# Abiotic conditions are not sufficient to predict spatial and interannual variation in abundance of *Ciona intestinalis* in Nova Scotia, Canada

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ABSTRACT: The vase tunicate *Ciona intestinalis* is an invasive sea squirt that poses several challenges for coastal marine ecosystems and human activities. Despite its widespread distribution, temporal and spatial variability in population abundances is high. We tested whether this variation could be explained by 4 abiotic variables: temperature, salinity, pH and water movement. We repeatedly measured these 4 variables and relative abundance of *C. intestinalis* at 11 sites along the Atlantic coastline of Nova Scotia, Canada, each month between May and October 2014 and 2015. Using nonlinear mixed-effects modelling, we found that salinity moderately explained abundance of *C. intestinalis* across sites in 2014, while in 2015 there were no strong associations between abundance and any measured abiotic variable. The combination of little explanatory power within years and inconsistency between years led us to conclude that none of the measured abiotic conditions was useful in predicting the abundance of *C. intestinalis*. This finding contrasts with previous studies in which temperature and salinity were effective at predicting the presence or absence of the species. Thus, we suggest that tolerances of these 2 factors may determine whether *C. intestinalis* can survive in a location, but that other factors predict the rate of population growth. Given that increased abundance exacerbates the negative effects of this invasive species, we advocate further study of alternative factors that lead to higher abundances of *C. intestinalis*, to help inform management decisions.

KEY WORDS: Population dynamics · Invasion biology · Sea squirt · Biofouling · Benthic invertebrate · Longitudinal · Tunicate · Ascidian

## 1. INTRODUCTION

Biotic responses to the abiotic environment are fundamental in understanding and predicting species' distribution and abundance (Guisan & Zimmermann 2000, Elith & Leathwick 2009, Kearney & Porter 2009). Models predicting species' distribution and abundance based on abiotic conditions have been frequently used in terrestrial environments, but less so in marine ones (Robinson et al. 2011). Yet, the increasing accessibility of large-scale abiotic data from remote sensing, data loggers, and hydrodynamic and biogeochemical models (Reiss et al. 2011) suggests that such data-informed models could partially transcend the limitations of directly surveying marine biota (e.g. surveys of settlement plates in harbours, trawls or remotely operated vehicles). If correlations between easily measured abiotic conditions and the survival and abundance of a species can be established, future monitoring and predictions based

on monitoring of abiotic conditions could be a more effective and less costly approach.

Like several other ascidians, the vase tunicate *Ciona intestinalis* (L., 1767) is a prominent marine invasive species (Lambert & Lambert 1998). Native to Europe (Bouchemousse et al. 2016), *C. intestinalis* is present in Nova Scotia (NS), Canada. Its range is still expanding in this region, having been most recently discovered in Newfoundland (Sargent et al. 2013). Little information is available regarding the impact of *C. intestinalis* beyond artificial habitats in NS; however, *Ciona* populations on the west coast of North America (likely to be *C. robusta*, but called *C. intestinalis* by Blum et al. 2007) have been shown to reduce species richness and change community composition. In addition to the possibility of detrimental effects on ecosystem function that arise with any marine invasion (Grosholz 2002, Sherman et al. 2016), the introduction of *C. intestinalis* has been particularly damaging to long-line mussel aquaculture in Atlantic Canada (Ramsay et al. 2008, Daigle & Herbinger 2009). Biofouling of mussels and infrastructure leads to crop losses (Comeau et al. 2012) and reduced efficiency of harvesting, and potentially reduces the aesthetic value of the mussels, all of which reduce profitability. Thus, understanding and predicting the population dynamics of this invasive species will have key practical benefits in managing their populations (Sephton et al. 2011). Our overall goal here was to test whether abiotic conditions could predict the abundance of *C. intestinalis*.

*C. intestinalis* was formerly considered a globally distributed species with a wide-ranging tolerance for abiotic conditions. Recent studies have revealed that it is, in fact, a species complex composed of at least 4 distinct taxa (Caputi et al. 2007, Nydam & Harrison 2007, 2010, 2011, Zhan et al. 2010, 2012, Sato et al. 2012, Roux et al. 2013, Brunetti et al. 2015, Pennati et al. 2015). Two of these taxa, *C. robusta* (Hoshino and Tokioka, 1967) (previously *C. intestinalis* spA) and *C. intestinalis* (previously *C. intestinalis* spB), are widespread biological invaders. The others (*C. intestinalis* spC and spD) are restricted to the Mediterranean (Zhan et al. 2010). We studied *C. intestinalis* (spB) in NS, and since the different species probably have at least some different responses to abiotic factors (given their differing ranges), we took care to distinguish among these species when appropriate in the planning of our monitoring design, in the interpretation of results and in the discussion.

The distribution and abundance of *C. intestinalis* throughout coastal NS is spatially and temporally heterogeneous (Sephton et al. 2011). Despite this phenomenon having been described for some time (Svane 1983), it has yet to be fully explained. Heterogeneity could be due to (1) patterns of anthropogenic activity, such as aquaculture practices and vessel movement (Zhan et al. 2012); (2) genetic differentiation that causes different responses to similar environmental conditions (Grosholz 2002); (3) variable biotic interactions, such as competition, predation and facilitation (Gulliksen & Skjæveland 1973, Paetzold et al. 2012, Lockwood et al. 2013); or (4) variable abiotic conditions throughout the province (Vercaemer et al. 2011). We focussed on the latter possibility in this study.

Abiotic conditions have been linked to the survival, growth and reproduction of *C. intestinalis*, but it is uncertain whether these conditions explain patterns in abundance in nature. Certainly, the short life cycle (Carver et al. 2006) and short dispersal distance of larvae (Petersen & Svane 1995) both permit the possibility that short-term changes in abiotic conditions could lead to local changes in abundance. Temperature and salinity have been strongly linked to the distribution of *C. intestinalis* (Dybern 1965, 1967), while laboratory-based studies have found that these factors also influence its survival and growth (Vercaemer et al. 2011, Madariaga et al. 2014). *C. intestinalis* ceases pumping water through its siphons during exposure to lower-salinity conditions, thus providing a potential mechanism to alter survival, growth and reproduction through inhibited respiration, feeding and excretion (Shumway 1978). In addition, temperature could influence the abundance of *C. intestinalis* by affecting survival, growth and reproduction (Howes et al. 2007, Vercaemer et al. 2011, Madariaga et al. 2014). *C. intestinalis* is typically found in sheltered locations with low hydrodynamic stress (Petersen & Svane 1995, Howes et al. 2007). This is possibly due to areas of higher hydrodynamic stress hindering fine-scale larval settlement processes, such as favourable hydrodynamic conditions induced by aggregated formations of conspecifics, as well as preferential orientation during settlement (Havenhand & Svane 1991, Rius et al. 2010). Less is known about the influence of pH on *C. intestinalis*, but lower pH has been suggested to benefit its survival and growth (Dupont & Thorndyke 2009). Whereas temperature and salinity have been used to develop estimates of habitat suitability and to predict presence and absence (Therriault & Herborg 2008, Lowen et al. 2016b), the association with abundance is less clear (Januario et al. 2015). To quantify the relationships between abiotic conditions and abundance, rather than presence/absence, we used a spatially un-

coupled gradient of abundance, sampling a range of sites with historic low to high abundances to compare against abiotic conditions.

To test whether variable abiotic conditions throughout the province are correlated with abundance of *C. intestinalis*, we conducted repeated measures of abundance at sites along the Atlantic coast of NS. At the same sites, we paired these measures with continuous *in situ* monitoring of temperature, salinity, relative water movement and pH. We chose a nonlinear growth model as the most appropriate to fit the response (Paine et al. 2012). We used relative abundance of *C. intestinalis* as a population growth proxy and incorporated random effects into a mixed-effects maximum likelihood model to account for variability in the abundance of *C. intestinalis* that may not be explained by abiotic conditions. This approach allowed us to investigate the relationships between longitudinal, repeated measures of abiotic conditions and the corresponding abundances of *C. intestinalis* over time to test the descriptive hypothesis that differences in abiotic conditions are associated with differences in the relative abundance of *C. intestinalis*.

## 2. MATERIALS AND METHODS

### 2.1. Field sites

We collected data from May to October in 2014 and 2015 at 11 sites in the nearshore environment of NS, predominantly on the Atlantic coast (Fig. 1). For
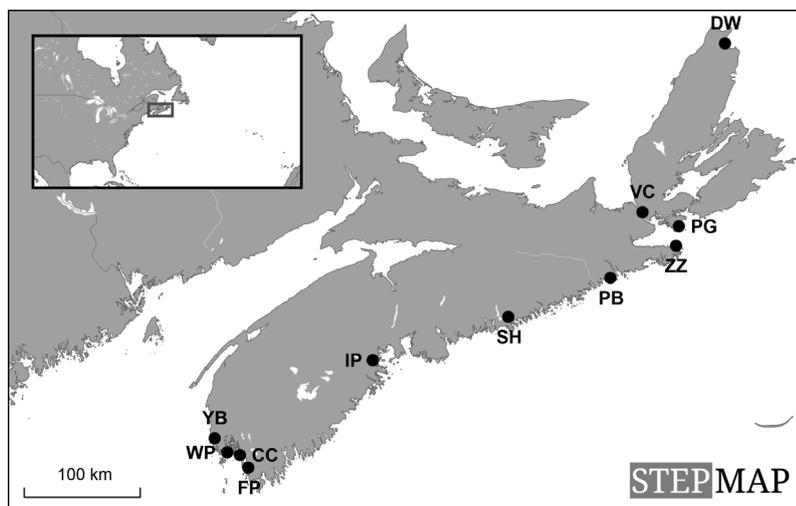


Fig. 1. Field sites (n = 11; see Table 1 for site name abbreviations and geographical coordinates) in Nova Scotia, Canada, where *Ciona intestinalis* abundance and abiotic conditions were monitored from May–October in both 2014 and 2015

*Ciona intestinalis* in this region, the timing of sampling captured the first spawning and recruitment period of both years, as well as encompassing the entire adult life cycle (Carver et al. 2003, 2006). Sites were harbours, mussel leases or sheltered bays, and all sites had floating docks or mussel aquaculture infrastructure on which to suspend monitoring equipment (Table 1). We chose these sites because they ranged along the longest contiguous axis of NS and because they provided a range of abundances of *C. intestinalis* (Table S1 in the Supplement at www.int-res.com/articles/suppl/m628p105_supp.pdf), based on historical records from the Department of Fisheries and Oceans Canada (unpubl.) and from Sephton et al. (2011, 2017).

### 2.2. Biotic data

Relative abundance of *C. intestinalis* was recorded at each site using settlement collectors similar to those described by Sephton et al. (2011). Percent cover was chosen as a measure of relative abundance, as it optimised the trade-off between total sample size (combining settlement plates per site and number of sites) and time for analysis (counting individual *C. intestinalis* in images with high densities would have been prohibitively time intensive with our sampling structure). Percent cover has previously been shown to be an appropriate measure of abundance (Wikum & Shanholtzer 1978, Foster et al. 1991, Drummond & Connell 2005, Rees et al. 2014), and we further verified this by confirming the close relationship between percent cover and abundance in a subsample of our settlement plates (Fig. S1). Each collector comprised 3 PVC settlement plates (10 × 10 × 0.3 cm), oriented horizontally in the water, tied at 25 cm intervals along a polypropylene rope (8 mm diameter), with a lead weight (0.4 kg) at the end, 25 cm below the bottom plate. Three collectors were deployed at each site in 2014 and 5 at each site in 2015. Collectors were attached to available infrastructure at each site, off the side of floating docks at harbours or directly tied to mussel farm lines. The collectors were submerged in the water so that the 3 plates were suspended approximately at 1.5, 1.75 and 2.0 m depth.

Table 1. Field site names, abbreviations, mean (± SD) relative abundance (rel. ab.) from the final measurement periods for 2014 and 2015, site types, and GPS coordinates. Deployment types were from docks in harbours partially enclosed by solid breakwaters, docks in sheltered bays or from mussel aquaculture infrastructure in sheltered bays

| Name | Abbr. | Rel. abundance (± SD) | | Type | Latitude (°N) | Longitude (°W) |
| --- | --- | --- | --- | --- | --- | --- |
| | | 2014 | 2015 | | | |
| Camp Cove | CC | 100 (0) | 100 (0) | Harbour | 43.7242 | 65.8399 |
| Cape Canso | ZZ | 73.9 (14.9) | 31.4 (35) | Harbour | 45.3348 | 60.9852 |
| Dingwall | DW | 1.3 (2.7) | 100 (0) | Harbour | 46.9031 | 60.4608 |
| Falls Point | FP | 100 (0) | 100 (0) | Harbour | 43.5311 | 65.7408 |
| Indian Point | IP | 100 (0) | 85.6 (10.1) | Mussel lease | 44.4571 | 64.3068 |
| Petit-de-Grat | PG | 82 (24) | 92.6 (9) | Sheltered bay | 45.5072 | 60.9607 |
| Port Bickerton | PB | 93.9 (7.7) | 46.1 (26.5) | Harbour | 45.1044 | 61.7219 |
| Ship Harbour | SH | 35.6 (14.3) | 99 (2.9) | Mussel lease | 44.8081 | 62.8466 |
| Venus Cove | VC | 4 (3.9) | 51.9 (17.7) | Sheltered bay | 45.6146 | 61.3901 |
| Wedgeport | WP | 3.7 (8.1) | 78.1 (30.6) | Harbour | 43.7140 | 65.9686 |
| Yarmouth Bar | YB | 0 (0) | 3.6 (9.6) | Harbour | 43.8158 | 66.1481 |

After deployment, we visited sites at approximately 4 wk intervals. During each visit, we photographed the underside of all plates. Thus, each image records the cumulative change in growth over the preceding ~4 wk measurement period. Each settlement collector (i.e. set of 3 settlement plates) was only removed from the water for photography for a maximum of 1 min per site visit. The plates were photographed by placing them upside-down in a custom holder covered with a light box (a plastic container with interior lighting from LED lights) to provide uniform and consistent lighting, with the added benefit of shading the settlement plates from exposure for the brief emersion time. The bottom of the plates was chosen for photography, as *C. intestinalis* displays a clear preference to settle and grow on this surface (Rius et al. 2010). We limited the temporal offset (usually 8–10 d) between the first and the last site sampled in any given measurement period. The order of site visits in each measurement period was also kept as consistent as possible to further minimise temporal discrepancies in the data among sites.

## 2.3. Biotic metric

To determine relative abundance of *C. intestinalis*, all images of plates were cropped and scaled to apply a uniform 10 × 10 grid-cell overlay on each image using ImageJ (Schindelin et al. 2015). The relative abundance was calculated from the number of grid cells in which *C. intestinalis* occupied 50% or more of the area, divided by the total number of cells (100 minus any grid cells obscured by collector rope).

## 2.4. Abiotic data

We chose to monitor temperature, salinity, pH and relative water movement (RWM). Data were recorded at 5 min intervals throughout the monitoring period beginning at the start of May and finishing at the start of October in both years. Data were then manually subdivided into sections between the start and end of each measurement period to exclude data collected during cleaning, calibration and data-uploading performed during site visits. Additional manual filtering eliminated any further aberrations due to occasional brief emersions from the water. In 2014, temperature and salinity were recorded by INW AquiStar® CT2X Conductivity Smart Sensors; pH and temperature were recorded by WTW WQL-pH Loggers; and relative water movement was recorded using Onset HOBO Pendant® G Acceleration Data Loggers. In 2015, temperature, salinity and pH were all recorded using YSI 600XLM Sondes, and relative water movement was recorded using Onset HOBO Pendant® G Acceleration Data Loggers. We calibrated conductivity and pH probes according to manufacturers' guidelines. Following the methods employed by Filip (2014), the positively buoyant accelerometers were tethered to the collectors to allow free 3-dimensional movement. The summed variance for each of the 3 dimensions of acceleration readings was used as a measure of RWM. Data loggers were submerged so that the thermometer/conductivity probe/pH probe/accelerometer was approximately at 1.5 m depth. We experienced significant data losses in 2014 (see Fig. 4) because of (ironically) bio-fouling (conductivity and pH loggers), water leaks into the data loggers resulting in malfunctions (pH

loggers) and the Atlantic Ocean (accelerometers washed away). In 2014, the overall quantity of missing measurement periods was: temperature = 1.8%; salinity = 25.5%; pH = 27.3%; and RWM = 27.3%. In 2015, we lost 1 measurement period of RWM data (1.8% of total; see Fig. 5).

## 2.5. Abiotic metrics

We calculated minimum (min), mean and maximum (max) metrics for temperature, salinity and pH. For RWM, we used the min, mean and max variance in acceleration. A value for each of these 12 metrics was calculated for every measurement period at every site, corresponding to the relative abundance value of *C. intestinalis* from that same site and measurement period. To calculate min and max metrics that were not subject to anomalous measurements, we used the min and max daily mean values for the metrics.

## 2.6. Statistical analysis

We conducted the statistical analysis separately for each year and then performed interannual predictions using the best models from the alternate year to test the predictive power outside of the sample data used to develop the models.

### 2.6.1. Abiotic metric selection

First, to reduce collinearity between abiotic metrics, we used a stepwise removal preselection process to choose just 1 metric (mean, min or max) per abiotic variable (temperature, salinity, pH, RWM). All abiotic metrics were compared in a pairwise fashion using Pearson's r correlation. For the pair with the highest correlation, we removed 1 metric based on *a priori* preference to prioritise the inclusion of mean temperature, minimum salinity and pH, and maximum RWM in the modelling process (when possible), as we assumed these would be most likely to influence growth. One exception to this was in 2014, when mean salinity was preferred to min salinity, due to the results of an extensive exploratory model development period prior to the formal bottom-up and top-down model development approaches that are described in Section 2.6.3. The stepwise removal process was repeated until 4 metrics, 1 for each abiotic variable, remained. The full process is described

in Tables S2 & S3. Any missing data were not interpolated, as the mixed-effects modelling we used adequately accommodates missing data. This allowed us to adhere to the principle of only using measures for which we had empirical values.

### 2.6.2. Growth model

We used a mechanistically derived logistic function (Eq. 1) to model the nonlinear response of the relative abundance of *C. intestinalis*. We then used nonlinear mixed-effects modelling to assess the longitudinal, repeated-measures data, allowing us to maximise data usage, given that missing data resulted in an unbalanced dataset. The logistic function had 3 parameters: the maximum population size (MaxPop); the time in days that it takes to reach half that maximum (D50); and a lag parameter that adjusted the slope through the inflection point of the growth curve (Lag). To ensure that the response function remained positive, MaxPop and D50 were included as exponents (consequently, interpreting parameter values needs to account for this). Days after deployment ($t = 0$) was used as the known time variable:

$$X(t) = \frac{\exp(\mathrm{MaxPop}) \times t^{\mathrm{Lag}}}{\exp(\mathrm{D50}) + t^{\mathrm{Lag}}} \qquad (1)$$

A mixed-effects modelling method was used to obtain fixed-effects and random-effects parameter estimates. We implemented this using the 'nlme' (Pinheiro et al. 2015) and 'boot' (Canty & Ripley 2012) packages in R, as seen in Eq. (2):

$$Y = \mathrm{nlme}(100 \times ((\mathrm{inv.logit}(X(t)) - 0.5) \times 2)) \qquad (2)$$

where $Y$ is the response variable (*C. intestinalis* %). In Eq. (2), the range of inv.logit() is [0, 1], but because the input value ($X(t)$) has a restricted range of [0, +inf], inv.logit($X(t)$) is restricted to a range of [0.5, 1]. Thus, Eq. (2) includes arithmetic adjustments to ensure the final range of the fitted response variable is between 0 and 100 (%).

To link our modelling language to the phenomena we studied, we used the following terminology for fixed and random effects. The fixed effects are the average response (relative abundance of *C. intestinalis*) that can be accounted for by observed variables (the monitored abiotic data) for the entire population studied (Subedi & Sharma 2011). We refer to these fixed effects as the 'species-level' effects, referring to the effects consistent across all populations measured in NS. In addition to variation in the response that can be accounted for by species-level ef-

fects, there are unknown within-site sources of variance in the abundance of *C. intestinalis*. Random effects (or modelled varying intercept; Bafumi & Gelman 2007) account for this unknown variability and, thus, we refer to these random effects as 'site-specific' effects. Incorporating the site-specific effects into the model parameter estimates broadens the relevance of this study beyond the 11 sites studied, as it provides an estimate of uncertainty for predictions at new sites.

### 2.6.3. Bottom-up and top-down model development

We began with a bottom-up model development approach. First, we fitted a 'base model', lacking abiotic covariates. After successful model convergence, we used a stepwise model selection process to identify the best combination of site-specific effects in addition to the species-level effects. We added site-specific effects to the model parameters in the following order: MaxPop, D50 and then Lag, first each individually and then cumulatively. Each new model with a different site-specific effect structure was compared to the previous one using Akaike's information criterion corrected for small sample sizes ($AIC_c$) to compare model likelihoods (Burnham & Anderson 2002). If model likelihood was not improved significantly by a more complex site-specific effect structure, the more parsimonious model was selected.

Next, we explored the inclusion of covariates in the model parameters. We centred the covariates to stabilise parameter estimation (Sequeira et al. 2014), except for the RWM metrics, which were already centred near 0. We introduced the abiotic metrics as covariates in the parameters in the following order: MaxPop, D50 and then Lag. We chose this sequence based on the relative effects of the 3 parameters on fitting carrying capacity abundance at each site, which was our priority. Covariates were introduced into parameters based on evidence of their effects on the growth of *C. intestinalis* and visualisation of the abiotic trends in our data. For example, salinity and temperature are considered to be the most important abiotic conditions (Dybern 1967, Therriault & Herborg 2008, Vercaemer et al. 2011, Lowen et al. 2016b), whereas preliminary data visualisation suggested that salinity displayed the strongest relationship with *C. intestinalis* in 2014. In this case, we always introduced the salinity metric into each of the model parameters first (in the order specified above). Each time a new model was fitted with different covariates in the model parameters, its goodness-of-fit

was assessed graphically (Klein et al. 2012), whereas model likelihoods were compared using $AIC_c$ for the 2 most recently specified models. Once model improvements plateaued, the candidate set of all models fitted was assessed based on the $AIC_c$ values and Akaike's weight ($w_i$) within the set.

We also carried out a complementary top-down model selection process. Model selection proceeded using all 4 preselected abiotic covariates in each of the 3 model parameters (MaxPop, D50 and Lag). We started with a 'global model', which was developed by selecting the most parsimonious site-specific structure with all abiotic covariates included (assessed in stepwise comparisons with $AIC_c$) (Burnham & Anderson 2002). Model designs were chosen *a priori* for the top-down process starting with the global model. Each of the abiotic covariates was systematically removed from the global model in a predefined order, consistent for all covariates. Each covariate was first excluded from parameters one at a time (MaxPop, D50 and Lag), creating 3 single-exclusion models per covariate. Subsequently, each covariate was excluded from 2 of the parameters at a time, creating 3 more double-exclusion models per covariate. Finally, each covariate was excluded from all model parameters (a full-exclusion model). For example, considering the salinity covariate, the first model excluded salinity from only the MaxPop parameter, followed by 2 more single-exclusion models with salinity excluded from only D50 or Lag. Double-exclusion models removed salinity from MaxPop and D50, MaxPop and Lag, or D50 and Lag. Finally, the reduced global model was run without the salinity covariate in any of the model parameters. This procedure was repeated for each abiotic covariate.

### 2.6.4. Model accuracy

Mean absolute error (MAE), root mean squared error (RMSE), modelling efficiency (MEF) and Spearman's rho ($\rho$) are the 4 measures of model accuracy recommended by Olsen et al. (2016) that we used to assess each model. MAE and RMSE were calculated using the 'hydroGOF' package (Zambrano-Bigiarini 2014), while 'JBTools' was used for MEF (Buttlar 2015). Interannual predictions were used to calculate the accuracy of out-of-sample predictions.

All plots were made using the 'Lattice' package (Sarkar 2008) and 'gridExtra' (Auguie 2012). All model development and assessment was carried out using R, version 3.2.2 (R Core Team 2015), run through RStudio, version 0.99.489 (RStudio Team 2015).

# 3. RESULTS

## 3.1. Observed growth

As expected, the relative abundance of *Ciona intestinalis* differed among sites in 2014 and in 2015. Final abundances ranged from 0–100% in 2014 and from 4–100% in 2015, with variable rates of increase in abundance (Figs. 2 & 3). Striking interannual differences in the final abundance of *C. intestinalis* within sites were also recorded. The sites with the most notable increases in abundance from 2014 to 2015 were Dingwall (DW: +99%), Ship Harbour (SH: +63%) and Wedgeport (WP: +74%), which are widely separated geographically (Fig. 1), and interspersed with sites having no such increases. The
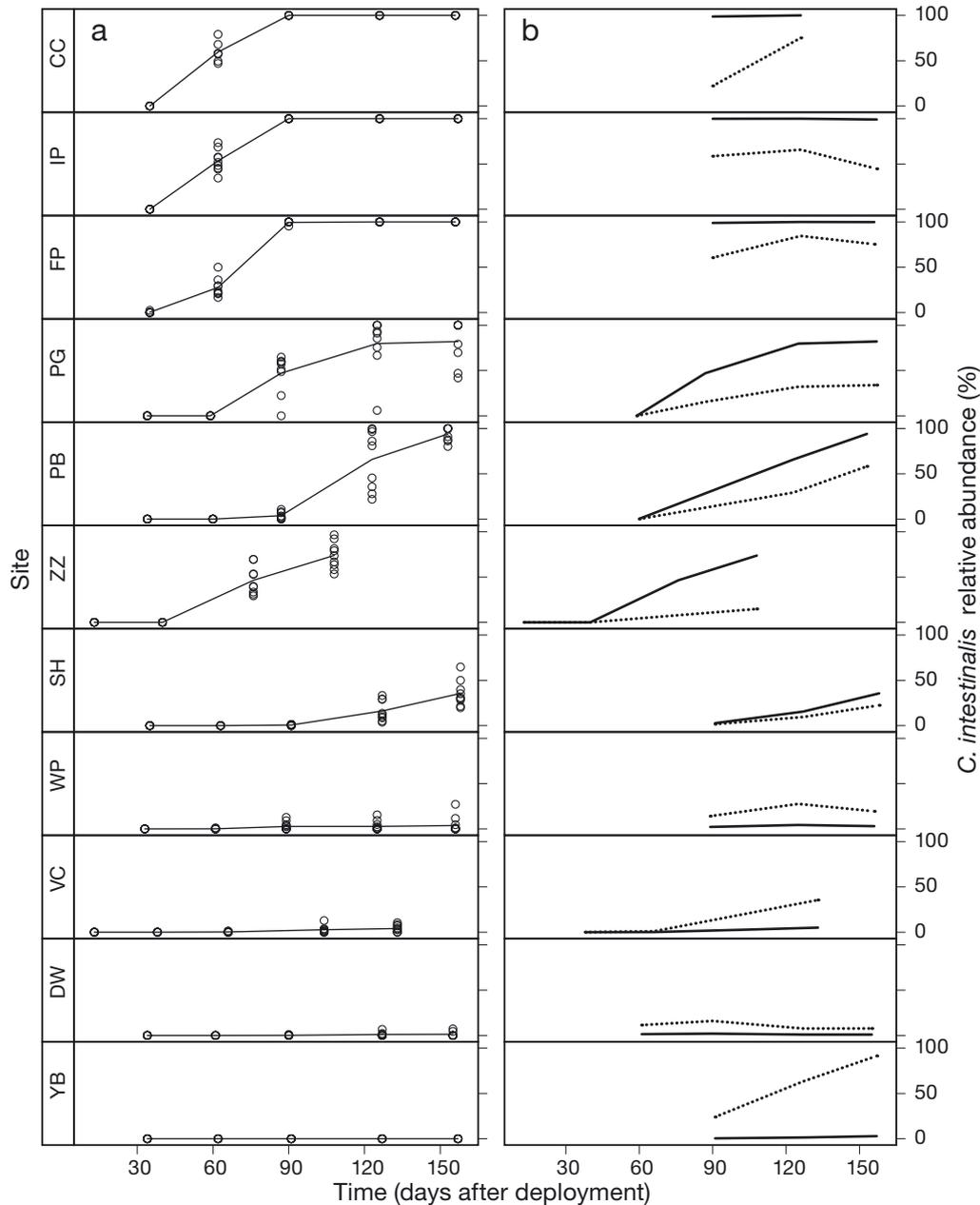


Fig. 2. (a) Observed *Ciona intestinalis* relative abundance (% cover) over time on individual settlement plates (black circles) and averaged for each site (solid black line) in 2014. (b) Fitted values from the best model (identical for best bottom up [BBU] and best top down [BTD] model development methods) in 2014, for the full-fit (solid lines) and for the species-level effects (dotted lines). The full-fit is the combination of species-level (fixed) effects and site-specific (random) effects. The relative contribution of the site-specific effects to the model fit for any site is the difference between the full-fit and the species-level fit. Site abbreviations as in Table 1
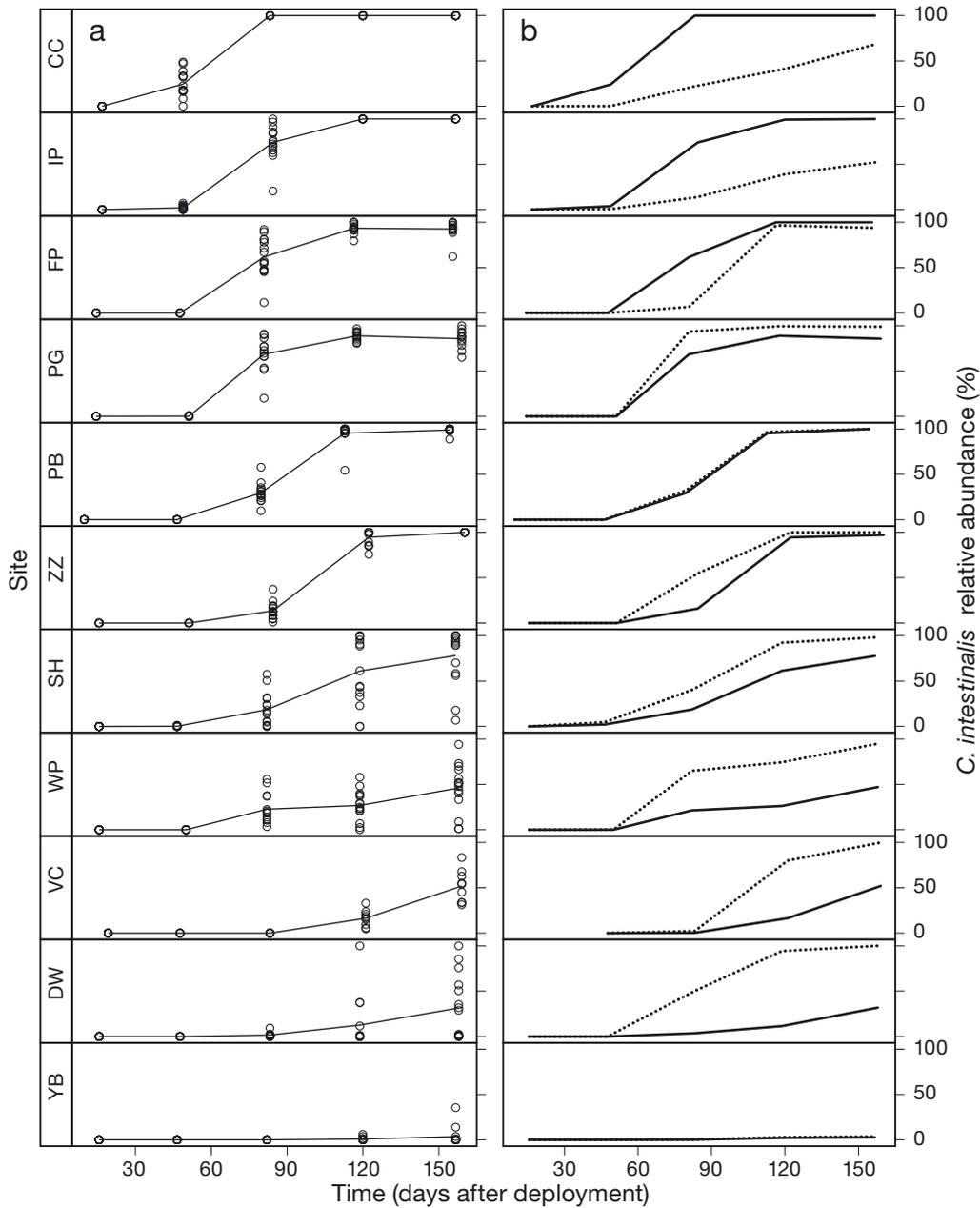
Fig. 3. As in Fig. 2, but for 2015. The fitted values in (b) are from the best model (best top down)

abundance of *C. intestinalis* also increased to a lesser degree at Venus Cove (VC: +48%) from 2014 to 2015 (Figs. 2 & 3). The sites at which we observed a reduction in abundance from 2014 to 2015 were Cape Canso (ZZ: −42%) and Port Bickerton (PB: −48%). The remaining sites had consistent relative abundance between the 2 years: Yarmouth Bar (YB: +4%), with consistently low abundance; and Camp Cove (CC: full saturation both years), Fall's Point (FP: full saturation both years), Indian Point (IP: −14%), and Petit-de-Grat (PG: +11%), all with consistently high abun-

dance. The observed relative abundance presented a spectrum from low to high, as well as large interannual differences, allowing us to fit models to a range of population growth dynamics.

## 3.2. Abiotic conditions and metric preselection

There were some differences in the temporal patterns of the abiotic conditions across measurement periods. Temperature increased over the first 4 meas-

urement periods at all sites, regardless of differences in initial and maximum temperatures (Figs. 4 & 5). Temperature at all sites peaked in the fourth measurement period (except YB in 2015), and then decreased by varying amounts in the final period. Salinity increased throughout the measurement periods at some sites, while at others it decreased slightly over time. Temporal variability in salinity was relatively high compared to the seasonal trend (more so than for temperature). Both pH and RWM had little or no temporal pattern but did exhibit substantial site-to-site variability (note that variation in 2015 RWM is less apparent in Fig. 5 due to inclusion of an extreme value at SH caused by a storm event).
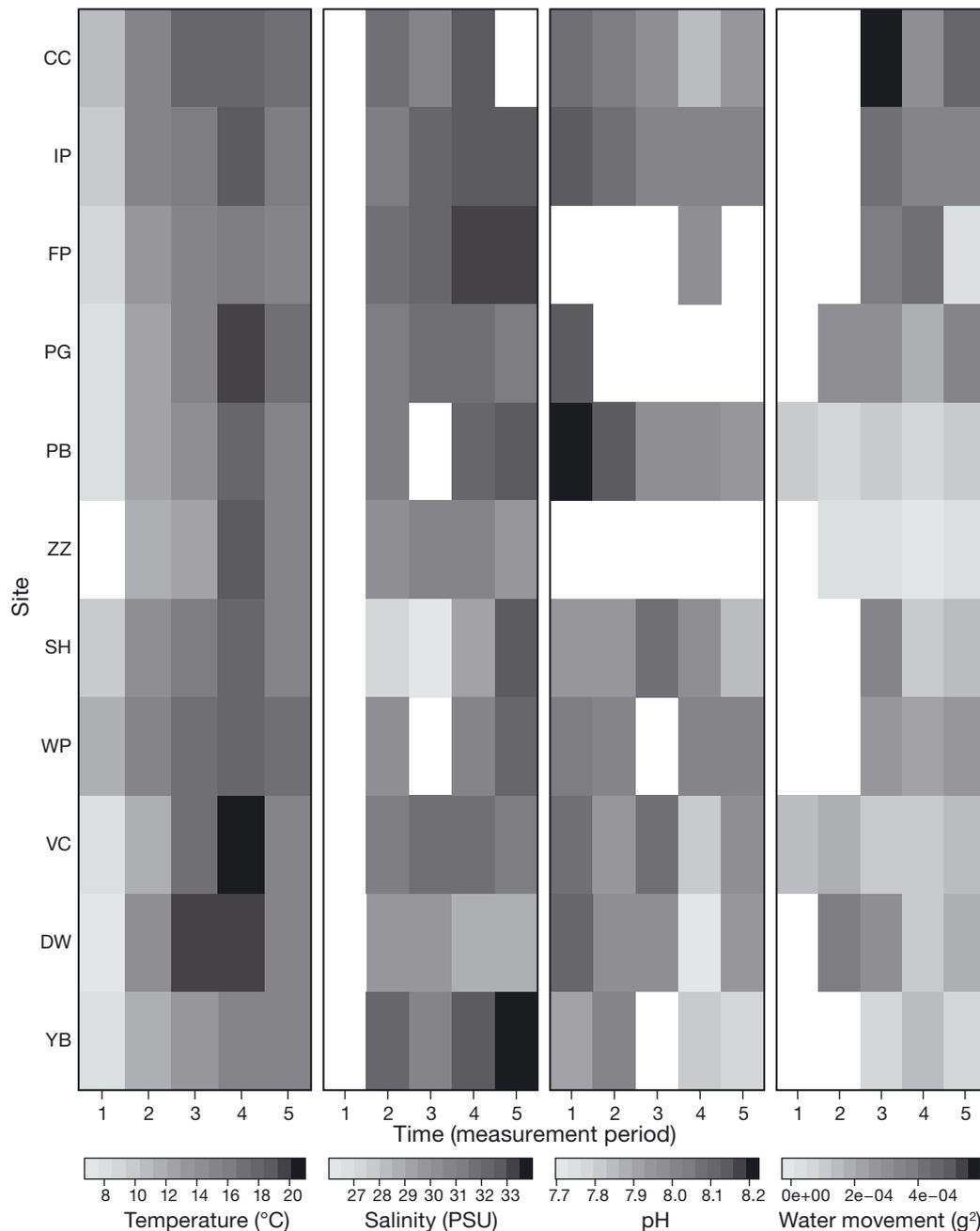


Fig. 4. Abiotic metrics for each of 5 measurement periods used in the 2014 model development. Sites are ordered top to bottom from highest to lowest overall mean *Ciona intestinalis* relative abundance. The metrics are presented using separate heatmap scales (bottom). White spaces are missing data
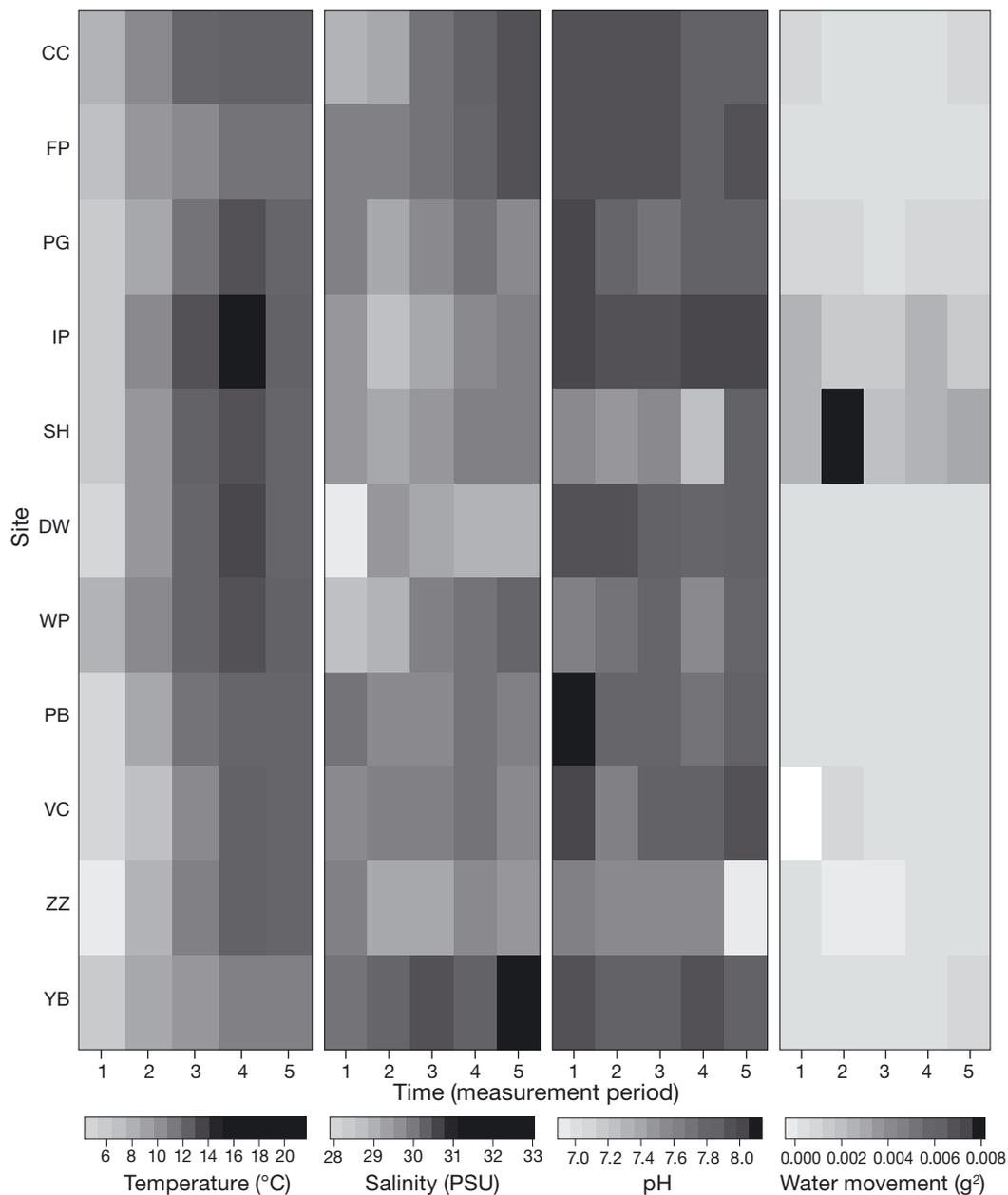
Fig. 5. As in Fig. 4, but for 2015

The stepwise preselection process (Tables S2 & S3) resulted in the selection of mean temperature, mean salinity, minimum pH and minimum variance in RWM for use in model fitting for the 2014 dataset (Fig. 4). Model convergence could not be achieved when minimum pH was included in the model (primarily due to gaps in the data in 2014 due to equipment failures). Mean temperature, maximum salinity, minimum pH and maximum variance in relative water movement were the 4 abiotic metrics selected in the 2015 data (Fig. 5).

### 3.2.1. Bottom-up and top-down candidate sets: 2014

The model with the most statistical support (lowest $AIC_c$ value) in 2014 was the same for both the bottom-up and the top-down methods of model development (Tables 2 & 3). The model parameter estimates can be found in Table S4. This model included mean salinity as a covariate in all 3 of the model parameters. All models in both candidate sets had site-specific (random) effects for the MaxPop parameter only. The lower $w_i$ for this best model amongst the top-down

Table 2. Top 5 ranked models from the bottom-up model development approach for 2014, ordered by decreasing Akaike's information criterion corrected for small sample sizes ($AIC_c$). The candidate set was composed of 30 models. The number of parameters ($k$), $AIC_c$ and the $AIC_c$ weight within the candidate set ($w_i$) are provided for each model. MaxPop was specified as a site-specific effect. The abiotic metrics used in model fitting for 2014 data were Salinity: mean salinity; and Temp: mean temperature. The 3 model parameters are the maximum population size (MaxPop), the time in days that it takes to reach half the MaxPop (D50) and a parameter that adjusts the slope through the inflection point of the growth curve (Lag)

| Model parameters | $k$ | $AIC_c$ | $w_i$ |
| --- | --- | --- | --- |
| MaxPop ~ Salinity, D50 ~ Salinity, Lag ~ Salinity | 8 | 2524.8 | 0.48 |
| MaxPop ~ Salinity, D50 ~ Salinity + Temp, Lag ~ Salinity | 9 | 2526.9 | 0.16 |
| MaxPop ~ Salinity, D50 ~ Salinity x Temp, Lag ~ Salinity | 10 | 2528.9 | 0.06 |
| MaxPop ~ Salinity × Temp, D50 ~ Salinity, Lag ~ Salinity | 10 | 2529.0 | 0.06 |
| MaxPop ~ Salinity, D50 ~ Salinity, Lag ~ Salinity × Temp | 10 | 2529.0 | 0.06 |

Table 3. Top 5 ranked models as in Table 2, but from the top-down model development approach for 2014. The candidate set was composed of 29 models. minRWM: minimum relative water movement; other details as in Table 2

| Model parameters | $k$ | $AIC_c$ | $w_i$ |
| --- | --- | --- | --- |
| MaxPop ~ Salinity, D50 ~ Salinity, Lag ~ Salinity | 8 | 2524.8 | 0.30 |
| MaxPop ~ Salinity + Temperature + RWM, D50 ~ Salinity + Temperature + minRWM, Lag ~ Salinity + minRWM | 13 | 2525.6 | 0.20 |
| MaxPop ~ Salinity + minRWM, D50 ~ Salinity + Temperature + minRWM, Lag ~ Salinity + Temperature + minRWM | 13 | 2526.0 | 0.16 |
| MaxPop ~ Salinity + minRWM, D50 ~ Salinity + Temperature + minRWM, Lag ~ Salinity + minRWM | 12 | 2526.6 | 0.12 |
| MaxPop ~ Salinity + Temperature + minRWM, D50 ~ Salinity + minRWM, Lag ~ Salinity + Temperature + minRWM | 13 | 2526.6 | 0.12 |

Table 4. Top 5 ranked models as in Table 2, but from the bottom-up model development approach for 2015. The candidate set was composed of 34 models. Salinity: maximum salinity; maxRWM: maximum relative water movement; pH: minimum pH; MaxPop and D50 were specified as site-specific effects; other details as in Table 2 but for 2015

| Model parameters | $k$ | $AIC_c$ | $w_i$ |
| --- | --- | --- | --- |
| MaxPop ~ Salinity, D50 ~ Salinity, Lag ~ Salinity + maxRWM + pH | 12 | 6111.7 | 0.25 |
| MaxPop ~ Salinity, D50 ~ Salinity, Lag ~ Salinity + pH | 11 | 6111.9 | 0.22 |
| MaxPop ~ Salinity, D50 ~ Salinity + maxRWM, Lag ~ Salinity + pH | 12 | 6112.7 | 0.15 |
| MaxPop ~ Salinity, D50 ~ Salinity + maxRWM, Lag ~ Salinity + maxRWM + pH | 13 | 6113.5 | 0.10 |
| MaxPop ~ Salinity + maxRWM, D50 ~ Salinity, Lag ~ Salinity + pH | 12 | 6113.6 | 0.10 |

candidate set of models indicates greater uncertainty in the top-down approach to identifying a best model. Regardless, both approaches yielded best models with relatively low values for $w_i$, suggesting that the best model is not clearly stronger than others in the candidate sets (Burnham & Anderson 2002). The models ranked second to fifth from the bottom-up candidate set included mean salinity in all 3 model parameters (same as the best model), while also having mean temperature included as a covariate in 1 model parameter (Table 2). The models ranked second to fifth from the top-down candidate set contained mean temperature and minimum variance in RWM (min-RWM) in at least 1 model parameter, in addition to mean salinity in all 3 (Table 3). Both development approaches resulted in the inclusion of mean salinity as a covariate in all 3 model parameters, suggesting that mean salinity was associated with the abundance of *C. intestinalis*. However, this only means that salinity provided the best fit to these data; it does not provide an indication of the overall amount of variability in abundance explained by salinity.

### 3.2.2. Bottom-up and top-down candidate sets: 2015

The best models fitted with the 2015 data differed between the bottom-up and the top-down methods. The best bottom-up (BBU) model, with the most statistical support (lowest $AIC_c$ ranking), contained minimum salinity as a covariate in all 3 model parameters, with maximum RWM (maxRWM) and minimum pH included as covariates in the Lag parameter (Table 4). All models in the 2015 bottom-up model fitting had site-specific (random) effects for the MaxPop and D50 model parameters. This suggests that there is greater variation in the rate of change of abundance in 2015 that is not ex-

Table 5. Top 5 ranked models as in Table 2, but from the top-down model development approach for 2015. The candidate set was composed of 29 models. Salinity: maximum salinity; pH: minimum pH; other details as in Table 2 but for 2015

| Model parameters | $k$ | AIC$_c$ | $w_i$ |
|---|---|---|---|
| MaxPop ~ Salinity + Temp + pH, D50 ~ Salinity + Temp + maxRWM + pH, Lag ~ Salinity + Temp + maxRWM + pH | 16 | 6100.2 | 0.47 |
| MaxPop ~ Salinity + Temp + maxRWM + pH, D50 ~ Temp + maxRWM + pH, Lag ~ Salinity + Temp + maxRWM + pH | 16 | 6101.5 | 0.24 |
| MaxPop ~ Salinity + Temp + maxRWM + pH, D50 ~ Salinity + Temp + maxRWM + pH, Lag ~ Salinity + Temp + maxRWM + pH | 17 | 6102.2 | 0.17 |
| MaxPop ~ Temp + maxRWM + pH, D50 ~ Salinity + Temp + maxRWM + pH, Lag ~ Temp + maxRWM + pH | 15 | 6103.3 | 0.09 |
| MaxPop ~ Salinity + Temp + maxRWM + pH, D50 ~ Temp + maxRWM + pH, Lag ~ Temp + maxRWM + pH | 15 | 6106.5 | 0.02 |

plained by the abiotic covariates (species-level effects). In contrast, the model with the most statistical support from the top-down method is like the global model, containing all 4 abiotic metrics as covariates in all 3 of the model parameters, except for maxRWM, which was not included in the MaxPop parameter (Table 5). Like the 2014 models, the top-down models in 2015 only had MaxPop site-specific effects. In 2015, the best top-down (BTD) model had a substantially lower AIC than the BBU model, as well as higher certainty among its candidate set ($w_i$ = 0.47, Table 5). Similar to 2014, this weight for the BTD model in 2015 does not suggest it is clearly the best model within its candidate sets (Burnham & Anderson 2002). In general, the 2 model development approaches do not agree upon the best model in 2015, suggesting that there was a lesser abiotic association in 2015 than in 2014. This difference is substantially reinforced by the analysis of how site-specific versus species-level effects contribute to model fit. The parameter estimates for the 2015 BBU and BTD models can be found in Table S5.

## 3.3. Contributions to model fits

In general, the full-fit (including site-specific and species-level effects) of the best models for 2014 and 2015 fit the observed average abundance of *C. intestinalis* well (Figs. 2 & 3). However, the proportion of the full-fit attributable to species-level effects and site-specific effects varies dramatically from site to site in 2014 and 2015. Visual inspection of the fitted values from the best 2014 model suggests that 2 sites are fitted well by species-level effects (DW and SH), others

are moderately fitted by both species-level and site-specific effects (VC, WP, PB, FP and IP), while the rest of the sites are almost exclusively fitted by site-specific effects (YB, ZZ, PG and CC) (Fig. 2). In 2015, 2 sites (YB and SH) are fitted well by the species-level effects alone, 4 sites (DW, IP, PG and WP) are moderately fitted by species-level and site-specific effects, while the remaining 5 sites (CC, FP, PB, VC and ZZ) are fitted mostly by site-specific effects. This indicates that the association between the abiotic covariates and the abundance of *C. intestinalis* is weaker or virtually absent at the sites where the site-specific effects are moderately to highly influential in the fitted values.

The best models from 2014 and 2015 have similar error rates, efficiency, and collinearity between fitted and observed values (Table 6). However, differences in model accuracy were evident when comparing observed data to the models' species-level effects only (Table 7). Inspecting the full model fit from 2014 and 2015, the lowest error rate (MAE and RMSE) and highest MEF is provided by the best model from

Table 6. Model accuracy results for the best model for 2014 (best bottom up/best top down, BBU/BTD) and 2015 (BTD), along with their corresponding base models (devoid of any abiotic covariates). The 4 tests of model accuracy are: mean absolute error (MAE), units = *Ciona intestinalis* %; root mean square error (RMSE), units = *C. intestinalis* %; modelling efficiency (MEF), a measure of variance explained, similar to $R^2$ (Amaro et al. 1998); and the Spearman rank correlation coefficient ($\rho$)

| Model | Year | MAE | RMSE | MEF | $\rho$ |
|---|---|---|---|---|---|
| Base | 2014 | 5.95 | 10.93 | 0.94 | 0.88 |
| BBU/BTD | 2014 | 4.86 | 9.97 | 0.95 | 0.90 |
| Base | 2015 | 6.57 | 13.08 | 0.90 | 0.90 |
| BTD | 2015 | 5.83 | 12.32 | 0.91 | 0.92 |

Table 7. Model accuracy results for species-level effects only for the best model for 2014 (BBU/BTD) and 2015 (BTD), along with their corresponding base models (devoid of any abiotic covariates). Abbreviations as in Table 6

| Model | Year | MAE | RMSE | MEF | $\rho$ |
|---|---|---|---|---|---|
| Base | 2014 | 34.41 | 42.18 | 0.06 | 0.35 |
| BBU/BTD | 2014 | 26.33 | 35.66 | 0.33 | 0.60 |
| Base | 2015 | 19.87 | 34.38 | 0.34 | 0.72 |
| BTD | 2015 | 20.08 | 34.17 | 0.34 | 0.77 |

2014, although the Spearman rank correlation is slightly lower than the 2015 best model. Variation in the abundance of *C. intestinalis* explained by the species-level effects alone for the best 2014 model is a marked improvement from the variation explained by the corresponding base model (with no abiotic covariates). When compared to the base model, the species-level effects alone from the best 2014 model reduced the error rate, improved the MEF by 0.27, and nearly doubled the correlation between fitted and observed values (Table 7). These improvements in model accuracy, when compared to the base model with no abiotic covariates, are therefore attributable to the inclusion of mean salinity as a covariate in each of the 3 model parameters. However, these improvements due to the inclusion of salinity still only explain a modest proportion of the observed variability in abundance (Table 7: improvements in the 4 model accuracy metrics for the 2014 best model [BBU/BTD model] in comparison to the base model).

In stark contrast, model accuracy assessments of the species-level effects alone from the best model in 2015 are nearly identical compared to the corresponding base model accuracy with no abiotic covariates (Table 7). The addition of the abiotic covariates in the BTD 2015 did not reduce error or improve the explanatory power. This suggests that while the inclusion of the abiotic covariates produced an overall better model fit in 2015 based on $AIC_c$, the associations between the abiotic metrics and the abundance of *C. intestinalis* were relatively weak in 2015 when compared to the more evident association with mean salinity in 2014. Regardless of the association with salinity and the better model accuracy in 2014 compared to 2015, the explanatory powers of the best models from either year are modest (Table 7), meaning the power of abiotic covariates as predictors of the abundance of *C. intestinalis* is low.

### 3.4. Interannual predictions

The different combinations of abiotic covariates included in the respective best models for 2014 and 2015 resulted in poor interannual predictions with the best models (Table 8). This was pronounced when predicting the 2014 data with the 2015 models (Table 8), with the poor results (negative MEF values) possibly exacerbated by the reduced dataset caused by missing data. Given that the most prominent associations between abiotic conditions and abundances of *C. intestinalis* appear to be different between the 2 years, this is not surprising.

Table 8. Accuracy of interannual predictions from using the model with 1 year's data to predict the other year's data. Abbreviations as in Table 6

| Model | Test data | MAE | RMSE | MEF | ρ |
|---|---|---|---|---|---|
| Best 2014 | 2015 | 21.4 | 35.67 | 0.27 | 0.67 |
| BBU 2015 | 2014 | 32.96 | 48.49 | −0.22 | 0.14 |
| BTD 2015 | 2014 | 36.10 | 53.65 | −0.50 | 0.40 |

## 4. DISCUSSION

### 4.1. Abiotic conditions fail to explain most variation in abundance of *Ciona intestinalis*

Our study shows that abiotic conditions in NS are not a consistent driver of the abundance of *C. intestinalis*. In 2014, there was an association between higher salinity and higher abundance. However, this was highly variable among sites, and consequently, models that included salinity explained only a moderate amount of the observed variability at the species level. In addition, this association did not persist between years, as no clear abiotic associations with abundance were observed in 2015. These findings for abundances do not align with past results for the influence of temperature and salinity on the distribution of *C. intestinalis*.

The links between the abundance of *C. intestinalis* and abiotic conditions are probably complex. Under controlled conditions in laboratory-based experiments, temperature and salinity have been shown to affect the survival and growth of *C. intestinalis* (Vercaemer et al. 2011, Madariaga et al. 2014). Meanwhile, several field studies have described correspondences between temperature or salinity and *C. intestinalis* population dynamics. Seasonal temperature changes were shown to (qualitatively) coincide with the timing of reproductive processes (gametogenesis and spawning) (Carver et al. 2003) and recruitment (Ramsay 2009). In contrast, another study found no correlation between temperature and the timing of recruitment peaks (Howes et al. 2007). In surveys of the distribution of *C. intestinalis*, populations were found to thrive across a wide range of average annual temperatures (Sephton et al. 2011), while they were absent only at sites with combined high temperatures and low salinities (the brackish Bras d'Or lakes in NS, which are unusual environments with regard to both temperature and salinity). Meanwhile, another survey of the distribution of *C. intestinalis* also found that it was absent from sites with very low annual average or minimum salinities (Dybern 1967). These studies provided the basis for including

temperature and salinity as predictors in species distribution modelling of *C. intestinalis* (Therriault & Herborg 2008, Januario et al. 2015, Lowen et al. 2016b). Some of these models for habitat suitability have performed well at predicting the presence or absence of *C. intestinalis.* However, when comparing habitat suitability and abundance, there was no relationship for *C. intestinalis* (analysis of *C. intestinalis* spB by Januario et al. 2015). Finally, a long-term study of natural populations of what is most likely *C. robusta* (*C. intestinalis* spA) also showed little correspondence between abundance and temperature or salinity (Caputi et al. 2015). Our findings are consistent with these latter results, with no clearly identified abiotic drivers of abundance in our measurements of *C. intestinalis* in NS.

Altogether, the evidence appears to suggest that the presence and absence of *C. intestinalis* may be driven by salinity or temperature, and that where present, abundance is driven by one or more interacting factors that have not been effectively documented yet. We suggest further efforts on 2 fronts: obtaining a mechanistic understanding of alternative processes that may drive population abundance, and making further empirical comparisons of abundances to models based on detailed measurements of environmental variables (biotic and abiotic).

Abundances of other invasive ascidians have similarly lacked clear associations with particular abiotic variables. As with *C. intestinalis,* salinity and temperature have been suggested as 2 of the more important factors influencing the distribution and success of other invasive ascidians. However, most of the evidence (primarily from *Botryllus schlosseri*, *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* spp.) has been based on laboratory tests of links between salinity and temperature and the species' survival, growth or reproduction (McCarthy et al. 2007, Epelbaum et al. 2009, Sorte et al. 2011, Zerebecki & Sorte 2011, Lord & Whitlatch 2015, Nagar & Shenkar 2016). Some field tests have found evidence for associations between abiotic variables (temperature and salinity) with biological metrics (recruitment and growth rates) (Stachowicz et al. 2002, Auker & Oviatt 2008, Valentine et al. 2009, Grey 2011, Sorte & Stachowicz 2011, Fletcher et al. 2013), but none, to our knowledge, have associated abundance with corresponding *in situ* abiotic monitoring throughout the adult life cycle. Moreover, Fletcher et al. (2018) found that temperature change had little effect on the relative abundance (percent cover) of *Didemnum vexillum*. Given our similar findings here, we suggest that caution should be exercised when using primarily lab-derived environmental tolerances to project abundances for all ascidians (Epelbaum et al. 2009). Instead, combining field-derived and lab-derived environmental tolerances for key biological processes is likely necessary to accurately model invasive ascidian population dynamics (Cockrell & Sorte 2013).

## 4.2.  Alternative hypotheses for heterogeneous abundance of *C. intestinalis*

Our results show that variability in the abundance of *C. intestinalis* across sites could not be explained well by the environmental factors we measured. Combining our results with those of past studies that monitored *C. intestinalis* at the same sites (Sephton et al. 2011, 2017) reveals that some sites have consistently higher or lower relative abundance, while others change from year to year (Table S1). Therefore, there must be alternative sources of variability that drive abundance. The alternate factor(s) could act either at the species level or in site-specific ways.

More generally, heterogeneity of several alternate environmental or community ecological factors could affect the abundance of *C. intestinalis.* For example, varying supplies of phytoplankton food for *C. intestinalis* (assuming non-toxic algae are blooming; Granmo et al. 1988) could affect its growth and survival. Phytoplankton densities have very different dynamics across our study region. Locations in southwestern NS tend to have sustained blooms throughout spring and summer, with lower spatial and interannual variation, whereas in eastern and northeastern NS, blooms in spring and autumn and greater spatial and interannual variation are influenced by local nutrient supply and remote forcing events (Tian et al. 2015). This heterogeneity spread across different spatial scales could account for some of the differences in *C. intestinalis* abundance. Similarly, dissolved oxygen, which can be reduced at least at one of our NS sites (Strain 2002), could affect survival of *C. intestinalis* (as it can in *C. savignyi*; Pool et al. 2013), although tolerances are not yet known for *C. intestinalis.* Alternately, spatiotemporal variation in the abundance and diversity of a predator community could explain variation in *C. intestinalis* population growth rates (Gulliksen & Skjæveland 1973, Lundälv & Christie 1987, Dumont et al. 2011). Finally, varying toxicity of pollutants at our different sites could also create heterogeneity (Johnston & Keough 2002, 2003, Gallo et al. 2011). In the context of our study, any of these factors could act directly on growth or survival on our settlement plates or indirectly by acting on local populations of *C. intestinalis* that supply the larvae that then settle on the plates.

Several other factors could only have affected larval supply and recruitment on our plates. In an experimental setting, turbidity affected settlement of *C. intestinalis* larvae, but not growth rates of juveniles (McLaughlin et al. 2013, Lowen et al. 2016a). Substrate availability, either anthropogenic or natural, for nearby source populations could produce differences in larval supply, especially given the relatively short dispersal distances of *C. intestinalis* (Havenhand & Svane 1991). Similarly, in a region with changeable patterns of ice cover from year to year (Petzold et al. 2014), ice cover and ice scour, which can affect subtidal benthic communities (Conlan et al. 1998, Gutt 2002), could affect the source populations, creating heterogeneity in recruitment and then abundance of *C. intestinalis*. Finally, interspecific competition by pre-existing colonial tunicates can influence *C. intestinalis* settlement but not subsequent growth rates (Paetzold et al. 2012), suggesting that temporal and spatial differences in other species observed on our plates (primarily *Botryllus schlosseri* and *Botrylloides violaceus*) could have caused heterogeneity in *C. intestinalis* recruitment, leading to differences in abundance.

Lastly, both plasticity and local adaptation could also contribute to the heterogeneity in *C. intestinalis* abundance. Responses to abiotic conditions in which parents live can affect the survival of *C. intestinalis* offspring (Renborg et al. 2014). In that study, salinity was the variable, consistent with the key predictor in one of our study years. In a separate study of *C. intestinalis* sampled in parallel at all of our sites in 2015, consistent DNA methylation patterns associated with different temperatures also suggest that some local accommodation is occurring (Ni et al. 2019). In addition, a similar study of *C. robusta* and *C. intestinalis* from globally distributed populations found associations between salinity and temperature and DNA methylation patterns, suggesting a potential role of environmental conditions in local accommodation over short periods of time (Ni et al. 2018). If such accommodations are varyingly successful at matching conditions over time, then heterogeneity will also arise. Alternately, propagule pressure (i.e. larval supply in *C. intestinalis*) and genetic diversity can determine the success of a species' introduction via local adaptation (Lockwood et al. 2013). However, this is expected to lead to measurable genetic structure in the population, and Zhan et al. (2012) showed that genetic differentiation is low ($F_{ST}$ values near 0) in pairwise comparisons among 3 subpopulations of *C. intestinalis* in NS. This suggests that, at the scale of our study, the explanatory scope of local adaptation for spatial and temporal heterogeneity in population growth is more limited.

In summary, there are numerous alternate mechanisms that could drive the heterogeneous population growth of *C. intestinalis* across NS. Future efforts to test specific mechanisms and incorporate them into a population growth model should focus on those factors that most directly affect growth on bare settlement plates: the number of competent larvae in the water and the propensity for growth in that location. Furthermore, monitoring of both local phytoplankton populations and *C. intestinalis* larval densities could help distinguish the possible mechanisms that act during recruitment versus growth.

### 4.3. Value and limitations of our approach: sampling and modelling

Our study is the first to simultaneously take continuous *in situ* measurements of abiotic conditions and the abundance of *C. intestinalis* on settlement plates, at multiple times during the species' adult life and spanning more than 1 yr. We chose to match the spatial scale of our study to a spatially uncoupled biotic gradient of low to high abundances found in NS. In this way, we attempted to better explain any relationship in our region at the species level between temperature, salinity, pH or water movement with abundance of *C. intestinalis*. Logistical constraints created a trade-off between the number of sites and temporal resolution for the abundance measurements. Our choice of 11 sites with an approximately monthly measurement interval (which also then determined the corresponding resolution for the abiotic metrics) provided a compromise with both moderately high spatial and temporal sampling in comparison to past studies. In addition to incorporating unbalanced data with missing values in our longitudinal abiotic measures, nonlinear mixed-effects modelling allowed us to fit a mechanistically derived response function to best fit abundance over time, with parameters that accounted for variable delays in initial growth, growth rates and maximum abundances between sites. Our goals were to ensure that spatial sampling provided multiple sites along the gradient of abundance, while also capturing the population growth dynamics at each location. Although we achieved these goals, it is possible that greater prioritisation of spatial sampling or temporal resolution (likely at the cost of the other) might better reveal subtle relationships between the abiotic conditions we measured and *C. intestinalis* population growth. In addition, solving the problems

caused by sea ice to allow sampling (at least of abiotic conditions) through the winter may also be valuable if such conditions have carry-over effects on population growth in the following summer.

It is possible that interannual differences in the associations between abiotic conditions and abundance may have been influenced by partially different sets of abiotic metrics used in the model development for the 2 study years. However, it is unlikely that this would make a notable difference, due to the high collinearity of the metrics that were excluded with the selected set. It is also possible that handling during data collection could influence the results of our study, but we limited emersion time of settlement collectors consistently across sites and measurement periods (see Section 2.2) and used a light box to control light for photography, with the added benefit of providing shade. Future studies could instead use a measurement without replacement method, provided deployment of the greater number of collectors could be feasibly achieved without causing substantial spatial environmental heterogeneities among collectors. In our study, we chose repeated measures of the same individuals as a powerful means of analysis (Lindstrom & Bates 1990) and the optimal choice, given the constraint of keeping our collectors close to the location of the abiotic data loggers at each site.

## 4.4. Conclusions

Despite clear salinity and temperature effects on the survival and growth of *C. intestinalis* in lab-based environmental tolerance experiments, as well as strong associations between abiotic conditions and presence/absence, temperature and salinity do not appear to be successful predictors of the abundance of *C. intestinalis*. Thus, our evidence suggests that management efforts for this invasive species cannot be focussed on particular sites with conditions that might be conducive for *C. intestinalis* growth. Rather, efforts should still prioritise limiting further spread of the species, particularly in uncolonised areas highlighted as suitable habitat for *C. intestinalis* presence by species distribution modelling. Our results for *C. intestinalis* also emphasise that the link from lab experiments to field measures of population dynamics may not be straightforward and highlight the importance of complementing laboratory studies with field testing. Finally, the search for alternate factors that might drive abundances of *C. intestinalis* and other invasive ascidians must continue.

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