



### Article Dissipation of a Polykrikos geminatum Bloom after Wind Events in Pearl River Estuary

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**Abstract:** Dinoflagellates is one dominant group in coastal marine phytoplankton communities and, on occasion, form blooms in estuaries and coastal ecosystems. While relationships between dinoflagellate bloom dynamics and nutrients are well-studied, information regarding bloom dissipation in estuaries is limited. We studied the dissipation of dinoflagellate *Polykrikos geminatum* blooms in the Pearl River Estuary, South China Sea, during August of 2011 using ecological, molecular, and satellite remote sensing data. We found that the dinoflagellate bloom was associated with water temperatures of 29.2–31 °C, salinities ranging 16.4–20, and ambient water nutrient concentrations that were not limited. The abundance of the ciliate *Euplotes rariseta*, which feeds on *P. geminatum* cell debris and bacteria, functions as an indicator species of *P. geminatum* bloom dissipation. In situ and satellite data indicate that bloom water masses were transferred from the central to inner estuary near Shenzhen Bay, driven by continuous, strong southerly winds; at which point in time, *P. geminatum* blooms dissipated to a high-salinity area near the estuary mouth driven by northerly winds and freshwater discharge, whereupon the blooms rapidly vanished. A low tolerance to low or high salinities resulted in *P. geminatum* bloom demise in the Pearl River Estuary. We propose that interactions among salinity, wind, and freshwater incursion result in *P. geminatum* bloom dissipation in the Pearl River Estuary.

Keywords: dinoflagellate; bloom dissipation; wind events; ciliate; salinity tolerance; estuary

#### 1. Introduction

Estuaries connect the ocean, land, and inland river systems [1] and are characterized by strong environmental gradients such as salinity because of river discharge [2–6]. In these environments, dinoflagellate blooms frequently occur [7,8], with typical species of estuaries including the gymnodiniacean taxa *Pheopolykrikos hartmannii* (Zimmermann) Matsuoka et Fukuyo and *Polykrikos geminatum* (Schütt) Qiu & Lin, which is formerly known as *Cochlodinium geminatum* Schütt [9–14]. Bloom is often defined according to algal cells concentrations that a lower limit may be set at ~>10 µg L<sup>-1</sup> chlorophyll a [15] or at  $3 \times 10^5$  cells L<sup>-1</sup> of cells abundance [16].

Previous studies on dinoflagellate bloom development and dissipation have focused on the effects of environmental factors, but the causes of most of dinoflagellate bloom dissipation are not well-understood. Weise et al. (2002) reported windy conditions could prevent the development of *Alexandrium tamarense* blooms or cause their dissipation in the St. Lawrence Estuary [17]. The mixing or disruption of a water mass, especially in combination with declining water temperatures, was also considered important in the dissipation of *Karenia brevis* blooms in the Gulf of Mexico [18,19]. The low salinity water from



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). the offshore is considered as the possible cause for the disappearance of *Margalefidinium polykrikoides* (Margalef) F. Gómez, Richlen & D.M. Anderson blooms in the Southern Sea of Korea [20]. Previous studies reported the riverine input and winds could be a noticeable driver of estuarine phytoplankton variability, affecting its water quality and ecosystem health of Chesapeake Bay, Caloosahatchee River Estuary [21,22].

Located at the southeast tip of China, Pearl River is second only in size to the Yangtze River, and the world's thirteenth largest river with an average annual discharge of  $3.3 \times 10^{11}$  m<sup>3</sup> [23]. The Pearl River Estuary has been significantly impacted and rendered highly eutrophic by urban runoff, transportation, waste disposal, and agriculture [5,6,24–28]. Dinoflagellate bloom events, including those of *P. geminatum*, have frequently occurred here in recent decades [11,12,14].

Previous studies on *P. geminatum* blooms have focused on their dynamics and relationships with nutrients [11,12,29,30]. The decrease in the water temperature and salinity may contribute to the dissipation of *P. geminatum* blooms [30]. However, the dissipation mechanisms of *P. geminatum* blooms in the Pearl River Estuary are still not well-understood. Few studies have examined the relationships between *P. geminatum* blooms, ciliates, wind, and salinity to quantitatively understand the factors that control *P. geminatum* bloom dissipation in estuarine systems [12].

We report that *P. geminatum* bloom dissipation was influenced by biological and hydrological factors in the Pearl River Estuary during the August of 2011. Our objectives are to describe the effects of the key factors on *P. geminatum* bloom dissipation and to propose possible mechanisms of *P. geminatum* bloom dissipation.

#### 2. Materials and Methods

#### 2.1. Study Sites, Sample Collection, and Culture

A bloom event occurred in the Pearl River Estuary during the August of 2011. Bloom water masses were tracked daily for sampling from August 19 to 26, 2011. Water samples were collected by a Niskin bottle from 0.5 m in bloom areas near Shenzhen and Zhuhai Bays from August 19 to 22 and 24 to 26, 2011, respectively (Figures 1 and 2a). Samples for the nutrients were filtered through GF/F filters, collected into 100 mL acid-cleaned (1 N HCl) polycarbonate bottles, and stored immediately at -20 °C. Samples for microscopic and DNA analysis were poured into 500 mL clean plastic bottles, preserved with Utermöhl's solution for microscopic examination [31], and stored in darkness at room temperature until analysis (within 1 month). Live algae and ciliate samples were collected in 500 mL clean plastic bottles and cultured using bloom water at Station 24-3. The bloom water samples were placed at 30 °C and under illumination (90  $\mu$ E·m<sup>-2</sup>·s<sup>-1</sup>, dark:light = 12:12 h) to promote the growth of blooms algae and ciliate. The feeding habits of ciliate and abundance of ciliate and algae were observed under microscope during the bloom water culture.

#### 2.2. Measurement of Temperature and Salinity

The surface water temperature and salinity at the sample stations were measured using a YSI instrument (Yellow Springs Instrument Co., Yellow Springs, OH, USA).

#### 2.3. Nutrients Analysis

Nutrients of the surface water, including nitrate, nitrite, silicate, and dissolved inorganic phosphate, were analyzed using an autoanalyzer (Lachat Instrument Inc., Loveland, CO, USA) following the standard colorimetric techniques [32].

#### 2.4. Microscopic Analysis

Bloom species were identified using both morphological and molecular characteristics in accordance with Qiu et al. (2013) [14]. Algal and ciliate cells were transferred to a glass microscope slide and covered by a coverslip. Morphology of the bloom species and live ciliates was observed with an Olympus BX51 epifluorescence microscope. Species



abundance and morphometric data were obtained from live individuals and Utermöhlfixed field samples. Ciliate identification and terminology followed Curds et al. (1974) [33].

Figure 1. Sampling locations (a) and study area (b): Pearl River Estuary, August 2011.



**Figure 2.** Bloom, *Polykrikos geminatum*, and ciliate cells. (a) Photograph of brown water in the Pearl River Estuary in *P. geminatum* bloom. Photomicrographs of a (b) *P. geminatum* dual-cell colony fixed in Utermöhl's solution, (c) ciliate cells (thin arrow) and *P. geminatum* cells (thicker arrow) and detritus (arrow head), and (d) ciliate cell apical and antapical views (thin arrow) and *P. geminatum* cell detritus (arrow head). Scale bars (b–d) =  $20 \mu m$ .

# 2.5. DNA Extraction, PCR Amplification and Sequencing of 18S rDNA Gene, and Phylogenetic Analyses

Twenty ciliate cells and twenty the bloom algae cells were isolated from the Utermöhlfixed samples, respectively, placed into separate 1.5-mL microtubes, resuspended in 0.5 mL DNA buffer, incubated overnight at 55 °C, and then shaken to ensure sufficient cell lysis. DNA was purified using the CTAB method [14,34,35]. Using 1  $\mu$ L of extracted DNA as the template, PCR was performed to amplify the subunit ribosomal RNA (rDNA) gene with universal 18S rDNA primers [13]. The DNA was purified and directly sequenced, as reported by Qiu et al. (2011) [36].

For the ciliate phylogenetic analysis, compiled sequences were aligned using CLUSTALW1.8 [37]. Neighbor joining (NJ) and maximum likelihood (ML) analyses were performed using Mega 5.0 with 1000 and 500 bootstrap replicates, respectively.

#### 2.6. Temporal Variation in Satellite Chlorophyll Concentrations and Wind

Chlorophyll time series data were constructed from Medium Resolution Imaging Spectrometer (MERIS) satellite imagery and collected daily at a resolution of 1 km throughout August 2011 [38]. Data were processed for atmospheric correction using MERIS Case-2 Regional Processor (version 1.6) in Basic ERS, Envisat, ATSR and Meris toolbox (BEAM) software (version 5.0) to obtain remote sensing reflectance [39]. QuikScat wind speed data in the Pearl River Estuary were produced by Remote Sensing Systems [40]. The average daily wind direction and speeds were calculated from the wind data at four positions (including 21°54′ N, 112°30′ E; 21°54′ N, 114°24′ E; 23°48′ N, 112°30′ E; and 23°48′ N, 114°24′ E) in and near the Pearl River Estuary during August 2011.

#### 2.7. Statistical Analysis

Pearson correlation analysis was conducted for the relationship between wind, nutrient concentrations, and *P. geminatum* or ciliate variables. Data analysis was performed using SPSS 24.0.

#### 3. Results

#### 3.1. In Situ Temperature, Salinity, Nutrients, and Polykrikos Geminatum

Throughout the bloom area, the temperature ranged 29.2–31 °C and salinity 16.4–20. The in situ surface temperature and salinity in bloom waters were temporally and spatially homogeneous (Table 1). The nutrient concentrations at different locations in bloom water masses from August 20 to 22 and 24 to 26 (Table 1) varied greatly: silicate (17.45–73.29  $\mu$ M SiO<sub>3</sub>), nitrate (0.12–73.48  $\mu$ M NO<sub>3</sub>), nitrite (0.06–34.98  $\mu$ M NO<sub>2</sub>), and phosphate (0.01–16.85  $\mu$ M PO<sub>4</sub>). The nutrient concentrations changed markedly at five stations on August 20, but after August 21, the concentrations of NO<sub>2</sub> and PO<sub>4</sub> were relatively homogeneous. The surface water nutrient concentrations were lower at stations near Zhuhai than Shenzhen Bay (Table 1). The DON and DOP concentrations at seven stations in the Pearl River Estuary were 6.45–31.18  $\mu$ M and 0.68–1.21  $\mu$ M, respectively.

**Table 1.** Environmental variables in *Polykrikos geminatum* bloom surface waters in the Pearl River Estuary, August 2011.

Study Areas	Date	Temperature (°C)	Salinity (PSU)	NO3 (μM)	NO2 (μM)	PO <sub>4</sub> (μM)	SiO <sub>3</sub> (μΜ)
Shenzhen bay	19 August 2011	29.3–29.8	18.9–19.3	ND	ND	ND	ND
	20 August 2011	29.5–30.3	16.4–19.1	4.40–73.48	10.99–34.98	0.17–16.85	59.08–73.29
	21 August 2011	30.0	17.9	1.17	0.06	0.38	54.28
	22 August 2011	29.9	17.6	0.12	0.75	1.64	47.37
Near Zhuhai	24 August 2011	30.3–31.0	17.0–20.0	26.60–39.29	3.81–4.85	0.01–0.28	31.93–40.79
	25 August 2011	30.3–30.5	17.7–18.8	15.63–27.88	2.30–3.77	0.04–0.04	17.45–29.70
	26 August 2011	29.2–29.6	18.0–20.2	32.05–32.21	5.74–5.97	0.01–0.13	34.41–34.67

A microscopic examination identified *Polykrikos geminatum* as the causative species of the bloom; a low abundance of *Skeletonema costatum* (0–3 × 10<sup>3</sup> cells L<sup>-1</sup>) and a few other algae (0–1 × 10<sup>2</sup> cells L<sup>-1</sup>) were present in the bloom water. Cells of *P. geminatum* were ellipsoidal, approximately 20.0–30.0 µm in length, 20.1–30.5 µm in width, and paired, without clear separation between neighboring zooids (Figure 2b). The cingulum of the cells wrapped around the cell body about 1.2 turns. Details of the morphological and molecular analyses are as described by Qiu et al. (2013) [14]. The sequence of 18S rDNA gene fragments from sorted cells of the bloom species were obtained (1513-bp length, GenBank accession number MW788712). BLAST analyses revealed the bloom species sequence to be identical to the one reported for *P. geminatum* Qiu and Lin (JX967270). With both morphological and molecular analyses, we attribute the bloom species to *P. geminatum* [14]. The abundance of *P. geminatum* changed slightly from August 19 to 22 (Table 2), was highest near Shenzhen Bay (stn 19-2) at 1.10 × 10<sup>7</sup> cells L<sup>-1</sup> on August 19, and reached

 $3.23 \times 10^6$  cells L<sup>-1</sup> (stn 19-1) and  $4.25 \times 10^6$  cells L<sup>-1</sup> (stn 19-3). The abundances of *P. geminatum* ranged from 0.00 (stn 20-5) to  $6.76 \times 10^6$  cells L<sup>-1</sup> (stn 20-3) and  $5.46 \times 10^6$  cells L<sup>-1</sup> (stn 21-1) on August 20 and 21, respectively (Table 2). The abundance decreased rapidly at stations near Zhuhai between August 24 and 26 ( $4.00 \times 10^4$  to  $5.93 \times 10^5$  cells L<sup>-1</sup> on August 24); considerable *P. geminatum* cell debris was also attached to our sampling net on August 24. The abundances near Zhuhai decreased gradually from  $2.67 \times 10^4$  to  $4.00 \times 10^4$  cells L<sup>-1</sup> on August 25 and  $0.00-4.44 \times 10^3$  cells L<sup>-1</sup> on August 26 (Table 2); no *P. geminatum* was detected at Station 26-2 on August 26.

**Table 2.** Abundance of *Polykrikos geminatum* and *Euplotes rariseta*, and ratios of *E. rariseta* to *P. geminatum* cells from *P. geminatum* bloom water in the Pearl River Estuary, August 2011.

Study Areas	Date	Station	Salinity	P. geminatum Abundance (cells L <sup>-1</sup> )	E. <i>rariseta</i> Abundance (cells L <sup>-1</sup> )	Ratios of <i>E. rariseta</i> to <i>P. geminatum</i> Abundance
		19-1	19.31	$3.23  imes 10^6$	$7.84  imes 10^3$	0.002
	19 August 2011	19-2	18.94	$1.10  imes 10^7$	0.00	0.000
		19-3	19.31	$4.25  imes 10^6$	$5.96  imes 10^5$	0.140
		20-1	19.07	$4.84  imes 10^6$	$3.16  imes 10^5$	0.065
Changhan have	20 August 2011	20-2	17.93	$2.11 imes10^6$	$2.89  imes 10^5$	0.137
Shenzhen bay		20-3	16.75	$6.76 imes10^6$	$2.36  imes 10^5$	0.035
		20-4	16.43	$1.16 imes10^6$	0.00	0.000
		20-5	18.16	0.00	0.00	0.000
	21 August 2011	21-1	17.85	$5.46  imes 10^6$	$8.00  imes 10^4$	0.015
	22 August 2011	22-1	17.59	1.47  imes 105	$1.02  imes 10^5$	0.694
		24-1	17.62	$5.93 imes10^5$	$6.67 imes10^4$	0.112
	24 August 2011	24-2	20.36	$4.00 imes10^4$	$4.44 imes10^4$	1.111
Near Zhuhai		24-3	17.56	$1.82  imes 10^5$	$2.22 \times 10^4$	0.122
	25 August 2011	25-1	18.87	$2.67  imes 10^4$	$2.67  imes 10^4$	1.000
		25-2	17.79	$4.00 imes10^4$	$1.33 imes10^4$	0.333
	26 August 2011	26-1	18.26	$4.44  imes 10^3$	$4.44  imes 10^3$	1.000
	26 August 2011	26-2	20.28	0.00	0.00	0.000

#### 3.2. Ciliate Morphology, Abundance, and Molecular Phylogeny

Ciliates measuring approximately 32–50 µm in length and 23–35 µm in width (n = 10), with an irregular ellipsoidal shape, rounded anterior and posterior ends, with 18–22 adoral zone of membranelles, 10 fronto-ventral cirri, 5 transverse cirri, 2 caudal cirri, 1 marginal cirrus, and 6 dorsal kineties matched descriptions of *Euplotes rariseta* [33]. Ciliates rapidly filtered bloom cell debris in ambient water with their cilia (Figure 2c,d). Cells of *E. rariseta* from 17 samples were counted during the bloom event (August 19–22 and 24–26), with the abundances ranging  $0.00-5.96 \times 10^5$  cells L<sup>-1</sup> (Table 2). The highest abundance of *E. rariseta* (5.96 × 10<sup>5</sup> cells L<sup>-1</sup>) occurred at Station 19-3 on August 19; the abundances reached  $6.67 \times 10^4$  cells L<sup>-1</sup> (stn 24-1),  $4.44 \times 10^4$  cell L<sup>-1</sup> (stn 24-2),  $2.220 \times 10^4$  cell L<sup>-1</sup> (stn 24-3) on August 24, and  $4.44 \times 10^3$  cells L<sup>-1</sup> (stn 26-1) and 0 cells L<sup>-1</sup> (stn 26-2) on August 26 (Table 2). In bloom water cultured in the laboratory, *E. rariseta* rapidly ingested bacteria and *P. geminatum* cell debris under microscope on day 3. Abundance *ciliate* and *P. geminatum* and ratios of *E. rariseta* to *P. geminatum* cells were  $1.02 \times 10^4$  cells L<sup>-1</sup>,  $7.60 \times 10^4$  cells L<sup>-1</sup>, and 7.471 on day 3 and  $4.40 \times 10^3$  cells L<sup>-1</sup>,  $1.60 \times 10^5$  cells L<sup>-1</sup>, and 36.364 showing clean water on day 5, respectively. Meanwhile, the ciliate abundance was increased shortly.

Ciliate cells were isolated from samples for DNA extraction; 18S rDNA gene fragments of 1739 bp in length were obtained. The alignment of 18S consisted of one ciliate sequence (GenBank accession number MW788713) differed by only 8 bp (0.46%) from that of *E. rariseta* (JX437134). BLAST analyses revealed the ciliate sequence to be identical with five

reported for *E. rariseta*: GenBank sequences JX437134, JX437135, AJ305248, MK028833, and MK050525, which verified the identification based on morphology.

#### 3.3. Ratios of E. rariseta to P. geminatum Cells

The ratios of *E. rariseta* to *P. geminatum* cells at stations near Shen Zhen Bay ranged 0–0.694, lower than between Zhuhai and Guishan Islands. The ratios from August 19 to 21 ranged 0–0.140. The second-highest ratio (0.140) occurred at Station 19-3, where the highest abundance of *E. rariseta* occurred. On August 21, the ratio at Station 21-1 was 0.015. After August 21, the abundance of *P. geminatum* decreased, and the ratio of *E. rariseta* to *P. geminatum* increased. The highest ratio (1.111) occurred at Station 24-2. The second-highest ratio (1.000) occurred at Stations 25-1 and 26-1, where *P. geminatum* was very low in abundance (Table 2).

#### 3.4. Daily Discharge, Winds, and Chlorophyll

The Daily Pearl River discharge at Gaoyao Station ranged 1920–3170 m<sup>3</sup> s<sup>-1</sup> from August 10 to 31 (Figure 3a). The total discharge from Pearl River in August 2011 was down ~60% of the volume discharged the previous year (August 2010) and ~70% the volume of the following year (August 2012) [41].



**Figure 3.** Daily discharge from Pearl River at Gaoyao Station (**a**), and wind speed and direction collected for the Pearl River Estuary (**b**), August 2011.

Between August 1 and 23, the winds blew from the south, then switched to northerlies from August 24 to 31. Southerly winds (approximately 1.5 m s<sup>-1</sup>) occurred from August 19 to 23, while northerly winds (1.5–4 m s<sup>-1</sup>) prevailed from August 24 to 26 (Figure 3b).

Based on variable chlorophyll concentrations, a bloom event occurred in August (Figure 4). While high chlorophyll concentrations were not observed in the Pearl River Estuary on August 1 (Figure 4a), a small site of high concentration occurred near the mouth of the Pearl River Estuary on August 4, which possibly represented the initial *P. geminatum* bloom (Figure 4b). A large area of high chlorophyll concentration (~17 µg L<sup>-1</sup>) with three northern branches occurred in the Pearl River Estuary on August 12 (Figure 4c). High chlorophyll concentration bloom water masses moved northward to areas near Shenzhen and Hong Kong on August 15 (Figure 4d). In situ investigations also reported large-scale blooms of *P. geminatum* in the center of the Pearl River Estuary on August 12 [42]. Smaller areas of high chlorophyll concentration moved between Zhuhai and Guishan Islands in the Pearl River Estuary on August 23 (Figure 4e).



**Figure 4.** Comparison of chlorophyll ( $\mu$ g L<sup>-1</sup>) derived from the Medium-Resolution Imaging Spectrometer in the Pearl River Estuary, August 2011. Images obtained on (**a**) August 1 (before bloom),

(**b**) August 4 (beginning of bloom), (**c**) August 12 (southerly winds during the bloom), (**d**) August 15 (southerly winds during the bloom), (**e**) August 23 (northerly winds during the bloom), and (**f**) August 29 (after bloom). High chlorophyll concentrations within red rectangles indicate *Polykrikos geminatum* bloom areas.

#### 3.5. Effects of Environmental Factors on P. geminatum and Ciliate Abundance

The statistical analysis showed a significant negative correlation between *P. geminatum* abundance and northerly winds and phosphate (Table 3, p < 0.01). The ratio of *E. rariseta* to *P. geminatum* abundance displayed a negative correlation with silicate (Table 3, p < 0.05) but a positive correlation with the northerly winds (Table 3, p < 0.05).

**Table 3.** Standardized regression coefficient and significance between *P. geminatum*, ciliate, temperature, salinity, and nutrients.

Variable	Number of Measurements	Temperature	Salinity	Northerly Winds	Westerly Winds	NO <sub>2</sub>	NO <sub>3</sub>	PO <sub>4</sub>	SiO <sub>3</sub>	<i>P. geminatum</i> Abundance
P. geminatum abundance	17	0.12	-0.14	-0.71 **	-0.01	-0.00	-0.29	-0.76 **	0.43	1.00
<i>E. rariseta</i> abundance	17	0.35	-0.04	-0.17	0.30	-0.35	-0.44	-0.52	0.09	0.45
Ratios of <i>E.</i> <i>rariseta</i> to <i>P.</i> <i>geminatum</i> abundance	17	0.36	0.09	0.52 *	0.16	-0.54	-0.34	-0.25	-0.68 *	-0.29

\* *p* < 0.05, \*\* *p* < 0.01.

#### 4. Discussion

We sought to assess causes of *P. geminatum* bloom dissipation using multivariate techniques, and we discuss how measured variables might affect algal cell growth and death in the bloom life cycle. We discuss relationships among *E. rariseta*, nutrients, winds, saline tolerance of *P. geminatum* cells, and bloom dynamics.

#### 4.1. Relationships between P. geminatum Blooms and Nutrients

Nutrient concentrations changed markedly from August 19 to 26, 2011. Pearson correlation coefficients reveal no significant effect of any single nutrient on *P. geminatum* bloom dissipation. Previous studies reported nutrient concentrations (silicate, nitrate, and nitrite) that did not limit *P. geminatum* bloom formation in the Pearl River Estuary [11,29].

#### 4.2. Relationships between Plankton and P. geminatum Bloom Dissipation

Zooplankton does not appear to play a key role in *P. geminatum* bloom decline. Macrozooplankton abundance was typically low throughout the bloom (1–8 indiv.  $m^{-3}$ , Hu and Liu, unpublished data). Some *P. geminatum* cells might be consumed by small heterotrophs (heterotrophic flagellates and invertebrate larvae) or degraded by heterotrophic bacteria and viruses.

Ciliates in marine ecosystems usually feed on bacteria, algae, and other protozoa [43]; they are important in linking material cycling and classical food chains [44,45]. The abundance of *P. geminatum* changed markedly in bloom areas where cell debris occurred from August 19 to 21. The ciliate *E. rariseta* rapidly ingested bacteria and *P. geminatum* cell debris, which provided it with nutrition for growth and reproduction. Previous studies have reported bacteria to be suitably sized for consumption and digestion by the ciliate *Euplotes* sp. [46]. Cells of *P. geminatum* produced considerable cell debris during bloom dissipation, which promoted the growth of *E. rariseta*.

Even though *E. rariseta* did not seem to be the cause of *P. geminatum* bloom demise, its rapid increase in abundance at the late bloom stage made it a promising indicator of *P. geminatum* bloom decline. High abundances of *E. rariseta* appeared near Shenzhen Bay on August 19 and 20 (Table 2), which suggests that *P. geminatum* cell breakdown

and bloom decline had commenced earlier. High *E. rariseta* to *P. geminatum* ratios were found consistently in the declining bloom samples. Potentially, this ratio can be used as a diagnostic index for declining blooms of *P. geminatum*.

## 4.3. Relationship between P. geminatum Blooms, Wind Speed and Direction, Salinity, and Temperature

Wind events drive water exchanges and water mass movements, which are often accompanied by changed salinity and nutrient concentrations. The daily discharge from the Pearl River was relatively stable during August 2011 (Figure 3a). Switching wind directions coincided with changes in the distribution of *P. geminatum* blooms. Southerly winds occurred from August 1 to 23, driving bloom water masses to the inner estuary. The large area of high chlorophyll concentration had three northward bands on August 12, which were caused by interactions between southerly winds and a northerly freshwater incursion. From August 24 to 26, the wind direction switched to northerly, with freshwater input pushing the force-driving water masses containing blooms from areas near Zhuhai to the outer estuary. The wind direction and freshwater input contributed to the expansion of high Chl water masses in the Pearl River Estuary [6].

The salinity and temperature of water masses containing *P. geminatum* blooms ranged 16.4–20 and 29.3–31 °C, respectively (Table 1). This species has been reported to favor salinities ranging 15–17.9 [10] and to form blooms in eutrophic waters of salinity and temperatures ranging 20–24 and 20.0–27.2 °C, respectively, October and November 2009 [12]; 20.5–23.1 and 26.5–27.1 °C, respectively, October 2009 [29]; and 19.6–20.3 and 24.3–25.4 °C, respectively, October and November 2018 [30]. Combined with our data, *P. geminatum* appears to favor salinities ranging 15–24 and temperature ranging 20.0–31 °C. Furthermore, isolated cells of *P. geminatum* cultured in water of salinity 32 shrank and stopped swimming.

Southerly winds drove water masses from near Zhuhai to the inner estuary, where wave action, which might cause the athecate dinoflagellate cells damage, broke some *P. geminatum* cells. Bloom water masses surrounding low-salinity waters exposed some *P. geminatum* cells to low salinity, with differences in the osmotic pressure possibly also leading to cell breakage. Many *P. geminatum* cells were broken or dead in low-salinity waters, which indicated bloom dissipation. Northerly winds drove bloom water masses from areas near Zhuhai to more saline areas, where high salinity accelerated *P. geminatum* bloom dissipation on August 24. Therefore, we consider that *P. geminatum* favors medium-salinity environments and that blooms transported in water mass from medium-salinity areas to those of lower or higher salinity experience accelerated bloom dissipation. The in situ results of *P. geminatum* bloom dissipation match the satellite results, with the chlorophyll concentrations decreasing from August 12 to 29 (Figure 4).

#### 4.4. Proposed Mechanism of P. geminatum Bloom Dissipation in Pearl River Estuary

Based on our results and historical data, we propose a mechanism for *P. geminatum* bloom dissipation in the Pearl River Estuary. Patterns of *P. geminatum* bloom dynamics vary in response to the wind direction and strength, freshwater input, and salinity (Figure 5). Favorable weather conditions increase both the *P. geminatum* cell growth and chlorophyll concentration, which indicates the locations of blooms near the estuary (Figure 5a). Continuous, strong southerly winds drive *P. geminatum* bloom water masses to the less-saline inner estuary, which results in bloom dissipation and ciliate growth (Figure 5b). Northerly winds with freshwater input then carry less-saline *P. geminatum* bloom water masses to more saline outer estuary waters and nearshore regions, where the *P. geminatum* abundance decreases, ratios of ciliates to dinoflagellate bloom cells increase, and the blooms dissipate and disappear (Figure 5c).



**Figure 5.** Conceptual pattern of *Polykrikos geminatum* bloom dynamics in the Pearl River Estuary. (a) High abundance of *P. geminatum* depicts the bloom in favorable weather conditions, (b) continuous and strong southerly winds drive *P. geminatum* bloom water masses to the inner estuary, and (c) northerly winds drive *P. geminatum* bloom water masses to the outer estuary. In high-salinity waters, *P. geminatum* cells break, and the bloom disintegrates. Changes in the environmental conditions shown in (**a**,**b**) are related to variations in the wind direction and seawater incursion. Conditions (**a**,**b**) change to (**c**) under the influence of northerly winds, freshwater input, and high salinity.

#### 5. Conclusions

While relationships between dinoflagellate bloom dynamics and nutrients are welldocumented, limited research has been undertaken on bloom dissipation. Three key factors significantly affect *P. geminatum* bloom dissipation in and near the Pearl River Estuary: *P. geminatum* salinity tolerance, wind direction and strength, and freshwater input. Wind is the first factor leading to *P. geminatum* bloom dissipation. Our results demonstrate that strong northerly or southerly winds result in water masses containing blooms being driven to areas of low salinity in the inner estuary or high salinity near the Pearl River Estuary mouth, respectively. Low-salinity waters are associated with *P. geminatum* cell breakage and bloom dissipation and with increased abundance of the ciliate E. rariseta. Low-speed northerly winds and freshwater incursion drive water masses containing blooms to areas near Zhuhai, and the *P. geminatum* abundance decreases. When bloom water masses move to near the estuary mouth driven by northerly winds, higher saline environments accelerate the P. geminatum bloom dissipation. Our results suggest that the ciliate E. rariseta is an indicator of *P. geminatum* bloom dissipation. Our proposed mechanism may provide a reference for subsequent studies of bloom dissipation in estuaries. This study confirmed the importance of meteorologic and hydrodynamic processes in affecting the bloom and dissipation of phytoplankton species in estuaries. Future studies on phytoplankton blooms and dissipation should include a more comprehensive effort on meteorological and hydrodynamic data gathering and numerical modeling to enable a more quantitative understanding of the dissipation mechanisms.

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