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Research article

Recruitment patterns and population development of the invasive ascidian *Ciona intestinalis* in Prince Edward Island, Canada

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Abstract

In 2004, an exotic tunicate, *Ciona intestinalis*, was detected in Montague River, Prince Edward Island. Since it was first detected, this exotic species has become an invasive nuisance species creating production problems in bivalve aquaculture industries including handling difficulties and resource competition with the cultured blue mussel, *Mytilus edulis*. *Ciona intestinalis* has become a challenging species to manage because of its long reproductive period and rapid biomass accumulation. Population development of *C. intestinalis* in a single season was investigated to inform the aquaculture management of this fouling species. The study focused on seasonal recruitment patterns and the subsequent development of the *C. intestinalis* population after settlement. Recruitment on experimental collectors occurred from mid-June until late November, with a peak in late August. A rapid increase in biomass was documented in late July, six weeks after the initial recruitment. No substantial increase in *C. intestinalis* biomass was observed in new recruits after mid-August.

Key words: ascidian, *Ciona intestinalis*, aquaculture management, recruitment, population development

Introduction

Culturing of the blue mussel, *Mytilus edulis* L. 1758, on Prince Edward Island (PEI) is facing its greatest challenge since its beginning over 20 years ago. Four exotic species have been introduced into PEI waters, and have become invasive, nuisance species (Ramsay et al. 2008). These four ascidians, *Styela clava* Herdman 1881 (clubbed tunicate), *Ciona intestinalis* L. 1767 (vase tunicate), *Botryllus schlosseri* Pallas 1766 (golden star tunicate), and *Botrylloides violaceus* Oka 1927 (violet tunicate), have caused significant fouling problems for the cultured mussel industry, especially in terms of increased production and processing costs (Thompson and MacNair 2004). As potential competitors for space and food resources, they may negatively impact mussel growth rates and meat yields. Mitigation strategies for eliminating tunicates from mussels and aquaculture gear have had inconsistent results and are costly (Carver et al. 2003; Thompson and MacNair 2004; Davidson et al. 2005). A long term strategy for the mitigation of tunicate impacts should include farm management practices that result in decreased biomass of tunicates on mussel gear and mussels. An understanding of the seasonal development and recruitment success of these organisms is an essential prerequisite of formulating management strategies.

The most recently identified tunicate, *C. intestinalis*, has proven to be highly competitive in its new environment. Ramsay et al. (2008) found that since its first detection in Montague River, PEI, in 2004, it rapidly spread and established itself in that river, as well as the adjacent Brudenell estuary. Prior to the introduction of *C. intestinalis* these rivers were dominated by *S. clava*, but this species has virtually disappeared with the increasing infestation by *C. intestinalis*.

The successful establishment of *C. intestinalis* is largely a result of its reproductive capability; it can produce gametes continually as long as temperatures are suitable (Carver et al. 2006). The lower limit for spawning activity was observed at 8°C in a Scandinavian population

(Dybern 1965; Gulliksen 1972) and in Nova Scotia, Canada (Carver et al. 2003). Carver et al. (2003) documented the presence of ripe eggs in the ovary from November (7°C) through January (1°C). Signs of egg resorption were evident in February and March (-1°C), with gamete production resuming in April, when water temperature increased to 4°C.

Observations on the life history of the *C. intestinalis* population in Lunenburg Bay, Nova Scotia by Carver et al. (2003) were consistent with a pattern of two generations or recruitment peaks per year (Dybern 1965). One recruitment event was observed in May-June and a second in August-September 2000 (Carver et al. 2003). Howes et al. (2007) conducted a monitoring program in the nearby Mahone Bay from 2002 to 2005 and also observed two recruitment peaks, generally lasting an additional month compared to those from Carver et al. (2003): May-July and August-October. In Carver et al.'s (2003) study, average growth rate was estimated at 20 mm mo^{-1} at 10-20°C growing to a maximum body length of 140 mm.

This study focused on documenting the timing of recruitment and the development of the *C. intestinalis* population in one estuary in PEI. This information could be used in conjunction with newly developed husbandry and mitigation techniques to lessen the impact of *C. intestinalis* on mussel aquaculture.

Methods

Study site

The confluence of the Brudenell and Montague Rivers, located at the eastern end of PEI (Figure 1), was chosen for this study because of the abundance of *C. intestinalis*. The associated rivers are very productive; in 2004, the growth rates for *M. edulis* were 2.25 to 2.32 mm mo^{-1} which is in the top 50% of all the mussel growing areas on PEI (Department of Fisheries and Oceans 2005). Montague and Brudenell Rivers accounted in 2004 for 10.4% of the total mussel production on PEI (Department of Fisheries and Oceans 2006). In 2006 the temperature ranged from -1.5°C under ice cover to 21°C in August while the salinity ranged from 15 psu in the fall to 30.1 psu in the spring. Levels of dissolved oxygen ranged from 4.9 to 10.1 mg L^{-1}, with the highest levels being observed in the spring. All environmental parameters were measured at a depth of 3 m.

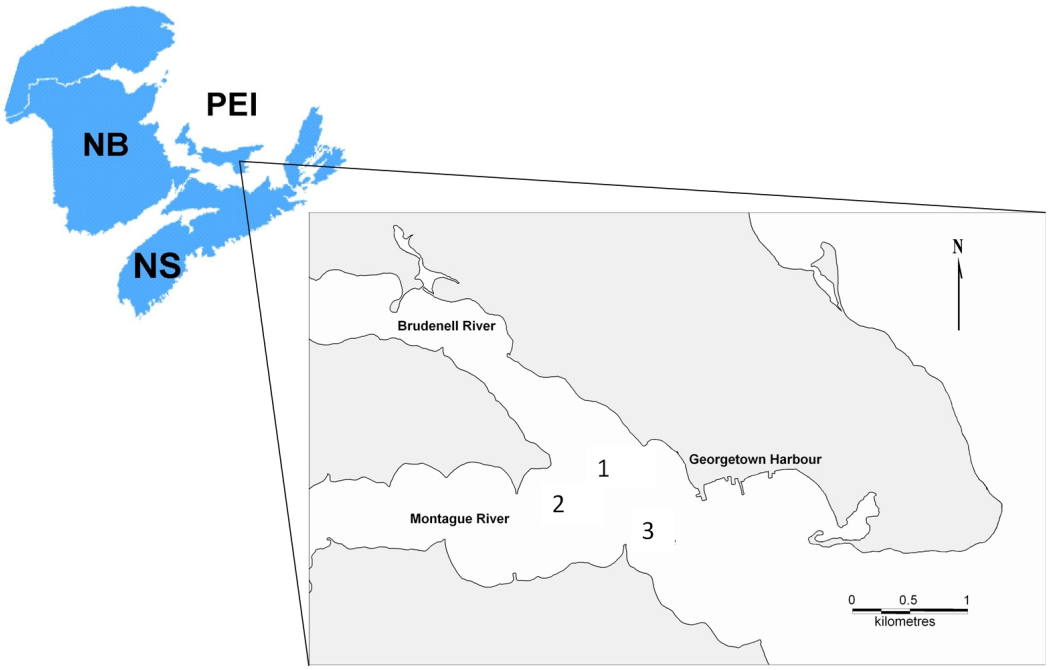

Figure 1. Confluence of the Brudenell and Montague rivers, located in eastern PEI on the east coast of Canada, indicating locations of sampling sites 1-3.

Recruitment

The magnitude of *C. intestinalis* recruitment during its reproductive season was estimated using grey 10 cm x 10 cm PVC collector plates suspended in a horizontal position from three mussel longlines at Sites 1-3 (see Figure 1). The longlines used for this study lacked mussel socks, however they were all located within a mussel lease where the surrounding longlines were in production and had an established population of *C. intestinalis*. Sites 1 and 2 were located in approximately 8 m water depth while Site 3 had 4 to 5 m water depth. Three collector plates were attached to a rope at depths of 2, 2.5 and 3 m. One rope of collector plates was tied to each of three mussel longlines for two weeks, at which time the plates were retrieved and preserved in 4% formalin. This was repeated every two weeks from May 1 to December 11 2006. In the laboratory, the number of ascidian recruits was estimated under a dissecting scope. A transparent grid was used to count juvenile tunicates on the collector plates. Species identification followed Bullard and Whitlatch (2004). In this study, collector plates were deployed without preconditioning, i.e., they were not soaked in sea water to allow biofilm to develop prior to deployment (Osman and Whitlatch 1995; Bourque et al. 2007). Preliminary assessment of the effect of preconditioning did not show a significant difference ($P = 0.216$).

Population development

Biomass accumulation of *C. intestinalis* during its reproductive season was investigated by deploying 15 collectors (rope with three collector plates) in early May on each of the three mussel longlines. One collector was retrieved by SCUBA diving every two weeks for the entire field season until the end of November 2006 to document changes in the biomass of *C. intestinalis*. Collector plates were removed from the collector rope and sealed in plastic ziplock bags for transport. Samples were quantified within 24 hours of removal from the water because it was difficult to preserve specimens and still obtain accurate measurements. Total tunicate mass (wet weight) was recorded for the entire collector plate after mean length and abundance were determined from ¼ of the collector plate. Hydroids, caprellid amphipods and other taxa found on the collector plates were removed before determining tunicate biomass by applying low-pressure water to the tunicates in a sieve. Only specimens > 5 mm in length from their holdfast to the branchial siphon were considered in analyses of tunicate abundance and mean length. *Ciona intestinalis* exhibited a tactile response during measuring and would contract, thereby reducing its length. To avoid false measurements tunicates were spread out on a tray and left for 15 minutes to relax before measuring.

In the second part of the population development study new collectors were successively deployed every two weeks from the beginning of May until mid-November 2006 on each mussel longline every two weeks and then retrieved for final assessment on November 28. Thus, this second experiment differed from the first cumulative time series in having a variable deployment date with a single retrieval date. The plates were quantified in the same manner as the collector plates deployed at the beginning of the field season.

Statistical analysis

Recruitment and temporal development (abundance, biomass and length) were analyzed using linear models (Christensen 1996) to determine significant differences between sample sites (Figure 1), collector position (top, middle, bottom) and sample/deployment dates. First order interactions were included but removed if non-significant. The model assumptions were assessed by residual analysis and Box-Cox analysis, and the dependent variables were subjected to transformation by the natural log, square root, or the cubic root transformations as appropriate to meet the model assumptions. In order to enable log transformations, values equal to zero were changed to half the value of the lowest, non-zero value included in the analysis prior to transformation. Sampling dates with zero or negligible occurrence of *C. intestinalis*, as well as extreme outliers, determined by the outlier detection test based on deletion residuals, were omitted from analysis. Pairwise comparisons between dates used the Bonferroni method (Christensen 1996). In the presence of a significant interaction between dates and sites, the interaction was modeled as a random effect to allow for overall comparisons between dates and sites. Estimates

are presented as least squares means with 95% confidence intervals, back-transformed to original scale as appropriate. The significance level was set at 0.05. Data were analyzed using Minitab 14 (Minitab Inc., Austin, TX) and SAS 9.1 (SAS Institute Inc., Cary, NC).

Results

Recruitment

The first observation of *C. intestinalis* occurred at all three sites on June 13 2006; a total of 0.3 individuals cm^{-2} had settled during the previous two weeks at a mean water temperature of 8.9°C (Figure 2a). Recruitment of *C. intestinalis* steadily increased throughout the summer until it peaked at 48.4 individuals cm^{-2} in the two weeks prior to August 22, where mean water temperature reached a high of 17.7°C. Approximately 240 individuals cm^{-2} were found on one plate from this collection interval. The last observed recruitment was on November 28 when *C. intestinalis* abundance decreased to 0.1 individuals cm^{-2}. At this time, mean water temperature had decreased to 8.5°C and continued to decrease to a mean of 6.1°C for the following two weeks. Recruitment levels were significantly different between sample dates ($P < 0.001$), the three collector positions ($P = 0.015$) and between the three sample sites ($P = 0.054$), with a significant sample date by site interaction ($P < 0.001$). Site 3 had significantly higher recruitment than the other two sites during the major recruitment period from July to mid-October (Figure 2b).

Population development

Ciona intestinalis was the dominant organism settling on the collector plates from the start of the study in June until the end in late November. Tunicates were first observed on June 13, with a density of 110 juveniles per collector plate detected with a dissecting microscope (Figure 3). By July 25, the tunicates could be quantified without a dissecting microscope, with densities reaching a mean of 224 individuals per collector plate averaged over the three sites. Abundance remained stable until early October when numbers increased to a mean of 429 individuals per collector plate. Total biomass of tunicates per collector plate remained relatively low (< 10 g) until July 11, thereafter, steadily increasing until the end of the field season, peaking at 2253

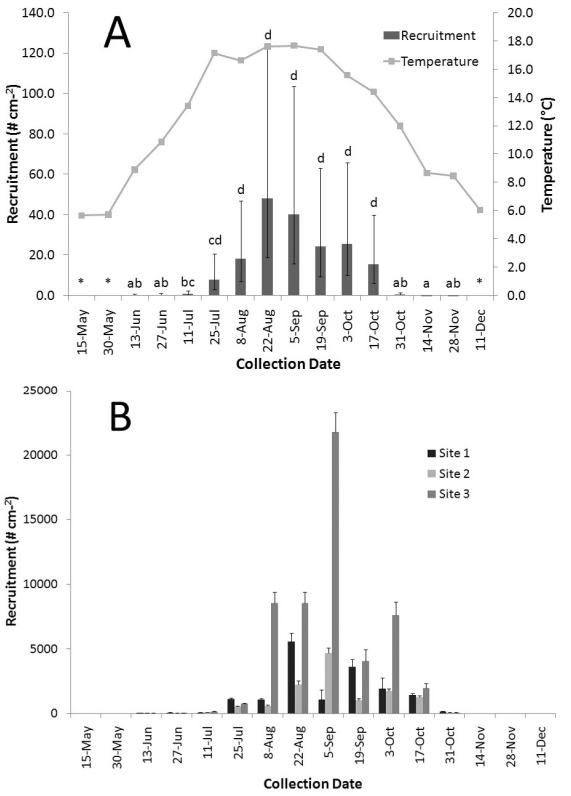

Figure 2. (A) Estimated *C. intestinalis* recruitment (with 95% CI) in 2006 in the Brudenell estuary; sample dates with the same letter were not statistically different following natural log-scale transformation. Mean 2-week temperature (°C) of each sample period is represented by the solid line. (B) Estimated *C. intestinalis* recruitment (± SE) in 2006 at each of the 3 study sites in the Brudenell estuary.

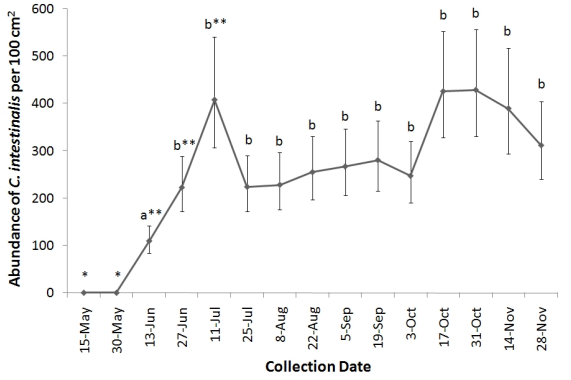

Figure 3. Estimated abundance (with 95% CI) of *C. intestinalis* accumulated over time in 2006 in the Brudenell estuary; sample dates with the same letter were not statistically different (natural log scale analysis). * Collection dates removed from analysis. ** Individuals were counted under a dissecting scope and are < 5 mm.

g per collector on October 31 (Figure 4). The decrease of approximately 10% in the mean biomass on the collector plates on the last two sampling dates was presumably caused by tunicates falling off. Mean tunicate length was first quantified on July 25 at 22 mm (Figure 5). Mean length continued to increase over the next month to 51.3 mm by August 22 (1.0 mm day^{-1}), reaching 69.4 mm by late November. Position on the collector had a significant effect on biomass ($P = 0.009$) and abundance ($P = 0.026$), but no effect on length ($P > 0.5$). Site 3 had greater tunicate abundance ($P < 0.001$) and biomass ($P < 0.001$), but tunicate length was not different between the three sites ($P > 0.5$). Abundance, biomass and length were different between retrieval dates in all analyses ($P < 0.001$) and there was a significant retrieval date by site interaction for abundance ($P = 0.036$), biomass ($P < 0.001$), and length ($P < 0.001$).

Investigation into the impact of recruitment time on the status of *C. intestinalis* at the end of the growing season showed that final abundance exceeded 200 individuals per plate on collectors which had been deployed in the period from May 1 to July 25 and retrieved on November 14 (Figure 6). Significant recruitment occurred on August 8 and 22 (Figure 2) but the final number for these plates was relatively low (<100 individuals per collector plate). Plates deployed in September and early October had 200-300 individuals per plate (>5mm) when assessed in mid-November. There were no individuals > 5 mm on plates deployed later in the season. Total biomass on collector plates was high on the plates deployed from May 1 to July 25, with weights between 878 g and 1970 g per collector plate (Figure 7). Tunicate biomass on plates deployed on August 8 had declined to 130 g per plate. Tunicate biomass was even lower on collector plates deployed between August 22 and the end of the field season (November 14) with a mean biomass below 75 g per plate. Abundance of *C. intestinalis* was greater at Site 3 ($P<0.001$), while position in the water column had some effect ($P=0.035$). There was no difference in biomass between the three sites ($P>0.5$), and a marginal difference between the water column positions was observed ($P=0.068$). The mean length of *C. intestinalis* on collector plates set from May 1 to July 25 ranged from 57-73 mm (Figure 8). Tunicate lengths on plates deployed after August 22 were < 20 mm and on those deployed after 17 October were < 5 mm. Tunicate length was smallest at Site 3 ($P<0.001$)

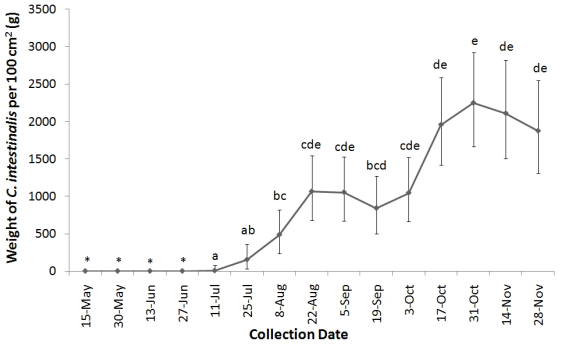

Figure 4. Estimated biomass (with 95% CI) of *C. intestinalis* accumulated over time in 2006 in the Brudenell estuary; sample dates with the same letter were not statistically different following square-root scale transformation. * Collection date removed from analysis.

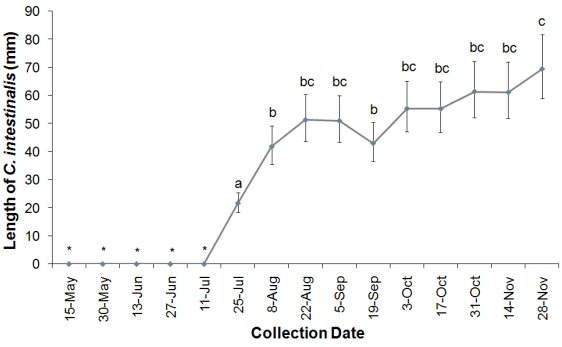

Figure 5. Estimated length (with 95% CI) of *C. intestinalis* accumulated over time in 2006 in the Brudenell estuary; sample dates with the same letter were not statistically different following natural log scale transformation. * Collection dates removed from analysis.

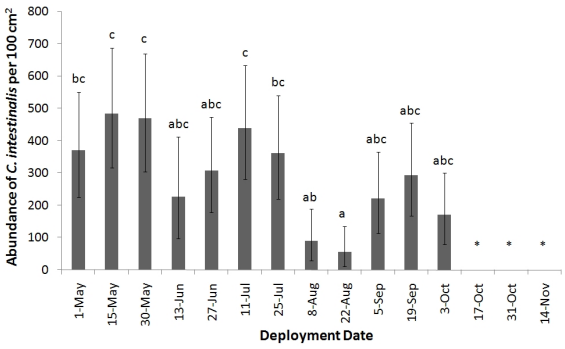

Figure 6. Estimated abundance (with 95% CI) of *C. intestinalis* accumulated over time, using different deployment dates, in 2006 in the Brudenell estuary; deployment dates with the same letter were not statistically different following square-root scale transformation. All samples were collected 28 November. * Deployment dates removed from analysis.

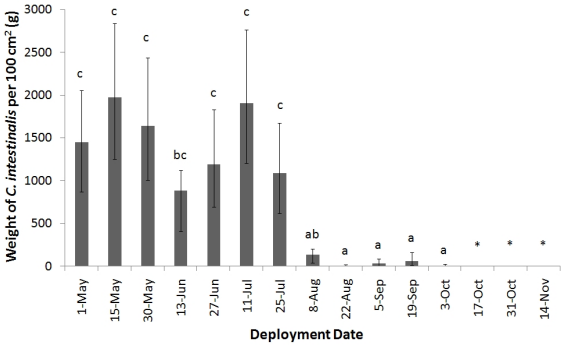

Figure 7. Estimated biomass (with 95% CI) of *C. intestinalis* accumulated over time, using different deployment dates, in 2006 in the Brudenell estuary; deployment dates with the same letter were not statistically different following cubic-root scale transformation. All samples were collected 28 November. * Deployment dates removed from analysis.

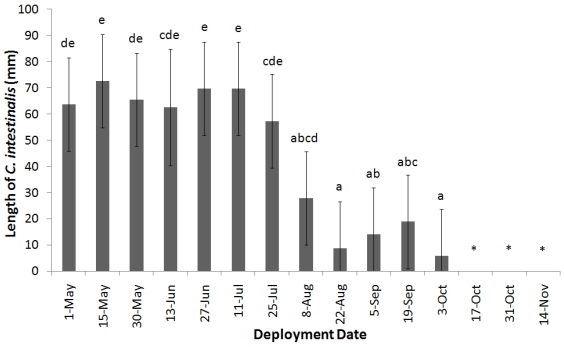

Figure 8. Estimated length (with 95% CI) of *C. intestinalis*, using different deployment dates, in 2006 in the Brudenell estuary; deployment dates with the same letter were not statistically different (square-root scale analysis). All samples were collected 28 November. * Deployment date removed from analysis.

and only a marginal difference was observed between water column positions (P=0.055). Abundance, biomass and length were different between retrieval dates in all analyses (P<0.001), and there was a significant deployment date by site interaction for biomass and length data (P<0.001).

Discussion

Early life history

In this study, the recruitment of *C. intestinalis* did not depend strongly on the position of the collector plates in the water column; however, location within the estuary did significantly affect recruitment patterns in the August-September period when water temperature was at its peak. In the absence of environmental data to explain this finding, we hypothesize it to be due to site differences in water depth, availability of alternative substrate in the vicinity of the collectors, and current flow patterns. The site with the highest recruitment levels (Site 3) during the peak recruitment period from late July until mid-October was near the shore, in relatively shallow water, and near a sandbar, which may have altered the water flow. In future studies it would also be of interest to deploy collectors which sample the entire water column to determine if there are differences in recruitment with depth. This study only sampled the upper 3 m of the water column, i.e., the zone where the mussels were being cultivated.

Recruitment patterns of *C. intestinalis* closely corresponded to water temperature. The first recruitment was observed when water temperatures rose above 8°C and ceased when water temperatures fell below 8°C. The recruitment levels increased with increasing temperature through the summer season. These results are consistent with the recruitment data reported by Carver et al. (2003) in Nova Scotia, although in PEI recruitment gradually increased to one major peak, while there were two peaks documented in Nova Scotia. Reasons for this may be the greater availability of seston and substrate availability in the water column in PEI due to the abundance of mussel crop in the vicinity. This would provide more food and space for the invasive tunicate to establish itself continually through the reproductive season. Temperature has also been shown to be an important environmental cue for the spawning of *S. clava*, another invasive tunicate in PEI (Bourque et al. 2007).

Effect on previously established tunicates

Before the introduction of *C. intestinalis*, *S. clava* was the primary invasive fouling tunicate of concern to the mussel industry. The reproductive period of *C. intestinalis* shown here is much greater than that of *S. clava* (Bourque et

al. 2007). Ramsay et al. (2008) have shown that the Brudenell estuary changed from a *S. clava* dominated system to a *C. intestinalis* dominated system within 2 years of the introduction of *C. intestinalis*. Both species of tunicate have caused significant production issues within the mussel aquaculture industry mainly because of the additional stress on mussel crops and associated gear due to the biomass of these organisms, however the fouling potential of *C. intestinalis* greatly exceeds that of *S. clava*. This information should be of concern to the mussel aquaculture industry in areas where *C. intestinalis* has not been introduced and highlights the need for monitoring, early detection and rapid response initiatives.

Temporal development

The design of the present study enabled us to determine the patterns of biomass accumulation for *C. intestinalis* from May through December 2006. This information is essential for determining when to apply mitigation techniques and how to appropriately alter husbandry practices. Important times in the seasonal dynamics of the tunicate are (1) late July when the population biomass begins to accumulate at an increasing rate, and (2) mid-August after which the population biomass of tunicates on newly deployed gear fails to attain a significant level by November. It appears that although peak recruitment happens in early September, these new recruits do not continue to grow at the rates observed by previously settled individuals and are possibly less viable later in the field season as water temperature begins to decline. While the first significant decline in biomass accumulation of *C. intestinalis* is observed in mid-August, the species is still recruiting until the end of November and biomass continues to accumulate until early October.

In 2006, severely decreased mussel productivity, due to the excessive fouling by *C. intestinalis*, was observed in the Brudenell estuary, PEI. Due to the cost and time requirements, the mussel aquaculture industry may consider one treatment for *C. intestinalis* in late July/early August, before new recruits have time to achieve considerable biomass. By early August, tunicate biomass was already 25% of that observed during mussel crop failures. This effort will stabilize the fouling biomass on mussel crop through the reproductive season of *C. intestinalis*. Significant winter mortality of *C. intestinalis* was observed in the spring of 2007, which, if observed in subsequent years, would additionally reduce the biomass of *C. intestinalis* on mussel crop to a more manageable level. *Ciona intestinalis* recruitment levels may be variable temporally and geographically, and winter mortality has been inconsistent between years, therefore the aquaculture industry needs to closely monitor the mussel crop for subsequent recruitment after treatment to determine the need for an additional treatment in late August/early September.

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