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Dynamics of co-occurring *Alexandrium minutum* (Global Clade) and *A. tamarense* (West European) (Dinophyceae) during a summer bloom in Cork Harbour, Ireland (2006)

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ABSTRACT

The dinoflagellate genus *Alexandrium* contains neurotoxin-producing species, which have adversely affected the aquaculture industry and fisheries worldwide. Seasonal toxic blooms of *Alexandrium* spp. occur on an annual basis in the North Channel area of Cork Harbour, Ireland, where resident populations of non-toxic *A. tamarense* (West European ribotype) and PSP toxin-producing *A. minutum* (Global Clade) co-occur. Field surveys were carried out throughout a bloom of *Alexandrium* spp. in the summer of 2006. Taxa-specific fluorescently labelled probes were used in a dual whole-cell fluorescent *in situ* hybridization (WC-FISH) assay for the simultaneous discrimination and quantification of *A. minutum* and *A. tamarense* in the water column. The bloom occurred following a weak spring tide in early June and *Alexandrium* cell concentrations exceeded 3×10^4 cells L^{-1}. *A. minutum* dominated numerically over *A. tamarense* throughout the sampling period (74% on average). The maximum cell concentration was $\sim 3.3 \times 10^5$ cells L^{-1} at the peak of the bloom and was localized at the eastern end of the North Channel. The bloom collapse coincided with increasing tidal flushing and significantly changing meteorological conditions (wind speed increase, lesser irradiance), which led to a water temperature drop of ~ 3 °C within a period of 7 days. GTX3 was the dominant PSP toxin variant and C-toxins were at times observed in samples. Assuming that *A. minutum* was the only microorganism synthesising PSP toxins, the internal toxin quota was on average 13.4 fmol $cell^{-1}$, a value similar to that observed in laboratory experiments. Monitoring of toxic *Alexandrium* species in Ireland will require the use of molecular methods for reliable discrimination and quantification.

1. Introduction

The distribution, intensity and frequency of harmful algal bloom (HAB) events have increased over the past decades worldwide (Anderson et al., 1989; Hallegraeff, 2003). These phenomena have had deleterious effects on flora and fauna in coastal areas and, through mass mortality of stocks or contamination with phycotoxins, have severely affected aquaculture operations in various countries (Shumway, 1990; Hallegraeff et al., 1998; FAO, 2004). The shellfish aquaculture sector is particularly sensitive to toxic algal blooms and as a result phytoplankton and biotoxin monitoring programs have been implemented to protect public health and limit economic losses (Andersen et al., 2003; Smayda, 2003). However, patchiness in the distribution of some toxic species and co-occurrences of morphologically similar organisms constitute difficulties that can hinder the efficiency of monitoring programs. The acquisition of reliable and accurate data through spatial and temporal surveys at sites where risks of HAB occurrences are high is necessary to parameterise predictive bio-physical models of harmful phytoplankton blooms (McGillicuddy et al., 2005).

Paralytic shellfish poisoning (PSP) is a potentially lethal intoxication syndrome that affects humans through consumption of shellfish contaminated with neurotoxins produced by some microalgal species (Wright, 1995; Cembella, 1998). PSP toxins constitute a group of about 20 low molecular weight alkaloid compounds referred to as saxitoxins, which impair the transmission of electric fluxes between muscular and nerve cells by inhibiting the voltage-gated sodium channels in synapses (Luckas et al., 2003). Symptoms in humans include nausea, dizziness, paresthesia, and severe intoxications can lead to death through respiratory failure (Kao, 1993).

The dinoflagellate genus *Alexandrium* comprises ~28 species, some of them with the ability to produce saxitoxins (Balech, 1995). The regulatory limit set for PSP toxins in shellfish tissues is typically 80 μg saxitoxin equivalent per 100 g (STX eq./100 g flesh) (FAO, 2004). Toxic populations of *Alexandrium* spp. have been associated with PSP events on most continents, with shellfish

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contamination levels greater than 3×10^3 μg STX eq. $100\ g^{-1}$ flesh in Scotland, Japan, South Africa or the US east coast having been observed (FAO, 2004). Morphological similarities between *Alexandrium* spp. do not often allow the discrimination between co-occurring species by light microscopy. Therefore, population dynamics studies carried out at the species level are not well documented (see John et al., 2003; Gribble et al., 2005; Anderson et al., 2005a for exceptions).

The increasing use of molecular biology methods in the last decade has contributed to the genetic characterization of several HAB species and the current understanding of relationships between genera, populations and strains (Scholin et al., 1995; Hansen et al., 2000; Edvardsen et al., 2003; John et al., 2004). A number of molecular assays based on the variability of rDNA genes between taxa have enabled the rapid detection and quantification of individual HAB species in complex environmental samples. Assays involving the use of whole-cell fluorescent *in situ* hybridization (WC-FISH), sandwich hybridization (SH), biosensors or real-time PCR have been developed for *Alexandrium* spp. and successfully applied to the analysis of natural populations (Anderson et al., 2005b; John et al., 2005; Dyhrman et al., 2006; Lazerges et al., 2006; Diercks et al., 2008). WC-FISH, which allows the specific detection of target taxa through the binding of oligonucleotide probes to the rRNA component of ribosomes present in actively growing cells, is a popular method for ecological studies (Sanz and Köchling, 2007; Wakeham et al., 2007). Assay design and protocol optimisation are less tedious compared to more sophisticated methods and involve relatively low costs. The non-disruption of cells in samples also offers the possibility of subsequent analysis by additional techniques.

In Ireland, PSP contamination of shellfish occurs on an annual basis in Cork Harbour, an inlet located on the south coast of the country, and seems to be caused by a population of *A. minutum* (Global Clade, Lilly et al., 2005), which co-occurs with a non-toxic population of the West European ribotype (W.E.) of *A. tamarense* there (Touzet, 2006). This clade has recently been termed "Group 2" by Lilly et al. (2007) because geographically based names can be misleading. Studies have shown that blooms originate from the North Channel area of the harbour, where high-density *Alexandrium* spp. resting cyst beds can be found (Ní Rathaille, 2007). However, the population dynamics of *A. minutum* and *A. tamarense* through bloom events are as yet unknown. This study documents the respective distribution and abundance of both species during a bloom in summer 2006 in Cork Harbour. Some environmental parameters (temperature, salinity, chlorophyll fluorescence) were monitored *in situ* in conjunction with the laboratory-based determination of PSP toxin, nutrient and *Alexandrium* spp. concentrations.

2. Methods

2.1. Study area

Samples were collected near low tide in the North Channel area of Cork Harbour, Ireland, during seven surveys at approximately weekly intervals from 25 May to 7 July 2006 (Table 1, Fig. 1). Cork Harbour is an industrialised (oil refinery, fret activities) natural harbour of $\sim 100\ km^2$ surface, which opens southward to the Celtic Sea. It is partitioned into three connected water basins: the main harbour, Lough Mahon and the North Channel. The main freshwater source is the Lee River, which flows from Cork City into Lough Mahon. The average tidal range for Cork Harbour is 3.7 and 2.0 m on spring and neap tides, respectively. It is a shallow harbour with hydro-dynamically scoured channels; the main channel is 10–20 m deep and the rest of the harbour has a depth comprised between 0 and 5 m at low water. Intertidal mudflats are exposed at low tide and cover a ~40% area of the North Channel. Cork Harbour is the only area in the Republic of Ireland where a ban on shellfish harvesting has been enforced due to the presence of PSP toxins.

Table 1
Details of the stations sampled during the surveys carried out in Cork Harbour in summer 2006.

Date (survey)	Station	Location	Time (local)	Latitude (N)	Longitude (W)
25 May 2006 (01)	4201	O	10:07	51°52.825′	8°15.046′
	4202	OP	10:36	51°52.883′	8°14.492′
	4203	P	10:58	51°52.922′	8°13.957′
	4204	PQ	11:19	51°52.911′	8°13.254′
	4205	Q	11:41	51°52.895′	8°12.891′
	4206	QR	12:06	51°52.941′	8°12.477′
	4207	R	12:26	51°52.928′	8°12.06′
	4208	T	13:11	51°52.169′	8°12.367′
31 May 2006 (02)	4307	T	13:56	51°52.190′	8°12.341′
	4308	R	14:23	51°52.914′	8°12.088′
	4309	Q	14:52	51°52.888′	8°13.004′
	4310	PQ	15:15	51°52.852′	8°13.508′
	4311	P	15:40	51°52.908′	8°14.031′
	4312	OP	16:00	51°52.906′	8°14.554′
	4313	O	16:20	51°52.817′	8°15.029′
8 June 2006 (03)	4403	OP	09:28	51°52.894′	8°14.585′
	4404	P	09:49	51°52.908′	8°14.030′
	4405	PQ	10:23	51°52.922′	8°13.501′
	4406	Q	10:57	51°52.928′	8°12.933′
	4407	R	11:22	51°52.922′	8°12.064′
	4408	T	11:57	51°52.168′	8°12.362′
15 June 2006 (04)	4508	T	13:32	51°52.176′	8°12.353′
	4509	R	14:07	51°52.917′	8°12.088′
	4510	Q	14:42	51°52.915′	8°12.939′
	4511	PQ	15:09	51°52.920′	8°13.474′
	4512	P	15:40	51°52.910′	8°13.970′
	4514	O	16:14	51°52.824′	8°15.026′
22 June 2006 (05)	4601	OP	08:41	51°52.933′	8°14.489′
	4602	P	09:04	51°52.910′	8°13.986′
	4603	PQ	09:34	51°52.938′	8°13.507′
	4604	Q	10:09	51°52.904′	8°13.011′
	4605	R	10:48	51°52.927′	8°12.071′
	4606	T	11:28	51°52.182′	8°12.320′
29 June 2006 (06)	4708	T	13:25	51°52.195′	8°12.344′
	4709	R	13:57	51°52.940′	8°12.092′
	4710	Q	14:41	51°52.897′	8°12.998′
	4711	PQ	15:07	51°52.925′	8°13.542′
	4712	OP	15:35	51°52.868′	8°14.428′
7 July 2006 (07)	4801	LAGOON[a]	10:00	51°53.050′	8°14.500′

[a] Sampling was carried out periodically at a fixed position from low to high tide; temperature, salinity and *Alexandrium* spp. concentrations only were measured.

2.2. Meteorological data and water column sampling

Daily values of incident irradiance data were obtained using a Licor LI-190 Quantum Sensor, which was located midway on the north shore of the North Channel. Wind speed data were obtained from Cork airport, west of Cork Harbour.

Temperature and salinity were measured *in situ* by means of a calibrated probe (WTW, Cond197i). The vertical profiling of chlorophyll fluorescence was carried out using a fluorometer (FL500 SeaTech, Oregon). Samples for chlorophyll-*a* analysis were obtained by filtering 500 mL seawater through GF/C filters. Filters were temporarily kept in a refrigerated box and stored at −32 °C in the laboratory until analysis. Seawater samples (50 mL) were passed through 0.45-μm syringe filters, kept in a refrigerated box and also stored at −32 °C for inorganic nutrient analysis (nitrate,