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ORIGINAL RESEARCH

Bloom of the two dinoflagellates *Ceratium furca* and *Diplopsalis lenticula* in a mangrove estuary of Thailand

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Abstract Knowledge of the planktonic species that appear in mangrove estuaries in tropical areas is still poor. We believe that case report data on the appearance of species is important for clarifying the ecosystem of the mangrove estuary. We report in this paper on the occurrence of blooms and discuss the factors that influence blooming, based on an observed bloom consisting of two dinoflagellates in the mangrove estuary of Samut Songkhram, Thailand, in December 2012. From fluorescence microscopic observation of the armors of the dinoflagellates after calcofluor white staining, we identified these dinoflagellates as *Ceratium furca* and *Diplopsalis lenticula*. In addition, from the annual data on air temperature and precipitation for that year, it was found that the bloom occurred in early dry season. Additionally, diatoms that inhibit the growth of dinoflagellates were observed at low densities. These results suggested that the two dinoflagellates, *C. furca* and *D. lenticula*, grew during disappearance of diatoms.

Keywords Dinoflagellate · *Ceratium furca* · *Diplopsalis lenticula* · Bloom · Mangrove estuary

Introduction

The dinoflagellates include many harmful and/or toxic species that cause damage to fisheries, and there are a large number of case reports about the distributions and the bloom they cause (Tangen 1977; Carder and Steward 1985; Azanza and Max Taylor 2001; Townsend et al. 2001; Su-Myat et al. 2012a, b). Some species have established laboratory culture strains and their growth characteristics have been investigated (Heaney and Eppley 1981; Parkhill and Cembella 1999; Hwang and Lu 2000; Navarro et al. 2006; Yamatogi et al. 2009). In general, it is considered that competition between organisms (predators, prey and niche) and environmental factors (water temperature, salinity, light, wind, ocean currents, nutrients, etc.) determine the growth of dinoflagellates (Bomber et al. 1988; Anderson 1994; Siu et al. 1997; Straile 1997; Paerl 1997; Smayda 2002; Yurimoto et al. 2006, 2007; Yamatogi et al. 2009). For example, in the case of the species that causes paralytic shellfish poisoning, *Gymnodinium catenatum*, it is involved in wind direction and estuary circulation in the

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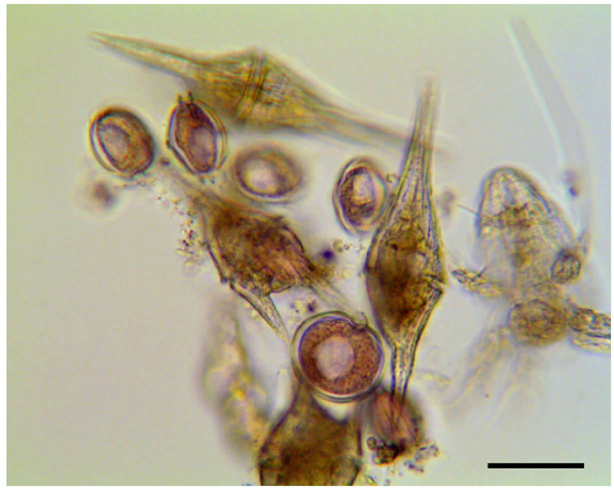

Fig. 1 Microphotograph of an accumulated plankton sample concentrated using a 20 µm mesh sieve, collected from St. 1 of Samut Songkhram in December 2012. *Scale bar* = 50 µm

inner part of the bay, to form a red tide when the growth rate is slow in winter (Abo and Miyamura 2005). In addition, it is easy to form a red tide because dinoflagellates and raphidophytes such as *Cochlodinium* spp., *Chattonella* spp. etc., have the ability to swim vertically, and so they can take advantage of a stratified environment to access nutrients, as is likely to occur in a thermocline or halocline in an estuary area during the rainy or summer seasons (Kimura et al. 1999; Park et al. 2001).

Mangrove forests in the tropics are formed around the intertidal zone of estuaries or the inner part of the bay (Lugo and Snedaker 1974). Fine mud is supplied from terrestrial areas through rivers, and it forms mud flats around the river mouth (Furukawa and Wolanski 1996). The coastal area experiences large environmental change due to the mixing of seawater and water flowing in from rivers, and turbidity is high in this area (Cyrus and Blaber 1992). Extreme environmental changes occur in the rainy season in the tropics due to the large climate change from the dry season (Goodbody 1961; Devassy and Goes 1988, 1989). For this reason, the appearance of phytoplankton around the estuaries of mangrove forests shows wide variation, because diatoms are likely to be dominated by the silicate supply from terrestrial areas in the rainy season, and dinoflagellates are likely to appear during low supply of silicate in the dry season (Biswas et al. 2010). However, little is known about the plankton species that appear in the tropical mangrove estuary, so data based on the accumulation of case reports on the occurrence of the various species is important to clarify the ecosystem of the mangrove estuary. In this paper, we report on the occurrence of blooms and discuss the factors that influence them, based on our observations of the blooms of two species of small- and large-celled dinoflagellates (Fig. 1) in the mangrove estuary of Samut Songkhram province in December 2012, with the aim of clarifying the dominance of dinoflagellates in a mangrove estuary as a changing environment from wet to dry seasons, and to find new research issues about plankton ecology in there.

Materials and methods

Survey area

Samut Songkhram province located in the inner west coast of the Gulf of Thailand (Fig. 2a), is a popular area for solar salterns in Thailand (Murakami 1983). In general, salterns require long sunshine hours and low rainfall. The province is known as a low rainfall region in Thailand because the monsoon from the Indian Ocean is blocked by mountains on the border of Thailand and Myanmar (Murakami 1983). Mangrove forests are present along wide area on the coast and huge mud flats and wide shallow coast are formed (Fig. 2c). For this reason, this area is popular for bivalve aquaculture. Aquaculture of the blood cockle is located in the tidal flat area close to the mangrove forests, and green mussels or oysters are farmed by hanging or pole cultures in the offshore area.

The research site is located approximately in the middle of two estuaries, formed by the Mae Klong River in the north and the Bang Tabun River in the south, both of them susceptible to fresh water (Fig. 2b). Three sampling stations depending on each different situation were set in the estuary (Fig. 2c): Station 1 was in an

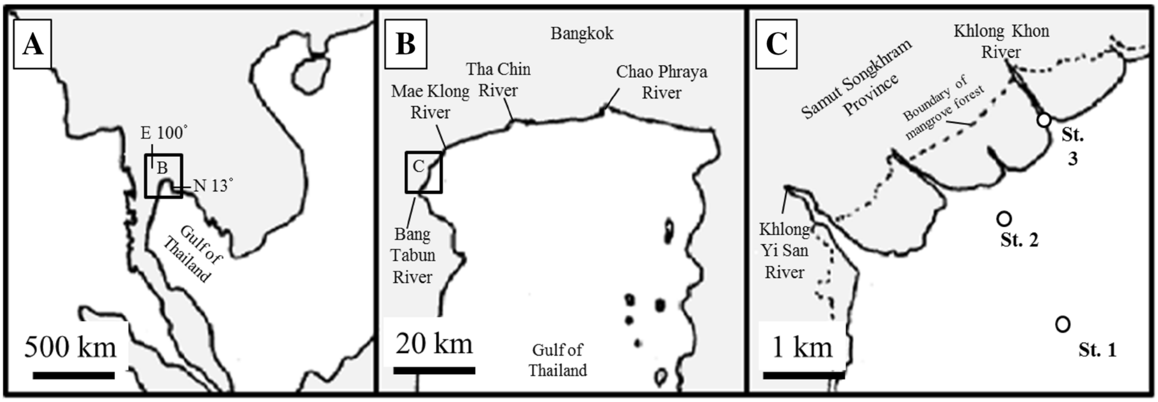

Fig. 2 Three sampling stations in the coastal waters of Samut Songkhram, Thailand, December 2012. **a** Location of the inner part of the Gulf of Thailand. **b** Location of the coastal area of Samut Songkhram. **c** Location of the three sampling stations in the coastal waters of Samut Songkhram

offshore area of the blood cockle aquaculture ground and near the mussels and oyster farming area (depth about 2 m), station 2 was in blood cockle aquaculture farm area near tidal flat (depth about 1 m) and station 3 was in a waterway in a mangrove forest (depth about 1 m).

Sample collection

Surface water for the plankton sample was taken with a 500 ml bottle from a fishing boat at three stations in coastal waters of Samut Songkhram province, Thailand, in December 2012 (Fig. 2). These samples were brought back to the laboratory, and each sample was concentrated in 2 ml of seawater using a sieve of 20 μm mesh width. These samples were fixed with formalin solution and stored at room temperature until used for observation by light or fluorescence microscopes.

Plankton observation

Two species of the dinoflagellates that was present in particularly high densities (Fig. 1) were observed after fluorescent staining of the armor forms on the cell surface with calcofluor white solution (Fritz and Triemer 1985). Cell densities were recorded using a Sedgewick-rafter counting chamber (1 ml) under a light microscope, and other plankton present was recorded at the genus level.

Weather information

For weather information in the survey area in January–December 2012, we referred to the annual climate data in Bangna Agromet near Bangkok City and close to Samut Songkhram (Japan Meteorological Agency http://www.data.jma.go.jp/gmd/cpd/monitor/index.html).

Results

Cell observation

Photo images from the light and fluorescence microscopes of the large-celled species of dinoflagellate are shown in Fig. 3a, b. A light microscope observation of this cell was compared with articles by Yamaji (1966) and Fukuyo et al. (1990). The upper body of the observed cell was long and narrow, and formed an apical horn. It became gradually thicker towards the base, where it formed an epitheca. In addition, the lower body was extending almost parallel to the rear, and two dorsal horns were observed in the

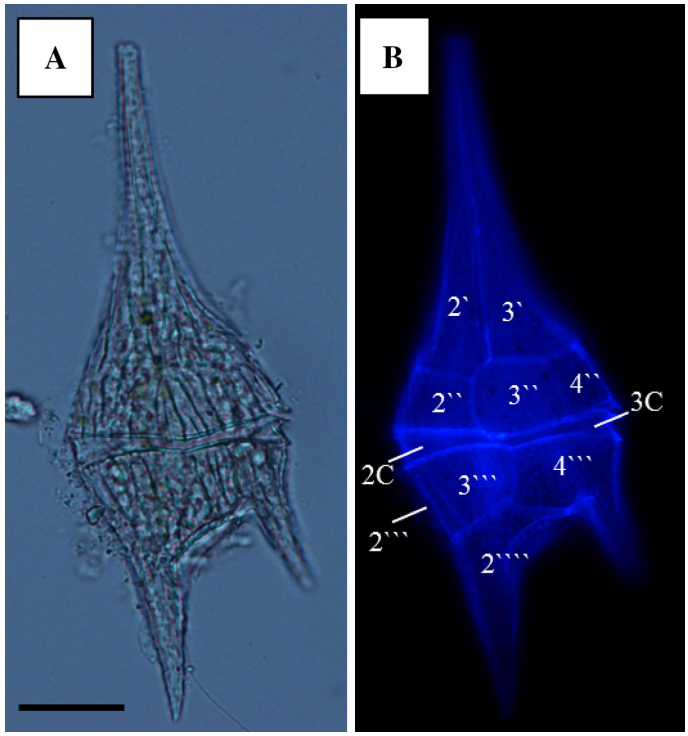

Fig. 3 Microphotographs of the large dinoflagellate identified as *Ceratium furca.* **a** The cell image from a light microscope. **b** The cell image from a fluorescence microscope. Kofoidian system of tabulation (Gómez et al. 2010): ` apical, `` precingular, ``` postcingular, ```` antapical and *c* cingular. *Scale bar* = 25 µm

hypotheca. The left dorsal horn was a little thicker and about 2-fold longer than the right horn. A fluorescence microscope image of the cell is shown in Fig. 3b. The armor forms were stained with the fluorescence solution. It was highly consistent with the illustration by Yamaji (1966), and this large cell was identified as *Ceratium furca*.

Photo images of the small-celled dinoflagellate with light and fluorescence microscopes are shown in Fig. 4a, b. Comparison of the morphology revealed by light microscope observation of the small cell was made with the descriptions of Lebour (1922), Yamaji (1966) and Gribble (2006). The observed cell was a flat disc shape (Fig. 4a), the side was oval, and the transverse groove was annular (Fig. 4b). In addition, the cytoplasm was a pink color.

The fluorescence microscope images are shown in Fig. 4c, d. The shape of each armor was compared with the illustrations and electron microscope photographs published by Lebour (1922) and Gribble (2006), respectively. The upper shell, consisting of three plates, met in the center and the second and third plates were almost the same form. Only the center plate was small. The small plate formed an isosceles triangle, such that the two upper sides were long and the bottom side was short (Fig. 4c). The hypotheca formed a large single plate (Fig. 4d). Based on these observations, this small-celled dinoflagellate was identified as *Diplopsalis lenticula*.

Number of cells

Cell numbers of the two species observed during the bloom are shown in Fig. 5. The results show significant differences in cell numbers between the stations. Although both *C. furca* and *D. lenticula* at St. 1 were present in high densities (almost the same, at about 2,000 cells/L) in the three stations, St. 2 *D. lenticula* was measured at over 3,000 cells/L and *C. furca* at only 200 cells. This result is indicating that *D. lenticula* comprises over 90 % in both the species. In addition, although *D. lenticula* was present at only 50 cells/L at St. 3, *C. furca* was measured at 680 cells/L. This result is indicating that *C. furca* comprises over 90 % in both the species.

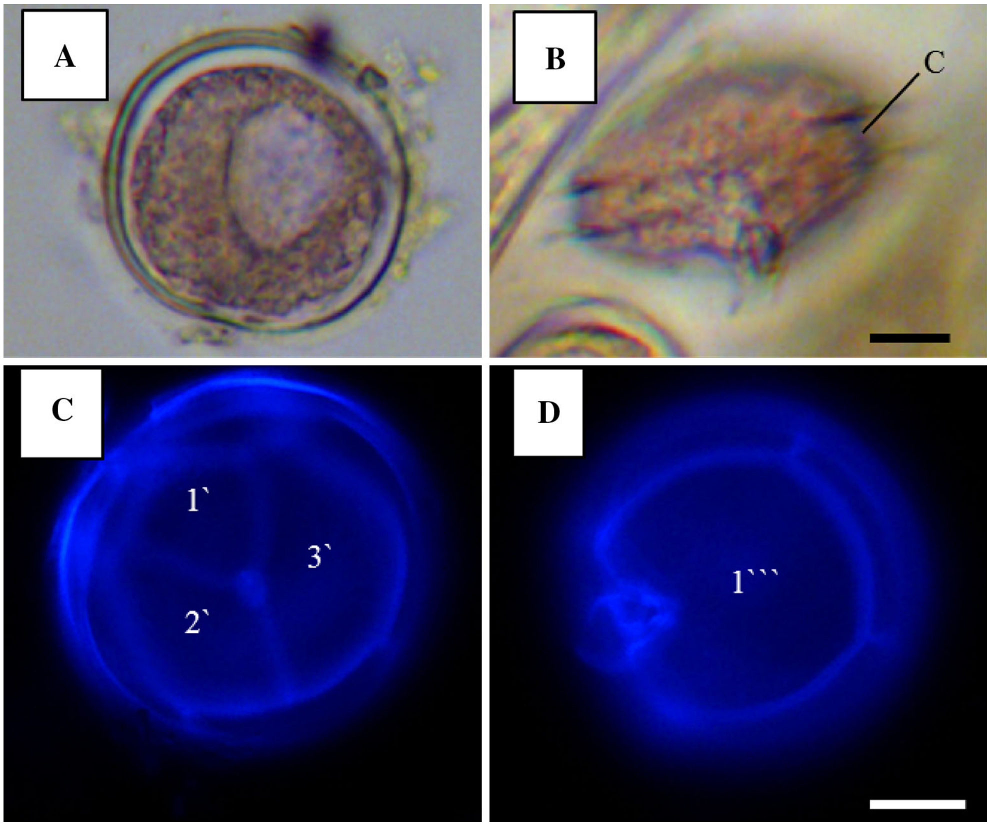

Fig. 4 Microphotographs of the small dinoflagellate identified as *Diplopsalis lenticula.* **a**, **b** The cell images from a light microscope. **c**, **d** The cell images from a fluorescence microscope. Kofoidian system of tabulation (Gómez et al. 2010): ` apical, ``` postcingular and *c* cingular. *Scale bar* = 25 μm

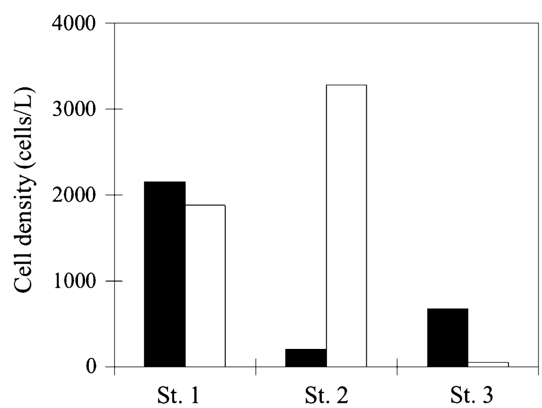

Fig. 5 Cell densities of the two dinoflagellates *Ceratium furca* (*filled square*) and *Diplopsalis lenticula* (*open square*) at each sampling station of Samut Songkhram in December 2012

Other plankton

Other plankton observed were almost exactly the same species at St. 1 and 2 and at low density (Fig. 6). Diatoms identified were *Chaetoceros* spp. (300 and 600 cells/L, Fig. 6a), *Pseudo-nitzschia* sp. (180 and 270 cells/L, Fig. 6b), *Rhizosolenia* sp.(10 and 20 cells/L, Fig. 6c), and *Leptocylindrus* sp.(30 and 50 cells/L, Fig. 6d), along with a zooplankton, *Copepoda* (160 and 500 ind./L, Fig. 6e). St. 3, located in the mangrove forests, was a different situation from the other two stations. No diatoms were observed, and *Copepoda* (30 ind./L, Fig. 6e) and detritus-like particles (50 μm < size particle; 60 pieces, Fig. 6f) was observed.

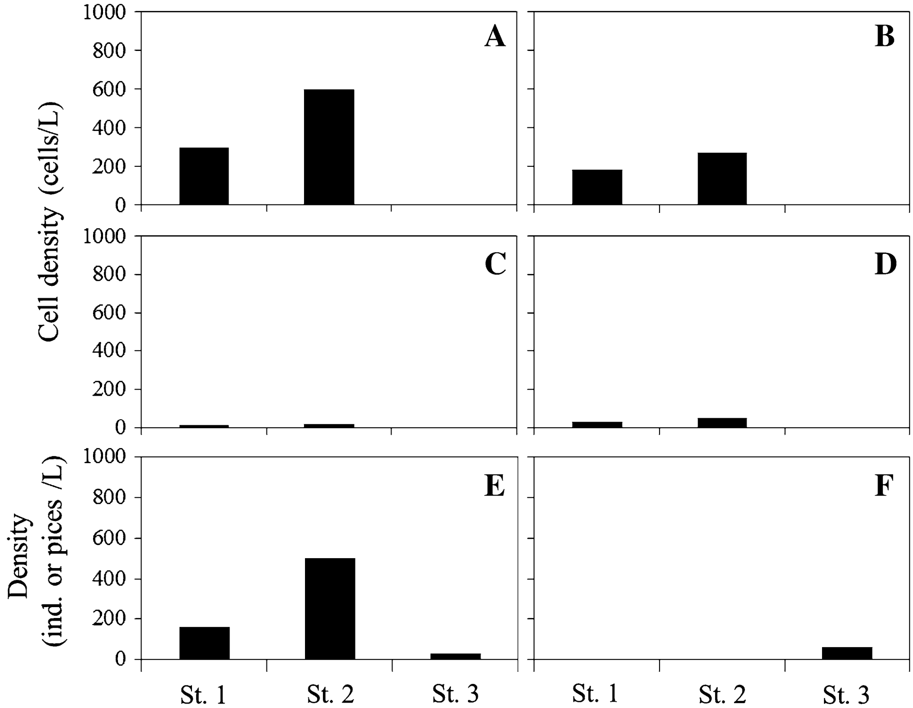

Fig. 6 Densities of phytoplankton, zooplankton and detritus-like particles in the samples from the three stations of Samut Songkhram in December 2012. **a** Cell densities of *Chaetoceros* spp. in the stations. **b** Cell densities of *Pseudo-nitzschia* sp. in the stations. **c** Cell densities of *Rhizosolenia* sp. in the stations. **d** Cell densities of *Leptocylindrus* sp. in the stations. **e** Densities of *Copepoda* in the stations. **f** Detritus-like particles (50 μm < size particle) in the stations

Weather information

The monthly average air temperature in 2012 showed the highest value of 31.1 °C in April and the lowest value of 28.0 °C in January, and in December it was 28.9 °C (Fig. 7). The monthly rainfall maximum was 455 mm in September and the minimum was 0 mm in December. The volume reduced from 226 to 128 mm during October and November.

Discussion

The weather information indicate that November was a transition period from rainy season to dry season, and that December, when sampling was carried out, can be classified as early dry season because the data show cool temperatures and low rainfall. Normally, because silicate is supplied from the rivers during the rainy season, diatoms predominate and dinoflagellates are in the minority (Wang et al. 2006). However, when the volume of silicate from the rivers is reduced in the dry season, the number of diatom cells is reduced (Wang et al. 2006). Dinoflagellates, which can swim and are able to take in nutrients efficiently, can form a bloom by growing in the niche vacated by the diatoms. We observed a bloom of *C. furca* and *D. lenticula* in Samut Songkhram during the early dry season, and the growth of these two species suggested that it was the result of the coastal environment changing due to seasonal variation. Although we could not collect weather information on the wind direction around Samut Songkhram during the blooming event, we discovered that the wind direction from the northeast dominates in this region in December, based on literature describing the monsoon in Thailand (Ogino 1967). This means the northeast monsoon highly develops as a dominant, because the tropical front move to the south, lower than the equator, and the

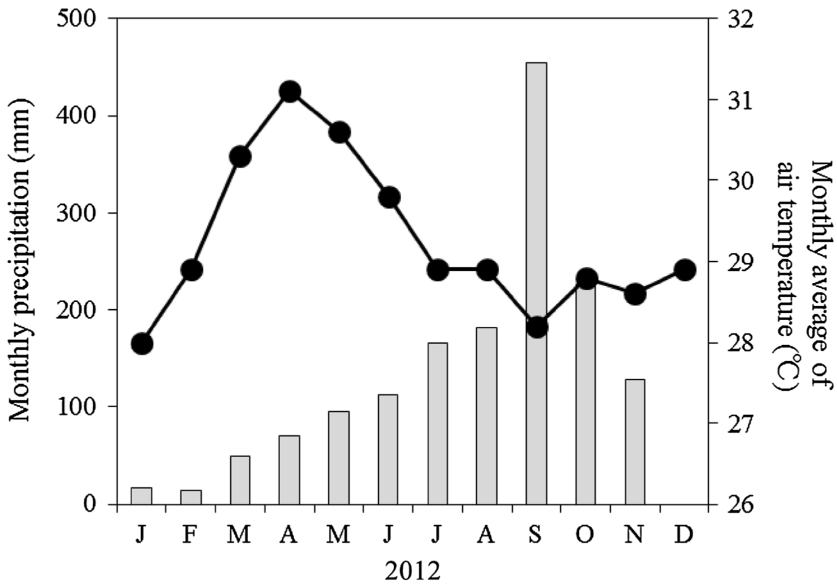

Fig. 7 Annual changes in monthly average air temperature (*line graph*) and monthly precipitation (*bar graph*) around Bangkok City (Bangna Agromet) in 2012. Data obtained from the Japan Meteorological Agency: http://www.data.jma.go.jp/gmd/cpd/monitor/index.html

northeast trade winds blow near the equator. Additionally, it is due to the pressure pattern changing that high pressure in the continental interior develops in low latitudes region of the Northern Hemisphere (Ogino 1967). Given the topography of the survey area and information on wind direction, because the waters are located on the west coast of the inner part of the Gulf of Thailand, it is highly possible that the formation of high cell densities of the two dinoflagellates species was due to drifting to the west coast caused by monsoon winds blowing towards the southwest from the northeast.

C. furca is distributed in oceans around the world, from tropical to frigid zones (Fukuyo et al. 1990). The genus *Ceratium* has been classified into surface distribution species (surface species) and deep layer distribution species (shade species) in a water column. *C. furca* is known as a surface species and often forms a red tide in the inner part of the bay (Fukuyo et al. 1990). This species is distributed across a wide temperature zone, confirmed as 6.6–29.4 °C in its natural habitat (Graham and Bronikowsky 1944; Fukuyo et al. 1990). The optimal growth conditions of this species have been confirmed by culture experiments as having a wide range: the optimal water temperature ranges from 18 to 28 °C and the salinity ranges from 17 to 34 PSU. For this reason, this species is considered to be always capable of growing in the environment of Samut Songkhram, and it seems likely that the growth observed was due to the vacated niche resulting from the disappearance of the diatoms in the conditions following the transition from rainy season to dry season. In addition, cell density of *C. fruca* that was observed in this study was about the 2,000 cells/L. In recent years, Karthik et al. (2014) had reported the extremely high density of the 20,000 cells/ml over *C. fruca* in the Andaman Sea. Our survey result was very low density compared with the case of Andaman Sea and the cells in Samut Songkhram was not affecting density in life and death on fish.

D. lenticula has also been found across a wide salinity distribution (Gopinathan 1972). For this reason, the low cell densities found at St. 3 were considered to be due to the physical dilution effect of river water rather than growth inhibition due to low salinity. *C. furca* is a mixotrophic dinoflagellate, which can obtain nutrition from both photosynthesis and predation of organisms, whereas *D. lenticula* is a heterotrophic dinoflagellate, which can feed only by predation. For this reason, *D. lenticula* requires organisms as food for growth. However, other than the two dinoflagellates observed, only some species of diatoms and zooplankton were observed at low densities. It was suspected predation of *C. furca* by *D. lenticula* for the reason, but we were not able to find the *D. lenticula* phagocyted *C. furca* in these samples. In addition, although it was suspected to involve nano and picoplankton in the growth of *D. lenticula*, we were not able to capture the occurrence of nano and picoplankton because the collected plankton sample was concentrated with a 20 μm mesh sieve in the sample preparation. For this reason, to make a clear understanding of the mutual relationship between *C. furca* and *D. lenticula*, we need to focus on these points in further study. In this paper, it is only captured one red tide event and each event is issue for a study. It is necessary to consider in a future study, other parameters to determine their influence in the occurrence of these events.

Conclusion

A bloom event comprising two species of dinoflagellate was observed in a mangrove estuary of the Samut Songkhram Province in Thailand in December 2012. These dinoflagellates were identified as *Ceratium furca* and *Diplopsalis lenticula*, and the bloom occurred during the early dry season. Additionally, diatoms that inhibit the growth of dinoflagellates were observed at low densities. These results suggested the two dinoflagellates, *C. furca* and *D. lenticula*, grew during disappearance of diatoms.

Author contribution T. Yurimoto carried out sampling and the plankton observation, and wrote the draft manuscript. D. Aue-umneoy, C. Meeanan and I. Tsutsui organized and supported the field survey, and discussed the results and the written manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest All authors declared that they have no conflict of interest.

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