J. Phycol. **41,** 74–83 (2005)

DOI: 10.1111/j.1529-8817.2005.04045.x

A STUDY OF THE SEXUAL REPRODUCTION AND DETERMINATION OF MATING TYPE OF *GYMNODINIUM NOLLERI* (DINOPHYCEAE) IN CULTURE[1]

Rosa Isabel Figueroa and Isabel Bravo[2]

Instituto Oceanogràfico Vigo, Cabo Estai-Canido, 36200 Vigo, Spain

Sexual reproduction of *Gymnodinium nolleri* (Ellegaard & Moestrup 1999) was studied by intercrossing experiments in all combinations of six clonal strains and backcrossing of five clonal F1 offspring. The results indicated that the conjugation of *G. nolleri* responded to the existence of more than two sexual types (complex heterothallism) and that compatibility between progeny of one cyst (inbreeding) was the rule. Sexual fusion, planozygote formation and development, cyst formation, and germination and planomeiocyte division were followed using time-lapse photography. This study revealed many similarities between the sexual stages and life cycle pattern of *G. nolleri* and the related *G. catenatum* and the existence under culture conditions of an alternative cycle between vegetative cells and zygotes without a hypnozygote stage. The fate of zygotes, division or encystment, was influenced by the nutritional status of the external medium. The division of *G. nolleri* planozygotes was promoted by high levels of external nutrients, whereas the maximum percentage of encystment was recorded when phosphates were reduced in the isolation medium. The division of zygotes might be different from both vegetative and planomeiocyte division because it resulted in two-cell chains with the cells not oriented in parallel.

Key index words: **Dinophyceae; encystment; gametes; *Gymnodinium nolleri*; life cycle; reproduction**

Abbreviations:**AV, average vigor; CI, compatibility index; RC, reproduction capability**

Living microreticulate cysts in northern Europe were first found in 1987 (Ellegaard et al. 1993). The excysted naked dinoflagellate, subsequently named *Gymnodinium nolleri* Ellegaard & Moestrup, was previously misidentified as the toxic chain-forming *G. catenatum* Graham. Microreticulate cysts have been reported from an increasing number of sites worldwide (Bolch and Reynolds 2002), and three microreticulate cyst-forming *Gymnodinium* species are now known: *G. catenatum* (Anderson et al. 1988), *G. nolleri* (Ellegaard and Moestrup 1999), and *G. microreticulatum* Bolch and Hallegraeff (Bolch et al. 1999). *Gymnodinium catenatum* is the only one that usually produces paralytic shellfish toxins (Ellegaard and Oshima 1998). Previous studies considered various aspects of the biology of the three species, such as cyst and cell morphology, reticulation pattern of cysts, pigments, and DNA sequence data. Molecular genetic studies situated *G. nolleri* closer to *G. catenatum* (Bolch et al. 1999). Notwithstanding their genetic proximity and morphological similarities, the two species show several differences: *G. nolleri* never forms chains longer than two cells, whereas *G. catenatum* produces long chains (Ellegaard et al. 1993, Nehring 1995); vegetative and cyst stages of *G. nolleri* are smaller and nontoxic (Ellegaard et al. 1998); and there are consistent differences at molecular and genomic levels (Ellegaard and Oshima 1998).

The relevance of sexuality and encystment in the life cycles and ecology of dinoflagellates has often been proved, because excystment is a process associated with bloom initiation, species dispersal, and genetic diversity (Wall 1971, Anderson and Wall 1978, Anderson et al. 1984). In this sense, having a detailed record of these processes is a fundamental aspect. Nevertheless, the life cycle of *G. nolleri* has never been studied in detail, and it is unknown whether there are differences between *G. nolleri* and *G. catenatum* in sexual stages morphology and mating behavior. The present work describes the sexual life cycle of *G. nolleri*, revealing similarities to, but also differences from, the life cycle pattern of *G. catenatum*.

MATERIALS AND METHODS

Strain isolation and maintenance. Strains of *G. nolleri* were isolated from individual wild cysts from Danish and Swedish waters (Table 1); VGO 632, VGO 633, and VGO 634 were from the culture collection of the Centro Oceanogràfico de Vigo and DK5, DK6, and 922I were kindly provided by Dr. M. Ellegaard, University of Copenhagen. Cultures were grown at 20° C, approximately 90 µmol photons $\cdot m^{-2} \cdot s^{-1}$, and a 12:12-h light:dark cycle. Culture stocks were maintained in Erlenmeyer flasks filled with 50 mL of L1 medium (Guillard and Hargraves 1993) without silica addition, prepared with Atlantic seawater adjusted to a salinity of 31 psu by the addition of sterile double-distilled water. Before running the experiments, one clonal strain was established from each of the cultures listed in Table 1; these strains are designated with the suffix "c".

The fate of fusing gamete pairs. Eighty-five fusing pairs from the cross DK5cxF1′c (F1′c is a clonal strain established by the isolation of one cell from the germination of one cyst of the cross DK5cxDK6c) in early stages of fusion joined with the girdles more or less perpendicular to each other by the sulcal

[1]Received 31 March 2004. Accepted 27 October 2004.

[2]Author for correspondence: e-mail isabel.bravo@vi.ieo.es.

TABLE 1. Parental strains of *Gymnodinium nolleri*.

Strain name	Source	Year of isolation	Culture origin	Isolator
VGO 632	Gullmar (Sweden)	2003	All products of a wild resting cyst	Present work
VGO 633	Gullmar (Sweden)	2003		Present work
VGO 634	Gullmar (Sweden)	2003		Present work
922I	Kattegat (Denmark)	1998		Ellegaard et al. (1998)
DK5	Øresund (Denmark)	1998		Ellegaard et al. (1998)
DK6	Øresund (Denmark)	1998		Ellegaard et al. (1998)

region were individually isolated, separately transferred to wells of tissue culture plates (6.4 mm diameter, Iwaki, Chiba, Japan) filled with fresh medium, and placed under the same conditions described previously for culture maintenance. The gamete pairs were crossed with an angle between 0 and 90 degrees, whereas dividing cells were parallel with each other; the two stages were thus impossible to confuse. Four different isolation media were used; L1 medium (L1), L1 medium without nitrates added (L-N), L1 medium without phosphates added (L-P), and L1 medium without nitrates or phosphates added (L-N-P). The evolution of the isolated pairs, planozygotes, and cells from cyst germination was examined at least daily during the time needed for them to fuse, encyst, or divide. Between 15 and 20 individuals of each sexual stage were photographed and measured at 630 × magnification using an inverted microscope (Axiovert 135, Zeiss, Jena, Germany) and an Image IPplus analyser (Media Cybernetics, Silver Spring, MD, USA). The minimum dormancy (minimum time from cyst formation to germination for any individual cyst) was also recorded for 27 cysts formed by the encysting planozygotes of the cross DK5cxF1′c by checking their germination every 2 days from the first day of formation to the day of the last germination.

Mating type and reproductive compatibility analysis. *Mating system:* Intercrosses and intracrosses (self-crosses) among all strains were conducted in duplicate sterile polystyrene petri dishes (16 mm diameter, Iwaki) filled with 3 mL of L1 medium with a 1:30 dilution of phosphates (P/30) and inoculated with exponentially growing cells (4000–6000 cells · mL^{-1}) to a final concentration of 700 cells · mL^{-1} (350 cells · mL^{-1} from each compatible strain). Cyst counting was performed at days 10, 15, 20, and 30 after crossing, and the highest cyst production was recorded for each cross. All cysts in each well were counted. Negative results were verified in a duplicate cross. Cyst production results were scored according to the criteria shown in Table 2.

For the study of the F1 generation, five clonal strains were established by the isolation of individual cells from the progeny of five cysts from the cross DK5cxDK6c. Duplicate backcrosses between the clonal F1 offspring with the parental strains were performed twice to verify the sexual pattern of the strains using the same experimental conditions described above. The cyst production recorded was the mean of four values.

TABLE 2. Scoring criteria for cyst production in *Gymnodinium nolleri* and equivalent concentration (based on Blackburn et al. 2001).

Score	Cyst concentration (cysts · L^{-1})
0	0
0*	0–3.0×10^2
1	$>3.0 \times 10^2$ to 2.0×10^3
2	$>2.0 \times 10^3$ to 1.0×10^4
3	$>1.0 \times 10^4$ to 1.0×10^5
4	$>1.0 \times 10^5$

Inbreeding: The nonclonal parental strains obtained from the germination of one cyst (Table 1) and strains obtained from the germination of cysts produced by crosses DK5cx922Ic and DK5cxDK6c were crossed to test intraclonal cyst production. The production of cysts was monitored as illustrated above. To make the interpretation of the experiments easier, we summarized the origins and uses of all the established strains in Table 3.

Reproductive compatibility: Three indices of reproductive success (Blackburn et al. 2001) were calculated from the data obtained in the studied crosses showed in Table 4:

- Compatibility index (CI): The number of compatible pairings resulting in a score ≥ 1 divided by the total number of possible crosses other than self-crosses
- Average vigor (AV): The average of the scores (0–5) for maximum cyst production for all successful crosses involving a particular strain
- Reproductive compatibility (RC): Calculated as the product of the CI and AV values

RESULTS

General description of sexual reproduction. Three days after crossing the parental strains, mating of gametes was observed. *Gymnodinium nolleri* gametes were morphologically indistinguishable from vegetative cells (hologamy), and both unequal-sized (anisogamy) and equal-sized fusing pairs (isogamy) were observed, though isogamy was more common. Fusing gametes could either be joined equatorially (Fig. 1a) or with the girdles more or less perpendicular to each other (Fig. 1b). In both cases, the point of initial contact was the sulcal region. These paired cells could persist for up to 8 days. Approximately 5 days after crossing, large, motile, heavily pigmented cells with dual trailing flagella (planozygotes) were observed (Fig. 1c). Transformation of the planozygote into a hypnozygote (resting cyst) involved loss of motility, shrinkage of cell contents, and the development of a thick wall within the outer membrane of the planozygote (Fig. 1d). The resting cyst of *G. nolleri* is brown, spherical (Fig. 1e, 21–43 μm diameter), or oval with a distinctive reticulate ornamentation. Hypnozygotes had a dormancy period of 33 ± 7 days. Cyst germination began by the drawing away of the protoplast from the cyst wall. The cyst wall ruptured (Fig. 1f), usually along the paracingular margin, and a single germling cell emerged inside a clear membrane formed by the inner cyst wall (Fig. 1g). The germling rotated inside this membrane for 5–20 min until it was finally released by the rupture

TABLE 3. Origins and uses of all the established strains.

Origin	Designation		Initial stage	Used for
Six original parental strains (first isolation)	See Table 1	Nonclonal	Cyst	Inbreeding (Table 6); Figure 1
One cell from each parental strain (second isolation)	"c" strains	Clonal	Vegetative cell	Mating type determination (Table 4); Backcrosses (Table 5)
From the germination of a cyst produced by crossing clonal parental strains (third isolation)	Three strains F and five strains F′	Nonclonal	Cyst	Inbreeding (Table 6)
From the isolation of a cell of the F and F′ strains (fourth isolation).	One strain Fc and five strains F′c	Clonal	Vegetative cell	Mating type determination (Table 4); Backcrosses (Table 5)
Cross of DK5c and F1′c	None	Nonclonal	Vegetative cells	Figures 2 ,3, and 5

of the bag. The resulting cell was characterized by a large size (41–63 μm long, 31–40 μm wide) ovoid shape with either a flattened or a pointed apex and a double longitudinal flagellum (Fig. 1h). A small orange accumulation body was often observed. Within 24 h to 4 days the planomeiocyte divided. This division was preceded by the striation and widening of the cell base, a feature that was observed for up to 3 days (Fig. 1i). The division process of the planomeiocyte (Fig. 1j) occurred by oblique binary fission and was similar to vegetative reproduction (Fig. 1k). The resulting two-cell chain had the apparently morphology of vegetative cells (Fig. 1l), though they usually were formed by larger cells that subsequently separated in two individual cells.

The fate of fusing gamete pairs. Figure 2 shows the sexual cycle of *G. nolleri*. The routes described in this drawing were established after monitoring the fusion and subsequently development of 85 fusing gamete pairs. The next description analyzes each step of this cycle.

Planozygote encystment: The fusion of gamete pairs (gametes, 31–38 μm long and 27–35 μm wide) was completed in less than 12–48 h after gamete pairing. Fusion of the basal areas and alignment of the cingula were the first processes in completing planozygote formation (Fig. 3, a–c). The young planozygote (first day from complete gamete fusion) was a longer (35–43 μm long, 27–30 μm wide) biconical cell with slightly flattened apices and double longitudinal flagellum (Fig. 3d). Older planozygotes (36–54 μm long, 28–38 μm wide) changed in shape, first elongating and then becoming more or less subspherical before finally undergoing encystment after 6–12 days (Fig. 3, e and f).

Planozygote division: Young planozygotes could either follow the general route described above and undergo encystment or divide within 4–7 days after the completion of gamete fusion, as shown by the sequential photographs (Fig. 4). Planozygote formation (Fig. 4, a–c) underwent the same steps as in the encystment case. Most of the planozygote divisions resulted in two-cell chains with cells oriented in a different way as compared with a normal vegetative chain. The cells were joined at a more or less perpendicular angle by the sulcal region of one cell and the apical region of the other. By means of chain rupture and subsequent division of its individual cells, a viable culture was produced. Figure 4e was obtained after the division of the planozygote in Figure 4d. Figure 4f corresponds to the product of a different planozygote division. In this latter case, the chain angle was slightly less than 90 degrees. This kind of chain was also observed in the original culture plates of gamete isolation, where no artifacts due to manipulation could be responsible of such process. Although not observed very often, planozygotes could also undergo a multiple division originating three cells (Fig. 5, a and b), never in a chain, a process that also resulted in a viable culture.

The percentage of planozygotes that underwent division was influenced by the nutritional status of the isolation medium, significant differences ($P < 0.05$) being found between media with or without phosphate limitation. Only 5%–8% of the planozygotes underwent encystment under replete phosphate concentrations (L1 and L-N media), whereas this percentage was between 45% and 55% in media with no phosphates added (L-P and L-N-P medium) (Fig. 6).

Mating type and reproductive compatibility analysis
Mating system: The matrix of intercrossing and selfcrossing compatibility for all possible crosses is presented in Table 4. Two main features were verified from these results: 1) Clonal strains were never selfcompatible, and 2) mating behavior did not respond to a simple + / − sexuality (e.g. DK5c was highly

TABLE 4. Results of *Gymnodinium nolleri* intercrossing experiments (see Table 2 for scoring criteria).

Strain	DK5c	VGO 632c	VGO 633c	F1′c	F1c	VGO 634c
DK5c	0	4	3	0	4	1
VGO 632c	4	0	4	4	4	4
VGO 633c	3	4	0	3	3	0
F1′c	0	4	3	0	3	1
F1c	4	4	3	3	0	3
VGO 634c	1	4	0	1	3	0

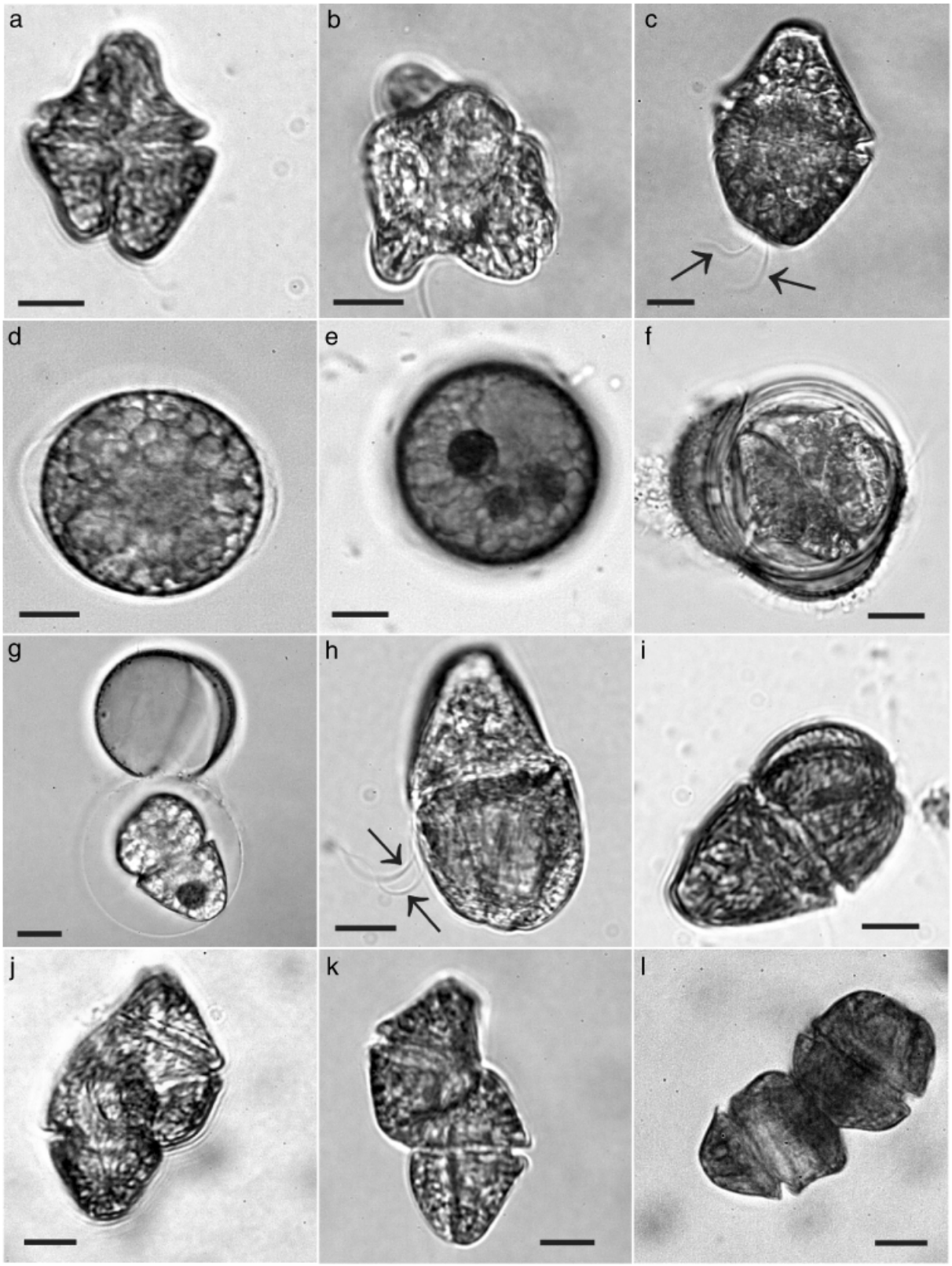

FIG. 1. Light micrographs of *Gymnodinium nolleri* sexual stages. (a) Equatorial and anisogamus fusing gamete pair. (b) Perpendicular fusing gamete pair. (c) Culture planozygote showing two longitudinal flagella (arrows). (d) Precyst stage. (e) Hypnozygote with three red spots. (f) Cyst germination. (g) Newly germinated planomeiocyte inside the excystment bag. (h) Planomeiocyte showing two longitudinal flagella (arrows). (i) Planomeiocyte showing striated forms in its base. (j) Planomeiocyte division. (k) Vegetative division. (l) Two cell chain formed by planomeiocyte division. Scale bars, 10 μm.

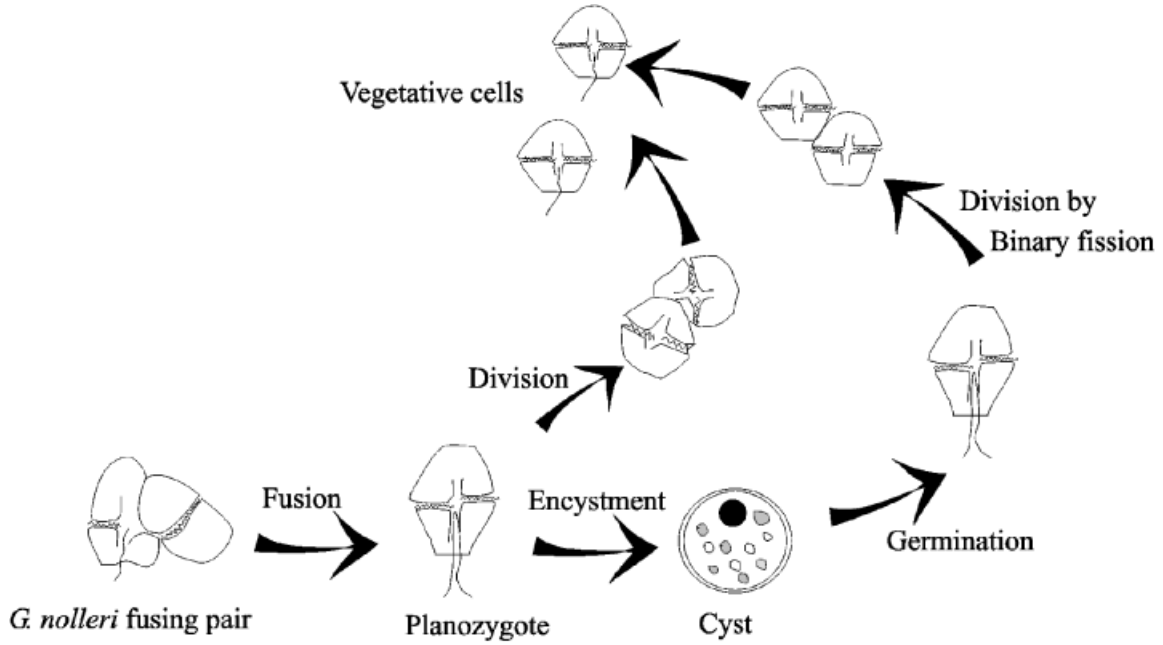

FIG. 2. *Gymnodinium nolleri* sexual cycle.

compatible with both VGO 632c and VGO 633c strains, which were also highly compatible between one another). This fact was also observed in the experiments testing sexual compatibility behavior of the offspring (Table 5). Clonal F1 offspring formed by the cross DK5cxDK6c presented different mating

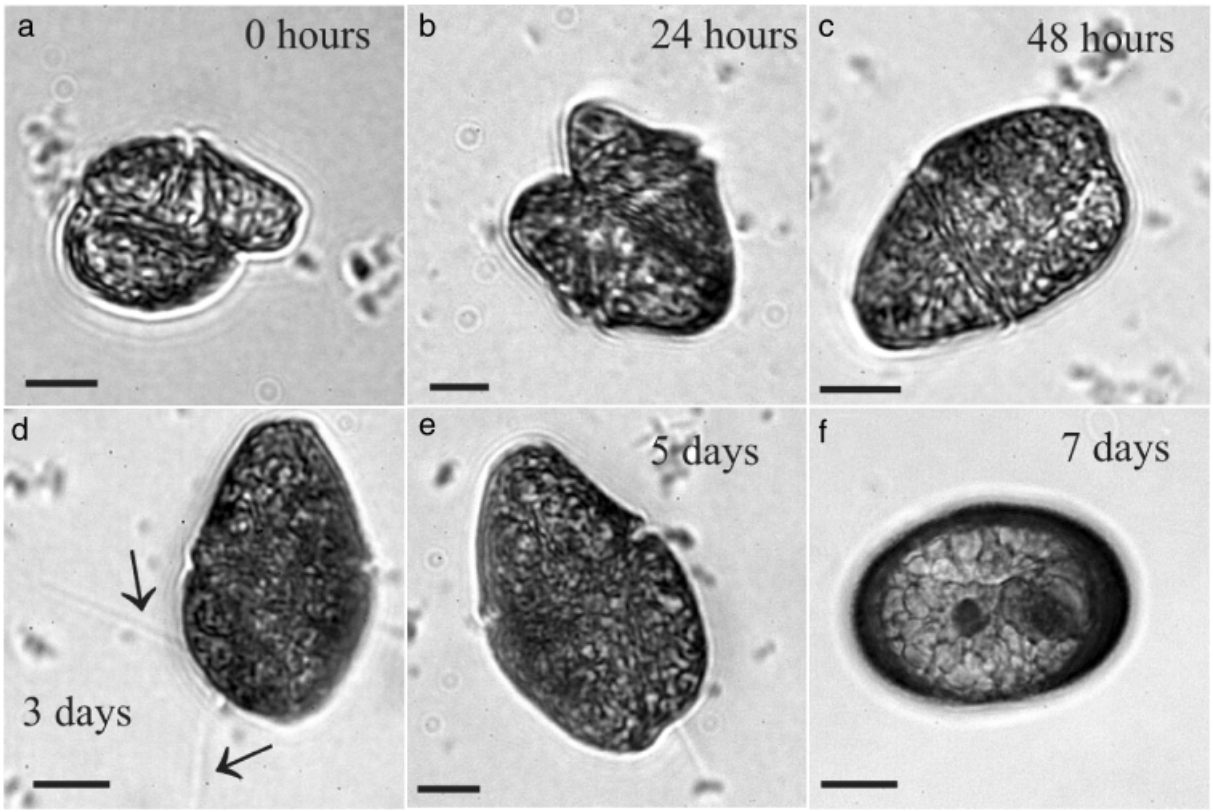

FIG. 3. Time-lapse photography of a fusing *Gymnodinium nolleri* gamete pair encystment. Only the more remarkable photographs are shown. (a) Isolated fusing pair forming 90 degrees between each other. (b) Partial gamete fusion. (c–e) Evolution over time of the planozygote morphology (the two training flagella are indicated by arrows). (f) Oval cyst with two red spots. Scale bars, 10 μm.

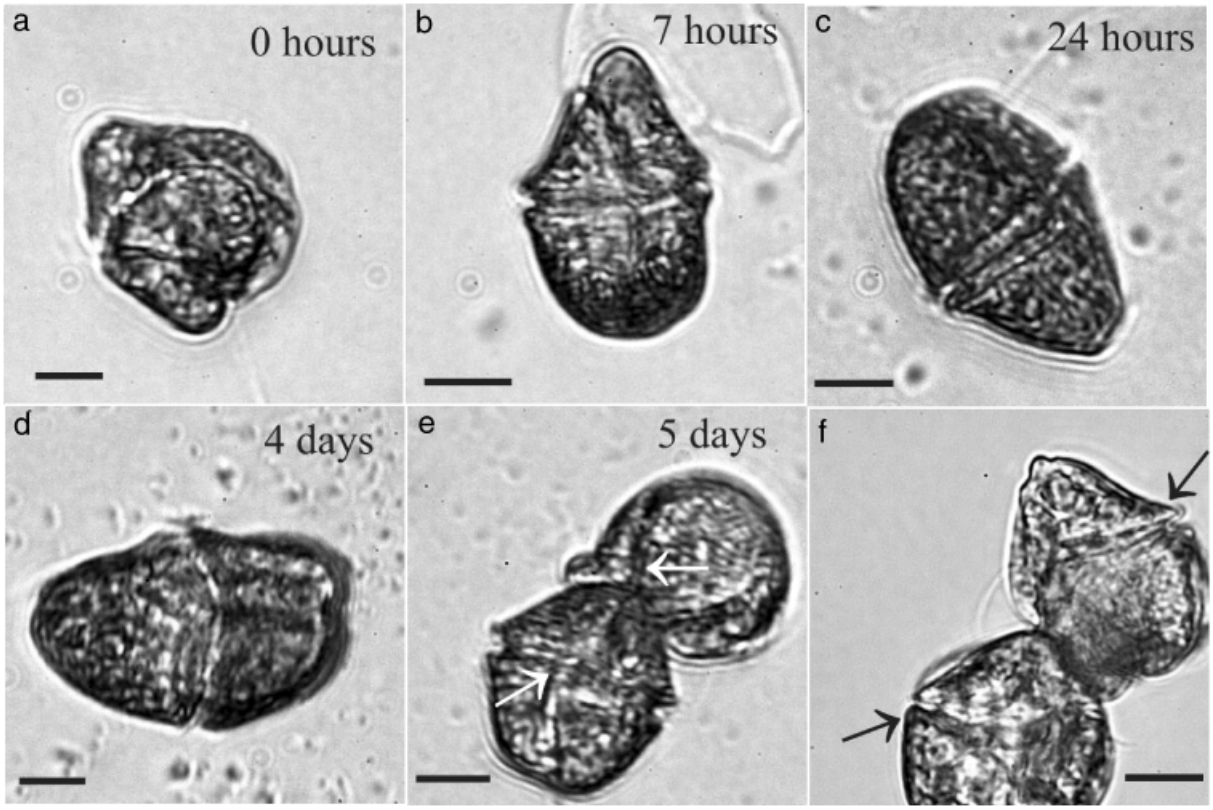

FIG. 4. Time-lapse photography of a *Gymnodinium nolleri* gamete pair fusion and planozygote division. Only the more informative photographs are shown. (a) Isolated fusing gamete pair forming an angle between 0 and 90 degrees. (b) Partial gamete pair fusion. (c and d) Evolution in time of the planozygote morphology. (e) Chain obtained from the division of the planozygote (d) (sulcal regions indicated by arrows). (f) Detail of the join between the two cells of a chain formed by planozygote division (sulcal regions indicated by arrows). Scale bars, 10 μm.

affinity covering three possibilities: 1) incompatibility or very low compatibility with both parental strains (F4′c), 2) high or low compatibility with only one of the parents (F1′c, F3′c, and F5′c), or 3) compatibility with both parental strains (F2′c). These experiments were performed four times and the same compatibility results were recorded, thus showing that each of the clonal strains has a fixed characteristic sexual pattern.

Inbreeding: Intracrosses (self-crosses) of parental strains ("Parents" in Table 3), showed that two (VGO 633 and VGO 632) of the six strains were self-com-

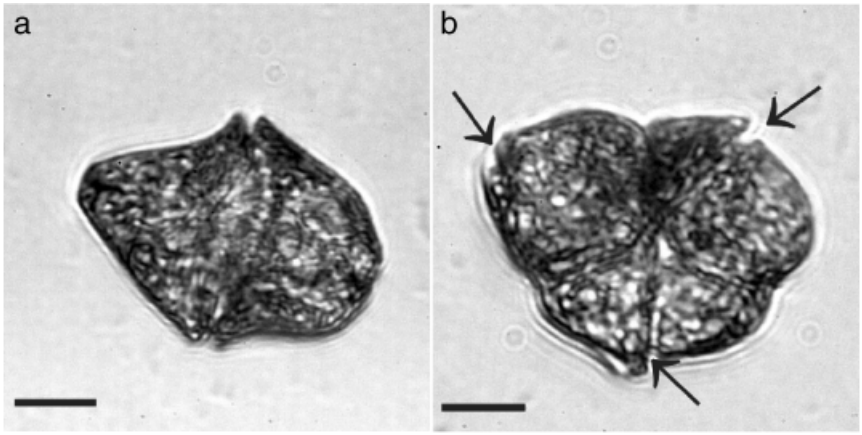

FIG. 5. *Gymnodinium nolleri* planozygote and the process of its multiple division. (a) Planozygote formed by isolated fusing gametes (original fusing pair is not shown). (b) Planozygote multiple division. The arrows indicate the sulcal regions of three cells. Scale bars, 10 μm.

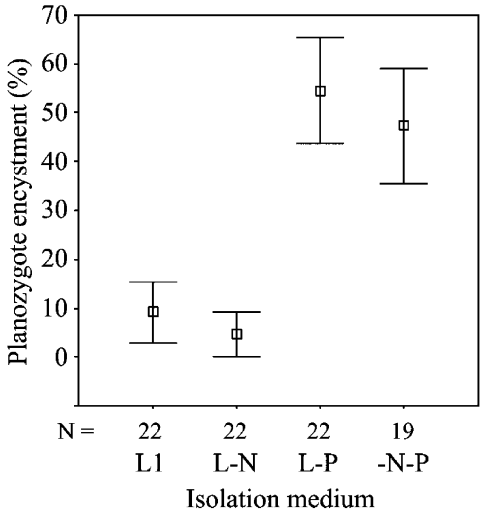

FIG. 6. *Gymnodinium nolleri* planozygote encystment (%) in the different media of fusing pairs isolation: L1, L-N, L-P, and L-N-P. Values are means ± SE.

patible with high levels of cyst production (Table 6). The self-crossing of the nonclonal sibling strains showed self-compatibility detected as cyst production in 75% of the cases. The high concentration of cysts recorded in some of these self-crosses was noteworthy, being the cysts formed mostly viable and capable of producing a viable offspring.

Reproductive compatibility: The variation in mating among strains was studied using the CIs shown in Table 7. High values of CIs in all strains were indicative of the large number of successful crosses that were possible among them. On the other hand, AV values that were also high provided evidence of the usually high cyst concentrations achieved in the compatible crosses.

DISCUSSION

Sexual stages. The results indicate that sexual phases of *G. nolleri* resemble patterns previously reported for other haplontic cyst-forming dinoflagellates, mainly the related *G. catenatum*. Gamete pairs have similar morphologies and couple in the same way, though isogamy is more common in *G. nolleri* and anisogamy in *G. catenatum* (Blackburn et al. 1989).

TABLE 5. Backcrossing results of *Gymnodinium nolleri* clonal offspring (see Table 2 for scoring criteria).

Strain	DK5c	DK6c
F1′c	0*	1
F2′c	4	1
F3′c	0	1
F4′c	0*	0
F5′c	3	0

Planozygote morphology is also very similar, except for the differences in size. A wide variety of life cycle patterns is possible among dinoflagellates (Pfiester and Anderson 1987, Pfiester 1989, Coats 2002). Although sexuality of hypnocyst-forming species was traditionally thought to be linked to resting stage formation, the capacity of planozygotes to undergo division has been cited in *Scrippsiella trochoidea* (Stein) Loeblich (Uchida 1991) and *Gyrodinium instriatum* Freudenthal et Lee (Uchida et al. 1996). We also recorded this process under culture conditions for *G. nolleri* planozygotes. As in the above-mentioned species, the planozygotes formed in our experiments may either reestablish the haploid phase or produce a resting cyst, though the factors determining these two patterns are not well understood (Uchida 2001).

It is uncertain whether nuclear fusion was completed before planozygote division. Nevertheless, though karyogamy occurred later than plasmogamy in many dinoflagellate species (Coats et al. 1984), it seems unlikely that nuclear fusion was not completed after 4–7 days, the average time interval elapsing before planozygote division. In fact, it was not possible to distinguish between encysting and dividing planozygotes, because their morphological development was similar. On the other hand, the percentage of zygotes that underwent division or encystment was influenced by the nutritional status of the external medium, a fact that rules out the possibility of a culture artifact affecting planozygote encystment. It has been hypothesized (Anderson et al. 1985, Olli and Anderson 2002) that cyst formation might be inhibited due to the nutritional status of planozygotes, because only those with enough reserves would form cysts. Our data may indicate that high levels of external phosphate had the same effect on encystment, in this case due to zygote division. This hypothesis is supported by the fact that only planozygotes formed in media without addition of phosphate encysted in significant percentages. Planozygote division, at present scarcely reported among dinoflagellates, has also been recently observed in *G. catenatum* (unpublished data).

Nevertheless, some morphological aspects have been found that differ in the development of the sexual stages of the two species. These differences apply mainly to planozygote and planomeiocyte division and can be summarized in two main points. First, the division of *G. nolleri* planozygotes under culture conditions resulted in cell pairs with a different morphology than those formed by vegetative division. This fact suggests that a process other than an oblique binary fission (normal vegetative reproduction) may take place. In contrast, *G. catenatum* planozygotes follow the same process as a vegetative division (oblique binary fission), and apparently normal products (two-cell chains) are obtained (unpublished data). Second, although the germination pattern is similar in both species, the division of *G. nolleri* planomeiocytes began with the striation of the basal cell area, which is progressively widened. In contrast, *G. catenatum* planomeiocytes do

TABLE 6. Result of *Gymnodinium nolleri* self-crossing experiments (see Table 2 for scoring criteria).

Parental strains						Offspring DK5cx922Ic			Offspring DK5cxDK6c				
VGO 633	VGO 632	VGO 634	DK5	DK6	922I	F1	F2	F3	F1′	F2′	F3′	F4′	F5′
4	3	0	0	0	0	3	3	0*	4	3	2	0	3

F strains, F1 offspring obtained from the germination of one cyst produced by the cross DK5cx922Ic; F′ strains, F1 offspring of one cyst of the cross DK5cxDK6c.

not suffer any morphological change before division, apart from cell enlargement (Blackburn et al. 1989).

Mating type and cyst production study. Mating type determination also shows evidence of great similarities between these *Gymnodinium* species, because both have a complex mating system that includes more than two sexual types (complex heterothallism). In the same way as has been proposed for *G. catenatum*, *G. nolleri* mating type may be influenced by multiple genetic factors segregating at meiosis, as is common in some fungal systems (Blackburn et al. 2001). Nevertheless, there are noticeable differences in this aspect. In *G. catenatum* outbreeding appears to be necessary to achieve cyst formation because cultures established from a single cyst are rarely self-compatible (Blackburn et al. 2001), whereas in *G. nolleri* cysts were produced both when crossing clonal strains and in cultures established from cyst germination (inbreeding). In addition, cyst production has never been recorded within a *G. nolleri* clone (homothallism), whereas this has been observed in few *G. catenatum* clonal strains (unpublished data). Analyzing the F1 offspring of one *G. nolleri* cross, we found noticeable differences in their compatibility pattern, which may indicate recombination of multiple mating factors, though not enough F1 progeny was available to determine whether it is two or more factors. The clonal strains used in the progeny study (strains Fc and F′c) were obtained by the isolation of one cell from self-compatible sibling strains (Tables 5 and 6). Sexual recombination, apart from the meiotic process occurring in the planomeiocyte, could have taken place in the F strains before the establishment of the clonal culture. This fact would justify a variety of sexual behaviors due to different genomic information affecting the compatibility of these clones.

TABLE 7. Reproductive compatibility of each *Gymnodinium nolleri* strain measured by compatibility index (CI), average vigor (AV), and reproductive compatibility (RC).

Strain	CI	AV	RC
DK5c	0.67	3.00	2.67
VGO 632c	0.83	4.00	3.33
VGO 633c	0.67	3.25	2.00
F1′c	0.67	2.75	2.00
F1c	0.83	3.40	2.50
VGO 634c	0.67	2.25	2.00

The reproductive compatibility indices calculated, CI, AV, and RC, indicate a general tendency of *G. nolleri* strains to be more compatible and to produce a greater concentration of cysts per cross than *G. catenatum* (reflected by higher CI and AV values, respectively). In this latter species, 81% of the interpopulation crosses studied by Blackburn et al. (2001) had CIs under 0.6, the minimum value achieved in our experiments. Following the same comparison, *G. catenatum* AV average values were always below 2.8, whereas more than 60% of our crosses exceed this figure. This tendency toward lower levels of cyst production and sexual compatibility in *G. catenatum* crosses is also enhanced by the already cited lack of inbreeding capacity of the progeny established from the germination of cyst. This is an important differential characteristic, because the size of cyst beds and the length of dormancy period are factors closely related to the onset of a bloom and therefore to a species presence in a given area.

Our data indicate that cyst formation and germination are faster processes in *G. catenatum* and not so evidently related to long-term survival as for *G. nolleri*. Although *G. nolleri*-fusing gamete pairs require more than 6 days to encyst, only an average time of 3–4 days is needed for the encystment of *G. catenatum* gamete pairs (unpublished data). For *G. nolleri* we estimated 26–40 days as the mean value of minimum dormancy. However, there is a notable intraspecific variability for which the range can be set between 15 and 70 days (unpublished data), whereas 13 days were estimated for *G. catenatum* (Blackburn et al. 1989). In this latter species, longer dormancy periods were only recorded for geographically distant crosses and were related to low progeny viability (Blackburn et al. 2001), suggesting that *G. catenatum* may produce cysts that excyst normally within a few days without accumulating seed beds (Dale and Amorim 2000).

The data presented suggest that the encystment process may have a different function in the life cycle of the two species. Concerning these species-specific encystment and excystment characteristics, the cyst-seeding mechanism may fit the *G. nolleri* better than *G. catenatum*. In general, the cyst of *G. catenatum* is extremely scarce to explain bloom events, as has been reported in Spain (Hallegraeff and Fraga 1998, Bravo and Ramilo 1999), Australia (Bolch and Hallegraeff 1990), and Japan (Matsuoka and Fukuyo 1994), although blooms do often occur. Blooms of *G. catenatum*

in South Africa (Pitcher et al. 1995) and Atlantic European coastal areas (Fraga et al. 1988, Moita 1993) have been related to oceanic circulation. Because of mass water movements, such as the relaxation of an upwelling process, efficient swimmers such as *G. catenatum* forming long-chain might be favored and transported to coastal waters (Fraga et al. 1988). This theory would undervalue the importance of cyst germination in explaining the presence of *G. catenatum* at a certain location, in favor of an external cell supply. No data are available concerning bloom dynamics of *G. nolleri*, but one of the main obvious differences between these species is the length of the chains formed. We have reported that, compared with *G. catenatum*, *G. nolleri* presented higher reproductive compatibility indices, the possibility of inbreeding, and a longer dormancy period.

All these factors are related with a more relevant paper of the cyst stage in the life cycle strategy, and they might be resources to cope with a smaller swimming capability. It has been proposed that *G. catenatum* may have evolved from a *G. nolleri*-like ancestor (Bolch 1999, Bolch and Reynolds 2002). In the evolution of sexuality, it seems likely that heterothallism came first and that homothallism arose later for those organisms for which the chances of finding an opposite mating type are restricted (Goodenough 1985). This hypothesis might support the theory of a more evolved G. *catenatum*. Within these lines, we observed that some clonal strains of *G. catenatum* are homothallic and that the planozygote division is a faster and probably a more improved process than in *G. nolleri*. These features might reflect the divergent historical evolution for these two species, which are related in many other aspects.

We thank Dr. Marianne Ellegaard for providing cultures for this study, Anna Godhe and Karin Rengefors for providing Swedish mud samples for cyst isolation, and I. Ramilo and A. Fernàndez-Villamarín for technical assistance. The research was supported by an Instituto Español de Oceanografía grant for training of research staff and for the project "New Strategy of Monitoring and Management of HABs in the Mediterranean Sea," proposal no. EVK32000.00621.

Anderson, D. M., Coats, D. W. & Tyler, M.A 1985. Encystment of the dinoflagellate *Gyrodinium uncatenum*: temperature and nutrient effects. *J. Phycol.* 21:200–6.

Anderson, D. M., Jacobson, D., Bravo, I. & Wrenn, J. H. 1988. The unique, microreticulate cyst of the naked dinoflagellate *Gymnodinium catenatum* Graham. *J. Phycol.* 24:255–62.

Anderson, D. M., Kulis, D. M. & Binder, B.J 1984. Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis*: cyst yield in batch cultures. *J. Phycol.* 20:418–25.

Anderson, D. M. & Wall, D 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. *J. Phycol.* 14:224–34.

Blackburn, S. I., Bolch, C. J. S., Haskard, K. A. & Hallegraeff, G. M. 2001. Reproductive compatibility among four global populations of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *Phycologia* 40:78–87.

Blackburn, S. I., Hallegraeff, G. M. & Bolch, C. J. 1989. Vegetative reproduction and sexual life cycle of the toxic dinoflagellate *Gymnodinium catenatum*. *J. Phycol.* 25:577–90.

Bolch, C. J. & Hallegraeff, G. 1990. Dinoflagellate cysts in recent marine sediments from Tasmania, Australia. *Bot. Mar.* 33:173–92.

Bolch, C. J. S., Negri, A. & Hallegraeff, G. 1999. *Gymnodinium microreticulatum* sp. nov. (Dinophyceae): a naked, microreticulate cyst-producing dinoflagellate, distinct from *Gymnodinium catenatum* and *Gymnodinium nolleri*. *Phycologia* 38:301–3.

Bolch, C. J. S. & Reynolds, M. J. 2002. Species resolution and global distribution of microreticulate dinoflagellate cysts. *J. Plankton Res.* 24:565–78.

Bravo, I. & Ramilo, I 1999. Distribution of microreticulate cysts from the Galician and Portuguese coast. *Sci. Mar.* 63: 45–50.

Coats, D. W. 2002. Dinoflagellate life-cycle complexities. *J. Phycol.* 38:417–9.

Coats, D. W., Tyler, M. A. & Anderson & D.M 1984. Sexual processes in the life cycle of *Gyrodinium uncatenum* (Dinophyceae): a morphogenetic overview. *J. Phycol.* 20:351–61.

Dale, B. & Amorim, A. 2000. Dinoflagellate resting cysts as seed beds for harmful algal blooms. *In* Hallegraeff, G. M., Blackburn, S. I., Bolch, C. J. & Lewis, R. J. [Eds.]. *Ninth International Conference on Harmful Algal Blooms*, Unesco, Tasmania, Australia, Abstract.

Ellegaard, M., Christensen, N. F. & Moestrup, Ø. 1993. Temperature and salinity effects on growth of a non-chainforming strain of *Gymnodinium catenatum* (Dinophyceae) established from a cyst from recent sediments in the Sound (Øresund), Denmark. *J. Phycol.* 29:418–26.

Ellegaard, M., Kulis, D. M. & Anderson, D. M. 1998. Cysts of Danish *Gymnodinium nolleri* Ellegaard et Moestrup sp. ined. (Dinophyceae): studies on encystment, excystment and toxicity. *J. Plankton Res.* 20:1743–55.

Ellegaard, M. & Moestrup, Ø. 1999. Fine structure of the flagellar apparatus and morphological details of *Gymnodinium nolleri* sp. nov. (Dinophyceae), an unarmored dinoflagellate producing a microreticulate cyst. *Phycologia* 38:289–300.

Ellegaard, M. & Oshima, Y. 1998. *Gymnodinium nolleri* Ellegaard et Moestrup sp. ined. (Dinophyceae) from Danish waters, a new species producing *Gymnodinium catenatum*-like cysts: molecular and toxicological comparisons with Australian and Spanish strains of *Gymnodinium catenatum*. *Phycologia* 37: 369–78.

Fraga, S., Anderson, D. M., Bravo, I., Reguera, B., Steidinger, K. A. & Yentsch, C. M. 1988. Influence of upwelling relaxation on dinoflagellates and shellfish toxicity in Ría de Vigo, Spain. *Estuarine Coastal Shelf Sci.* 27:349–61.

Goodenough, U. 1985. An essay on the origins and evolution of eukaryotic sex. *In* Halvorns, H. O. & Monroy, A. [Eds.] *The Origin and Evolution of Sex, Marine Biological Laboratory Lectures in Biology.* Vol. 7. Publisher:Alan R. Liss, Inc., New York, pp. 123–40.

Guillard, R. R. L. & Hargraves, P. E. 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycologia* 32:234–6.

Hallegraeff, G. M. & Fraga, S. 1998. Bloom dynamics of the toxic dinoflagellate *Gymnodinium catenatum*, with emphasis on Tasmanian and Spanish coastal waters. *In* Anderson, D. M., Cembella, A. D. & Hallegraeff, G. M. [Eds.] *Physiological Ecology of Harmful Algal Blooms*. Springer-Verlag, Berlin, Heidelberg, pp. 59–80.

Matsuoka, K. & Fukuyo, Y. 1994. Geographical distribution of the toxic dinoflagellate *Gymnodinium catenatum* Graham in Japanese coastal waters. *Bot. Mar.* 37:495–503.

Moita, M. T. 1993. Development of toxic dinoflagellates in relation to upwelling patterns of Portugal. *In* Smayda, T. J. & Shimizu, Y. [Eds.] *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishers B.V., pp. 299–304.

Nehring, S. 1995. *Gymnodinium catenatum* Graham (Dinophyceae) in Europe: a growing problem? *J. Plankton Res.* 17:85–102.

Olli, K. & Anderson, D. M. 2002. High encystment success of the dinoflagellate *Scrippsiella* cf. *Lachrymosa* in culture experiments. *J. Phycol.* 38:145–56.

Pfiester, L. A. 1989. Dinoflagellate sexuality. *Int. Rev. Cytol.* 114:249–72.

Pfiester, L. A. & Anderson, D. M. 1987. Dinoflagellate reproduction. *In* Taylor, F. J. R. [Ed.] *The Biology of Dinoflagellates*. Botanical Monographs. Blackwell Scientific Publications, Oxford, pp. 611–48.

Pitcher, G., Agenbag, J., Calder, D., Hortsman, D., Jury, M. & Taunton-Clark, J. 1995. Red tides in relation to the meteorology of southern Benguela upwelling system. *In* Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P. & Marcaillou-Le Baut, C. [Eds.] *Harmful Marine Algal Blooms*. Lavoisier, Paris, pp. 657–62.

Uchida, T. 1991. Sexual reproduction of *Scrippsiella trochoidea* isolated from Muroran Harbor Hokkaido. *Nippon Suisan Gakkaishi/Bull. Jap. Sci. Fish* 57:1215.

Uchida, T. 2001. The role of cell contact in the life cycle of some dinoflagellate species. *J. Plankton Res.* 23:889–91.

Uchida, T., Matsuyama, Y., Yamaguchi, M. & Honjo, T. 1996. The life cycle of *Gyrodinium instriatum* (Dinophyceae) in culture. *Phycol. Res.* 44:119–23.

Wall, D. 1971. Biological problems concerning fossilizable dinoflagellates. *Geos. Man* III:1–15.