Preliminary results of the MIDTAL project: a microarray chip to monitor toxic in the Orkney Islands, U.K.

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Abstract

Harmful algae can cause economic damage to fisheries and tourism. Additionally, toxins produced by harmful algae and ingested via contaminated shellfish can be potentially fatal to humans. Monitoring these harmful algae can be difficult as determining cell morphology by light microscopy may be insufficient to give definitive species attribution. The goal of the EU FP7 project MIDTAL (microarrays for the detection of toxic algae) was to achieve rapid species identification using species specific probes for rRNA genes in a microarray chip format. Field samples from the Orkney Islands, an area of the U.K. that has a number of nuisance and toxic species were tested with the second generation of the microarray chip. Species specific probes were looked at for the toxin producing dinoflagellates *Alexandrium tamarense* Group III (North American clade) and *Dinophysis acuta* and also general class probes for Dinophyta, Heterokontaphyta and Prymnesiophyta over the course of a year. These were compared with light microscopy cell counts. A good agreement in determining presence and absence between the methods was found. The second generation microarray is potentially more sensitive than cell counts. However, further work is needed to ensure that the microarray signal for each species provides an accurate quantitative assessment.

Blooms of toxic microalgae referred to as harmful algal blooms (HABs), represent a significant threat to fisheries resources and human health throughout the world. (Hallegraeff 1993; 1995). Harmful algae are a taxonomically diverse group and produce a variety of different toxins. However, the dinoflagellates are one of the most genetically diverse groups of phytoplankton (Stern *et al.* 2010) that contain several toxic species (Hallegraeff 1993). In U.K. waters the occurrence of toxic dinoflagellate species can be quite common (Higman *et al.* 2001; Smayda 2006; Davidson & Bresnan 2009). These algae represent a potential threat to tourism and the economy (Hoagland *et al.* 2002; Hoagland & Scatasta 2006) and present a real danger to human health due to the potential consumption of contaminated seafood. Therefore, monitoring programmes that measure toxins, which 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 also mandatory (Borja 2005). Intensive and costly monitoring programmes are undertaken for the surveillance of phytoplankton and their toxins in coastal areas. These monitoring programs traditionally use light microscopy to count cells. However, this process requires a high degree of expertise and can be time consuming. Morphological analysis can often be insufficient to give definitive species and toxin attribution. Particularly in the case of some dinoflagellate species such as *Alexandrium tamarense* where morphological identification between the toxic group III (North American clade) and non-toxic group I (Western European clade) is difficult (Leaw *et al.* 2005). Currently the methodology used in UK to monitor for *Alexandrium* states the threshold level of *Alexandrium* for shellfishery closure is the mere presence of *Alexandrium* in the water column (AFBI, 2006a; 2006b; CEFAS, 2011). Being able to distinguish between toxic and non-toxic strains would save a great deal of money each year, avoiding unnecessary shellfishery closure.

Molecular techniques can provide a tool for preliminary detection of harmful algae before toxins pass a safety threshold level as well distinguishing between toxic and non-toxic strains. A variety of methods based on the sequencing of nucleic acids have been developed over the past decade (Karlson *et al.* 2010) which have considerably improved our ability to accurately identify organisms to the species level. Microarrays are a state of the art technology in molecular biology for the processing of bulk samples for the detection of target RNA/DNA sequences. Microarrays are a collection of microscopic spots of DNA attached to a solid surface; each spot is composed of an aliquot of an oligonucleotide probe that is a specific sequence, which targets one species or taxon. RNA or DNA can then be extracted from field

samples, labelled with fluorophores such as Cy-5 and Cy-3 and hybridised to the microarray chip. The chip is then scanned and when target RNA or DNA has bound to the probes on the spots they fluoresce indicating the presence of a particular species. The Phylochip differs from most microarrays in that the probes target a range of different species. Phylochips have previously shown to be an accurate, effective and reproducible technique to monitor algae (Metfies & Medlin 2004; 2008; Metfies *et al.* 2007; Gescher *et al.* 2008, 2010).The EU project MIDTAL-Microarrays for the detection of toxic algae- aims to automate detection of harmful algae through the use of rRNA probes, targeting both the 18S and 28S rRNA genes in such a phylochip format. Existing rRNA probes for toxic algal species have been adapted for use in a microarray format on a glass slide.

An initial prototype chip was produced for the project (first generation chip) and was tested. Improvements to this chip included addition of more probe sequences, refinement of non-specific sequences, reassignment of sequences to different species and removal of unsuitable sequences. The second generation of the chip (the main focus of this study) has also been subject to extensive testing by all partner institutes on the MIDTAL project. Further re-development of the probes has led to the 3rd version of the chip, which represents the final version.

To examine the reliability and performance of the 2nd generation MIDTAL chip for detection of harmful phytoplankton populations, samples were collected for cell counts and RNA extraction from the Orkney Islands U.K., throughout the course of a year. To assess the monitoring potential in individual species that have proven difficult to monitor in the past, the study focussed on *Alexandrium tamarense* and *Dinophysis acuta* comparing microscope cell counts with the microarray data.

Materials and Methods

Monthly seawater samples were taken from Scapa Flow, Orkney (58°54'23.46"N 3°13'27.72"W) throughout 2009-2010. Water samples were taken using a tube sampler of 3 m depth and a measured volume (1 L) and were first pre-filtered through a mesh of 80 µm to remove large particles then filtered through nitrocellulose filters (Whatman, U.K.) with a pore size of 3 µm. The filter was then immediately submersed into 1 mL of Tri-Reagent

(Ambion, U.K.) and the material was stored at -80 °C. In addition to this 200 ml of seawater taken for microscope cell counts was preserved with acidic lugol's iodine.

Before extraction an aliquot of *Dunaliella tertiolecta* (5 x 10^5 cells) was added as an internal control for the RNA extraction process. RNA extraction was carried out first by bead beating (60 sec at 4800 oscillations/min) the sample in the tri-reagent to lyse cells, then 1-Bromo-3-chloro-propane (0.1 mL) was then added and the mixture added to phase lock columns (5Prime, USA) and centrifuged to separate organic and aqueous phases, the aqueous phase was removed and RNA was precipitated in isopropanol (-20^{o}C), followed by a wash with 75 % ethanol. After drying, the pellet was suspended in RNA Storage Solution (50 µL, Ambion, U.K.) and stored at -80 °C.

The RNA was then labelled using a Platinum Bright 647 Infrared Nucleic Acid kit (Kreatech, USA), fragmented in a salt buffer (Gescher *et al.* 2010) and hybridised to a pre-activated epoxysilane-coated microarray chip at 65 °C. Un-hybridised RNA was removed from the chip surface using 3 washing steps with increasing buffer stringency. The slide was first washed in a low stringency buffer (2x SSC/10 mM EDTA/0.05% SDS), then a second, more stringent buffer (0.5x SSC/10 mM EDTA) was applied. Both of these washes were performed at room temperature. Finally a third most stringent wash (0.2 x SSC/10 mM EDTA) was performed at 50^{o}C to minimise background noise. The chip was scanned (Genepix 4000B Axon Inc.) with a resolution of 10 µm and an excitation wavelength of 635 nm. The scanned images were then analysed with Genepix analyser software (Axon, Inc, U.S). The total fluorescence signal intensity from each probe was calculated by measuring the pixel intensity in the defined area for that probe minus the background fluorescence. Each microarray slide contained two arrays, samples were run in duplicate on separate slides. Each array contained 8 spots for each probe. Therefore for each probe a mean value was calculated for the 16 spots specific for that probe over the two arrays. Data was normalised to an internal positive control added after the labelling step. The cut off for those deemed to be a positive hit for a particular probe was a normalised signal >0.2.

The internal control was probes specific for a TATA box. The internal control was added to the hybridisation mixture prior to the hybridisation. The control probes target was a PCR amplicon produced by PCR amplification from a DNA extract of *Saccharomyces cerevisiae* using the primers TBP-F (5´ TTTTCAGATCTAACCTGCACCC 3´) and TBP-R-CY5 (5´ ATGGCCGATGAGGAACGTTTAA 3´).

Cell counts were carried out using the Utermöhl method used by monitoring agencies in the U.K. (AFBI, 2006a; AFBI 2006b; CEFAS 2011), to compare existing sampling methods for the Orkney Islands with the microarray method. Acidic lugol's preserved seawater collected at the time of sampling was sedimented in Utermöhl settling chambers (20 mL). The chamber was viewed under an inverted microscope and species/ genus or class identification and counts performed for the whole area for species of low abundance, or average of 10 fields of view taken for species of high abundance. Counts were performed in duplicate. In the case of *Alexandrium tamarense* counts were assumed to be either group I or group III as identification further than this under light microscopy is difficult.

Results and discussion

Members of the Dinophyta and Heterokontaphyta were present in the cell counts throughout the year (Fig. 1 A). This was also reflected in the microarray data (Fig. 1 B.) where there was a positive signal for probes specific for the Dinophyta and Heterokontaphyta in all months. This shows that the microarray is an effective tool at determining the presence and absence of these broad taxonomic groups. Relationships between cell numbers showed significant correlation with the microarray signal for the Heterokontaphyta (Fig. 2 A) (r=0.85, $p=0.05$) whereas the Dinophyta showed a strong positive relationship between cell numbers and signal (r=0.97, $p=0.05$) (Fig. 2B)

In both the microarray data and the cell counts there was the same seasonal pattern documented, with a summer maximum in abundance for Dinophyta, Heterokontophyta both in cell numbers and in the microarray. Dinoflagellates rose in numbers in April with small gymnodiniods being dominant. In May this was followed by increases in species from *Scrippsiella, Dinophysis, Prorocentrum, Alexandrium* and *Protoperidinium* with maximum species richness and numbers in July. Dinoflagellate numbers and richness tailed away after August back to low levels in November. This seasonal pattern has been shown in previous studies in the Orkney Islands (Higman *et al.* 2001; Fraser et al, 2006 and in the North Sea (Reid *et al.* 1990).

Absence of the Prymnesiophytes (Fig. 1A) from the cell counts was likely caused by ineffective preservation of the Prymnesiophytes with acidic lugol's which is the standard fixative within monitoring programmes and although in some months some small flagellates

were observed under light microscopy it was difficult to distinguish Prymesiophytes from members of the Heterokontaphyta. In this way the microarray chip is more inclusive of more taxonomic algal groups than the current monitoring methods. The microarray may also be a more sensitive method than that used by monitoring agencies for light microscopy counts. However, further work is required to validate this.

The settling chambers usually used in U.K. monitoring programs are either 20 mL or 50 mL in volume (in this study we chose a standard size of 20 mL) so even counting in triplicate this represents a maximum sampling pool of 150 mL. Therefore the limit of detection is a minimum of 50 cells litre^{-1} and at such low numbers the probability of counting 1 in a random 20 mL may be quite small. Of course some monitoring programs in other countries may use larger volumes but currently in the U.K. this is not the case and processing of large volumes brings with it additional challenges. In contrast the microarray data represents direct analysis of 1 litre (or more depending on how much is filtered before the filter is clogged). This is important because monitoring programmes are required to detect cells at certain thresholds. For example 100 cells litre^{-1} for *Dinophysis acuta*, which is at the limit of detection for current light microscopy counting methodologies in the U.K (AFBI 2006b) because this represents 2 cells per 20 mL.

Calibration curves for the majority of species on the chip have been developed using cultured cells in order to back calculate from the microarray signal to cell number and these will be made available on the MIDTAL database when it is made public at the close of the project. Comparisons between cell counts for *Alexandrium tamarense* and microarray data for *Alexandrium tamarense* Group III (Fig. 2 C) showed a positive correlation (r=0.70, $p=0.05$). In April and September *Alexandrium tamarense* were absent from the cell counts but present in the array. However, in these two months it is likely that cell numbers would have been low and perhaps not detectable in cell counts. Certainly in other studies, *Alexandrium* shows increased numbers in late spring and summer months (Brown *et al.* 2001; Töbe et al 2001; Smayda 2006; Lilly *et al.* 2007; Davidson & Bresnan 2009). Cells L^{-1} values calculated from the microarray data produced using a calibration curve for *Alexandrium tamarense* Group III slightly underestimated cell numbers, although light microscopy counts do not discriminate group I from group III. Another explanation could be low efficiency of the hybridisation step or a low RNA extraction efficiency. Further work is needed to accurately calibrate the 3rd generation of the chip to enable effective back calculation from signal to cell number for each of the probes. The 3rd generation of the chip is currently being

tested. Improvements to the sample preparation have been made including improved extraction and purification of RNA, hybridisation of RNA to the array, and specificity of binding of the probes.

Highest abundance of *Dinophysis acuta* was seen in May and July for both the cell counts and microarray data where cell numbers reached 150 cells litre^{-1} and the comparisons in the cell counts and microarray data for *Dinophysis acuta* (Fig. 3 A, B) showed good agreement for presence and absence apart from in June and August where it was detected by the microarray but was absent from cell counts. It is that unlikely counting errors were made because of the distinctive nature of this species and absence of any other dinophysioids in the counts in these months. It is more likely therefore that cell numbers in the water column were below the cell count detection limit indicating that the microarray detection limit is lower than that of cell counts for this species. The increase in abundance of *D. acuta* in the water column in July and the summer months has been seen in other studies (Davidson & Bresnan 2009; Raine *et al.* 2010, Farrell *et al.* 2012). Correlations between probes specific for *D. acuta* and cell counts still showed a positive correlation (r=0.66, $p=0.05$) (Fig. 2 D) but this was weaker than for the class probes and *Alexandrium tamarense*. At this stage data has not been produced for calibration curves to convert signal to cell numbers for *Dinophysis* species due to the difficulty in culturing this species.

This preliminary study shows that there is good agreement between presence of species in the cell counts and microarray data for the dinoflagellate species examined and also between the class level probes particularly when cell numbers were high. The microarray methodology makes analysis of larger volumes of water possible giving direct analysis of 1 L or more without lengthy processing or counting protocols. For cells such as *Dinophysis* that are present at low densities which require additional sampling such as plankton net trawls this will be highly useful. In this respect the microarray could be a valuable tool in the identification of harmful species in the water column before they reach threshold levels.

This study further supports the evidence that microarrays are an effective technique for species detection (Metfries & Medlin 2004; 2008). and the in the case of this chip, effective for harmful species The overall aim of the MIDTAL project to develop a working a chip to detect a wide range of species using rRNA probes, has almost been met. The MIDTAL project has now applied this research to the monitoring of harmful algal blooms. However, the chip still requires extensive testing to determine whether potential false positives

(fluorescent signals detected when species are not detected by cell counts) in field samples are the result of cross-reactivity of the probes with other species, or result from the presence of target species below detection limits of the cell counting protocol. The majority of the probes have been tested with pure cultures to get lower limits of detection and also calibration but in the case of some probes such as those specific for *Dinophysis* species this is ongoing work.

References

AFBI 2006a Standard operating procedure for the collection of water samples for analysis of potential toxin producing phytoplankton cells in compliance with EU reg. 2004/854 Online publication accessed 16th May 2012 http://www.afbini.gov.uk/marine-biotoxins-nrl-phytoplankton-collection-sop-v2.pdf

AFBI 2006b Standard operating procedure for the identification and enumeration of potential toxin-producing phytoplankton species in samples collected from UK coastal waters using the Utermöhl method. Online publication accessed 16th May 2012 http://www.afbini.gov.uk/marine-biotoxins-nrl-phytoplankton-enumeration-sop-v4.pdf

Borja, A., 2005. The European Water Framework Directive: a challenge for nearshore, coastal and continental shelf research. *Continental Shelf Research,* **25**, 1768–1783.

Brown, J., Fernand, L., Horsburgh, A.E. & Read, J.W. 2001. Paralytic shellfish poisoning on the east coast of the UK in relation to seasonal density-driven circulation. *Journal of Plankton Research*, **23**, 105-116

CEFAS 2011 Protocol for the collection of water for the Algal Biotoxin Official Control Monitoring Programme under EU Regulation 854/2004. Online publication accessed 16th May 2012 http://www.cefas.defra.gov.uk/media/506022/protocol%20for%20collection%20of%20water%20e&w.pdf

Davidson, K. & Bresnan, E. 2009 Shellfish toxicity in UK waters: a threat to human health? *Environmental Health,* 8, 4 doi:10.1186/1476-069X-8-S1-S12

Farrell, H., Gentien, P., Fernand L., Lunven, M., Reguera, B., González-Gil, S., & Raine, R. 2012 Scales characterising a high density thin layer of *Dinophysis acuta* Ehrenberg and its transport within a coastal jet. *Harmful Algae,* **15**, 36-46

Fraser, S., Brown, L., & Bresnan, E. 2006 Monitoring programme for toxin producing phytoplankton in Scottish coastal waters April 2004 – 31 March 2005. Fisheries Research Services Contract Report No 03/06 www.scotland.gov.uk/Uploads/Documents/Coll0306.pdf

Gescher, G., Metfies K. & Medlin, L.K. 2010 Microarray hybridization for quantification of microalgae In: Karlson, B., Cusack, C. and Bresnan, E. editors. Microscopic and molecular methods for quantitative phytoplankton analysis. *IOC Manuals and Guides, no. 50,* Intergovernmental Oceanographic Commission of UNESCO, pp. 77-86

Gescher C., Metfies K. & Medlin L.K. 2008 The ALEX CHIP—Development of a DNA chip for identification and monitoring of *Alexandrium*. *Harmful Algae,* **7**,485-494

Hallegraeff, G. M. 1993 A review of harmful algal blooms and their apparent global increase. *Phycologia,* **32**, 79-99.

Hallegraeff, G. M. 1995 Harmful algal blooms: a global overview. In *Manual on Harmful Marine Microalgae* Hallegraeff, G. M., Anderson, D.M., Cembella, A.D. Eds UNESCO, Paris, pp. 1–22.

Higman W. A., Stone D. A. & Lewis J. M. 2001 Sequence comparisons of toxic and non-toxic *Alexandrium tamarense* Dinophyceae isolates from UK waters. *Phycologia,* **40**, 256-262.

Hoagland P., Anderson D. M., Kaoru Y. & White A.W. 2002 The economic effects of harmful algal blooms in the United States: estimates, assessment issues, and information needs. *Estuaries*, **25**, 819–837.

Hoagland, P. & Scatasta, S. 2006 The economic effects of harmful algal blooms. In *Ecology of Harmful Algae*; Granéli, E., Turner, J. T., Eds.; Springer-Verlag: Berlin, 391-401.

Karlson, B., Cusack, C. & Bresnan, E. editors 2010 Microscopic and molecular methods for quantitative phytoplankton analysis. Paris, UNESCO. IOC Manuals and Guides, no. 55. IOC/2010/MG/55 110 pages.

Leaw, C. P., Lim, P. T., Ng, B. K., Cheah, M. Y., Ahmad, A. & Usup, G. 2005 Phylogenetic analysis of *Alexandrium* species and *Pyrodinium bahamense* Dinophyceae based on theca morphology and nuclear ribosomal gene sequence. *Phycologia,* **44**,550–65.

Lilly, E. L., Halanych, K. M. & Anderson, D. M. 2007 Species boundaries and global biogeography of the *Alexandrium tamarense* complex Dinophyceae. *Journal of Phycology*, **43**, 1329–1338.

Metfies, K., & Medlin. L. K. 2004 DNA microchips for phytoplankton: the fluorescent wave of the future. *Nova Hedwigia,* **79**, 321-327.

Metfies, K. & Medlin, L. K. 2008 Feasibility of transferring fluorescent in situ hybridization probes to an 18S rRNA gene Phylochip and mapping of signal intensities. *Applied and Environmental Microbiology,* **74**, 2814– 2821.

Metfies K., Berzano M., Mayer C., Roosken P., Gualerzi C., Medlin L.K. & Muyzer G. 2007 An optimized protocol for the identification of diatoms, flagellated algae and pathogenic protozoa with phylochips. *Molecular Ecology Notes*, **7**, 925–936

Raine, R. , McDermott, G., Silke, J., Lyons, K., Nolan, G. & Cusack, C. 2010 A simple short range model for the prediction of harmful algal events in the bays of southwestern Ireland. *Journal of Marine Systems*, **83**, 150-157.

Reid, P.C., Lancelot, C., Gieskes, W.W.C., Hagmeier, E. & Weichart, G. 1990 Phytoplankton in the North Sea and its dynamics: a review. *Netherlands Journal of Sea Research*, **26**, 295–331.

Stern, R. F., Horak, A. Andrew RL, Coffroth, M. A., Andersen, R. A., *et al.* 2010. Environmental barcoding reveals massive dinoflagellate diversity in marine environments, *PLOS One*, **5**, 1932-6203.

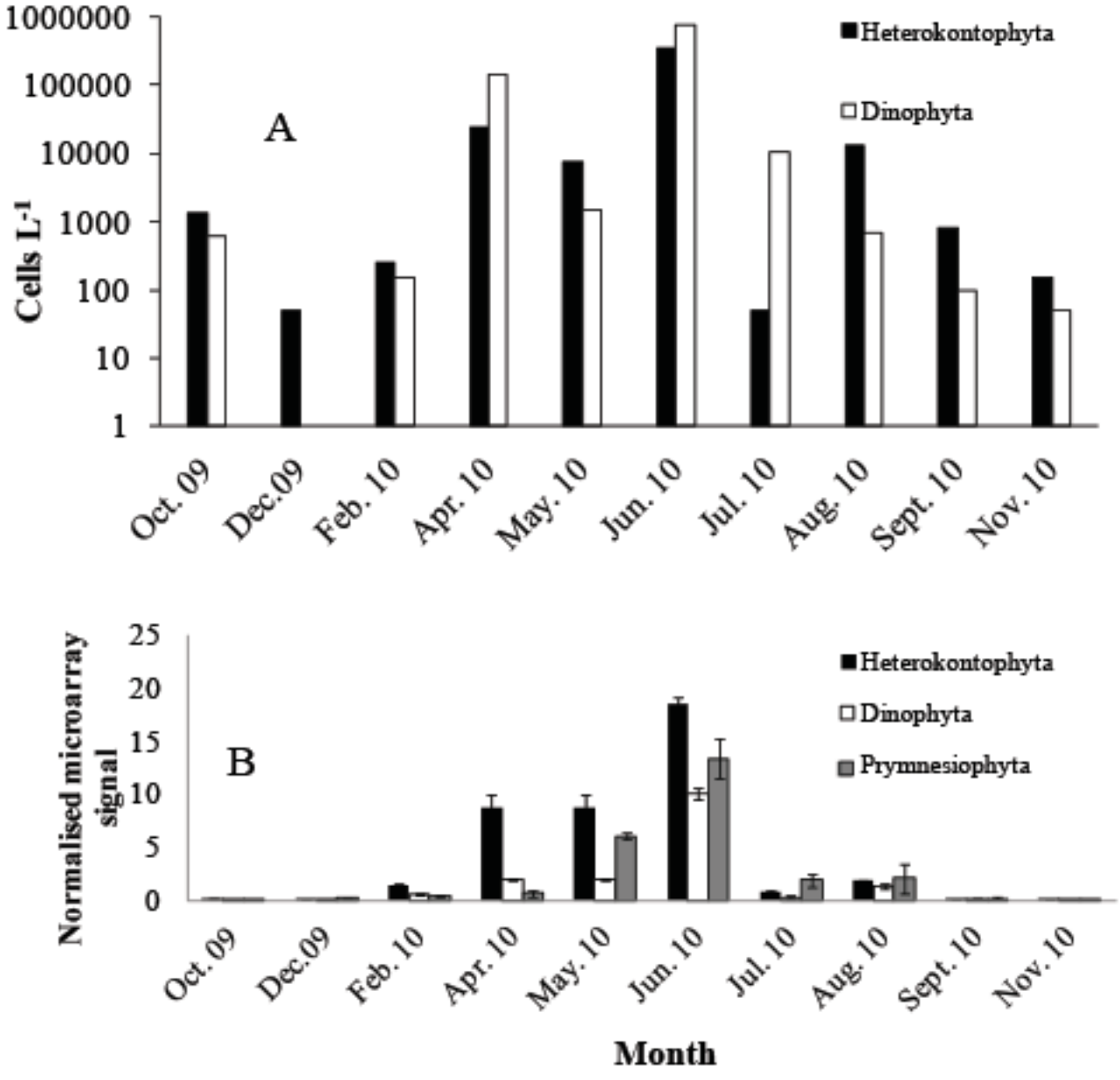
Smayda, T.J. 2006 Harmful Algal Bloom Communities in Scottish Coastal Waters: Relationship to Fish Farming and Regional Comparisons—A Review; Paper 2006/3. Scottish Executive, Scottish Environmental Protection Agency SEPA Online publication http://www.scotland.gov.uk/Publications/2006/02/03095327

Töbe, K. Ferguson, C., Kelly, M., Gallacher, S. & Medlin L.K. 2001 Seasonal occurrence at a Scottish PSP monitoring site of purportedly toxic bacteria originally isolated from the toxic dinoflagellate genus *Alexandrium*. *European Journal of Phycology*, **36**, 243-256.

Figure 1: A) Cell counts (Cells L^{-1}) of cells belonging to Heterokontophyta and Dinophyta in water sampled from Scapa flow, Orkney UK in months throughout 2009-2010. B) Normalised microarray signal for probes specific for Heterokontophyta, Dinophyta and Prymnesiophyta, values are means ±S.E of all probes on the chip specific for those groups (*n*=16) in water sampled from Scapa flow, Orkney UK in months throughout 2009-2010.

Figure 2: A) Cells (Cells L^{-1}) of Heterokontophyta against normalised microarray signal for probes specific for Heterokontophyta in water sampled from Scapa flow, Orkney UK in months throughout 2009-2010. B) Cells (Cells L^{-1}) of Dinophyta against mean normalised microarray signal for probes (*n=16*) specific for Dinophyta in water sampled from Scapa flow, Orkney UK in months throughout 2009-2010. C) Cells (Cells L^{-1}) of *Alexandrium tamarense* against normalised microarray signal for probes specific for *Alexandrium tamarense* NA in water sampled from Scapa flow, Orkney UK in months throughout 2009-2010. D) Cells (Cells L^{-1}) of *Dinophysis acuta* against mean normalised microarray signal for probes specific for *Dinophysis acuta* in water sampled from Scapa flow, Orkney UK in months throughout 2009-2010.

Figure 3: A) Cell counts (Cells L^{-1}) for *Dinophysis acuta.* B) Mean normalised microarray signal for probes specific for *Dinophysis acuta*, shaded bars represent months where *Dinophysis acuta* was present in counts and open bars when it was absent in water tube samples from the top 3 m of surface waters in Scapa flow, Orkney UK in months throughout 2009-2010.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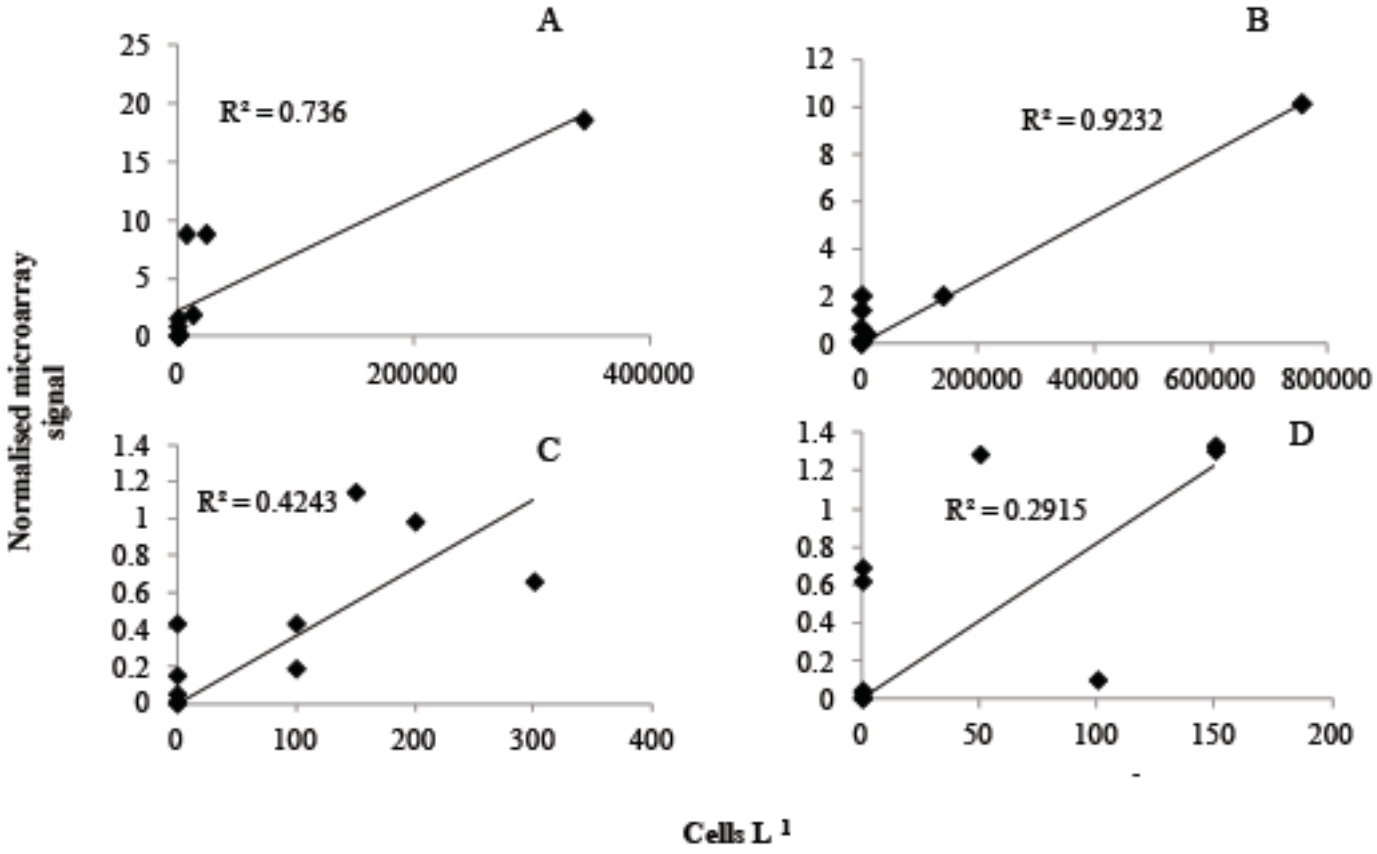
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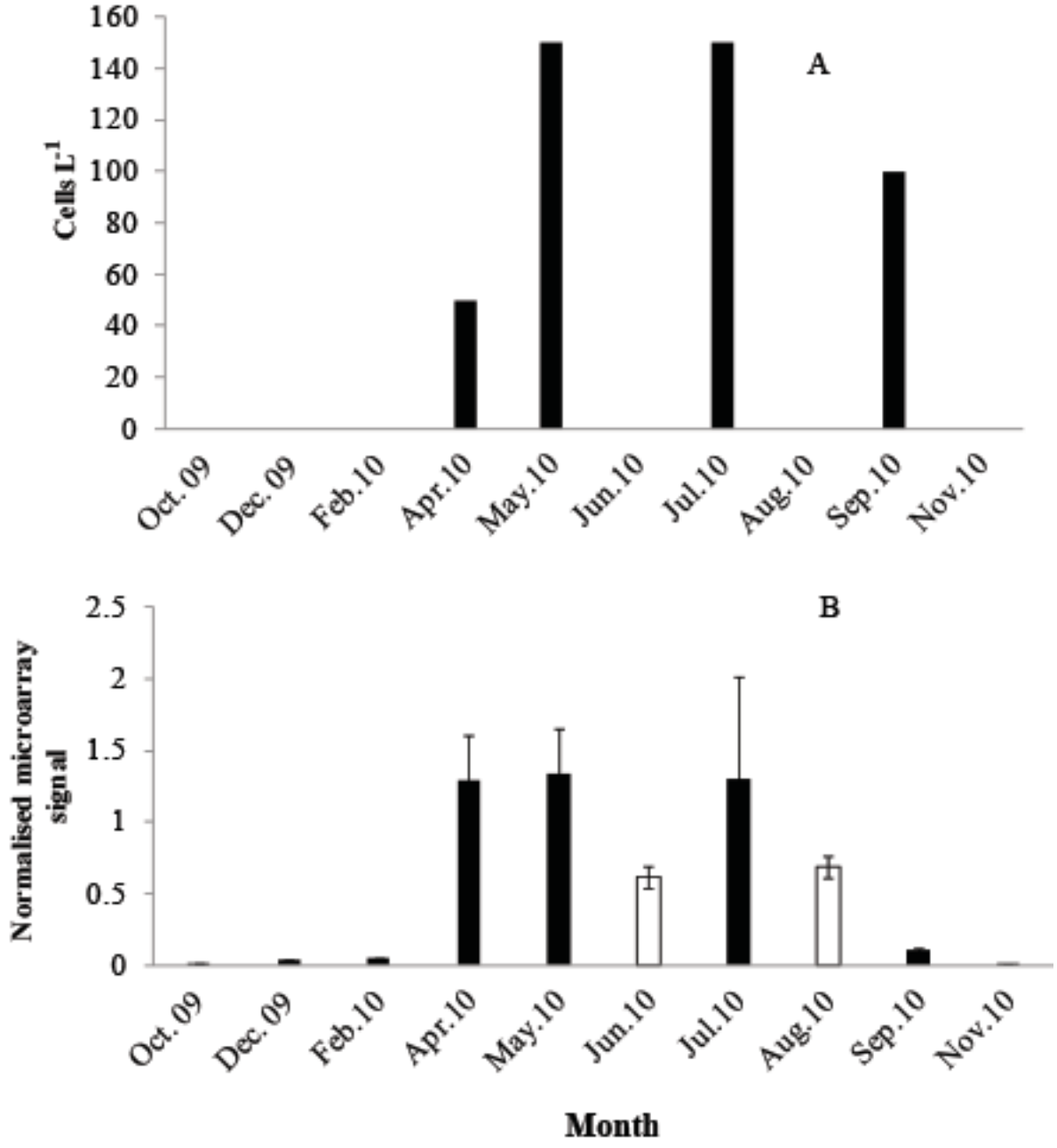
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