023/A:1015876701363>.
- Ichimi, K., Suzuki, T., Ito, A., 2002. Variety of PSP toxin profiles in various culture strains of *Alexandrium tamarense* and change of toxin profile in natural *Alexandrium tamarense* population. *J. Exp. Mar. Biol. Ecol.* 273(1), 51–60. [http://doi.org/10.1016/s0022-0981\(02\)00137-5](http://doi.org/10.1016/s0022-0981(02)00137-5).
- Ichimi, K., Yamasaki, M., Okumura, Y., Suzuki, T., 2001. The growth and cyst formation of a toxic dinoflagellate, *Alexandrium tamarense*, at low temperatures in northeastern Japan. *J. Exp. Mar. Biol. Ecol.* 261(1), 17–29. [http://doi.org/10.1016/s0022-0981\(01\)00256-8](http://doi.org/10.1016/s0022-0981(01)00256-8).
- IPCC, 2023. Synthesis report of the IPCC. Sixth Assessment Report (AR6), 84 pages, website last accessed on 27/04/2023: <https://www.ipcc.ch/report/sixth-assessment-report-cycle/>.
- Jang P.G., Jang M.C., Lee W.J., Shin, K., 2010. Effects of nutrient property changes on summer phytoplankton community structure of Jangmok Bay. *Ocean Polar Res.* 32(2), 97–111. <http://doi.org/10.4217/opr.2010.32.2.097>.
- Jeong, H.J., Shim, J.H., Kim, J.S., Park, J.Y., Lee, C.W., Lee, Y., Feeding by the mixotrophic thecate dinoflagellate *Fragilidium* cf. *mexicanum* on red tide and toxic dinoflagellate. *Mar. Ecol. Prog. Ser.* 176, 263–277.
- Jeong, H.J., Yoo, Y.D., Kim, J.S., Seong, K.A., Kang, N.S., Kim, T.H., 2010. Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in Marine planktonic food webs. *Ocean Sci. J.* 45(2), 65–91. <http://doi.org/10.1007/s12601-010-0007-2>.
- John, E.H., Flynn, K.J., 2000. Growth dynamics and toxicity of *Alexandrium fundyense* (Dinophyceae): the effect of changing N:P supply ratios on internal toxin and nutrient levels. *Eur. J. Phycol.* 35(1), 11–23. <http://doi.org/10.1017/s0967026200002572>.

- Karp-Boss, L., Boss, E., Jumars, P.A., 2000. Motion of dinoflagellate in a simple shear flow. *Limnol. Oceanogr.* 45(7), 1594–1602. <http://doi.org/10.4319/lo.2000.45.7.1594>.
- Kibler, S.R., Litaker, R.W., Holland, W.C., Vandersea, M.W., Tester, P.A., 2012. Growth of eight *Gambierdiscus* (Dinophyceae) species: effects of temperature, salinity and irradiance. *Harmful Algae* 19, 1–14. <http://doi.org/10.1016/j.hal.2012.04.007>.
- Kim, H.S., Park, H.J., Wang, H., Yoo, H.Y., Park, J.Y., Ki, J.S., 2021. Low temperature and cold stress significantly increase saxitoxins (STXs) and expression of STX biosynthesis genes *stxA4* and *sxtG* in the dinoflagellate *Alexandrium catenella*. *Mar. Drugs* 19(6), 291. <http://doi.org/10.3390/md19060291>.
- Kim, J.H., Oh, S.J., Kim, S.-Y., 2019. The effect of temperature, salinity and irradiance on the growth of *Alexandrium affine* (Dinophyceae) isolated from southern sea of Korea. *Korean Soc. Mar. Environ. Saf.* 25(2), 229–236. <http://doi.org/10.7837/kosomes.2019.25.2.229> [In Korean, with English abstract].
- Kjørboe, T., 1993. Turbulence, phytoplankton cell size, and the structure of pelagic food webs. *Adv. Mar. Biol.* 29, 1–72. [http://doi.org/10.1016/s0065-2881\(08\)60129-7](http://doi.org/10.1016/s0065-2881(08)60129-7).
- Kodama, M., 1990. Possible links between bacteria and toxin production in algal blooms. In Graneli, E., Sundstrom, B., Edler, L., Anderson, D.M. (Eds.) *Toxic Marine Phytoplankton*. Elsevier, N.Y., United States, pp. 52–61.
- Laabir, M., Collos, Y., Masseret, E., Grzebyk, D., Abadie, E., Savar, V., Sibat, M., Amzil, Z., 2013. Influence of environmental factors on the paralytic shellfish toxin content and profile of *Alexandrium catenella* (Dinophyceae) isolated from the Mediterranean Sea. *Mar. Drugs* 11(5), 1583–1601. <http://doi.org/10.3390/md11051583>.
- Lassus P., Chomérat, N., Hess, P., Nézan, E., 2016. *Toxic and Harmful Microalgae of the World Ocean. / Microalgues toxiques et nuisibles de l’océan mondial*. Denmark, International Society for the Study of Harmful Algae / Intergovernmental Oceanographic Commission of UNESCO. IOC Manuals and Guides, Denmark. 523 p.
- Lee, K.H., Jeong, H.J., Kang, C.H., Ok, J.H., You, J.H., Park, S.A., 2019. Growth rates and nitrate uptake of co-occurring red-tide dinoflagellates *Alexandrium affine* and *A. fraterculus* as a function of nitrate concentration under light-dark and continuous light conditions. *Algae* 34(3), 237–251. <http://doi.org/10.4490/algae.2019.34.8.28>.
- Lee, S.W., Park, C., Lee, D.B., Lee, J.G., 2014. Effects of freshwater discharge on plankton in Cheonsu Bay, Korea during the rainy season. *Ocean Sci. J.* 19(1), 41–52. <http://doi.org/10.7850/jkso.2014.19.1.41>.
- Lee, T.C.H., Kwok, O.T., Ho, K.C., Lee, F.W.P., 2012. Effects of different nitrate and phosphate concentrations on the growth and toxin production of an *Alexandrium tamarense* strain collected from Drake Passage. *Mar. Environ. Res.* 81, 62–69. <http://doi.org/10.1016/j.marenvres.2012.08.009>.
- Lee, Y.S., 2006. Factors affecting outbreaks of high-density *Cochlodinium polykrikoides* red tides in the coastal seawaters around Yeosu and Tongyeong, Korea. *Mar. Pollut. Bull.* 52(10), 1249–1259.

<http://doi.org/10.1016/j.marpolbul.2006.02.024>.

- Lewis, A.M., Coates, L.N., Turner, A.D., Percy, L., Lewis, J., 2018. A review of the global distribution of *Alexandrium minutum* (Dinophyceae) and comments on ecology and associated paralytic shellfish toxin profiles, with a focus on Northern Europe. *J. Phycol.* 54, 581-598. <https://doi.org/10.1111/jpy.12768>
- Lewis, A.M., Dean, K.J., Hartnell, D.M., Percy, L., Turner, A.D., Lewis, J.M., 2022. The value of toxin profiles in the chemotaxonomic analysis of paralytic shellfish toxins in determining the relationship between British *Alexandrium* spp. and experimentally contaminated *Mytilus* sp.. *Harmful Algae* 102131. <https://doi.org/10.1016/j.hal.2021.102131>.
- Li, J., Gilbert, P.M., Alexander, J.A., Molina, M.E., 2012. Growth and competition of several harmful dinoflagellates under different nutrient and light conditions. *Harmful Algae* 13, 112–125. <http://doi.org/10.1016/j.hal.2011.10.005>.
- Li, J., Gilbert, P.M., Zhou, M., Lu, S., Lu, D., 2009. Relationship between nitrogen and phosphorus forms and ratios and development of dinoflagellate blooms in the East China Sea. *Mar. Ecol. Prog. Ser.* 383, 11–26. <http://doi.org/10.3354/meps07975>.
- Li, P., Ma, Q., Xu, S., Liu, W., Ma, Z., Ni, G., 2021. Opposite growth responses of *Alexandrium minutum* and *Alexandrium catenella* to Photoperiods and temperatures. *Plants* 10(6), 1056. <http://doi.org/10.3390/plants10061056>.
- Li, Z., Mertens, K.N., N'ezan, E., Chom'erat, N., Bilien, G., Iwataki, M., Shin, H.H., 2019. Discovery of a new clade nested within the genus *Alexandrium* (Dinophyceae): morpho-molecular characterization of *Centrodinium punctatum* (Cleve) F.J.R. Taylor. *Protist* 170(2), 168–186. <http://doi.org/10.1016/j.protis.2019.02.003>.
- Lie, H.J., Cho, C.H., 1994. On the origin of the Tsushima Warm Current. *J. Geophys. Res.* 99(C12), 25081–25091. <http://doi.org/10.1029/94jc02425>.
- Lim, P. T., Ogata, T., 2005. Salinity effect on growth and toxin production of four tropical *Alexandrium* species (Dinophyceae). *Toxicon* 45(6), 699–710. <http://doi.org/10.1016/j.toxicon.2005.01.007>.
- Lim, P.T., Leaw, C.-P., Kaga, S., Sekiguchi, K., Ogata, T., 2007. Growth responses of five non toxic *Alexandrium* species (Dinophyceae) to temperature and salinity. *Mar. Res. Indonesia* 32(2), 189–195. <http://doi.org/10.14203/mri.v32i2.454>.
- Lim, P.T., Leaw, C.-P., Usup, G., Kobiyama, A., Koike, K., Ogata, T., 2006. Effects of light and temperature on growth, nitrate uptake, and toxin production of two tropical dinoflagellates: *Alexandrium tamiyavanichii* and *Alexandrium minutum* (Dinophyceae). *J. Phycol.* 42(4), 786–799. <http://doi.org/10.1111/j.1529-8817.2006.00249.x>.

- Lim, P.T., Leaw, C.P., Sato, S., Thuoc, C.V., Kobiyama, A., Ogata, T., 2011. Effect of salinity on growth and toxin production of *Alexandrium minutum* isolated from a shrimp culture pond in northern Vietnam. *J. Appl. Phycol.* 23, 857-864. DOI 10. 1007/s10811-010-9593-8.
- Lim, Y.K., Baek, S.H., Lee, M.J., Kim, Y.Y., Choi, K.H., 2019. Phytoplankton composition associated with physical and chemical variables during summer in the southern sea of Korea: Implication of the succession of the two toxic dinoflagellates *Cochlodinium* (a.k.a. *Margalefidinium*) *polykrikoides* and *Alexandrium affine*. *J. Exp. Mar. Biol. Ecol.* 516, 51–66. <http://doi.org/10.1016/j.jembe.2019.05.006>.
- Lin, Z.R., Gen, H.X., Zhang, Q.H., Chen, Z.F., Dai, L., Yu, R.C., 2022. Toxin production of dinoflagellate *Gymnodinium catenatum* isolated from East China Sea. *Harmful Algae* 113, 102188. <http://doi.org/10.1016/j.hal.2022.102188>.
- Lundholm, N.; Churro, C.; Fraga, S.; Hoppenrath, M.; Iwataki, M.; Larsen, J.; Mertens, K.; Moestrup, Ø.; Zingone, A. (Eds) (2009 onwards). IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. Accessed at <https://www.marinespecies.org/hab> on 2023-02-15. <https://doi.org/10.14284/362>.
- MacIntyre, J., Cullen, J., Cembella, A., 1997. Vertical migration, nutrition and toxicity in the dinoflagellate *Alexandrium tamarense*. *Mar. Ecol. Prog. Ser.* 148, 201–206. <http://doi.org/10.3354/meps148201>.
- Matsuda, A., Nishijima, T., Fukami, K., Adachi, M., 2006. Growth kinetics and paralytic shellfish poisoning toxin production in phosphorus limited cultures of *Alexandrium catenella*. *Nippon Suisan Gakkaishi.* 72(2), 193–200. <http://doi.org/10.2331/suisan.72.193>.
- Mertens, K.N., Adachi, M., Anderson, D.M., Band-Schmidt, C.J., Bravo, I., Brosnahan, M.L., Bolch, C.J.S., Calado, A.J., Carbonell-Moore, M.C., Chomérat, N., Elbrächter, M., Figuera, R.I., Fraga, S., Gárate-Lizárraga, I., Gracés, E., Gu, H., Hallegraeff, G., Hess, P., Hoppenrath, M., Horiguchi, T., Iwataki, M., John, U., Kremp, A., Larsen, J., Leaw, C.P., Li, Z., Lim, P.T., Litaker, W., MacKenzie, L., Masseret, E., Matsuoka, K., Moestrup, Ø, Montresor, M., Nagai, S., Nézan, E., Nishimura, T., Okolodkov, Y.B., Orlova, T.Y., Reñe, A., Sampedro, N., Satta, C.T., Shin, H.H., Siano, R., Smith, K.F., Steidinger, K., Takano, Y., Tillmann, U., Wolny, J., Yamaguchi, A., Murray S., 2020. Morphological and phylogenetic data do not support the split of *Alexandrium* into for genera. *Harmful Algae* 98, 101902. <https://doi.org/10.1016/j.hal.2020.101902>.
- Murata, A., Leong, S.C.Y., Nagashima, Y., Taguchi, S., 2006. Nitrogen: phosphorus supply ratio may control the protein and total toxin production of dinoflagellate *Alexandrium tamarense*. *Toxicon* 48(6), 683–689. <http://doi.org/10.1016/j.toxicon.2006.08.004>.
- Navarro, J.M., Muñoz, M.G., Contreras, A.M., 2006. Temperature as a factor regulating growth and toxin content in the dinoflagellate *Alexandrium catenella*. *Harmful Algae* 5(6), 762–769. <http://doi.org/10.1016/j.hal.2006.04.001>.
- Nguyen-Ngoc, L., 2004. An autecological study of the potentially toxic dinoflagellate *Alexandrium affine* isolated from Vietnamese waters. *Harmful Algae* 3(2), 117–129. <http://doi.org/10.1016/s1568->

9883(03)00062-3.

- Nhu Bui, Q.T., Kim, H., Park, H.J., Ki, J.S., 2021. Salinity affects saxitoxins (STXs) toxicity in the dinoflagellate *Alexandrium pacificum*, with low transcription of STX-biosynthesis genes *sxtA4* and *sxtG*. *Toxins* 13, 733. <http://doi.org/10.3390/toxins13100733>.
- Ogata, T., Ishimaru, T., Kodama, M., 1987. Effect of water temperature and light intensity on growth rate and toxicity change in *Protogonyaulax tamarensis*. *Mar. Bio.* 95(2), 217–220. <http://doi.org/10.1007/bf00409008>.
- Oh, S.J., Park, J.A., Kwon, H.K., Yang, H.S., Lim, W.A., 2012. Ecophysiological studies on the population dynamics of two toxic dinoflagellates *Alexandrium tamarensis* and *Alexandrium catenella* isolated from the southern coast of Korea. I. Effects of temperature and salinity on the growth. *J. Kor. Soc. Mar. Environ. Eng.* 15(2), 133–141. <http://doi.org/10.7846/jkosmee.2012.15.2.133>.
- Okolodkov, Y., Dodge, J.D., 1997. Morphology of some rare and unusual dinoflagellates from the north-eastern Atlantic. *Nova Hedwigia* 64, 353-365.
- Orr, R.J.S., Stüken, A., Rundberget, T., Eikrem, W., Jakobsen, K.S., 2011. Improved phylogenetic resolution of toxic and nontoxic *Alexandrium* strains using a concatenated rDNA approach. *Harmful Algae* 10(6), 676–688. <http://doi.org/10.1016/j.hal.2011.05.003>.
- Oshima, Y., Blackburn, S.I., Hallegraeff, G.M., 1993. Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodinium catenatum* from three different countries. *Mar. Biol.* 116(3), 471–476. <http://doi.org/10.1007/bf00350064>.
- Oshima, Y., Hasegawa, M., Yasumoto, T., Hallegraeff, G., Blackburn, S., 1987. Dinoflagellate *Gymnodinium catenatum* as the source of paralytic shellfish toxins in Tasmanian shellfish. *Toxicon* 25(10), 1105–1111. [http://doi.org/10.1016/0041-0101\(87\)90267-4](http://doi.org/10.1016/0041-0101(87)90267-4).
- Parkhill, J.P., Cembella, A.D., 1999. The effects of salinity, light and inorganic nitrogen on the growth and toxigenicity of the marine dinoflagellate *Alexandrium tamarensis*. *J. Plank. Res.* 21(5), 939–955. <http://doi.org/10.1093/plankt/21.5.939>.
- Perini, F., Galluzzi, L., Aversano, C.D., Iacovo, E.D., Luciana, T., Ricci, F., Forini, M., Ciminiello, P., Penna, A., 2014. *SxtA* and *sxtG* gene expression and toxin production in the Mediterranean *Alexandrium minutum* (Dinophyceae). *Mar. Drugs* 12(10), 5258–5276. <http://doi.org/10.3390/md12105258>.
- Ramilo, I., Figueroa, R.I., Rayón-Viña, F., Cuadrado, Á., Bravo, I., 2021. Temperature-dependent growth and sexuality of the ciguatoxin producer dinoflagellate *Gambierdiscus* spp. in cultures established from the Canary Islands. *Harmful Algae* 110, 102130. <http://doi.org/10.1016/j.hal.2021.102130>.
- Sakamoto, S., Kotani, Y., 1998. Variation of paralytic shellfish contents and composition in *Alexandrium tamarensis* collected at Kure Bay, Hiroshima Prefecture. *Bull. Nansei. Natl. Fish. Inst.* 31, 45–52.
- Shi, J., Liu, Y., Gu, H., Li, C., 2022. Variation in the growth and toxin production of *Gymnodinium catenatum*

- under different laboratory conditions. *Mar. Biol.* 169, 130. <http://doi.org/10.1007/s00227-022-04115-x>.
- Shin, H.H., Baek, S.H., Li, Z., Han, M.S., Oh, S.J., Youn, S.H., Kim, Y.S., Kim, D., Lim, W.A., 2014. Resting cysts, and effects of temperature and salinity on the growth of vegetative cells of the potentially harmful species *Alexandrium insuetum* Balech (Dinophyceae). *Harmful Algae* 39, 175–184. <http://doi.org/10.1016/j.hal.2014.07.012>.
- Shin, H.H., Li, Z., Réveillon, D., Rovillon, G.A., Mertens, K.N., Hess, P., Kim, H.J., Lee, J.H., Lee, K.W., Kim, D.K., Park, B.S., Hwang, J.I., Seo, M.H., Lim, W.A., 2020. *Centrodinium punctatum* (Dinophyceae) produces significant levels of saxitoxin and related analogs. *Harmful Algae* 100, 101923. <http://doi.org/10.1016/j.hal.2020.101923>.
- Shin, H.H., Li, Z., Seo, M.H., Soh, H.Y., Lim, W.A., Park, J.W., 2019. Harmful dinoflagellate dinoflagellate *Prorocentrum donghaiense* Lu is widely distributed along the East China Sea and Korean coastal area. *Ocean Sci. J.* 54(4), 685–691. <http://doi.org/10.1007/s12601-019-0028-4>.
- Sommer, H., Whedon, W.F., Kofoed, C.A., Stohler, R., 1937. Relation of paralytic shellfish poison to certain plankton organisms of the genus *Gonyaulax*. *Arch. Pathol.* 24, 537–559.
- Sparrow, L., Momigliano, P., Russ, G.R., Heimann, K., 2017. Effects of temperature, salinity and composition of the dinoflagellate assemblage on the growth of *Gambierdiscus carpenteri* isolated from the Great Barrier Reef. *Harmful Algae* 65, 52–60. <http://doi.org/10.1016/j.hal.2017.04.006>.
- Stefels, J., 2000. Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. *J. Sea. Res.* 43, 183–197.
- Sullivan, J.M., Swift, E., 2003. Effects of small-scale turbulence on net growth rate and size of ten species of marine dinoflagellates. *J. Phycol.* 39(1), 83–94. <http://doi.org/10.1046/j.1529-8817.2003.02094.x>.
- Sun, Y., Youn, S.H., Kim, Y., Kang, J.J., Lee, D.B., Kim, K.W., Jang, H.K., Jo, N., Yun, M.S., Song, S.K., Lee, S.H., 2022. Interannual variation in phytoplankton community driven by environmental factors in the northern East China Sea. *Front. Mar. Sci.* 9, 769497. <http://doi.org/10.3389/fmars.2022.769497>.
- Tawong, W., Yoshimatsu, T., Yamaguchi, H., Adachi, M., 2015. Effects of temperature, salinity and their interaction on growth of benthic dinoflagellates *Ostreopsis* spp. from Thailand. *Harmful Algae* 44, 37–45. <http://doi.org/10.1016/j.hal.2015.02.011>.
- Taylor, F.J.R., Fukuyo, Y., Larsen, J., Hallegraeff G.M., 2003. Taxonomy of Harmful Dinoflagellates. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds.), *Manual on Harmful Marine Microalgae*. UNESCO Publishing, Paris, pp. 389–432. Monographs on oceanographic methodology 11.
- Teague, W.J., Jacobs, G.A., Ko, D.S., Tang, T.Y., Chang, K.I., Suk, M.S., 2003. Connectivity of the Taiwan, Cheju, and Korea straits. *Cont. Shelf Res.* 23(1), 63–77. [http://doi.org/10.1016/s0278-4343\(02\)00150-4](http://doi.org/10.1016/s0278-4343(02)00150-4).
- Tomas, W.H., Tynan, C.T., Gibson, C.H., 1997. Turbulence phytoplankton interrelationships. *Progr. Phycol. Res.* 12, 283–324.



- Tomas, W.H., Vernet, M., Gibson, C.H., 1995. Effects of small scale turbulence on photosynthesis, pigment, cell division, and cell size in the marine dinoflagellate *Gonyaulax polyedra* (Dinophyceae). *J. Phycol.* 31(1), 50–59. <http://doi.org/10.1111/j.0022-3646.1995.00050.x>.
- Touzet, N., Franco, J.M., Raine, R., 2007. Influence of inorganic nutrition on growth and PSP toxin production of *Alexandrium minutum* (Dinophyceae) from Cork Harbour, Ireland. *Toxicon* 50(1), 106–119. <http://doi.org/10.1016/j.toxicon.2007.03.001>.
- Ustup, G., Kulis, D.M., Anderson, D.M., 1994. Growth and toxin production of the toxic dinoflagellate *Pyrodinium bahamense* var. *compressum* in laboratory cultures. *Nat. Toxins* 2(5), 254–262. <http://doi.org/10.1002/nt.2620020503>.
- Wng, D.Z., Hsieh, D.P.H., 2005. Growth and toxin production in batch cultures of a marine dinoflagellate *Alexandrium tamarense* HK9301 isolated from the South China Sea. *Harmful Algae* 4, 401–410. <http://doi.org/10.1016/j.hal.2004.07.002>.
- Wang, D.Z., Zhang, S.G., Gu, H.F., Chan, L.L., Hong, H.S., 2006. Paralytic shellfish toxin profiles, and toxin variability of the genus *Alexandrium* (Dinophyceae) isolated from the Southeast China Sea. *Toxicon* 48(2), 138–151. <http://doi.org/10.1016/j.toxicon.2006.04.002>.
- Wang, Y., Bi, R., Zhang, J., Gao, J., Takeda, S., Kondo, Y., Chen, F., Jin, G., Sachs, J.P., Zhao, M., 2022. Phytoplankton distribution in the Kuroshio-Oyashio region of the Northwest Pacific Ocean: Implications for marine ecology and carbon cycle. *Front. Mar. Sci.* 9, 865142. <http://doi.org/10.3389/fmars.2022.865142>.
- Wang, Y.F., Yu, R.C., Lu, D.D., Lu, S.H., Zhang, C.S., Yan, T., Zhang, Q.C., Zhou, M.J., 2018. Recurrent toxic blooms of *Alexandrium* spp. in the East China Sea-Potential role of Taiwan Warm Current in bloom initiation. *J. Ecol. Toxicol.* 2, 115.
- White, A.W., 1978. Salinity effects on growth and toxin content of *Gonyaulax excavata*. A marine dinoflagellate causing paralytic shellfish poisoning. *J. Phycol.* 14(4), 475–479. <http://doi.org/10.1111/j.1529-8817.1978.tb02472.x>.
- Wiese, M., D'Agostino, P.M., Mihali, T.K., Moffitt, M.C., Neilan, B.A., 2010. Neurotoxic alkaloids: saxitoxin and its analogs. *Mar. drugs* 8(7), 2185–2211. <http://doi.org/10.3390/md8072185>.
- Xu, J., Ho, A.Y.T., He, L., Yin, K., Hung, C., Choi, N., Lam, P.K.S., Wu, R.S.S., Anderson, D.M., Harrison, P.J., 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. <http://doi.org/10.1016/j.hal.2012.02.006>.
- Yamamoto, T., Tarutani, K., 1997. Effects of temperature, salinity and irradiance on the growth of toxic dinoflagellate *Alexandrium tamarense* isolated from Hiroshima bay, Japan. *Jpn. J. Phycol.* 45, 95–101.
- Yasuda I., 2003. Hydrographic Structure and Variability in the Kuroshio-Oyashio Transition Area. *J. Oceanogr.* 59, 389–402. <http://doi.org/10.1023/A:1025580313836>.

- Yñiguez, A.T., Lim, P.T., Leaw, C.P., Jipanin, S.J., Iwataki, M., Benico, G., Azanza, R.V., 2020. Over 30 years of HABs in the Philippines and Malaysia: What have we learned? *Harmful Algae* 102, 101776. <http://doi.org/10.1016/j.hal.2020.101776>
- Yoo, Y.D., Jeong H.J., Kim, M.S., Kang, N.S., Song, J.Y., Shin, W.G., Kim, K.Y., Lee, K.T., 2009. Feeding by phototrophic red-tide dinoflagellates on the ubiquitous marine diatom *Skeletonema costatum*. *J. Eukaryot. Microbiol.* 56, 413-420.
- Yoshida, T., Sako, Y., Uchida, A., 2001. Geographic differences in paralytic shellfish poisoning toxin profiles among Japanese populations of *Alexandrium tamarense* and *A. catenella* (Dinophyceae). *Phycological Research* 49(1), 13–21. <http://doi.org/10.1111/j.1440-1835.2001.tb00228.x>.
- Yoshimatsu, T., Yamaguchi, H., Iwamoto, H., Nishimura, T., Adachi, M., 2014. Effects of temperature, salinity and their interaction on growth of Japanese *Gambierdiscus* spp. (Dinophyceae). *Harmful Algae* 35, 29–37. <http://doi.org/10.1016/j.hal.2014.03.007>.
- Yuan, D., Zhu, J., Li, C., Hu, D., 2008. Cross-shelf circulation in the Yellow Sea and East China Seas indicated by MODIS satellite observations. *J. Mar. Syst.* 70(1–2), 134–149. <http://doi.org/10.1016/j.jmarsys.2007.04.002>.
- Zhou, M., Yan, T., Zou, J., 2003. Preliminary analysis of the characteristics of red tide area in Changjiang River estuary and its adjacent sea. *Chin. J. Appl. Ecol.* 14, 1031–1038 [in Chinese].
- Zhou, C., Place, A.R., Yan, X., Xu, J., Luo, Q., William, E., 2015. Interactions between *Karlodinium veneficum* and *Prorocentrum donghaiense* from the East China Sea. *Harmful Algae* 49, 50–57. <http://doi.org/10.1016/j.hal.2015.08.004>.
- Zhou, M.J., Shen, Z.L., Yu, R.C., 2008. Responses of a coastal phytoplankton community to increased nutrient input from the Changjiang (Yangtze) river. *Cont. Shelf. Res.* 28(12), 1483–1489. <http://doi.org/10.1016/j.csr.2007.02.009>.
- Zhou, Z.X., Yu, R.C., Sun, C., Feng, M., Zhou, M.J., 2019. Impact of Changjiang River discharge and Kuroshio intrusion on the diatom and dinoflagellate blooms in the East China Sea. *JGR: Oceans* 124(7), 5244–5257. <http://doi.org/10.1029/2019jc015158>.

## Figure legends

**Fig. 1.** Hydrographic waters and their circulations in the East China Sea, modified by Hu and Yang (2001) and Yuan et al., (2008). A red circle indicates the station that *Centrodinium piunctatum* was isolated. CDW: Changjiang Diluted Water; ECSCC: East China Sea Coastal Current; KCC: Korean Coastal Current; TWC: Tsushima Warm Current; TWSC: Taiwan Wram Sea Current.

**Fig. 2.** Growth curves of *Centrodinium piunctatum* cultivated at different temperatures (A) and salinities (B). The error bars represent standard deviations. Note: the rapid decline of cell numbers at 30°C and the slow decline of cell numbers at 5 and 10°C.

**Fig. 3.** Toxin profiles and proportions in *Centrodinium piunctatum* cultivated at different temperature and salinity levels. Note: the increase in STX-proportion with increasing temperature.

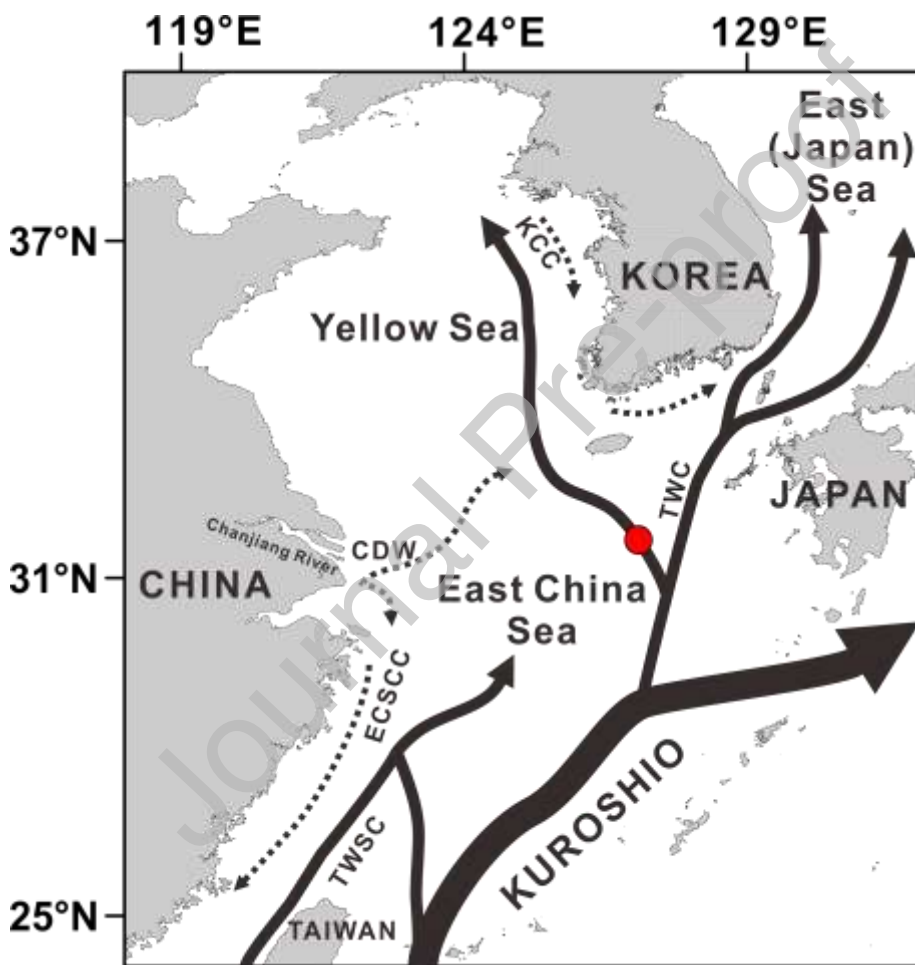


Fig. 1.

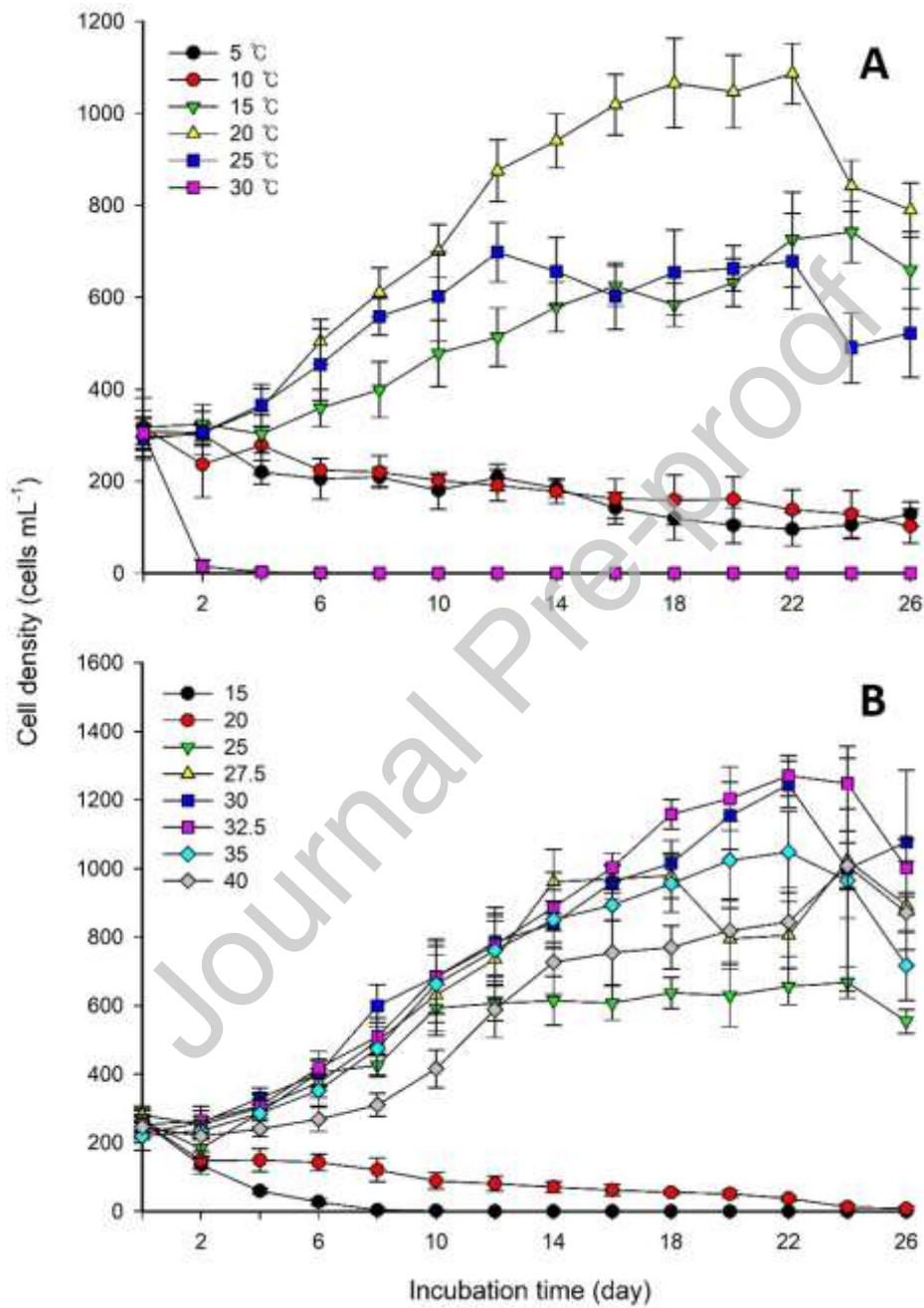


Fig. 2.

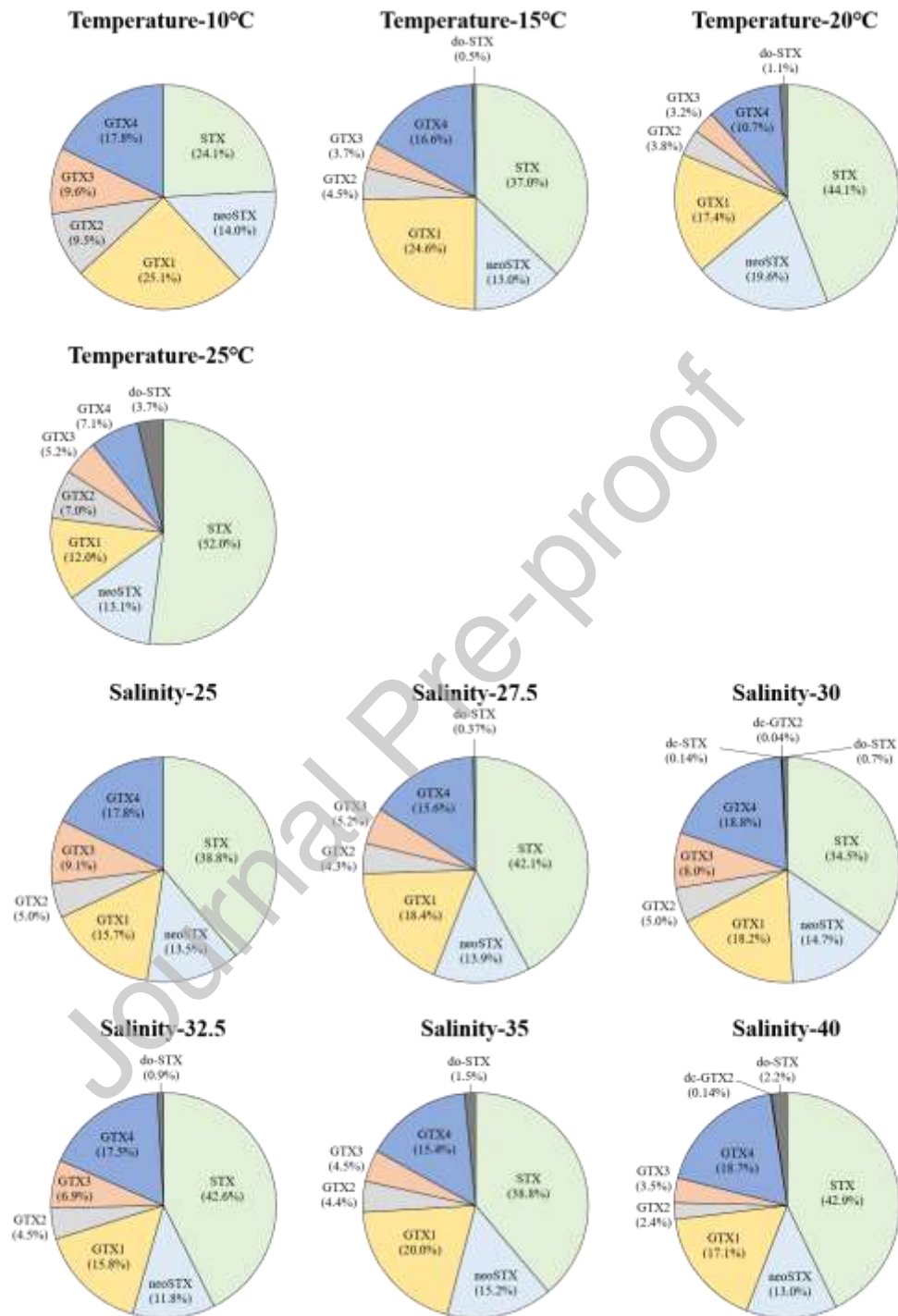


Fig. 3.

## Tables

**Table 1.** Growth rates and maximum cell densities of *Centrodinium punctatum* cultivated at different temperatures and salinities.

	Growth rate (day <sup>-1</sup> )	Maximum cell density (cells mL <sup>-1</sup> )
Temperature-5	-	303 ± 25
Temperature-10	-	318 ± 37
Temperature-15	0.06 ± 0.01	743 ± 67
Temperature-20	0.08 ± 0.01	1087 ± 66
Temperature-25	0.08 ± 0.01	698 ± 65
Temperature-30	-	304 ± 36
Salinity-15	-	262 ± 19
Salinity-20	-	262 ± 44
Salinity-25	0.08 ± 0	668 ± 46
Salinity-27.5	0.08 ± 0	1024 ± 85
Salinity-30	0.07 ± 0.01	1245 ± 68
Salinity-32.5	0.08 ± 0	1271 ± 59
Salinity-35	0.07 ± 0	1048 ± 118
Salinity-40	0.07 ± 0	1008 ± 65

**Table 2.** Mean toxin contents (fmol cell<sup>-1</sup>) of *Centrodinium punctatum* cultivated at different temperatures and salinities.

	ST X	neoST X	GTX 1	GTX 2	GTX 3	GTX 4	dc- STX	dc- GTX2	doSTX** *	Tota l
Temperature-10	55	32	57	22	22	41	<LOQ	<LOD	<LOD	228
Temperature-15	107	37	71	13	11	48	<LOQ	<LOQ	1	288
Temperature-20	93	41	37	8	7	23	<LOQ	<LOQ	2	210
Temperature-25	118	30	27	16	12	16	<LOQ	<LOQ	8	226
Salinity-25	74	26	30	10	17	34	<LOQ	<LOD	<LOQ	191
Salinity-27.5	116	38	51	12	14	43	<LOQ	<LOQ	1	276
Salinity-30	69	29	36	10	16	38	0.29*	0.08*	1	200
Salinity-32.5	115	32	43	12	19	47	<LOQ	<LOQ	2	270
Salinity-35	88	34	45	10	10	35	<LOQ	<LOQ	4	227
Salinity-40	48	14	19	3	4	21	<LOQ	0.15**	2	111

LOQ: limit of quantification; LOD: limit of detection

\*detected in only one replicate (<LOQ for the other)

\*\*detected in only one replicate (<LOD for the other)

\*\*\*expressed in dc-STX equivalent

## Highlights

1. *Centrodinium punctatum* prefers warm waters and an oceanic habitat for its growth.
2. Toxin contents of *Centrodinium punctatum* do not change significantly with temperature.
3. A salinity of 40 can decrease the toxin contents of *Centrodinium punctatum*.



**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof