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In situ dynamics of cyst and vegetative cell populations of the toxic dinoflagellate *Alexandrium catenella* in Ago Bay, central Japan

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Temporal changes in the *in situ* germination flux of cysts and the abundance of vegetative cells of the toxic dinoflagellate *Alexandrium catenella* were investigated in Ago Bay, central Japan from July 2003 to December 2004. The *in situ* germination flux (cells m^{-2} day^{-1}) was measured using 'plankton emergence trap/chambers (PET chambers)'. Germination of the cysts in the sediments occurred continuously during the study, ranging from 52 to 1753 cells m^{-2} day^{-1}, with no temporal trend. This germination pattern appeared to be promoted by a short mandatory dormancy period for newly formed cysts coupled with a broad temperature window for germination. For the vegetative populations, high abundances ($>10^5$ cells m^{-2}) were recorded in the water column from spring to summer and from autumn to early winter. The size of the vegetative populations did not correlate with the cyst germination flux but rather larger vegetative populations were often observed when the water temperature was around 20°C, indicating that bloom development was mainly regulated by the temperature. Nonetheless, the continuous germination pattern of *A. catenella* is advantageous enabling the germinated cells to immediately exploit favorable bloom conditions.

KEYWORDS: *Alexandrium catenella*; cyst; *in situ* germination; bloom formation; population dynamics

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INTRODUCTION

Sexual reproduction occurs in the life cycles of many dinoflagellates, with resting cysts (hypnozygotes) being formed as a result of this process (e.g. Dale, 1983; Walker, 1984; Pfiester and Anderson, 1987; Blackburn *et al.*, 1989; Kita *et al.*, 1993; Figueroa and Bravo, 2005a,b; Kremp and Parrow, 2006; Figueroa *et al.*, 2006, 2008). Under certain conditions, vegetative cells produce gametes that fuse to form a motile zygotic cell (planozygote) which will eventually transform into a resting cyst. The resting cysts possess a mandatory dormancy period during which germination is inhibited endogenously. Once the cysts have passed this maturation period, their germination depends on environmental triggers, such as temperature, light and oxygen (Dale, 1983; Walker, 1984; Pfiester and Anderson, 1987; Montani *et al.*, 1995; Anderson *et al.*, 1987, 2003). Since the germination of cysts can lead directly to the initiation of blooms of vegetative cells, it is important to investigate the ecology and the physiology of both cysts and vegetative cells to acquire a better understanding of the population dynamics of cyst-forming dinoflagellates.

The dinoflagellate *Alexandrium catenella* (Whedon and Kofoid) Balech is widely distributed in temperate coastal waters throughout the world (Hallegraeff, 1993) and is responsible for many outbreaks of paralytic shellfish poisoning (PSP). In Japan, *A. catenella* occurs mainly in the western Pacific coast region and the Seto Inland Sea (Imai *et al.*, 2006). This organism is also found in Ago Bay, located on the southeast coast of Kii Peninsula, central Japan. Here, PSP contamination in the noble scallop, *Mimachlamys nobilis*, has been occasionally caused by *A. catenella* at a level exceeding quarantine limits (Hata *et al.*, 2013).

The physiological characteristics of *A. catenella* cysts, in relation to dormancy and germination, have been studied extensively in the laboratory (Hallegraeff *et al.*, 1998; Figueroa *et al.*, 2005; Joyce and Pitcher, 2006). However, little is known about *in situ* germination dynamics of *A. catenella* in natural benthic conditions, and such conditions are impossible to reproduce in a laboratory setting. To investigate this, the 'plankton emergence trap/chamber (PET chamber)' was developed to collect freshly germinated cells from microalgal cysts (Ishikawa *et al.*, 2007) and was used to conduct these investigations to monitor the *in situ* germination of *A. catenella* cysts from Ago Bay bottom sediments. Results obtained from July to October 2003 have been previously reported (Ishikawa *et al.*, 2007) and, in this report, we provide additional information gathered from November 2003 to December 2004 to better understand the temporal relationship between *in situ* germination of *A. catenella* cysts and the vegetative cell populations in the water column. Finally, this study evaluates the quantitative importance of cyst germination on *A. catenella* population dynamics in Ago Bay.

METHOD

Ago Bay is heavily used for pearl aquaculture. It has an area of 25 km^2 with limited fresh water input and a narrow inlet (ca. 2 km) connecting it to the Pacific Ocean (Fig. 1). All samples were collected once or twice a month from July 2003 to December 2004 at a station (34°16.30′ N; 136°48.40′ E), located at the southern extent of the bay (Fig. 1). The average depth at the sampling station is 11 m and the bottom sediments are composed of fine mud. All sampling was conducted before noon (09:00–12:00 h).

Water sampling

Water samples were collected with a Van-Dorn sampler at 0, 2, 4, 6, and 8 m and 1 m above the bottom. The samples from the 6 depths were used to enumerate vegetative cells of *A. catenella*. Those at 1 m above the bottom were also used to determine the dissolved oxygen (DO) concentration by the Winkler method (Strickland and Parsons, 1972). Vertical profiles of temperature and salinity were measured by a Multi-probe (QUANTA, Hach-Hydrolab).

For vegetative cell counts, 500 mL samples were immediately fixed by adding borax-buffered formaldehyde at a final concentration of 1% (v/v). These preserved samples were settled for at least 24 h and thereafter concentrated into 10 mL by siphoning. From this concentrated sample, a 1 mL aliquot was transferred to a Sedgewick-Rafter chamber and *A. catenella* were enumerated using an inverted microscope (NIKON ECLIPSE TE-300 equipped with epifluorescence optics) at ×200 magnification. All cell counts were made in triplicate and *A. catenella* cells were identified at ×400–600 magnification under ultra violet excitation by confirming their plate tabulation with Calcofluor White M2R (Sigma-Aldrich Co., St Louis, MO, USA) which was previously added to the concentrated sample (Fritz and Triemer, 1985). The cell densities (cells L^{-1}) were plotted against their respective depths, and these data were then integrated to obtain the total abundance of vegetative cells in the water column per m^2 (cells m^{-2}).

Sediment sampling

Sediment sampling was carried out using the method of Yokoyama and Ueda (Yokoyama and Ueda, 1997), in which an acrylic core tube (6.4 cm diameter, 23 cm length) was installed in the bucket of an Ekman grab

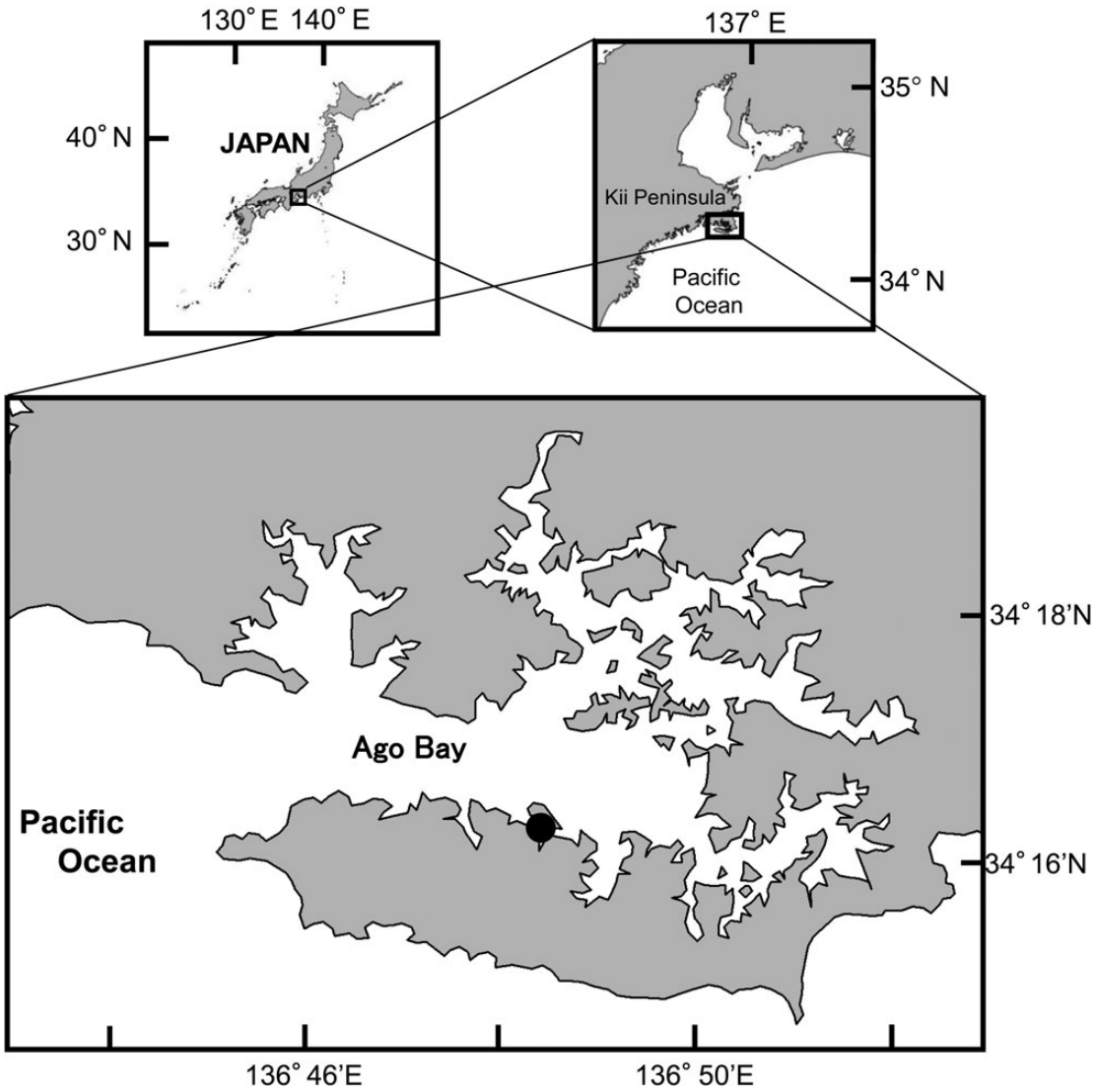

Fig. 1. Location of the sampling station (closed circle) in Ago Bay, Japan.

(see Ishikawa *et al.*, 2007) to collect an undisturbed sediment core sample. Typically, nine core samples were randomly obtained from a ca. 10 m × 10 m area surrounding the station. Three of these cores were used for counting *A. catenella* cysts, while the remaining six cores were used for measuring *in situ* germination flux of *A. catenella* within PET chambers. Immediately after the grab was recovered, the temperature of the sediment was measured with a mercury thermometer at a depth of 1 cm in one of the cores used for cyst counting.

Cyst counting

For enumerating *A. catenella* cyst concentrations in the sediment, the top 3 cm of three cores were sliced off and pooled together. The sediment samples were then processed following the primuline technique developed by Yamaguchi *et al.* (Yamaguchi *et al.*, 1995). For this, the sediment was homogenized and a 2.5 g subsample suspended in ca. 50 mL of distilled water and sonicated for 1 min. The suspension was passed through 200 and 20 μm sieves, and the fraction retained on the 20 μm sieve was resuspended in 5 mL of distilled water and fixed by adding 1 mL of 5% glutaraldehyde for 30 min. After fixation, the suspension was centrifuged at 700×*g* for 15 min. and the supernatant was removed. Five mL of 99% methanol were then added, the sample was mixed well and refrigerated at ca. 4°C for 1 day. Following this, the methanol was removed by centrifugation, as described above, and was replaced with 10 mL of distilled water. One mL of a 2 mg mL^{-1} stock solution of primuline (Sigma-Aldrich Co.) was added and the

sample was allowed to stain for 30 min. After staining, the suspension was again centrifuged and the supernatant was removed and replaced with 5 mL of distilled water. From this stained sediment suspension, a 0.1 mL aliquot was spread over a Sedgewick-Rafter counting chamber along with 0.9 mL distilled water and the cysts were counted under blue light excitation using an epifluorescence inverted microscope at ×100. The fluorescently labeled cysts were easily identified based on their characteristic bright green labeled fluorescence as well as their distinct morphological characteristics which include an elongated and ellipsoidal shape with rounded ends (Fukuyo, 1985). When a fluorescent cyst was seen, it was further examined under normal light to determine whether the cytoplasmic contents of the cyst were intact; only these cysts were included in the tally. While it is impossible to distinguish *A. catenella* and *A. tamarense* cysts from one another based on their morphology and size (Fukuyo, 1985), it is possible to morphologically differentiate vegetative cells of these species from one another on the basis of their thecal plate tabulation. During this study, no vegetative cells of *A. tamarense* were observed in any of the water samples examined from Ago Bay, thus all of the cysts counted were definitely *A. catenella*. The densities of cysts per g wet sediment were converted into cysts per cm^3 of wet sediment by measuring the specific gravity of the original sediment according to the method of Kamiyama (Kamiyama, 1996). All counts were done in triplicate and the average was calculated for each sample.

In situ germination flux

Measurement of the *in situ* germination flux of *A. catenella* cysts was carried out using plankton emergence trap/chambers (PET chambers) (Ishikawa *et al.*, 2007). The sediments from the cores (see above) were individually transferred into six PET chambers as soon as the cores were collected according to the handling procedure described in Ishikawa *et al.* (Ishikawa *et al.*, 2007). These were placed onto a stainless steel incubation platform without delay so as to minimize the effects of being out of water, and the platform was lowered to a bottom depth of ∼11 m. After 24 h, the chambers were retrieved and the water above the sediment in the top cylinder of the chamber was collected. Prior to sample collection, the outside of the cylinder and base plate were thoroughly washed by brush scrubbing with fresh water to prevent contamination of vegetative cells which might attach to the outside of the PET chamber from the surrounding ambient seawater during incubation. There were situations where only four or five of the six PET chambers were successfully retrieved. The water samples (ca. 500 mL) were fixed with borax-buffered formaldehyde at a final concentration of 1% (v/v) and concentrated into 10 mL volume by repeated aspiration following settling (normally for >24 h). An aliquot of the concentrated samples was spread over the base plate of the combined plate chamber (see Hasle, 1978) and the germinated cells were counted at 200× using an inverted epifluorescence microscope. This process was repeated until the entire 10 mL volume was examined for every sample. The concentrated samples contained silt and clay particles resulting from a small amount of sediment resuspension that occurred during the PET chamber sampling process. The amount of sediment in the samples sometimes prevented detection of germinated *A. catenella* cells using normal illumination. As a result, the germinated cells were enumerated by observing their autofluorescence under blue light epifluorescence excitation and finally identified on the basis of their thecal plate tabulation using the calcofluor white staining method as described above. In the samples, large *A. catenella* cells (ca. 60 μm length) and elongated in shape, which is typically indicative of a planomeiocyte, were observed; however, smaller cells (<50 μm) with a more rounded shape were also observed and these could be vegetative cells resulting from planomeiocyte division while the PET chamber was being incubated. In this situation, every two vegetative cells that were counted were assumed to originate from one planomeiocyte. The cell numbers in the PET chambers were first averaged to provide a daily germination number per chamber (i.e. cells $chamber^{-1}$ day^{-1}) and then these were converted into daily germination value or 'germination flux' per m^2 (i.e. cells m^{-2} day^{-1}) by taking the sediment surface opening area on the base plate of the PET chamber (i.e. 32.2 cm^2, see Ishikawa *et al.*, 2007). In one instance, from July to October 2003 except for 9 August, samples were obtained with the PET chambers for two consecutive days (see Ishikawa *et al.*, 2007). In this case, for this study, the germination flux was calculated using the mean value of the cell numbers counted from all the PET chambers of the two consecutive days. The detection limit of the flux was 52 cells m^{-2} day^{-1}, when all six chambers were successfully retrieved and this limit was reduced to 78 cells m^{-2} day^{-1} when only four chambers were retrieved. It is assumed that grazing by zooplankton on the germinated cells in the PET chamber during incubation was negligible, since large zooplankton was rarely observed in the samples. Furthermore, it should be noted that the germination flux obtained in this study did not result from sediment resuspension as there was no significant turbulence within the PET chamber.

To evaluate the contribution of germinated cysts to the abundance of cysts per unit area in a day, a germination

rate (% day^{-1}) was calculated for each sampling as follows: the *in situ* germination flux (cells m^{-2} day^{-1}) was divided by the abundance of the cysts in 1 cm thickness of the sediment per m^2 (cysts m^{-2}) which was enumerated using the cyst densities (cysts cm^{-3}) in the pooled top 3 cm sediment (see above) and then multiplied by 100.

RESULTS

Environmental conditions

Temperature in the water column showed a clear temporal pattern as generally observed in temperate coastal waters with high values in summer and low values in winter (Fig. 2a). Temperatures exceeding 25°C were often recorded from June to September in 2003 and 2004. Temperatures in the water column were lower than 15°C from December 2003 to March 2004. The highest temperatures in 2003 and 2004 were recorded at 0 m (the surface) in September (29.5°C) and July (29.6°C), respectively. A minimum temperature of 11.1°C was recorded at 1 m above the bottom in March 2004. Thermal stratification was evident from July to September in 2003 and from May to September in 2004. The temporal change in temperature of the sediment showed a similar trend to that in the water column, with a maximum of 26.8°C in August 2004 and a minimum of 10.2 in February 2004.

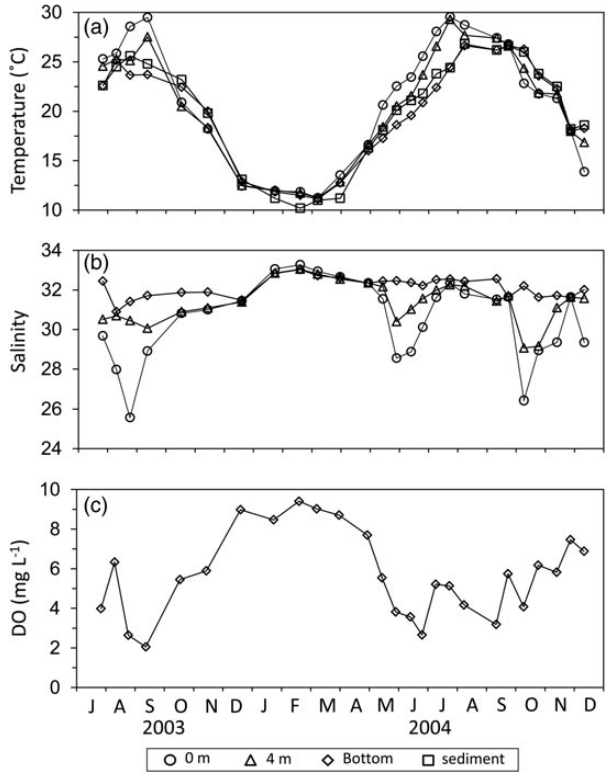

Fig. 2. Temporal changes of (**a**) temperature at different depths in the water column and at 1 cm depth in the sediment, (**b**) salinity at different depths in the water column, and (**c**) DO concentration at 1 m above the bottom (represented as Bottom in the legend), at the sampling station in Ago Bay.

During this time frame, salinity values ranged from 25.6 to 33.3 with salinities lower than 30 measured at the surface but almost always from 28 to 33 (Fig. 2b). Lower salinities, particularly <30, were caused by heavy rainfall. In general, temporal pattern in salinity was not obvious.

Concentrations of DO at 1 m above the bottom were occasionally lower than 4 mg L^{-1} in the warmer seasons (from July to September in 2003 and from May to September in 2004) when the water column was thermally stratified (Fig. 2c). Higher DO values were recorded in the colder seasons (from December 2003 to April 2004) when the water column was vertically mixed. The minimum DO concentration of 2.1 mg L^{-1} was measured in September 2003 and the maximum of 9.4 mg L^{-1} in February 2004.

Vegetative cells

Vegetative cells of *A. catenella* were observed in the water column with distinct patterns, with cell abundances >10^5 cells m^{-2} in October and December 2003, and from the middle of May to the middle of June 2004 in addition to the end of November 2004 (Fig. 3a, Table I). The maximum abundance of 1.1 × 10^7 cells m^{-2} was recorded at the end of May 2004. Vegetative cells were not detected in the warmest months (from late in August to September 2003, early in July 2004 and from August to October 2004), or in the coldest months (from January to early in March 2004 and in December 2004). When all of the cell density (cells L^{-1}) data obtained from each sampling depth (i.e. 0, 2, 4, 6 and 8 m and 1 m above the bottom) in this study are plotted against temperature and salinity recorded at the corresponding depth on T-S diagram (Fig. 4), it is evident that *A. catenella* occurred at a relatively high density (>300 cells L^{-1}) at the range of salinities between 28 and 33 (Fig. 4). Although the cells were detected at the temperatures of 12.5–29.6°C (which was almost equivalent to the year-round temperature range of 11.1–29.6°C), the high densities of >300 cells L^{-1} were often found associated with a narrow range of the temperatures, between 18 and 23°C, with peak densities (>1900 cells L^{-1}) at around 20°C (Fig. 4).

Cysts and *in situ* germination

The cyst density of *A. catenella* in the top 3 cm of the sediment varied from 35 to 133 cysts cm^{-3} throughout this

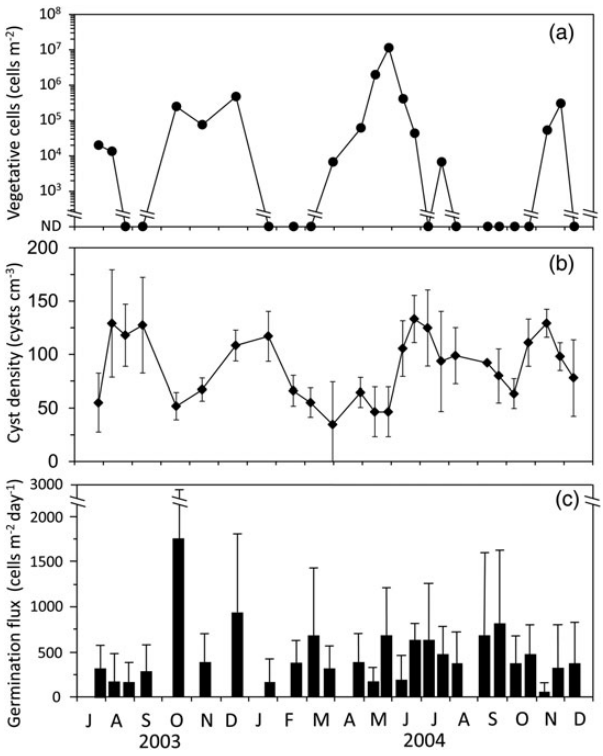

Fig. 3. Temporal changes in (**a**) integrated number of vegetative cells of *A. catenella* throughout the water column (from the surface to 1 m above the bottom), (**b**) density of *A. catenella* cysts in the top 3 cm of the sediment, and (**c**) *in situ* germination flux of *A. catenella* cysts from the surface sediment, at the sampling station in Ago Bay. ND in panel (**a**) denotes that the cells were not detected. Bars in (**b**) and (**c**) represent ±SD and +SD, respectively.

study period (Fig. 3b, Table I). The cyst density, in general, tended to increase during and/or after the bloom of vegetative cells and decreased during non-bloom periods or with development of the bloom except for one case late in October 2004.

Germination of *A. catenella* cysts from surface sediments occurred continuously throughout the study period (Fig. 3c, Table I). The germination fluxes were highly variable (from 52 to 1753 cells m^{-2} day^{-1}), and a temporal trend in these fluxes was not observed, as in all cases, standard deviations surrounding the flux values were quite large. The average germination flux throughout the study period was 461 cells m^{-2} day^{-1} and the germination rate varied from 0.004 to 0.337% day^{-1} (Fig. 5, Table I).

DISCUSSION

PET chamber and *in situ* germination data

The PET chamber is designed to prevent contamination of motile cells (vegetative cells, planomeiocytes and planozygotes) in the surrounding ambient waters from entering the chamber during incubation (Ishikawa *et al.*, 2007). However, external contamination has been observed on PET chambers when incubated in an environment of high cell densities of *A. fundyense* (>10 000 cells L^{-1}, D.M. Kulis, unpublished data), even when the chambers were rinsed well prior to harvest. This contamination was so significant that the PET chamber data could not be used in some studies. In this study, motile cells were occasionally found in the bottom waters at our station throughout the study period but in very low densities (<20 cells L^{-1}, data not shown). Therefore, the possibility of cell contamination can be dismissed for this study. Nonetheless, it should be noted that there were large standard deviations surrounding the germination fluxes (Fig. 3c, Table I). Since the sampling efficiency of the PET chamber to collect germinated cells is supposed to be at 100%, a possible source of the large standard deviations could be attributed to the significant heterogeneity of cyst germination from the sediments, in addition to the highly variable nature of the cyst densities in the PET chamber core sediments even though the samples were collected from a relatively small sampling area (ca. 10 m × 10 m).

In addition, since it is generally agreed that germination in natural sediments only involves the cysts within the first few mm of the sediment surface, the absolute value of the germination rate that was derived from pooled cyst counts from the top 3 cm of sediment in this study should be carefully reconsidered; however, it can be useful to evaluate the relative extent of germination and to understand the temporal trend of germination.

Factors related to *in situ* cyst germination

It has been reported for some dinoflagellate species, including those in the genus *Alexandrium*, that sexual reproduction is enhanced by high cell population densities in the water column, resulting in mass cyst formation and subsequent deposition into the sediment (Anderson *et al.*, 1983; Takeuchi, 1994; Ishikawa and Taniguchi, 1996; Anglès *et al.*, 2012a,b). In this study, the cyst abundance in the sediment increased during and/or after blooms of *A. catenella*, except for one case late in October 2004, for which we have no explanation (Fig. 3a and b). The general trend in the dynamics between cyst and vegetative cell abundances observed confirms that newly formed cysts were responsible for the enhanced cyst concentrations in the sediments. After being produced, dinoflagellate cysts are required to pass through a mandatory dormancy period before they are capable of germination (Dale, 1983; Walker, 1984; Pfiester and Anderson, 1987). For example, cysts of *Alexandrium tamarense* exhibit a dormancy period of 1–12 months for various strains (e.g.

Table I. Date of sampling in 2003 and 2004, integrated number of vegetative cells of A. catenella throughout the water column (from the surface to 1 m above the bottom), cyst density (± SD) in the top 3 cm of the sediment, in situ *germination flux (± SD) from the surface sediment and germination rate, at the sampling station in Ago Bay*

Date	Vegetative cells (cells m^{-2})	Cysts (cysts cm^{-3})	Germination flux (cells m^{-2} day)	Germination rate (% day^{-1})
25 Jul 03	2.0×10^4	55 (±27)	311 (±254)	0.057
9 Aug 03	1.3×10^4	129 (±50)	156 (±311)	0.012
23 Aug 03	ND	118 (±29)	156 (±220)	0.013
11 Sep 03	ND	127 (±45)	280 (±309)	0.022
16 Oct 03	2.5×10^5	52 (±13)	1753 (±1174)	0.337
13 Nov 03	7.6×10^4	67 (±11)	373 (±341)	0.056
18 Dec 03	4.7×10^5	108 (±14)	933 (±880)	0.086
22 Jan 04	ND	117 (±23)	156 (±260)	0.013
18 Feb 04	ND	66 (±14)	373 (±260)	0.057
6 Mar 04	ND	55 (±14)	684 (±743)	0.124
29 Mar 04	6.7×10^3	35 (±40)	311 (±254)	0.089
28 Apr 04	6.1×10^4	65 (±14)	373 (±341)	0.057
13 May 04	2.0×10^6	46 (±23)	156 (±180)	0.034
27 May 04	1.1×10^7	46 (±23)	674 (±536)	0.147
12 Jun 04	4.1×10^5	105 (±26)	187 (±278)	0.018
24 Jun 04	4.3×10^4	133 (±22)	622 (±197)	0.047
8 Jul 04	ND	125 (±36)	622 (±622)	0.050
22 Jul 04	6.7×10^3	94 (±47)	467 (±326)	0.050
7 Aug 04	ND	99 (±26)	363 (±364)	0.037
10 Sep 04	ND	92 (±0)	674 (±910)	0.073
22 Sep 04	ND	80 (±25)	809 (±811)	0.101
8 Oct 04	ND	63 (±14)	363 (±306)	0.058
23 Oct 04	ND	111 (±22)	467 (±326)	0.042
12 Nov 04	5.3×10^4	129 (±13)	52 (±127)	0.004
26 Nov 04	3.0×10^5	98 (±13)	311 (±482)	0.032
10 Dec 04	ND	78 (±36)	363 (±458)	0.047

ND denotes that cells were not detected.

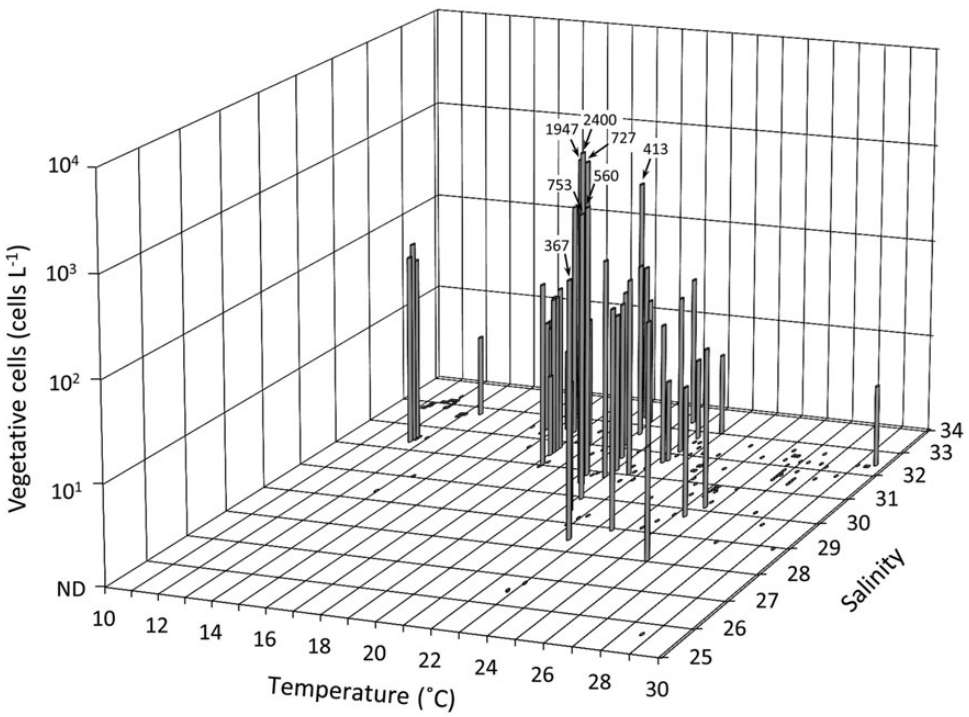

Fig. 4. Vegetative cell density of *A. catenella* at various conditions of temperature and salinity at the sampling station in Ago Bay. All data of the cell density obtained from different depths (0, 2, 4, 6 and 8 m and 1 m above the bottom) in the water column were plotted with temperature and salinity recorded at the corresponding depth. Cell density above 300 cells L^{-1} is indicated with arrows. ND denotes that cells were not detected.

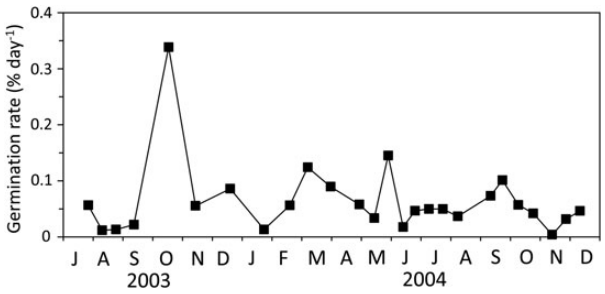

Fig. 5. Temporal change in germination rate of *A. catenella* at the sampling station in Ago Bay.

Dale *et al.*, 1978; Turpin *et al.*, 1978; Anderson, 1980; Perez *et al.*, 1998). *Alexandrium catenella*, on the other hand, has a relatively short dormancy period, although different geographic strains of this species also exhibit different dormancy periods (Hallegraeff *et al.*, 1998); 28–55 days for strains from Australia (Hallegraeff *et al.*, 1998), 15–18 days from South Africa (Joyce and Pitcher, 2006), 5–65 days from Spain (Figueroa *et al.*, 2005) and 10–14 days from Japan (Yoshimatsu, 1992; Takeuchi, 1994). Thus, it is reasonable to expect that newly formed cysts of *A. catenella* in Ago Bay would mature soon after their deposition on the surface sediments and be poised to germinate.

Following their mandatory dormancy period, the germination of the dinoflagellate cysts can be regulated by various environmental factors (e.g. Dale, 1983; Pfiester and Anderson, 1987; Anderson *et al.*, 2003). Temperature has been shown as the principal regulator in water bodies where seasonal bottom water temperature changes occur (e.g. Dale, 1983; Pfiester and Anderson, 1987). The temperature window for germination (permissive temperature range for cyst germination; see Pfiester and Anderson, 1987; Anderson *et al.*, 2003) of *A. catenella* cysts varies among geographically different strains. Cysts from South Africa germinate over a temperature range from 4 to 22°C with the highest germination at 10°C (Joyce and Pitcher, 2006), whereas Takeuchi (Takeuchi, 1994) reported that the temperature window for cysts from Tanabe Bay in central Japan is from 10 to 30°C, although the germination is minimal at 30°C. A preliminary laboratory experiment also revealed that the temperature window for germination of *A. catenella* cysts in Ago Bay was from 10 to 30°C with an optimal range of 15–25°C (A. Ishikawa, unpublished data). These temperatures span the annual temperature variation within the sediments recorded at the study site during this study period (i.e. 10.2–26.8°C; Fig. 2a), allowing for *in situ* germination of *A. catenella* cysts throughout the year (Fig. 3c, Table I). Interestingly, the germination rates measured (Fig. 5, Table I) were not always robust when the sediment temperature was in the optimum range for germination from July to November 2003 and from April to December 2004 (Fig. 2a), thus, it appears that, although temperature is a basic factor regulating germination, there appear to be other drivers that enhance or impede *A. catenella* germination in Ago Bay.

It is well known that anoxic conditions inhibit the germination of many dinoflagellate cysts (Endo and Nagata, 1984; Anderson *et al.*, 1987; Kremp and Anderson, 2000). Moreover, it is also suggested that low DO concentrations regulate the germination of dinoflagellate cysts (Ishikawa and Taniguchi, 1994; Montani *et al.*, 1995; Kremp and Anderson, 2000). Although the values of DO concentration at the surface sediment where cyst germination can occur could not be measured in this study, it should be reasonable to consider that the temporal trend of DO at the surface sediment was similar to that observed at 1 m above the bottom throughout the year and, furthermore, the values were much lower at the surface sediment when the water column was thermally stratified. At our sampling site, the germination rate of *A. catenella* cysts was reduced late in August 2003, in September 2003 and in the middle of June 2004 (Fig. 5, Table I), when DO concentrations at 1 m above the bottom were low (2.1–3.6 mg L^{-1}; Fig. 2c). However, the germination rate was relatively high despite low DO (3.8 mg L^{-1}) at the end of May 2004. Thus, the effect of low DO on the *in situ* germination of *A. catenella* cysts was not evident in this study. Light is not always essential to trigger germination, but it can boost germination rates of dinoflagellate cysts (Endo and Nagata, 1984; Anderson *et al.*, 1987; Kremp and Anderson, 2000). Although light values were not measured, it is assumed that it could penetrate to the 11 m bottom depth of the study site despite significant variation of the attenuation and intensity throughout the year. Further investigation using the PET chambers could clarify these interacting, but confounding, factors on the germination of these cysts.

Germination of cysts and formation of vegetative cell populations

There were periods of time when vegetative cells of *A. catenella* were not present in the water samples collected during the study period (Fig. 3a, Table I). In contrast, cyst germination occurred continuously in surface sediments throughout the year (Fig. 3c, Table I). When comparing germination flux and the integrated abundance of the vegetative cells in the water column (Table I), there was no significant positive relationship between them ($r = 0.11$, $n = 26$, $p > 0.05$), indicating that the magnitude of the bloom was not regulated by the germination flux. If we calculate the daily inoculum from cyst germination into the water column (11 m depth) using the average germination flux of 461 cells m^{-2} day^{-1}, an inoculum of

0.04 cells L^{-1} day^{-1} would result. Even when the maximum flux of 1753 cells m^{-2} day^{-1} recorded in this study is considered, only 0.16 cells L^{-1} day^{-1} would be delivered into the water column. These appear to be very low inoculation rates.

Takeuchi (Takeuchi, 1994) reported that the temperature range for optimal growth of *A. catenella* isolated from Tanabe Bay, Japan, was 20–25°C. Furthermore, Siu *et al.* (Siu *et al.*, 1997) determined that a temperature of 20–25°C and a salinity of 30–35 promoted the best growth for an *A. catenella* isolate established from Tai Tam Bay, Hong Kong. Likewise, in our laboratory, it has been confirmed that the optimal salinity for growth of *A. catenella* isolated from Ago Bay was between 25 and 35, and at salinity of 30, the higher growth rates were obtained at a temperature range between 17.5 and 27.5°C (A. Ishikawa, unpublished data). During this study, temporal changes in salinity were commonly observed between 28 and 33 (Fig. 2b), suggesting that the occurrence of vegetative cells in the field does not appear to be effectively restricted by this parameter which is supported by relatively large populations of *A. catenella* being observed at these recorded salinity values (Fig. 4). In contrast, the optimal temperature range for growth (17.5–27.5°C) was within the year-round temperature range of 11–30°C (Fig. 2a) with the highest concentrations of the vegetative cells actually observed over a relatively narrow window of water column temperatures (18–23°C) (Fig. 4). The high concentrations of *A. catenella* were also found for almost the same temperature range (16–24°C) in monitoring data from Ago Bay collected by the Mie Prefecture Fisheries Research Institute over the course of 13 years (Hata *et al.*, 2013). Together, these data suggest that temperature is a major factor in controlling the growth of the cells in Ago Bay.

Overall, the magnitude of the vegetative population of *A. catenella* is dependent on vegetative cell growth and not a direct consequence of cyst germination fluxes; only a small inoculum of germinated cells from the sediment is sufficient to promote a bloom when growth conditions are favorable. As witnessed for other dinoflagellate species for which *in situ* germination has been investigated including *Scrippsiella trochoidea* (Ishikawa and Taniguchi, 1996), *Ensiculifera carinata*, *Gonyaulax spinifera*, *G. verior*, *Protoperidinium claudicans*, *P. conicoides* and *P. conicum* (Ishikawa and Taniguchi, 1997), *Alexandrium fundyense* (Anglès *et al.*, 2012a) and *A. minutum* (Anglès *et al.*, 2012b), cyst germination is primarily responsible for 'seeding' bloom formation. This concept is also demonstrated in a mathematical model analyzed for *A. minutum* (Estrada *et al.*, 2010). These findings help explain the critical, but sometimes limited role of cyst germination in bloom initiation that has been suggested for many cyst-forming dinoflagellate species (e.g. Wall, 1971, 1975; Dale, 1983; Anderson, 1984, 1998).

Ecological implications of germination pattern for population dynamics

Ishikawa and Taniguchi (Ishikawa and Taniguchi, 1996, 1997) recognized three different cyst germination patterns, i.e. continuous, sporadic and synchronous, for photosynthetic and heterotrophic dinoflagellate species in a field study using a 'germinating cell trap/sampler' in Onagawa Bay, Japan. A continuous germination pattern was found for *S. trochoidea* cysts (Ishikawa and Taniguchi, 1996) and also for *A. minutum* cysts (Anglès *et al.*, 2012b), characterized by a peak or peaks at particular time points. Given the criteria established by the above studies, the germination pattern of *A. catenella* in Ago Bay should be categorized as continuous but as a subtype without a marked peak (Fig. 3c, Table I) as there appears to be no time interval at which germination is consistently more pronounced over the course of this 18-month investigation. The continuous germination of *A. catenella* cysts in Ago Bay can be attributed to their short mandatory dormancy period coupled with a broad 'temperature window' for germination. Since this continuous germination behavior allows *A. catenella* to immediately exploit favorable growth conditions within the water column, it is advantageous for this species in that large populations of vegetative cells can be established, over other phytoplankton species, in coastal temperate environments where both biotic and abiotic conditions are variable. Therefore, from an ecological viewpoint, *A. catenella* can be considered an opportunistic species. Further investigations of *in situ* cyst germination using the PET chambers in different locations would allow for a better understanding of the opportunistic behavior of *Alexandrium catenella* in addition to population bloom dynamics as well as establishing a better relationship between cyst densities and germination fluxes.

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