Vol. 301: 67–79, 2005 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published October 11

Taxon-specific analysis of growth and mortality rates of harmful dinoflagellates during bloom conditions

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ABSTRACT: Growth and mortality rates of natural single *Alexandrium* spp. cells were measured by the Landry-Hassett dilution technique during different phases of blooms. Taxon-specific experiments were conducted between May and October 2002 during 3 intense blooms of *A. taylori* and *A. catenella* at different locations of the Mediterranean Sea. In addition, dilution experiments using chlorophyll *a* as a proxy for total phytoplankton biomass were used to estimate daily rates of net growth and mortality of the total phytoplankton community. *A. taylori* growth rates ranged from 0.04 to 0.67 d^{-1} and mortality rates from −0.20 to −0.65 d^{-1}. Growth rates of *Gymnodinium* sp., an accompanying dinoflagellate species during the *A. taylori* bloom studied, were similar to those measured for *A. taylori*, whereas their mortality rates (−0.58 to −0.82 d^{-1}) were slightly higher. *A. catenella* growth and mortality rates were balanced (0.24 and 0.44 d^{-1} compared with −0.25 and −0.44 d^{-1}, respectively). The highest mortality rates (−0.65 d^{-1}) were measured during the decline phase of 2 *A taylori* blooms. At the decline of the blooms, *A. taylori* and *A. catenella* showed considerable mortality, but microzooplankton grazing was not confirmed to be the main cause of the bloom termination. In general, growth was not limited by nutrients in the experiments. There were a few cases of a potential nutrient limitation in these areas and, in general, blooms were not conditioned by nutrients. When changes in biomass (chlorophyll *a*) were measured, non-linearity of data due to saturation was observed. The interpretation of these results required a split-function model. Saturated grazing (G_s) was 28.9 µg chl *a* l^{-1} d^{-1}, during which the saturating phytoplankton population represented a chl *a* concentration of 16 µg l^{-1} (P_s).

KEY WORDS: Dilution technique · Growth rate · Mortality rate · Dinoflagellates · HAB · *Alexandrium* · *Gymnodinium*

INTRODUCTION

Blooms of noxious phytoplankton are recurrent events in coastal waters. Such harmful algal blooms (HABs) can be local phenomena or affect large areas. In either case, they may endanger human health, marine ecosystems and resources such as tourism, fisheries and aquaculture. Efforts to model HAB dynamics are important in order to predict the location and timing of HAB events. However, the required data on the key biological processes in bloom dynamics, such as vegetative growth and loss rates due to mortality, are almost non-existent during natural bloom conditions (Garcés & Masó 2001). Studies reporting *in situ* specific growth rates during major dinoflagellate proliferations (Chang & Carpenter 1988, Reguera et al. 1996) are more abundant than studies of mortality rates (Agusti et al. 1998, Agusti & Duarte 2000). The lack of data on the required variables severely limits the modelling processes and therefore the prediction of HAB events.

The dilution technique has been broadly applied in aquatic ecosystems to estimate growth and grazing impact rates on phytoplankton (Landry & Hassett 1982, Landry et al. 1995). This approach has been critically

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analyzed (Gallegos 1989, Landry et al. 1995). The dilution technique can only provide unbiased estimates of phytoplankton growth and mortality rates when several assumptions are met: (1) growth rate of phytoplankton must not be altered by dilution per se; (2) growth must not be limited by available nutrients; (3) phytoplankton must grow exponentially; and (4) consumption rates of the microzooplankton must be linear with respect to the phytoplankton concentration. The first assumption is not difficult to test or control in practice (Landry et al. 1995). As for the second one, the growth rate of phytoplankton can be estimated by comparing the rates measured in undiluted samples with or without nutrient additions (Landry & Hassett 1982, Andersen et al. 1991, Landry et al. 1995). The most important assumption is that grazing impact must vary in direct proportion to the dilution of the grazer population density. This assumption can be easily altered when the clearance rate of individual grazers and/or growth response of their total population vary significantly with food concentration over the course of the incubation. Finally, the consumption rates of the microzooplankton could be non-linear with respect to the phytoplankton concentration (Gallegos 1989, Evans & Paranjape 1992, Redden et al. 2002). In spite of these limitations, the number of published records based on dilution experiments is increasing due to the fact that it is a useful and simple method for estimating biological rates (Dolan et al. 2000, Calbet & Landry 2004).

The genus *Alexandrium* is the group of dinoflagellates associated with most HABs in the Mediterranean Sea (coastal locations), and the species *A. taylori* and *A. catenella* are two of the most problematic members. The first bloom event described for *A. taylori* occurred at La Fosca beach (1994), a pocket beach situated on the Catalan coast (NW Mediterranean) (Delgado et al. 1997). However, it is known that blooms of this species have occurred at Mediterranean beaches since the 1980s (Garcés et al. 1998, Giacobbe & Yang 1999). The ability of *A. taylori* to produce and maintain high densities ($>10^5$ cell l^{-1}) in protected regions leads to a greenish-brown discoloration of the water masses during the summer months (June to August). Recently, paralytic shellfish poisoning (PSP) and novel protein toxins have been described for this species (Emura et al. 2004, Lim et al. 2005). The *A. taylori* bloom studied was unusual considering its extremely high biomass levels and its relatively long duration (Garcés et al. 1999). The long maintenance phase of the bloom was due to a low population loss rate related to a critical coupling between local circulation patterns (breeze conditions) and biological strategies (vertical migration of the organisms) (Basterretxea et al. 2004). The *A. taylori* bloom at La Fosca was characterized by high cell numbers of the accompanying dinoflagellate *Gymnodium* sp. This species is an unarmoured non-toxic planktonic species known to produce high cell numbers at beaches along the coasts of Catalonia and the Balearic Islands. Cells range in size from 35 to 40 µm in length. The epitheca and hypotheca are nearly equal in size and the cells have numerous large, reddish-yellow-brown chloroplasts. HPLC analysis of pigments revealed the presence of the marker pigment peridinin.

The toxic (PSP containing) *Alexandrium catenella* is generally considered to be a cold-water species, but in the Mediterranean Sea it is mainly found during the summer months (Vila et al. 2001b, Lugliè et al. 2003). Since the first Mediterranean blooms of this species in the harbors of Valencia and Barcelona (1994 and 1996 respectively), there has been growing evidence of its geographical expansion along coastal areas of the NW Mediterranean Sea. In addition to the possibly allochthonous origin of *A. catenella* in the region, a high probability of anthropogenic introduction (probably by means of ballast water) has been suggested for this species, which is closely related to Japanese strains (Lilly et al. 2002, Penna et al. 2003). In the Mediterranean, this species may reach high cellular concentrations ($>10^5$ to 10^6 cells l^{-1}) in harbors for periods of 2 to 3 wk. Widespread blooms covering both confined and non-confined water have also been detected (Vila et al. 2001a).

The aim of the present study was to evaluate the importance of growth and mortality rates as regulating mechanisms for the development, maintenance and decline phases of harmful algal blooms. We analyzed the taxon-specific growth and mortality rates by applying the dilution method during 3 different bloom events, including an almost monospecific bloom of *Alexandrium catenella* (Tarragona harbour, Catalan coast) and 2 *A. taylori* blooms (La Fosca beach, Catalan coast and Vulcano beach, Sicily). Daily growth and mortality rates of the total phytoplankton community were estimated using chlorophyll *a* as a proxy for their biomass.

MATERIALS AND METHODS

Dilution experiments were performed during the temporal evolution of 3 individual dinoflagellate blooms, dominated by target species, i.e. *Alexandrium taylori* at La Fosca beach (Catalan coast, Spain) and Vulcano beach (Sicily, Italy) and *Alexandrium catenella* in Tarragona harbor (Catalan coast, Spain).

Coastal monitoring stations. The study area, La Fosca beach, is located on the Costa Brava (41° 50′ N, 3° 08′ E, NW Mediterranean). Its dimensions are 525

× 300 m (approximately rectangular) and it opens towards the SE. Detailed information on *Alexandrium taylori* blooms at this location has been previously described (Garcés et al. 1999, 2002). During the summer of 2002, surface samples were collected at a fixed point (1 m water depth) every 3 to 4 d.

The West Bay of Vulcano (Sicily, 38° 25′ N, 14° 57′ E), a tourist locality of the Aeolian Islands (Tyrrhenian Sea), is a shallow, semicircular beach of approximately 300 × 100 m that opens towards the NW and covers about 7 ha. Recurrent summer blooms of *Alexandrium taylori* have been detected at several points of the bay with evident water discoloration (see Penna et al. 2002 for bloom details and topography of the area). A dilution experiment was conducted at a sampling point frequently affected by a persistent, dense patch of *A. taylori*. Between June and October 2002, the surface layer of this location (1 m water depth) was sampled on a weekly basis. Sampling usually took place between 12:00 and 17:00 h.

Tarragona harbor (41° 51′ N, 1° 13′ E, Catalonia) is an industrial and fishing harbor situated on the Costa Daurada (NW Mediterranean). It covers about 154 ha (4.5 km long; 600 m to 2 km wide; 21 m maximum depth), and is therefore one of the largest in the Mediterranean. A fixed point, located in the inner part of Tarragona harbor, was sampled every 3 to 5 d from September to November 2002.

The parameters measured during the 3 blooms studied included quantification of target species and other phytoplankton, chlorophyll *a*, salinity, temperature and inorganic nutrients.

Dilution experiment. Estimates of phytoplankton growth rates and microzooplankton grazing rates were based on changes in total chlorophyll *a* and abundance of target species observed in a series of incubations in which the original water sample was diluted with filtered water from the same site following the methods described in Landry & Hassett (1982). During the *Alexandrium taylori* blooms at the La Fosca and Vulcano beaches, a subset of 7 dilution experiments were performed during the 3 bloom phases: development, maintenance and decline (see Table 1). During the *A. catenella* bloom in the Tarragona harbor, 2 experiments covering the maintenance and decline phases of the bloom were performed.

Surface water (not pre-filtered) was diluted (7 levels) with filtered seawater (Whatman GF/F filter) collected from the same location. Treatments ranged from 100 to 2% of unfiltered water in duplicate 2 l polycarbonate bottles. All material employed was pre-cleaned with 10% HCl Milli-Q water and rinsed (3 times) with distilled water. For each experiment, 14 bottles were used for the nutrient-enriched dilution series. Two additional bottles were filled with natural seawater only and employed as control samples without nutrients. Nutrient additions were based on specific volumes of SIGMA f/2 artificial, Guillard's (f/2) Marine Water Enrichment Solution (containing 882.46 µM $NaNO_3$, 36.23 µM $NaH_2PO_4 \cdot H_2O$, 52.78 µM $Na_2SiO_3 \cdot 9H_2O$). For the La Fosca and Tarragona experiments, the bottles were usually incubated in a culture chamber for 24 h at 24°C, under a 12:12 h L:D cycle and irradiance conditions of 120 µmol photons m^{-2} s^{-1}. During the *Alexandrium taylori* experiment at Vulcano, the bottles were incubated *in situ*, fixed at 1 m depth. These incubations started at 14:00 h local time and lasted for 24 h. The photoperiod for this specific period was 14:10 h (L:D), with a mean of daily irradiance of 800 µmol photons m^{-2} s^{-1} (National Weather Institute).

Table 1. Dilution experiments conducted at La Fosca beach, Vulcano beach and Tarragona harbour in summer–autumn 2002. Chl_0: initial chlorophyll *a* concentration. Cell $density_0$: initial cell concentration of target species (*Alexandrium taylori*, *Gymnodinium* sp., *A. catenella*). % target species, relative contribution of the target species over the total dinoflagellate abundance. nd: not determined

Locality Experiment	Species	Date	Chl_0 (µg l^{-1})	Cell $density_0$ of target species (cell l^{-1})		% target species over total dinoflagellate community		Dominant phytoplankton
La Fosca				*A. taylori*	*Gymnodinium* sp.	*A. taylori*	*Gymnodinium* sp.	
At 1	*A. taylori/Gymnodinium* sp.	08 Jul	15	6.83×10^4	4.38×10^4	62	38	Diatoms
At 2	*A. taylori/Gymnodinium* sp.	22 Jul	3.2	1.31×10^5	9.01×10^4	72	26	Dinoflagellates
At 3	*A. taylori/Gymnodinium* sp.	25 Jul	8.3	1.00×10^5	1.22×10^4	43	56	Dinoflagellates
At 4	*A. taylori/Gymnodinium* sp.	29 Jul	30.4	2.34×10^6	4.71×10^5	84	15	Dinoflagellates
At 5	*A. taylori/Gymnodinium* sp.	04 Sep	8.7	1.18×10^4	4.43×10^5	2	96	nd
Vulcano				*A. taylori*	*A. catenella*			
At 6	*A. taylori*	08 Aug	3.5	3.42×10^4	0	nd	nd	Diatoms
Tarragona harbour				*A. catenella*		*A. catenella*		
Ac 7	*A. catenella*	19 Sep	32.9	1.16×10^4		82		Nanoflagellates
Ac 8	*A. catenella*	25 Sep	31.8	1.84×10^6		100		Dino- & nano-flagellates
Ac 9	*A. catenella*	08 Oct	57	2.7×10^4		81		Diatoms

Subsampling and analyses. Initial and final 60 ml subsamples for the quantification of chlorophyll *a* (chl *a*) were filtered on 25 mm Whatman GF/F glass fiber filters. Chl *a* samples were extracted in 10 ml 90% acetone and measured with a Turner Designs fluorometer. Parallel aliquots (150 ml) were fixed with lugol (1% final concentration) for phytoplankton and microzooplankton quantification using the microscopic procedures outlined below. The general procedure for identifying and quantifying phyto- and microzooplankton cells involved sedimentation of a subsample in a 50 ml settling chambers for 24 h and counting of cells in an appropriate area (Throndsen 1995) using an inverted Leica microscope. With this approach, the minimum species abundance detected was 20 cells l^{-1}. Nutrient samples were frozen immediately after collection, and concentrations of nitrate, nitrite, ammonia, phosphate and silicate were measured with an autoanalyzer following (Grassoff et al. 1983).

Data analyses. Net rates of changes (k) in measured variables (total chl *a*, dinoflagellate cell numbers) were determined assuming an exponential growth, using the following equation:

$$k_i = 1/t \ln(C_t / C_0) \quad (1)$$

where k_i is net growth rate at dilution level i, C_0 and C_t are initial and final concentrations of each species or of chl *a*, and t is the duration of the experiment (1 d).

Cell aggregation induced variations in C_0 during the filling of the experiment bottles. Rather than being replicates of the same theoretical dilution (7 levels), each bottle (n = 14) was therefore considered as an individual experiment.

In the case of non-saturated grazing, phytoplankton and species-specific growth (μ_n, d^{-1}) and grazing mortality (m, d^{-1}) were calculated according to the linear regression model proposed by Landry & Hassett (1982):

$$k_i = \mu_n - mD_i \quad (2)$$

where k_i is the net growth rate at dilution level i, μ_n is the instantaneous rate of phytoplankton growth with added nutrients, m is the phytoplankton mortality rate and D_i is the dilution factor at level i. The statistical significance of deviations from linearity in the dilution experiments was tested for all series. Linear response curves were only considered for occasions on which the model (Eq. 2) was significant with a 95% confidence level. When the assumption of linearity was not met (as required for the methods described in Landry & Hassett 1982) due to the saturated feeding kinetics observed in the functional response of the microzooplankton, a split-function model of microzooplankton grazing was used to estimate growth and grazing rates (Redden et al. 2002). Phytoplankton growth rates and microzooplankton grazing rates were obtained using conventional analysis with dilutions sufficient to ensure that the grazing rate was proportional to the phytoplankton concentration. These conditions were ensured by including a very high dilution treatment (0.05%).

The procedures employed also allowed estimations of the phytoplankton concentration (P_s) that saturated grazing:

$$P_s = \frac{\mu[C_t - C_0 \exp(\mu t)]}{mD\,[1 - \exp(\mu t)]} \quad (3)$$

where the variables μ and m have already been obtained from more diluted samples for which Eq. (2) applied. Note that Eq. (3) solves P_s for a single variable, so only 1 saturated sample needs to be calculated, although many concentrations were calculated in order to confirm that grazing was truly saturated. In this way, a mean P_s value and a standard error were obtained. The maximum rate at which microzooplankton consumed phytoplankton (G_s) was estimated in conditions of saturated grazing, using the equation $G_s = m \times P_s$. The balance between mortality and phytoplankton growth was estimated as $P_b = mP_s/\mu$.

The influence of nutrient addition on the growth rates of target species and total phytoplankton were investigated by including dilution experiments without nutrient additions. Growth limitation by nutrient availability in the experiments was evaluated using μ_0:μ_n ratios, where μ_n and μ_0 are the growth rate with nutrients and without nutrient addition, respectively, as determined with Eq. (1).

Mortality rates calculated by the dilution method were compared with an estimate of grazing mortality (G_{est}). G_{est} was calculated as the decrease of a target species with respect to its initial concentration due to grazing by microzooplankton (Table 2). The average maximum and minimum ingestion rates were calculated using data presented in published records (Stoecker et al. 2000, Kamiyama & Arima 2001, Jeong et al. 2002, 2003, Stoecker et al. 2002, Calbet et al. 2003). G_{est} was calculated using the following equation:

$$G_{est} = \ln [(C_0 - C_{tp}/C_0)] \quad (4)$$

where C_0 is the initial concentration of each target species and C_{tp} is the final concentration of each target species according to the ingestion rates applied.

RESULTS

Alexandrium taylori bloom event at La Fosca beach

During the 4 mo of sampling, concentrations of *Alexandrium taylori* at La Fosca revealed the common phases described for blooms (Fig. 1a). The develop-

Table 2. *Alexandrium* sp. and *Gymnodium* sp. Initial cell concentration of dominant microzooplankton (cells l^{-1}) during the dilution experiments conducted at La Fosca beach and Tarragona harbour in summer–autumn 2002. Tintinida >20 µm include the genus *Tintinopsis* and *Favella* in Tarragona sampling. Others include nauplii and bivalve larvae

Locality Experiment	Species	Date	Dominant microzooplankton (cells l^{-1})							
			Heterotrophic dinoflagellates	Aloricated ciliates *Mesodinium*	Aloricated ciliates Ciliates >20 µm	Aloricated ciliates Ciliates <20 µm	Loricated ciliates Tintinnida >20 µm	Loricated ciliates Tintinnida <20 µm	Rotifera	Other
La Fosca										
At 1	*A. taylori/Gymnodinium* sp.	08 Jul	0	0	0	455	0	0	0	0
At 2	*A. taylori/Gymnodinium* sp.	22 Jul	40	0	320	910	200	40	0	0
At 3	*A. taylori/Gymnodinium* sp.	25 Jul	0	0	910	0	0	0	0	0
At 4	*A. taylori/Gymnodinium* sp.	29 Jul	80	0	440	0	40	40	0	40
At 5	*A. taylori/Gymnodinium* sp.	04 Sep	0	0	0	0	0	0	0	0
Tarragona harbour										
Ac 7	*A. catenella*	19 Sep	160	150	40	605	800	300	20	240
Ac 8	*A. catenella*	25 Sep	440	0	1160	910	2220	2730	120	120
Ac 9	*A. catenella*	08 Oct	60	910	5460	910	540	910	220	40

ment phase, characterized by increases in cell numbers from 10 to 10^5 cells l^{-1}, was observed from June to mid-July, when surface temperatures increased from 18 to 22°C. The maintenance phase (from mid-July to August, 19 to 24°C) was characterized by cell densities of between 10^5 and 10^6 cells l^{-1}. During this period, a cell density peak (2.7×10^6 cells l^{-1}) was recorded. A sharp decrease in cell concentration, detected in early September, marked the decline phase of the bloom. *A. taylori* was the most abundant dinoflagellate detected, but was closely followed by *Gymnodinium* sp. (up to 10^5 cell l^{-1}) (Table 1). The 2 species showed similar temporal patterns (Fig. 1b), but cells of *Gymnodinium* sp. appeared 1 mo later than those of *A. taylori*, whereas its development phase, characterized by increases in cell numbers from 10 to 10^4 cells l^{-1}, occurred earlier (early July). Concentrations of dissolved inorganic nitrogen (DIN) were 3 µM during the development phase, reaching a maximum during maintenance (Fig. 1c). A declining tendency was observed later on. Concentrations of PO_4 ranged from 0 to 0.8 µM, but a slight increase was observed during the bloom development. The observed chl *a* maximum (30 µg l^{-1} on 29 July) coincided with the maximum abundances recorded for both *A. taylori* and *Gymnodinium* sp. (Fig. 1d).

Alexandrium taylori bloom event at Vulcano

The 4 mo of study performed at the Vulcano station only revealed the maintenance and decline phases of an *Alexandrium taylori* bloom, because this event had already started prior to the sampling period (Fig. 2a). During the maintenance phase of this bloom (from 15 June to 7 July, 27 to 28°C) cell densities varied within 1 order of magnitude, from 10^6 to 10^7 cells l^{-1}, with a maximum of 2.3×10^7 cells l^{-1} (mid-June). From mid-July a clear decline in *A. taylori* densities was observed, during which cell numbers dropped to 10^2 cells l^{-1}. A modest increase to 10^5 cells l^{-1} was detected on 3 August. The total disappearance of *A. taylori* cells detected later marked the complete termination of the bloom. *Gymnodinium* sp., the most abundant dinoflagellate accompanying *A. taylori* at La Fosca beach, was absent in these samples. In mid-June, a PO_4 maximum (5.5 µM) was observed, followed by a decrease to <1 µM. These low values remained constant during the rest of the monitored period. Concentration of DIN showed a variable pattern (from 1 to 6 µM) during the entire bloom period (Fig. 2b). These values were similar to those observed at La Fosca beach. The temporal evolution of chl *a* at Vulcano followed the pattern observed for *A. taylori* cell numbers (Fig. 2c).

Alexandrium catenella bloom event in Tarragona harbour

During the 3 mo of sampling, the concentrations of *Alexandrium catenella* also showed the 3 common bloom phases (Fig. 3a). The development phase, characterized by increases in cell numbers from 10^2 to 10^5 cells l^{-1}, was observed from late August to late September when the water temperature was between 27 and 21°C. A sharp increase in inorganic nutrient concentrations (DIN 60 µM and PO_4 2.5 µM) (Fig. 3b) was detected before maximum cell concentrations were attained (21 September). The maintenance phase was short and characterized by cell densities ranging from

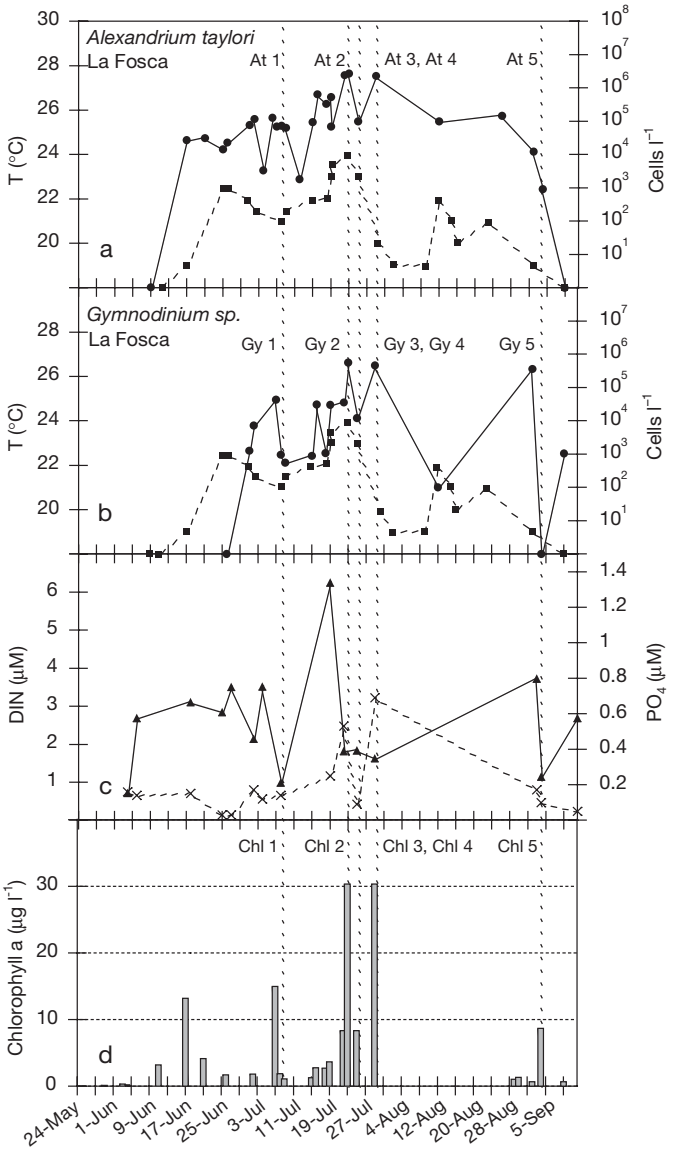

Fig. 1. *Alexandrium taylori* and *Gymnodium* sp. (a) Cell density of *A. taylori* (●) and water temperature (■) at La Fosca beach (Catalan coast) from May to September 2002. (b) Cell density of *Gymnodinium* sp. (●) and water temperature (■). (c) Values of DIN (▲) and PO_4 (×). (d) Chlorophyll *a*. Dilution experiments are marked with vertical dashed lines

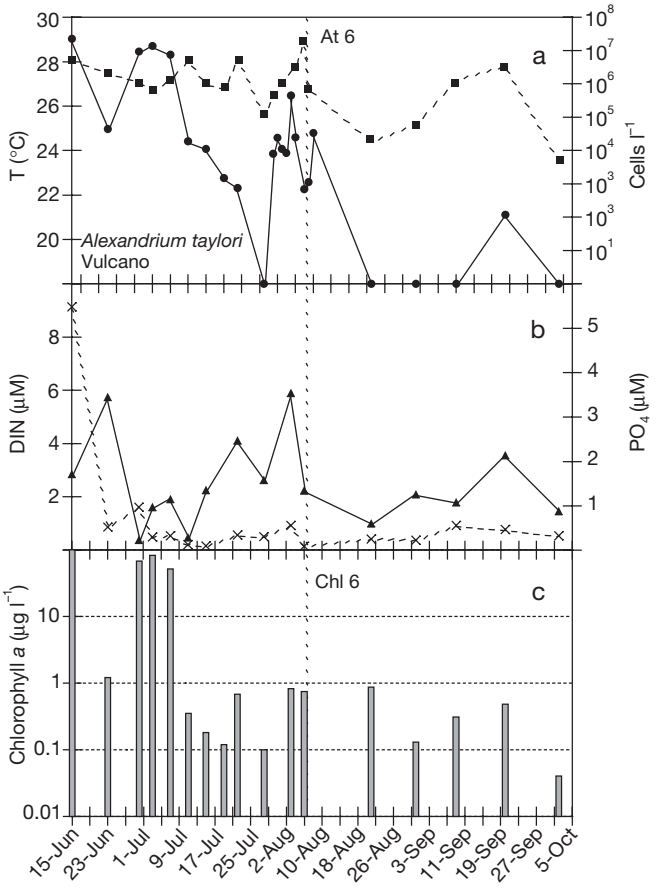

Fig. 2. *Alexandrium taylori.* (a) Cell density of *A. taylori* (●) and water temperature (■) at Vulcano beach (Sicily) from June to September 2002. (b) Values of DIN (▲) and PO_4 (×). (c) Chlorophyll *a*. Dilution experiments are marked with vertical dashed lines

10^5 to 10^7 cells l^{-1}. A sharp decrease in cell concentrations, detected during early October, marked the decline phase of the bloom. Concentrations of chl *a* were not related to the abundance of *A. catenella* (Fig. 3c), because other phytoplankton groups contributed to the overall concentrations of this biomass proxy. *Gymnodinium impudicum* was the dominant dinoflagellate species (10^2 to 10^6 cells l^{-1}) during the development phase of *A. catenella* bloom. However, during the maintenance phase, the *A. catenella* bloom was almost monospecific (see Table 1).

The composition of dominant microzooplankton observed during the 3 dinoflagellate blooms studied is shown in Table 2. Generally, the *Alexandrium taylori* bloom was characterized by very low densities of microzooplankton, clearly dominated by small naked ciliates. In particular, Expt At 5 was characterized by a concentration of microzooplankton below the detection limit of the microscope analyses due to the high cell densities of the dinoflagellates. Heterotrophic dinoflagellates, ciliates, tintinnida, rotifera, bivalve larvae and other zooplankton (mainly nauplii) were more abundant in the *A. catenella* than in the *A. taylori* bloom. The main genera of heterotrophic dinoflagellates were *Ceratium*, *Gyrodinium*, *Protoperidinuim* and *Polykrikos*.

Species specific growth and loss rates

The results obtained during the dilution experiments for the different species are summarized in Table 3. Estimated *Alexandrium taylori* growth rates ranged

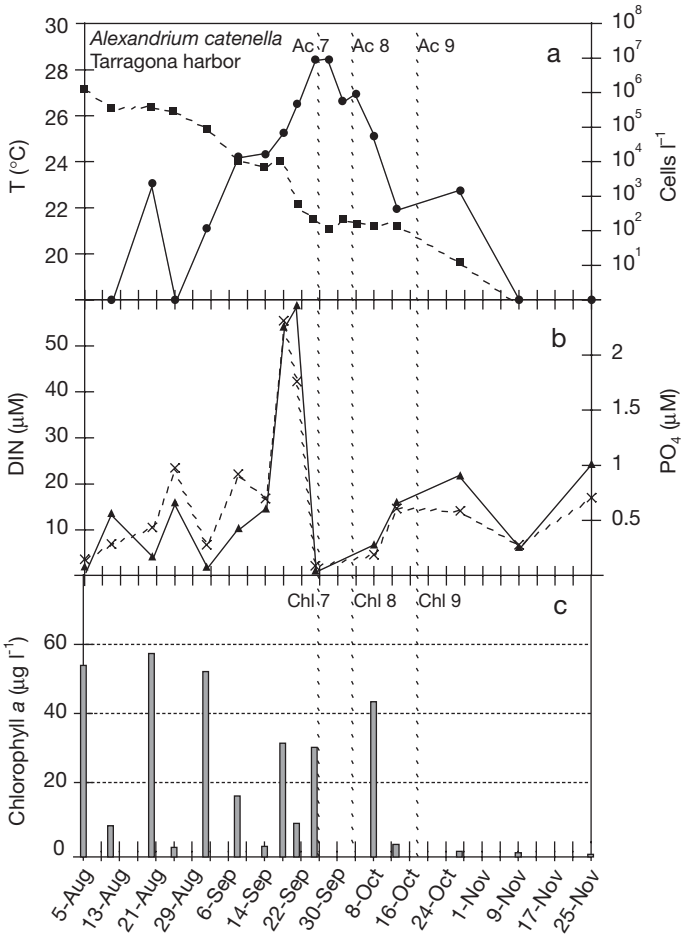

Fig. 3. *Alexandrium catenella.* (a) Cell density of *A. catenella* (●) and water temperature (■) at Tarragona harbour (Catalan coast) from August to November 2002. (b) Values of DIN (▲) and PO_4 (×). (c) Chlorophyll *a*. Dilution experiments are marked with vertical dashed lines

from 0.04 to 0.67 d^{-1}, whereas mortality rates ranged from −0.20 to −0.65 d^{-1}. Similar growth rates were observed for *Gymnodinium* sp., but mortality rates (−0.58 to −0.82 d^{-1}) were slightly higher. Growth and mortality rates for *A. catenella* were balanced (0.24 to 0.44 d^{-1} and −0.25 to −0.44 d^{-1}, respectively).

The results for the temporal evolution of growth and mortality rates versus target species evolution are presented in Fig. 4. Maximum dinoflagellate growth rates were attained during the development phase of the *Alexandrium taylori* bloom at La Fosca beach but values were also high during the decline phase of this bloom. Moderate growth rates were measured during the maintenance phase of both the *A. taylori* and the *A. catenella* blooms. Mortality rates of *Alexandrium* species ranged from −0.20 to −0.65 d^{-1}, with maximum values during the decline phase of the *A. taylori* bloom at La Fosca beach. At Vulcano, the decline phase of the *A. taylori* bloom also showed a high mortality rate.

For cases in which saturated grazing was not observed, all dilution levels were considered for calculations of µ and *m* (Fig. 5a,b). However, some dilution experiments (e.g. Gy 2, Gy 3 and Ac 7) showed a scattered data pattern and could not be interpreted by the linear method. The observed scattering of data did not show a typical saturation curve, so the method proposed by Gallegos (1989) and Redden et al. (2002) was not applied.

Phytoplankton growth and microzooplankton grazing: the dilution method for measuring saturated grazing

The results of the dilution experiments obtained for the total phytoplankton biomass (chl *a*) are summarized in Table 4. For cases in which saturated

Table 3. Target species mortality rates (*m*), growth rate (μ_n) determined from dilution experiments amended with nutrients and net growth rates (μ_0) without nutrients. r^2 proportion of variance accounted for. Grazing mortality estimated (G_{est}) through the *in situ* concentration of microzooplankton in each experiment. nd: not determined

Locality Experiment	Species	Date (d^{-1})	*m* (d^{-1})	μ_n (d^{-1})	r^2	G_{est} (d^{-1})	μ_0	Dinoflagellate bloom phase
La Fosca								
At 1	*Alexandrium taylori*	08 Jul	−0.20	0.67	0.53	−0.83	0.78	Development
At 2	*Alexandrium taylori*	22 Jul	−0.52	0.24	0.59	−1.30	0.06	Maintenance
At 3	*Alexandrium taylori*	25 Jul	−0.44	0.30	0.64	−0.31	0.44	Maintenance
At 4	*Alexandrium taylori*	29 Jul	−0.29	0.04	0.92	−0.04	0.32	Maintenance
At 5	*Alexandrium taylori*	04 Sep	−0.65	0.64	0.79	0	0.61	Decline
Gy 1	*Gymnodinium* sp.	08 Jul	−0.58	0.20	0.45	−2.60	0.72	Development
Gy 5	*Gymnodinium* sp.	04 Sep	−0.82	0.66	0.76	0	0.78	Decline
Vulcano								
At 6	*Alexandrium taylori*	08 Aug	−0.57	0.17	0.56	nd	0.22	Decline
Tarragona harbour								
Ac 8	*Alexandrium catenella*	25 Sep	−0.25	0.24	0.42	−0.66	0.36	Maintenance
Ac 9	*Alexandrium catenella*	08 Oct	−0.44	0.44	0.55	3.5	0.54	Decline

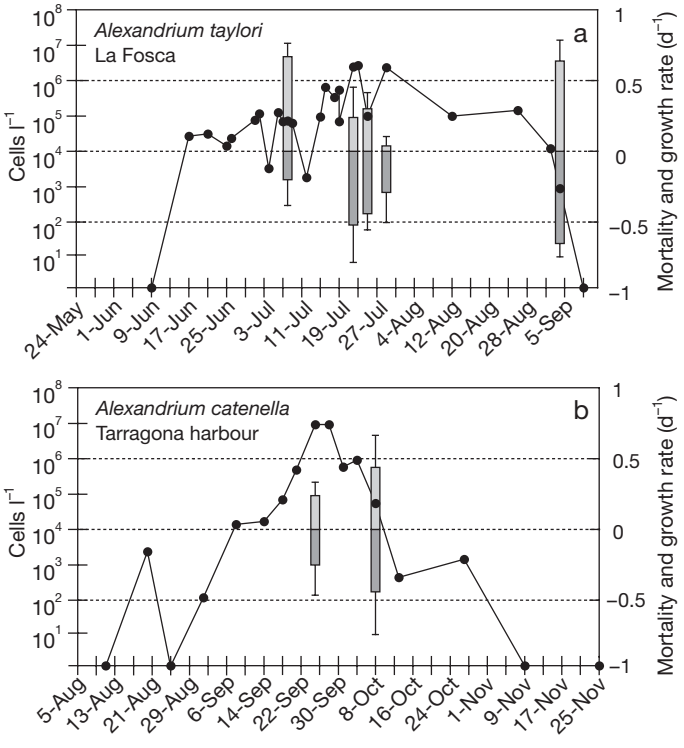

Fig. 4. *Alexandrium taylori* and *A. catenella.* Cell density of target species (●) and species mortality rate (*m*) and growth rate (μ_n) with nutrients determined from dilution experiments. (a) *A. taylori* from May to September 2002 at La Fosca beach (Catalan coast). (b) *A. catenella* from August to November 2002 at Tarragona harbour (Catalan coast)

grazing was not observed, all dilution levels were considered for calculations of μ and *m* (Fig. 5c). In 4 experiments, the apparent phytoplankton growth rate was independent of the dilution level, because grazing was saturated. The linear fit was obtained from more diluted samples in which saturation was not observed (Fig. 5d). The *y*-intercept was μ and the slope was *m*. The phytoplankton concentration at which grazers become saturated (P_s) and the saturated grazing rates (G_s) were estimated, as indicated. Most of the saturated cases corresponded to the experiments with chl *a* concentrations >15 μg l^{-1}. The maximum rate at which microzooplankton consumed phytoplankton in the experiments was 28.99 μg chl *a* l^{-1}d^{-1} measured in Tarragona harbour on 25 September. The balance between mortality and phytoplankton growth ($P_{b)}$ showed maximum values of 31.31 μg chl *a* l^{-1}.

There was no significant correlation between initial abundances of target dinoflagellates and their corresponding growth or mortality rates (n = 23). Higher concentrations of chl *a* (initial conditions) were generally associated with higher growth rates, but this tendency was not significant. There was no significant correlation between initial chlorophyll *a* values and mortality rates. No significant relation between the growth rate of the phytoplankton stock (chl *a*) and the growth rate of the target species was observed either. A higher mortality of target species was usually associated with a higher mortality of phytoplankton (chl *a*), but the tendency was not significant.

Estimated impact of microzooplankton on dinoflagellates

The grazing impact of microzooplankton (G_{est}) was estimated using the data on target species and micro-

Table 4. Phytoplankton growth (chlorophyll *a*). Initial phytoplankton concentrations (Chl_0). Mortality rates (*m*) and growth rate (μ_n) with nutrients determined from dilution experiments. Average value of the phytoplankton concentration that saturates grazing (P_s) and standard deviation (n = 4). Saturated cases are shown (*), G_s: saturated grazing; SD: standard deviation; P_b: balance between grazing and phytoplankton growth

Locality Experiment		Date	Chl_0 (μg l^{-1})	*m* estimate (d^{-1})	μ_n estimate (d^{-1})	P_s ± SD (μg l^{-1})	G_s ± SD (μg l^{-1} d^{-1})	P_b (μg l^{-1})
La Fosca								
Chl 1	Chlorophyll *a*	08 Jul	15	−0.97	0.96	*10.92 ± 0.77	10.60 ± 0.75	11.03
Chl 2	Chlorophyll *a*	22 Jul	3.2	−0.45	0.85			1.69
Chl 3	Chlorophyll *a*	25 Jul	8.3	−1.33	1.09			10.13
Chl 4	Chlorophyll *a*	29 Jul	30.4	−1.27	1.24	*11.28 ± 0.96	14.32 ± 1.22	11.55
Chl 5	Chlorophyll *a*	04 Sep	8.7	−1.22	0.89			8.80
Vulcano								
Chl 6	Chlorophyll *a*	08 Aug	3.5	−0.87	0.96			3.17
Tarragona harbour								
Chl 7	Chlorophyll *a*	19 Sep	32.9	−0.98	0.52	*16.61 ± 2.34	16.28 ± 2.30	31.31
Chl 8	Chlorophyll *a*	25 Sep	31.8	−1.07	1.24	*12.27 ± 0.34	28.99 ± 0.80	10.59
Chl 9	Chlorophyll *a*	08 Oct	45	−0.88	1.48			29.6

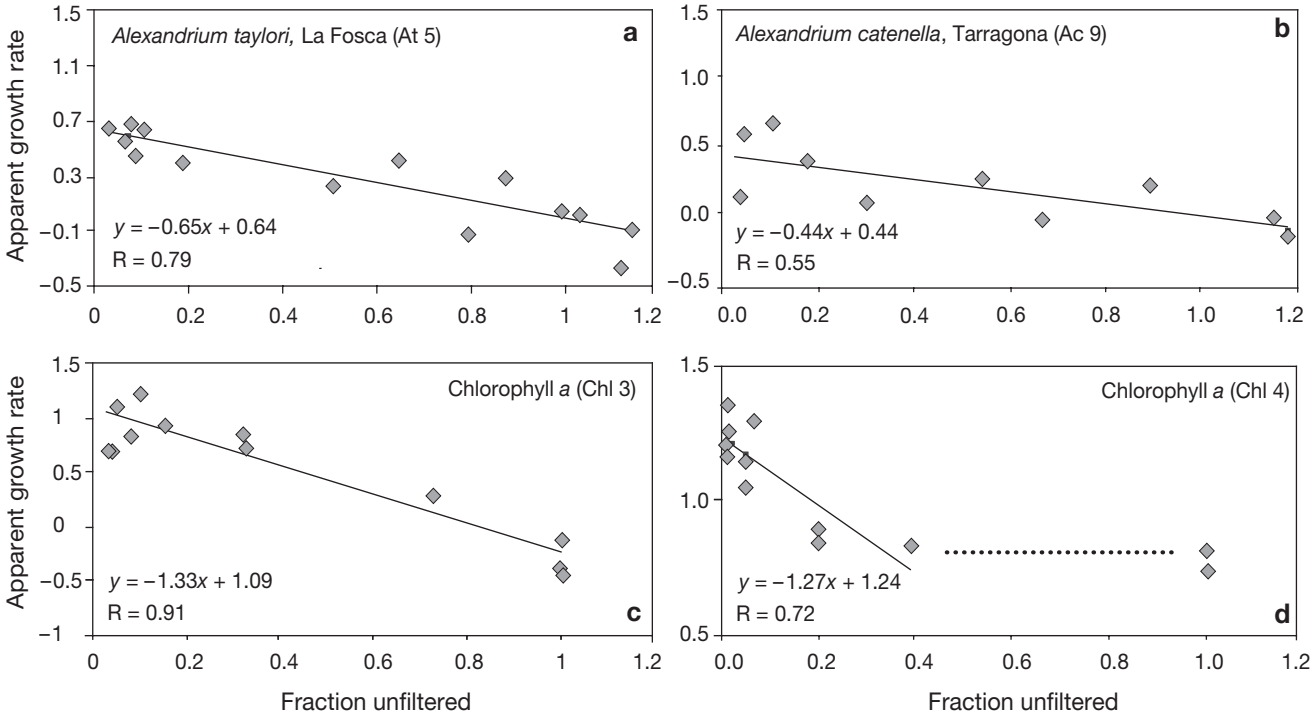

Fig. 5. *Alexandrium taylori* and *A. catenella*. Apparent growth rates of target species as a function of the dilution factor in the dilution experiments at the stations indicated. (a) *A. taylori* (At 5). (b) *A. catenella* (Ac 9). Dilution method for measuring saturated and non-saturated grazing. (c) Apparent growth rate of chlorophyll *a* (Chl 3) in a non-saturated grazing case. (d) Chlorophyll *a* (Chl 4) in a saturated grazing case. Solid line shows the best fit using linear grazing when the initial (C_0) and final value (C_t) of the diluted phytoplankton concentration (chlorophyll *a*) are both less than the saturation concentration (P_s). The *y*-intercept is μ and the slope is *m*. Dotted line shows the best fit using the saturated grazing model when C_0 and C_t value of the diluted phytoplankton concentration (chlorophyll *a*) are both greater than P_s

zooplankton cell concentration collected during the experiments (Table 3), as well as published records of ingestion rates. During Expts At 3 (*Alexandrium taylori*, July 25), At 4 (*A. taylori*, July 29), At 5 (*A. taylori*, September 4) and Gy 5 (*Gymnodinium* sp., September 4) calculated mortality rates from the dilution experiments exceeded those of microzooplankton grazing. In the case of *A. catenella*, the mortality calculated by the 2 methods employed was significantly different at the decline of the bloom.

Effects of nutrient addition

The experiments revealed a significant correlation between growth rate estimates with (μ_n) and without (μ_0) nutrient additions ($\mu_n = 0.96\mu_0 - 0.19$, $r^2 = 0.52$). Only 1 taxon-specific experiment (At 2, *Alexandrium taylori*, 22 July) showed a different (μ_0/μ_n) ratio, whereas 1 experiment (Chl 9, 8 October) showed phytoplankton growth limitation. These deviating cases coincided with a decrease of *in situ* DIN concentrations.

DISCUSSION

Studies on species-specific growth and mortality rates arising from natural blooms provide information for the interpretation of phases and the proper understanding of HAB development. Single measurements of species-specific growth and mortality rates of toxic dinoflagellates have been published previously (Calbet et al. 2003, Collos et al. 2004), but the present study is the first attempt to measure these rates at different phases of blooms.

Before discussing the results obtained, several methodological aspects should be evaluated. The study included the basic methodological assumption that growth rates should increase linearly with dilution rates, by using the r^2 calculated from linear regressions of the dilution data. However, in 4 cases, the results departed significantly from linearity, and interpretations of these experiments with linear regression were therefore inappropriate. The most problematic species regarding such nonlinear data scattering was *Gymnodinium* sp. The data scattering might have been due to low grazing rates, which are difficult to detect with

regression analysis, but a possible violation of the basic assumption cannot be fully excluded. However, in our case, the existence of non-linear relationships between phytoplankton mortality rates and the dilution factor does not seem to be the most plausible explanation for the data scattering. In the case of *Gymnodinium* sp., the species appeared to grow independently of the dilution factor. It should be noted that *Alexandrium taylori* has been described to produce hemolytic exotoxin compounds (Emura et al. 2004), and the abundance of this species possibly induced the erratic growth rates observed for *Gymnodinium* sp. Allelopathic compounds excreted by some phytoplankton species have been shown to affect the growth of other species (Sukenik et al. 2002, Fistarol et al. 2003). Non-linear behavior has been observed previously in other dilution experiments (Gallegos 1989, Landry et al. 1993, Lessard & Murrell 1998, Worden & Binder 2003), occasionally leading to uninterpretable data (Dolan et al. 2000). In spite of these results, the non-linearity of data by no means invalidates the dilution approach in general terms.

Our results showed considerable variability in both growth and mortality rates of dinoflagellates during the bloom event. Growth and mortality rates based on chl *a* measurements also showed considerable variability. The initial conditions of the experiments performed in the present study were variable. The variation included (1) the phase of the bloom, (2) the initial cell density, (3) the composition of the accompanying phytoplankton community, (4) the nutrient availability, and (5) the composition and abundance of microzooplankton.

The development phase of the *Alexandrium taylori* bloom at La Fosca beach extended from early June to mid-July, and was characterized by high growth (0.67 d^{-1}) and very low mortality (–0.2 d^{-1}). This is the only case in which the ratio between mortality and growth resulted in an increase in the population. During the maintenance phase, characterized by cell concentrations $>10^5$ cells l^{-1}, growth rates were moderate or low (Expts At 2, At 3 and At 4), and mortality rates slightly exceeded growth in all the cases. Employing the mitotic index, we previously calculated potential growth rates of 0.5 d^{-1} for vegetative cells in the maintenance phase (Garcés et al. 1998). These results closely match estimates of the At 1 and At 5 experiments described in the present study, but it should be noted that irradiance conditions (both quality and quantity) most certainly influenced growth during dilution experiments. Most experiments described in the present study were carried out in the laboratory, employing artificial light sources, but the Vulcano beach experiment was performed *in situ*. Reduced light levels or changes in the spectral composition are expected to influence the estimated values of μ, but should not alter results for *m*. It is therefore not unlikely that the laboratory experiments underestimated the true μ values in the field.

Maintenance of a bloom obviously implies that cell losses are balanced by gains, but during this phase mortality rates exceed the growth. It is possible that additional processes such as cell accumulation and biological aggregation increase the apparent population growth, thus enabling the persistence of high cellular densities shown in the temporal evolution of the bloom. The coupling between migration strategies and local physical circulation were shown to play a key role in the maintenance of high cell densities of the *Alexandrium taylori* bloom (Basterretxea et al. 2004).

In the decline phase of the *Alexandrium taylori* bloom, the growth rate was high (0.64 d^{-1}) and similar to the mortality rate (–0.65 d^{-1}), therefore leading to a null net balance. Since the population nevertheless declined, it can be assumed that an additional source(s) of cell mortality (other than grazing) existed. This might include cell lysis (Garcés & Masó 2001), microbial infection by virus (Suttle & Chan 1995) or bacteria (Imai et al. 2001), parasite attack (Juneau et al. 2003, Lawrence & Suttle 2004), self-shading and encystment (sexual reproduction) (Garcés et al. 2004). The hypothesis that losses of dinoflagellates are not related to grazing by microzooplankton is only supported by the experiments showing an estimated grazing lower than the mortality rate measured by the dilution method.

The dilution experiment of *Alexandrium taylori* at Vulcano was conducted during the decline phase of the bloom. The estimated low growth rates in this experiment, in combination with considerable mortality rates, were in agreement with the temporal pattern of cell concentrations in the field.

Two coinciding dinoflagellate species, *Alexandrium taylori* and *Gymnodinium* sp., varied over more than 1 order of magnitude during the La Fosca bloom. Although the temporal coverage of the experiments is limited, differences between the growth rates and mortality of these species may have affected their relative abundances. For example, the growth rates of *Gymnodinium* sp. were inversely related to the density of *A. taylori*, which supported the influence of allelopathy. Such differences obviously affect the overall composition of the phytoplankton population.

The 2 *Alexandrium catenella* experiments revealed compensating growth and mortality rates, according to which stable cell concentrations were expected for the bloom period studied. This prediction coincided with the *in situ* temporal evolution of the bloom during the maintenance phase, but the lack of any apparent relationship between the growth rates and the decline

phase, as in the case of *A. taylori* at the end of their bloom, could suggest that the values of species-specific growth rates obtained in a single experiment might reflect the previous history of the population, rather than the immediate response to prevailing environmental conditions (Reguera et al. 2003).

Considering the concentrations of microzooplankton and calculated *m*/μ ratios, the grazing impact on the *Alexandrium catenella* bloom was considerable during all the experiments and sufficient to control dinoflagellate increase by growth. However, during the maintenance phase decreases in dinoflagellate cell numbers due to grazing were small compared to the initial concentrations. This was due to the high cell numbers reached by the dinoflagellates. In the decline phase of the bloom, the estimated mortality should be applied at least 1 wk to explain the decreases in cell concentrations observed *in situ*.

No nutrient limitation, except in *Alexandrium taylori* in At 2 and in chl *a* on October, 8 was found. The latter case suggested that other phytoplankton groups present in the community, rather than the target dinoflagellates, might have been nutrient-limited. There was no relationship between growth and nutrient availability in the experiments. It should be noted that La Fosca and Vulcano beaches are situated in highly urbanized areas and Tarragona harbor receives freshwater inflows from the River Francolí. There were few cases of a potential nutrient limitation in these areas and the blooms were not conditioned by nutrients (Giacobbe et al. 2004, Vila et al. 2004).

The lack of any apparent relationship between the growth rate of total phytoplankton (chl *a*) and the specific rates of target species (cell numbers) reflected differences between the taxonomic groups (or species) comprising the population studied. This was observed previously by Collos (2004) during a bloom of *Alexandrium catenella*, and illustrates the limitations of chl *a* for estimates of species-specific growth rates. Interpretation of grazing impact on the target species using general parameters like chl *a* could also be ambiguous (Waterhouse & Welschmeyer 1995) because at this point we have no knowledge on specific rates.

Measurements of chl *a* indicated that microzooplankton was saturated on several occasions, but this was not confirmed by changes in the cell numbers obtained during the dinoflagellate experiments. These discrepancies coincided with the expected lower grazing pressure on toxic species like *Alexandrium*. It is known that many heterotrophic and mixotrophic protists do not ingest toxic phytoplankton (Jeong et al. 1999a,b), but an effective predation on such groups was observed for others (Jeong et al. 2001, Stoecker et al. 2002). A modified grazing pressure on *Alexandrium* was also supported by the comparison between grazing mortality estimated from the concentration of microzooplankton and mortality rates calculated from the dilution technique. This comparison suggested that, in general, microzooplankton could potentially graze more than the dilution predicted.

High biomass blooms, such as those of *Alexandrium*, are caused by a combination of factors that enhance growth rates, suppress mortality, or both (Smayda 1997, Stoecker et al. 2000). Our experiments revealed that the HAB species were affected by different values of mortality during the development of the population. However, when bloom densities of *A. taylori* and *A. catenella* were attained, grazing mortality had little effect on their abundance. At the decline of the blooms, *A. taylori* and *A. catenella* showed considerable mortality, but microzooplankton grazing was not confirmed to be the main cause of the bloom termination, in which no single mechanism was apparently involved.

Acknowledgements. The authors wish to thank the personnel of the Agencia Catalana de l'Aigua de la Generalitat de Catalunya in charge of routine monitoring, for their co-operation and assistance; J. Riba for his co-operation in Tarragona harbour; K. vanLening for the pigment analyses; R. Ventosa for nutrient analyses; and E. Flo, M. Cousin and B. Moli for providing technical support. The comments by A. Calbet, K. vanLening and 3 anonymous reviewers greatly helped to improve an earlier version of the manuscript. This study was supported by the EU-financed Research Project STRATEGY EVK3-CT-2001-00046 and by the contract between Agencia Catalana de l'Aigua (Departament de Medi Ambient, Generalitat de Catalunya) and CSIC awarded to J. Camp.

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Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

Submitted: October 19, 2004; Accepted: April 28, 2005
Proofs received from author(s): September 7, 2005