JOURNAL OF PLANKTON RESEARCH | VOLUME 27 | NUMBER 2 | PAGES 199–204 | 2005

SHORT COMMUNICATION

Development of specific rRNA probes to distinguish between geographic clades of the *Alexandrium tamarense* species complex

UWE JOHN, LINDA K. MEDLIN AND RENÉ GROBEN*†

ALFRED WEGENER INSTITUTE FOR POLAR AND MARINE RESEARCH, AM HANDELSHAFEN 12, 27570 BREMERHAVEN, GERMANY

†PRESENT ADDRESS: CENTRE FOR ECOLOGY & HYDROLOGY, LANCASTER ENVIRONMENT CENTRE, LIBRARY AVENUE, BAILRIGG, LANCASTER LA1 4AP, UK.

*CORRESPONDING AUTHOR: rgro@ceh.ac.uk

Received August 20, 2004; accepted in principle October 1, 2004; accepted for publication November 8, 2004; published online November 22, 2004

The globally occurring Alexandrium tamarense/fundyense/catenella species complex consists of toxic and non-toxic strains that are morphologically difficult to distinguish. We developed four specific ribosomal RNA probes that can identify the entire species complex, the strains of the toxic North American clade and the strains of the two non-toxic clades from Western Europe and the Mediterranean Sea by DNA dot blot and fluorescence in situ hybridization. These probes are a first step for the development of an early warning system for the presence of A. tamarense.

INTRODUCTION

The dinoflagellate genus *Alexandrium* Halim (Balech, 1995) contains some important producers of paralytic shellfish poisoning (PSP) toxins, among them, the three species *Alexandrium tamarense*, *Alexandrium catenella*, and *Alexandrium fundyense* (Cembella, 1998; Scholin, 1998; Taylor and Fukuyo, 1998). The three species are morphologically difficult to distinguish and, therefore, are often combined under the term of '*A. tamarense* species complex'. The characteristics that are used for the identification of these species include cell shape, geometry of the apical pore complex (APC), presence (*A. tamarense*) or absence (*A. catenella*/*A. fundyense*) of a ventral pore on the apical plate (1′), and whether the cells show a tendency for chain forming (*A. catenella*) or not (*A. tamarense*/*A. fundyense*). However, these morphological features are often difficult to determine because they can be influenced by environmental factors and culture conditions (Taylor and Fukuyo, 1998), and, in addition, intermediate morphological forms have been discovered (Cembella and Taylor, 1985; Sako *et al.*, 1990).

To clarify the phylogenetic relationship among the morphotypes and strains of the '*A. tamarense* species complex' and develop a reliable system for their identification, sequence analyses of the small subunit of the ribosomal gene (SSU rDNA) (Scholin *et al.*, 1995; John *et al.*, 2003b), the large subunit (LSU rDNA) (Scholin *et al.*, 1994, 1995; Medlin *et al.*, 1998; Higman *et al.*, 2001; John *et al.*, 2003b) and internal transcribed spacer (ITS) regions of the rDNA operon (Adachi *et al.*, 1996a) have been done. These analyses have shown that phylogenetic relationships do not reflect a separation into the three morphotypes *A. tamarense*, A. *fundyense* and *A. catenella* but instead separate the strains into phylogenetic clades according to their geographic origin. These geographically and genetically distinct clades, also called 'ribotypes', are the Temperate Asian (TA), Tropical Asian (TROP), Tasmanian (TASM), West European (WE), and North American (NA) clades, the last one also includes strains from Asian waters (Scholin *et al.*, 1994) and from the Orkney Islands (Medlin *et al.*, 1998). Recently, John and co-workers (John *et al.*, 2003b) described an additional ribotype consisting of strains from the Mediterranean Sea (ME). Whereas strains of the *A. tamarense* morphotype occur globally and are found in all those clades, the *A. fundyense* morphotype

doi:10.1093/plankt/fbh160, available online at www.plankt.oupjournals.org

only occurs in North American waters (NA clade) while *A. catenella* strains are exclusive to the NA and TA clades. Cembella *et al.* (Cembella *et al.*, 1987) found that the '*A. tamarense* species complex' contains toxic and non-toxic strains that occur in different areas of the world. So far, all strains belonging to the TA, TROP and NA ribotypes have been shown to be potentially toxic, expression of toxicity depending on nutrient status (Flynn *et al.*, 1994; John and Flynn, 2000). In contrast, no strains in the WE and ME clades have been found to ever be toxic (Scholin *et al.*, 1995; Tillmann and John, 2002; John *et al.*, 2003b). It should be taken into account though that only four strains of the ME clade originating from the same location are available so far, and that toxic strains belonging to that clade might be discovered in the future with high spatial sampling. John *et al.* (John *et al.*, 2003b) developed a hypothesis about the evolution of the '*A. tamarense* species complex' that led to the global distribution of toxic and non-toxic strains.

A visual identification of toxic members of the *A. tamarense* species complex can only be done if cells are of the *A. fundyense* or *A. catenella* morphotype, whereas toxic and non-toxic *A. tamarense* strains are morphologically indistinguishable (Cembella, 1998; Scholin, 1998). One possible solution for the problem of a confident identification of morphologically indistinguishable taxa is the use of molecular probes that bind specifically to a certain species or strain and can, therefore, be used to identify them (for a review of this technique see Amann and Ludwig, 2000). Probes can be used by DNA dot blot and fluorescence *in situ* hybridization (FISH), and they can be applied in a potential automated early warning system for potentially toxic phytoplankton blooms and to track introductions of species or strains from foreign waters. The value of a probe-based identification system is not decreased by the fact that the level of toxicity changes with varying nutrient status of the cell (John and Flynn, 2000), because the probes identify ribotypes which, for *A. tamarense*, are always either non-toxic or potentially toxic. For example, all strains of the NA clade are potentially toxic and their secure identification is very useful because a change in the nutrient levels might trigger toxicity quickly, and those strains would then become harmful. Also, no strain from a non-toxic clade has ever been reported to become toxic.

In this study, specific molecular probes have been developed for the entire species complex and three different geographic clades (NA, WE and ME) that occur in European waters.

RESULTS AND DISCUSSION

Ribosomal RNA (rRNA) oligonucleotide probes were designed as previously described (Simon *et al.*, 2000) by using the ARB (http://www.arb-home.de/) software package in combination with a database of more than 450 published and unpublished algal SSU rDNA sequences and a database of 150 sequences of the variable D1/D2 region of the LSU rDNA. These datasets have partially been used for a revised phylogeny of the '*A. tamarense* species complex' (John *et al.*, 2003b), and our probes target the strains of the genetic clades given in that paper. One molecular probe has been developed for the entire '*A. tamarense* species complex' based on SSU rDNA sequences (ATAM01), whereas three ribotype-specific probes were designed from LSU rDNA sequences. Those were ATNA02 (specific for the NA clade), ATWE03 (specific for the WE clade) and ATME04 (specific for the ME clade). All probes showed 100% specificity to their group of target organisms, and at least one mismatch to all known non-target organisms. For probe ATNA02, we redesigned the previously published NA clade specific probe NA1 (Miller and Scholin, 1998) by shifting it five bases upstream of its target sequence, because a computer analysis (OLIGO, Hitachi, Japan) showed that NA1 was likely to produce a hairpin fold that may prevent correct binding under certain conditions. Nevertheless, both probes are specific and can be used to detect the NA clade. A general eukaryote-specific probe EUK1209R (Giovannoni *et al.*, 1988) was used as a positive control in all experiments. All probes used in this study are listed in Table I.

The probes were tested for specificity with DNA dot blots by using PCR amplification products from 24 target and non-target species/strains (Table II). Unialgal cultures were grown in 500-mL Erlenmeyer flasks in IMR½ growth medium (Eppley *et al.*, 1967), supplemented with 10 nM selenite for *A. tamarense*, *A. catenella*, *A. fundyense*, *Alexandrium pseudogonyaulax*, *Alexandrium taylori*, *Alexandrium minutum*, *Alexandrium lusitanicum* and *Prorocentrum* species, or in K medium (Keller *et al.*, 1987) for *Alexandrium affine* and *Alexandrium ostenfeldii*. All cultures were maintained at 15°C in a growth chamber with a 14 : 10 hour light : dark photocycle, at a photon flux density of 150 μmol m^{-2} s^{-1}, except for A. *ostenfeldii* (90 μmol m^{-2} s^{-1}). Total DNA extractions were carried out from 500 mL of culture in logarithmic growth phase by using the PAN Plant kit (PAN Biotech, Aidenach, Germany) according to the manufacturer's instructions. Total DNA concentration was measured spectrophotometrically at 260 nm, and DNA quality was verified by agarose–gel electrophoresis. PCR primers and conditions to amplify the SSU rDNA gene and D1/D2 regions of the LSU rDNA gene were performed as described in Medlin *et al.* (Medlin *et al.*, 1988) and Scholin *et al.* (Scholin *et al.*, 1994), respectively.

Table I: Probes used in this study

Probe name	Probe location (gene/region)	Specific for	Probe sequence [5′–3′]	Hybridization temperature for dot blots	Formamide concentration for *in situ* buffer
EUK1209R[a]	18S rDNA/helix 36	Eukaryotes	GGGCATCACAGACCTG	50°C[b]	0% (v/v)
ATAM01	18S rDNA/helix E21-7	*Alexandrium tamarense* species complex	TTCAAGGCCAAACACCTG	56°C	20% (v/v)
ATNA02	28S rDNA/D1-D2	*A. tamarense* species complex North American/Orkney strains	AACACTCCCACCAAGCAA	56°C	40% (v/v)
ATWE03	28S rDNA/D1-D2	*A. tamarense* species complex Western European strains	GCAACCTCAAACACATGG	54°C	40% (v/v)
ATME04	28S rDNA/D1-D2	*A. tamarense* species complex Mediterranean strains	CCCCCCCACAAGAAACTT	54.5°C	40% (v/v)

[a]Giovannoni *et al.* (1988).
[b]Probe is specific from 50 to 56°C.

Table II: Designation and geographical origin of strains used in this study

Species (geographic clade)	Strain	Origin	Collector
Alexandrium affine	CCMP 112	Rio de Vigo (Spain)	I. Bravo
Alexandrium catenella (Temperate Asian)	BAHME 215	Tarragona (Spain)[a]	M. Delgado
Alexandrium fundyense (North American)	GT 7	Bay of Fundy (USA)	A. White
Alexandrium lusitanicum	BAHME 91	Laguna de Obidos (Portugal)	E. Silva e Sousa
Alexandrium minutum	Al5T	Gulf of Trieste (Italy)	A. Beran
Alexandrium ostenfeldii	BAHME 136	Timaru (New Zealand)	N. Berkett
Alexandrium pseudogonyaulax	AP2T	Gulf of Trieste (Italy)	A. Beran
Alexandrium tamarense (North American)	AL18b	St. Lawrence (Canada)	A. Cembella
	BAHME 181	Orkney Islands (UK)	M. Elbrächter
	BAHME 182	Orkney Islands (UK)	M. Elbrächter
	BAHME 184	Orkney Islands (UK)	M. Elbrächter
	BAHME 200	Orkney Islands (UK)	M. Elbrächter
	GTTP01	Perch Pond, Falmouth, MA (USA)	D. Kulis
	OF84423D	Ofunata Bay (Japan)	M. Kodama
A. tamarense (Mediterranean)	SZN 01	Gulf of Naples (Italy)	M. Montresor
	SZN 08	Gulf of Naples (Italy)	M. Montresor
	SZN 19	Gulf of Naples (Italy)	M. Montresor
	SZN 21	Gulf of Naples (Italy)	M. Montresor
A. tamarense (West European)	31/4	Cork Harbour (Ireland)	W. Higman
	31/9	Cork Harbour (Ireland)	W. Higman
	CCMP 115	Tamar Estuary (UK)	I. Adams
	UW42	Belfast (UK)	W. Higman
Alexandrium taylori	Ay1T	Lagoon of Marano (Italy)	A. Beran
Prorocentrum lima	CCMP 1743	Gulf of Maine (USA)	M. Faust
Prorocentrum micans	BAHME 04	Helgoland (Germany)	G. Drebes
Prorocentrum minimum	BAHME 137	Vigo (Spain)	I. Bravo

[a]Most likely an introduction by ballast water from Asia (John *et al.*, 2003b).

DNA dot blot experiments followed the protocol of John *et al.* (John *et al.*, 2003a) using the non-radioactive Digoxigenin system (Roche, Mannheim, Germany). The only modification to the method by John *et al.* (John *et al.*, 2003a) was that all washing steps consisted of two 5-min washes with 2× SSC/0.1% SDS at room temperature, and two 15-min washes with 1× SSC/0.1% SDS at hybridization temperature. The probe dependent hybridization temperatures are given in Table I. Under these conditions, all probes showed strong hybridization signals with their respective target sequences. This allowed unambiguous identification (Fig. 1).

FISH of whole cells from laboratory cultures was carried out as previously described (John *et al.*, 2003a; Groben and Medlin, 2005) using fluorescein-labelled probes. The formamide concentrations in the hybridization buffer that are necessary for specific binding of the various probes to their respective target cells, are given in Table I.

Different strains of *A. tamarense* were tested in FISH assays with the specific rRNA probes developed in this study and with the eukaryote-specific probe EUK1209R. These strains were an *A. tamarense* strain from the American east coast (GTPP01) and one from the Orkney Islands (UK) (BAHME 181), both representing the NA clade, an *A. tamarense* strain from the WE clade (31/9), an *A. tamarense* strain from the ME Clade (SZN08) and one strain of each other species given in Table II. Under optimized FISH conditions, all probes gave strong signals with their specific target strains but not with any non-target cell (Figs 2 and 3). The positive signals of ATWE03 looked different from those of other probes. Instead of a rather uniform distribution of the signal inside the cell, where probes bind to the ribosomes in the cytosol, the signal was mainly detected near the centre of the U-shaped nucleus (Fig. 3), which is presumably the location of the nucleolus as previously discussed by Adachi *et al.* (Adachi *et al.*, 1996b). We assume that this results from binding of the probe to pre-ribosomal structures in the nucleolus before they are processed into functional ribosomes. The ATWE03 probe molecules were only able to bind weakly to the mature ribosomes either because of conformational changes in the rRNA molecule or because the probe binding site was blocked by ribosomal proteins. More investigations will be done concerning this aspect of the probe binding, anyway, as ATWE03 shows this kind of signal only with their target strains it can be considered as strain-specific.

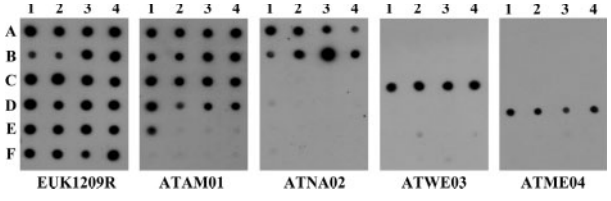

Fig. 1. Dot-blot hybridization of PCR amplified rRNA sequences with Digoxigenin-labelled oligonucleotide probes (Table I). Spotted samples are *Alexandrium tamarense* North American clade strains: A1, BAHME 181; A2, BAHME 182; A3, BAHME 184; A4, BAHME 200; B1, GT-7; B2, AL18b; B3, OF84423D; B4, GTPP01; *A. tamarense* West European clade strains: C1, CCMP 115; C2, 31/4; C3, 31/9; C4, UW42; *A. tamarense* Mediterranean Sea clade strains: D1, SZN 01; D2, SZN 08; D3, SZN 19; D4, SZN 21; E1, *Alexandrium catenella* (BAHME 215); E2, *Alexandrium lusitanicum* (BAHME 91); E3, *Alexandrium minutum* (Al5T); E4, *Alexandrium pseudogonyaulax* (AP2T); F1, *Alexandrium ostenfeldii* (BAHME 136); F2, *Alexandrium taylori* (AY1T); F3, *Alexandrium affine* (CCMP 112); F4, *Prorocentrum minimum* (BAHME 137).

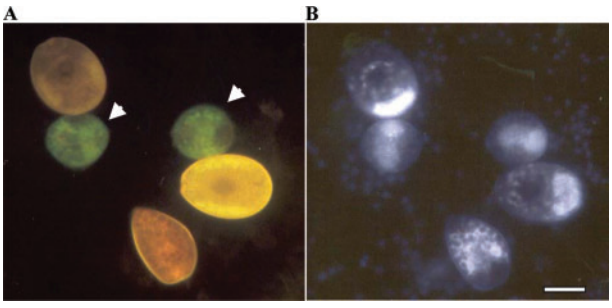

Fig. 2. (**A**) *In situ* hybridization of *Alexandrium tamarense* (SZN 01), *Prorocentrum micans* (BAHME 04) and *Prorocentrum lima* (CCMP 1743) with probe ATAM01. Arrows indicate the positive green signal of the *A. tamarense* cell. (**B**) DAPI (4′,6′-diamidino-2-phenylindole) counterstain of the cells. Scale bar represents 20 μm.

A problem that can occur in FISH assays is the masking of hybridization signals by strong autofluorescence of the cells. The occurrence of autofluorescence is species specific, e.g. *A. taylori* exhibited strong autofluorescence, whereas *A. tamarense* showed hardly any and is also influenced by the physiological condition of the cells. Therefore, laboratory cultures were harvested in exponential growth phase to obtain cells for our experiments that would show a strong probe fluorescence in case of a positive signal because they would contain more ribosomes than cells in stationary phase. When this is not possible, i.e. for field samples, an elongated fixation time is recommended, in which the ethanol extracts more chlorophyll (Chl) and hence minimizes the autofluorescence. Another possibility is a 1-h incubation of the filter in 50% dimethylformamide at room temperature after fixing the cells (Groben and Medlin, 2005). This step removes remaining Chl, with a decrease in autofluorescence of most cells; this procedure makes it easier to identify probe signals of cells in stationary phase. Even after these treatments, there might be some remaining autofluorescence, so it is not recommended to use fluorochromes, like Cy3, that emits light in the same wavelength as Chl as this might create false-positive results, even when these labels might give a higher signal intensity than the fluorescein we used in

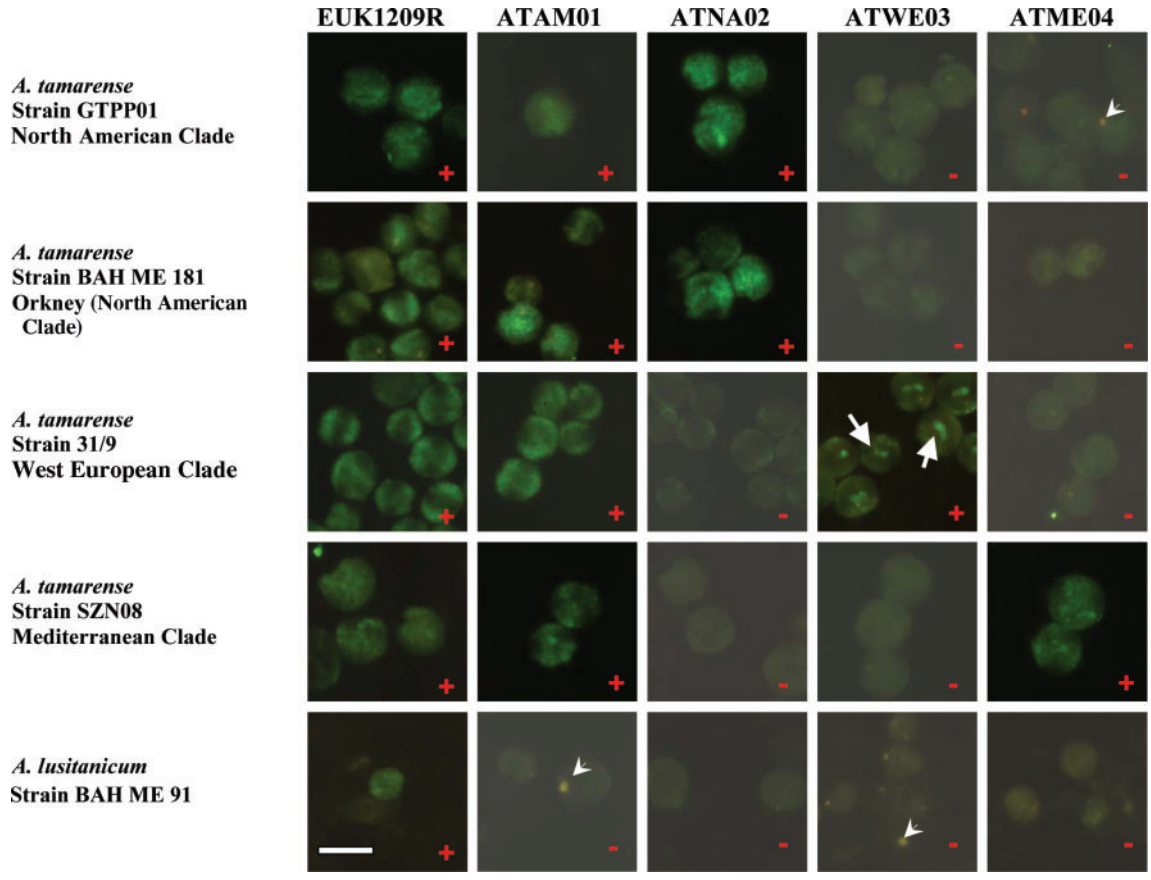

Fig. 3. Array of five different *Alexandrium* strains hybridized with probes listed in Table I. Pictures of negative non-target cells (–) were enhanced in brightness and contrast compared to those of positive target cells (+), as the non-target cells would otherwise not have been visible in the array. Arrows show binding of ATWE03 to the nucleolus of *Alexandrium tamarense* (West European). Arrowheads mark yellow fluorescent bodies inside cells that are visible in some cases independent of hybridization and should not be mistaken for hybridization signals. Scale bar represents 30 µm.

our experiments. Instead, other possibilities to enhance the signal to background ratio are the use of microscopy filter sets that partially cut off the autofluorescence, or the use of the Tyramide Signal Amplification system that increases fluorescence intensity 10–20-fold (Not *et al.*, 2002).

Molecular phytoplankton probes have already been successfully used to analyse field samples. The abundance of various taxa has been estimated by DNA dot blot hybridization, i.e. for Bolidophyceae in the ME and Pacific Ocean (Guillou *et al.*, 1999) or for groups of Prymnesiophytes in the Pacific Ocean (Moon-van der Staay *et al.*, 2000). Parsons and co-workers (Parsons *et al.*, 1999) applied fluorescent-labelled probes to analyse field samples for the occurrence of *Pseudo-nitzschia* species in Louisiana coastal waters, and John and co-workers (John *et al.*, 2003a) used FISH probes for *A. ostenfeldii* in combination with *A. tamarense* probes to investigate the co-occurrence of those species around the Orkney Islands. Currently, our probes are used in various on going research projects.

All these examples have proven the high potential of molecular probes for monitoring programmes and studying the occurrence, abundance and ecological niche of phytoplankton taxa. These newly developed probes for the '*A. tamarense* species complex' make another important phytoplankton group accessible to be analysed by these techniques.

ACKNOWLEDGEMENTS

The authors thank Helga Mehl for her technical assistance. The work was supported in part by the BMBF project TEPS (Project no. 03F0161) and by the EU project AIMS (MAS3-CT97-0080).

REFERENCES

Adachi, M., Sako, Y. and Ishida, Y. (1996a) Analysis of *Alexandrium* (Dinophyceae) species using sequences of the 5.8S ribosomal DNA and internal transcribed spacer regions. *J. Phycol.*, **32**, 424–432.

Adachi, M., Sako, Y. and Ishida, Y. (1996b) Identification of the toxic dinoflagellates *Alexandrium catenella* and *A. tamarense* (Dinophyceae) using DNA probes and whole-cell hybridization. *J. Phycol.*, **32**, 1049–1052.

Amann, R. and Ludwig, W. (2000) Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology. *FEMS Microbiol. Rev.*, **24**, 555–565.

Balech, E. (1995) The Genus. *Alexandrium* Halim (Dinoflagellata). Sherkin Island Marine Station, Cork, Ireland.

Cembella, A. D. (1998) Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae. In Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. (eds), *Physiological Ecology of Harmful Algal Blooms*. Springer-Verlag, Heidelberg, pp. 381–404.

Cembella, A. D., Sullivan, J. J., Boyer, G. L. *et al.* (1987) Variation in paralytic shellfish toxin composition within the *Protogonyaulax tamarensis/catenella* species complex; red tide dinoflagellates. *Biochem. Syst. Ecol.*, **15**, 171–186.

Cembella, A. D. and Taylor, F. J. R. (1985) Biochemical variability within the *Protogonyaulax tamarensis catenella* species complex. In Anderson, D. M., White, A. W. and Baden, D. G. (eds.), *Toxic Dinoflagellates*. Elsevier, New York, pp. 55–60.

Eppley, R. W., Holmes, R. W. and Strickland, J. D. H. (1967) Sinking rates of the marine phytoplankton measured with a fluorochrometer. *J. Exp. Mar. Biol. Ecol.*, **1**, 191–208.

Flynn, K., Franco, J. M., Fernandez, P. *et al.* (1994) Changes in toxin content, biomass and pigments of the dinoflagellate *Alexandrium minutum* during nitrogen refeeding and growth into nitrogen or phosphorous stress. *Mar. Ecol. Prog. Ser.*, **111**, 99–109.

Giovannoni, S. J., DeLong, E. F., Olsen, G. J. *et al.* (1988) Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial-cells. *J. Bacteriol.*, **170**, 720–726.

Groben, R. and Medlin, L. K. (2005) *In situ* hybridisation of plankton using fluorescently-labelled rRNA probes. *Methods Enzymol.*, 395 in press.

Guillou, L., Moon-Van der Staay, S. Y., Claustre, H. *et al.* (1999) Diversity and abundance of Bolidophyceae (Heterokonta) in two oceanic regions. *Appl. Env. Microbiol.*, **65**, 4528–4536.

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

John, U., Cembella, A. D., Hummert, C. *et al.* (2003a) Discrimination of the toxigenic dinoflagellates *Alexandrium tamarense* and *Alexandrium ostenfeldii* in co-occurring natural populations from Scottish coastal waters. *Eur. J. Phycol.*, **38**, 25–40.

John, U., Fensome, R. A. and Medlin, L. K. (2003b) The application of a molecular clock based on molecular sequences and the fossil record to explain biogeographic distribution within the *Alexandrium tamarense* species complex. *Mol. Biol. Evol.*, **20**, 1015–1027.

John, E. H. and Flynn, K. J. (2000) Growth dynamics and toxicity of *Alexandrium tamarense* (Dinophyceae): the effect of changing N:P supply ratios on internal toxin and nutrient levels. *Eur. J. Phycol.*, **35**, 11–23.

Keller, M. D., Selvin, R. C., Claus, W. *et al.* (1987) Media for the culture of oceanic ultraphytoplankton. *J. Phycol.*, **23**, 633–638.

Medlin, L. K., Elwood, H. J., Stickel, S. *et al.* (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, **71**, 491–499.

Medlin, L. K., Lange, M., Wellbrock, U. *et al.* (1998) Sequence comparison links toxic European isolates of *Alexandrium tamarense* from the Orkney Islands to toxic north American stocks. *Eur. J. Protistol.*, **34**, 329–335.

Miller, P. E. and Scholin, C. A. (1998) Identification and enumeration of cultured and wild *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targeted fluorescent probes and filter-based whole cell hybridization. *J. Phycol.*, **34**, 371–382.

Moon-van der Staay, S. Y., van der Staay, G. W. M., Guillou, L. *et al.* (2000) Abundance and diversity of Prymnesiophyceae in picoplankton communities from the equatorial Pacific Ocean inferred from 18S rDNA sequences. *Limnol. Oceanogr.*, **45**, 98–109.

Not, F., Simon, N., Biegala, I. C. *et al.* (2002) Application of fluorescence *in situ* hybridization coupled with tyramide signal amplification (FISH-TSA) to assess eukaryotic picoplankton composition. *Aquat. Microbial Ecol.*, **28**, 157–166.

Parsons, M. L., Scholin, C. A., Miller, P. E. *et al.* (1999) *Pseudo-nitzschia* species (Bacillariophyceae) in Louisiana coastal waters: Molecular probe field trials, genetic variability, and domoic acid analyses. *J. Phycol.*, **35**, 1368–1378.

Sako, Y., Kim, C. H., Ninomiya, H. *et al.* (1990) Isozyme and cross analysis of mating populations in the *Alexandrium catenella/tamarense* species complex. In Granéli, E., Sundström, B., Edler, L. and Anderson, D. M. (eds), *Toxic Marine Phytoplankton*. Elsevier, New York, pp. 320–323.

Scholin, C. A. (1998) Morphological, genetic, and biogeographic relatioships of the toxic dinoflagellates *Alexandrium tamarense, A. catenella*, and *A. fundyense*. In Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. (eds), *Physiological Ecology of Harmful Algal Blooms*. Springer-Verlag, Heidelberg, pp. 13–27.

Scholin, C. A., Hallegraeff, G. M. and Anderson, D. M. (1995) Molecular evolution of the *Alexandrium tamarense* species complex (Dinophyceae) dispersal in the North American and West Pacific regions. *Phycologia*, **34**, 472–485.

Scholin, C. A., Herzog, M., Sogin, M. *et al.* (1994) Identification of Group- and Strain-Specific Genetic Markers for Globally Distributed *Alexandrium* (Dinophyceae). 2. Sequence Analysis of a Fragment of the LSU rRNA Gene. *J. Phycol.*, **30**, 999–1011.

Simon, N., Campbell, L., Ornolfsdottir, E. *et al.* (2000) Oligonucleotide probes for the identification of three algal groups by dot blot and fluorescent whole-cell hybridization. *J. Euk. Microbiol.*, **47**, 76–84.

Taylor, F. J. R. and Fukuyo, Y. (1998) The neurotoxigenic dinoflagellate genus *Alexandrium* Halim: General Introduction. In Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. (eds), *Physiological Ecology of Harmful Algal Blooms*. Springer-Verlag, Heidelberg, pp. 381–404.

Tillmann, U. and John, U. (2002) Toxic effects of Alexandrium spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content. *Mar. Ecol. Prog. Ser.*, **230**, 47–58.