POPULATION DYNAMICS

Population dynamics of *Alexandrium fundyense* in the Gulf of Maine: outlook for improved management and forecasting

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Abstract

Paralytic shellfish poisoning (PSP) is a recurrent and widespread problem in the Gulf of Maine (GOM) caused by the dinoflagellate *Alexandrium fundyense*. Blooms have been the subject of more than a decade of investigation through the ECOHAB-GOM and GOMTOX research programs. Multiple large-scale field surveys have provided data that were combined with mooring observations, satellite-tracked drifters, and numerical model simulations to document the complex dynamics of *A. fundyense* blooms within this region. A conceptual model of *A. fundyense* bloom dynamics and PSP toxicity in the region is summarized here, highlighting key physiological, behavioral, and environmental or oceanographic factors underlying blooms. A numerical model has also been developed and evaluated against cruise observations and other data. The status of those modeling efforts is discussed, including recent efforts to provide seasonal forecasts of *A. fundyense* bloom magnitude, and near-real time hindcasts and forecasts of use to resource managers.

Introduction

Paralytic shellfish poisoning (PSP) toxicity is a recurrent and widespread problem in the Gulf of Maine (GOM; Fig. 1), affecting vast expanses of the region's nearshore and offshore shellfish (Shumway *et al.* 1988; Anderson 1997). Toxicity is not uniform, but instead reflects *Alexandrium fundyense*[1] growth and toxin accumulation in several separate zones or habitats defined by circulation patterns and the temporal distribution of the dinoflagellate (Anderson 1997). This biogeographic diversity in *A. fundyense* blooms has been the subject of sustained investigation through the ECOHAB-GOM (Anderson *et al.* 2005d) and GOMTOX (www.whoi.edu/gomtox/) research programs. A series of large-scale field surveys provided data that were combined with mooring observations, drifter tracks, and numerical model simulations to document the complex dynamics of blooms within this region.

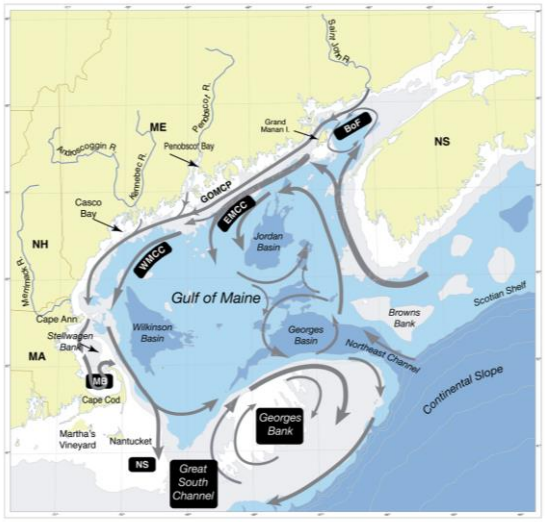

Fig.1. Map of Gulf of Maine showing ocean currents.

[1] Both *A. tamarense* and *A. fundyense* occur in the Gulf of Maine region. We consider these to be varieties of the same species (Anderson et al. 1994; Brosnahan et al. 2010). Neither antibody nor oligonucleotide probes can distinguish between them, and only detailed analysis of the thecal plates on individual cells can provide this resolution. This is not practical for field samples. Accordingly, for this study, the name *A. fundyense* is used to refer to both forms.

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This paper highlights major features and mechanisms underlying the widespread, coastal blooms that can close hundreds of kilometers of coastline for shellfish harvesting. A numerical model that provides realistic simulations of these blooms is also presented, emphasizing progress towards short-term (weekly) and seasonal (annual) forecasts of bloom dynamics and toxicity.

The *Alexandrium fundyense* conceptual model

A dominant feature underlying *A. fundyense* regional bloom dynamics is the Maine Coastal Current or MCC (Fig. 1; Lynch *et al.* 1997) - a composite of multiple segments and branch points. The two major transport features in this system are the eastern and western segments of the MCC, hereafter termed the EMCC and WMCC. Conceptual models of *A. fundyense* bloom dynamics within the MCC have been provided by Anderson *et al.* (2005c) and McGillicuddy *et al.* (2005).

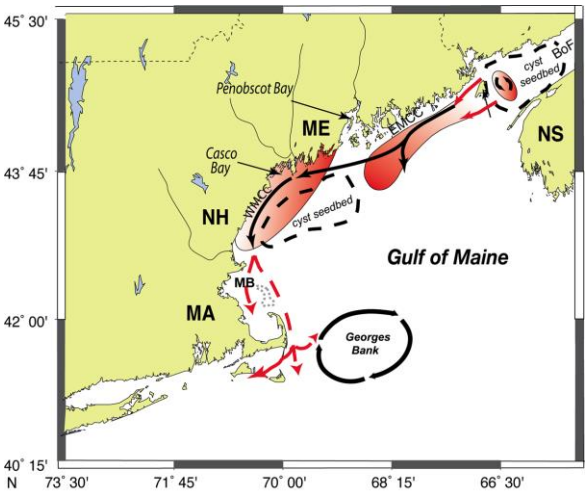

Fig.2. Map of cyst beds.

Key features in the models are two large cyst "seedbeds"- one in the Bay of Fundy and the other offshore of mid-coast Maine (Fig. 2; Anderson *et al.* 2005c). Cysts germinate from the BOF seedbed, causing recurrent coastal blooms that are self-seeding with respect to future outbreaks in that area. In effect, the BOF is an incubator for localized populations in that area, but the incubator is leaky, as cells escape into the EMCC, where they bloom, particularly at the distal end of that coastal current, where waters warm and stratify (Townsend *et al.* 2001). Some cells travel south and west with the EMCC, while others deposit cysts in the mid-coast Maine seedbed. In subsequent years, these latter cysts (combined with vegetative cells from populations within the EMCC) inoculate WMCC blooms that cause toxicity in western portions of the Gulf and in offshore waters as well. Toxicity in southerly and western regions of the GOM such as Massachusetts Bay is regulated by coastal current transport, with northeasterly winds accelerating the alongshore and cross shor movement of the populations (Anderson et al. 2005a).

Hindcasting and forecasting efforts

A coupled physical/biological model of *A. fundyense* population dynamics in the Gulf of Maine has been developed that is consistent with the above conceptual model (e.g., McGillicuddy *et al.* 2005; Anderson *et al.* 2005c; He *et al.* 2008; Li *et al.* 2009). The model is initiated from large-scale maps of cyst distribution, with germination rates parameterized through laboratory experiments. Likewise, the growth of the resulting vegetative cells is regulated by light, temperature, and salinity, again parameterized using laboratory cultures. In a novel application of this model, observations were combined with model hindcast simulations to identify the dominant factor leading to a 2005 *A. fundyense* bloom considered to be the largest in at least three decades (He *et al.* 2008). Anderson *et al.* (2005b) proposed three factors to explain the historic 2005 outbreak: 1) high abundance of resting cysts that provided a large inoculum; 2) storms with strong northeast winds that carried toxic cells towards, and along the coast; and 3) abundant fresh water runoff, providing macro- and micro-nutrients, a stratified water column, and an alongshore (towards the southwest) transport mechanism. These factors were evaluated using a sensitivity analysis that utilized field observations in the *A. fundyense* population dynamics model (He *et al.* 2008). A snapshot from the 2005 hindcast simulation that used the 2004 cyst data (hereafter termed the central hindcast) illustrates the bloom's regional-scale characteristics (Fig. 3A). Recently germinated cells swimming upward from the western GOM and BOF cyst seedbeds are evident in vertical transects. Germinated cells inoculate the coastal current system,

which flows from northeast to southwest and then spreads offshore in the south. Large-scale characteristics of the simulation are generally consistent with field observations.

Initial conditions of the three sensitivity experiments were identical to the central hindcast in all respects except: experiment 1 utilized the 1997 cyst map instead of 2004; experiment 2 was forced by winds from a more typical year (2004) instead of the strong downwelling-favorable winds of 2005; experiment 3 used riverine discharge from a typical year (2004) instead of the anomalously large discharge of 2005.

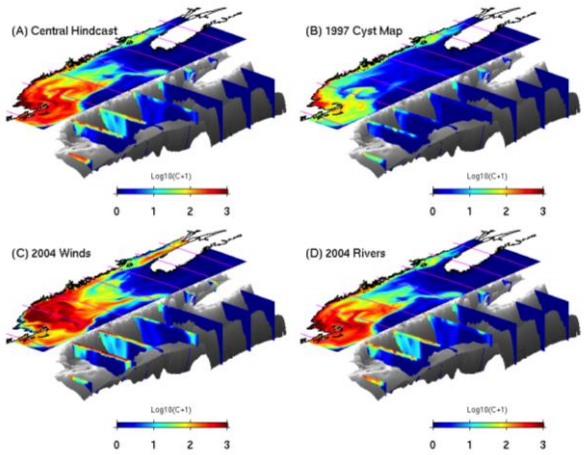

Fig. 3. Bloom predictions based on a. hind casting;b.cyst beds; c. wind forcing; d. riverine discharge.

This sensitivity analysis suggested that high cyst abundance in the WGOM was the main cause of the 2005 bloom. Wind forcing was an important regulator, in the form of both episodic bursts of northeast winds and the downwelling-favorable mean condition, causing onshore advection of offshore populations. Anomalously high river runoff enhanced alongshore transport near the coast. These and other results demonstrate that model simulations initiated from *A. fundyense* cyst distributions capture large-scale seasonal patterns in the distribution and abundance of vegetative cells. Cyst abundance is a first-order predictor of regional bloom magnitude the following year in the WGOM, suggesting that cyst abundance may hold the key to interannual forecasts of PSP severity, recognizing that other factors will determine the extent of population growth and delivery to shore. This is a major finding that is of significant importance in terms of bloom management and forecasting in the region.

Weekly and annual forecasts

The model has been used to produce near-real-time quasi-operational nowcasts and forecasts for 2006 - 2010. Each of these synoptic simulations was initiated using a regional map of *A. fundyense* cyst abundance in the GOM (e.g., Anderson *et al.* 2005c) obtained in the winter before the next bloom season. Each year, weekly model updates were made available to a listserve of more than 150 managers and other officials and scientists involved with PSP outbreaks in the northeastern US. These weekly updates allowed the listserve members to go to a website where they could view the latest model simulations of that year's *Alexandrium* bloom, extended one week forward in time using weather forecasts. An example forecast can be seen at http://omglnx3.meas.ncsu.edu/yli/08forecast/dino_08.htm. Forecasts were also sent to researchers at sea to aid in the planning of sampling activities. Readers are also encouraged to visit the forecasting web site cited above to scrutinize the comparisons between simulated and predicted *A. fundyense* concentrations in 2008 as one example of the skill of the model. Other analyses of model skill are given in Stock *et al.* (2005) and He *et al.* (2008). The reception for this information has been highly positive, as it gave managers a view of the entire bloom in the Gulf through weekly updates during the bloom season. This information was complementary to shellfish toxicity measurements made on a weekly basis at scattered locations along the coast. Seasonal or annual forecasts have also been made. This effort began when a cyst survey in late 2007 revealed that cyst abundance offshore of mid-coast Maine was 30% higher than in fall 2004, just prior to the historic bloom of 2005. Those cyst maps are shown in Fig. 4. The 2008 field season thus offered an exceptional opportunity for testing the hypothesis that the magnitude of the bloom in the western Gulf of Maine is set by the abundance of resting cysts. In advance of the bloom season, the coupled physical-biological model was used to make a seasonal

forecast using an ensemble of scenarios based on archived hydrographic simulations from 2004-2007 model runs.

The ensemble forecast was made available to resource managers on the web at http://omglnx3.meas.ncsu.edu/yli/simulation_new/08forecast/dino_08.htm. The simulations were initialized with zero cell concentration throughout the domain and the cyst map prescribed from fall 2007 observations. Each member of the ensemble was based on the hydrodynamic hindcast for each specific year, which affected the abundance and distribution of *A. fundyense* cells through environmental influences on germination, growth, mortality, and transport. Although the hindcasts for 2004-2007 did not span the range of all possible outcomes, they provided contrasting conditions including one with strong downwelling-favorable winds and anomalously high river discharge in May (2005) and one with near climatological conditions (2004). They also spanned the range from major PSP outbreak (2005) to moderate (2006, 2007) to low (2004) levels of regional toxicity.

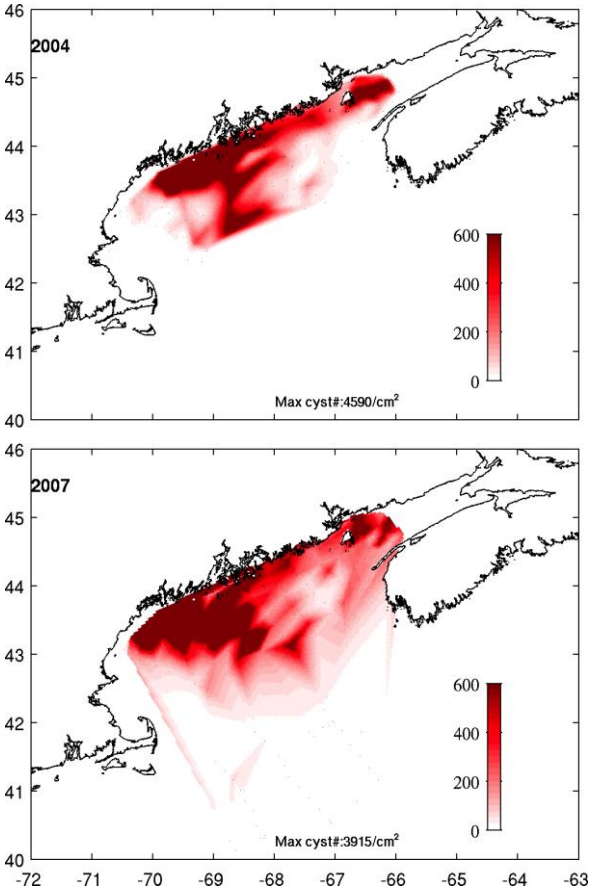

Fig.4. Cyst maps for 2004 and 2007.

All of the simulations indicated a severe bloom in the western GOM, on par with the historic bloom of 2005. A press release was issued (http://www.whoi.edu/page.do?pid=9779&tid=282&cid=41211&ct=162). This information was used by resource managers in staffing decisions in advance of the bloom and was seen by many as a major factor in the controlled and moderate response of the public and press during the outbreak, and thus in the reduced economic impacts compared to the 2005 event. The seasonal forecast was confirmed when a major bloom occurred, extending from Maine through New Hampshire and much of Massachusetts, leading to federal emergency assistance to these three states because of the "failed fishery".

This seasonal forecast of the 2008 outbreak is a major breakthrough, as it represents the first prediction of a red tide or HAB on a regional scale, and speaks to the advanced nature of our understanding of the *A. fundyense* bloom dynamics in the GOM, and to the sophistication and accuracy of our numerical model.

For 2009, a "moderately large" outbreak was forecast, based on the cyst abundance observed in fall, 2008 (http://www.whoi.edu/page.do?pid=24039&tid=282&cid=56567). This forecast was generally accurate, since the toxicity was more limited in scale than in the previous year, extending only to the middle of Massachusetts Bay. However, a resurgence of toxicity in June and July occurred in Maine, leading to very high and prolonged toxicity in that state. This second wave of toxicity could not have been anticipated in the seasonal forecast, and reflected unusual wind patterns in June and July.

For 2010, the forecast that was issued was similar to that for 2008– i.e., a. "significant" *A. fundyense* bloom was anticipated since even more cysts were documented in late 2009 than were present in 2007, immediately before the large-scale 2008 outbreak (http://www.whoi.edu/page.do?pid=24039&tid=282&cid=69586). This forecast was, however, not borne out by the subsequent bloom that year. Relatively small sections of the Maine coast were closed because of toxicity, with no closures in coastal New Hampshire or Massachusetts. GOMTOX

research cruises documented very low *A. fundyense* cell abundances in both nearshore and offshore waters of the GOM, so the issue was not a lack of onshore transport, but rather the overall lack of a bloom. Our working hypothesis is that a mesoscale GOM water mass change occurred that lies outside the envelope of observations from the six years used as the basis of the 2010 ensemble forecast.

This hypothesis is currently being evaluated using GOMTOX survey data for 2010, satellite measurements of ocean color, as well as moored observations from instrumented buoy networks (McGillicuddy *et al.* in prep.) Preliminary analyses suggest that the deep basins of the GOM were fresher and warmer than was observed in prior years. This water mass anomaly would have affected intermediate and surface waters, the latter being where *A. fundyense* resides. For example, surface waters were several degrees warmer than in 2008, when a large *A. fundyense* bloom took place. Stratification, nutrient concentrations, grazers, and other factors critical to *A. fundyense* growth could all have been affected. Should we be able to deduce the mechanisms responsible for the lack of a bloom in the WGOM in 2010, those processes could then be included in the population dynamics model. Furthermore, we note that the water mass changes mentioned above relate to the large-scale circulation of the northwest Atlantic, and therefore are observable months in advance of the *A. fundyense* season using moored instruments in ocean observing systems. Therefore, it is conceivable that forecasts can be made taking into account this type of variability. It is indeed fortunate that GOMTOX cruises were scheduled for 2010, as this will allow us to understand the factors that prevented a bloom and thereby allow us to improve our model.

Overview

The conceptual and numerical models described herein are a result of more than a decade of detailed study of *A. fundyense* dynamics over a large area in the GOM. The models are extraordinarily useful research and management tools that help to guide decisions about closures and re-openings of harvest sites, support forecasts and predictions that are of use to shellfish industry and resource managers, and that in general, provide a context against which blooms and toxicity observations can be viewed. Looking back, one can highlight the information needs and analytical approaches that can help other countries or regions develop similar models for HABs and their waters. First and foremost, one needs a detailed understanding of the hydrography of the area under investigation, including adjacent waters that influence the localized flows. Major current systems need to be identified and characterized, as well as the episodic movements of water associated with storm runoff, upwelling, downwelling, and other factors. Moored instruments and survey cruises are needed to characterize this hydrography to provide data to numerical models that are critical in the development of an understanding of HAB dynamics. Initially, the numerical models should focus entirely on the physics of the region, but ultimately, biological elements can be added (e.g. Stock *et al.* 2005) that can be very useful in understanding HAB dynamics. For a cyst-forming HAB species like *Alexandrium fundyense*, much of the biological model formulation has already been accomplished, and can be adapted to the strains of this or related species in a different area following laboratory studies to derive growth rate and germination rate as a function of temperature, light, and salinity. More sophisticated efforts might include nutrient uptake kinetics, as this can be useful in forecasting the decline of blooms in the locations where cysts will be formed and deposited. This is important as the initial condition for physical/biological models for cyst-forming species. Grazing may need to be considered as well, but this is a difficult issue to parameterize in any detail. In our formulations, we have utilized a mortality rate that varies with temperature according to a Q_{10} formulation (He *et al.* 2008). It is simplistic, but thus far, does an adequate job with bloom termination judging from the match between our simulations and observations. This is one example where a simple approximation can replace a complex submodel and still provide acceptable simulations.

Another key feature in the development and application of conceptual models like that

described here is the documentation of the nutrient environment that the HAB species will occupy. Survey cruises will provide large-scale snapshots of the nutrient fields, but these change constantly, and are quickly out of date. We have found it useful to utilize "climatological" or long term average nutrient fields for the modeling efforts. These have been derived for the GOM region on the basis of numerous shipboard surveys conducted throughout the years, with those data being compiled and related to parameters such as temperature and salinity. The development of climatological nutrient fields is thus an important priority for those wishing to develop models in a particular region. However, as demonstrated in 2010 in the GOM, there may be years in which the nutrient fields differ dramatically from the climatology

Our numerical model for *A. fundyense* in the GOM will undoubtedly be refined and modified through time. In its present form, however, it is already proving very useful as a management tool and as a means to communicate the nature of the HAB phenomenon to the public, the press, and to agency officials. Development of such models for HABs in other regions requires a systemic approach whereby the key hydrographic and biological features of the system are identified, characterized, and ultimately modeled. Conceptual models and numerical models are best formulated in parallel, as each provides information and insights to the other. Effective management and mitigation of HABs are greatly facilitated by these efforts.

Acknowledgements

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Interannual variability in *Alexandrium* spp. cyst densities in Cork Harbour, Ireland and it's relation to bloom intensity

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Abstract

The distributions of *Alexandrium* cysts in the sediments of Cork Harbour, Ireland, have been investigated since 2003. Cyst densities of *A.minutum* and *A.tamarense* were examined during the winter dormant season in the North Channel of Cork Harbour, where blooms are known to initiate. The means and variances of the horizontal cyst distribution in the top 1 cm of sediment in the North Channel were compared between years, and the data were also analysed in conjunction with the maximum observed vegetative cell densities in subsequent summers. The results show a decreasing trend in cyst densities since 2003. An analogous decreasing trend in the maximum observed bloom cell density was also apparent since 2004 when an exceptional *Alexandrium* bloom ($5*10^5$ cells l^{-1}) occurred. The potential of the winter cyst density in controlling the intensity of summer blooms is discussed.

Introduction

Shellfish aquaculture is a significant component of the economy of the southwest coast of Ireland (Parsons 2006). The occurrence of Harmful Algal Blooms has hindered the development of the industry, in particular through contamination of shellfish with algal biotoxins (McMahon and Silke 1998). Cork Harbour, Ireland's most industrialised harbour located on the southern Irish coast, has a history of episodic contamination of shellfish with Paralytic Shellfish Toxins, and blooms of *Alexandrium* spp. can occasionally reach high cell density (ca. 10^3 ml^{-1}; Ni Rathaille 2007). The first documented outbreak occurred in 1987, and subsequently there have been frequent bans on shellfish harvesting in the area. Notable exceptions have been the years 1999 and 2001. Cork Harbour is the only site in the Republic of Ireland where PSP contamination has occurred, and toxin contamination usually occurs between mid-June to mid-July. The *Alexandrium* genus is large (~28 species) and to date *A. minutum*, *A. tamarense* and *A. ostenfeldii* have been identified in Cork Harbour (Touzet *et al*. 2008). It was originally thought that *A. tamarense* was the causative organism of these toxic events. However, toxin profiles of cultures isolated from the estuary have indicated that the causative organism is *A. minutum* (Touzet *et al*. 2007a). Isolates had a toxin profile of the two potent gonyautoxins GTX2 and GTX3 which have been characteristic of contaminated stock (Furey *et al*. 1998; Touzet *et al*. 2007).

The life cycle of *Alexandrium* has a resting cyst stage, in which state it can overwinter or stay dormant for several years in adverse conditions (Anderson *et al.* 1997). Excystment into the planktonic vegetative form initiates *Alexandrium* blooms. Knowledge of the distribution and cyst density is therefore potentially vital information if the effects of toxic *Alexandrium* blooms are to be managed and mitigated. This study examined the variation in the distribution and density of *Alexandrium* cysts in Cork Harbour since 2003.

Materials and Methods

Cyst surveys were carried out in the north-eastern section of Cork Harbour known as the North Channel (Figure 1). It is in this part of the estuary, which has dimensions 8 km by 0.5 km, where *Alexandrium* blooms initiate and where the highest dinoflagellate cyst densities can be found (Ni Rathaille 2007). Sub-tidal sediment samples were obtained using a manually operated Ekman Birge bottom sampler (HydroBios, Kiel). Triplicate sub-

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samples of surface sediment were taken from this using sawn off 50 ml syringes that were filled to the depth of the sediment in the sampler. Intertidal samples were collected from the shoreline manually, also using 50 ml core syringes. The sediment was kept in the dark at 4º C until further analysis in the laboratory. In order to quantify the number of cysts in the sediment, the surface 1 cm of each sediment core was removed, mixed to ensure homogeneity, and from this a measured, weighed volume (usually 0.4 ml) was put into a beaker of water, ultrasonicated, and the cysts were extracted using density gradient centrifugation with sodium polytungstate. Prior to 2007 Ludox was used for the density gradient. The number of *Alexandrium* cysts was counted using a Sedgwick-Rafter cell at x100 magnification. Cyst surveys were carried out between mid-September and mid-April when *Alexandrium* can be expected to be in the dormant phase (Table 1).

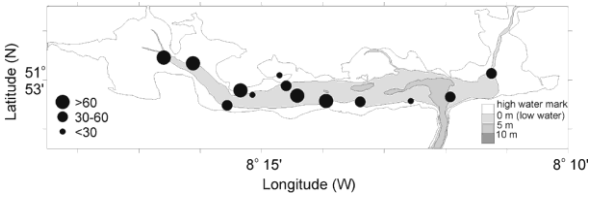

Fig. 1. Distribution of *Alexandrium* cyst densities, North Channel, April 2010. Units are in cysts g dry sediment^{-1}.

Results

The most recent survey carried out on 6-7 April 2010 involved thirteen sampling sites, three of which were intertidal (Figure 1). Cyst densities of *A. tamarense* ranged from 5-20 cysts . g dry wt sediment^{-1}. Cyst densities of *A. minutum* were more variable and generally higher in the range of 5-80 cysts . g dry wt sediment^{-1}. Higher cyst densities were found in the western (inner) end of the North Channel (Figure 1). Mean cyst densities of *A. tamarense* and *A. minutum* were 11 and 38 cysts g^{-1} respectively. These data can be compared with those obtained during the dormant season in previous years (Table 1). A decline in *A. tamarense* and *A. minutum* cyst densities is evident in years proceeding 2003. Results from the annual surveys identified six locations containing notably increased cyst densities (>mean + 2*s.d) during the annual dormant season (Figure 2). These were located in the regions of the North Channel with the most retention near the furthest east and west of the channel and in a lagoon situation along the north shore. The other three locations were where gyres exist on ebb and flood tides near the mouth of the channel. Cysts can be regarded as passive, positively buoyant particles within the water column, and might be expected to form 'seed shadows', analogous to terrestrial systems (Wyatt 2003).

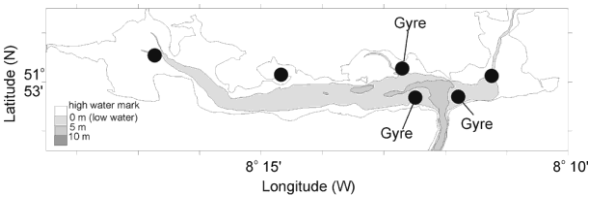

Fig. 2. Locations where increased *Alexandrium* cyst densities have been recorded, North Channel, Cork Harbour.

Thus characteristic water movement patterns could influence the distribution of the cysts within the sediment. Figure 3 illustrates the variation with time in the mean observed *Alexandrium* resting cyst densities in the North Channel. It is evident that densities never achieved as great a value as was found in the winter of 2003/2004. *A. minutum* cyst densities of up to 1680 cysts . g dry wt sediment^{-1} were found during the winter of 2003/2004, with a mean value of 437 cysts . g dry wt sediment^{-1}. In the following September, after the summer bloom, the highest count recorded for this species was 301 (mean 88) cysts . g dry wt sediment^{-1}. A similar pattern was observed for *A. tamarense* cyst densities, although counts were relatively lower than *A. minutum* (Table 1). Maximum and mean recorded densities of 956 and 302 cysts . g dry wt sediment^{-1} in the winter of 2003/2004 fell to 239 and 54 cysts . g dry wt sediment.$^{-1}$ respectively in September of 2004.

When these data were plotted against observed *Alexandrium* vegetative cell densities, the decrease in *Alexandrium* cyst densities corresponded with a decrease in the vegetative cell densities after the winter period of 2003-2004. A bloom of 620,000 cells l^{-1} was observed in the western half of the N. Channel in the summer of 2004. Yet during the same period in 2005, the maximum count recorded was only 31,000 cell l^{-1}. With the exception of the year 2009 cell densities were recorded annually, but never peaked above 55,000 cells^{-1}.

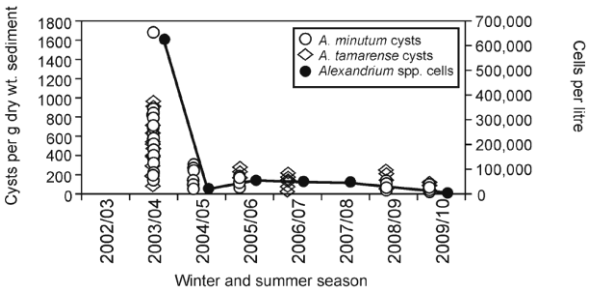

Fig 3. *A. minutum* and *A. tamarense* cyst densities and *Alexandrium* spp. bloom cell densities, North Channel, Cork Harbour 2003-2010.

The data derived from four subsequent winter cyst surveys and summer bloom studies all showed low densities of both species (Table 1; Figure 3).

Table 1. *A. tamarense* and *A. minutum* cyst density data (cysts/g dry sediment), North Channel (2003-2010).

Dormant Season	Date	*A. tamarense*			*A. minutum*		
		max	mean	sd	max	mean	sd
2003/2004	22-25 Oct 2003 9-12 Mar 2004	959	302	232	1680	437	325
2004/2005	21 Oct 2004	239	54	77	301	88	91
2005/2006	16 Sept 2005	264	88	85	175	96	64
2006/2007	3-5 Oct 2006	213	75	51	-	-	-
2008/2009	Oct 2008	247	108	69	140	39	32
2009/2010	6-7 Apr 2010	20	11	5	76	38	23

Discussion

The monitoring of HABs is crucial in order to provide enough information to predict bloom events and, if possible, their intensity. There are, however, relatively few time series of cyst surveys. In the Gulf of Maine, studies have been carried out on *Alexandrium* blooms for over two decades. In 2005, a massive *A. fundyense* bloom occurred there. It has been suggested, through modelling, that the bloom intensity was linked to an unusually high cyst density, which had been measured the previous autumn (He *et al*. 2008). Furthermore, these observed high cyst levels reflected deposition from an autumn bloom of *A. fundyense* that occurred in late 2004 (Anderson *et al*. 2005). A parallel case can be made for the situation in Cork Harbour. In September 2003, cell densities of *Alexandrium* of the order 20,000 cells l^{-1} were observed in samples in the North Channel (Ni Rathaille 2007). Such levels would be unusually for this time of year. The late summer bloom could have deposited fresh cysts causing increased densities in the sediment, as measured in October of that year, which then promoted a large bloom the following spring. No September *Alexandrium* bloom has however been observed since, and cyst densities have remained low. On the other hand, the 2004 *Alexandrium* bloom is known to have provided a substantial number (ca. 5000 cm^{-2}) of cysts to the western section of the North Channel through direct measurement with sediment traps (Ni Rathaille 2007). Such high levels were not evident in the autumn cyst survey of 2004. This could have been due to their burial, export or some may even have been lost through germination after a mandatory dormancy period. Increasing the cyst density by a factor of two in a model of *Alexandrium* blooms in Hiroshima Bay did not alter the bloom intensity but did bring the timing forward by about a week (Yamamoto and Seike 2003). It might be expected that if environmental conditions were favourable then planktonic cell numbers would be more influenced by the speed of vegetative reproduction than by excystment rate. If the size of the inoculum of cells (provided by excystment) is not important, then substantial blooms could occur in any year. There has not however been a substantial bloom of *Alexandrium* in Cork Harbour since 2004.

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Influence of environmental variables on *Alexandrium catenella* motile stage and other harmful taxa in southern Chile (43° - 55° S) (January – December 2009)

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Abstract

Phytoplankton records during the last decade in Southern Chile (41º-55º S) show that *Alexandrium catenella* blooms are recurrent, have an annual frequency in the area between 43º50’ and 55º00’, but sporadic (2002, 2006 y 2009) between latitudes 42º20’and 43º20’. These blooms are always associated with the presence of Paralytic Shellfish Poison (PSP) in molluscs. The temporal and geographical distribution by *A. catenella* during each event seems to be associated with the characteristics of different sectors of the study area. Remarkable hydrographic and meteorological differences occur in this vast geographical area. Based on biological, toxicological, hydrographic and meteorological information generated during 2009, the structure and interrelationships of recorded variables are analyzed to identify key variables linked to *A. catenella* blooms, which will serve to generate explanatory models for these phenomena.

Introduction

Phytoplankton records during the last decade in the fjords of Southern Chile (41º-55º S.L.) show that *Alexandrium catenella* blooms are recurrent, have an annual frequency between 43º50’ and 55º00’ S, but occur sporadically (2002, 2006 and 2009) along latitudes of 42º20’and 43º20’. These blooms are always associated with Paralytic Shellfish Poisons (PSP) in sentinel molluscs (Guzmán et al., 1975, 2007, 2009). The temporal and geographical distribution of *A. catenella* during each event seems to be associated to the features of different sectors of the study area. Remarkable hydrographic and meteorological differences can be found in this vast geographical area. At least three important physical phenomena that affect *A. catenella* abundance and distribution have been identified. The first refers to abundance gradients of this dinoflagellate, frequently found in fjords and channels connected to the continental shelf, showing an abundance increase from the head to the mouth of the fjord (Guzmán et al. 1975), the second is related to dispersion phenomena forced by winds, tides and currents that spread the motile stage of this organism, and the third is associated with advection phenomena and shear probably

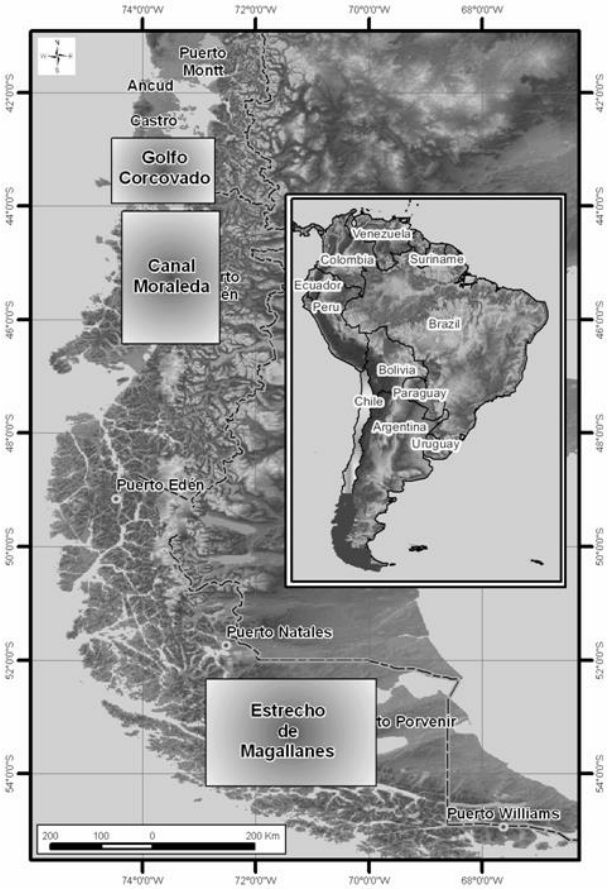

Fig. 1. Sampling area. forced by winds, tides and currents that lead to a local higher dinoflagellate

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concentration generating toxicity hotspots, such as the Magellan region (Guzmán et al., 2002, 2009).

Material and Methods

During 2009 27 biological, toxicological, hydrological and meteorological variables were monitored under a quasi-monthly sampling scheme along 151 stations located in southern Chile between 41° and 55° S (Fig. 1). Quantitative phytoplankton was obtained by hose sampling from two layers (surface to 10m and 10m to 20m depths) and qualitative phytoplankton was sampled by vertical hauls between surface and 20m using a 23µm net. Toxicological information was obtained from bivalves by mouse bioassay (PSP and DSP) and HPLC (ASP). Hydrographic information and oxygen were obtained by CTD and meteorological data were measured with a thermal anemometer and weather stations. The data were sorted, transformed to base 10 logarithms and analyzed using principal components analysis (PCA) without rotation. The study area was divided into 3 sectors: Corcovado Gulf (37 variables), Moraleda Channel (35 variables) and Strait of Magellan (16 variables). Variables were: Relative abundances of *A. catenella*, *Dinophysis acuta*, *D. acuminata*, *Pseudo-nitzschia cf. australis*, *P. cf. pseudodelicatissima*, *A. ostenfeldii* and *Protoceratium reticulatum*, besides G Index, phytoplankton species richness, transparency, air temperature, air pressure, wind speed, cloudiness, wind direction, PSP, diarrhetic shellfish poison (DSP), surface, 5m, 10m and 20m water temperatures, salinities and oxygen concentrations, and phytoplankton abundance.

Results and Discussion

PCA results show that the proportion of the explained variance for the first two components in the three analyzed situations is between 39.5 and 49.8%. However the eigen values of the significant variables are above 0.75 reflecting a strong association between them. The principal PCA results are summarized in Table 1 and Figs. 2-4.

Table 1. Principal Component Analysis statistics.

% explained variance first component	% explained variance second component	Determinant correlation	Kaiser-Meyer-Olkin
Corcovado Gulf			
24,99	18,67	$7{,}84 * 10^{-5}$	0,73
Moraleda Channel			
26,93	12,61	$6{,}56 * 10^{-10}$	0,77
Strait of Magellan			
31,69	18,15	$2{,}11*10^{-19}$	0,81

Results for the analyzed sectors show that air and surface water temperatures are correlated with phytoplankton and consequently with harmful phytoplankton abundance. This correlation is primarily due to higher irradiance during Spring and Summer. In contrast, surface salinity shows a close association with species richness and phytoplankton abundance.

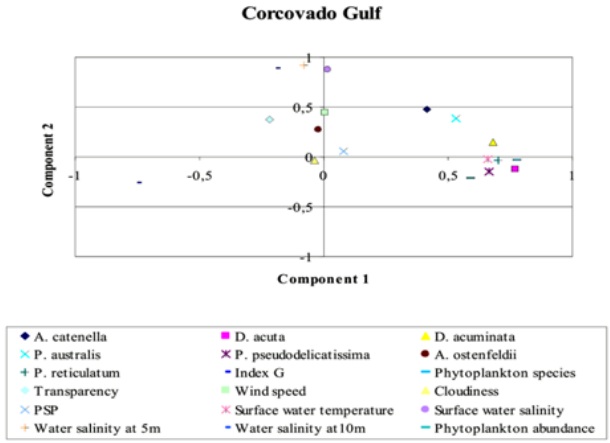

Fig. 2. Results of principal component analysis applied to information of Corcovado Gulf.

At a more localized level, the Corcovado Gulf show that surface water temperature is associated with species richness and phytoplankton abundance, but not always with harmful taxa abundances, since *Pseudo-nitzschia* species show a stronger relationship, in contrast to dinoflagellates speciesfor which this relation is less clear. Salinity is associated to microalgal diversity. During Spring and Summer, along the Moraleda Channel the air temperature is related to water temperature and favours freshwater inputs due to snow and ice melting, affecting surface salinity and water transparency due to particulate matter input, but transparency is also affected by phytoplankton abundance. Apparently, freshwater runoff

favours also the phytoplankton development, as is shown by PCA reflecting an increase of species richness and phytoplankton abundance, including an increment of harmful taxa concentrations. As was expected *A. catenella* relative abundance segregates together with PSP concentrations.

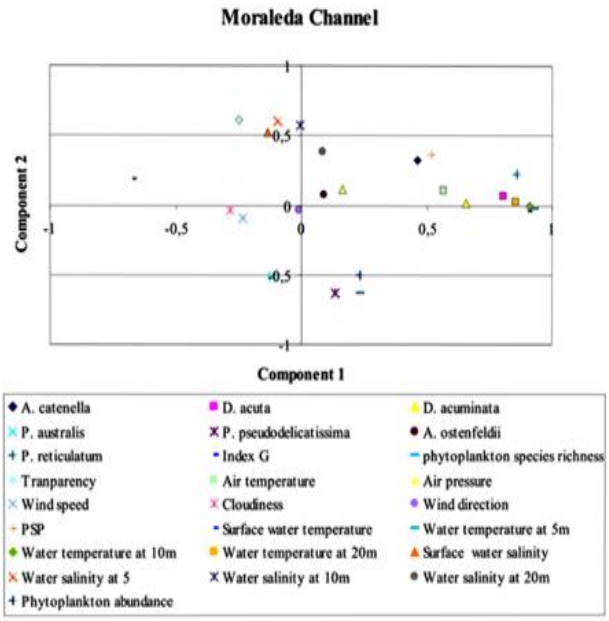

Fig. 3. Results of principal component analysis applied to information of Moraleda Channel.

In the Strait of Magellan, although temperature shows the narrowest variability, the air and water temperatures are positively correlated to species richness and phytoplankton abundance, as well as, to the relative abundances of *A. catenella*, *D. acuminata*, *P. cf. australis* and *P. cf. pseudodelicatissima*.

Conclusions

The meteorological and hydrographic forcing affects the geographical distribution of *A. catenella* and also other harmful species, with air temperature and water temperatures the most important. The greatest abundance of *A. catenella* occurs when the rest of the phytoplankton is also more abundant.

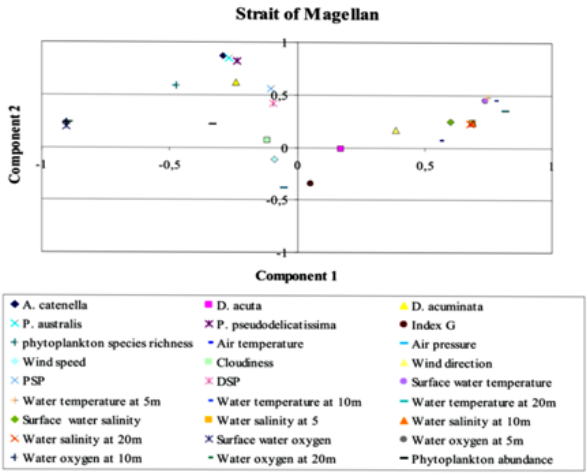

Fig. 4. Results of principal component analysis applied to information of the Strait of Magellan.

Acknowledgements

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Spatial and temporal variability of *Alexandrium catenella* and PSP in southern Chile (43° - 55° S) (May 2006 – July 2010)

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Abstract

Since 1972 *Alexandrium catenella* and Paralytic Shellfish Poison (PSP) are known from Magellan Strait, being sporadic and restricted to the Magellan region until 1990. Afterwards, recurrent annual blooms and PSP outbreaks and a northern expansion occurred. *A. catenella* was detected in the Aysén region in 1992 and in 1998 in the southeast of Chiloé Island. In October 2009 it reached its most northern distribution at Calbuco (41°48'S; 73°10'W) and was cited at the Pacific coast of Chiloé Island. The blooms - PSP outbreaks - show different annual patterns encompassing vast geographical areas. In its northern area of distribution, they are sporadic, reaching densities of 1,000 cells l^{-1} and a toxicity of 1,419 µg STX eq. 100 g^{-1} shellfish meat. In the southern area, densities have been up to 53,800 cells l^{-1}, toxicity has reached 27,159 µg, nevertheless the highest densities, 1,132,200 cells l^{-1}, the highest relative abundances and the greatest mean PSP levels have been observed in the region of Aysén. The interannual variability and its wide geographic coverage, including sectors and periods with higher probabilities to detect the microalga and PSP, suggest that the bloom initiations and PSP outbreaks are of climatic - oceanographic origin.

Introduction

Since October 1972 *Alexandrium catenella* blooms and PSP outbreaks are known for the Magellan region (Guzmán et al., 1975a). Since the nineties, blooms increased in frequency, annual toxicity outbreaks occurred and an expanded distribution to the North was observed. The microalga was detected in the region of Aysén in 1992 (Muñoz et al., 1992) and PSP in 1994 (Guzmán et al., 2002) and in the region of Los Lagos, it was detected in 1998 in the southeast area of Chiloé Island (Lembeye et al., 1998) and PSP detected in 2002 (Mardones et al., 2010). Today this microalga is present along the fjords between 41° and 55° S, but PSP encompasses from 43° to 55° (Guzmán et al., 2010). These facts triggered the initiation in May 2006 of a phytoplankton and marine toxin monitoring program to protect public health and minimise impacts to productive activities. This contribution presents an analysis of *A. catenella* and PSP temporal and geographic patterns along the Chilean fjords and channels from May 2006 to July 2010.

Material and Methods

A total of 151 sampling sites have been established between 41° and 55° S (Fig. 1). Sampling sites are visited monthly in Spring, Summer and Autumn, and during Winter about every 40 days. Sampling was conducted since May 2006, with gaps between March-October 2007, October-November 2009 and January 2010. The variables considered here are relative abundance and density of *A. catenella* and PSP concentrations in bivalve molluscs. Relative abundance is estimated from net samples (23 µm), collected by vertical tows from 20 m depth to the surface. At each site three replicates at two points are collected, integrating a single sample from the six hauls. Relative abundance

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estimations are made on sedimented samples counting the microalgal vegetative phase. Details on relative abundance scale and its estimations are presented in Guzmán et al. (this proceeding). Density was estimated from samples taken with a 2.5 diameter hose, in two strata: surface - 10 m and 10 - 20 m depths. Density was estimated by the Utermöhl (1958) method. Paralytic Shellfish Poison (PSP) was estimated by mouse bioassay (A.O.A.C., 1990) in *Aulacomya ater*, *Mytilus chilensis* and *Venus antiqua* and analyses conducted at the Ministerial Regionals Secretariat for Health.

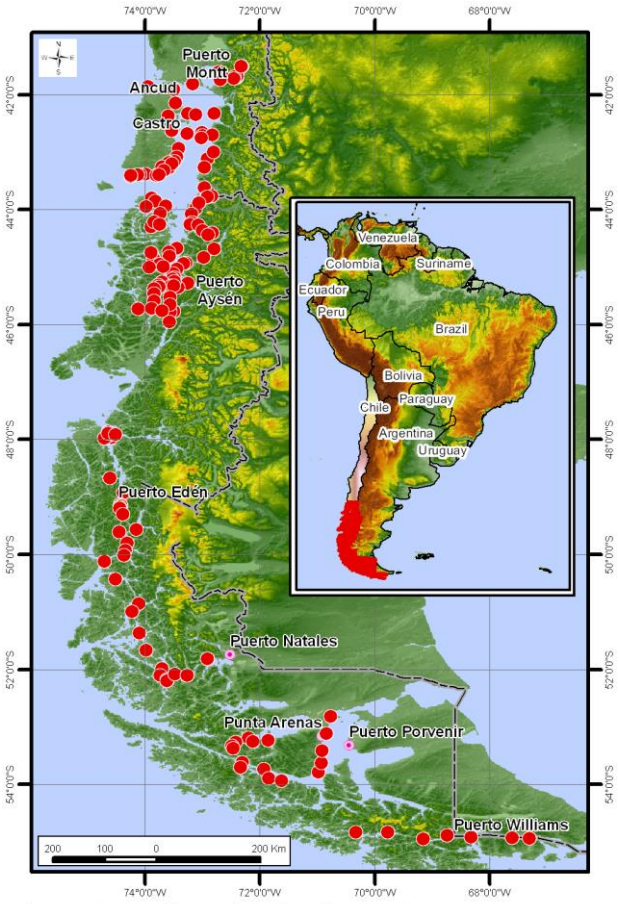

Fig. 1. Sampling sites in the study area.

Results and Discussion

The relative abundance in comparison to density estimates for *A. catenella* reflects more appropriately variations in shellfish toxicities but the three variables show similar tendencies, particularly during periods of increase. Toxicity peaks are preceded or coincident with relative abundance peaks and in some occasions with density peaks (Figs.2-4). The region of Aysén and the Magellan region are the geographic areas with greater persistence and spatial coverage of blooms of *A. catenella* and PSP outbreaks, the first presents on average highest toxicities, but the highest records have occurred in the Magellan region (Figs. 2-3). This region has the longest historical record, with toxicities as high as 52.920 µg at the Magellan Strait (Uribe et al. 1995).

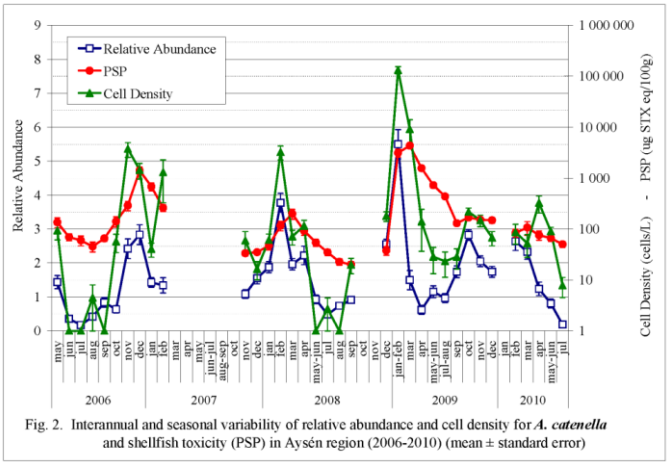

Fig. 2. Interannual and seasonal variability of relative abundance and cell density for ***A. catenella*** and shellfish toxicity (PSP) in Aysén region (2006-2010) (mean ± standard error)

In the region of Los Lagos, blooms and outbreaks are sporadic, and preferably cover the far southern and eastern coast of Chiloé Island, not reaching densities, relative abundances and toxicities as high as in the other regions (Fig. 4). Also an increase with latitude of toxicity highest records along the study area exists. Previous to 2009, *A. catenella* blooms and PSP outbreaks were characterized by its initiation during spring in the Magellan region, and one or two months later the bloom and PSP outbreak appeared in the region of Aysén, and in some years reached the southern tip of Chiloé island, 2002, 2006, 2009. However, during the summer of 2009 (March) occurred a very intense bloom of *A. catenella* and PSP toxic outbreak covering almost all the Aysén region, not affecting the Magellan region, which subsequently, during spring presented a toxic bloom reaching the highest toxicities of that period and independently on what was happening in the region of Aysén, excluding its southernmost area. During 2010 the conditions in microalga abundance and toxicity in bivalve

molluscs have been milder (Figs. 2-4). There are interannual differences, with periods of toxicity levels and *A. catenella* abundance relatively low (e.g. 2007), as well as periods of intense blooms and PSP outbreaks with very high toxicity and abundances of the microalga (e.g. 2009) (Figs. 2-4).

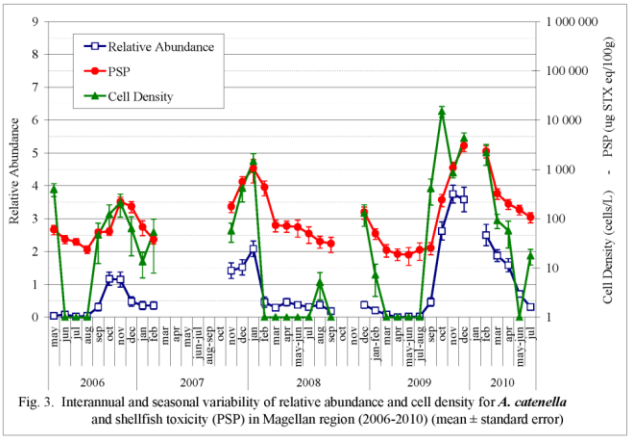

Fig. 3. Interannual and seasonal variability of relative abundance and cell density for ***A. catenella*** and shellfish toxicity (PSP) in Magellan region (2006-2010) (mean ± standard error)

In the Aysén region *A. catenella* blooms usually occur during summer (January to March) but can occur also in Spring (October and December), whereas in the Magellan region, may occur in Spring, Summer and Fall, depending on the sector in this region. In the region of Los Lagos blooms have occurred only in late Spring (December) and Summer (January to March) (Figs. 2-4). The microalga is not present or appears at very low concentrations during the winter months (July-August), but toxic shellfish can be detected during this period as a result of previous blooms and toxin levels reached in previous months (Fig. 2-4).

Conclusions

A. catenella blooms and toxicity outbreaks not always coupled between regions and may even show differences within each region in the distribution and abundance of *A. catenella* and consequently in PSP distribution. The blooms of the microalga and the PSP outbreaks show interannual differences, characterized by mild and intense periods. Within the Chilean fjords, the extensive geographic coverage of *A. catenella* blooms associated to PSP outbreaks suggests that climatic oceanographic factors are responsible for the initiation of these phenomena.

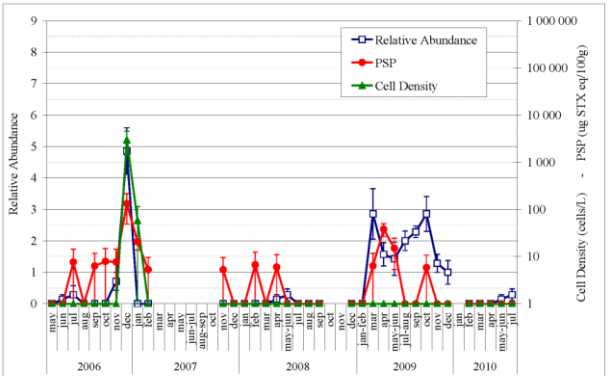

Fig. 4. Interannual and seasonal variability of relative abundance and cell density for ***A. catenella*** and shellfish toxicity (PSP) in Chiloé Island Southern area (2006-2010) (mean ± standard error)

Acknowledgements

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The effect of Light on Growth Rate and Primary Productivity in *Pseudo-nitzschia australis* and *Pseudo-nitzschia turgidula*

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Abstract

A neritic isolate of *Pseudo-nitzschia australis* and an oceanic isolate of *P. turgidula* were assessed for rates of growth and primary production as a function of photosynthetic photon flux density (PPFD). Maximal exponential growth rates were 1.44 d^{-1} for *P. australis* and 1.00 d^{-1} for *P. turgidula*, and were attained at the highest PPFD examined (334 µmol quanta m^{-2} s^{-1}). Maximum carbon (^{14}C) uptake rates (P^B_m), determined via photosynthesis versus irradiance (PE) curves, were 0.707 µg C (µg Chl *a*)$^{-1}$ h^{-1} for *P. australis* and 0.563 µg C (µg Chl *a*)$^{-1}$ h^{-1} for *P. turgidula*. Reduced carbon uptake rates for the oceanic *P. turgidula* isolate were not unexpected, as previous work has demonstrated a reduced photosynthetic efficiency for this oceanic species. The higher growth rates for the neritic species *P. australis* may demonstrate an ability to adapt to rapidly changing coastal environments, in contrast to the more invariant oceanic environment.

Introduction

Access to light is an absolute requirement for phytoplankton metabolism and the synthesis of organic molecules needed for their growth and reproduction. Photosynthesis, as the essential process needed for primary metabolism, requires visible light (photosynthetically active radiation [PAR]; 400-700 nm wavelengths), which in the natural environment is highly variable on both temporal and spatial scales. Here, we investigate the effects of light on the exponential growth rate and primary productivity in two species of the toxigenic diatom genus *Pseudo-nitzschia* – a neritic isolate of *P. australis* and an oceanic isolate of *P. turgidula*. Each species' photosynthetic response is quantified using exponential growth rate (µ) and short-term ^{14}C uptake measurements (photosynthesis versus irradiance [PE] curves) to derive parameters which characterize the light- and dark-dependent reactions of photosynthesis, thereby gaining an assessment of these species' response to photosynthetic photon flux densities (PPFDs). The present study provides a comparative analysis of the effects of light on two *Pseudo-nitzschia* species isolated from widely contrasting environmental habitats.

Materials and Methods

P. turgidula (unialgal strain NWFSC 254) was collected at Ocean Station PAPA (OSP: 50°N, 145°W) in the subarctic, northeast Pacific Ocean in June 2006. *P. australis* (unialgal strain 0771B) was collected at Point Reyes, California in March 2007. Maintenance of stock cultures, growth medium, sample fixation and enumeration, chlorophyll *a* and *in vivo* fluorescence determination are described in detail by Bill (2011). Briefly, all experiments were conducted at 13°C (± 0.2°C), illuminated on a 12:12 hr, light:dark cycle and cell growth was monitored twice daily for cell abundance and *in vivo* fluorescence. Specific growth rates for the eight PPFDs tested were determined for the exponential portion of the growth curves using least-squares linear regression of the natural logarithm of *in vivo* fluorescence as a function of time. Prior to the PE experiments, cultures of each species were subdivided and grown for a minimum of two weeks under sub-saturating (50 µmol quanta $m^{-2}s^{-1}$) and saturating (225 µmol quanta m^{-2} s^{-1}) PPFDs. PE curves were obtained using short-term (30 min) incubations of $NaH^{14}CO_3$ using a temperature-controlled photosynthetron equipped with white (halogen) light at 16 PPFDs between 0-800 µmol quanta m^{-2} s^{-1}. The carbon uptake rates obtained

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from liquid scintillation counting of incorporated ^{14}C were normalized to Chl *a* concentration, and fitted to the three-parameter PE model of Platt and Gallegos (1980) using a non-linear, least-squares regression technique to obtain maximum carbon fixation (P^B_m) and initial slope (α).

Results

The maximum exponential growth rates (μ_{max}) obtained for *P. australis* (1.44 d^{-1}) and *P. turgidula* (1.00 d^{-1}) were observed at the highest PPFD examined (334 µmol quanta m^{-2} s^{-1}; Fig. 1). Both cultures exhibited a linear, positive relationship between growth rate and PPFD at the lower PPFDs - *P. australis* from 21 to 71 µmol quanta m^{-2} s^{-1} and *P. turgidula* from 21 to 52 µmol quanta m^{-2} s^{-1}. At PPFDs above these ranges, the relationship between growth rate and PPFD gradually plateaued, approaching their respective saturation values. PPFD saturation for *P. australis* was approximately 200 µmol quanta m^{-2} s^{-1} while *P. turgidula* PPFD saturation was approximately 100 µmol quanta m^{-2} s^{-1}, indicating that PPFDs at or above these irradiances should be used to achieve maximal growth rates in culture.

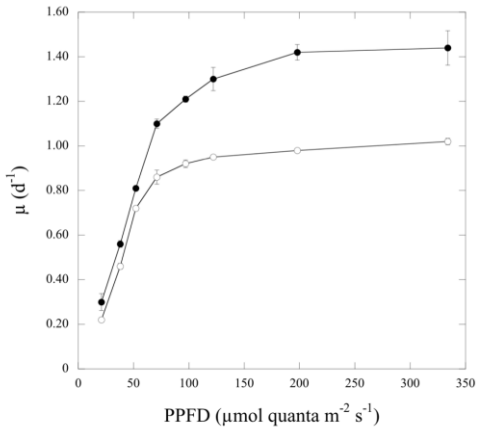

Fig. 1. The mean exponential specific growth rate (µ: d^{-1}) of replicate cultures (n=2) plotted as a function of PPFD for *P. australis* (●) and *P. turgidula* (○). Error bars are the range of replicates.

The PE curves of *P. australis* and *P. turgidula* cultures grown under saturating (225 µmol quanta m^{-2} s^{-1}) and sub-saturating (50 µmol quanta m^{-2} s^{-1}) PPFD are shown in Figure 2. Cultures of *P. australis* previously acclimated to saturating PPFD reached a maximum carbon (^{14}C) uptake rate (P^B_m) more than double than that achieved for *P. turgidula* (0.707 and 0.316µg C (µg Chl *a*)$^{-1}$ h^{-1}, respectively). Sub-saturating PPFD-acclimated cultures of *P. australis* attained a lower P^B_m of 0.572 µg C (µg Chl *a*)$^{-1}$ h^{-1} compared to the saturating PPFD-acclimated culture while the reverse was observed for *P. turgidula* where the sub-saturating PPFD-acclimated culture attained a higher P^B_m of 0.563 µg C (µg Chl *a*)$^{-1}$ h^{-1} than the saturating PPFD-acclimated culture.

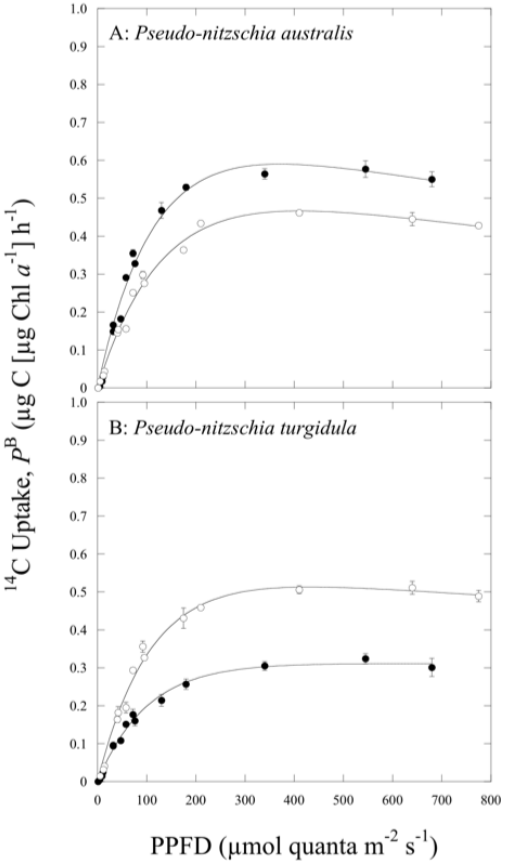

Fig. 2. Photosynthesis versus irradiance (PE) curves: mean carbon (^{14}C) uptake rate normalized to Chl *a* as a function of PPFD for replicate (n=2) cultures of A: *P. australis* and B: *P. turgidula* acclimated to saturating PPFD (●) and sub-saturating PPFD (○). Error bars are the range of replicates.

Discussion

Maximum growth rates (μ_{max}) attained at saturating PPFD ranged from 1.29 - 1.44 d^{-1} for *P. australis* and from 0.92 - 1.00 d^{-1} for *P. turgidula*, and are similar to those reported previously for strains of these two species

(Lundholm *et al*. 2004; Marchetti *et al*. 2006; Howard *et al.* 2007). Maximum growth rates for *P. australis* were consistently greater (*ca.* 35 - 45%) than those achieved for *P. turgidula* at the same saturating PPFD employed. The difference in the *in situ* light environment typically experienced by these two species may explain the consistently higher growth rates achieved by *P. australis* over *P. turgidula* at saturating PPFDs. *P. australis* is considered primarily a neritic species and the isolate used here was collected from a coastal California upwelling system. Phytoplankton in such systems typically experience larger and more frequent fluctuations in environmental parameters such as light and nutrient availability than oceanic areas (Kudela *et al.* 2010). In addition, physical parameters such as vertical and horizontal water movement, via fast moving currents, can have large impacts on the availability of PPFD to free-floating organisms such as phytoplankton. *P. australis* may have adapted to these abrupt and inconsistent changes in its light environment by developing the capacity for higher growth rates over a wider range of PPFDs.In contrast to the coastal environment described above, the oceanic waters of OSP where *P. turgidula* was isolated, is situated in the high-nutrient, low-chlorophyll (HNLC) region of the subarctic northeast Pacific Ocean. The surface waters of this region are characterized by less intense variability in water movement, PPFD, and nutrient form and concentration, and likely provide this isolate of *P. turgidula* with a relatively stable growth environment. The PE curves generated here were obtained from both a sub-saturating PPFD-acclimated (low light-adapted) culture and a saturating PPFD-acclimated (high light-adapted) culture. The uptake curves generated for *P. australis* are typical of those generally observed when comparing low and high light-adapted cells; high light-adapted cultures yield greater values for the initial slope (α) of the PE curve, and higher maximum C uptake rates (P^B_m) than low light-adapted cultures. However, the opposite was observed for *P. turgidula*. Here the low light-adapted culture yielded greater values of both α and P^B_m than the high light-adapted culture. This may be a result of the overall reduced photosynthetic efficiency observed for the oceanic species, *P.* cf. *turgidula* (Marchetti *et al.* 2006). The results of the present laboratory study indicate that maximum growth rates for *P. turgidula* do not increase substantially above 100 µmol quanta m^{-2} s^{-1}, suggesting that maintenance of these cells for multiple generations near 225 µmol quanta m^{-2} s^{-1} (an *in situ* PPFD not normally expected at OSP) may have compromised their photosynthetic capabilities, resulting in depressed photosynthetic rates upon exposure to elevated rates of PPFD in culture. This study provides evidence for differential maximum growth and carbon uptake rates for two species of *Pseudo-nitzschia* from contrasting environments. These differences are likely attributed to the variable oceanographic abiotic parameters experienced by these isolates and highlights the need for comparative studies of genera with cosmopolitan distributions.

Acknowledgements

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Annual cycle of *Pseudo-nitzschia* species in Outer Oslofjorden, Norway

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Abstract

The annual cycle of the diatom genus *Pseudo-nitzschia* was examined in Outer Oslofjorden, Norway. Vertical net tows (2 5- 0 m depth) and seawater samples (1m depth) were collected monthly for one year (June 2009 -June 2010). The diversity of *Pseudo-nitzschia* species were recorded from live and preserved material with light microscopy and from acid cleaned samples viewed in scanning (SEM) and transmission electron microscopy (TEM). *Pseudo-nitzschia* species were present in every sample collected during the year. Nine species of the genus were identified, of which eight are potentially toxic: *Pseudo-nitzschia delicatissima, P. pseudodelicatissima, P. calliantha, P.* cf. *cuspidata, P. pungens, P. multiseries, P. frau-dulenta* and *P. seriata.* Two species, *Pseudo-nitzschia calliantha* and *P. delicatissima,* were the most frequently observed and present in 9 out of 11 samples. The highest concentration of *Pseudo-nitzschia* spp. was recorded in January 2010 (1, 61 x 10^6 cells L^{-1}).

Introduction

Species of the genus *Pseudo-nitzschia* H. Peragallo (H. & M. Peragallo, 1900) are widely distributed and present in all biogeographic zones (Hasle 2002; Casteleyn *et al.* 2008). Earlier investigations from Norwegian waters have shown a considerable variation in the species composition of the genus *Pseudo-nitzschia,* both geographically and seasonally (Hasle *et al.* 1996). The genus contains more than 30 species and several of them are found in Norwegian waters (e.g. *P. delicatissima, P. fraudulenta, P. granii, P. heimii, P. pungens, P. mul-tiseries, P. calliantha, P. seriata, P. obtusa, P. americana).* Twelve species of *Pseudo-nitzschia* have been documented to produce domoic acid (DA) a neurotoxin that causes amnesic shellfish poisoning (Moestrup 2005; Moschandreou *et al* 2010). The ability to produce DA varies among species, thus an exact identification and knowledge about their geographical and seasonal occurrence at the species level is important. Monitoring of microalgae (including toxic species like *Pseudo-nitzschia*) is based on light microscopy; nevertheless a precise identification of *Pseudo-nitzschia* at the species level requires verification by electron microscopy and/or molecular biological tools. The genus *Pseudo-nitzschia* is a common component of the phytoplankton in Norwegian waters. The aim of the present study is to examine the composition and abundance through the annual cycle of *Pseudo-nitzschia* in Outer Oslofjorden in the Northern Skagerrak.

Materials and methods

Samples were collected monthly during one year period from June 2009 to June 2010, at station OF2 (59.186668N, 10.691667E) in Northern Skagerrak, Norway. Seawater samples collected at 1 m depth were preserved in Lugol's solution (1% of final concentration) and cell counts were made according to the protocol of Uthermöhl (1958). Vertical net-tows (20 µm mesh size) from 25 - 0 m depth were preserved with formaldehyde (2% final concentration) and with Lugol's solution (1% final concentration). In order to remove organic material the formaldehyde preserved net samples were acid cleaned (Throndsen *et al.* 2007). The cleaned frustules were mounted on stubs and grids and viewed in a Hitachi FEG S-4800 SEM and Philips CM-1000 TEM.

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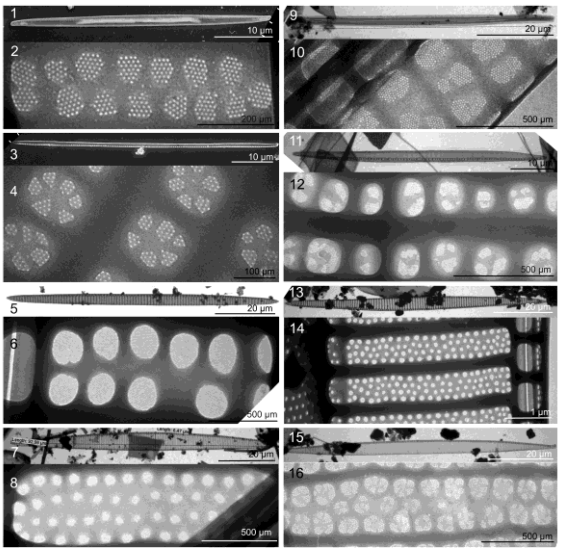

Fig. 1. SEM and TEM micrographs of eight potentially toxic *Pseudo-nitzschia* species; *delicatissima* – group: (1, 2) *P. delicatissima*; (3, 4) *P. calliantha*; (9, 10) *P. pseudodelicatissima*; (11, 12) *P.* cf. *cuspidata.; seriata* – group: (5, 6) *P. pungens*; (7, 8) *P. seriata;* (13, 14) *P. multiseries*; (15, 16) *P. fraudulenta.*

Results

Occurrence and cell counts

Pseudo-nitzschia species were present in all samples with densities ranging from 500 cells L^{-1} in June 2010 to 1.61 million cells L^{-1} in January 2010 (Table 1). In January 2010, the high cell density was mainly related to a bloom of *P. delicatissima* and *P. calliantha*. The *delicatissima*-group (species with valve width less than ca. 3 µm) dominated all samples except the April and May samples of 2010.

Species diversity and identification

Pseudo-nitzscia species identification was based on morphometric characteristics (Hasle *et al.*1996, Lundholm *et al.* 2003).

A total of ten species of the genus *Pseudo-nitzschia* were found, nine of them were identified: *P. calliantha, P. delicatissima, P. pseudodelicatissima, P.* cf. *cuspidata*, *P. fradulenta*, *P. seriata*, *P. pungens*, *P. multiseries* and *P. americana*. An unidentified *Pseudo-nitzschia* species resembles *P. pseudodelicatissima* in all morphometric characteristics but differs in poroid structure. *Pseudo-nitzschia calliantha* and *P. delicatissima* were the most frequently observed and present in 9 out of the 11 examined samples. The species composition changed through the year and the number of species present in one sample varied from one in June 2010 to seven in June and October 2009 (Table 1).

Table 1. Abundance and monthly occurrence of *Pseudo-nitzschia* spp. during June 2009- June 2010 in Outer Oslofjord, Norway.

***Pseudo-nitzschia* species**	**22.06 2009**	**05.08 2009**	**22.09 2009**	**20.10 2009**	**17.11 2009**	**09.12 2009**	**21.01 2010**	**11.03 2010**	**13.04 2010**	**11.05 2010**	**22.06 2010**
***Pseudo-nitzschia* spp. cells L^{-1}**	**99100**	**15000**	**217500**	**91500**	**150100**	**11400**	**1615900**	**141200**	**1400**	**800**	**500**
***delicatissima*- group**											
P. calliantha	x	x	x	x	x	x	x	x		x	
P. delicatissima	x			x	x	x	x	x	x	x	x
P. pseudodelicatissima	x			x							
*P.*cf. *cuspidata*					x	x					
Pseudo-nitzschia sp.	x			x							
***seriata*- group**											
P. fraudulenta	x	x		x						x	
P. seriata	x	x						x	x	x	
P. pungens	x		x	x	x	x				x	
P. multiseries				x	x						
P. americana						x	x				

Discussion

Occurrence and cell density of *Pseudo-nitzschia* spp. varied through the seasons, with a more or less gradual increase from June 2009 until January 2010 (bloom) followed by a subsequent decrease. The *delicatissima*- group dominated the samples and the most common *Pseudo-nitzschia* species were *P. calliantha* and *P. delicatissima* (the only representative of the genus in June 2010). Eight potentially toxic *Pseudo-nitzschia* species were detected in the present study: *Pseudo-nitzschia pungens, P. multiseries, P. frau-dulenta, P. seriata, P. delicatissima, P. pseudodelicatissima, P. calliantha* and *P.* cf. *cuspidata* (Fig. 1). Seven of the nine identified *Pseudo-nitzschia* species have earlier been encountered regularly in Norway; the exceptions are P. *cuspidata* and *P. pseudodelicatissima* that are recent additions to the Skagerrak phytoplankton flora.

Acknowledgments

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An integrated approach for the assessment of HAB dynamics in two NW Mediterranean bays from a GEOHAB perspective

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Abstract

Alfacs and Fangar Bay in the Ebro Delta, NW Mediterranean are the major sites in Catalonia for shellfish cultivation. These bays are subject to occasional closures in shellfish harvesting due to the presence of phycotoxins. Fish kills have also been associated with harmful algal blooms. The comparison of phytoplankton dynamics in both bays offers the opportunity to reveal differences in bloom patterns of species known to be harmful for the ecosystem and aquaculture activities. Field research is underway under the GEOHAB framework within the Core Research Project on HABs in Fjords and Coastal Embayments. The overall objective of this study is to improve our understanding of HAB biogeographical patterns, and key elements driving bloom dynamics in time and space within these semi-constrained embayments. Via the comparative approach we aim to improve the prediction for monitoring purposes, with a focus on *Karlodinium* spp. associated with massive kills of aquaculture species. This objective is addressed by incorporating long-term time series of phytoplankton identification and enumeration with the first results of recent field work in both bays. The latter includes the application of optical sensors, to yield a complementary view with enhanced spatial and temporal resolution of bloom phenomena.

Introduction

The two semi-enclosed embayments Alfacs and Fangar Bay in the Ebro Delta system, NW Mediterranean, are the major aquaculture sites in Catalonia. Due to the presence of phycotoxins, both bays are subject to occasional harvesting closures. The ichthyotoxic species *Karlodinium veneficum* and *K. armiger* (in this area previously referred to as *Gyrodinium corsicum* (Garcés *et al*. 2006)) have been found in Alfacs Bay since 1994. In 2010 the species were also detected in Fangar Bay. In spite of their proximity and similar climatic conditions, Alfacs and Fangar Bay profoundly differ in HAB dynamics. Circulation patterns and retention time of water in both bays are differently affected by winds, coastal currents, and freshwater inflow from agriculture. The comparison of environmental forcing functions and bloom characteristics in both bays therefore provides the opportunity to improve our understanding of the key element

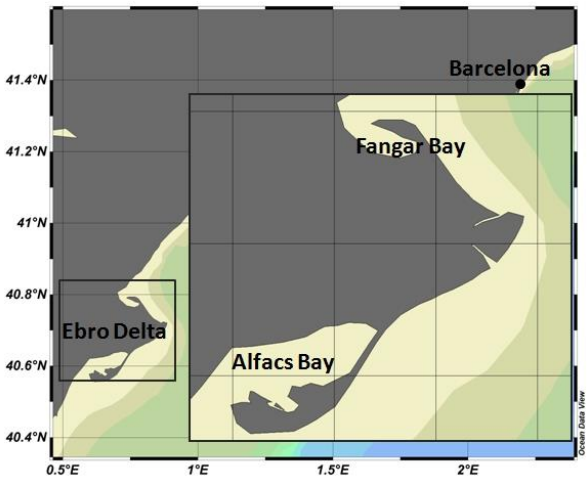

Fig.1 Study area in the Ebro Delta, Spain, NW Mediterranean.

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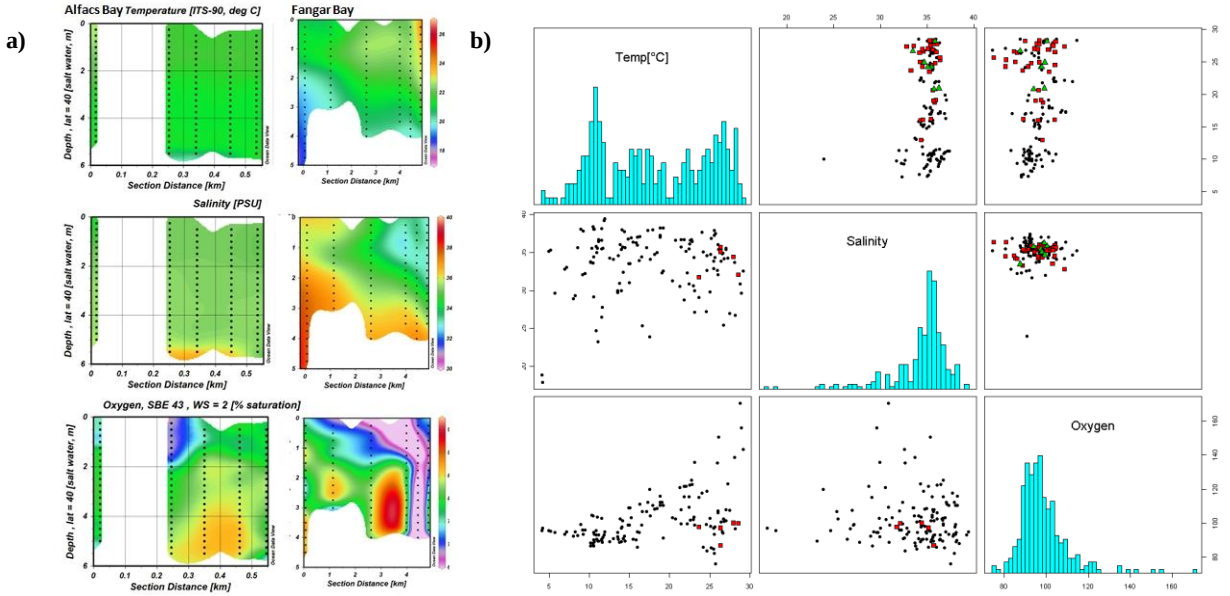

Fig.2 a) CTD casts on a transect of sampling stations (vertical dotted lines) in Alfacs (left) and Fangar (right) Bay. The first station is at section distance 0km). b) Pair plot of *Karlodinium* spp. detection in three abundance classes (<1000cells L-1=black dots; <10,000 cells L-1 =red squares; >10,000 cells L-1=green triangles) from 4 Jan – 30 Aug 2010 in Alfacs (upper three scatter plots) and Fangar (lower three scatter plots) with respect to temperature, salinity and oxygen saturation. The distribution of total *Karlodinium* spp. abundances in environmental ranges of both bays is given in the bar charts.

that drive bloom dynamics in time and space.Via the comparative approach we aim to improve the prediction for monitoring purposes, with a focus on *Karlodinium* spp. associated with massive kills of aquaculture species (Delgado *et al.* 1995). This objective is addressed by incorporating long-term time series of phytoplankton identification and enumeration with the first results of recent field work in both bays. The latter includes the application of oceanographic and optical sensors, to yield a complementary view with enhanced spatial and temporal resolution of bloom phenomena. The objective of the presented work is the comparison of high *Karlodinium* spp. abundances in both bays.

Material and Methods

Cell numbers for abundance class generation of *Karlodinium* spp., temperature, salinity, and oxygen values were taken within the regular monitoring project in five stations in each of both bays for the time between January and August 2010. Complementary, an intensive depth resolved comparative field study was conducted between May and July, here only CTD casts along a transect of regular monitoring stations in both bays are taken into account.

Results and Discussion

Variation in time and space of the environmental conditions in both bays (temperature, salinity, and oxygen saturation) as well as maximum abundances of *Karlodinium* spp. are reported. The highest abundances and maximum cell concentrations were reached in Alfacs Bay. At the time of maximum abundance of *Karlodinium* spp. in Alfacs Bay and first detection in Fangar Bay, we identify differences in environmental characteristics between bays (Fig. 2a). At the stations of high algal abundance in each bay (first station in the transects, at 0km section distance), we report a difference of >1 °C in temperature and >1 in salinity from surface to bottom, indicating a certain degree of stratification (Fig. 2a). A trend of *Karlodinium* spp. blooms in stratified waters was recognized in a 20 years´ time series of monitoring in the Ebro Delta Bays

(Fernandéz-Tejedor 2010). During the summer months, dams are open for rice field irrigation in the Delta area. Consequently flow of freshwater increases from the rice fields to the bays and stratification predominates in both bays. This is due to a lateral fresh water inflow through a series of channels of the main land, and sea water inflow from the Mediterranean as a salt wedge along the bottom (Camp and Delgado 1987).

As Fangar Bay is smaller than Alfacs Bay, the influences of freshwater inflow, as well as from the southwesterly coastal currents, have a stronger effect on this bay. In 2010, *Karlodinium* spp. have been detected throughout a wide range of environmental conditions (Fig. 2b). Higher abundances, however, occur in the range of salinity of 32-35, and temperature of 20-27°C in both bays. The combination of these small ranges in salinity and temperature may not be the key elements that trigger high *Karlodinium* spp. abundances, but provide an environmental setting of this year´s bloom patterns. This can be an indication of the presence of proxies for algal proliferations that can be used for the early detection of blooms, e.g. by means of optical sensors.

Outlook on coupling of environmental- and HAB patterns:

- Inclusion of additional environmental parameters such as nutrients and turbidity
- Analysis of high depth resolution of biological and physical parameters from May to July
- Application of an optical sensor system and derivation of physical-optical bloom proxies
- Incorporation of bio-optical datasets into oceanographic models
- Setup of a long-term environmental observatory

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Role of cyst germination in the bloom initiation of harmful algal species in Korean waters

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Abstract

To clarify the role of cyst germination in the bloom initiation of harmful algal species, isolation and germination experiments on single cysts were carried out in four harmful algal bloom (HAB) species biweekly or monthly. Germination maxima of *Alexandrium tamarense* were observed in winter, but little or no germination occurred in summer. In the case of *Scrippsiella trochoidea*, mass encystments were detected in the water column in July and August when the vegetative population flourished. The vegetative population of Nostocales flourished from August to November; however, the most active akinete germination occurred from the end of March to early April. In *Peridinium bipes*, the cysts collected at higher temperatures germinated more quickly than those seeded at lower temperatures, while cysts collected in the fall and early winter had a higher cumulative excystment rate than those from the spring or summer. From these results, the types of germination patterns showed that there is a temporal discrepancy between the peak of germination success and the bloom of the vegetative population. These results indicate that active germination is not likely to be a direct trigger that forms the blooms of HABs.

Introduction

A number of phytoplankton species present a dormant resting stage that allows survival under adverse environmental conditions in their life histories. Dinoflagellates resting cysts were found to sink to the bottom of sediments or the near-bottom nepheloid layers, when environmental conditions are unsuitable for growth (Prakash 1967; Wall 1971). The cyst population can provide an inoculum for a new bloom after a specific dormancy period (Kremp & Heiskanen 1999). Certain cyanobacteria of Nostocales, including the noxious bloom-forming species *Anabaena* and *Aphanizomenon*, form a thick-walled reproductive structure termed akinete which may serve as a dormant cell under adverse conditions (Baker & Bellifemine 2000). When favorable conditions return, akinetes germinate to produce trichomes (Hori et al. 2002; van Dok & Hart 1996), which act as inocula for the new blooming (Hori et al. 2002; Kravchuk et al. 2002; van Dok & Hart 1996). There are no doubts that cysts including akinetes play an important role as the seed of the vegetative population of phytoplankton (Anderson & Wall 1978; Hansson 1996). Sometimes, however, bloom initiation does not correspond to a direct consequence of *in situ* germination patterns of cysts. (Ishkawa & Taniguchi 1996, 1997). Therefore, the relationship between algal bloom formation and cyst germination rates remains unclear.

However, it is important to identify the effect of encystment and excystment on algal blooming initiations. In this study, I focused on the seasonality relationship between the development of vegetative populations and the cyst germination modes.

Material and Methods

Sampling sites were located in a coastal area (Masan and Youngil Bay) and an inland lake (Juam Reservoir and Seokchon Pond) in South Korea. Vegetative cell and cyst abundance were analyzed by using an optical microscope (Axioplan, Zeiss, Germany). The excystment rates of *Alexandrium*, *Scrippsiella*, *Peridinium* and Nostocales such as *Anabaena* and *Aphanizomenon* were measured by a cyst germination experiment. The isolated cysts were inoculated one by one (using a microcapillary) into individual wells of a 96-well tissue culture plate (Falcon, USA) containing filtered water. The inoculated cysts were incubated at water temperatures similar to those of the sampling station bottom layer, under 12–30 μmol photons m^{-2} s^{-1} with a 12 h light: 12 h dark cycle.

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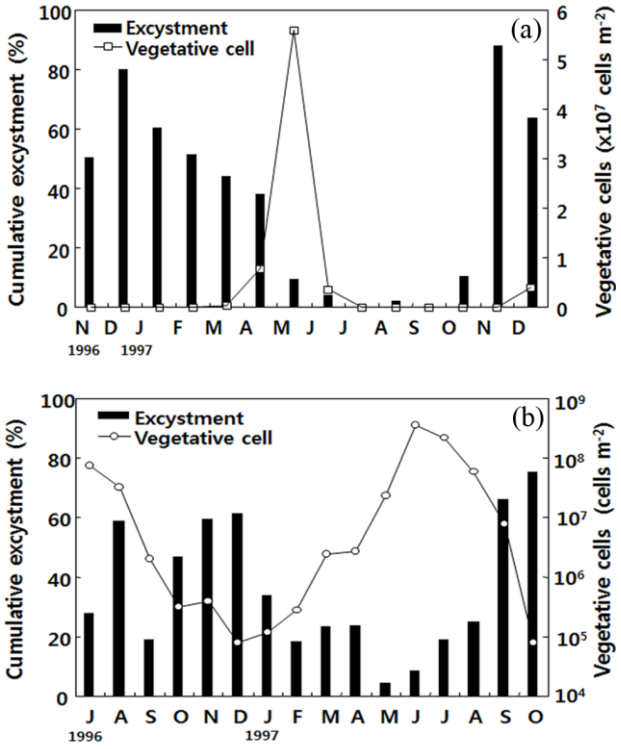

Fig. 1. Seasonal changes of excystment (%) and vegetative cell numbers of HAB species (a) *Alexandrium tamarense* (Kim *et al*. 2002), (b) *Scrippsiella trochoidea* (Kim & Han 2000)

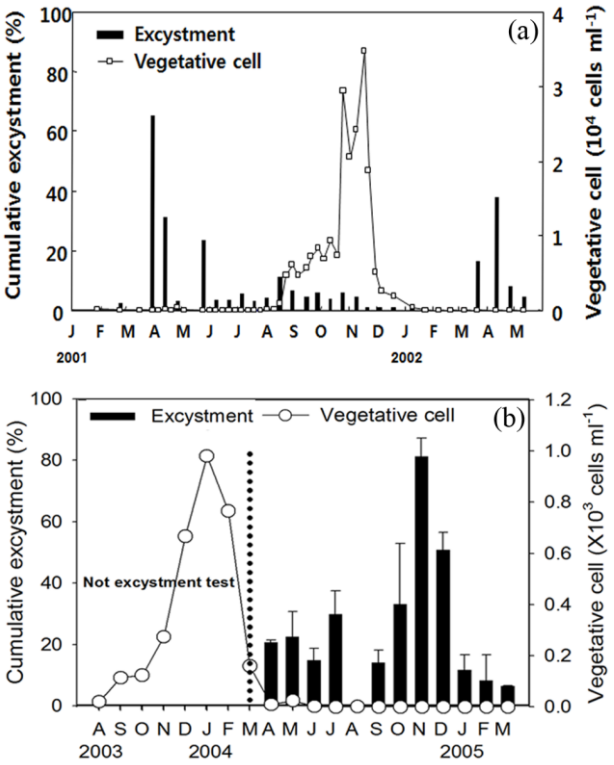

Fig. 2. Seasonal changes of excystment (%) and vegetative cell numbers of HAB species (a) species of Nostocales (Kim *et al*. 2005), (b) *Peridinium bipes* (Kim *et al*. 2007)

Results and Discussion

The vegetative population of *Alexandrium tamarense* peaked in spring, but germination success was not higher. Germination maxima (80–90%) were observed during the winter season when the *A. tamarense* vegetative cells were almost not detectable in the water column (Fig. 1a). Similar germination results for *A. tamarense* were observed in Hiroshima Bay (Itakura & Yamaguchi 2001).

In *Scrippsiella trochoidea*, the vegetative population peaked in summer; however, the germination ratio was much lower in the bloom season. Active germination was observed in autumn after 3–4 months of blooming and the vegetative population decreased dramatically (Fig. 1b).

The highest vegetative population of Nostocales was observed in November after 7 months of active germination. Of this group, the most active germination was observed from the end of March to early April; however, the vegetative cells were extremely difficult to detect in the water column (Fig. 2a). In the excystment test of *Peridinium bipes*, cysts obtained from samples collected at higher temperatures (over 15 °C) germinated more quickly than those seeded at lower temperatures, while cysts collected in the fall and early winter had a higher cumulative excystment rate than those collected in the spring and summer, suggesting that cysts deposited at higher temperatures may act as a seed population for the winter blooms (Fig. 2b).

In Korean waters, some HAB species have shown a temporal discrepancy between the peak of the germination success and the bloom of the vegetative population, meaning germination success occurs very early and not immediately prior to the bloom of the vegetative population. These results suggest that active germination does not trigger directly an increase to the vegetative population of HABs. In conclusion, cyst-forming phytoplankton species possess their own seasonality in diverse germination modes as a survival strategy.

Acknowledgement

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Growth and toxin production of *Azadinium spinosum* in batch and continuous culture

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Abstract

Azaspiracids are lipophilic marine biotoxins causing gastrointestinal symptoms similar to DSP toxins. Since 1995, azaspiracids have been encountered in Europe, Africa and more recently in North and South America and Japan. The biological primary producer remained undiscovered during many years and has now been identified as *Azadinium spinosum*. The organism was grown using K modified medium, at 18°C with a PFD of 200 µmol.m^{-2}.s^{-1} and a photoperiod of 16L/8D. Batch cultures were carried out using 75mL and 10L flasks, while continuous cultures were produced in 100L chemostats. Cells were recovered using centrifugation or filtration. Different extraction solvents and procedures as well as evaporation modes were evaluated for yield. Quantitation was carried out using LC-MS-MS. *A. spinosum* had a maximum growth rate of 0.6 d^{-1} with K modified medium, and reached maximum cell concentration of 300000 cells.mL^{-1}. Toxins were mostly intracellular, with 5 to 10% toxin in the culture medium. Analogues detected included AZA1, -2 and the methyl esters of AZA1 and -2, AZA1 being the predominant toxin.

Introduction

Harmful algal blooms might cause severe human illness due to the consumption of bivalves, as microalgae are the principle food for bivalve mollusks. In 1995, contaminated mussels (*Mytilus edulis*) from Killary harbor (Ireland) were consumed in the Netherlands (McMahon and Silke, 1996). The toxic agent caused diarrhea, nausea, vomiting and stomach cramps in consumers, symptoms typical for diarrheic shellfish poisoning (DSP). A new toxin was discovered and was named azaspiracid after structural identification by (Satake *et al.*, 1998). Since then azaspiracids have been encountered in Europe, Africa and more lately in South and North America and Japan (Twiner *et al*, 2010). Recently, the biological source of azaspiracids (AZAs), *Azadinium spinosum* (strain 3D9) a small dinoflagellate, was discovered. This organism produces Azaspiracid-1 and 2 (Krock *et al.*, 2009; Tillmann *et al.*, 2009).

Growth and toxicity of dinoflagellates are dependent of various environmental and nutritional factors. Among various environmental factors, salinity and aeration might affect dinoflagellate growth and toxicity.

This early work on *A. spinosum* aims to assess the effect of salinity and aeration on growth and toxicity; to evaluate the cell growth and toxin production in pilot scale chemostats and to assess analytical scale extraction procedures for best yield and suppression of AZA1 and AZA2 methyl esters, two possible artefacts of extraction.

Materials and methods

Evaluation of protocols for extraction of azaspiracid from *A. spinosum*

Cells were recovered using centrifugation or filtration, different extraction solvents (methanol, acetone, ethanol, acetonitrile, dichloromethane, H_2O) and mixtures thereof as well as evaporation modes were evaluated for yield. Methyl ester formation on AZA1 and -2 was studied using methanol-d_4 for extraction and or reconstitution after evaporation.

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Culture condition

A. spinosum (3D9) was grown using K modified medium (Keller *et al.*, 1987), without NH_4Cl and with Na_2SeO_3 (1.72 $mg.L^{-1}$), at 18°C with a PFD of 200 $\mu mol.m^{-2}.s^{-1}$ and a photoperiod of 16L/8D, in batch and continuous culture. For experimental purpose, the strain was grown in triplicate at six different salinities (10, 20, 30, 32, 35, 40 psu) adjusted by dilution with Milli-Q® water or evaporation, in sterile 70mL polystyrene flasks. Salinity was checked with a refractometer. The initial culture cell concentration was 5000 $cell.mL^{-1}$ and the culture was grown until stationary phase for each condition.To evaluate the effect of aeration for a future growth in chemostat, *A. spinosum* was cultured in triplicate in 10 L flat bottomed flasks with and without aeration at 35 psu at the same initial cell concentration.After adaptation to aeration and agitation the microalgae were inoculated in a 100 L medium scale chemostat to evaluate cell growth and toxin production. The flow rate was 0.15 d^{-1}. When the first bioreactor was at steady state it was connected to a second bioreactor to increase cell concentration and toxicity.

Cell growth

Every day or two 1 mL sample was collected to asses cell concentration with a particle counter. A Gompertz model was applied to follow growth kinetics in batch culture, to determine the maximum growth rate (μ_{max} in d^{-1}), the maximum cell concentration (A expressed as $\ln(X/X_0)$ with X in $cells.mL^{-1}$) and the latency time (λ in days). The model follows the equation:

$$f(x) = A*\exp(-\exp(\mu_{max}*\exp(1)/A*(\lambda-x)+1))$$

Where x is the time (days).

Extraction procedure used and LC-MS-MS analysis

At the end of the experiment on salinity, every two days for the experiment on aeration and twice a week for the continuous culture, triplicate samples (10 mL) were collected in all flasks and bioreactors for toxin analysis. Samples were centrifuged (2500 g, 20 min, 4°C). The supernatant was discarded and the pellet suspended with 500 µL of acetone 90% and sonicated. After sonication the aliquot were centrifuged (15000 g, 10 min, 4°C). Each supernatant were transferred into a 5 mL glass tube and gently evaporated under nitrogen at 35°C. The pellets were resuspended in 500 µL of acetone 90%, homogenised and centrifuged again. These steps were repeated three times in total. After evaporation of the supernatants, they were reconstituted in 500 µL methanol 90%. Subsequently, the samples were filtered (Whatman nanopore 0.2 µm) at 15000 g, 15 min, 4°C, and transferred into HPLC vials with inserts. The samples were then analysed by LC/MS-MS following method C described in Rehmann *et al.*, (2008).

Results and discussion

Extraction procedure

Less formation of AZA1 and AZA2 methyl esters were measured with centrifugation compared to filtration and with extraction with acetone compared to methanol. Highest azaspiracid yield was obtained with methanol/H_2O and acetone/ H_2O ratio of 100, 90 and 80%, compared to lower ratios and other solvents.

Better recovery was obtained when using 5 mL glass tubes during the evaporation procedure compared to 1.5 mL HPLC vials for the evaporation.AZA1 and 2 methyl esters are believed to be artefacts of extraction due to methanol (figure 1) as they are formed mainly during the extraction and in a lower amount during the reconstitution.

Physiology

Growth of *A. spinosum* was observed between 30 and 40 psu, with a μ_{max} assessed between 0.37 and 0.73 d^{-1}. From 10 to 20 psu all cells died between the beginning and the second day of the experiment. The best cell concentration was obtained at 35 psu and the highest toxicity with 30 and 40 psu (table 1).

Table 1. Summary of results from the experiment on salinities

Salinity (psu)	30	32	35	40
μ_{max} (day^{-1})	0.37	0.52	0.52	0.73
Latency time (days)	1.53	1.65	1.77	4.26
A ($cells.mL^{-1}$)	48 813	55 701	71 379	28 934
AZA1 ($fg.cell^{-1}$)	17.77	16.63	12.47	20.56
AZA2 ($fg.cell^{-1}$)	13.21	12.10	9.22	16.12

A. spinosum had a μ_{max} situated around 0.6 d^{-1} with K modified medium, and reached maximum cell concentration of 200000 to 300000 cells.mL^{-1} with aeration and of 80000±5000 cells.mL^{-1} without aeration, azaspiracid final concentration per cell was also higher with aeration than without aeration, i.e 39±4 and 19±4 fg.$cell^{-1}$, respectively.
The two pilot scale bioreactors (2*100 L, = R1 and R2) were run in series at 0.15 d^{-1} (10 mL.min^{-1}). At steady state, bioreactor 1 had 194 000 ± 6000 cells.mL^{-1} with a toxicity of 62±1 fg.$cell^{-1}$ and bioreactor 2 had 215000±3000 cells.mL^{-1} with a toxicity of 98±5 fg.$cell^{-1}$. Chemostats in series increased algal concentration and toxicity in the second bioreactor compared to the first one and gave a stable concentration of toxins along time at steady state.

Conclusion

Medium scale bioreactors in series allowed for the continuous production of toxic *Azadinium spinosum* in large volumes. It can be used as source of AZA1 and -2 for contamination of bivalves, for toxicological studies, and for purification of azaspiracid-1 and 2.
Extraction procedure of azaspiracid from *A. spinosum* with methanol, the solvent currently used for the extraction of lipophilic toxins from bivalves produced two artefacts of extraction. Although previously reported by Rehmann *et al.* (2008), in mussels, these artefacts were not observed in such high concentrations after extraction of mussels. Thus, with *A. spinosum* acetone must be used in place of methanol.

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Effect of Light Intensity on Five Species of *Gambierdiscus*

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Abstract

The effect of light intensity on growth of 5 species of *Gambierdiscus*, the dinoflagellate genus that causes ciguatera fish poisoning, was tested in a series of laboratory experiments. Growth data showed *G. carolinianus* required the lowest irradiance for positive growth (I_{min} = 5 $\mu E \cdot m^{-2} \cdot s^{-1}$), but grew poorly at irradiances >400 $\mu E \cdot m^{-2} \cdot s^{-1}$. In contrast, *G. ruetzleri* and *G. caribaeus* had maximum growth irradiances (μ_{max}) near 200 $\mu E\ m^{-2} \cdot s^{-1}$ and were able to tolerate light levels >700 $\mu E \cdot m^{-2} \cdot s^{-1}$. Projected growth data indicated all 5 species should be able to maintain growth to depths >100 m in optically clear tropical waters.

Introduction

Light penetration through the water column is a critical regulator of primary production in the ocean and limits the distribution of benthic microalgae, including dinoflagellates. One genus of benthic dinoflagellates, *Gambierdiscus*, is of considerable interest because of its causative association with ciguatera fish poisoning (CFP). CFP causes more human illness than all other seafood consumption maladies combined (Lewis and Holmes 1993). CFP events are spatially and temporally unpredictable, probably due to inherently different toxicities among the *Gambierdiscus* species or strains that dominate blooms (Litaker *et al.* 2010). Species dominance is in turn influenced by environmental factors such as temperature, salinity, irradiance, dissolved nutrients, and substrate availability. The extent to which these environmental factors affect the growth of individual *Gambierdiscus* species is unclear. Previous physiological studies examining the effects of environmental factors (e.g. Bomber *et al.* 1988; Chinain *et al.* 1999) were completed before it was possible to accurately identify *Gambierdiscus* species (Litaker *et al.* 2009; 2010). Additional studies using taxonomically defined species will therefore be required to fully characterize the physiology of *Gambierdiscus* species. This study examines how light availability may define the depth distributions and overall habitat space occupied by five different *Gambierdiscus* species.

Methods

Cultures of Gambierdiscus Ribotype 2 (CCMP 1655) and G. pacificus (CCMP1650) were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP). G. carolinianus, G. caribaeus and G. ruetzleri were established from the Caribbean region or the SE US Atlantic coast (Table 1; Litaker et al. 2009).

Table 1. *Gambierdiscus* species used in this study.

Species	Location
G. carolinianus	Outer shelf, North Carolina, USA
G. caribaeus	Carrie Bow Cay, Belize, Caribbean
G. Ribotype 2	Martinique, Caribbean
G. pacificus	Moorea, Society Islands, Pacific Ocean
G. ruetzleri	Carrie Bow Cay, Belize, Caribbean
G. belizeanus	St. Barthelemy Island, Caribbean
G. carpenteri	Guam, Northern Mariana Islands, Pacific Ocean

Five replicate cultures of each *Gambierdiscus* species were inoculated into flasks containing modified K medium as outlined in Litaker *et al.* (2009). The flasks were incubated at 27°C under full spectrum fluorescent lights at irradiances of 4-664 $\mu E \cdot m^{-2} \cdot s^{-1}$ (12:12 light:dark cycle). At 2-3 day intervals, each replicate flask was shaken and the culture was transferred to a glass screw-capped centrifuge tube. *In vivo* fluorescence was quantified

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immediately using a Turner fluorometer (Turner Designs Inc., Sunnyvale, California, USA) and each culture was poured back into its flask and returned to the incubator. Growth rates, μ (d^{-1}), were determined from the slope of the *ln* fluorescence vs. time curve. Preliminary studies showed that growth rates calculated with fluorescence data are equivalent to those based on cells counts. Growth rate vs. irradiance (*I*) data were fitted to log normal curves using SigmaPlot software (Systat Software Inc., San Jose California, USA). Maximum growth rate (μ_{max}), the corresponding irradiance (I_{max}),

$$z_{max} = \frac{-ln\left(\frac{I_{min}}{I_{surf}}\right)}{K_d} \quad (1)$$

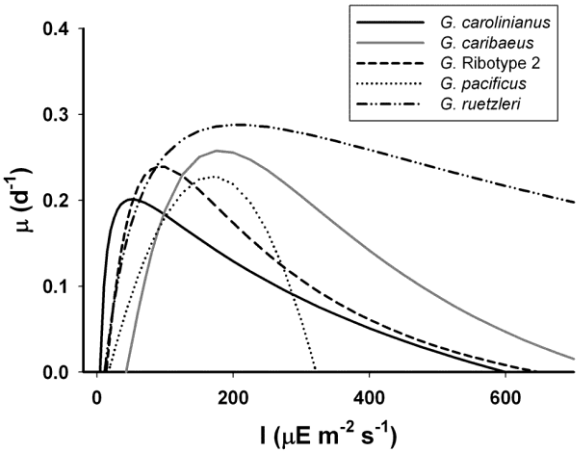

Fig. 1. Growth rates of *Gambierdiscus* species vs irradiance levels

as well as the minimum and maximum irradiances that yielded positive growth (I_{min}, I_{high}), were then determined using the fitted curves. Eq. 1 was used to estimate the maximum projected depth range (z_{max}) for each species after Kemp *et al.* (2004), where K_d (m^{-1}) is the diffuse attenuation coefficient and I_{surf} = irradiance at the ocean surface. K_d values were selected to represent a range of water types from optically clear ocean water (K_d = 0.01 m^{-1}) to highly turbid estuarine water (K_d = 1.5 m^{-1}).

Results

The growth experiments revealed *Gambierdiscus* species exhibited remarkably little variation in μ_{max} (0.2-0.3 d^{-1}, Table 2). The irradiances levels required to achieve μ_{max}, however, varied considerably. For example, under nutrient replete growth conditions, I_{max} ranged from 51 μE·m^{-2}·s^{-1} in *G. carolinianus* to 206 μE·m^{-2}·s^{-1} in *G. ruetzleri*. The I_{min} level was uniformly low for all species tested, varying from 5 μE·m^{-2}·s^{-1} in *G. carolinianus* to 45 μE·m^{-2}·s^{-1} in *G. caribaeus* (Fig. 1, Table 2). These I_{min} values are equivalent to 0.25% and 2.3% of typical irradiance levels at the ocean's surface. *Gambierdiscus* species also exhibited a wide variation in their ability to tolerate high irradiance levels. For example, the species with the lowest tolerance, among those tested was *G. pacificus*. Growth in this species saturated at 170 μE·m^{-2}·s^{-1} and cells began dying when irradiances exceeded 320 μE·m^{-2}·s^{-1} (Fig. 1, Table 2). *G. carolinianus* and *G.* Ribotype 2 achieved maximal growth at lower light levels (<100 μE·m^{-2}·s^{-1}) than *G. pacificus*, but could maintain positive growth to ~600 μE·m^{-2}·s^{-1}, at which point the cells began to die. In contrast, *G. caribaeus* reached maximum growth at ~200 μE m^{-2}·s^{-1} and maintained positive growth at irradiances >700 μE·m^{-2}·s^{-1}. The greatest tolerance to light was shown by *G. ruetzleri*, which reached maximum growth at 206 μE·m^{-2}·s^{-1} and was projected to maintained positive growth at irradiances of >900 μE·m^{-2}·s^{-1} (Fig. 1, Table 2).

Table 2. Maximum growth rates (μ_{max}, d^{-1}), corresponding irradiances (I_{max}), minimum (I_{min}) and maximum (I_{high}) required light levels (μE·m^{-2}·s^{-1}) for *Gambierdiscus* species

Species	μ_{max}	I_{max}	I_{min}	I_{high}
G. carolinianus	0.20	51	5	554
G. caribaeus	0.26	196	45	>700
G. Ribotype *2*	0.24	93	15	589
G. pacificus	0.23	169	21	318
G. ruetzleri	0.29	206	13	>900

When I_{min} was utilized to calculate the maximum projected depth, z_{max} (Eq. 1), the results showed all *Gambierdiscus* species were projected to have a relatively similar depth range. In optically transparent oligotrophic ocean water (K_d<0.05 m^{-1}), all species exhibited a z_{max} >70 m (Fig. 2). Maximum projected depth increased to 90-150 m for water with a K_d of <0.04 m^{-1}. In highly turbid estuarine/coastal water (K_d>1 m^{-1}), z_{max} for each species was <6 m (Fig. 2).

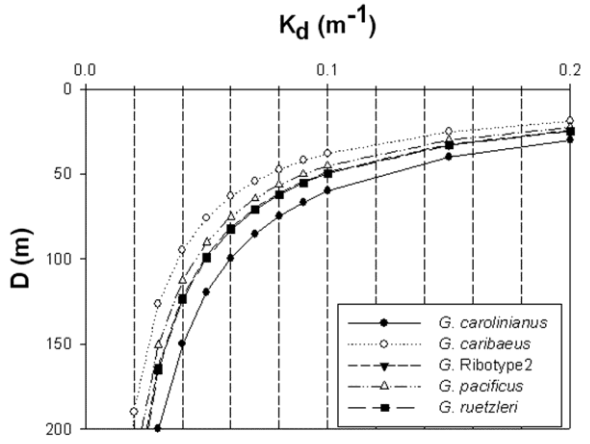

Fig. 2. The diffuse attenuation coefficient K_d (m^{-1}) vs maximum projected depth for each *Gambierdiscus* species

Discussion

The results of the μ vs. *I* experiments indicated *Gambierdiscus* species may be distributed across tropical and subtropical shelf environments to depths of >100 m in oligotrophic waters. There were substantial differences in the tolerance of each species to high irradiances and in their minimum light requirements. With an I_{max} of 51 $\mu E \cdot m^{-2} \cdot s^{-1}$, an I_{min} of only 5 $\mu E \cdot m^{-2} \cdot s^{-1}$ (0.25% surface light) and poor growth above 400 $\mu E \cdot m^{-2} \cdot s^{-1}$, *G. carolinianus* is best adapted to low light/deep environments. In contrast, *G. ruetzleri* was better adapted to high light, but could also grow in very low light (I_{min}=13 $\mu E \cdot m^{-2} \cdot s^{-1}$, I_{max}=206 $\mu E \cdot m^{-2} \cdot s^{-1}$, I_{high} >900 $\mu E \cdot m^{-2} \cdot s^{-1}$). For waters with K_d values ≤0.04 m^{-1}, all *Gambierdiscus* species were projected to maintain positive growth to depths of 90-150 m (Fig. 2). Such low attenuation coefficients are not uncommon in the Caribbean region and have been measured in the Gulf of Mexico, Florida Strait s, Lesser Antilles, and across the Caribbean Sea, with slightly higher attenuation coefficients in near shore waters (0.05-0.07 m^{-1}) (NASA 2010).

If *Gambierdiscus* species do commonly occur at the projected depths (Fig. 2), then prevailing ideas about habitat space should be expanded. The current literature suggests that *Gambierdiscus* tends to be most abundant in shallow habitats (<30 m) (Taylor 1985 and references therein), though there are reports of significant population densities to depths of 45 m (Carlson 1985). We have routinely isolated *G. carolinianus* from macrophytes collected at >40 m on the outer shelf off North Carolina, USA, an area influenced by the Gulf Stream. These latter observations are consistent with the fact that *Gambierdiscus* species are likely distributed to greater depths than previously recognized. A future CFP research challenge will be to quantify *Gambierdiscus* cell densities in deeper water habitats and to determine if those populations are contributing significantly to the overall flux of ciguatoxins into the food chain.

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