# *BioProbe* - towards a sensor-based "Taxonomist on a *SmartBuoy*"

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## Introduction

Bioprobe concerns the development of an automated system for the recognition of species based on the detection of specific nucleic acid sequences. The starting point is phytoplankton and in particular toxic algae implicated in harmful algal blooms (HAB), though the concept extends to any biological target that can be collected from seawater at remote locations and lysed *in situ*, such as other planktonic organisms, bacteria or viruses.

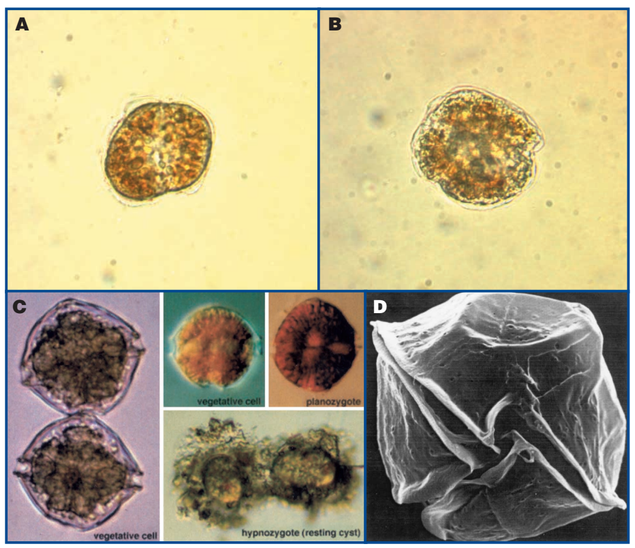
Reasons for the development of such a system include:

- more comprehensive and fine scale monitoring data to detect changes in species composition
- to provide a better assessment of biodiversity and thus develop an improved understanding of the marine ecosystem on the microscopic scale.
- an early warning system for the occurrence of harmful organisms

## The target organism

*Alexandrium tamarense* was chosen as a first target, since it is a producer of saxitoxin (the causative agent of paralytic shellfish poisoning) and blooms in UK waters. The *Alexandrium* species complex consists of at least 27 species, 10 of which produce toxins. The different organisms are morphologically too similar to be differentiated by traditional optical microscopy (Figure 1). Sequence data for ribosomal RNA of the *Alexandrium* species complex is available and an interesting correlation between phylogenetic lineage/geographic origin and ability to produce toxins has been observed (C. Scholin *et al.*, 1994, *J. Phycol*, **30**, 999-1011). Molecular genetics based taxonomy is a promising tool for identification and enumeration of HAB species.

*Figure 1:* Alexandrium tamarense




*A. Alexandrium tamarense CCAP1119/1 (non-toxic isolate from Plymouth) x80*
*B. Alexandrium tamarense WAH 4 (toxic isolate from Weymouth) x80*
*C. Alexandrium tamarense (photo by Yasuwo Fukuyo)*
*D. Alexandrium tamarense (electron microscope 30µm wide)*

## The SmartBuoy system

*SmartBuoy* is a versatile, moored platform designed to take high frequency time-series measurements of biological, chemical and physical variables for extended periods in harsh environments (Figure 2). Depending on intended application, the payload of the platform can be configured by selecting from a range of modular instruments. For example sensors for conductivity, temperature, pressure, fluorescence, optical back-scatter, irradiance and dissolved oxygen are available as well as *in situ* nutrient analysers (NAS-2E) and a programmable water samples that stores up to 50 samples for subsequent laboratory analysis (Aqua Monitor). Data from the instruments is archived by a multi-channel logger (Eco-System Monitor) and can be relayed back to the lab in real time using telemetry.

**It is the aim of this project to provide an instrument for molecular taxonomy - the *Bioprobe* - for integration into the SmartBuoy system.**

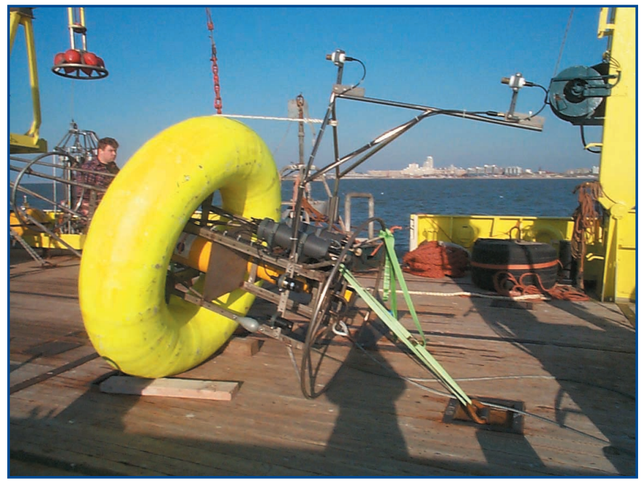


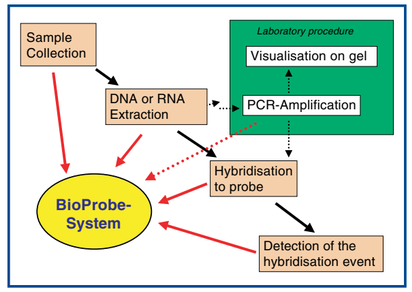
*Figure 2a SmartBuoy with a range of sensors before deployment. (photo D. Sivyer)*

*Figure 2b SmartBuoy with a range of sensors after recovery. (photo D. Sivyer)*

## The BioProbe system

Figure 3 summarises the steps involved in the analytical procedure. In the laboratory a method of detection based on lineage specific primers and nested PCR followed by gel-electrophoresis for the visualisation of the amplification product has been established (Figure 4).

*Figure 3*
*The analytical procedure*



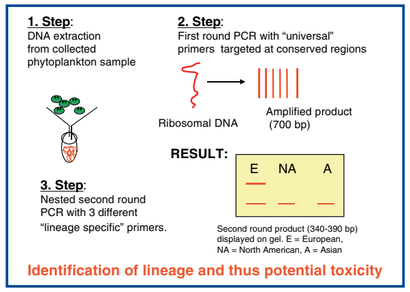




*Figure 4*
*Identification by nested PCR*

Detection the hybridisation between extracted ribosomal RNA and an immobilised detection probe either directly through impedance spectroscopy or indirectly through the amperometric detection of an enzyme label following a sandwich hybridisation assay are being evaluated at present (Figure 5). The direct detection method has the advantage of reducing the number of steps required, but the use of a sandwich format introduces additional safeguards against false positive results and allows signal amplification. Should the detection limit not be sufficient for the envisaged application, integration of PCR amplification of the target sequence into the automated device will also be considered.

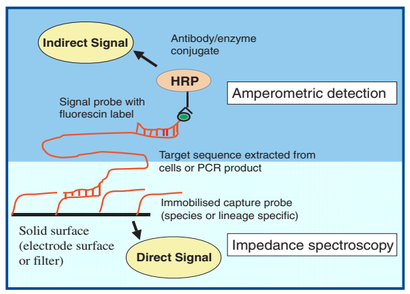



*Figure 5 The detection mechanism*

## Future work

The challenge for future work is the integration of the individual components into a autonomous system capable of working under the harsh conditions at sea. Issues that have to be addressed in more detail are sensitivity, selectivity and accuracy of the selected detection method and general system requirements such as reagent stability, resistance to biofouling and system robustness.

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