

Factors influencing the initiation of blooms of the raphidophyte *Heterosigma akashiwo* and the diatom *Skeletonema costatum* in a port in Japan

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Abstract

We investigated how environmental factors initiate *Heterosigma akashiwo* and *Skeletonema costatum* blooms from resting stages in bottom sediments in a shallow port over 2 yr. Using field-collected sediments, we also conducted laboratory experiments on how light intensity affects germination of resting stages and growth of the germinated cells. Both phytoplankton species bloomed only in summer, when water temperature and solar radiation were high enough for growth. All three blooms of *H. akashiwo* and the earliest bloom of *S. costatum* in a year occurred right after transmission of strong light ($>200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) to the bottom layer and a peak occurred in dissolved inorganic phosphorus (DIP). In the laboratory, resting stages of *H. akashiwo* and *S. costatum* germinated even in dim light (20 and $65 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively), but germinated cells required stronger light of >130 and $280 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively, for rapid growth. This value is much higher than the threshold for survival, and is higher than the half-saturating light intensity for growth of vegetative cells. Abundance of the resting stages of both species in the sediments rapidly increased during blooms and logarithmically decreased during nonbloom periods, suggesting that resting stages are continuously consumed. For both species, our results suggest that blooms initiate when transmission of sufficient light permits: first, germination of cells from the sediment; second, rapid growth of these germinated cells. Temperature and DIP must also exceed a facilitating threshold.

Many phytoplankters have resting stages in their life cycle. The resting stages usually inhabit bottom sediments when conditions are unfavorable for the survival and growth of vegetative cells (Anderson and Wall 1978). Germination of resting stages is usually regulated by environmental factors such as water temperature (Anderson and Rengefors 2006) and light (McQuoid and Hobson 1996), and studies have suggested that the release of

environmental constraints allows germination of the resting stages and often initiates a bloom (Anderson and Wall 1978).

Along the shore of Hakata Bay is located Fukuoka City, Kyushu, Japan, which has 1.4 million residents. The bay receives Fukuoka's municipal and industrial wastewater and experiences frequent phytoplankton blooms, especially in its semienclosed section (Fig. 1). The dominant phytoplankton taxa in Hakata Bay are the raphidophyte *Heterosigma akashiwo* and diatoms, including *Skeletonema costatum*. Both are meroplanktonic.

H. akashiwo is a member of the class Raphidophyceae, which causes prodigious red tide blooms in eutrophic coastal waters from the subpolar to the subtropical zone (Pratt 1966; Honjo 1993; Smayda 1998). Physiological and ecological features of *H. akashiwo* were reviewed by Honjo (1992, 1993) and Smayda (1998). *H. akashiwo* inhabits

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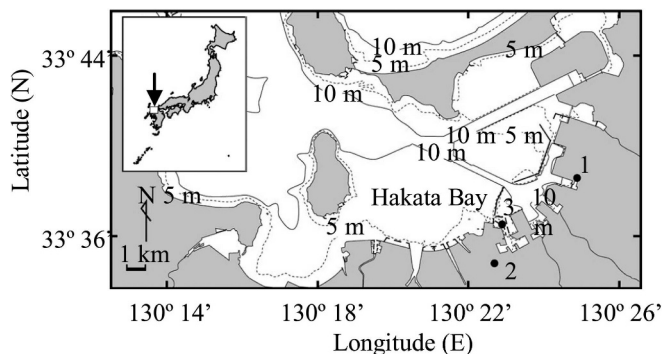


Fig. 1. A map of Hakata Bay indicating the locations of the Hakozaiki Fishing Port (1), the weather station (2), and Sta. H (3).

bottom sediments in several forms that allow survival under low water temperature and dark conditions, and these forms have been referred to as “benthic stage cells” (Tomas 1978b; Yamochi 1989), “cysts” (Imai et al. 1993), and “resting cells” (Han et al. 2002). A benthic stage cell differs from a cyst in the number of chloroplasts (Smayda 1998), whereas a resting cell is immotile and unarmored and has two flagella (Han et al. 2002). Unlike a cyst, which must rest for at least 2 weeks before germinating (Itakura et al. 1996), a resting cell has no mandatory dormancy period (Han et al. 2002). Of the three cell types, however, only cysts have been identified in natural sediments (Imai et al. 1993). Hence, in this study we considered the *H. akashiwo* cells living in natural sediments to be cysts.

Cyst germination and the survival of germinated *H. akashiwo* cells are strongly controlled by temperature and are markedly activated when bottom water temperature exceeds 15°C or 16°C (Yamochi and Joh 1986; Imai and Itakura 1999; Shikata et al. 2007). In the temperate coastal waters inhabited by *H. akashiwo*, water temperatures of the bottom layer exceed 15°C for at least a few months each year, yet *H. akashiwo* blooms usually are not consecutive and multimodal but rather unimodal in early summer (Honjo 1993) or bimodal in early summer and autumn (Tomas 1980). Shikata et al. (2007) examined the effects of light on the germination of *H. akashiwo* in the laboratory and found that cysts can germinate under either light or dark conditions, but darkness markedly decreases the survival rates of motile cells just after germination. Thus, in addition to water temperature, light conditions at the bottom layer may regulate *H. akashiwo* population dynamics.

Like *H. akashiwo*, most diatoms dominant in coastal areas, such as *S. costatum*, *Thalassiosira* spp., and *Chaetoceros* spp., inhabit bottom sediments in forms that allow survival under cold, dark conditions (Hargraves and French 1975; Durbin 1978; Itakura et al. 1997). These forms have been referred to as “resting spores” and “resting cells” (Hargraves and French 1975; Garrison 1981; Itakura et al. 1992). For some diatoms, temperature has been found to influence the length of time it takes a resting spore to germinate, but heat alone does not cause dormancy to break (von Stosch and Fecher 1979). In contrast, light is an important trigger for germination of

diatom resting stages (Hollibaugh et al. 1981; French and Hargraves 1985; Sicko-Goad et al. 1989). Therefore, light intensity may strongly regulate the initial phases of diatom blooms in the field.

Although these previous laboratory studies indicated that light levels are likely to be closely associated with the initial phases of blooms of both *H. akashiwo* and meroplanktonic diatoms, direct field studies have not yet been conducted. Because *H. akashiwo* and *S. costatum* are cosmopolitan, numerous studies have examined the mechanisms of their blooms. However, the contribution of *H. akashiwo* cysts and *S. costatum* resting cells (Itakura et al. 1992) to their bloom dynamics has scarcely been discussed. Therefore, we investigated the dynamics of *H. akashiwo* and *S. costatum* planktonic cells in relation to environmental factors, including the temperature and light environment at the bottom layer in a fishing port of Hakata Bay. Populations of *H. akashiwo* and *S. costatum* develop rapidly as the species bloom and then blooms terminate rapidly, while related environmental factors such as light and nutrient concentrations in the water column also change quickly. Therefore, we conducted high-frequency field sampling to follow the changes in population structure and environmental factors. In addition, we conducted laboratory experiments on the effects of light intensity on germination, survival, and growth of the two species using bottom sediments and cultured strains isolated from the fishing port. For simplicity, hereafter, we have termed both *H. akashiwo* cysts and *S. costatum* resting cells indiscriminately as “resting stages.”

Methods

Study site and investigation rationale—Hakata Bay is a small, shallow bay (Fig. 1, east–west: 20 km, north–south 10 km; maximum depth 23 m; tidal range 2 m). The southeastern area of the bay is closed off by a large water-control dike. Our investigation was mainly conducted in Hakozaiki Fishing Port (33°37′30″N, 130°25′00″E; Fig. 1-1; water depth range from 2.5 to 4.5 m), which is located inside the control dike in the southeastern area of the bay. This port receives negligible freshwater drainage, and has not been dredged since 1980.

We sampled seawater for planktonic cell counting and nutrient concentration measurements, and sediments for resting-stage counting, and we investigated environmental factors (water temperature, salinity, and underwater light intensity) in Hakozaiki Fishing Port, and also got daily data of precipitation, solar radiation, and average wind speed from January 2004 to April 2006 from a weather station, the Japan Meteorological Agency (<http://www.jma.go.jp/jma/indexe.html>; Fig. 1) about 6 km away from Hakozaiki Fishing Port.

Sampling in Hakozaiki Fishing Port—Seawater sampling was conducted daily from January 2004 to April 2006 in Hakozaiki Fishing Port. Seawater was sampled using a plastic bottle (volume 1 liter) with a small weight. When sampling bottom seawater, we slowly sank the bottle closed with a rubber plug attached to a string so as not to

resuspend bottom sediments, and then opened the plug. Between 10:30 h and 11:00 h on each sampling date, environmental conditions were measured and seawater was sampled from the surface and the bottom layer (30 cm above the bottom); samples were brought to the laboratory within 15 min. The water samples were used for counting phytoplankton and subsequent measurement of concentrations of dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP), and silicate. Details of these measurements are given below.

Sediment sampling was conducted every month from April 2004 to April 2006 except for August 2005 in Hakozaki Fishing Port. Sediments were sampled using two gravity core samplers of 10- and 20-cm inner diameter (DIK-180A, Daiki Rika Kogyo; core sampler, Kaken). Because we found that the gravity core of 20-cm diameter was easier to use than one of 10-cm diameter, we changed gravity core in the course of the investigation. Four or five cores were collected, separately wrapped in aluminum foil just after sampling, and brought to the laboratory. The cores were placed vertically in a refrigerator (4°C) for 1 d to allow slightly disturbed particles to settle. The top 1 cm or 3 cm of each sediment core was then removed, put into a plastic container, and mixed thoroughly. The plastic container was quickly covered with aluminum foil and stored at 4°C for 3 to 6 months.

Counting phytoplankton—Planktonic cells of *H. akashiwo* and *S. costatum* were counted in seawater sampled from Hakozaki Fishing Port. The sample bottle was gently turned upside down five times before counting. Vegetative cells of *H. akashiwo* and *S. costatum* were counted in 500 μL of the sample in a counting slide with engraved lines (Rigosha) with an upright light microscope. The counting was replicated. Resting and vegetative cells of *S. costatum* were distinguished by the presence or absence of condensed cytoplasm (Itakura et al. 1992; McQuoid 2005) and we counted only vegetative cells. For *H. akashiwo*, only motile cells were counted as vegetative cells. Before counting phytoplankton, the seawater samples were not fixed, as counting occurred within 1 h of collecting each sample.

The densities of resting stages of *H. akashiwo* and *S. costatum* in each sediment sample were determined by the extinction dilution method (most probable number [MPN] method); the MPN methods followed the protocols of Imai and Itakura (1999) and Itakura et al. (1997), respectively. MPN incubations were conducted at 25°C for *H. akashiwo* or 20°C for *S. costatum* at a photon flux density of 400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for 12 h each day. In 2004, the enumeration of resting stages of *S. costatum* was conducted on the top 3-cm layer of bottom sediments, but in 2005 and 2006 they were made on the top 1-cm layer. The reason for the change in the investigation layer thickness to 1 cm was to catch a clearer fluctuation in the concentration of *S. costatum* resting stages, particularly as *S. costatum* vegetative cell numbers in the water column fluctuated frequently in 2004.

Environmental factors in Hakozaki Fishing Port—In Hakozaki Fishing Port, water temperature and salinity,

and underwater light intensity were measured daily from January 2004 to April 2006, and from April 2004 to April 2006 except for periods from 1 to 23 January and from 1 to 31 March 2005, respectively. Water temperature and salinity at the surface and bottom layer were measured using a thermosalinity meter (model 85, YSI/Nanotech). Underwater light intensity was measured from the surface to the bottom layer at intervals of 0.5 m using an underwater light photon meter (ALW-CMP, Alec Electronics). The average extinction coefficient (k) was calculated with the following equation (Kirk 1994):

$$I(z) = I_0 e^{-kz} \quad (1)$$

where $I(z)$ is photon flux density at the depth of z , and I_0 is photon flux density at the surface layer.

Analyses of DIN, DIP, and silicate concentrations in Hakozaki Fishing Port were conducted on the seawater samples taken daily from April 2004 to April 2006 except for August 2005. The seawater samples were filtered with a 0.22- μm syringe filter (Millex-GV, Millipore) and then frozen (−30°C) for subsequent analyses of DIN (NO_2^- , NO_3^- , and NH_4^+), DIP (PO_4^{3-}), and silicate concentrations with an autoanalyzer (TRAACS 800, Bran + Luebbe) using the method of Strickland and Parsons (1968).

Testing the effects of photon flux density on release of planktonic cells from bottom sediments—The sediment used in this experiment was sampled in August 2006 at Sta. H (Fig. 1: 10-m depth) using the same method noted above. This sediment core sample was chosen because it had a high density of resting stages of both *H. akashiwo* and *S. costatum*. The sample was taken from the top 1 cm of each sediment core, mixed, and stored at 4°C in the dark for 7 months.

The sediment was cultured for 4 d to monitor the germination of resting stages and subsequent vegetative growth. The seawater used for culture medium was collected from the Tsushima Warm Current around Oki Island (34°24'58"N, 130°12'20"E), about 70 km away from Hakata Bay, in open waters. These seawater samples were aged in the laboratory for more than 1 yr. The seawater was autoclaved after enrichment with modified SWM-3+GeO₂ (final concentration: 0.2 mg L^{−1}) for *H. akashiwo* culture and enrichment with modified SWM-3 medium (Yamasaki et al. 2007) for *S. costatum* culture. For *H. akashiwo* treatments, 10 g (wet weight) of sediment was sieved with a stainless-steel 20- μm mesh in complete darkness to remove zooplankton; sediment was then mixed with modified SWM-3+GeO₂ medium, the suspension was adjusted to 1 liter, and 30 mL of the sediment suspension was poured into a 50-mL Nunc flask ($n = 21$, three replicates of seven treatments). For *S. costatum* treatments, 1.0 g (wet weight) of sediment was sieved with a stainless-steel 50- μm mesh in complete darkness, mixed with modified SWM-3 medium, the suspension adjusted to 1 liter, and 30 mL of the sediment suspension poured into a 50-mL Nunc flask ($n = 21$, three replicates of seven treatments). Because samples were prepared in the dark, to see the display of the electric balance when sediment

samples were weighed we used a night-vision scope (headlamp type; λ -300EX, Kenko) with infrared light. To avoid exposing the sample to the infrared radiation, the electronic balance was placed in a box, with only the display outside. Although we observed the sediment suspensions under a light microscope just after making them, at that time there were no motile cells or vegetative cells of *H. akashiwo* or *S. costatum*.

To test the effects of light intensity on release of the resting cells, the culture suspensions were incubated at 22.5°C at seven photon flux densities (15, 30, 40, 65, 130, 280, or 400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) for 12 h each day. The various photon flux levels were achieved by placing individual sample flasks into boxes with windows made of polyester screens with different transparencies. Photon flux levels were measured with a quantum scalar laboratory irradiance sensor (QSL-2101, Biospherical Instruments). Concurrently, we also investigated densities of viable (able to germinate) resting stages of *H. akashiwo* and *S. costatum* in the suspension samples with the MPN method. The MPN incubations were conducted at 22.5°C with 400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for 12 h each day.

The sediment density in the *H. akashiwo* culture suspensions was high, and so it was difficult to observe *H. akashiwo* cells. Therefore, on days 2 and 4 after starting the *H. akashiwo* culture incubation, about 25 mL of the solution above the sediment on the bottom was gently removed by a pipette, temporarily transferred to an empty Nunc flask, and shaken in a dimly lit room ($<1 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). One milliliter of the mixed solution was sampled to count motile cells, and the rest was returned to the culture flask. For *S. costatum*, on days 2 and 4 each culture suspension was shaken and 1 mL of it was directly sampled to count vegetative cells. Motile cells of *H. akashiwo* and vegetative cells of *S. costatum* in 500 μL of each suspension sample were counted in a counting slide with carved lines (Rigosha) under an upright light microscope. The counting for both *H. akashiwo* and *S. costatum* was replicated. Resting and vegetative cells of *S. costatum* were distinguished by the presence or absence of condensed cytoplasm (Itakura et al. 1992; McQuoid 2005).

Testing the effects of photon flux density on growth of vegetative cells—The *H. akashiwo* and *S. costatum* clones (not axenic) used in this experiment were isolated from surface-layer seawater samples from Hakozaki Fishing Port and washed with a micropipette. These strains were subcultured at 22.5°C and 400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for 12 h each day. An exponential phase culture was transferred to darkness, incubated for 3 d, and used for the main tests. The aims of incubation in the dark were twofold: first, to compare light requirement for survival in vegetative cells with that in cells germinated from resting stages and tested in the sediment incubations; second, to eliminate energy for growth from any intracellular nutrients taken up and accumulated under strong light during the subculture period. As soon as the cell growth stopped in each culture, a proportion of the culture was inoculated into 50-mL Nunc flasks containing 30 mL of modified SWM-3 medium, and the flasks were placed under seven levels of

photon flux density (15, 30, 40, 65, 130, 280, or 400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) ($n = 21$: seven treatments in triplicate). Temperature and photoperiods were the same as those in the subculture. Precise photon flux density levels were achieved with the same method used in the sediment culture experiments. One milliliter of the culture was sampled daily from each flask and cells in the sample were counted under an upright light microscope. The method of counting was the same as the sediment culture experiment.

The specific growth rate (μ ; d^{-1}) was calculated using the method of Guillard (1973). Equation 2, modified from an equation developed by Lederman and Tett (1981), was used to describe the relationship between growth rate and photon flux density:

$$\mu = \mu_m \frac{I - I_0}{(K_s - I_0) + (I - I_0)} \quad (2)$$

where μ_m is the maximum growth rate (d^{-1}), I is the photon flux density ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$), I_0 is the photon flux density at the light compensation point ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$), and K_s is the photon flux density at $\mu_m/2$ (the half-saturating photon flux density).

Results

Variations in cell density in the water column—Figure 2a shows temporal fluctuations in the density of motile cells of *H. akashiwo* (mean value of surface and bottom layers) in the Hakozaki Fishing Port. Motile cells of *H. akashiwo* were intermittently present from March to November 2004 and from April to October 2005. *H. akashiwo* cell densities increased to $>10^3$ cells mL^{-1} twice in 2004 and once in 2005. The periods of these blooms and the maximum cell densities are summarized in Table 1.

Figure 2b shows temporal fluctuations in vegetative cells of *S. costatum* in the port. Vegetative cells of *S. costatum* were present in all seasons. During each period from 01 January to 13 April 2004, from 20 November 2004 to 28 February 2005, from 16 March to 06 April, and 07 November to 15 April 2005, however, concentrations of the vegetative cells were always <30 cells mL^{-1} , and for periods always less than 8 d. That is, in winter and early spring (November–March, except for early March 2005) they were observed at very low densities and frequencies. The density of *S. costatum* increased to $>10^3$ cells mL^{-1} seven times in 2004 and five times in 2005. These bloom periods and maximum cell densities are listed in Table 1.

Variations of resting-stage density in bottom sediments—The resting-stage density of *H. akashiwo* in bottom sediments of Hakozaki Fishing Port fluctuated from 1.4×10^1 to 1.7×10^4 MPN g wet sediment $^{-1}$ (Fig. 2c). In both 2004 and 2005, the resting-stage density in the bottom sediments sharply increased from the time of a bloom peak until the decline phase of a bloom in the water column (May–June and July 2004, May–June 2005) and logarithmically decreased during the other periods.

The resting-stage density of *S. costatum* in bottom sediments of the port fluctuated from 4.6×10^3 to 1.3×10^6 MPN g wet sediment $^{-1}$ in 2004 and from 3.3×10^3 to $3.3 \times$

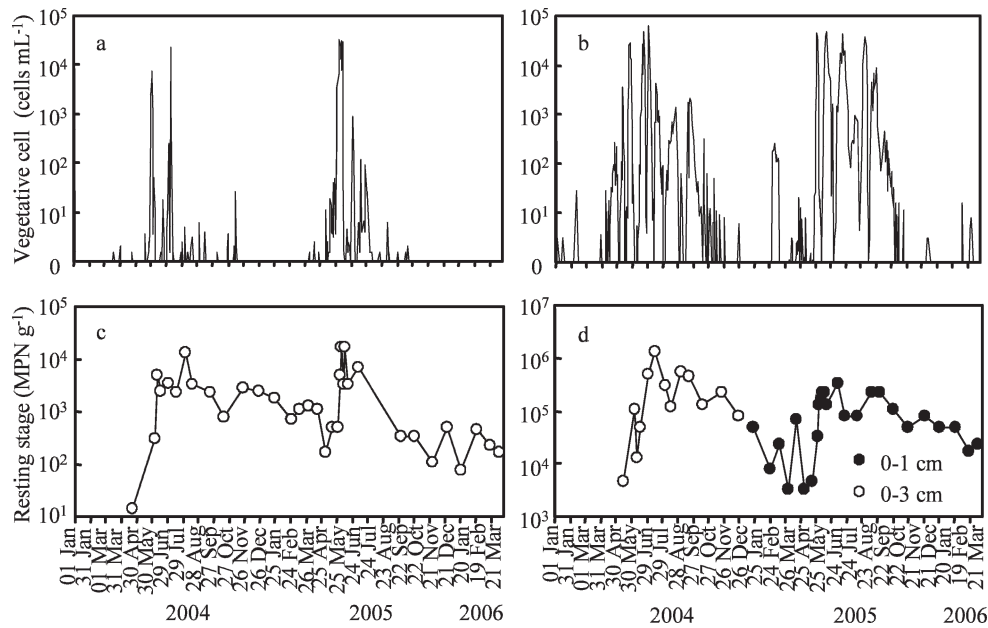


Fig. 2. Fluctuations of (a, b) vegetative cell density and (c, d) resting-stage density (MPN g wet sediment⁻¹) of *Heterosigma akashiwo* (panels a and c) and *Skeletonema costatum* in the sediments (panels b and d) in Hakozaki Fishing Port from early January 2004 to mid-April 2006. The vegetative cell density data represent the average values of the surface and bottom layers.

10⁵ MPN g wet sediment⁻¹ in 2005 (Fig. 2d). Similar to *H. akashiwo*, in both 2004 and 2005, the density of *S. costatum* resting stage in the bottom sediment sharply increased from the time of the peak to the decline phase of a bloom in the water column (May–June and July 2004, May–June 2005) and logarithmically decreased during the other periods.

Fluctuations in environmental conditions in the water column—Fluctuations in water temperatures in Hakozaki Fishing Port from January 2004 to April 2006 are shown in Fig. 3a. Water temperatures at the surface and bottom layers, respectively, varied from 7.0°C to

31.5°C and 7.0°C to 28.9°C in 2004, 6.1°C to 31.5°C and 6.5°C to 30.2°C in 2005, and 6.8°C to 17.1°C and 7.0°C to 14.8°C in January–March 2006. Water temperatures increased from February (2004 and 2005) or January (2006) until July and decreased from August until January (2004) or December (2005).

Fluctuations of salinities at the surface and bottom layer ranged from 9.1 to 33.3 and 14.8 to 33.7, respectively (Fig. 3b). Except for the lowest salinity on 05 December 2004, however, salinities at the bottom layer fluctuated within the narrow range of 24.0 to 33.7. Precipitation fluctuated from 0 to 79 mm in the weather station from early January 2004 to mid-April 2006 (Fig. 3c). Decreases in salinity followed rainfall events (Fig. 3b,c).

We calculated the seawater density from the data of water temperature and salinity. The seawater density ranged from 1004 to 1025 kg m⁻³ during the investigation. In all years, a difference in the seawater densities between the surface and bottom layer began in March and disappeared in October (Fig. 3d), indicative of density stratification during these periods.

The relationship among phytoplankters, water temperature, and salinity is shown in Fig. 4. *H. akashiwo* appeared in the ranges of water temperature and salinity at the surface and bottom layers, respectively, from 13.2°C to 31.2°C and from 18.0 to 33.1 and from 12.9°C to 29.0°C and from 29.4 to 33.7, and bloomed in the ranges of water temperature and salinity at the surface and bottom layers, respectively, from 21.3°C to 25.5°C and from 19.6 to 33.1 and from 20.0°C to 23.5°C and 31.9 to 33.7. *S. costatum* appeared in the ranges of water temperature and salinity at the surface and bottom layers, respectively, from 7.0°C to 31.5°C and from 9.1 to 33.3 and from 7.5°C to 30.2°C and

Table 1. Timing and maximum density of blooms observed.

	Bloom period		Maximum density (cells mL ⁻¹)
	Year	Month and day	
<i>Heterosigma akashiwo</i>	2004	30 May–2 Jun	7.5×10 ³ (1 Jun)
		7–9 Jul	2.2×10 ⁴ (8 Jul)
	2005	28 May–7 Jun	3.2×10 ⁴ (1 Jun)
<i>Skeletonema costatum</i>	2004	12 May	3.6×10 ³ (12 May)
		22–29 May	4.7×10 ⁴ (23 Jun)
		17–25 Jun	2.8×10 ⁴ (27 May)
	2005	30 Jun–5 Jul	6.3×10 ⁴ (1 Jul)
		17–18 Jul	4.3×10 ³ (17 Jul)
		23 Aug	1.4×10 ³ (23 Aug)
		16–24 Sep*	2.1×10 ³ (23 Sep)
		26 May–1 Jun	4.6×10 ⁴ (28 May)
		13–23 Jun	4.8×10 ⁴ (17 Jun)
7–26 Jul	4.3×10 ⁴ (18 Jul)		
26 Aug–4 Sep	3.6×10 ⁴ (1 Sep)		
12–24 Sep	8.8×10 ⁴ (23 Sep)		

* Except for 18 Sep.

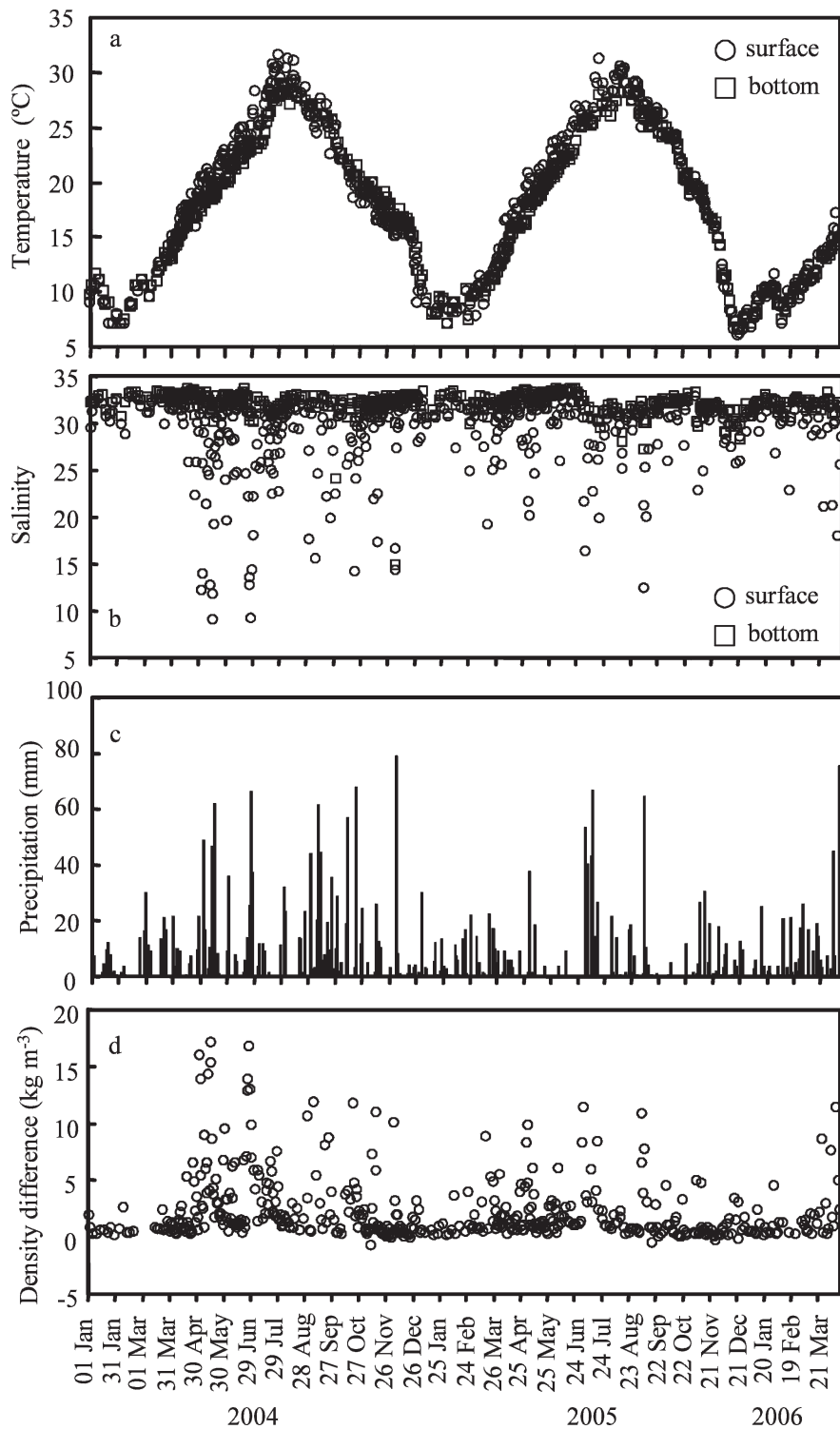


Fig. 3. Fluctuations of (a) water temperatures and (b) salinities at the surface (open circle) and bottom layers (open square) in Hakozaki Fishing Port, (c) precipitation recorded in the weather station near Hakozaki Fishing Port, and (d) the density difference between the surface and bottom layers (bottom minus surface) in the port, from January 2004 to mid-April 2006.

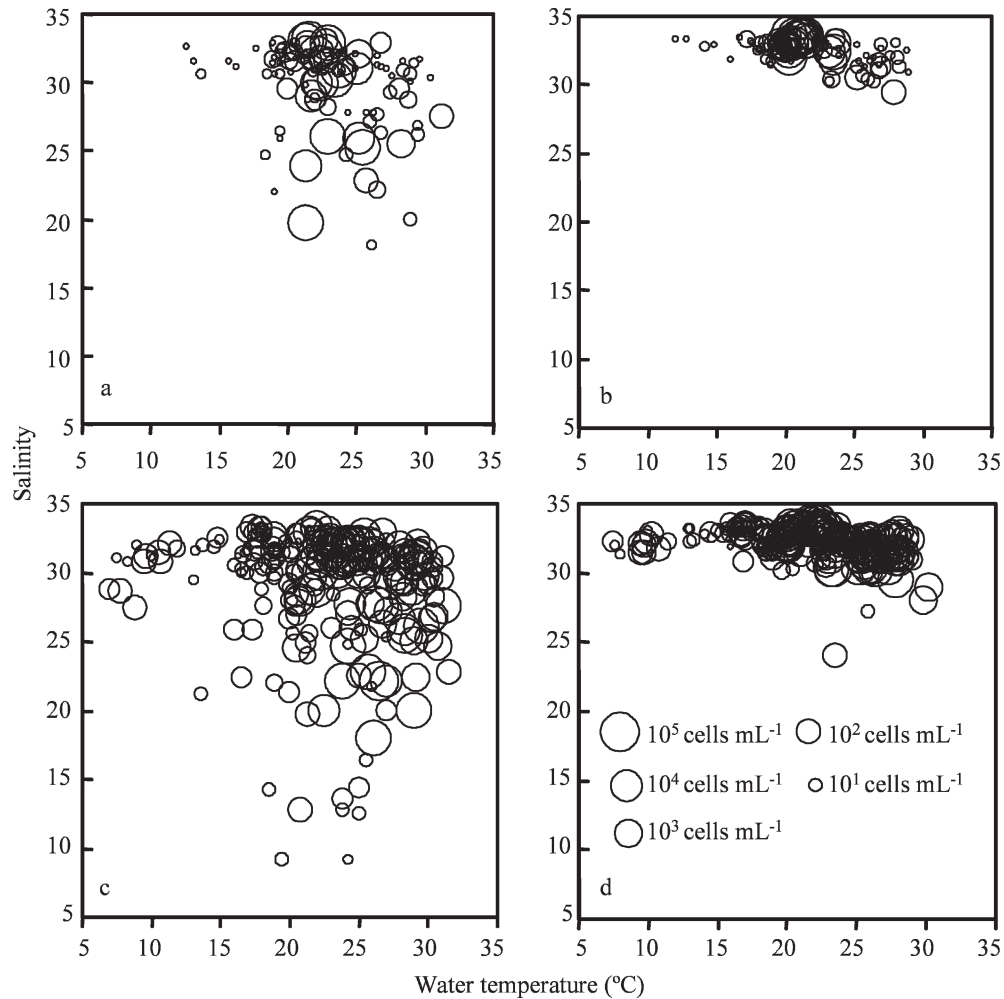


Fig. 4. The relationship between water temperature and salinity at (a, c) the surface and (b, d) the bottom layer vs. vegetative cell abundance of *Heterosigma akashiwo* (panels a and b) and *Skeletonema costatum* (panels c and d) in Hakozaki Fishing Port. The bubble size represents the average values of vegetative cell densities at the surface and bottom layers.

from 24.0 to 33.7, and bloomed in the ranges of water temperature and salinity at the surface and bottom layers, respectively, from 20.7°C to 31.2°C and from 18.0 to 33.3 and from 18.6°C to 28.3°C and 29.4 to 33.7.

From early January 2004 to mid-April 2006, average DIN, DIP, and silicate concentrations at the surface and bottom layer fluctuated from 5.00 to 134 $\mu\text{mol L}^{-1}$, <0.03 to 3.67 $\mu\text{mol L}^{-1}$, and 2.32 to 162 $\mu\text{mol L}^{-1}$, respectively (Fig. 5).

Solar radiation ranged from 1.3 to 30.7 MJ m^{-2} at the weather station near Hakozaki Fishing Port from January 2004 to mid-April 2006 (Fig. 6a). Photon flux density in the water column varied from 27.2 to 2264 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at the surface and 1.31 to 412 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at the 2.5-m depth layer (Fig. 6b,c). The photon flux density at the surface often exceeded 1000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ between April and October but remained below this value at other times. At the bottom layer the photon flux density was the highest from the beginning of April to the middle of May in both 2004 and 2005 and in June 2005, continuously exceeding 200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Fig. 6c). The extinction coefficient ranged from

0.28 to 4.11 (Fig. 6d). The solar radiation at the weather station (S) was correlated with the photon flux density at surface layer in Hakozaki Fishing Port (I_0), and we obtained a linear approximation with a high regression coefficient (R^2) as follows:

$$I_0 = 51.202S + 3.1656 \quad (R^2 = 0.617) \quad (3)$$

The first bloom of *H. akashiwo* and *S. costatum* occurred right after strong light continuously penetrated to the bottom in early summer in both 2004 and 2005.

Daily mean wind speed ranged from 0.8 to 9.8 m s^{-1} in the weather station from January 2004 to mid-April 2006 (Fig. 7). The wind speed was sometimes $>6 \text{ m s}^{-1}$ in summer–autumn (31 July, 19 August, 30 August, 29 September, and 20 October in 2004, and 06 September in 2005) and winter (01 February and 22 December in 2005). High winds at the weather station in summer were associated with typhoons. However, any temporal relationship between the wind speed and blooms of *H. akashiwo* and *S. costatum* was obscure.

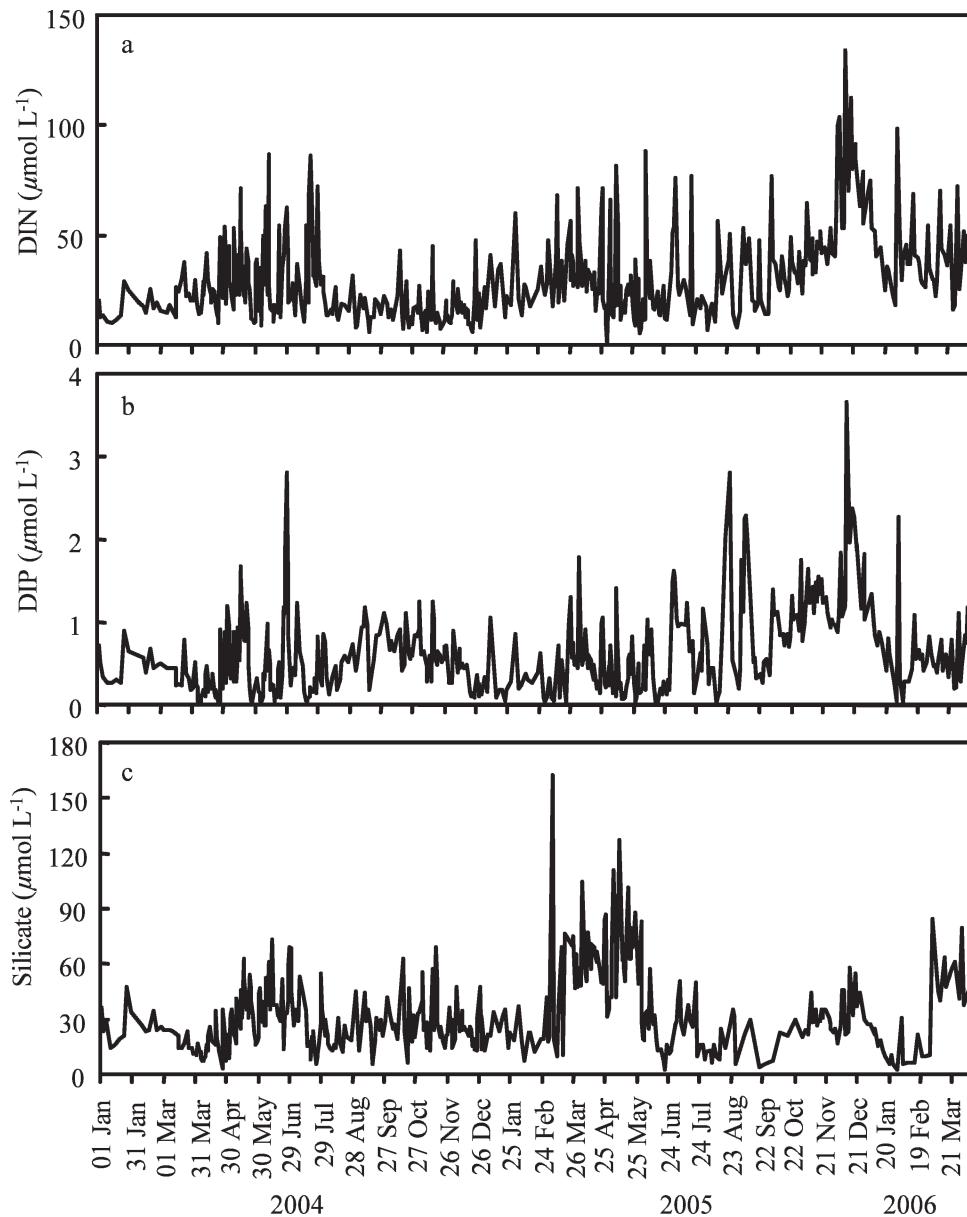


Fig. 5. Fluctuations of (a) DIN, (b) DIP, and (c) silicate concentrations in Hakozaiki Fishing Port from January 2004 to mid-April 2006. The data represent the average values of the surface and bottom layers.

Effects of photon flux density on release of planktonic cells from bottom sediments—Table 2 shows variations in the numbers of motile or vegetative cells when sediment samples including the resting stages were incubated at different light intensities. The density of viable resting stages of *H. akashiwo* and *S. costatum* in the sediment suspension were 27 and 114 MPN mL⁻¹, respectively. Motile cells of *H. akashiwo* appeared under all photon flux densities on day 2, but the number of motile cells decreased between days 2 and 4, with those incubated under dim light ($\leq 65 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) having fewer than the original number of viable resting stages on day 4. Furthermore, the number of motile cells increased much more rapidly at 280 and 400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ than at 130 $\mu\text{mol quanta}$

$\text{m}^{-2} \text{s}^{-1}$. Vegetative cells of *S. costatum* were observed in cultures incubated only at $\geq 65 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. On day 4, however, the number of vegetative cells in the 65 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ treatment was still less than the original number of viable resting stages and the 130 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ treatment had only slightly more than that number. In contrast, the number of vegetative cells far exceeded the original number of viable resting stages in the 280 and 400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ treatments on day 4.

Effects of photon flux density on growth rates of vegetative cells—Motile and vegetative cells of *H. akashiwo* and *S. costatum* could grow at photon flux densities of ≥ 40 and $\geq 30 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively. Approxima-

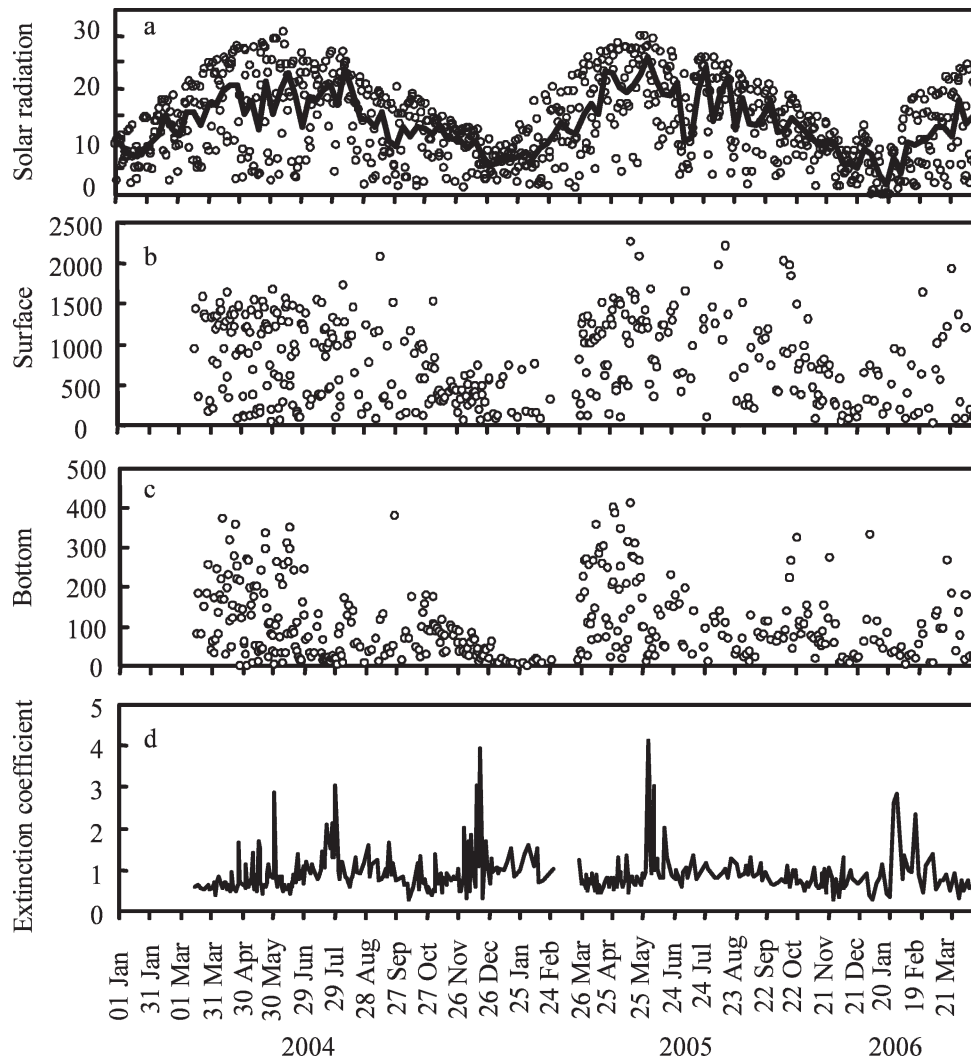


Fig. 6. Fluctuations of (a) solar radiation ($\text{MJ m}^{-2} \text{d}^{-1}$) at the weather station from early January 2004 to mid-April 2006, and photon flux densities ($\mu\text{mol m}^{-2} \text{s}^{-1}$) at (b) the surface, (c) 2.5-m depth layer, and (d) the mean extinction coefficient over all depth layers in Hakozaki Fishing Port from mid-March 2004 to mid-April 2006. The solid line (panel a) indicates the 1-week moving average. The extinction coefficient was calculated from the photon flux densities measured at 0.5-m intervals from the surface to 2.5-m depth.

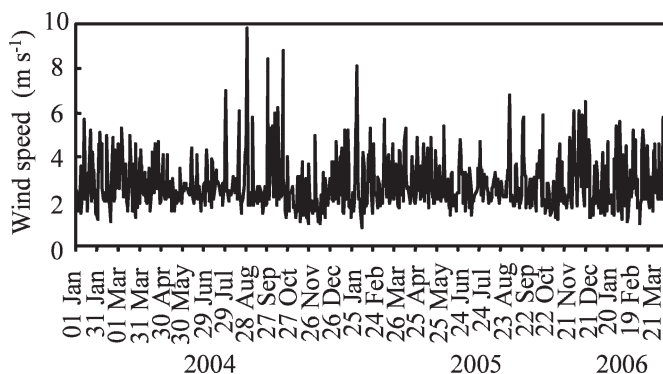


Fig. 7. Fluctuations of average wind speed at the weather station from early January 2004 to mid-April 2006.

tion of parameters by means of the nonlinear least-squares method gave values for μ_m , K_s , and I_0 of 1.33 d^{-1} , 152.77 , and $33.81 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ for *H. akashiwo* and 1.90 d^{-1} , 138.70 , and $8.62 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ for *S. costatum*, respectively.

By applying the parameter K_s obtained in this laboratory experiment into Eq. 1, we calculated the deepest water depth in Hakozaki Fishing Port at which the photon flux density underwater satisfied K_s for growth of *S. costatum*, which cannot actively swim to the surface layer, unlike *H. akashiwo* (Fig. 8). The computationally deepest water depth ranged from -0.28 to 7.2 m . In general, the depth was often greater than the water depth ($2.5\text{--}4.5 \text{ m}$) of the port from March to November, that is, the light stronger than K_s occurred at the bottom at that time. Conversely, the depth was continuously low from December to February.

Table 2. Effect of photon flux density ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) on germination of resting stages in the sediment and survival and growth just after germination in *Heterosigma akashiwo* and *Skeletonema costatum*. The values on days 2 and 4 are cell densities (cells mL^{-1}) of motile cells of *H. akashiwo* and vegetative cells of *S. costatum* in suspension under various photon flux densities. Each value (mean \pm SD) represents the average of triplicate measurements.

Photon flux density ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$)	<i>H. akashiwo</i>		<i>S. costatum</i>	
	Day 2	Day 4	Day 2	Day 4
400	11 \pm 3	192 \pm 93	167 \pm 55	13,433 \pm 4450
280	9 \pm 2	127 \pm 18	100 \pm 75	4900 \pm 1353
130	7 \pm 3	35 \pm 11	3 \pm 6	277 \pm 90
65	5 \pm 4	5 \pm 6	0 \pm 0	30 \pm 5
40	1 \pm 1	1 \pm 1	0 \pm 0	0 \pm 0
30	2 \pm 1	1 \pm 2	0 \pm 0	0 \pm 0
15	0 \pm 1	0 \pm 0	0 \pm 0	0 \pm 0

Discussion

Three blooms of *H. akashiwo* occurred in Hakozaki Fishing Port from early January 2004 to mid-April 2006, two of which occurred from the end of May to the beginning of June (Fig. 2a). Blooms of *H. akashiwo* in early summer (May–July) also occur in some other coastal areas of Japan (Yamochi 1989; Imai and Itakura 1999), Narragansett Bay in the United States (Tomas 1980), and

the Strait of Georgia in Canada (Taylor and Haigh 1993). In contrast, *S. costatum* usually blooms several times a year (Karentz and Smayda 1984; Han et al. 1992; Itakura et al. 1997), as noted in our sampling area as well (Fig. 2b). The annual bloom dynamics of *S. costatum* in Hakozaki Fishing Port was characterized by a first occurrence in early summer (May) with repeated decline-and-bloom cycles until autumn (September or October). What environmental conditions drive these annual rhythms of *H. akashiwo* and *S. costatum*?

Environmental factors for seeding from resting stage germination—H. akashiwo and *S. costatum* generally appeared and then bloomed in early summer, following the winter–spring period when they hardly appeared at all in Hakozaki Fishing Port. Even if environmental conditions favorable for growth occurred, these species cannot bloom without a seed population in the water column. We deduce that for blooms to initiate, an inoculation of seed population in the water column is necessary. Past studies have asserted the importance of resting-stage germination in seeding the water column and thus in initiating phytoplankton bloom (e.g., Anderson and Rengefors 2006).

Imai and Itakura (1999) investigated the effect of temperature on resting-stage germination of *H. akashiwo* in the laboratory and found that the resting stages actively germinated at temperatures $\geq 15^\circ\text{C}$ in the laboratory.

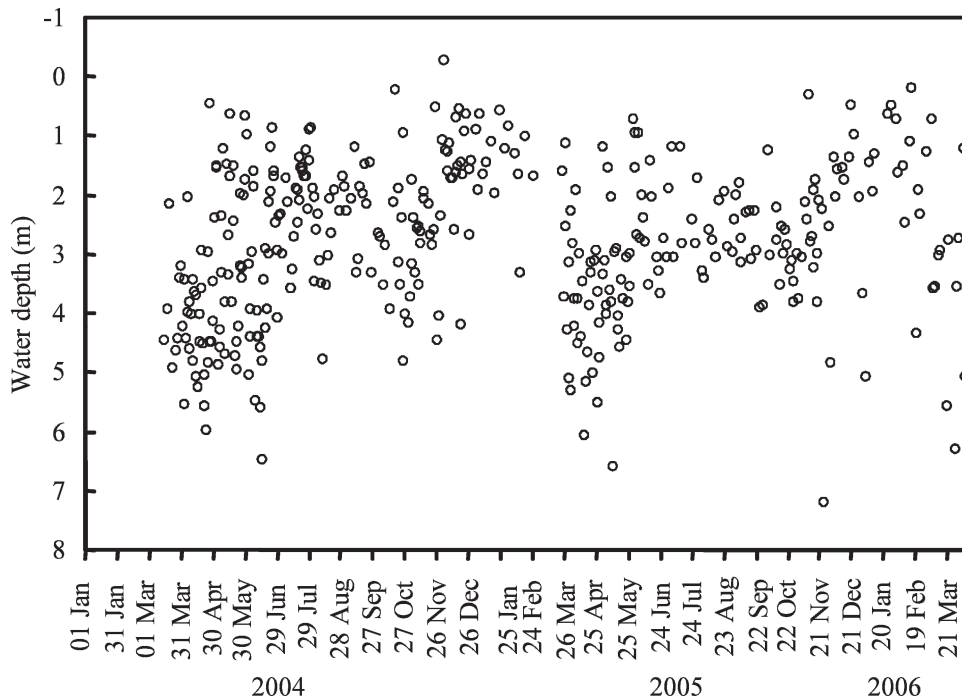


Fig. 8. Variations in the computationally deepest water depth in Hakozaki Fishing Port at which the photon flux density from solar radiation satisfied K_s for growth of *Skeletonema costatum*. Conversion from energy ($\text{MJ m}^{-2} \text{d}^{-1}$) to photon flux density ($\text{mol quanta m}^{-2} \text{d}^{-1}$) in solar radiation followed the method of Thimijan and Heins (1983). The water depth is a calculational value, and so, when the depth was over the water depth, 2.5–4.5 m, of Hakozaki Fishing Port, if only light condition is considered, it is presumed that *S. costatum* could grow at a rate more than K_s in all layers from surface to bottom.

Shikata et al. (2007) restudied the relationship between temperature and resting-stage germination in *H. akashiwo* and found that temperature does not switch resting-stage germination on or off, but rather controls the speed of germination, measured as the time from induction of germination to the appearance of motile cells. Shikata et al. (2007) found that the speed of germination was greatly increased and the appearance of motile cells from sediments containing resting stages was synchronized by the temperature attaining $\sim 16^{\circ}\text{C}$. Shikata et al. (2007) also found that cells having germinated at temperatures $\geq 16^{\circ}\text{C}$ can survive better than those germinated below this temperature. These laboratory works are consistent with the fact that motile cells regularly appeared when the bottom water temperature reached 15°C , and blooms occurred 2 to 3 weeks later in northern Hiroshima Bay (Imai and Itakura 1999); the relationship between a water temperature of 15°C and the appearance of motile *H. akashiwo* cells has also been observed in Osaka Bay (Yamochi and Joh 1986), Narragansett Bay (Tomas 1980), and the Georgia Strait (Taylor and Haigh 1993). Furthermore, motile cells of *H. akashiwo* appeared at bottom water temperatures $\geq \sim 13^{\circ}\text{C}$ and bloomed when conditions ranged from 20°C to 24°C even in Hakozaki Fishing Port (Fig. 4b). The strong relationship between high temperature ($\geq \sim 15^{\circ}\text{C}$) and occurrence of *H. akashiwo* blooms in early summer in many coastal areas may be due to higher temperature facilitating the initial phase of a *H. akashiwo* bloom—synchronization of resting-stage germination and high survival rate of germinated cells—once the waters reach about 15°C .

However, the seasonal fluctuations of *H. akashiwo* cannot be explained by temperature alone, because synchronization of resting-stage germination, high survival rate of germinated cells, and active growth of motile cells occur over remarkably wide temperature ranges. Although surface and bottom water temperatures in Hakozaki Fishing Port remained $\geq 15^{\circ}\text{C}$ from March to December 2004 and March to November 2005 (Fig. 3a), only three blooms occurred in 2004 to 2005. Thus, environmental factors other than temperature must be playing a role.

On the other hand, although low temperature can considerably delay germination in some diatoms (von Stosch and Fecher 1979), our preliminary examination showed that germination of *S. costatum* resting stages was delayed only 1 or 2 d when incubated at 10°C as compared with those at 25°C (data not shown). *S. costatum* has been observed across a wide range of water temperatures in the Seto Inland Sea (Itakura et al. 1997), Narragansett Bay (Karentz and Smayda 1984), and Sechart Inlet (Haigh et al. 1992). Similarly, we also recorded this species across a wide range of bottom water temperatures in Hakozaki Fishing Port (Fig. 4d). Hence, unlike in the case of *H. akashiwo*, water temperature would not affect seeding from resting stages in bloom initiation of *S. costatum* in early summer.

In Hakozaki Fishing Port, *H. akashiwo* blooms followed intermittent or continuous transmission of strong light to the bottom layer in early summer from the beginning of April to mid-May (Fig. 6), when water temperature was also high enough for rapid germination and high survival rates of the germinated cells. From observations of resting-

stage germination in the light and dark every 6 h, Shikata et al. (2007) found that *H. akashiwo* resting stages can germinate in the dark, but these germinated cells die soon afterward. The same authors then proposed that as soon as a motile cell germinates it requires light for morphogenesis to a vegetative cell, which can proliferate. According to our laboratory experiments, the intensity of the light required for survival of newly germinated cells and more rapid growth just after germination is quite high ($\geq 280 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) (Table 2). In the laboratory, we sieved the bottom sediments and added a high concentration of GeO_2 to the medium to inhibit diatom growth, but these conditions do not exist in the field. Thus, in natural environments *H. akashiwo* cannot elude competition with meroplanktonic diatoms and predation by zooplankton that can germinate at the same time. Other environmental factors that inhibit the success of the initial phase of an *H. akashiwo* bloom include resource competition with simultaneously germinating meroplankters, attacks (nutrient removal, allelopathy, grazing, parasitism, and infection) from organisms already living in the water column, and physical factors such as washout. Therefore, in addition to adequately high water temperature, when the *H. akashiwo* cells, germinated from bottom sediment, suddenly appear as a new population, strong light is another minimum requirement for active seeding from resting stages in bottom sediments.

The same should apply to *S. costatum*, which bloomed just after the penetration of strong light to the bottom layer in early summer. The resting cells of *S. costatum* in sediments germinated under dim light but rapidly grew only under strong light of $\geq 280 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. *S. costatum* may also require strong light to initiate vegetative growth after germination. Therefore, like *H. akashiwo*, the first bloom of *S. costatum* may occur virtually simultaneously in early summer. However, most mid- to late-summer blooms of *S. costatum* occurred without strong light. Initiations of *S. costatum* blooms after the first one in early summer may not be explained solely by conditions for seeding from the resting stage.

In Hakozaki Fishing Port, the densities of resting stages of *H. akashiwo* and *S. costatum* in the bottom sediments sharply increased just after blooms and logarithmically decreased over the nonbloom periods (Fig. 2c,d). Imai and Itakura (1998) reported a surge of *H. akashiwo* resting-stage density just after a bloom at a sampling station (water depth ca. 10 m) in northern Hiroshima Bay, but the investigation during nonblooming periods was not conducted. Moreover, Itakura et al. (1997) and Imai and Itakura (1999) reported that, at sampling stations (water depth 10–22 m) in the same bay, the fluctuations of resting stages of *H. akashiwo* and *S. costatum* did not show the obvious seasonal rhythm observed in Hakozaki Fishing Port. Both *H. akashiwo* and *S. costatum* have a relatively short dormancy period (Itakura et al. 1992, 1996) in comparison with other marine meroplanktonic species such as *Chattonella* spp. (Imai and Itoh 1987) and *Alexandrium tamarense* (Anderson 1980). *H. akashiwo* resting stages can germinate only if resuspended (Shikata et al. 2007); *S. costatum* requires light for germination (Itakura et al. 1992)

in addition to resuspension, but we observed germination under dim light of $65 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Table 2). However, the germinated cells require conditions such as high temperature and strong light for survival and keeping the population by immediate rapid growth. Therefore, although the resting stages can survive in sediments for long periods (Itakura et al. 1997; Imai and Itakura 1999; Lewis et al. 1999), on the basis of the conditions required for germination of *H. akashiwo* and *S. costatum*, the number of resting stages in the sediment should decrease during nonbloom periods in shallow Hakozaki Fishing Port.

However, for the resting stages' population in the sediments to decrease by these germinations, it is necessary that the resting stages be resuspended. Blowing wind generally is a factor for the resuspension of resting stages from the bottom in summer when thermal stratification is established, but no relationship between wind speed and fluctuations in resting-stage density in the bottom could be found. However, Hakozaki Fishing Port (2.5–4.5 m) is much shallower than the sampling stations in northern Hiroshima Bay (10–22 m), and fishing and leisure boats frequently come and go in Hakozaki Fishing Port. Thus, even if sufficiently strong wind events do not happen, the resting stages originally in the sediments may easily and frequently become resuspended in the water column and germinate in the port in comparison with deeper sampling stations such as northern Hiroshima Bay.

Environmental factors for growth of vegetative cells—Even if a sufficient seed population for bloom exists in the water column, no bloom occurs without the environmental conditions required for vegetative cell growth there. The temperature–bloom relationship in *H. akashiwo* in the field has been also demonstrated by some laboratory studies showing that optimum water temperature of vegetative cell growth is $\geq 15^\circ\text{C}$ (Smayda 1998).

Salinity changes sometimes affect vegetative cell growth in phytoplankton. *H. akashiwo* and *S. costatum* can tolerate wide ranges of salinity: from 2 to 50 and 8.7 to 40, respectively (Tomas 1978a; Shimura et al. 1979). Salinity varied from 9.1 to 33.3 in the surface layer during this investigation (Fig. 3b), so survival and growth of these phytoplankters in the water column should have been scarcely affected by the salinity changes. Actually we found no obvious correlation between salinity and the appearance and bloom of either *H. akashiwo* or *S. costatum* (Fig. 4).

Irradiance is generally an important condition for bloom development of diatoms including *S. costatum* (Erga and Heimdal 1984), which cannot swim actively to the surface layer with strong light, unlike flagellates such as *H. akashiwo*. In northern Kyushu, where our study was conducted, the solar radiation in winter (December–February) is the lowest among the four seasons (Fig. 6a). The water depth at which K_s was met (on the basis of our experiments) in *S. costatum* tended to be continuously shallower from November to February (Fig. 8). As conditions at which *S. costatum* can actively proliferate spread to deeper water, the biomass of *S. costatum* can develop more rapidly. Hence, together with water temper-

ature, irradiance would also strongly drive the blooms of *S. costatum* in summer. The relationship between blooming and underwater light often has been discussed with regard to vertical mixing (Diehl et al. 2002) because it is assumed that strong mixing results in high algal losses from the euphotic layer (Gaedke et al. 1998a,b). In Hakozaki Fishing Port, fluctuations in the density difference indicated that vertical mixing frequently occurred from October to February (Fig. 3d), a period when *S. costatum* rarely appeared and never bloomed (Fig. 2b).

The minimum cellular nitrogen concentration required for survival of vegetative cells is 24 pg cell^{-1} (Hosaka 1992), meaning that an *H. akashiwo* bloom (cell density $\geq 1000 \text{ cells mL}^{-1}$) requires at least $1.71 \mu\text{mol DIN L}^{-1}$ to be maintained. In Hakozaki Fishing Port, DIN concentration varied from 5.00 to $134 \mu\text{mol L}^{-1}$ (Fig. 5a), so adequate nitrogen for the maintenance of a *H. akashiwo* bloom existed continuously during our study. Considering the half-saturation constant of $0.40 \mu\text{mol L}^{-1}$ for nitrate uptake of *S. costatum* (Eppley et al. 1969), which is much lower than the $1.47\text{--}2.45 \mu\text{mol L}^{-1}$ for *H. akashiwo* (Tomas 1979; Herndon and Cochlan 2007), the same applies to the maintenance of a *S. costatum* bloom. The half-saturation concentration for silicate uptake of *S. costatum* is $0.80 \mu\text{mol L}^{-1}$ (Paasche 1973), and diatom populations are generally maintained irrespective of season if silicate concentration exceeds a threshold of approximately $2.00 \mu\text{mol L}^{-1}$ (Egge and Aksnes 1992). In Hakozaki Fishing Port, silicate concentration fluctuated from 2.32 to $162 \mu\text{mol L}^{-1}$ (Fig. 5c) and thus always exceeded the threshold. Hence, our findings indicate that DIN and silicate concentrations have little effect on the population dynamics of *H. akashiwo* and *S. costatum* in this port.

On the other hand, the minimum cellular phosphorus concentrations required for survival of *H. akashiwo* and *S. costatum* vegetative cells are 0.095 and $0.0028 \text{ pmol cell}^{-1}$ (Watanabe and Nakamura 1984; Tarutani and Yamamoto 1994), such that their blooms ($\geq 1000 \text{ cells mL}^{-1}$) require at least 0.095 and $0.0028 \mu\text{mol L}^{-1}$ to be maintained, respectively. In Hakozaki Fishing Port, DIP concentration varied from <0.03 (below the measurable limit) to $3.67 \mu\text{mol L}^{-1}$ (Fig. 5b), and so it is likely that low phosphorus levels might sometimes inhibit the development of both *H. akashiwo* and *S. costatum* blooms. The half-saturation coefficients for phosphate uptake of *H. akashiwo* and *S. costatum* are $1.00\text{--}1.98 \mu\text{mol L}^{-1}$ (Tomas 1979) and $0.68 \mu\text{mol L}^{-1}$ (Tarutani and Yamamoto 1994), respectively. Thus, the uptake rate of *H. akashiwo* and *S. costatum* depend on extracellular DIP when DIP concentration is below 2.00–3.96 and $1.36 \mu\text{mol L}^{-1}$, respectively. On the basis of our data, the DIP uptake rates of the two species in Hakozaki Fishing Port would nearly always depend on extracellular DIP concentration, and the growth rates, which are regulated by intracellular phosphorus quotas, also would be indirectly regulated by this environmental factor. On the basis of these findings, we conclude that phosphorus limitation regulates the growth of *H. akashiwo* and *S. costatum* in the port, whereas the nutrients nitrogen and silicate appear to be nonlimiting factors because of sufficient levels for growth.

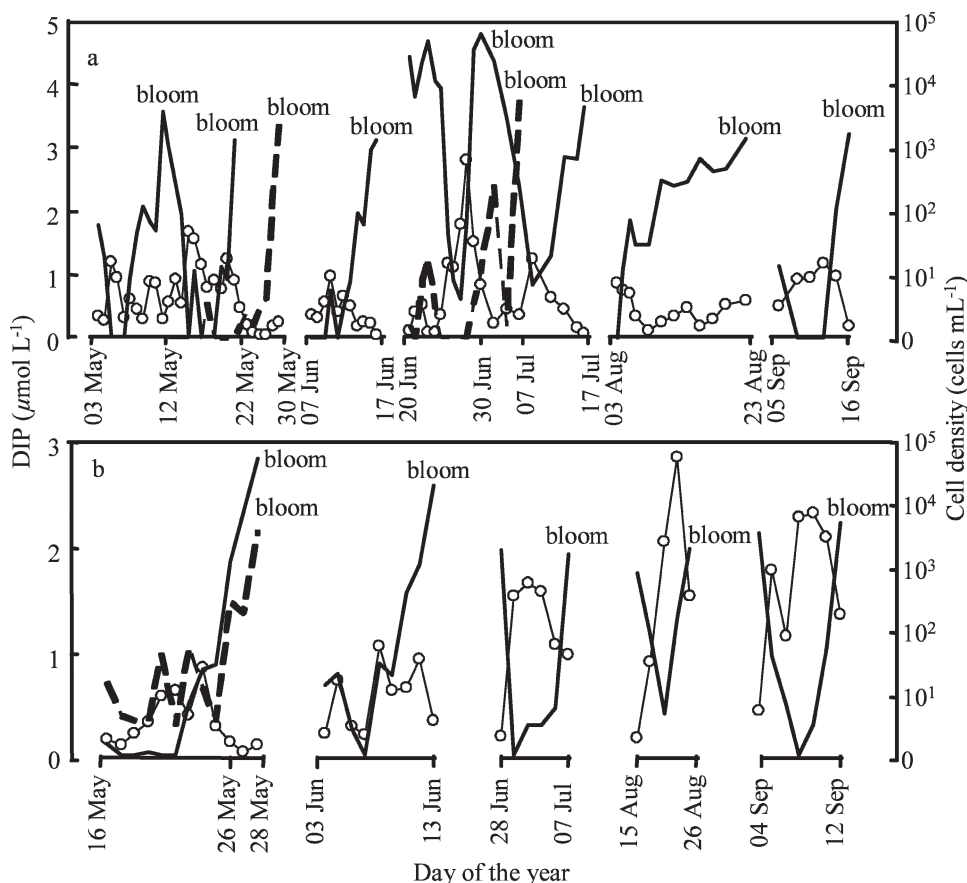


Fig. 9. Short-term fluctuations of DIP concentration (open circle) and cell abundances of *Heterosigma akashiwo* (hatched line) and *Skeletonema costatum* (solid line) for about 10 d before blooms in (a) 2004 and (b) 2005.

We noted fluctuations of cell densities and DIP for about 10 d before all the recorded blooms of *H. akashiwo* and *S. costatum* in Hakozaki Fishing Port (Fig. 9), but a peak in DIP concentration always occurred within 1 week before a bloom. Thus, our findings indicate that, in addition to water temperature and irradiance, DIP concentration affects when *H. akashiwo* and *S. costatum* can bloom in the port. As described above, although *S. costatum* summer blooms subsequent to the earliest one did not always follow strong light penetration, variations of these blooms completely followed peaks of DIP concentration instead. These blooms occurred within a short time after the species' previous respective blooms and so in these cases may have arisen from a seed population of a few vegetative cells in the water column remaining from the previous bloom.

Seeding by resting stages at the bottom vs. vegetative cell growth: Contributions to the bloom event—High-frequency data collection in this shallow port revealed in great detail the relationships between the bloom dynamics of *H. akashiwo* and *S. costatum* and environmental factors. Bloom period and fluctuations of important environmental conditions, as referred to above, in Hakozaki Fishing Port are summarized in Fig. 10.

The environmental conditions, such as water temperature, irradiance, and phosphorus source, for active

vegetative cell growth of *H. akashiwo* and *S. costatum* were suitable right before the blooms, and so the blooms could develop. Consequently, the environmental conditions for vegetative cell growth explain the occurrence of *H. akashiwo* and *S. costatum* blooms in summer when water temperature and irradiance are high, and as well as the summer blooms of both species, which always follow peaks of DIP concentration. However, the period when the conditions were suitable was wide, and so this does not completely explain why the bloom of both species first coincided in early summer (May–June) through the 2 yr. The fact may imply that a sufficient seed population in the water column is required for bloom initiation because vegetative cells of each species fluctuated at low density and low frequency from winter to spring. On the basis of earlier studies and results of our laboratory experiments, the resting stages of *H. akashiwo* and *S. costatum* can germinate at low temperatures and under low light intensity, but *H. akashiwo* requires both high temperature and strong light and *S. costatum* requires strong light for survival and immediate growth of the germinated cells. That is, for germinated cells to survive in both species, strong light is required. The light intensities are much higher than the threshold (I_0) for the survival of vegetative cells, and are higher than the half-saturating photon flux density (K_s). Continuous light strong enough for survival

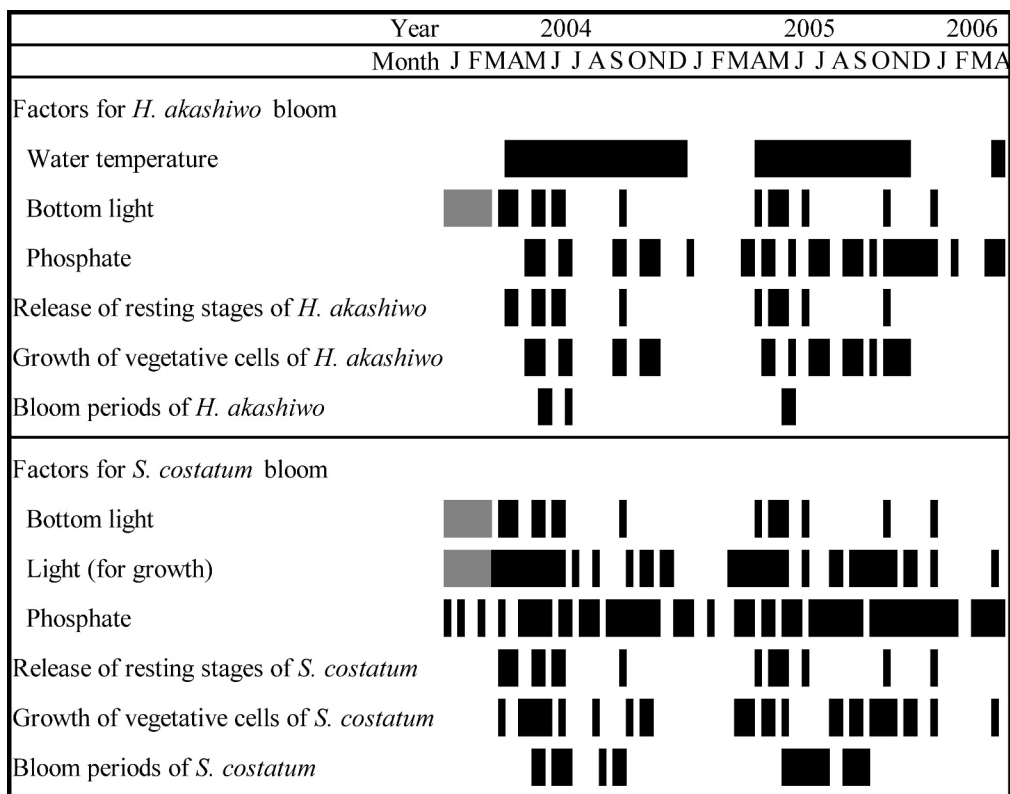


Fig. 10. Summary of bloom periods of *Heterosigma akashiwo* and *Skeletonema costatum*, and fluctuations in important environmental factors during the investigation. Black bars on the periods of water temperature and bottom light, and light and phosphate represent the period for which the factors were favorable for release of resting stages from sediments and growth of vegetative cells, respectively. The bars of water temperature and bottom light present the periods when mean water temperature was $>15^{\circ}\text{C}$, and bottom light intensity peaked $>200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively. The bars of light (for growth) and phosphate represent the periods when mean computationally deepest water-saturated K_s of *S. costatum* peaked $>2.5 \text{ m}$, and phosphate concentration peaked over K_s for uptake rates of *H. akashiwo* and *S. costatum*, respectively. The periods when all factors needed for each species for active release of resting stages or active growth of vegetative cells coincided are also presented with black bars. Gray bars represent “not investigated.”

and immediate growth of the germinated cells penetrates to the bottom only in early summer even in shallow Hakozaki Fishing Port. This indicates that blooms of *H. akashiwo* and *S. costatum* initiate when high water temperature (only for *H. akashiwo*) and strong light exist, and develop when high water temperature (only for *H. akashiwo*), high irradiance (only for *S. costatum*), and high DIP concentration are adequate for growth of the two species, and the season when environmental conditions for bloom initiation and development is suitable is only in early summer. Our study showed that *H. akashiwo* and *S. costatum* can bloom when the conditions for bloom initiation and development coincide and we strongly suggest that the temporally close link between bloom initiation from successful seeding by resting-stage germination and survival and immediate growth of the germinated cells and bloom development by vegetative cell growth is a key in blooming mechanisms of the two species in Hakozaki Fishing Port.

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