Epiphytic abundance and toxicity of *Prorocentrum lima* populations in the Fleet Lagoon, UK

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Introduction

Prorocentrum lima is a weakly swimming dinoflagellate (Figure 1) that primarily inhabits sandy sediments, or is epiphytic typically on debris, macroalgae or macrophytes (Tindall and Morton, 1998; Levasseur *et al.*, 2003). *Dinophysis* spp. and *P. lima* produce okadaic acid (OA) and dinophysistoxins (DTX) that may accumulate in the edible flesh of shellfish feeding on microalgae, which can result in the poisoning syndrome diarrhetic shellfish poisoning (DSP) in human consumers (Anderson *et al.*, 1998).

P. Lima loosely attaches by a coating of mucus or lives freely within the interstices of macroalgal thalli and macrophytes (Vila *et al.*, 2001) (Figure 2). Underestimation of toxic dinoflagellate abundance may be because of lack of sampling of species with epiphytic and epibenthic strategies (Levasseur *et al.*, 2003). Previously DSP events have resulted in temporary closure of the *Crassostrea gigas* (oyster) farm in the lagoon. In the absence of *Dinophysis*, *P. lima* are the most likely causative organism.

Macroalgae and macrophytes (seagrass) from the Fleet were sampled for *P. lima* during summer 2002.

Figure 1: P. lima microscopy images; cell length 31-47 µm, width 22-40 µm (Yasuwo Fukuyo, Westpac-HAB, 2005)

Figure 2: Mucilaginous matrix of epiphytic dinoflagellates under: (1A) light microscope, (1B) scanning electron microscope. (Vila et al., 2001)

Methods

Study site

The Fleet lagoon, Dorset, is a natural shallow basin of 3 to 5 m depth (Figures 3 & 4). *C. gigas* are cultivated in the eastern lagoon (Figure 5). The coarse sediments of the inlet channel are colonised by brown and red algae (Figure 6), whereas the soft mud beds of the lagoonal basin support green algal meadows (Figures 7 & 8) and the macrophytes *Zostera* and *Ruppia* spp. (seagrass).

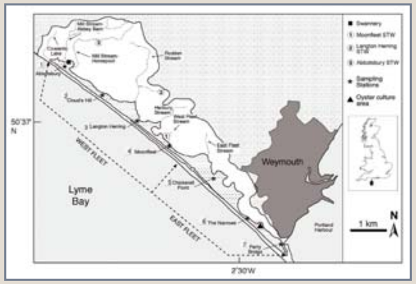

Figure 3: The Fleet Lagoon, showing sampling stations 1-7, the oyster farm, sewage treatment works and the drainage basin

Figure 4: The Fleet Lagoon

Figure 5: Oyster farm trestles

Figure 6: Variety of macroalgae at The Narrows, Sta. 6

Figure 7: Exposed seagrass at Chickerell Point, Sta. 5

Figure 8: Seagrass and opportunistic macroalgae at Moon Fleet, Sta. 4

Sample collection and processing

A survey was conducted every 3 to 4 weeks during summer 2002 at 7 stations in the Fleet Lagoon (Figure 3). Oysters were collected for toxin extraction.

A field assay was employed for separating microalgal epiphytes (Yasumoto *et al.*, 1979; Vila *et al.*, 2001; Foden *et al.*, 2005). Dominant macrophytes and macroalgae species were identified. Cut samples were placed in sealable plastic bags with filtered lagoon water and shaken vigorously for 1 min to dislodge epiphytic microalgae. A sub-sample was preserved for microscopic identification and enumeration. The remainder were concentrated onto filters and frozen for toxin extraction and analysis by LC-MS.

Results

Variability in macroalga, macrophyte and *P. lima* abundance

Macroalga and macrophyte (seagrass) diversity increased over the 4 month period and they varied in abundance. The mean densities of *P. lima* cells on all macroalgae and all macrophytes are presented in Figure 9. The range in concentration was from 0 (i.e. undetectable) to 1.25 x 10^4 *P. lima* cells g^{-1} FW macroalgae or macrophytes. *P. lima* cell density increased as the season advanced.

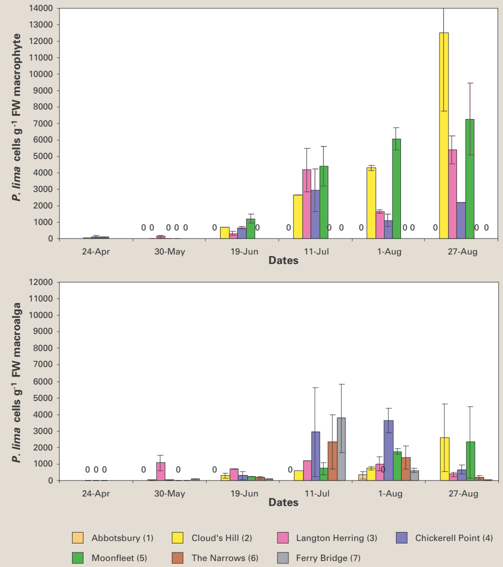

Figure 9: Mean P. lima cells g⁻¹ FW (A) macrophytes and (B) macroalgae; Sta. 1 – 7. Standard error bars shown

DSP toxins

Table 1 presents the mean OA and DTX-1 toxin concentrations and ratios found in *P. lima* at 3 stations. OA and DTX-1 were not detected by LC-MS analysis in any of the oyster sample extracts.

Table 1: DSP toxin concentrations (pg) $cell^{-1}$ and cellular OA/DTX-1 ratios (means and standard deviation) in *P. lima* cells

			Mean (pg) toxin $cell^{-1}$				Ratio
Station	**Species**	**Date**	**OA**	**St. dev.**	**DTX-1**	**St. dev.**	**OA/DTX-1**
6	*Ceramium* sp.	11/07/02	0.60	0.08	2.3	0.060	0.27
		01/08/02	1.2	0.09	5.4	0.18	0.22
		27/08/02	0.51	0.21	3.3	0.68	0.16
5	*Zostera* sp.	19/06/02	1.0	0.27	1.8	0.12	0.57
		11/07/02	0.42	0.21	0.88	0.65	0.48
		01/08/02	0.68	0.20	1.4	0.44	0.48
		27/08/02	0.68	0.26	1.8	0.62	0.39
3	*Enteromorpha* sp.	19/06/02	0.85	0.57	3.7	2.6	0.23
		11/07/02	0.15	0.020	0.35	0.050	0.43
		01/08/02	0.70	0.14	1.2	0.19	0.60
		27/08/02	1.5	0.29	2.6	0.61	0.58

Conclusions

- The field assay and the concentration of cells on filters proved highly successful for separating *P. lima* from supporting substrata (Foden *et al.*, 2005).
- *P. lima* abundance varied spatially and temporally in association with different species of macroalgae and macrophytes (seagrass). Greatest densities of *P. lima* were associated with 3-dimensional surfaces of *Zostera*, *Ruppia* and *Enteromorpha* species, in the microtidal, moderate to low energy systems of mid Fleet.
- Wild cells contained the DSP toxins OA and DTX-1 in ratios known to be characteristic of *P. lima* strains from the Fleet lagoon (Nascimento *et al.*, 2005).
- Absence of DSP toxins in *C. gigas* flesh implies quantities of *P. lima* dislodged into the water column were too low for ingestion to lead to sequester of toxins, during the sampling period. This indicates the capacity of the methods applied in this study to monitor *P. lima* at very low densities, and their potential for prediction of a DSP event.

Acknowledgements

This research was partly supported by the E.U. Harmful Algal Blooms Expert System (HABES) project (contract EVK2-CT-2000-00092 awarded to D.A.P)

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