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Encystment and Excystment of *Gyrodinium instriatum* Freudenthal et Lee

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In the present study, we have investigated the conditions influencing encystment and excystment in the dinoflagellate *Gyrodinium instriatum* under laboratory conditions. We incubated *G. instriatum* in modified whole SWM-3 culture medium and in versions of modified SWM-3 from which NO_3^-, PO_4^{3-}, NO_3^- + PO_4^{3-}, or Si had been omitted and observed encystment. Percentage encystment was high in the media without N and without P, while the percentage encystment in the medium lacking both N and P was highest. Moreover, to investigate N or P concentration which induced the encystment, *Gyrodinium instriatum* was also incubated in media with different concentrations of inorganic N and P; the concentrations of NO_2^- + NO_3^- and PO_4^{3-} were measured over time. The precursors of cysts appeared within 2 or 3 days of a decrease in NO_2^- + NO_3^- or PO_4^{3-} concentration to values lower than 1 μM or 0.2 μM, respectively. When cysts produced in the laboratory were incubated, we observed excystment after 8–37 days, without a mandatory period of darkness or low temperature. We incubated cysts collected from nature at different temperatures or in the dark or light and observed excystments. Natural cysts excysted at temperatures from 10 to 30°C, in both light and dark, but excystment was delayed at low temperatures. These studies indicate that *G. instriatum* encysts in low N or P concentration and excysts over a wide temperature range, regardless of light conditions, after short dormancy periods.

Keywords:
· Encystment,
· excystment,
· dinoflagellate,
· *Gyrodinium instriatum*,
· cyst,
· nutrient,
· temperature.

1. Introduction

Most planktonic dinoflagellates produce cysts (Blackburn and Parker, 2005), a process that allows their populations to persist during harsh periods until conditions improve. Excystment often initiates blooms (Anderson and Wall, 1978; Rengefors and Anderson, 1998).

One such dinoflagellate, *Gyrodinium instriatum* Freudenthal et Lee, often forms red tides and damages coastal fisheries (Jimenez, 1993). It can grow well in a wide range of temperatures and salinities, with notable tolerance to salinity as low as 5 psu (Nagasoe *et al*., 2006). Its cysts are egg-shaped, transparent or brownish, and covered with gelatin-like mucus. They have been reported from around Japan in Senzaki Bay, the area south of Harima Nada (Matsuoka, 1985), Lake Hamana (Kojima and Kobayashi, 1992), and the Ariake Sea and Hakata Bay (Shikata *et al*., unpublished). Uchida *et al*. (1996) investigated the life cycle of *G. instriatum*. Conjugation of this organism is homothallic, and the planozygote, after a period of 6 days, divides to form two flagellate cells, which later also divide. On the other hand, cyst precursors or "pre-cysts", which are furnished with two longitudinal flagella and are dorsoventrally flattened and pale greenish-brown, were transformed directly into cysts within a few days of the start of observations by Uchida *et al*. (1996), unlike planozygotes of other dinoflagellates such as *Gymnodinium nolleri* (Figueroa and Bravo, 2005), which can also transform into other life forms (vegetative cell) than cysts. These authors suggested that cysts

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Table 1. The five media used in the culture experiments.

Treatment	Abbreviation
Modified SWM-3	SWM
Modified SWM-3 without $NaNO_3$	SWM-*N*
Modified SWM-3 without NaH_2PO_4-$2H_2O$	SWM-*P*
Modified SWM-3 without $NaNO_3$, without NaH_2PO_4-$2H_2O$	SWM-*N*-*P*
Modified SWM-3 without $NaSiO_3$-$9H_2O$	SWM-*Si*

were of zygote origin because the pre-cysts have two longitudinal flagella, as do planozygotes, but the process of transformation from planozygote to pre-cyst was not observed. Interestingly, by simply incubating *G. instriatum* vegetative cells for 2 to 3 weeks in modified SWM-3 medium (Itoh and Imai, 1987), which is rich in N and P, they achieved encystment. When the cysts were left for about 1 month under the cold and dark conditions, a number excysted. However, it is still not known in detail what triggers encystment and excystment.

Given that N or P deficiency triggers encystment in many dinoflagellate species (Pfiester and Anderson, 1987), we investigated the effects of both N and P deficiency on encystment in *G. instriatum*. Moreover, by using cysts produced in the laboratory and collected from nature, we were able to investigate maturity and excystment in *G. instriatum*.

2. Materials and Methods

2.1 Culture maintenance

The clonal and axenic strains of *Gyrodinium instriatum* used were isolated from Hakozaki Fishing Port, in Hakata Bay, Japan (lat. 33°7′30″ N, long. 130°5′00″ E). The strains were used for all experiments within 6 months of isolation. They were tested for bacterial contamination by the fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) staining method (Porter and Feig, 1980); all were verified as axenic.

Cultures were maintained in 200-mL flasks, containing 100 mL of modified SWM-3 medium (Itoh and Imai, 1987) without calcium pantothen, nicotinic acid, ρ-aminobenzonic acid, inositol, folic acid or thymine addition (Yamasaki *et al*., 2007), with a salinity of 30 psu, at 25 or 20°C under 350 or 530 μmol photons $m^{-2}s^{-1}$ of cool-white fluorescent illumination on a 12 h:12 h light:dark cycle. Irradiance in the incubator was measured with a Quantum Scalar Laboratory Irradiance Sensor (QSL-2101, Biospherical Instruments Inc., San Diego, CA, USA).

2.2 Effects of nutrient deficiency on encystment

Gyrodinium instriatum was incubated to a density of 4000 cells mL^{-1}, including a few pre-cysts (<1 cell mL^{-1}), in modified SWM-3 medium (hereafter referred to as SWM). Subsequently 0.15 mL of the culture was inoculated into five 6-well microplates with 3.85 mL of SWM lacking NO_3^- (hereafter referred to as *N*); lacking PO_4^{3-} (hereafter referred to as *P*); *N* and *P*; or SiO_3^{2-} (hereafter referred to as *Si*); or lacking nothing (Table 1). Three replicates were used for each treatment. The inoculated microplates were incubated for 3 weeks at 25°C under 350 μmol photons $m^{-2}s^{-1}$ of cool-white fluorescent illumination on a 12 h:12 h light:dark cycle. For each of the five treatments, three wells were used for counting motile cells and the other three were used for counting cysts. Every 3 days, 300 μL of the culture was collected from each well, mixed, and counted for motile cells under a light microscope. The cysts attached to the bottom of each well were counted with an inverted microscope, because the cysts of *G. instriatum* show strong adhesion to the bottom of the well, and few cysts occur in suspension. Finally, maximum yields of the cells + cysts during the investigation periods were calculated. Percentage cyst formation was also calculated by dividing the cyst yield by the maximum yield of motile cells under the assumption that one cyst was formed from one cell. Considering that Uchida *et al*. (1996) observed small cells which conjugate and transform to planozygotes, the small cells (gametes) may be formed by meiosis of a vegetative cell. If so, cyst would have the same nuclear phase as vegetative cell as well as planozygotes and pre-cyst. Therefore, the assumption that one cyst was formed from one cell was applied to the calculation of the percentage of cyst formation.

2.3 Effects of inorganic nitrogen and phosphorus concentrations on encystment

For observations of encystment it was considered ideal to follow the number of cysts. The cysts of *G. instriatum*, however, are associated with viscous material and stick to the bottom of the flasks, making quantitative estimation impossible. Moreover, in this experiment we needed to follow nutrient concentrations, and therefore incubation in a large-volume chamber was necessary for nutrient analysis. A pre-cyst is a motile cell that is dorsoventrally flattened and has orange droplets in the central region. These morphological characteristics allow

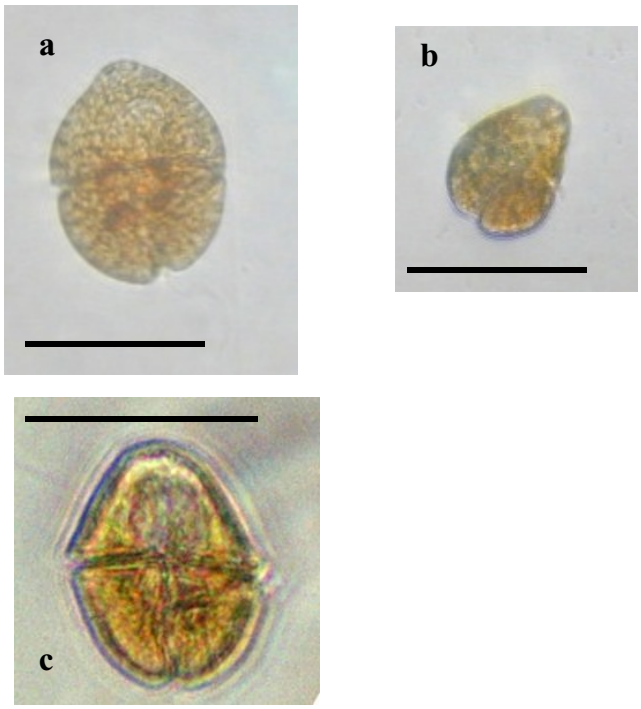

Fig. 1. Life cycle stages of *Gyrodinium instriatum*. Scale bars = 25 μm. (a) Pre-cyst; (b) planozygote; (c) vegetative cell.

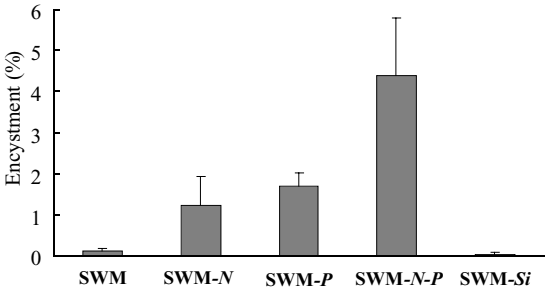

Fig. 2. Encystment percentages in different media. Each point (means ± SD) represents the average of triplicate measurements. Encystment percentage was also calculated by dividing the cyst yield by the maximum yield of motile cells under the assumption that one cyst was formed from one cell.

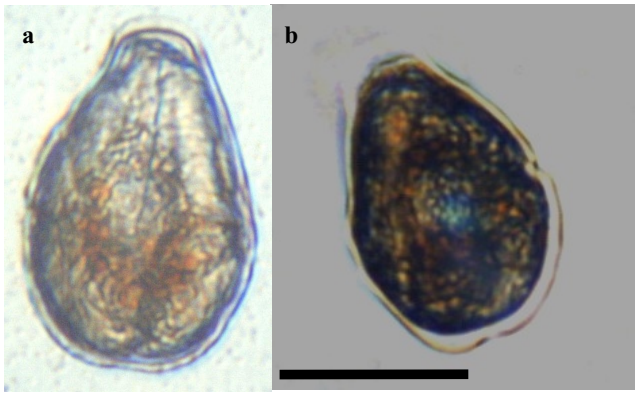

Fig. 3. Cysts produced in SWM-*N* (a) and SWM-*P* (b). Scale bar = 25 μm.

the pre-cyst (Fig. 1a) to be easily distinguished from other life forms such as planozygotes (Fig. 1b) and vegetative cells (Fig. 1c). Uchida *et al*. (1996) reported that pre-cysts transformed into cysts within a few days after they were isolated and placed in fresh medium (modified SWM-3; Imai and Itoh, 1987). Furthermore, we conducted a preliminary experiment in which 180 pre-cysts, formed in SWM-*P*, were isolated in a 48-well plate with 750 μL of SWM-*P* and their morphological changes observed for 3 days. Of the 180 pre-cysts, 152 metamorphosed to cysts, 18 died, and 10 retained the pre-cyst conformation. Because this showed that a significant proportion of pre-cysts are destined to metamorphose into cysts and not into other life forms, we can consider that the formation of pre-cysts represents the induction of encystment. Therefore, in this experiment, we counted pre-cysts suspended in the Erlenmeyer flasks as a measure of encystment instead of counting cysts.

Cultures of *G. instriatum* containing 4000 cells mL^{-1} of motile cells but few pre-cysts (<1 cell mL^{-1}) were con-

centrated by gravity filtration without vacuum with a filter (pore diameter 0.45 μm) to a concentration of 20000 cells mL^{-1}. Concentrated *G. instriatum* sample (2 mL) was inoculated into each 500-mL Erlenmeyer flask with 400 mL of seawater, which originally contained 3.5 μM of DIN and 0.08 μM of DIP, enriched with a chemical component of SWM with different concentrations of NO_3^- (0, 10, 100, or 1000 μM, with a PO_4^{3-} concentration of 50 μM), or PO_4^{3-} (0, 1, 10, or 50 μM, with a NO_3^- concentration of 1000 μM). Three replicate flasks were used for each treatment. *Gyrodinium instriatum* were incubated for 2 weeks at 20°C under 530 μmol photons m^{-2}s^{-1} of cool-white fluorescent illumination on a 12 h:12 h light:dark cycle, and 12 mL of the cell suspension was sampled daily. The reason why conditions of temperature and light intensity were changed from those in the experiment described in Subsection 2.2 was that the encystment percentage at 20°C under 530 μmol photons m^{-2}s^{-1} appeared to be scarcely higher than that a 25°C under 350 μmol photons m^{-2}s^{-1} based on the results of our preliminary experiments. An aliquot of the 12-mL culture (2 mL) was used for counting motile cells and pre-cysts. The remainder was filtered with a 0.22-μm syringe filter, and then frozen (–80°C) for analysis of N and P. $NO_2^- + NO_3^-$ and PO_4^{3-} concentrations were subsequently determined with an autoanalyzer (TRAACS 800, Bran + Luebbe Co., Hamburg, Germany) in accordance with the method of Strickland and Parsons (1968).

2.4 *Observations of excystment of cysts produced in the laboratory*

As noted above, cysts of *G. instriatum* are associated with viscous material and stick to the bottom of the flasks. Therefore, to isolate cysts in this experiment, SWM-*N* including pre-cysts of *G. instriatum* (pre-cysts:total cells = 1:9) was mixed with autoclaved (121°C, 15 min) sediments from Hakozaki Fishing Port in an Erlenmeyer flask. After the suspension had been incubated for 3 days, the surface of sediments deposited on the bottom of the flask was gently taken off with a pipette. Cysts in the suspension (45–55 in number) were isolated individually into four 48-well microplates, each containing 750 μL of SWM, and incubated in four series of 25°C or 10°C and in dim light (80 μmol photons m^{-2}s^{-1}) or in darkness. The cysts at 25°C and dim light were checked for excystment every day from the start of incubation. After the other cysts had been stored under their respective conditions of temperature and light or dark for 16 days, they were moved to 25°C and light, and subsequently checked for excystment every day, as evidenced by the presence of motile cells and opening of the archeopyle. A cyst that completely blanched due to disappearance of protoplasm without opening of the archeopyle was counted as a dead cyst.

2.5 *Observation of excystment of cysts in nature*

Bottom sediments were collected from Hakozaki Fishing Port on 2 July 2004. The surface 0 to 3 cm of a core was cut off and stored at 4°C in darkness for 80 days. In a refrigerated room (4°C), 20 cysts of *G. instriatum* extracted from this stored sediment were isolated into six 48-well microplates, each filled with 750 μL SWM. The cysts were incubated at five temperatures (10, 15, 20, 25, or 30°C) in either dim light (80 μmol photons m^{-2}s^{-1}) or darkness (20°C only). Cysts kept in the light were checked for excystment every 3 days; those kept in the dark were checked on day 12 only.

3. Results

3.1 *Encystment in medium without added N or P*

The maximum yield of motile cells was high in SWM at 32800 cells mL^{-1} (day 15), while in SWM without *Si* (SWM-*Si*) it was 26600 cells mL^{-1}; yield was low in SWM without *N* (SWM-*N*) at 6490 cells mL^{-1} (day 21), in SWM without *P* (SWM-*P*) at 4780 cells mL^{-1} (day 9–12), and in SWM without *N* and *P* (SWM-*N*-*P*) at 4710 cells mL^{-1} (day 9–12). The number of cysts produced was low in SWM (40 cysts mL^{-1} on day 21), and in SWM-*Si* (51 cysts mL^{-1} on day 21), but high in SWM-*N* (75 cysts mL^{-1} on day 9–15) and SWM-*P* (82 cysts mL^{-1} on day 15) and highest in SWM-*N*-*P* (206 cysts mL^{-1} on day 9–12). Expressed as percentages, the encystment frequencies were 0.11%, 1.2%, 1.7%, 4.4% and 0.03%, in SWM, SWM-*N*, SWM-*P*, SWM-*N*-*P* and SWM-*Si*, respectively; the encystment frequency in SWM-*N*-*P* was therefore the highest (Fig. 2). The shape of cysts was the same among media, but, the color of cysts in SWM-*P* was greenish-brown while in other media they were pale (Fig. 3).

3.2 *Relationship between encystment and extracellular N and P*

We tested the relationship between N concentration and encystment (Fig. 4). In the media with N, additional concentrations of 0 (N-0), 10 (N-10), or 100 μM (N-100), the pre-cyst concentration increased within 1 or 2 days after the total motile cell (pre-cysts and other motile cells) concentration had reached the stationary phase. The color of cytoplasm of pre-cysts formed in N-0, N-10 and N-100 is pale greenish-brown. The concentration of N decreased steadily until the growth of motile cells stopped. Within 2 or 3 days after the concentration of N was < 1 μM, pre-cyst numbers increased sharply. There was little or no decrease in P concentration. However, in the medium with an N concentration of 1000 μM (N-1000), no clear surge in pre-cyst concentration was observed, and the remaining concentrations of N and P were respectively 442 and 23 μM on day 13, when the experiment finished.

We then tested the relationship between P concen-

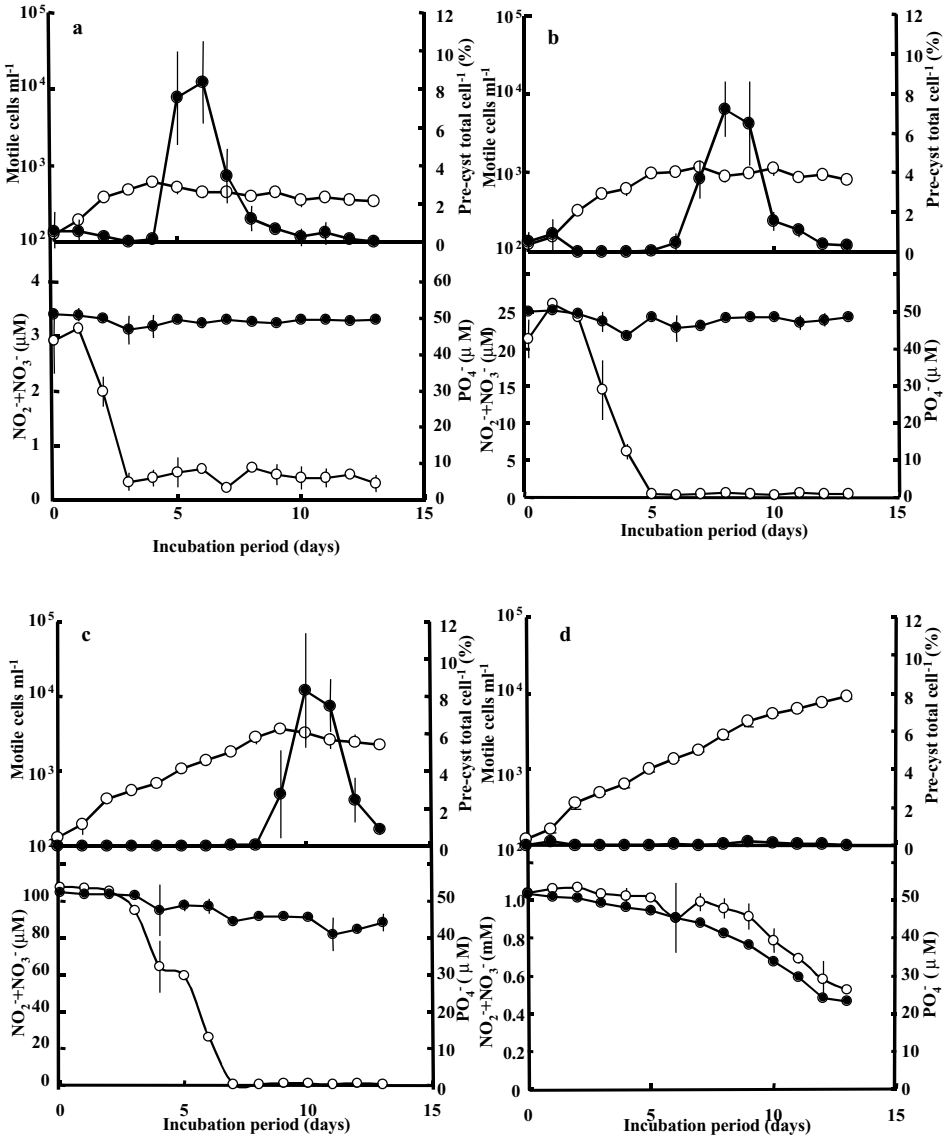

Fig. 4. Time course of changes in motile cells (upper, open circle) and pre-cysts (upper, filled circle) and concentrations of $NO_2^- + NO_3^-$ (lower, open circle) and PO_4^{3-} (lower, filled circle) in N-0 (a), N-10 (b), N-100 (c), and N-1000 (d) μM. Each point (means ± SD) represents the average of triplicate measurements.

tration and encystment (Fig. 5). The concentration of motile cells increased progressively, and the stationary phase was less clearly defined than that in the experiment with N. In media with P concentrations of 0 μM (P-0) and 1 μM (P-1), the number of pre-cysts with dark greenish-brown cytoplasm increased sharply within 3 days after the concentration of P became <0.2 μM. However, the maximum percentage of pre-cysts in P-1 was 2%, which was lower than that in P-0 (6%). No obvious decrease in N concentration was observed in either P-0 or P-1. In the medium with an additional P concentration of 10 μM (P-10), no surge in pre-cyst concentration was observed, although the P concentration had decreased to 0.15 μM by day 10. At P-10, N concentration started to decrease on day 8 and the remaining N concentration was 540 μM on day 13, when the experiments finished. In the medium with additional P concentrations of 50 μM (P-50), again no surge in pre-cyst concentration was observed, and the concentrations of the remaining N and P were respectively 522 and 23 μM on day 13.

3.3 Excystment of cysts produced in the laboratory

We examined the time course of excystment at different temperatures and in the light or dark (Fig. 6). Cysts

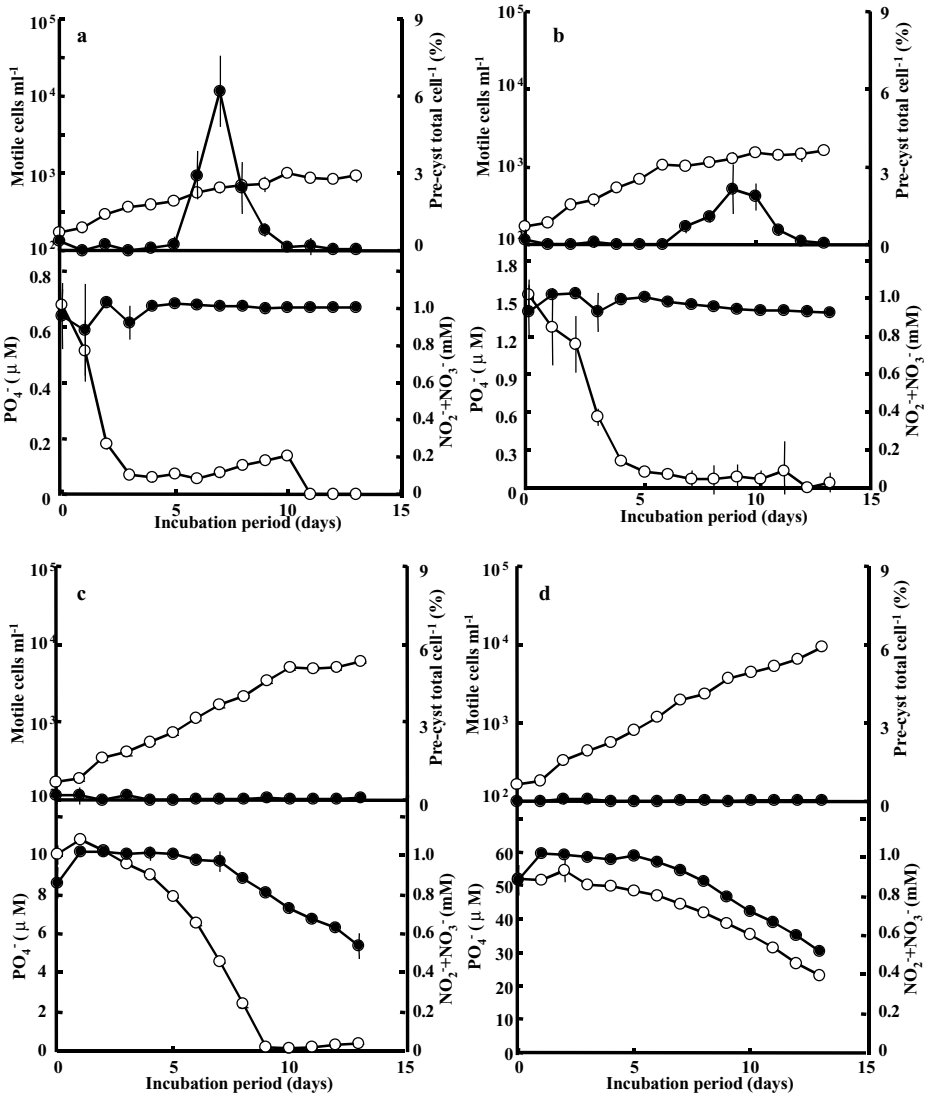

Fig. 5. Time course of changes in motile cells (upper, open circle) and pre-cysts (upper, open circle) and concentrations of PO_4^{3-} (lower, open circle) and $NO_2^- + NO_3^-$ (lower, filled circle) in P-0 (a), P-1 (b), P-10 (c), and P-50 (d) μM. Each point (means ± SD) represents the average of triplicate measurements.

incubated at 25°C in the light excysted from days 8 to 37, a few at a time. In the cysts stored at 25°C in the dark, at 10°C in the light, or at 10°C in the dark, excystment was observed from days 16 to 39, days 20 to 35, and days 19 to 36, respectively. Thereafter, the cysts continued to excyst slowly, similarly to the cysts at 25°C in the light. The final excystment percentage at 10°C in the dark was higher (48.9%) than that at 25°C in the light (28.6%), at 25°C in the dark (22.9%), or at 10°C in the light (25.5%). Concurrently, the number of dead cysts also increased progressively. Finally, all cysts that did not excyst died without opening the archeopyle by the end of the experiments.

3.4 Excystment of cysts in nature

Most cysts excysted at temperatures in ≥20°C within 3 days, but it took 9 days to excyst at 15°C and at 10°C (Fig. 7). The final excystment percentages at 10, 15, 20, 25, and 30°C were 70%, 65%, 80%, 65%, and 55%, respectively; hence no systematic relationship was observed between incubation temperature and excystment in the light. All cysts that could not excyst in the light died without opening the archeopyle. The excystment rate at 20°C in the dark was 65%, with 5% of cells remaining without excystment; these values compared with those occurring at 20°C in the light.

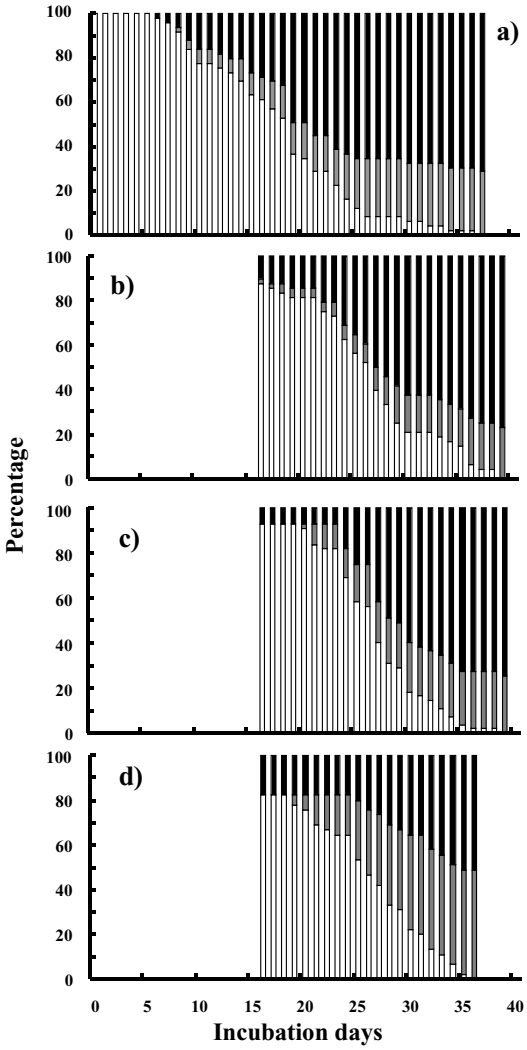

Fig. 6. Time course of changes in percentages of cysts (white), excysted cysts (gray), and dead cysts (black) after incubation at 25°C and in continuous light (a), and after storage at 25°C in the dark (b), or at 10°C in the light (c), or dark (d).

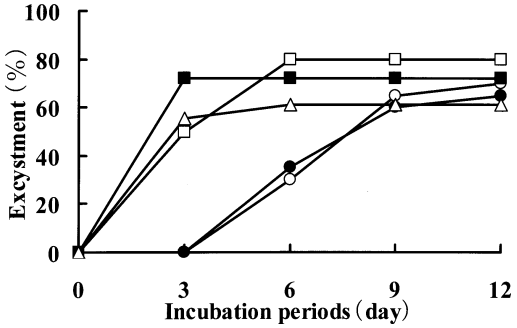

Fig. 7. Time course of excystment of natural cysts at temperatures of 10 (open circle), 15 (filled circle), 20 (open square), 25 (filled square) and 30°C (open triangle). Each point represents a percentage of the total number of isolated cysts.

4. Discussion

We investigated the effects of N and P deficiency on encystment in *Gyrodinium instriatum* in batch culture. It is already well known that N and P deficiencies are important triggers of encystment in dinoflagellates (Pfiester, 1975, 1976, 1977; Walker and Steidinger, 1979; Yoshimatsu, 1981; Sako *et al*., 1984, 1987; Anderson *et al*., 1985).

We found encystment in all the media studied. However, the encystment frequency was much higher in SWM-*N*, SWM-*P*, and SWM-*N*-*P* than in SWM or SWM-*Si* (Fig. 2). The color of the cysts formed in SWM-*P* was different from those formed in other media (Fig. 3), suggesting that the process of encystment induced by P deficiency may be different from that induced by N deficiency. These results show that either N or P deficiency can a trigger encystment in *G. instriatum*, but encystment may be furthermore promoted by both N and P deficiency as the encystment percentage in SWM-*N*-*P* was higher than those in either SWM-*N* or SWM-*P*.

To investigate the relationship between N or P concentration and encystment, we followed the progress of encystment in relation to N and P concentration. In the experiment where N concentrations varied, the pre-cyst concentration sharply increased within a few days of extracellular concentration of N in N-0, N-10, and N-100 decreasing to <1 μM (Fig. 4). In contrast, in N-1000, in which the extracellular concentration of $NO_2^- + NO_3^-$ remained high, no surge of pre-cysts occurred (Fig. 4). Organic nitrogen was not measured, but it is already known that *G. instriatum* cannot utilize organic nitrogen for growth (Nagasoe, 2006), and we found that a concentration of N (i.e. inorganic nitrogen) < 1 μM promotes *G. instriatum* encystment.

In the experiment where P concentrations varied, a surge in pre-cyst concentration occurred in P-0 and P-1 within 3 days of P concentrations decreasing to <0.2 μM (Fig. 5). Although *G. instriatum* can utilize some organic phosphorus (Nagasoe, 2006), the organic phosphorus concentrations in open seawater (used as the base of our medium) were less than the detection limit (0.01 μM). Hence, a low concentration of P (<0.2 μM) may be also trigger *G. instriatum* encystment. However, the maximum percentage of pre-cysts in P-1 was lower than that in P-0 (Fig. 5). Moreover, although P decreased to <0.2 μM in P-10, no surge in pre-cyst concentration was apparent in this case (Fig. 5).

Another species of *Gyrodinium*, *G. uncatenum* Hulburt, forms planozygotes and partly encysts after extracellular nutrient levels decrease below the detection limit and the intracellular pool of nutrients has been de-

Table 2. Variations of nutrient cell quota for one cell in medium different from the initial concentration of N and P in the experiment shown in Figs. 4 and 5.

Medium	Day	N cell^{-1} (nmol)	Medium	Day	P cell^{-1} (nmol)
N-0	2	3.02	P-0	2*[4]	0.87
	3*[1]	3.49		3	0.23
	4	trace*[5]		4	0.02
	5*[2]	trace*[5]		5	trace*[5]
				6*[2]	0.04
N-10	4	13.8	P-1	3	1.08
	5*[1]	5.73		4*[4]	0.60
	6	0.15		5	0.08
	7*[2]	trace*[5]		6	0.03
				7*[2]	0.03
N-100	6	24.0	P-10	9*[4]	0.61
	7*[1]	14.4		10	0.02
	8	trace*[5]		11	trace*[5]
	9*[2]	trace*[5]		12	trace*[5]
				13*[3]	trace*[5]
N-1000	10	14.7	P-50	9	0.85
	11	14.5		10	0.58
	12	6.48		11	0.65
	13*[3]	10.9		12	0.59
				13*[3]	0.43

*[1]The day when extracellular $NO_2^- + NO_3^-$ concentration decreased to <1.0 μM.
*[2]The day when pre-cysts increased.
*[3]The final day of this experiment.
*[4]The day when extracellular PO_4^- concentration decreased to ≤0.2 μM.
*[5]"Trace" means that it was a negative value.

pleted (Anderson *et al*., 1985). Although we did not measure the intracellular nutrient quota, we roughly estimated nutrient quota (Q; nmol cell^{-1}) distributed to a cell on a sampling day (t) from the experimental results shown in Figs. 4 and 5 by the following equation:

$$Q = \frac{S_{t-1} - S_t}{N_t}, \quad (1)$$

where S is N or P concentration (μmol L^{-1}) and N is cell density (cells mL^{-1}). The calculation results are shown in Table 2. The N quota distributed to one cell suddenly decreased to a low level on the previous day before pre-cysts sharply increased in N-0, N-10 and N-100, after which a surge of pre-cysts was observed. Similarly, in P-0 and P-1, the P quota suddenly decreased to a low level two or three days before the surge of pre-cysts. However, in P-10, P quota decreased to a low level but the surge of pre-cysts did not occur on the following days. Therefore, the encystments in media different from initial N concentrations in *G. instriatum* might be related to deficiency of intracellular N quota by low extracellular concentration. On the other hand, the encystments in media different from initial P concentrations might not be directly induced by deficiency of intracellular P quota resulted from low extracellular P concentrations, or may not occur with an extra- and intra-cellular P deficiency alone. Nagai *et al*. (2004) found that cyst yields of *Alexandrium tamarense* were higher with lower N and P concentrations, but higher in higher metals concentrations. In the batch incubation used in our experiments, together with N and P, concentrations of other chemical components such as metals and vitamins will also decrease gradually. Moreover, differences in cyst color lead us to assume that encystments in low N and P concentrations may result from different metabolic path ways, and there is a possibility that N deficiency by itself induced encystment, but encystment by P-deficiency also requires a sufficient amount of the other chemicals such as metals.

However, Uchida *et al*. (1996) succeeded in producing cysts of *G. instriatum* without controlling N or P lev-

els in the medium. In our experiments, although its frequency was lower than with low N and P concentrations, encystments actually occurred, even with high N and P concentrations. As ascribed by Uchida *et al*. (1996), an increase in the number of cells and the pheromones that they excreted can also trigger encystment of *G. instriatum*.

The mandatory dormancy period of *G. instriatum* cysts in the N-limited medium was short, as in *Gymnodinium catenatum* (Blackburn *et al*., 1989) and *Alexandrium catenella* (Joyce and Pitcher, 2006), but it showed individual variation (Fig. 6). Moreover, the dormancy period of *G. instriatum* cysts was scarcely affected by temperature, like *Gymnodinium catenatum* (Bravo and Anderson, 1994), or light changes. But this is unlike the cysts of many other dinoflagellates, such as *Alexandrium tamarese* (Anderson and Wall, 1978), *Peridinium* sp. (Endo and Nagata, 1984), *Ceratium hirundinella* and *Peridinium aciculiferum* (Rengefors and Anderson, 1998). The excystment percentage of cysts stored at low temperature (10°C) and in the dark was higher than in cysts stored at high temperature or in light, and the tendency was similar to the percentage of cysts produced in nutrient-rich medium (Uchida *et al*., 1996). However, the rates of germination and survival of cysts we found were higher than those found by Uchida *et al*. (1996). This may be caused by differences in the ways cysts were prepared; we created cysts under much lower nutrient concentrations, and, unlike Uchida *et al*. (1996), we created cysts attached to sediment particles. Cysts formed and stored under low nutrient concentrations and attached to sediment particles, which are close to conditions in the field, may have a higher survival and germination potential. Moreover, the different strains may also be related to the difference in germination and survival rates of cysts.

Cysts after mandatory dormancy (mature cysts) collected in the field excysted with similar percentages at different temperatures (Fig. 7), whether they were stored in the light or dark. Germination rates of natural cysts were higher than those of cysts formed in the laboratory, but it should be stated that death rates of cysts in the laboratory were higher than those of natural cysts. The deaths of cysts immediately following encystment as occurred in the laboratory (Fig. 7), usually occur even in the field but we might simply not observe the process in natural cysts because we tried to observe the germination of natural cysts after storage for a long period. Our results confirm that *G. instriatum* has a wide "temperature window" for excystment (Anderson and Rengefors, 2006) and an ability to excyst in the dark. Excystment of this organism occurred at low temperatures (10 and 15°C), but was delayed, as was also observed in the raphidophyte *Heterosigma akashiwo* (Shikata *et al*., 2007). In nature, if the temperature is >20°C, mature cysts of *G. instriatum* might excyst synchronously.

Like many other inshore plankton organisms, such as the dinoflagellates *Alexandrium* spp. (Wyatt and Jenkinson, 1997; Anderson *et al*., 2005) and *Pyrodinium bahamense* (Azanza *et al*., 2004), the raphidophyte *Heterosigma akashiwo* (Itakura *et al*., 1996), tintinnids (Kamiyama and Tsujino, 1996), diatoms (McQuoid and Hobson, 1996), and copepods—notably *Acartia* spp., *Eurytemora affinis* (Poppe), *Temora longicornis* Müller and *Centropages hamatus* (Lilljeborg) (Katajisto, 1996; Engel and Hirche, 2004), *G. instriatum* is meroplanktonic. Although the encystment percentage of *G. instriatum* (c.a. 4–5% in SWM-*N-P* at 25°C under 350 μmol photons $m^{-2}s^{-1}$ shown in Fig. 2) was lower than that (10–20%) of many dinoflagellates (Anderson *et al*., 1984; Binder and Anderson, 1987; Montresor and Marino, 1996), they all encyst when extracellular N and P nutrient levels fall to concentrations too low to support vegetative reproduction (Pfiester, 1975, 1976, 1977; Walker and Steidinger, 1979; Yoshimatsu, 1981; Sako *et al*., 1984, 1987; Anderson *et al*., 1985). From the moment the vegetative forms can no longer grow, they quickly produce pre-cysts and subsequently sticky benthic cysts when the water column becomes temporarily unfavorable, as under such conditions they risk predation, parasitic or pathogenic infection, and/or lateral advection to a permanently unfavorable sea area. Our findings are compatible with the widely distributed seed-bed strategy (Wyatt and Jenkinson, 1997; Garcés *et al*., 2004; Anderson *et al*., 2005) in many species of dinoflagellate (e.g. *Alexandrium* spp.). Moreover, *G. instriatum* can mature in short periods without low temperature and dark treatment and can excyst over a wide range of temperatures, whether in the light or dark. This organism can excyst more frequently than many other dinoflagellates, which require mandatory dormancy periods which are often long (Rengefors and Anderson, 1998; Kremp and Anderson, 2000), or which have a narrow "temperature window" (Anderson and Rengefors, 2006). However, the individual variation in the dormancy period, as well as variation in the delay in excystment at low temperatures, would prevent synchronization of cyst germination. Moreover, the viscous and sticky properties of cysts allow the cysts to attach to particles such as bottom sediment and sinking particles, and may become isolated from the overlying, oxygen-rich water, and therefore suffer germination inhibited by oxygen deficiency, as generally found in dinoflagellates (Anderson *et al*., 1987). Finally, these would act to reduce "wastage" of the seed population caused by weakness of regulation by temperature and light on cyst germination in sediments (Shikata *et al*., 2007). Such wastage by mismatch in timing between excystment and the optimum in environmental resources is comparable to the wastage or failure of fish stocks caused by a similar mismatch between timing of fish-egg hatching and that of

the occurrence of their food (Cushing and Horwood, 1994). Although the seeding strategy of this organism is typical of dinoflagellates, the germination strategy may be less so.

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