Proceedings of the 3rd International Invasive Sea Squirt Conference, Woods Hole, USA, 26–28 April 2010

Research Article

Predicting larval dispersal of the vase tunicate *Ciona intestinalis* in a Prince Edward Island estuary using a matrix population model

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Editor's note:
This paper is a contribution to the proceedings of the 3rd International Invasive Sea Squirt Conference held in Woods Hole, Massachusetts, USA, on 26–28 April 2010. The conference provided a venue for the exchange of information on the biogeography, ecology, genetics, impacts, risk assessment and management of invasive tunicates worldwide.

Abstract

Despite sporadic observations of the vase tunicate, *Ciona intestinalis*, on boats and mooring structures in Charlottetown Harbour, Prince Edward Island, the species has not established a population in the harbour nor dispersed the ~12 km downstream to Hillsborough Bay, an important source of blue mussel (*Mytilus edulis*) spat for the PEI aquaculture industry. A population matrix model used in conjunction with an oceanographic model suggests that advection of larvae from the harbour to the spat production area requires more than one or two generations, and the use of intermediate settlement nodes, such as navigational aids and aquaculture sites located in the upper part of Hillsborough Bay, as 'stepping stones'. Maintaining potential settlement nodes in a tunicate-free condition could delay oceanographic dispersal of *C. intestinalis* within the estuary. According to observations of colonial tunicate dispersal in 2010, most likely originating from colonies established in the same locations where *C. intestinalis* inoculations have been detected, dispersal was occurring in the vicinity of one of the nodes identified by the model as priorities to be monitored for early detection of tunicate settlement, but had not yet reached the other node. A major finding is that the dispersal of solitary tunicates by oceanographic processes, often considered uncontrollable, is evidently amenable to management through monitoring and cleaning of the intermediate settlement nodes.

Key words: ascidian, dispersal model, oceanographic model, invasive species, early detection, management, monitoring

Introduction

The blue mussel (*Mytilus edulis* Linnaeus, 1758) aquaculture industry in Prince Edward Island (PEI), Canada, supplies 80% of the blue mussels in the North American market (DFO 2006), and is adversely affected by invasive tunicates, especially the vase tunicate *Ciona intestinalis* (Linnaeus, 1767). Tunicate fouling on suspended aquaculture equipment in PEI has increased production and processing costs for blue mussels due to the need for control measures, and the

logistical and personnel requirements to manage the increased weight and volume of tunicate-fouled equipment (Locke et al. 2007; Ramsay et al. 2009). Consequently, intensive management of certain anthropogenic vectors of tunicate dispersal has been undertaken with the goal of preventing spread of tunicates into estuaries not currently infested (Locke et al. 2009). Vectors associated with bivalve aquaculture industries have been the most amenable to management using existing regulatory authorities (National Code on Introductions and Transfers of Aquatic Organisms 2003).

Figure 1. Map of Prince Edward Island, Canada, showing the location of Hillsborough Bay, place names mentioned in the text, and study area (box).
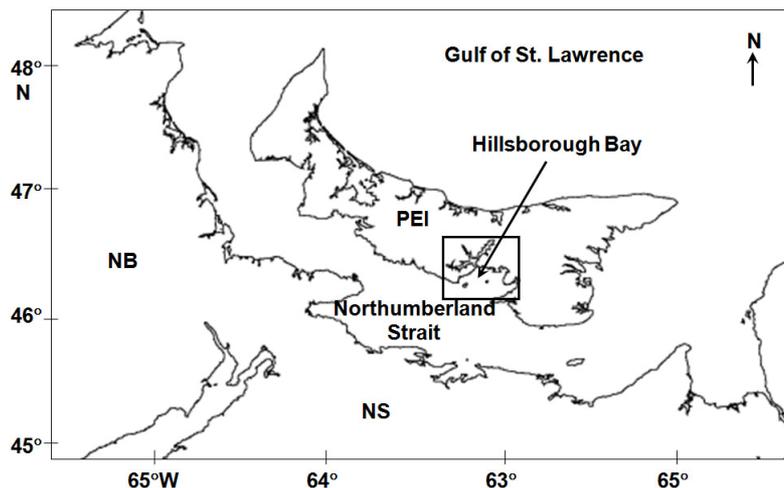
Tunicate management in PEI, through the Introductions and Transfers permitting process, has been successful in limiting or at least slowing the spread of solitary tunicates between estuaries, but does not address the issue of dispersal of tunicates within an estuary (Locke et al. 2007). Nor does the permitting process address vectors outside of the aquaculture industry, whether anthropogenic (e.g., boating, shipping and fishing), or natural (e.g., advection by oceanographic currents). Advection is often assumed by scientists, resource managers and aquaculture growers to be an uncontrollable vector, particularly at the within-estuary scale (Locke, pers. obs.). Moreover, it has sometimes been suggested that, if oceanographic vectors are expected to transport a species to a given area, attempting to control dispersal by other vectors to that area is at a best a delaying tactic and at worst a wasted effort (Locke, pers. obs.). Such an assumption serves to discourage the development of management strategies for potentially controllable anthropogenic vectors - after all, why bother to invest the time and resources to protect an uninfested area if the species will be carried there by natural processes anyway.

The potential for intra-estuarine dispersal of tunicates by natural processes is of particular concern in the Hillsborough Bay tidal system, including Charlottetown Harbour and the connected Hillsborough Bay (Figure 1). *C. intestinalis* has been observed several times on boats or wharves in Charlottetown Harbour, but is not yet established either in the harbour or in the vicinity of Nine Mile Creek, located in Hillsborough Bay, approximately 12 km from the harbour (Locke, unpub. data). Nine Mile Creek is an important production area for mussel spat, which is collected by the aquaculture industry and distributed to mussel aquaculture leases in estuaries throughout PEI. However, Nine Mile Creek is located "downstream" from Charlottetown Harbour and might be at risk as a receiving zone for tunicate propagules dispersed on falling tides. Under PEI's tunicate management policies, because solitary tunicates are presently absent from Hillsborough Bay, mussel spat produced in the Nine Mile Creek area can be transferred to any estuary in PEI. If *C. intestinalis* were to become established in Hillsborough Bay, only estuaries that already contained *C. intestinalis* would be allowed to receive the spat, potentially resulting in shortages of supply for the other estuaries. Consequently, Hillsborough Bay is monitored in autumn for the presence of *C. intestinalis* before mussel spat transfers are permitted.

C. intestinalis produces short-lived planktonic larvae (Table 1), but their transport by tidal currents is arguably the least manageable of the vectors that could disperse *C. intestinalis* in Hillsborough Bay. Understanding the role of the oceanographic vector in larval transport from Charlottetown Harbour to Hillsborough Bay could help inform decisions about the management of anthropogenic vectors. Understanding the patterns of larval dispersal from

Table 1. Duration of egg and larval development of *Ciona intestinalis* related to water temperature. Eggs decompose if not fertilized within 30 h (Carver et al. 2003). The potential duration of drifting unfertilized eggs therefore ranges from 0 (for eggs retained in mucus strings) to the tabled value (for eggs spawned without mucus strings and which drift throughout the pre-fertilization period). Duration of drifting embryonic development (period between egg fertilization and hatching) varies between 0 (for eggs retained in mucus strings) to the tabled value for eggs that drift throughout this period. Duration of tadpole larval phase ranges from 0 (for larvae retained in mucus strings) to tabled value (for eggs spawned without mucus strings or where the larvae have escaped from mucus strings). It is assumed that larvae are competent to settle as juveniles immediately upon hatching.

Temperature (C)	Duration of egg drift until fertilization (h)	Duration of drifting embryonic development (h)	Duration of drifting or swimming tadpole larval phase (d)	Location of study	Reference
9	--	63	--	Scandinavia	Svane and Havenhand 1993
10-12	--	48	4-5	Scandinavia	Dybern 1965
15	--	24-36	1	Nova Scotia	Carver et al. 2003
15	--	27-33	<1-5	Scandinavia	Havenhand and Svane 1991
16	30	26	--	Scandinavia	Svane and Havenhand 1993
18	--	12	--	Japan	Na and Lee 1977
18-20	--	18	--	New England	Cirino et al 2002
18-20	--	<24	1-1.5	Scandinavia	Dybern 1965
20	--	22	--	New England	Bullard and Whitlatch 2004
Not reported	--	--	0.5-1.5	Britain	Millar 1952
Not reported	--	--	6	Scandinavia	Svane and Young 1989

Charlottetown Harbour could also assist in designing more effective monitoring programs for tunicates.

We constructed a matrix population model of *Ciona intestinalis* and combined it with an oceanographic model of the Hillsborough Bay area to predict population growth and spread from a hypothetical inoculation in Charlottetown Harbour. A matrix model uses the life cycle of an organism in order to describe the dynamics and stage-dependent structure of the population (Caswell 2001). We examined the role of currents and the distribution of settlement structures in the system to predict the rate and direction of movement of the tunicate throughout the system and to determine whether management of settlement substrates might slow this dispersal. Tunicate occurrences on navigation buoys in Hillsborough Bay and Charlottetown Harbour were investigated at the end of the 2010 growing season as a test of the model predictions.

Methods

Study area and oceanographic model

The Hillsborough Bay watershed is an estuary located on the south shore of PEI, draining three major rivers (Hillsborough, North and West rivers) through Charlottetown Harbour into Hillsborough Bay, which in turn flows into the Northumberland Strait. The domain considered in the mathematical model was bounded by latitudes 46°24′N and 46°03′N, and longitudes 62°51′W and 63°20′W. This area includes Charlottetown Harbour and the lower reaches of the tidal rivers and extends to the mussel spat collection area in Hillsborough Bay near Nine Mile Creek (Figure 1).

Ocean current data were obtained from a three-dimensional numerical hydrodynamic circulation model that covers the whole of Northumberland Strait with a horizontal resolution of 200 m. It is nested within the Gulf of St. Lawrence Ocean Model, a model covering the Gulf of St. Lawrence and Scotian Shelf with a horizontal resolution of 4 km (Chassé and Miller 2010). The Northumberland Strait model has eight vertical layers with finer resolution (2m) close to the surface. The model makes use of partial cells, so that water depth in the deepest layer is equal to the actual depth at that location. It includes a turbulence closure scheme and advection-diffusion equations for the temperature and salinity fields. Environmental forcing was incorporated by using the National Centers for Environmental Prediction reanalysis data provided by the U.S. National Oceanic and Atmospheric Administration- Cooperative Institute for Research in Environmental Sciences Climate Diagnostics Center (Boulder, Colorado,
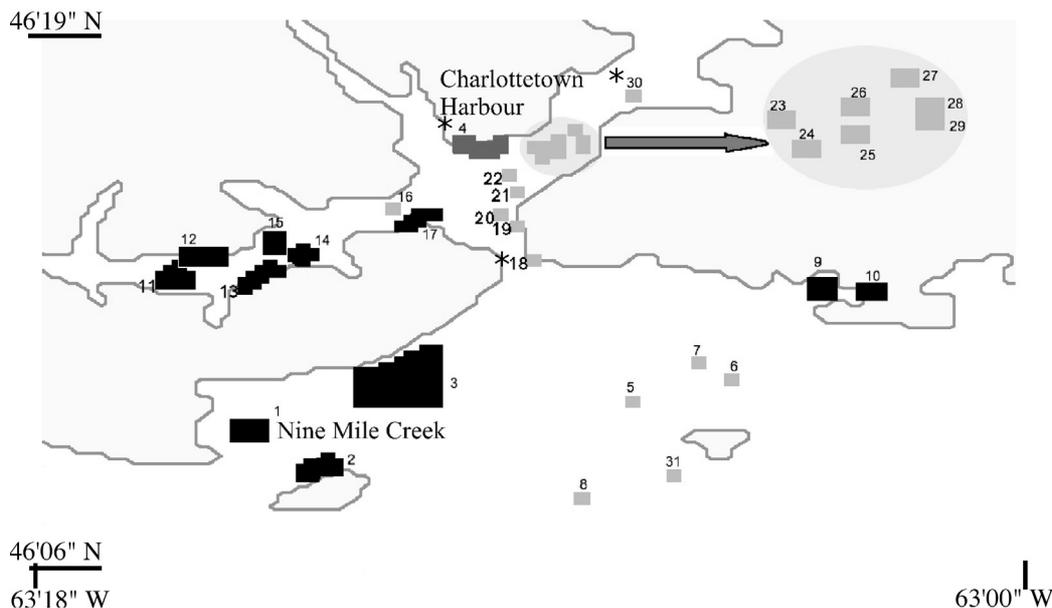
Figure 2. Areas of suitable substrate ('nodes') for settlement of *Ciona intestinalis* within Hillsborough Bay. Black areas represent aquaculture leases, grey areas represent buoys, and the one dark grey area (node 4) represents a harbour. Nodes are numbered for tracking purposes. Nodes where *C. intestinalis* have been detected are marked with an asterisk.

USA) and includes the wind, air temperature, cloud cover and precipitation rate from which the heat fluxes were calculated. The large scale model is also forced by the tides coming from the Atlantic and by the runoff from larger rivers entering the system; their signals propagate to the high resolution nested model through the boundaries. The model is initialized with climatological temperature and salinity fields for the starting day of the simulation. It uses hourly physical inputs and one-minute time steps to solve the momentum equations and the advection-diffusion relationships for temperature and salinity.

The domain was divided into blocks corresponding to the horizontal resolution of the oceanographic model within the Hillsborough Bay watershed (200 m × 200 m), forming a grid of 195 blocks in the east-west direction by 190 blocks in the north-south direction. The oceanographic model generates velocities for every grid point, in each east–west and north-south direction, at each hour for the top 2 m surface layer. The time frame for generating average transition matrices (discussed later) for the model was 8 August 8 to 9 October 2005,

giving a total of 103,147,200 velocities at each of the 37,050 gridpoints.

Substrates used by *Ciona intestinalis* for settlement include docks, moored aquaculture equipment, and navigational and mooring buoys (Petersen and Svane 1995; Locke and Nadeau, pers. obs.). Hillsborough Bay contains at least 31 distinct patches of potential settlement area, identified in the model as nodes (Figure 2, Table 2). The submersed surface area for settlement presented by navigational buoys was obtained from the Canadian Coast Guard (A. Smith, Fisheries and Oceans Canada, pers. comm.). Underwater surface area of floating wharves was conservatively estimated as the product of length and width of the wharf. The surface areas available at aquaculture lease nodes were estimated by multiplying the surface area of a sock by mussel sock density within the lease by the area of each lease. Midpoints of the ranges of values surveyed in PEI mussel farms by Drapeau et al. (2006) were used: for sock density, 93.05 socks/100 m^2 (range 6.2 to 179.9 socks/100 m^2) and for sock length, 2.1 m (range 1.2 to 3.0 m). We estimated the diameter of each mussel sock as 25 cm, slightly larger than the 20 cm used by

Table 2. Characteristics of settlement nodes used in the model of Hillsborough Bay. Node types are L (aquaculture lease), B (buoy) and D (dock). For buoys, the site and buoy number are indicated in parentheses (corresponding to Canadian Coast Guard (2009), and Canadian Hydrographic Service Chart Nos. 4460 – Charlottetown Harbour and 4466 – Hillsborough Bay.

Node	Node Type	Node Surface Area (m^2)
1	L	635,211
2	L	635,211
3	L	412,874
4	D (downtown Charlottetown docks)	18,000
5	B (Spithead buoy C5)	54
6	B (Squaw Point buoy B)	54
7	B (Squaw Point buoy A)	54
8	B (Fitzroy Rock buoy C4)	54
9	L	476,408
10	L	317,605
11	L	529,342
12	L	529,342
13	L	476,408
14	L	264,671
15	L	317,605
16	B (North Point entrance to West River buoy CH4)	54
17	L	370,539
18	B (Blockhouse Point buoy C7)	54
19	B (Battery Point buoy C8)	54
20	B (Canceaux Spit buoyC9)	54
21	B (Rosebank Point Outer buoy C12)	54
22	B (Old Battery Point buoy C13)	54
23	B (Charlottetown Harbour buoy C15)	54
24	B (Rosebank Point Centre buoy C14)	54
25	B (Yacht Club race buoy Y or PRIV1)	54
26	B (Prince St. Wharf Ruins buoy C17)	54
27	B (Railway Wharf East Flats buoy C19)	54
28	B (Charlottetown Railway Wharf C18)	54
29	B (Southport Wharf Ruins buoy C)	54
30	B (Hillsborough/East River buoy C20)	54
31	B (Governor's Island buoy CD)	54

Characteristics of settlement nodes used in the model of Hillsborough Bay. Node types are L (aquaculture lease), B (buoy) and D (dock). For buoys, the site and buoy number are indicated in parentheses (corresponding to Canadian Coast Guard (2009), and Canadian Hydrographic Service Chart Nos. 4460 – Charlottetown Harbour and 4466 – Hillsborough Bay.

McKindsey et al. (2009), but our estimate provides for a somewhat larger surface area associated with mussel growth on the outside of the sock, as compared to the surface of an unpopulated sock. The same values were used to estimate the surface areas at oyster aquaculture leases, lacking data on which to base a more accurate estimate. Since oyster leases are typically located in shallower water, we likely overestimated their surface area.

Ciona intestinalis has been detected in Charlottetown Harbour on five occasions: two small (possibly juvenile) individuals on a cage moored near node 18 (Figure 2) and one on a floating dock near node 30 in 2008; and one individual on a floating dock at node 4 and approximately 100 individuals on hulls of two boats moored at two other docks in node 4 in 2009. All of these sightings occurred on structures that were later removed from the harbour and it is not known whether reproduction occurred. Node 4, which included both marinas in Charlottetown Harbour and was the site where the largest number of *C. intestinalis* was detected as well as the highest frequency of sightings, was used as the most likely starting point for a hypothetical inoculation. Nodes 1, 2, and 3, representing the aquaculture lease areas near Nine Mile Creek, were the dispersal endpoints of interest.

Biological input to the matrix population model

The matrix population model incorporated both the spatial distribution of substrates suitable for settlement, and stage-dependent life-history estimates (fecundity, mortality and settling rates, and maturation time) for a complete life cycle of *Ciona intestinalis* from the release of the egg through the non-feeding planktonic larval stage,

settlement, and maturation to release of the next generation of eggs. Simulations of the model were done using MATLAB 7.6.0 (Matlab 2008).

Essentially, the model was a quantitative description of the fate (survival and settlement) of a cohort of the eggs and larvae initially released from a starting node (for simplicity, both stages were combined and referred to as 'larvae'). The assumptions of this process were:

(1) larvae can drift in the water column for at most N hours before expending their stored energy and becoming incompetent to settle;

(2) drifting eggs and larvae that are preyed upon, and larvae that do not settle before expending their energy store die, giving an effective settlement rate s;

(3) once settled a tunicate will not relocate and settle upon other substrate;

(4) settled larvae mature in q hours;

(5) mature adults release larvae into the water column at a constant rate r and die at a rate d.

Mathematically, this process can be expressed in the following manner:

A_t is a vector representing the number of adult *C. intestinalis* at time t for each node. Dispersal and establishment of tunicates is modelled using the following matrix model:

$$A_t^* = (1-d)A_{t-1} + \sum_{n=1}^{N} rsP_n A_{t-n-q} \quad \text{(equation 1)}$$

$$A_t = \min\{A^*_t, K\} \qquad \text{(equation 2)}$$

where in addition to the parameters described above, P_i is a transition matrix (described below), and the vector K is the adult carrying capacity for each node. Carrying capacity is modelled as a simple saturation: at each time step, if the value of A_t computed in equation 1 is larger than K, it is truncated to K, since additional larvae can not settle at a node once the carrying capacity is reached (Barbeau and Caswell 1999). Note that equation 2 is an entrywise comparison.

Each entry in the transition matrix, P_i, represents a probability of transitioning from one node to another by advection. To simplify the model, only the passive dispersal of larvae by ocean currents (advection) was considered, and the contribution of active swimming was ignored. This seemed reasonable given that in laboratory studies, *C. intestinalis* larvae swim at mean speeds of 0.6–4 mm/s during bursts of 5–20 sec (Tsuda et al. 2001; Tsuda et al. 2003; McHenry and Patek 2004; Zega et al. 2006).

Compared to the oceanographic currents in the Hillsborough Bay system, on the order of 27 mm/s, the effects of swimming by larvae would be negligible. Each of the N transition matrices was computed for each one-hour time step over the average life span of tunicate larvae (N hours) using the water velocities predicted by the oceanographic model. Each entry in the matrix is a transition probability, determined as the sum of all transitions for which larvae land within a substrate node divided by the total number of transitions within the bay (regardless of landing position) generated over i hours (where $i=1,…,N$) to obtain a probability of landing in that node. This procedure was performed for each node over the larval life span. Thus, the row m column n entry of P_i is the probability that a larvae released at node n disperses to node m after i hours.

The model starts with the introduction of a single reproducing adult at node 4 in Hillsborough Bay, thus all simulations were standardized to this starting point. Apart from the advantages of expressing population growth in a standardized manner, the selection of a single adult as a starting point for the population is not biologically unrealistic; while most *C. intestinalis* are protandrous hermaphrodites, 15% of those occurring in the Gulf of Naples are self-fertile (Rosati and Santis 1978). Each simulation was run for a six-month duration, representing the period 1 May to 31 October. Life history information needed for the matrix model was obtained from published literature, and unpublished experiments conducted in PEI. The selection of parameter values used in the model (Table 3) is further discussed in the following paragraphs.

N (Duration of planktonic stage): The duration of the planktonic stage, N, was the average time required for egg drift, fertilization, embryonic development of the fertilized egg and larval development, and was estimated to be 72 hours. Another term for N could be 'larval life expectancy'. *C. intestinalis* disperses by drifting eggs, egg-strings and swimming larvae, but in addition, fertilized eggs and developing larvae may be retained in mucus strings (Petersen and Svane 1995). In the laboratory, 40–60% of the larvae escape from the mucus string after hatching (Svane and Havenhand 1993); the others may settle near the parent. These variations in early life history complicate the selection of an appropriate planktonic drift duration. Development rates are also temperature

Table 3. Values of *Ciona intestinalis* life history characteristics and number of settlement nodes used in the model, and ranges of values used for sensitivity analyses.

Parameter	Description	Value and Unit	Range of values for sensitivity analyses
N	Duration of planktonic stage	72 hours	0–72
r	Per capita reproduction rate	100 larvae/tunicate/hour	
s	Per capita settling rate	0.10	0.02–0.20
d	Post-settlement per capita mortality rate	1/4,380 individual/hour	
q	Average age for maturation	1,008 hours	806–4,032
m	Total number of nodes	31 nodes	
K_i	Tunicate carrying capacity of node i	Area of node i (from Table 2)·240 adults/m^2	

dependent. Most settlement in PEI took place at temperatures from 12 to 18° C (Ramsay et al. 2009). Literature values for the maximum period of combined embryonic (egg) and larval development at these temperatures varied from 1.5 to 10 days. We assumed for the model that the upper bound of these development times rarely occurs in the field. The free swimming larval stage can last up to 6 days in the laboratory, but is generally much shorter in the field, on the order of minutes to hours, and most settlement occurs in less than 24 hours (Havenhand and Svane 1991; Svane and Havenhand 1993). Many tunicate species appear capable of extending the larval duration, especially in the laboratory, but the ability of these larvae to settle (competence) may be compromised. The competent period of larvae may be only 5–40% of the lifespan of the longest-lived larva (van Duyl et al. 1981; Worcester 1994). No pre-competent period has been demonstrated for *C. intestinalis* (Svane and Havenhand 1993), therefore the lower bound of duration of the free-swimming stage is assumed to be 0 hours.

r (Per capita reproductive rate): The hourly larval release rate was calculated using the daily average release. We used a per capita reproductive rate *r* of 2,351 larvae per day per adult tunicate, which was determined during an experiment conducted on *C. intestinalis* in eastern PEI (Bourque, unpub. data). This exceeds the maximum daily production observed in *C. intestinalis* from the Lunenburg, NS, area of 1,998 eggs per day per adult tunicate in late May (Carver et al. 2006). The maximum fecundity at Lunenburg, averaged over the last two weeks of May (temperatures between 6 and 9° Celsius), was 533 eggs per day per adult tunicate (Carver et al. 2006).

s (Per capita settling rate): In the absence of empirical data, we followed Petersen and Svane (1995)'s estimate that approximately 10% of *C. intestinalis* larvae survive to settlement; thus, we set *s* = 10%. This proportion was derived from Petersen and Svane's (1995) calculation of population spawning potential (based on observed population density and individual size, and a literature-based value of 10,000 eggs/individual) and observed recruitment rates in Kertinge Nor, Denmark. The magnitude of their estimate of larval mortality is consistent with presumed rates of natural mortality of the larvae of benthic invertebrates, although these have been difficult to quantify (Metaxas and Saunders 2009). We were unable to locate any direct measures of per capita settling rate of *C. intestinalis* or any other solitary tunicates in the literature, and direct observations of mortality in the larvae of colonial tunicates vary widely. During dispersal of *Lissoclinum patella*, 72% of the larvae were consumed by fish (Olson and McPherson 1987), but for *Podoclavela muluccensis*, larval mortality was only 3–18% (Davis and Butler 1989). Larval mortality of *Diplosoma similis* was 3–5% per minute (Stoner 1990). It is unlikely, however, that mortality rates for these colonial species, typically determined by divers visually following individuals of brightly coloured, large, tunicate larvae in clear tropical waters, are comparable to that of smaller and less conspicuous *C. intestinalis* larvae dispersing in turbid PEI waters. We assume for the model that most of the mortality of *C. intestinalis* larvae is not due to predation, but occurs when larvae expend their energy stores before a suitable settlement substrate has been located. Thus, we expressed per capita settling rate as an instantaneous survival rate at the time of settling, rather than an attrition of larvae over the duration of the planktonic stage. A sensitivity analysis of per capita settling rate was conducted, varying *s* from 0.02 to 0.20.

d (Per capita post-settlement mortality rate):
Mortality rate after settlement has not, to our
knowledge, been determined for *C. intestinalis*.
Life spans of *C. intestinalis* were 2–6 months for
Japanese and Mediterranean populations (Dybern
1965; Yamaguchi 1975) and 1–2 years for
British populations (Berrill 1947); however, life
spans cited by these authors probably indicate
the maximum duration of survival and do not
reflect attrition of the population. Assuming that
C. intestinalis in PEI survive on average one
growing season (~6 months) and that mortality
occurred uniformly over this period, we
estimated the fraction of settled juveniles and
adults dying each hour to be $d= 1/4,380$ adult
tunicates per hour.

q (Age of maturation): The age of maturation
was estimated to be six weeks post-settlement (q
$= 1,008$ hours), averaging the observations of
Yamaguchi (1975) that sexual maturity occurred
in one month in summer and two months in
winter in Japan. In both PEI and Nova Scotia,
individuals that settled in May-June were
capable of initiating egg production and
spawning by August of the same year (Carver et
al. 2006; Ramsay et al. 2009), suggesting that
maturity might be similar or a little slower than
in Japanese populations. In Nova Scotia,
individuals grow at 20 mm per month between
July and September and commence spawning at
approximately 40 mm in length (Carver et al.
2003), suggesting two months to maturity, but in
waters colder than those in PEI. A sensitivity
analysis was conducted, varying q between 806
and 4,032 hours.

K (Tunicate carrying capacity of node): The
settling capacity K_i of each node (tunicates/node)
was calculated using a standard capacity of 240
tunicates/m^2 (Petersen and Riisgård 1992;
Riisgård et al. 1996) multiplied by the estimated
settlement areas of each of the 31 nodes (Table
2). The value of 240 tunicates/m^2 was chosen as
representative of the late-summer population
density of *C. intestinalis* which is bracketed by
low values measured in spring (20–76
tunicates/m^2 in March-May) and higher values in
late autumn (450 tunicates/m^2 in November).

Modelling the management of nodes

A detailed examination of the pattern of
settlement of *C. intestinalis* on nodes between
Charlottetown Harbour and Nine Mile Creek was
undertaken in order to identify highest-priority
nodes for monitoring, or those for which removal

or cleaning could be used to interrupt the spread
of tunicates in the event that dispersal from
Charlottetown to Nine Mile Creek could not be
accomplished by a single generation of tunicates.
To establish which nodes between Charlottetown
Harbour and Nine Mile Creek would be
influential in the dispersal and settlement of
larvae, a sensitivity analysis was performed by
systematically reducing the settlement rate at
individual nodes to represent treatment (20–80%
reduction) or removal (100% reduction) while
holding the settling rate at other nodes constant
(0% reduction).

The goal was to determine the minimum
number of nodes that would need to be removed
or treated in order to prevent the transport of
larvae between Charlottetown Harbour and Nine
Mile Creek. All possible paths between the two
locations were considered over an *N* hour period
where *N* represents the average life span of
larvae in the water column. Let

$$Q = \sum_{n=1}^{72} P_n \qquad \text{(equation 3)}$$

be an adjacency matrix, the matrix derived by
summing all transition matrices. If entry $Q[m,n]$
of the adjacency matrix is greater than zero, then
there exists a directed path from node *n* to node
m over a larva's life span, otherwise a path does
not exist. Hence, a graph of the connections
between nodes can be constructed by Q. Using
MATLAB, the minimum number of nodes
(intermediate steps) required for a path to exist
between one node and another was calculated,
equivalent to the number of generations required
for tunicate dispersal over this path.

Comparison of model results to empirical observations

The Charlottetown marinas in node 4 (Charlotte-
town Yacht Club and Peake's Quay) were
visually inspected for tunicates opportunistically
throughout the summer and surveyed in detail
using an underwater video camera on 29
September 2010. Visual surveys for tunicates
were conducted throughout the Nine Mile Creek
area from 30 September to 6 October 2010. Node
3 (mussel lease area) was surveyed by paired
divers who swam six transects and by a team
who lifted 11 sections of grow-out mussels and
five sections of seed mussels with a hydraulic
boom and visually inspected the mussels and
gear. Nodes 1 and 2 (oyster leases near Nine
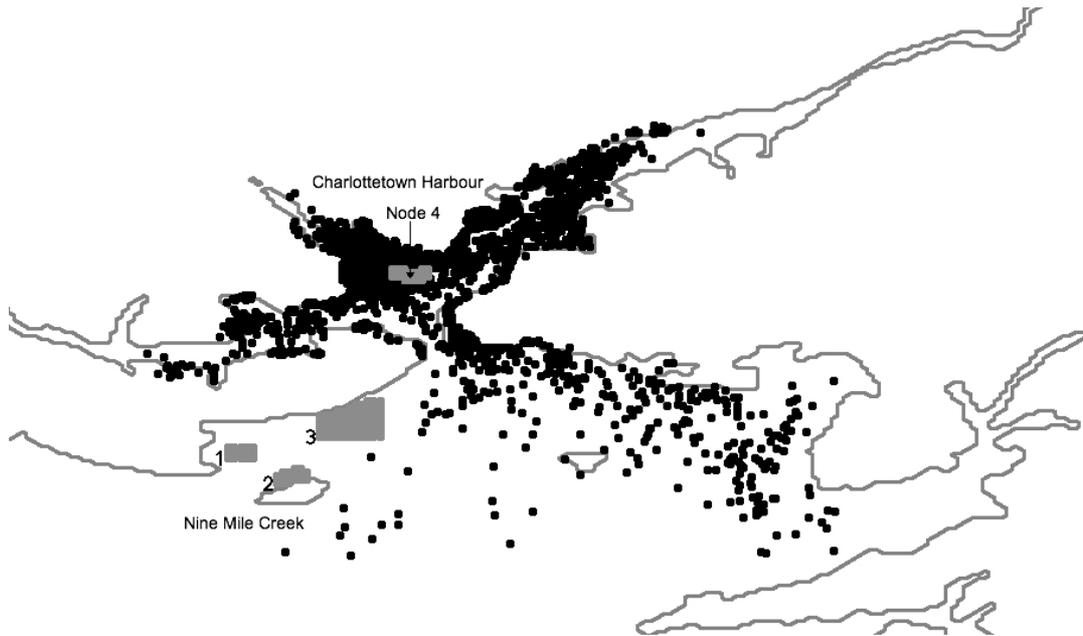Mile Creek) were visually inspected by a team

Figure 3. Hourly `landing' points (black dots) of larvae of *Ciona intestinalis* as predicted by the mathematical model, after leaving Charlottetown Harbour (node 4). There are no `landings' on any of the Nine Mile Creek nodes (nodes 1, 2 and 3).

Table 4. Sensitivity analyses of the effects on the model of reducing or increasing the average age of maturation (q) of *Ciona intestinalis*, with concomitant changes in number of generations per six-month growing season. Results are expressed relative to a standardized value of 1 tunicate representing the estimate of adult tunicates established at Nine Mile Creek after six months of dispersal, based on a model using q=1,008 hours, corresponding to production of four generations/growing season.

	Average age of maturation, q (hours) [generations/season]				
	806 [5]	1008 [4]	1344 [3]	2016 [2]	4032 [1]
Standardized abundance (adult tunicate)	4.85	1	0.01	0	0

which lifted buoys and lines by hand from a boat. The wharf and boats at Nine Mile Creek were surveyed using the underwater video camera. Comparable surveys were conducted in each of these areas in 2008 and 2009.

Navigational buoys in Charlottetown Harbour and Hillsborough Bay were examined when the Canadian Coast Guard removed them for winter storage on 1–2 December 2010. Thirteen buoys were visually inspected for tunicates upon arrival at the wharf in Charlottetown.

Results

The model results indicated that the oceanographic vector would be unlikely to disperse *Ciona intestinalis* directly from Charlottetown Harbour to the aquaculture leases at Nine Mile Creek during a single generation (72 hours of planktonic larval drift) (Figure 3). As demonstrated in the map of hourly 'landing points', prevailing currents carried propagules from node 4 throughout the tidal river portions of the

Table 5. Sensitivity analysis determing the effect on model predictions of reducing the estimated duration of the planktonic stage (N) of *Ciona intestinalis*. Results are expressed relative to a standardized value of 1 tunicate representing the estimate of adult tunicates established at Nine Mile Creek after six months of a dispersal, based on a model using $N = 72$ hours.

	Duration of planktonic stage, N (hours)						
	0	12	24	36	48	60	72
Standardized abundance (adult tunicate)	0	0.02	0.24	0.46	0.65	0.83	1

Table 6. Sensitivity analysis determining the effect on model predictions of a range of values for settlement rate (s) of *Ciona intestinalis*. Results show a standardized estimate of adult tunicates at Nine Mile Creek resulting from four generations of tunicates dispersing over six months, relative to a value of 1 adult tunicate (representing the estimate with s=0.10).

	Settlement Rate, s									
	0.02	0.04	0.06	0.08	0.1	0.12	0.14	0.16	0.18	0.20
Standardized abundance (adult tunicate)	0.06	0.27	0.50	0.75	1	1.26	1.53	1.81	2.10	2.40

estuary and along the northeastern portions of Hillsborough Bay, but did not carry propagules along the western shore toward Nine Mile Creek and nodes 1, 2 and 3.

Sensitivity analysis of the average age for maturation (q) which in turn controlled the number of generations of *C. intestinalis* per growing season in the model, similarly indicated that dispersal from Charlottetown Harbour to Nine Mile Creek could not be accomplished in one or two generations, even for a model run over a six month period (Table 4). *C. intestinalis* dispersal to Nine Mile Creek required a minimum of three generations. The three-generation model resulted in the establishment of 1% as many tunicates as the standardized result of 1 adult tunicate for $q = 1,008$ h, or four generations.

Modifying the biological parameters used in the model changed the abundance of adult tunicates established at Nine Mile Creek after six months of dispersal using the four-generation model, but tunicates were still able to disperse to Nine Mile Creek in these scenarios as long as duration of the planktonic stage (N) and settlement rate (s) exceeded zero (Tables 5 and 6). Fewer tunicates reached Nine Mile Creek when the planktonic duration was shortened, most likely because the distance of dispersal of each generation was also shorter (Table 5). Doubling the settlement rate on all nodes from the initial value of s=0.10 more than doubled the estimate of adult tunicates at Nine Mile Creek

(Table 6). Reducing the settlement rate by 80% to s=0.02 reduced the establishment of tunicates to 6% of the standardized establishment value with s=0.10.

The occurrence of multiple generations within a growing season could allow nodes intermediate in location between Charlottetown Harbour and Nine Mile Creek to be used as 'stepping stones' or intermediate steps in dispersal to reach Nine Mile Creek within a single growing season. Removing specific nodes in the system or reducing their settling capacity could interrupt the dispersal of *C. intestinalis* from Charlottetown Harbour to Nine Mile Creek (Table 7). Systematically reducing the settling rate on individual nodes to represent treatment (20–80% reduction) or removal (100% reduction) of that node indicated only nodes 1, 2, 3, 18 and 26 influenced dispersal of *C. intestinalis* (Table 7). The individual removal or reduction in settlement area of any of the other 26 nodes reduced the population at Nine Mile Creek by < 1%. Because nodes 1, 2, and 3 are the leases located at Nine Mile Creek, nodes 18 and 26 would be the most important nodes to monitor for early detection of tunicates dispersing from Charlottetown Harbour. The greatest change associated with removal of an individual node would occur following the removal of node 18, the Blockhouse Point buoy (11% reduction in tunicate population at node 3), or node 26, the Prince Street Wharf Ruins buoy (4%). Decreasing the tunicate settlement on each buoy
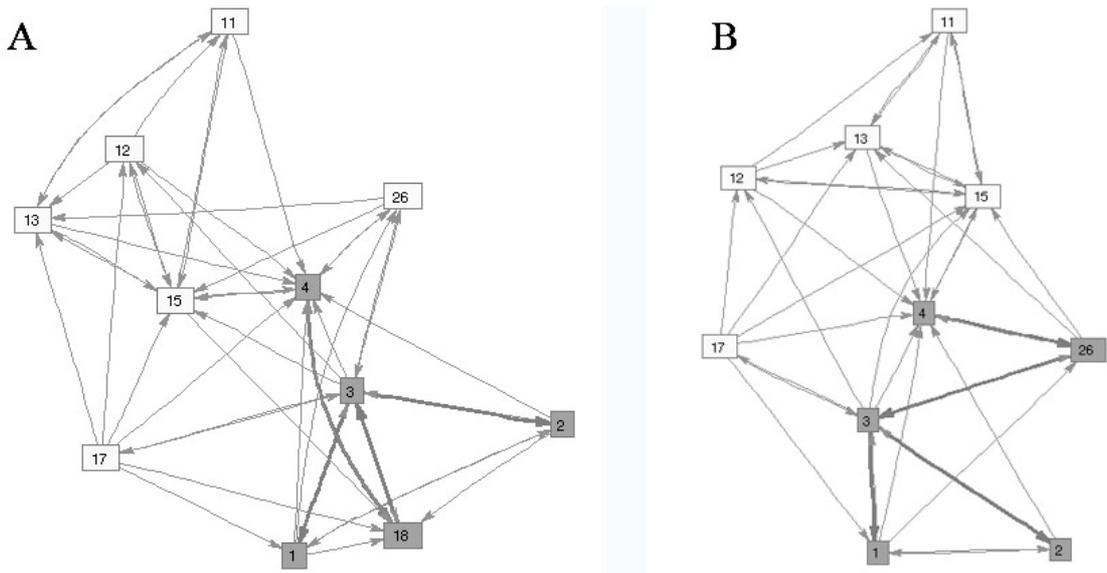
Figure 4. Directed paths of *Ciona intestinalis* dispersal between nodes, showing possible routes from Charlottetown Harbour (node 4) to Nine Mile Creek (nodes 1, 2 and 3). Only non-zero paths (i.e., non-zero entries in the adjacency matrix in equation (3)) are shown. Connecting arrows between the nodes show the direction of dispersal. (**A**) Pathways connecting Charlottetown Harbour to node 18 and then to the Nine Mile Creek nodes are shown in bold, including the two-generation pathways (4→18→2) and (4→18→3). A two-generation pathway (4→26→3) and a three-generation pathway (4→15→18→3) also exist in this model. (**B**) With removal of node 18 from the model, the only remaining pathway connecting Charlottetown Harbour to Nine Mile Creek is the two-generation pathway: (4→26→3).

to 80% of its current value would reduce the tunicate population at node 3 by 8% and 3%, respectively.

Non-zero entries in the adjacency matrix developed for all possible paths between Charlottetown Harbour (node 4) and Nine Mile Creek (nodes 1, 2 and 3) identified directed pathways involving 11 of the 31 nodes: nodes 1, 2, 3, 4, 11, 12, 13, 15, 17, 18 and 26 (Figure 4A). While nine nodes had pathways leading toward node 4, relatively few pathways led from node 4 to other nodes. Direct connections from node 4 were identified only for nodes 15, 18 and 26, none of which were located at Nine Mile Creek. Thus, *C. intestinalis* would not be able to disperse from Charlottetown Harbour to Nine Mile Creek in a single generation. Three two-generation pathways connected node 4 to a node at Nine Mile Creek: (4→18→3), (4→18→2) and (4→26→3). Transport via node 15 required an additional generation: (4→15→18→3). Node 18, identified as an influential node by the analysis summarized in Table 7, therefore also provided two of the most direct (two-generation) paths

between node 4 (Charlottetown Harbour) and nodes 1, 2, and 3 (Nine Mile Creek) (Figure 4A). With the removal of node 18 from the analysis, pathways from node 4 led to only nodes 26 and 15 (Figure 4B). In the absence of node 18, there was no pathway from node 15 to node 3. Only one pathway led from node 4 to Nine Mile Creek: the two-generation pathway (4→26→3).

No solitary tunicates (*C. intestinalis*, *Styela clava*) were detected during monitoring of potential settlement sites conducted in the period September 29 – December 2 (Table 8). Colonial tunicates *Botrylloides violaceus* and *Botryllus schlosseri* were detected in Charlottetown Harbour and at Nine Mile Creek, but nowhere else in Hillsborough Bay. No tunicates were found on node 18 in Hillsborough Bay, or on other buoys investigated in its vicinity. Colonial tunicates were present on all buoys sampled from Charlottetown Harbour: *B. violaceus* on all but one buoy (node 23, buoy C15), which was the only buoy where *B. schlosseri* was found. We were unable to sample node 26 but all the buoys

Table 7. Modelled results of managing dispersal of *Ciona intestinalis* to Nine Mile Creek (node 3), by removing or reducing settlement opportunities on individual nodes in Hillsborough Bay, PEI. Settlement rate was adjusted to values representing removal ($s =0$) or reduction in carrying capacity ($s = 0.02$ represents a reduction of 80%, $s =0.08$ represents a reduction of 20%) for each individual node, while holding all other nodes at $s=0.10$ (i.e., at full carrying capacity). Results show the proportion of the population established at Nine Mile Creek (node 3) at the end of a six-month growing season. Only nodes which contributed to a population change are listed in the table (nodes 1, 2, 3, 18 and 26). Results are expressed as settlement on node 3 relative to a standardized value of 1 representing adult tunicates established at node 3 when $s=0.10$ at all nodes.

Node i	Settlement Rate, *s* at node *i* [Reduction in carrying capacity of node *i*]					
	0 [100%]	0.02 [80%]	0.04 [60%]	0.06 [40%]	0.08 [20%]	0.10 [0%]
1	0.51	0.63	0.72	0.81	0.91	1
2	0.95	0.98	0.99	0.99	1	1
3	0	0.36	0.56	0.71	0.86	1
18	0.89	0.92	0.95	0.97	0.99	1
26	0.96	0.97	0.98	0.99	0.99	1

Table 8. Summary of presence (+) and non-detection (0) of non-indigenous tunicates on nodes monitored for tunicates at end of growing season, 2010, in Hillsborough Bay, PEI. Nodes not listed in the table were not monitored. No *Ciona intestinalis* or *Styela clava* were detected.

1	Node Type	*Botrylloides violaceus*	*Botryllus schlosseri*
1	L (oyster; Nine Mile Creek)	+	+
3	L (mussel; Nine Mile Creek)	+	+
4	D (downtown Charlottetown docks)	+	+
5	B (Spithead buoy C5)	0	0
6	B (Squaw Point buoy B)	0	0
7	B (Squaw Point buoy A)	0	0
8	B (Fitzroy Rock buoy C4)	0	0
18	B (Blockhouse Point buoy C7)	0	0
19	B (Battery Point buoy C8)	+	0
20	B (Canceaux Spit buoy C9)	+	0
21	B (Rosebank Point Outer buoy C12)	+	0
22	B (Old Battery Point buoy C13)	+	0
23	B (Charlottetown Harbour buoy C15)	0	+
24	B (Rosebank Point Centre buoy C14)	+	0
28	B (Charlottetown Railway Wharf C18)	+	0
31	B (Governor's Island buoy CD)	0	0

in its vicinity were infested with one or the other colonial tunicate. Both colonial species were abundant at the Charlottetown Harbour docks at node 4, as well as in the Nine Mile Creek area at nodes 3 and 1 (mussel and oyster leases, respectively), and on the dock structures and boats moored at Nine Mile Creek Wharf. Our observations of colonial tunicates since 2008 suggest that the Nine Mile Creek area and Charlottetown area populations originated from separate inoculation events, first detected at the Nine Mile Creek wharf (*B. schlosseri*) and node 1 (*B. violaceus*), and at node 4 in Charlottetown Harbour (both species), respectively.

Discussion

Our prediction that the oceanographic dispersal of *Ciona intestinalis* in the Hillsborough Bay/ Charlottetown Harbour system would occur only over short distances (at most, mean dispersal of 6 km per generation under the initial conditions of the model) is consistent with previous literature suggesting that the planktonic dispersal of *Ciona intestinalis* is quite limited. Petersen and Svane (1995) noted that Scandinavian populations were very localized, and their model of dispersal in a Danish fjord-cove system predicted retention within the cove, which was about 5 km in length.

Their prediction was supported by the distribution of adult *C. intestinalis* and planktonic eggs and larvae, which were restricted to the cove. Whether this limitation resulted solely from dispersal, or was related to a lack of suitable habitat outside the cove, is not apparent from the publication. Validation of larval dispersal models is a non-trivial problem (Metaxas and Saunders 2009), but our finding of limited oceanographic dispersal from the wharves in Charlottetown is also supported by the observation that colonial tunicates, apparently originating from inoculation of the wharf area, are now distributed on aids to navigation throughout Charlottetown Harbour but have apparently not dispersed to upper Hillsborough Bay immediately outside of Charlottetown Harbour (Table 8). The ability of the colonial species to drift as rafting colonies (Worcester 1994) is likely to afford them a greater range of dispersal than *C. intestinalis*.

Accurate predictions of dispersal of an invasive species in a new area are, however, confounded by many factors. Dispersal results from a complex interaction between physical and biological factors including spawning time, spawning location, physical transport processes (advection, dispersion or eddy diffusion, vertical position of the propagule in a flow field with sheer), planktonic duration, planktonic survival, mode of propagule development, propagule behaviour, and alternative mechanisms of dispersal (e.g., drifting adults or juveniles, or floating reproductive fragments) (Kinlan and Gaines 2003; Edwards et al. 2007). Factors that may facilitate the establishment and spread of invasive tunicates include climate change, eutrophication, disturbances in natural communities and human transport related activities (Lambert 2007). Environmental conditions such as water temperature, water quality, nutrient levels, and food quality and availability all affect reproductive and growth rates which would affect the dispersal of *C. intestinalis*. Rates of growth and development relative to temperature vary among populations of *C. intestinalis* (Dybern 1965), so introduction of new genetic material or selection for different growth rates in the existing population could alter the generation time. One or two generations per year is typical of *C. intestinalis* in most northern European or North American populations (Sweden, England, Nova Scotia) (Dybern 1965; Carver et al. 2006). In PEI, recruits reached 40 mm, which according to Carver et al. (2003) is a size capable of

spawning, by 8 August (Ramsay et al. 2009). This suggests that the production of two generations per year should be possible before water temperatures in PEI drop below 8°C, the lower limit of spawning (Dybern 1965; Gulliksen 1972; Carver et al. 2006), typically in October (Locke, pers. obs.). In Plymouth, England, three generations were occasionally observed "in favourable years", and routinely occurred in Venice Lagoon and the eastern Mediterranean (Dybern 1965). An increase in the number of generations per year of *C. intestinalis* could increase the likelihood of dispersal from Charlottetown to Nine Mile Creek.

The finding that *C. intestinalis* is unlikely to be dispersed in one generation from Charlottetown to Nine Mile Creek could be modified by extreme natural events not represented in the current model. Indeed, the occurrence of high-energy storms may combine with the presence of drifting or rafting adults, resuspended juveniles, or floating reproductive material to greatly extend the dispersal scales of organisms (Kinlan and Gaines 2003). Meteorological phenomena that result in stronger oceanographic currents, or currents in directions counter to their usual patterns, could also extend the normal dispersal range of planktonic larvae; for example, an unusually strong northbound current associated with El Niño was suggested as the most likely vector dispersing green crabs (*Carcinus maenas*) a distance of 650 km from Oregon to Vancouver Island in 1997–1998 (Jamieson et al. 2002). Estimates of dispersal based on movement of invasion fronts are often higher than direct estimates of dispersal, because they are sensitive to the tails of dispersal distributions, i.e., the longest distances propagules can successfully travel and establish (Kinlan and Gaines 2003), which may occur only rarely.

The involvement of potential vectors not included in the model could also extend the dispersal range of *C. intestinalis* in the Hillsborough Bay system beyond the range attainable by planktonic larvae. *C. intestinalis* routinely settles onto a number of anthropogenic substrates in PEI estuaries that could readily be advected by currents, including buoys or floating rope (Locke and Bourque, pers. obs.). We have only rarely observed small *C. intestinalis* attached to drifting eelgrass (*Zostera marina* L.) in PEI (Locke and Bourque, pers. obs.), although large numbers of *C. intestinalis* occur on eelgrass in Scandinavia (Petersen and Svane 1995). Svane

and Havenhand (1993) also reported finding advanced juveniles attached to drifting sediment particles, and noted the ability of these juveniles (and, to a lesser extent, adults) to reattach to hard substrates. For the most part, these means of dispersal would function in a similar manner to passively dispersing particles, and therefore could be predicted through the use of our model.

Treating the larva of *Ciona intestinalis* as a passively dispersing particle, however, most likely biased the model to overestimate larval dispersal. Empirical measures of realized larval dispersal distance are consistently shorter than the potential maximum dispersal distance for marine invertebrates, including tunicates, due to a number of factors including behaviour of the larva and attrition of larval numbers through predation (van Duyl et al. 1981; Worcester 1994; Shanks et al. 2003). Genetic dispersal estimates indicate that null models of passively dispersing propagules adequately predict dispersal distance for marine organisms with propagule duration <1 day, but overestimate dispersal distances for those with longer propagule duration (Shanks 2009). Given that we assumed a larval duration of 3 days for *C. intestinalis*, Shanks' analysis would suggest that excluding behaviour from the model might result in an overestimate of dispersal. On the other hand, Edwards et al. (2007) modelled the influence of three different vertical migration strategies chosen to maximize differences in vertical distribution associated with larval behaviour, and found that behaviour accounted for ≤2% of the variation in dispersal.

Anthropogenic vectors also have the potential to carry *C. intestinalis* to the Nine Mile Creek area. Nine of 85 recreational vessels surveyed by Darbyson et al. (2009a) in 2003–2004, in eastern PEI ports where *C. intestinalis* is now abundant, were bound for Charlottetown as their next port of call. We were told by staff at one of the wharves in Charlottetown Harbour (node 4) that a boat with *C. intestinalis* fouling had arrived several weeks before one of our surveys. To reach Charlottetown Harbour from other ports in PEI, vessels have to sail past Nine Mile Creek. Release of propagules from an adult attached to a vessel or even loss and reattachment of juveniles carried on the hull could account for the two *C. intestinalis* that settled near the entrance to Charlottetown Harbour (node 18) in 2008.

The finding that *C. intestinalis* should not be able to disperse by oceanographic drift directly from Charlottetown Harbour to Nine Mile Creek without intermediate (anthropogenic) settlement nodes, and that all other means of transportation presently identified are anthropogenic, suggests that dispersal of the tunicate is amenable to control. Darbyson et al. (2009b) determined that anti-fouling treatments of boat hulls were effective in preventing settlement of the clubbed tunicate, *Styela clava*, and we think this would also reduce the risk of transport of *C. intestinalis*. Regular inspection and cleaning of boat motors and aluminum hulls commonly used by mussel growers, neither of which are treated with anti-fouling paints (Darbyson et al. 2009b), would also reduce the risk. Another option to reduce the risk would be the removal of some of the more influential nodes that could be used as 'stepping stones' to Nine Mile Creek; however, all identified nodes are necessary for aquaculture or navigational purposes. Given that removal of these nodes would not likely be feasible, a suggested mitigation practice in the Hillsborough Bay would be inspection of all nodes from the bay at least once, and preferably twice per season, followed by cleaning if necessary. For some nodes, such as the navigational buoys maintained by the Canadian Coast Guard, removal and cleaning is already carried out each autumn. Periodic inspection (and cleaning if necessary) of the buoys earlier in the settlement season would also help discourage larval dispersal. Ramsay et al. (2009) were able to quantify recruits on settling plates in PEI without a microscope by 25 July, suggesting that an *in situ* inspection of buoys by divers might become feasible by mid-August. Inspection of structures on aquaculture leases would also be advisable. As indicated in Figure 4 and Table 7, nodes 1, 2, 3, 18, and 26 would be the highest-priority sites to be monitored during the growing season.

Controlling the dispersal of invasive species is certainly not an easy task, but this model suggests that, if Charlottetown Harbour serves as the main point of origin of *C. intestinalis* propagules from moored vessels, interruption of the dispersal path to Nine Mile Creek is feasible. To strengthen the model, it would be advisable to examine the predicted dispersal of larvae under a greater range of oceanographic conditions, including extreme events; to include settlement nodes not presently in the model (including the Nine Mile Creek wharf, which was excluded because inspections have not identified it as a source of *C. intestinalis*); and to simulate the model with additional node points of release of *C. intestinalis* larvae.

Acknowledgements

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