Development of a Conceptual Warning System for Toxic Levels of *Alexandrium fundyense* in the Bay of Fundy based on Remote Sensing Data

Running page head: Remote sensing of *A. fundyense*

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Abstract: Harmful algal blooms (HABs) present a potential danger for human health and commercial activities, especially in coastal regions. Observing systems increasingly rely on remote sensors to monitor and possibly predict the locations and intensity of such blooms. Here we present a novel approach for detecting HABs of *Alexandrium fundyense* in the Bay of Fundy, Canada. *A. fundyense* is considered toxic for individuals who consume shellfish when cell abundances adjacent to shellfish harvesting areas are as low as 200 cells•L^{-1}, making it difficult to use direct remote sensing techniques to assess the threat in the early stages of the development of the bloom. Using *in situ A. fundyense* cell abundance measurements, together with satellite observations of sea-surface temperature and the occurrence of diatom-dominated phytoplankton populations, a warning system was developed based on three levels of alerts: green (low abundance of *A. fundyense*), orange (possible threat of *A. fundyense*) and red (high probability of *A. fundyense* concentrations that would result in shellfish toxicity above safe levels for human consumption). Combined information on diatom phenology and variations in sea-surface temperature are key to the timing of *A. fundyense* blooms: our data reveal that the termination of the diatom spring bloom, associated with the warming of the water, can trigger an increase in *A. fundyense* cell abundance. The objective criteria for a HAB warning system was developed and tested in the Bay of Fundy using two different datasets: one to develop the algorithm (data collected between **1998** and **2007**) and one to assess its performance (data collected in 2011). The warning system is based on the cautionary principle that a false negative (warning not issued when it should have been) is far more serious than a false positive (warning issued when it should not have been). The overall success of the algorithm when tested on the validation dataset is about 70%

using a threshold of 150 *A. fundyense* cells•L^{-1}, with a low occurrence of false negative red alerts (<8%). The satellite-data-based warning can be used to optimize an *in situ* monitoring system, which can be designed to be more intensive when the warning status is orange or red. This study demonstrates that combined satellite information on phytoplankton phenology and sea-surface temperature can help predict low abundances of toxic *A. fundyense* cells. It also highlights the importance of an integrated approach combining satellite and *in situ* observations to monitor HABs.

1. Introduction

Harmful algal blooms (HABs) continue to be of concern throughout the world and research is focusing on the development of tools for their early detection. Toxic algae occur in all phytoplankton groups, including diatoms (e.g., *Pseudo-nitzschia* spp.), dinoflagellates (e.g., *Alexandrium* spp.) and cyanobacteria (e.g., *Trichodesmium* spp.). This work focuses on a single species, *Alexandrium fundyense*, from a particular location, the Bay of Fundy, eastern Canada.

The dinoflagellates, *Alexandrium fundyense* is known to produce paralytic shellfish poisoning (PSP) toxins in the Bay of Fundy and neighboring Gulf of Maine. PSP toxins can accumulate in shellfish through filter-feeding, and are potentially fatal to vertebrate consumers (Prakash et al. 1971, Martin and Richard 1996, Hamer et al. 2012). This harmful algal species affects wild fisheries as well as finfish and shellfish aquaculture in the region. *A. fundyense* has been responsible for Atlantic salmon (*Salmo salar*) poisoning and mortalities in the Bay of Fundy (Martin et al. 2008), when concentrations reached 2.0 x 10^5 cells•L^{-1} (Burridge et al. 2010). *A. fundyense* was also responsible for Atlantic herring (*Clupea harengus harengus*) mortalities in 1976 and 1979 (White 1977, 1980).

A. fundyense is considered to be harmful for vertebrate consumers of shellfish even when cells are present at low densities in natural waters, with counts of 200 cells•L^{-1} being considered to be the level at which toxicity can be detected in shellfish in the Bay of Fundy (Martin et al. 2010b; J.L. Martin, pers comm). Values of around 500 cells•L^{-1} can lead to levels of shellfish toxicity above the threshold accepted for human consumption (80 µg STX equiv. 100 g meat), leading to closures of shellfish harvesting areas (Page et al. 2004, J.L. Martin pers. comm.). Many countries throughout the world, including Canada, have extensive programs to monitor toxins in shellfish to comply with domestic and international regulations that ensure safe products for consumers.

Variables including the timing of the *Alexandrium* spp. blooms (temporal and spatial variations in abundance), and environmental conditions prior to, during, and after these occurrences, are being studied in some areas, to understand the population dynamics of this species. These observations have revealed a complex pattern of seasonal variations, with the species generally beginning to appear in the water column in late spring (May) and subsiding in late summer (July/August) (Martin et al. 2010b). There is strong interannual variability in the timing, intensity, and regional distribution of the cells (Page et al. 2004). During the winter resting period, cysts of *A. fundyense* tend to occur in high concentrations in areas of mud/clay bottoms rather than gravel/rocky bottom sediments. A high abundance of resting cysts in the winter does not correlate with large blooms of *A. fundyense* in the summer months (Martin et al., 2014). Many years of observations have shown that *A. fundyense* cells first appear in the water column when the water temperature begins to increase, and the population multiplies exponentially in low-wind and low-light conditions associated with fog (Martin et al. 2014a). The identification of the exact environmental conditions that trigger the occurrences and propagation of *A. fundyense* cells continues to be elusive (Townsend et al., 2005, 2014). These complexities have made it difficult to model and predict *A. fundyense* blooms with a view to minimizing risks to seafood consumers and damage to the aquaculture industries.

It is difficult, if not impossible, to develop a large-scale warning system that relies solely on *in situ* measurements. For instance, automated moorings and buoys, despite measuring water properties with high temporal resolution are not able to capture changes that may occur several kilometers upstream or downstream of their location. This has led to the investigation of the use of satellite remote sensing based on sea-surface temperature to detect blooms of *A. tamarense* in the Gulf of Maine (e.g., Keafer and Anderson, 1993). In other regions of the world, algorithms have been developed to identify HABs using satellite ocean-colour measurements (Sathyendranath et al. 1997; Subramaniam et al. 1999, 2002, Kahru et al. 1997, Babin et al. 2005, Hu et al. 2010, Tomlinson et al. 2004, 2009). Growing interest in the detection of HABs has led to the creation of an international program (the IOC-SCOR GEOHAB programme) that co-ordinates and builds on national, regional and international efforts in HAB research within an ecological and oceanographic context.

In this study we begin with the method of Keafer and Anderson (1993) and consider whether a combination of variables measured by remote sensing could provide information complementary to what we observe from *in situ* measurements. Recently, Mc Gillicuddy et al. (2015) investigated the use of the Medium Resolution Imaging Spectroradiometer (MERIS) ocean colour data to obtain information on *A. fundyense* blooms in the Bay of Fundy. They concluded that satellite-retrieved chlorophyll-a concentration, an index of phytoplankton biomass, could be useful to track the presence of high phytoplankton biomass. Direct observation of the presence of *A. fundyense* at harmful levels using remote sensing remains difficult, especially considering that levels of toxins in shellfish are considered unsafe even at very low cell concentrations (around 500 cells•L^{-1}), when *A. fundyense* is not the dominant species in the water column. Furthermore, in the Bay of Fundy, the optical signature of the species closely resembles that of other common phytoplankton species, including diatoms and other dinoflagellates (M.-H. Forget, unpublished data).

We explore the potential to detect *A. fundyense* indirectly, using indicators of the marine ecosystem that are accessible through remote sensing. In particular we exploit the species succession dynamics evidenced by Townsend et al. (2005) in the Gulf of Maine, where *A. fundyense* blooms tend to follow the decline of diatom blooms. Since *A. fundyense* appears when the temperatures increase after the winter minimum, we also used sea-surface temperature (SST) as a proxy for *A. fundyense* observation. The focus of the study is on *A. fundyense* cells present in the surface waters - we do not address the issue of overwintering *A. fundyense* resting cysts, which can be responsible for some winter time shellfish toxicity – either through re-suspension of cells due to digging activities, wave movement or other disturbances. We also investigate whether using a threshold of 200 *A. fundyense* cells•L^{-1} is a realistic target for a remote sensing approach using our *in situ* dataset to determine the optimum threshold for a warning system.

2. Material and Methods

2.1 *In situ* data

A monitoring program was initiated in 1987 with water samples collected at four/five stations on a monthly basis during the colder months (from November to March each year) and on a weekly basis between April and October to monitor phytoplankton population dynamics, including the toxic algae *A. fundyense* (Martin et al. 2001,2006, 2014b), amongst other objectives. From this archive of five stations (See Figure 1, Martin et al. 2014b), we selected data from the most offshore station, i.e., Wolves station (Figure 1), between 1998 and 2007 (corresponding to the period of satellite observations), to develop a satellite-based approach to monitor blooms of *A. fundyense*. In 2011, two additional offshore stations were added to the initial sampling plan (stations 46 and 57, Figure 1, Table 1) to create an *in situ* dataset independent of the data used for algorithm development. These three stations were used because of their offshore location, which made them better suited for satellite observations (i.e., reduced contamination of the marine signal by land) than in-shore stations. We limited the original dataset from early May to mid-July to focus our study on the timing of *A. fundyense* blooms. Station Wolves is located 7.5 and 16.5 km from stations 46 and 57 respectively while station 46 and 57 are separated by 9.5 km.

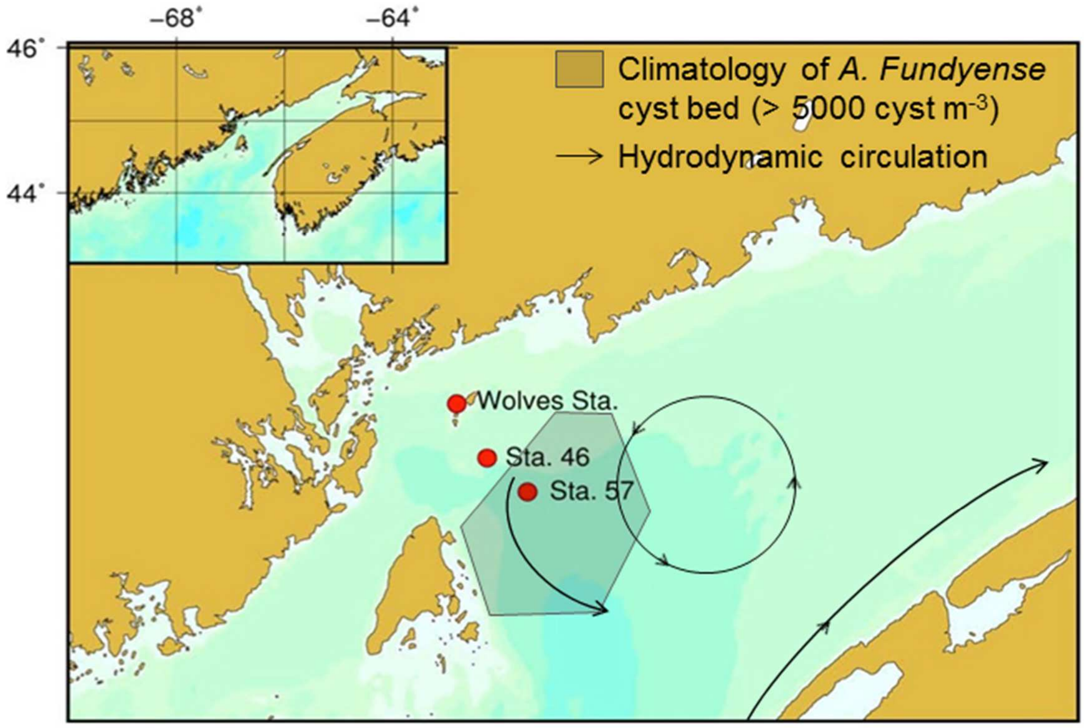

Figure 1: Sampling stations in the Bay of Fundy, eastern Canada: The Wolves and off shore stations 46 and 57.

Sampling was conducted aboard the Canadian Coast Guard Research Vessel, *Viola M. Davidson* and on the Huntsman Marine Science Center vessel *Fundy Spray*.

Phytoplankton samples were collected from the water surface by bucket for all three stations. During the summer months a 10 m vertical plankton haul was made with a 20-µm mesh net, 0.3 m in diameter. A subsample was preserved with formalin:acetic acid (1:1 by volume) for plankton identification. Water samples (250 mL) were preserved with 5 mL formalin:acetic acid. Samples were brought back to the laboratory and 50-mL subsamples were allowed to settle in Zeiss counting chambers for 16 h before microscopic examination. All phytoplankton of size greater than 5 µm were identified and enumerated (as cells•L^{-1}) using a Nikon inverted microscope.

Table 1: Station name, station number, location, period of sampling

Name	N	Longitude	Latitude (°W)	Period of sampling
Wolves Islands	16	-66.73	44.97	1998-2011
Off shore 1	46	-66.67	44.89	2011
Off shore 2	57	-66.59	44.84	2011

2.2 Satellite Data

2.2.1 Satellite datasets for algorithm development

Daily remote sensing reflectance (level 2) data from the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) were downloaded from the NASA ocean-color website (http://oceancolor.gsfc.nasa.gov). Images over the Bay of Fundy covering the *in situ* sampling stations were downloaded from April to July between 1998 and 2007.

Chlorophyll-a concentration, an index of phytoplankton biomass, was derived using the OC4 algorithm (O'Reilly et al., 1998). The algorithm developed by Sathyendranath et al. (2004) was applied to the remote-sensing reflectance to derive, on a pixel-by-pixel basis, the probability of the occurrence of diatoms, expressed on a scale from 0 to 1 over an eight-day period of observation. In brief, the algorithm is based on look-up-tables to derive chlorophyll-a concentration from remote-sensing reflectance for two possible populations of phytoplankton, one dominated by diatoms and one made of a mix of phytoplankton. Each of these two phytoplankton populations has different absorption properties, which were derived by studying the relationships between the composition and concentration of pigments (i.e., chlorophyll-a, fucoxanthin and chlorophyll-c_3, Sathyendranath et al, 2004). Using a semi-analytical reflectance algorithm (Sathyendranath and Platt, 1997, 1998), reflectance ratios 490:670 and 510:555 are computed for chlorophyll-a ranging from 0.01 to 64 mg m^{-3} for the two possible populations (see Figure 3 Sathyendranath et al. 2014). On a pixel-per-pixel basis, the two chlorophyll-a concentrations (i.e., chl-$a_{490:670}$ and chl-$a_{510:555}$) are computed for each population (i.e., diatom and mixed) giving a total of four chlorophyll-a concentrations. The lowest coefficient of variation, CV_i, where *i* stands for diatom or mixed population, computed as:

$$\mathrm{CV}_i = \left(\frac{\sigma(\mathrm{chl{-}a}_{490:670}, \mathrm{chl{-}a}_{510:555})}{\overline{(\mathrm{chl{-}a}_{490:670}, \mathrm{chl{-}a}_{510:555})}}\right)_i, \quad (1)$$

provides the population in the pixel. A value of 1 is returned if the population of phytoplankton is dominated by diatoms ($CV_{diatom} < CV_{mixed}$) and 0 if this is not the case. Because sediment loads and colored dissolved organic matter would impact the four chlorophyll-a concentrations, and therefore the coefficient of variation (CV), in a similar fashion, we believe that the diatom algorithm remains valid in coastal waters, as attested by the phenology of diatoms which is consistent with species succession recorded in this region (Johnson et al., 2017). Daily satellite passes were averaged into eight-day composite images using the arithmetic mean of all available values for a given pixel during that time period, which yielded a probability of occurrence of diatoms (subsequently expressed in %) for each pixel. The probability of occurrence of diatoms and chlorophyll-a concentration were projected on an equal-area grid with a 1.5 km resolution (2.25 km^2 per pixel).

Daily Sea Surface Temperature (SST) data from the Advanced Very High Resolution Radiometer (AVHRR) pathfinder version 5.2 (PFV5.2) time series were downloaded from the NASA Jet Propulsion Laboratory archive (http://podaac.jpl.nasa.gov) for the same time period and region as the ocean-colour data. The PFV5.2 data are an updated version of the Pathfinder Version 5.0 and 5.1 collections described in Casey et al. (2010).

Global L3 SST data were averaged over an eight-day period and projected on the 1.5 km grid from the initial 4 km resolution (0.044°). An example of satellite-derived probability of diatom occurrence (PDO) and SST for the Bay of Fundy is shown in Figure 2.

For each satellite dataset (i.e., eight-day composite images of SST, diatom occurrence and chlorophyll-a concentration), 5x5 matrices centered on the Wolves station were extracted for the times series. Eight-day composites were selected to reduce the impact of cloud cover while keeping a temporal resolution consistent with the *in situ* sampling. A total of 74 match-ups between satellite data and *in situ* measurements were obtained, over the years 1998 – 2007. Note that the initial *in situ* dataset had 118 samples where the abundance of *A. fundyense* was enumerated, but 44 were not exploitable due to missing information on SST, diatoms or both sets of satellite data because of cloud cover, despite the 8-day binning.

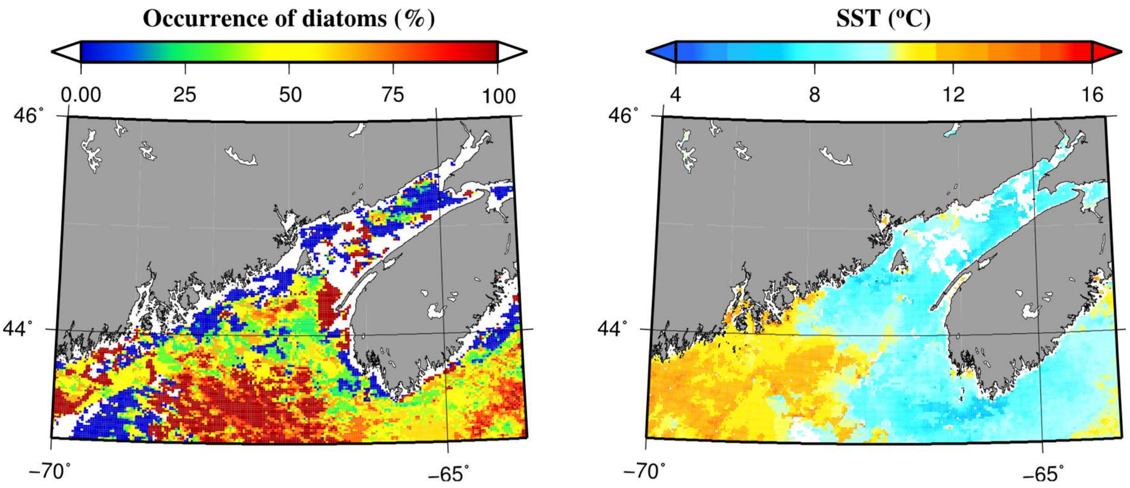

Figure 2: Composite (24-31 May 2006) image of the Bay of Fundy showing the probability of occurrence of diatoms derived from the SeaWiFS sensor and sea-surface temperature (SST) derived from the AVHRR sensor for the same period, and same location. Occurrence of diatoms is given as a percentage probability (0 – 100) of finding diatom-dominated populations in that area during that time interval.

2.2.2 Satellite dataset for algorithm validation

The algorithm was developed using SeaWiFS data for the years 1998 to 2007. Because of intermittent failure of the sensor from 2007 to 2010, NASA terminated the mission in 2010. For the year 2011, data from ESA's MERIS sensor were used to estimate the occurrence of diatoms (sea-surface temperature was inferred from AVHRR data for the 1998-2007 time series). The diatom algorithm was adapted from SeaWiFS to MERIS instead of the Moderate Resolution Imaging Spectroradiometer (MODIS) because it required only minor tuning of the algorithm to account for 5 nm shifts in two of the wavebands (560 and 665 nm) used to discriminate diatoms, whereas no band shifting was required for the other two wavebands (490 and 510 nm). Daily, full-resolution (300 m at nadir) level-2 MERIS data from January to August 2011 over the Bay of Fundy were downloaded from the NASA Ocean Color website (http://oceancolor.gsfc.nasa.gov). These data were composited into eight-day images with a resolution of 1.5 km, as was done for the SeaWiFS dataset. Satellite data coincident with *in situ A. fundyense* cell counts were extracted at the Wolves, Sta. 57 and Sta. 46 for the year 2011, which yielded a total of 25 data points for validation of the algorithm.

2.3 Warning system for possible toxic levels of *A. fundyense*

Given the difficult task of inferring *A. fundyense* cell concentrations using satellite remote sensing information, a simple model that uses three levels of warning (safe = green; inconclusive = orange; harmful = red) was developed based on sea-surface temperature and the probability of diatom occurrence (PDO). In agreement with the information provided by the climatology, the three threshold levels that are used to detect favourable conditions (or not), for the presence of *A. fundyense,* as illustrated in Figure 3 are as follows:

- PDO above a given threshold, PDO_T, indicates safe conditions (no toxic *A. fundyense* cell concentrations):
- PDO below PDO_T and SST below a lower threshold, SST_{LT}, indicates safe conditions (no toxic *A. fundyense* cell concentrations):

- SST above an upper threshold, SST_{UT} and PDO below PDO_T indicates likelihood of high concentrations of toxic *A. fundyense* cells
- PDO below PDO_T and SST between SST_{LT} and SST_{UT} - no conclusive indication of toxic level of *A. fundyense.*

In developing the warning system, optimum values have to be found for the thresholds PDO_T, SST_{LT} and SST_{UT} that lead to the best performance of the algorithm (see Figure 3 schematic).

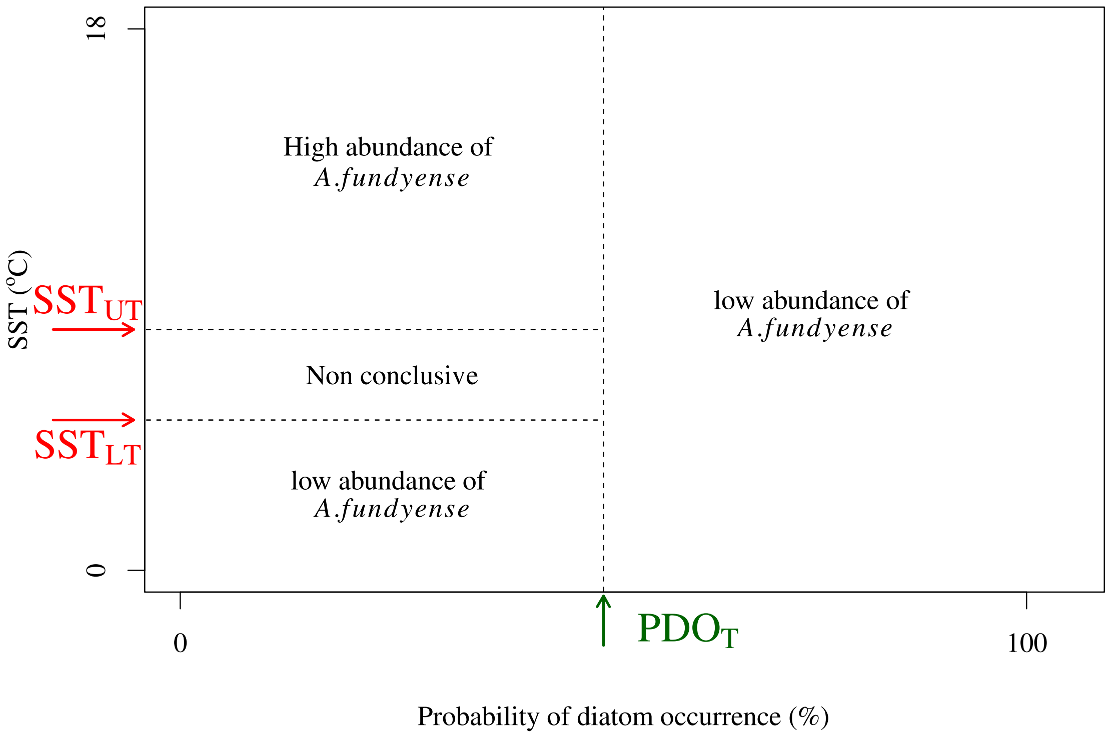

Figure 3: Schematic of the satellite-based warning system that relies on two SST thresholds and one PDO threshold to infer safe, non-conclusive and toxic level of A. fundyense.

A sensitivity study was carried out to find the optimum thresholds that provided the best performance (i.e., success rate) of the algorithm. Abundance of *A. fundyense* was varied between 100 and 600 cells•L^{-1} (step of 50 cells•L^{-1}), PDO was varied between 30 and 80% (step of 5%) to find the optimum threshold PDO_T, and SST was varied between 4.4 and 6° C (step of 0.1° C) to find the lower temperature threshold, SST_{LT}, and the upper SST was varied between 6 and 9° C (step of 0.1° C) to find the upper temperature threshold, SST_{UT}. This led to a total number of 63,767 possible cases. The objective of this sensitivity study was to simultaneously find the lowest *A. fundyense* cell concentration that can be most successfully predicted by proxy using SST and PDO, and the corresponding SST and PDO criteria. For each case of this sensitivity study, a weighted score was assigned according to a comparison of the satellite warning system prediction versus the actual *in situ* measurements of *A. fundyense* abundance as follows:

- *In situ A. fundyense* abundance is potentially toxic and satellite response is red: 1.25 points,
- *In situ A. fundyense* abundance is not potentially toxic and satellite response is green: 1 point,
- *In situ A. fundyense* abundance is potentially toxic and satellite response is orange: 0.5 point,
- *In situ A. fundyense* abundance is not potentially toxic and satellite response is orange: 0.25 point,
- *In situ A. fundyense* abundance is potentially toxic and satellite response is green (false negative): -1 points,
- *In situ A. fundyense* is not potentially toxic and satellite response is red (false positive): 0 points.

This scoring system was weighted to emphasize the ability to predict potential toxic levels of *A. fundyense* cells; in that respect, agreement between the satellite and *in situ* data for potential toxic levels of *A. fundyense* is weighted at 1.25 points and false negatives are given a negative score (-1).

<u>3. Results</u>

3.1 Phenology and spatial variation of A. fundyense, diatom occurrence and Sea-Surface Temperature

Phytoplankton phenology in the Bay of Fundy derived from satellite data follows the pattern commonly observed at temperate latitudes, with a strong bloom in late March/early April (Vargas et al. 2009, Platt et al. 2009, Racault et al. 2012, Martin et al. 1995, 1999, 2001, 2006) with the probability of diatom-dominated populations being consistently high in early Spring (Figure 4). Climatological data of satellite-derived occurrence of diatoms and *in situ* measurements of *A. fundyense* abundance reveals an interesting succession in the development and termination of blooms of these two species. Analysis of the climatological data suggests that the development of *A. fundyense* blooms may be related to the decrease in diatom abundance, as well as the increase in SST and a change in wind patterns. McGillicuddy (2010), studying *A. fundyense* in neighbouring Gulf of Maine, reported similar relationships with temperature and winds. Termination of the *A. fundyense* bloom is not discussed in the present study; however, it often coincides with the onset of the fall diatom bloom when the probability of diatom occurrence increases monotonically to greater than 40% (day 292). Extreme events such as storms and persistent strong winds are also known to end *A. fundyense* blooms. It is noteworthy that a high PDO (~80%) occurs in the winter months when chlorophyll-a concentration is moderately high (~1.2 mg m^{-3}), while the probability of diatom occurrence at the chlorophyll-a maximum (~ 2.9 mg m^{-3}on day 130) is only about 40%.

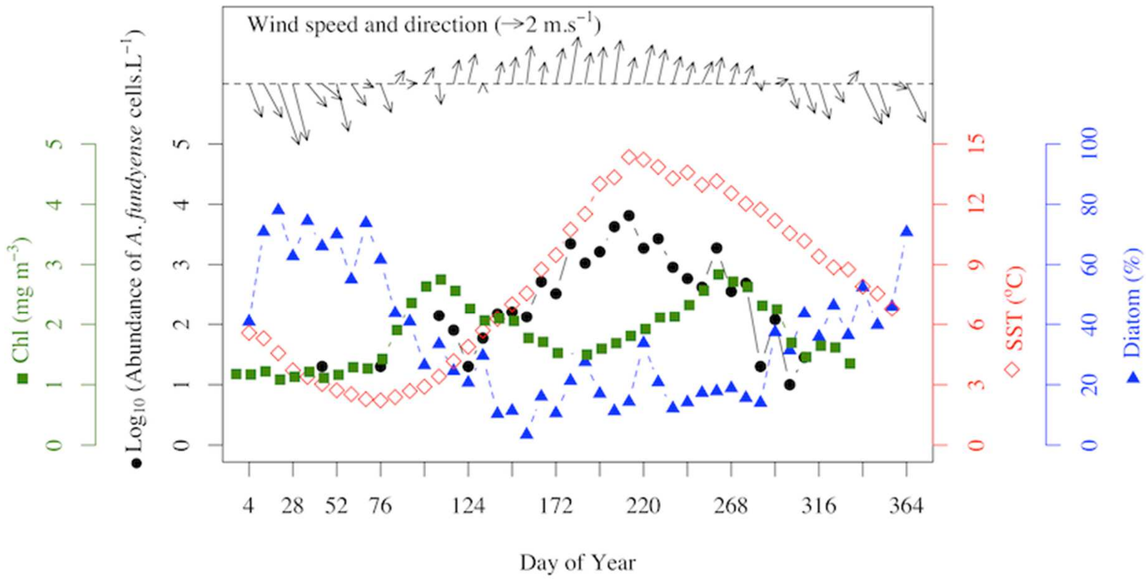

Figure 4: Eight-day climatology (1998-2007) of satellite-derived SST (red diamonds), probability of occurrence of diatoms (blue triangles), chlorophyll-a concentration (green squares) and wind speed and direction, as well as in situ cell abundance of A. fundyense (black Circle) in the Bay of Fundy (Wolves station).

The diatom algorithm relies on spectral differences in the remote sensing reflectance and is independent of the biomass at the first order, whereas the chlorophyll-a algorithm, based on band-ratios, depends mainly on the magnitude of the remote sensing spectra, and therefore biomass.

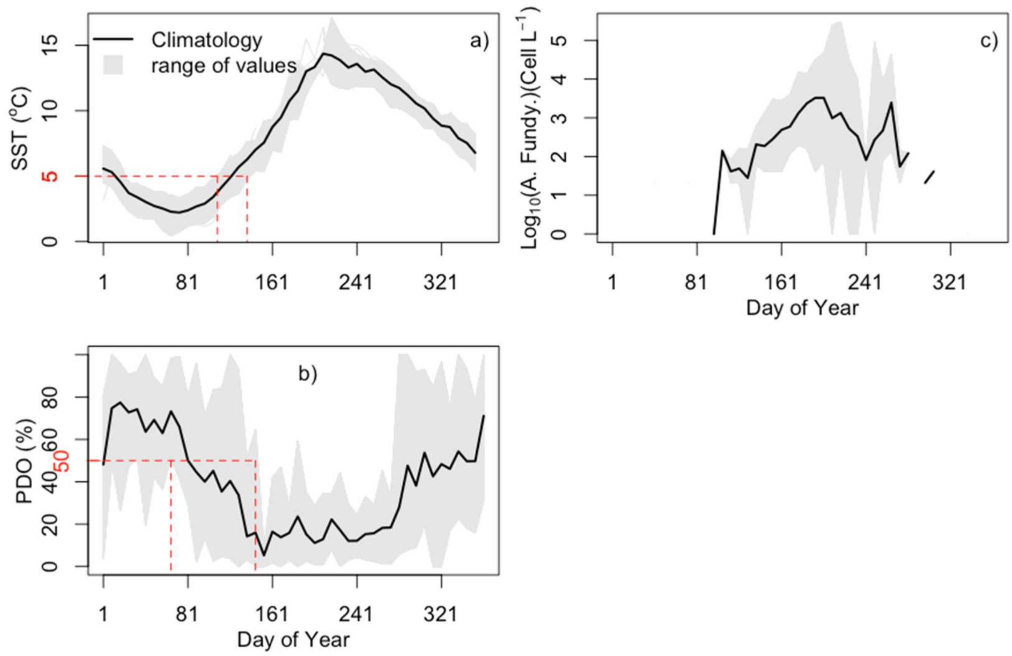

Figure 5: Eight-day climatology (1998-2007) and range of variation of a) satellite-derived SST, b) probability of occurrence of diatom and c) in situ cell abundance of A. fundyense at the Wolves station.

Sea-surface temperature and probability of occurrence of diatoms show a strong interannual variability (Figure 5 a and b). As an indicator, we have selected the first time that sea-surface temperature is warmer than 5 degrees during the spring, which occurs between early (~ 1st) and late (~ 21st) June, showing a temporal variability of three weeks, in agreement with the high variability observed with the timing of *A. fundyense*. The termination of the phytoplankton diatom spring bloom shows an even wider range of variation than the SST. This is not surprising given the number of environmental factors that can explain shift and successions in phytoplankton population (e.g., nutrient availability, strength of stratification and depth of the mixed layer). As an indicator of the timing of the termination of the diatom dominance, we have recorded the day after which probability of occurrence of diatoms remains below 50% for three successive eight-day composite images. This occurs between the end of March (~ 21^{st}) and end of June (~30^{th}), such that the decline in diatom occurrence is spread over a 12-week period. The earliest initial occurrence of potentially toxic concentrations of *A. fundyense* cells (> 200 cells•L^{-1}) was recorded on 13 May 2005 while the latest initial occurrence was recorded on 7 June 2007 at the Wolves station (Figure 5c). This corresponds to a four-week window in the onset of the *A. fundyense* bloom for the period of observation in the study area. The probability of diatom occurrence reached levels of up to 80% in late winter /early spring (day of year 28 to 84) whereas concentrations of *A. fundyense* remain low during the same time period (less than100 cells•L^{-1}). Following the sharp decrease in the PDO around days 85 to 116, an increase in *A. fundyense* concentrations was observed starting around day-of-year 124 (i.e., beginning of May, 100 cells•L^{-1}) and reaching a maximum in summer, with abundances often greater than 10,000 cells•L^{-1} (Figure 4). During the sampling period, the onset of the exponential growth phase of *A. fundyense* coincided with an increase in SST, and a change in wind patterns from a southeastward direction to a northeastward direction (Figure 4). Information on the phenology of SST and PDO reveals that the decline of the diatom bloom is a necessary but not sufficient condition for the development of *A. fundyense*. It is also noteworthy that diatom dominance shows a strong spatial variability compared to SST (Figure 2), perhaps in a similar fashion to *A. fundyense*. This pattern is revealed when comparing the properties at the three offshore stations and notably the correlation, or absence of correlation, between

SST, POD and *A. fundyense* over the period of sampling between late April (~ 23rd) and late July in (~20th) 2011 (Table 2).

Table 2: Correlation coefficients (r^2) between stations Wolves, 46 and 57 for sea-surface temperature, probability of occurrence of diatom and abundance of A. fundyense.

	SST			POD			*A. fundyense abundance*		
	Wol.	St. 46	St. 57	Wol.	St. 46	St. 57	Wol.	St. 46	St. 57
Wol.	1			1			1		
St. 46	0.94	1		0.32	1		0.96	1	
St. 57	0.93	0.98	1	0.32	0.54	1	0.42	0.39	1

Sea-surface temperature shows a high positive correlation coefficient between the three stations (> 0.93), which is consistent with the rather homogeneous spatial distribution of SST and is also in agreement with the seasonal and interannual variation of SST (Figure 5), which showed the least variation (e.g., reaching the 8 degree threshold over a 3-week period between 1998 and 2007 at the Wolves station). The correlation coefficients between the 3 stations for POD and *A. fundyense* abundance show weaker relationships (r^2 < 0.54) than ones observed for SST, except for *A. fundyense* between stations 46 and Wolves (r^2 = 0.96). The mean *A. fundyense* abundance at stations Wolves, 46 and 57 are 1053(±1926), 370 (±803) and 313 (±439) cell.L^{-1} respectively and exhibit a decreasing gradient from the coast towards the center of the Bay of Fundy. The mean SST remains homogeneous between all three stations: Wolves = 10.4 ± 3.1 °C, station 46 = 10.7 ± 4.4 and station 57 = 10.5 ± 3.3 °C. The POD shows similar means at stations Wolves and 46

(44 ± 21% and 47 ± 21 %), which are slightly higher than the mean POD at station 57 (40 ± 21%), despite similar average conditions over the period of observation (almost 2 months).

3.2 SST and PDO thresholds for *A. fundyense* warning system

The weighted scoring system described in the methods section was applied to the development dataset (N=74) for all the possible sets of thresholds (i.e., SST_{LT}, SST_{UT} and PDO_T). This resulted in scores that varied between 25.75 (worse performance) and 55.75 (best performance). The best score (55.75) was obtained for a detection level of *A. fundyense* counts of 150 cells•L^{-1} and corresponded to thresholds of 60% for the probability of occurrence of diatoms, 5.5°C for the lower sea-surface temperature threshold and 6.8° for the upper SST threshold (the same score was also obtained for a SST_{LT} of 5.6°C). Figure 6 shows the application of these thresholds together with *A. fundyense* cell counts and corresponding SST and PDO measurements for each sample.

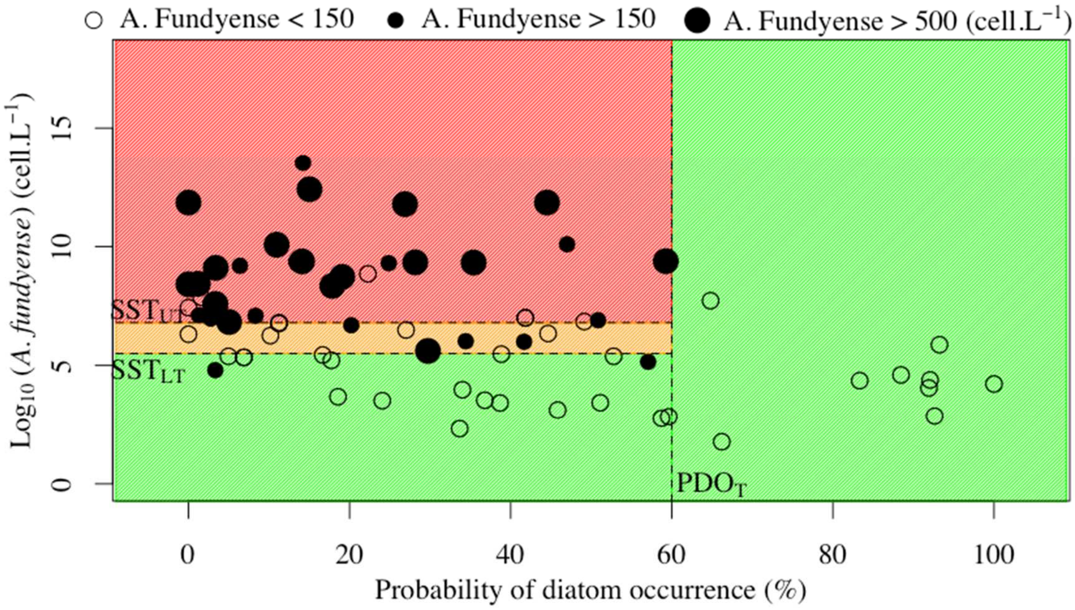

Figure 6: A. fundyense cell abundance as a function of SST and probability of diatom occurrence using the development dataset. Warning alerts (threshold 1 to 3) are colour-coded from green, to orange to red for low, inconclusive and high risk of toxicity, respectively.

The success rate of the algorithm under these criteria is 73% (Table 3), or 54 out of 74 cases. Among these 54 well-identified cases, 28 were classified as toxic levels of *A. fundyense* and 26 were correctly identified as safe levels of *A. fundyense* abundance. Only 3% (2 out of 74 cases) resulted in false negative warnings and 9% in false positive alerts (7 out of 74 cases). Of the remaining cases, 15% (or 11 out of 74) were not conclusive (orange warning level), when adding these 11 cases to the proper assessment of *A. fundyense* level, the algorithm reaches a success rate of 88%. When applied to the validation dataset (N=25), the success rate remained similar to that of the development

Table 3: Matchup between satellite and in situ warning levels of A. Fundyense cell concentrations for the development dataset (N=74). The threshold for A. fundyense concentration for satellite detection is 150 cell.L^{-1}.

		Satellite		
		Toxic	Safe	
In situ	Toxic	28 11	2	3% False negative
	Safe	7	26	
		9% False positive		88% success

Table 4: Matchup between satellite and in situ warning levels of A. Fundyense cell concentration for the validation dataset (N=25). The threshold for A. fundyense concentration for satellite detection is 150 cell.L^{-1}.

		Satellite		
		Toxic	Safe	
In situ	Toxic	12 1	4	16% False negative
	Safe	1	7	
		4% False positive		80% success

dataset with an overall success rate of 76% (Table 4), which reaches 80% when accounting for the orange level. The percentage of false negative warnings is 16%, false positive warnings and inconclusive cases were both equal to 4%.

Variation of the scores as a function of the lower SST threshold shows a continuous decrease between 4.4 and 5.2 ºC followed by a sharp increase to reach the maximum score at a temperature of 5.5 or 5.6 ºC (Figure 7a). Beyond the SST_{LT} value of 5.6 ºC, one observes a monotonic decrease. The score remains fairly high for the upper SST threshold from 6 to 6.8 ºC, reaching the maximum value at 6.8 ºC and then decreases rapidly as SST increases (Figure 7b). These results demonstrate the narrow ranges of lower and upper temperature thresholds at which the algorithm performs best. For the probability of diatom occurrence threshold (Figure 7c), the score increases from 30 to 60% PDO followed by a small decrease, then remains constant past a value of 65%, a function of the training dataset, which contains only few cases with high PDO.

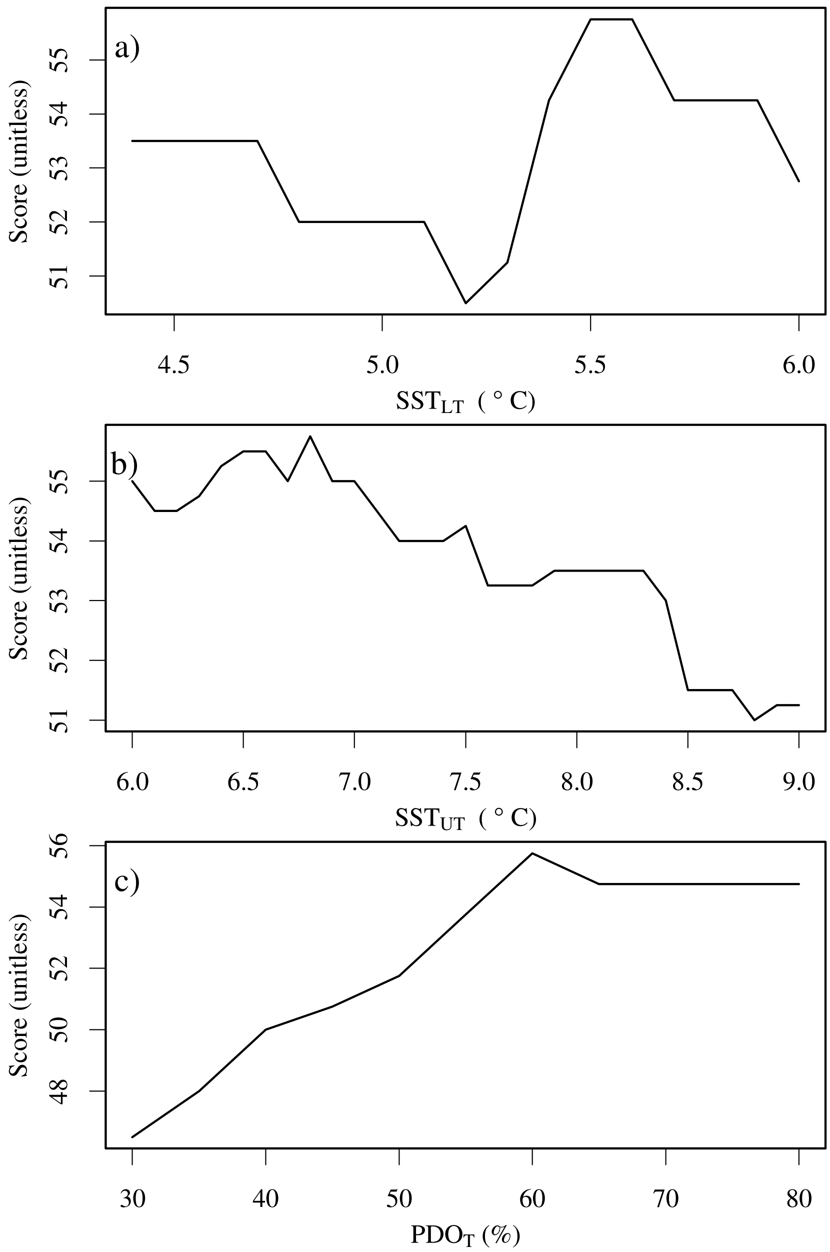

Figure 7: Performance score of the warning algorithm as a function of a) lower threshold of sea-surface temperature (SST_{LT}), b) upper threshold of sea-surface temperature (SST_{UT}) and c) threshold of probability of occurrence of diatom (PDO_T).

3.3 Detection of the onset of *A. fundyense blooms*

Even if the algorithm has demonstrated a good overall performance (i.e., ~80% success rate for both the development and validation dataset), a critical assessment of the algorithm lies in its ability to detect the onset of *A. fundyense* exponential growth and variation in timing. Blooms of *A. fundyense* follow a life cycle that can be described in four stages starting with 1) the release of cysts from the bottom of the ocean (excystement) in early spring, 2) a vegetative period, 3) the germination and blooming of *A. fundyense* in late spring and early summer and 4) the sedimentation of newly formed cysts in the fall. Although no direct relationship between the number of cysts and the abundance of cells has been demonstrated (Anderson et al. 2014, Martin et al. 2005, Martin et al. 2014a), the spatial extent of *A. fundyense* cyst beds in the fall/winter drives the spatial distribution of the bloom the following spring. The sediment beds in the central part of the Bay of Fundy (Figure 1) host one of the two major cyst beds in the Gulf of Maine/Bay of Fundy system (Anderson 2014). It has been shown that light and temperature trigger the release of the cysts (Anderson et al. 2005, 2014) into the water column, especially at depths less than 50 m and where transport plays a major role in the distribution of the bloom. Cysts in sediments at depths greater than 100 m tend to have a two-week time lag in germination, compared to those at shallower depths (Vahtera et al. 2014), which is in agreement with the timing observed at stations 46 and 57 (Figure 8b-c, respectively), where the first level of abundance greater than 150 cell.L^{-1} occurs three to four weeks later than at station Wolves (Figure 8a). Station Wolves is located in a relatively shallow part of the Bay of Fundy (60 m) compared to station 46 (111 m), which is located on a strong bathymetric gradient, and station 57 (126 m). Some cysts that are released in the central part of, or close to, the gyre of the Bay of Fundy (stations 46 and 57), are leaked at its edges and are carried towards the east (i.e., Gulf of Maine) by the coastal current. In that respect, stations 46 and 57 are located at the edge of the gyre such that the cysts in that area are subject to lateral transport. Our algorithm uses a pixel-based approach that relies on water mass properties to model favourable conditions for *A. fundyense* growth. It does not identify the initiation of the bloom per se but rather an abundance of cells for given environmental conditions. The chronological mapping of the warning level in late spring early summer is consistent with known hydrodynamic patterns and bathymetry of the Bay of Fundy, however, it does not provide information on transport and germination of the cells, which are subject to complex, and not fully understood, forcing. Our model rather relies on identification of water masses. Despite this, the algorithm is able to detect the first toxic levels of *A. fundyense* at all stations and provide information on temporal variation. At the Wolves station (Figure 8a), the first occurrence of *A. fundyense* cell concentrations over 150 cells•L^{-1} occurs in early May, and is correctly identified as an orange warning by the satellite-based algorithm. The algorithm also records the decrease in *A. fundyense* abundance in late May in agreement with the *in situ* measurements. Again, the algorithm detects the increase in the toxic algae the following week (red level the first week of June) and remains at the red level from early June to mid-July as *A. fundyense* abundance is greater than the 150 cell.L^{-1} threshold. The satellite warning system is in agreement with *in situ* measurements. In late July, conditions were identified as safe (green) for an abundance of 320 cells•L^{-1}. In that instance, we observed a sharp decrease in *A. fundyense* abundance, but it was not below the safe level.

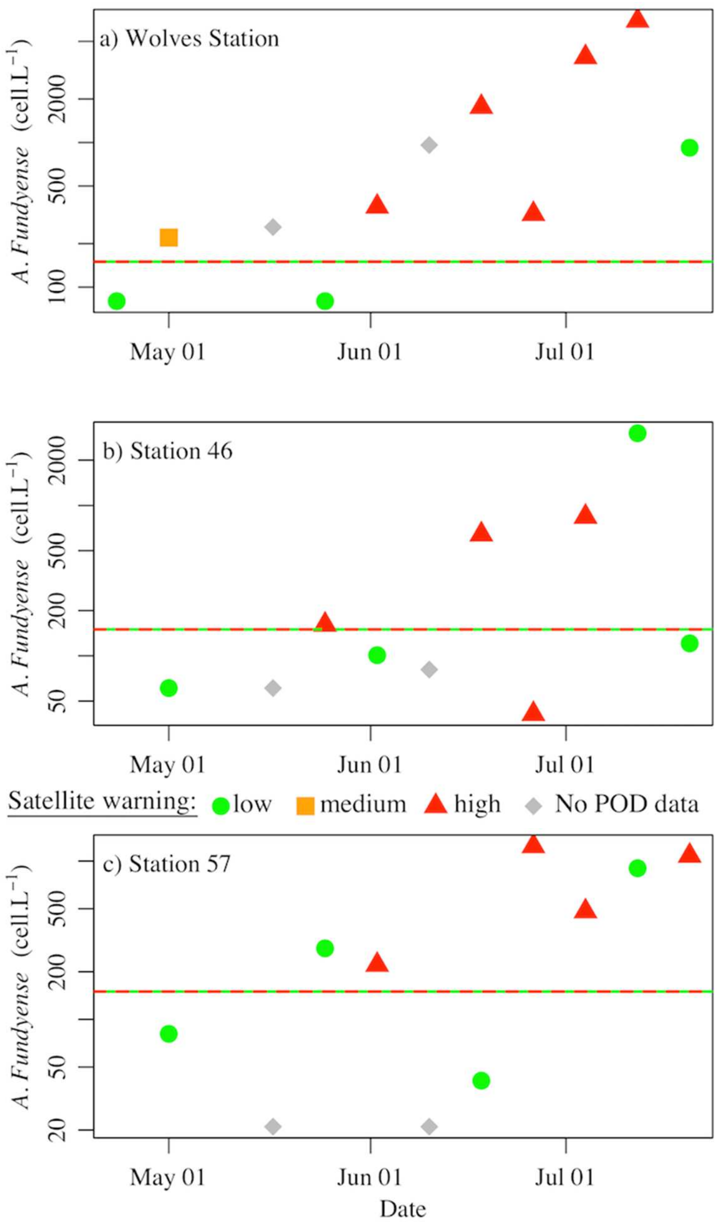

Figure 8: A. fundyense cell abundance as a function of time at station Wolves (a), station 57 (b) and station 46 (c) for the year 2011. Solid circles are color-coded as a function of satellite warning level, i.e., green, orange and red for low, inconclusive and high risk of toxic level of A. fundyense cell abundance respectively.

Stations Wolves and 46 show very similar temporal variation in *A. fundyense* abundance (r^2 = 0.96, Table 2) and the algorithm provides a similar response, recording the first increase in *A. fundyense* at toxic level (May 25th), followed by a decrease below the toxic level (green level, June 2nd) and another increase (June 18th). However, the warning system shows a false positive on the 25th of June. In July, the algorithm is in agreement with the *in situ* measurements, except for a false negative occurring for high abundance of *A. fundyense* (~ 3000 cell.L^{-1}). At station 57, the warning system records the first high level of *A. fundyense* with a one week lag, i.e., false negative on the 25th of May. However, the *A. fundyense* abundance is 281 cell.L^{-1}, which is well below the 500 cell.L^{-1} threshold when shellfish are considered unsafe for human consumption. Similarly to stations Wolves and 46, the algorithm detects the sudden decrease in abundance of *A. fundyense* (mid-July) to below the toxic level.

3.4 Spatial distribution of *A. fundyense* bloom

We selected the year 2011 to derive information on the development and spatial distribution of *A. fundyense* blooms in the Bay of Fundy (Figure 9) from the 26th of April to the 7th of July (see supplementary material for years 1998 to 2007). The first two weeks of observation show low levels of *A.* fundyense, in agreement with the *in situ* measurements except for a slight elevation of *A.* fundyense abundance (220 cell.L^{-1}) at the Wolves station the last week of April. The first possible toxic level of *A. fundyense* detected by satellite observations occurred mid-May in the central part of the Bay of Fundy (orange/red) in addition to a small area flagged as possible high abundance of *A. fundyense* (> 150 cells•L^{-1}, red) close to the shore. This large patch of potential *A. fundyense* cells with abundance greater than 150 cells•L^{-1} spreads during the second half of May. However, the lack of valid pixels in the last week of May prevents comparisons with *in situ* measurements, which show an increase from the previous week with values over the threshold of 150 cells•L^{-1} at the offshore stations (i.e., 46 and 57). By early to mid- June, in agreement with *in situ* measurements at all stations, the entire region of interest is categorized as orange and red levels of *A. fundyense* abundance, except for two small areas in the northeast and southwest. By the end of June, the satellite observations show the patchy nature of *A. fundyense* bloom, with both low and high levels of warning juxtaposed. Again, the satellite warning system agrees with *in situ* observations. Late June, most of the region is classified as possible high levels of *A. fundyense* abundance except for the central part. It is remarkable that the *in situ* measurements are in agreement with the satellite warning, with stations Wolves and 57 noted as red levels with abundance of *A. fundyense* of 320 and 1241 cell. L^{-1} respectively, whereas station 46 (41 cell. L^{-1}) is located in a region of low abundance of the toxic algae. Finally, for the first week of July, the number of pixels classified as red decreases again, notably from the northeast part of the region of interest. The development of *A. fundyense* in 2011 is similar to observations from previous years (Supplementary material), with a bloom that usually begins in the central part of the Bay of Fundy and spreads towards the coastal areas with high interannual variability in the spatial distribution of the bloom.

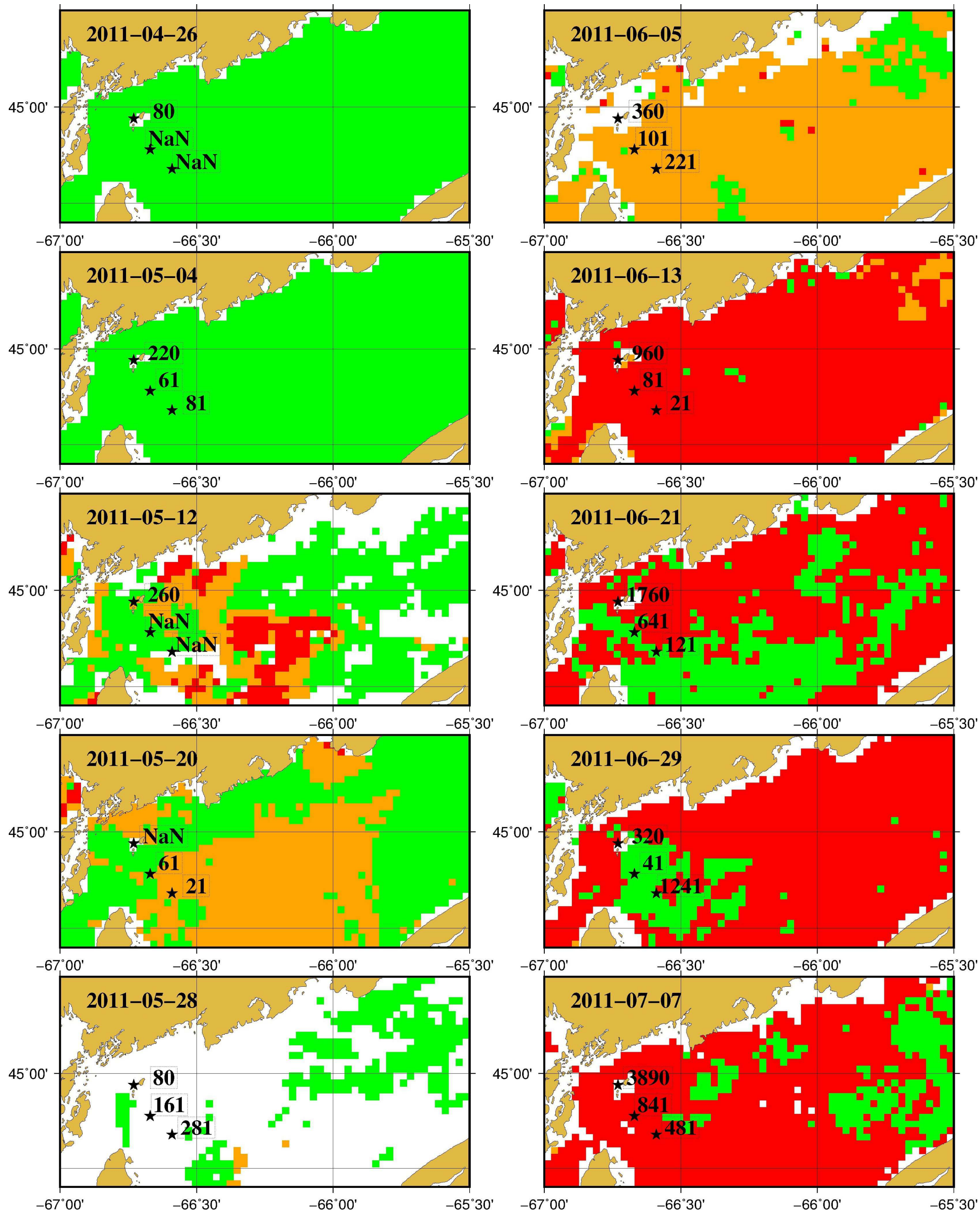

Figure 9: Eight-day composite image of A. fundyense warning system for the entire Bay of Fundy from the 22 April to 7 July 2011. The color-coding represents low (green ≤ 150 cells•L^{-1}), medium (orange, inconclusive information on A. fundyense toxicity level) and high (>150 cells•L^{-1}) A. fundyense cell abundance. Numbers in boxes indicate the cell abundance of A. fundyense at the Wolves station (black solid star).

4. Discussion and conclusion

Our approach to detect *A. fundyense* blooms relies on the theory of habitat suitability that is extensively used in ecosystem management. One of the main assumptions of this ecological approach is that there is no need to directly detect the organism of interest but rather inform on favorable environmental conditions. This type of approach has been used for a wide range of organisms, ranging from whales in the pelagic environment to habitat mapping in the near-shore. Previous studies have also demonstrated that habitat modeling was useful to derive information on harmful algal blooms, such as the production of domoic acid off the coast of California in the United States of America (Anderson et al., 2016), the detection of the harmful algae *K. mikimotoi* in Ireland using satellite-derived chlorophyll concentration and sea-surface temperature (Raines et al. 2010) and forecasting the risk of harmful algal blooms on the Atlantic coast of Europe using satellite remote sensing and other information associated with marine conditions (Davidson et al. 2016).

A. fundyense cells produce toxins that causes paralytic shellfish poisoning (PSP toxins) and can harm humans that consume shellfish that have accumulated PSP toxins at levels equal or higher than 80 µg toxin per 100 gram of bivalve shellfish edible tissue. It is commonly agreed that 200 cells•L^{-1} corresponds to the threshold above which toxicity will be detected in shellfish from the Bay of Fundy. Such a low number of cells at which *A. fundyense* cells can result in detectable levels of toxins in shellfish, (compared to an average abundance of phytoplankton of 10^3 to 10^6 cell per liter), as well as an optical signature similar to other phytoplankton species, makes it impossible to directly detect abundance of *A. fundyense* with confidence. Here, we have chosen to use a warning system that can indicate possible high levels of *A. fundyense* abundance in the spring and early summer, a critical time when the rapid increase in *A. fundyense* abundance represents a cause for concern. The warning system uses information on satellite-sea surface temperature and probability of diatom occurrence to infer the possible levels of toxic *A. fundyense* blooms. Rather than attempting to provide a number of cells, we have chosen an approach that will rely on three different levels of warning, for which the probability of occurrence of *A. fundyense* increases from low risks to high risks.

Our development dataset of *A. fundyense* cell counts, SST and PDO was used to refine PDO and SST thresholds, and estimate the "most detectable" level of *A. fundyense* cell concentrations using a scoring system that favours the detection of high concentrations of *A. fundyense* cells using satellite-retrieved proxies, and penalises the detection of false negative responses (i.e., toxic levels of *A. fundyense* at which shellfish have detectable levels of toxins but no warning by the satellite system). A sensitivity study identified the best set of criteria to infer potentially toxic concentrations of *A. fundyense* cells: our algorithm performed best at 150 cells•L^{-1} rather than the recommended concentration of 200 cells•L^{-1}, which also contributes toward a precautionary approach (information on sea-surface temperature as well as probability of occurrence of diatoms contributes to the flagging of potential toxic levels of *A. fundyense* cells). The PDO_T criteria (> 60%) is more indicative of a safe level of *A. fundyense* (6 out of 8 possible cases) whereas the SST_{UT} threshold (> 6.8°C) appeared to be a better indicator of high concentrations of *A. fundyense* (16 out of 18 possible cases). Note that the thresholds in SST and PDO seem to be sensitive to any small variations (Figure 7) such that the performance of the algorithm can rapidly degrade if, for instance, the lower SST threshold changes by half a degree Celsius. Overall, the algorithm was successful in detecting the timing of *A. fundyense* exponential growth phase apart from the offshore station 46. The poor performance of the algorithm at station 46 is explained by high sea-surface temperature (above 11°C and up to 19°C) associated with a low probability of occurrence of diatoms (between 0 and 53% with a mean of 28%) whereas stations 57 and Wolves show levels of diatoms higher or equal to 60% in many instances with means of 40 and 43% respectively. However, the algorithm tends to produce false negatives, which is consistent with the principle of the precautionary approach. The results at station 46 suggest that the algorithm should be adapted for the central waters of the Bay of Fundy where diatom abundance might be lower than on the coast. This next step could be achieved with a larger dataset collected in the central part of the Bay of Fundy spanning a longer time period than the dataset used in this study.

The spatial development of *A. fundyense* blooms is in agreement with other studies in the area (see Townsend et al., 2001, 2005) as well as the hydrodynamic circulation of the Bay of Fundy (Aretxatabatela et al., 2008, 2014), including the transport and sedimentation of cysts, despite an absence of relationship between cysts and cell abundance of *A. fundyense* (Martin et al. 2014). As was found in the Aretxatabatela et al. studies (2008,2014), the first toxic level of *A. fundyense* occurs in the central and eastern part of the Bay of Fundy, following input of cold, nutrient-rich waters from the Scotian Shelf and contributing to the eastern Maine coastal current. These waters enter the central part of the Bay of Fundy (i.e. the gyre) and spread toward the southwestern regions. Our analysis of the satellite time series of occurrence of toxic levels of *A. fundyense* abundance also reveals the very patchy nature of *A. fundyense* abundance. The ability of the algorithm to detect harmful concentrations of *A. fundyense* constitutes a valuable synoptic complement to *in situ* measurements. Operationally, its application can provide a broad view of the entire basin allowing managers to focus on areas of concern that may not necessarily be included under a monitoring program. This algorithm also provides the ability to detect the first occurrence of high abundance of *A. fundyense* in the central part of the Bay of Fundy.

A limitation of the algorithm is that it does not account for uncertainties in both *in situ* and satellite measurements. Whereas this might not be an issue in extreme cases (high abundance of *A. fundyense*, low or high SST and high PDO), this might decrease the ability of the algorithm to properly identify a given level of warning. For instance, abundance of *A. fundyense* of 121 cells•L^{-1} occurred on 22 June 2011, when satellite ocean-colour information indicated a level of occurrence of diatoms of 58.3% and temperature of 16.6 ^{o}C. The PDO is very close (less than 2%) to the threshold between safe level of *A. fundyense* abundance (i.e. 60%) and the cell count is close to the detection threshold of 150 cells•L^{-1}, such that the difference between safe and toxic concentration of *A. fundyense* cells is very slim. Similar situations also occur on 13 May 2005 where both SST (5.2 ^{o}C) and PDO (57%) are close to the thresholds SST_1 and PDO_1 respectively, and on 7 July 2007 when PDO (59%) is close to the safe threshold. .

There is a continued and growing commitment by the international community to monitor HABs due to their socio-economic impact on human activities (e.g., fisheries, tourism, human health). Numerous studies have led to regional approaches, which combine *in situ* and remotely sensed measurements of several parameters (Cannizzaro et al. 2008, Hu et al. 2010, Tomlinson et al. 2009, Carvalho et al. 2010, 2011). Here, we developed a monitoring system for *A. fundyense* in the Bay of Fundy that relies only on satellite remote sensing data. The criteria used to delineate the level of alert for possible presence of *A. fundyense* were defined using SST and occurrence of diatoms. The algorithm showed consistent performance using both the development as well as test datasets, and was in agreement with *in situ* counts of *A. fundyense* in two out of three stations. The algorithm has the tendency to produce false positives which, given the possible impact of *A. fundyense* and resulting shellfish toxicity on human consumers, favors the precautionary approach.

In early spring, levels of *A. fundyense* are very low to zero, whereas in summer, they are usually well above the minimum threshold of toxicity for shellfish consumption. Satellite observations tend to clearly identify these two periods. The critical time is the onset of the bloom, which is very difficult to predict using *in situ* measurements of *A. fundyense* abundance due to its high spatial and temporal variability. In this respect, the use of satellite remote sensing proxies (SST and probability of occurrence of diatoms) appears as an emerging tool to map the development of *A. fundyense* in time and space. A limitation of the satellite method is the inability to observe the surface of the ocean under cloudy conditions. This limitation can be overcome for SST measurements by using data from the Group for High Resolution Sea Surface Temperature (GHRSST), which combines data from multiple sensors as well as *in situ* data to provide quality-controlled SST for operational applications. The rapid development of the bloom would also require a higher temporal observation window than the eight-day composite used in this study. The consistency between the two dataset (AVHRR/SeaWiFS (1998-2007) and MODIS/MERIS (2011)) demonstrates the robustness of the algorithm and its potential for recent and future sensors such as the Sea and Land Surface Temperature Radiometer (SLSTR) and Ocean and Land Colour Imager (OLCI) on board the Sentinel-3A platform launched by the European Space Agency (ESA) in February 2016. ESA is planning to launch a second sensor (Sentinel-3B) in the coming year that would help to provide the high temporal resolution required for early detection of *A. fundyense* blooms. Our study also reveals the need for local information on *A. fundyense* to be coupled with satellite observations to extrapolate measurements to the entire Bay of Fundy. Ideally, *in situ* measurements, model simulation and satellite observations should be integrated in a comprehensive observational system that would allow local and regional observation and prediction of *A. fundyense* abundance and growth in the Gulf of Maine and Bay of Fundy regions.

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List of Figure Captions:

Figure 1: Sampling stations in the Bay of Fundy, eastern Canada: The Wolves and off shore stations 46 and 57.

Figure 2: Composite (24-31 May 2006) image of the Bay of Fundy showing the probability of occurrence of diatoms derived from the SeaWiFS sensor and sea-surface temperature (SST) derived from the AVHRR sensor for the same period, and same location. Occurrence of diatoms is given as a percentage probability (0 – 100) of finding diatom-dominated populations in that area during that time interval.

Figure 3: Schematic of the satellite-based warning system that relies on two SST thresholds and one PDO threshold to infer safe, non-conclusive and toxic level of A. fundyense.

Figure 4: Eight-day climatology (1998-2007) of satellite-derived SST (red diamonds), probability of occurrence of diatoms (blue triangles), chlorophyll-a concentration (green squares) and wind speed and direction, as well as in situ cell abundance of A. fundyense (black Circle) in the Bay of Fundy (Wolves station).

Figure 5: Eight-day climatology (1998-2007) and range of variation of a) satellite-derived SST, b) probability of occurrence of diatom and c) in situ cell abundance of A. fundyense at the Wolves station.

Figure 6: A. fundyense cell abundance as a function of SST and probability of diatom occurrence using the development dataset. Warning alerts (threshold 1 to 3) are colour-

coded from green, to orange to red for low, inconclusive and high risk of toxicity, respectively.

Figure **7**: Performance score of the warning algorithm as a function of a) lower threshold of sea-surface temperature (SST_{LT}), b) upper threshold of sea-surface temperature (SST_{UT}) and c) threshold of probability of occurrence of diatom (PDO_{T}).

Figure 8: A. fundyense cell abundance as a function of time at station Wolves (a), station 57 (b) and station 46 (c) for the year 2011. Solid circles are color-coded as a function of satellite warning level, i.e., green, orange and red for low, inconclusive and high risk of toxic level of A. fundyense cell abundance respectively.

Figure 9: Eight-day composite image of A. fundyense warning system for the entire Bay of Fundy from the 22 April to 7 July 2011. The color-coding represents low (green ≤ 150 cells•L^{-1}), medium (orange, inconclusive information on A. fundyense toxicity level) and high (>150 cells•L^{-1}) A. fundyense cell abundance. Numbers in boxes indicate the cell abundance of A. fundyense at the Wolves station (black solid star).