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Research article

The invasive colonial ascidian *Didemnum vexillum* on Georges Bank — Ecological effects and genetic identification

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Abstract

Since the discovery of the invasive colonial tunicate *Didemnum vexillum* Kott, 2002 on Georges Bank in 2002, research has focused on investigating the spread of the tunicate invasion, evaluating its potential impact on the benthic community, identifying it to species level, and determining its region of origin. The percent cover of *Didemnum vexillum*, measured from bottom photographs, ranges from 0-100% in individual photos and between 0-79% when averaged within photo transects. Individual photos represent an area of the seabed measuring ~ 0.39 m^2 while photo transects range from ~ 700-1000 meters in length. Hydroids are the second most abundant epifaunal taxon. The macrofauna identified in bottom photo analysis comprises 21 different taxa, of which burrowing and non-burrowing anemones are the most numerous. Our detailed analysis of bottom photographs suggests that *Didemnum vexillum* is able to out-compete other epifaunal and macrofaunal taxa. An Analysis of Similarity (ANOSIM) test on macrofauna abundance data collected with a Naturalist dredge from 1994 to 2006, indicates that *Didemnum vexillum* has had a significant impact on the species composition of the benthic community. The abundance of two polychaete species, *Nereis zonata* Malmgren, 1867 and *Harmothoe extenuata* Grube, 1840, increased significantly in infested areas compared with uninfested areas, according to two-way Analysis of Variance (ANOVA). We found four distinct nucleotide sequences of the 18s rDNA gene among 17 samples of *Didemnum* species, three from Georges Bank and one from New Zealand. Two of the Georges Bank sequences were identified as *Didemnum albidum* Verrill, 1871, a species native to the northeast United States. The third sequence represents the invasive *Didemnum vexillum* from Georges Bank, and the fourth sequence an undescribed species from New Zealand (not *D. vexillum*).

Key words: continental shelf, *Didemnum*, epifauna, Georges Bank, gravel habitat, macrofauna, seafloor photographs, 18s rDNA gene

Introduction

Georges Bank has served as important commercial fishing grounds for many species of groundfish dating back to the 18th century (German 1987). Located east of Cape Cod, Massachusetts, Georges Bank is a shallow submarine plateau 150 km wide by 280 km long (Uchupi and Austin 1987), encompassing an area of ~ 35,000 km^2 inside the 100 m isobath (Backus 1987). Since 2002, these fishing grounds have been host to the invasive colonial ascidian *Didemnum vexillum* Kott, 2002 (Valentine et al. 2007).

D. vexillum has been found in coastal areas of New England since the late 1980s, growing on hard substrates including rock outcrops, boulders, gravel, dock pilings, floating docks, and other materials (Bullard et al. 2007a). The means of introduction of *D. vexillum* onto Georges Bank remains unclear. Since its introduction, *D. vexillum* has colonized at least 230 km^2 of pebble gravel habitat in two adjacent areas, generating large concern about its potential effects on fisheries and benthic habitats (Valentine et al. 2007). *D. vexillum* can be quite variable in morphological appearance (U.S. Geological Survey 2008). Colonies on Georges Bank often appear pink, yellow, beige, or white in color, can exist as thin encrusting layers, or may possess tendrils protruding from thick encrusting mats.

D. vexillum is capable of both sexual and asexual reproduction. Throughout the *Didemnum*

genus, larvae produced in sexual reproduction are generally short-lived, swimming for only a few hours before attaching to the substrate (Berrill 1935; Olson 1983). This short, free-swimming larval stage likely does not last long enough for larvae to be carried great distances by ocean currents, and we suggest sexual reproduction of *D. vexillum* contributes only to local spread on Georges Bank.

Asexual reproduction and fragmentation however, may play a much more critical role in the spread of this species. Asexual reproduction through budding often creates the long tendrils observed in *D. vexillum* colonies (Figure 1). These fragile tendrils can break off from parent colonies and be transported long distances by ocean currents. Semidiurnal tidal currents on Georges Bank range up to 100 cm/s. Fragments may be able to re-attach within six hours after being in contact with the substrate (Bullard et al. 2007b). Thus, *D. vexillum* fragments have the potential to disperse ~ 20 km before re-attaching to the substrate. In our view, fragmentation, whether by natural causes or by fishing disturbance, appears to be the most likely mechanism for the further spread of *D. vexillum* on Georges Bank.

There are several potential impacts associated with the invasion of *D. vexillum* on Georges Bank. *D. vexillum* can turn a heterogeneous pebble gravel habitat into a homogeneous tunicate mat. Previous work has indicated that the pebble gravel habitat on Georges Bank may play an important role in the survival and success of juvenile Atlantic cod (*Gadus morhua* Linnaeus, 1758) and haddock (*Melanogrammus aeglefinus* Linnaeus, 1758) (Lough et al. 1989) and is important in the settlement of sea scallop (*Placopecten magellanicus* Gmelin, 1791) larvae (Thouzeau et al. 1991). Consequently, the invasion of *D. vexillum* on Georges Bank pebble gravel sites may not only result in profound changes to the substrate, but may also negatively impact the benthic community and the associated fisheries.

The colonization of *D. vexillum* on Georges Bank is the first documented off-shore invasion by this species. The aims of the present research are to investigate the spread of *D. vexillum* through video and photographic imagery, to measure the ecological impacts on the benthic community through sampling with a Naturalist dredge, and to use genetic analysis and taxonomy to identify this species.

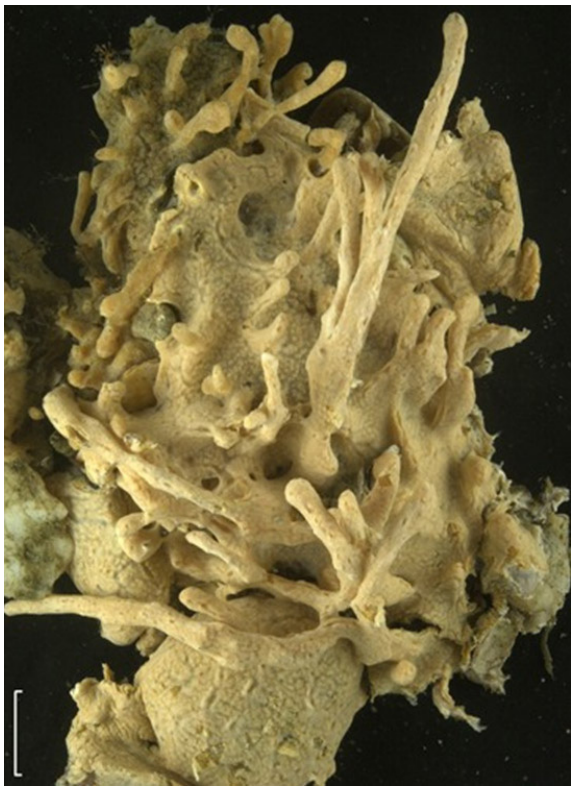

Figure 1. Colony of *D. vexillum* over-growing mussel shell on northern Georges Bank demonstrating the long tendrils that can form through asexual budding. Collected November 1, 2003, by: Jeremy Collie, Page Valentine, and Robert Reid. Photo credit: Dann Blackwood, U.S. Geological Survey. The horizontal scale bar is 2 cm.

Materials and methods

Study area

Samples for this study came from four areas of pebble gravel habitat on Georges Bank with contrasting levels of bottom fishing and *D. vexillum* infestation (Figure 2, Table 1). All of the *Didemnum* we observed and sampled in areas 18 and 19 is the invasive species, *D. vexillum*. *D. albidum* Verrill, 1871 is native to Georges Bank, but we have only observed it in very small colonies (~2 cm^2) in areas 20 and the MB on the Canadian side of the bank.

Video and photo imagery

Video and still photography was taken with the USGS SEAbed Observation and Sampling System (SEABOSS). Video imagery from Area 18 (open to fishing) was used to make visual estimates of tunicate percent cover aboard the

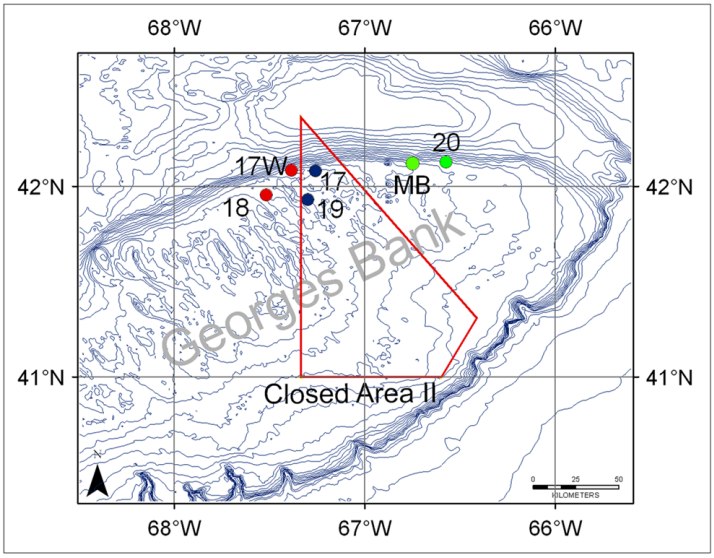

Figure 2. Map of Georges Bank off New England showing location of study areas and Closed Area II. Areas 18 and 19 have been colonized by *D. vexillum*, and Areas 17 and 17W have not. See Table 1 for water depths. In U.S. waters, red areas (17W, 18) are open to fishing; blue areas (17, 19) are closed to fishing. Green areas are in Canada. Samples used in genetic analysis are from Areas, 18, 19, 20, and MB (see Table 2).

Table 1. Description of study areas.

Study Area	17	17W	18	19	MB	20
Latitude (N)	42°04.6'	42°04.9'	41°57.2'	41°55.78'	42°07.19'	42°07.5'
Longitude (W)	067°15.6'	067°21.3'	067°31.0'	067°17.94'	066°44.91'	066°34.5'
Depth range (m)	44-49	50-51	41-65	52-55	66-80	82-86
D. vexillum	Absent	Absent	Present	Present	Present	Present
Fishery status	Closed	Open	Open	Closed	Closed	Closed

research vessel. Still photos of the sea floor, taken in 2003 and 2004, were analyzed with a Mathworks Matlab R2006a program. A total of 516 bottom photos from 26 transects (18-20 photos per transect) were analyzed. The bottom substrate was classified with a binary index (Hixon et al. 1991), whereby the dominant sediment category occupies at least 50% of the area and the subdominant category occupies at least 20% of the remaining area. Percent cover of colonial epifauna was estimated with a random-dot method whereby 70 random points are projected onto each bottom photo which represents an area of the seabed measuring 76 x 51 cm. Each random point is classified by the user as one of six categories of attached epifauna: *D. vexillum*, *Filograna implexa* Berkeley, 1828 (a tubiculous polychaete worm), hydroid, bushy bryozoan, sponge, or other. The choice the user makes for each point, along with the location of each point, is automatically recorded into a text file. These data are then used to determine percent cover of the six epifauna categories for each photo, and along each transect. Free-living macrofauna present in each photo are also recorded.

Spearman rank correlations were calculated between the percent cover of *D. vexillum* and the incidence of macrofauna and other epifaunal taxa. Permutation tests were performed to calculate the probability levels associated with these rank-order correlations. The PRIMER 6 software package was used to perform the following analyses (Clarke and Warwick 2001). A Bray-Curtis similarity matrix was calculated from the root-transformed abundances of 21 macrofaunal taxa, aggregated over transects. A non-metric Multi-Dimensional Scaling (MDS) analysis of this similarity matrix was used to ordinate the photo transects. The routine BIOENV was used to calculate the rank correlation between the similarity matrix of macrofaunal taxa and the percent cover of *D. vexillum*, averaged over transects. The Analysis

of Similarity (ANOSIM) routine was used to test for differences in the macrofaunal assemblages between samples collected in 2003 and 2004.

Naturalist dredge data

A 1-m Naturalist dredge was used on research cruises to collect samples of benthic macrofauna from 1994-2006 in Area 18 (open to fishing), and from 2002-2006 in Area 19 (closed to fishing). See Collie et al. (2005) for more details. Generally, 3-4 benthic samples were collected from the pebble gravel habitat in each area in each year sampled. The dredge was lowered to the seabed and towed at a speed of 1-1.5 kt for 60 to 120 seconds to avoid overfilling the dredge and damaging the collected fauna. The benthic samples were preserved in 5% buffered formaldehyde in seawater and brought back to the laboratory for analysis. In the laboratory organisms were sorted and identified to the lowest possible taxonomic level. For each taxa identified, a count and dry weight were obtained and the data were standardized per unit volume of sediment.

The abundance data of 120 species, known to be sampled quantitatively, were imported into the PRIMER 6 program and a Bray Curtis similarity matrix was created from the square-root transformed abundance data. Species that were not sampled quantitatively were excluded from the analysis. An MDS plot, ANOSIM test, and similarity percentages (SIMPER) were calculated from the same Bray Curtis similarity matrix. Two-way Analysis of Variance (ANOVA) was used to determine if the abundance of organisms identified with SIMPER increased significantly after the *D. vexillum* infestation, relative to reference areas without the tunicate. The response variable for the ANOVAs was the square root of abundance and the fixed factors were (before/after infestation) and treatment (infested/uninfested). A significant ($p<0.05$) time×treatment interaction (with 1 degree of freedom) indicates that the abundance of the particular species changed following *D. vexillum* infestation.

Taxonomic and genetic identification

DNA was extracted from 16 samples of *Didemnum* from Georges Bank and one sample from New Zealand preserved in 95% ethanol (Table 2). Three of the *Didemnum* samples (3, 13, and 15) from Georges Bank were taxonomically identified by Gretchen Lambert (personal

Table 2. *Didemnum* samples from which DNA was extracted for this study. See Figure 2 for location of study areas.

Sample no.	Species	Station number	Study area	Region	Year collected	Collector	DNA sequence
1	*D. vexillum*	33	18	Georges Bank	2004	URI/GSO	C
2	*D. vexillum*	3	18	Georges Bank	2005	URI/GSO	C
3	*D. vexillum*	5	18	Georges Bank	2005	URI/GSO	C
4	*D. vexillum*	176	19	Georges Bank	2005	URI/GSO	C
5	*D. vexillum*	177	19	Georges Bank	2005	URI/GSO	C
6	*D. albidum*	111A	MB	Georges Bank	2005	URI/GSO	A
7	*D. albidum*	111B	MB	Georges Bank	2005	URI/GSO	B
8	*D. albidum*	133A	20	Georges Bank	2005	URI/GSO	B
9	*D. albidum*	133B	20	Georges Bank	2005	URI/GSO	A
10	*D. vexillum*	8	18	Georges Bank	2006	URI/GSO	C
11	*D. vexillum*	9	18	Georges Bank	2006	URI/GSO	C
12	*D. vexillum*	26	19	Georges Bank	2006	URI/GSO	C
13	*D. albidum*	67	MB	Georges Bank	2006	URI/GSO	A
14	*D. albidum*	83	MB	Georges Bank	2006	URI/GSO	A
15	*D. albidum*	104	20	Georges Bank	2006	URI/GSO	B
16	*D. albidum*	105	20	Georges Bank	2006	URI/GSO	A
17	*Didemnum* sp. B	NZ	n/a	Doubtful Sound, New Zealand	2006	Gretchen Lambert	D

communication) who also provided the sample of an undescribed species of *Didemnum* from New Zealand. Prior to extraction, 15-25 mg of *Didemnum* tissue was frozen overnight at -20ºC. Frozen samples were diced with a scalpel and pulverized with a pestle. The standard protocol for the Qiagen DNeasy Tissue Kit was followed for DNA extraction, except that we found extraction was improved by allowing samples to incubate in a 50ºC water bath overnight and eluting them with 50µl Buffer AE (instead of the recommended 200µl). DNA concentration and absorbance were measured with a NanoDrop ND-1000 Spectrophotometer.

A total of four different primer sets were used in this study. Two of the four primer sets were taken from Price et al. (2005), and the other two sets were designed in Integrated DNA Technologies (IDT) PrimerQuest software based on a *Didemnum* sp. sequence from GenBank (NCBI ID# AB211072). Each designed primer set consisted of both a reverse and forward primer. The primer sets ordered were F16 with R497 (Price et al. 2005), F476 with R917 (Price et al. 2005), F899 with R1617, and F1594 with R1988. Table 3 shows the sequences of all four primer sets.

The polymerase chain reaction (PCR) was used to amplify portions of the 18s rDNA gene. All PCR reactions contained 100-200 ng DNA, 1-2µl of the forward primer, 1-2µl of the reverse primer, 25µl of Qiagen Taq PCR Master Mix Kit, and enough water to bring the reaction to 50µl. Samples were loaded into an Eppendorf Master Cycler and run at 94ºC for 2 minutes, followed by 30 cycles of 94ºC for 30 seconds, 56ºC for 30 seconds, 72ºC for 30 seconds, and a final elongation step of 72º for 10 minutes. Samples were then run on a 5% poly-acrylamide gel to see if the amplification was successful. Each well contained 1µl of Promega Blue/Orange Loading Dye and 4-5µl of PCR product. A Fisher BioReagents 50bp Mini DNA Ladder (5µl per well) was used to estimate PCR product size. Gels were then stained with a solution of ethidium bromide and viewed on a UV transilluminator.

Prior to sequencing, all samples were purified with the Qiagen QIAquick PCR Purification Kit. The standard protocol was followed except we found that purification was improved if the samples were spun at 13,000 rpm instead of the recommended 10,000 *g* (~10,081 rpm). Total DNA concentration and absorbance were measured with a NanoDrop ND-1000 Spectrophotometer. Samples were sequenced at the University of Rhode Island Genomics Sequencing Center with an Applied Biosystems 3130xl Genetic Analyzer. F16 sequences were obtained for samples 1-17 from Table 2, while F16-R497, F476-R917, F899-R1617, and F1594-R1988 sequences were obtained for samples 6, 7, 11, and 17.

Table 3. Primer Sequences; F=forward, R=reverse.

Primer Name	Sequence
F16	AAGCCATGCAAGTGCAAGTACGAG
R497	CTGAAATGGGTAATTTGCGCGCCT
F476	GCGCGCAAATTACCCATTTCAGAC
R917	AACGACTCTCGACGTGCAACTT
F899	GTTCGCAGGTTGCCCTGAAGAAAT
R1617	ACCAGACGAATCGTTCCACGAACT
F1594	AGTTCGTGGAACGATTCGTCTGGT
R1988	GGTTTCGTTTGGTTTCACGAGCCT

Sequence analysis was performed with DNAStar Lasergene 7 software. Analysis began by performing a multiple sequence alignment (MSA) with the F16 sequences of samples 1-17. All MSA's were produced in MegAlign using the Clustal W method with default parameters. Sequences obtained from the four primer sets mentioned above for samples 6, 7, 11, and 17 were assembled in SeqMan and SeqBuilder to create a near-complete 18s sequence for each sample. These four sequences were imported into MegAlign where an MSA and similarity/diversity matrix was created.

Results

The sediment composition of the bottom photos was uniformly pebble gravel. All but five of the photos had pebbles as both the dominant and sub-dominant sediment categories (pebble-pebble). Of these five, three were pebble-sand, one pebble-boulder, and one boulder-boulder. The percent cover of *D. vexillum*, measured from the bottom photos, ranged from 0-100% in individual photos and between 0-79% when averaged within transects. The percent cover of hydroids, the second most abundant epifaunal taxon, was inversely correlated with the percent cover of *D. vexillum* (Figure 3). The Spearman

rank correlation coefficient (ρ) was -0.176 (p=0.002).

Likewise, the number of non-colonial macrofauna was inversely related to the percent cover of *D. vexillum* (Figure 4); the correlation coefficient was negative (ρ=-0.253) and highly significant (p<0.001).

The benthic macrofauna identified in bottom photo analysis included 21 different taxa, of which burrowing and non-burrowing anemones were the most numerous, followed by seastars and sea scallops (Figure 5).

The MDS analysis provided an acceptable ordination of the photo transects, which appears to be related to the percent cover of *D. vexillum* (Figure 6). Three clusters of transects are apparent in the MDS plot. The top cluster of three transects (2004) had almost no *D. vexillum* and high numbers of burrowing anemones. The five transects on the bottom right (2003, 2004) had the highest percent cover of *D. vexillum* and lowest macrofauna (8-20 animals per transect). The larger group of transects on the bottom left (2004) had intermediate levels of *D. vexillum* and macrofauna.

An analysis of the macrofaunal data with the BIOENV routine indicated that the similarity matrix was significantly correlated with the percent cover of *D. vexillum* (ρ=0.41, p=0.001). An ANOSIM test was performed which indicated that the year effect on the macrofaunal assemblage was significant (Global R=0.638, p=0.002). Therefore the MDS was repeated with the 2003 transects removed. With 2004 data only, the correlation between the macrofaunal assemblage and percent cover of *D. vexillum* was lower but still significant (ρ=0.21, p=0.03).

Naturalist dredge data

An MDS plot (Figure 7) revealed clear differences in benthic species composition between 1994-2001 (pre-*D. vexillum*) and 2002-2006 (post-*D. vexillum*). The benthic fauna from Area 19 (closed to fishing) is very similar to that from Area 18 (open to fishing) since colonization by *D. vexillum*. The ANOSIM analyses based on abundance data indicated a significant difference (Global R=0.357, p=0.001) between the species composition of the before and after samples.

The SIMPER test identified two polychaete species, *Nereis zonata* Malmgren, 1867 and *Harmothoe extenuata* Grube, 1840, as largely responsible for the change in species

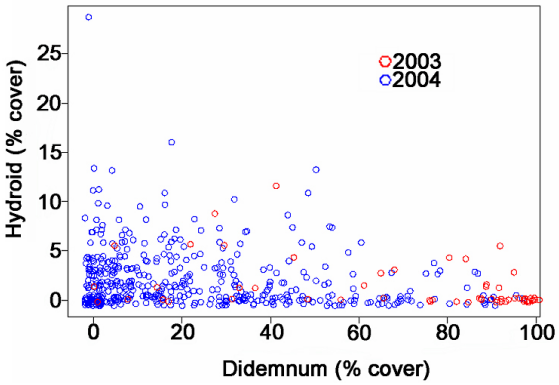

Figure 3. Percent cover of hydroids and *D. vexillum* on pebble gravel as observed in bottom photos from Area 18 in 2003 (red) and 2004 (blue). The points were jittered slightly to remove overlap.

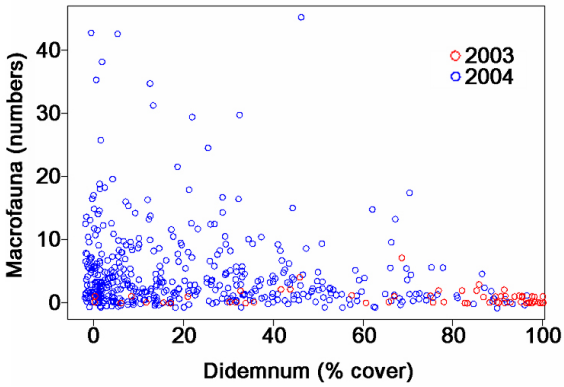

Figure 4. Number of free-living macrofauna and percent cover of *D. vexillum* in bottom photos from Area 18 in 2003 (red) and 2004 (blue). The points were jittered slightly to remove overlap.

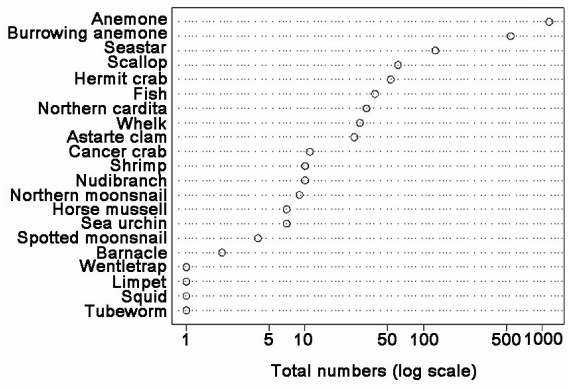

Figure 5. Total numbers of benthic macrofauna observed in bottom photographs from Area 18, sorted by abundance.

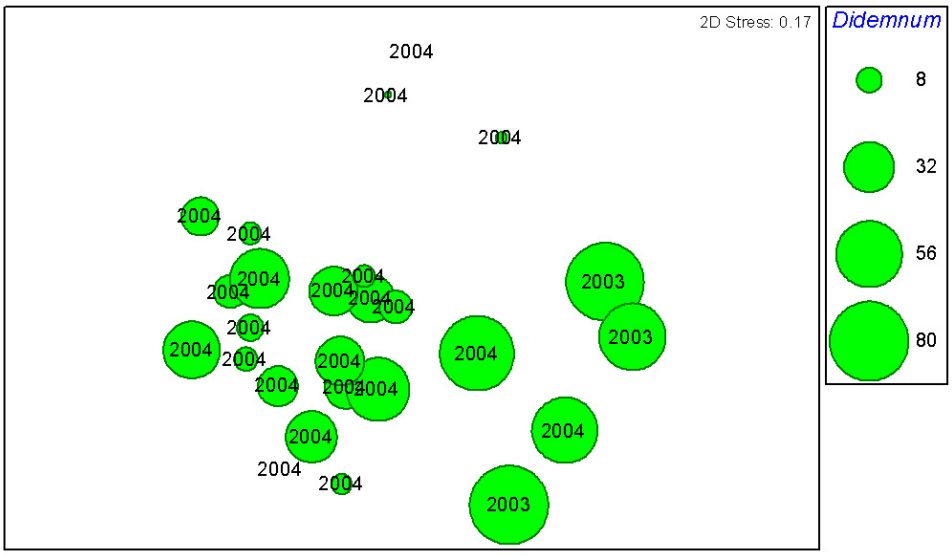

Figure 6. Non-metric Multi-Dimensional Scaling (MDS) plot of the abundance of 21 benthic macrofaunal taxa in 26 photo transects from Area 18. The label identifies the year (2003 or 2004). Bubble area is proportional to the percent cover of *D. vexillum*. See text for further explanation.

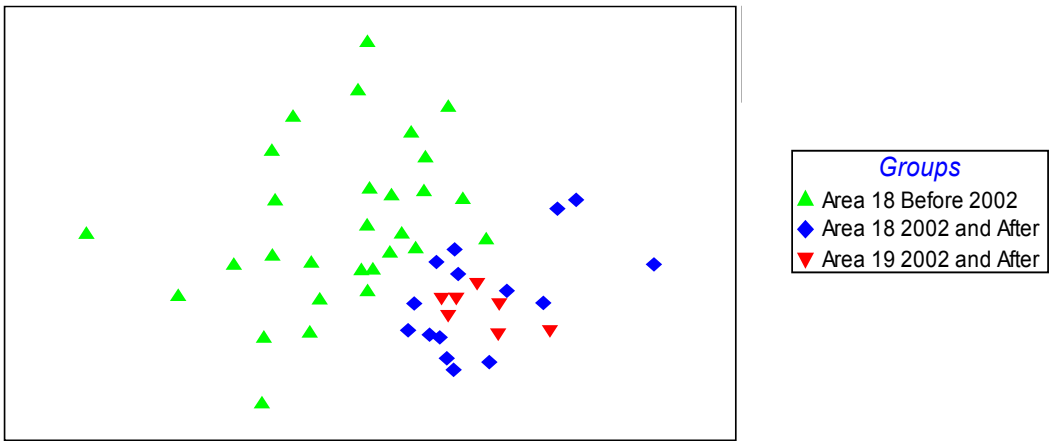

Figure 7. Non-metric Multi-Dimensional Scaling (MDS) plot based on the abundance data of 120 species in Naturalist dredge samples from Area 18 (open to fishing) and Area 19 (closed to fishing). *D. vexillum* appeared in 2002. An analysis of Similarity (ANOSIM) test indicated significantly different species compositions between groups sampled before and after the appearance of *D. vexillum* (p=0.001).

composition. The time×treatment interactions in the two-way ANOVAs were significant ($p<0.001$), indicating that the abundance of these two polychaete species increased significantly from 2002-2006 at Sites 18 and 19 relative to two reference areas (17 and 17W) without *D. vexillum* (Figure 8).

Genetic and taxonomic identification

Approximately 481 base pairs of the 18s gene for samples 1-17 were sequenced with the primer F-16. The MSA created from these sequences showed 4 distinct nucleotide sequences among samples 1-17. Samples 6, 9, 13, 14, and 16 all had the same sequence referred to as sequence A.

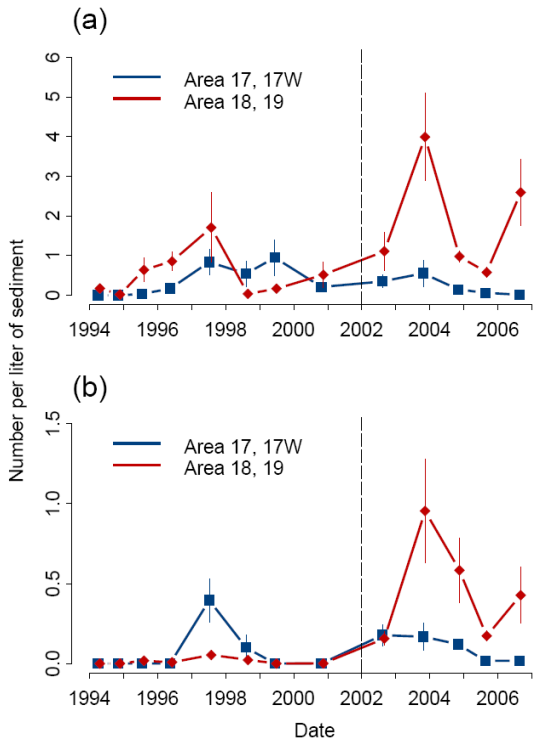

Figure 8. Number per liter of sediment of two polychaete species *Nereis zonata* (a) and *Harmothoe extenuata* (b) collected in Naturalist dredge samples from Areas 18 and 19 colonized by *D. vexillum* and Areas 17 and 17W not colonized by *D. vexillum*. Vertical lines are 95% confidence intervals and vertical dashed lines indicate when *D. vexillum* infestation began in Areas 18 and 19.

Samples 7, 8, and 15 all had the same sequence referred to as sequence B. Samples 1, 2, 3, 4, 5, 10, 11, and 12 all had the same sequence referred to as sequence C. Finally, sample 17 had it's own unique sequence referred to as sequence D. From these four groups, samples 6, 7, 11, and 17 were chosen as representative samples based on the amount of extracted DNA required for further sequencing. Near-complete 18s rDNA sequences (~2000 bps) were obtained for sample 6 (sequence A), sample 7 (sequence B), sample 11 (sequence C), and sample 17 (sequence D). GenBank accession numbers for sequences A, B, C, and D are EU337058, EU337059, EU337060, and EU337061, respectively. A diversity/similarity matrix created from the MSA of sequences A, B, C, and D indicated that sequences A and B were highly similar to each other, with a divergence of 3.7 percent. By contrast, sequences C and D were less closely related, with a divergence of 8.2 percent (Table 4). When the two pairs of sequences (A and B vs. C and D) were compared, they had an average divergence of 13.78 percent.

Figure 9. Colony of *D. vexillum* encrusting pebble gravel habitat and hydroid colony on Georges Bank. Collected November 1, 2003, by: Jeremy Collie, Page Valentine, and Robert Reid. Photo credit: Dann Blackwood, U.S. Geological Survey. Image represents an area of seabed measuring 76 x 51 cm.

Table 4. Diversity and similarity of sequences A, B, C, D of the 18s rDNA gene. The upper triangle contains the percent similarity and the lower triangle the percent divergence among pairs of sequences.

Sequence	A	B	C	D
A		96.3	86.9	87.2
B	3.7		87.5	87.9
C	14.3	13.6		92.2
D	14.0	13.2	8.2	
Sample no.	6	7	11	17

Samples 13 and 15 from Georges Bank belonging to sequences A and B were taxonomically identified as *Didemnum albidum*, a relatively rare species native to the east coast of North America (G. Lambert, personal communication). Sample 3 belonging to sequence C from Georges Bank exhibits all the physical characteristics of the invasive *D. vexillum*, although gonads and brooding larvae were not present in the specimens that we sequenced. We believe it is the invasive *D. vexillum* because earlier samples from this site were confirmed as *D. vexillum* and we found only one genotype at this site. Sample 17 from New Zealand belonging to sequence D was identified as an unidentified species of *Didemnum,* but not *D. vexillum*, from New Zealand.

Discussion

Analysis of video and photo transects showed that as of 2007, *D. vexillum* continues to thrive on Georges Bank. Preliminary analyses of these data show that although the percent cover appears to be decreasing in Area 18, it is increasing in Area 19 inside Closed Area II. Both areas have a pebble gravel substrate which has been shown to be important in the life cycle of juvenile Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) (Lough et al. 1989) and is important in the settlement of sea scallop (*Placopecten magellanicus*) larvae (Thouzeau et al. 1991). Given that Area 18 is heavily dredged for scallops, it is highly likely that fragmentation of colonies occurs, which facilitates further spread of the tunicate. In Area 18, *D. vexillum* has been seen in bottom photos encrusting many bivalves including sea scallops. Therefore any scallop shells from this area discarded overboard in un-infested areas after scallops are processed, could potentially aid in the spread of the tunicate as well. Spreading through this method could be reduced by the adoption of better fishing practices.

Detailed analysis of the bottom photographs suggests that *D. vexillum* is able to out-compete other epifaunal and macrofaunal taxa. For example, the tunicate is often observed overgrowing hydroids in the bottom photographs (Figure 9). Interestingly, anemones were observed at high (10-339 individuals) densities in transects with moderately high percent cover of *D. vexillum* (13-45%). Anemones are one of the few groups of animals that appear able to resist overgrowth by the tunicate.

Our analysis of the dredge samples shows that the invasion of *D. vexillum* has significantly changed the species composition of the benthic community over time and confirms the results of our preliminary studies (Valentine et al. 2007). This change in species composition is due to the significant increase in the abundance of two polychaete species, *Nereis zonata* and *Harmothoe extenuata*. These increases suggest that *D. vexillum* is acting as a facilitator by creating a habitat that is more favorable to these two polychaete species. While little is known about the behavior of these two polychaetes, it is possible that the tunicate mat provides protection from predators, allowing the dramatic increase in their abundance. Stomach-content analysis of several groundfish species native to Georges Bank has shown that polychaetes are an abundant prey item (Bowman et al. 2000). Although these polychaetes have increased significantly, they may not be accessible to bottom feeders through the dense tunicate mat. We have observed tunicate fragments in the stomachs of several species of demersal fish, but it is unlikely that the fish derive nutritive value from this feeding (Valentine et al. 2007).

Our genetic analysis confirms the usefulness of the 18s rDNA gene in distinguishing different species of *Didemnum* (Swalla et al. 2000; Stach and Turbeville 2002). The genetic analysis and taxonomic identification have shown there are at least two species of *Didemnum* on Georges Bank, one of which is the invasive *D. vexillum* and the other *D. albidum*, a relatively rare native to the northeast United States.

Based on our findings, we suggest that future research be focused on the spread and impacts of the invasion, developing management strategies, and creating outreach programs to the commercial fishing industry to limit its spread.

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