ALEXANDRIUM CATENELLA (WHEDON & KOFOID) BALECH, 1985, IN MAGELLAN WATERS, CHILE.

ALEXANDRIUM CATENELLA (WHEDON & KOFOID) BALECH, 1985, EN AGUAS MAGALLÁNICAS, CHILE.

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ABSTRACT

Alexandrium catenella is a dinoflagellate that produces Paralytic Shellfish Poison (PSP). It is widely distributed in coastal waters around the world, and forms large devastating blooms in southern Chile. Several strains were collected from Magellanic fjords and channels to study its life cycle and to assess its growth rate at various levels of salinity. Compared with field-collected cells, vegetative cells cultured in the laboratory were smaller, rounder, and formed shorter chains. Pellicle cysts formed rapidly under stress conditions when observed on microscope. This type of cyst was found in the gut of shellfish collected in the field. The sexual life cycle included isogametes that formed a large planozygote, which formed into a thick-walled resting cyst. All stages presented minor morphological differences compared with others closely related species in the genus. Formation of gametes was mainly restricted to the stationary phase of growth, and was probably induced by nutrient depletion. The maximum growth rate attained in cultures simulating summertime water conditions (*i.e.*, 11 °C and salinity of 15–30 psu) was low, ranging from 0.18 to 0.35 divisions day^{-1}. These data support the hypothesis that massive excystment is the main cause of the intense blooms in the Magellanic fjords and channels. The localization and study of cyst reservoirs and the factors that promote excystment should be a priority in terms of understanding the processes that trigger extensive blooms in Magellanic waters.

Key words: Dinoflagellates, cyst, subantarctic, life cycle.

RESUMEN

Alexandrium catenella es un dinoflagelado productor del denominado Veneno Paralizantes de los Mariscos, que se encuentra presente en muchas zonas costeras del planeta. En el Sur de Chile esta especie forma grandes floraciones que han causado serios problemas económicos y de salud. Varias cepas unialgales, colectadas en las aguas marinas interiores de Magallanes, se usaron para estudiar su ciclo de vida y evaluar su tasa de crecimiento a distintas salinidades. Las células que se observaron en cultivo fueron más pequeñas, redondeadas y formaron cadenas más cortas que las obtenidas en terreno.

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Quistes temporales, similares a los observados en el estómago de bivalvos, se formaron rápidamente en condiciones de estrés. El ciclo de vida sexual de la especie incluyó la formación de isogametos que formaron un gran planocigoto que finalmente originó un quiste bentónico de dormancia. Todos los estadios encontrados presentaron apenas leves diferencias morfológicas con los descritos en otras especies del mismo género. Los gametos se formaron principalmente en la fase de crecimiento estacionario, por lo que se deduce que la disminución de nutrientes estimula su formación. Las máximas tasas de crecimiento alcanzadas en cultivos que simularon las condiciones de verano de las aguas magallánicas, es decir temperatura de 11 °C y salinidades de 30 a 15 psu, fueron de 0,18 a 0,36 div. día^{-1}. Estos datos apoyan la hipótesis de la germinación masiva de quistes bentónicos como causa principal de las grandes floraciones de esta especie en los fiordos y canales magallánicos. La localización de los reservorios de quistes y los factores que promueven su germinación deberían ser una prioridad de investigación.

Palabras clave: Dinoflagelados, quiste, subantártico, ciclo de vida.

INTRODUCTION

Dinoflagellates are one of the most important components of coastal plankton worldwide, except in Antarctica. This wide geographic distribution is partly attributable to their life cycle, which includes a dormant stage that helps them survive in adverse conditions (Dale 1983). These protists have a haplontic life cycle with sexual stages in which vegetative cells transform into gametes (Pfiester 1989, Wyatt & Jenkinson 1997). A motile planozygote forms after gamete fusion (Pfiester 1989). The planozygote settles into an overwintering stage—a benthonic thick-walled resting cyst—which can survive for several years in the fine sediment of the marine seafloor (Dale 1983). The cyst requires a dormancy period to reach maturity and excyst (Anderson 1998). The planomeiozygote forms after excystment and divides into four vegetative cells by meiosis. Vegetative cells can also form a temporary cyst (pellicle cyst) without a sexual process. The two types of cysts are morphologically different (Anderson & Wall 1978).

Alexandrium is one of the most widely studied genera of dinoflagellates because some of its species produce potent neurotoxins. These toxins can be accumulated by filter-feeding shellfish and can cause human death, even at low concentrations. *A. catenella* was the first species in the genus reported to be toxic, after an exhaustive study in Californian waters (Sommer *et al.* 1937). This species is also present in Japan, South Africa, and Korea(Balech 1985). More recently, it has been also found in France (Thau Lagoon) and the Mediterranean Spanish coast (Vila et al. 2001). In Chile, *A. catenella* has been recorded from Chiloé Island (42°S) to the southern part of the Magallanes Region (55.5°S) (Guzmán *et al.* 1975, Lembeye 1998[1]). At the beginning of the 1990s, mesoscale blooms of this species covered hundreds of kilometers, and caused the death of more than 20 people in Magellanic waters. The origins of these blooms are elusive, but we propose two contrasting hypotheses: a massive cyst germination event or a high growth rate during the vegetative stage.

Water mass movements and the biology of the organism, particularly its life cycle, play key roles in the development of large-scale red tide blooms (Anderson 1995). The source area may be distant from affected areas (Tyler & Seliger 1978, Tester *et al.* 1991). As the bloom moves, the changing conditions can either stimulate or inhibit its development. The bloom can be inhibited when the growth rate falls below a critical value, or when the population is forced to form other stages such as gametes or cysts (Persson et al. 2008, Wang *et al.* 2007). Salinity is the main environmental parameter that affects the physiology of the organisms and that controls its distribution in estuaries (Day *et al.* 1989). Magellanic fjords and channels have an estuarine hydrographic structure (Valdenegro & Silva 2003), and it is unknown how local strains of *A. catenella* are affected by salinity changes in these waters. Here, we document for the first time the stages of the life cycle of *A. catenella* from the Magallanes Region. In addition, to test the two hypotheses presented above regarding the origin of *Alexandrium* blooms, we measured growth rates in cultures under simulated summertime water conditions.

MATERIAL AND METHODS

Two strains of *A. catenella* obtained from the Strait of Magellan were used to examine the life cycle. The isolates were identified based on their

plate characteristics. A detailed taxonomical analysis will be presented elsewhere (Uribe *et al.* in prep.). One strain was collected off-shore at Buena Bay (53°30'S) in February 1994, and the other was collected off-shore at Ciervos River (53°11'S) in January 2004. Non-axenic multiclonal cultures were grown in batch mode in 250 cc Erlenmeyer flasks. Each flask contained 100 ml f/2 medium without silicate (Guillard 1975), prepared with 0.45 µm filtered seawater. Culture conditions were as follows: salinity, 30.5 psu; temperature, 11 °C; photoperiod, 16 h light/8 h dark. Light was provided by daylight fluorescent tubes at an intensity of 84 µE m^2 s^{-1}. For observations, individual cells were selected from cultures in stationary or decline phase. Different stages were identified by bright-field microscopy according to the descriptions by Anderson & Wall (1978), Turpin *et al.* (1978), and Benavides *et al.* (1983).

For growth-rate experiments, the Buena Bay strain was grown under the conditions described above. Lower salinities of 25, 20, and 15 psu were obtained by diluting seawater from the Strait of Magellan with distilled water. Two flasks at each salinity level were examined. For quantification, cells were fixed using Lugol's iodine and three replicate samples from each flask were counted every 3–4 days under an inverted microscope. More than 200 cells were counted each time. Division rates were calculated from the exponential growth phase (Guillard 1973).

RESULTS

Vegetative cell: Field-collected vegetative cells showed slight anteroposterior compression. The cingular wings were prominent, which resulted in the general aspect having a more compressed appearance (Figs. 1–3). The transdiameter and height varied from 29 to 42 µm, and cells differed in size even within the same chain (Fig. 3). Long chains formed by 16 or more cells were not uncommon. Cultured cells had a more rounded appearance, because their cingular wings were less prominent. On average, they were smaller in size than field-collected cells, and chains were composed of no more than four cells (Fig. 4). Cell division took place in a characteristic oblique manner (Fig. 5).

Vegetative growth: Field-collected vegetative cells thrived and grew easily in the laboratory when cultured within 4–5 days of collection. At 11 °C, *A. catenella* grew at salinities of 15–30.5 psu. Division rates ranged from 0.36 div day^{-1} at 20 psu to 0.15 div day^{-1} at 15 psu (Table 1). Cells cultured in the two flasks at each salinity showed similar growth rates (Table 1).

In the stationary phase, the highest concentration was 16,000 cells l^{-1} at 25 psu and the lowest was approximately 6,000 cells l^{-1} at 20 psu (Table 1). There was no lag phase at 30 psu salinity, but at 25 psu the acclimation to the new salinity level took several days. At 20 psu, there was no detectable lag phase, but at 15 psu the lag phase extended for approximately 2 weeks.

Temporary cyst: Under mechanical stress, the vegetative cell lost its normal wall cell (ecdysis) and transformed into a spherical cyst (Figs. 6, 9). This also occurred under combined conditions of osmotic and temperature stress, which result when a is observed under the microscope. This stage was also observed in the gut of filter-feeding shellfish, even several days after collection (Figs. 7, 8). The cysts had a thin translucent wall without appreciable ornamentation (Fig. 10).

Sexual cycle: In cultures maintained for some weeks and that had reached the stationary phase, we observed a high proportion of smaller, less pigmented cells that represented the gametic stages (Fig. 12). The cells were isogametes, as they were all approximately the same size. The gametes were identified as such because they were attracted by other gametes. Once in contact, cells fused together with the anteroposterior axis of one cell angled at approximately 45° in relation to the other cell's axis (Figs. 11, 13). The fusion of cytoplasms began at the ventral zone of both cells as a thin cytoplasmic bridge that widened slowly until the two cytoplasms were indistinguishable. The newly formed cell was larger than the vegetative cell, and had a longer anteroposterior axis (Figs. 13–16). Bright-field microscopy did not allow us to observe the two typical flagella present in this stage. The planozygote moved slowly and tended to remain at the bottom of the culture container.

Gametes and planozygotes collected from the flask and kept at 4 °C did not produce cysts after 15, 30, or 45 days in the dark. However, a few resting cysts were observed at the bottom of the culture vessels that were maintained for some weeks in the light. The cysts that formed in culture had a characteristic oblong form with rounded poles

TABLE 1. Growth parameters of *Alexandrium catenella* cultures. The two values
in each cell represent data obtained from two experimental flasks.

	30 psu	25 psu	20 psu	15 psu
Division rate	0.23–024	0.23–0.27	0.34–0.36	0.15–0.18
Maximum cell concentration (cells/ml)	14,780–12,810	16,310–13,460	7,420–5,860	12,030–9,452

(Figs. 17, 18, 19, 21). Compared with field-collected cysts, they were slightly more rounded (Figs. 19, 20). A thick wall consisting of two layers was clearly visible. The inner layer was thick and translucent and the outer layer was thin and opaque (Figs. 19, 20). The cell contained many spherical bodies and a characteristic large orange-red spot. Neither the cultured nor field-collected cysts had a mucilaginous coating. Germination experiments could not be carried out because of the small number of cysts obtained.

DISCUSSION

A. catenella is one of the most widely distributed species within the genus *Alexandrium*. Originally described in the Gulf of California and once considered a cold temperate species, it has since been reported from many areas around the world, even in Mediterranean waters. Despite being one of the most potent producers of marine toxins, little is known about its life cycle; consequently, the ecology of the species is poorly understood. This species forms part of a complex that includes *Alexandrium tamarense* and *Alexandrium fundyense* (Uwe *et al.* 2003). The species are difficult to separate taxonomically based on their morphological features, because the diagnostic characters are subtle (Lilly *et al.* 2007). For this reason, the stages depicted in this report are morphologically similar to those of *A. tamarense*, the most studied species in the group (Anderson & Wall 1978, Turpin *et al.* 1978). The gametes form directly from vegetative cells, and are smaller, pale and show a thinner covering (possibly a theca), which was not analyzed in this study. The pattern of gamete fusion is similar to that described for most other dinoflagellates (Pfiester 1984, Figueroa *et al.* 2005). Gametes were indistinguishable morphologically and behaviorally, suggesting that the studied strains are isogamous. As reported in other similar studies (*e.g.*, Anderson 1998), gametes formed abundantly in the stationary phase after several days of exponential growth, suggesting that nutrient depletion stimulates formation of gametes and triggers initiation of the sexual cycle. Some aberrant forms observed in this phase were probably cells at final stages of the fusion process. The planozygote is large, and would not be confused with vegetative cells.

Given that the cultures used in this study were not clonal, it was not possible to establish the thallism of *A. catenella* from southern Chile. *A. catenella* from Japanese waters is heterothallic and requires two different clones to form a zygote (Yoshimatsu 1981). Homothallic and heterothallic clones can be found within one species, as demonstrated for *A. excavatum* from the lower Saint Lawrence (Quebec, Canada) (Destombe & Cembella 1990). Further detailed studies are required to clarify this point.

Although many gametes and planozygotes were found in the culture vessels, the final number of cysts was quite low. Poor nutrient conditions prevent encystment—the step from planozygote to cyst (Anderson 1998). As demonstrated in *A. catenella* from Mediterranean waters, a high yield of cysts depends on nutrient levels, particularly that of phosphate (Figueroa *et al.* 2005). Cyst formation in cultured *A. catenella* from Japanese waters is stimulated or inhibited by several bacterial strains in natural seawater (Adachi *et al.* 2001, 2003). Our cultures were not axenic, and we did not conduct experiments on the effects of varying nutrient levels. Therefore, we cannot exclude the possibility that nutrients or bacteria negatively affected cyst production. The resting cysts formed in the laboratory were morphologically similar to those described by Lembeye (2004), although they were slightly less oblong than *A. catenella* cultured from Spanish waters (Figueroa *et al.* 2005) and *A. tamarense* from North America (Anderson & Wall 1978, Turpin *et al.* 1978).

In general, vegetative cells of *Alexandrium catenella* were easily cultured under laboratory conditions. The strains described in this report, as well as several other strains, were occasionally maintained in collection bottles for 5 days at high concentrations at air temperatures *of ca.* 8–15 °C before inoculation. However, some strains were not as easily cultivated.
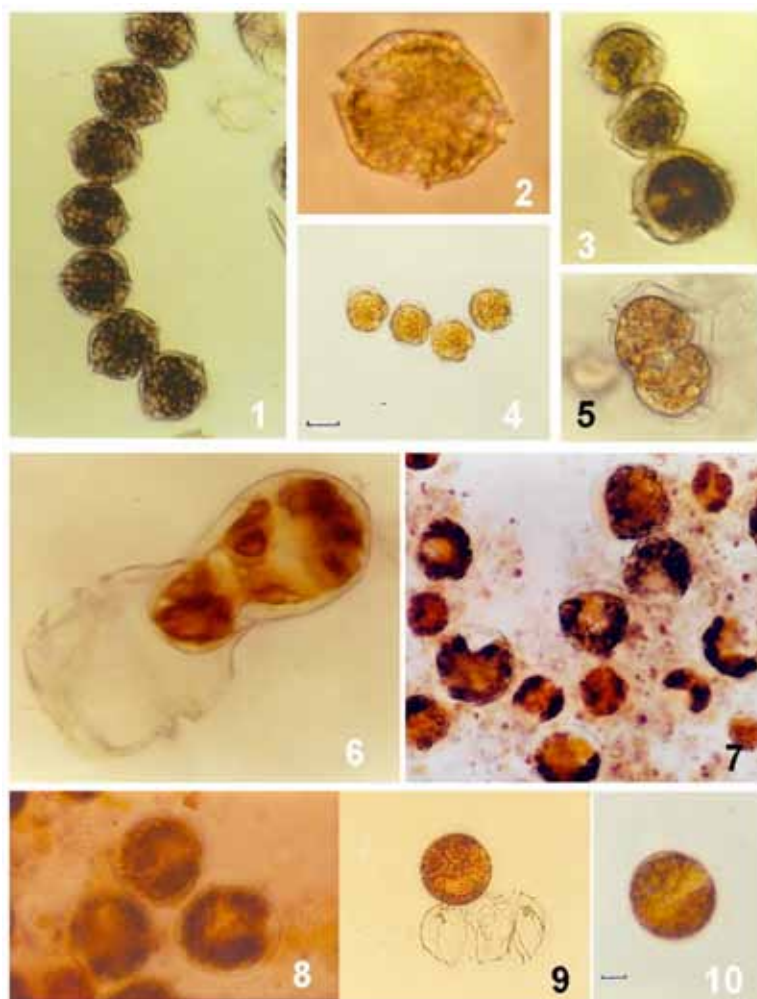
PLATE 1. Vegetative stages of *A. catenella.*

Fig. 1. Chain from field sample.
Fig. 2. Contour of field-collected cell.
Fig. 3. Differently sized cells within a chain.
Fig. 4. Short chain of cultured cells.
Fig. 5. Vegetative division.
Fig. 6. Release of pellicle cyst from theca.
Fig. 7. Pellicle cyst from shellfish gut 4 days after collection.
Fig. 8. Detailed view of pellicle cyst obtained from shellfish gut.
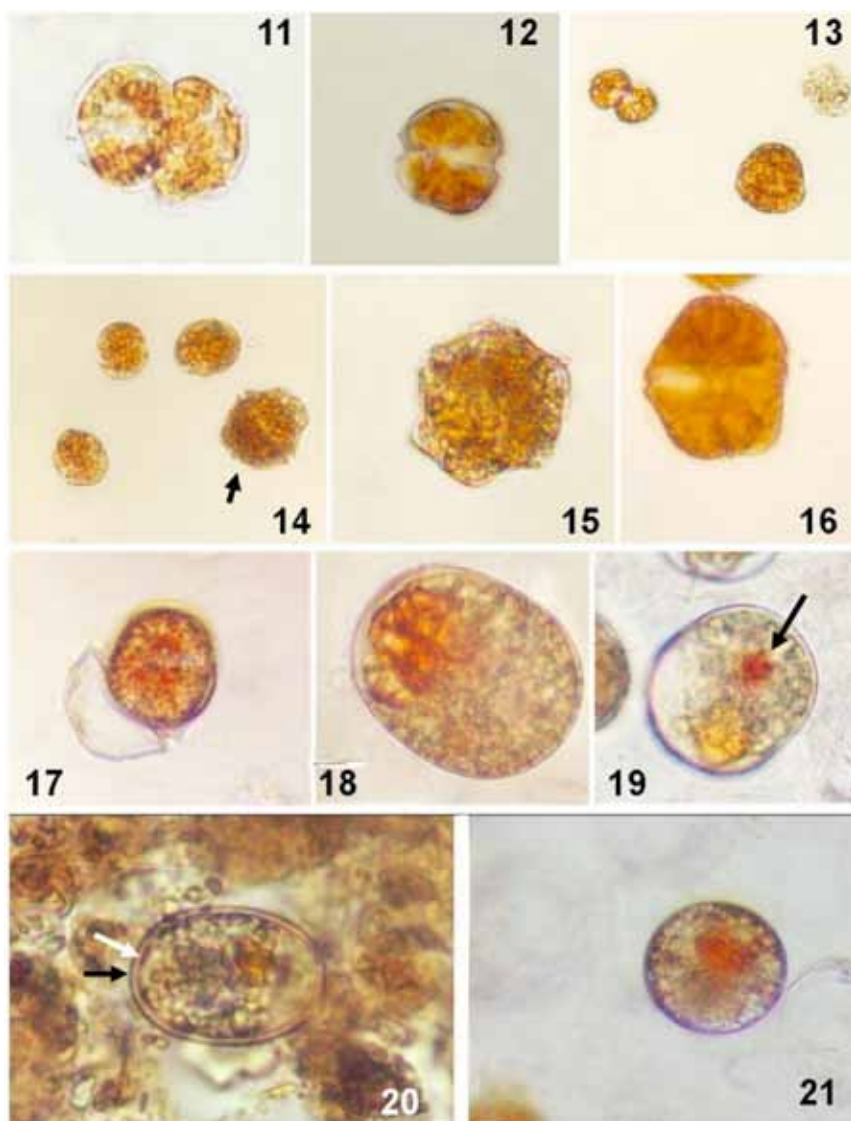Figs. 9, 10. Cultured pellicle cyst.

PLATE 2. Sexual stages of the A. *catenella* life cycle.

Fig 11. Pair of fusing isogametes.
Fig. 12. Gametic cell with theca.
Fig. 13. Fusing gamete pair and planozygote.
Fig. 14. Three vegetative cells and one planozygote (black arrow).
Fig. 15. Morphology of planozygote.
Fig. 16. Healthy planozygote. Note the large anteroposterior axis.
Fig. 17. Release of resting cyst.
Fig. 18. Immature resting cyst.
Fig. 19. Mature resting cyst. Note the characteristic red spot (black arrow).
Fig. 20. Resting cyst collected in the field. Wall with thick inner
layer (white arrow) and thin outer layer (black arrow).
Fig. 21. Cultured cyst viewed from one pole.

We made several attempts to cultivate strains from Puerto Zenteno, located in the Strait of Magellan, close to the Atlantic Ocean, but we were never able to maintain cultures, even when samples were cultured within 24 hours of collection. One possible explanation for this difficulty is that the strains were stressed populations carried far from their origin, as *A. catenella* blooms in the Pacific sector of the Strait. A sample containing small cells (presumably gametes) of *Alexandrium* was collected off Buena Bay, but we were unable to cultivate cysts or vegetative cells from it. Evidently, strains react differently in culture, and there may be sufficient genetic variability in regional populations of *Alexandrium* to produce contrasting growth characteristics and physiology (Anderson 1998, Figueroa *et al.* 2005).

As shown in this study, the pellicle cyst remains intact and in good condition even after several days in the digestive tract of *Mytilus*. After passing through the digestive tract, these cysts could eventually reinoculate the surrounding waters, producing vegetative cells and maintaining the population long after its main peak. This type of situation, in which pellicle cysts of *Alexandrium taylori* were harbored in sediments, is thought to have caused an extended bloom in Costa Brava, Catalonia, Spain (Garcés *et al.* 2002). The cells trapped in shellfish guts could potentially be used to inoculate cultures, since *Alexandrium* blooms are elusive and commonly difficult to detect. We did not use this approach in the present study because abundant *Alexandrium* samples were available within inland Magellanic seawaters in the 1990s.

The division rates of *Alexandrium* from around the world range from 0.3 to 0.7 div day^{-1} (Anderson 1998, Band-Schmidt *et al.* 2003). The growth rates obtained for the stain examined in the present study are at the lower end of this range at the salinities tested. In the 1990s, extensive blooms and world-record levels of PSP toxin in shellfish (some figures exceeding 100,000 µg saxitoxin eq/100 g shellfish tissue) were found in Magellanic waters. A very high density of *Alexandrium* is required for such extreme toxic burdens in shellfish; such densities cannot be explained solely by the vegetative growth of *A. catenella*. Instead, a massive excystment process is required at the initiation phase of such megablooms. Systematic assessments of cyst beds and detailed studies of factors that affect the excystment process must be undertaken to understand the dynamics of *Alexandrium* blooms in southern Chile.

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