Rapid Assessment for *Didemnum vexillum* in Southwest New Brunswick

J.L. Martin, M.M. LeGresley, J.A. Cooper, B. Thorpe, A. Locke, N. Simard, D. Sephton, R. Bernier, I. Bérubé, B. Hill, J. Keays, D. Knox, T. Landry, T. Lander, A. Nadeau and E.J. Watson

Science Branch, Maritimes Region
Fisheries and Oceans Canada
St. Andrews Biological Station
531 Brandy Cove Road, St. Andrews, NB
E5B 2L9

2010

Canadian Technical Report of Fisheries and Aquatic Sciences 2882

Fisheries and Oceans Canada | Pêches et Océans Canada

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of Fisheries and Oceans Canada, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in the data base *Aquatic Sciences and Fisheries Abstracts*.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. II n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications à part entière. Le titre exact figure au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques.*

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Canadian Technical Report of
Fisheries and Aquatic Sciences 2882

March 2010

Rapid Assessment for *Didemnum vexillum* in Southwest New Brunswick

by

J.L. Martin, M.M. LeGresley, J.A. Cooper, B. Thorpe[1], A. Locke[2], N. Simard[3], D. Sephton[4], R. Bernier[2], I. Bérubé[3], B. Hill[1], J. Keays[4], D. Knox, T. Landry[2], T. Lander, A. Nadeau[2] and E.J. Watson[2]

Fisheries and Oceans Canada, Maritimes Region, St Andrews Biological Station,
531 Brandy Cove Road, St. Andrews, New Brunswick, Canada E5B 2L9

[1] New Brunswick Department of Agriculture and Aquaculture, 107 Mount Pleasant Road, St. George, NB Canada E5C 3S9
[2]Gulf Fisheries Centre, Fisheries and Oceans Canada, 343 Université Ave, P.O. Box 5030, Moncton, NB Canada E1C 9B6
[3]Institut Maurice-Lamontagne, Pêches et Océans Canada, 850 route de la mer, C.P. 1000, Mont-Joli, QC Canada G5H 3Z4
[4]Bedford Institute of Oceanography, Fisheries and Oceans Canada, 1 Challenger Drive, P.O. 1006, Dartmouth, NS Canada B2Y 4A2

This is the two hundred and ninety-first Technical Report of
the Biological Station, St. Andrews, NB

Cat. No. Fs 97-6/2882E ISSN 0706-6457 (print version)

Cat. No. Fs 97-6/2882E-PDF ISSN 1488-5379 (online version)

Correct citation for this publication:

Martin, J.L., LeGresley, M.M., Cooper, J.A., Thorpe, B., Locke, A., Simard, N., Sephton, D., Bernier, R., Bérubé, I., Hill, B., Keays, J., Knox, D., Landry, T., Lander, T., Nadeau, A. and Watson, E.J. 2010. Rapid Assessment for *Didemnum vexillum* in southwest New Brunswick. Can. Tech. Rep. Fish. Aquat. Sci.2882: iv + 16p.

TABLE OF CONTENTS

ABSTRACT

Martin, J.L., LeGresley, M.M., Cooper, J.A., Thorpe, B., Locke, A., Simard, N., Sephton, D., Bernier, R., Bérubé, Hill, B., Keays, J., Knox, D., Landry, T., Lander, T., Nadeau, A. and Watson, E.J. 2010. Rapid Assessment for *Didemnum vexillum* in southwest New Brunswick. Can. Tech. Rep. Fish. Aquat. Sci.2882: iv + 16p.

Invasive species are a great threat to habitat and natural biodiversity. The colonial ascidian, *Didemnum vexillum*, has been documented to smother wharfs, floating docks, moorings and hard substrate. In recent years it has rapidly expanded its distribution on both the east and west coasts of North America. In the northwest Atlantic it has been observed as far south as New York and its distribution extends to northern Maine. Although it is suspected that this species was introduced into the Damariscotta River in southern Maine in the early 1970s, its presence was not officially confirmed until the 1980s. *D. vexillum* was detected in 2003 on structures in Eastport, Maine, very close to Canada. In September 2009, a rapid assessment to search for *D. vexillum* using divers, underwater camera systems and scallop drags in Canadian waters around Campobello and Deer Islands, adjacent to Eastport, did not detect colonies of the organism.

RÉSUMÉ

Martin, J.L., LeGresley, M.M., Cooper, J.A., Thorpe, B., Locke, A., Simard, N., Sephton, D., Bernier, R., Bérubé, I., Hill, B., Keays, J., Knox, D., Landry, T., Lander, T., Nadeau, A. and Watson, E.J. 2010. Rapid Assessment for *Didemnum vexillum* in southwest New Brunswick. Can. Tech. Rep. Fish. Aquat. Sci. 2882: iv + 16p.

Les espèces envahissantes menacent la biodiversité naturelle ainsi que les habitats. Le tunicier colonial, *Didemnum vexillum*, peut recouvrir les quais, pontons, bouées d'amarrage et les substrats durs. Récemment, son aire de distribution s'est répandu rapidemment sur les côtes est et ouest de l'Amérique du Nord. Dans l'Atlantique Nord-Ouest il a été observé du sud de New York jusqu'au nord du Maine, aux États-Unis. Malgré qu'on soupçonne qu'il a été introduit dans la rivière Damariscotta au Maine au début des années 1970, sa présence n'a été confirmée officiellement que dans les années 1980. Puisque *D. vexillum* a été découvert à Eastport au Maine, en 2003, une évaluation rapide pour le repérer dans les eaux canadiennes adjacentes a été entreprise en septembre 2009 en utilisant des plongeurs, des appareils de photographie sous-marine et des dragues à pétoncles près des Îles Deer et Campobello. Aucune colonie de *D. vexillum* a été dépistée durant cette étude.

INTRODUCTION

Throughout the world the field of invasive species research is growing in attention and becoming more of a priority with increasing documentation of alien species' invasions (Carlton and Geller, 1993, Leppäkasoski et al. 2003). Unfortunately when established, most invasions are irreversible and can impact the environment, human health and economies.

Aquatic invasive species (AIS) can pose threats to native plants and animals with moderate to severe ecological and economical impacts through a number of actions such as: 1) predation, 2) niche space competition with natural populations, and 3) alteration of habitat. Many regions throughout the world have been experiencing more and more threats from introductions and spread of AIS in recent years and are conducting rapid assessment surveys such as those carried out in the northwest Atlantic (Mathieson et al. 2008), California (Cohen et al. 2005) and eastern Canada (A. Locke, Gulf Fisheries Centre, P.O. Box 5030, Moncton, NB Canada E1C 9B6, pers. comm.; N. Simard, Institut Maurice-Lamontagne, 850 route de la mer, C.P. 1000, Mont-Joli, QC Canada G5H 3Z4, pers. comm.; C. McKenzie, Northwest Atlantic Fisheries Center, East White Hills Road, St. John's, NL Canada A1C 5X1, pers. comm.).

A species of major concern to many regions of the world including Canada's east coast is the colonial ascidian or "sea squirt", *Didemnum vexillum* Kott, 2002 (Kott 2002, 2004, Lambert 2009). Major concerns associated with *D. vexillum* invasions include: ecological traits it shares with other species, high growth rates, ability to spread by fragmentation, tolerance to a wide range of environmental conditions, lack of predators, and ability to alter the abundance and composition of benthic habitat (Dijkstra et al. 2007a, Daley and Scavia 2008, Collie et al. 2009). *D. vexillum* has been documented to be an aggressive and rapidly spreading colonial ascidian (Bullard et al. 2007a; Lengyel et al. 2009).

D. vexillum has been identified in New Zealand as a serious bio-security risk (Sinner and Coutts 2003; Coutts and Forrest 2007). They observed a rapid spread from the hull of a barge first moored in northern New Zealand in 2001 which was moved to Shakespeare Bay where *D. vexillum* colonies then settled onto wharves, moorings, seabeds, adjacent vessels and structures. In 2008, *D. vexillum* was first reported in Wales (and thought to have been introduced within the previous 5 years) in a marina and was found to be covering algae, ascidians, pontoons, chains and ropes (Kleeman 2009).

Due to concerns that *D. vexillum* on Canada's Pacific coast may expand its range and the fact that it may extend its range further north into Canada on the Atlantic coast, a biological synopsis (Daniel and Therriault 2007) was prepared prior to a risk assessment workshop held in Charlottetown, PEI, 2007. Participants from both the national and international research community discussed and documented *D. vexillum* biology, native and non-native distributions in Canada and assessed the risk for Canadian waters (DFO 2007).

In the northeastern United States, colonies of *D. vexillum* (Fig. 1) have been documented from Eastport, northern Maine to New York (Daley and Scavia 2008) and on Georges Bank (Valentine et al. 2007a, 2007b, Lengyel et al. 2009). Although it was first officially documented on the east coast of the US in 1988, there are anecdotal reports from the 1970s (Bullard et al.

2007a) and it has been suggested that *D. vexillum* was first introduced to the Gulf of Maine through Pacific oyster (*Crassostrea gigas*) culture which began in the early 1970s in the Damariscotta River, Maine. Since *D. vexillum* was detected, its overall abundance has increased considerably with highest percent cover observed during the fall and winter (Dijkstra et al. 2007a). Bullard et al. (2007) have suggested that in areas where it blankets the seafloor, it could affect fisheries by smothering bivalves, altering the biodiversity of the seafloor and possibly killing organisms that provide food for fish and other bottom feeders.

Since 2006, an invasive ascidian sampling program in the Canadian portion of the Bay of Fundy has not yet detected *D. vexillum* on regularly deployed plate collectors (LeGresley et al. 2008; Murielle LeGresley, Biological Station, 531 Brandy Cove Road, St. Andrews, New Brunswick, Canada E5B 2L9, pers. commun.). However, due to the close proximity of *D. vexillum* populations in Eastport, Maine (US), to several important Canadian fisheries, aquaculture, and ecologically and biologically significant areas (Buzeta and Singh 2008) in and around Deer Island and Campobello there is concern that plate monitoring alone may not be adequate for early detection. Therefore, researchers from New Brunswick, Nova Scotia and Quebec gathered in southwest New Brunswick to conduct a rapid assessment with the objective of surveying a number of locations in Passamaquoddy Bay, New Brunswick in late September 2009, to search for colonies of *D. vexillum*.

MATERIALS AND METHODS

Tunicate plate collectors have been deployed since 2006 at the following locations including wharves and aquaculture sites in southwestern New Brunswick (LeGresley et al. 2008;, Murielle LeGresley, Biological Station, 531 Brandy Cove Road, St. Andrews, New Brunswick, Canada E5B 2L9, pers. comm.): Fairhaven (Clam Cove area) and Leonardville on Deer Island, Harbour de Loutre, Head Harbour, Roosevelt Provincial Park and Wilsons Beach on Campobello Island and Indian Island. Other collectors were suspended in Ingalls Head, North Head and Seal Cove (Grand Manan Island), Brandy Cove, St. Andrews Harbour, Hog Island in Passamaquoddy Bay, Back Bay, Charlie Cove, Letete, Lime Kiln, Wallace Cove, and further along the coast at Beaver Harbour, Dipper Harbour, Musquash, Saint John and the Kennebacasis Yacht Club. (Fig. 2). All collector lines were suspended 1 metre below the surface and in sufficient water so that the collectors were above bottom. Collectors were suspended from compensator buoys or floating docks at all sites. Two collectors remained suspended from May through November (full season), with additional collectors deployed from May to August (early season) and also from August to November. Some full season collectors were lost but the early and late season collectors provided some data at those sites.

On September 23, 2009 to assist in the identification for the rapid assessment in Canadian waters, researchers (Fig. 3) from New Brunswick, Nova Scotia and Quebec observed, took still photographs and captured underwater videos of live populations of *D. vexillum* colonies at wharf and floating dock sites in Eastport, Maine that were previously recorded by Daley and Scavia (2008) and Bullard et al. (2007a). Samples were also collected for reference and preserved in 10% seawater-formalin buffered with sodium borate and 95% ethanol for preservation of calcium carbonate spicules (Lambert, Appendix 1).

Sampling on September 24th was conducted under near ideal conditions with some sun and cloud and a light breeze of <10 knots. On September 25th the weather was not as conducive to sampling and observation with rain and increasing winds from the northeast (~20 knots), throughout the morning. Seas in Head Harbour Passage were averaging 1 metre.

On September 24th and 25th, the following three boats were used as part of the rapid assessment survey: 1) the Department of Fisheries and Oceans vessel *Salar*, 2) the Huntsman Marine Science Centre vessel *Fundy Spray* and 3) the New Brunswick Dept. of Agriculture and Aquaculture vessel. On September 24th, all three boats went to a number of locations off Deer Island (Clam Cove, Cummings Cove and Leonardville), Indian Island and Campobello Island (Head Harbour, Wilsons Beach, Curry Cove, Harbour de Loutre, Welshpool, Friars Head and Lower Duck Cove) (Fig. 3, Table 1). Two diving teams (2 divers each) searched the bottom at low tide at Clam Cove, Indian Island and Cummings Cove on September 24th looking for *D. vexillum* and collecting samples of interest. On September 25th one dive team searched at Lower Duck Cove.

The original intent was to deploy a snorkelling team on September 25th in coves around Campobello but due to excessive winds, it was not deemed safe to undertake this portion of the survey.

A Seabird Model 25 CTD profiler was used to collect vertical profiles of temperature, salinity and fluorescence at each site. Salinity results are reported on the Practical Salinity Scale (psu).

Ascidian or "tunicate-like" samples were retrieved either from scallop drags or diving; put in Ziplock bags with menthol crystals and kept cool on ice for return to the laboratory at the St. Andrews Biological Station (SABS). Upon return to the lab, samples were examined live, sorted and identified with a Nikon dissecting microscope. Specimens of interest were preserved in 10% formalin and treated according to G. Lambert's method (Appendix 1). Any *Didemnum* samples were also preserved in 95% ethanol for spicule identification. Specimens that were called "unknowns" were taken to Mont Joli (Institut Maurice Lamontagne) for further identification at a tunicate training workshop by C. and G. Lambert (October 5-9, 2009).

Underwater surveillance videos around wharfs and floating docks were made using: Micro Video (TM) colour submersible tube cameras using Cyberlink and Microsoft Windows Movie Maker software.

Bottom dragging was undertaken at five locations (Table 1, Fig. 4) aboard the *Fundy Spray*. One 18 inch Grand Manan "Miracle Gear" scallop drag was used with each drag lasting five to eight minutes.

Table 1. List of CTD and scallop drag locations

Date	Time	Station	Latitude (N) Degree dec. minutes	Longitude (W) Degree dec. minutes	Depth (m)
24-Sept. 2009	11:08	Clam Cove	44 57.818	67 00.540	25.6
24-Sept. 2009	13:17	Harbour de Loutre	44 55.0	66 56.5	26.2
24-Sept. 2009	14:02	Curry Cove	44 55.500	66 56.587	30.5
25-Sept. 2009	09:15	Friars Head	44 52.785	66 58.310	24.4
25-Sept. 2009	10:20	Welshpool	44 53.119	66 57.853	24.4

Additionally, researchers from New Brunswick, Nova Scotia and Quebec provided expertise and equipment for sampling and identification (Fig. 2).

RESULTS AND DISCUSSION

Collector plates deployed as part of a regular monitoring programme for invasive tunicates in southwest New Brunswick have not as yet detected the presence of *D. vexillum* (LeGresley et al. 2008, Murielle LeGresley, Biological Station, 531 Brandy Cove Road, St. Andrews, New Brunswick, Canada E5B 2L9, pers. comm.). There was concern that this method may not have been extensive enough to detect colonies on other substrates. As a result, this particular rapid assessment was undertaken to look solely for *D. vexillum*. Although a number of specimens including sponges and *Ciona intestinalis* were retrieved through the exercise, *D. vexillum* was not detected throughout the rapid assessment at any of the Canadian sites.

The salinity during the rapid assessment ranged from 32.35-32.58 psu. Annual salinities in the region can range between 21.19 psu in the spring to 33.39 psu in late fall. (Martin et al. 2006). This is well within the tolerance levels for *D. vexillum* from the literature that indicate that it can tolerate wide fluctuations in salinity above 20 psu but it is rarely found in locations less than 20 psu (Lambert 2002, Lambert 2005).

Surface temperatures during the rapid assessment ranged from 11.5-12.0°C. Surface water temperatures for the region tend to be highest in mid-Passamaquoddy Bay (17^{o}C) in September and lowest (-1.0°C) in Passamaquoddy Bay in February (Martin et al. 2006). Temperature ranges for *D. vexillum* is from -2°C to 24°C (Lambert 2005) with a preference for either cooler summer temperatures or an environmental factor related to temperature (Osman and Whitlatch 2007). Daley and Scavia (2008) suggest that temperature ranges along the northeast coast of the United States are ideal for *D. vexillum* to produce new colonies and spread.

D. vexillum has been detected in Eastport, Maine (Dijkstra et al. 2007a, Bullard et al 2007a). Its range has been documented to be from Eastport to New York and onto Georges, Stillwagen and Tellies Banks with 230 km^2 coverage on Georges Bank with 50-90% cover in some areas (Bullard et al. 2007a, Lengyel et al. 2009, Valentine et al. 2007b). The Bay of Fundy has a native *Didemnum* species (*D. albidum*) that has been present for many years and is often mistaken for the invasive *D. vexillum* (Daniel and Therriault 2007).

D. vexillum grows on substrates such as rocks, gravel, boulders, hydroids, scallops, mussels, oyster and barnacles (Bullard et al. 2007b, Valentine 2007a and 2007b, Daniel and Therriault 2007). Much of this habitat is typical of bottom substrates for much of Passamaquoddy Bay (near the Eastport populations) further suggesting that *D. vexillum* could successfully invade the region. Additionally *D. vexillum* is able to invade in both shallow and deep waters (Osman and Whitlatch 2007).

The biology and life cycle of *Didemnum* spp. has been widely documented (Bullard et al. 2007a, Therriault and Herborg 2007, Lambert 2005, Kott 2002). Although *D. vexillum* is hermaphroditic and produces tadpole larvae, they only swim in the water for minutes to hours before settling (Lambert and Lambert 2005) reducing the distances that they can be transported and suggesting that this might not be the preferred method of transport and introduction although this would facilitate maintenance of a population once established in a particular area (Osman and Whitlatch 1998, 2007). It is thought that the more obvious means of transport for *D. vexillum* in our region would be asexual division through fragmentation (or rope-like growth) and transport through hull fouling, aquaculture, currents, waves or tides (Bullard et al. 2007b, Dijkstra et al. 2007a). Fragments have been known to survive for more than four weeks (M. Carman, Woods Hole Oceanographic Institution, 266 Woods Hole Rd., MS# 08, Woods Hole, MA 02543, pers. comm.). The fragments that are able to survive can reattach to suitable substrates. Experiments by Bullard et al. (2007b) have shown that many fragments can reattach in 6-12 hr with 75-80% reattaching in 30 hr.

Once established, *D. vexillum* does not seem to be reduced by predators (Osman and Whitlatch 2007). Attempts at removal have shown that *D. vexillum* is extremely difficult to remove from structures (Therriault and Herborg 2007). A concern is that once established, *D. vexillum* could be responsible for a shift in species diversity or a displacement of native species, which would pose a threat to the local biodiversity, marine protected areas, traditional fisheries and aquaculture (Dijkstra et al 2007b, Dijkstra and Harris 2009; Lengyel et al. 2009, Osman and Whitlatch 1995). *D. vexillum* has been observed as having a significant impact on species composition in the benthic community and out-competing both epifaunal and macrofaunal communities. It has been known to overgrow other ascidians, sponges, macroalgae, hydroids, anemones, bryozoans, scallops, mussels, polychaetes and crustaceans as well as reduce food supply, inhibit settlement for other organisms (Bullard et al. 2007a). *D. vexillum* has few predators (Bullard et al. 2007a). Dijkstra and Harris (2009) conducted two studies – one from 1979-1982 and the other from 2003-2006 following the detection of *D. vexillum*. They found that the co-existence had shifted from a community in which the diversity was maintained by secondary substrates to a community where the diversity was maintained by primary substrates. For example, the mussels that were historically a dominant species on their collector panels and ropes experienced a significant decline suggesting a reduction in mussel abundance. A change in species presence/absence can result in patches of “free space” and allow or enable settlement of species such as invasives.

Asexual reproduction and fragments that break off seem to play an important role in the spread of *D. vexillum*. Some colonies have long appendages that can separate from the colony and be transported for long distances through currents and water movements (Lengyel et al. 2009). With

the associated large tides and currents in Passamaquoddy Bay and the St. Croix estuary flushing times have been estimated at about 6-8 days for the St. Croix estuary and 8-17 days for Passamaquoddy Bay (Ketchum and Keen 1953; Trites 1962). These conditions and the fact that only the St. Croix River (<2 km wide at Eastport) separates Canada from the populations in Eastport, ME suggest the provision of an opportunity for transport, retention and settlement of *D. vexillum* segments through the region.

Another likely vector for the transport of *D. vexillum* from Cape Cod as far north to Maine (Carman 2007) is through ship traffic, hull fouling and the movement between regions of the Northeast US. Herborg et al. (2009) indicated in their forecasting of the spread of *D. vexillum* that slow vessels, aquaculture fishing vessels, small vessels and large commercial vessels could act as vectors. There is a definite possibility in the Passamaquoddy Bay region that *D. vexillum* could be transported in a similar manner as a result of the high volume of all these types of vessels travelling freely through the area.

Therriault and Herborg (2007) determined from results of an on-line survey of AIS and tunicate experts that the level of impact from an invasion of *D. vexillum* was high. *D. vexillum* was ranked among the highest threats in terms of environmental and industrial impacts. They found that there are a number of areas in Atlantic Canada that would be favourable for the arrival, settlement, survival and capability to reproduce. One of the areas identified to be high risk is the Bay of Fundy – especially due to its close proximity to populations in the US. This particular area is known for its scallop fishery and the dragging of the bottom for scallops, which could facilitate fragmentation of *D. vexillum*. If *D. vexillum* becomes established in the region there would be further concern for the spread of fragments.

Other countries, such as New Zealand, have experienced first hand the devastation and implications of an invasion of *D. vexillum* (Coutts and Forrest 2007). Eradication has so far been unsuccessful although they did learn some important lessons and did develop some response tools that might work in the future for different substrates. In June 2008, *D. vexillum* was detected in Wales and they are still working on eradication measures that are to be undertaken in 2010 (Kleeman 2009).

An attempt has been made to remove colonies from the wharf in Eastport, ME, but with little success to date (Harris and Dijkstra, University of New Hampshire, 149 Spaulding Life Science Center College Road, Durham, NH 03824, pers. comm.). Throughout the world there is increasing concern as to location and spread of this invasive species. From a global perspective, there are suggestions that risk assessments are becoming more and more popular as management tools. Attention in New Zealand and Australia is moving forward with establishing guidelines and quarantines for Health Standards for imports to prevent introductions such as *D. vexillum* (Hewitt and Campbell 2007). They are implementing biosecurity strategies and legislation both externally and locally to aid in preventing and managing AIS invasions.

It is important that an effort be continued to ensure the early detection of *D. vexillum* populations in the local area. A rapid assessment should be conducted annually and a rapid response should be implemented for the very real possibility that *D. vexillum* could spread to Atlantic Canadian waters.

ACKNOWLEDGEMENTS

Funding for the analyses was provided through Fisheries and Oceans Canada (Aquatic Invasive Research). We thank P. Fitzgerald (Huntsman Marine Science Centre), Capt. R. Longmire and D. Craig of the *Fundy Spray* (Huntsman Marine Science Centre) who have helped with the field work. B. Chang and L. Cooper reviewed the manuscript. B. Chang did the maps. C. McAdam edited and J. Power proof-read the manuscript. Thanks to S. Taylor for media coverage and taking photos during the assessment.

REFERENCES

Bullard, S.G., Lambert, G., Carman, M.R., Byrnes, J., Whitlatch, R.B., Ruiz, G., Miller, R.J., Harris, L., Valentine, P.C., Collie, J.S., Pederson, J., McNaught, D.C., Cohen, A.N., Asch, R.G., Dijkstra, J. and Heinonen, K. 2007a. The colonial ascidian *Didemnum* sp. A: Current distribution, basic biology and potential threat to marine communities of the northeast and west coasts of North America. J. Exp. Mar. Biol. Ecol. 342(1): 99-108.

Bullard, S.G., Sedlack, B., Reinhardt, J.F., Litty, C., Gareau, K. and Whitlatch, R.B. 2007b. Fragmentation of colonial ascidians: Differences in reattachment capability among species. J. Exp. Mar. Biol. Ecol. 342(1): 166-168.

Buzeta, M-I. and Singh. R. 2008. Identification of ecologically and biologically significant areas in the Bay of Fundy, Gulf of Maine. Volume 1: Areas identified for review, and assessment of the Quoddy Region. Can. Tech. Rep. Fish. Aquat. Sci. 2788: 87 p.

Carlton, J.T. and Geller, B. 1993. Ecological roulette: the global transport of nonindigenous marine organisms. Sci. 261(5117): 78-82.

Carman, M.R. 2007. Benthic foraminifera associated with the invasive ascidian, *Didemnum* sp. A. J. Exp. Mar. Biol. Ecol. 342(1): 147-153.

Cohen, A.N., Harris, L.H., Bingham, B.L., Carlton, J.T., Chapman, J.W., Lambert, C.C., Lambert, G., Ljubenkov, J.C., Murray, S.N., Rao, L.C., Reardon, K. and Schwindt, E. 2005. Rapid assessment survey for exotic organisms in southern California bays and harbors, and abundance in port and non-port areas. Biol. Invasions. 7(6): 995-1002.

Collie, J.S., Hermsen, J.M. and Valentine, P.C. 2009. Recolonization of gravel habitats on Georges Bank (northwest Atlantic). Top. Stud. Oceanogr. Deep-Sea Res. II 56(19-20): 1847-1855.

Coutts, A.D.M. and Forrest, B.M. 2007. Development and application of tools for incursion response: Lessons learned from the management of the fouling pest *Didemnum vexillum*. J. Exp. Mar. Biol. Ecol. 342(1): 154-162.

Daley, B.A. and Scavia, D. 2008. An integrated assessment of the continued spread and potential impacts of the colonial ascidian, *Didemnum* sp. A, in U.S. waters. NOAA Technical Memorandum NOS NCCOS 78, 61 p.

Daniel, K.S. and Therriault, T.W. 2007. Biological synopsis of the invasive tunicate *Didemnum* sp. Can. Manuscr. Rep. Fish Aquat. Sci. 2788: 59 p.

DFO. 2007. Proceedings of the national peer-review workshop for the risk assessment for two solitary and three colonial tunicates in both Atlantic and Pacific Canadian waters. PRO2009/045.

Dijkstra, J., Harris, L.G. and Westerman, E. 2007a. Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. J. Exp. Mar. Biol. Ecol. 342(1): 61-68.

Dijkstra, J., Sherman, H. and Harris, L.G. 2007b. The role of colonial ascidians in altering biodiversity in marine fouling communities. J. Exp. Mar. Biol. Ecol. 342(1): 169-171.

Dijkstra, J. and Harris, L.G. 2009. Maintenance of diversity altered by a shift in dominant species: implications for species coexistence. Mar. Ecol. Prog. Ser. 387: 71-80.

Herborg, L-M., O'Hara, P. and Therriault, T.W. 2009. Forecasting the potential distribution of the invasive tunicate *Didemnum vexillum*. J. Appl. Ecol. 46(1): 64-72.

Hewitt, C.L. and Campbell, M.L. 2007. Mechanisms for the prevention of marine bioinvasions for better biosecurity. Mar. Pollut. Bull. 55(7-9): 395 -401.

Ketchum, B.H. and Keen, D.J. 1953. The exchanges of fresh and salt waters in the Bay of Fundy and in Passamaquoddy Bay. J. Fish. Res. Board Can. 10: 97-124.

Kleeman, S.N. 2009. *Didemnum vexillum* – feasibility of eradication and/or control. CCW Science report. No: 875, 53 p. CCW, Bangor, Wales, UK.

Kott, P. 2002. A complex didemnid ascidian from Whangamata, New Zealand. J. Mar. Biol. Assoc. U.K. 82(4): 625-628.

Kott, P. 2004. A new species of *Didemnum* (Ascidiacea, Tunicata) from the Atlantic coast of North America. Zootaxa 732: 1-10.

Lambert, G. 2002. Nonindigenous ascidians in tropical waters. Pac. Sci. 56(3): 291-298.

Lambert, G. 2005. Ecology and natural history of the protochordates. Can. J. Zool. 83(1): 34-50.

Lambert, G. 2009. Adventures of a sea squirt sleuth: unraveling the identity of *Didemnum vexillum*, a global ascidian invader. In Proceedings of the 2nd International Invasive Sea Squirt Conference, October 2-4, 2007, Prince Edward Island. Edited by A. Locke and M. Carman. Aquatic Invasions (Spec. Iss.) 4(1): 5-28.

Lambert, G. and Lambert, C. 2005. Key to the commonest harbour ascidians in Washington October 2005. In Lambert and Lambert 2005. Ascidian Identification Workshop. November 4, 2005. Nanaimo, BC. pp. 9-10.

LeGresley, M.M., Martin, J.L., McCurdy, P., Thorpe, B. and Chang, B.D. 2008. Non-indigenous tunicate species in the Bay of Fundy, eastern Canada. ICES J. Mar. Sci. 65(5): 770-774.

Lengyel, N.L., Collie, J.S. and Valentine, P.C. 2009. The invasive colonial ascidian *Didemnum vexillum* on Georges Bank – Ecological effects and genetic identification. In Proceedings of the 2nd International Invasive Sea Squirt Conference, October 2-4, 2007, Prince Edward Island. Edited by A. Locke and M. Carman. Aquatic Invasions (Spec. Iss.) 4(1): 143-152.

Leppäkoski, E., Gollasch, S. and Olenin, S. 2002. Alein species in European Waters. In Invasive Aquatic Species in Europe. Edited by E. Leppäkoski, S. Gollasch and S. Olein. Kluwer Academic Publishers. The Netherlands. pp. 1-6.

Martin, J.L., LeGresley, M.M. and Strain, P.M. 2006. Phytoplankton monitoring in the southwest Bay of Fundy during 1993-96. Can. Tech. Rep. Fish. Aquat. Sci. 2629: 92 p.

Mathieson, A.C., Pederson, J. and Dawes, C.J. 2008. Rapid assessment of fouling and introduced seaweeds in the northwest Atlantic. Rhodora. 110(944): 406-478

Osman, R.W. and Whitlatch, R.B. 1995. Predation on early ontogenetic life stages and its effect on recruitment into a marine epifaunal community. Mar. Ecol. Prog. Ser. 117: 111-126.

Osman, R.W. and Whitlatch, R.B. 1998. Local control of recruitment in an epifaunal community and the consequences to colonization processes. Hydrobiologia. 375/376: 113-123.

Osman, R.W. and Whitlatch, R.B. 2007. Variation in the ability of *Didemnum* sp. to invade established communities. J. Exp. Mar. Biol. Ecol. 342(1): 40-50.

Sinner, J. and Coutts, A.D.M. 2003. Benefit-cost analysis of management options for *Didemnum vexillum* in Shakespeare Bay (New Zealand). Prepared for Port Marlborough New Zealand Limited. Cawthron Report No. 924. 29 p.

Therriault, T.W. and Herborg, L-M. 2007. Risk Assessment for two solitary and three colonial tunicates in both Atlantic and Pacific Canadian waters. CSAS Res. Doc. 2007/063, 64 p. http://www.dfo-mpo.gc.ca/csas/

Trites, R.W. 1962. Temperature and salinity in the Quoddy Region of the Bay of Fundy. J. Fish. Res. Board Can. 19: 975-978.

Valentine, P.C., Carman, M.R., Blackwood, D.S. and Heffron, E.J. 2007a. Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat. J. Exp. Mar. Biol. Ecol. 342(1): 109-121.

Valentine, P.C., Collie, J.S., Reid, R.N., Asch, R.G., Guida, V.G. and Blackwell, D.S. 2007b. The occurrence of the colonial ascidian *Didemnum* sp. on Georges Bank gravel habitat – Ecological observations and potential effects on groundfish and scallop fisheries. J. Exp. Mar. Biol. Ecol. 342(1): 179-181.

Fig. 1. *D. vexillum* from Eastport Maine shown in “pancake batter” form and with finger-like lobes.

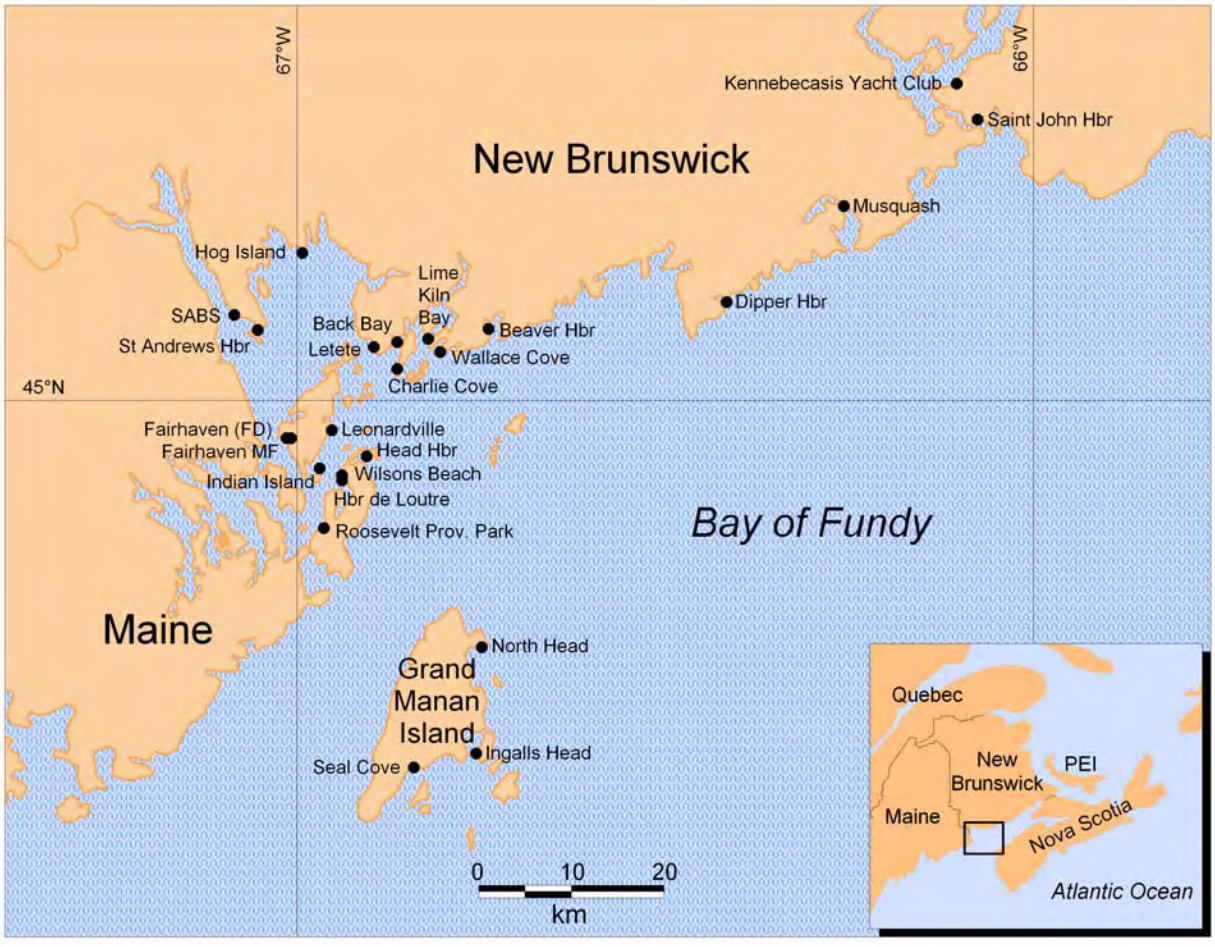

Fig. 2. Locations where tunicate collectors have been deployed.

Fig. 3. Participants in the rapid assessment for *D. vexillum*. Back row – Andrew Cooper, Terralynn Lander, Thomas Landry, Pat Fitzgerald, Dawn Sephton; middle row – Renée Bernier, Murielle LeGresley, Erica Watson, André Nadeau, Joanne Keays; Front row – Andrea Locke, Jennifer Martin, Isabelle Bérubé and Nathalie Simard. (Missing from photo – Derek Knox, Barry Hill, Bruce Thorpe).

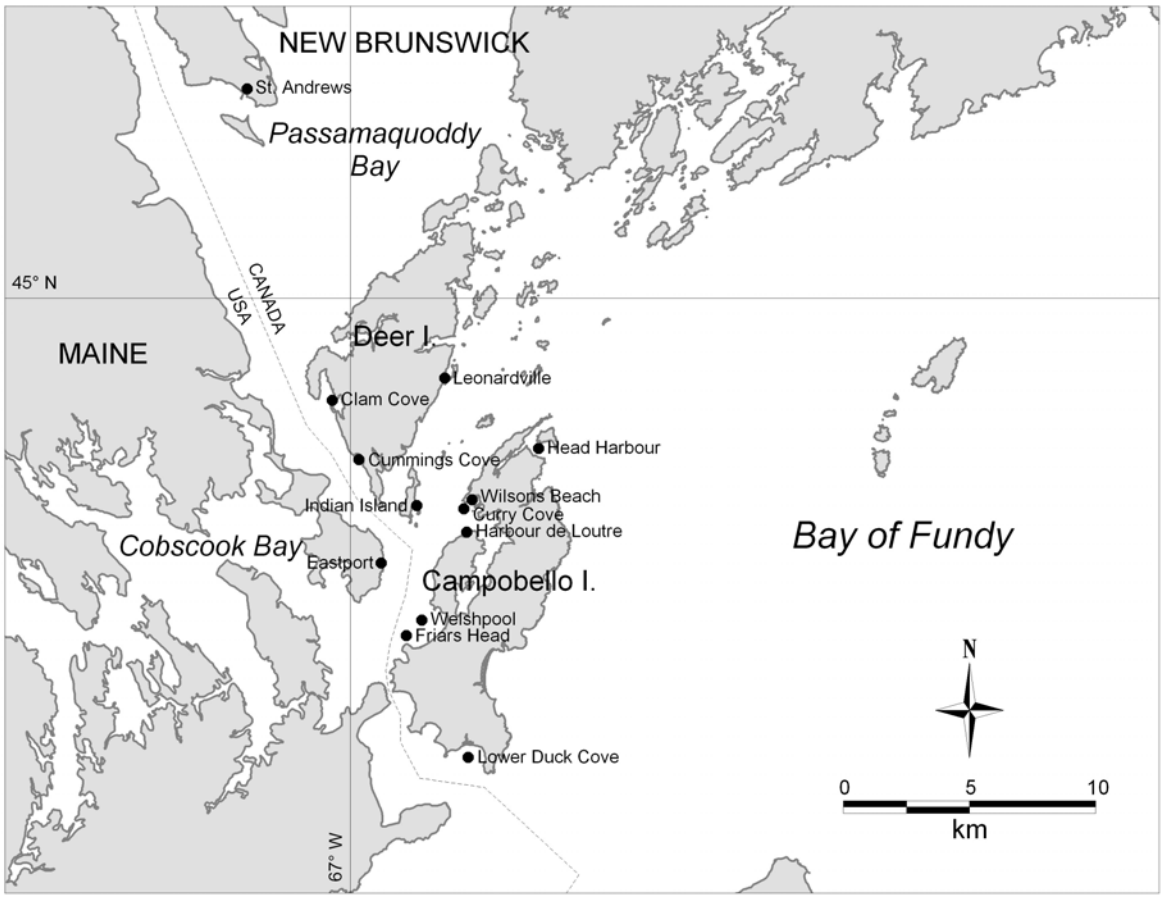

Fig. 4. Map showing St. Andrews, the location of the Biological Station and locations surveyed for *D. vexillum* during the rapid assessment 2009.

Appendix 1. Relaxing and preserving ascidians for taxonomy

http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/htm/page41.htm

Gretchen Lambert
University of Washington Friday Harbor Laboratories
glambert@fullerton.edu

Relaxing and fixing ascidians for taxonomy

The ascidians should first be relaxed, then fixed in 10% seawater formalin buffered with sodium borate to help preserve spicules and color.

The formula for 1 liter of fixative:

- 100 ml of full strength formaldehyde (37%), 850 ml of seawater, and 50 ml of distilled water (or reverse osmosis water, or tap water if it does not have a lot of minerals in it; sometimes I get a precipitate with tap water). It is necessary to use the 50 ml of distilled water instead of all sea water so that the solution will not be hypertonic. [Ethanol is definitely NOT a good preservative for taxonomy, and ascidians should never be placed directly into ethanol, except a subsample for molecular analysis. Ethanol makes the tissues opaque and brittle, and it removes all color.]

- To this add 1 gram of sodium borate. Mix thoroughly before use. The borate is not very soluble, so it takes a while to dissolve; thus I make the solution a few hours or even a day ahead of when I want to use it.

Two methods for relaxing ascidians:

- An easy way to relax ascidians is with menthol crystals. You can carry a small vial of crystals in the field with you, place a few crystals in a ziploc bag with the sample in sea water and seal tightly. By the time you get back to the lab, the animals will usually be at least partially relaxed. If not, keep them in the bags for a bit longer, or proceed as follows.

- A second method for relaxing ascidians uses menthol in ethanol, a technique I learned from Don Abbott. Fill a small bottle (10-20 ml or so) with crystals of menthol. Then fill the bottle with 95% ethanol and shake to dissolve the menthol, which is much more soluble in ethanol than in water. Place the ascidian in a dish of seawater (or the menthol/seawater from your ziploc bag). Then add about 5 drops of the menthol/ethanol and QUICKLY cover the dish tightly (I use a small sheet of glass or plastic with a weight on top) to prevent evaporation of the menthol. Every 10 minutes or so add another few drops of the menthol/ethanol until when you insert a sharp probe into an open siphon there is absolutely no response. Use a hand lens or microscope to be sure about this! Relaxation may be achieved in as little as 10-15 minutes or so for some species, but may take several hours for others.

Fixing relaxed ascidians:

- Fill a dish with fresh seawater and have a jar of the 10% seawater/formalin fixative ready. Lift off the crystallized menthol that will be floating on the water surface (I put it on a paper, dry it and put it back into the bottle for re-use). Transfer the relaxed specimens to the dish of fresh seawater, rinse briefly to remove the extra menthol crystals (you may have to do this twice) and then quickly transfer to the jar of fixative and cover. If it's a large solitary specimen, hold it upside down to let the seawater drain out of the open siphons before immersing it into the fixative with the siphons pointing upward so that the animal will quickly fill with the fixative.

- If you won't be returning to the lab after collecting specimens, take the formalin, bottles, etc in the field with you. Leave the relaxing samples in the ziploc bags with menthol for several hours, then rinse in sea water or blot on paper towels and transfer to formalin. You can of course fix the ascidians directly in the 10% seawater formalin without relaxation but it makes identification especially of colonial species very difficult.

Fixing ascidians for molecular analysis and preservation of calcium carbonate spicules

Fixation in 95% ethanol and freezing:

- Before placing a specimen into 95% ethanol, if you can, rinse in tap or distilled water to remove as much seawater as possible, because the seawater will cause precipitates to form in the ethanol. At the very least, blot on paper towels before placing in the ethanol. Do this in the field with fresh specimens. Don't use colonies that have been relaxing in menthol for several hours and may be half dead by the time you get to the lab.

- With colonial species, cut into small pieces before placing in the ethanol. With solitary species, remove the tunic and discard, and blot the body thoroughly (or rinse and then blot) to remove as much liquid as possible before placing it in ethanol. You can also dissect out the gonads and preserve only the gonads in ethanol. Store the specimens in a freezer as soon as you can.

Long-term storage of specimens

Opinions vary as to how to store ascidians long-term for museum vouchers. I and the Monniots in Paris leave them in the formalin forever, but even buffered formalin slowly becomes acidic and needs to be replaced periodically. Patricia Kott in Australia transfers specimens to 70% ethanol for permanent storage, after a minimum of 4 months' fixation in formalin.

Because ascidian taxonomy is very difficult, and many species, especially colonial ones, resemble one another morphologically, it is imperative that we all begin assembling a permanent subset of tissue for molecular analysis that is preserved directly into 95% ethanol and stored in a freezer. This will also be of tremendous value in helping to determine the point of origin of invasive non-indigenous species. Some new techniques are being developed to even be able to

utilize dried and formalin-fixed museum specimens (Yue and Orban 2001). Rapid isolation of DNA from fresh and preserved fish scales for polymerase chain reaction. We must augment 19th century style taxonomy's total reliance on morphology with the molecular systematics of the 21st century. Museums, now the repositories of the world's species, should set up facilities for storage of companion vouchers for molecular analysis.

I agree with Patricia Kott that "no power on earth will maintain the living colour of ascidians after they are collected - so nothing can replace: (1) colour notes on living specimens before they are removed from the substrate; and/or (2) in situ photographs." To this, I would add notes on the general appearance of the living animal or colony. For example, the atrial languets of many aplousobranchs are highly contractile, and their short stubby appearance in preserved zooids bears little resemblance to their appearance in living zooids.

Larval morphology is of great importance in the identification of colonial forms; care should be taken to preserve brooded embryos and any swimming tadpoles.

REFERENCE

Yue, G.H. and Orban, L. 2001. Rapid isolation of DNA from fresh and preserved fish scales for polymerase chain reaction. Mar. Biotechnol. 3 (3): 199-204.