Environmental control of harmful dinoflagellates and diatoms in a fjordic system

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Abstract

Fjordic coastlines provide an ideal protected environment for both finfish and shellfish aquaculture operations. This study reports the results of a cruise to the Scottish Clyde Sea, and associated fjordic sea lochs, that coincided with blooms of the diarrhetic shellfish toxin producing dinoflagellate *Dinophysis acuta* and the diatom genus *Chaetoceros*, that can generate finfish mortalities. Unusually, *D. acuta* reached one order of magnitude higher cell abundance in the water column (2840 cells L^{-1}) than the more common *Dinophysis acuminata* (200 cells L^{-1}) and was linked with elevated shellfish toxicity (maximum 601 ± 237 µg OA eq/kg shellfish flesh) which caused shellfish harvesting closures in the region. Significant correlations between *D. acuta* abundance and that of *Mesodinium rubrum* were also observed across the cruise transect potentially supporting bloom formation of the mixotrophic *D. acuta*. Significant spatial variability in phytoplankton that was related to physical characteristics of the water column was observed, with a temperature-driven frontal region at the mouth of Loch Fyne being important in the development of the *D. acuta*, but not the *Chaetoceros* bloom. The front also provided significant protection to the aquaculture located within the loch, with neither of the blooms encroaching within it. Analysis based on a particle-tracking model confirms the

28 importance of the front to cell transport and shows significant inter-annual differences in advection
29 within the region, that are important to the harmful algal bloom risk therein.

30 Keywords: Harmful Algal Bloom, Dinoflagellates, Diatoms, Biotoxins, Aquaculture, Front

31 1. Introduction

32 Harmful algal blooms (HABs) are a recurrent problem for marine aquaculture. While some blooms
33 are anthropogenically generated, often related to elevated water column nutrient concentrations
34 (Davidson et al., 2014; Glibert et al., 2005; Gowen et al., 2012), many are natural events that exhibit
35 great spatial and temporal variability.

36 HABs can be harmful to aquaculture in a number of distinct ways. High biomass blooms are a threat
37 to finfish aquaculture. While some of these blooms may generate toxins or water column
38 deoxygenation, blooms of diatoms can often be harmful to fish by virtue of heavily silicified and
39 barbed setae. These setae can irritate or damage fish gills when concentrations are high enough,
40 sometimes leading to mortality (Davidson et al. 2011).

41 In temperate waters, human poisoning is typically related to the consumption of shellfish
42 contaminated with algal toxins. Algal toxins are most frequently produced by selected dinoflagellate
43 genera. These organisms can potentially be harmful at relatively low cell concentrations (e.g. <2000
44 cells L^{-1} for *Alexandrium tamarense* (Lebour) Balech (Davidson and Bresnan, 2009)) when consumed
45 by bivalves that concentrate the toxins in their flesh (Davidson and Bresnan, 2009). Important
46 amongst these is the genera *Dinophysis* (Ehrenberg) that produces potent lipophilic toxins that
47 generate severe gastrointestinal illness in consumers of contaminated shellfish (Reguera et al., 2012).
48 Incidents of *Dinophysis* generated shellfish toxicity (e.g. Whyte et al., 2014) have generated
49 significant and indiscriminate negative publicity for the aquaculture industry as a whole.

50 Understanding the (potentially different) environmental conditions that promote blooms from both of
51 these different harmful genera is therefore important for the sustainable development and
52 management of aquaculture. Given the importance of fjordic regions to aquaculture worldwide

(Norway, Chile, New Zealand, Scotland), such understanding is particularly important in these environments (Cembella et al., 2010, 2005). Worldwide, these locations are often relatively remote and free from the anthropogenic nutrient loading that can sometime generate high biomass harmful algal blooms (HABs) in more urban locations. However, even these low anthropogenic impact environments experience temporally and spatially variable naturally occurring HAB events that have the potential to negatively impact both shellfish and finfish aquaculture.

Out of the greater than 200 identified species of the globally occurring genus *Dinophysis*, only 12 of these have been classified as toxin producers (Reguera et al., 2012). These *Dinophysis* species have been associated with the production of okadaic acids (OAs), dinophysistoxins (DTXs [analogues 1-4]) and pectenotoxins (PTX) (Reguera et al., 2012). A low abundance (<100 cells L^{-1}) of *Dinophysis* spp. are present as a background in the regular phytoplankton community but high abundance blooms can occur (Reguera et al., 2012). Blooms are most common in summer and, in Scottish waters, can reach abundances of 10^3 cells L^{-1} (Swan and Davidson, 2012) and 10^4 cells L^{-1} (S. Swan, pers. comm,), although abundances of 10^5 cells L^{-1} have been observed worldwide, probably aggregated by water movements rather than *in-situ* cell growth (Smayda, 2006). Six *Dinophysis* species appear in Scottish waters, the majority of which are toxin producers, the most common being *Dinophysis acuminata* (Claparède & Lachmann) followed by *Dinophysis acuta* (Ehrenberg) (Tett and Edwards, 2002, Swan and Davidson, 2012).

Analysis of plankton data from the Continuous Plankton Recorder has shown spatial and temporal shifts in the distribution of *Dinophysis* in the North Sea over recent decades (Edwards et al., 2006). There has been an observed reduction in the mean annual abundance of *Dinophysis* off the east coast of the United Kingdom, while an increase occurred in west Norwegian coastal waters. Edwards, et al. (2006) speculate that the role of increased sea surface temperature (SST) and reduced salinities due to climate change off the Norwegian coast may be important in promoting *Dinophysis* growth. Indeed, there has been an observed reduction in salinity and an increase in water temperatures of Norwegian coastal waters in recent years (Saetre et al., 2003).

Dinophysis blooms are recurrent features in UK waters and have been observed for over 100 years (Davidson et al., 2011). Shellfish toxicity is common and while regulatory monitoring has generally been successful in protecting humans, DSP (Diarrhetic Shellfish Poisoning) incidents do occur. The first reliable record of this was in 1997 when 49 people in London became ill after consuming contaminated shellfish (Scoging and Bahl, 1998). This DSP outbreak represented the first recorded illnesses from UK shellfish in 30 years (Scoging and Bahl, 1998). See Tett and Edwards (2002) for a summary of shellfish toxicity outbreaks in Scotland.

The most recent UK outbreak of DSP happened in 2013 when 70 people were recorded as suffering from symptoms in London. Whyte et al. (2014) argue that this bloom, and another in 2006, was related to a rapid a change in the dominant mean wind direction around the Shetland Islands where the contaminated shellfish were grown. This hypothesis is supported by research carried out into "wind-driven water exchange" onto the southwest Irish shelf and links to recurrent HAB events, including *Dinophysis* blooms (Raine et al., 2010). These *Dinophysis* spp. cells are carried along a wind-initiated coastal jet current off the Irish west coast into Bantry Bay (Farrell et al., 2012; Raine, 2014), on the Irish south-west coast, an area responsible for 80% of mussels and 50% of oysters in total Irish aquaculture (Raine et al., 2010). Once inside the bay the cells are able to proliferate in toxic blooms which close shellfish harvesting sites for months of the year resulting in inconvenience and economic loss (Raine et al., 2010).

The toxin DTX-2 is a dinophysistoxin and its production is often linked with the presence of *D. acuta* (Aune et al., 2007; MacMahon and Silke, 1996; Vale and Sampayo, 2000). This toxin may be depurated from shellfish flesh more slowly than other lipophilic toxins causing a build-up of DTX-2 relative to OA (Vale, 2004) thus potentially prolonging closures of shellfish harvesting areas. While *D. acuta* may be less frequently observed than *D. acuminata* in Scottish waters, it has the potential for greater impact on the shellfish industry. Shellfish toxicity, however, may not have a simple relationship to *D. acuta* cell abundance due to variable cellular toxin contents or toxin dilution within shellfish from other food sources (Dahl and Johannessen, 2001).

While negative impact of blooms of the diatom *Chaetoceros* (Ehrenberg) is not so frequently documented there are a number of reports relating to *Chaetoceros* mediated kills of farmed fish (Bruno et al., 1989; Treasurer et al., 2003). Diatom mediated fish kills are increasingly being reported by aquaculture businesses in Scotland with weekly alert reports now being produced for some areas of the country to provide early warning of these events (K. Davidson, unpublished data). Oceanographic studies on the western Scottish shelf demonstrate the frequent presence of *Chaetoceros* and its potential for advection to the coast (Fehling et al., 2012; Siemering et al., 2016) where it can impact on aquaculture activities.

Oceanic species typically have larger spines and setae than coastal species (Tomas, 1997) which may cause more irritation to fish gills at lower concentrations due to spines with barbs breaking off, remaining inside fish gills even after a bloom has passed (Bruno et al., 1989; Hallegraeff, 2004). Fish can be killed through capillary haemorrhage, upset to gas exchange in gills, suffocation from excess mucus production or by secondary disease from open wounds. In British Columbia *Chaetoceros convolutus* (Castracane) and *Chaetoceros concavicornis* (Mangin) caused mass fish mortalities (2.4 tonnes) in cultured salmonids at only 5000 cells L^{-1} (Albright et al., 1993; Hallegraeff, 2004). In Scotland, *Chaetoceros wighami* (Brightwell) caused losses of 44 tonnes of salmonids (Bruno et al., 1989; Treasurer et al., 2003).

The genus *Chaetoceros* is often the most abundant phytoplankton community member (Bresnan et al., 2009; Fehling et al., 2012; Moschonas et al., 2017) and is a particularly species-rich genus (Rines and Hargraves, 1987). Typically, in inshore Scottish locations the coastal morphotype is most common and peaks in spring and summer (Moschonas et al., 2017). Gowen et al. (1983) found *Chaetoceros decipiens* to be common throughout spring and summer in the well-mixed Scottish Loch Ardbhair. In Narragansett Bay, USA, *Chaetoceros* blooms in early spring and again in autumn; the most abundant species being *Chaetoceros debilis* (Cleve), *Chaetoceros compressus* (Lauder) and *Chaetoceros didymus* (Ehrenberg). (Rines and Hargraves, 1987). Tomas (1997) states that *C. wighami* is present mainly in brackish water, whereas *C. convolutus* and *C. concavicornis* are cosmopolitan to northern temperate and cold-water regions. As many as fifteen different species can be observed together

(Rines and Hargraves, 1987), which can make identification difficult, therefore the separation of species into groups (as in Tomas (1997) and Fehling, et al. (2012)) is useful.

In common with other fjordic regions that support an aquaculture industry the Scottish west coast is characterised by complex hydrography. Currents are split around many small islands and water exchange into fjords is restricted by shallow entrance sills (Booth, 1987). In addition, conditions undergo short-term periods of intense change (Bresnan et al., 2016), flushing coastal regions and breaking down stratification. These many variables mean that prediction of the occurrence of HAB events is challenging in fjordic regions and requires further investigation into the interaction between the organisms and their physico-chemical environment.

This study reports the results of a research cruise in the fjordic Scottish Clyde sea region during summer 2015 at a time when blooms of highly toxic *D. acuta* and potentially fish killing *Chaetoceros* were both present. By conducting a transect through different water masses within the restricted exchange environment of Loch Fyne and out into the more open Clyde Sea the relationships between the environmental conditions and the different harmful phytoplankton present were studied hence allowing evaluation of potential drivers for the blooms and their location.

2. Methods

2.1 Study Site

The Clyde Sea study site is located on the west coast of Scotland (Figure 1) and is a large area which connects the River Clyde and several sealochs to the North Channel, above the Irish Sea (McIntyre et al., 2012). Marine inflow water comes mainly from the Irish Sea and the Malin Shelf, and is dependent on their respective compositions (Grantham and Tett, 1993). It is isolated from the Irish Sea and the rest of the Scottish west coast by a front across the Great Plateau which separates the high salinity waters of the North Channel (>34) from those inside the Clyde Sea (<33) (Edwards et al., 1986).

Celtic Sea water flowing north past the Irish east coast is influenced by freshwater runoff (reduced salinity/ increased nutrients) and enters the North Channel. Malin shelf water is held offshore by the Islay front (Simpson et al., 1979), created by North Channel outflow north into the Inner Hebrides. The Great Plateau front prevents direct exchange of currents in and out of the Clyde Sea, and provides vertically homogeneous water masses there (Edwards et al., 1986). Tidal speeds rarely exceed 0.5 ms^{-1} within the Clyde Sea, this is reduced to 0.2 ms^{-1} for the sealochs (McIntyre et al., 2012). Most water flows northwards in an anti-clockwise direction, at depth, around Arran and into the northern sealochs and channels. Water transit time is ~1 month with a mean northwards current speed, at depth, of 0.03 ms^{-1} (Edwards et al., 1986). A smaller branch of water diverts clockwise up Kilbrannan Sound and mixes at the north of Arran with Loch Fyne and Kyles of Bute outflows.

Surrounding the main basin are several restricted exchange fjordic sealochs (Lochs Fyne, Riddon, Striven, Holy, Long, Goil, Ryan and the Gareloch), as well as the islands of Arran, Bute and the Cumbraes (Connor and Little, 1998). Loch Fyne, the largest of the associated sea lochs, is situated on the north-west corner of the basin, generates a 1.3 x 10^9 m^3 annual freshwater outflow, from an 894 km^2 catchment area, to the Clyde Sea region (Gillibrand, 2001). Nitrate-salinity concentrations are indicative of unpolluted runoff with highest DIN concentrations <20 µM (Grantham and Tett, 1993).

The Loch has two sills (located at Otter Ferry and Minard) which restrict exchange between in- and outflows (Gillibrand, 2001). See McIntyre et al. (2012) for a detailed review of the study area.

2.2 Field Campaign

In situ sampling was conducted on 8-9 September 2015 aboard the RV Seòl Mara. Samples were collected from 12 stations that spanned Loch Fyne and the Clyde Sea, east of Arran (Figure 1). At each station, the vertical profile of the water column was analysed for salinity, temperature, fluorescence and oxygen concentration using a SBE 19 CTD profiler (Sea-Bird Electronics) with SBE 43 (Sea-Bird Electronics) oxygen and Wetlab Wetstar (Sea-Bird Electronics) fluorometer sensors. The fluorescence data were used to guide discrete sampling at three depths in the upper water column corresponding to (1) surface, (2) chlorophyll maximum (if present, else 5 m) and (3) below the chlorophyll maximum (BCM). Table 1 records each sampled depth and the total water column height at each station. Food Standards Scotland (FSS) regulatory biotoxin and harmful phytoplankton monitoring sites are also marked (Figure 1), where samples were collected and analysed prior to, and concurrently with, the cruise for shellfish biotoxins. The diatom genus *Chaetoceros* was not enumerated in these regulatory samples as it does not present a specific threat to human health.

2.3 Satellite Imaging

Satellite SST scenes were acquired and processed from the NOAA Advanced Very-High Resolution Radiometer (AVHRR) sensor, cloud-masked, and mapped to the study area at 1.1 km resolution in geographic projection (Miller et al., 1997). In addition, ocean colour data were acquired from the NASA Aqua-MODIS and NASA/NOAA Suomi-VIIRS sensors, but these data are not presented as the relatively low surface cell concentrations did not allow distinction of phytoplankton types during the rare glimpses of the ocean due to considerable cloud cover during the study.

2.4 Phytoplankton

Phytoplankton samples were collected by a Niskin bottle at three depths per site (see Table 1) and decanted into opaque 500 ml plastic bottles then fixed to ~1% final concentration of acidified Lugol's iodine. Samples were stored on deck in a cool box and on return to the laboratory, they were stored at 4 °C in the dark. For analysis, a 50 ml sub sample was dispensed into a Hydro-Bios settling chamber

and allowed to settle overnight before enumeration on a Zeiss Axio S100 inverted microscope (Utermöhl, 1958).

The phytoplankton community (including diatoms, dinoflagellates and ciliates) were enumerated, where possible, to species level. Nanoplankton, including cryptophytes, were not counted in this study. The diatom *Pseudo-nitzschia* (Peragallo) was grouped as either large *seriata* or small *delicatissima* groups following Fehling et al. (2006) and *Chaetoceros* was recorded as oceanic or coastal group (Tomas, 1997). When the community is considered by cell type (diatoms, dinoflagellates or ciliates) samples from each site (surface, chlorophyll maximum and BCM) are averaged and their standard deviation calculated. Otherwise, error bars are not present since points are a single sample count.

2.5 Pigments

For pigment analysis, 0.5 L of seawater was vacuum filtered onto 47 mm GF/F filters, then immediately flash frozen in liquid N and stored at -80 °C. Each sample was processed in duplicate. Extraction was carried out in 5 ml 90% acetone solution using an ultrasonic probe for 35 s at 50 W. Extracts were clarified and analysed by reverse phase HPLC using a Thermo Accela Series HPLC system with chilled autosampler (4 °C) and photodiode array detector. The instrument was calibrated with standards purchased from DHI (Denmark). Pigments were identified based on retention time and spectral match using photodiode array.

2.6 Weather Data

Wind data from Prestwick Airport, site number 01007, were accessed from the Met Office Integrated Data Archive System (MIDAS, n.d.).

2.7 Nutrients

Samples for the determination of inorganic nutrients were taken from each sampled depth at all sites (see Table 1) and immediately filtered using pre-combusted glass fibre filters (25 mm GF/F, Whatman, 6 hours at 450 °C) and stored in clear polyethylene bottles at -20 °C. In the laboratory,

samples were defrosted and analysed on a five channel QuAAtro autoanalyser (Seal Analytical) for Total Oxidised Nitrogen (nitrate and nitrite (TOxN)), phosphate, ammonium and silicate content.

2.8 Multivariate Data Analysis

Data were statistically analysed using the software package PRIMER. The multivariate analyses Multidimensional Scaling (MDS) and Hierarchical Agglomerative Clustering with SIMilarity PROFiles (HAC, SIMPROF) were carried out on similarity matrices of fourth-root transformed phytoplankton community count data (see Clarke (1993); Clarke and Ainsworth (1993); Clarke and Green (1988)). The Bray-Curtis similarity coefficient (Equation 1) effectively compares different stations to determine which are most similar according to their phytoplankton community structures. For ease of interpretation, samples were binned according to cell type (diatoms, dinoflagellates, ciliates) with separate analyses conducted on each *a-priori* assigned group, as well as the phytoplankton as a whole. Significant sample groupings, determined from HAC with SIMPROF (plots not shown), were used to encircle MDS sample groups to aid interpretation.

$$S_{jk} = 100\left\{1 - \frac{\sum_{i=1}^{p}|y_{ij}-y_{ik}|}{\sum_{i=1}^{p}|y_{ij}+y_{ik}|}\right\} \quad (1)$$

2.9 Food Standards Scotland Data

Publicly available local area shellfish biotoxin data from around the cruise period were accessed from the results of FSS regulatory monitoring programme (Food Standards Scotland, n.d.) carried out under contract by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS). Shellfish samples were collected and analysed for biotoxins as described in Stubbs, et al. (2014).

2.10 Modelling Study

An unstructured grid based biophysical particle tracking model was run to determine the likely spread and mixing of phytoplankton cells in late summer of 2015, coincident with the cruise and, for comparison, in 2014 when *D. acuta* and *Chaetoceros* blooms were not evident.

The model was based on previous studies in the area (Adams et al., 2016, 2014), with the underlying hydrodynamics derived from a well-established and comprehensively tested implementation of the Finite Volume Community Ocean Model (FVCOM) (Chen et al., 2011). This simulated the

hydrographic conditions in our study region in different years, deriving oceanographic boundary conditions from a larger-scale ocean shelf model (Dabrowski et al., 2014) and a tidal inversion solution (Egbert et al., 2010), with major river inputs and meteorological forcing being obtained from a linked implementation of the Weather Research and Forecasting Model at 2 km resolution. The hydrodynamic model's horizontal resolution varied from 130 m in complex coastal areas to 4.6 km at the open boundaries. Its configuration, testing and validation have been described in more detail previously in Aleynik et al. (2016).

The movement of phytoplankton (as passive organisms) was modelled occupying the surface layer of the water column (mean depth = 1.48 m, standard deviation = 1.70 m) in both 2014 and 2015 to compare conditions in two separate years. Three separate four week long simulations were undertaken (start dates staggered by two weeks, beginning 13th August) in both years. In each case, the transport of 1000 particles that were released from each of 17 locations throughout the Clyde Sea was simulated (Figure 12). The 17 locations were chosen to evaluate cell transport in different parts of the sealoch, specifically around the frontal system observed in 2015 at the entrance to Loch Fyne, the FSS sampling sites, at the Clyde Sea entrance and some transition stations. By following the location of particles throughout each four week simulation, the degree of spread and mixing of cells in the water column was identified.

3. Results

3.1 Oceanographic Conditions: Field and Satellite Data

Stations at the head of Loch Fyne (Stations 1-3) had elevated temperature (>12.5 °C, Figure 2B) and slightly reduced salinities (<31) compared with the rest of the loch (Figure 2B). These stations were coincident with a patch of increased florescence in the surface layer (spans stations 1-5; 3.21-6.22 mg m^{-3}) (Figure 2E). There is a shallow sill at Station 3 (<50 m water depth at station 3, Figure 2) which limits exchange of water between the upper and lower basins of the loch, resulting in an isolated deep water mass (9.60-10.76 °C, Figure 2A) in the upper basin. Elevated oxygen (Figure 2D) is restricted to the upper water column (<40 m) but is elevated deeper further into the Clyde Sea (8.19 mg L^{-1} at 5 m for stations 1-3 to 7.12 mg L^{-1} at 50 m for station 12, Figure 2D). A patch of high fluorescence (up to 7.23 mg m^{-3}) between stations 10, 11 and 12 was observed in the Clyde Sea (Figure 2E), maximum depth 18 m, and corresponds with a surface patch of lower salinity (31.44).

A change in water mass signature is evident between stations 4 and 6 in the temperature/salinity (T/S) plot (Figure 3). This can also be seen in the surface waters in the temperature (Figure 2A), salinity (Figure 2B), density (Figure 2C) oxygen (Figure 2D) and fluorescence (Figure 2E) plots, with a clear temperature front at station 6 separating the water within Loch Fyne from the outer Clyde Sea water. The least cloud covered satellite SST scene during the study period confirms the presence of the temperature front to the northwest of Arran near stations 7 and 8 (Figure 4).

The wind in the Clyde is predominantly South-Westerly (plots not shown). This is consistent with the prevailing wind in the region and the approximately North-South orientation of the Clyde Sea and sealochs funnelling wind. Wind speed can increase to up to 34.92 ms^{-1} in isolated events, however most wind is between 1-5 m s^{-1}. Wind direction and speed did not correlate with cell abundance of *D. acuta* in the Clyde (r: -0.02, p: >0.5 and r: 0.01, p: >0.1 respectively), and did not undergo any large changes in speed or direction in the period before the bloom. The lack of routine monitoring of *Chaetoceros* in the cruise area meant there was insufficient data to conduct this analysis for this genus.

3.2 Nutrient Conditions

TOxN, phosphate and silicate are not markedly increased by the front, but do peak just inside the front's edge, at station 6, across all depths (4.08-4.96 µM, 0.49-0.51 µM and 3.31-3.86 µM respectively). Surface layer depletion of TOxN, phosphate and silicate is evident in upper Loch Fyne (stations 1-3; 0-0.09 µM, 0.04-0.08 µM and 0.98-1.34 µM respectively), however concentrations of all nutrients here remain elevated at the chlorophyll maximum and BCM.

At station 7, seaward of the front, concentrations of TOxN, phosphate and silicate were markedly lower than station 6. Here, and elsewhere in the Clyde sea, nutrient concentrations do not vary much with depth (0.48-1.01 µM TOxN, 0.15-0.21 µM phosphate, 0.59-1.15 µM silicate, Figure 5 B-D) in contrast to the observations made in upper Loch Fyne (stations 1-3). Moving seaward, nutrient concentrations gradually increased to stations 9 and 10 (1.26-3.18 µM for TOxN, 0.26-0.44 µM for phosphate and 1.82-2.68 µM for silicate). Subsequently, concentrations gradually decreased from stations 10-12. Ammonium does not share the same pattern to other forms of N, maintaining low concentrations (0.01-0.17µM at all depths) until station 12 where concentrations increased markedly (0.26-0.48 µM, Figure 5A).

3.3 Phytoplankton Community & Pigments

Surface waters in Loch Fyne have increased total chlorophyll (chlorophyll a + chlorophyll b + chlorophyll c + chlorophyllide a) (maximum 7.2 µg L^{-1} station 3), which decreases steadily approaching the front at station 7 (Figure 6). Total chlorophyll is generally lowest at BCM depth, apart from around the the front where BCM concentrations were slightly increased (station 7 4.2 µg L^{-1}) compared to the surface layers. In the outer Clyde Sea, concentrations were much higher than anywhere else on the transect, at all depths (maximum 9.9 µg L^{-1} station 11).

Sampling stations were mostly dominated by diatoms (maximum abundance 1.2 $x10^6$ cells L^{-1}) except for stations 7 and 9 where more dinoflagellates were recorded (4.6 $x10^4$ ± 2869 cells L^{-1} and 1.9 $x10^4$ ± 1844 cells L^{-1} dinoflagellates respectively) (Figure 7). Outer Clyde Sea stations (10-12) had the highest concentrations of diatoms overall (3 $x10^6$ – 1.2 $x10^6$ cells L^{-1}) (Figure 7). Stations above the front (1-6) were also diatom dominated (3.2 $x10^5$ – 6.1 $x10^4$ cells L^{-1}). Ciliates decreased in

concentration steadily from the top of Loch Fyne to the front edge (station 6) where concentrations increased again from station 7 at the front seaward edge (Figure 5). The diatoms *Chaetoceros* (coastal group), *Thalassiosira* (Cleve), *Skeletonema* (Greville) and *Katodinium* (Fott) were the four genera observed to reach the highest abundance during the study (1.3×10^6, 1.8×10^5, 2.6×10^4 and 1.5×10^4 cells L^{-1} respectively, Figure 8).

Dinophysis Species

At all cruise stations three *Dinophysis* species were identified: *D. acuta, D. acuminata* and *Phalacroma rotundatum* (*Dinophysis rotundata* (Claparède & Lachmann)). There was a bloom of *D. acuta* at stations 7 and 8, positioned just seaward of the identified front (Figure 8B). The bloom was evident at all sampled depths, with the deep sample at station 7 exhibiting the highest abundance (2840 cells L^{-1}). In contrast, *D. acuminata*, while exhibiting some degree of increased abundance near the front, reached significantly lower abundances (maximum = 200 cells L^{-1}). A second minor increase was also evident at the chlorophyll maximum of station 11 (Figure 8B). There was a general low abundance of *P. rotundatum*; it only occurred in three samples and reached a maximum concentration of 40 cells L^{-1} at station 3 (Figure 8C).

Results from FSS regulatory monitoring for shellfish toxins were consistent with the presence of elevated *D. acuta* in the region at the time of the cruise. At Campbeltown monitoring site toxins reached their highest concentration at the time of the cruise (7th Sept 2015, 601 ± 237 µg OA eq/kg shellfish flesh) which then steadily reduced to 37 ± 15 µg OA eq/kg shellfish flesh on 16th November 2015 (Figure 9A). Ardkinglas site toxicity peaked (457 ± 159 µg OA eq/kg shellfish flesh) on 14th July 2015, however did not show a pronounced increase in shellfish toxins around the time of the cruise (148 ± 58 µg OA eq/kg shellfish flesh, 8th September 2015) (Figure 9B). Otter Ferry site, being the only location with Pacific Oysters (*Magallana gigas* (Thunberg)) instead of Blue Mussels (*Mytilus edulis* (*Linnaeus*)), had very little toxicity throughout the year (Figure 9C) with no instances above the regulatory limit (regulatory limit = 160 µg OA eq/kg shellfish flesh, above which shellfish harvesting is closed). Loch Riddon site did not increase above the regulatory limit, however its maximum value

348 (159 ± 62 µg OA eq/kg shellfish flesh, 8th September 2016, Figure 9D) was coincident with the cruise
349 date and observed *D. acuta* bloom. Loch Striven had elevated toxicity during the cruise period (371 ±
350 129 µg OA eq/kg shellfish flesh, 15th September 2015, Figure 9E). The Sound of Gigha, located
351 outside of the Clyde Sea on the west coast of the Mull of Kintyre peninsula, did not have toxicity
352 more than 62 ± 25 µg OA eq/kg shellfish flesh (27th July 2016) (Figure 9F).

353 *Diatoms*

354 At station 10 there were large abundances of *Chaetoceros* coastal group (, $1.3 \times 10^6 - 6.4 \times 10^5$ cells L^{-1}),
355 however, cells did not occur inside Loch Fyne or around the front (1-7, Figure 8H). At stations 11
356 and 12 concentrations remain elevated, however only in deep samples (7.8×10^5 and 6.9×10^5 cells L^{-1}
357 respectively). The *Chaetoceros* coastal group bloom corresponds with a patch of elevated
358 fluorescence (6.7 mg L^{-1}, Figure 2E) and reduced salinity (31.44, Figure 2B). Oceanic group of
359 *Chaetoceros* reached four orders of magnitude lower cell abundance than the coastal group
360 (maximum 300 cells L^{-1}). The oceanic group, however did occur inside Loch Fyne at station 4 (200
361 cells L^{-1}).

362 Potentially toxic members of the *Pseudo-nitzschia seriata* group reached maximum abundance (1200
363 cells L^{-1}) in the surface of station 10 (Figure 8G), however this only occurs at a single station at one
364 depth. The presumed non-toxic *Pseudo-nitzschia delicatissima* group was more abundant throughout
365 the transect. The common spring bloom diatom genus *Skeletonema*, peaked only in station 6 located
366 inside the front, with maximum concentration at the chlorophyll maximum (2.6×10^4 cells L^{-1}, Figure
367 8F).

368 *Non-harmful dinoflagellates and ciliates*

369 Dinoflagellate abundance increased at the seaward front edge (station 7) and was lower elsewhere
370 (Figure 7). The most abundant dinoflagellate species observed was *Katodinium* spp. reaching a
371 maximum abundance at station 12 (1.5×10^4 cells L^{-1} at the chlorophyll maximum) within the Clyde
372 Sea (Figure 8D). The maximum abundance of *Tripos furca* (Ehrenberg) was observed just after the
373 front at station 7 (maximum abundance 5240 cells L^{-1}) and was similar in distribution to *D. acuta*

(Figure 8E). Concentrations of ciliates were lower in Loch Fyne, high abundance at station 7 and 8, and lower concentrations in the outer Clyde Sea, except for surface Loch Fyne samples having high abundance (stations 1-3, 8460-1 x10^4 cells L^{-1}). In Loch Fyne stations 1-6, *Strombidium* (<50 µm, Claparède & Lachmann) dominated, especially in surface waters, with small peaks of *Leegaardiella* (Lynn & Montagnes) and *Mesodinium* (von Stein) at the surface stations 2-4 (not shown). *Strombidium* (<50 µm) is still a dominant ciliate outside of Loch Fyne, however at most stations *Mesodinium* was the most abundant ciliate member particularly at stations on the Clyde Sea side of the front (7 and 8) where *D. acuta* blooms were observed. There is a strong correlation (r^2: 0.79, p = <0.001) between *D. acuta* abundance and the ciliate *Mesodinium* from all stations at all depths (Figure 10A). There is a weak, but nevertheless significant, correlation (r^2: 0.22, p = <0.01) between *D. acuta* and total ciliate abundance (Figure 10B), however once *Mesodinium* abundance is removed from that of total ciliates, then there is no correlation (r^2: 0.003, p = >0.5) (Figure 10C).

3.4 Multivariate Analysis

Based on the phytoplankton community structure, transect sites were found to occupy distinct groups related to their similarity of species composition. The distance between points on the MDS ordination reflect their biological similarity to each other. The HAC plots with ANOSIM procedure are not shown, but statistically significant groupings are overlaid as bubbles on MDS plots. Stress coefficients were low for all MDS plots (Figure 10A-D), i.e. a 2D plot preserves the distances between points well.

Total phytoplankton

Statistically significant groups were Loch Fyne, the front region and outer Clyde Sea stations (Figure 10A). Stations in the outer Clyde Sea (10-12) group away from other samples in a HAC at 60% similarity, these stations can be further grouped at 75% similarity. Loch Fyne and stations on both sides of the front (1-9) separate at 60% similarity. The stations immediately seaward of the front (7-9) group at 75% similarity. Stations above the front (1-6) have some smaller, but still statistically significant, 75% similarity groups, except for a sample from station 1 (BCM sample) which separates from all the other samples at 56% similarity.

Diatoms

Samples were statistically separated with 50% similarity at the front edge (Figure 10B). Stations 7-9 largely group at 70% similarity away from Loch Fyne stations (1-6) which occur above the front. Seaward of the front, stations 10-12 are grouped together and form 2 significant groups at 75% similarity.

Dinoflagellates

Overall, these form a single significant group which is 60% similar (Figure 10C). Three samples from stations 1 (BCM), 2 (chlorophyll maximum) and 3 (BCM) form another group. Front stations were grouped together (75% similarity) with stations on the seaward side of the front (7-11), with the exception of station 10 which forms another 75% similarity group. Loch Fyne stations (1-6) were largely grouped at 75% similarity in the centre of the plot.

Ciliates

Ciliates form no significant groups in HAC with SIMPROF analysis, therefore all samples can be considered as one group (Figure 10D).

3.5 Modelling Study

The model allowed us to explore how differences in hydrography between different years impacted the transport of (harmful) phytoplankton, and hence the role of the front into whether cells could have been suspended in one location leading to *in-situ* growth. The front is potentially a transient feature at the mouth of Loch Fyne and the model can enquire about hydrodynamics of the region between different years which may have promoted its formation. Figure 12A and B show cumulative model outputs over the month-long model simulations from 13th August 2015 – 10th September 2015, coincident with our cruise and the significant *D. acuta* and *Chaetoceros* blooms, and between 14th August 2014 – 11th September 2014, for comparison, when no *D. acuta* bloom was evident. The 17 model seeding points are strategically placed to show transport of cells from the entrance to Loch Fyne (D-I), the FSS regulatory monitoring sites (A, C, M), at the Clyde Sea entrance (N, P, Q) and other transition stations. Seed sites were identically placed for both model runs.

Simulations in 2015 show cells seeded within Loch Fyne remaining within the loch (A, B, Figure 12B). Seed points around the proposed frontal region (D-I) appear to cluster their cells within the area east of the Isle of Bute, with some transport into upper Kilbrannan Sound (J). Cells seeded at Campbeltown site (M) were transported across the south end of Arran and into the cell plume originating from the inner Firth (K). These cells do not appear to be carried into the river Clyde or Kyles of Bute regions, therefore it is unclear how cells would exchange with the Loch Striven site (C) in this instance. Sites located near in the Great Plateau undergo a greater degree of mixing (particle spread) than in the 2014 model simulation, with more exchange of particles occurring between the Clyde Sea and the North Channel.

Simulations from 2014, in contrast, indicate that a distinctive frontal region was not present near the mouth of Loch Fyne, with particles from all seeding points, including those originating within the loch travelling toward the exit of the Clyde Sea (Figure 12A). The seeding points across the Great Plateau (N, P, Q) either travel out into the North Channel or remain stationary, as if suspended in the Great Plateau front. Particles seeded at Campbeltown site (M) are transported out of the Clyde Sea, not fuelling blooms within the area. The proposed frontal region at the entrance to Loch Fyne is indistinct with cells transported freely from inside Loch Fyne and into Kilbrannan Sound (B, E, H), where there is some confinement of their transport with most travelling down the east coast of Arran (F, G, I). In common with those seeded in Loch Fyne, there is free-flow of particles from Loch Striven (C) and the mouth of Loch Fyne to the inner Firth (K).

4. Discussion

The major oceanographic feature of the Clyde sea area evident from our 2015 survey was the temperature front located near the mouth of Loch Fyne (Figure 2A), supported by satellite data (Figure 4), with a salinity front also evident near the head of Loch Fyne (Figure 2B). This water mass arrangement is common in fjords and estuaries which have freshwater inflow at their head and a shallow sill at their entrance restricting offshore water exchange (Largier, 1993; Parsons et al., 1983). Frontal systems are often associated with high phytoplankton biomass (Franks, 1992a) with patches of

elevated chlorophyll often following the pycnocline in the surrounding stratified regions (Franks, 1992b). A clear example of this was in the low salinity stratified water at stations 1-4 near the head of Loch Fyne (Figure 2B), with taxonomic analysis indicating this was associated with a bloom that was primarily composed of the diatom *Thalassiosira* (Figure 8K). This genus is usually considered not harmful to aquaculture, although Kent et al. (1995) reported gill lesions and fish mortality associated with a dense bloom of *Thalassiosira* spp. in British Columbia. Cell abundance data were not reported in that study, but were likely to be much greater than those found in this study which are typical of Scottish coastal waters (Fehling et al. 2006, 2012) without reported harm to farmed fish.

The pronounced temperature front at the mouth of Loch Fyne did not exhibit the high phytoplankton biomass typical of these structures elsewhere (Franks, 1992a) with relatively low fluorescence and total chlorophyll concentrations being evident at, and immediately adjacent to, the front. Dinoflagellate blooms, in particular, often occur at fronts (Franks, 1992a, 1992b; Parsons et al., 1983) which do not require a particularly high cell abundance to harm aquaculture operations when toxins are produced, which appears to be the case here. The greatest abundance of dinoflagellates was found at station 7 on the seaward side of the front, with this peak being dominated by shellfish toxin producing *D. acuta,* and the non-harmful taxa *Katodinium* and *Tripos furca*. The front also promoted the highest abundance of *D. acuminata* found in the transect, but this was an order of magnitude less than that of *D. acuta* (Figure 8).

Nutrient availability at frontal regions is often linked to enhanced phytoplankton biomass and productivity. As noted above, enhanced biomass was not evident at the Loch Fyne temperature front. This is, however, consistent with the lack of significantly elevated nutrient concentrations. The most dominant dinoflagellates at the front (*Katodinium, D. acuta* and *T. furca),* are now known to have phagotrophic capabilities (Naustvoll, 2000; Reguera et al., 2012; Smalley and Coats, 2002). Various authors have demonstrated that *Dinophysis* spp. feed on the ciliate *Mesodinium* in laboratory culture (Nishitani et al., 2008; Park et al., 2006; Reguera et al., 2012), with *Dinophysis* spp. and *Mesodinium* spp. having been observed to aggregate together in the field in thermally stratified thin layers (Sjöqvist and Lindholm, 2011). The greatest abundance of ciliates in the transect was also found at the

480 temperature front, with *Mesodinium* being the most abundant genus. The total ciliate population, and
481 *Mesodinium* alone, were significantly correlated with *D. acuta* over the transect as a whole (Figure
482 10A, B). However, when the *Mesodinium* population was removed from total ciliate abundance then
483 the correlation with *D. acuta* was not significant (Figure 10C), showing that *Mesodinium,* specifically,
484 is linked to *D. acuta* abundance. Hence, our results are consistent with the hypothesis that
485 *Mesodinium* is an important prey species for *Dinophysis.*

486 According to the literature, *Dinophysis* may be part of a food chain involving cryptophytes and
487 ciliates: the internal plastids in *Dinophysis* have been found to be molecularly similar, or identical, to
488 those found in *Geminigera cryophilia* (Takishita et al., 2002) and *Teleaulax amphioxia* (Janson,
489 2004). It has been suggested that *Dinophysis* gained these plastids of cryptophyte origin through
490 ingestion of ciliates which had previously fed on cryptophytes (Janson, 2004). Therefore, even though
491 cryptophytes were not specifically enumerated in this study, an avenue of useful research in the future
492 would be to observe co-occurring abundances of *Dinophysis* spp., ciliates and cryptophytes in the
493 field.

494 The most abundant *Dinophysis* species in Scottish waters is *D. acuminata*, with the *D. acuta* bloom
495 event being sporadic in the region (Stubbs et al., 2014). The frontal *Dinophysis* bloom observed
496 during this study was composed of both *D. acuta* and *D. acuminata*, the order of magnitude larger
497 concentration of the former suggests that *D. acuta* blooms later in the year, when the thermocline is
498 deeper, a hypothesis supported by many studies (see Dahl and Johannessen (2001); Díaz et al. (2016);
499 Escalera et al. (2006); Reguera et al. (2012, 1993)). Raine (2014) and Reguera et al. (2012) suggest
500 that a fine balance between aggregation of cells, their retention time in the system and the potential
501 for *in-situ* growth is required for *Dinophysis* bloom development, with blooms having been previously
502 linked with upwelling systems (Diaz et al., 2013; Escalera et al., 2010; Pazos et al., 1995) and
503 hydrographic features such as thin layers (Reguera et al. 2012) and coastal jets (Farrell et al. 2012).
504 Thermally-driven stratification patterns are an important driver for general seasonal phytoplankton
505 growth (Raine, 2014), have been linked to *Dinophysis* blooms (Lassus et al., 1988) and were even
506 observed to shift *Dinophysis* assemblages in favour of *D. acuta* in warmer years (Escalera et al.,

2006). Our results also suggest that coastal temperature fronts may be important in its bloom development.

During the survey, *D. acuta* was only present in significant concentrations on the Clyde Sea side of the front, suggesting that it provided conditions suitable to promote a bloom of those cells that then aggregated against it. In contrast, *D. acuminata* was more dispersed and occurred, albeit at lower abundance, across the length of Loch Fyne and the Clyde Sea with cells present at all sampled depths. This cosmopolitan distribution suggests a different growth strategy to *D. acuta*.

The lack of enhanced nutrient availability at the front may explain the relatively low observed diatom abundance in this part of the transect, with the most abundant diatom *Chaetoceros* coastal group reaching only 2.9 $x10^4$ cells L^{-1} at station 6 and even lower in concentration at station 7 (5280 cells L^{-1}). The front did promote the highest abundance of silicoflagellates over the transect with an abundance of ~ 2000 cells L^{-1} at station 7, suggesting that these organisms, rather than diatoms, best utilised what silicate was available there.

An additional community structuring factor which cannot be overlooked is that of zooplankton grazing impact. One of the largest losses to the phytoplankton community is through top-down zooplankton control (Lampert et al., 1986), however the impact of zooplankton grazing on the formation and demise of harmful algal blooms is still an under-researched area (Campbell et al., 2005). Zooplankton have been shown to be selective in their grazing (Porter, 1973; Stoeker et al., 1981; Teegarden et al., 2001) and their preference for certain prey species will shape the phytoplankton community potentially by promoting blooms of certain species. Studies have shown that zooplankton may selectively avoid toxic cells (Teegarden, 1999; Teegarden et al., 2001), however other studies have shown no impact on grazing through the presence of toxic species (Kozlowsky-Suzuki et al., 2006). Zooplankton certainly have the ability to bioaccumulate toxins (Maneiro et al., 2000) and transfer them to higher trophic levels potentially causing wide-reaching harm to the local marine food web. Of course, the vast array of size classes and trophic preferences within the

zooplankton will affect what impact their grazing has on different harmful species (Kozlowsky-Suzuki et al., 2006).

Univariate phytoplankton data show key differences in cell abundance between diatoms and dinoflagellates on different sides of the frontal region indicating that it provided an important barrier to cell transport, potentially protecting the aquaculture sites located on the landward side. As noted above, potentially harmful *Chaetoceros* coastal group were abundant at outer Clyde Sea stations, and did exhibit a small increase at the front, but its abundance at stations 1-5 was below 1000 cells L^{-1}, indicating that this potentially harmful diatom was not transported further towards the within-loch aquaculture sites.

Diatoms, however, were not absent within the loch with *Skeletonema* occurring at station 6 (maximum = 2.5 $x10^4$ cells L^{-1}), on the Loch Fyne side of the front, and *Thalassiosira* being important in upper Loch Fyne (maximum 1.6 $x10^5$ cells L^{-1}) where the freshwater inflow at the loch head mixes with the loch body water mass. Cells of the *Pseudo-nitzschia delicatissima* group, that is thought to be non-toxic in Scottish waters (Fehling et al., 2004), also only reached ~1000 cells L^{-1} in the Loch. In addition, oceanic group of Chaetoceros was present at 200 cells L^{-1} at the chlorophyll maximum at station 4. With the exception *Chaetoceros* oceanic group, these genera were more abundant within, rather than outside of, the loch again suggesting that the front was limiting exchange.

While dinoflagellates were most numerous at the front the composition of the community was different on both sides of the front. Pronounced blooms were less obvious than for diatoms, but the multivariate analysis demonstrated, in common with diatoms, two somewhat distinct dinoflagellate communities on different sides of the front.

The transfer of water and cells between the open coastal ocean and areas of restricted exchange is potentially key to governing HAB events therein. For example, Raine et al. (2010) demonstrated the important impact of wind driven transfer on harmful blooms of *Dinophysis* in Ireland's Bantry Bay. Advective transport as a mechanism of promoting harmful blooms has also been demonstrated in Scottish waters by Gillibrand et al. (2016) for *Karenia mikimotoi* (Hansen & Moestrup), Whyte et al.

(2014) for *Dinophysis* and Aleynik et al. (2016) through hydrodynamic modelling. Smayda (2006) hypothesised that most *Dinophysis* blooms in Scottish waters are the result of wind aggregations and possible further *in-situ* growth. Sometimes these aggregation events correlate with a change in wind conditions (e.g. Whyte et al. (2014)), but comparison of meteorological data across years indicated that no substantive changes to normal wind conditions occurred in the Clyde around the time of the 2015 survey and hence neither anomalous wind speed nor wind direction appear to be the immediate promotors of the 2015 blooms. It is possible that a lag effect prevents the immediate response of *Dinophysis* occurrence from wind directional changes in the sheltered Clyde Sea area. Offshore cells may be initially transported into the Irish Sea or onto the Malin Shelf, then coastal currents transport them within the Clyde Sea sometime later where they were able to proliferate. Similarly, rainfall may increase north or west of the Clyde Sea and this water subsequently enters the Clyde Sea through rivers which may affect front formation. Calculating exact drivers for the difference in Clyde Sea characteristics would require further observational weather and current studies across a wide area.

Data from the FSS shellfish toxicity monitoring, do suggest that advection is important in providing the seed population for the observed *Dinophysis* bloom. Clyde Sea waters are thought to circulate anti-clockwise around the Isle of Arran, some water entering the inshore areas north of the Isle of Bute and Great Cumbrae and potentially another branch travelling up Loch Fyne (Edwards et al., 1986). This progression of offshore water in the Clyde Sea can be seen in Figure 2 as a warm oxygenated tongue of water decreasing with depth at it travels inshore. Consistent with this model of water movement is the marked increase in DSP toxin concentration in shellfish at the Campbeltown FSS monitoring site from approximately 2 weeks prior to the cruise (Figure 9A). Ardkinglas site, located at the head of the loch, appears protected from DSP toxin contamination during this *D. acuta* bloom (Figure 9B), however suffered from toxicity earlier in the year (July) when there may have been no front preventing cell transport inside the loch. Toxicity was accumulated at the Loch Striven site throughout the year, reaching a peak during the September *D. acuta* blooms (Figure 9E). As Loch Striven is located to the east of the Clyde Sea basin, and on the seaward side of the Loch Fyne front, it is entirely reasonable to assume that this loch is always open to toxin contamination with, or without,

585 the presence of a front, and began accumulating toxin from the supposed previous *Dinophysis* bloom
586 which reached Ardkinglas. The Otter Ferry site, also located within Loch Fyne, also appears to have
587 not accumulated toxins during the *D. acuta* bloom in September (Figure 9C), however this site
588 harvests Pacific Oysters (all other sites farm Blue Mussels). Mussels are known to accumulate more
589 DSP than other bivalve species (Vale and Sampayo, 2002; Yasumoto et al., 1978), and even appear to
590 selectively ingest dinoflagellates, in particular *Dinophysis spp.* (Sidari et al., 1998). As oysters are
591 cultivated on intertidal trestle beds in Scotland, as opposed to mussels, which are hung from ropes in
592 the upper water column, this may further limit their exposure to certain types of toxic algae (Mcleod,
593 2014). Loch Riddon mussels did not accumulate substantial DSP toxins (Figure 9D), which is
594 surprising given their proximity to Loch Striven. It is possible that the location of the Isle of Bute
595 limits water exchange between the Kyles of Bute and the outer Clyde Sea basin. Sound of Gigha site
596 is located outside of the Clyde Sea and shows that the *Dinophysis* toxic events of summer 2015 are
597 restricted to the Clyde Sea region (Figure 9F).

598 Particle tracking modelling was consistent with the conclusions drawn above (Figure 12). In both
599 modelled years, there is some aggregation of particles around the mouth of Loch Fyne (particularly at
600 sites 5-9), however there is much more defined exchange of these modelled cells between the loch and
601 the Clyde Sea in 2014 indicating that, in absence of the front cells, were more easily able to exchange
602 between the open sea and the loch. The model does not, however, take into account any *in-situ* growth
603 of cells which would allow localised blooms to form, or any mixing out of the surface layer. Inclusion
604 of some biological parameters to simulate a growth/death response is a necessary next step but is
605 currently limited by the difficulty in parameterising the growth and mortality of important HAB
606 genera, in particular heterotrophs such as *Dinophysis*. Detailed laboratory growth studies are required
607 to achieve this.

608 The model highlights the difference of Clyde Sea circulation patterns between 2014 and 2015
609 suggesting that the conditions which promoted the 2015 *D. acuta* bloom are not annually recurring in
610 the region. In addition, we observed that, in both years water in the outer Clyde sea at the location of
611 the large 2015 *Chaetoceros* bloom, was not exchanged with Loch Fyne.

5. Conclusions

This study demonstrates the occurrence of an unusual *D. acuta* bloom in the Scottish Clyde Sea which was associated with a temperature front at the mouth of a sealoch. FSS regulatory data show the accumulation of DSP toxins inside Clyde Sea shellfish during the time of the bloom. Toxins appear to be transported around the Clyde Sea in a similar mode to that suggested in Edwards, et al. (1986). The bloom's associated toxicity resulted in prolonged periods of closure for the region's shellfish harvests. A strong relationship was evident between the occurrence of the ciliate *Mesodinium* and *D. acuta* abundance, further supporting the hypothesis that *Mesodinium* is a preferred prey species for heterotrophic *Dinophysis*. The potentially harmful diatom *Chaetoceros* was also found to bloom to considerable concentrations on the Clyde Sea side of the front. The front appeared to separate phytoplankton communities between the Loch and the outer Clyde Sea, preventing the exchange of harmful phytoplankters thereby protecting aquaculture activities in the loch. The presence of a front, however, could easily trap harmful algae within the loch causing blooms to occur near aquaculture activities.

Particle tracking modelling showed that the frontal region is likely a transient feature which requires an unknown specific set of conditions to form. Further research into the hydrodynamics of the region and growth parameters of harmful algae to improve future implementations of the model are both important areas of future research to drive understanding of HAB events in these complex coastal regions.

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Table and Figure Captions

Table 1: Field stations and sampled depths. Referred to throughout the text as surface, chlorophyll maximum and below the chlorophyll maximum (BCM)

Table 2: Cell abundance (cells L^{-1}) data of phytoplankton genera which contribute to at least 5% of any single sample. Blank cell = zero abundance; Chloro. Max. = Chlorophyll Maximum.

Figure 1: The Clyde Sea area showing field sampling stations (circles), Food Standards Scotland biotoxin monitoring sites (squares) and other points of reference (italic text)

Figure 2: CTD contour plots from cruise transect (A) Temperature (°C), (B) salinity, (C) density (kg m^{-3}), (D) oxygen concentration (mg L^{-1}) and (E) fluorescence (mg m^{-3}). Water depth is <50m. For full colour interpretation, please see the electronic version of this publication.

Figure 3: Temperature salinity plot depicting different water masses across the transect (all data is <50 m water depth, as in Figure 2)

Figure 4: Sea surface temperature satellite data taken from the time of the cruise (16 September 2015) showing a temperature front to the north of Arran. Scale in degrees Celsius. Black areas are cloud cover or land

Figure 5: Nutrients at stations (A) Ammonium, (B) Total Oxidised Nitrogen (TOxN), (C) Phosphate, (D) Silicate. Solid line = surface, dotted line = chlorophyll maximum, dot-dash line = below the chlorophyll maximum. See Table 1 for sample depth information

Figure 6: Total chlorophyll concentration; solid line = surface, dotted line = chlorophyll maximum, dot-dash line = below chlorophyll maximum (See Table 1 for sample depth information). Total chlorophyll = chlorophyll a + chlorophyll b + chlorophyll c + chlorophyllide a

Figure 7: Total ciliates (black), diatoms (grey) and dinoflagellates (white) in cells L^{-1}. Each bar is the mean between the three sampled depths (See Table 1) at each station. Therefore, n=3 and error bars are 1 standard deviation

Figure 8: Important phytoplankton species plot; solid line = surface, dotted line = chlorophyll maximum, dot-dash line = below chlorophyll maximum (See Table 1 for sample depth information) (A) *Dinophysis acuminata* (B) *Dinophysis acuta* (C) *Phalacroma rotundatum* (D) *Katodinium* sp. (E) *Tripos furca* (F) *Pseudo-nitzschia delicatissima* group (G) *Pseudo-nitzschia seriata* group (H) *Chaetoceros* coastal group (I) *Chaetoceros* oceanic group (J) *Skeletonema* sp. (K) *Thalassiosira* sp.

Figure 9: Total toxin content, measured in total OA/DTXs/PTXs (µg OA eq/kg), of sites in and around the Clyde Sea (A) Campbeltown, Blue Mussels (B) Ardkinglas, Blue Mussels (C) Otter Ferry, Pacific Oysters (D) Loch Riddon, Blue Mussels (E) Loch Striven, Blue Mussels (F) Sound of Gigha, Blue Mussels. Note regulatory limit for OA/DTXs/PTXs in shellfish flesh is 160 µg OA eq/kg, above which shellfish harvest areas are closed. Zero values are not plotted

Figure 10: Correlations between (A) *D. acuta* and *Mesodinium* presence: $r^2 = 0.78$ and $p = <0.001$; (B) *D. acuta* and total ciliate presence: $r^2 = 0.19$ and $p = <0.01$; (C) *D. acuta* and ciliate abundance (without *Mesodinium* abundance): $r^2 = -0.03$ and $p = >0.5$

Figure 11: MDS of stations phytoplankton community with HAC clusters showing statistically significant simprof sections. For all plots: triangle = surface sample, circle = chlorophyll maximum sample and cross = below chlorophyll maximum sample. (A) Total Phytoplankton (solid = 60% similar, dashed = 75% similar), (B) Diatoms (solid = 50% similar, dashed = 70% similar), (C) Dinoflagellates (solid = 60% similar, dashed = 70% similar), and (D) Ciliates, no significant clustering

Figure 12: Particle tracking model simulations run in two years: (A) 2014, key geographic locations labelled (B) 2015. Points A-Q mark model seed simulation points. Both panels show the cumulative particle distribution at the end of the model run. Each seed point has a different colour distribution for ease of interpretation

Table 1

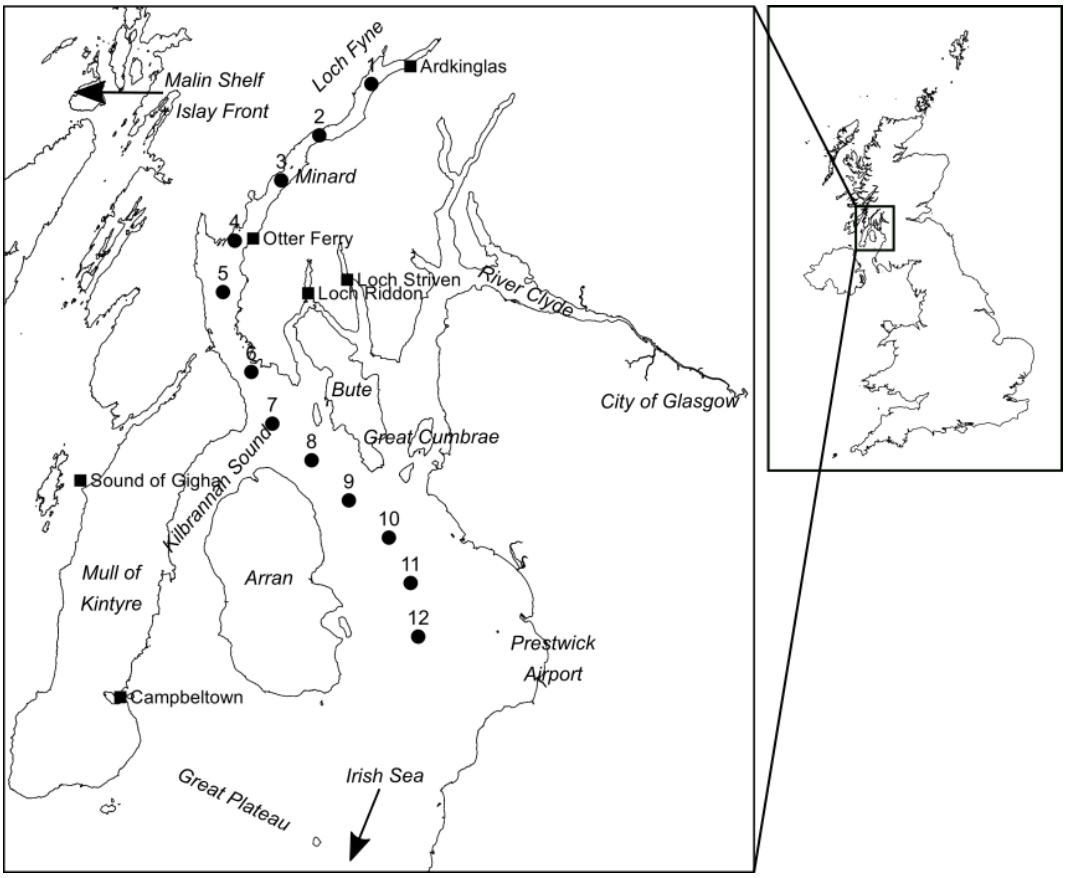
Field Station (Figure 1)	Latitude (Decimal)	Longitude (Decimal)	Depths Sampled (m)	Water Column Depth (m)
1	56.2	-5.07	2, 5, 10	44
2	56.1	-5.18	2, 6, 10	136
3	56.1	-5.26	2, 7, 10	65
4	56.0	-5.36	2, 5, 10	43
5	55.9	-5.38	2, 5, 10	145
6	55.8	-5.31	1, 3, 10	134
7	55.8	-5.26	1, 4, 10	132
8	55.7	-5.17	1, 4, 10	123
9	55.7	-5.08	1, 4, 8	146
10	55.6	-4.99	1, 4, 12	80
11	55.6	-4.93	2, 6, 10	90
12	55.5	-4.91	1, 12, 15	77

Table 2

	1			2			3			4			5			6		
	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth
Pseudo-nitzschia delicatissima group	1060	620	540	60	80		280	100	80	100	40	80	740	1040	540	680	1300	1180
Skeletonema spp.	80	40	100			160	100		60	540	200	40	40	120	220	11720	25640	2320
Thalassiosira spp.	21200	20600	10000	89890	54151	1320	181947	54151	10840	114258	183571	17560	8960	10920	40072	2340	1080	2660
Cylindrotheca spp.	960	660	400	240	360	120	420	320	160	660	1100	500	440	640	600	380	700	560
Chaeotoceros sp. coastal group	780	560	680	260	300		160	680	140	440	840	980	540	740	720	800	29160	2440
Navicula spp.	340	220	260	60	140	40	140	140	40	320	400	140	460	480	140	380	980	820
Tripos furca	180	100	20	20	40		100		20	80	100		180	160	20	100		40
Dinophysis acuta		140		140	20		60		20	20	40		120	60	20	40		40
Prorocentrum gracile	500	380	220	80	100		140		20	480	100	60	240	400	240	240	140	120
Protoperidinium spp.	260	300	140	240	140	280	340	100	80	180	120	80	220	240	180	140	200	420
Gymnodinium <50um	740	1220	1240	1580	1140	220	1200	1260	1080	3520	2020	1020	640	400	480	520	800	880
Katodinium spp.	440	340	920	580	280	160	480	780	620	680	580	980	680	380	740	340	460	520
Silicoflagellate		620	100		60	20	200	80	40	180	140	40	820	860	220	500	200	340
Strombidium <50um	1980	2160	740	8880	1720	360	6280	780	560	10040	2660	880	1560	1220	780	760	400	820
Mesodinium	340	460	180	700	180	40	1000	160	60	780	320	140	140	340	300	160	500	320

	7			8			9			10			11			12		
	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth	Surface	Chloro. Max.	Depth
Pseudo-nitzschia delicatissima group	340	280	220	440	140	220	140	340	240		40	300	240	360	400	60		220
Skeletonema spp.	640	620	220	320	80	640	140	40	160	100	440	240	380			500		
Thalassiosira spp.	100	40	100	160	140	40	300	840	1560	1440	1840	280	320	560	1440	100		80
Cylindrotheca spp.		20	20	20	40	100	100	140	760	520	920	500	220	360	680		100	160
Chaeotoceros sp. coastal group	2100	1840	5280	320	360	141875	820	1000	1880	1305560	1040602	636847	285211	374398	776201	86100	386636	694213
Navicula spp.	100	60	80	260	60	120	220	140	880	960	1080	380	300	560	1000	160	120	220
Tripos furca	4860	3480	5240	2020	2460	120	3520	1320	360	720	320		1560	720	200	960	140	40
Dinophysis acuta	2020	1200	2840	1180	1380	40	120	340	280	200	80		100	480	80	60	100	20
Prorocentrum gracile	780	960	700	1020	1080	100	1620	960	400				1400	640	120	2700	120	
Protoperidinium spp.	560	460	940	440	600	200	620	340	360	2200	1360	900	760	1400	960	400	360	280
Gymnodinium <50um	20	140	120	140	180	260	140	120	520	320	280	100	2200	720	480	620	320	860
Katodinium spp.	1420	4720	5120	4200	3300	940	640	780	760	360	600	100	740	1160	1240	260	15240	4880
Silicoflagellate	2040	1580	1260	1780	1900	300	680	740	520	1000	360	100	1120	1000	360	800	540	260
Strombidium <50um	2560	1840	1900	2380	2580	2400	1480	1880	2160	880	1440	440	1820	1840	600	1120	3440	480
Mesodinium	5840	3380	3720	4040	3640	600	1200	1120	600	960	840	80	1000	2280	1040	1180	840	760

Figure 1


Malin Shelf
Islay Front
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Loch Riddon
River Clyde
Bute
City of Glasgow
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8
Great Cumbrae
Sound of Gigha
Kilbrannan Sound
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Mull of
Kintyre
Arran
Prestwick
Airport
Campbeltown
Irish Sea
Great Plateau

Figure 2 – greyscale in print

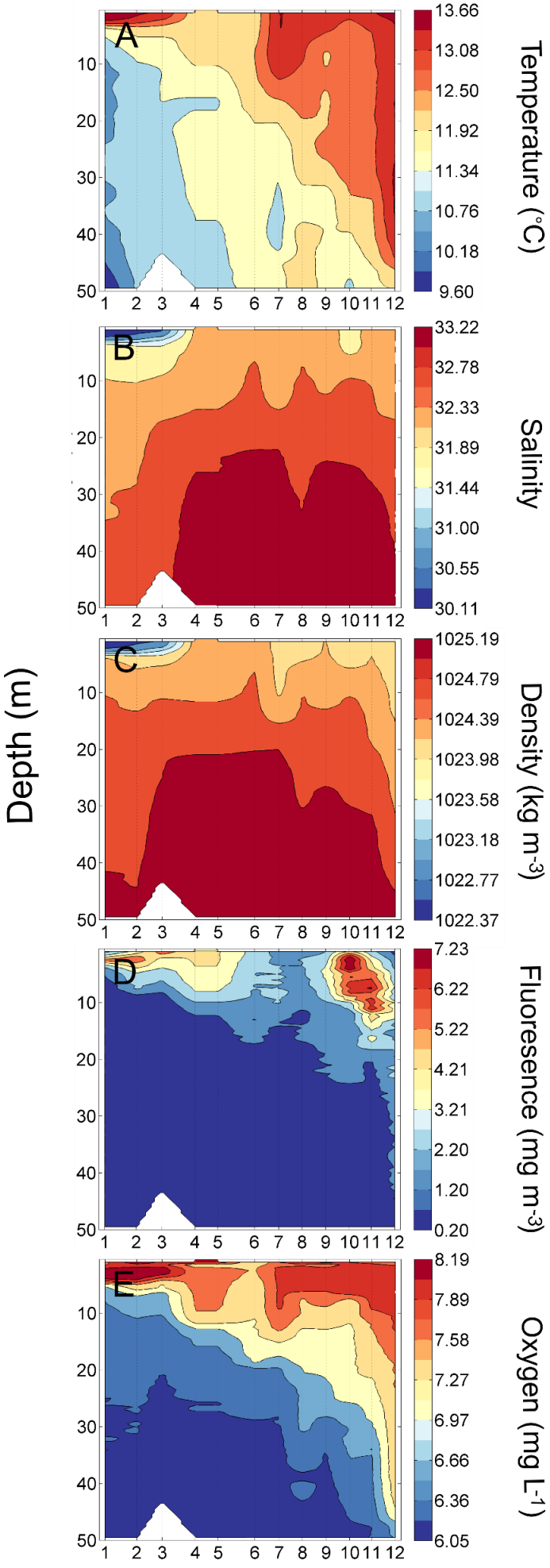

Figure 3 - colour

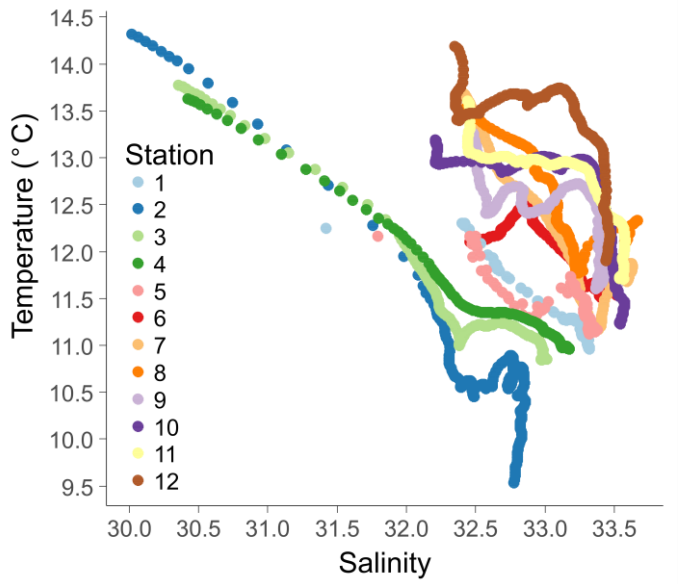

Figure 4 - colour

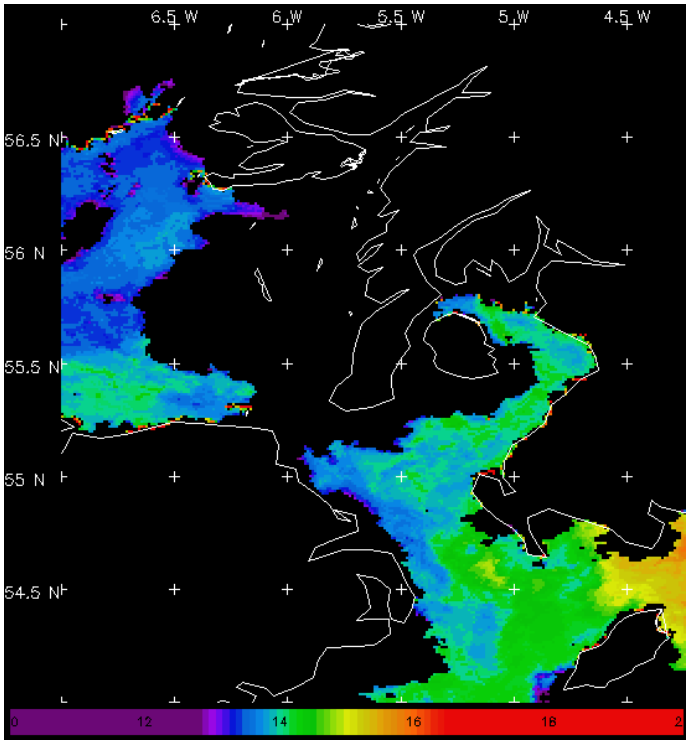

Figure 5

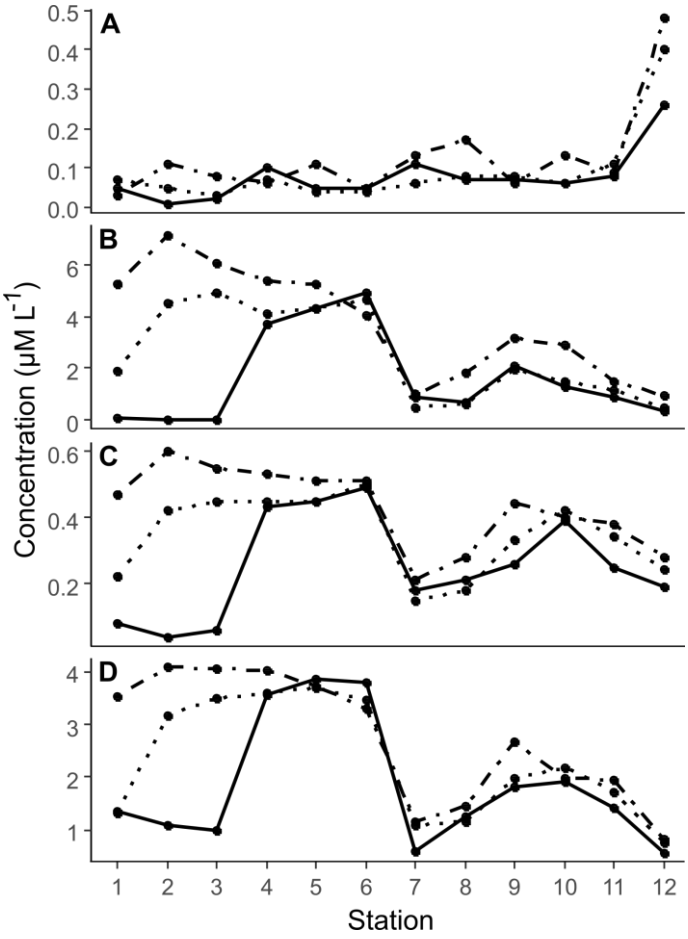
A
B
C
D
Concentration (μM L-1)
Station
0.5
0.4
0.3
0.2
0.1
0.0
6
4
2
0
0.6
0.4
0.2
4
3
2
1
1
2
3
4
5
6
7
8
9
10
11
12

Figure 6

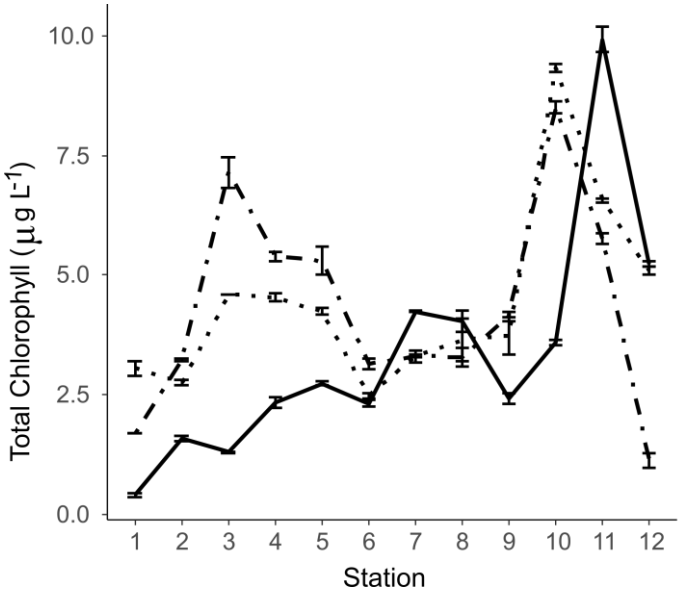
10.0
7.5
5.0
2.5
0.0
Total Chlorophyll (µg L-1)
1
2
3
4
5
6
7
8
9
10
11
12
Station

Figure 7

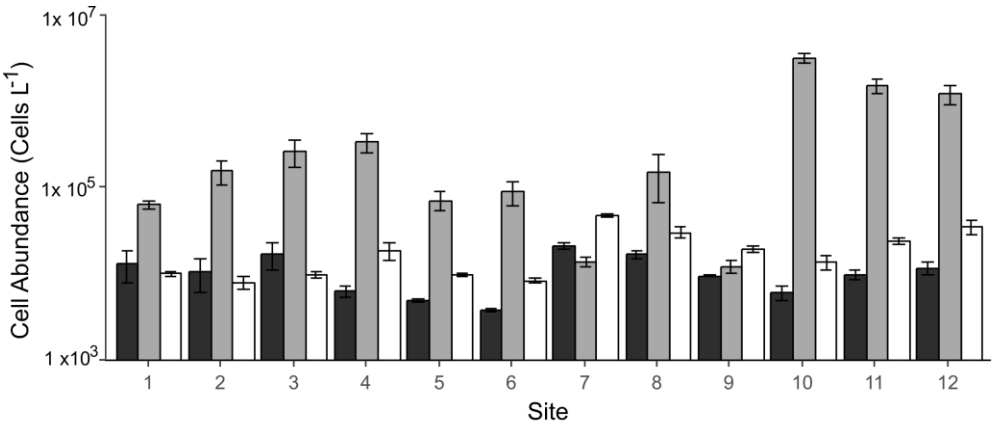
1x 10^7
1x 10^5
1 x10^3
Cell Abundance (Cells L^-1)
1
2
3
4
5
6
7
8
9
10
11
12
Site

Figure 8

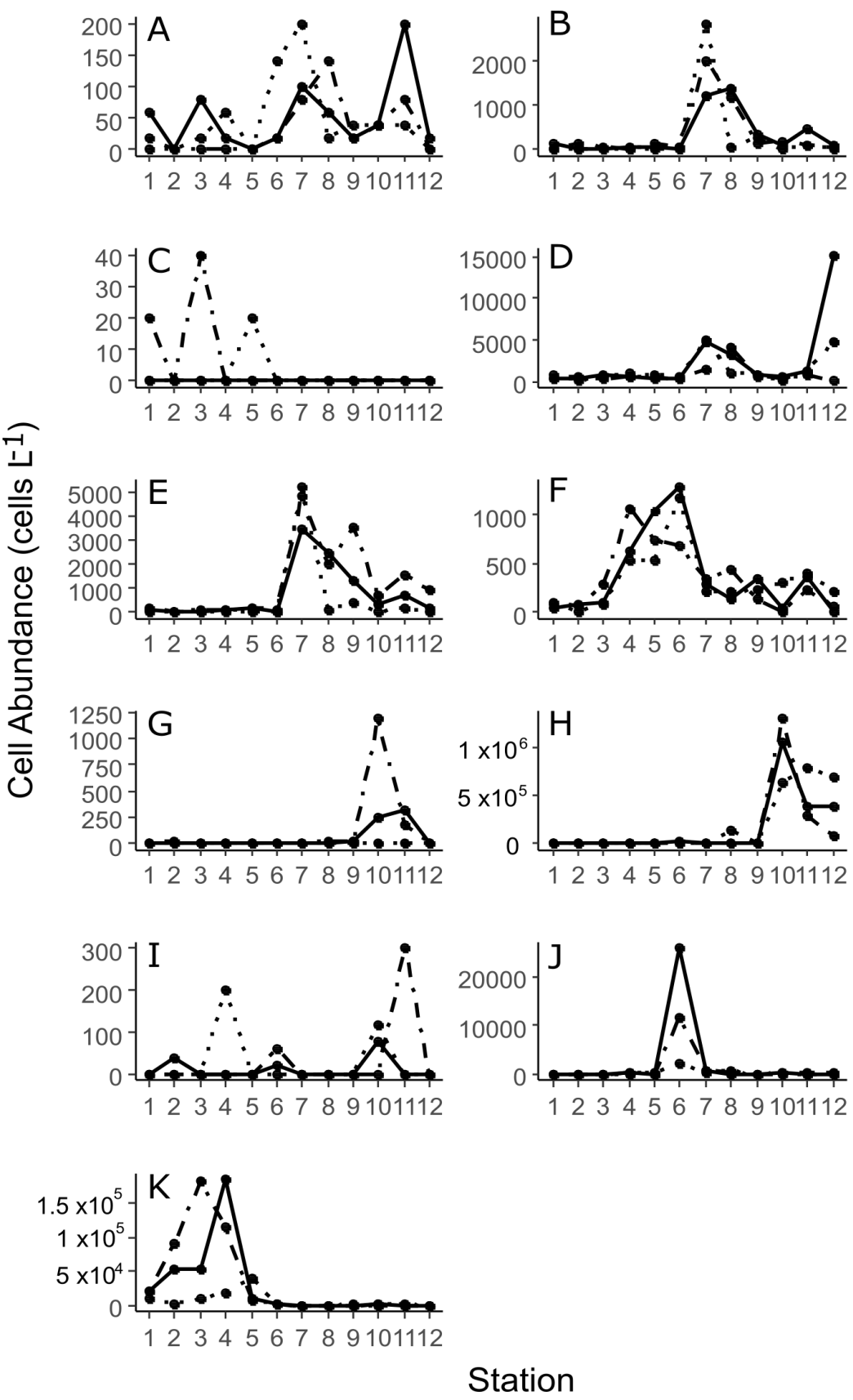

Figure 9

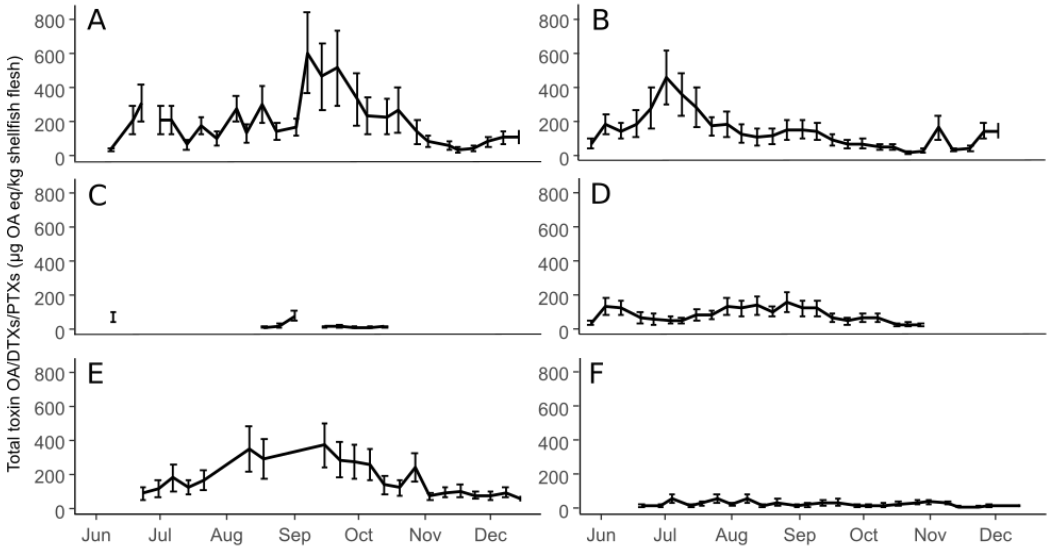
A
B
C
D
E
F
800
600
400
200
0
Total toxin OA/DTXs/PTXs (µg OA eq/kg shellfish flesh)
Jun
Jul
Aug
Sep
Oct
Nov
Dec

Figure 10

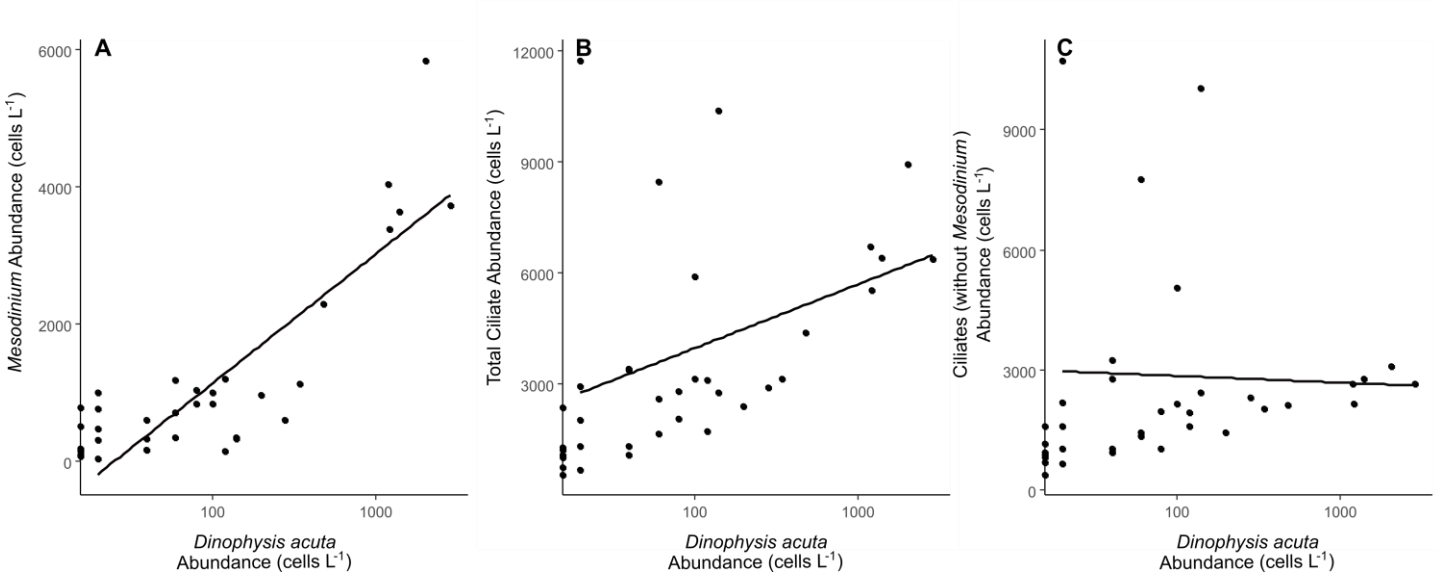
A
6000
4000
2000
0
100
1000
Mesodinium Abundance (cells L-1)
Dinophysis acuta
Abundance (cells L-1)
B
12000
9000
6000
3000
100
1000
Total Ciliate Abundance (cells L-1)
Dinophysis acuta
Abundance (cells L-1)
C
9000
6000
3000
0
100
1000
Ciliates (without Mesodinium)
Abundance (cells L-1)
Dinophysis acuta
Abundance (cells L-1)

Figure 11

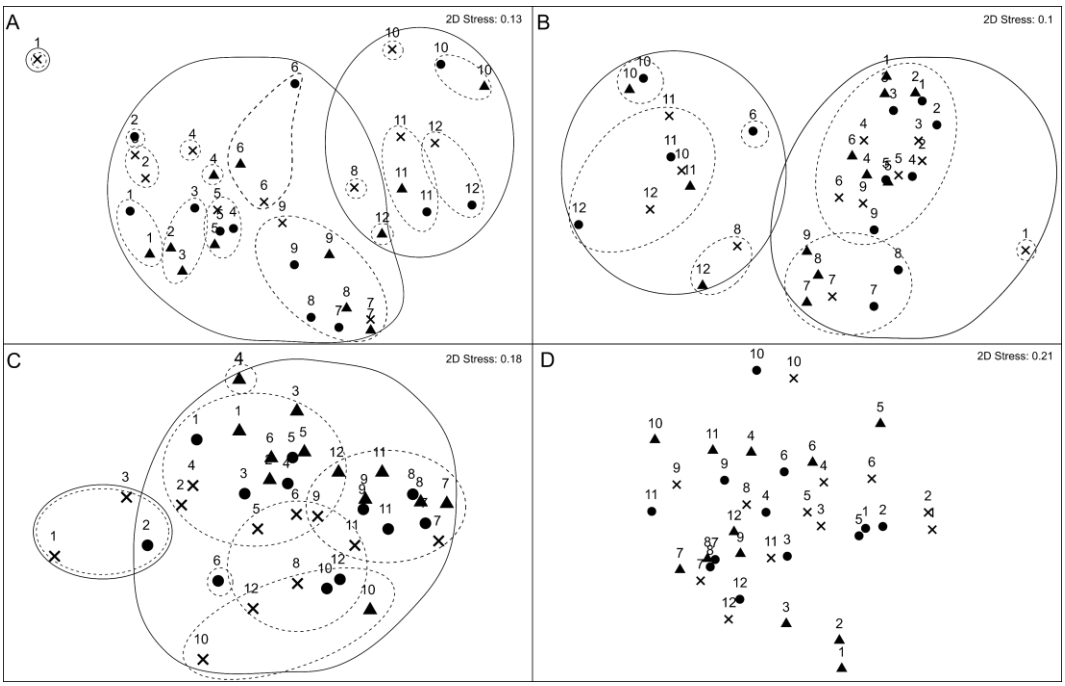

Figure 12 - colour