JOURNAL OF PLANKTON RESEARCH | VOLUME 26 | NUMBER 12 | PAGES 1459–1470 | 2004

Effects of growth medium, temperature, salinity and seawater source on the growth of *Gymnodinium catenatum* (Dinophyceae) from Bahía Concepción, Gulf of California, Mexico

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Received 19 April, 2004; accepted in principle 2 June, 2004; accepted for publication 2 August, 2004; published online August 20, 2004

Laboratory studies were performed to determine the effect of temperature, salinity, seawater sources and culture media on the vegetative growth of clonal cultures of Gymnodinium catenatum *isolated from Bahía Concepción, Mexico. These isolates were heterothallic and isogamous. Exponential growth rates of* G. catenatum *in f/2 with different selenium concentrations and soil extract and GSe media were moderate. Maximum cell yields were obtained in GSe and f/2 media with selenium (10^{-8} and 10^{-7} M), while in f/2 medium with soil extract cell yields were considerably lower. The highest percentage of long chains was found in f/2 media supplied with selenium (10^{-8} M). The optimal temperature range for growth was 11.5–30°C, with the highest growth rates between 21 and 29°C. The range of salinity tolerated by* G. catenatum *changed with seawater source. With seawater from Vineyard Sound (Massachusetts, USA),* G. catenatum *grew at salinities from 15 to 36, with an optimal growth rate obtained at salinities between 26 and 30. With seawater from Bahía Concepción, this species tolerated salinities from 25 to 40, with optimal growth at salinities between 28 and 38. Ecophysiological measurements reported here are consistent with the environment of the bay, which has limited input of humic materials from runoff and high salinity and temperature. These data, when viewed with data from studies of globally distributed* G. catenatum, *demonstrate the ability of this species to live in a broad array of habitats.*

INTRODUCTION

Gymnodinium catenatum Graham was first described from the Gulf of California by Graham (Graham, 1943). This species was linked to paralytic shellfish poisoning (PSP) in the Galician rias in Spain (Estrada *et al.*, 1984) and later in the Bahía de Mazatlán (Mee *et al.*, 1986) and many other locations worldwide (Hallegraeff and Fraga, 1998). Within the last decade, it has been reported in other localities along the Mexican Pacific coastline (Fig. 1). This apparent increase might be the result of the increased awareness from the higher number of researchers studying harmful algal blooms or could reflect a recent dispersal of the species to new areas.

PSP is the most serious shellfish-related toxin syndrome in Mexico. To date, this poisoning is the most dangerous, causing 72% of the seafood poisoning outbreaks that have occurred during the last decade. Three of these events represent 87% of the poisoning cases (460 individuals poisoned, with 32 deaths), in Mazatlán, Guerrero and Oaxaca, the most affected areas [Secretaria de Salubridad y Asistenica (SSA), Procaduria Federal de Protección al Ambiente (PROFEPA) in (Sierra-Beltrán *et al.*, 1998)]. In addition, great numbers of marine animals (fish and turtles) were also affected by PSP outbreaks (Sierra-Beltrán *et al.*, 1998).

doi:10.1093/plankt/fbh133, available online at www.plankt.oupjournals.org

Fig. 1. Map of Mexico showing coastal areas where *Gymnodinium catenatum* occurs (black circles) and where paralytic shellfish poisoning (PSP) outbreaks were linked with this species (blank circles) recorded from 1943 to 2003 (Graham, 1943; Osorio-Tafall, 1943; Mee *et al.*, 1986; Manrique and Molina, 1997; Cortés-Altamirano *et al.*, 1999; Licea *et al.*, 1999; Ramírez-Camarena *et al.*, 1999; Cabrera-Mancilla *et al.*, 2000; Morales-Blake *et al.*, 2000; Figueroa-Torres and Zepeda-Esquivel, 2001; Gárate-Lizárraga *et al.*, 2001a, 2004; Góngora-González, 2001; Herrera-Galindo, 2002; Gárate-Lizárraga and Siqueiros-Beltrones, 2003).

In general, *G. catenatum* abundances are high in the Gulf of California (Mee *et al.*, 1986; Cortés-Altamirano *et al.*, 1999), and particularly in Bahía Concepción, moderate abundances of the motile (32 000–570 000 cells L^{-1}) and resting stage (1–4% total abundance) of *G. catenatum* have been recorded (Morquecho and Lechuga-Devéze, 2003; Gárate-Lizárraga *et al.*, 2004; Morquecho, L. and Lechuga-Devéze, C. H. 2004). The toxins neosaxitoxin, decarbamoyl saxitoxin, decarbamoyl gonyautoxin 2, B1-2 and C1-C3 have been found in phytoplankton and scallop samples (Gárate-Lizárraga *et al.*, 2001b; Band-Schmidt *et al.*, in press). Total concentration of paralytic shellfish toxins found in *Argopecten ventricosus* and *Pinna rugosa* has been higher than the threshold limit for human consumption [80 μg eq. Saxitoxin (SXT) 100 g^{-1} meat] (Sierra-Beltrán *et al.*, 1996; Lechuga-Devéze and Morquecho-Escamilla, 1998).

Studies of the influence of environmental factors on the physiological and life-history processes related to cell growth have been undertaken only in few clones of *G. catenatum*. Most of those studies have been done on Tasmanian and Spanish clones [e.g. (Oshima *et al.*, 1987, 1993; Blackburn *et al.*, 1989; Hallegraeff *et al.*, 1991; Bravo and Anderson, 1994; Flynn *et al.*, 1996; Doblin *et al.*, 1999a,b)]. Strain-specific responses have been identified which could explain in part the variable bloom patterns of the same species in different environments (Doblin *et al.*, 2000).

In spite of being responsible for high toxin levels in shellfish at several localities along the Mexican Pacific coastline, no studies have been conducted thus far on the growth and environmental tolerances and preferences of Mexican strains of *G. catenatum*, even though they have been responsible for high toxin levels in shellfish at several localities along the mexican pacific coastline. In Bahía Concepción, higher abundances of toxic dinoflagellates are found during spring (Lechuga-Devéze and Morquecho-Escamilla, 1998), a transition period from a mixed to a stratified water column (Lechuga-Devéze and Morquecho-Escamilla, 1998; Palomares-García *et al.*, 2002). The hydrographic conditions during this transition period are characterized by a rise in water temperature (from 17–21°C to 20–26°C), increases in nutrient concentration (nitrates, nitrites, phosphates and silicates) below 20 m and low concentrations of dissolved oxygen below 15 m of depth, generation of H_2S and accumulation of organic matter (Gilmartin and Revelante, 1978; Lechuga-Devéze and Morquecho-Escamilla, 1998).

Here we investigate the effect of temperature, salinity, seawater source and culture medium composition on the vegetative growth of *G. catenatum* isolated from Bahía Concepción and relate these observations to the environmental conditions at the times of blooms of this species in the bay.

METHOD

The organism

Gymnodinium catenatum clonal strain (GCCV-10) used during this study was established in April 2000 from vegetative cells collected by vertical tows using a 20 μm phytoplankton net from an area located in the central basin of Bahía Concepción Gulf of California, Mexico (26°33′–26°53′ N, 111°42′–112°56′ W) (Fig. 2). The cell concentrate was sieved through a 60 μm mesh to eliminate larger organisms. Filtrate was enriched with f/2 medium (Guillard and Ryther, 1962) modified by addition of H_2SeO_3 (10^{-8} M) and by reducing the concentration of $CuSO_4$ to 10^{-8} M (Anderson *et al.*, 1984). In the laboratory, vegetative cells of *G. catenatum* were isolated from the enrichments with micropipettes under an inverted microscope (Carl Zeiss Axiovert 100). Single cells were transferred to 96 well plates with modified f/2 medium and maintained at 20 ± 1°C with overhead illumination of 150 μmol m^{-2} s^{-1} supplied with cool-white fluorescent lights (12:12 h L:D cycle).

Growth in different media

Growth of *G. catenatum* was tested in modified f/2 medium (as above) supplied with different concentrations of selenium (0, 10^{-6}, 10^{-7} and 10^{-8} M), f/2 medium with soil extract and GSe medium (Doblin *et al.*, 1999a) using Bahía Concepción seawater at a salinity of 35. Triplicate 25 mL batch cultures were grown in polycarbonate vials and maintained in conditions previously described. Cultures were pre-adapted for at least two growth cycles in batch mode. Every fifth day, 2 mL subsamples were fixed in Lugol's iodine and counted in a 1 mL Sedgwick-Rafter counting slide under an optical microscope (Leica, DMLS), and the number of cells per chain was recorded. Cell density was used to calculate exponential growth rates according to Guillard (Guillard, 1973). The average growth rate in each medium was determined from the average of four replicates.

Effect of temperature on growth

Temperature effects on growth rate were determined in a temperature-gradient bar (Watras *et al.*, 1982) ranging from 5 to 35°C, at 20 different temperature levels. For a description of this device, see (Rengefors and Anderson, 1998). Light was supplied by four cool-white fluorescent lamps set at a 10:14 h L:D cycle, positioned under the test tubes. Irradiance values inside test tubes were 230 μmol m^{-2} s^{-1}. Test tubes were filled with 25 mL of modified f/2 medium using Vineyard Sound seawater (salinity ≈ 30). Prior to transfer into the temperature-gradient bar, cultures were acclimated stepwise, transferring to higher and lower temperatures, with two transfers made during exponential growth. Culture growth was determined by *in vivo* fluorescence monitored daily with a fluorometer (Turner Designs Model 10-AU). Fluorescence data were used to calculate exponential growth rates according to Guillard (Guillard, 1973).

Effect of seawater source and salinity on growth

Salinity effects on growth were determined using two sources of seawater: Vineyard Sound and Bahía Concepción. The seawater was diluted with distilled water or was

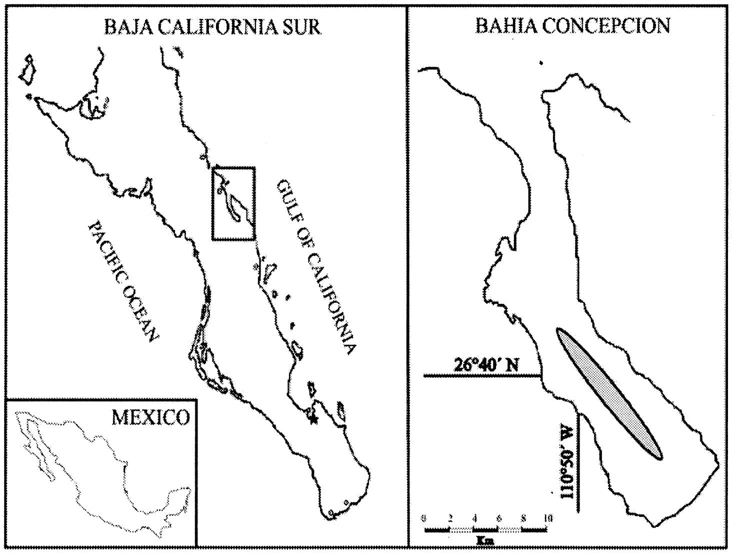

Fig. 2. Map of Bahía Concepción showing the collection site for phytoplankton samples.

evaporated to obtain salinities from 15 to 45. Fifty-milliliter culture tubes with 25 mL of modified f/2 medium at each salinity were used. Light was supplied with fluorescent lamps in a 10:14 h L:D cycle with Vineyard Sound water and at 12:12 h L:D cycle with seawater from Bahía Concepción. Both experiments were performed at 20 ± 1°C and 150 μmol m^{-2} s^{-1}. Cultures were previously acclimated stepwise, transferring gradually to the different salinities of the experiment, with two transfers made during exponential growth. Culture growth was determined from the time course of *in vivo* fluorescence or by cell density as described above.

Sexual induction

For crossing experiments, 11 strains of *G. catenatum* isolated from single cells from Bahía Concepción were inoculated in 25 mL of f/2 phosphate-deficient medium (all other major nutrients, trace metals and vitamins at f/2 concentrations) at 20°C and 150 μmol m^{-2} s^{-1}, 12:12 h L:D cycle (Anderson *et al.*, 1984). The isolates were mixed together and kept clonal to determine whether *G. catenatum* is heterothallic or homothallic.

Statistical analyses

Differences in growth rates, cells yields and length of chains in the different treatments were tested using ANOVA. The analyses were calculated using Statistica v. 5.0 (StatSoft, Inc.) at a significance level of $\alpha = 0.05$.

RESULTS

Effect of media on growth rate

Exponential growth rates of *G. catenatum* in the different media were not significantly different ($P > 0.05$) (0.15–0.19 day^{-1} at a salinity of 35) (Fig. 3a), except for f/2 without selenium, where growth and survival were poor after three transfers (data not shown). However, final cell yields in GSe and f/2 plus selenium (10^{-8} and 10^{-7} M) were significantly higher ($P > 0.05$), with maximum cell densities between 1559 and 1970 cells mL^{-1} (Fig. 3b). Cell yields were considerably decreased in f/2 medium with soil extract (394 cells mL^{-1}).

A high percentage (46–62%) of short chains (two to three cells) were observed in all media after 5 days of culture. As cultures aged, a higher percentage (26–30%) of cells forming chains were observed in GSe and f/2 media with different selenium concentrations and soil extract (Fig. 3c). Only 5–20% of the cells formed long chains (longer than four cells) between 15 and 25 days of culture. A higher percentage of long chains were observed in f/2 media with the lowest concentration of selenium (10^{-8} M) after 5 and 15 days of culture.

Temperature effects on growth

Growth of *G. catenatum* at different temperatures was examined using Vineyard Sound water enriched with f/2 medium at a salinity of ~30. Growth of *G. catenatum* was observed between 11.5 and 30°C, with optimal exponential growth rates of 0.14–0.21 day^{-1} between 15 and 29°C (Fig. 4). The highest rate (0.18–0.21 day^{-1}) was observed between 21 and 29°C.

Effect of seawater source and salinity on growth

Gymnodinium catenatum growth in Vineyard Sound seawater enriched with modified f/2 enrichments was tested at salinities between 15 and 36 (Fig. 5). The highest growth rates (0.24 day^{-1}) were obtained at salinities from 26 to 30, with a significant difference ($P > 0.05$). Growth and survival were poor at salinities of 10 and 40, with no survival after a few days in culture.

Gymnodinium catenatum grew in seawater from Bahía Concepción at salinities from 26 to 40 (Fig. 5). Growth and survival were poor at salinities of 20 and 45, with no survival after a few days in culture. The highest growth rates (0.28–0.31 day^{-1}) occurred at salinities between 28 and 38 ($P > 0.05$). Significant differences ($P > 0.05$) in the growth rates between Vineyard Sound seawater and Bahía Concepción water were found at salinities of 26, 28 and 30. At salinities of 28 and 30, the highest growth rates were obtained using seawater from Bahía Concepción.

Vegetative cells, gametes, planozygotes and cysts

Vegetative cells of *G. catenatum* in exponential phase cultures usually form chains of two to four cells in length (Fig. 6a). In culture, chains as long as 18 cells have been observed. As cultures age, cells become narrower (Fig. 6b) and a higher percentage of two-cell chains and single cells were observed (Fig. 6b–d). On the basis of observations of fusing cells, gametes of *G. catenatum* are morphologically indistinguishable from vegetative cells and are thus isogamous (Fig. 6e). Only in the crossing experiments of two strains was sexual reproduction observed. After sexual reproduction (Fig. 6f and g), large planozygotes were observed in cultures after 15 days. These strains are heterothallic, as we obtained resting cysts or hypnozygotes only from non-clonal cultures after 20 days of incubation. Live cysts are thick walled, spherical and orange-brown in color and have a red pigmented body (Fig. 6h). The diameter of the cysts ranged from 36.9 to 47.6 μm. The percentage of cyst formation in culture conditions was low (0.5–1).

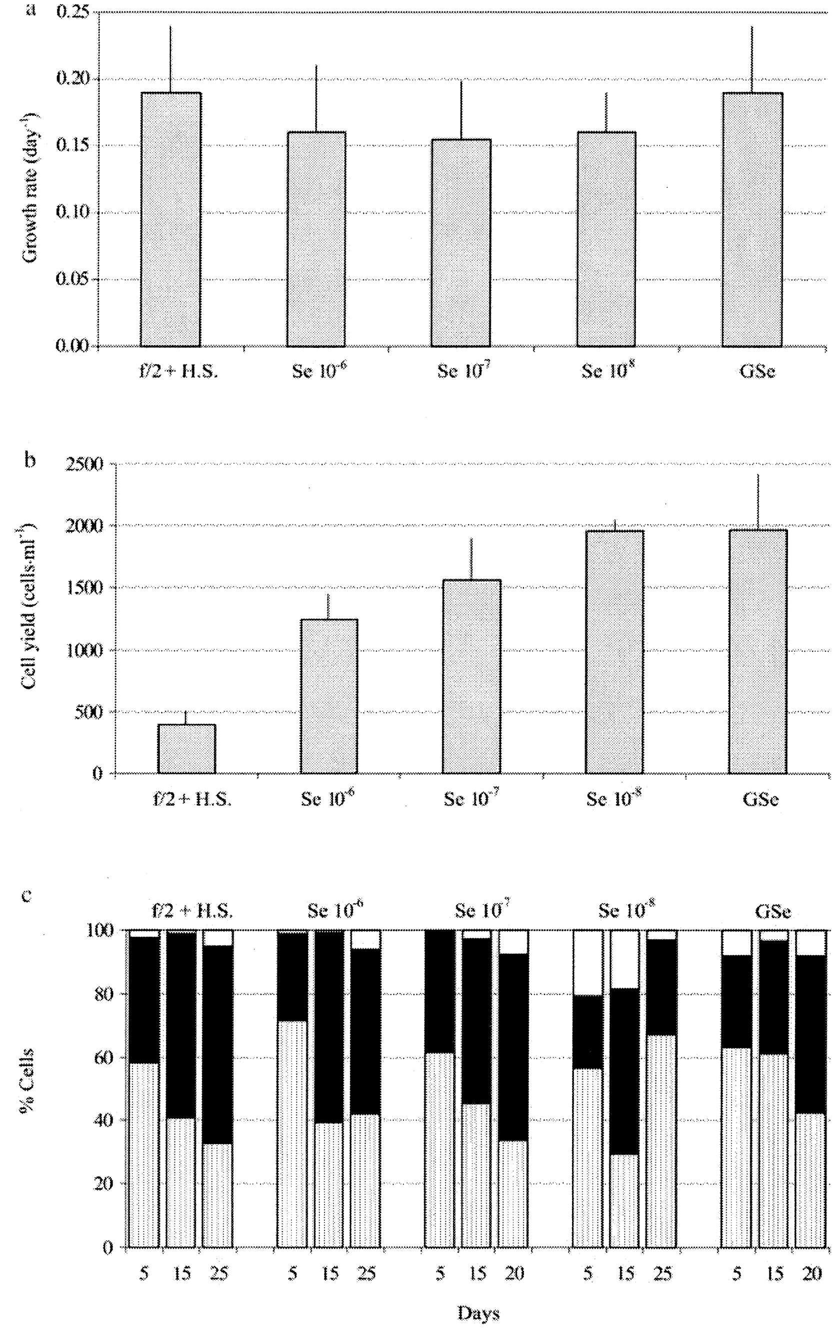

Fig. 3. *Gymnodinium catenatum.* Growth responses in different media: modified f/2 with different selenium concentrations (10^{-8}, 10^{-7} and 10^{-6} M), modified f/2 with humic substances (H.S.) and GSe media: (**a**) average exponential growth rates, (**b**) average maximum cell yields, (**c**) chain lengths at 5, 15 and 20 days of culture. Empty bars: single cells. Diagonal lines: chains of two or three cells. Dotted bars: chains with four or more cells. Error bars = SE.

DISCUSSION

This species has been detected at a growing number of locations along the Mexican Pacific coast, concurrent with the increase in the known global distribution of the PSP producing dinoflagellate *G. catenatum* (Fig. 1). This could be due to more vigilant recognition of the species

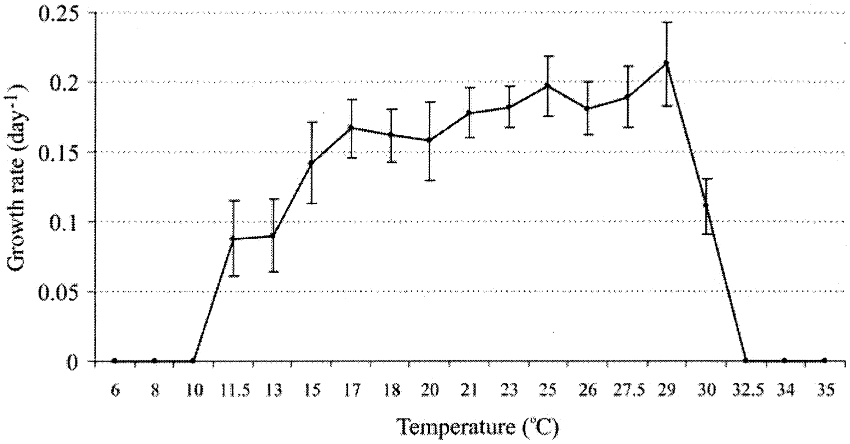

Fig. 4. *Gymnodinium catenatum.* Growth responses to temperature. Average exponential growth rates. Error bars = SE.

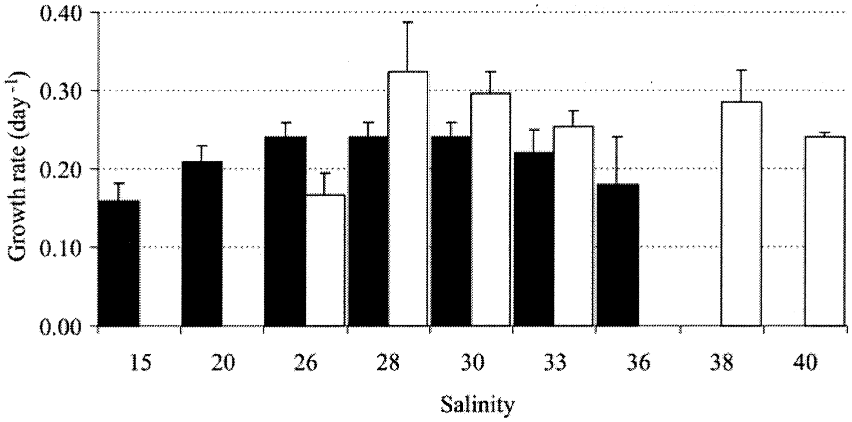

Fig. 5. *Gymnodinium catenatum.* Growth responses to salinity and different seawater sources. Average exponential growth rate at different salinities using seawater from Bahía Concepción (empty bars) and Vineyard Sound seawater (solid bars). Error bars = SE.

by scientists or a sign of species dispersal within the region. In Bahía Concepción, an arm of the Gulf of California, *G. catenatum* has been linked to PSP (Gárate-Lizárraga *et al.*, 2004; Band-Schmidt *et al.*, in press). However, no *in vitro* studies have been conducted on the growth of Mexican strains of *G. catenatum.* Here we describe the results of culture studies on a strain of *G. catenatum* from Bahía Concepción to help explain the bloom dynamics of this species in the bay.

Growth medium effects

There were differences in growth characteristics in the medium types that were tested (f/2 with added selenium, added soil extract and normal GSe media), the most significant being maximum cell yield and chain length. The exponential growth rates in all media were moderate, and no significant difference was observed. The highest cell yields were obtained in f/2 media with selenium (10^{-7} and 10^{-8} M) and GSe media which contain selenium and soil extract. GSe medium has been reported to promote better growth than f/2 medium for other strains of *G. catenatum* (Bravo, 1986; Blackburn *et al.*, 1989). Previous studies have shown that soil extract can stimulate the growth of diatoms and dinoflagellates (Prakash *et al.*, 1973; Usup *et al.*, 1994; Imai *et al.*, 1998; Maas *et al.*, 2000). The stimulation in growth is thought to result from several mechanisms: chelation of trace metals and provision of nutrients (particularly nitrogen and selenium) (Usup and Azanza, 1998). However, in f/2 media with soil extract, the cell yield was very low (<500 cells mL^{-1}). A higher percentage (20%) of long chains were observed in f/2 media with low selenium concentration (10^{-8} M), suggesting that those cells were in good physiological condition. Based on the observations of higher cell yields and

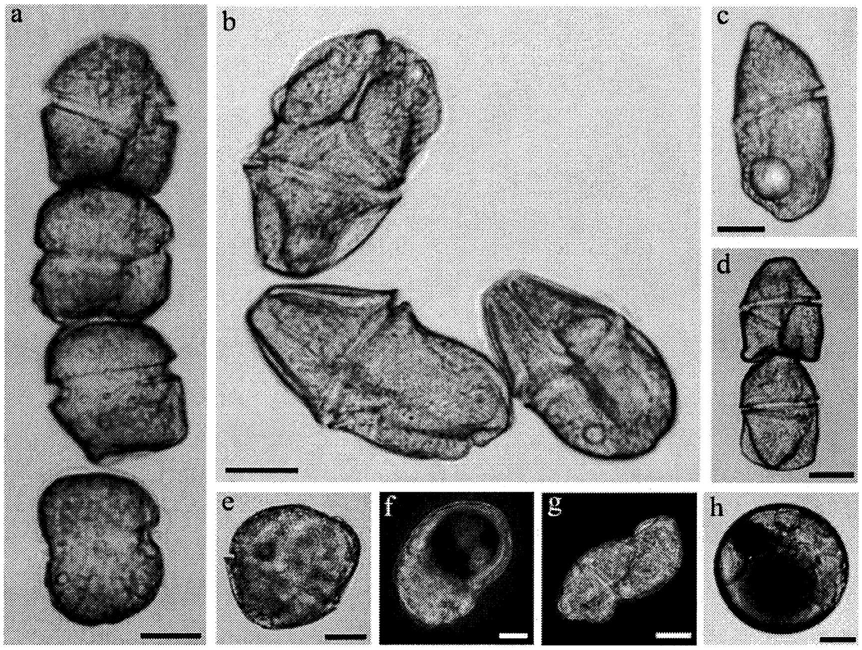

Fig. 6. Life-cycle stages of *G. catenatum* in culture. Scale bar = 20 µm. (**a**) Vegetative cell separating from four-cell chain during exponential phase, (**b–c**) narrow single cells, (**d**) two-cell chain, (**e**) swimming gametes during sexual reproduction, (**f–g**) large planozygotes, (**h**) non-clonal cysts formed in nutrient-deficient medium with red pigmented body.

higher percentage of long chains, these data suggest that low selenium concentrations favor the growth of *G. catenatum*, but at concentrations $>10^{-8}$ M this micronutrient becomes toxic, differing from selenium requirements observed in other strains of *G. catenatum*. A Tasmanian strain of *G. catenatum* (GCDE08) had a requirement for selenium (Doblin *et al.*, 1999a). In selenium-deficient medium, a decline in growth rate and biomass yield was observed. Increase in growth rates and biomass yields was directly proportional to the addition of selenium (10^{-9} and 10^{-7} M) in the seawater medium (Doblin *et al.*, 1999a). Doblin *et al.* (2000) documented different selenium requirements for multiple geographic strains of *G. catenatum*, evidenced by differences in growth rates and cell yield. Three different responses to selenium deficiency were observed: (i) decrease of exponential growth rate and biomass yields, (ii) decrease in biomass yields only and (iii) no decrease in growth or biomass yields. In the *G. catenatum* strain from Bahía Concepción, no significant changes in the growth rate were observed and the biomass yields decreased proportionally with the addition of selenium in the seawater medium. Selenium plays a role which is well understood in algal nutrition and toxicity (Kaplan *et al.*, 1986). It can stimulate the growth of marine chrysomonads (Pintner and Provasoli, 1968) and seems to be necessary for some freshwater dinoflagellates and diatoms (Fries, 1982). Selenium is present in natural waters as selenate (Se^{VI}), selenite (Se^{IV}) and organoselenium compounds (Boyer and Brand, 1998). In the cell, selenium can be found as selenocysteine which functions as an antioxidant in enzymes and can degrade peroxides non-enzymatically (Gennity *et al.*, 1985; Boyer and Brand, 1998).

In some areas where *G. catenatum* forms blooms, population development seems to be related to heavy rainfall or the presence of rivers that contribute material similar to soil extract from land runoff (Hallegraeff *et al.*, 1995). However, the strain from Bahía Concepción would have little exposure to land runoff, given the scarce annual rainfall (112–155 mm) mainly during the summer (García, 1981). This species might therefore not be physiologically adapted to take advantage of this growth factor, consistent with the decrease in cell yield that was observed when soil extract was added to its culture medium.

Temperature effects

Growth rate of *G. catenatum* varied significantly with temperature. The temperature–growth response of *G. catenatum* presented a wide plateau, culminating in a sudden decline from the optimum. As expected, the highest growth rates (0.18–0.21 day^{-1}) were obtained at the upper end of this range, between 21 and 30°C. This coincides with the temperature range over which this species is found in Bahía Concepción, 18–29°C

(Gárate-Lizárraga *et al.*, 2001a; Góngora-González, 2001; Morquecho and Lechuga-Devéze, in press). Interestingly, Graham (1943) reported the occurrence of *G. catenatum* at a surface temperature of 14–17°C with salinities of 35.07–35.50 at 29°N in the Gulf of California. This bloom thus occurred near the lower end of the species temperature tolerance. Further south, along the coast of Colima, *G. catenatum* has been found at temperatures of 23–25°C (Morales-Blake *et al.*, 2000).

For comparative purposes, the optimal growth temperatures for geographically distinct *G. catenatum* isolates are compiled in Table I. The temperature tolerances and preferences of the strain of *G. catenatum* from Bahía Concepción are similar to those of the Spanish strain, which can grow from 14 to 29°C, with a maximal growth rate between 22 and 28°C (Bravo and Anderson, 1994). In nature, however, *G. catenatum* is found along the Spanish coast at suboptimal temperatures (12–18°C) for its growth (Bravo and Anderson, 1994). Optimal temperatures determined in culture generally do not coincide with the temperature of maximum abundances in the natural environment, as maximal growth rates are not a prerequisite for population success if other factors (e.g. grazing) are favorable.

Temperature can determine the biogeography of different ecotypes (Hallegraeff and Fraga, 1998). The Japanese (Sensaki Bay) strain of *G. catenatum* has a narrow temperature range for growth (15–25°C; Ogata *et al.*, 1989). The Hiroshima Bay strain has a wider temperature range (20–30°C), with moderate growth rates exceeding 0.2 day^{-1} (Yamamoto *et al.*, 2002). Along the western coast of Japan, however, blooms of *G. catenatum* have been observed over a wide range of temperatures (6–27°C) (Kotani *et al.*, 2000). The Tasmanian strain tolerates colder temperatures in culture, from 14.5 to 20°C (Blackburn *et al.*, 1989), although in nature it has the same temperature range as the Spanish strain (12–18°C) (Hallegraeff *et al.*, 1995). On the coast of Uruguay, *G. catenatum* blooms during warm summer and autumn months when temperatures are 17–25°C (Méndez and Ferrari, 2002). In Venezuela, *G. catenatum* is present across a temperature range of 22–28°C, forming blooms when the water is >25°C (La Barbera-Sánchez and Gamboa-Maruez, 2001). Tropical populations from Palau, Philippines and Thailand are present at temperatures between 25 and 30°C (Hallegraeff and Fraga, 1998). Clearly, there are varieties of this species, each adapted to a particular region. The Mexican strain falls at the tropical end of these temperature tolerances.

Minor differences were also observed in maximal growth rates (Table II), mainly in comparison with the isolate from Galicia, Spain, which grew almost twice as

Table I: Temperature range and optimum temperature for the growth of Gymnodinium catenatum populations in the natural environment (N) and in laboratory culture (C)

Location	Range	Optimum	Reference
Mexico			
Bahía Concepción	18–29°C (N)	20–29°C (N)	Gárate-Lizárraga *et al.* (2001a)
	11.5–29°C (C)	15–29°C (C)	Present study
West Coast of B.C.	13–17°C (N)		Gárate-Lizárraga and Siqueiros-Beltrones (2003)
Manzanillo Bay	+23°C (N)	25°C (N)	Morales-Blake *et al.* (2000)
Bay of Mazatlán	16–24°C (N)	17.5–20°C (N)	Cortés-Altamirano *et al.* (1999)
Gulf of California	14–17°C (N)		Graham (1943)
Puerto Libertad		16–17°C (N)	Cortés-Altamirano *et al.* (1999)
Japan			
Sensaki Bay	6–27°C (N)		Kotani *et al.* (2000)
	15–25°C (C)		Ogata *et al.* (1989)
Hiroshima Bay	20–30°C (C)	25–30°C (C)	Yamamoto *et al.* (2002)
Spain	14–29°C (C)	22–28°C (C)	Bravo and Anderson (1994)
	12–18°C (N)		
Tasmania	12–18°C (N)		Hallegraeff *et al.* (1995)
	14.5–20°C (C)		Blackburn *et al.* (1989)
Uruguay	17–25°C (C)		Méndez and Ferrari (2002)
Venezuela	22–28°C (N)		La Barbera-Sánchez and Gamboa-Maruez (2001)
Palau, Philippines and Thailand	25–30°C (N)	25–28°C (N)	Hallegraeff and Fraga (1998)

Table II: Growth rates of Gymnodinium catenatum strains under different growth conditions

Strains	Source	Growth rate (division day^{-1})	Growth conditions	Reference
Not specified	Galicia, Spain	0.53	K medium, 24°C, 150 μmol m^{-2} s^{-1}, 14/10 h L/D	Bravo and Anderson (1994)
GC21V	Vigo, Spain	0.25–0.30	K medium with 100 μm of nitrate and 20 μm of phosphate, 18°C, 180 μmol m^{-2} s^{-1}, 12/12 h L/D	Flynn *et al.* (1996)
GCDE08	Derwent Estuary, Tasmania	0.24	GPM with Se (10^{-9}, 10^{-7}M), 18°C, salinity 28, 180 μmol m^{-2} s^{-1}, 12/12 h L/D	Doblin *et al.* (1999a)
		0.19–0.25	Same as above with standard humic acid or dissolved organic matter with/or without EDTA, 150 μmol m^{-2} s^{-1}, Se 10^{-9} M	Doblin *et al.* (1999b)
GCDE9305	Derwent Estuary, Tasmania	0.34	GPM 1/10 with Se (10^{-9} M), 18°C, salinity 28, 180 μmol m^{-2} s^{-1}, 12/12 h L/D	Doblin *et al.* (2000)
GCHU02	Huon Estuary, Tasmania	0.28	Same as above	Doblin *et al.* (2000)
GCJP10	Sensaki Bay, Japan	0.24	Same as above	Doblin *et al.* (2000)
GCSP01	Ria de Vigo, Spain	0.24	Same as above	Doblin *et al.* (2000)
Not specified	Hiroshima Bay, Japan	0.31	f/2 with Se (1 nM), 25°C, salinity 30, 180 μmol m^{-2} s^{-1}, 12/12 h L/D, pH 8.2	Yamamoto *et al.* (2002)
GCCV-10	Bahía Concepción, Mexico	0.14–0.21	f/2 medium, 15–29°C, salinity 30, 230 μmol m^{-2} s^{-1}, 10/14 h L/D	Present study
GCCV-10	Bahía Concepción, Mexico	0.24	f/2 medium, 20°C, Vineyard Sound seawater, salinity 26–30, 150 μmol m^{-2} s^{-1}, 10/14 h L/D	Present study
GCCV-10	Bahía Concepción, Mexico	0.28–0.31	f/2 medium, 20°C, Bahía Concepción seawater, salinity 28–38, 12/12 h L/D	Present study
GCCV-10	Bahía Concepción, Mexico	0.15–0.19	f/2 with Se (10^{-6} M, 10^{-7} M, 10^{-8} M) and GSe medium, 20°C, salinity 35, 150 μmol m^{-2} s^{-1}, 12/12 h L/D	Present study

fast as the Bahía Concepción isolate. Most other isolates of *G. catenatum* grew at rates that were at the high end of those observed for the Bahía Concepción isolate (~0.3 day^{-1}). The low growth rates recorded for the Bahía Concepción strain of *G. catenatum* could explain the generally moderate cell densities of this species observed in the bay. Experiments on additional isolates are needed to determine whether low growth rates are a stable characteristic of Mexican *G. catenatum* or simply reflect a suitable, but non-optimal growth medium.

Salinity and seawater source effects

Gymnodinium catenatum was grown in two sources of seawater across a broad salinity range. With Vineyard Sound seawater enriched with f/2, the highest growth rates (0.24 day $^{-1}$) were attained at salinities from 26 to 30, while using seawater from Bahía Concepción, with the same f/2 formulation, the maximum growth rate (0.30 day^{-1}) was observed at salinities from 28 to 38. The high salinity tolerance of the Mexican strain of *G. catenatum* is expected, since in its natural environment, this strain is present at salinities that are consistently close to 35 throughout the year (34.6–37) (Félix-Pico and Sánchez, 1976).

Few studies have examined the effect of salinity on growth for strains of *G. catenatum*. The salinity range for the strains of *G. catenatum* that have been studied is narrower than that for the strains of *G. catenatum* from Bahía Concepción. A Tasmanian strain of *G. catenatum* tolerates salinities of 23–34 (Blackburn *et al.*, 1989), similar to the Japanese (Hiroshima Bay) strain that tolerates salinities between 20 and 32 (Yamamoto *et al.*, 2002). A Venezuela strain grew at salinities from 33.2 to 39, blooming in natural waters over a salinity range of 36.5–38 (La Barbera-Sánchez and Gamboa-Maruez, 2001). Blooms of *G. catenatum* in Spanish waters were observed at a salinity of 35.3 (Fraga *et al.*, 1993).

Doblin *et al.* (Doblin *et al.*, 2000) attributed growth differences for a single strain of *G. catenatum* in different seawater batches to variations in micronutrient requirements such as selenium. In the southern part of Bahía Concepción where the higher cyst densities are found (Morquecho and Lechuga-Devéze, 2003) and thus presumably *G. catenatum* blooms originate, the sediment is mainly biogenic, with high concentrations of Ca, Sr, Cr, La, Ce and Th (Shumilin *et al.*, 1996). The average concentration of Se in the bay is 4.6 p.p.m. (Shumilin *et al.*, 1996). In seawater from Bahía Concepción, the concentration of Mg was the highest at 247.6 p.p.m., followed by Fe (54.8 p.p.m.) and Zn (5 p.p.m.). No traces of Cd, Cu, Ni and Pb were found (data not shown). Recent investigations indicate the presence of hydrothermal venting (Greene and Forrest, 2002) which can produce a unique micronutrient composition in the seawater of this bay. This could promote better growth of *G. catenatum* compared to other seawater sources.

Sexuality and cyst formation

Life-cycle stages of *G. catenatum* from Bahía Concepción coincide with previous descriptions for this species (Blackburn *et al.*, 1989). Sexuality and cyst production were achieved in both N- and P-limited media, as also reported by Blackburn *et al.* (Blackburn *et al.*, 1989) and Bravo and Anderson (Bravo and Anderson, 1994). Compatible strains of opposite mating types were also required for sexual reproduction (Blackburn *et al.*, 1989). We conclude that the Bahía Concepción strain of *G. catenatum* is heterothallic, as we only obtained resting cysts from compatible strains in nutrient-deficient medium. Fusing gametes were indistinguishable from vegetative cells.

Ecological implications

In Bahía Concepción, low abundances (<2500 cells L^{-1}) of *G. catenatum* cells can be observed from January to August, with maximum abundances (3.2×10^4 cells L^{-1}) present from April to May (Morquecho and Lechuga-Devéze, in press). During this period, water temperatures range from 17 to 30°C (Gárate-Lizárraga *et al.*, 2001a; Góngora-González, 2001; Morquecho and Lechuga-Devéze, in press) and salinities are ~35.

When water temperatures are >30°C, *G. catenatum* disappears from the water column (Góngora-González, 2001). In the present study, optimal temperature for growth was between 21 and 29°C, with a very sharp decline at higher temperatures, consistent with the observed pattern in natural waters. However, low abundances might be observed during colder months, since cells can grow at lower temperatures (down to 11.5°C).

The high salinity tolerance of this Mexican strain is consistent with the high salinity of the bay from which it was isolated. There is thus general agreement between the culture data and field conditions. Purely on the basis of temperature and salinity tolerances and preferences, we would expect *G. catenatum* blooms to be seasonal (from late winter to early summer) in Bahía Concepción, disappearing when the water becomes either too warm (>30°C) or too salty (>38). Both of these situations are observed. Other possible scenario could be that this species remain, at moderate abundances, for a longer period (until late summer) in subsurface waters (10–20 m). Superimposed on these environmental constraints, macronutrient and micronutrient (e.g. selenium) limitations are expected to influence population dynamics as well. In late summer, anoxic conditions, generation of H_2S and accumulation of organic matter <20 m (Bustillos-Guzmán *et al.*, 2000; Lechuga-Devéze *et al.*, 2001) are probably limiting the abundance of vegetative cells in the water column and/or restricting their presence to subsuperficial layers, where temperature range oscillates between 20 and 29°C (Morquecho and Lechuga-Devéze, in press).

In spite of the moderate cell abundance of *G. catenatum* in Bahía Concepción, high concentrations of paralytic shellfish toxins are found in mollusks (Sierra-Beltrán *et al.*, 1996; Lechuga-Devéze and Morquecho-Escamilla, 1998). The Bahía Concepción isolates produce paralytic shellfish toxins, and their toxin profile correlates with that found in molluscs (Gárate-Lizárraga *et al.*, 2004; Band-Schmidt *et al.*, in press).

In summary, *G. catenatum* from Bahía Concepción seems to have evolved physiological and nutritional requirements that differ from those of other strains of this species. No significant changes in the growth rate were observed at different selenium concentrations, but biomass yields decreased proportionally with the addition of selenium in the seawater medium. The isolate from this bay exhibits a good growth at high salinities, does not require soil extract for growth, tolerates high temperatures and has better growth using seawater from this bay. Strains from Bahía Concepción should prove to be interesting in further studies, given their adaptation to high-salinity, low-runoff environment, in contrast to the moderate-salinity, runoff-dominated systems like those in the Huon estuary in Tasmania (Blackburn *et al.*, 1989).

ACKNOWLEDGEMENTS

The authors thank D. Kulis for his assistance in the growth experiments and D. Góngora and F. Zapata for their assistance during field work. They also thank CIBNOR Graduate Program and the National Council of Science and Technology (CONACyT) for financial and logistical support (grant 144382 to C.J.B.-S.). This research was supported in part by CIBNOR Institutional Research Projects AYCG-8, AYCG-11 (1999–2000) and CONACyT grant R33598-B. This work was also supported by NOAA Grant no. NA96OP0099 and NSF Grant no. OCE-9808173 (to D.M.A.) under the U.S. ECOHAB Program sponsored by NOAA, EPA, NSF, NASA and ONR. This is Contribution Number 11205 from the Woods Hole Oceanographic Institution and 114 from the ECOHAB Program.

REFERENCES

Anderson, D. M., Kulis, D. M. and Binder, B. J. (1984) Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis*: cyst yield in batch cultures. *J. Phycol.*, **20**, 418–425.

Band-Schmidt, C. J., Bustillos-Guzmán, J., Gárate-Lizárraga, I. *et al.* (in press) Paralytic shellfish toxin profile of the dinoflagellate *Gymnodinium catenatum* and the scallop *Argopecten ventricosus* from Bahía Concepción, Gulf of California, Mexico. *Harmful Algae.*

Blackburn, S. I., Hallegraeff, G. M. and Bolch, C. J. (1989) Vegetative reproduction and sexual life cycle of the toxic dinoflagellate *Gymnodinium catenatum* from Tasmania, Australia. *J. Phycol.*, **25**, 577–590.

Boyer, G. L. and Brand, L. E. (1998) Trace elements and harmful algal blooms. In Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. (eds), *Physiological Ecology of Harmful Algal Blooms.* NATO ASI Series G: Ecological Sciences. Vol. 41. Springer-Verlag, Berlin, pp. 489–508.

Bravo, I. (1986) Cyst germination, cultivation and encystment of *Gymnodinium catenatum* Graham. *Invest. Pesq.*, **50**, 313–321.

Bravo, I. and Anderson, D. M. (1994) The effects of temperature, growth medium and darkness on excystment and growth of the toxic dinoflagellate *Gymnodinium catenatum* from northwest Spain. *J. Plankton Res.*, **16**, 513–525.

Bustillos-Guzmán, J., López-Cortés, D., Hernández, F. *et al.* (2000) Pigment signatures associated with an anoxic zone: Bahía Concepción, Gulf of California. *J. Exp. Mar. Biol. Ecol.*, **249**, 77–88.

Cabrera-Mancilla, E., Ramírez-Camarena, C., Muñoz-Cabrera, L. *et al.* (2000) Primer registro de *Gymnodinium catenatum* Graham (Gymnodiniaceae) como causante de marea roja en la Bahía de Acapulco, Gro. México. In Ríos-Jara, E., Juárez-Carrillo, E., Pérez-Peña, M., López-Uriarte, E., Robles-Jarero, E. G., Hernández-Becerril, D. U. and Silva-Briano, M. (eds), *Estudios sobre el plancton en México y el Caribe.* Sociedad Mexicana de Planctología y Universidad de Guadalajara, Guadalajara, pp. 85–86.

Cortés-Altamirano, R. L., Nuñez-Pasten, A. and Pasten-Miranda, N. (1999) Abundancia anual de *Gymnodinium catenatum* Graham dinoflagelado tóxico de la costa Este del Golfo de California. *Cienc. Mar.*, **3**, 50–56.

Doblin, M., Blackburn, S. I. and Hallegraeff, G. M. (1999a) Comparative study of selenium requirements of three phytoplankton species: *Gymnodinium catenatum*, *Alexandrium minutum* (Dinophyta) and *Chaetoceros* cf. *tenuissimus* (Bacyllariophyta). *J. Plankton Res.*, **21**, 1153–1169.

Doblin, M., Blackburn, S. I. and Hallegraeff, G. M. (1999b) Growth and biomass stimulation of the toxic dinoflagellate *Gymnodinium catenatum* (Graham) by dissolved organic substances. *J. Exp. Mar. Biol. Ecol.*, **236**, 33–47.

Doblin, M. A., Blackburn, S. I. and Hallegraeff, G. M. (2000) Intraspecific variation in the selenium requirements of different geographic strains of the toxic dinoflagellate *Gymnodinium catenatum. J. Plankton Res.*, **22**, 421–432.

Estrada, M., Sánchez, F. J. and Fraga, S. (1984) *Gymnodinium catenatum* (Graham) en las rias gallegas (NO de España). *Invest. Pesq.*, **48**, 31–40.

Félix-Pico, E. F. and Sánchez, R. S. (1976) Tercer informe final del programa de orientación técnica para aprovechamiento de los recursos naturales existentes y prácticas de maricultivo en Bahía Concepción y Ensenada de La Paz. *Sec. de Asent. Rec. Hidrául.*, 20 pp.

Figueroa-Torres, M. G. and Zepeda-Esquivel, M. A. (2001) Mareas rojas del puerto interior, Colima, México. *Sci. Nat.*, **3**, 39–52.

Flynn, K. J., Flynn, K., John, E. H. *et al.* (1996) Changes in toxins, intracellular and dissolved free amino acids of the toxic dinoflagellate *Gymnodinium catenatum* in response to changes in inorganic nutrients and salinity. *J. Plankton Res.*, **18**, 2093–2111.

Fraga, S., Bravo, I. and Reguera, B. (1993) Poleward surface current at the shelf break and blooms of *Gymnodinium catenatum* in Ria de Vigo (NW Spain). In Smayda, T. J. and Shimizu, Y. (eds), *Toxic Phytoplankton Blooms in the Sea.* Elsevier, New York, pp. 245–249.

Fries, L. (1982) Selenium stimulates growth of marine macroalgae in axenic culture. *J. Phycol.*, **18**, 328–331.

Gárate-Lizárraga, I. and Siqueiros-Beltrones, D. A. (2003) Infection of *Ceratium furca* by the parasitic dinoflagellate *Amoebophrya ceratii* (Amoebo-(Amoebophryidae) in the Mexican Pacific. *Acta Bot. Mex.*, **65**, 1–9.

Gárate-Lizárraga, I., Bustillos-Guzmán, J. J. and Alonso-Rodríguez, R. (2001a) Distribution of *Gymnodinium catenatum* (Graham 1943) in coastal waters of México. *Harmful Algae News*, **22**, 7 (IOC Newsletter on toxic algae and algal blooms).

Gárate-Lizárraga, I., Hernández-Orozco, M. L., Band-Schmidt, C. J. *et al.* (2001b) Red tides along the coasts of Baja California Sur, México (1984–2001). *Oceánides*, **16**, 127–134.

Gárate-Lizárraga, I., Bustillos-Guzmán, J. J., Alonso-Rodríguez, R. *et al.* (2004) Comparative paralytic shellfish toxin profiles in two marine bivalves during outbreaks of *Gymnodinium catenatum* (Dinophyceae) in the Gulf of California. *Mar. Pollut. Bull.*, **48**, 397–402.

García, E. (1981) Modificaciones al sistema de clasificación climática de Köppen (Adaptada para la República Mexicana). Instituto de Geografía de la UNAM, Mexico.

Gennity, J. M., Bottino, N. R., Zingaro, R. A. *et al.* (1985) A selenium-induced peroxidation of glutathione in algae. *Phytochemistry*, **24**, 2817–2821.

Gilmartin, M. and Revelante, N. (1978) The phytoplankton characteristics of the barrier island lagoons for the Gulf of California. *Estuarine Coastal Mar. Sci.*, **7**, 29–47.

Góngora-González, D. T. (2001) Estructura microfitoplanctónica y condiciones hidrológicas relacionadas con la presencia de dinoflagelados tóxicos en bahía Concepción, B.C.S. México. BS Thesis. Universidad Autónoma de Baja California Sur, La Paz, B.C.S., Mexico, 55 pp.

Graham, H. W. (1943) *Gymnodinium catenatum*, a new dinoflagellate from the Gulf of California. *Trans. Am. Microsc. Soc.*, **62**, 259–261.

Greene, H. G. and Forrest, M. J. (2002) Shallow water hydrothermal venting along an onshore-offshore fault, Bahía Concepción, Baja California Sur, México and its influence on seafloor geology and biology. VI. Reunión Internacional sobre Geología de la Península de Baja California, La Paz, B.C.S., Mexico, 39 pp.

Guillard, R. R. L. (1973) Division rates. In Stein, J. R. (ed.), *Handbook of Phycological Methods.* Cambridge University Press, London.

Guillard, R. R. L. and Ryther, U. H. (1962) Studies on marine planktonic diatoms *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve). *Gran. Can. J. Microbiol.*, **8**, 229–239.

Hallegraeff, G. M. and 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. and Hallegraeff, G. M. (ed.), *Physiological Ecology of Harmful Algal Blooms.* NATO ASI Series. Vol. G 41. Springer-Verlag, Berlin, pp. 59–80.

Hallegraeff, G. M., Nichols, P. D., Volkman, J. K. *et al.* (1991) Pigments, fatty acids, and sterols of the toxic dinoflagellate *Gymnodinium catenatum*. *J. Phycol.*, **27**, 591–599.

Hallegraeff, G. M., McCausland, M. A. and Brown, R. K. (1995) Early warning of toxic dinoflagellate blooms of *Gymnodinium catenatum* in southern Tasmanian waters. *J. Plankon Res.*, **17**, 1163–1176.

Herrera-Galindo, J. E. (2002) Composición, abundancia y distribución de los dinoflagelados en la zona cercana a la línea de costa y marina adyancente al río Copalita, en bahías de Huatulco, Oaxaca (dic. 1997–oct. 1998). MS Thesis. Universidad del Mar., Puerto Angel, Oaxaca, Mexico, 158 pp.

Imai, I., Yamaguchi, M. and Wantanabe, M. (1998) Ecophysiology, life cycle and bloom dynamics of *Chatonella* in the Seto Inland Sea, Japan. In Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. (eds), *Physiological Ecology of Harmful Algal Blooms.* NATO ASI Series G: Ecological Sciences. Vol. 41. Springer-Verlag, Berlin, pp. 95–112.

Kaplan, D., Richmond, A. E., Dubinsky, Z. *et al.* (1986) Algal nutrition. In Richmond, A. (ed.), *Handbook of Microalgal Mass Culture.* CRC Press, Boca Raton, FL, pp. 147–198.

Kotani, Y., Mastuyama, Y. and Sakamoto, S. (2000) Biological and ecological characteristics of *Gymonidinum catenatum* in Japan. In *Ninth International Conference on Harmful Algal Blooms.* (conference abstracts).

La Barbera-Sánchez, A. and Gamboa-Maruez, J. (2001) Distribution of *Gymnodinium catenatum* Graham and shellfish toxicity on the coast of Sucre State, Venezuela, from 1989–1998. *J. Shellfish Res.*, **20**, 1257–1261.

Lechuga-Devéze, C. H. and Morquecho-Escamilla, M. L. (1998) Early spring potentially harmful phytoplankton in Bahía Concepción, Gulf of California. *Bull. Mar. Sci.*, **63**, 503–512.

Lechuga-Devéze, C. H., Reyes-Salinas, A. and Morquecho-Escamilla, M. L. (2001) Anoxia in a coastal bay: a case of study of a seasonal event. *Rev. Biol. Trop.*, **49**, 525–534.

Licea, S., Gómez-Aguirre, S., Cortés-Altamirano, R. *et al.* (1999) Notas sobre algunos florecimientos algales y la presencia de especies tóxicas en cinco localidades del Pacífico Mexicano (1996–1999). In Tresierra-Aguilar, A. E. and Culquichicón-Malpica, Z. G. (eds), *VIII COLACMAR.* Tomo I. Trujillo, Peru.

Maas, E. W., Thiele, J., Thompson, C. *et al.* (2000) Modifications of an algal culture medium for sustained growth of a saxitoxin-producing isolate of *Alexandrium minutum*. *J. Appl. Phycol.*, **12**, 95–98.

Manrique, F. A. and Molina, R. E. (1997) Occurrence of red tides in the Bacochibampo, Guaymas, Sonora, Mexico. *Hidrobiología*, **77**, 81–84.

Mee, L. D., Espinosa, M. and Díaz, G. (1986) Paralytic shellfish poisoning with *Gymnodinium catenatum* red tide on the Pacific coast of Mexico. *Mar. Environ. Res.*, **19**, 77–92.

Méndez, S. and Ferrari, G. (2002) Floraciones algales nocivas en Uruguay: antecedentes, proyectos en curso y revisión de resultados. In Sar, E. A., Ferrario, M. E. and Reguera, B. (eds), *Floraciones algales nocivas en el cono sur americano.* Instituto Español de Oceanografía, Pontevedra, pp. 269–288.

Morales-Blake, A., Hernández-Becerril, D. and Cavazos-Guerra, C. (2000) Registros de mareas rojas en las bahías de Manzanillo, Colima, México. In Ríos-Jara, E., Juárez-Carillo, E., Pérez-Peña, M., López-Uriarte, E., Robles-Jarero, E. G., Hernández-Becerril, D. U. and Silva-Briano, M. (eds), *Estudios sobre el plancton en México y el Caribe.* Sociedad Mexicana de Planctología y Universidad de Guadalajara, Guadalajara, pp. 81–82.

Morquecho, M. L. and Lechuga-Devéze, C. H. (2003) Dinoflagellates in recent sediments from Bahía Concepción, Gulf of California. *Bot. Mar.*, **46**, 132–141.

Morquecho, L. and Lechuga-Devéze, C. H. (2004) Seasonal occurrence of plankton dinoflagellates and cyst production in relationship to environmental variables in subtropical Bahía Concepción, Gulf of California. *Bot. Mar.*, **47**, 313–322.

Ogata, T., Kodama, M. and Ishimaru, T. (1989) Effect of water temperatures and light intensity on growth rate and toxin production of dinoflagellates. In Okaichi, T., Anderson, D. M. and Nemoto, T. (eds), *Red Tides: Biology, Environmental Science, and Toxicology.* NATO ASI Series Springer, Belgium, pp. 423–426.

Oshima, Y., Blackburn, S. I. and Hallegraeff, G. M. (1993) Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodinium catenatum* from three different countries. *Mar. Biol.*, **116**, 471–476.

Oshima, Y., Hasegawa, M., Yasumoto, T. *et al.* (1987) Dinoflagellate *Gymnodinium catenatum* as the source of paralytic shellfish toxins in Tasmanian shellfish. *Toxicon*, **25**, 1105–1111.

Osorio-Tafall, B. F. (1943) El Mar de Cortés y la productividad fitoplanctónica de sus aguas. *Anales de la Escuela Nacional de Ciencias Biológicas.* IPN, Mexico, **3**, 73–118.

Palomares-García, R., Martínez-López, A. and Gárate-Lizárraga, I. (2002) Plankton community changes in Bahía Concepción, Mexico. *Oceánides*, **17**, 113–128.

Pintner, I. J. and Provasoli, L. (1968) Heterotrophy in subdued light of 3 *Chrysochromulina* species. *Bull. Misaki Mar. Biol. Inst.*, **12**, 25.

Prakash, A., Rashid, M. A., Jensen, A. *et al.* (1973) Influence of humic substances on the growth of marine phytoplankton: diatoms. *Limnol. Oceanogr.*, **18**, 516–524.

Ramírez-Camarena, C., Cortés-Altamirano, R. and Muñoz-Cabrera, L. (1999) Mareas Rojas provocadas por el dinoflagelado *Gymnodinium catenatum* (Gymnodiniales: Gymnodiniaceae) en bahía de Mazatlán, Sin., México, en 1997. *Rev. Biol. Trop.*, **47**, 77–80.

Rengefors, K. and Anderson, D. M. (1998) Environmental and endogenous regulation of cyst germination in two freshwater dinoflagellates. *J. Phycol.*, **34**, 568–577.

Shumilin, E., Godinez-Orta, L., Cruz-Orozco, R. *et al.* (1996) Características litólogo-geoquímicas de los sedimentos superficiales de Bahía Concepción, B.C.S. Instituto Nacional de Geoquímica, A.C. Mexico. *Actas INAGEQ*, **2**, 79–84.

Sierra-Beltrán, A. P., Morquecho-Escamilla, M. L., Lechuga-Devéze, C. H. *et al.* (1996) PSP monitoring program at Baja California Sur, Mexico. In Yasumoto, T., Oshima, Y. and Fukuyo, Y. (eds), *Harmful and Toxic Algal Blooms.* IOC UNESCO, Paris, pp. 105–108.

Sierra-Beltrán, A., Cruz, A., Núñez, E. *et al.* (1998) An overview of the marine food poisoning in Mexico. *Toxicon*, **36**, 1493–1502.

Usup, G. and Azanza, R. V. (1998) Physiology and bloom dynamics of the tropical dinoflagellate *Pyrodinium bahamense*. In Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. (eds), *Physiological Ecology of Harmful Algal Blooms.* NATO ASI Series G: Ecological Sciences. Vol. 41. Springer-Verlag, Berlin, pp. 81–94.

Usup, G., Kulis, D. M. and Anderson, D. M. (1994) Growth and toxin production of the toxic dinoflagellate *Pyrodinium bahamense var. compressum* in laboratory cultures. *Nat. Toxins*, **2**, 254–262.

Watras, C. J., Chisholm, S. W. and Anderson, D. M. (1982) Regulation of growth in an estuarine clone of *Gonyaulax tamarensis*: salinity-dependent temperature responses. *J. Exp. Mar. Biol. Ecol.*, **62**, 25–37.

Yamamoto, T., Oh, S. J. and Kataoka, Y. (2002) Effect of temperature, salinity and irradiance on the growth of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae) isolated from Hiroshima Bay, Japan. *Fish. Sci.*, **68**, 356–363.