View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Repositorio Institucional Digital del IEO



# Field testing for toxic algae with a microarray: initial results from the MIDTAL project

McCoy, G.R.[1], Raine, R.[1], Medlin, L.K.[2], Chen, J.[2], Kooistra, W.[3], Barra, L.[3], Ruggiero, M.V.[3], Graneli, E.[4], Hagström, J.A.[4], Salomon, P.S.[4], Reguera, B.[5], Rodríguez, F.[5], Escalera, L.[5], Edvardsen, B.[6], Dittami, S.M.[6], Eikrem, W.[6], Lewis, J.M.[7], Berzano, M. [7], Elliott, C.T.[8], Campbell, K. [8] & Pazos, Y.[9]

[1]Martin Ryan Institute, National University of Ireland, Galway, Ireland: g.mccoy3@nuigalway.ie, [2]Marine Biological Association of UK, The Laboratory, Citadel Hill, Plymouth, UK: medlin@obs-banyuls.fr, [3]Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy: kooistra@szn.it, [4]Linnaeus University, Marine Ecology Department, SE-39182 Kalmar, Sweden: edna.graneli@lnu.se, [5]Instituto Español de Oceanografía, Cabo Estai, Canido, 36390 Vigo, Spain: beatriz.reguera@vi.ieo.es, [6]University of Oslo, Department of Biology, Blindern 0316 Oslo, Norway: bente.edvardsen@bio.uio.no, [7]School of Life Sciences, University of Westminster, London W1W 6UW, UK: lewisjm@westminster.ac.uk, [8]Institute of Agri-Food and Land Use, Queens University of Belfast, N. Ireland: chris.elliott@qub.ac.uk, [9]INTECMAR, Peirao de Vilaxoán, Villagarcía de Arosa, 36611 Spain: ypazos@intecmar.org.

## Abstract

One of the key tasks in MIDTAL (MIcroarrays for the Detection of Toxic ALgae) is to demonstrate the applicability of microarrays to monitor harmful algae across a broad range of ecological niches and toxic species responsible for harmful algal events. Water samples are collected from a series of sites used in national phytoplankton and biotoxin monitoring across Europe. The samples are filtered; rRNA is extracted, labelled with a fluorescent dye and applied to a microarray chip. The signal intensity from >120 probes previously spotted on the chip is measured and analysed. Preliminary results comparing microarray signal intensities with actual field counts are presented.

## Introduction

Blooms of toxic or harmful microalgae (HABs), represent a significant threat to fisheries resources and human health throughout the world. Since many HABs have significant economic impacts, monitoring programmes which measure toxins that have accumulated in shellfish flesh have become a necessity. In Europe, this requirement for monitoring is established in a series of directives in which monitoring of coastal waters for potentially harmful phytoplankton is mandatory. Traditionally phytoplankton identification and enumeration is carried out using LM. This technique requires a high degree of skill of operator, and is time-consuming. Furthermore, the morphological similarity between different species within or even across phytoplankton genera has meant that light microscopy alone is at times insufficient to assess the potential toxicity of a water sample. A variety of methods based on the sequencing of nucleic acids have been developed which have considerably improved our ability to accurately identify organisms to the species level. These have been outlined recently in a manual for phytoplankton analysis (Karlson *et al*. 2010). Microarrays are state of the art molecular biology for the processing of bulk samples for detection of target RNA/DNA sequences. In MIDTAL, existing rRNA (18S, 28S) probes and antibodies for toxic algal species and their toxins have been adapted for use in a microarray format. This paper presents the first field trial results .

## Materials and Methods

Water samples are taken and a measured volume is filtered through nitrocellulose filters (pore size 1-3 µm). The volume of sample filtered depends on turbidity of the water: 0.5-2 l is usually filtered up to when the filter starts to clog. The filter is then immediately submersed in 1ml of Tri-Reagent (Ambion, UK) and an aliquot of *Dunaliella tertiolecta* (5 .$10^6$ cells) added as an internal control for the RNA extraction process. The material is then stored at -80 °C. RNA extraction is carried out through cell lysis, sequential extraction with 1-

Pagou, P. and Hallegraeff, G. (eds). Proceedings of the 14th International Conference on Harmful Algae. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO 2013

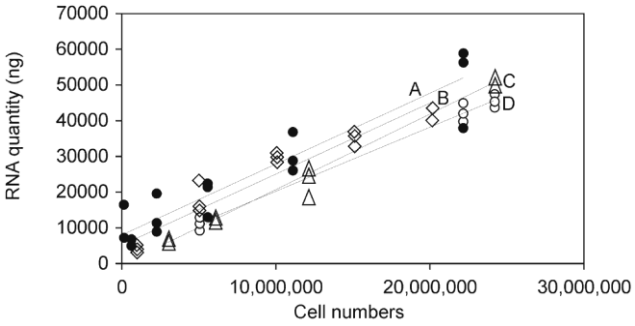


**Fig. 1**. Comparison of RNA extraction efficiencies on cultures of *Dunaliella tertiolecta* between four MIDTAL partners A ($R^2$ = 0.8836), B ($R^2$ = 0.9243), C ($R^2$ = 0.9848) and D ($R^2$ = 0.9912).

Bromo-3-chloro-propane (BCP) and isopropanol, followed by ethanol wash. After the final centrifugation step, the pellet is suspended in RNase free water and stored at -80 °C. The RNA is labelled using Platinum Bright 647 Infrared Nucleic Acid kit, fragmented and hybridised to pre-activated epoxysilane-coated microarray chip at 65°C. Unlabelled RNA is removed from the chip surface using 3 washing steps, with different stringency involving EDTA, minimising background noise. The chip, pre-spotted with over 120 oligonucleotide probes corresponding to a taxonomic hierarchy (kingdom, class, genus and species) is scanned (Genepix 4000B Axon Inc.) and fluorescence intensity from each probe measured. Results are compared with LM of original water sample. This ongoing process will be carried out over 2 years. Preliminary results comparing microarray signal intensities with actual field counts are presented.

## Results and Discussion

*RNA extraction efficiency.* Good yields of high quality RNA were extracted from *D. tertiolecta* cells when a preliminary standard curve was made (Fig. 1). The relationship between cell numbers and RNA content was linear with satisfactory coefficient of determination from four randomly selected project partners.

*Sensitivity of the hybridisations.*The sensitivity of hybridisations onto the microarray were investigated by testing a range of probes which should be highlighted by a particular organism growing under different environmental conditions. Fig. 2 shows results from probes for prymnesiophytes tested on a culture of *Prymnesium parvum.* These probes were adapted for the microarray from those by Lange *et al*. 1996; Simon *et al*. 1997; 2000; Töbe *et al*. 2006; Eller *et al*. 2007. A NanoDrop Spectrophotometer was used to quantify the RNA after the labelling and RNA clean-up steps to determine the exact labelled RNA amount when approximately 1 , 5 , 25 and 100 ng were hybridised to the chip. A pre-selected signal: noise ratio threshold level was applied so that the limit of quantification was represented by a signal of 2. Thus if the optimum probe for prymnesiophytes (PrymS02_25; Lange *et al*. 1996) is applied, then the microarray can not accurately detect RNA amounts below 5 ng (Fig. 2a). Example of image intensities is also shown in Fig. 2b.

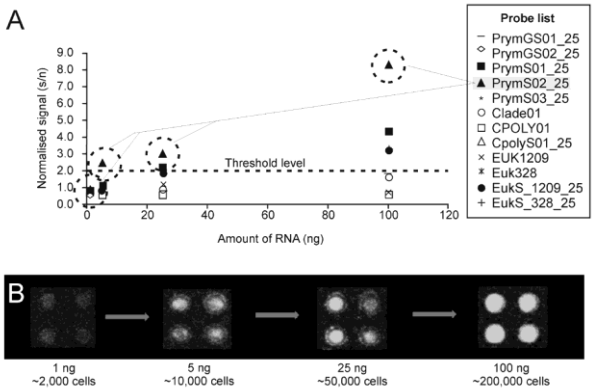


**Fig. 2.** Standardisation of the *Prymnesium parvum* signal. (A) Calibration curve of RNA (1, 5, 25 and 100ng) against signal intensity for a range of probes. (B) Images of the optimum probe PrymS02_25 when increasing amounts of RNA are hybridised to the microarray. Increasing signal intensity represents increasing cell numbers.

*Development of microarray chip.* A 1st chip designed for a specified range of HAB species produced weak signals for several species-probe combinations. A 2nd generation chip was designed in which the probes were increased in length to 25 base pairs. This meant a higher melting point temperature was required and thus hybridisation temperature was increased from 58 to 65 °C. This temperature was adopted as standard between all project partners and will be further optimised for the next generation of chip.

*Light microscopy and microarray field results.* Examples of microarray results are shown in

Figs 3 and 4. Fig 3 compares data obtained from the 1st and 2nd generation chips.

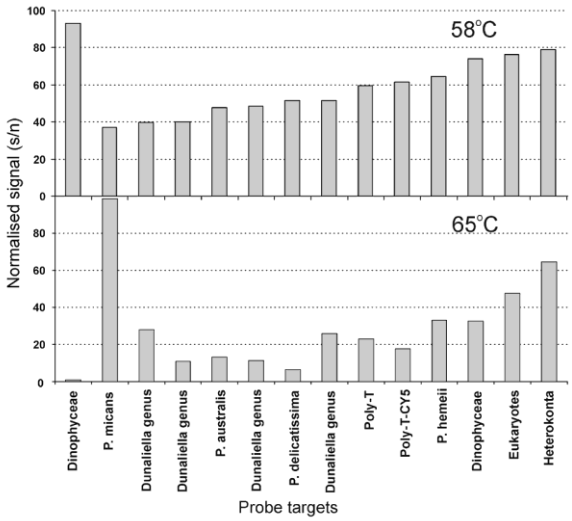


**Fig. 3.** Microarray results of 1st and 2nd generation chips both hybridised with the same Bell Harbour field extract at temperatures of 58 and 65 °C. Note the difference between the Pmica02 and PmicaD02_25 probe species specific for *P. micans*.

The sample was taken in Bell Harbour, Ireland 2009, during a bloom of *Prorocentrum micans*. LM showed a cell density of 360,000 cells $l^{-1}$. The *P. micans* probe used on the 2nd generation chip (PmicaD02_25 (98.53 s/n ratio); L.K. Medlin unpubl.) gave a vastly stronger signal to its complement (Pmica02 (37.15 s/n ratio)) on the 1st generation microarray, which was 7 base pairs shorter. A general agreement between microarray signal results and cell counts was obtained. There is also an elevated signal from the class level probe for Dinoflagellates. The strongest signals in Fig.3 signify eukaryotes, heterokonts, dinoflagellates, as well as chlorophyte *Dunaliella* and Poly-T-CY5 used as controls. Cross-reactivity with *P, heimii* will need to be addressed on the 3rd generation chip because it reacts with many target species. A second comparison between LM counts and a selection of 2nd generation microarray results from a sample from Killala Bay, August 2009, is shown in Fig. 4. An assemblage of *P. seriata* group numerically dominated the sample (112,000 cells $l^{-1}$) (Fig. 4a). The microarray data could identify these as *P. fraudulenta, P. seriata, P. australis,* and *P. multiseries.* A variety of *Alexandrium* probe signals were also evident, which could not be resolved by LM (Fig. 4b) and require EM to confirm the species.

## Conclusions

The aim of MIDTAL is to provide a new method to support toxic algal monitoring and reduce the need for mouse bioassay. Demonstration of its capabilities is the first step towards this goal. These field results indicate that there remains further development work to be done but point towards the potential of a 'universal' HAB microarray.

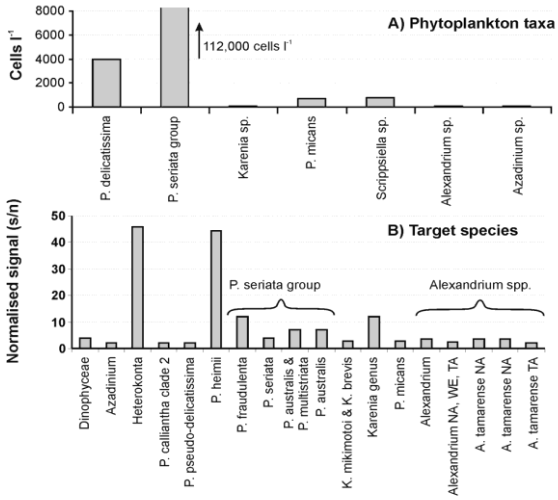


**Fig. 4.** (A) Cell counts and (B) 2nd generation microarray chip hybridised with RNA at temperature of 65°C from Killala Bay field extract on 15 Aug 2009.

**Acknowledgements.** MIDTAL is funded through EU 7th Framework (*FP7*-ENV-2007-1-*MIDTAL*-201724).

## References

Eller, G., Töbe, K. & Medlin, L.K. (2007). J. Plankton Res 29: 629-640.

Lange, M., et al. (1996). J. Phycol 32: 858-868.

Lim, E.L., et al. (1993). Appl. Environ. Microbiol. 59: 1647-1655.

Moon van der Staay, S.Y., et al. (2000). Limnol. Oceanogr. 45: 98-109.

Simon, N., et al. (2000). J. Eukaryot. Microbiol. 47: 76-84.

Simon, N., (1997). Eur. J. Phycol 32: 393-401.

Töbe, K., Eller, G. & Medlin, L.K (2006). J. Plankton Res 28: 643-657.

IOC-UNESCO. (2010) Karlson, B., Cusack, C. & Bresnan, E. (eds). IOC Manuals and Guides no. 55. Paris. 110 pp