

Toxic phytoplankton response to warming in two Mediterranean bays of the Ebro Delta

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ABSTRACT

The analysis of phytoplankton and environmental parameters of the Alfacs and Fangar bays time series (northwestern Mediterranean) from 1990 to 2009 reveals certain trends. There is an increase in the average water column temperature by 0.11, 0.01, 0.80 and 0.23 °C for spring, summer, fall and winter respectively in Alfacs Bay and by 1.76, 0.71, 1.33, 0.89 °C for spring, summer, fall and winter in Fangar Bay. The trends in phytoplankton populations show a shift in the timing of occurrence of *Karlodinium* spp. blooms and an increase of *Pseudo-nitzschia* spp. abundance. There is no correlation between the average seasonal temperatures and toxic phytoplankton abundance.

INTRODUCTION

The linear warming trend of global surface temperature over the 50 years from 1956 to 2005 is 0.13 [0.10 to 0.16] °C per decade (IPCC, 2007). In the Western Mediterranean, during the same period of time the linear trend is 0.002 - 0.009 °C per year, 0.025 °C per year for the period 1973-2005 at a station in Estartit on the Catalan coast (Vargas-Yáñez *et al.*, 2008). In other areas, Peperzak (2003) suggests an increase in toxic phytoplankton blooms due to climate change as a conclusion of different laboratory studies. Analysis of data from the Continuous Plankton Recorder in the northeast Atlantic shows that climate oscillations and warming play an important role in governing fluctuations of some harmful algal species, from exceptional blooms to long-term decadal trends (Edwards *et al.*, 2006).

Increase in temperature is a factor to consider affecting the seasonal composition of phytoplankton and the position of biogeographic boundaries; each species has a temperature window for survival and optimal growth, although we know that many phytoplankton species from coastal regions tolerate changes in temperature. Other factors such as stratification, upwelling, freshwater run-off from land, and cloud cover, need to be considered as indirect effects of climatic variations, and these factors may be as important as temperature increase alone to explain responses of phytoplankton to climate change (Dale *et al.*, 2006). Other factors to consider are pH - surface ocean pH has decreased by ~ 0.12 units to 8.2 (Hays *et al.*, 2005) - and the increase in atmospheric CO₂ from a pre-industrial level of 280 µatm to the present 370 µatm.

Time series of environmental parameters and phytoplankton abundance are needed to assess trends in phytoplankton populations. Unfortunately, most toxic phytoplankton time series in the

Mediterranean have started only recently (since the 1990s), associated with European legislation on shellfish growing areas; and longer series, more than 30 years are necessary to reveal convincing relationships between climate and harmful algal blooms (Dale *et al.*, 2006).

Alfacs Bay, located in the south of the Ebro delta, has a surface of 50 km² and a maximum depth of 6 m. Fangar Bay, located in the north of the Ebro delta, has a surface of 12 km² and a maximum depth of 4 m (Camp and Delgado, 1987). Both are important aquaculture areas and receive freshwater through irrigation channels used for rice cultivation. The first studies of phytoplankton in the Ebro delta area, outside the bays, were by Herrera and Margalef (1963) and Margalef and Herrera (1964), and later by Estrada (1972) and López and Arté (1973) in Fangar Bay; and by Delgado (1987) in both Alfacs Bay and Fangar Bay. In August 1987 a programme was established to monitor the quality of the shellfish growing areas in Catalonia; since then phytoplankton and oceanographic parameters have been monitored weekly.

Toxic events in these bays are related to the presence of *Alexandrium minutum*, *A. catenella*, *Dinophysis sacculus*, *D. caudata*, *Protoceratium reticulatum*, *Karlodinium veneficum*, *K. armiger*, and *Pseudo-nitzschia* spp. (Delgado, 2003; Delgado *et al.*, 1996; 2000; 2004; Diogène *et al.*, 2008; Fernández-Tejedor *et al.*, 2004; 2008; 2009; Garcés *et al.*, 2006). Species of *Pseudo-nitzschia* found in both bays are *P. calliantha*, *P. delicatissima*, *P. fraudulenta* and *P. pungens* (Quijano-Scheggia *et al.*, 2008). There is seasonality in the bloom period for the different phytoplankton species (Fernández-Tejedor *et al.*, 2008). Moreover shellfish mortalities occur in both bays associated with warm events in summer (Lleti *et al.*, 1995; Ramón *et al.*, 2007). Water discolorations due to *Noctiluca scintillans* were observed in the area in 1971 (López and Arté, 1971) but toxic phytoplankton events were not reported before 1989.

The first PSP (Paralytic Shellfish Poisoning) event in the Ebro Delta area occurred in May 1989 (Delgado *et al.*, 1990), associated with a bloom of *Alexandrium minutum*. *Alexandrium* was known to be linked to PSP since 1927 (Sommer and Meyer, 1937); the species *A. minutum* was described from samples taken in Alexandria Harbour in 1956 (Halim, 1960b). The first PSP event on the Mediterranean coast of Spain occurred in 1987 (Bravo, 1993). PSP was not reported in the Mediterranean before 1970 (Hallegraeff, 2003). DSP (Diarrhetic Shellfish Poisoning) is known since 1978 (Yasumoto *et al.*, 1978) and was linked to *Dinophysis* in 1980 (Yasumoto *et al.*, 1980a). The first DSP event on the Spanish Catalan coast occurred in 1998 in Alfacs Bay; DSP was reported on the French Catalan coast in 1987 (Belin and Raffin, 1998). *Dinophysis caudata* was abundant in the western Mediterranean during the Thor cruises in 1909-1910 (Navarro and Bellón Uriarte, 1945), but only since 1980 has it been known that certain cases of diarrhoea in consumers of molluscs are not due to bacteria or viruses, but to DSP toxins, as in the Adriatic (Ciminiello *et al.*, 2003).

This study analyzes the phytoplankton and environmental parameters of the time series from Alfacs and Fangar bays (north western Mediterranean) from 1990 to 2009.

METHODS

In Alfacs Bay and Fangar Bay water temperature, salinity and oxygen have been measured every week since May 1990 at surface and bottom at one station at the centre of each bay. Since January 1992 these parameters are also measured at every meter in the water column, and in June 1992 more sampling stations were added to the weekly monitoring. Since May 1990 phytoplankton species composition has been determined every week at the same stations. The depths for phytoplankton samples are surface, bottom and integrated water column for the central station, but only at the surface for the rest. In 2006, surface samples were replaced by integrated water column samples to adapt the sampling scheme to the European legislation (EC, 2004). Phytoplankton samples are preserved using formalin, and the Utermöhl procedure is used for identification and quantification using magnifications 100x, 200x and 400x (Hasle, 1978). The main references used for the identification are Tomas (1997), Thronsen *et al.* (2007) and the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae (Moestrup *et al.*, 2009). Since October 2000, chlorophyll was measured in duplicate samples, unpreserved and maintained in darkness, by *in vivo* fluorescence using a TURNER fluorometer (Lorenzen, 1966; Jeffrey and Welschmeyer, 1997).

The average temperature of the water column at the central stations of each bay (Alfacs $n = 8-24$, Fangar $n = 6-18$, per week, 8/6 depths per sampling day and 1-3 sampling days per week) was calculated for each week and used to obtain the seasonal average for each year between 1990 and 2009 (6762 and 4661 temperature measurements in total for Alfacs and Fangar bays respectively). Linear regression was used to evaluate the increase or decrease in seasonal average temperature. Winter average refers to the year when the season begins.

Water temperature anomalies were calculated for each week and depth, the water column average of the anomalies was calculated for each week, and used to obtain seasonal anomalies for each year. The mean of all the standard deviations for each week and depth per season was used to determine which years were different from the average due to the lack of significant differences between different years for each season. The Kruskal-Wallis One Way Analysis of Variance on Ranks result was $H = 18$ with 18 degrees of freedom ($P = 0.456$) for spring, fall and winter; $H = 19$ with 19 degrees of freedom ($P = 0.457$) for summer.

The temperature and salinity measurements were used to calculate sigma-t for each week and depth. The difference between sigma-t at 6m and at 0.5 m depth was used as a stratification index. The average stratification index was calculated for each season.

The average phytoplankton species abundances of the surface and bottom weekly samples at the central station of each bay were used to calculate the seasonal average per year.

The Spearman Rank Order Correlation was employed to test the correlation between the seasonal temperature and species abundances or the seasonal stratification index and species abundances.

RESULTS

Temperature

In Alfacs Bay, average seasonal temperatures for spring, summer, fall and winter in the period covered were respectively 18.9 ± 0.5 , 26.1 ± 0.6 , 17.4 ± 1.1 , 11.3 ± 1.1 °C (mean \pm SD, $n = 19-20$ years). In Alfacs Bay the linear trend from spring 1990 to spring 2009 shows a non-significant increase of 0.006, 0.0003, 0.042, 0.012 °C per year ($R^2 = 0.00, 0.00, 0.05, 0.00$) for spring, summer, fall and winter respectively (Figure 1). In Fangar Bay, average seasonal temperatures were 18.3 ± 0.9 , 25.3 ± 0.7 , 16.6 ± 1.4 , 10.9 ± 1.1 °C (mean \pm SD, $n = 19-20$ years). The linear trend shows a non-significant increase of 0.093, 0.037, 0.070, 0.047 °C per year ($R^2 = 0.33, 0.10, 0.08, 0.06$) for spring, summer, fall and winter respectively (Figure 2). In these 19 years, from 1990 to 2009, the seasonal water column temperature increased 0.11, 0.01, 0.80 and 0.23 °C for spring, summer, fall and winter respectively in Alfacs Bay. In Fangar Bay the increase was 1.76, 0.71, 1.33, 0.89 °C for spring, summer, fall and winter. The means of the standard deviations of the seasonal water temperatures were 1.8, 1.4, 1.3, 1.9 in Alfacs Bay and 1.8, 1.8, 2.4, 2.1 in Fangar Bay for spring, summer, fall and winter respectively; these means were used to determine the years with positive and negative seasonal temperature anomalies. From summer 1990 to summer 2009, in Alfacs Bay, positive water temperature anomalies occurred in fall 1995 and 2006 and negative temperature anomalies occurred in fall 1993 and 1999 and winter 2004-2005. Positive temperature anomalies also occurred in Fangar Bay during winter 1996-1997 and fall 2006. The average water column temperature was higher than 28°C during 10 weeks for the decade 1990-1999 and 17 weeks for the decade 2000-2009.

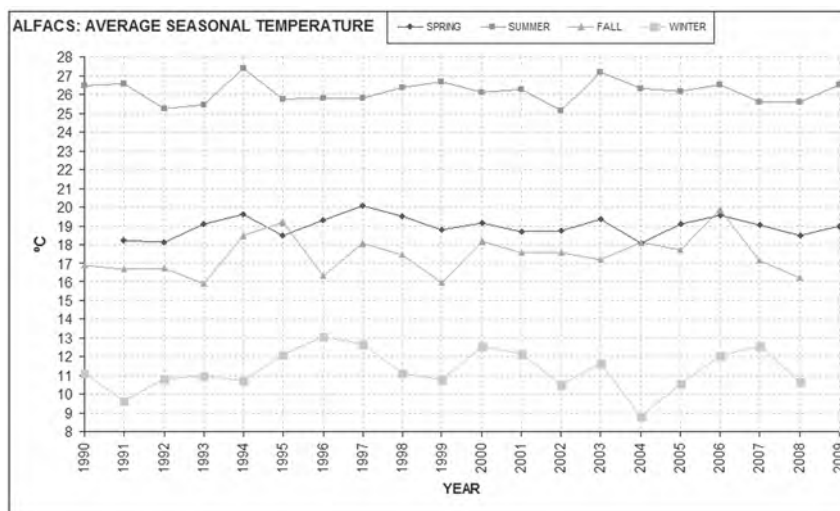


Figure 1. Average seasonal temperature for the period spring 1990-summer 2009 in Alfacs Bay (n = 12-14 weeks).

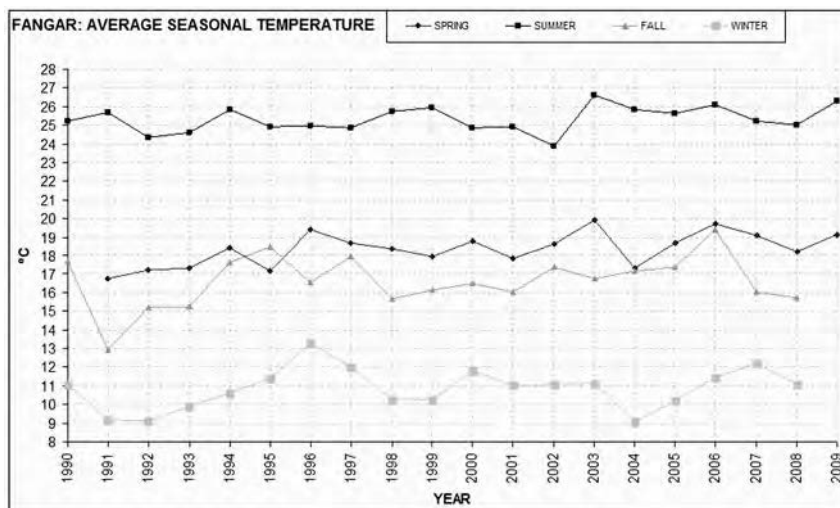


Figure 2. Average seasonal temperature for the period spring 1990-summer 2009 in Fangar Bay (n = 12-14 weeks).

Salinity

For the period 1990-2009, salinity in Alfacs Bay has increased 0.019, 0.030, 0.010 units per year, for spring, summer and fall and decreased 0.014 units for winter, this negative value of the linear trend in winter is due to very low salinities in winter 2008-2009, while the linear trend for winter is positive, 0.039 units for the period 1990-2007.

Stratification

In Alfacs Bay mean seasonal stratification indices were 1.83 ± 0.44 , 2.06 ± 0.36 , 1.83 ± 0.43 , 1.16 ± 0.58 for spring, summer, fall and winter. The maximum was measured in spring 2003, stratification was also strong in winter 1996, summer and fall 1992 (3.2, 3.1, 2.9, 3.0 respectively).

Toxic phytoplankton

In Alfacs Bay there is a shift in the time of occurrence of *Karlodinium* spp. blooms, from winter in 1994-1999 to spring in 2000 and spring-summer in 2003-2009 (Figure 3). Positive correlation exists between *Karlodinium* spp. abundance and the stratification index in spring (Spearman rank 0,682 P= 0,003). There is a trend to an increase in the abundance of *Pseudo-nitzschia* spp. (Figure 4), more important in summer and fall. *D. sacculus* and *A. minutum* are more abundant in spring and winter. There is a lack of correlation for the averaged temperatures of each season per year and the phytoplankton cell abundance. In Fangar Bay there is a decrease in *D. sacculus* abundance during all seasons of the year, while *D. caudata* increases the abundance during the summer season. In Fangar Bay *Pseudo-nitzschia* spp. blooms do not show the increase in abundance observed in Alfacs Bay.

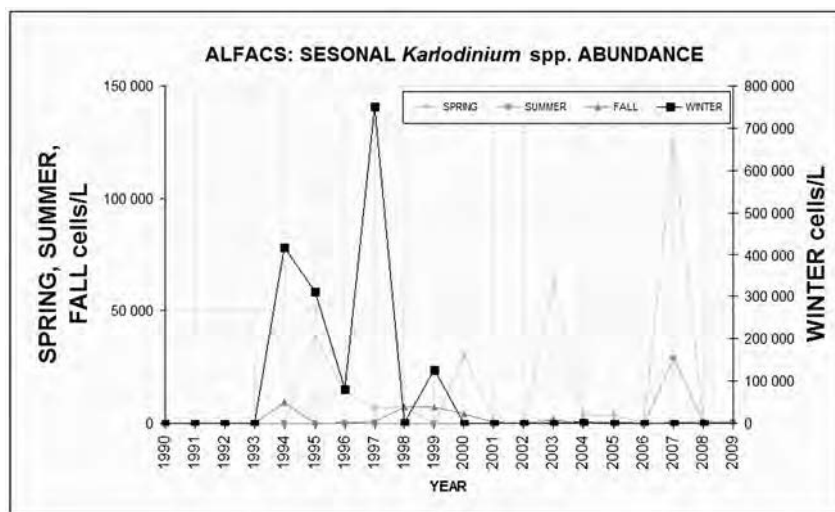


Figure 3. Seasonal *Karlodinium* spp. cell abundance in Alfacs Bay for the period 1990-2009 (n= 12-14 weeks).

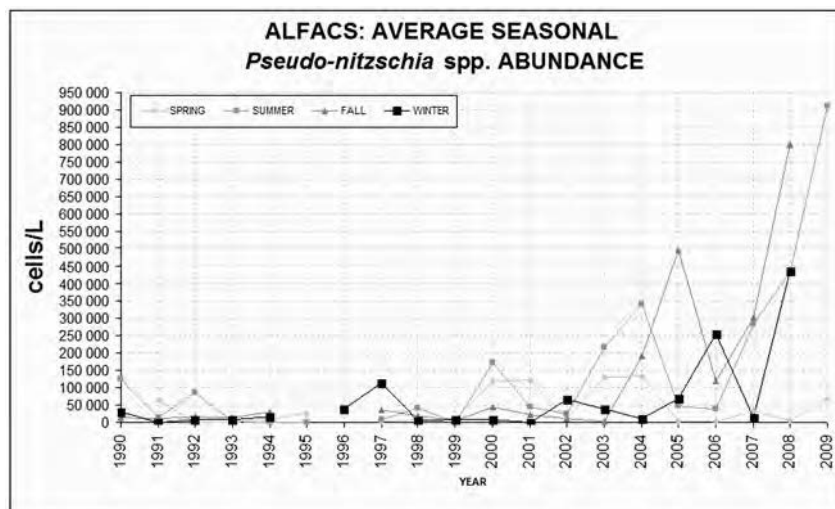


Figure 4. Seasonal *Pseudo-nitzschia* spp. abundance in Alfacs Bay for the period 1990-2009 (n = 12-14 weeks).

DISCUSSION

Long-term changes related to global warming are usually statistically modeled by means of linear regression. Vargas-Yáñez *et al.* (2009) showed that the results reported in different studies on the trends for salinity and temperature in the western Mediterranean depend on the period of time considered and on the data analysis methods used. With linear regression, we detect a significant increase of temperature for the 1990-2009 time series in Alfacs and Fangar bays.

Warming is more intense in Fangar Bay than in Alfacs Bay for all seasons of the year, especially in summer and spring. In Thau Lagoon (Mediterranean coast of France), water temperature increased 0.091, 0.061, 0.052 °C per year for spring, summer and fall for the time series from 1972 to 2006 (Collos *et al.*, 2009). The increases in temperature in Thau Lagoon for spring and fall are similar to those in Fangar Bay but the summer increase in water temperature in Fangar Bay is only half that in Thau.

Average seasonal water column temperatures for the period 1990-2009 were 18.3, 25.3, 16.6, 10.9 °C for spring, summer, fall and winter in Fangar Bay. For comparative purposes, López and Arté (1973), who measured the water column temperature at the same station during the years 1968-1971 calculated seasonal water column temperatures as 17.9, 24.5, 14.2, 10.7 °C for spring, summer, fall and winter. Comparison between the averages for the periods 1968-71 and 1990-2009 shows respective increases of 0.5, 0.8, 2.4, 0.1 °C (Figure 5).

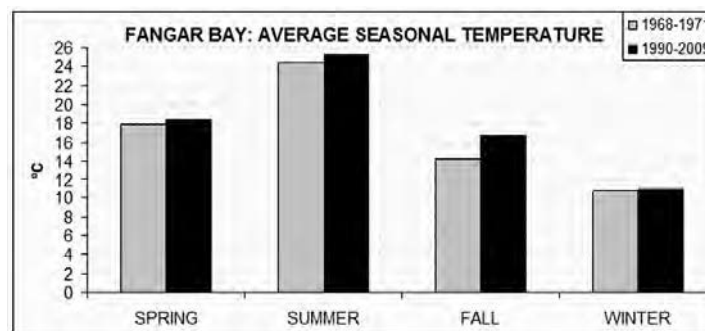


Figure 5. Average seasonal temperature in Fangar Bay. The average values for the period 1968-1971 are shown by grey bars, black bars show the average for the period 1990-2009. The frequency of measurements was monthly for 1968 and bimonthly for 1969-71, in total 27 days of measurements in 4 years.

Positive thermal anomalies, even of short duration, may represent an important physiological change for some organisms such as *Mytilus galloprovincialis*. During warm events, a reduction in grazing together with an increase in nutrients could lead to the high phytoplankton abundance observed in 2003 in Fangar Bay (Ramón *et al.*, 2007). The effect of water temperature on mussel (*M. galloprovincialis*) mortality was studied in laboratory experiments by Anestis *et al.* (2007), who showed that animals started to die as water temperature reached 26°C, and that when temperature exceeds 25°C filtration falls significantly. In Alfacs and Fangar bays, summer shellfish mortalities occur due to high water temperatures (Lleti *et al.*, 1995; Ramón *et al.*, 2007). The average water column temperature in summer for the period 1990-2009 for Alfacs and Fangar bays were 26.1 ± 0.6 and 25.3 ± 0.7 (mean \pm SD), exceeding the threshold for filtration rate in both bays and for mortality in Alfacs Bay.

There is no correlation between the averaged temperatures of each season per year and toxic phytoplankton cell abundances. The analysis of the toxic phytoplankton species present in Alfacs and Fangar show a shift in the bloom period for the ichthyotoxic *Karlodinium* (Figure 3), and an increase in *Pseudo-nitzschia* (Figure 4) densities in the last seven years. Spring *Karlodinium* blooms are correlated with stratification. The main factor controlling stratification in Alfacs Bay is the status of the irrigation channels discharging into the bay (Solé *et al.*, 2009). The management of the channels is controlled by the associations of rice producers and shellfish producers, which generates two main period, an 'open channel' - (more stratified water column) and a 'closed channel' - (less stratified water column).