Ciona intestinalis environmental control points: field and laboratory investigations

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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

Life history processes, such as reproduction, survival and growth, are known to be strongly affected in ascidians by different types of environmental factors including temperature and salinity. In a field study conducted from 2005 to 2009 in southern Nova Scotia, an area affected by invasions of Ciona intestinalis, low winter and high summer temperatures were shown to be strongly associated with intra- and inter-annual variation in larval recruitment. No clear patterns of association were seen with other environmental variables such as chlorophyll and indices of nutrient concentrations. In a 12 week challenge experiment in the laboratory, survival and growth of juvenile C. intestinalis were affected by both salinity and temperature. Individuals exposed to high temperature (25°C) and low salinity (20) did not survive the sustained exposure. In addition, Individual Specific Growth Rates were shown to decrease as salinity decreased. Temperature and salinity are factors which will subsequently influence distribution, persistence and potential for spread of adult populations. New favourable temperature and salinity conditions (e.g., potentially resulting from global climate change) will likely alter the distribution patterns of C. intestinalis.

Conversely, infestation management techniques or site selection could benefit from unfavourable sustained temperature and salinity conditions.

Key words: vase tunicate, larval recruitment, survival, growth rate, environmental conditions, aquaculture

Introduction

The solitary ascidian Ciona intestinalis (Linnaeus, 1767) is distributed in northern Europe, on both coasts of North America and South America, in South Africa, in Australia and New Zealand, and along the Asian shore from Indonesia to Japan (Therriault and Herborg 2008). This successful invader is considered cryptogenic in the Mediterranean Sea and parts of eastern Canada, including Nova Scotia. C. intestinalis can exhibit dominant growth patterns relative to other species in its native and introduced ranges, to the point that it has become a prominent fouling species interfering with shellfish aquaculture in Atlantic Canada: in Nova Scotia since 1997 (Cayer et al. 1999; Clancy and MacLachlan 2004) and in Prince Edward Island since 2004 (Locke et al. 2007). Indeed, these large solitary tunicates are found in sheltered shallow coastal waters and can form dense and heavy aggregations on shellfish aquaculture gear and products, as well as completely foul artificial structures, such as docks and boat hulls.

The distribution of this ascidian is patchy in southern Nova Scotia (Sephton et al. 2011), although vectors and pathways for spread are widely available. Survival and reproduction are associated with the physiological tolerances of a species and characteristics of the environment (Guisan and Thuiller 2005), therefore, it is important to assess those traits and their interaction with environmental conditions in
order to understand the present distribution, persistence and potential for spread of \textit{C. intestinalis}.

Reports of the environmental (mostly restricted to temperature and salinity) tolerances for \textit{C. intestinalis} indicate wide ranges of conditions (\textit{i.e.}, -1 to 30°C and salinities of 12 to 40). These ranges clearly represent conditions for various populations or ecotypes from different locations and/or transient environmental conditions and, therefore, may overestimate the environmental tolerance of a particular population. In addition, these environmental conditions are reported for populations in their native ranges. At present, it is not clear whether local environmental conditions are the driving forces behind survival, growth and reproduction of the patchy \textit{C. intestinalis} populations in southern Nova Scotia.

Spawning and subsequent recruitment of \textit{C. intestinalis} have been found to be dependent on light (Svane and Havenhand 1993) and temperature (Carver et al. 2003; Howes et al. 2007). It has also been suggested that productive areas with high nutrient loads are susceptible to invasions by tunicates (Locke et al. 2007), and that artificial or enhanced natural (\textit{e.g.}, mussels) hard substrates contribute to their success (Wasson et al. 2005; Tyrrell and Byers 2007). Improving our understanding of environmental factors associated with survival, growth and reproduction of patchy \textit{C. intestinalis} populations in southern Nova Scotia will allow us to better predict secondary introductions and spread. Furthermore, understanding the environmental cues, and their interactions that influence such population growth, also has the potential to improve mitigation and control measures applied to limit the impact of \textit{C. intestinalis} infestations.

The goal of this study was to better understand the environmental factors influencing the recruitment and growth of \textit{C. intestinalis} through long-term investigations, both in the field and in the laboratory. Concurrent with on-going larval recruitment monitoring in the field, we monitored a suite of environmental parameters during the infestation period to try to account for intra- and inter-annual variation in recruitment, and to compare current conditions with previous environmental monitoring at the same location during pre-infestation years (Keizer et al. 1996). In the laboratory, we set up a factorial experimental design to examine the joint effects of temperature and salinity on survival and growth, in order to characterize the environmental tolerances of a population from southern Nova Scotia.

**Methods**

**Study site and water quality**

The study site was comprised of three different mussel leases in Indian Point, Nova Scotia (44°27.25′N; 64°19.00′W) where \textit{Ciona intestinalis} recruitment has been monitored since 2003 using collectors immersed at the same depth (Figure 1). The three leases have different \textit{C. intestinalis} infestation histories: Lease 1 and...
Lease 2 have been fouled for several years and are sheltered from wind and wave exposure while Lease 3 is more exposed. Lease 3 was fallowed (i.e., complete removal of substrate) in 2003 for the duration of one year and Lease 1 was fallowed later in 2005, also for one year, therefore Lease 2 will serve as an unfallowed reference.

Water samples and temperature/salinity measurements were taken biweekly in the field at the same location and depth as the collectors. In addition, water temperature was recorded hourly with a Minilog 8-TR (VEMCO, Halifax, NS) data logger from 2008 onward. Otherwise, water temperature and salinity were measured using YSI probes and meters (Yellow Springs Instruments, Yellow Springs, OH, Model 85 or ProPlus). From 2007 onward, water samples were taken using a 2L Niskin bottle and samples were held on ice during transport to the lab at the Bedford Institute of Oceanography (BIO). Raw water samples for relative nutrients analysis were not filtered in the field and were frozen upon arrival at the lab. Indices of dissolved reactive nutrient (soluble silicates, nitrate and ortho-phosphate) concentrations were determined with an AAII Technicon Segmented Flow Analyzer according to Industrial Methods #186-72W-1973, 158-71W-1972 and 155-71W-1973, respectively, while ammonia was determined fluorometrically following the method developed by Kerouel and Aminot (1997). Chlorophyll was determined with a Turner-Designs bench fluorometer following the method described in Mitchell et al. (2002).

**Recruitment**

Larval recruitment of *Ciona intestinalis* was monitored from June to December, in 2005 to 2010, with collectors deployed following the methods in Howes et al. (2007). The collectors, deployed at 5m depth, consisted of three inverted clear plastic Petri plates (surface area of 78.54 cm²) arranged horizontally under a plastic flowerpot saucer to provide the shading preferred by *C. intestinalis* larvae (Gulliksen 1972). In addition, the lip of the saucer prevented any “edge” effect (i.e., light, micro-hydrodynamics) detrimental to larval recruitment. Three collectors were equally spaced throughout each lease. Every second week, the Petri dishes were retrieved from the field collectors and replaced with new Petri dishes that had accumulated a biofilm for two weeks in the laboratory at the Bedford Institute of Oceanography, as the presence of a biofilm facilitates recruitment (Wieczorek and Todd 1997). The Petri dishes were placed in plastic bags filled with sea water and transported in a cooler from the field site to the laboratory. *C. intestinalis* recruits were identified and counted for every Petri dish using a dissecting microscope at 15× magnification within 24 h of collection.

As bi-weekly larval recruitment of *C. intestinalis* seemed to closely follow temperature, regardless of collector location at the study site, temperature data were extracted from Minilog files and averaged the same amount of time as the collectors deployment period as a proxy for the number of degree-days. Regressions of log(larval count) vs. log(temperature) were determined for the years 2007 to 2009 at Lease 1, years of complete environmental monitoring. No Minilog data were available in 2007, so temperature data collected using a YSI probe when the Petri dishes were retrieved were used instead.

**Condition and Gonadosomatic index**

A sample of 30 adult animals (30 to 100mm relaxed length) were regularly collected from nearby mussel lines on the study site in 2007 to determine the relationship between relaxed length (RL) or contracted length (CL) and dry weight (DW), measured to the nearest 0.1mg. Relaxed animals were obtained naturally, without treatment. Condition index \( CI = (DW_{viscera} + DW_{gonad})/DW_{total} \) (Petersen et al. 1995) and Gonadosomatic index \( GSI = DW_{gonad}/DW_{viscera} \) (D. Bourque, pers. comm.) were additionally calculated in 2008.

**Temperature and salinity tolerances**

Juvenile *Ciona intestinalis* recruits were obtained from the field site in order to study survival and growth under controlled environmental conditions. Fouling panels made of 15 cm × 15 cm PVC were deployed on a separate line at 5m depth at Lease 1 in August 2007 (Figure 1). Ninety-six panels were retrieved after 8 weeks and carefully transferred, immersed in coolers, to the quarantine laboratory at BIO where environmental conditions could be manipulated. Panels with an approximate coverage of 10 juvenile *C. intestinalis* were labelled and acclimated at ambient temperature of 15°C and salinity of 30.5 for four weeks. This extended acclimation period was necessary to
ensure that *C. intestinalis* individuals were not stressed by handling and general laboratory rearing conditions, which could make them more vulnerable to subsequent treatment effects.

Nine combinations of temperature and salinity (three levels of temperature: low 8, medium 16 and high 24°C and three levels of salinity: low 20, medium 25 and high 35) were set up in duplicate for a total of eighteen static tanks. The different salinities were prepared by mixing filtered fresh water and salt water or by adding the required amount of aquarium grade sea salt (Instant Ocean®, VA, USA). The different temperatures were obtained by immersing a cold water coil in the tank or by using a small adjustable heater. The fouled experimental panels with acclimated *C. intestinalis* individuals were randomly assigned to the different tanks which were brought gradually to experimental conditions over a period of two weeks (with adjustment of temperature and salinity of 2°C and 2 every 24 hours). Tanks were monitored daily for dissolved oxygen concentration, temperature and salinity. They were scrubbed clean three times per week when water was changed. Photoperiod was maintained at 15 h light / 9 h dark. Individuals were fed six times a week with a 1:1:1 mix of *Tetraselmis* sp., *Isochrysis* sp., *Thalassiosira weissflogii* (Instant Algae® Reed Mariculture, CA) to supplement ambient sea water to a final concentration of 10,000 cells ml⁻¹. This algal concentration is in the range of optimal conditions reported in growth and filtration rate experiments of *C. intestinalis* in laboratory (Peterson et al. 1995; Daigle and Herbinger 2009).

Panels were removed from the tanks at the end of the acclimation period and every three weeks thereafter, placed on a custom made stand fitted with a reference ruler, photographed with a Canon Powershot A620 camera (Canon Canada Inc., ON) and placed back in the tanks. This method allowed regular monitoring of survival and growth of individual ascidians over 12 weeks (4 periods of 3 weeks each). Since some individuals did not relax completely at the time of photography, contracted length was measured. To induce contraction, each individual was gently stimulated by touch and all were contracted at the time of photography. Contracted length measurements were appropriate since each tunicate was individually monitored over time for growth rate. In addition, previous measurements of relaxed and contracted length obtained for individuals sampled from the same study site in 2007 showed that relaxed length (RL) can also be derived from contracted length (CL) as: RL = 2.1711(CL)⁻⁰.⁹¹¹¹ (n=297, r²=0.83). Survival of individuals was assessed by examining live animals (i.e., observation of contraction) and pictures taken of individual panels, and Survival Rate (SR) was calculated for each panel as: SR=n_{t+1}/n_t where n_t is the number of live individuals on the panel at time t. Rates were then transformed as arcsin√(n_{t+1}/n_t)/t for normalisation. Individual length measurements were taken from the digital pictures for each period and Individual Specific Growth Rate (ISGR) were calculated as: ln(L_{t+1}/L_t)/t, where L_t is the individual length at day t. To test for significance of the effect of treatments on SR and on ISGR, two-way ANOVAs were performed for each period using Minitab 15 (Minitab Inc., Austin, TX, USA). When data were balanced with observations in each combination of temperature and salinity, a full model was fitted, with effects of temperature, salinity, interaction between temperature and salinity, and replicate tank effect nested within temperature and salinity. Temperature and salinity were treated as fixed effects and replicate tank as a random effect. When data were unbalanced without observations in some subset without observations in some subset, a simpler reduced model with temperature and salinity effects was fitted to the full dataset.

**Results**

**Water quality**

Summer temperatures recorded at Lease 1 at 5m depth were relatively higher in 2008 and in 2009 compared with 2007 (Figure 2). Salinity averaged around 31.2 and decreased to 26.8 following heavy rains in the spring and fall, particularly in 2009. Dissolved oxygen varied seasonally between 6.7 and 11.5 mg L⁻¹. Chlorophyll a concentrations were consistently low in 2007 (< 1.0 µg L⁻¹) and were lower than in 2008 and 2009 when chlorophyll a concentration occasionally rose to 6.0 µg L⁻¹. Relative nutrient concentrations ranged from 0.1 to 3.0 µM for ammonia with lowest concentrations in 2008. Nitrate concentrations were very low, close to 0 µM with late fall- winter
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Figure 2. Environmental parameters recorded at Lease 1, Indian Point, Nova Scotia, for years 2007, 2008 and 2009. Note that the right panels represent indices of nutrient concentrations. (November) peaks of 3.0 µM in. Phosphate concentration varied seasonally between 0.2 and 1.0 µM, and silicate concentration between 0.3 and 8.3 µM.

Recruitment
Lease 3, which was fallowed in 2003, showed ~50% cumulative recruitment of Ciona intestinalis in 2003, 2004 and 2005 when compared with the reference Lease 2 (Figure 3). By 2006, the level of recruitment was again similar to Lease 2. Similarly, Lease 1, which was fallowed in 2005, showed lower recruitment than Lease 2 from 2005 to 2007, at which point the three leases had similar levels of larval recruitment. In 2008, there was a 10 fold increase in recruitment compared with previous years, and this increase occurred for all leases. Larval recruitment was also very strong in 2009 for all three leases.

Figure 3. Cumulative yearly recruitment (total number of Ciona intestinalis recruited/Petri dish over a year) indexed at 100 for the reference Lease 2, Indian Point, Nova Scotia, in 2003. Note logarithmic scale.
Figure 4. Annual variation in *Ciona intestinalis* larval recruitment and temperature at Lease 1, Indian Point, Nova Scotia. Figures for 2003 and 2004 are modified from Howe et al. (2007). Temperature data from 2003 to 2007 are discrete measurements. Temperature data from 2008 to 2010 are computed from Minilog recordings. Note logarithmic scale of larval recruitment.

Bi-weekly recruitment at Indian Point showed a consistent yearly pattern, with usually two distinct peaks, closely associated with variation in water temperature. This pattern is very similar for the three leases, thus results are shown for Lease 1 only (Figure 4). The intensity of recruitment at Lease 1 varied greatly between years, from an average peak of 54, 54 and 40 larvae per Petri dish, in 2004, 2005 and 2007 respectively (low infestation years), to 197 and 110 in 2003 and 2006 (medium infestation years) to 1138, 340 and 599 in 2008, 2009 and 2010 (high infestation years), respectively. Larval recruitment during the summer of 2008 was high, particularly at Lease 1.

Regressions of log(larval count) vs. log(temperature) determined at Lease 1 were significant in 2007 and 2008 but not in 2009 (Figure 5). Larval recruitment of *C. intestinalis* was variable among the three collectors at Lease 1, especially when the numbers of recruits were high.

Condition and Gonadosomatic index

In 2008, the early decrease of the CI of the resident population, from 56% to 37%, seemed to correspond to the first increase in larval recruitment in early summer; however, CI increased again before the second peak of recruitment (Figures 6A and 6B) and from then slowly decreased until the end of the following winter. In contrast, as summer progressed, the GSI of the resident population slowly but continuously decreased from 15% in early June to a plateau of 5% in late fall (Figure 6C).

For the Indian Point *C. intestinalis* population, the relation between DW, WW and RL was established with individuals sampled in 2007 as $\text{DW} = 0.00003(RL)^{2.275}$ ($n=307$, $r^2 = 0.80$) and $\text{WW} = 0.0013(RL)^{2.2427}$ ($n=337$, $r^2 = 0.83$).

Temperature and salinity tolerances

In the experimental study, *Ciona intestinalis* individuals survived all treatment combinations for at least nine weeks except for the low salinity (20)-high temperature (24°C) treatment, where 100% mortality occurred in the first three weeks (Figures 7 and 8A). The first period (weeks 1 to 3) was thus the only period when all treatment combinations could be tested with a full model including salinity and temperature interaction. We observed significant effect of salinity ($F_{2,79}=21.93$ $P<0.001$) and temperature ($F_{2,79}=33.82$ $P<0.001$) on survival rate, but there was no significant interaction between the two factors and no significant effect of replicate tank.
Overall survival rate decreased during the first period with increasing temperature and with decreasing salinity. The same overall pattern was observed for the next three periods, weeks 4 to 6, 7 to 9 and 10 to 12 (Figure 8A). Because the low salinity (20)-high temperature (24°C) treatment was missing, a full model could not be fitted to the complete dataset. With six balanced data subsets (created by excluding either the salinity of 20 or the 24°C treatments for three periods), no significant salinity-temperature interaction was observed. A significant effect of replicate tank within treatment was observed in only two instances due to 100% mortality of one replicate in the 24°C-salinity 25 treatment and one replicate in the 24°C- salinity 35 treatment. However, these total mortality episodes appear to have been chance events due to the very low number of individuals alive in these replicate tanks at the beginning of the period. Hence, fitting a reduced model to the complete dataset with just the effect of salinity and temperature appeared warranted. There were significant effects of salinity ($F_{2,69}=9.89, P<0.001$ for weeks 4 to 6, $F_{2,58}=6.99, P=0.002$ for weeks 7 to 9 and $F_{2,49}=25.70, P<0.001$ for weeks 10 to 12) and temperature ($F_{2,69}=3.75, P=0.028$ for weeks 4 to 6, $F_{2,58}=10.09, P<0.001$ for weeks 7 to 9 and $F_{2,49}=12.69, P<0.001$ for weeks 10 to 12) on survival rate. As observed in the first period, survival rate decreased with increasing temperature and with decreasing salinity. Over the full 12 week challenge, 100% mortality occurred for tunicates exposed to a salinity of 20 regardless of temperature, or exposed to 24°C regardless of salinity. Mortality was particularly rapid for individuals exposed to both a salinity of 20 and 24°C. Only individuals exposed to low or medium temperature (8 or 16°C) and medium or high salinity (25 or 35) survived the full 12 week exposure.

Over the course of the experiment, mean Individual Specific Growth Rates of *C. intestinalis* individuals maintained in the different treatments were low (<1.0% per day) and sometimes negative as the weeks progressed, especially at low salinity (Figure 8B). Since all *C. intestinalis* individuals died within the first period (weeks 1 to 3) at the low salinity-high temperature (20 – 24°C) combination, our data set was generally unbalanced. Therefore, the full model with interaction was tested on six balanced data subsets (created by excluding either the salinity of 20 or the 24°C treatments) for the first three periods. No significant salinity-temperature interaction was observed, except in the specific case of the first period excluding the 24°C treatment. Similarly, the effect of replicate tank within treatment was not significant except for the specific case of the second period excluding the 24°C treatment. Fitting a reduced model to the complete dataset with just the effect of salinity and temperature appeared therefore warranted. There was a significant effect of salinity ($F_{2,272}=16.05, P<0.001$ for weeks 1 to 3, $F_{2,204}=4.18, P=0.017$ for weeks 4 to 7 and $F_{2,150}=5.40, P=0.005$) and temperature ($F_{2,272}=13.01, P<0.001$ for weeks 1 to 3, $F_{2,204}=6.09, P=0.002$ for weeks 4 to 7 and $F_{2,150}=3.66, P=0.029$) on growth rate.
Figure 6. Seasonal variation of *Ciona intestinalis* larval recruitment at one collector on Lease 2, Indian Point, Nova Scotia, for 2008 (A) and Condition index CI=(DWviscera + DWgonad)/DWtotal) (B) and Gonadosomatic index (GSI=DWgonad /DWviscera) (C) of mature individuals sampled at same location on Lease 2 in 2008. 

$P=0.050$ for weeks 10 to 12) on ISGR. A significant effect of temperature was only observed for the third period ($F_{2,154}=3.54\ P=0.031$). ISGRs decreased as salinity decreased during the first three periods. The effect of temperature in the third period seemed to be driven by a slightly higher growth at 24°C. For the last period, weeks 10 to 12, there were no observations for any treatment involving either a salinity of 20 or 24°C; therefore the full model was tested on the remaining 4 combinations. There was a nearly significant effect of salinity ($F_{1,82}=4.72\ P=0.068$), a significant effect of temperature ($F_{1,82}=29.39\ P=0.001$), and a strong interaction ($F_{1,82}=31.77\ P=0.001$) but no significant effect of replicate.

Discussion

Long-term studies of population dynamics are important because they often reveal seasonal variations. Yearly recruitment of *Ciona intestinalis* at Indian Point from 2003 to 2010, typically occurred in two distinct peaks: a smaller peak in the early summer, followed by a stronger peak in early fall. The highest density was observed in mid-September 2008, reaching 14.5 ind·cm$^{-2}$ over 2 weeks. This two-peak pattern has already been documented in Nova Scotia in studies lasting one or two years (Carver et al. 2003; Howes et al. 2007) and this long-term study confirms the pattern.

Counts of *Ciona intestinalis* individuals from PVC recruitment panels deployed in San Francisco Bay in 2002 also showed a strong seasonal pattern with a first peak in August and a second smaller peak in October (Blum et al. 2007), but density was low compared to Nova Scotia (2.7 ind·cm$^{-2}$ in 1 month). In eastern PEI, however, Ramsay et al. (2009) found that *C. intestinalis* recruitment gradually increased over the summer, reaching a single major peak in August 2006 with 48.4 ind·cm$^{-2}$ recruited to a 10 cm x 10 cm PVC panel over two weeks. In 2008, when *C. intestinalis* abundance peaked in Nova Scotia, the peak density on the PEI panels was 30.0 ind·cm$^{-2}$ over two weeks in mid-August (A. Ramsay, pers. comm.). In 2009, in Boughton Bay, PEI, densities on PVC plates were as high as 100 ind·cm$^{-2}$ (S. Collin, pers. comm.). Recruitment densities in PEI may even have been higher than reported, as fewer *C. intestinalis* larvae settle on PVC compared with plastic (polystyrene) Petri dish (B. Vercaemer, unpub. data).

It should be noted that the recruitment density and the cumulative recruitment observed here probably represent settlement of only a portion of the pool of *C. intestinalis* larvae present in the water column. According to Svane and Havenhand (1993), about half of the larvae which hatch from eggs retained in mucus strings may escape to the plankton. In a recruitment study in a Danish fjord, in *C. intestinalis* native range, maximum mean recruitment was 9.56 ind·cm$^{-2}$ over 2 weeks in early June (calculated from Peterson and Svane 1995), clearly representing a lower density for a native persistent population, compared to invasive populations such as in NS and PEI. From a management perspective, it is interesting to note...
that following a lease for one year resulted in lower infestation (up to 50%) for at least two subsequent years, relatively to unfallowed leases (Figure 3). However, this method, derived from terrestrial pest management control, was used during low infestation years and its validity has yet to be tested with high level of infestation and in locations where individuals could find refuge on other surrounding substrates.

*Ciona intestinalis* recruitment in both NS and PEI gradually increased with increasing temperature (Carver et al. 2003; Howes et al. 2007; Ramsay et al. 2009; this study). Furthermore, at Indian Point, a positive, relatively well-fitted exponential relationship was observed between the intensity of recruitment and temperature, particularly in 2008 when recruitment peaked. However, when there is high recruitment variability within a lease or a slight lag between recruitment and temperature peaks, such as in 2009, the relationship is not significant. Since many developing larvae are retained in adhesive mucus strings which create dense aggregations of both adults and newly settled *C. intestinalis* (Svane and Havenhand 1993), peak of recruitment generally translated two months later in peaks of adult infestation on nearby mussel lines (P. Darnell, pers. comm) where food is abundantly available and predation is minimal.

Previous studies have reported that temperature is an important cue for sexual maturation, spawning and recruitment, the latter displayed either in distinct peaks in cooler regions (Marin et al. 1987; Carver et al. 2003; Howes et al. 2007) or year round in warmer regions (Dybern 1965; Gulliksen 1972). The pattern seen here is consistent with cold temperate populations which produce two overlapping generations per year, the first generation being recruited as soon as the temperature reached \~8°C, reaching maturity later in the summer with increasing CI, and producing a second generation. The two generations co-exist through the winter and spawn as soon as the water temperature is suitable again, at which time the older individuals die (Dybern 1965; Carver et al. 2003; Howes et al. 2007). Non-feeding swimming larvae settle within 48h, therefore lag time between spawning and recruitment is minimal. Sexual maturity was particularly high for individuals collected at the study site in 2008, ranging from 75 to 85% of mature ovocytes present in the gonads of adults from May to October (D. Bourque, pers. comm.). The CI and GSI decreasing profiles (Figures 4B and C) would indicate that spawning and subsequent recruitment started in early June, which indeed was reflected in the recruitment profile (Figure 4A), but the two indices decreased in a different manner. The GSI decreased steadily from early June to a plateau in late fall until it increased again the following spring. CI actually reflects both spawning events (affecting DW_gonad) and growth activities (affecting DW_viscera) and is thus more complex to interpret. The profile observed here would then represent first the spawning activity of a fully grown, mature generation in early summer. This spawning resulted in a new wave of recruits.
Figure 8. Survival rate (A) and Specific Growth Rate (B) ±SE of Ciona intestinalis exposed to temperature and salinity combinations in the laboratory.

Table 1. Comparison of relative nutrient and chlorophyll concentrations between pre-infestation and this study.

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<td>Silicate (µM)</td>
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<td>Chlorophyll a (µg.L-1)</td>
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1 Data modified from Keizer et al (2006).
that mostly grew in late summer (hence the increase in CI), but by fall, both generations could be spawning, resulting in a plateau and a slow decrease in CI until the end of the following winter. Carver et al. (2003) also noted the difficulty of linking CI with concurrent spawning and growing activities in _C. intestinalis_. Depending on temperature and/or food availability, the second generation could spawn the first year in the fall if maturity is not delayed, and contribute in the subsequent year to the first recruitment and possibly the second recruitment peak. This incremental recruitment could explain the 2008, 2009 and 2010 outbreaks in the Indian Point population, although other populations have displayed irregular intense peaks which were not apparently linked to changes in environmental conditions (Keough 1983; Cayer et al. 1999). Nevertheless, not only was the water temperature in the summer of 2008 substantially higher than the previous summer, but the winter of 2008 was particularly mild. As winter water temperature was not closely followed until 2008, air temperature data for the area from Environment Canada was examined instead. The winters of 2004, 2005 and 2007 (identified as low infestation years) were indeed colder winters with average minimum/minimum temperature of -10.0/-20.5°C, -9.1/-17.6°C and -9.4/-21.0°C respectively, compared to an average minimum/minimum temperature of -8.0/-16.3°C, -9.3/-17.6°C and -5.2/-15.8°C in 2008, 2009 and 2010 (the warmest winter in the decade), respectively, years identified with high levels of infestation.

A recent environmental niche model (Therriault and Herborg 2008), based on the documented distribution of _Ciona intestinalis_ in Canadian waters, allowed predictions of potential distribution using layers of environmental conditions. Quarterly variations in temperature and salinity and annual oxygen and chlorophyll a concentrations represented layers which each contributed between 8 and 16% to the predictive model. It is worth noting that temperature overall contributed 46.4%, and salinity 30.7%, to the model. However, winter temperature (October to March) contributed 28.3% to the model, further emphasizing the need to closely monitor winter temperatures.

The environmental parameters measured from 2007, 2008 and 2009, a period corresponding to low, high and high levels of _C. intestinalis_ recruitment, respectively, were well within the range of values collected using the same protocols during a monitoring program at the same site from 1992 to 1994, prior to tunicate infestation (Keizer et al. 1996). During those years, at similar depth, temperature ranged from -2 to 17°C and salinity averaged around 30.5. Chlorophyll a, an integrator of nutrients, concentrations ranged from 0.2 to 4.0 µg L⁻¹ with lower values occurring during the winter months and an occasional peak to 7.9 µg L⁻¹. Relative nutrient concentrations were also very similar compared to pre-infestation concentrations (Table 1). In 2007, summer temperatures were lower than in 2008 or 2009, but fall temperatures were similar. Dissolved oxygen concentrations were lower in 2008 than other years, presumably associated with higher temperatures, as were the peaks in chlorophyll a. Salinity was reduced by a few heavy rainfall events in 2007 and 2009, while such events were very limited in 2008. This almost constant salinity in 2008 (i.e., limited influx of fresh water) seemed to be associated with lower, but not limiting, relative nutrient concentrations that year, in particular ammonia and silicate. In contrast, the rainfall events in 2009 appeared to correspond to influx of freshwater with nutrients such as ammonia, which in turn seemed to be associated with the large peak of chlorophyll a in the fall. Overall, when comparing environmental conditions in 2007, 2008 and 2009, with the exception of very low chlorophyll a concentrations in 2007 and low, but not limiting ammonia concentrations in 2008, no obvious pattern emerged, except for the differences in temperature. In fact, lower than normal temperatures in 2007 were probably not particularly conducive to high rates of primary production. Hence, low temperatures and low food availability might have been compounded factors associated with low recruitment of _C. intestinalis_ larvae that particular year.

Many environmental factors, such as food quality and availability, photoperiod and hydrodynamics are known to affect survival, growth and reproduction of ascidians, but temperature and salinity are the two most important (Dybern 1965; Guliksen 1972; Bates 2005; Lambert 2005; Carver et al. 2006). The different salinities and temperature tested in the experimental challenge are within the range of values recorded during the course of the Aquatic Invasive Species monitoring program at sites in coastal Nova Scotia where _C. intestinalis_ was present.
(Sephton et al. 2011). While these temperatures and salinity values are discrete, short-term measures, they clearly correspond to potential conditions which current or future populations could encounter and adapt. *C. intestinalis* individuals survived long-term (up to 12 weeks) exposure to some environmental conditions that are not commonly encountered for such duration in their current distribution range in Nova Scotia. At 8°C, a typical spring and fall temperature, there was a 35 and 65% survival rate at salinities of 25 and 35, respectively, after 12 weeks of exposure. At 16°C, a temperature commonly encountered in summer in sheltered bays of NS, the survival rate was 16 and 50% at salinities of 25 and 35, respectively. Sustained summer temperature of 24°C is probably unlikely in most locations in NS, except maybe in a few saltwater lakes and semi-enclosed estuaries along the Gulf of St. Lawrence coast (A. Locke, pers. comm.). Sustained salinities around 20 could occur in some NS estuaries. The size of the individuals which endured the experimental conditions for 12 weeks was well within the range of mature and spawning individuals in the field. In fact, *C. intestinalis* can mature early, at 8-10 weeks of age (Carver et al. 2003). The results of the challenge experiment suggest that sustained temperatures of 24°C or salinities of 20 would not allow long term survival of *C. intestinalis*. The *C. intestinalis* ecotype of southern Nova Scotia seems to display environmental tolerances that are narrower than the native populations of northern Europe (-1 to 20°C, salinities of 12 to 30), however these ranges are consistent with environmental conditions in much of Atlantic Canada.

The increase in body length in solitary ascidians may not be an ideal measure of growth, but in our opinion it is acceptable when there is a good relationship between relaxed length (RL) or contracted length (CL) and dry weight (DW) or wet weight (WW) as we observed in this study. The relationships established in this study (see Results) are very similar to those reported in Carver et al. (2003) and Carver et al. (2006). During the experimental combined temperature-salinity challenge, individual growth rates showed high variability and negative growth was observed almost systematically in the last three-week period before death. Negative growth was also observed preceding winter die-off in the solitary tunicate *Ascidia mentula* (Svane and Lundalv 1981) or in sub-optimal conditions (turbidity) for *C. intestinalis* (Peterson et al. 1997). Laboratory growth studies of *C. intestinalis* in Denmark showed specific growth rates varying from 0 to 2.41% length per day at ambient conditions (10°C and salinity of 20) over a 16 day experiment with low food availability (0 to 2,000 cells mL⁻¹) (Peterson et al. 1995). Specific growth rate measurements made in the field varied from -0.3% to a maximum of 1.3% length per day in a turbid eelgrass meadow environment over a 15 day study (Peterson et al. 1997). The specific growth rates established in those studies were calculated using relaxed length (ISGRRL) while contracted lengths (ISGRCL) were used in this study. The two measures may be compared using the formula ISGRRL = 0.9111 * ISGRCL where 0.9078 is the power coefficient of the relation established between RL and CL for the Indian Point population (see Methods). Here, in laboratory sustained low/high temperature and low/high salinity conditions, the maximum mean specific growth rate of *C. intestinalis* was ~1% length per day. However, even under these sub-optimal conditions, 62 of the 278 measured individuals grew between 1 and 2% length per day and 14 grew more than 2% length per day in the first 3 weeks. After 12 weeks, only 4 individuals were growing at more than 1% length per day. We did not observe growth rates equal to those reported by Petersen et al. (1995) who recorded optimum mean growth for *C. intestinalis* between 2–3% length per day. However, growth rates measured in the field can be highly variable; ISGRCL were measured in the field at Indian Point in September 2010 and varied from -1.58 to 1.97% length (n=38) during a 42 day period at 13–20°C (D. Sephton, pers. obs.). Nonetheless, the mean ISGRs displayed in this experimental challenge clearly reflected suboptimal temperature and salinity conditions.

Growth exhibited in laboratory may not necessarily reflect patterns observed in the field, even in suboptimal conditions (e.g., low salinity, high temperature) as food quality and abundance may be inferior to those in the field. In addition, without a true control (same salinity and temperature conditions as the acclimation period), the patterns observed in this laboratory study reflected relative tolerance to different salinity and temperature combinations. Nevertheless, they reveal physiological capabilities and consequently environmental tolerance ranges. Overall, the most challenging conditions for long-term survival and growth of *C. intestinalis* were low salinity (20) and high temperature.
(24°C) and, more importantly, the combination of those two factors.

Combining environmental current and potential conditions and physiological tolerance information is important, in particular, for management and control of *C. intestinalis* infestation. Yet, one must differentiate between transient and sustained exposure to different environmental conditions. For example, *C. intestinalis* individuals found near the surface can die out with a sustained decrease in salinity during September-November rainfalls, while individuals living deeper in the water column survive and their recruits recolonize shallower substrate the following spring (B. Vercaemer, pers. obs.). This observation has also been made for the *C. intestinalis* population of San Francisco in winter (Blum et al. 2007). Extreme reductions in temperature have also been implicated as a cause of death (see review by Millar 1971) in *C. intestinalis* populations. In other perennial solitary ascidians, such as *Ascidia mentula*, younger individuals seems to withstand extreme temperatures more successfully than older individuals and continue to grow but at a reduced rate (Svane and Lundalv 1981; Marin et al. 1987). Such extreme events likely control population dynamics only temporarily. However, less extreme, but sustained conditions, such as long-term exposure of juveniles to temperature and salinities similar to this challenge experiment, would allow survival, minimal growth and potentially reproduction of individuals; this may lead to local adaptations to new conditions or new sites. This is particularly important in the context of environmental and anthropogenic changes (Stachowicz et al. 2002).

With low rates of predation and inter-specific competition and high substrate availability around a typical mussel farm, variation in recruitment may be the most important factor regulating the abundance of *C. intestinalis*. Other external recruitment sources would be limited as larval dispersal has been shown, in a native Swedish *C. intestinalis* population, to be restricted (Svane and Havenhand 1993).

Suitable winter and summer temperatures and spring and fall salinities will greatly favour survival, growth and reproduction of *C. intestinalis* individuals. Infestation could then be controlled by modifying substrate availability (*e.g.* fallowing), by re-locating leases or fouled shellfish so as to take advantage of local brackish salinities within the environmental tolerance range of the production species, (N. D’Eon, pers. comm.) or local hydrodynamics such as wave exposure (Howes et al. 2007), or by biotic management (*e.g.*, lowering mussel socks to allow for controlled crab predation, P. Darnell, pers. comm.). These options are somewhat satisfactory but at present, mechanical (high pressure) or chemical methods are preferred in high infestation areas such as in eastern PEI (Locke et al. 2009).

In South Africa, Australia and New Zealand, countries within the invaded range of *C. intestinalis* where infestations have occurred on shellfish aquaculture sites, the initially dense populations have declined over the years (Kott 1990; McDonald 2004; Carver et al. 2006). However no single explanatory factor has been identified. In contrast, in southern Nova Scotia and in eastern PEI, the levels of *C. intestinalis* infestations do not yet show any sign of abating.

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**References**


