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## *In situ* production of dissolved organic carbon (DOC) by phytoplankton blooms (*Cochlodinium polykrikoides*) in the southern sea of Korea

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## ABSTRACT

We measured dissolved organic carbon (DOC) concentration in the surface seawater of Gamak Bay located in the southern Korea and groundwater nearby the bay in August 2007 to determine effect of harmful algal blooms on DOC distribution in the surface waters. The average of DOC concentration in the bloom stations ( $157 \pm 25 \mu\text{M}$ ) was 1.6-fold higher than that of the non-bloom stations ( $96 \pm 15 \mu\text{M}$ ). The DOC concentrations in groundwater and offshore seawater were significantly lower than those in the bloom stations but showed similar values in the non-bloom stations. The facts that salinity showed no significant relationship with DOC but Chl-*a* showed a significant correlation with DOC, indicate that the major source of DOC in the bloom stations may be *in situ* phytoplankton production rather than groundwater and/or river water. In addition, DOC showed a strong relationship with peridinin (biomarker of dinoflagellate) but showed no relationship with fucoxanthin (biomarker of diatom). These results suggest that the DOC distribution in Gamak Bay seems to be controlled by *in situ* phytoplankton production by dinoflagellate rather than diatom. Therefore, distributions of DOC in the southern sea of Korea during bloom season may be controlled by biological processes such as the intensity and duration of dinoflagellate.

## 1. Introduction

Dissolved organic carbon (DOC) is the largest organic carbon reservoirs in the ocean. The amount of carbon in this pool is almost similar to carbon content in the atmosphere (Williams and Druffel, 1987; Emerson and Hedges, 1988; Amon and Benner, 1996). DOC in the marine environment is an important component of the global carbon cycle. In the coastal zones, the primary source of DOC is *in situ* biological production such as extracellular release by phytoplankton, excretion by zooplankton, cell lysis from viral infection, egestion of microzooplankton, dissolution of fecal pellets, and bacterial transformation. In addition, riverine and submarine groundwater discharge (SGD) derived DOC is an important source of allochthonous DOC to coastal environments (Meybeck, 1993; Cauwet, 2002; Santos et al., 2009; Kim et al., 2012). Therefore, distributions of DOC in the coastal areas could be largely influenced by autochthonous and allochthonous sources.

Many studies have conducted about production and distribution of DOC associated with phytoplankton biomass and community composition. Lomas and Bates (2004) suggested the seasonal accumulation of

DOC in surface water should be controlled by phytoplankton community composition in the Atlantic Ocean. The DOC production could be affected by primary production, the size of dominant algal groups, bacteria productivity, and food web structure inclusive of zooplankton (Baines and Pace, 1991; Norrman et al., 1995; Agusti et al., 1998; Carlson et al., 1998; Anderson and Ducklow, 2001; Teira et al., 2001; Maranon et al., 2004). Especially, phytoplankton bloom has a significant production of DOC (Norrman et al., 1995; Wetz and Wheeler, 2007; Spilling et al., 2014). Norrman et al. (1995) reported that DOC concentrations increased from  $130 \mu\text{M}$  to  $250 \mu\text{M}$  for 3 days by an experimental diatom bloom, with higher C:N ratio of dissolved organic matter ( $> 22$ ). Wetz and Wheeler (2007) found that DOC release rates were significantly higher in the exponential or transition growth phase than in the stationary growth phase in axenic batch cultures of five coastal diatom species. On the other hand, in the stationary growth phase of the mesocosm experiments, release of DOC was two times higher in diatom-dominated communities than in dinoflagellate-dominated communities (Spilling et al., 2014).

Although most researches on phytoplankton DOM production concentrated in diatom bloom due to the largest contributor to oceanic new

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and primary production (Dugdale and Wilkerson 1992; Sarthou et al., 2005), the distribution, production mechanism, and cycle of DOC linked to phytoplankton bloom is not clearly understood. Harmful algal blooms have occurred every summer in the southern sea of Korea since 1982 (Cho et al., 2001; Cho and Costas, 2004). The harmful algal bloom in this study region is caused by a dinoflagellate called *Cochlodinium polykrikoides* (*C. polykrikoides*), the dominant harmful algal bloom species. It has also been known as *Gyrodinium impudicum* (Jeong et al., 2000). In general, the first bloom-forming dinoflagellate occurs off Oenarodo Island, off Kohung in Cholla province, Korea, and then spreads toward the southeastern coast of Korea along Kuroshio Current (Lee et al., 2010). Thus, in this study, we aim to determine the concentrations of DOC in harmful algal bloom patch and non-patch areas of the southern sea of Korea and to evaluate DOC production by *in situ* dinoflagellate algal bloom.

## 2. Materials and methods

### 2.1. Study area

Gamak Bay is an oval and semi-enclosed bay and located in middle part of the southern sea, Korea. Mean water depth and area of the bay is 9 m and 112 km<sup>2</sup>, respectively (Lee and Park, 2004; Kim et al., 2014). Annual averages of precipitation and temperature are approximately 1170 mm and 14 °C (Lee et al., 2010), respectively. Tidal range is about 3 m in spring tide (Lee and Park, 2004). The bay extends 9 km in east-west direction and 15 km in south-north direction (Kim et al., 2014). The surface sediment of the bay consists of mainly silt (40–60%) and clay (30–50%) and characterizes as clayey silt facies through determination of sediment and core samples (Lee et al., 1995). Even though there is no information for source and distribution of DOC in this bay, river water and groundwater discharged into this bay may be potential sources of DOC. In this bay, many aquaculture industries of oysters, ark shells, rockfish, sea bream, and flounder have been developed since 1960s. Red tide bloom (*C. polykrikoides* bloom) has occurred since 1984, especially July and August and has resulted in fisheries damage almost every year since 1995 (Kang et al., 2002). The fisheries damage by *C. polykrikoides* blooms from 1993 to 2012 was 121 million dollars (Park et al., 2013). Lee and Kim (2007) reported that the dominant phytoplankton of the observed stations outside Yeolja Bay located near Gamak Bay were diatoms (47%–52% on average) and dinoflagellates (4%–19% on average) in 2003. Particularly, dinoflagellates were up to 60% of the total phytoplankton community in red-tide areas (Lee and Kim, 2007).

### 2.2. Sampling

The surface seawater was collected in the coastal areas in Gamak Bay on 17th, 20th, 23rd, and 25th August 2007 during day-time (10:00–15:00). Seawater samples were obtained from the surface (~0.5 m below the surface) using a submersible pump on shipboard. To compare the DOC concentration in different bloom conditions, we checked conditions of seawater by eyes and collected seawater samples in visible dinoflagellate red-tide bloom areas (open-circle) and non-bloom areas (filled circles) (Fig. 1). For DOC endmember concentrations of offshore seawater, seawater samples were collected outside the bay (S1 and S2, Fig. 1).

In order to determine the groundwater endmember of DOC concentration, fresh and brackish groundwater samples were taken from Yeolja Bay which is located near Gamak Bay (opened-square,  $n = 6$ , Fig. 1) as we did not collect groundwater samples in Gamak Bay. The groundwater samples were collected from shallow (< 50 cm) wells below the sandy sediment surface around Yeolja Bay. Briefly, surface seawater and groundwater samples for DOC measurement were filtered using Whatman GF/F filters (pore size: 0.7 μm) and stored in fire-sealed glass ampoules after acidifying the samples to pH 2 using 6 M HCl and

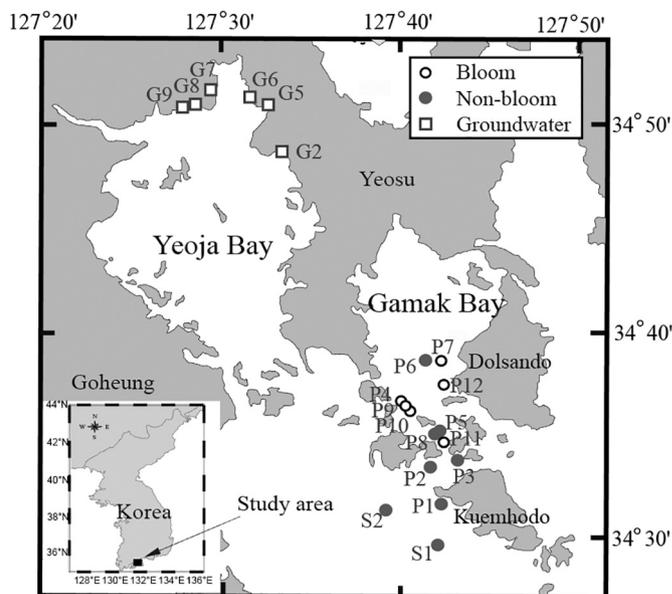


Fig. 1. Surface water (open and closed circles) and groundwater (open square) sampling stations for dissolved organic carbon (DOC) in the Gamak Bay and the Yeolja Bay, Korea. The open and closed circles indicate the red-tide bloom area and the non-bloom area, respectively (modified from Lee et al., 2010). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in room temperature until analysis. All filters and ampoules were pre-combusted at 500 °C for 5 h (Kim et al., 2015).

### 2.3. Analytical methods

Temperature and salinity in surface seawater were measured directly on shipboard using a portable conductivity-temperature-depth (CTD) probe (OceanSeven-302, Idronaut Srl., Italy) (Lee et al., 2010). Temperature and salinity in groundwater were measured using a portable probe (YSI Pro series).

The DOC concentration was measured using a TOC-V<sub>CPH</sub> (Shimadzu, Japan). Five point standard curves of acetanilide were used to standardize DOC measurements. In the injection system, the acidified seawater samples were bubbled with high-purity air gas (purity: 99.999%) to completely remove inorganic carbon species. Carrier gas was passed at a controlled flow rate of 150 mL min<sup>-1</sup> through a combustion tube filled with thermal decomposition catalyst heated to 720 °C. The samples of DOC were oxidized to form CO<sub>2</sub>, which was detected by a non-dispersive infrared detector. The reliability of the measurements was verified on a daily basis by comparing the measured values with a DOC-certified seawater sample (DSR: 44–46 μM for DOC, University of Miami) and procedural blanks. The results were in good agreement with certified DSR values (deviation: < 5%).

## 3. Results

Temperature, salinity, and concentrations of peridinin (a marker pigment of dinoflagellates), fucoxanthin (a marker pigment of diatoms), and chlorophyll *a* data in surface seawater and groundwater used in this study were obtained from Lee et al. (2010), and sampling stations and periods of these data agree with those of DOC measurement. Temperature and salinity of surface seawater in Gamak Bay ranged from 24.8 °C to 28.3 °C and from 31.5 to 32.1 in the dinoflagellate bloom stations, respectively. In the non-bloom stations, temperature and salinity ranged from 24.1 °C to 28.8 °C and from 29.3 to 31.8, respectively (Table 1.). The concentration of DOC ranged from 129 μM to 199 μM with an average of 157 ± 25 μM in the dinoflagellate bloom

**Table 1**

Temperature, salinity, and dissolved organic carbon (DOC) concentration in surface seawater of the bloom stations and non-bloom stations in Gamak Bay and offshore seawater and groundwater nearby Gamak Bay in August 2007. Temperature and salinity data were obtained from Lee et al. (2010).

Date	Station	Temp. (°C)	Sal.	DOC (μM)	Date	Station	Temp. (°C)	Sal.	DOC (μM)
<b>Bloom station</b>					<b>Groundwater</b>				
17 Aug.	P4	24.8	31.5	199	5 Aug.	G2	26.5	25.2	125
20 Aug.	P7	28.1	31.5	161		G7	24.2	25.5	137
23 Aug.	P9	27.5	31.8	169	19 Aug.	G5	17.8	23.5	69
	P10	27.5	31.8	128	22 Aug.	G2	30.4	19.8	81
25 Aug.	P11	26.4	32.1	136		G6	27.6	28.8	137
	P12	28.3	31.9	148		G8	16.4	0.8	75
						G9	28.4	1.7	122
	Average	27.1	31.8	157		Average	25.1	19.0	107
	St. deviation	1.3	0.2	25		St. deviation	5.3	11.2	30
<b>Non-bloom station</b>					<b>Offshore seawater</b>				
17 Aug.	P1	26.3	32.3	108	17 Aug.	S1	25.4	32.1	78
	P2	24.1	31.5	76	20 Aug.	S1	27.3	32.2	91
	P3	24.5	29.3	95	23 Aug.	S1	27.1	32.2	98
20 Aug.	P5	26.4	32.0	100	25 Aug.	S1	28.8	32.2	96
	P6	28.0	31.3	102		Average	27.1	32.2	91
23 Aug.	P8	27.3	31.9	87		St. deviation	1.4	0.0	9
					17 Aug.	S2	26.4	32.1	101
					20 Aug.	S2	27.8	32.2	134
					23 Aug.	S2	26.9	32.2	76
					25 Aug.	S2	28.2	32.2	100
	Average	26.1	31.4	95		Average	27.3	31.1	102
	St. deviation	1.5	1.1	11		St. deviation	0.8	0.1	24

stations, and from 76 μM to 134 μM with an average of  $96 \pm 15 \mu\text{M}$  in the non-bloom stations, respectively (Table 1). These DOC concentrations in the bloom stations were higher than those in the surface layer (0–200 m) of the East/Japan Sea ( $68 \pm 6 \mu\text{M}$ ) (Kim et al., 2015) and were lower than those in groundwater (Hampyeong Bay;  $185 \pm 52 \mu\text{M}$ ) and river waters (Mankyong River; 265–530 μM and Han River; 73–270 μM) in Korea (Park et al., 2006; Kim et al., 2012; Kim et al., 2013).

The averages of DOC concentrations were  $91 \pm 9 \mu\text{M}$  and  $102 \pm 23 \mu\text{M}$  in S1 and S2 (offshore seawater), respectively. Groundwater temperature and salinity ranged from 16.4 °C to 30.4 °C (mean  $\pm$  standard deviation;  $25.1 \pm 5.3$  °C) and from 0.8 to 28.8 ( $19.0 \pm 11.2$ ). The average of DOC concentration in groundwater was  $107 \pm 30 \mu\text{M}$ , ranging from 69 to 137 μM.

#### 4. Discussion

Scatter plots between DOC concentration and salinity in the bloom and non-bloom seawater, offshore seawater, and groundwater showed no significant relationship (Fig. 2). Such a trend was found in surface waters off Tongyeong, Korea, where outbreaks of *C. polykrikoides* bloom often occur (Kwon et al., 2018). Generally, major sources for DOC in coastal zones, such as river mouths and bays are freshwater-associated (riverine and groundwater-derived) DOC input, resulting in DOC concentrations decreasing with increasing salinity (Guo et al., 1999; Abril et al., 2002). On contrast, there was no correlation between DOC and salinity in this bay (Fig. 2). The DOC concentrations in the fresh groundwater (salinity: 0.8 and 1.7) were similar to those in the brackish groundwater (salinity > 20) except for one groundwater sample, indicating that DOC concentrations in groundwater were fairly homogeneous in this area regardless of salinity. The DOC concentrations in the non-bloom stations showed similar values to those in the open ocean seawater and/or groundwater, inferring that the DOC distribution in the non-bloom stations was mainly affected by open ocean seawater and/or groundwater entering into the bay. Furthermore, these results indicate that fresh groundwater and river water are not primary sources of DOC at this study area.

The DOC concentrations (avg.:  $157 \pm 25 \mu\text{M}$ ) in the bloom stations were significantly higher than those (avg.:  $96 \pm 15 \mu\text{M}$ ) of the non-

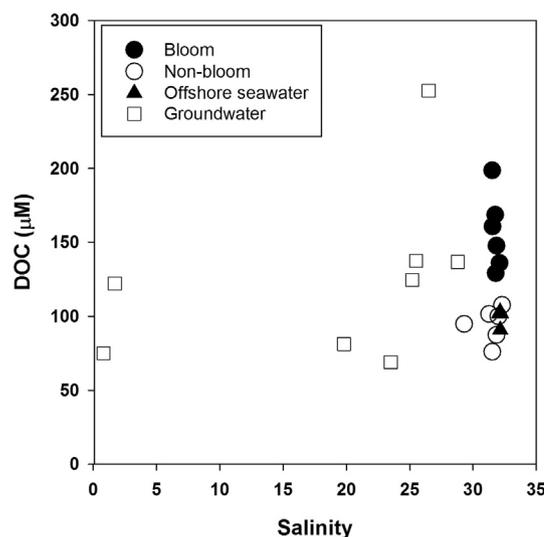


Fig. 2. Plots of salinity versus dissolved organic carbon (DOC) in surface seawaters and groundwaters in the Gamak Bay and the Yeolja Bay, Korea.

bloom stations ( $t$ -test,  $p < .05$ ). These high DOC concentrations in the bloom stations may not be influenced by groundwater-derived DOC from land into the bay because the DOC concentrations in groundwater were significantly lower than those in the bloom stations ( $t$ -test,  $p < .05$ ). Generally, DOC concentrations in groundwater and river water were higher than those in seawater (Oh et al., 2017; Park et al., 2006). However, there is small amount of surface runoff due to no large river that enters into this bay. Furthermore, a very strong correlation ( $r = 0.96$ ,  $n = 14$ ,  $p < .05$ ) was observed between DOC and Chl-*a* in the surface water (Fig. 3). These results with the fact that there is no relationship between salinity and DOC suggest that the major source of DOC in the bloom stations may be *in situ* phytoplankton production rather than groundwater and/or river water. Similarly, Nakatsuka et al. (2004) reported that, based on the relationship between DOC and Chl-*a*, DOC distributions in the surface and subsurface waters (0–200 m) of the Sea of Okhotsk were influenced by DOC produced by *in situ*

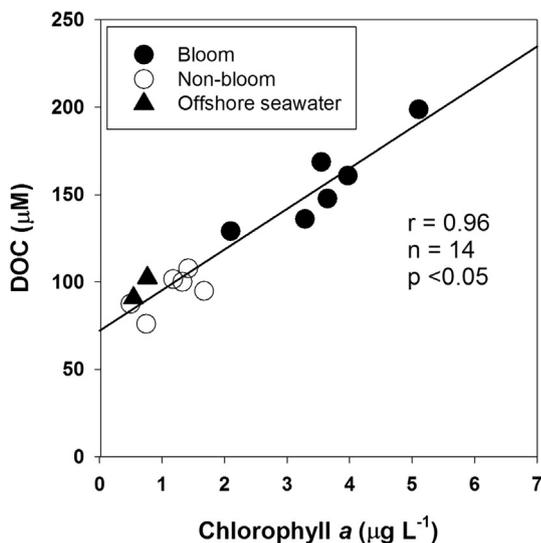


Fig. 3. Plots of chlorophyll *a* versus dissolved organic carbon (DOC) in surface seawaters in the Gamak Bay, Korea. The solid line indicates the linear regression line.

phytoplankton as well as DOC input from the ambient river.

The previous studies reported that harmful algal blooms (HABs) have occurred in Gamak Bay (Lee et al., 2010). After first occurrence of HABs in 1984, algal blooms occurred 46 times and 23 casual species were determined until 2006 in this bay (NIFS, 2008). The most common species are *Prorocentrum* sp., *Cochlodinium polykrikoides*, *Chaetoceros* sp., *Skeletonema costatum*, and *Heterosigma akashiwo*. The correlations between DOC and pigments (peridinin and fucoxanthin) in the surface water and significant differences of pigment concentrations between in the bloom station and non-bloom station seem to provide important information for controlling species of the DOC distribution. The average of peridinin concentration in the bloom stations (avg.:  $5.5 \mu\text{g L}^{-1}$ ) was approximately 5-fold higher than that in the non-bloom station (avg.:  $1.0 \mu\text{g L}^{-1}$ ). On the other hand, the average of fucoxanthin concentration in the bloom stations (avg.:  $1.0 \mu\text{g L}^{-1}$ ) showed a similar value with that in the non-bloom station (avg.:  $1.3 \mu\text{g L}^{-1}$ ). In addition, concentrations of DOC showed a very significant relationship ( $n = 14$ ,  $r = 0.91$ ,  $p < .05$ ) with concentrations of peridinin, however there was no significant correlation ( $n = 14$ ,  $r = 0.01$ ,  $p = .766$ ) between DOC

and fucoxanthin (Fig. 4). These results suggest that the DOC distribution in Gamak Bay seems to be controlled by *in situ* phytoplankton production by dinoflagellate rather than diatom.

Excess DOC, difference of the average DOC concentration between the bloom station and non-bloom station, are approximately  $60 \mu\text{M}$  (Table 1). This excess DOC may be resulted from excess peridinin concentration ( $4.4 \mu\text{g L}^{-1}$ ) produced by *in situ* dinoflagellate production. According to previous culture and mesocosm experiments, maximum release of DOC was found during stationary growth phase by dinoflagellate (*Alexandrium tamarense*) culture experiments (Chen and Wangersky, 1996). Spilling et al. (2014) showed that DOC release in dinoflagellate-dominated communities sharply increased during the stationary growth phase from multiyear mesocosm experiments. Thus, higher DOC concentrations in the dinoflagellate bloom patch areas could be effectively produced *in situ* during the stationary and/or declining growth phase. Fukuzaki et al. (2014) reported that net increase of DOC concentrations in two dinoflagellate species (*Heterocapsa circularisquama* and *Alexandrium catenella*) was  $38.6 \pm 14.8 \text{ mg CL}^{-1}$  for *H. circularisquama* and  $24.1 \pm 5.5 \text{ mg CL}^{-1}$  for *A. catenella* in axenic incubation experiments.

Lee et al. (2010) suggested that outbreaks of dinoflagellate algal bloom in the southern sea of Korea were associated with the elevated dissolved organic nitrogen (DON) under dissolved inorganic nitrogen (DIN)-limited conditions. The average concentrations of DON, DIN, and dissolved inorganic phosphate (DIP) in the bloom stations were  $5.9 \mu\text{M}$ ,  $3.5 \mu\text{M}$ , and  $0.11 \mu\text{M}$ , respectively, and these relatively higher DON concentrations were favorable for blooming of dinoflagellates in competition with diatoms (Lee et al., 2010). The level of DON in the offshore harmful algal bloom areas might have determined by transformation of DIN introduced by SGD (Lee et al., 2010). According to previous studies, *C. polykrikoides*, one of the major dinoflagellate algal bloom species in this region, can take up dissolved organic phosphate (DOP) and DON (Kim et al., 2007; Kudela et al., 2008) and even can engulf smaller size cryptophytes using their sulcus (Jeong et al., 2004). Thus, carbon-rich dissolved organic matter can be effectively produced by dinoflagellate algal blooms after DIN depletion.

## 5. Conclusions

We used *in situ* DOC data to determine an influence of phytoplankton biomass on DOC distribution in Gamak Bay located in the southern coast, Korea. The results that the DOC concentrations at the visible bloom areas were relatively higher than those at the non-bloom

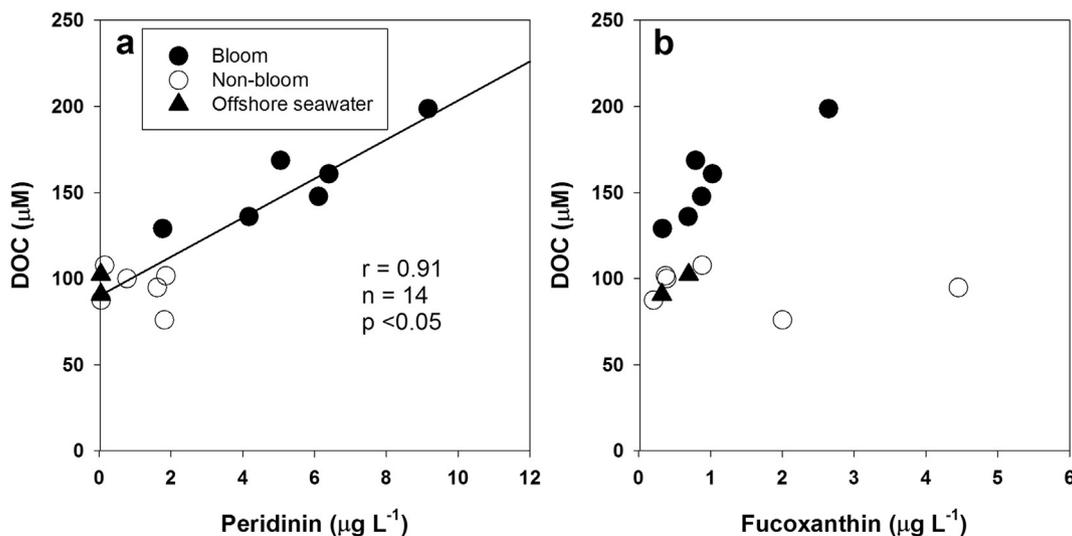


Fig. 4. Plots of concentrations of dissolved organic carbon (DOC) versus concentrations of (a) peridinin and (b) fucoxanthin in surface seawaters in the Gamak Bay, Korea. The solid line indicates the linear regression line.

areas and no significant relationship between salinity and DOC concentration was observed suggest that DOC distribution may be controlled by autochthonous sources rather than allochthonous sources such as riverine and groundwater discharge. Extensive studies, including biogeochemical parameters, such as colored dissolved organic matter (CDOM), amino acids,  $\delta^{13}\text{C}$ , and  $\delta^{14}\text{C}$  are necessary in the future in order to understand the biogeochemical behavior of DOC in the coastal oceans during harmful algal blooms.

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