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Influence of temperature, salinity and irradiance on the growth and cell yield of the harmful red tide dinoflagellate *Alexandrium catenella* colonizing Mediterranean waters

MOHAMED LAABIR[1]*, CECILE JAUZEIN[2], BENJAMIN GENOVESI[3], ESTELLE MASSERET[1], DANIEL GRZEBYK[1], PHILIPPE CECCHI[4], ANDRÉ VAQUER[1], YVON PERRIN[1] AND YVES COLLOS[1]

[1]ECOLOGIE DES SYSTÈMES MARINS CÔTIERS, UMR5119, UM2, CNRS, IRD, IFREMER, UM1, UNIVERSITÉ MONTPELLIER 2, CC 093, 34095 MONTPELLIER, FRANCE, [2]THE UNIVERSITY OF TEXAS, MARINE SCIENCE INSTITUTE, 750 CHANNEL VIEW DRIVE, PORT ARANSAS, TX 78373, USA, [3]INSTITUT DES SCIENCES DE L'EVOLUTION UMR 5554 UM2-CNRS-IRD, UNIVERSITÉ MONTPELLIER 2, CC065, PLACE E. BATAILLON, 34095 MONTPELLIER CEDEX 05, FRANCE AND [4]IRD UMR G-EAU, 361 RUE JEAN FRANÇOIS BRETON, BP 5095, 34196 MONTPELLIER, FRANCE

*CORRESPONDING AUTHOR: mohamed.laabir@univ-montp2.fr

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In a laboratory study, we determined the influence of temperature, salinity and irradiance on the growth of the paralytic shellfish poisoning (PSP) toxin producer *Alexandrium catenella*, which can form toxic blooms in the Thau lagoon (western Mediterranean Sea). The strain studied, ACT03, was grown in an artificial seawater medium. The influence of temperature and that of salinity were analysed using 48 different combinations of 6 salinities (10–40 psu) and 8 temperatures (9–30°C) under saturating irradiance (100 μmol photons m^{-2} s^{-1}). ACT03 appeared to be an euryhaline strain that can survive at salinities as low as 10 psu and can grow at salinities up to 40 psu. This strain can grow between 15 and 30°C. The highest growth rates (>0.4 day^{-1}) were observed between 35 and 40 psu and 15 and 27°C. The influence of irradiance on growth and cell pigment content was tested between 10 and 260 μmol photons m^{-2} s^{-1} at 20°C and 38 psu. The results revealed both a low compensation irradiance and that light saturation was reached at 90 μmol photons m^{-2} s^{-1}. Temperature had the greatest influence on growth. The ecophysiological characteristics reported here are consistent with the environmental conditions encountered in the Thau lagoon. *A. catenella* exhibited important adaptive capacities over the large range of tested physical factors. This flexibility helps us to explain its ability to bloom seasonally on the Mediterranean coast, where the physico-chemical environmental conditions are characterized by high seasonal variations.

KEYWORDS: *Alexandrium catenella*; toxic blooms; Thau lagoon; growth; cell yield; temperature; irradiance; salinity

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INTRODUCTION

Proliferation of toxic dinoflagellates has a negative impact on marine resources, water quality and human health (Smayda, 1990). The frequency and distribution of harmful algal blooms (HABs) appears to be increasing globally in coastal environments (Hallegraeff, 1993; Glibert *et al.*, 2005). Among these dinoflagellates, *Alexandrium catenella*, a paralytic shellfish poisoning (PSP) toxin producer, is becoming widely distributed around the world (Lilly *et al.*, 2007). This species has been reported as causing extensive blooms in various marine waters, such as the Pacific Ocean off North America (Scholin and Anderson, 1994), Chile (Cordova and Muller, 2002), New Zealand (MacKenzie *et al.*, 2004), Japan, China, South Korea (Adachi *et al.*, 1996; Yeung *et al.*, 2002) and the western Mediterranean sea since 1994 (Vila *et al.*, 2001; Penna *et al.*, 2005). Large, frequent *A. catenella* blooms in Mediterranean waters seem to be restricted to harbours or confined zones (Garcès *et al.*, 1999; Penna *et al.*, 2005; Bravo *et al.*, 2008). For the first time, in 1998 a bloom of this species was observed in the Mediterranean lagoon of Thau (France) (Abadie *et al.*, 1999; Lilly *et al.*, 2002). Since then, several extensive blooms of *A. catenella* have occurred in the spring and in autumn during the last decade (with up to 15×10^6 cells L^{-1} observed in autumn 2004), which have impacted the nearby shellfish farms (Genovesi *et al.*, 2011).

Top-down control on harmful algae including grazing (Turner and Tester, 1997), viral lysis (Suttle, 1994) and algicidal bacteria (Doucette *et al.*, 1999) are involved in loss processes. Blooms of harmful dinoflagellates are affected by multiple environmental factors. Apart from the bottom-up controls associated with the availability of nutrient resources (see Collos *et al.*, 2007; Leong *et al.*, 2010), many studies have investigated the influence of physical factors on the biology and physiology of several *Alexandrium* species (Table I). Temperature impacts many physiological processes in HABs, such as division rate, photosynthesis, toxin production and respiration (Cembella, 1998; Nagasoe *et al.*, 2006; Fu *et al.*, 2008; Xu *et al.*, 2010). This environmental parameter strongly influences the dynamics of HABs by regulating the rate of cyst germination (Genovesi *et al.*, 2009) and vegetative growth (Navarro *et al.*, 2006). Salinity influences growth and cell toxin content (e.g. Giacobbe *et al.*, 1996; Parkhill and Cembella, 1999; Hwang and Lu, 2000; Grzebyk *et al.*, 2003; Band-Schmidt *et al.*, 2004; Kim *et al.*, 2004; Wang and Hsieh, 2005). Irradiance is also an important environmental factor that influences physiological reactions in microalgae; growth rate can be reduced under low irradiance, and photoinhibition can occur at a high light intensity (Kim *et al.*, 2004; Baek *et al.*, 2008). Other studies have reported on the influence of the culture medium and origin of the water used for cultivation on the growth of dinoflagellates, highlighting specific requirements regarding certain trace elements, such as selenium (Band-Schmidt *et al.*, 2004).

The adaptive and ecophysiological flexibility of dinoflagellates helps us to explain their fitness in fluctuating environments, such as may be encountered in semi-confined coastal ecosystems where they regularly proliferate. Thau (43°25′N, 03°39′E) is a shallow Mediterranean lagoon (mean and maximum depths of 4 and 10 m, respectively) permanently open to the sea and exposed to runoff, which can episodically influence water column salinity (Plus *et al.*, 2003). The rain pattern is characterized by strong inter-annual variability (200–1000 mm $year^{-1}$). The seasonal climate fluctuations impose a wide range of water temperatures (3–29°C) and salinities (27–40 psu) (Mazouni *et al.*, 1998). Water temperature and wind regime (direction and strength) are likely the most important environmental factors leading to or precluding *A. catenella* blooms in the Thau lagoon, via control of the water column stability (Laanaia *et al.*, 2008). Studies on the HABs species (and of *A. catenella* in particular) that investigate the physiological impact from combined abiotic factors remain scarce (Kudela *et al.*, 2010). Such an approach has never been carried out for the *A. catenella* strains blooming in Mediterranean waters. This is the aim of the present study, which examines the influence of salinity, temperature and irradiance on the growth and cell yield of *A. catenella* cultures grown under nutrient-replete conditions.

METHOD

Culture of *A. catenella* and growth rate measurements

The *A. catenella* strain ACT03 used in this study was obtained after isolation of a single vegetative cell from a seawater sample that was collected during the toxic bloom event in October 2003 in Thau. Since then, ACT03 has been maintained in batch cultures on the sterilized artificial medium ESAW (enriched artificial sea water), without silicates (Harrison *et al.*, 1980) at 38 psu, at a temperature of 20°C and under a cool-white fluorescent illumination (100 μmol photons m^{-2} s^{-1}) under a 12 h:12 h light:dark cycle. The experiments were carried out using sterile flasks, each containing 30 mL of culture medium inoculated with

Table I: Summary of main physico-chemical parameters most favourable for Alexandrium growth as determined in various in vitro *and in situ studies. Growth rate and/or maximum cell concentration correspond to the highest reported values in these studies. For* in vitro *studies, strain name is provided near the species name when available*

Species and studied area	Maximum cell density ($\times 10^6$cells L^{-1})	Maximum growth rate (day^{-1})	Temperature (°C) for optimal growth, [c]fixed temperature	Salinity (psu) for optimal growth, [c]fixed salinity	Light (μmol m^{-2} s^{-1}), CI: compensation irradiance, SI: saturating irradiance, [c]fixed irradiance	Reference
Mediterranean waters						
A. catenella ACT03 (Thau)	22	0.4–1.0	20–27	30–38	SI: 90	This study
A. tamarense/catenella complex (Thau)[a]	16	0.59–0.9	18–22	36–38	[b]	Collos *et al.* (Collos *et al.*, 2004, 2007)
A. catenella (Tarragona Harbor, Spain)	10	0.44	21–22	[b]	[b]	Garcés *et al.* (Garcés *et al.*, 2005)
A. catenella (Catalan coast, Spain)[a]	26	[b]	21–24	34–37	[b]	Bravo *et al.* (Bravo *et al.*, 2008)
A. minutum (Catalan coast, Spain)[a]	33	[b]	12–14	34–36	[b]	Bravo *et al.* (Bravo *et al.*, 2008)
A. minutum (Syracuse Bay, Sicily, Italy)[a]	0.1	[b]	16–24	32–37.7	[b]	Vila *et al.* (Vila *et al.*, 2005)
A. minutum (Ganzirri lagoon, Sicily, Italy)[a]	0.06	[b]	17–21	29–30	[b]	Giacobbe *et al.* (Giacobbe *et al.*, 1996)
A. minutum (Greece)[a]	0.22	0.25	20[c]	38.5[c]	60[c]	Ignatiades *et al.* (Ignatiades *et al.*, 2007)
Asian waters						
A. catenella (Hong Kong, China)	18	0.28	20–25	30–35	120[c]	Siu *et al.* (Siu *et al.*, 1997)
A. catenella TNY7 (Japan)	2	0.51	20–25	25–35	SI: 56	Matsuda *et al.* (Matsuda *et al.*, 2006)
A. catenella (Japan)[a]	[b]	[b]	10–15	32	[b]	Iwasaki (Iwasaki, 1992)
A. tamarense (Japan)[a]	0.076	0.23	7.5–9.0	31–33	[b]	Ichimi *et al.* (Ichimi *et al.*, 2001)
A. tamarense ATHS-92 (Japan)	[b]	0.54	17	25	SI:250	Hamasaki *et al.* (Hamasaki *et al.*, 2001)
A. tamarense ATHS-93 (Japan)	[b]	0.42	17[c]	29	350[c]	Hamasaki *et al.* (Hamasaki *et al.*, 2001)

A. tamarense ATHS-95 (Japan)	[b]	0.35	17	36.5[c]	SI:160	Hamasaki *et al.* (Hamasaki *et al.*, 2001)
A. tamarense HK9301 (Hong Kong, China)	21	0.6	23[c]	25–30	80–220	Wang and Hsieh (Wang and Hsieh, 2005)
A. tamarense AtPA01 (Malaysia)	>10	0.42	25[c]	25–30	140[c]	Lim and Ogata (Lim and Ogata, 2005)
A. tamiyavanichii AcMS01 (Malaysia)	~2.5	0.35	25[c]	25	140[c]	Lim and Ogata (Lim and Ogata, 2005)
A. peruvianum ApKS01 (Malaysia)	~7	0.35	25[c]	15	140[c]	Lim and Ogata (Lim and Ogata, 2005)
A. affine (Vietnam)	35	0.35	24	30	25[c]	Nguyen-Ngoc (Nguyen-Ngoc, 2004)
A. minutum T1 (Taiwan)	200	[b]	25	15	120	Hwang and Lu (Hwang and Lu, 2000)
A. minutum AmKB06 (Malaysia)	~45	0.46	25[c]	10–30	140[c]	Lim and Ogata (Lim and Ogata, 2005)
North American Atlantic waters						
A. fundyense GtMP (Gulf of Maine, USA)	14	0.37	20	[b]	SI: 200	Anderson *et al.* (Anderson *et al.*, 1984)
A. fundyense MI (Gulf of Maine, USA)	[b]	0.7	15	25–30	≥175 (CI: 15)	Etheridge and Roesler (Etheridge and Roesler, 2005)
A. fundyense BoF (Bay of Fundy, USA)	[b]	0.5	15	25	≥175 (CI: 15)	Etheridge and Roesler (Etheridge and Roesler, 2005)
A. tamarense Pr18b (St Lawrence Estuary, Canada)	10	0.5	[b]	25	SI: 150	Parkhill and Cembella (Parkhill and Cembella, 1999)
South American waters						
A. catenella ACC02 (South of Chile)	29.5 (12°C)	0.3 (14°C)	14	30	59.53[c]	Navarro *et al.* (Navarro *et al.*, 2006)
A. tamarense AT-D3 (Argentina)		0.3	15	30–35	SI: 130	Fulco and Gayoso (Fulco and Gayoso, 2004)
European Atlantic waters						
A. minutum AM89BM (Brittany, France)	4.4	0.6	18[c]	25	100[c]	Grzebyk *et al.* (Grzebyk *et al.*, 2003)
A. ostenfeldii LF37 (Denmark)	50	0.24	20	15–20	75[c]	Jensen and Moestrup (Jensen and Moestrup, 1997)

[a] *In situ* studies.
[b] No data available.
[c] Fixed environmental parameter used during the laboratory experiments.

200 cells mL^{-1}. All of the experiments were carried out in triplicate cultures.

In the first set of experiments, the growth rates were studied using a crossed factorial design with 48 different conditions, obtained from combining eight temperatures (9, 12, 15, 18, 21, 24, 27 and 30°C) and six salinities (10, 15, 20, 30, 35 and 40 psu) under an irradiance of 100 μmol photons m^{-2} s^{-1}. To prevent any shock to the inocula due to changes in temperature and salinity, the cultures were pre-acclimated to the desired experimental conditions via stepwise transfer over a defined period (≥25 days), following Yamaguchi and Honjo (Yamaguchi and Honjo, 1989) and Kim *et al.* (Kim *et al.*, 2004).

In a second set of experiments dedicated to investigating the effect of irradiance, the inoculum culture was grown at 20°C and 100 μmol photons m^{-2} s^{-1}. Eight light intensities were then tested: 10, 30, 50, 70, 90, 130, 200 and 260 μmol photons m^{-2} s^{-1}. These intensities were obtained by attenuating the cool-white fluorescent light with absorbing vinyl screens and further controlled using a quantum light meter (LI-COR Quantum/Photometer). For this second set of experiments, the salinity was set at 38 psu, and the cultures were incubated at 20°C.

For both experiments, every day over 2 weeks, the experimental flasks were gently shaken and 500 μL samples were fixed using Lugol's iodine solution. Cell concentration was monitored daily by direct microscopic counts using a Nageotte counting chamber. Following Guillard (Guillard, 1973), the specific growth rate (μ; day^{-1}) was calculated from the slope of a linear regression over the entire exponential phase of growth by the least square fit of a straight line to the data after logarithmic transformation; $\mu = (\mathrm{Ln}N_t - \mathrm{Ln}N_o)/(t_1 - t_o)$ in units of day^{-1} where N_o and N_t represent the cell density in cells mL^{-1} at the start, t_o, and end, t_1, of the exponential phase, respectively. Growth was monitored either for at least 15 days or until the maximum cell concentration (cell yield) was reached.

Cell morphology, size and pigment analyses

To assess the influence of salinity and irradiance on cell size and morphology, at least thirty fixed cells from each flask were photographed during the exponential growth phase using a digital video camera connected to an optical microscope (AxioLab, Zeiss) with a ×10 lens. The photographs were digitally processed using the software Optilab Pro 2.6.1, which automatically extracted the mean diameter in micrometre. Morphological features, including cellular anomalies and the formation of temporary cysts, were recorded.

For pigment analysis, the concentrations of Chlorophyll a (Chl *a*), Chlorophyll c (Chl *c*) and Phaeophytin (Phae *a*) were spectrofluorometrically determined in accordance with Neveux and Lantoine (Neveux and Lantoine, 1993). Five millilitre from each culture in exponential growth phase were filtered using a 25-mm diameter GF/F filter on the same day in which the morphometric analyses were carried out. Pigments were extracted using 6 mL of 90% acetone for 24 h at 4°C after disrupting the filter with a 10-s sonication. The samples were centrifuged (at 3500 rpm, 3°C for 7 min) and a 3 mL subsample was analysed using a Perkin Elmer LS50B spectrofluorometer. Pigment concentration was expressed as pg $cell^{-1}$, using the cell concentration values that were obtained the same day from cell counts.

Modelling temperature and salinity influences

The growth rates and cell yield data sets obtained from our experiments were used to infer dimensionless functions, which allowed us to model the growth and biomass response at different combinations of temperature and salinity. These functions varied between 0 and 1 and modulated the maximal value (μ_{max} for growth rate and D_{max} for cell density). The same model was used for both data sets and based on the following equation:

$$X = X_{max} \times f_T \times f_S \quad (1)$$

where the variable X is expressed as a function of the maximal value X_{max} (μ_{max} in day^{-1} or D_{max} in cell mL^{-1}), which is regulated by a temperature-response function f_T and a salinity-response function f_S.

For modelling the limitation equations, f_T and f_S, formulations were based on parameters emphasizing the joined influence of temperature and salinity on the growth of ACT03 via the integration of specific criteria [equations (2) and (4)]. With respect to our data sets, the Thébault function (Thébault, 1985; Andersen and Nival, 1988; Tian, 2006) appeared to be the most efficient for modelling the combined influences from temperature and salinity; it uses a few, though robust parameters suitable for fitting growth and cell yield.

Dependence on temperature took the form of a Thébault function, varying between 0 and 1 [equation (2)]:

$$f_T = 2 \times (1 + \alpha) \times \frac{\beta}{(\beta^2 + 2 \times \alpha \times \beta + 1)} \text{ with } \beta = \frac{(T - T_{thr})}{(T_{opt} - T_{thr})} \quad (2)$$

where T is the temperature (in °C), T_{thr} (in °C) is the threshold temperature under which the biological rate is zero (e.g. no growth), T_{opt} (in °C) is the optimal temperature and α is a constant. Additional constraints were added to optimize the adjustment of the model to our experimental data. The model was adapted given that the optimal temperature (T_{opt}) depends on salinity [equations (3a)–(3c)]:

$$T_{opt} = T_{opt-min} \quad \text{if } S \leq 20\,\text{psu} \tag{3a}$$

$$T_{opt} = \times\, T + b \quad \text{if } 20\,\text{psu} < S < 30\,\text{psu} \tag{3b}$$

$$T_{opt} = T_{opt-max} \quad \text{if } S \geq 30\,\text{psu} \tag{3c}$$

where $T_{opt\text{-}min}$ (in °C) and $T_{opt\text{-}max}$ (in °C) are the optimal temperatures obtained for lower and higher salinity conditions (S in psu), respectively. The constants "a" and "b" were calculated as follows: $a = (T_{opt\text{-}max} - T_{opt\text{-}min})/(30-20)$; $b = T_{opt\text{-}min} - 20 \times a$.

The salinity-response function f_S was represented using a modified Thébault function that varied between a minimal positive value and 1, as follows:

$$f_S = f_{So} + (1 - f_{So}) \times f' \tag{4}$$

with $f' = 2 \times (1+\alpha') \times \beta'/(\beta' + 2 \times \alpha' \times \beta' + 1)$ and $\beta' = (S - S_{thr})/(S_{opt} - S_{thr})$where f_{So} is the minimum value obtained when the salinity (S in psu) is lower than the threshold value S_{thr} (in psu), S_{opt} (in psu) is the optimal salinity and α′ is a constant.

A representation of the temperature-response function f_T and the salinity-response function f_S estimated from these data is shown in Fig. 1a and b.

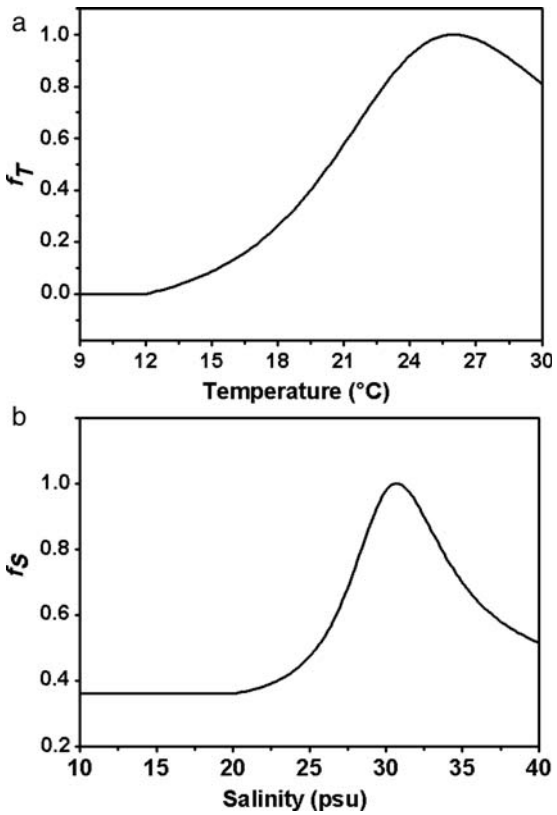

Fig. 1. Evolution of the temperature-response (f_T at 30 psu, a) and salinity-response (f_S at 20°C, b) functions used for the modelling of growth rates. These functions are described in equations (2), (3a–c) and (4).

Modelling the influence of irradiance

From the light experiment data, the trends observed along the irradiance gradient were simulated using either linear regression or equations derived from the Michaelis–Menten model. If an exponential increase in the parameter value was observed with increasing irradiance, the original Michaelis–Menten equation was used, taking into account a potential x-intercept or y-intercept as described in equations (5) (Kim *et al.*, 2004) and (6), respectively:

$$Y = Y_{max} \times \frac{(I - I_0)}{(I + (K_S - 2 \times I_0))} \tag{5}$$

$$Y = Y_0 + (Y_{max} - Y_0) \times \frac{I}{(I + K_S)} \tag{6}$$

where the parameter value Y (growth rate and cell yield) is a function of the maximal value (Y_{max}, Y units), the irradiance I (μmol photons m^{-2} s^{-1}), the half-saturation constant K_S (μmol photons m^{-2} s^{-1}), the x-intercept value I_0 (μmol photons m^{-2} s^{-1}) and the y-intercept value Y_0 (Y units).

When an exponential decrease was observed along the irradiance gradient, the modelled data were determined using the reverse Michaelis–Menten relation (Varela and Harrison, 1999):

$$Y = Y_{max} \times \left(1 - I_{n-max} \times \frac{I}{(I + K_{In})}\right) \tag{7}$$

where Y is the parameter value (cell pigment content), I is the irradiance (μmol photons m^{-2} s^{-1}), Y_{max} is the maximal value corresponding to the y-intercept (Y units), I_{n-max} is the maximal inhibition (values from 0 to 1) and K_{In} is the inhibition constant (irradiance at which the inhibition rate corresponds to $I_{n-max}/2$, in μmol photons m^{-2} s^{-1}).

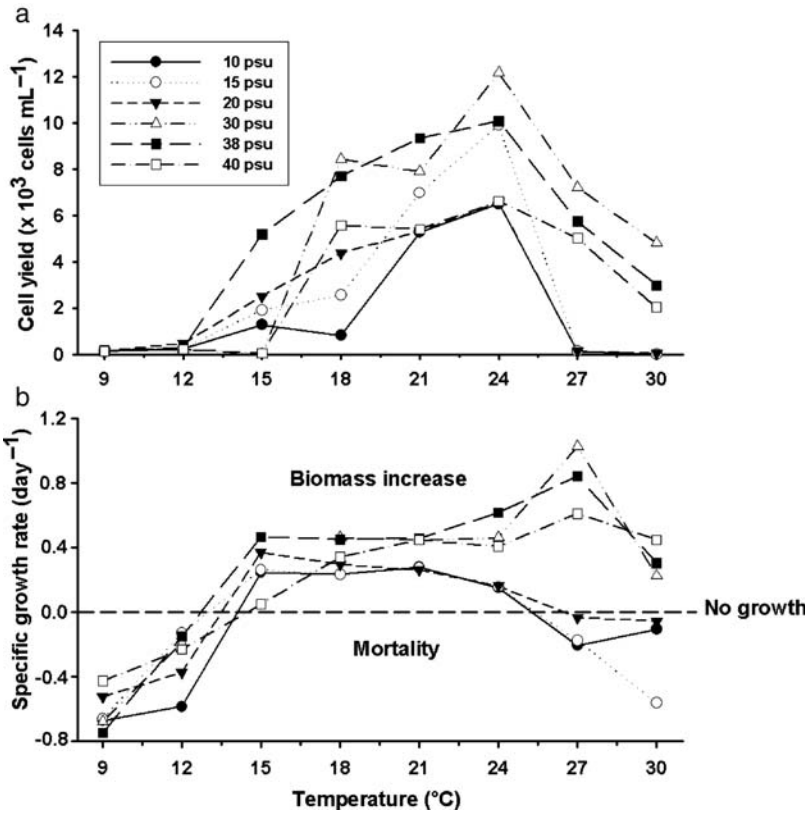

Fig. 2. Influence of salinity and temperature on the (a) cell yield and (b) specific growth rate for *A.catenella* at 48 combinations of salinity and temperature. Negative growth rates correspond to a decrease in cell concentration (with respect to inoculation time) due to mortality.

Statistics

To compare growth rate or cell yield data sets obtained from each experimental condition, we used either ANOVAs followed by Tukey's multiple comparison tests or unpaired *t*-tests, depending on the number of the environmental conditions tested. Statistical analyses were performed using Prism software (GraphPad Software, Inc.). Parameterization of the temperature, salinity and irradiance influences was accomplished using MATLAB (Mathworks).

RESULTS

Growth and cell yield at different temperatures and salinities

Temperature had a predominant influence on both the maximum cell concentration (Fig. 2a) and specific growth rate (Fig. 2b) for *A. catenella*. No growth occurred at temperatures $<12^{\circ}$C (Fig. 2b) and, even, cell densities decreased after inoculation suggesting mortality (Fig. 2a). Numerous temporary cysts were observed at 12°C. Growth occurred at all of the salinities tested between 15 and 24°C. Between 27 and 30°C, growth occurred only at the higher salinities (>20 psu). At 10 psu, morphological deformations of the cells were observed, but the cells swam actively. The highest growth rates were observed at 27°C at a salinity $\geq$30 psu, the highest value reaching 1 day^{-1} at 30 psu. However, the highest cell yield was observed at 24°C and the same salinity (30 psu). At salinities <20 psu, the maximum growth rates ($\sim$0.37 day^{-1}) were obtained at 18–21°C. The response-surface contours for the growth rate and cell yield data obtained from the combined temperature and salinity experiment are shown in Fig. 3a and b. Corresponding modelled surface contours following equation (1) are shown in Fig. 3c and d, respectively. For growth rate data, the optimal fit ($r^2 = 0.59$) was obtained for the following parameter values: $T_{thr} = 12^{\circ}$C; $S_{thr} = 20$ psu; $T_{opt} = 17^{\circ}$C when $S \leq 20$ psu and $T_{opt} = 26^{\circ}$C when $S \geq 30$ psu; and $S_{opt} = 30.7$ psu (Fig. 3c). For the cell concentration data, the entire data set did not allow a convergence with the model. However, when using data obtained from the salinity range 30–40 psu only, a convergence of the model ($r^2 = 0.76$) was obtained, where $T_{thr} = 12^{\circ}$C, $S_{thr} = 20$ psu, $T_{opt} = 23.2^{\circ}$C and $S_{opt} =$

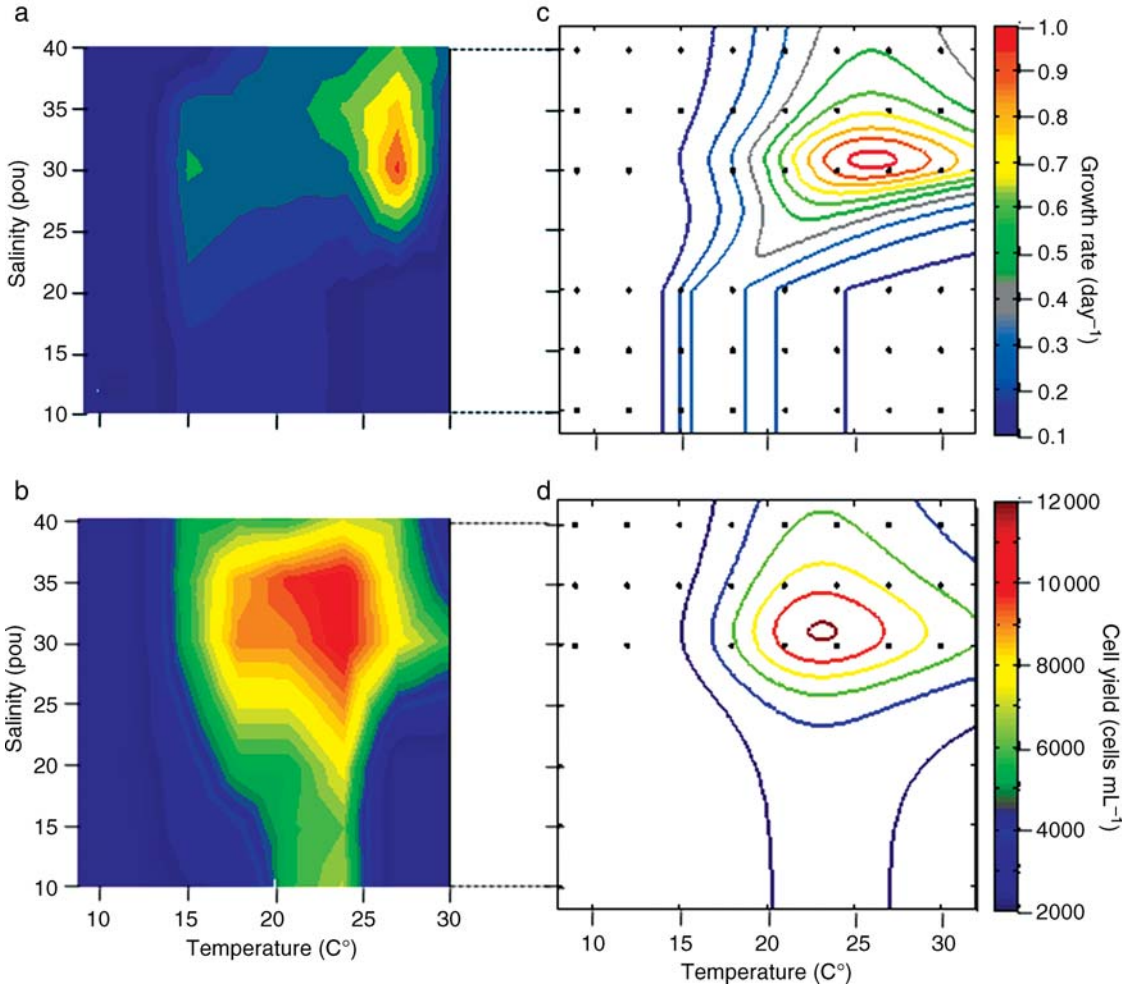

Fig. 3. Experimental data for the specific growth rate (in day^{-1}) (a) and cell yield (in cells mL^{-1}) (b) from 48 combinations of salinity and temperature. Modelled data for growth (c) and cell yield (d) were obtained from equations (1), (2), (3a, b, c) and (4).

30.7 psu. Using these parameter values in the model applied to the entire cell density data plot provided an $r^2 = 0.69$ (Fig. 3d).

Influence of irradiance

The specific growth rate of *A. catenella* was 0.14 day^{-1} at 10 μmol photons m^{-2} s^{-1}, the minimum irradiance tested in this study. The average growth rate at 30 μmol photons m^{-2} s^{-1} was 0.22 day^{-1}. Growth appeared to level off at 90 μmol photons m^{-2} s^{-1}, judging from the range of growth rates at higher irradiance levels (Fig. 4b). The optimum irradiance for growth was thus ≥90 μmol photons m^{-2} s^{-1}. Photoinhibition did not occur at irradiance levels of 260 μmol photons m^{-2} s^{-1}, which was the maximum irradiance tested in our study. Cell yield increased significantly with irradiance until 90 μmol photons m^{-2} s^{-1} and then reached a plateau at $18 \pm 2.5 \times 10^3$ cells mL^{-1} (Fig. 4a).

Michaelis–Menten equations (5) and (6) were applied to describe the exponential growth rate and increase in cell yield, respectively. The calculated compensation irradiance (I_0) was 8 μmol photons m^{-2} s^{-1} for both parameters measured. The maximum growth rate (μ_{max}) and half-saturating irradiance (K_S) were 0.5 day^{-1} and 23 μmol photons m^{-2} s^{-1}, respectively. The maximum cell yield (D_{max}) and half-saturating irradiance (K_S) were 23 450 cells mL^{-1} and 56.4 μmol photons m^{-2} s^{-1}, respectively.

Effect of salinity and irradiance on pigment content and cell size

Chl *a*, Chl *c* and Phae *a* cell content all decreased significantly (one-way ANOVA, $P < 0.0001$) with

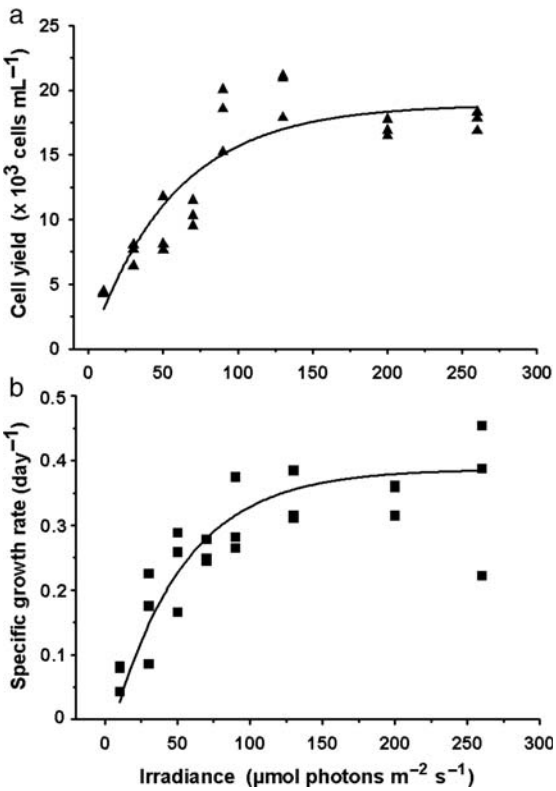

Fig. 4. Final cell concentration (a) and specific growth rate (b) for *A. catenella* strain ACT03 at various irradiance values (temperature = 20°C and salinity = 38 psu). The solid lines represent the modelled data obtained from equation (6) for final cell concentration and (5) for growth. The parameter values obtained were: (a) Y_{max} = 23 450 cell mL^{-1}, K_S = 56.4 µmol photons m^{-2} s^{-1}, Y_0 = 0 cell mL^{-1}, r^2 = 0.79; (b) μ_{max} = 0.50 day^{-1}, K_S = 23.7 µmol photons m^{-2} s^{-1}, I_0 = 8 µmol photons m^{-2} s^{-1}, r^2 = 0.77.

irradiance (Fig. 5a–c), by 78, 79 and 85%, respectively, between 10 and 260 µmol photons m^{-2} s^{-1}, corresponding to a ~5-fold decrease per cell. The Phae *a*/Chl *a* ratio decreased significantly (one-way ANOVA, $P < 0.0001$), from 0.10 to 0.07, as irradiance increased. The Chl *a* content decreased significantly (one-way ANOVA, $P = 0.0184$) with increasing salinity (Fig. 5d). A similar decrease in the minor pigment, Chl *c* (Fig. 5e), allowed the (Phae *a*/Chl *a*) ratio to remain relatively constant, between 0.07 and 0.08.

Cell diameter (Fig. 6a) decreased significantly (one-way ANOVA, $P = 0.0003$) with increasing salinity, from 29.1 ± 0.83 to 24.53 ± 0.93 µm when salinity increased from 20 to 40 psu. The data followed an inverse linear relationship ($r^2 = 0.76$). Cell diameter exhibited a significant decrease in ~30% (unpaired *t*-test, $P = 0.0047$) between light-saturating conditions and cells grown at 10 µmol photons m^{-2} s^{-1} from 25.91 ± 0.51 to 23.54 ± 0.51 µm, respectively (Fig. 6b).

DISCUSSION

For the ACT03 strain, light saturation was reached at ~90 µmol photons m^{-2} s^{-1}, as growth rate and cell size levelled off under higher irradiances at a fixed temperature (20°C, Figs. 4b and 6b). For other *Alexandrium* species grown under temperature and salinity values close to those used in our study, saturation irradiance ranged from 120 to 130 µmol photons m^{-2} s^{-1} (Siu *et al.*, 1997; Fulco and Gayoso 2004). Nevertheless, moderate growth rates were obtained at lower irradiances (≥0.3 day^{-1} under >30 µmol photons m^{-2} s^{-1}), with a compensation irradiance (I_0) estimated at 8 µmol photons m^{-2} s^{-1}. Furthermore, simultaneous changes in cell pigment content indicated that ACT03 adapts well to low light conditions. Decreasing light intensity (from 260 to 10 µmol photons m^{-2} s^{-1}) induced a decrease in cell size (Fig. 6b), whereas photoacclimation was responsible for a large increase in photosynthetic pigments (up to ~5-fold per cell). Chl *a* and Chl *c* increased from 15.9 ± 10.8 to 71.8 ± 7.8 pg cell^{-1} and from 1.2 ± 0.7 to 5.7 ± 0.6 pg cell^{-1}, respectively (Fig. 5a and b). Similar changes have been commonly observed in many phytoplankton studies, as smaller cells with increased light-harvesting capabilities are more likely to capture a low-light flux (Falkowski *et al.*, 1981; Falkowski and Raven, 1997). Nevertheless, our data suggested that photosynthetic activity decreased for light-limited cells, and subsequently carbon fixation, which in turn resulted in a decrease in cell size and growth rate.

The range of irradiance we used (10–260 µmol m^{-2} s^{-1}) did not allow for the detection of a photoinhibition threshold. However, no evidence for such a phenomenon was detected in a Chilean strain of *A. catenella* (Carignan *et al.*, 2002) grown between 100 and 800 µmol photons m^{-2} s^{-1}, confirming that the genus *Alexandrium* in general is adapted to high irradiances (Smayda, 2008). In contrast, photoacclimation to low light resulted in a nearly 3-fold increase in Chl *a* per cell, from 16 to 40 pg cell^{-1} in the Chilean strain (Carignan *et al.*, 2002), a smaller change than in our strain. To clarify the photoinhibition effect for our *A. catenella* isolate, further experiments must be carried out under higher irradiances. In the Thau lagoon, the maximum irradiance in the surface layer can be very high (e.g. 1736 µmol photons m^{-2} s^{-1} recorded in July 1999 at noon, Bec *et al.*, 2005). During the spring and autumn of 1999, which corresponds to the seasons when *A. catenella* typically blooms, the mean values for irradiance integrated over a 7-m depth were 380 and 184 µmol photons m^{-2} s^{-1}, respectively. Given that *A. catenella* cells concentrate at the sub-surface (0.5–1.5 m,

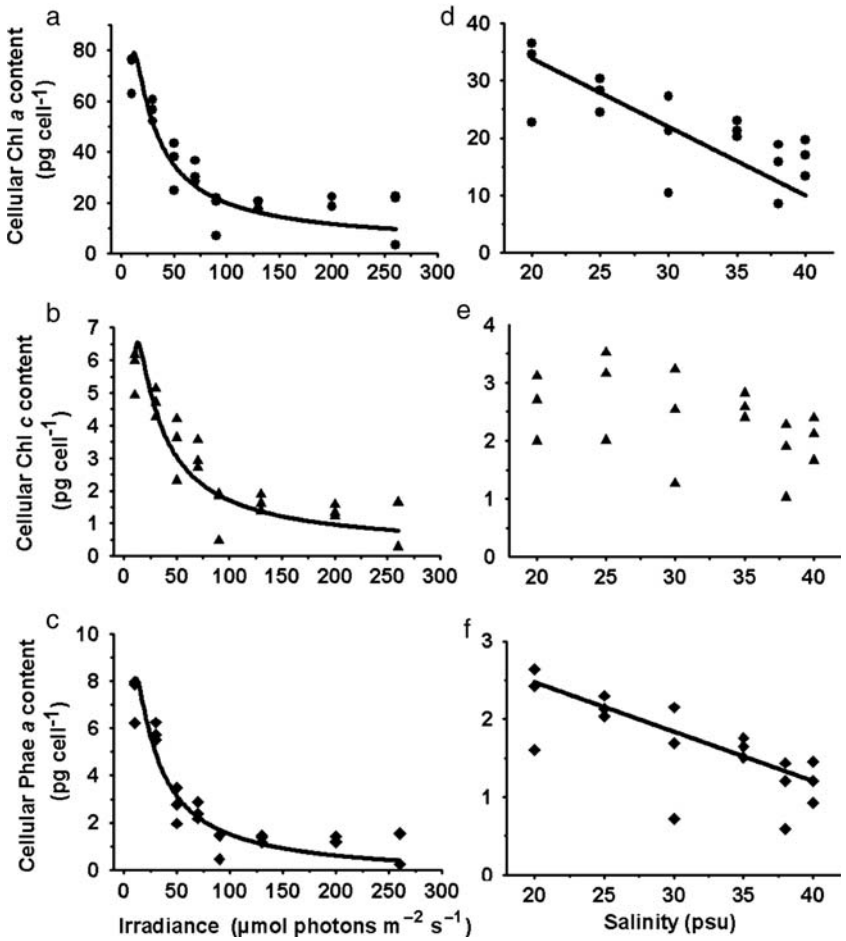

Fig. 5. Influence of irradiance (a–c) and salinity (d–f) on Chlorophyll a, Chlorophyll c and Phaeophytin—a specific cell concentration of *A. catenella*. Modelled data were obtained from equation (7) for (a), (b) and (c) and from linear regression for (d) and (f). The parameter values obtained were: (a) $\Upsilon_{max} = 101.0$ (pg cell^{-1}), $K_{In} = 25.2$ µmol photons m^{-2} s^{-1}, $I_{n\text{-}max} = 0.95$, $r^2 = 0.86$; (b) $\Upsilon_{max} = 7.32$ (pg cell^{-1}), $K_{In} = 41.4$ µmol photons m^{-2} s^{-1}, $I_{n\text{-}max} = 1$, $r^2 = 0.85$; (c) $\Upsilon_{max} = 11.4$ (pg cell^{-1}), $K_{In} = 20.0$ µmol photons m^{-2} s^{-1}, $I_{n\text{-}max} = 1$, $r^2 = 0.88$; (d) $\Upsilon = -1.19 \times S + 57.73$, $r^2 = 0.66$; (f) $\Upsilon = -0.63 \times S + 3.73$, $r^2 = 0.9$. There was no significant relationship between cellular Chlorophyll c content (e) and salinity.

unpublished data), the shallow depth in the lagoon (<10 m) and even <4 m in Angle Creek where blooms occur, and the low I_0 estimated in ACT03, this species seems well fitted to water column light conditions in Thau.

With respect to salinity, the optimal growth of the strain studied occurs between 30 and 40 psu. Therefore, this organism is well adapted to the salinity conditions recorded for the Angle Creek in the Thau lagoon, where salinity is usually ≥35 psu and can increase in ~39 psu in the summer, remaining high when the blooms appear in autumn. Within this range of variation, salinity does not seem to be a critical factor for *A. catenella* blooms in this Mediterranean lagoon. Our results demonstrate that the size of *A. catenella* cells increases when salinity decreases. This could be explained as an adaptation to the ambient osmotic pressure (Mayfield and Gates, 2007; Chen and Jiang, 2009). Moreover, the growth rate being weaker at low salinities, cell divisions are fewer and, consequently, cell size increases.

With respect to temperature, ACT03 seems to be thermophilic, but the experimental data show a strong interaction between temperature and salinity. The strain exhibited contrasting growth rates at high temperature–high salinity versus at high temperature–low salinity (Fig. 2b): it appears adapted to higher salinities when temperature ranged between 15 and 27°C. Our modelling approach, using growth rate and cell yield data, reflected the respective and combined influences of temperature and salinity on this organism. From growth rate data, the optimized model required that optimal temperature T_{opt} depends on salinity. Our model provided a growth temperature threshold (T_{thr}), optimal

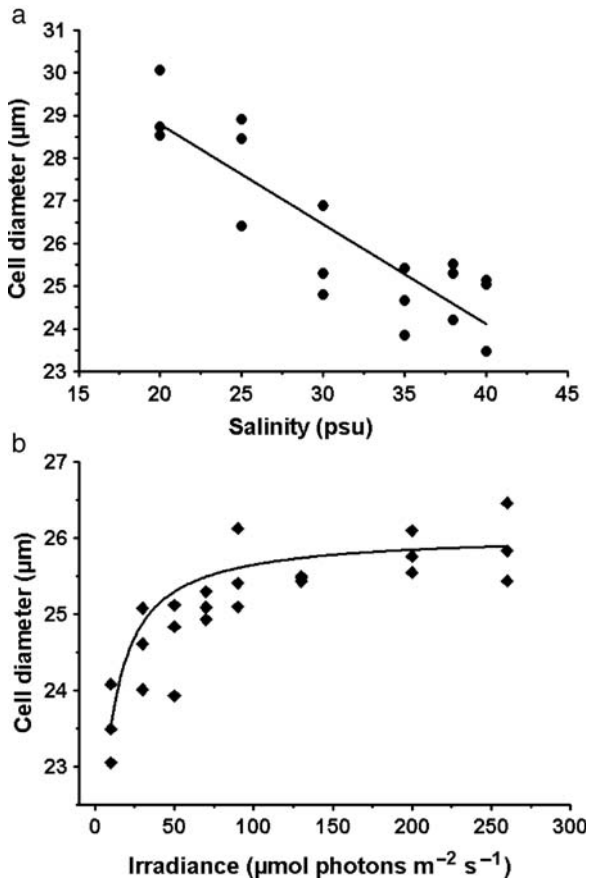

Fig. 6. Change in the cell diameter of the ACT03 strain during the exponential growth phase, as a function of salinity (a) and irradiance (b). Modelled data were obtained from linear regression and equation (6) for (a) and (b), respectively. The parameter values obtained were: (a) $D = -0.23 \times S + 33.51$, $r^2 = 0.76$; (b) $D_m = 26.3$ µm, $K_S = 35.8$ µmol photons m^{-2} s^{-1}, $D_O = 22.8$ µm, $r^2 = 0.78$.

temperature (T_{opt}) and optimal salinity (S_{opt}), which fitted well with the experimental data set, indicating that not only growth rates but also cell yields should be considered when experimentally investigating the optimal environmental growth conditions that might determine the *in situ* development of blooms.

In Thau lagoon, *A. catenella* blooms usually appear in spring and autumn at temperatures between 18 and 24°C (Laanaia *et al.*, 2008; Collos *et al.*, 2009), which correspond to the growth values of ~0.45–0.62 day^{-1} measured in the present study. The maximum growth rate determined here (~1 day^{-1}) corresponds rather closely to the maximum growth rate of 0.90 day^{-1}, determined *in situ* for nutrient-replete *A. catenella* (Collos *et al.*, 2007). Garcés *et al.* (Garcés *et al.*, 2005) reported somewhat lower values for the *A. catenella* populations sampled in Tarragona harbour (0.24–0.44 day^{-1}). Interestingly, the two sets of salinity and temperature conditions that prevailed for the *Alexandrium* blooms in the Thau lagoon during spring and autumn (18–24°C and 36–38 psu) are similar to those identified for other Mediterranean populations of *A. catenella* that bloom in Tarragona harbour where surface temperatures vary between 21 and 25°C and salinities range between 34 and 37 psu (Table I, Bravo *et al.*, 2008). *Alexandrium* cells, however, have been observed in the water column of Thau lagoon when the temperature is between 11°C (in autumn after the water cools) and 26–27°C (in July at the end of the spring bloom and at the end of August, which corresponds to an early autumn bloom period). While water temperature in the middle of summer might be optimal for growth, *Alexandrium* cells have never been detected (threshold 100 cells L^{-1}) in the water column by the phytoplankton monitoring network (REPHY) of Ifremer (E. Abadie, Ifremer, personal communication) from mid-July to the end of August. This discrepancy, between the optimum temperature allowing the higher growth rate determined *in vitro* and *in situ* bloom conditions, has been previously noted for other coastal phytoplankters (e.g. Grzebyk and Berland, 1996) and could be due to top-down factors such as grazing by micro-zooplankton and herbivorous copepods (Turner and Tester, 1997; Smayda, 2008). As seen *in vitro*, the much lower cell yield obtained at 27°C suggests that metabolic processes (e.g. respiration) might be sufficiently high as to render the organism less competitive in the summer with respect to other, less favourable environmental conditions that remain to be identified. As such, nutrient availability, including inorganic and organic nitrogen and phosphorus nutrient availability, might play an important role (Collos *et al.*, 2004; 2007; 2009; Jauzein *et al.*, 2010). Blooms of *A. catenella* in Thau lagoon have been shown to be nitrogen-limited (Collos *et al.*, 2004; 2007), at least on the basis of "classical" nutrients (e.g. nitrate), especially as blooms can occur during dry periods when no such nutrients (including nitrate and phosphate) are available. However, the mixotrophic character of this species (Burkholder *et al.* 2008) indicates that it may not experience nutrient limitation in a classical sense with respect to inorganic nutrients.

Data from studies on the globally distributed *A. catenella* show the ability of this dinoflagellate to develop in a wide range of marine areas (Table I). This species is ubiquitous and develops in Mediterranean, South American and Asian waters, and it exhibits cell densities up to 29.5×10^3 cells mL^{-1}. Matsuda (Matsuda *et al.*, 2006) reported optimal salinities between 20 and 28 psu for a strain of *A. catenella* from Tanabe bay in Japan. A study undertaken in Hong-Kong on *A. catenella* (Siu *et al.*, 1997) demonstrated an optimal salinity range between 30 and 35 psu, which matched their field

observations. The maximum growth rate ranged from 0.3 to 1 day^{-1} (Table I), with the highest values recorded for *A. catenella* strains that colonize Mediterranean coastal waters.

With respect to temperature, the optimum for growth of *A. catenella* strains ranged between 10 and 15°C for Japanese and Chilean strains and 20 and 27°C for strains living in the warmer Mediterranean waters. *In situ* bloom temperatures for *A. catenella* in the Mediterranean waters (between 18 and 24°C) are also among the highest reported in field data (Table I). Altogether, these observations suggest that Mediterranean *A. catenella* might represent ecotypes adapted to higher temperatures and/or higher salinities when compared with other world areas.

When considering the influence of abiotic factors on bloom dynamics, given that *A. catenella* has a life cycle that alternates between benthic resting cysts and vegetative planktonic cells, it is interesting to compare the environment conditions favourable for cyst germination with those allowing the growth of vegetative cells, and with respect to the time frame of bloom developments in the Thau lagoon. A previous study reported that the excystment of the resting cysts from the *A.catenella/tamarense* population in Thau lagoon was more successful between 12 and 28°C at an appropriate salinity (30–38 psu), but the excystment success markedly decreased <9 or >30°C (Genovesi *et al.*, 2009). These temperature conditions favouring excystment matched well our experimental growth data and water temperatures for which *Alexandrium* cells were observed in Thau lagoon (Collos *et al.*, 2009). Hence, in the spring, germination of resting cysts may be triggered at >12°C, the whole process taking about a week while the water is warming, so that planozygotes can be released at a temperature suitable for survival or slow vegetative growth. A few days or weeks later, *Alexandrium* cells are detected near the surface (at ~0.7 m sampling depth) when water temperature reaches 14–15°C, and the bloom may subsequently develop as water temperature rises (Collos *et al.*, 2009; unpublished data). Conversely in autumn, germination of resting cysts may start at a temperature suitable for optimum growth (e.g. 22–25°C), which might favour a more rapid bloom development, thus explaining why denser blooms occur during this season, and with the help of longer periods of time (up to several weeks), when compared with spring, under NW wind <4 m s^{-1} (Laanaia *et al.*, 2008).

It can be concluded that the highly adaptive capacity to temperature, salinity and irradiance of the *A. catenella* strain studied may be among the reasons favouring the large blooms of *A. catenella* in Thau. Furthermore, with respect to its euryhalinity, this organism may have the potential to spread to other Mediterranean lagoons, including nearby lagoons along the French Mediterranean coast that are characterized by broad fluctuations in salinity and water temperature. Further study using a large number of *A. catenella* strains would be necessary to obtain an array of ecophysiological responses to environmental abiotic conditions prevailing in Thau lagoon that would be representative of this *A. catenella* population. This would result in a better understanding of the influence of abiotic factors on the *A. catenella* bloom dynamics in this lagoon.

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