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Research Article

Seasonal reproduction of the non-native vase tunicate *Ciona intestinalis* (Linnaeus, 1767) in Nova Scotia, Canada, in relation to water temperature

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Abstract

Intra-annual and inter-annual reproductive periodicity for *in situ* populations of *Ciona intestinalis* were documented from October 2013 to August 2015 in the small vessel marina at the Bedford Institute of Oceanography in Dartmouth, NS. Three metrics of reproduction were monitored: (i) larval settlement, (ii) gonad development and (iii) gamete viability. *In situ* settlement was observed between June and November 2014. Gonad development during spring consisted of a sharp increase in the proportion of males followed by development into hermaphrodites, which resulted in a near entirely-hermaphroditic population throughout the summer and fall. The proportion of males and hermaphrodites began to decline by late fall; hermaphrodites were absent by early winter (i.e., late December or January), whereas males persisted at low abundance throughout the winter. *In-vitro* fertilization assays demonstrated that gametes became non-viable by early December. The seasonal changes in development stages were compared with respect to ambient seawater temperature and growing degree days. The spring maturation in 2014 occurred 3 weeks earlier than observed in 2015 (i.e., May 2014 and June 2015), likely due to a 2.0 °C higher mean seawater temperature than the same period in 2015. The effect of temperature on development rate was confirmed in a mesocosm experiment where the 3 °C elevated temperature treatment resulted in earlier sexual maturation by ten days for males and eight days for hermaphrodites compared to those in the unheated treatment. These results demonstrate the potential for an extended reproductive window, and perhaps extended geographic range, in response to predicted increases in ambient sea surface temperatures in Atlantic Canada in the next few decades.

Key words: aquatic invasive species, temperature, growing degree days, larval settlement, reproductive cycles, sexual maturation

Introduction

The vase tunicate *Ciona intestinalis* (Linnaeus, 1767) is a solitary ascidian that settles on hard substrate and lives as a sessile filter feeder (Carver et al. 2006). It is native to the North East Atlantic, but is now found as an invasive species in temperate regions world-wide (Dybern 1965; Carver et al. 2006). The vase tunicate has been present on the Atlantic coast of North America since the mid-1800s; however, recent population outbreaks and range expansions have caused concern in both Canada and the United States (Carver et al. 2006; Ramsay et al. 2008; Therriault and Herzborg 2008; Carman et al. 2010).

The introduction of *C. intestinalis* to non-native regions has been damaging to native species and ecosystems. *C. intestinalis* has a life history strategy characterized by early and rapid growth and development, and high reproductive output, making it an effective competitor and invader (Carver et al. 2006). It reduces species richness in benthic ecosystems by out-competing native species for both food and space, often overgrowing pre-existing benthic communities (Blum et al. 2007; Lutz-Collins et al. 2009). The rapid spread and increase in *C. intestinalis* abundance in eastern Canada over the past 20 years has also caused problems in the aquaculture industry (Ramsay et al. 2008; Daigle

and Herbinger 2009; Lutz-Collins et al. 2009). Biofouling on aquaculture equipment and mussel lines is costly to remove and reduces productivity (Daigle and Herbinger 2009; Carman et al. 2010; Aldred and Clare 2014). As a harmful invasive species, its distribution and abundance will depend on its ability and capacity to reproduce under ambient conditions in its non-native range.

Ciona intestinalis are protandrous hermaphrodites with three distinct gonad maturation stages: without gametes (hereafter referred to as blank), filled sperm duct (male), and filled sperm duct and oviduct (hermaphrodite) (Millar 1952; Dybern 1965). They are oviparous and reproduce by broadcast spawning and external fertilization. Larvae hatch after one to two days of embryonic development, then undergo one to five days of planktonic development before settling on hard substrates and metamorphosing (Berrill 1947; Dybern 1965; Svane and Havenhand 1993). With a 12 to 18 month lifespan in Nova Scotia, individuals grow and reproduce from late spring through early fall, then enter a state of dormancy through winter (Carver et al. 2003). A regression in sexual development stage occurs, resulting from a cessation of gamete production and resorption of remaining gametes in the ovaries and testicular follicles (Millar 1952; Dybern 1965; Carver et al. 2003). If the tunicates survive the winter, they may re-initiate gamete production and reproduce again in early spring prior to senescence and death (Millar 1952; Dybern 1965).

The initiation and duration of reproduction in marine invertebrates is affected by a combination of endogenous factors (e.g., internal cycles of growth and development) and external factors (e.g., temperature, salinity, food availability) that cause variation in growth and development (Giese 1958). Previous studies have shown temperature to influence *C. intestinalis* growth, with individuals in warmer water reaching critical size for maturation in less time and at a smaller size (Millar 1952; Dybern 1965; Yamaguchi 1975). Furthermore, the duration of the breeding season increases with decreasing latitude (Millar 1952; Dybern 1965; Yamaguchi 1975) and, within a region, an extended reproductive window and increased recruitment have been observed in warmer water (Reinhardt et al. 2013). The present study documents local reproductive patterns in Halifax, Nova Scotia (NS), Canada, over two reproductive seasons to determine inter-annual variation with respect to temperature.

Growing degree days (GDD) provides a measure of integrated temperature exposure, or thermal time, to standardize temperature comparisons between years (Trudgill et al. 2005). Poikilothermic invertebrates, like *C. intestinalis,* have a rate of development proportional to the environmental temperature (Sharpe and DeMichele 1977; Trudgill et al. 2005). The relationship between growth rate and temperature is linear between the base temperature, where development initiates, and the thermal optimum, where the maximum rate of development is achieved. The required temperature exposure to reach a given stage of development is a constant; therefore, individuals exposed to higher temperatures will develop faster and reach maturity earlier, but at similar GDD than those exposed to lower temperatures (Trudgill et al. 2005). This measure of physiological time has been shown to explain more of the variation in growth and embryonic development rates than calendar time for various freshwater and marine fish (Lange and Greve 1997; Neuheimer and Taggart, 2007). To date, GDD has been used to compare inter-annual *C. intestinalis* settlement with respect to temperature and to measure maturation in other invasive tunicates (Epelbaum et al. 2009; Reinhardt et al. 2013). This study applies GDD to compare sexual development in *C. intestinalis* between years and between experimentally elevated temperature treatments.

Due to the effects of temperature on reproductive patterns, predicted increases in sea surface temperature (Loder and Wang 2015) have the potential to alter annual biological cycles. Increased temperatures can alter the timing, duration, and capacity for reproduction in invasive species, potentially changing their competitive ability with native species (Stachowicz et al. 2002). Warmer waters could additionally promote range expansion into environments that were not previously suitable for a sustained population (Hellmann et al. 2008). By documenting the current, local patterns of development and reproduction, and the effects of temperature on these cycles, we provide a reference for comparison with other regions and future conditions.

The main objectives of this study were to describe the local reproductive periodicity of *C. intestinalis* and assess the effect of temperature on the reproductive timing by means of *in situ* observations and a controlled mesocosm experiment. Three components of the reproductive cycle were investigated. Weekly *in situ* settlement rates were estimated to determine the timing of settlement initiation and termination and the relative magnitude of reproductive output. The timing of sexual development in the *in situ* population was compared between the two study years in conjunction with the annual temperature patterns. *In-vitro* fertilization assays were conducted to verify the reproductive viability of sexually mature individuals. Lastly, the effect of temperature

on the timing of sexual development was tested directly in a mesocosm experiment with unheated and heated water temperature treatments.

Material and methods

In situ reproductive periodicity

Field sampling was conducted in the small vessel marina at the Bedford Institute of Oceanography (BIO) in Halifax Harbour, Dartmouth, NS (44.6828ºN; 63.6119ºW) (hereafter the BIO site) from October 2013 through August 2014. A population of *C. intestinalis* has been present at the BIO site since at least 2006 (Sephton et al. 2011); however, there was a mass mortality during the winter of 2014–2015 associated with above normal precipitation and snow melt runoff that reduced the site salinity to < 20. A population was transplanted from Sambro Harbour, NS (44.4794ºN; 63.6089ºW) to the BIO site (about 30 km) on 16 April 2015. Sambro Harbour was selected as a source of *C. intestinalis* based on previous comparisons of reproductive development showing high correspondence with Halifax Harbour (AM Moore, pers. comm.).

Ciona intestinalis settlement was monitored *in situ* with artificial settlement collectors deployed off the floating dock at the BIO site. Strings of three 10 cm × 10 cm PVC settlement plates were assembled with the plates at 25 cm, 75 cm and 125 cm below the surface. The plates were texturized on the downward side and were conditioned in 90-µm filtered seawater to establish a biofilm to facilitate *C. intestinalis* settlement (Wieczorek and Todd 1997). Two strings of plates were deployed and collected weekly from May to November 2014. Retrieved plates were placed in laboratory tanks of 90-µm filtered seawater heated 2 °C to 3 °C above ambient, which provided a food supply and promoted faster growth, while preventing the introduction of new larvae. Once large enough, the settlers were identified and counted under a dissecting microscope. The mean (± 95% CI) settlement was standardized over two-week intervals to increase statistical power, and a one-way analysis of variance (ANOVA) followed by Tukeys all-pairwise comparisons test was used to detect significant differences between means. As there was no significant difference in settlement between depths, each plate was treated as a replicate for a total of 12 replicates per two-week interval (Underwood 1997).

From October 2013 to December 2014, weekly to biweekly samples of twenty to thirty adult *C. intestinalis* were collected haphazardly from the *in situ* population and sexually staged based on the presence of gametes in the gonaducts (Dybern 1965). Following the relocation from Sambro Harbour in April 2015, approximately 100 individuals were placed in a pearl net (35 cm × 35 cm pyramidal net, 0.25 cm^2 mesh) suspended from the floating docks at the BIO site. Semiweekly, twenty individuals were selected haphazardly and staged using non-destructive, on site sampling after which all tunicates were returned to the net. In order to test for significant differences in the proportions of blanks, males and hermaphrodites at each sample date, 95% confidence intervals were calculated for the proportions of each sexual stage.

Surface temperature at the BIO site was recorded every 15 min from October 2013 to December 2015 with a SeaBird CTD (SBE 37) deployed with the sensor at a depth of 1 m.

Cumulative growing degree days (GDD) was calculated according to the following equation (McMaster and Wilhelm 1997),

$$GDD = \sum(T_\theta - T_B) \quad (1)$$

where T_θ is the daily average temperature calculated from the SeaBird time series, and T_B is the base temperature at which gonad development initiates, set to 3 °C in this study based on previous observations of Nova Scotia populations (Carver et al. 2003). As the adult tunicates present in the spring population may have been spawned anywhere from early spring to late summer, GDD calculations were initiated on 1 January of each year. Therefore, the GDD presented here refers to the thermal exposure necessary for sexual development from the winter dormancy stage, not from settlement. The GDD have been presented as a range between sample dates, during which the stage may have developed. The maximum is the GDD of a sample in which the stage was first observed, and the minimum is the GDD from the previous sample date. Comparisons of GDD ranges were done between the maximum value of the lower range and the minimum value of the higher range.

Weekly *in-vitro* fertilization assays were conducted in spring (2014 and 2015) and fall (2014) to assess the onset and termination of gamete viability associated with seasonal changes in reproductive stage. Eggs were extracted from 10 to 15 tunicates within 20 to 30 minutes of collection from the BIO site, and placed in 250 mL of 25-µm filtered seawater maintained at ambient temperature. About 100 µL of sperm was extracted from each of 5 individuals and diluted in 50 mL of filtered seawater. The egg solution was divided into 3 replicate beakers and diluted to 200 mL with filtered seawater. A 5-mL

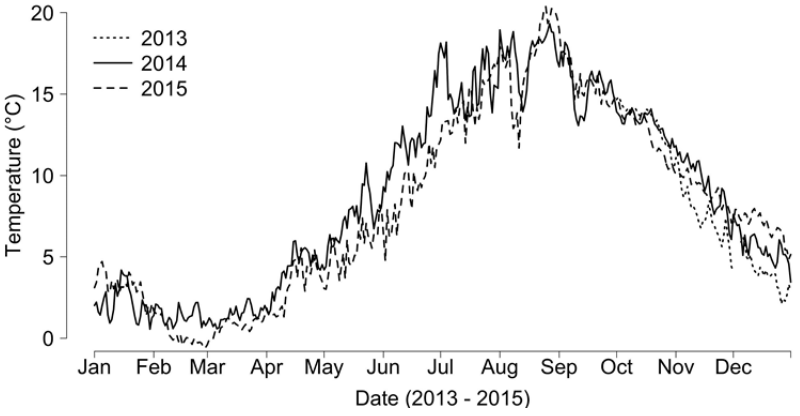

Figure 1. Annual daily average temperatures at the BIO site from 1 October 2013 to 31 December 2015. Temperature was recorded every 15 minutes with a Seabird CTD (SBE 37-SM RS-232) deployed at 1 m depth.

sample was collected to determine initial egg density. About 500 µL of sperm solution was stirred into each beaker and left to fertilize for 1 hour. Preliminary assays monitored over 24 hours determined maximum fertilization success is achieved within the first hour. Following the fertilization period, the solution was rinsed over a 45-µm sieve to remove excess sperm and prevent polyspermy, which is a lethal condition. Rinsed eggs were suspended in 200 mL of ambient, 25-µm filtered seawater. A 5-mL sample was collected from each beaker and preserved in 4% formalin 3 hours after fertilization, based on observations of maximum fertilization within one to two hours. All samples were examined under a stereomicroscope and percent fertilization success was determined from the number of fertilized blastula *versus* the total number of eggs.

Mesocosm experiment

The effects of elevated seawater temperature on the time required for *C. intestinalis* to reach sexual maturation was assessed with a 4-tank mesocosm array deployed in the BIO marina. Each tank (250 L capacity) had constant flow through of ambient seawater filtered to 300 µm, with complete turnover every three hours. Two tanks were unheated while two other tanks were heated to 3 °C above the unheated tank temperature. The temperature offset was near the maximum capacity for mesocosm heaters. Temperature in each mesocosm was monitored with a Ponsel C4E sensor and temperature in the heated treatments adjusted every 15 minutes. On 28 April 2015, four pearl nets each containing 50 *C. intestinalis* were placed in separate mesocosm tanks and sub-sampled weekly. One additional pearl net was also maintained outside of the mesocosm (*in situ*) to assess any artifacts of mesocosm tanks. The temperature of the heated tanks was increased gradually over 24 hours after the tunicates had been introduced, and tunicates were allowed to acclimate for 48 hours. Twice a week, 20 individuals from each treatment (heated tanks, unheated tanks and *in situ*) were sampled haphazardly and staged. Sampling and staging continued until samples consisted of 100% hermaphrodites in all treatments. A Chi-square goodness of fit was used to test for significant differences in the proportion of hermaphrodites to males at each sample date.

Results

In 2014, the daily average temperature ranged from 0.56 °C on 30 January to 19.32 °C on 27 August (Figure 1). In 2015, the daily average temperature ranged from −0.60 °C on 27 February to 20.37 °C on 25 August. In general, 2014 was warmer than 2015 from April through June, with an average difference of 1.99 °C and a maximum difference of 5.96 °C. The GDD in 2014 began to surpass that of 2015 in May, when mean seawater temperature exceeded 3 °C (T_B). For the remainder of the calendar year, the 2014 GDD remained greater than the 2015 GDD on any given date. The annual cumulative GDD was 2262 in 2014 compared to 2074 in 2015.

In situ reproductive periodicity

In situ settlement of *C. intestinalis* was observed between 12 June 2014 and 13 November 2014 (Figure 2) and differed significantly between dates (ANOVA, $F_{(12,135)} = 5.44$, $P < 0.001$). There were no significant differences in settlement between the beginning (21 May through 18 July) and end (26 September through 13 November) of the reproductive window.

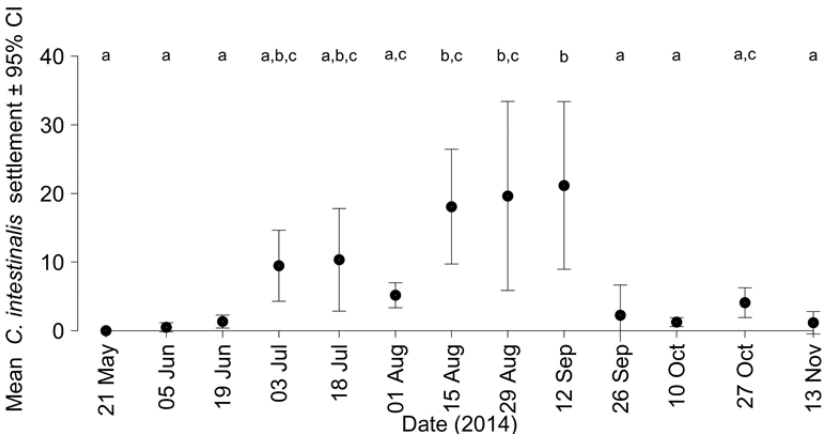

Figure 2. Mean (±95% CI, n = 12) *C. intestinalis* settlement levels (number per 100 cm^2) in 2014 on PVC settlement plates at three depths (0.25 m, 0.75 m, and 1.25 m) and two locations along the floating dock at the BIO site. The date indicated represents the middle of the biweekly interval. Significant differences between dates as determined by ANOVA and the all-pairwise Tukey test are indicated with a, b, and c.

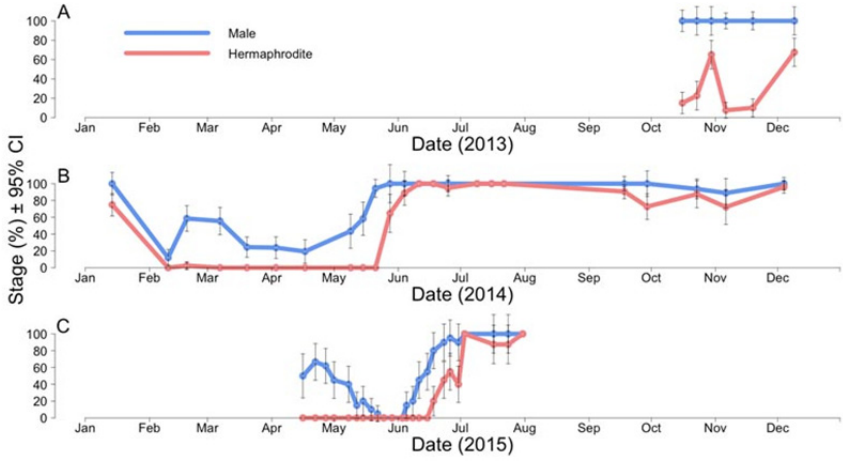

Figure 3. Mean (± 95% CI) proportions of blank, male, and hermaphroditic stages of *C. intestinalis* sampled *in situ* at the BIO site (A) from 16 October to 31 December 2013; (B) from 01 January to 4 December 2014; (C) from 16 April to 31 July 2015.

Settlement began to increase significantly on 3 July, and exceeded the beginning and end season settlement rates from 10 August through 12 September.

In the spring, a sharp increase in the proportion of males was observed, followed by an increase in the proportion of hermaphrodites (Figure 3). Both males and hermaphrodites developed earlier in 2014 than in 2015. Males were initially observed on 9 May 2014 and four weeks later in the year, on 5 June 2015 (Table 1; Figure 3B and C). The first appearance of males occurred 58 GDD earlier in 2014 relative to 2015, between the minimum of the higher range and the maximum of the lower range (Table 1). Hermaphrodites were initially observed on 28 May 2014 and three weeks later in the year on 18 June 2015 (Table 1; Figure 3B and C). The first appearance of hermaphrodite occurred within 27 GDD (Table 1). Once each stage was initially observed, the population rapidly transitioned to be composed entirely of that stage. In 2014, males reached 94.4% by 21 May (12 days) and hermaphrodites reached 100% by 11 June (14 days) (Figure 3B). In 2015, males reached 95% of the population by 26 June (21 days) and hermaphrodites comprised 100% of the population by 3 July (15 days) (Figure 3C).

Gamete viability assays were initiated in the spring of 2014 and 2015 with the initial appearance of hermaphrodites. Average fertilization success was 71.4 % in 2014 and 78.7% in 2015.

In both years, hermaphrodites exceeded 70% of the population throughout the summer and early fall, and a decrease was observed in early winter (Figure 3). The population of *C. intestinalis* staged in the fall (October through December) of 2013 and 2014 was at least 90% male or hermaphroditic, and less than 10% blank (Figure 3A, B). In January and February 2014, a sharp decrease in the proportion of males (25.0 to 11.9%), hermaphrodites (75.0 to 0%) and a corresponding increase in the proportion of blank individuals (0 to 88.1%) was observed; these proportions were maintained until mid-April 2014 (Figure 3B). A similar decrease was observed in the winter of

Table 1. Dates and cumulative growing degree days (GDD; $T_B = 3$ °C) at the time of first observed male and hermaphroditic *C. intestinalis* in the *in situ* population at the BIO site in 2014 and 2015 and in the unheated and heated mesocosm tanks (2015). GDD was calculated using daily average temperature from 1 January of each year. The range of GDD in which the stage may have developed is represented by the GDD at the time of first observation (maximum) and the GDD immediately following the previous sample (minimum).

	Male		Hermaphrodite	
	Date	GDD	Date	GDD
2014 *in situ*	9 May	21 – 67	28 May	129 – 162
2015 *in situ*	5 June	125 – 128	18 June	189 – 202
2015 Unheated	29 May	113 – 130	23 June	280 – 314
2015 Heated	19 May	118 – 140	15 June	349 – 386

2015, culminating in the mortality of the entire accessible *C. intestinalis* population at the sample site. In April 2015, the relocated population contained similar proportions of blanks and males, and no hermaphrodites. The proportion of males decreased from mid-April to mid-May 2015 resulting in a population briefly composed entirely of blank individuals from mid-May to early June (Figure 3C).

In association with the observed decrease in the proportion of mature individuals, fertilization assays in the fall of 2014 indicated decreasing gamete viability compared to spring assays (reduced to 48.50%). Gametes tested on 8 December 2014 were non-viable despite the presence of apparently healthy gametes in the gonaducts until 19 December 2014.

Mesocosm experiment

The daily average temperature in the unheated and heated mesocosm tanks were 3.74 °C and 6.73 °C, respectively, on 30 April and had increased to 16.40 °C and 19.48 °C, respectively, on 3 July. The daily average temperature range recorded in the marina was 2.71 °C on 30 April and 13.09 °C on 3 July. An average difference of 2.92 °C was maintained between the unheated and heated tanks, and the unheated tanks were on average 2.19 °C warmer than the *in situ* temperature. Following the activation of the heaters on 27 April, the cumulative GDD in the heated tanks was 648 by the end of the experiment compared to 450 GDD in the unheated tanks.

At the onset of the mesocosm experiment, there were similar proportions of males and blanks, whereas hermaphrodites were absent (Figure 4). A decrease in the proportion of males was observed in both the unheated and heated treatments, from the initiation of the experiment through 15 May. Both males and hermaphrodites developed earlier in the heated treatment than in the unheated treatment. Males were initially observed on 19 May in the heated tanks and on 29 May in the unheated tanks, developing within the same range of GDD (Table 1). Males in the unheated tanks also developed within the same GDD range as the *in situ* tunicates, though one week earlier. Males comprised over 90% of the population by 5 June and 18 June in the heated and unheated tanks, respectively, and this did not occur until 26 June outside the mesocosm (Figure 3C, Figure 4).

Hermaphrodites were initially observed on 15 June in the heated tanks and on 23 June in the unheated tanks, within 35 GDD of each other (Table 1; Figure 4). Hermaphrodites in the unheated tanks developed five days and 78 GDD later than the *in situ* tunicates (Table 1). Chi-square goodness of fit tests at each sample date indicated that the proportions of males and hermaphrodites were significantly greater in the heated tanks from 15 May to 5 June, and on 15 and 18 June (χ^2-tests, df = 2; $P < 0.01$).

Discussion

The settlement pattern observed at the BIO site was bi-modal, a pattern observed at some nearby locations (Carver et al. 2003; Howes et al. 2007; Vercaemer et al. 2011) while a uni-modal pattern has also been documented on Nova Scotia's South Shore (Carver et al. 2003). The present study also noted maximum settlement from late-August to mid-September, which may result from the spring cohort reaching maturity (Dybern 1965; Howes et al. 2007). Uni-modal, bi-modal, and continuous spawning have been documented in various regions globally and are thought to be related to seawater temperature (Dybern 1965; Reinhardt et al. 2013). Warmer regions such as Japan and the Mediterranean Sea experience continuous spawning, producing up to four generations annually (Yamaguchi 1975; Caputi et al. 2015). Cooler temperate regions including Great Britain, southern Scandinavia, and Atlantic Canada typically experience two spawning events between June and November (Millar 1952; Petersen and Svane 1995; Vercaemer et al. 2011), but may also present a single peak in settlement (Millar 1952; Carver et al. 2003; Ramsay et al. 2009).

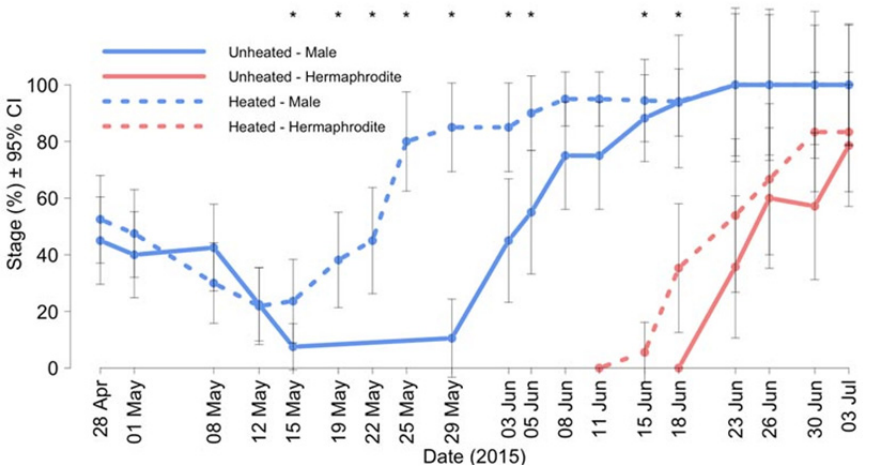

Figure 4. Mean (± 95% CI) proportions of blank, male, and hermaphroditic stages of *C. intestinalis* sampled from the unheated and heated mesocosm treatments from 28 April to 3 July 2015. Significant ($P < 0.05$) differences in the proportions between treatments as determined by a Chi-square test are indicated with an asterix.

Subarctic regions such as northern Scandinavia experience only one settlement event in July (Dybern 1965). The effect of temperature on timing and duration of spawning events was further demonstrated in a study by Reinhardt et al. (2013) in comparisons among multiple sites in New England. Earlier *C. intestinalis* settlement, a longer settlement window, and higher total recruitment was observed at sites with warmer annual temperatures.

In early December, prior to the decline in hermaphrodite proportions, gametes observed in the gonaducts were found to be non-viable. Previous studies have also documented a cessation in gamete production and a regression in sexual development stage as a result of resorption of gametes in the ovaries and testicular follicles during the winter months (Millar 1952; Dybern 1965; Carver et al. 2003). Gametes remaining in the gonads over winter were observed to have low viability; fertilization success with the few mature eggs present was less than 10% and sperm were observed to rapidly lose motility (Carver et al. 2003). The present study tested gamete viability earlier, prior to the shift in the population composition, and found that otherwise healthy looking gametes were non-viable. This suggests that macroscopic sexual staging alone may not accurately indicate the reproductive potential of an individual, particularly at the end of the reproductive season.

In addition to gamete resorption, the dramatic changes in the observed proportions of sexually mature individuals are likely in part attributable to senescence among the older individuals, which is prompted by environmental stresses such as salinity or temperature extremes (Millar 1952; Petersen and Svane 1995; Caputi et al. 2015). A mass mortality event was observed in February of 2014 and was essentially 100% in February 2015, which is when temperature and salinity were lowest. It has been previously demonstrated that exposure to salinity less than 20 results in rapid mortality (Shumway 1978; Vercaemer et al. 2011). Similar declines in abundance have been previously observed in the winter and spring (Petersen and Svane 1995). With a 12 to 18 month lifespan in Nova Scotia, those individuals spawned in the spring or fall of the previous year are expected to die, leaving the individuals spawned in the most recent summer and fall to grow and reproduce (Millar 1952; Dybern 1965; Carver et al. 2006).

The delay in maturation timing between 2014 and 2015 corresponded to annual temperature trends, and the attainment of a GDD threshold, which supports the hypothesis that timing of sexual development is influenced by temperature exposure and is not dependent on time of year alone. However, variation in other factors, such as food availability or environmental stress (e.g., salinity), between the two study years also may have contributed to the delayed onset in maturation observed in 2015 (Shumway 1978; Petersen et al. 1995). Under the controlled conditions of the mesocosm, though an increase in temperature advanced the time of maturation for both stages, male development occurred at the same GDD, and hermaphroditic development occurred at similar GDD between treatments. However, complete sexual development occurred earlier and at fewer GDD in tunicates outside the mesocosm, compared to those in the unheated tanks, further suggesting an influence by other environmental factors. Although

the mesocosm was designed to mimic ambient conditions as closely as possible, more so than a laboratory setting, differences in circulation and food availability could arise between the outside environment and the tanks since the turnover was on the order of three hours. Further studies are required to determine the effects of these factors relative to each other and to temperature.

The inter-annual GDD calculations were conducted from 1 January of each year, to assess the thermal exposure required for adults to reach sexual maturation from a state of winter dormancy. Though indistinguishable in terms of size, variation in the exact age (i.e., spawned in spring or late summer) and origin (i.e., Halifax or Sambro) of the tunicates may have resulted in different early life growth histories, which were not accounted for in this study.

Taken together, information on the timing of adult sexual development, gamete viability and larval settlement provide insight into the reproductive pattern of *C. intestinalis* and the effects of temperature on its reproductive potential. In addition to demonstrating earlier development in a warmer year, sexual maturation was artificially accelerated with elevated temperatures under controlled conditions. Furthermore, fertilization assays demonstrated that the presence of mature gametes does not guarantee reproductive viability, particularly prior to sexual regression in the early winter. By documenting the current, local timing of sexual maturation and settlement windows, we have provided a baseline for comparison with other regions and future conditions. For example, predicted increases in sea surface temperatures over the next several decades (Loder and Wang 2015) may result in an earlier and a prolonged reproductive window. It may also result in range shifts or range expansions of tunicates into adjacent regions that currently do not support tunicate development. For example, with regards to the East Coast of Canada, environmental suitability models indicate Newfoundland and perhaps Labrador could represent suitable habitat for *C. intestinalis* (Therriault and Herzborg 2008). It is instructive then that more recent work has indeed documented colonization of southern Newfoundland by *C. intestinalis* with further geographic spread expected (Sargent et al. 2013). Finally, the effects of climate change may alter the competitive ability of *C. intestinalis*, as well as other aquatic invasive species and native species. A prolonged reproductive season, increased reproductive output, or range expansion for *C. intestinalis* resulting from increased mean seawater temperature could result in further ecological and economic damages, especially to the bivalve aquaculture industry.

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