UNIVERSITÉ DU QUÉBEC À RIMOUSKI

IDENTIFICATION DES ASSEMBLAGES DE KYSTES DE DINOFLAGELLÉS DANS LES PORTS DE LA CÔTE EST CANADIENNE

ANALYSE DE L'INFLUENCE DU TRANSPORT MARITIME ET DU DEVERSEMENT D'EAU ET DE SEDIMENTS CONTENUS DANS LES BALLASTS

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RÉSUMÉ

Le transport d'organismes marins dans les réservoirs de ballasts de navires est maintenant reconnu comme étant un vecteur important pour l'introduction d'organismes nuisibles dans un nouvel environnement. Les dinoflagellés, de par leur faculté de produire des floraisons d'algues nuisibles (HAB), constituent une menace réelle lorsqu'ils sont introduits dans un nouvel environnement. En effet, alors que certaines espèces sont capables de produire une prolifération massive de cellules qui peut causer des dommages à la vie marine, d'autres produisent des toxines dangereuses pour les organismes marins et les humains. De plus, 15% de tous les dinoflagellés sont reconnus comme étant capable de produire des kystes de résistances dans le cadre de leur cycle vital. Ces kystes peuvent facilement être transportés d'un environnement à un autre dans les ballasts de navires et peuvent ainsi aboutir, lors du déballastage, dans les ports des régions côtières. Cette étude porte sur les assemblages de kystes de dinoflagellés retrouvés dans les dépôts sédimentaires de surface de certains ports de la Nouvelle-Écosse. Les sédiments ont été récoltés lors du mois de juillet 2008 à l'aide d'une benne benthique d'Ekman. Les concentrations moyennes varient entre 1346 et 83 351 kystes g^{-1} de sédiment sec alors que la diversité spécifique varie entre 14 et 40. Les résultats ont démontré la présence de trois groupes distincts d'assemblages de kystes de dinoflagellés qui diffèrent selon la concentration de kystes et la composition spécifique: groupe 1 (Yarmouth et Liverpool), groupe 2 (Shelburne, Halifax et Sheet Harbour) et groupe 3 (détroit de Canso, Little Narrows et Sydney). Les concentrations moyennes de kystes de dinoflagellés dans les ports ont démontré une augmentation significative en fonction du nombre de navires fréquentant ces ports et du volume d'eau de ballast provenant de navires qui n'ont pas effectué d'échange d'eau de ballast. Un important lit de kystes du complexe d'espèces *Alexandrium tamarense*, dinoflagellés potentiellement toxiques et capable de former des HAB, a été découvert dans le bassin de Bedford du port d'Halifax (concentrations maximales : 220 872 ± 148 086 kystes g^{-1}séd. sec). Cette étude constitue le premier effort pour examiner les relations entre le trafic commercial maritime et les concentrations de kystes de dinoflagellés dans les ports de la Nouvelle-Écosse.

Mots clés : Kystes de dinoflagellés, assemblages, distribution, Nouvelle-Écosse, ballasts, navires, espèces non indigènes, HAB

ABSTRACT

The transport of marine organisms in ballast tanks of cargo ships is now recognized as an important vector for the introduction of marine pests in the environment. With their ability to produce Harmful Algal Blooms (HAB), dinoflagellates represent a serious threat when they are introduced to new environments. Indeed, while some species are able to produce massive algal proliferation that may cause coastal anoxia threatening marine life, others generate a variety of toxins that can cause harm and even death to marine organisms and humans. Also, 15% of all dinoflagellates produce resistant dormant cysts as part of their life cycle. These cysts can easily be transported from one environment to another in ships ballasts and they can be discharged in ports during deballasting. This study focuses on identifying the dinoflagellate cyst assemblages found in the surface sediment deposits of selected ports of Nova Scotia. Samples were collected in July 2008 from eight ports in Nova Scotia using an Ekman bottom grab sampler. The average concentrations range from 1346 to 83 351 cysts g^{-1} dry sed. and the species richness index ranges from 14 to 40. Results show that there are three distinct groups of dinoflagellate cyst assemblages: group 1 (ports of Yarmouth and Liverpool), group 2 (ports of Shelburne, Halifax and Sheet Harbour) and group 3 (ports of Canso Strait, Little Narrows and Sydney). Dinoflagellate cyst concentrations averaged over each port showed a statistically significant increase with the number of ships visiting these ports and with the volume of ballast water from ships that did not undertake a ballast water exchange. High cyst concentrations (220 872 ± 148 086 cysts g^{-1} dry sed.) of the potentially toxic and bloom forming *Alexandrium tamarense* species complex were found in Bedford Basin of the port of Halifax. This study constitutes the first attempt to examine the relationships between shipping and dinoflagellate cysts concentrations in ports of Nova Scotia.

Keywords : Dinoflagellate cysts, assemblages, distribution, Nova Scotia, ballasts, ships, non-indigenous species, HAB

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INTRODUCTION GÉNÉRALE

Le transport des organismes aquatiques par les navires commerciaux transocéaniques et domestiques est devenu un vecteur dominant pour l'introduction d'espèces exogènes envahissantes (EEE) au Canada depuis le début des années '60 (Duggan et al. 2005). Chaque année, des milliers de navires en provenance de l'extérieur circulent dans les eaux portuaires canadiennes, offrant de nombreuses opportunités à ces espèces de s'installer. Ainsi, depuis l'ouverture de la voie maritime du Saint-Laurent, environ 170 espèces non indigènes se sont établies dans la région des Grands Lacs (Duggan et al. 2005).

Ces organismes sont, pour la majeure partie, transportés dans les eaux et les sédiments des réservoirs de lest ou ballast ('ballast tanks') des navires. La présence d'espèces exogènes dans ces réservoirs ne signifie toutefois pas qu'il y aura automatiquement un établissement de celles-ci dans le nouvel environnement. En ce qui concerne les kystes de dinoflagellés, huit étapes sont nécessaires afin que l'introduction soit un succès (Hallegraeff 1998). Ces étapes peuvent être résumées en quatre grands points, dont le premier consiste en la prise d'eau et potentiellement de sédiments dans les réservoirs de ballast lors d'un bloom saisonnier de plancton dans le port d'origine. Toutefois, la probabilité que des dinoflagellés toxiques soient pris lors de la prise d'eau dans les réservoirs de ballasts est fortement dépendante : (1) des habitudes de navigation (i.e. des routes fréquemment empruntées), (2) de la saisonnalité des blooms de phytoplancton dans les ports où la prise d'eau de ballast s'effectue et, (3) de la présence locale d'un lit de kystes dans les sédiments (Hallegraeff 1998). Les organismes doivent par la suite survivre aux processus de ballastage, aux longs voyages à l'obscurité et à des conditions de transport généralement difficiles (Lavoie et al. 1999). La troisième étape comprend le transfert du navire au milieu côtier du port d'arrivée, suivi de la germination des kystes, la croissance et la reproduction du dinoflagellé dans le nouvel environnement

(Hallegraeff 1998). Finalement, suite à l'établissement les organismes peuvent se propager régionalement via les courants côtiers ou le transport domestique, causant potentiellement de sérieux problèmes écologiques, économiques et même de santé publique (Carlton and Geller 1993, Hallegraeff 1998).

Il ne faut toutefois pas se fier au nombre d'espèces introduites pour évaluer la gravité du problème. En effet, une seule espèce peut causer des dommages importants et souvent irréversibles à l'environnement (Bourgeois et al. 2001). L'introduction d'une espèce phytoplanctonique capable de produire des floraisons nuisibles (« harmful algal blooms », HAB) dans un nouvel environnement en constitue un bon exemple. Ce type de floraison est devenu un fléau à l'échelle mondiale suite à l'accroissement de leur dispersion géographique dû, notamment, au transport des microorganismes dans les eaux de ballast (Hallegraeff et al. 1997, Vila et al. 2001).

Les espèces d'algues toxiques sont nuisibles pour l'environnement, soit par la production de toxines ou à travers d'autres effets nocifs comme l'obstruction des branchies de poisson ou la réduction d'oxygène liée aux accroissements de biomasse algale. La plupart des espèces impliquées dans les HAB sont des algues photosynthétiques ainsi que quelques espèces de protozoaires hétérotrophes (Anderson et al. 2002). Les dinoflagellés comptent environ 100 taxons capables d'engendrer des proliférations d'algues nuisibles (Sournia 1995, Smayda 1997). Parmi ceux-ci, seulement un petit nombre sont capables de produire des kystes de résistance, les plus connus étant *Gymnodinium catenatum* et *Alexandrium* spp. La plupart de ces espèces peuvent produire des substances toxiques pour l'Humain et les animaux (Doblin and Dobbs 2006).

Un intérêt particulier doit donc être porté aux dinoflagellés potentiellement toxiques qui forment des kystes de résistance pouvant être transportés dans les sédiments de ballast. En effet, ces kystes peuvent demeurer viables dans le sédiment pendant de nombreuses années (Doblin and Dobbs 2006). Ils sont extrêmement résistants aux conditions difficiles (obscurité prolongée, faibles concentrations d'O_2) des réservoirs de ballast dans lesquels ils peuvent se retrouver (Hallegraeff 1998). En effet, des kystes de dinoflagellés ont été observés dans les sédiments des réservoirs de ballasts de navires arrivant dans les ports d'Australie, de la Nouvelle-Zélande, des États-Unis, du Canada, de l'Écosse et de l'Angleterre (Hamer et al. 2000 et références citées). Lors de la vidange des réservoirs avant le chargement de marchandises au port, les kystes peuvent être relâchés et potentiellement s'établir dans un nouvel environnement. De nombreuses espèces ont pu être ainsi introduites dans plusieurs ports (Carlton and Geller 1993).

Il n'est cependant pas facile de démontrer qu'une population de dinoflagellés a récemment été introduite dans une nouvelle zone. Pour ce faire, il faut d'abord prouver que cette population est effectivement nouvelle dans cette zone (Lilly et al. 2002), tâche qui peut s'avérer très ardue. Ceci se fait généralement au moyen de carottes de sédiment, par l'examen des populations de dinoflagellés présentes dans le passé (Hallegraeff et al. 1997) afin d'établir si une nouvelle espèce a pu s'installer à cet endroit. Toutefois, des quelques 2000 espèces de dinoflagellés marins, seulement 15 % des espèces produisent des kystes au cours de leur cycle vital (Head 1996). Il sera donc possible de prouver l'introduction d'une nouvelle espèce seulement si cette dernière produit un kyste qui se conservera quelques dizaines ou centaines d'années.

Très peu d'études ont été effectuées sur la diversité des espèces de kystes et de formes végétatives de dinoflagellés retrouvées dans les zones portuaires canadiennes (Bérard-Therriault et al. 1999, Carver and Mallet 2001). Il y a donc peu d'information concernant la composition des espèces retrouvées à ce jour. L'obtention de ce type d'information devient essentielle pour contrôler l'arrivée de nouvelles espèces dans les eaux canadiennes et ainsi déterminer si les mesures prises pour enrayer ce problème sont efficaces.

Cette étude vise à déterminer les assemblages de kystes de dinoflagellés présents dans les sédiments de surface des ports de la côte est canadienne et d'examiner l'influence du trafic maritime (i.e. navires marchands) sur ces assemblages. Pour ce faire, la distribution des assemblages de kystes de dinoflagellés a premièrement été observée à l'intérieur des huit ports sélectionnés selon un gradient croissant du nombre de navires et du volume d'eau déchargée pour ces ports. Par la suite, la distribution à l'intérieur des ports a été établie afin de déterminer si elle est homogène à l'intérieur d'un même port. Finalement, l'influence du trafic maritime sur la composition des assemblages de kystes a été déterminée en analysant le nombre de navires visitant chaque port ainsi que la quantité d'eau de ballast déversée sur l'abondance moyenne ainsi que le nombre d'espèces de kystes de dinoflagellés retrouvées dans cette étude. Ce projet fait partie du réseau canadien sur les espèces marines envahissantes (Canadian Aquatic Invasive Species Network ou CAISN). Le texte qui suit est présenté sous la forme de deux publications scientifiques (un article et une note) qui seront soumis au journal Marine Ecology Progress Series.

CHAPITRE 1
LES ASSEMBLAGES DE KYSTES DE DINOFLAGELLÉS DANS LES SÉDIMENTS DE SURFACE DES PORTS DE LA NOUVELLE-ÉCOSSE, CANADA : EXISTE-T-IL UNE INFLUENCE PROVENANT DU TRAFIC MARITIME?

1.1 Résumé en français du premier article

Dans le but de vérifier si le trafic commercial maritime influence la concentration et la composition des assemblages de kystes de dinoflagellés de la côte est canadienne, des échantillons de sédiments de surface ont été prélevés dans les ports de la Nouvelle-Écosse en juillet 2008. Les ports ont été choisis selon la fréquence du trafic maritime (de 0 à plus de 1 000 navires annuellement). Les résultats ont démontré la présence de trois groupes distincts d'assemblages de kystes de dinoflagellés qui diffèrent selon la concentration de kystes et la composition spécifique: groupe 1 (Yarmouth et Liverpool), groupe 2 (Shelburne, Halifax et Sheet Harbour) et groupe 3 (détroit de Canso, Little Narrows et Sydney). Les concentrations moyennes dans les ports ont varié entre 1 346 et 83 351 kystes g^{-1} de séd. sec et l'indice de richesse spécifique a varié entre 14 et 40. Les concentrations moyennes de kystes de dinoflagellés dans les ports ont démontré une augmentation significative en fonction du nombre de navires et du volume d'eau de ballast provenant de navires qui n'ont pas effectué d'échange d'eau de ballast. Ces résultats suggèrent que les navires semblent jouer un rôle dans le transport et le transfert des kystes de dinoflagellés dans les ports où l'eau de ballast est déchargée. Ces relations sont toutefois fortement influencées par les résultats d'un port en particulier, Halifax, qui est le port qui reçoit le plus grand nombre de navires (dominé par le trafic local qui ne nécessite pas d'échange d'eau de ballast avant d'entrer dans le port). La distribution des assemblages de kystes de dinoflagellés à l'intérieur des ports était assez homogène (six sites par port). Cette étude constitue le premier effort pour examiner les relations entre le trafic commercial maritime et les concentrations de kystes de dinoflagellés dans les ports canadiens.

Mots clés : Kystes de dinoflagellés, distribution en surface, transport maritime, eau de ballast, sédiment de ballast, Canada, côte est

Ce premier article, intitulé « Dinoflagellate cyst assemblages in surface sediments from ports of Nova Scotia, Canada: Is there an influence of shipping traffic?», fut co-rédigé par moi-même ainsi que par les professeurs André Rochon et Suzanne Roy. Il sera soumis dans quelques jours à la revue Marine Ecology Progress Series. En tant que premier auteur, ma contribution à ce travail fut l'essentiel de la recherche sur les assemblages de kystes de dinoflagellé, l'échantillonnage, l'exécution du travail de laboratoire, les analyses statistiques et la rédaction de l'article. Les professeurs André Rochon et Suzanne Roy, second et troisième auteur, ont fourni l'idée originale et ont contribué à la révision de l'article. Une version abrégée de cet article a été présentée lors de la réunion annuelle du Canadian Aquatic Invasive Species Network (CAISN) à Québec en avril 2011.

1.2 Dinoflagellate cyst assemblages in surface sediments from ports of Nova Scotia, Canada: Is there an influence of shipping traffic?

Manuscrit en préparation pour une publication dans la revue Marine Ecology Progress Series

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1.2.1. ABSTRACT

We collected surface sediment samples from eight ports in Nova Scotia in July 2008 in order to obtain basic information on the dinoflagellate cyst assemblages in the sedimentary deposits from ports on Canada's East coast and examine if shipping activity influences the nature and abundance of the dinoflagellate cyst assemblages in this environment. These ports cover a gradient from low to high shipping traffic. Results show that there were three distinct groups of dinoflagellate cyst assemblages: group 1 (Yarmouth and Liverpool), group 2 (Shelburne, Halifax and Sheet Harbour) and group 3 (Canso Strait, Little Narrows and Sydney), which differed based on the dinoflagellate cyst concentrations and species composition. Average concentrations over all ports ranged from 1346 to 83 351 cysts g^{-1} dry sed and the species richness index ranged from 14 to 40. Dinoflagellate cyst concentrations averaged over each port showed a statistically significant increase with the number of ships and the volume of ballast water from ships that did not undertake a ballast water exchange, suggesting that ships may play a role in the transportation and seeding of dinoflagellate cysts in receiving ports. However, these relations were influenced strongly by the results from one port: Halifax, which receives the greatest number of ships among all the ports considered here (dominated by domestic ships with no ballast water exchange). The within-port distribution of the dinoflagellate cyst assemblages was quite homogeneous (six sites per port). This study constitutes the first attempt to examine the relationships between shipping and dinoflagellate cysts concentrations in Canadian ports.

Keywords : Dinoflagellate cysts, surface distribution, shipping traffic, ballast water, ballast sediment, Canada, East coast

1.2.2. INTRODUCTION

The transport of marine organisms in ships' ballast tanks has been recognized as an important vector for the introduction of marine pests in the environment (e.g. Hallegraeff and Bolch 1992, Carlton and Geller 1993, Subba Rao et al. 1994, Niimi 2004, Kipp et al. 2010). The impacts of these introduced organisms are numerous, and they can either be economical, ecological or even public health-related. Dinoflagellates are microscopic algae with their ability to produce Harmful Algal Blooms (HAB) and present a serious threat because they can impact all three sectors mentioned above. Indeed, while some species are able to produce massive algal proliferations that may cause anoxia and cell death, others generate a variety of toxins that can cause harm and even death to marine organisms and humans.

Dinoflagellates are either autotrophic, heterotrophic, mixotrophic or even parasitic (Gaines and Elbrachter 1987). Together with the diatoms and coccolithophorids, they play an important role in the primary production of aquatic ecosystems (e.g. Rochon et al. 1999). About 200 species of dinoflagellates, which represent approximately 15% of living dinoflagellate species, produce a hypnozygote or organic-walled resting cyst as part of their life cycle (Head 1996). Once the cysts are formed in the water column, they behave as fine silt particles and sink to the seafloor (Dale 1976). They then remain dormant for a mandatory period after which the cells will excyst and start a new life-cycle (Evitt 1985). The cell wall of most of these resting cysts is composed of dinosporin, a resistant material that allows the cyst to be preserved in the sediments for long periods of time (Fensome et al. 1993). The cysts can survive in unfavourable environmental conditions and thus play an essential role in the dispersal and survival of the species (Anderson and Wall 1978).

These dinoflagellate cysts can easily be transported across oceans in ships ballast water and sediments. With their ability to survive in harsh conditions and their long viability in sediments (months to years: Anderson et al. 2004), cysts of dinoflagellate species that are known to produce HABs constitute a serious threat for coastal marine environments. It is thus important to monitor their introduction from shipping activities.

Determining if a new cyst-producing algal species has been introduced normally requires the analysis of time series from sediment cores, which is beyond the scope of the present study. However, we can examine if differences have taken place among ports with different shipping traffic, assuming that ships can be a source of dinoflagellate cysts (e.g. Hallegraeff and Bolch 1992). Previous studies have suggested that increases in the number of ships visiting a port and the volume of ballast water discharged could influence the concentrations of marine organisms and species diversity in ports (Bourgeois et al. 2001, Ruiz and Carlton 2003 and references therein, Lawrence and Cordell 2010). To examine this, we used eight ports from Nova Scotia that differ in terms of shipping traffic. This study, which is part of the Canadian Aquatic Invasive Species Network (CAISN) program, will also provide information on the dinoflagellate cysts assemblages in local ports, including their within-port spatial distribution. In addition, it establishes a baseline for the recognition of newly introduced dinoflagellate cysts in the future. The main objective of this work was thus to identify the dinoflagellate cysts assemblages present in the recent sedimentary deposit of the eight ports chosen along a gradient from low to high shipping traffic and assess the potential influence of commercial shipping traffic on the cyst assemblages.

1.2.3. Material and methods

1.2.3.1. Study area

Sampling was done during the port sampling campaign of the Canadian Aquatic Invasive Species Network (CAISN) program in July 2008. Surface sediments were collected in triplicate from 48 stations located in eight different ports (6 stations port^{-1}) located along the south and eastern shores of Nova Scotia and Cape Breton, Canada (Figures 1.1 and 1.2). The distance between each port was at least 50 km. The ports were chosen according to the number of ships visiting each of them and the amount of ballast water being discharged. They were selected to reflect a gradient from low to high shipping traffic for the average of the years 2005 (data from the Canadian Ballast Water Database) and 2006 (M.G. Deneau and S.A. Bailey, DFO, unpublished data). Yarmouth represents the port at the lower end of the gradient (i.e., no commercial ship deballasting) and Halifax the upper end member with 1012 ships visiting from the US and other countries (Table 1.1).

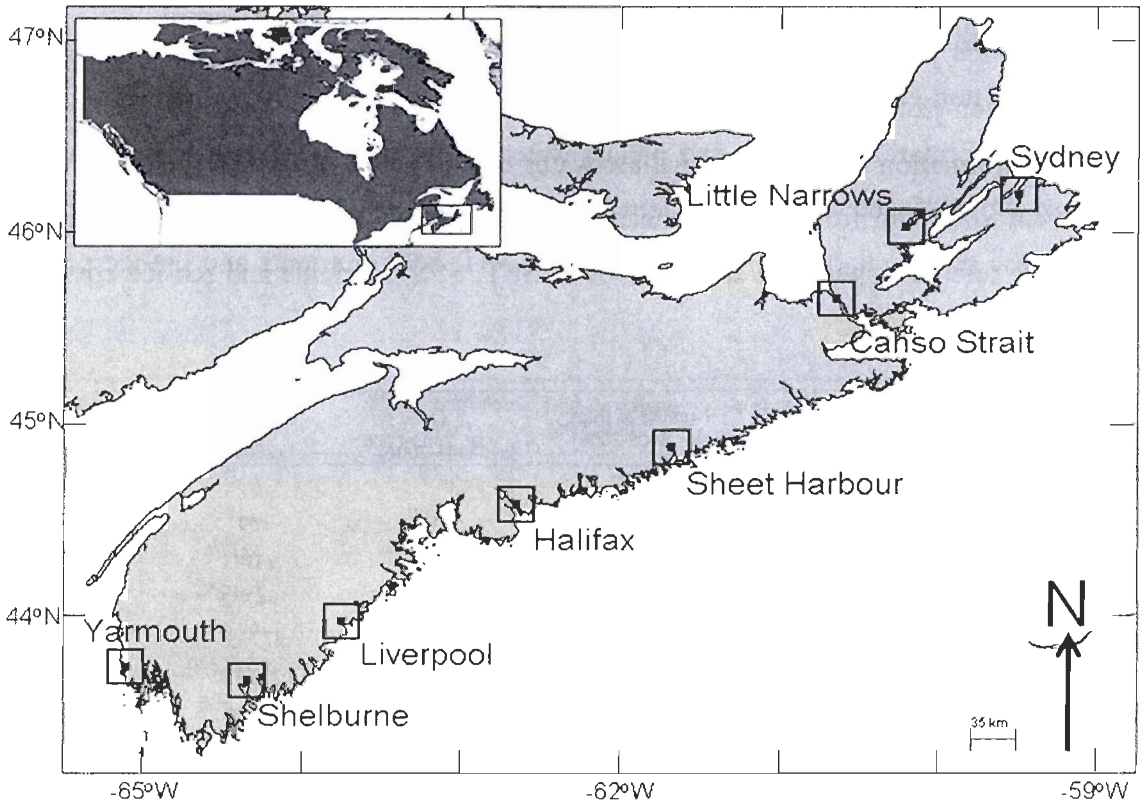

Figure 1.1: Location of the eight sampled ports in Nova Scotia, Canada.

Table 1.1. Dinoflagellate cyst species richness (number of species observed), average cyst concentrations (cysts g^{-1} dry sed.), average number of ships and volume of discharged ballast water (in metric tons, MT) for the years 2005-2006 in the eight ports of Nova Scotia examined in this study. The volumes of discharged ballast water which were exchanged (EXCH) and non-exchanged (NEXCH) (in metric tons, MT) are only available for the year 2006. The information on ships and ballast water come from the Canadian Ballast Water Database for 2005 and from M.G. Deneau and S.A. Bailey, DFO for 2006 (unpublished data). Please refer to appendix VII for values of SST, SSS, nutrients and sea-ice cover for the Scotian Shelf.

Ports	Species richness	Av. concent ± SD	Number of ships (av. 2005-2006)	Discharged volume (MT) (av. 2005-2006)	EXCH (MT) (2006)	NEXCH (MT) (2006)
Yarmouth	14	1346 ± 874	0	0	0	0
Shelburne	39	14 467 ± 11 487	51	560	109	0
Liverpool	19	4717 ± 3472	13	17 550	2319	616
Halifax	40	81 606 ± 81 632	1 012	2 061 266	704 552	736 892
Sheet Harbour	31	6313 ± 2431	6	47 696	67 339	0
Canso Strait	22	2034 ± 885	340	5 969 114	5 312 330	311 546
Little Narrows	18	2077 ± 1284	33	228 703	62 936	50 230
Sydney	37	25 573 ± 42 260	83	10 599	7319	12 027

1.2.3.2. Sediment sampling

We randomly collected sediment samples in triplicate at six stations in each port (Figure1. 2). Samples were collected using an Ekman bottom grab sampler (12.5 x 12.5 x 22 cm). In order to collect the most recent cysts we collected only the sediment that was in the oxic layer (when present), i.e. approximately the top 2 cm layer or less, which represents roughly the past 20 years based on a sediment accumulation rate of 1 cm per 10 years for Bedford Basin, Halifax, N.S. (Miller et al. 1982). The sediment was put into plastic bags and tagged with GPS coordinates, time and date. The overlying seawater in the grab was also transferred by pipette with the sediments because cysts may get resuspended from surface sediments during sampling. Samples were refrigerated (4°C) and kept in the dark until laboratory processing (from a few months to a year).

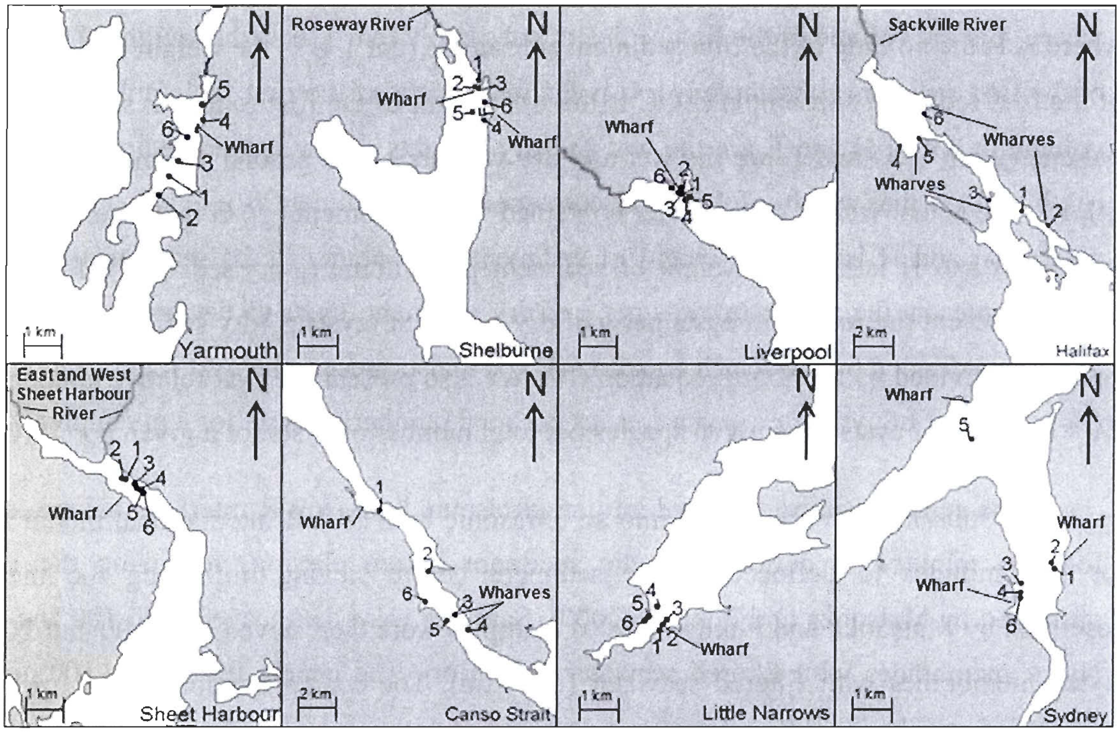

Figure 1.2. Location of the six sites randomly selected from the eight sampled ports. Note differences in scale for Halifax and Canso Strait.

1.2.3.3. Sample processing and analysis

Whole sediment samples were first homogenized by gently stirring the sediment in the bag with a spatula. A volume of 1 cm^3 of sediment was then collected by displacement of an equal volume of seawater in a graduated cylinder. A second subsample of sediment was weighed and dried to calculate the proportion of water using the following equations:

(1) $C = B/A$

(2) $F = D - E$

(3) $G = (F/D) \times 100$

(4) $H = C \times (100-G)/100$

where A is the volume of the first sediment subsample (cm^3), B is the weight of the volume of the first sediment subsample (g), C is the wet weight of the first sediment subsample per volume ($g\ cm^{-3}$), D and E are the wet and dry weights of the second sediment subsample (g), F is the actual weight of the water contained in the sediment (g), G is the percentage of water (%) and H is the dry weight of sediment per volume (g dry sed. cm^{-3}). Finally, in order to present the results in cysts per g of dry sediment (cysts g^{-1} dry sed.), the cysts cm^{-3} value was divided by the fourth equation (H). We also present the cyst relative abundances (% = (number of cysts for a given species per total number of cysts for a given port)*100)).

The subsamples were placed into an ultrasonic bath (VWR international model 50T) for two minutes to deflocculate the sediment before sieving, following the method described by Matsuoka and Fukuyo (2000). Samples were then sieved onto 100 and 20 μm Nytex membranes with filtered seawater (0.45 μm). The coarse fraction (> 100 μm) was discarded and the 20-100 μm fractions was kept and transferred into a 50 ml beaker using a wash bottle. Fine particles were suspended and concentrated by gentle swirling motions. The supernatant was poured on a 20 μm mesh Nytex membrane then transferred into a Petri dish (Fisherbrand, 60 x 15 mm). The 20-100 μm fractions was observed under an inverted microscope (Nikon Eclipse TE2000-U) at 100X magnification and all the cysts present were counted. Identification was made at 400X magnification with the help of several references from the literature (Rochon et al. 1999, Matsuoka and Fukuyo 2000, Head et al. 2001, Marret and Zonneveld 2003, Matsuoka et al. 2009) or with the use of a scanning electron microscope (SEM). When possible, the cysts were identified to the species level. Unknown cysts were isolated and incubated in multi-well plates (COSTAR®3524, Corning Incorporated) with f/2 medium without silica (Guillard and Ryther 1962, Guillard 1975) to try to identify the vegetative form of the unidentified cyst.

1.2.3.4. Statistical analysis

Prior to the laboratory analysis, all samples (i.e. 3 replicates (A, B, C) x 6 sites x 8 ports) were randomly divided into three groups, each group containing one of the three replicates from each sample site. In order to obtain an estimate of the dinoflagellate cyst concentrations and species richness in the ports we did a preliminary analysis of the first group. This preliminary analysis revealed that 4 ports had the highest cyst concentrations: Sheet Harbour, Shelburne, Sydney, and Halifax (ranging between 10 275 and 83 351 cysts g^{-1} dry sed.) (Table 1.2). Since microscopy is time-consuming, we decided to examine all three replicates only for samples from these 4 ports. Results of the preliminary analysis from the eight ports were used to compare the diversity and specific abundances between the ports. Results from the 4 selected ports were used to evaluate the variation in diversity and specific abundances within a given port.

Non-metric multidimensional scaling (nMDS) and cluster analyses were used to examine the cyst assemblages between and within the ports. nMDS results were done with a Bray Curtis similarity index for the dinoflagellate cyst assemblages after a fourth root transformation. These analyses were done with PRIMER 6 with PERMANOVA+ (PRIMER-E ltd 2008). A linear regression was used to test the influence of shipping traffic (i.e. number of ships and volume of ballast water discharged) on the average concentrations and species richness of dinoflagellate cysts. All linear regressions were performed in JMP 7 (SAS Institute 2007).

1.2.4. Results

A total of 53 dinoflagellate cyst taxa were observed throughout the eastern and south-eastern coast of Nova Scotia, including Cape Breton, 36 of which were identified to the species level. Two of these species are from dinoflagellates known to produce toxins: the cysts of the *Alexandrium tamarense* complex (i.e., *Alexandrium tamarense, A. fundyense* and *A. catenella*) can produce saxitoxin (STX), a toxin known for its paralytic shellfish poisoning (PSP), and *Operculodinium centrocarpum* (= *Protoceratium reticulatum*) can produce yessotoxin (YTX) (Satake et al. 1997). Also, *Scrippsiella trochoidea* is known to produce harmful algal blooms (HAB). In addition, we identified 10 taxa to the genus level (e.g. *Brigantedinium* spp.) and 7 taxa remain unidentified (labelled unidentified cyst A, B, C, etc.). Table 1.2 gives the complete list of the dinoflagellate cyst species with their average abundance in cysts per gram of dry sediments (cysts g^{-1} dry sed.) and their relative abundance (%) found in this study.

Table 1.2. Dinoflagellate cyst average concentration (cysts g^{-1} of dry sed.) with standard deviation (SD) and relative abundance (% = (number of cysts for a given species/total number of cysts for a given port)*100)) in the eight Nova Scotia ports with the number (n) of cysts counted for a given port. (*Harmful/toxic species. ‡Potentially exotic and/or toxic species)

	Yarmouth		Shelburne		Liverpool		Halifax		Sheet Harbour		Canso Strait		Little Narrows		Sydney	
Cyst name	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)
n = number of cysts counted for a given site	n = 1199		n = 4030		n = 1414		n = 14 460		n = 1851		n = 1319		n = 516		n = 4375	
Autotrophs : Heterotrophs ratio	4 : 7		21 : 16		12 : 10		18 : 17		20 : 11		11 : 8		8 : 9		13 : 19	
Autotrophic species																
*Cyst of *Alexandrium tamarense* complex	108 ± 50	8	3197 ± 2322	1	9 ± 14	< 1	57 959 ± 82 072	70	28 ± 28	< 1	8 ± 22	< 1	0 ± 0	0	0 ± 0	0
Ataxiodinium choane	0 ± 0	0	28 ± 20	< 1	0 ± 0	0	3 ± 4	< 1	3 ± 6	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0
Bitectatodinium tepikiense	0 ± 0	0	1298 ± 569	9	1 ± 0	0	10 659 ± 10 962	13	4057 ± 2089	39	104 ± 96	5	322 ± 168	15	492 ± 453	3
Impagidinium spp.	0 ± 0	0	2 ± 3	< 1	0 ± 0	0	4 ± 6	< 1	3 ± 4	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	< 1
cf. *I. patulum*	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	2 ± 3	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0
I. strialatum	0 ± 0	0	1 ± 2	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0
cf. *Gymnodinium impudicum*	0 ± 0	0	20 ± 19	< 1	0 ± 0	0	0 ± 0	0	42 ± 36	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0
Nematosphaeropsis labyrinthus	0 ± 0	0	2 ± 4	< 1	0 ± 0	0	0 ± 0	0	4 ± 9	< 1	0 ± 0	0	14 ± 17	< 1	0 ± 0	0
**Operculodinium centrocarpum*	8 ± 12	< 1	560 ± 184	4	51 ± 117	1	2068 ± 2140	2	320 ± 72	3	0 ± 0	0	0 ± 0	0	17 ± 17	< 1
Cyst of *Pentapharsodinium dalei*	0 ± 0	0	1096 ± 265	7	0 ± 0	0	1300 ± 1463	2	84 ± 50	< 1	10 ± 13	< 1	0 ± 0	0	133 ± 109	< 1
Cyst of *Scrippsiella* spp.	0 ± 0	0	0 ± 0	0	0 ± 0	0	27 ± 56	< 1	0 ± 0	0	50 ± 50	2	0 ± 0	0	12 ± 25	< 1
Cyst of *Scrippsiella* sp. 1	1 ± 2	< 1	13 ± 24	< 1	0 ± 0	0	516 ± 522	< 1	5 ± 5	< 1	50 ± 24	2	268 ± 491	13	7230 ± 12 202	43
Cyst of *S. lachrymosa*	0 ± 0	0	43 ± 28	< 1	5 ± 12	< 1	1139 ± 1192	1	254 ± 117	2	0 ± 0	0	0 ± 0	0	0 ± 0	0
‡Cyst of *S.* cf. *regalis*	0 ± 0	0	2 ± 4	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0
*Cyst of *S. trochoidea*	11 ± 11	< 1	216 ± 61	1	67 ± 91	1	21 ± 28	< 1	0 ± 0	0	483 ± 748	24	11 ± 18	< 1	162 ± 128	< 1
Spiniferites spp.	0 ± 0	0	563 ± 123	4	0 ± 0	0	42 ± 50	< 1	176 ± 80	2	15 ± 22	< 1	159 ± 207	8	92 ± 55	< 1
S. bulloideus	0 ± 0	0	54 ± 132	< 1	5 ± 12	< 1	10 ± 24	< 1	32 ± 27	< 1	5 ± 9	< 1	15 ± 31	< 1	6 ± 6	< 1
S. delicatus	0 ± 0	0	60 ± 52	< 1	0 ± 0	0	19 ± 27	< 1	102 ± 56	< 1	11 ± 21	< 1	7 ± 11	< 1	43 ± 38	< 1

Table 1.2 (continued). Dinoflagellate cyst average concentration (cysts g^{-1} of dry sed.) with standard deviation (SD) and relative abundance (% = (number of cysts for a given species/total number of cysts for a given port)*100)) in the eight Nova Scotia ports with the number (n) of cysts counted for a given port. (*Harmful/toxic species. ‡Potentially exotic and/or toxic species)

	Yarmouth		Shelburne		Liverpool		Halifax		Sheet Harbour		Canso Strait		Little Narrows		Sydney	
Cyst name	Av. conc. ± SD	Rel. Abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)
n = number of cysts counted for a given site	n = 1199		n = 4030		n = 1414		n = 14 460		n = 1851		n = 1319		n = 516		n = 4375	
S. elongatus	0 ± 0	0	265 ± 130	2	1 ± 0	0	150 ± 162	< 1	66 ± 30	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0
S. frigidus	0 ± 0	0	599 ± 480	4	2 ± 0	0	356 ± 366	< 1	165 ± 66	2	0 ± 0	0	0 ± 0	0	0 ± 0	0
S. hyperacanthus	0 ± 0	0	0 ± 0	0	3 ± 0	0	0 ± 0	0	0 ± 1	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0
S. membranaceus	0 ± 0	0	107 ± 76	< 1	4 ± 0	0	15 ± 18	< 1	817 ± 602	8	2 ± 6	< 1	0 ± 0	0	4 ± 9	< 1
S. mirabilis	0 ± 0	0	14 ± 13	< 1	3 ± 8	< 1	87 ± 90	< 1	40 ± 29	< 1	0 ± 0	0	0 ± 0	0	8 ± 9	< 1
S. ramosus	0 ± 0	0	95 ± 31	< 1	11 ± 20	< 1	57 ± 56	< 1	96 ± 52	< 1	19 ± 17	< 1	2 ± 6	< 1	23 ± 23	< 1
Heterotrophic species																
Brigantedinium spp.	797 ± 518	59	2769 ± 1207	18	2491 ± 1672	53	3175 ± 1371	4	1405 ± 519	14	310 ± 221	15	170 ± 149	8	2755 ± 1629	16
B. cariacoense	112 ± 83	8	242 ± 117	2	556 ± 451	12	892 ± 425	1	144 ± 57	1	55 ± 47	3	26 ± 36	1	144 ± 129	< 1
B. irregulare	0 ± 0	0	0 ± 0	0	0 ± 0	0	2 ± 5	< 1	0 ± 0	0	2 ± 4	< 1	0 ± 0	0	0 ± 0	0
B. simplex	253 ± 187	19	2079 ± 691	14	1439 ± 1394	31	1047 ± 623	1	1523 ± 408	15	530 ± 493	26	12 ± 15	< 1	1062 ± 519	6
‡*Cochlodinium* sp. 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	1 ± 3	< 1	0 ± 0	0	2 ± 5	< 1	36 ± 63	2	186 ± 206	1
‡*Cochlodinium* sp. 2	0 ± 0	0	0 ± 0	0	0 ± 0	0	12 ± 18	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	6 ± 6	< 1
Dubridinium lenticulatum	1 ± 3	< 1	226 ± 60	1	3 ± 7	< 1	45 ± 37	< 1	71 ± 29	< 1	19 ± 18	< 1	18 ± 30	< 1	181 ± 130	1
Dubridinium sp. 1	35 ± 35	3	44 ± 33	< 1	22 ± 55	< 1	821 ± 685	< 1	185 ± 118	2	0 ± 0	0	0 ± 0	0	92 ± 39	< 1
‡*Echinidinium* sp. 1	0 ± 0	0	22 ± 27	< 1	0 ± 0	0	7 ± 16	< 1	196 ± 116	2	0 ± 0	0	0 ± 0	0	35 ± 32	< 1
‡*Echinidinium* sp. 2	15 ± 12	1	320 ± 85	2	11 ± 13	< 1	439 ± 215	< 1	289 ± 101	3	12 ± 24	< 1	0 ± 0	0	2333 ± 1766	14
I. minutum	0 ± 0	0	12 ± 17	< 1	0 ± 0	0	100 ± 184	< 1	0 ± 0	0	0 ± 0	0	3 ± 8	< 1	214 ± 150	1
Lejeunecysta cf. *sabrina*	0 ± 0	0	6 ± 8	< 1	3 ± 8	< 1	162 ± 171	< 1	6 ± 8	< 1	0 ± 0	0	0 ± 0	0	57 ± 55	< 1
Cyst of *Polykrikos kofoidii*	0 ± 0	0	58 ± 52	< 1	3 ± 7	< 1	720 ± 887	< 1	21 ± 23	< 1	0 ± 0	0	0 ± 0	0	5 ± 8	< 1
Cyst of *Protoperidinium* spp.	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	11 ± 26	< 1

Table 1.2 (continued). Dinoflagellate cyst average concentration (cysts g^{-1} of dry sed.) with standard deviation (SD) and relative abundance (% = (number of cysts for a given species/total number of cysts for a given port)*100)) in the eight Nova Scotia ports with the number (n) of cysts counted for a given port. (*Harmful/toxic species. [‡]Potentially exotic and/or toxic species)

	Yarmouth		Shelburne		Liverpool		Halifax		Sheet Harbour		Canso Strait		Little Narrows		Sydney	
Cyst name	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)	Av. conc. ± SD	Rel. abund. (%)
n = number of cysts counted for a given site	n = 1199		n = 4030		n = 1414		n = 14 460		n = 1851		n = 1319		n = 516		n = 4375	
Cyst of *P. americanum*	0 ± 0	0	529 ± 254	4	11 ± 13	< 1	241 ± 204	< 1	73 ± 47	< 1	19 ± 16	< 1	0 ± 0	0	63 ± 64	< 1
Cyst of *P. nudum*	0 ± 0	0	3 ± 5	< 1	0 ± 0	0	69 ± 102	< 1	0 ± 0	0	0 ± 0	0	3 ± 8	< 1	29 ± 33	< 1
Cyst of *P. stellatum*	0 ± 0	0	6 ± 11	< 1	0 ± 0	0	1 ± 1	< 1	0 ± 0	0	0 ± 0	0	2 ± 6	< 1	4 ± 7	< 1
Quinquecuspis concreta	0 ± 0	0	2 ± 4	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0
Selenopemphix nephroides	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	32 ± 11	< 1
S. quanta	2 ± 4	< 1	469 ± 103	3	22 ± 39	< 1	699 ± 623	< 1	64 ± 31	< 1	0 ± 0	0	0 ± 0	0	43 ± 29	< 1
Votadinium calvum	0 ± 0	0	4 ± 10	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	7 ± 18	< 1	23 ± 33	< 1
V. spinosum	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	< 1
Unidentified cysts																
Unidentified cyst A	3 ± 6	< 1	3 ± 8	< 1	0 ± 0	0	84 ± 187	< 1	0 ± 0	0	165 ± 87	8	480 ± 645	23	1047 ± 1225	6
Unidentified cyst B	0 ± 0	0	0 ± 0	0	0 ± 0	0	38 ± 47	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0
Unidentified cyst C	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	157 ± 91	8	520 ± 395	25	137 ± 109	< 1
Unidentified cyst D	1 ± 2	< 1	0 ± 0	0	5 ± 12	< 1	349 ± 566	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	16 ± 16	< 1
Unidentified cyst E	0 ± 0	0	0 ± 0	0	0 ± 0	0	10 ± 23	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0
Unidentified cyst F	1 ± 1	< 1	0 ± 0	0	1 ± 2	< 1	4 ± 10	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0
Unidentified cyst G	0 ± 0	0	0 ± 0	< 1	0 ± 0	0	0 ± 0	0	0 ± 0	0	5 ± 6	< 1	0 ± 0	0	171 ± 381	1

MDS ordination of dinoflagellate cyst assemblages among the ports included in this survey shows that there were three distinct groups of ports: group 1 (Yarmouth and Liverpool), group 2 (Shelburne, Halifax and Sheet Harbour) and group 3 (Canso Strait, Little Narrows and Sydney) (Figure 1.3). Apart from Sydney, all the other ports shared a 60% similarity level. Only the ports of Shelburne and Sheet Harbour shared a 70% similarity between their cyst assemblages. The low stress value of 0.01 indicates that the ordination respects relatively well the distances between the different assemblages. In group 1 (Yarmouth and Liverpool), the average cyst concentration is 3032 ± 2383 cysts g^{-1} dry sed. (Table 1.1) and the cyst assemblages were dominated by the *Brigantedinium* group. In Yarmouth, the cyst of the *Alexandrium tamarense* complex represented the most important accompanying species. The dinoflagellate cyst assemblages in the second group of ports (Shelburne, Halifax and Sheet Harbour) were characterized by the presence of four dominant genera/species: the *Brigantedinium* group, the cyst of the *Alexandrium tamarense* complex, *Bitectatodinium tepikiense* and the *Spiniferites* group. The average dinoflagellate cyst concentration for this group was 34 128 ± 41 318 cysts g^{-1} dry sed. Finally, in the third group of ports (Canso Strait, Little Narrows and Sydney), the average cyst concentration was 15 193 ± 13 578 cysts g^{-1} dry sed. and the dinoflagellate cyst assemblages were mainly composed of the *Brigantedinium* group, the cysts of *Scrippsiella* group, the unidentified cysts (unidentified cyst A, C, D and G) group and *B. tepikiense*. All of these, with the exception of the cysts of *Scrippsiella* group (undetectable with standard palynological methods – see above) have been found in other studies from the same region (e.g. de Vernal et al. 1997, Rochon et al. 1999, Devillers and de Vernal 2000).

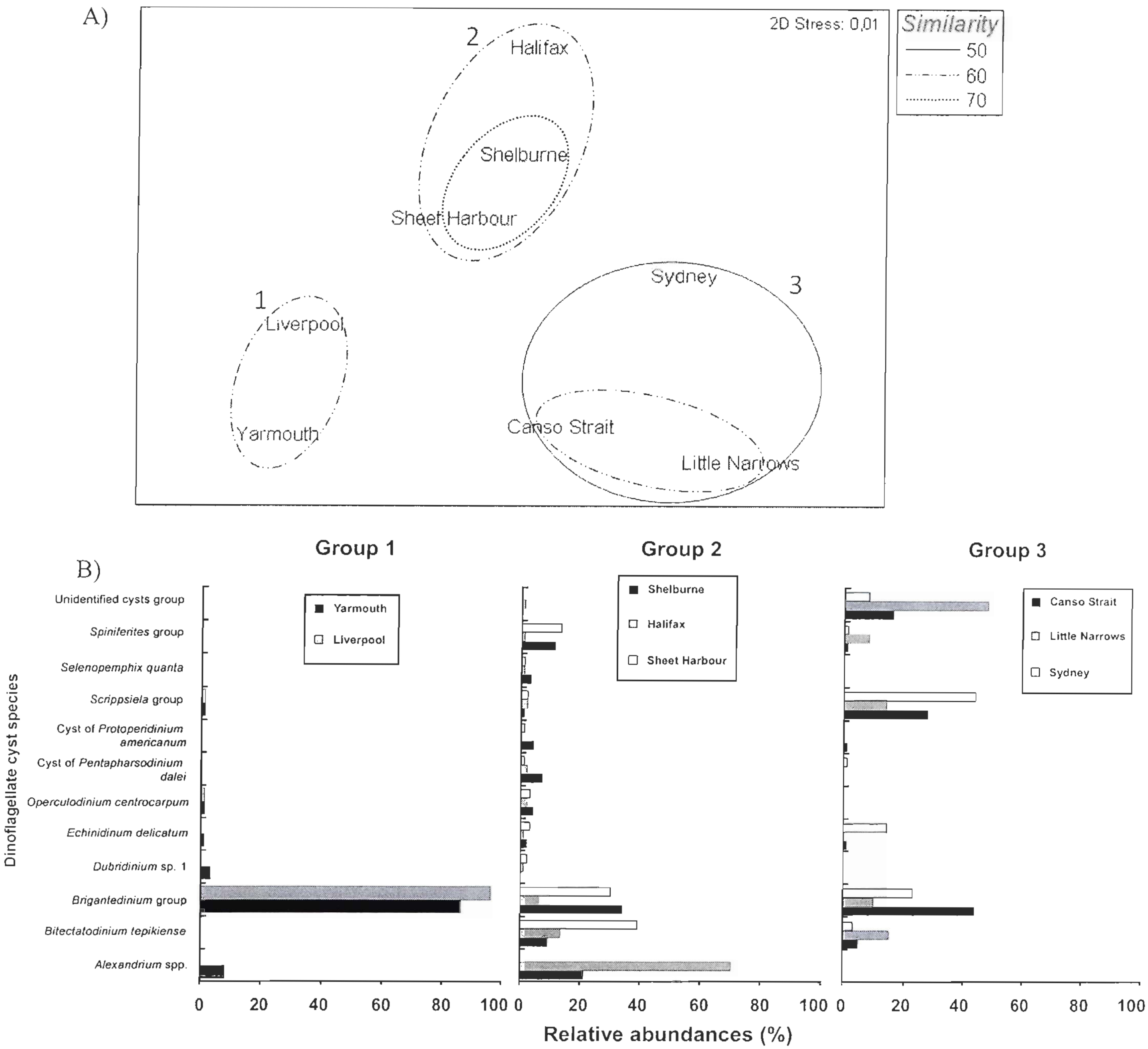

Figure 1.3. A) nMDS results using a Bray Curtis similarity index for the dinoflagellate cyst assemblages of the eight sampled ports after a fourth root transformation; B) relative abundances (5) of the dominant dinoflagellate cysts for the 3 groups of ports.

Dinoflagellate cyst assemblages found in the eight ports (Table 1.2) shows some general trends when it comes to the dominant (i.e. single species or group of species representing more than 90% of the assemblage) and accompanying species (i.e. species representing more than 1% but less than 90% of the assemblage) (see Figure 1.3B). For example, the *Brigantedinium* group, which comprises *Brigantedinium* spp., *B. cariacoense*, *B. irregulare* and *B. simplex*, was part of the dominant group in 6 of the 8 ports (Yarmouth, Shelburne, Liverpool, Sheet Harbour, Canso Strait and Sydney). Some species were also found in all the ports. For instance, the *Brigantedinium* spp. group represented 6 to 95% of the dinoflagellate cyst assemblages in all of the ports. The cysts of *Scrippsiella* spp. group (*Scrippsiella* spp., *Scrippsiella* sp. 1, *S. lachrymosa*, *S.* cf. *regalis* and *S. trochoidea*) were also present in all the ports. Their relative abundance ranged from <1% to 44% in the ports of Yarmouth and Sydney respectively. However, the maximum relative abundances for that group were found in Cape Breton: Canso Strait (29%), Little Narrows (13%) and Sydney (44%). Another species found throughout Nova Scotia, with the exception of Yarmouth, was *Bitectatodinium tepikiense*. Its relative abundance ranged from <1% to 39% in the ports of Liverpool and Sheet Harbour respectively. Finally, although found at a relatively low abundance in a few ports (<1% in Liverpool, Sheet Harbour and Canso Strait), the cysts of the *Alexandrium tamarense* complex were present throughout the eastern and south-eastern shores of Nova Scotia, with the exception of the ports of Little Narrows and Sydney.

The number of dinoflagellate cyst taxa found in each port ranged from 14 to 40. The highest values of species richness were found for the ports of Shelburne (39) and Halifax (40) (Table 1.1). The average concentration of dinoflagellate cysts in the ports ranged from 1346 to 83 351 cysts g^{-1} dry sed., and the highest concentrations were found in Sydney (16 869 cysts g^{-1} dry sed.) and Halifax (83 351 cysts g^{-1} dry sed.) (Table 1.1). Table 1.1 also gives the number of ships visiting each port as well as the volume in metric tonnes (MT) of ballast water being discharged in the ports, including volumes of non-exchanged and exchanged ballast water (for the year 2006).

To examine the influence of shipping traffic, the average cyst concentrations were regressed against the number of ships visiting the ports studied. Linear regression shows that there was a highly significant influence of the number of ships on the average concentrations of dinoflagellate cyst (average cyst concentrations = 3829.6 – 69.89 * number of ships; $r^2 = 0.89$; ANOVA model: $F_{1,6} = 24.27$; $p = 0.003$) (Figure 1.4a) but not between the number of ships and the number of dinoflagellate cyst species (species richness) observed in the ports (species richness = 24.86 + 0.01 * number of ships; $r^2 = 0.21$; ANOVA model: $F_{1,6} = 1.60$; $p = 0.25$) (Table 1.1). Results also show that there was no influence of the discharged volume of ballast water (irrespective of whether ballast water was exchanged or not during the trip) on the average cyst concentrations (average cyst concentrations = 15 502.92 + 0.001 * discharged ballast volume; $r^2 = 0.01$; ANOVA model: $F_{1,6} = 0.07$; $p = 0.80$) nor on the species richness index (species richness = 27.80 - $2.92e^{-7}$ * discharged ballast volume; $r^2 = 0.003$; ANOVA model $F_{1,6} = 0.02$; $p = 0.89$) (Table 1.1). There was no influence of the discharged volume of ballast water from ships that undertook a ballast water exchange on the average dinoflagellate cyst concentrations or the species richness index (Table 1.1). However, when considering only the ships that did not undertake a ballast water exchange (domestic traffic), there was a highly significant influence of the discharged volume of ballast water on the average dinoflagellate cyst concentrations (average cyst concentrations = 4585.77 + 0.09 * non-exchanged ballast volume; $r^2 = 0.86$; ANOVA model: $F_{1,6} = 13.55$; $p = 0.01$) (Figure 1.4b) but not on the species richness index. However, these significant relations were influenced strongly by the results from one port: Halifax, which receives the greatest number of domestic traffic ships among all the ports considered here, and had the highest cyst concentrations.

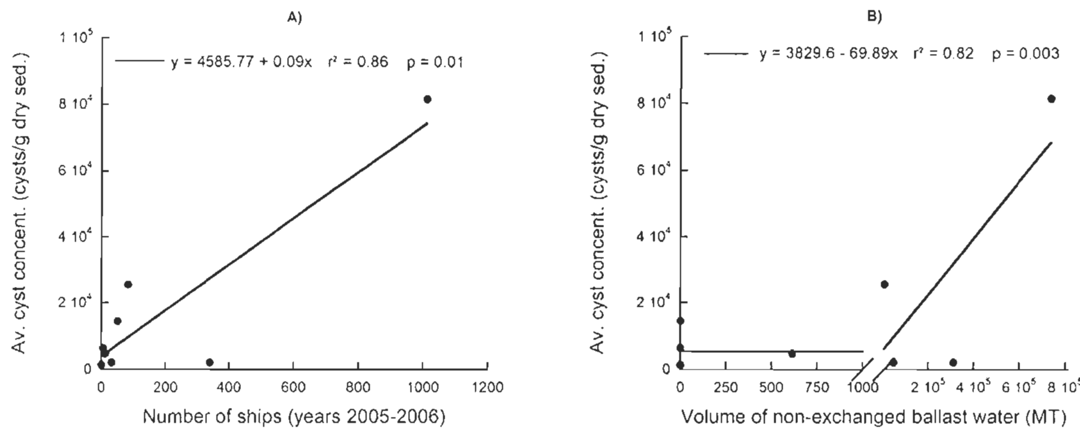

Figure 1.4. Results from the linear regressions between A) the number of ships and the average concentration of dinoflagellate cysts (cysts g^{-1} dry sed.), and B) the volume of non-exchanged discharged ballast water in metric tonnes (MT) and the average concentration of dinoflagellate cysts (cysts g^{-1} dry sed.). Note that the scales of the x axis are different.

The spatial distribution of dinoflagellate cysts within ports was examined only for the four ports that had the highest species richness index as well as the highest cyst concentrations: Shelburne, Halifax, Sheet Harbour and Sydney. The average concentration of dinoflagellate cysts (cysts g^{-1} dry sed.) and the species richness for each of the sites and ports are found in Table 1.3 while the relative abundances of cyst species are shown in Table 1.4. Figure 1.5 gives the cluster results from the non-metric MDS ordination of dinoflagellate cyst assemblages. It shows that the similarity between the sites within ports varies between 65% (for Sydney, station lying outside the 70% similarity outline in Figure 1.5) and 80%. The relatively low stress value of 0.07 indicates that the ordination respects relatively well the distances between the assemblages.

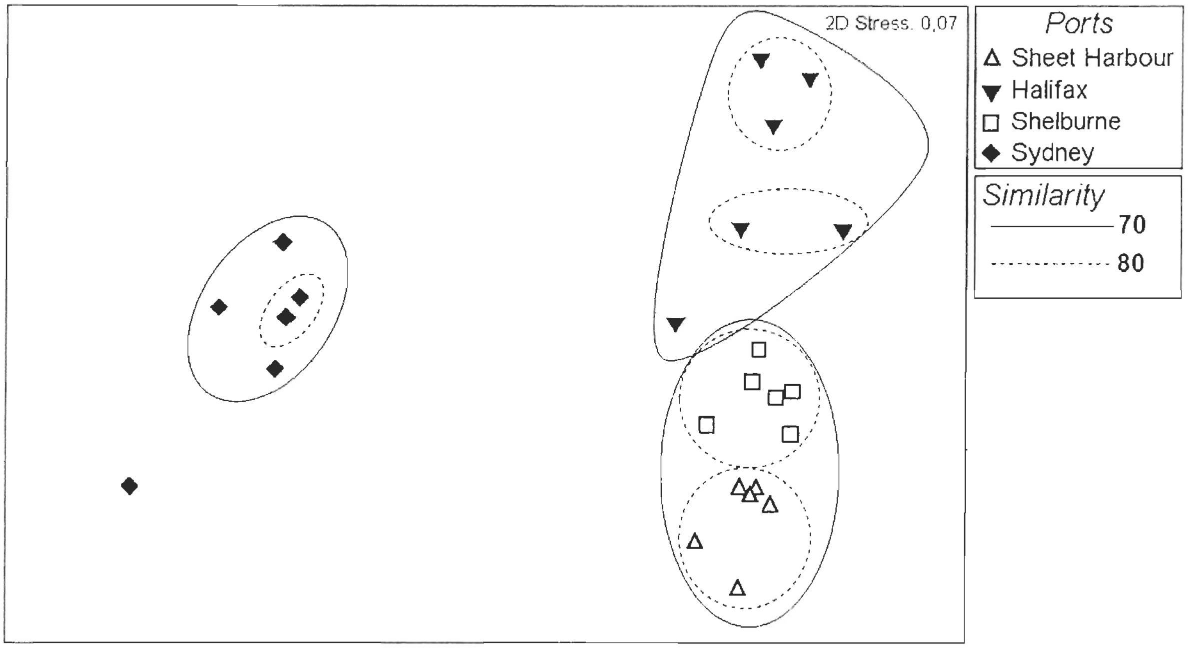

Figure 1.5. nMDS results using a Bray Curtis similarity index for the dinoflagellate cyst assemblages for the ports of Halifax (▼), Sydney (♦), Shelburne (□) and Sheet Harbour (Δ) (6 sites ports^{-1}) after a fourth root transformation.

Within the port of Shelburne, the average cyst concentrations ranged between 9552 and 21 963 cysts g^{-1} dry sed. for sites 4 and 1 respectively; species richness ranged between 25 and 33 for sites 5, 6 and 1 respectively (Table 1.3). The similarity between sites 2, 3, 4, 5 and 6 reached 85% (not shown in Figure 1.5); site 1 shares an 80% similarity with the other sites. The differences between the sites reside in the difference between the assemblages, either in the cyst concentrations or the species diversity. For example, the cysts of the *Alexandrium tamarense* complex are not part of the dominant species at site 2 and *Bitectatodinium tepikiense* is not considered a dominant species at sites 5 and 6 (see Table 1.4 and Figure 1.6 for more details).

Table 1.3. Within-port cyst information: average concentration (cysts g^{-1} dry sed.) with standard deviation (SD) and species richness (number of species observed) per sampling site for the ports of Shelburne, Halifax, Sheet Harbour and Sydney.

Sites	Shelburne		Halifax		Sheet Harbour		Sydney	
	Av. concent. ± SD	Species richness	Av. concent. ± SD	Species richness	Av. concent. ± SD	Species richness	Av. concent. ± SD	Species richness
Site 1	21 963 ± 11 964	33	25 279 ± 21 700	27	11 547 ± 4069	26	14 758 ± 13 270	31
Site 2	15 199 ± 9029	26	11 131 ± 5010	29	12 670 ± 2870	28	14 198 ± 15 505	28
Site 3	12 966 ± 3798	27	29 435 ± 8649	28	5940 ± 2266	23	6153 ± 580	26
Site 4	9552 ± 4300	29	113 377 ± 35 540	29	4593 ± 2922	27	14 417 ± 8591	29
Site 5	18 401 ± 1279	25	64 168 ± 31 360	32	14 575 ± 6832	26	3329 ± 2584	23
Site 6	12 125 ± 1698	25	256 715 ± 172 838	27	12 327 ± 5542	26	48 359 ± 52 141	31

The average cyst concentrations in the port of Halifax ranged from 11 131 to 256 715 cysts g^{-1} dry sed. for sites 2 and 6 respectively, and the species richness varied between 27 in sites 1 and 6 and 32 in site 5 (Table 1.3). As seen in Figure 1.5, the similarity between the sites in Halifax was 70% but two groups of sites (sites 1 and 3 and sites 4, 5 and 6) shared a similarity of 80%. In this port, the cysts of the *Alexandrium tamarense* complex were part of the dominant species at all sites. It was the only dominant species for site 6 with a relative abundance of 86%. *Bitectatodinium tepikiense* was also a dominant species for sites 1, 4 and 5. For sites 2 and 3, the other dominant species was the *Brigantedinium* group (see Table 1.4 and Figure 1.6 for more details).

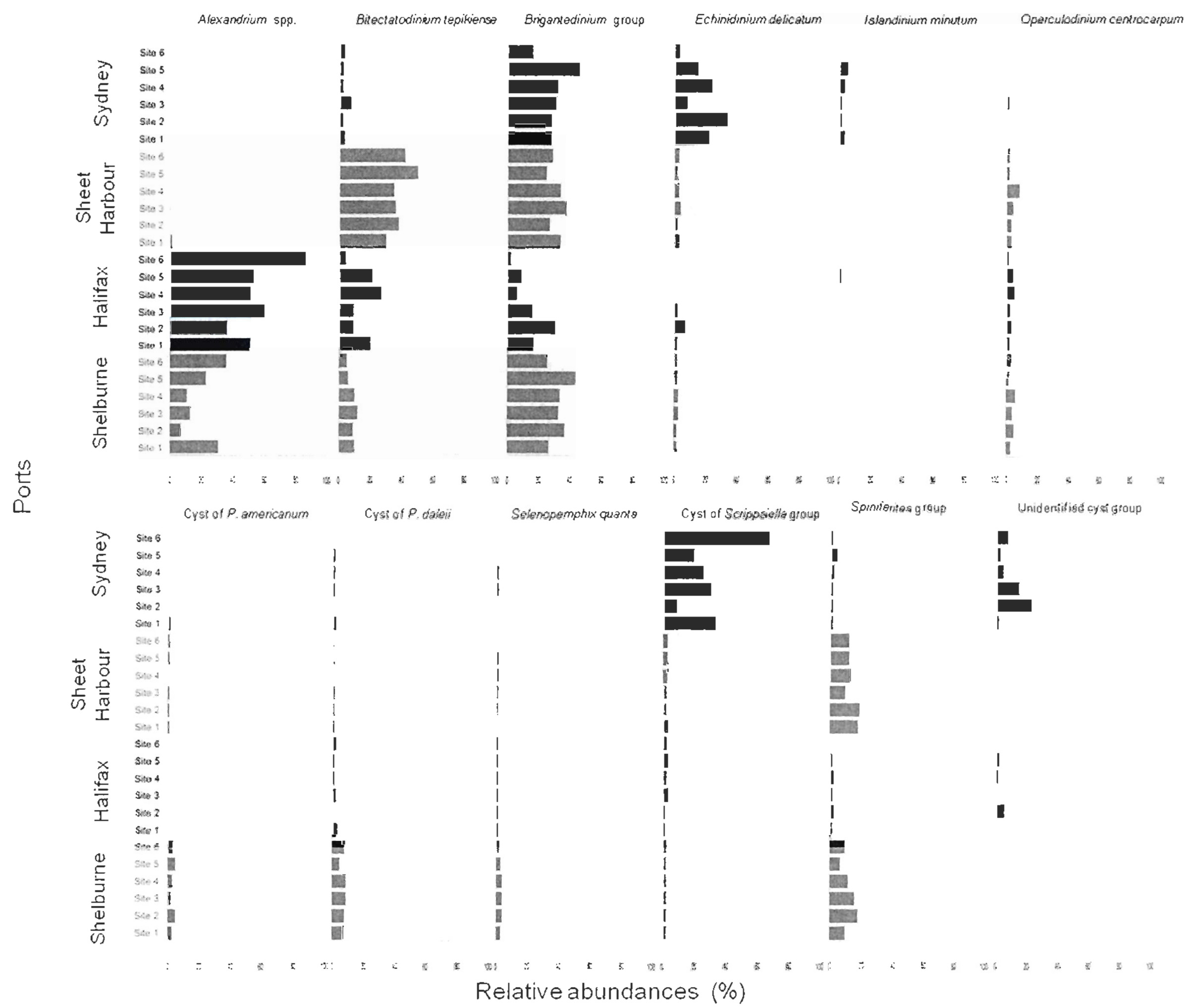

Figure 1.6. Relative abundances (%) of the dominant dinoflagellate cyst taxa for each site in the port of Shelburne, Halifax, Sheet Harbour and Sydney.

In Sheet Harbour, the average cyst concentrations varied between 4593 and 12 670 cysts g^{-1} dry sed. in sites 4 and 2 respectively, while the species richness varied between 23 and 28 in sites 2 and 3 respectively (Table 1.3). Like the port of Shelburne, the similarity between the six sites of the port of Sheet Harbour reached 80% (Figure 1.5). However there were two groups of sites that shared an 85% similarity level (not shown in Figure 1.5): sites 3 and 4 and sites 1, 2, 5 and 6. Two species mostly dominated the assemblages of all the sites: *Bitectatodinium tepikiense* and the *Brigantedinium* group. The *Spiniferites* group (i.e.

Spiniferites spp., *S. bulloideus*, *S. delicatus*, *S. elongatus*, *S. frigidus*, *S. hyperacanthus*, *S. membranaceus*, *S. mirabilis* and *S. ramosus*) was also part of the dominant species but only for sites 1, 2 and 4 (Figure 1.6).

The average cyst concentrations in the port of Sydney varied between 3329 and 48 359 cysts g^{-1} dry sed. at sites 5 and 6 respectively. The species richness varied between 23 at site 5, and 31 for sites 1 and 6 (Table 1.3). This port had the lowest similarity index of the four ports examined with values ranging between 65 and 70%. Site 5, located a few kilometres away from the others (see Figure 1.2), shared only 65% similarity with the rest of the sites. For all six sites, the *Brigantedinium* group was part of the dominant species and it was accompanied either by *Echinidinium* sp. 2 at sites 1, 2, 4 and 5, or by the cyst of *Scrippsiella* group at sites 1, 3, 4, 5 and 6, or by the unknown cyst group (i.e. unidentified cysts A to G) at sites 2 and 3 (see Table 1.4 and Figure 1.6 for more details).

Table 1.4. Within-port cyst information: relative abundance (% = (number of cysts for a given species/total number of cysts for a given site)*100) of dinoflagellate cysts within four ports of Nova Scotia with the number (n) of cysts counted for a given site. (*Harmful/toxic species. ‡Potentially exotic and/or toxic species)

	Shelburne						Halifax						Sheet Harbour						Sydney					
Cyst name	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
n = number of cysts counted for a given site	n = 772	n = 558	n = 574	n = 523	n = 615	n = 627	n = 1841	n = 676	n = 1639	n = 6037	n = 2900	n = 6625	n = 492	n = 388	n = 344	n = 447	n = 497	n = 697	n = 412	n = 224	n = 306	n = 487	n = 165	n = 1686
Autrotrophs : Heterotrophs ratio	19:13	15:11	16:11	16:12	13:12	15:10	12:12	14:11	13:12	14:14	15:14	14:12	16:10	18:10	13:10	18:9	15:11	15:11	11:16	10:14	7:16	9:18	8:12	10:18
Autotrophic species																								
*Cyst of *Alexandrium tamarense* complex	31	7	13	11	23	36	51	36	60	51	53	86	<1	<1	0	<1	<1	<1	0	0	0	0	0	0
Ataxiodinium choane	<1	<1	<1	<1	0	<1	0	<1	0	0	0	<1	<1	0	0	<1	0	0	0	0	0	0	0	0
Bitectatodinium tepikiense	10	9	12	10	6	5	20	9	9	27	21	4	30	38	36	35	50	42	3	2	7	2	2	3
Impagidinium spp.	0	0	<1	<1	0	0	<1	0	<1	0	0	0	0	<1	0	0	<1	<1	<1	0	0	0	0	0
cf. *I. patulum*	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0
I. strialatum	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
cf. *Gymnodinium impudicum*	<1	<1	0	<1	<1	<1	0	0	0	0	0	0	<1	<1	<1	<1	<1	<1	0	0	0	0	0	0
Nematosphaeropsis labyrinthus	<1	0	0	0	0	0	0	0	0	0	0	0	0	<1	0	<1	0	0	0	0	0	0	0	0
**Operculodinium centrocarpum*	3	5	4	6	2	3	2	3	2	5	4	1	3	3	4	8	2	2	<1	<1	<1	<1	<1	0
Cyst of *Pentapharsodinium dalei*	7	8	9	9	5	8	3	<1	2	1	1	2	<1	<1	<1	<1	<1	1	2	<1	<1	1	1	<1
Cyst of *Scrippsiella* spp.	0	0	0	0	0	0	0	0	0	<1	<1	0	0	0	0	0	0	0	0	<1	0	0	2	0
Cyst of *Scrippsiella* sp. 1	<1	0	0	<1	0	0	0	<1	1	<1	1	<1	<1	<1	0	<1	0	0	31	8	28	24	17	66
Cyst of *S. lachrymosa*	<1	<1	<1	<1	<1	<1	1	<1	2	1	2	1	3	2	2	3	3	3	0	0	0	0	0	0
‡ Cyst of *S.* cf. *regalis*	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
* Cyst of *S. trochoidea*	1	2	1	2	1	1	<1	<1	<1	0	0	<1	0	0	0	0	0	0	2	<1	2	1	1	<1
Spiniferites spp.	2	4	6	6	3	4	0	<1	<1	<1	<1	0	1	3	2	3	<1	1	<1	<1	<1	1	1	<1
S. bulloideus	1	0	0	0	0	0	0	0	0	<1	<1	0	<1	<1	<1	<1	<1	<1	<1	0	0	<1	0	<1
S. delicatus	<1	<1	<1	<1	<1	<1	0	0	0	<1	<1	<1	1	<1	<1	3	<1	1	<1	<1	0	<1	3	<1
S. elongatus	2	2	1	3	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	2	<1	<1	0	0	0	0	0	0

Table 1.4 (continued). Within-port cyst information: relative abundance (% = (number of cysts for a given species/total number of cysts for a given site)*100) of dinoflagellate cysts within four ports of Nova Scotia with the number (n) of cysts counted for a given site. (*Harmful/toxic species. ‡Potentially exotic and/or toxic species)

	Shelburne						Halifax						Sheet Harbour						Sydney					
Cyst name	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
n = number of cysts counted for a given site	n = 772	n = 558	n = 574	n = 523	n = 615	n = 627	n = 1841	n = 676	n = 1639	n = 6037	n = 2900	n = 6625	n = 492	n = 388	n = 344	n = 447	n = 497	n = 697	n = 412	n = 224	n = 306	n = 487	n = 165	n = 1686
S. frigidus	2	9	7	<1	2	4	<1	<1	<1	<1	<1	<1	1	2	3	1	1	1	0	0	0	0	0	0
S. hyperacanthus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	0	0	0	0	0	0	0	0
S. membranaceus	1	<1	<1	<1	<1	<1	<1	<1	0	0	<1	<1	1	12	2	1	6	8	0	0	0	0	0	<1
S. mirabilis	<1	<1	<1	0	0	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	0	0	0	<1
S. ramosus	<1	<1	<1	1	<1	<1	<1	<1	<1	<1	<1	<1	1	1	<1	1	<1	<1	<1	<1	<1	<1	0	<1
Heterotrophic species																								
Brigantedinium spp.	17	17	16	16	26	15	10	13	7	4	6	2	17	12	16	13	12	14	21	16	24	21	30	12
B. cariacoense	<1	3	2	2	2	<1	2	4	2	1	2	<1	1	<1	3	1	1	2	<1	2	<1	1	<1	<1
B. irregulare	0	0	0	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B.simplex	10	17	15	16	17	10	5	14	6	<1	1	<1	16	14	19	19	12	14	7	10	7	10	15	3
‡*Cochlodinium* sp. 1	0	0	0	0	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	2	0	<1	1	<1	1
‡*Cochlodinium* sp. 2	0	0	0	0	0	0	<1	0	0	<1	<1	<1	0	0	0	0	0	0	0	<1	<1	<1	0	<1
Dubridinium lenticulatum	1	2	1	2	<1	2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	2	<1	1	2	<1	<1
Dubridinium sp. 1	<1	<1	<1	<1	<1	<1	1	<1	3	2	<1	<1	2	3	1	<1	1	2	<1	<1	1	1	2	<1
‡*Echinidinium* sp. 1	0	<1	0	<1	<1	0	0	<1	0	0	0	0	3	2	2	<1	2	3	<1	<1	0	<1	0	<1
‡*Echinidinium* sp. 2	2	2	3	3	2	2	2	7	2	<1	<1	<1	3	2	4	3	2	3	22	34	8	24	15	3
I. minutum	<1	0	0	<1	<1	0	0	0	0	<1	<1	<1	0	0	0	0	0	0	3	<1	<1	3	5	<1
Lejeunecysta cf. *sabrina*	<1	0	<1	<1	0	0	<1	<1	<1	<1	<1	<1	0	0	<1	0	<1	<1	1	<1	<1	<1	<1	<1
Cyst of *Polykrikos kofoidii*	<1	<1	<1	<1	<1	<1	<1	2	1	<1	<1	<1	<1	<1	0	0	<1	<1	0	0	<1	<1	0	<1
Cyst of *Protoperidinium* spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	<1
Cyst of *P. americanum*	3	5	2	3	5	3	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1	<1	<1	<1	<1	0	<1

Table 1.4 (continued). Within-port cyst information: relative abundance (% = (number of cysts for a given species/total number of cysts for a given site)*100) of dinoflagellate cysts within four ports of Nova Scotia with the number (n) of cysts counted for a given site. (*Harmful/toxic species. ‡Potentially exotic and/or toxic species)

	Shelburne						Halifax						Sheet Harbour						Sydney					
Cyst name	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
n = number of cysts counted for a given site	n = 772	n = 558	n = 574	n = 523	n = 615	n = 627	n = 1841	n = 676	n = 1639	n = 6037	n = 2900	n = 6625	n = 492	n = 388	n = 344	n = 447	n = 497	n = 697	n = 412	n = 224	n = 306	n = 487	n = 165	n = 1686
Cyst of *P. nudum*	0	0	0	0	<1	<1	<1	0	<1	<1	<1	0	0	0	0	0	0	0	<1	0	<1	<1	0	<1
Cyst of *P. stellatum*	<1	0	<1	0	0	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	<1	0	<1	0	0
Quinquecuspis concreta	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Selenopemphix nephroides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	<1	<1	<1	<1	<1
S. quanta	3	4	4	4	3	2	<1	<1	<1	1	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Votadinium calvum	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	0	<1	<1	0	<1
V. spinosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	0	0	0	0	0
Unidentified cysts																								
Unidentified cyst A	<1	0	0	0	0	0	0	4	<1	0	0	0	0	0	0	0	0	0	<1	20	10	3	<1	5
Unidentified cyst B	0	0	0	0	0	0	<1	<1	<1	<1	0	0	0	0	0	0	0	0	<1	0	0	0	0	0
Unidentified cyst C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	2	3	<1	<1	<1
Unidentified cyst D	0	0	0	0	0	0	<1	<1	<1	<1	2	<1	0	0	0	0	0	0	<1	<1	0	0	1	<1
Unidentified cyst E	0	0	0	0	0	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified cyst F	0	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified cyst G	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	<1	1	0	0	2

1.2.5. Discussion

This study provides the first data on dinoflagellate cyst concentrations and species composition of dinoflagellate cyst assemblages, including HAB species, for ports of Nova Scotia, Canada. To our knowledge, very few studies have examined dinoflagellate cysts in this region. Most of the previous work dealt with the vegetative form of specific dinoflagellates (Cembella et al. 1999, 2000, Carver and Mallet 2001, Cembella et al. 2002). Furthermore, this project represents the first attempt to relate shipping traffic with the dinoflagellate cyst assemblages in ports from this region of Canada.

A few authors have worked on dinoflagellate cyst assemblages in surrounding regions of Nova Scotia, e.g. Atlantic Ocean and US coastal waters (Miller et al. 1982, de Vernal and Giroux 1991, de Vernal et al. 1997, Rochon et al. 1999, Devillers and de Vernal 2000, Pospelova et al. 2004, Pospelova et al. 2005). However, these data cannot be used for direct comparison with our results because of differences in methodology, notably the use of strong acids (HCl and HF) used in standard palynological preparations to concentrate the cysts. Calcareous cysts (e.g. *Scrippsiella trochoidea*) or delicate cysts such as those of the *Alexandrium tamarense* complex are destroyed during the process, and thus are not found in these assemblages. Furthermore, some of the studies mentioned above present their results in cysts cm^{-3} of wet sediment, which will vary according to the amount of water contained in the sediment. In the present study, potentially living dinoflagellate cysts (i.e. with cellular content) were found at all 96 stations. The dinoflagellate cyst concentrations observed in this study are highly variable and maximum values were found in the port of Halifax. We note a similarity with dinoflagellate cyst concentrations obtained for a nearby station, on the continental shelf (de Vernal et al. 1997), even though preparation methods differ and concentrations are expressed per unit of volume.

Three distinct groups of dinoflagellate cyst assemblages were observed in the ports of Nova Scotia: group 1 (Yarmouth and Liverpool), group 2 (Shelburne, Halifax and Sheet Harbour), and group 3 (Canso Strait, Little Narrows and Sydney). We examined possible reasons for the grouping of ports observed here. The sites are grouped geographically:

group 1 is located in the southern part of Nova Scotia, group 2 is in the central section and group 3 is in the northern part of Nova Scotia. Also, visual analysis of some of the collected sediment samples from the ports of Yarmouth and Liverpool indicated that they were strongly anoxic, especially around the wharfs. In Yarmouth, the sediments near the wharf contained a lot of fish waste (e.g. fish scales) coming mostly from the local fish plant, while in Liverpool, wood fibres originating from the pulp and paper industry constituted a predominant deposit at the sampling sites (Stewart and White 2001). For the second group, a common characteristic is the presence of a river discharging freshwater: the Roseway River in Shelburne, the Sackville River in Halifax and the East and West River Sheet Harbour in Sheet Harbour. Freshwater can benefit dinoflagellates either through stratification of surface waters or the input of growth-promoting humic substances. For the third group, there seems to be no obvious common environmental characteristic apart from the fact that all three ports are located in Cape Breton. Canso Strait is characterized by a fjord-type water circulation (Stewart and White 2001), the port of Little Narrows is located in Bras d'Or Lake which is characterized by limited water circulation and low salinity, while the port of Sydney has estuarine-like salinity and receives freshwater from the Sydney River (Stewart and White 2001). Some of these ports will be discussed in more details below.

The distribution of the dinoflagellate cyst assemblages in these Nova Scotia ports shows that some species are more abundant in a particular region of the province. Such is the case for the cysts of the *Alexandrium tamarense* complex, which was more abundant in the western part, and the cysts of *Scrippsiella* group, which was more abundant in the eastern part. In the case of cysts of the *Alexandrium tamarense* complex, our results correspond rather well with the locations of sites affected by paralytic shellfish poisoning (PSP) along the Canadian East coast, suggesting the presence of cells from the *Alexandrium tamarense* complex (Anderson et al. 1994, Bates 1997). One interesting difference between our results and those studies is that Bates (1997) reported that the region at the mouth of the port of Sydney was affected by PSP but we did not find any cysts of the *Alexandrium tamarense* complex in this port. Anderson et al. (1994) also reported that a region in the south of Cape Breton was affected by PSP. A study by Worms et al. (1993) has shown that the north shore of Cape Breton and the north entrance of Canso Strait were free of PSP. However, these studies looked at saxitoxin concentrations in molluscs; they did not look at the presence of cysts in sediment. It is thus possible that cysts of the *Alexandrium tamarense* complex are present at several other locations in Nova Scotia, but this has not yet been detected because no outbreaks of PSP have been reported and no dinoflagellate cysts survey have been done prior to the present one. The highest concentrations of the cysts of *Scrippsiella* group were found in the port of Sydney. The dominant species in that group is the cyst of *Scrippsiella* sp. 1. This species remains unidentified but given the high concentration of this species in one site, it should be considered as a species potentially capable of producing HAB (Subba Rao et al. 1994, Wang et al. 2007, Terenko and Terenko 2009).

Another potentially toxic species found in this study is *O. centrocarpum*. The species producing this cyst (*P. reticulatum*) is known to produce yessotoxin (YTX), a shellfish toxin recognized for its damage to the shellfish industry (Reinecke 1967, Satake et al. 1997, Eiki et al. 2005). It has been found in six of the eight ports but the highest concentrations were found in the ports of group 2 (Shelburne, Halifax and Sheet Harbour). *Operculodinium centrocarpum* is considered to be a cosmopolitan species (Edwards and Andrle 1992, Rochon et al. 1999, Marret and Zonneveld 2003). There was also another potentially toxic species found in the port of Halifax and in the ports located in Cape Breton: *Cochlodinium* sp. 1 and sp. 2. Species of the *Cochlodinium* genus have been implicated in fish kills of wild and impounded fish around the globe and it seems to be expanding its range (Gobler et al. 2008 and references therein). Very little is known about these dinoflagellate cysts. Excystment experiments of identical cysts collected in various localities along the west coast of Portugal produced *Gymnodiniales* sp. vegetative cells (A. Amorim and S. Anglès personal communication), suggesting that the cysts are not *Cochlodinium* spp. Therefore, because we are uncertain of the identity of these cysts, we have kept the name *Cochlodinium*. This may explain why there are no records of *Cochlodinium* species in other local plankton studies. For safety reasons, we have decided to classify those two taxa as potentially toxic and/or exotic. Other taxa of interest include *Echinidinium* sp. 1 and *Echinidinium* sp. 2. The description of the genus *Echinidinium* is fairly recent (Zonneveld 1997) and it has not been reported in previous studies for this region or for the Northwest Atlantic Ocean. Due to morphological similarities between the genera *Echinidinium* and *Islandinium*, it is possible that *Echinidinium* specimens may have been identified as *Islandinium* prior to the formal description of the genus by Zonneveld (1997). Indeed, *Islandinium minutum* and *Islandinium* var. *cezare* contribute up to ~22% of dinoflagellate cyst assemblages in the Cabot Strait area (GEOTOP 2011). Because of the uncertainties on the identity and distribution of these two taxa we can only classify them as potentially exotic species.

In this study we found a positive relationship between the dinoflagellate cyst concentrations and the number of ships in the ports of Nova Scotia. Furthermore, we also found a positive relationship between the volume of discharged ballast water from ships that had not undertaken a ballast water exchange and the average dinoflagellate cyst concentrations in bottom sediments; even though this was influenced by a single data point the port of Halifax. These results suggest that dinoflagellate cyst concentrations are somehow favoured by the presence of ships or that some species may originate from them. To our knowledge, this is the first time that this type of relationship has been demonstrated for dinoflagellate cysts in marine commercial ports in Canada. The volume of discharged ballast water (Table 1.1) shows that Canso Strait is the port that receives the highest volume of ballast water followed by the port of Halifax. However, the port of Halifax receives a greater volume of ballast water from ships that had not undertaken a ballast water exchange than Canso Strait and it is also the port that receives the largest number of ships. It is also in this port that the dinoflagellate cyst concentrations and species richness index were the highest. Nevertheless, other statistical analyses have shown that when the port of Halifax is taken out of the regressions there were no significant relationships between the variables. Our findings thus seem to suggest that shipping traffic has some influence on the local populations of dinoflagellate cysts found in bottom sediments of these ports. Ruiz and Carlton (2003, and references therein) suggested that an increase in propagule abundance and frequency of inoculation should increase the chances of successful invasion. Furthermore, in their study, Verling et al. (2005) suggested that propagule supply to an individual port is a complex function of ship types, ship numbers, and source regions of these ships and their associated discharge patterns. Lawrence and Cordell (2010) reported that ships that undergo trans-oceanic ballast water exchange release fewer propagules, and thus ships from domestic ports represent a greater threat because of reduced transit time and therefore less propagule mortality.

The size and speed of cargo vessels increased considerably during the past forty years. Along with the increased eutrophication of coastal water in many regions around the world, this has increased the chances of successful transfer of dinoflagellate species across oceanic boundaries (Hallegraeff and Bolch 1992). In Canada, several studies have noted the transport and the introduction of dinoflagellate species (e.g. Subba Rao et al. 1994, Gosselin et al. 1995, Carver and Mallet 2001). In a study on dinoflagellates in ballast water of ships arriving to the East Coast of Canada, Roy et al. (submitted) found more than 150 different dinoflagellate species in a total of 63 commercial ships. Approximately 30% of these species are capable of producing resistant dormant cysts and can thus possibly end up in bottom sediments of ports. Furthermore, Casas-Monroy et al. (2011) found 65 dinoflagellate cyst taxa in ballast sediments from ships (with or without ballast water exchange) arriving to the East Coast of Canada. About 70% of the dinoflagellate cysts taxa found in their study were also observed in the present study. It is possible that more species are already present in the study area but the analysis of sediment samples for dinoflagellate cysts can only prove the presence of a species, not the absence (Smayda 2007). Moreover, in an ongoing study by Weise et al. (in prep.) it appears that ballast sediments can be released during the deballasting process, suggesting a way that dinoflagellate cysts might be discharged in a receiving port.

The spatial distribution of dinoflagellate cysts within ports of Nova Scotia was quite homogeneous, which implies that future surveys for shipping-related aquatic invasive species introductions could be done with a spatially limited sampling effort. Results have shown that the distribution of the dinoflagellate cyst assemblages in a given port is at least 70% similar, with the exception of site 5 in the port of Sydney, which will be discussed later.

Several factors, such as the hydrographic conditions in the ports or biological and ecological factors, can influence the distribution of dinoflagellate cysts in the environment. As mentioned earlier, the cysts behave as fine silt particles (Dale 1976). Hence the hydrographic conditions where the cysts are formed constitute a major factor influencing their distribution (e.g. Pospelova et al. 2005). These conditions may also influence the sedimentation rate and sediment transport, which can in return also influence the cyst concentrations in the sediment. Furthermore, because the cyst formation derives from biological and ecological factors, the cysts distribution will also depend on the spatial distribution of the motile form. The latter is in turn influenced by biotic and abiotic factors which differ according to the groups of dinoflagellates: heterotrophic, autotrophic or mixotrophic (Jacobson and Anderson 1994, Dale 1996, Pospelova et al. 2005). For example, the distribution of heterotrophic dinoflagellates will depend on the availability of prey (diatoms, ciliates and small flagellates) while the distribution of autotrophic dinoflagellates relies upon the availability of dissolved nutrients (Taylor and Pollingher 1987, Pospelova et al. 2005).

Autotrophic dinoflagellate cysts dominated the assemblage at all six sites in the port of Shelburne. The sampling sites were located between the mouth of the Roseway River and a salmon fish farm. Hence, the nutrients coming from those two sources, along with water stratification induced by a layer of low salinity water could explain the dominance of autotrophic dinoflagellates (Dale et al. 1999). A potential explanation for the relatively high abundance of the cysts of the *Alexandrium tamarense* complex in this port could be that cysts of the species were transported away from the bloom that occurred nearby in June 2000 (Cembella et al. 2002).

In the port of Halifax, the autotrophic: heterotrophic ratio was ~1. However, the highest concentrations of cysts in this port are mainly due to the contribution of the autotrophic *Alexandrium tamarense* complex. Possible explanations for this ratio can include, for instance, high local abundances of dinoflagellates and/or a high rate of encystment (Pospelova et al. 2005). Contamination of the water and sediment from sewage wastes (Stewart and White 2001) may provide enough nutrients to sustain a high rate of autotrophic dinoflagellate productivity. Also, the Bedford Basin receives the outflow of the Sackville River, which likely favours the sustained growth of *Alexandrium tamarense* complex through the input of humic substances (Granéli and Moreira 1990) and through increased stratification (Margalef et al. 1979, Stewart and White 2001). Also, as seen earlier, this study indicates that shipping traffic influences dinoflagellate cyst concentrations and it this thus possible that the dinoflagellate cyst concentrations observed in this study are influenced by both shipping traffic and natural environment factors.

The port of Sheet Harbour was dominated by two species: *Bitectatodinium tepikiense* and the *Brigantedinium* group. *Bitectatodinium tepikiense* (= *Gonyaulax digitale*; autotrophic taxon) is known to be a common species in silled basins along the eastern and southeastern shores of Nova Scotia (Miller et al. 1982). Marret and Zonneveld (2003) noted that *Brigantedinium* spp. (= *Protoperidinium* spp.) can dominate the cyst assemblages in most environments and were not restricted to any specific environmental conditions (e.g. temperature, salinity, phosphate and nitrate concentrations), other than the presence of their prey. Indeed, *Brigantedinium* spp. is known to produce a vegetative dinoflagellate form that is heterotrophic: *Protoperidinium* spp. (e.g. *B. cariacoense* = *P. avellanum*; *B. simplex* = *P. conicoides*), for which the distribution is affected by the availability of their preferred prey (diatoms, ciliates and small flagellates) (Dale 1996).

Out of the four ports analyzed to evaluate the spatial distribution of dinoflagellate cyst assemblages within a port, the port of Sydney was the one showing the least homogeneity. Site 5 contributed mainly to the differences between the sites. This site is located a few kilometres from the other sites, at the confluence of the two arms of the port of Sydney while the rest of the sites are located in the south arm. Also, site 5 is situated further from the shore than the other sites; it can thus be influenced by different hydrographic conditions. The south arm of the port has an estuarine two-layer circulation with a surface layer of low-salinity water that flows toward the sea and a bottom layer of more-saline water coming from the sea. Waters in the north arm are well mixed horizontally and vertically (Stewart and White 2001). Furthermore, as Weise et al. (2002, and references therein) pointed out, a combination of factors, such as favourable temperature and salinity conditions, the supply of humic substances and the increase of water column stability can improve cell growth. Hence the lower dinoflagellate cyst concentrations and species richness index of site 5 may be explained by the fact that this site is located in a region less suitable for dinoflagellate growth.

1.2.6. Conclusion

This study constitutes the first attempt to link the dinoflagellate cyst assemblages in surface sediments with the shipping traffic of ports from Nova Scotia, eastern Canada. We identified a total of 53 dinoflagellate cysts taxa, two of which are known to be toxic species: *Alexandrium* spp. and *Operculodinium centrocarpum* (= *Protoceratium reticulatum*), and possibly 3 if we include *Cochlodinium* spp. The number of dinoflagellate cyst taxa found in each port ranged from 14 to 40 while the average dinoflagellate cyst concentrations ranged from 1346 to 83 351 cysts g^{-1} dry sed. These concentrations are relatively high when compared with other studies on dinoflagellate cysts in surface sediments (e.g. Holzwarth et al. 2007, Radi et al. 2007, Krepakevich and Pospelova 2010, Pospelova and Kim 2010, Rubino et al. 2010). The distribution of dinoflagellate cysts was quite homogenous within each port, suggesting that fewer samples of sediments might be required for future studies. Dinoflagellate cyst concentrations were positively correlated with the number of ships and the volume of ballast water discharged from non-exchanged ships, suggesting that ships (mainly domestic ones) may play a role in the transportation and seeding of dinoflagellate cysts in receiving ports. It is likely that the dinoflagellate cyst populations found in this study constitute a combination of indigenous and introduced species. Monitoring the dinoflagellate cysts population in sediments of ports (particularly those with intensive shipping activity) should occur on a regular basis to ensure that new species of potentially harmful dinoflagellate not being introduced. Some form of regulation on the discharge of non-exchanged ballast water should be applied (or ballast water should be treated before discharge) since it has been shown that this category of ships can pose a significant threat to local dinoflagellate cyst populations by contributing to the increase of toxic species concentrations, or by introducing new HAB species to the environment.

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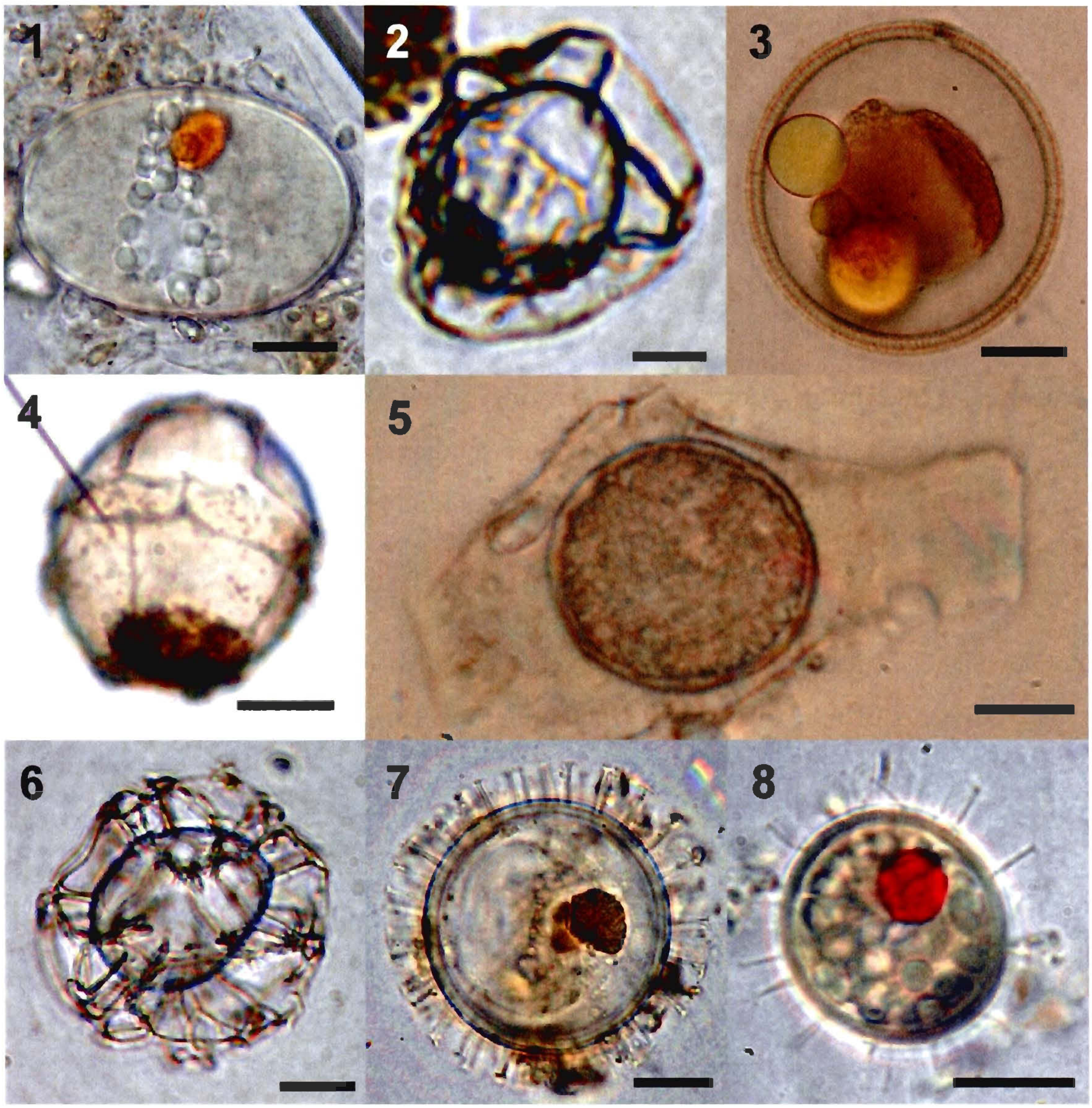
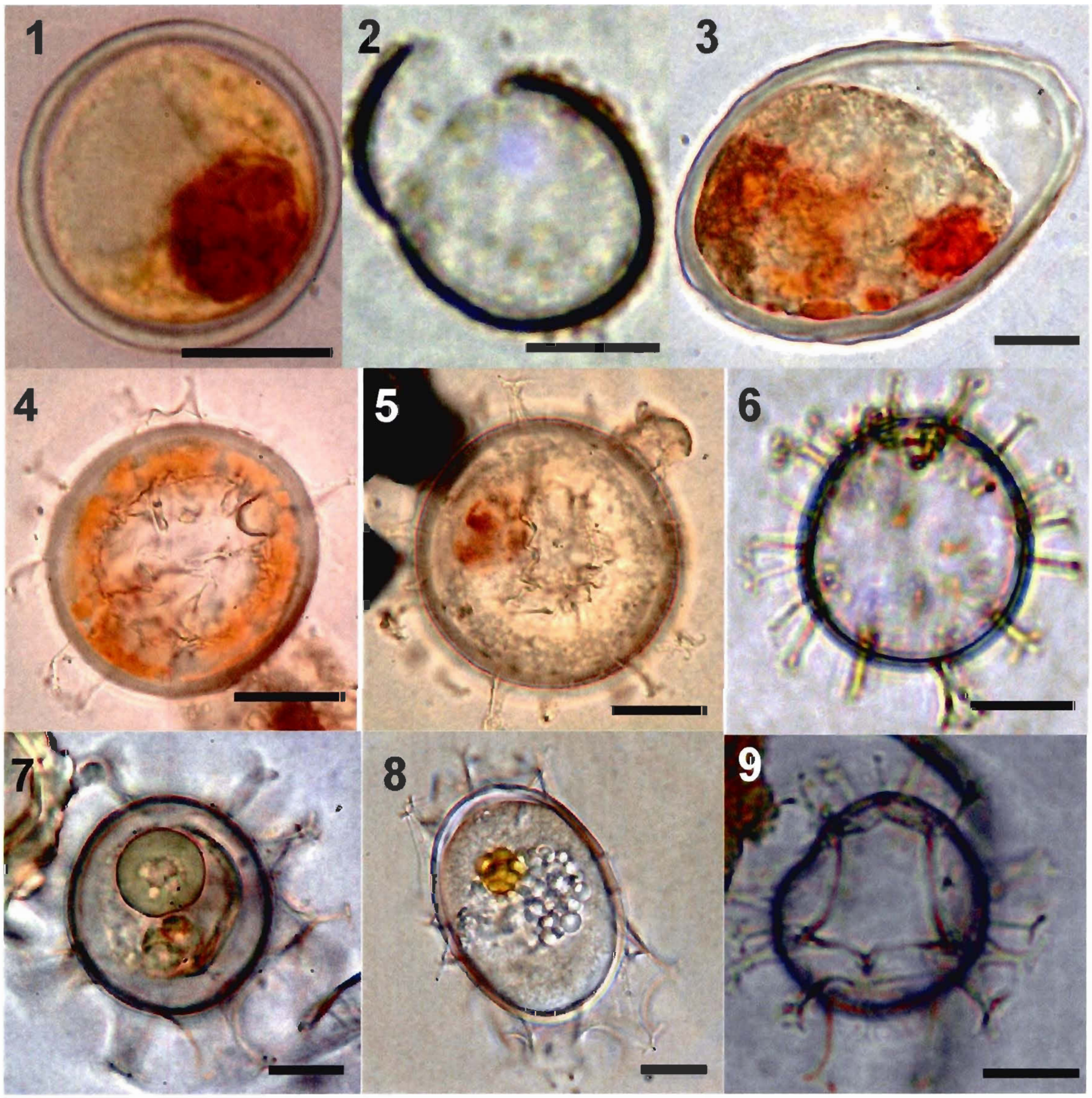
APPENDIX I



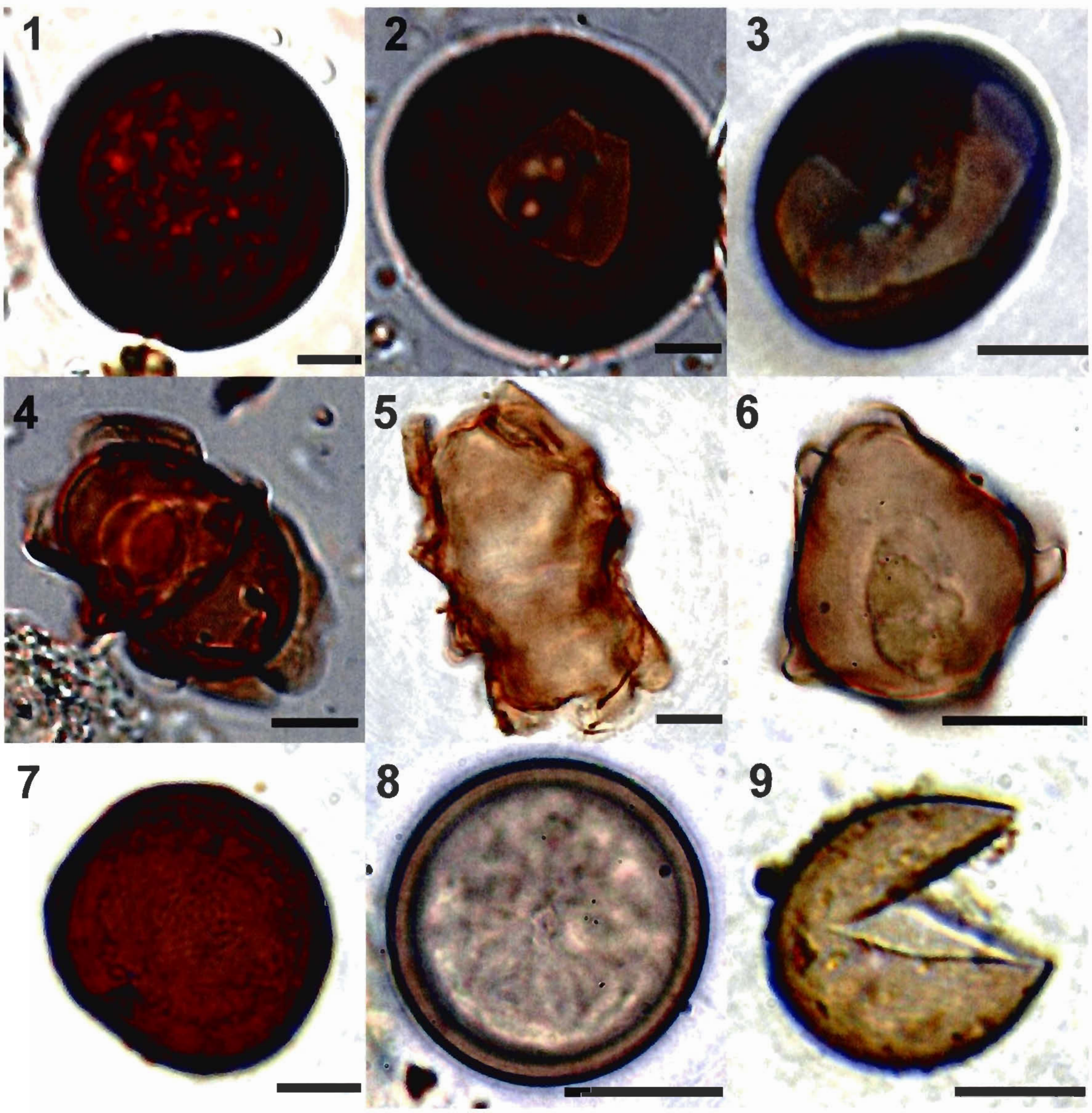
1) Cyst of the *Alexandrium tamarense* species complex, 2) *Ataxiodinium choane*, 3) *Bitectatodinium tepikiense*, 4) *Impagidinium* spp., 5) cf *Gymnodinium impudicum*, 6) *Nematosphaeropsis labyrinthus*, 7) *Operculodinium centrocarpum*, 8) cyst of *Pentapharsodinium dalei.* Scale bar = 10 µm.

APPENDIX II



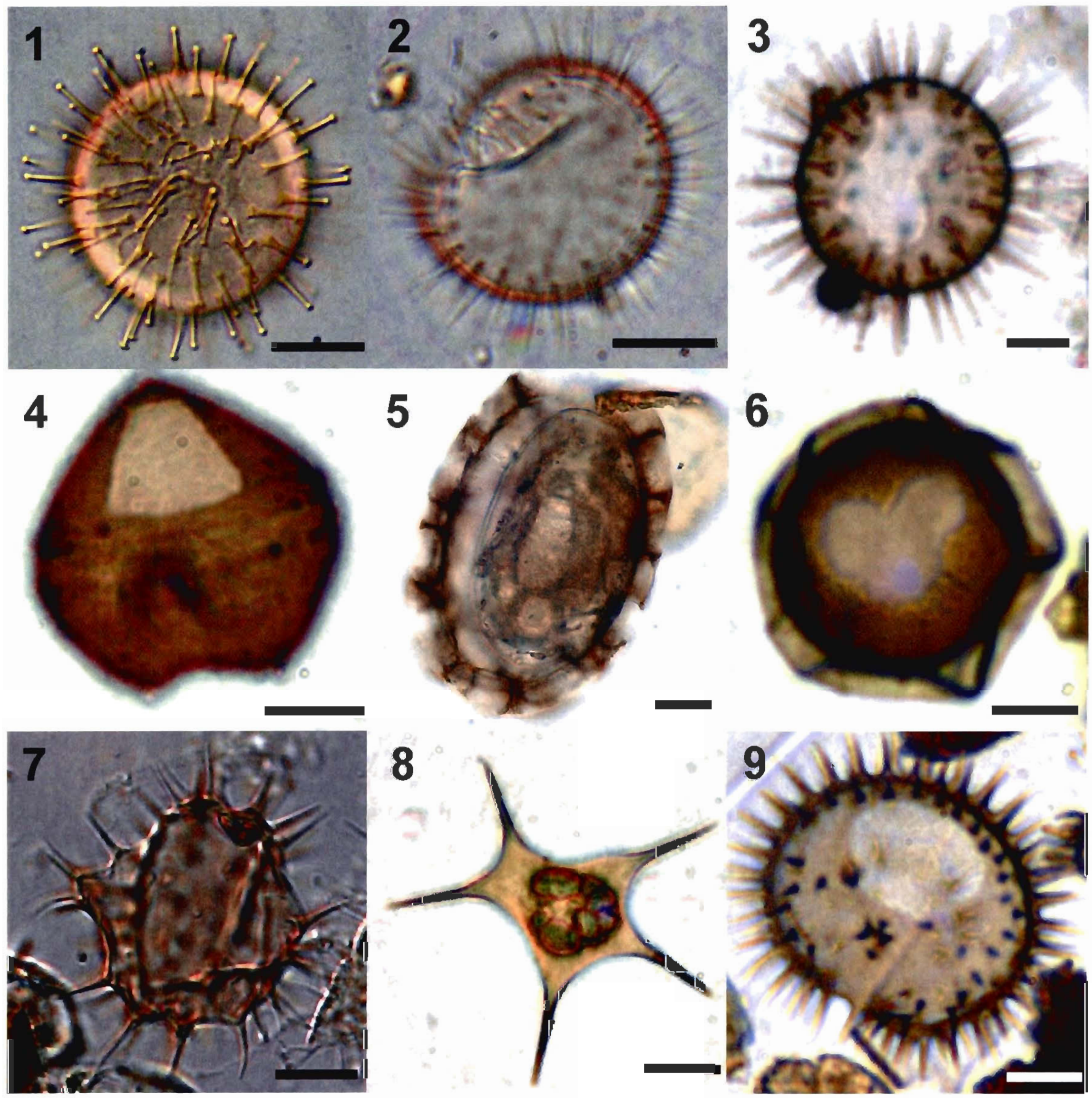
1) Cyst of *Scrippsiella* sp. 1 (with cellular content), 2) cyst of *Scrippsiella* sp. 1 (without cellular content), 3) cyst of *Scrippsiella lachrymosa*, 4) *Spiniferites* spp., 5) *Spiniferites* spp., 6) *Spiniferites bulloideus*, 7) *Spiniferites delicatus*, 8) *Spiniferites frigidus*, 9) *Spiniferites ramosus*. Scale bar = 10 μm.

APPENDIX III



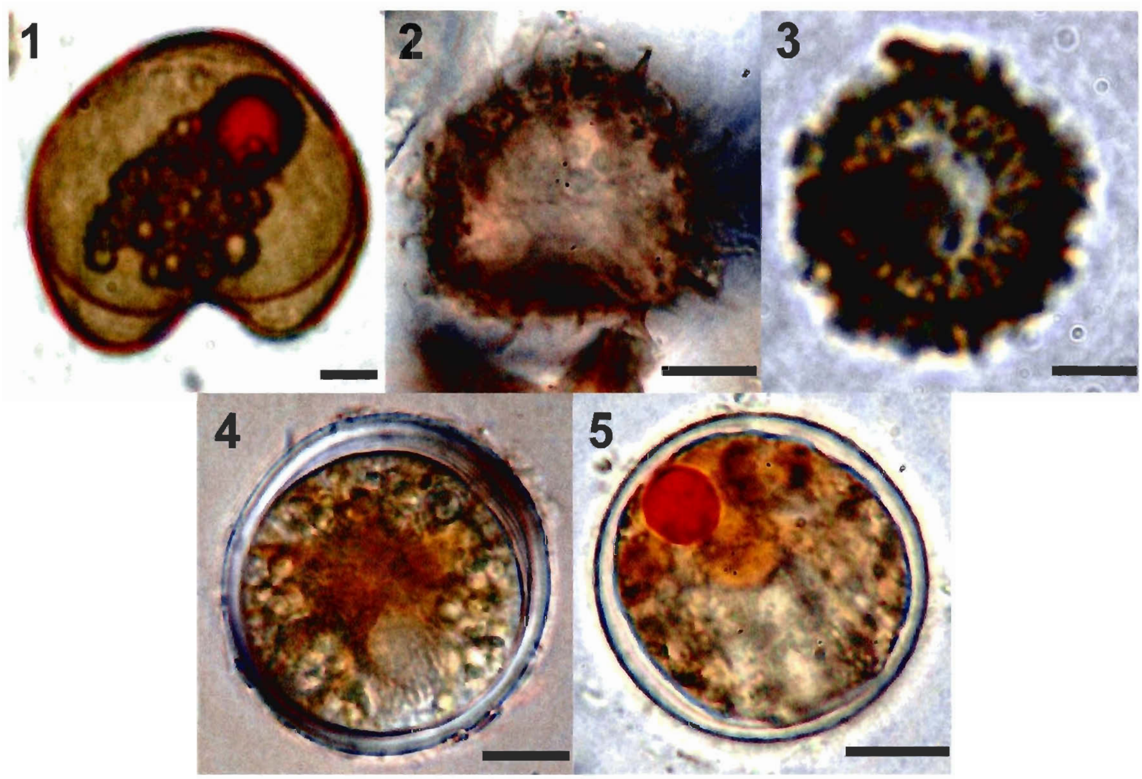
1) *Brigantedinium* spp., 2) *Brigantedinium simplex*, 3) *Brigantedinium irregulare*, 4) *Cochlodinium* sp. 1 (with cellular content), 5) *Cochlodinium* sp. 1 (without cellular content), 6) *Cochlodinium* sp. 2, 7) *Dubridinium lenticulatum*, 8) *Dubridinium* sp. 1 (with cellular content), 9) *Dubridinium* sp. 1 (without cellular content). Scale bar = 10 μm.

APPENDIX IV



1) *Echinidinium* sp. 1, 2) *Echinidinium* sp. 2, 3) *Islandinium minutum*, 4) *Lejeunecysta* cf. *sabrina*, 5) cyst of *Polykrikos kofoidii*, 6) cyst of *Protoperidinium americanum*, 7) cyst of *Protoperidinium nudum*, 8) cyst of *Protoperidinium stellatum*, 9) *Selenopemphix quanta*. Scale bar = 10 μm.

APPENDIX V



1) *Votadinium calvum,* 2) unidentified cyst A, 3) unidentified cyst B, 4) unidentified cyst C, 5) unidentified cyst D. Scale bar = 10 µm.

APPENDIX VI. Complete list of the dinoflagellate cyst species found in Nova Scotia, Canada.

Cyst name (= motile cell name)	Cyst name (= motile cell name)
Cyst of *Alexandrium tamarense* species complex	*Spiniferites elongatus* (=*Gonyaulax elongata*)
Ataxiodinium choane (=*Gonyaulax* sp.)	*Spiniferites frigidus* (=*Gonyaulax* sp.)
Bitectatodinium tepikiense (=*Gonyaulax digitale*)	*Spiniferites hyperacanthus* (=*Gonyaulax* sp.)
Brigantedinium spp. (=*Protoperidinium* spp.)	*Spiniferites membranaceus* (=*Gonyaulax* sp.)
Brigantedinium cariacoense (=*Protoperidinium avellanum*)	*Spiniferites mirabilis* (=*Gonyaulax spinifera*)
Brigantedinium irregulare (=*Protoperidinium denticulatum*)	*Spiniferites ramosus* (=*Gonyaulax* sp.)
Brigantedinium simplex (=*Protoperidinium conicoides*)	*Votadinium calvum* (=*Protoperidinium oblongum*)
Cyst of *Cochlodinium* sp. 1	*Votadinium spinosum* (=*Protoperidinum claudicans*)
Cyst of *Cochlodinium* sp. 2	unidentified cyst A
Dubridinium lenticulatum (=*Diplopsalis* sp.)	unidentified cyst B
Dubridinium sp. 1 (=*Diplopsalis* sp. 1)	unidentified cyst C
Echinidinium aculeatum	unidentified cyst D
Echinidinium delicatum	unidentified cyst E
Impagidinium spp. (=*Gonyaulax* spp.)	unidentified cyst F
cf. *Impagidinium patulum* (=*Gonyaulax* sp.)	unidentified cyst G
Impagidinium strialatum (=*Gonyaulax* sp.)	
Islandinium minutum (=*Protoperidinium* sp.)	
cf. *Gymnodinium impudicum* (=*Gyrodinium impudicum*)	
Lejeunecysta cf. *sabrina* (=?*Protoperidinium leone*)	
Nematosphaeropsis labyrinthus (=*Gonyaulax spinifera*)	
Operculodinium centrocarpum (=*Protoceratium reticulatum*)	
Cyst of *Pentapharsodinium dalei*	
Cyst of *Polykrikos kofoidii*	
Cyst of *Protoperidinium americanum*	
Cyst of *Protoperidinium nudum*	
Cyst of *Protoperidinium stellatum*	
Quinquecuspis concreta (=?*Protoperidinium leone*)	
Cyst of *Scrippsiella* spp.	
Cyst of *Scrippsiella* sp. 1	
Cyst of *Scrippsiella lachrymosa*	
Cyst of *Scrippsiella* cf. *regalis*	
Cyst of *Scrippsiella trochoidea*	
Selenopemphix nephroides (=*Protoperidinium subinerme*)	
Selenopemphix quanta (=*Protoperidinium conicum*)	
Spiniferites spp. (=*Gonyaulax* spp.)	
Spiniferites bulloideus (=*Gonyaulax scrippsae*)	
Spiniferites delicatus (=*Gonyaulax* sp.)	

APPENDIX VII. Values of SST, SSS, nutrients and sea-ice cover for the Scotian Shelf for the year 2007.

Values of SST, SSS and nutrients for the Scotian Shelf for the year 2007.

	SST (°C)	SSS (psu)	Phosphate (mmol m^{-3})	Nitrite-Nitrate (mmol m^{-3})
Winter	0	30.9	0.8	5.5
Spring	3	31.3	0.4	0.7
Summer	17	30.8	0.6	3.5
Fall	13	30.6	0.4	1.15

Sea-ice cover of the Scotian Shelf. Average for the years 1971 to 2000.

	1971-2000 Mean (Km^2)
January	1600
Febuary	14000
March	18000
April	5800

CHAPITRE 2
CONCENTRATION ÉLEVÉE DE KYSTES DU DINOFLAGELLÉ POTENTIELLEMENT TOXIQUE *ALEXANDRIUM TAMARENSE* DANS LE BASSIN DE BEDFORD, HALIFAX, NOUVELLE-ÉCOSSE, CANADA

2.1 RÉSUMÉ EN FRANÇAIS DU DEUXIÈME ARTICLE

Une concentration élevée de kystes du dinoflagellé potentiellement toxique *Alexandrium tamarense* a été découverte dans les sédiments du port d'Halifax en Nouvelle-Écosse, Canada. Les concentrations moyennes pour cette espèce varient entre 4033 ± 2647 et 220 872 ± 148 086 kystes g^{-1} de sédiment sec pour les six sites considérés et les concentrations les plus élevées sont retrouvées près des terminaux de navires commerciaux dans le bassin Bedford. Cette espèce est considérée comme étant endémique à la région d'étude, mais nos travaux suggèrent qu'une quantité significative de kystes dans le bassin Bedford ait pu être introduite suite au processus de déballastage d'eau et de sédiments de ballast.

2.2 High cyst concentrations of the potentially toxic dinoflagellate *Alexandrium tamarense* species complex in Bedford Basin, Halifax, Nova Scotia, Canada

Manuscrit en préparation pour une publication

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2.2.3. Abstract

We report a large cyst bed of the potentially toxic and bloom-forming dinoflagellate *Alexandrium tamarense* species complex in bottom sediments from the port of Halifax, Nova Scotia, Canada. The average cyst concentrations of that species ranged from 4033 ± 2647 and 220 872 ± 148 086 cysts g^{-1} of dry sediments and the highest concentrations were found near ship terminals in Bedford Basin. Although this species is endemic to this region, our work strongly suggests that some of the cysts of *A. tamarense* species complex found in the port of Halifax were introduced through discharged ballast water and sediments.

2.2.4. Introduction

Dinoflagellates are the second largest group of phytoplankton in marine environments in terms of species diversity and abundance, and it is estimated that there are approximately 1500 species of free-living marine dinoflagellates around the world (Sournia et al. 1991, Gomez 2005). Some dinoflagellates are known to produce cysts at different stages of their life cycle, but only about 15% of them will produce a resistant dormant cyst (Head 1996), called a hypnozygote, as part of their life cycle (Dale 1983). One of the roles played by the cyst is to help the cells survive during unfavourable conditions, but they can also serve as seeds for the initiation of blooms (Dale 1983). Over 100 species of dinoflagellates are capable of forming harmful algal blooms (HAB), 50 of which are known to be toxic (Sournia 1995).

Dinoflagellate cysts range in size from 10 to 100 μm, and thus they behave as fine silt particles in the natural environment (Dale 1976, Dale et al. 1978). Therefore, they can be easily transported by surface and/or bottom currents. Other vectors of transport of live cysts include dredging activities in ports (Anderson and Wall 1978, Dale 1986) and commercial shipping traffic with the discharge of ballast water and sediments (Dale 1986, Carlton and Geller 1993, Hallegraeff 1998, Lavoie et al. 1999, Niimi 2004). Sediments can be found in relatively large quantities in ballast tanks when ballast water is being pumped from shallow areas at low tide or when sediments are suspended in the water column during stormy conditions (Hallegraeff and Bolch, 1992). Hence, dinoflagellate cysts can be transferred along with sediment during ballast uptake, or they can be produced directly in the ballast tanks by dinoflagellates present in the water. Indeed, in a study by Hallegraeff and Bolch (1992), the authors observed the presence of more than 300 million cysts of toxic *Gymnodinium catenatum* and *Alexandrium* spp. in one single ballast tank of a ship under investigation. This ship had taken ballast water during a bloom of toxic dinoflagellates, resulting in the transport and potential transfer of millions of viable toxic cysts to the destination port.

Alexandrium tamarense, Alexandrium fundyense and *Alexandrium catenella* are morphologically similar species of toxic dinoflagellates (considered as a "species complex": Balech 1985) that produce morphologically identical cysts (Turgeon et al. 1990, Cembella 1998, Kennaway and Lewis 2004, Head et al. 2006) as part of their life cycle. The toxins they produce (e.g. saxitoxins and neosaxitoxins) are neurotoxin well known for causing paralytic shellfish poisoning (PSP), which is a syndrome responsible for human illness and even death following the consumption of contaminated shellfish (Anderson et al. 1994, Ciminiello et al. 2000). *Alexandrium tamarense* is endemic to the eastern part of Canada and north-east USA coastal waters but it is also present in other parts of the world, in temperate waters of both northern and southern hemispheres (Taylor et al. 2008). Cysts of cf. *Alexandrium tamarense* have been reported from several of these environments, including the coasts of Sweden (Person et al. 2000), Spain (Blanco 1995), North America (Anderson et al. 1994), and Tasmania and Southern Australia (Bolch and Hallegraeff 1990). Although this species is known for causing PSP, its first description by Lebour in 1925 referred to a species found in the Tamar Estuary, which appeared to be non-toxic (Balech, 1995). Non-toxic strains of this species have also been found in Australia, the Gulf of Thailand, and Northern Europe, but most of the North American and Japanese strains are exclusively toxic (Hallegraeff et al. 1991 and references therein, Hallegraeff 1993, Lilly et al. 2007).

The present study is part of the port survey included in the Canadian Aquatic Invasive Species Network (CAISN) program. It complements another study on the distribution of dinoflagellate cyst assemblages by the same authors (Lacasse et al. in prep.). The work presented here reports the discovery of a large cyst bed of *Alexandrium* spp. in bottom sediments from Bedford Basin in the port of Halifax, Nova Scotia, Canada, and the possible implications for shipping in this port.

2.2.5. Material and methods

Sampling was done in July 2008. Sediment samples were randomly collected in triplicate at six stations in Halifax harbour (n = 18) (Figure 2.1) using an Ekman bottom grab sampler (12.5 x 12.5 x 22 cm). In order to collect the most recent cysts we collected only the sediment within the oxic layer (when present), i.e. approximately the top 2 cm layer or less, which represents roughly the past 20 years based on a sediment accumulation rate of 1 cm per 10 years for Bedford Basin, Halifax, N.S. (Miller et al. 1982). Samples were refrigerated (4°C) and kept in the dark until processed in the laboratory (within 12 months).

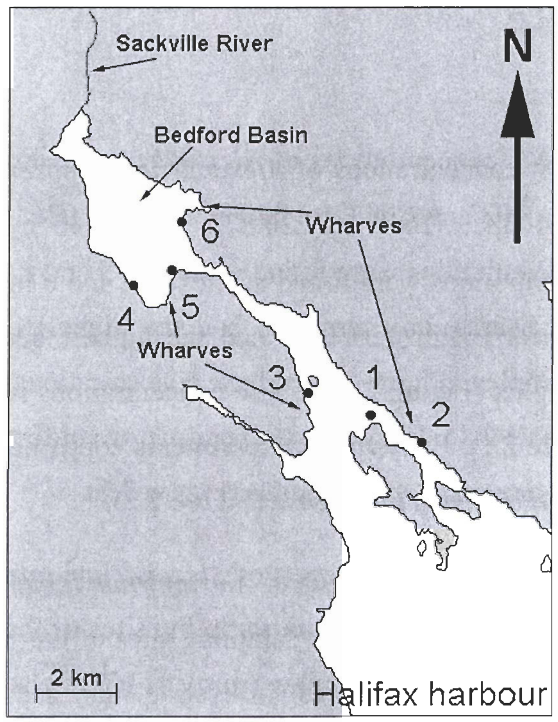

Figure 2.1. Location of the six sampling sites randomly selected from the port of Halifax, Nova Scotia, Canada.

Whole sediment samples were first homogenized by gently stirring the sediment in the bag with a spatula. A volume of 1 cm^3 of sediment was then collected by displacement of an equal volume of sea water in a graduated cylinder. Samples were sieved onto 100 and 20 µm Nytex membranes with filtered seawater (0.45 µm) in order to retain the fraction between 20-100 µm. This fraction was observed under an inverted microscope (Nikon Eclipse TE2000-U) at 100X magnification and all the cysts present were counted.

Identification was made under 400X magnification with the help of several references from the literature (Rochon et al. 1999, Matsuoka and Fukuyo 2000, Head et al. 2001, Marret and Zonneveld 2003) or with the use of a scanning electron microscope (SEM). When possible, the cysts were identified to the species level. Unknown cysts were isolated and incubated in multi-well plates (COSTAR®3524, Corning Incorporated) with f/2 medium without silica (Guillard and Ryther 1962, Guillard 1975) to identify the vegetative form to the species level if cyst germination occurred. See Lacasse et al. (in prep.) for more details.

2.2.6. Results

The average cyst concentrations of *Alexandrium tamarense* complex in the port of Halifax ranged from 4033 ± 2647 to 220 872 ± 148 086 cysts g^{-1} of dry sediments (Table 2.1). High concentrations were found at sites 4, 5 and 6 in Bedford Basin. However, site 6, a site located near a ship terminal, had the highest concentration of cysts of *A. tamarense* species complex (Figure 2.1). These concentrations represent >50% of all cysts found in this port (Table 2.1). Excystment experiments confirmed that some of these cysts were indeed cysts of *Alexandrium tamarense* (Figure 2.2).

Table 2.1. Average concentrations of cysts of *Alexandrium tamarense* species complex and of all dinoflagellate cysts found in the six sampling sites of the port of Halifax. Values are given as average of triplicate subsamples (in cysts g^{-1} dry sed.) with standard deviation (SD) and the relative abundance (%) of *Alexandrium tamarense* species complex.

Site	Cysts of *A. tamarense* conc. ± SD	All dino. cyst conc. ± SD	Rel. abund. of *A. tamarense*
1	12 944 ± 10 803	25 279 ± 21 700	51%
2	4033 ± 2647	11 131 ± 5010	36%
3	17 521 ± 7293	29 435 ± 8649	60%
4	58 263 ± 22 342	113 377 ± 35 540	51%
5	34 116 ± 18 934	64 168 ± 31 360	53%
6	220 872 ± 148 086	256 715 ± 172 838	86%

2.2.7. Discussion

This study is the first to document the occurrence of cysts of the *Alexandrium tamarense* complex in Bedford Basin in the port of Halifax. Here we explore possible explanations for the presence of such a large quantity of *Alexandrium* cysts in the basin. Although this species has been known to produce recurrent blooms in the port of Halifax during the months of May and June every year for at least the past 15 years (Claire Carver pers. communication), we could not find any previously published information regarding these blooms (likely not major toxic events) nor on the presence and concentrations of cysts of this potentially toxic species in the port of Halifax. There is no available information from palynological surveys because cysts of *Alexandrium* have a relatively low potential for geological preservation (Head 1996).

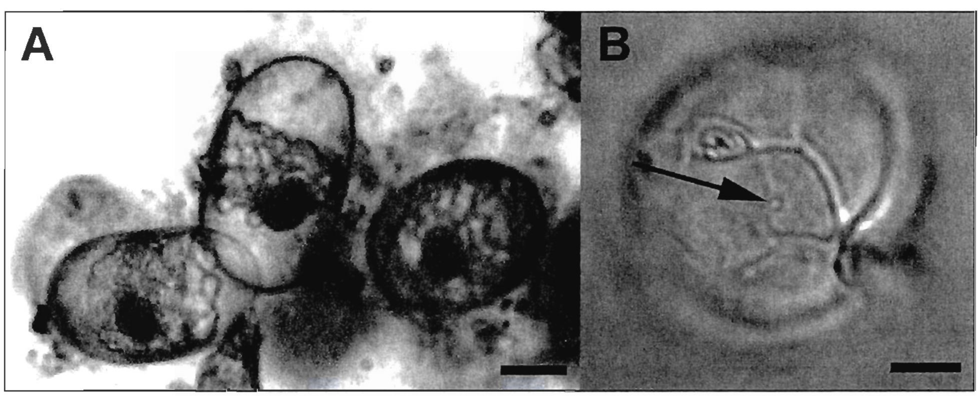

Figure 2.2. A) Cysts of the *Alexandrium tamarense* species complex; B) vegetative cell of *Alexandrium tamarense*. Scale bar = 10 µm. Arrow pointing to the pore located on the suture of plate 1'.

Given the high concentrations of the cysts of the *Alexandrium tamarense* species complex found here and since no significant blooms of this species have been reported in the past few years, it is possible that cysts have been introduced through ballast water discharged in the port of Halifax. Indeed, several studies have shown the presence and the subsequent transport of dinoflagellate cysts and vegetative forms in ballast tanks (e.g. Hallegraeff and Bolch 1992, Hamer and Collin 2000, Hallegraeff and Gollasch 2006). Locally, three studies have examined ballast water introductions: Carver and Mallet (2001) found vegetative cells of *Alexandrium* spp. in ballast tanks of ships visiting ports of Nova Scotia, such as the port of Halifax. In the CAISN program, Casas-Monroy et al. (2011) identified over 65 dinoflagellate cysts taxa, including cysts of *Alexandrium*, in sediments of ballast tanks from commercial ships on the Canadian East coast, and Roy et al. (submitted) found living cells of the *Alexandrium tamarense* complex in ballast water of ships visiting the port of Halifax. Furthermore, Lacasse et al. (in prep) found a positive relationship between the volume of discharged ballast water from ships that had not undertaken a ballast water exchange and the average dinoflagellate cyst concentrations in bottom sediments. These ships are thus more susceptible of transporting viable dinoflagellate cyst that can be discharged along with ballast water and sediments. Indeed, most of these ships come from regions located in the northeast USA, where harmful algal blooms already occur. The port of Halifax receives annually over 1000 ships and over half (>700 000 metric tons) of the ballast water discharged in this port comes from ships that had not undertaken a ballast water exchange during their trip. The largest concentrations of cysts were found near important ship terminals in Bedford Basin (stations 4 to 6), suggesting that the cysts might come from ballast water and sediments being discharged in the port, or in proximity to the sewage discharge sites. From conversations with ship officials, ballast discharge is frequently initiated as ships enter the port of Halifax (or before) so that it is nearly complete when ships reach the terminals. This procedure may favour the discharge of greater concentrations of cysts near the terminals, since more ballast sediment were discharged near the end of deballasting (Weise et al. in prep.).

Coastal embayment, such as Bedford Basin, often plays an important role as accumulation sites for cyst-forming species, allowing them to expand geographically (Anglès et al. 2010). Bedford Basin is a 70 m deep and roughly circular basin located at the upstream end of the port of Halifax. It is separated from the rest of the port by a narrow channel (the Narrows, 0.4 km) with 20 m shallow water at its inner end (Stewart and White, 2001). Water circulation in the port of Halifax is generally a three layer circulation: a freshwater flow from the Sackville River at the surface, a return flow at mid-depth, and a deep near-bottom layer in Bedford Basin with reduced circulation (Stewart and White, 2001). Weakest currents are found in Bedford Basin (where high cyst concentration stations are found: stations 4, 5 and 6) while the strongest occur in the Narrows where surface outflow is from 1.6 to 2.4 cm s^{-1} and incoming bottom currents from 0 to 6 cm s^{-1} (Petrie and Yeats, 1990). These hydrographic conditions likely help to retain the cysts in Bedford Basin and, eventually promote the formation of a potentially toxic bloom of the *Alexandrium tamarense* complex. Furthermore, the presence of the Sackville River at the north-western tip of the Basin (Figure 2.1) may also contribute to create favourable conditions for growth of this species, through freshwater runoff (e.g. Giaccobe et al. 1996, Weise et al. 2002), increased water column stability (Margalef et al. 1979) and humic substances (Granéli and Moreira 1990). Also, the contamination of the water and sediments due to long term sewage waste pollution in Bedford Basin (Stewart and White 2001) has created anaerobic conditions in the sediment, which are ideal for the preservation of dinoflagellate cysts (Dale et al. 1999). However, the fact that no significant bloom of *Alexandrium tamarense* has been reported, despite the favourable conditions found in Bedford Basin, suggests that a large quantity of these cysts may have been introduced through discharged ballast water and sediments.

2.2.8. Conclusion

This study highlights the presence of large quantities of dinoflagellate cysts from the potentially toxic *Alexandrium tamarense* species complex in the bottom sediments of the port of Halifax. The highest concentrations of *Alexandrium* cysts were found in Bedford Basin, at stations located near two important ship terminals. The large volume of discharged ballast water in this port, notably from domestic ships that have not undertaken ballast water exchange, may contribute to the high cyst concentrations seen here. The environmental characteristics of Bedford Basin may favour the retention and growth of this species, although large toxic blooms have not been reported from this environment. The presence of important cyst beds of the *Alexandrium tamarense* species complex in the port of Halifax is also a concern for the uptake of these toxic cells when ships upload ballast water in their tanks, which can result in the exportation of toxic strains of *Alexandrium* spp. to other regions of the world. This can also be a concern when no cells are found in the water column, since cysts can remain viable for months, or even years (Anderson et al. 2004) and they can be uploaded in ballast tanks along with sediment, particularly during storms or windy conditions. Further studies should monitor this port more closely for toxic algal species, especially if increases in harmful algal blooms occur in the northeast USA regions since much of the domestic traffic originates from this region.

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CHAPITRE 3
CONCLUSION GÉNÉRALE

Cette étude avait pour principal objectif d'identifier les assemblages de kystes de dinoflagellés retrouvés dans les différents ports de la Nouvelle-Écosse et de déterminer si le trafic maritime influence la composition et les concentrations de kystes de ces assemblages. Ainsi, ce travail établit les bases relatives à la composition et les concentrations des assemblages de kystes retrouvés dans huit ports de la Nouvelle-Écosse. De plus, cette étude a démontré pour la première fois que le trafic maritime semble exercer une influence visible sur les assemblages des kystes de dinoflagellés de la Nouvelle-Écosse. Cette relation positive a été observée entre les navires n'ayant pas effectué d'échange d'eau de ballast lors de leur voyage et leur effet sur les concentrations de kystes de dinoflagellés dans les sédiments des ports.

Bien qu'il existe des ouvrages portant sur la distribution des assemblages de kystes de dinoflagellés dans la région du Nord-Est Atlantique (e.g. Miller et al. 1982, de Vernal et al. 1997, Rochon et al. 1999, Devillers et de Vernal 2000), aucun ne portait sur les zones portuaires de la région spécifique à l'étude. De plus, il n'était pas possible de comparer les résultats obtenus ici avec ceux publié dans le passé, puisque les méthodes de traitement du sédiment employées pour ces travaux détruisent les kystes délicats tels les kystes d'*Alexandrium* spp. ou les kystes composés de carbonate de calcium, tels ceux du genre *Scrippsiella*.

Cette étude a permis de mettre en évidence que les assemblages de kystes de dinoflagellés dans les zones portuaires de la Nouvelle-Écosse sont soumis aux conditions environnementales retrouvées dans ces ports, mais subissent également l'influence du trafic maritime. En effet, trois groupes d'assemblages ont pu être observés et des ressemblances propres à chacun des groupes ont été identifiées. Le premier groupe, constitué des ports de Yarmouth et Liverpool, est situé dans la partie Ouest de la Nouvelle-Écosse. Ces deux ports semblent tous deux soumis à des pollutions d'origine anthropique, que ce soit suite au rejet de déchets de poissons à Yarmouth ou de fibres de bois à Liverpool, sources de pollution qui causent vraisemblablement l'anoxie dans les sédiments. Les ports du second groupe (Shelburne, Halifax et Sheet Harbour) ont en commun la présence de rivières qui créent un environnement propice à la croissance des dinoflagellés notamment grâce à l'apport de substances humiques et à une couche d'eau de surface plus douce. Finalement le troisième groupe est composé des ports situés au Cape Breton. La situation géographique de ces ports semble être le point commun qui les unit. De plus, ces trois groupes étaient caractérisés par la présence d'espèces dominantes propres à chacun. L'assemblage de kystes de dinoflagellés du groupe 1 était dominé par un seul groupe d'espèces : le regroupement *Brigrantedinium* (i.e. *Brigantedinium* spp., *B. cariacoense, B. irregulare* et *B. simplex*). L'assemblage du groupe 2 était, quant à lui, dominé par plusieurs espèces : le regroupement *Brigantedinium*, le complexe d'espèces *Alexandrium tamarense*, *Bitectatodinium tepikiense* et le regroupement *Spiniferites* (i.e. *Spiniferites* spp., *S bulloideus, S. delicatus, S. elongatus, S. frigidus, S. hyperacanthus, S. membranaceus, S. mirabilis* et *S. ramosus*). Finalement, l'assemblage du groupe 3 était également dominé par plusieurs espèces : le regroupement *Brigantedinium*, le regroupement *Scrippsiella* (i.e. *Scrippsiella* spp., *Scrippsiella* sp. 1, *S. lachrymosa, S.* cf. *regalis* et *S. trochoidea*), le regroupement des kystes non identifiés (i.e. unidentified cysts A, C, D et G) et *B. tepikiense*.

Tel que mentionné précédemment, cette étude a permis de mettre en évidence l'influence du trafic maritime sur les assemblages de kystes. Toutefois, la disponibilité réduite des données relatives au trafic maritime, i.e. le nombre de navires et le volume d'eau déversé qui étaient disponibles pour deux années seulement, a rendu l'interprétation de cette relation difficile. Il serait intéressant, lors de prochaines recherches, de refaire l'analyse mais avec une base de données plus considérable afin de valider l'influence du trafic maritime. La provenance des navires pourrait également permettre d'identifier les régions sources des espèces retrouvées dans nos échantillons.

Néanmoins, la relation positive a seulement été observée pour le port d'Halifax. C'est dans ce port que les plus grandes concentrations de kystes ont été retrouvées, ainsi que la plus grande diversité d'espèces de dinoflagellés capables de produire des kystes. De plus, c'est également dans le port d'Halifax, plus précisément dans le bassin Bedford, qu'un important lit de kystes a été découvert. Des concentrations élevées de kystes du complexe d'espèces *Alexandrium tamarense*, groupe d'espèces potentiellement toxiques et capables de former des floraisons nuisibles, ont été retrouvées près de terminaux de navires. La présence d'un aussi grand nombre de kystes en ces lieux alors qu'aucune floraison notable n'a été observée dans le passé suggère qu'une quantité non négligeable de kystes puisse provenir de l'eau et du sédiment des ballasts de navires.

Finalement, l'observation de la distribution spatiale des assemblages de kystes à l'intérieur d'un même port a permis d'établir que leur distribution est relativement homogène. Cette information facilitera les recherches futures qui n'auront pas à fournir un aussi grand effort d'échantillonnage pour identifier les assemblages. Il serait intéressant de faire une étude similaire à celle-ci dans quelques années afin d'observer si de nouvelles espèces de dinoflagellés capables de produire des kystes de résistance se seront établies dans les eaux côtières canadiennes.

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