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Eckford-Soper, Lisa; Bresnan, Eileen; Lacaze, Jean-Pierre; Green, David; Davidson, Keith

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1 The competitive dynamics of toxic *Alexandrium fundyense* and non-toxic  
2 *Alexandrium tamarense*: the role of temperature

3 Lisa K. Eckford Soper<sup>1,3,\*</sup>, Eileen Bresnan<sup>2</sup>, Jean-Pierre Lacaze<sup>2</sup>, David H Green<sup>1</sup>,  
4 Keith Davidson<sup>1</sup>

5 <sup>1</sup>Scottish Association for Marine Science, Scottish Marine Institute, Oban, PA37  
6 1QA, UK

7 <sup>2</sup>Marine Scotland Science, 375 Victoria Road, Aberdeen, AB11 9DB.

8 <sup>3</sup>Marine Biological Section, Department of Biology, University of Copenhagen,  
9 Universitetsparken 4, DK- 2100 Copenhagen Ø, Denmark

10 \*Corresponding author. Email: [Lisa.eckfordsoper@bio.ku.dk](mailto:Lisa.eckfordsoper@bio.ku.dk)

11 Highlights

- 12 • Yield, growth and toxin production of *A. fundyense* and *A. tamarense* were  
13 studied.
- 14 • Observed growth rates varied with temperature.
- 15 • Cell/biomass yield was greater for *A. tamarense* at all temperatures.
- 16 • Species interactions were temperature dependent.
- 17 • *A. fundyense* toxin production was inhibited in co-culture with *A. tamarense*.

18

19 Key words:

20 *Alexandrium fundyense*, *Alexandrium tamarense*, biogeography, competitive  
21 interactions, toxicity, Scotland, FC-FISH

## 1 **Abstract**

2 The dinoflagellate *Alexandrium* produces paralytic shellfish poisoning toxins. The  
3 genus is globally distributed, with Scottish waters being of particular interest due to the  
4 co-occurrence of different species and strains. In Scottish waters, *Alexandrium* was  
5 historically thought to be dominated by the highly toxic (Group I) *A. fundyense*.  
6 However, the morphologically indistinguishable (Group III) *A. tamarense* has recently  
7 also been found to co-occur, raising important questions in relation to *Alexandrium*  
8 biogeography. To begin to address these, we investigated *Alexandrium* growth, yield  
9 and toxin production in a range of temperature conditions characteristics of present and  
10 potential future conditions, using a recently developed flow cytometry method that  
11 allowed, for the first time, simultaneous enumeration of the cryptic species in co-  
12 culture. Experiments were undertaken in a range of temperatures (12, 15, 18 and 21 °C)  
13 in the phosphate (P) limiting conditions that promotes *A. fundyense* toxicity.  
14 Cell/biomass yield was greater for *A. tamarense* at all temperatures, with observed  
15 growth rates varying with temperature. Growth rather and yield were different in mono-  
16 and co-culture with the outcome of these interactions also being temperature dependent.  
17 For toxic *A. fundyense*, GTX-3, STX and NEO were the dominant analogues, but total  
18 toxicity, toxicity per cell and the number of, and relative proportion of, toxin analogues  
19 changed in relation to the onset of P limitation and also as a function of temperature,  
20 with the highest toxin concentrations per cell being observed at 12 °C. Toxin  
21 concentrations were approximately double in P limited stationary phase compared to  
22 exponential growth. Toxin concentrations were lower in the co-cultures, indicating  
23 inhibition of production in the presence of non-toxic *A. tamarense*. The strong  
24 performance of *A. tamarense* is in co-culture at odds with the historical understanding  
25 that Scottish waters were dominated by *A. fundyense* and indicates that changes in water

1 temperatures, and also potentially alleopathic interactions, will influence *Alexandrium*  
2 populations and hence the PSP toxicity threat to humans from shellfish.

3

#### 4 **Introduction**

5 The observed frequency, intensity, and geographical distribution of observed harmful  
6 algal bloom (HAB) events have increased over the last few decades (Gowen et al.,  
7 2012; Hallegraeff, 1993; Lilly et al., 2007) with the genus *Alexandrium* being of global  
8 importance due to its widespread distribution and synthesis of potent neurotoxins that  
9 are associated with Paralytic Shellfish Poisoning (PSP). Humans, birds, sea mammals  
10 and fish can all be affected by PSP toxins, with the usual exposure route for humans  
11 being through the consumption of contaminated shellfish (Smayda., 2004).

12 The majority of *Alexandrium* based toxicity events are thought to have been  
13 caused by the morphospecies *A. tamarense*, *A. catanella* and *A. fundyense*, which make  
14 up the *A. tamarense* species complex (Scholin et al., 1994). The taxonomy of this  
15 complex has been questioned (Anderson et al., 1994; Scholin et al., 1995). Initially  
16 Scholin et al., (1994) demonstrated a correlation between strains and their geographical  
17 origin with five main phylogenetic clades defined: North American, Temperate Asian,  
18 Western European, Tropical Asian and Tasmanian. Subsequently Lilly et al. (2007)  
19 demonstrated these clades to have genetically distinct lineages and named them Group  
20 I-V. Previously in North West Europe, *A. tamarense* has been reported to occur as either  
21 the biotoxin producing Group I (North American) ribotype or the non-toxin producing  
22 Group III (Western European) ribotype, with their occurrences being geographically  
23 separated. Recently John et al. (2014) have reclassified Group I *A. tamarense* as *A.*  
24 *fundyense*, it is this reassignment that we use within this paper.

1           In Scottish (and northern English) waters, monitoring programmes have  
2 demonstrated that the genus *Alexandrium* is a common constituent of phytoplankton  
3 communities in spring and summer (Stubbs et al., 2014). Regional hotspots include:  
4 Orkney, Shetland, the Western Isles, the west coast and Northern England (Collins et al.,  
5 2009), however information on the biogeography of specific *Alexandrium* spp. in the  
6 region remains limited. Medlin et al. (1998) analysed the D1/D2 region of the LSU  
7 rRNA gene from number of isolates from the Orkney Islands and found them all to be *A.*  
8 *tamarense* (using the taxonomy of the time) rather than *A. fundyense*. The periodic high  
9 toxicity levels in Scottish shellfish, the lack of records of the non-toxic Group III  
10 *Alexandrium* above 55 °N (Töebe et al., 2013), and that UK monitoring programs only  
11 identify *Alexandrium* to genus level, led to the assumption that *Alexandrium* populations  
12 in Scottish waters were exclusively (toxin producing) Group I. Recent observations  
13 question this, as Group III cysts and vegetative cells have now been shown to have a  
14 wide geographical distribution in Scottish waters (Brown et al., 2010; Collins et al.,  
15 2009) and Group I and Group III cells have been co-observed in the water column, both  
16 in the Shetland Isles at 60 °N by Touzet et al. (2010), and in the North Sea at ~ 57 °N by  
17 Töebe et al. (2013). The spatially extensive distribution of Group III cells in the latter  
18 study clearly indicates that these observations are more than transient events, and  
19 coincide with the decline in the occurrence of PSP toxins in Scottish shellfish in recent  
20 years (Bresnan et al., 2008).

21           Töebe et al. (2013) and Touzet. (2010) have observed Group I *A. fundyense* and  
22 Group III *A. tamarense* co-occurring in the water column, with hybrid cysts having also  
23 been identified in Scottish sediment samples (Eckford-Soper, 2013), indicating that  
24 there is no *a priori* reason to assume that blooms of Group I and Group III cells will  
25 occur in isolation, and hence that both mono- and multi-species blooms might be

1 expected in Scottish waters. Lacking molecular studies of the sediment record, we can  
2 only speculate if these new observations relate to a change in the biogeography of the *A.*  
3 *tamarensis* species complex, a change in their relative composition, a historical  
4 misunderstanding of community composition, or are simply related to historical genus  
5 based monitoring. However, given the Group I *A. fundyensis* / Group III *A. tamarensis*  
6 co-occurrence it is clear that laboratory studies of their physiology should consider their  
7 co-occurrence, competition and interactions.

8         The aim of our study was therefore to investigate cell yield, growth rate, and  
9 toxin production of Group I *A. fundyensis* and Group III *A. tamarensis* obtained from  
10 Scottish waters in isolation and under co-culture. Experiments were conducted with  
11 strains maintained at a range of environmentally relevant temperatures and the nutrient  
12 conditions likely to promote toxicity of the *A. fundyensis*.

13

## 14 **Materials and Methods**

### 15 *2.1. Culture methods and cell acclimation*

16 Two Group I *A. fundyensis* (CCAP 1119/24 and 1119/28) and two Group III *A.*  
17 *tamarensis* (CCAP 1119/31 and 1119/33) strains that had previously been isolated by  
18 Marine Scotland Science from Scottish waters (Scapa, Orkney) and subsequently  
19 deposited in the Culture Collection of Algae and Protozoa (CCAP) at SAMS, were  
20 initially chosen for our study. Cultures were grown in 1litre Erlenmeyer flasks in batch  
21 mode, acclimated to a modified phosphate (3  $\mu$ M) L1 medium (Guillard and Hargraves.,  
22 1993) in which all other nutrients were available in excess. Experiments were conducted  
23 at four different temperatures: 12 °C, 15 °C, 18 °C and 21 °C. The three lower  
24 temperatures were chosen to represent the range of conditions that an *Alexandrium* cell  
25 might experience in Scotland presently or in future scenarios due to climate warming:

1 12 °C reflects the current sea surface temperatures of a typical Scottish spring; 15 °C the  
2 current sea surface temperatures of a typical Scottish summer; and 18 °C was chosen as  
3 a hypothetical summer temperature in the coming decades (Harrison et al., 2001;  
4 Hughes, 2007). It is unlikely Scottish seas will reach a temperature of 21 °C and hence  
5 only temporal changes in cell yields were determined at this temperature. A light  
6 intensity of 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and 12h:12h light:dark cycle was used in all  
7 experiments. Stock cultures were maintained in exponential growth by regular sub-  
8 culturing every 10 days. During acclimatisation to the different temperature the growth  
9 of the cultures was monitored using *in vivo* fluorescence (RFU) as a proxy for cell  
10 numbers (Turner Trilogy, UK). Experiments were only conducted when stock cultures  
11 exhibited a consistent and reproducible exponential growth rate when assessed using a  
12 General linear Model (GLM). Acclimation took between 4-7 re-inoculations depending  
13 on the temperature.

14

## 15 2.2. *Mono- and co-culture experiments*

16 Initial experiments were undertaken to evaluate any influence of agitation associated  
17 with sampling and the similarity of growth rates between different *A. fundyense* and *A.*  
18 *tamarensis* strains. Given the results of these experiments (detailed below in section 3)  
19 and logistical constraints of incubator space, subsequent mono-culture and co-culture  
20 experiments were conducted in triplicate on one toxic *A. fundyense* (1119/28) and one  
21 non-toxic *A. tamarensis* (1119/33) strain.

22 In the mono-cultures experiments *A. fundyense* and *A. tamarensis* were studied  
23 separately, and in the co-culture experiments they were grown in the same flasks.  
24 Inoculums for all experiments were taken from acclimated late exponential phase stock  
25 cultures. The inoculum cell density in the mono-cultures was  $\approx 400 \text{ cells ml}^{-1}$ , while in

1 the co-cultures each strain was added at an initial concentration of  $\approx 200$  cell ml<sup>-1</sup>. The  
 2 same modified L1 medium described in section 2.1 was used. Cells were allowed to  
 3 grow in batch mode until the post stationary phase of the culture had become  
 4 established.

### 5 2.3. Enumeration of total cell abundance & growth rate determination

6 Daily sub-samples were removed aseptically from each flask. Cells were preserved with  
 7 Lugol's iodine solution (1% final concentration) and enumerated using a 1 ml  
 8 Sedgewick-Rafter counting chamber. Growth rate was calculated as divisions per day.  
 9 Using the logistic growth equation (Verhulst, 1838) we were able to take into account  
 10 the impact of the carrying capacity on growth, Equation 1:

$$\frac{dN}{dt} = \mu N \left( \frac{K - N}{K} \right) \quad (1)$$

11  
 12 Where  $dN/dt$  is the expected rate of change in the population size at any point in time  
 13 given the population size (N), the carrying capacity of the environment (K), and the  
 14 intrinsic growth rate ( $\mu$ ). The intrinsic rate of growth is the rate of growth when  
 15 individuals are not constrained by environmental limits. The integrated form of the  
 16 logistic equation, Equation 2 (Ferris and Wilson, 1987) was fitted (using a least  
 17 squares approach):

$$N_{(t)} = \left( \frac{N_0 K}{N_0 + (K - N_0) e^{-\mu t}} \right) \quad (2)$$

### 19 20 2.4. Discrimination and enumeration of *A. fundyense* and *A. tamarensis* in co-culture

21 Daily sub-samples were collected for fluorescent *in situ* hybridisation based  
 22 enumeration of the different cryptic species (Eckford-Soper et al., 2013). A subsample  
 23 of culture (10 ml) was removed and made up to 40 ml using autoclaved filtered



1 seawater. This sample was fixed with formalin (1 % final concentration) and left to rest  
2 for one hour before being centrifuged (4000 g, 10 mins). The supernatant was then  
3 discarded. Ice cold methanol (10 ml) was added to the cell pellet to extract the pigments  
4 and nucleic acids (Touzet et al., 2010). The sample was stored at -20 °C until analysis.

5       The taxa specific oligonucleotide probes TamA (for *A. tamarensis*) and  
6 TamToxC (for *A. fundyensis*) (Touzet et al., 2010) were used to fluorescently label the  
7 different strains. Samples were removed from the -20 °C freezer and centrifuged (4000  
8 g, 5 min) and the methanol supernatant aspirated off. Hybridisation buffer (5X SET and  
9 0.1 % IGEPAL) was added (500 µl) to the cell pellet and the cells re-suspended to rinse  
10 off the methanol. The samples were centrifuged (4000 g, 5 min) and the supernatant  
11 removed. This process was repeated to ensure that the methanol was removed from the  
12 sample. Cells were re-suspended in 500 µl of hybridisation buffer containing 1 µl of  
13 each of the taxa specific probes. After this time the samples were kept in the dark by  
14 wrapping the tubes in aluminium foil and incubated in a dark incubator (55 °C, 60 min).  
15 After hybridisation, the cells were pelleted by centrifugation (4000 g, 5 min) and the  
16 supernatant discarded. Samples were washed with 500 µl of preheated (55 °C) 0.2X  
17 SET buffer to remove the excess unbound probes. Samples were then centrifuged (4000  
18 g, 5 min) one final time and the supernatant removed. Finally, the cells were re-  
19 suspended in 2.5 ml of autoclaved filtered seawater for subsequent enumeration.

20       Flow cytometric identification and enumeration of the labelled cells was  
21 conducted using a FACSort (Beckton Dickinson) instrument fitted with a blue argon  
22 (488 nm) laser and a 150 µm instrument aperture. Discrimination and enumeration of  
23 the two ribotypes was achieved based on side scatter (SSC) and fluorescence detection  
24 of the TamToxC and TamA probes within the FL1 (515-545 nm) channel (Eckford-  
25 Soper et al., 2013). Aliquots (1 ml) were analysed with fluorescent acquisition, gated by

1 light scatter parameters. The sample was analysed rapidly (within two minutes) as the  
2 probes are quickly degraded by light.

3

4

#### 5 *2.5. Toxin analysis*

6 Toxin analysis was only carried out on samples incubated at 12, 15 and 18°C. Samples  
7 were collected during the exponential and stationary phases of growth. Aliquots were  
8 harvested aseptically containing a known concentration (typically ~ 250,000 cells) and  
9 centrifuged (3000 g, 20 min). The supernatant was removed and the cell pellet was  
10 stored at – 20 °C. Subsequent analysis was conducted by the post-column oxidation  
11 (PCOX) that uses reversed phase liquid-chromatography (LC) with post-column  
12 oxidation and fluorescent detection (Van De Riet et al., 2009). Two analyses were  
13 performed per sample, firstly to analyse GTX and STX toxins and secondly to analyse  
14 the C-toxins. The samples were tested for GTX-1, GTX-2, GTX-3, GTX-4, de-GTX-3,  
15 dc-GTX-2, dc-STX, STX, C-1 and C-2.

16 Prior to analysis cell pellets were thawed and 1ml of autoclaved filtered seawater  
17 was added to the sample and vortexed. The solution was transferred to an Eppendorf  
18 micro-centrifuge tube. To ensure all cells were transferred the process was repeated  
19 using an additional 200 µl of filtered seawater. The cell solution was centrifuged (3000  
20 g, 20 min), the supernatant discarded, and acetic acid (500 µl of 0.5 M) was added to the  
21 cell pellet. Toxin extraction was carried out by freezing at -80 °C (30 min) and thawing  
22 at room temperature (30 min) three times. The samples were vortexed and centrifuged  
23 (8000 g, 20 min). Aliquots (200 µl) of the toxin extracts were transferred to a Millipore  
24 Ultrafree MC 0.2 µm filter unit and centrifuged (6000 g, 3 min) (Van De Riet et al.,  
25 2009). The contents were then transferred to glass reaction vials.

1           The working solutions were prepared from individual PSP standards purchased  
2 from NRC (Canada). The stock standard mixture and serial dilutions for the instruments  
3 linear calibration were prepared as described in Van De Riet et al. (2009). All  
4 calibration curves demonstrated good linearity with the  $r^2$  value being greater than 0.99.  
5 To ensure instrument reliability a previously quantified sample was analysed before the  
6 culture samples.

7           The analysis system consisted of: the LC system, post column reaction system,  
8 reaction coil, fluorescent detector and LC columns. The mixed working solution and  
9 serial dilutions (10  $\mu$ L for GTX and STX toxins and 5  $\mu$ L for C-toxins) were injected  
10 into the system and separated chromatographically. Part of the extract was  
11 chromatographed with a step gradient using a heptane sulfonic acid/phosphoric acid  
12 buffer system for the analysis of GTX and STX analogues. The extract was also  
13 chromatographed for the C-toxins using an isocratic tetrabutylammonium phosphate  
14 buffer for the C-toxin analogues. The toxins were detected by PCOX of the analytes at  
15 85 °C with a phosphoric acid periodic acid buffer solution followed by fluorescence  
16 detection (excitation: 330 nm, emission: 390 nm). Integration and data analysis was  
17 conducted using ChromQuest 2.6.1 software. To calculate the concentration of the  
18 toxins as STX equivalents, the concentrations of toxins (in nM) were converted to  
19  $\mu$ moles and multiplied by the relative toxicities of each individual toxin (Van De Riet et  
20 al., 2009).

21

## 22 *2.6. Other parameters*

23           The pH of each flask could be measured using a SevenEasy pH meter and  
24 calibrated using two solutions with known pH values. Every second day samples were  
25 removed aseptically for the determination of intracellular biomass and extracellular

1 nutrient concentrations. For particulate carbon and nitrogen (POC, PON), samples were  
2 collected by filtering 60-100 ml of culture (depending on the cell density) through a 25  
3 mm diameter pre-combusted (450 °C for 4h) GF/F filter (Fehling et al., 2005), which  
4 was then frozen at -20 °C for subsequent analysis. Aliquots (50 ml) of the filtrate were  
5 removed and stored at -20 °C for extracellular nutrient analysis (total phosphorus (TP)  
6 and total nitrogen (TN)). After defrosting TP and TN were analysed using a Lachat  
7 QuikChem auto analyser with Omnion 3.0 software (Davidson et al., 2007). POC  
8 measurements were made as described in (Flynn and Davidson, 1993) using a 20-20  
9 stable isotope mass spectrometer (PDZ Europa) with an ANCA-NT prep system  
10 calibrated with isoleucine.

11

## 12 **3. Results**

### 13 *3.1 Preliminary experiments*

14 Initial monoculture experiments allowed comparison of the growth rate of the two *A.*  
15 *fundyense* and two *A. tamarensis* strains. Growth rates of both *A. fundyense* and both *A.*  
16 *tamarensis* strains were found to not be statistically significantly different (GLM  $P > 0.05$ ),  
17 with no influence of agitation on the calculated rates. Hence, only one *A. fundyense* strain  
18 (CCAP 1119/28) and one *A. tamarensis* strain (CCAP 1119/31) were used for all further  
19 experiments.

### 20 *3.2. Mono-culture: temporal dynamics*

21 *A. fundyense* and *A. tamarensis* were studied in the triplicated mono-culture experiments at  
22 the four experimental temperatures, and both exhibited net positive growth at all the  
23 temperatures studied (Fig. 1). The linear phase of logarithmic plots of cell abundance were  
24 examined, and in all cases exponential growth ceased a few days after the concentrations of

1 P (the yield limiting nutrient) reached its minimum value (data not shown). However,  
2 differences in response were evident between *A. fundyense* and *A. tamarensis* at different  
3 temperatures. In general, the length of the exponential phase increased with temperature  
4 until 18 °C, and at all temperatures the duration of the exponential phase of cell division was  
5 longer for *A. tamarensis*.

6

### 7 3.3. Mono-culture: cell yields and growth rates

8 Spearman Rank correlation analysis found statistically significant linear correlations  
9 between cell abundance and POC for both ribotypes at all temperatures evaluated (12 °C: *A.*  
10 *fundyense*,  $r = 0.954$ ,  $p = 0$ ; *A. tamarensis*  $r = 0.939$ ,  $p = 0$ . 15 °C . *A. fundyense*  $r = 0.939$ ,  $p =$   
11  $0$ ; *A. tamarensis*  $r = 0.973$ ,  $p = 0$ . 18 °C, *A. fundyense*,  $r = 0.918$ ,  $p = 0$ , *A. tamarensis*,  $r =$   
12  $0.923$ ,  $p = 0$ ), demonstrating that cell numbers were correlated with biomass.

13 Mean peak cell density of the replicate *A. tamarensis* cultures exceeded that of *A.*  
14 *fundyense* at all temperatures (Fig. 1). This difference was significant at all temperatures  
15 except 15 °C (Mann-Whitney,  $p < 0.05$ ). While the extracellular limiting P decreased through  
16 uptake to a low concentration, it was not always completely eliminated from the cultures,  
17 and between 0.01 – 0.22  $\mu\text{M}$  remained (data not shown). Fig. 2 therefore displays peak cell  
18 yields normalised per  $\mu\text{M}$  of P utilised. This confirms that the *A. tamarensis* strain was able  
19 to create a greater cell yield (and biomass) per unit of resource ( $p < 0.05$ ).

20 Mean cell specific growth rate calculated is plotted as a function of temperature in  
21 Fig. 3, and is tabulated in Table 1. Growth rates exhibited a generally “humped” response for  
22 both strains, with highest rates at the intermediate temperatures of 15 and 18 °C and lowest at  
23 12 °C. The bell shaped curve was more pronounced for *A. fundyense*, which had temperature  
24 optima of 18 °C with a sharp decline in growth rate either side of this. *A. tamarensis* had a  
25 broader tolerance range at all temperatures except 12 °C. *A. tamarensis* had a maximum SGR

1 that was statistically significantly higher (GLM  $p > 0.05$ ) than that of *A. fundyense* at 12 °C,  
2 but at 18 °C the reverse was true (Fig. 3).

3

#### 4 3.4 Mono-culture: PSP toxins

5 The amount and composition of STX produced by *A. fundyense* varied with both growth  
6 phase and temperature (Fig. 4). At all temperatures, cellular toxin concentrations  
7 increased considerably once cells entered stationary phase, with a  $\geq 2$ -fold increase in  
8 comparison to the exponential phase of growth. This increase was due to an increase in  
9 individual analogue concentrations, and at 12 °C and 18 °C an increase in the total  
10 number of analogues detected. STX equivalents per cell were greatest at 12 °C (Fig. 4a),  
11 with concentrations in the exponential phase being approximately one to two thirds  
12 higher than at 15 °C (Fig. 4b) and 18 °C (Fig. 4c).

13 While STX was the main toxin analogue found at all temperatures (Fig. 4), a  
14 range of others were present. In exponential growth at 12 °C the analogues GTX-1,  
15 GTX-3, GTX-2, GTX-5 dc-GTX-3, NEO, STX, C1 and C2 were found. GTX-1, GTX-  
16 2, dc-GTX-3, GTX-3, NEO, STX and C-1, C-2 were all present at 15 °C. Fewer  
17 analogues were present at 18 °C with: dc-GTX-3 GTX-3, NEO, STX, C-1 and C-2  
18 being observed. On entering stationary growth the number of analogues remained  
19 unchanged at 15 °C but increased at both 12°C (to include GTX-2) and 18 °C with the  
20 number of analogues now including: GTX-1, GTX-3, dc-GTX-3, NEO, STX, C-1 and  
21 C2 (Fig. 4). GTX-2 and GTX-5 were not found at this temperature and the analogues  
22 GTX-4, dc-GTX-2 and dc-STX were not found at any temperature.

23 Cellular toxicity did not translate into total toxicity per unit of culture volume.  
24 For example, although cellular toxicity was greatest at 12 °C, the total toxicity per unit  
25 of culture volume was lowest at 12 °C (2845 pg STX diHCleq ml<sup>-1</sup>) and highest at 15

1 °C (5583 pg STX diHCleq ml<sup>-1</sup>) with no significant linear relationship with temperature  
2 ( $r^2 = 36.9$ ,  $p = 0.584$ ).

3

#### 4 3.5 Co-culture: cell yields and growth rates

5 Growth rate estimates calculated by light microscopy (Sedgwick Rafter) and flow  
6 cytometry are compared in Table 1. Growth rates calculated by each method were found  
7 to not be statistically significantly different (GLM  $P > 0.05$ ).

8 Fig. 1 shows the changes with time of the combined abundance of *A. fundyense*  
9 and *A. tamarensis* enumerated by FISH-FC in the co-culture experiments (in comparison  
10 to their growth in monoculture). Fig. 5, explores the dynamics of these culture in more  
11 detail by displaying the temporal evolution of cell densities of each of the competing  
12 cryptic species.

13 At all temperatures interaction between the two strains was evident. The effect  
14 was most clearly seen at 12 °C where *A. tamarensis* dominated to the near exclusion of  
15 *A. fundyense*. In this case, the peak yield of *A. tamarensis* was 87 % of the combined  
16 peak cell yield. While net growth of the *A. fundyense* did occur, it increased from 200  
17 cells ml<sup>-1</sup> to a mean peak density of only 599 cells ml<sup>-1</sup> and did not exhibit clear  
18 exponential growth. A competitive dominance of *A. tamarensis*, although not to such a  
19 great extent, was also evident at 15 °C and 21 °C. In these cases, the peak yield of *A.*  
20 *tamarensis* was 68% and 67% of the respective totals. However, at 18 °C these  
21 interactions were more balanced with similar peak densities (*A. fundyense* achieving  
22 51% of the total). At all temperatures, except 18 °C where yields were similar, the  
23 dominance of the *A. tamarensis* peak cell density over *A. fundyense* was statistically  
24 significant (Mann-Whitney  $p > 0.05$ ). In co-culture the peak cell densities achieved by  
25 *A. tamarensis* were relatively constant at all temperatures, varying from 4156 cells ml<sup>-1</sup>

1 at 18 °C to 5350 cells ml<sup>-1</sup> at 21 °C. However, the peak cell density of *A. fundyense*  
2 varied much more markedly with temperature from 599 cells ml<sup>-1</sup> at 12 °C to 2847 cells  
3 ml<sup>-1</sup> at 18 °C.

4 Maximum specific growth rates of the co-cultures (Fig. 3) exhibited a similar  
5 'humped response' for *A. fundyense* as was evident in monoculture. For *A. tamarensis*,  
6 the response was similar to monoculture at the three higher temperatures, but a  
7 markedly higher growth rate was evident in co-culture at 12 °C.

8

### 9 3.6. Co-culture: toxins

10 In co-culture, the pattern of increased toxicity from exponential to stationary phase  
11 evident in the *A. fundyense* monocultures was conserved (Figs. 4a-c). However the toxin  
12 per (*A. fundyense*) cell was markedly lower than in monoculture at all of the  
13 temperatures studied. At 15 °C the same toxin analogues were present as in  
14 monoculture. However, at 12 °C only GTX-3, NEO, STX, C-1 and C-2 were present  
15 during exponential growth with the addition of GTX-1, GTX-3, GTX-5, dc-GTX-3,  
16 STX and C-2 in stationary phase. At 18°C GTX-1 was absent from the mixed culture  
17 samples with GTX-3, NEO, STX, C-1 and C-2 being present during exponential  
18 growth, plus dc-GTX-3 in the stationary phase.

19 The observed toxin concentration normalised per unit culture volume was  
20 consistently lower in co-culture culture compared to monoculture. Concentrations  
21 exhibited a clear linear relationship with temperature ( $r^2 = 99.8$ ,  $p = 0.025$ ), increasing  
22 from (305 pg STX diHCl<sub>eq</sub> ml<sup>-1</sup>) at 12 °C to (2517 pg STX diHCl<sub>eq</sub> ml<sup>-1</sup>) at 18 °C.

23

## 24 4. Discussion

### 25 4.1 *A. tamarensis* in Scottish waters



1 There are no major physical barriers to transport of phytoplankton cells in the waters  
2 surrounding the British Isles, as evidenced by examples of large distance transport of  
3 *Dinophysis* spp. and *Karenia mikimotoi* (Davidson et al., 2009; Farrell et al., 2012;  
4 Whyte et al., 2014). The coastal current of the Scottish west coast (Simpson and Hill,  
5 1986) provides a mechanism of northwards transport of cells, with the fjordic sea lochs  
6 that dominate the Western and Northern coastline of Scotland providing an ideal refuge  
7 for cyst forming organisms such as the *Alexandrium*. However, biological barriers to  
8 range expansion may exist, with temperature tolerance being thought to be one of these  
9 (Hinder et al., 2011). For example, we have previously found strains of *A. minutum* to  
10 be unable sustain net positive growth at 12 °C (Davidson et al., 1999), an observation  
11 that may in part explain the lack of *A. minutum* in the relatively cold Scottish waters.  
12 Temperature is a key driver of ecological processes and a key abiotic driver of  
13 ecological systems; yet, the effects of temperature are complex. A review of 688 species  
14 by Tylianakis et al. (2008) found that temperature alterations affect a number of species  
15 to species interactions, including competition. Community structure is not just affected  
16 by the direct impacts of temperature on physiology but also on how these direct impacts  
17 affect other processes (Reuman et al., 2014). A better general understanding of how the  
18 physiological influences of temperature will give us a better understanding of species to  
19 species interactions and ultimately community dynamics. For example, Tilman et al.  
20 (1981) studied the competition interactions between two freshwater diatoms  
21 *Asterionella formosa* and *Synedra ulna* (Nitzsch). They found that that due to their  
22 ability to reduce environmental silica at different temperatures *A. formosa* was able to  
23 displace *S. ulna* at temperatures below 20 °C with the reverse being true above 20 °C.

24 To ensure the results of our study were not an artefact of rapidly changing  
25 temperature regimes, cultures were first acclimated to the different temperatures.

1 Experiments were only conducted when the stock cultures exhibited a consistent and  
2 reproducible exponential growth rate. Although Scottish waters do not reach 21 °C, the  
3 inclusion of this temperature allowed us to study the growth and interaction response of  
4 these globally important organisms over a wider range of temperatures. Our  
5 observations of positive net growth of the different *tamarensis* strains at all temperatures  
6 studied, and that the growth rate and cell yield of *A. tamarensis* exceeded that of *A.*  
7 *fundyensis*, at the lowest experimental temperature in our study (12 °C) demonstrates  
8 that temperature should not be an ecophysiological barrier to Group III *A. tamarensis*  
9 survival in Scottish waters. Results at 12 °C are consistent with the study of Touzet et  
10 al. (2010) who found co-existence of Group I and Group III cells in the water column at  
11 this temperature. Both ribotypes were observed at even lower temperatures (10 °C) by  
12 Töbe et al. (2013). The “appearance” of Group III *A. tamarensis* in Scottish waters is  
13 therefore likely not related to a climate-mediated northward increase in temperature,  
14 suggesting that Group III *A. tamarensis* blooms may have been much more spatially and  
15 temporally extensive than previously recognised.

16

#### 17 4.2 Yield and growth rates

18 Differences in cell yield between the different strains occurred at all  
19 temperatures. Cell densities were lower than those that might be expected to be  
20 impacted by light limitation through self-shading (Agusti., 1991). Furthermore, while  
21 growth in culture can often be inhibited by pH, it did not exceed 8.4 in our experiments  
22 (data not shown) and *A. tamarensis* can typically tolerate a pH of above 9 (Hansen,  
23 2002; Schmidt and Hansen, 2001).

24 It is well known that different species of phytoplankton can display a wide range  
25 of cellular composition with plasticity in cellular nutrient content and ratios, with

1 nutrient limiting conditions typically extending the range of C:N:P stoichiometry  
2 through increases in C:N or C:P ratios for N and P limited cells respectively (Flynn et  
3 al., 1993; Geider and La Roche., 2002). Hence the greater mean cell yield (and given  
4 the correlation between cells and C, of biomass yield) that was achieved by *A.*  
5 *tamarensis* at all temperatures indicates a more efficient utilisation of P.

6         The lower yield of *A. fundyensis* raises the question of whether the production of  
7 toxin has an energetic cost to the cell that diverts resources from biomass production.  
8 However, the fraction of cellular N associated with PSP toxins is small, and in a  
9 modelling study Flynn (2002) found toxin production to have no significant metabolic  
10 cost to the cell. Hence, a direct influence of toxin producing capability on the lower  
11 yield of *A. fundyensis* seems unlikely. The difference in cell/biomass yield achieved with  
12 the same unit of resource (P) is therefore consistent with these being co-existing  
13 sympatric cryptic species (Töebe et al., 2013; John et al., 2014).

14         While the observed increase in maximum cell yield with increasing  
15 (monoculture) temperature exhibited by the different strains was not statistically  
16 significant, this response was similar to *Alexandrium catenella* isolated from the Thau  
17 lagoon in Southern France (Laabir et al., 2011), that exhibited an increase in peak cell  
18 yield per unit limiting nutrient over the range of 12 to 24 °C.

19         The optimal temperature for *A. fundyensis* growth in monoculture was 18 °C,  
20 with a sharp decline in growth rate either side of this optima. *A. tamarensis* exhibited a  
21 lower maximum but a broader tolerance range, perhaps indicating a different growth  
22 strategy. Laabir et al. (2011) summarised published growth rates for different  
23 *Alexandrium* species, with the values obtained in our experiments falling within the  
24 observed range for *A. tamarensis*. Taylor et al. (2014) observed growth rates ranging  
25 from 0.1-0.5 div day<sup>-1</sup> depending on the strain and culture conditions. Average growth

1 rates for Scottish Group I isolates were 0.2 +/- 0.2 in comparison to 0.24 +/- 0.03 for  
2 English Group III isolates. Other studies carried out on some tropical strains of *A.*  
3 *tamarensis* exhibited maximum growth rates at considerably higher water temperatures  
4 than those in our experiments (Lim and Ogata, 2005). Our observed temperature  
5 response, with an optimal of 15-18 °C for both strains, is consistent with the response  
6 one might expect for other dinoflagellates from temperate northern latitudes (Eppley,  
7 1972), including Danish isolates of *A. ostenfeldii* (Jensen and Moestrup, 1997). The  
8 preference of both strains for the intermediate water temperatures is also consistent with  
9 the observation that most Scottish *Alexandrium* blooms occur in summer when upper  
10 water column water temperature is typically 14-16 °C (Fehling et al., 2006). While *A.*  
11 *fundyensis* growth rates in particular were depressed at the lowest temperature (12 °C),  
12 growth did occur, consistent with the ability of *Alexandrium* to sometimes bloom during  
13 the Scottish spring.

14

#### 15 4.3 Co-culture interactions

16 Given that *A. fundyensis* and *A. tamarensis* are morphologically identical, experiments to  
17 study their competitive interaction on a temporal basis have previously been  
18 impractical. While the development of taxa-specific molecular assays, such as  
19 fluorescent *in situ* hybridisation (FISH) probes targeting rDNA regions, has allowed the  
20 identification and enumeration of cryptic species in field samples (Touzet et al., 2010),  
21 the time intensive nature of the fluorescence microscopy based cell enumeration that  
22 forms part of this technique, makes it impractical to apply to time course laboratory  
23 culture studies that generate large numbers of samples. However, by combining FISH  
24 based labelling of cells with their flow cytometric enumeration (Eckford-Soper et al.,  
25 2013) we were able to achieve rapid discrimination and enumeration of the different

1 strains. Such an approach makes, for the first time, mixed community experiments  
2 practicable. It could easily be extended to incorporate other species of *Alexandrium* or  
3 to study the dynamics other genera that contain morphologically indistinct species.

4 A clear finding from our experiments was that the interaction of the different  
5 toxic and non-toxic strains had the potential to influence the composition and toxicity of  
6 the *Alexandrium* “bloom” as a whole. At all temperatures, the cell yield in co-culture  
7 was approximately mid-way between the respective higher and lower values exhibited  
8 by *A. fundyense* and *A. tamarensis* in monoculture, but as demonstrated by Fig. 5 this  
9 mixed community did not contain an equal abundance of the different cryptic species.  
10 Nor did it achieve the same ratio of *A. fundyense* and *A. tamarensis* cells that would be  
11 expected from their relative performance in the monocultures. One possible cause is the  
12 production of growth inhibiting substances that was first considered by Harder (1917) in  
13 *Nostoc punctiforme*, and has now been shown to be common (Ikawa et al., 1997). These  
14 substances can either affect other organisms (hetero-inhibition) or themselves (auto-  
15 inhibition) (Stephens et al., 2010). Such alleopathy has been demonstrated for  
16 *Alexandrium* (Fistarol et al., 2004), but no attempt was made to evaluate alleopathic  
17 chemicals here.

18 Temperature has also previously been demonstrated to have an important  
19 influence on the outcome of competition between phytoplankton species in both the  
20 laboratory and outdoor mass culture (Goldman and Mann, 1980; Goldman, 1977) and  
21 we found this parameter to influence the competitive yields of *A. fundyense* and *A.*  
22 *tamarensis*. The interaction effect was most pronounced at 12 °C where *A. tamarensis*  
23 out-competed *A. fundyense* by a large margin. *A. tamarensis* was also dominant at 15 °C  
24 and 21 °C, but at 18 °C, the different strains had approximately equal maximum yields.  
25 In co-culture *A. tamarensis* exhibited higher or similar maximum specific growth rate

1 that *A. fundyense* at three out of the four temperatures, with the exception being 18 °C.  
2 This suggests that changes of just a few degrees could have a large impact on  
3 interaction between the two cryptic species.

4 The results discussed above are consistent with recent field evidence that,  
5 should a suitable seed population be present, non-toxic group III *A. tamarense* blooms  
6 could develop in Scottish waters to the competitive detriment of toxic group I *A.*  
7 *fundyense* should they co-occur. Given that Brosnahan et al. (2010) found that the  
8 zygotes formed from mixing Group I and Group III cells were not viable, and hence that  
9 co-existence is likely to persist, the competitive interactions within mixed populations  
10 are likely to be important to the toxicity of *Alexandrium* blooms in Scottish waters.

11

#### 12 4.4 Toxins

13 Different *Alexandrium* species/strains produce PSP toxins in differing  
14 compositions and concentrations with cellular toxin also varying as a function of  
15 nutrient limitation (Boyer et al., 1987; John and Flynn., 2000), growth stage (Proctor et  
16 al., 1975; Anderson et al., 1990a; Flynn et al., 1994), water temperature (Ogata et al.,  
17 1987) and salinity and senescence (Landsberg, 2002). No saxitoxin or analogues were  
18 produced by *A. tamarense* in any phase of growth, consistent with the reported lack of  
19 toxicity of these group III cells. In contrast, toxin production by *A. fundyense* occurred  
20 during exponential growth, but with a marked increase in total toxicity (approximately  
21 double) in the P limited stationary phase. Such observations are consistent with other  
22 *Alexandrium* species (*A. minutum* and *A. fundyense*) (Boyer et al., 1987; Anderson et  
23 al., 1990b; Flynn et al., 1994; Lippemeier et al., 2003).

24 Characteristic toxin profiles of individual isolates or strains have been suggested  
25 as a phenotypic markers (Cembella and Taylor, 1986; Anderson et al., 1994; Flynn et

1 al., 1994). A number of studies have observed that *A. tamarense* exhibits a “relatively  
2 stable” profile of different STX analogues (Boyer et al., 1987; Cembella et al., 1987).  
3 Others have observed significant changes to the toxin profile with life cycle stage,  
4 irradiance, temperature and nutrient availability (John and Flynn. 2000), with large  
5 changes in toxin composition of individual isolates having been observed in *A.*  
6 *tamarense* and *A. catenalla* (Boczar et al., 1988), *A. fundyense* (Anderson et al., 1990b)  
7 and *A. minutum* (Lippemeier et al., 2003; Xu et al., 2012). Our study is consistent with  
8 these latter observations, with toxin composition varying with changing nutritional  
9 status and, as discussed below, temperature and competition. STX, NEO and GTX-3  
10 were the dominant analogues produced by our cultures, with other analogues found in  
11 lower concentrations. STX, NEO, GTX-3, as well as GTX-4 were also the dominant  
12 analogues in the toxin profile of two different Group I strains isolated from Scottish  
13 waters (Collins et al., 2009). However, the toxin profiles observed by Collins et al.  
14 (2009) and Medlin et al. (1998) had more analogues than our study. The roles of  
15 individual toxin analogues have not been established so it difficult to hypothesise why,  
16 under different conditions, different toxin analogues were synthesised in differing  
17 combinations.

18       There are a number of theories as to why some harmful algal species produce  
19 toxins. One is that toxin production is adaptation by some dinoflagellates to overcome  
20 the ecological disadvantage of low nutrient affinity. Frangopulos et al. (2004) observed  
21 that there was a negative relationship between toxin content per cell and P-uptake  
22 efficiency, suggesting that as toxin production is enhanced under P-limitation, grazing  
23 pressure would be redirected towards the Group III cells (Guisande et al., 2002). The  
24 elevated toxin concentrations we observed at 12 °C are apparently consistent with the  
25 finding of Proctor et al. (1975), Sui et al. (1997), Anderson. et al. (1990) and Hamasaki

1 (2001), who demonstrated that toxic dinoflagellates have a higher cellular toxicity at  
2 low temperatures. It has been hypothesised that lower temperatures act to reduce protein  
3 synthesis, resulting in an excess of arginine which is thought to be a precursor of toxin  
4 biosynthesis (Anderson et al., 1990b). Arginine availability and thus toxin  
5 concentrations are thought to increase further in low P but high N conditions, such as  
6 those used in this study (Flynn et al., 1994). Alternatively, the higher cellular toxin  
7 content at low temperature could be an adaptation to lower cell numbers as it has been  
8 proposed that PSP toxins may act as a chemical signal, stimulating mating in  
9 *Alexandrium* spp., and therefore the higher PSP toxin concentrations in non-optimum  
10 conditions may be a strategy to increase the chances of mating to compensate for their  
11 lower cell densities (Lim and Ogata., 2005; Wyatt and Jenkinson., 1997). Nevertheless,  
12 despite the higher cellular toxin concentrations in our cultures at 12 °C, the total toxin  
13 concentration per unit culture volume were higher at the temperatures where higher cell  
14 yields were evident (15-21 °C).

15 A surprising finding was that the total toxicity per ml and per cell was lower in the  
16 cultures grown in mixed culture, suggesting some form of inhibition of toxin  
17 production. This difference was most evident at 12 °C and 15 °C where the total toxicity  
18 per ml of co-cultured *A. fundyense* cells was approximately four times less than that in  
19 monoculture. Guisande et al. (2002) suggested that toxin production acts to inhibit  
20 competing co-occurring species. While direct evidence of this remains limited, as noted  
21 above a number *Alexandrium* species are thought to synthesise allelochemicals that may  
22 have a number of negative ecological functions. These chemicals appear to not be  
23 related to PSP toxicity but rather appear to have other modes of action such as lytic  
24 compounds (Ma et al., 2009; Tillmann and John., 2002; Fistarol et al., 2004). Some



1 form of alleopathic interaction is therefore a potential explanation for the observed  
2 suppression of *A. fundyense* toxicity when in co-culture.

3

4

## 5 **5. Conclusions**

6 Using a novel application of FISH and flow cytometry we were able, for the first time,  
7 to conduct the first laboratory growth, competition and toxin production study on  
8 morphologically identical toxic Group I *A. fundyense* and non-toxic Group III *A.*  
9 *tamarensis*.

10 The influence of temperature on a range of parameters was evident from  
11 monoculture experiments. Alterations in temperature had a marked effect on toxicity  
12 and toxin profile. Our results therefore suggest that changing water temperatures will  
13 influence the growth, competition and hence biogeography of the *A. tamarensis* species  
14 complex in Scottish waters. Despite this, temperature cannot exclusively explain all the  
15 interactions we observed between cells grown together, especially the impact of co-  
16 culture on toxicity. This would suggest that there are other interactions occurring  
17 between the cells that we are not yet able to quantify.

18

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## **References**

- Agusti, S., 1991. Light environment within dense algal populations: cell size influences on self-shading. *J. Plankton Res.* 13, 853–871.
- Anderson, D.M., Kulis, D.M., Doucette, G.J., Gallagher, J.C., Balech, E., 1994. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. *Mar. Biol.* 120, 467–478.
- Anderson, D.M., Kulis, D.M., Sullivan, J., Hall, S., 1990. Toxin composition variations in one isolate of the dinoflagellate *Alexandrium fundyense*. *Toxicon* 28, 885–893.
- Anderson, D.M., Kulis, D.M., Sullivan, J., M., Hall, S., Lee, C., 1990b. Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. *Mar. Biol.* 104, 511–524.
- Boczar, B.A., Beitler, M.K., Liston, J., Sullivan, J.J., Cattolico, R.A., 1988. Paralytic Shellfish Toxins in *Protogonyaulax tamarensis* and *Protogonyaulax catenella* in Axenic Culture. *Plant Physiol.* 88, 1285–90.
- Boyer, G.I., Sullivan, J., Andersen, R.J., Harrison, P.J., Taylor, F.J.R., 1987. Effects of limitation on toxin production and composition in the marine dinoflagellate *Protogonyaulax tamarensis*. *Marine Biol.* 128, 123–128.
- Bresnan, E., Turrel, E.A., Fraser, S., 2008. Monitoring PSP toxicity and *Alexandrium* hotspots in Scottish waters, in: Moestrup Ø., Doucette G., Enevoldson H., Godhe A., Hallegraeff G., Luckas B., Lundholm N., Lewis J., Rengefors K., Sellner K., Steidinger K., Tester, P., Zingone A. (Eds.) Proceedings of the 12th International Conference on Harmful Algae. Intergovernmental Oceanographic Commission of UNESCO, Copenhagen. pp 76-79,
- Brosnahan, M.L., Kulis, D.M., Solow, A.R., Erdner, D.E., Percy, L., Lewis, J., Anderson, D.M., 2010. Outbreeding lethality between toxic Group I and non-toxic Group III *Alexandrium tamarensis* spp. isolates: Predominance of heterotypic encystment and implications for mating Interactions and biogeography. *Deep. Res. II* 57, 175–189.

- 1 Brown, L., Bresnan, E., Graham, J., Lacaze, J.-P., Turrell, E., Collins, C., 2010. Distribution,  
2 diversity and toxin composition of the genus *Alexandrium* (Dinophyceae) in Scottish  
3 waters. *Eur. J. Phycol.* 45, 375–393.
- 4 Cembella, A., Sullivan, J.M., Boyer, G.I., Taylor, F.J.R., Anderson, D.M., 1987. Variations in  
5 paralytic shellfish composition within *Protogonyaulax tamarensis/catenella* species  
6 complex: Redtide dinoflagellates. *Biochem Syst Ecol* 15, 171–186.
- 7 Cembella, A.D., Taylor, F.J.R., 1986. Electrophoretic variability within the *Protogonyaulax*  
8 *tamarensis/catenella* species complex: Pyridine-linked dehydrogenases. *Biochem Syst*  
9 *Ecol* 14, 311–323.
- 10 Collins, C., Graham, J., Brown, L., Bresnan, E., Lacaze, J.-P., Turrell, E. a., 2009.  
11 Identification and Toxicity of *Alexandrium tamarensis* (Dinophyceae) in Scottish  
12 Waters. *J. Phycol.* 45, 692–703.
- 13 Davidson, K., Gareth, W., Flynn, K.J., John, E.H., 1999. An investigation of non steady state  
14 algal growth. I. An experimental model ecosystem. *J. Plankton Res.* 21, 811–837.
- 15 Davidson, K., Gilpin, L.C., Hart, M.C., Fouilland, E., Mitchell, E., Calleja, I.A., Laurent, C.,  
16 Miller, A.E.J., Leakey, R.J.G., 2007. The influence of the balance of inorganic and  
17 organic nitrogen on the trophic dynamics of microbial food webs. *Limnol. Oceanogr.* 52,  
18 2147–2163.
- 19 Davidson, K., Miller, P., Wilding, T.A., Shutler, J., Bresnan, E., Kennington, K., Swan, S.,  
20 2009. A large and prolonged bloom of *Karenia mikimotoi* in Scottish waters in 2006.  
21 *Harmful Algae* 8, 349–361.
- 22 Eckford-Soper, L., 2013. The competitive dynamics of toxic and non-toxic ribotypes of the  
23 harmful dinoflagellate *Alexandrium tamarensis* in Scottish waters. University of  
24 Aberdeen, SAMS, UHI.
- 25 Eckford-Soper, L.K., Davidson, K., Bresnan, E., 2013. Identification and quantification of  
26 toxic and nontoxic strains of the harmful dinoflagellate *Alexandrium tamarensis* using  
27 fluorescence in situ hybridization and flow cytometry. *Limnol. Oceanogr. Methods* 11,  
28 540–548.
- 29 Eppley, R.W., 1972. Temperature and phytoplankton growth in the sea. *Fish. Bull.* 70, 1063–  
30 1085.
- 31 Farrell, H., Gentien, P., Fernandez, L., Lunven, M., Raguera, B., Gonzalez-Gil, D., Rain, R.,  
32 2012. Scales characterising a high density thin layer of *Dinophysis acuta* Ehrenberg and  
33 its transport within a coastal jet. *Harmful Algae* 15, 36–46.
- 34 Fehling, J., Davidson, K., Bates, S.S., 2005. Growth dynamics of non-toxic *Pseudo-nitzschia*  
35 *delicatissima* and toxic *P. seriata* (Bacillariophyceae) under simulated spring and summer  
36 photoperiods. *Harmful Algae* 4, 763–769.
- 37 Fehling, J., Davidson, K., Bolch, C., Tett, P., 2006. Seasonality of *Pseudo-nitzschia* spp.  
38 (Bacillariophyceae) in western Scottish waters. *Mar. Ecol. Prog. Ser.* 323, 91–105.

- 1 Ferris, H., Wilson, L.T., 1987. Concepts and Principles of Population Dynamics, in: Dickson,  
2 D.W., Veech, J.A. (Eds.), *Vistas on Nematology*. Society of Nematologists, Hyattsville,  
3 Md, pp. 372–376.
- 4 Fistarol, G., Legrand, C., Selander, E., Hummert, C., Stolte, W., Granéli, E., 2004.  
5 Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal  
6 monocultures. *Aquat. Microb. Ecol.* 35, 45–56.
- 7 Fistarol, G.O., Legrand, C., Selander, E., Hummert, C., Stolte, W., Graneli, E., 2004.  
8 Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal  
9 monocultures. *Mar. Ecol. Prog. Ser.* 35, 45–56.
- 10 Flynn, K., Davidson, K., 1993. Predator-prey interactions between *Isochrysis galbana* and  
11 *Oxyrrhis marina*. I. Changes in particulate  $\delta^{13}\text{C}$ . *J. Plankton Res.* 15, 455–463.
- 12 Flynn, K., Franco, J., Fernández, P., 1994. Changes in toxin content, biomass and pigments  
13 of the dinoflagellate *Alexandrium minutum* during nitrogen refeeding and growth into  
14 nitrogen or phosphorus stress. *Mar. Biol.* 111, 99–109.
- 15 Flynn, K.J., 2002. Toxin production in migrating dinoflagellates: a modelling study of PSP  
16 producing *Alexandrium*. *Harmful Algae* 1, 147–155.
- 17 Flynn, K.J., Davidson, K., Leftley, J., 1993. Carbon-nitrogen relations during batch growth of  
18 *Nannochloropsis oculata* (Eustigmatophyceae) under alternating light and dark. *J. Appl.*  
19 *Phycol.* 5, 465–475.
- 20 Frangópulos, M., Guisande, C., deBlas, E., Maneiro, I., 2004. Toxin production and  
21 competitive abilities under phosphorus limitation of *Alexandrium* species. *Harmful*  
22 *Algae* 3, 131–139.
- 23 Geider, R., La Roche, J., 2002. Redfield revisited: variability of C:N:P in marine microalgae  
24 and its biochemical basis. *Eur. J. Phycol.* 37, 1–17.
- 25 Goldman, J.C., 1977. Steady state growth of phytoplankton in continuous culture:  
26 Comparisons of internal and external nutrient equations. *J Phycol.* 13, 251–258.
- 27 Goldman, J.C., Mann, R., 1980. Temperature influenced variations in speciation and chemical  
28 composition of marine phytoplankton in our-door mass culture. *Journal Exp. Mar. Biol.*  
29 *Ecol.* 46, 29–39.
- 30 Gowen, R., Tett, P., Bresnan, E., Davidson, K., McKinney, A., Harrison, P.J., Milligan, S.,  
31 Mills, D., Silke, J., Crooks, A.M., 2012. Antropogenic nutrient enrichment and blooms  
32 of harmful phytoplankton, in: Gibson, R.N., Atkinson, R.J.A., Gordon, J.D.M. (Eds.),  
33 *Oceanography and Marine Biology: An Annual Review*. pp. 65–126.
- 34 Guillard, R.R.L., Hargraves, P.E., 1993. *Stichochrysis immobilis* is a diatom, not a  
35 chrysophyte. *Phycologia* 32, 234–236.

- 1 Guisande, C., Frangópulos, M., Maneiro, I., Vergara, A.R., Riveiro, I., 2002. Ecological  
2 advantages of toxin production by the dinoflagellate *Alexandrium minutum* under  
3 phosphorus limitation 225, 169–176.
- 4 Hallegraeff, G.M., 1993. A review of harmful algae blooms and their apparent global  
5 increase. *Phycologia* 32, 79–99.
- 6 Hamasaki, K., 2001. Variability in Toxicity of the Dinoflagellate *Alexandrium tamarense*  
7 Isolated from Hiroshima Bay, Western Japan, as a Reflection of Changing  
8 Environmental Conditions. *J. Plankton Res.* 23, 271–278.
- 9 Hansen, P.J., 2002. Effect of high pH on the growth and survival of marine phytoplankton:  
10 Implications for species succession. *Aquat. Microb. Ecol.* 28, 279–288.
- 11 Harder, R., 1917. Ernährungsphysiologische Untersuchungen an Cyanophyceen,  
12 hauptsachlich dem endophytischen *Nostoc punctiforme*. *Z. Bot.* 9, 145.
- 13 Harrison, P.A., Berry, P.M., Dawson, T.P., 2001. Climate Change and Nature Conservation  
14 in Britain and Ireland: Modelling natural resource responses to climate change (the  
15 MONARCH project). UKCIP Technichal report. Oxford.
- 16 Hinder, S.L., Hays, G.C., Brooks, C.J., Davies, A.P., Edwards, M., Walne, A.W., Gravenor,  
17 M.B., 2011. Toxic marine microalgae and shellfish poisoning in the British isles:  
18 history, review of epidemiology, and future implications. *Environ. Health* 10, 54.
- 19 Hughes, S.L., 2007. Services, Fisheries Research. Scottish Ocean Climate Status Report 2004  
20 and 2005. Aberdeen.
- 21 Ikawa, M., Sasner, J.J., Haney, J.F., 1997. Inhibition of *Chlorella* growth by degradation and  
22 related products of linoleic and linolenic acids and the possible significance of  
23 polyunsaturated fatty acids in phytoplankton ecology. *Hydrobiologia* 70, 271–276.
- 24 Jensen, S., Moestrup, J., 1997. Autecology of the toxic dinoflagellate *Alexandrium*  
25 *ostenfeldii*: life history and growth at different temperatures and salinities. *Eur. J.*  
26 *Phycol.* 32, 9–18.
- 27 John, E.H., Flynn, K.J., 2000. Growth dynamics and toxicity of *Alexandrium fundyense*  
28 (Dinophyceae): the effect of changing N:P supply ratios on internal toxin and nutrient  
29 levels. *Eur. J. Phycol.* 35, 11–23.
- 30 John, U., Litaker, R.W., Montresor, M., Murray, S., Brosnahan, M.L., Anderson, D.M., 2014.  
31 Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae)  
32 taxonomy: the introduction of five species with emphasis on molecular-based (rDNA)  
33 classification. *Protist* 165, 779–804.
- 34 Laabir, M., Jauzein, C., Genovesi, B., Masseret, E., Grzebyk, D., Cecchi, P., Vaquer, a.,  
35 Perrin, Y., Collos, Y., 2011. Influence of temperature, salinity and irradiance on the  
36 growth and cell yield of the harmful red tide dinoflagellate *Alexandrium catenella*  
37 colonizing Mediterranean waters. *J. Plankton Res.* 33, 1550–1563.

- 1 Landsberg, J., 2002. The effects of harmful algal blooms on aquatic organisms. *Rev. Fish.*  
2 *Sci.* 113–300.
- 3 Lilly, E.L., Halanych, K.M., Anderson, D.M., 2007. Species boundaries and global  
4 biogeography of the *Alexandrium tamarens* complex (Dinophyceae) 1. *J. Phycol.* 43,  
5 1329–1338.
- 6 Lim, P., Ogata, T., 2005. Salinity effect on growth and toxin production of four tropical  
7 *Alexandrium* species (Dinophyceae). *Toxicon* 45, 699–710.
- 8 Lippemeier, S., Frampton, D.M.F., Blackburn, S.I., Geier, S.C., Negri, A.P., 2003. Influence  
9 of phosphorous limitation on toxicity and photosynthesis of *Alexandrium minutum*  
10 (Dinophyceae) monitored by in-line detection of variable chlorophyll fluorescence. *J.*  
11 *Phycol.* 331, 320–331.
- 12 Ma, H., Krock, B., Tillmann, U., Cembella, A., 2009. Preliminary characterization of  
13 extracellular allelochemicals of the toxic marine dinoflagellate *Alexandrium tamarens*  
14 using a *Rhodomonas salina* bioassay. *Mar. Drugs* 7, 497–522.
- 15 Medlin, L.K., Lange, M., Wellbrock, U., Donner, G., Elbrächter, M., Hummert, C., Luckas,  
16 B., 1998. Sequence comparisons link toxic European isolates of *Alexandrium tamarens*  
17 from the Orkney Islands to toxic North American stocks. *Eur. J. Protistol.* 34, 329–335.
- 18 Ogata, T., Ishimaru, T., Kodama, M., 1987. Effect of water temperature and light intensity on  
19 growth rate and toxicity change in *Protogonyaulax tamarens*. *Mar. Biol.* 95, 217–220.
- 20 Proctor, N.H., Chan, S.I., Trevor, A.J., 1975. Production of saxitoxin by cultures of  
21 *Gonyaulax catenella*. *Toxicon* 13, 1–9.
- 22 Reuman, D.C., Holt, R.D., Yvon-Durocher, G., 2014. A metabolic perspective on  
23 competition and body size reductions with warming. *J. Anim. Ecol.* 83, 59–69.
- 24 Schmidt, L., Hansen, P., 2001. Allelopathy in the prymnesiophyte *Chrysochromulina*  
25 *polylepis*: effect of cell concentration, growth phase and pH. *Mar. Ecol. Prog. Ser.* 216,  
26 67–81.
- 27 Scholin, C.A., Hallegraeff, G.M., Anderson, D.M., 1995. Molecular evolution of the  
28 *Alexandrium tamarens* “species complex” (Dinophyceae): Dispersal in the North  
29 American and West Pacific regions. *Phycologia* 472–485.
- 30 Scholin, C.A., Herzog, M., Sogin, M., Anderson, D.M., 1994. Identification of Group- and  
31 Strain-specific markers for the globally distributed *Alexandrium* (Dinophyceae). II  
32 sequence analysis of a fragment of the LSU rRNA gene. *J. Phycol.* 30, 999–1011.
- 33 Simpson, J., Hill, A.E., 1986. The Scottish coastal current, in: Skreslet, S. (Ed.), *Coastal*  
34 *Marine Ecosystems*. Nato. ASI. Series., pp. 295–308.
- 35 Smayda, T.J., 2004. Harmful algal bloom communities in Scottish Coastal waters:  
36 Relationship to fish farming and regional comparisons- A Review.

- 1 Stephens, E., Ross, I.L., Mussgnug, J.H., Wagner, L.D., Borowitzka, M. a., Posten, C., Kruse,  
2 O., Hankamer, B., 2010. Future prospects of microalgal biofuel production systems.  
3 Trends Plant Sci. 15, 554–564.
- 4 Stubbs, B., Swan, S., Davidson, K., Turner, A., de Campos, C., Algoet, M., 2014. Annual  
5 report on the results of the Biotoxin and Phytoplankton Official Control Monitoring  
6 Programmes for Scotland - 2013. Annual report to FSAS.
- 7 Sui, G.K.Y., Young, M.L.C., Chan, D.K.O., 1997. Environmental and nutritional factors  
8 which regulate population dynamics and toxin production in the dinoflagellate  
9 *Alexandrium catenella*. Hydrobiologia 352, 117–140.
- 10 Taylor, J.D., Kegel, J.U., Lewis, J.M., Medlin, L.K., 2014. Validation of the detection of  
11 *Pseudo-nitzschia* spp. using specific RNA probes tested in a microarray format:  
12 Calibration of signal based on variability of RNA content with environmental  
13 conditions. Harmful Algae 37, 183–193.
- 14 Tillmann, U., John, U., 2002. Toxic effects of *Alexandrium* spp. on heterotrophic  
15 dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content.  
16 Mar. Ecol. Prog. Ser. 230, 47–58.
- 17 Tilman, D., Mattson, M., Langer, S., 1981. Competition and nutrient kinetics along a  
18 temperature gradient: An experimental test of a mechanistic approach to niche theory.  
19 Limnol. Oceanogr. 26, 1020–1033.
- 20 Toebe, K., Alpermann, T.J., Tillmann, U., Krock, B., Cembella, A., John, U., 2013.  
21 Molecular discrimination of toxic and non-toxic *Alexandrium* species (Dinophyta) in  
22 natural phytoplankton assemblages from the Scottish coast of the North Sea. Eur. J.  
23 Phycol. 48, 12–26.
- 24 Touzet, N., Davidson, K., Pete, R., Flanagan, K., McCoy, G.R., Amzil, Z., Maher, M.,  
25 Chapelle, A., Raine, R., 2010. Co-occurrence of the West European (Gr.III) and North  
26 American (Gr.I) ribotypes of *Alexandrium tamarense* (Dinophyceae) in Shetland,  
27 Scotland. Protist 161, 370–84.
- 28 Tyljanakis, J.M., Didham, R.K., Bascompte, J., Wardle, D.A., 2008. Global change and  
29 species interactions in terrestrial ecosystems. Ecol. Lett. 11, 1351–1363.
- 30 Van De Riet, J.M., Gibbs, R.S., Chou, F.W., Muggah, P.M., Rourke, W.A., Burns, G.,  
31 Thomas, K., Quilliam, M.A., 2009. Liquid Chromatographic Post-Column Oxidation  
32 Method for Analysis of Paralytic Shellfish Toxins in Mussels, Clams, Scallops, and  
33 Oysters: Single-Laboratory Validation. J. AOAC Int. 92, 1690–1704.
- 34 Verhulst, P.F., 1838. A note on population growth. Corresp. Math. Phys. 10, 113–121.
- 35 Whyte, C., Swan, S., Davidson, K., 2014. Changing wind patterns linked to unusually high  
36 *Dinophysis* blooms around the Shetland Islands, Scotland. Harmful Algae 39, 365–373.
- 37 Wyatt, T., Jenkinson, I.R., 1997. Notes on *Alexandrium* population dynamics. J. Plankton  
38 Res. 19, 551–575.

1 Xu, J., Ho, A.Y.T., He, L., Yin, K., Hung, C., Choi, N., Lam, P.K.S., Wu, R.S.S., Anderson,  
2 D.M., Harrison, P.J., 2012. Effects of inorganic and organic nitrogen and phosphorus on  
3 the growth and toxicity of two *Alexandrium* species from Hong Kong. Harmful Algae  
4 16, 89–97.

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6



1 Table 1: Maximum specific growth rates (divisions day<sup>-1</sup>) for the group I *A. fundyense*  
 2 and group III *A. tamarensis* grown in mono and co-culture.

3

<b>Strain</b>	<b>12°C</b>	<b>15°C</b>	<b>18°C</b>	<b>21°C</b>
<i>A. fundyense</i>	0.12	0.22	0.27	0.16
<i>A. tamarensis</i>	0.16	0.21	0.22	0.16
<b>Co-culture (combined)</b>	0.16	0.18	0.20	0.15
<b>Co-Culture (<i>A. fundyense</i>)</b>	0.09	0.20	0.26	0.16
<b>Co-Culture (<i>A. tamarensis</i>)</b>	0.23	0.24	0.21	0.16

4

5

6

1 Legends

2

3 Figure 1: Cell densities ( $\text{ml}^{-1}$ ) at 12°C (a), 15°C (b), 18°C (c) and (d) 21°C. Group I *A.*  
4 *fundyense* (●), co-culture (◇) and group III *A. tamarensis* (○) strains. All results are  
5 means of triplicate flasks. Error bars represent SE.

6

7 Figure 2: Maximum cell yields ( $\text{ml}^{-1}$ ) normalised per  $\mu\text{m PO}_4$  consumed for *A.*  
8 *fundyense* (●), and *A. tamarensis* (○) strains in monoculture.

9

10 Figure 3: Maximum Specific Growth Rate (SGR)  $\text{div day}^{-1}$  at 12°C, 15°C, 18°C and  
11 21°C for *A. fundyense* (●), *A. tamarensis* (○), co-cultured *A. fundyense* (▼) and *A.*  
12 *tamarensis* (▽).

13

14 Figure 4: PSP Toxin concentrations in saxitoxin equivalents per cell at 12°C (a), 15°C  
15 (b) and 18°C (c) in Exponential (Ex) and Stationary (Sta) phases of growth for the  
16 group I strain in mono-culture (T) and in co-culture culture (M).

17

18 Figure 5: Cell densities ( $\text{ml}^{-1}$ ) at 12°C (a), 15°C (b), 18°C (c) and (d) 21°C for the co-  
19 culture experiments calculated using a flow cytometer (FC) and (for comparison) a  
20 Sedgwick Rafter (SR). *A. fundyense* (■), *A. tamarensis* (□), total flow cytometer counts  
21 (◆) and Sedgwick rafter counts (◇). All results are means of triplicate flasks. Error bars  
22 represent SE.

23