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Research article

Temporal and spatial development of an infestation of *Styela clava* on mussel farms in Malpeque Bay, Prince Edward Island, Canada

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Abstract

The first confirmed report of the invasive solitary ascidian *Styela clava* (clubbed tunicate) in the blue mussel (*Mytilus edulis*) production area of March Water in Malpeque Bay, Prince Edward Island (PEI), occurred in September of 2002. Mussel farms in eastern PEI waters have been challenged with heavy infestations of this tunicate since 2000, causing both production and processing problems for the industry. A multi-year study was initiated in June of 2003 to document the spread of *S. clava* within the mussel producing areas of Malpeque Bay. The study design consisted of the establishment of a series of 4 transect lines extending outwards from the initial area of detection and the subsequent deployment of PVC collector plates at predetermined distances. Collection plates were retrieved in late fall of each of 4 study years, and *S. clava* specimens were quantified for abundance and body length. Mean recruitment levels of *S. clava* per collector increased from 0.4 individuals in 2003 to 370.8 in 2006. By November 2006, the geographical spread within Malpeque Bay reached approximately 12 km from the initial area of detection. This study demonstrated that within 3 years of detection, a few individual *S. clava* expanded to a population at nuisance levels for the mussel industry of PEI.

Key words: invasion process, mussel production, recruitment, spatial, *Styela clava*, temporal

Introduction

In September 2002, mussel producers in the March Water area of Malpeque Bay, Prince Edward Island (PEI) reported a low abundance of the clubbed tunicate (*Styela clava* Herdman 1882) on their mussel crop. *Styela clava* is a solitary, hermaphroditic ascidian native to the northwestern Pacific waters of Japan, Korea and China (Millar 1960; Lützen 1999; Dupont et al. 2006) and is considered to be an aggressive invader throughout the world (Clarke and Therriault 2006). Fertilization occurs externally (Svane and Young 1989), resulting in larvae which are planktonic for ~ 24 hours before attaching to a hard substrate (Svane 1984). Recruitment occurs in PEI waters from late June to late October, with maximum recruitment occurring in late September (Bourque et al. 2007). Although present in several other mussel production areas of PEI since 1998, this exotic tunicate had not previously been detected in March Water. This ascidian species was initially identified on a mussel farm in the Brudenell River in January 1998 and by 2001 had spread to several additional areas in the southeastern waters of PEI (Thompson and MacNair 2004). Each new detection of *S. clava* in mussel producing estuaries of eastern PEI rapidly developed into a substantial infestation within 2 to 3 years, resulting in increased costs associated with growing, harvesting and processing the mussel crop (Thompson and MacNair 2004; Canada DFO 2006).

Successful establishment of *S. clava* into new areas depends largely on conditions affecting its growth and reproduction (Minchin et al. 2006). Necessary factors enabling *S. clava* to flourish include the availability of hard substrate for attachment (Lützen 1999) combined with an abundance of phytoplankton as a food source (Locke et al. 2007). The infrastructure in the water column associated with PEI mussel farms (Bourque et al. 2007), combined with the nutrient rich estuarine waters of PEI (Locke et al. 2007), provided optimal conditions for establishment and population growth of this ascidian species to unprecedented levels (G. Lambert, University of Washington Friday Harbor Laboratories, pers. comm.).

The invasion process leading from the initial establishment of *S. clava* on the mussel farms on eastern PEI from 1998 to 2002 was not well documented. During this time the primary focus was on the development of management strategies to reduce the impacts of this new invader (Davidson et al. 2005). The detection of *S. clava* in March Water in the fall of 2002 presented an opportunity to document the establishment and subsequent spread of a new exotic tunicate in a mussel growing area from a very early stage in the invasion process. This study was initiated early in the summer of 2003 with the aim of documenting the invasion process over a 4 year period. The results will provide insight into predicting the rate of establishment of a newly detected exotic species and can be utilized for the selection and evaluation of mitigation strategies to prevent or impede the spread affecting mussel aquaculture (Johnson and Padilla 1996).

Methods

Study site

This study was conducted in the March Water area of Malpeque Bay, located on the north-western coast of PEI, Canada, 46.51432°N, 63.73565°W (Figure 1). Since 1998, the March Water area has been used extensively for mussel production (DFO Leasing Division, Charlottetown, PEI. pers. comm.), utilizing the long-line culture method. This area now supports approximately 500 ha of production leases. The mean water depth is 4.5 m and it is usually ice-covered from December to April each year. The average salinity is ~ 28 ‰ and water temperatures range from about 6°C to 26°C from mid-June to early November.

Experimental design

Following the initial detection of *S. clava* in September 2002, a comprehensive SCUBA diving survey of all the mussel leases in the March Water area was conducted to determine the extent of this new infestation. This initial survey resulted in determining the epicentre of the original infestation. A series of 4 transect lines were plotted extending from the epicentre,

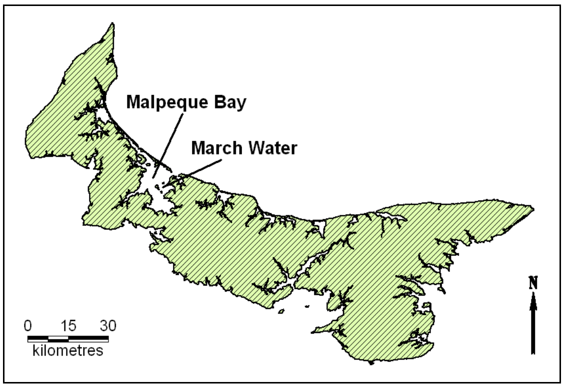

Figure 1. Location of Malpeque Bay (with March Water in northeast), Prince Edward Island.

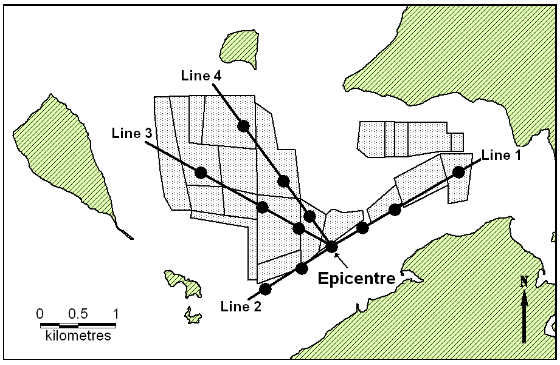

Figure 2. Location of transect lines and collector deployments in March Water. Line 1 (excluding the most eastern collector) and line 2 were considered adjacent to the mussel leases. Lines 3 and 4 were considered within the mussel leases.

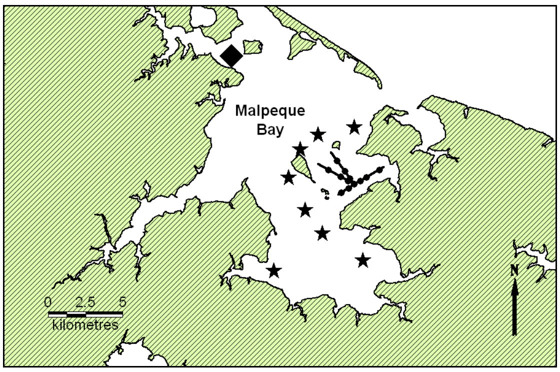

Figure 3. Additional site locations (stars) within Malpeque Bay for the final study year. In September, 2006 *S. clava* was detected 12 km to the northwest (diamond).

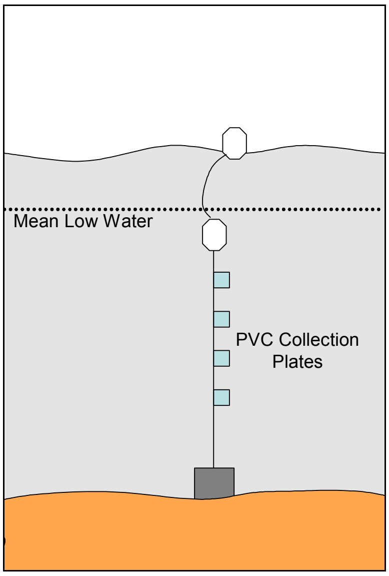

Figure 4. Schematic diagram of collector deployment. Collector size was 10 cm × 10 cm.

2 of which were immediately adjacent to the mussel leases and 2 positioned within the mussel leases (Figure 2). In mid-June of each study year, collectors were deployed at sites on the transect lines and left in the water until early November, corresponding to the recruitment phase of *S. clava* (Thompson and MacNair 2004; Bourque et al. 2007). In 2003, collector plates were deployed at the epicentre and at distances of 0.5 km and 1.0 km on each of the 4 transect lines. An additional set of collectors was added to transect lines 1, 3 and 4 at 2.0 km from the epicentre during 2004 - 2006. During the final study year (2006), several additional sets of collectors were deployed at distances extending 4 km from the epicentre on each of the 4 transects in addition to several other sites in Malpeque Bay to monitor further potential spread outside of the March Water study area (Figure 3). All of these additional collectors were at least 1.5 km distant from the mussel leases in March Water. Collector sets were assembled using a concrete block for an anchor and attaching 4 (10 cm × 10 cm) dark grey PVC plates to 7 mm polypropylene rope, as described in Bourque et al. (2007). This array was supported with a 20 cm buoy placed at 0.5 m below Mean Low Water (MLW) as described in Wyatt et al. (2005), and with a rope leading to a surface buoy for retrieval (Figure 4). The PVC plates were attached to the rope vertically using cable ties at 0.5 m intervals, resulting in plates located in the water column at 1 m, 1.5 m, 2.0 m and 2.5 m from the surface at MLW.

Laboratory analysis

Annual recruitment was quantified at the end of each study period (early November) by removing, counting and measuring body lengths of individual *S. clava* specimens from each collector. Body lengths were determined using electronic calipers to measure the distance from the most anterior surface of the specimen to the surface of the holdfast.

Statistical analysis

Statistical analysis was performed using SigmaStat 3.0.1 and included Kruskal-Wallis One Way Analysis of Variance on Ranks to compare annual *S. clava* recruitment between each study year, distance from the epicentre, proximity to mussel leases and depth of collector in the water column. *Styela clava* body length was also compared between each study year. All estimates are recorded as mean ±SE.

Results

Dive survey

Fewer than 15 individual *S. clava* specimens were detected during the SCUBA diving survey of all mussel leases in October 2002. These specimens were concentrated within a small section in the southeastern part of the mussel leases within a diameter of approximately 100 m. This area is referred to as the epicentre of the initial introduction.

Analysis of collectors

The mean annual *S. clava* recruitment per collector for all sites combined during each study year increased significantly ($P < 0.001$) from 0.4 ±0.2 individuals in 2003 to 3.4 ±1.0, 77.7 ±10.7 and 370.8±42.9 for the years 2004, 2005 and 2006, respectively (Figure 5). Recruitment between sites based on distance from the epicentre combined for all years were not significantly different ($P>0.5$) between the epicentre, 0.5 km, 1.0 km and 2.0 km, respectively (Figure 6). Collectors located within mussel leases had significantly higher ($P<0.002$) recruitment at 198.9±30.5 compared to 65.2±14.4 for collectors deployed adjacent to mussel leases (Figure 7). Depth of collectors within the water column did not significantly affect recruitment (P > 0.5), with mean values of 148.5±42.0, 139.0±36.9, 124.3±31.1 and 129.7±35.7 for depths of 1.0 m, 1.5 m, 2.0 m and 2.5 m, respectively (Figure 8). Mean *S. clava* body length increased from 17.6±8.2 mm to 29.7±2.9 mm and 30.6±1.5 mm for the years 2003, 2004 and 2005, respectively. The mean body length in 2006 of 40.9±1.4 mm was significantly higher (P<0.001) than the 3 previous years (Figure 9).

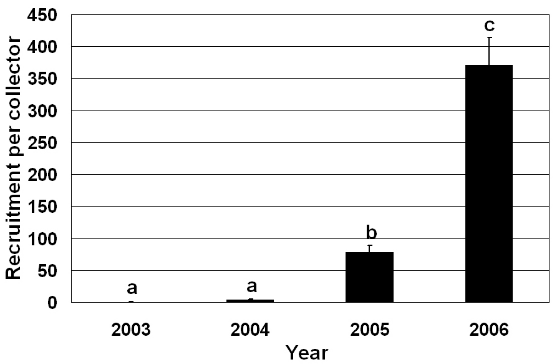

Figure 5. Mean annual recruitment compared by year (P<0.001). Significant differences in recruitment between years are indicated by different letters.

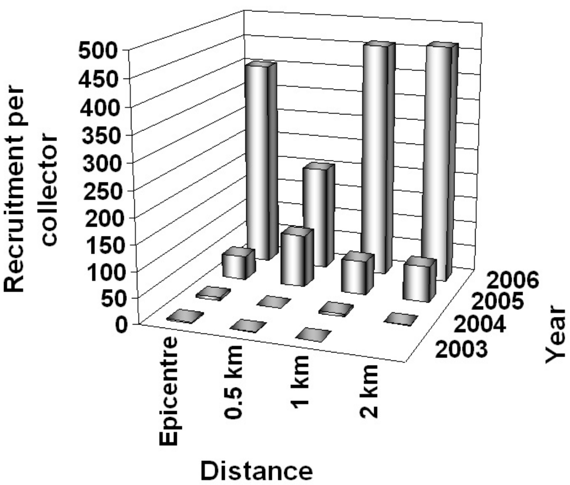

Figure 6. Mean annual recruitment compared by distance from epicenter.

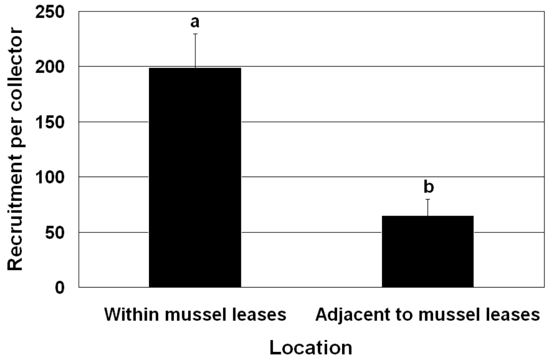

Figure 7. Mean recruitment per collector for all years combined compared by proximity to mussel leases (P<0.002). Significant differences in *S. clava* recruitment are indicated by different letters.

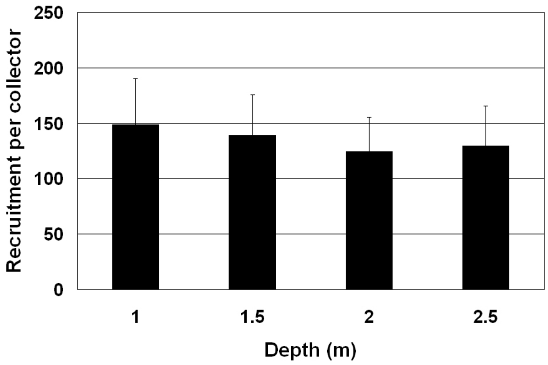

Figure 8. Mean annual recruitment compared by depth in water column (P>0.5).

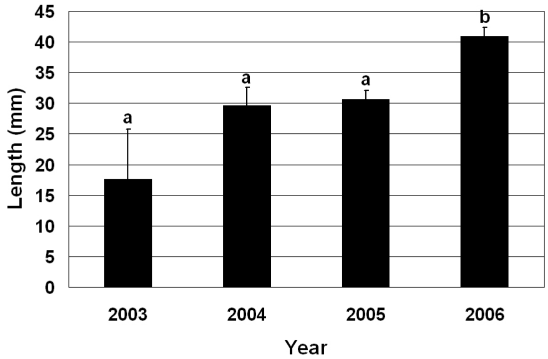

Figure 9. Mean annual *S. clava* length compared by year (P<0.001). Significant differences in length between years are indicated by different letters.

Styela clava recruitment was observed on only three of eight collector sets deployed outside of the March Water study area during the 2006 study period. These collectors were located at a distance of 4 km on transect lines 3 and 4 (Figure 2) and at the site 4 km to the north of the epicentre (Figure 3), and had a mean recruitment of 279.4 ± 41.1, 173.8 ± 21.5 and 42.0 ± 6.8, respectively. The combined mean recruitment of these collectors was 161.9 ± 29.5, which was significantly less ($P<0.001$) than the recruitment within the March Water mussel leases (483.5±60.22) and not significantly different than the recruitment on the collectors adjacent to the leases (213.1±38.6).

In early September 2006, the authors observed low abundances of *S. clava* on mussel leases located on the far side of Malpeque Bay, 12 km northwest of the March Water study area (Figure 3).

Discussion

The results of this study suggest that the exotic species *S. clava* can readily establish itself within the context of a long-line mussel culture operation, and develop into a full scale infestation within 3 years of initial detection. What began with a few individual *S. clava* specimens in the fall of 2002 soon manifested as a thriving population by 2004 and developed to a nuisance invader, creating serious problems on mussel farms in March Water by 2005. By September 2007, this invasive ascidian was entirely overgrowing untreated mussel socks and gear (Figure 10).

Figure 10. Heavy infestation of *S. clava* on mussel socks and gear in September, 2007.

Study results showed significant increases in annual mean recruitment on PVC collectors by factors of approximately 10, 20 and 5 as compared to the previous year during 2004, 2005 and 2006, respectively. The successful establishment of the *S. clava* population in Malpeque Bay was largely dependent on the availability of suitable habitat, namely hard substrate, which would not have been available without the infrastructure present within the mussel leases (Bourque et al. 2007). Significant differences in recruitment were not demonstrated between site distances from the epicentre but rather between site locations within or adjacent to mussel leases. The collectors located adjacent to the mussel leases which were not entirely surrounded by substrate would not have provided as much habitat and propagule pressure as the collectors positioned within the mussel leases. These factors most likely resulted in a larger inoculant pool within the leases, increasing the chance of gametes encountering each other and developing into larvae (Carlton 1996).

It is evident from study observations that factors other than habitat and propagule pressure also play a significant role in the temporal and spatial spread of this tunicate species. Collectors located at 4 km from the epicentre demonstrated only slightly less recruitment than collectors positioned adjacent to the mussel leases during 2006, even though they were located more than 1.5 km away from the nearest lease and were in areas of mud or sandy bottom with limited hard substrate present. These collectors were located north and northwest of March Water and in close proximity to the channel at the entrance of Malpeque Bay. Interestingly, several other collectors to the west and south and at similar distances from the epicentre revealed no recruitment in 2006 which further demonstrates the sporadic nature of the invasion process in this area.

Prevailing winds during the reproductive phase of *S. clava* are from the south-southwest (Environment Canada 2008) and may have been a factor facilitating the movement of the propagules generated from the established population within the mussel leases, to the collectors outside of the March Water area. Another variable which may have contributed to this dispersal is water flow patterns in this area of Malpeque Bay. Both of these factors warrant future investigation which would provide insight leading to a better understanding of the invasion process of a new exotic tunicate species.

The source of the *S. clava* population detected on the mussel farms on the opposite side of Malpeque Bay in 2006 is unknown. Considering that *S. clava* adults are sessile and all natural dispersal takes place at the gamete and larval stages (Lambert 2005) and that *S. clava* larvae are only viable for ~ 24 hours in the water column, it is questionable whether this introduction was caused by natural dispersal from the March Water population 12 km away. Other potential sources for this introduction are movement of boats, gear or product associated with the aquaculture industry or commercial fisheries within Malpeque Bay.

Accurate prediction of spread and densities of a new invasive tunicate is confounded by several factors including: influence of oceanographic currents and tides; meteorological events such as prevailing winds or storm events; environmental factors including water temperature, water quality, nutrient levels, food quality and availability; and anthropogenic activity, including management and mitigation practices on mussel farms within an affected area.

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