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# Winter Population Dynamics of the Intertidal Amphipod *Corophium volutator* in the Bay of Fundy

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*Corophium volutator* is a dominant invertebrate inhabiting U-shaped burrows on the large intertidal mudflats of the upper Bay of Fundy, Canada. *C. volutator* has been extensively studied in the summer, but little is known about winter dynamics. To understand its yearly life cycle, we must characterize the abiotic conditions on the mudflats through the winter, assess responses of *C. volutator* populations during this time of high abiotic stress, and evaluate possible adaptations used by *C. volutator* to survive the winter. To characterize the mudflats and assess population responses, we sampled two mudflats during the winters of 2009-2010 and 2010-2011 To determine how *C. volutator* may have adapted to winter conditions, we monitored their vertical distribution in the mud and measured responses to prolonged exposure to cold temperatures. Densities of *C. volutator* declined gradually through the winter. *C. volutator* did not shift its vertical distribution deeper in the mud in response to winter conditions; it was mostly found within the first 1.5 cm of mud, where temperatures are coldest and exposure to abiotic stress highest. The ability of *C. volutator* to survive short-term cold exposure (-3 and -8$^{o}$C) was good, but declined with longer exposure time and colder temperature. This study provides insight as to why population densities of *C. volutator* vary between sites and between years as a consequence of variability in winter severity.

## 1. Introduction

Winters in aquatic and marine habitats in New Brunswick, Canada, can be a time of high stress. Abiotic stresses such as ice cover, ice scour, and low temperature extremes may cause declines in density of important species. Unfortunately, these extreme conditions have also often inhibited researchers from systematically assessing the effect of winter on species dynamics. Without investigating this aspect of ecology, researchers are left with an incomplete picture of the factors influencing the dynamics of a study organism. This is particularly important because winter at high latitudes constitutes a significant proportion of the life cycle of some organisms.

### *1.1 Corophium volutator*

*Corophium volutator* is a benthic marine amphipod that ranges in size between 1 and 10 mm. It occupies "U"-shaped burrows which it digs using its long secondary antennae (Meadows and Reid, 1966). *C. volutator* is a dominant invertebrate found on the mudflats in the upper Bay of Fundy, with densities exceeding 50,000 ind. $m^{-2}$ (Peer et al., 1986; Wilson, 1989). Its life cycle typically involves two generations per year (Gratto et al., 1983; Peer et al., 1986; Wilson, 1989). One generation is born in spring and reproduces during the summer, and the second generation is born in mid to late summer and individuals must survive winter before reproducing in the following spring (Peer et al., 1986). *C. volutator* is ecologically important because it is a key component of the food web; for example, it is a main food for semipalmated sandpipers (*Calidris pusilla*; Hicklin and Smith, 1979; Hicklin and Smith, 1984) during their stopover in the Bay of Fundy before migrating to South America for the winter (Hicklin, 1987). Densities of *C. volutator* were reported to vary from mudflat to mudflat (Barbeau et al., 2009), which in turn affects habitat use by shorebirds (Sprague et al., 2008). Variation in winter mortality has been suggested as an important factor explaining variation in summer densities.

Studies on the ecology of *C. volutator* were mostly conducted during the summer months. To our knowledge, no study has focused on *C. volutator* during the winter with respect to spatial variability and population demographics. From comparisons between fall and spring samplings, it is known indirectly that the overwintering cohort of *C. volutator* grows little during the winter (Wilson, 1989; Drolet, 2009), and that the population declines during this period (Barbeau et al., 2009; Drolet, 2009).

Ambient air temperatures in the Bay of Fundy can reach extremes of -30°C during the winter months (Loomis, 1995), but water temperatures reach a minimum of +1°C (Campbell and Stasko, 1986). Cold temperatures result in the formation of blocks of drift ice, ranging from a few $cm^3$ to a few $m^3$, and may be present from December to April (Gordon and Desplanque, 1983; Partridge, 2001). The surface of the mudflats can freeze, producing a layer of surface ice ranging from 0.5 to 30 cm in thickness (Gordon and Desplanque, 1983). Because of semidiurnal tides, mudflat invertebrates are alternately exposed to both the cold ambient temperatures and warmer ocean water twice daily. As well, scouring of the mudflat surface by ice blocks may cause stress and mortality. The effects of temperature on mortality have been observed in European populations of *C. volutator* (Mills and Fish, 1980), but not in North American populations of *C. volutator*. Because *C. volutator* is exposed to extremes in cold, and populations have been observed to decline in the winter (Peer et al., 1986; Barbeau et al., 2009; Drolet, 2009), it is important to assess the survival ability of individuals to low temperature stresses.

### *1.2 Objectives*

Our main objective was to study the biology of *C. volutator* in winter. We first described trends in density of *C. volutator* by sampling two mudflats during the winters of 2009-2010 and 2010-2011. We then investigated behavioural and physiological adaptations of *C. volutator* to cold conditions by measuring their vertical distribution in the mud during the winter of 2009-2010, and testing their tolerance to cold exposure by freezing them in the laboratory.

## 2. Methods

### *2.1 Study area*

Our study area was in the upper Bay of Fundy, known for its high tidal amplitude (up to 15 m) and extensive intertidal mudflats mostly composed of silt and clay. We selected two representative mudflats, Grande Anse and Pecks Cove, in Chignecto Bay (Fig. 1).

### *2.2 Population trends over winter*

To evaluate temporal changes in density of *C. volutator* over winters 2009-2010 and 2010-2011, the two mudflats were sampled at 3-week intervals between December and March. At each site, we sampled along three transects (600-m long) running perpendicular to the low tide line, and starting at the end of a salt marsh (Pecks Cove) or rocky beach (Grande Anse). Along each transect, five 40-m long zones were randomly selected (spaced by a minimum of 40 m), and 4 core samples were randomly taken within each zone.  Mud samples were taken using a 6.5-cm diameter corer, which was pushed into the mud until the anoxic layer was reached (Drolet, 2009), such that all *C. volutator* specimens within the cored area were collected.  Each core was sieved through 250-µm mesh, to retain all *C. volutator* (Crewe et al., 2001), and the remains preserved in 95% ethanol.  Samples were later sorted under a dissecting microscope and the number of *C. volutator* individuals counted.

Spatial and temporal variation in density of *C. volutator* was quantified using a mixed-model ANOVA, with Year (2009-2010 and 2010-2011), Round (5 sampling occasions within each year), and Site (Grande Anse and Pecks Cove) as fixed factors, and Transect (3 replicates nested in Site), and Zone (5 replicates nested in Transect) as random factors. Denominators for F-ratios are presented in Table 1 and were determined according to Underwood (1997). Tukey post-hoc comparisons were conducted on significant fixed effects.  A variance component analysis was done to evaluate the relative importance of random sources of variation in explaining the variability in *C. volutator* density (Table 2).

*2.3 Vertical distribution in the mud*

We evaluated depth selection of *C. volutator* in the mud during the winter and potential shifts in vertical distribution at different temporal scales by measuring densities within different layers of mud. This was done during winter 2009-2010 at Pecks Cove, in a 20 x 20 m zone located 250 m from shore. Pecks Cove was chosen because it had high *C. volutator* densities. To assess the long-term seasonal changes in the vertical distribution of *C. volutator*, three discrete sampling rounds were conducted: in 1) mid-December, before ice appeared, 2) late February, when ice was present and 3) mid-March, after ice melted. Within each round, sampling was conducted on three different days to assess shorter-term responses to variation in temperature. On each sampling day, samples were collected at five discrete times following exposure of the study site as the tide receded: 0, 30, 60, 120 and 240 min after initial exposure. This was done to evaluate if there was any change in the vertical distribution as a response to short-term variation in temperature due to the cycle of inundation.

Within each exposure time, five replicate samples were taken using vertical corers (Coulthard and Hamilton, 2011), consisting of a section of PVC pipe, 7.5 cm in diameter and ~5.5 cm in length. Each core had four partially sectioned slits at 0.5, 1.5, 3.0, and 5.0 cm. Aluminum fins were fashioned to fit into the slits along the PVC cores; the metal fins were used to section the mud and ensure that no movement between layers was possible. An inverted petri dish cover was placed over the top to ensure no *C. volutator* escaped. Layered mud cores containing *C. volutator* were sieved within 6 hours of collection. Every layer was sieved separately using a 250-µm mesh (Crewe et al., 2001). Remaining *C. volutator* were collected in plastic vials, preserved in 95% ethanol and later sorted as described above.

A split-plot design was used to analyze the proportion of *C. volutator* present in each layer with Round (mid-December, late February and mid-March), Time after initial exposure (0, 30, 60, 120 and 240 min), and Layer (top 0-0.5, 0.5-1.5, 1.5-3 and bottom 3-5 cm) considered fixed factors, and Day (3 replicate days nested within Round) as a random factor. Denominators for the F-ratios (Table 2) were determined according to Underwood (1997). Tukey's post-hoc comparison was performed on significant fixed effects.

*2.4 Temperature Stress Test*

To measure the effect of cold stress on survival of *C. volutator*, 125 cores (5.0 cm diameter, 7.0 cm length) were collected on 7 March 2011 at 250 m from shore at Pecks Cove. Cores were transported to the laboratory. Five cores were immediately processed to evaluate survival after collection. The other cores were placed in six Styrofoam coolers (to limit temperature variation in a freezer), which were then placed in six different freezers. To mitigate the possibility of cores desiccating during the experiment, each core had 5.0 cm of ocean water covering the mud (which froze into an ice layer in the freezers); this also served to simulate conditions that would be encountered in the field. Three freezers had been set to -3°C (maximum coefficient of variation was 21.5%), and three freezers had been set to -8°C (maximum coefficient of variation was 17.9%). Three cores were removed from each freezer 1, 2, 4, 7, 10, and 14 days later. Cores were acclimated in a +2°C refrigerator for 12 h, and to room temperature for 4 h prior to sieving. Each core was sieved through a 250-µm sieve (Crewe et al., 2001). The contents and *C. volutator* were placed in a petri dish and live individuals were separated from dead ones under a dissection microscope and preserved separately. Individuals were considered alive if they exhibited any active movement, or response to prodding. Later, preserved samples were processed as described earlier.

An ANOVA was used to evaluate the survival of *C. volutator* through time and at different temperatures. Exposure time (1, 2, 4, 7, 10, and 14 days) and Temperature (-3 and -8 °C) were used as fixed factors, and Freezer (3 replicates nested in Temperature) was considered a random variable. F-ratios were calculated according to Underwood (1997). Tukey post-hoc comparisons were used to assess specific differences between treatments for significant fixed effects.

## 3. Results

### *3.1 Population trends over winter*

Analysis of *C. volutator* density over the two winters detected a significant effect of the interaction between the fixed factors Year, Round and Site (Table 1). We thus conducted post-hoc comparisons on this interaction to evaluate the effect of round within each Site x Year combination. In one case, Grande Anse in 2009-2010, there was no change in density throughout the winter (Fig. 2). The following year at Grande Anse, there was a gradual decrease in density and the first two rounds were significantly different from the last two rounds. In Pecks Cove, there was a gradual decrease in density for both years. When focusing on the effect of year within each Site x Round combination, Pecks Cove densities were higher during the first winter than the second winter for every round except the third. At Grande Anse, densities were significantly higher for the first two rounds of the second year and similar for the following 3 rounds. Finally, when evaluating the effect of site within each Year x Round combination, *C. volutator* density was a significantly higher for every round of each year at Pecks Cove than at Grande Anse.

Based on the variance component analysis, the majority, 60%, of the random variation in *C. volutator* density was attributed to differences between cores, 33% to differences between zones, and 5% to the Year x Round x Transect(Site) interaction (Table 1). Transect and the other random interactions accounted for 1.5% or less of the random variation.

### *3.2 Vertical distribution in the mud*

Analysis of vertical distribution of *C. volutator* during the winter of 2009-2010 detected a significant interaction between the two fixed factors of Round and Layer (Table 2). There was a significant difference in the proportion of individuals found in the first two layers between mid-December and late February, and between late February and mid-March. In late February, we found a higher proportion of individuals in the top layer (0-0.5 cm deep) and a smaller proportion in the second layer (0.5-1.5 cm deep) when compared to the other two rounds (Fig. 3). There were also a significantly higher proportion of individuals in the third layer (1.5-3 cm deep) during the first round compared to the second round. There was no significant round effect for the deepest layer (3-5 cm). Overall, between 40 and 60 % of *C. volutator* are found within the first 0.5 cm of the mud, about 20-40 % of the population is found between 0.5 – 1.5 cm of mud, and only about 20% of the population is found between 1.5 – 5.0 cm.

*3.3 Temperature stress test*

Analysis of survival of *C. volutator* exposed to different temperatures for different lengths of time indicated that there was a significant interaction between Exposure and Temperature (Table 3). Post-hoc comparisons to evaluate the effect of exposure time within each temperature treatment indicated that the decline in the proportion of surviving *C. volutator* over time was not significant at -3°C (Fig. 4). Note that variation amongst freezers (the appropriate unit of replication here) was high, lowering our power to detect small and intermediate differences in survival. In the -8°C treatment, survival was high for the first two days, significantly declined between days 2 and 7, and remained consistently low afterwards. Post hoc comparisons to assess differences in survival between the two temperatures indicated that survival was similar for the first two days of the experiment, but then was higher at -3°C than at -8°C from day 4 to day 14.

## 4. Discussion

There has been little investigation into the population dynamics of *C. volutator* during the winter (Partridge, 2001). What we know about trends has mostly been determined from comparisons between the end of one sampling season (early spring to late fall) and the beginning of the next (Peer et al., 1986; Barbeau et al., 2009; Drolet, 2009). It was suggested that winter dynamics may be an important predictor of summer densities, and thus influence the key ecological roles of *C. volutator*. Here, we provided the first detailed study of trends in density over the winter months, and explored behavioural and physiological adaptations of *C. volutator* that allows some individuals to survive through very harsh conditions.

Our sampling, following densities over two consecutive winters at two representative mudflats, showed that densities tend to decline linearly. This suggests that a constant number of individuals died between each sampling round, rather than a constant proportion. Mortality over the course of the winter may be predominantly temperature-driven, although sharp declines in density did not appear following extreme cold events. Surprisingly, at Grande Anse in 2009-2010, there was no detectable decline in density over the entire winter, maybe as a result of low densities (many samples had no *C. volutator*) making detection of a potential decline difficult. Pecks Cove did show a clear decline of *C. volutator* density through the winter, and this decline appears to be similar between the two years. Based on the variance components analysis, the spatial distribution of *C. volutator* during the winter varied most at the scale of cores and zones, and little at the scale of transects. This is similar to the distribution observed at other times of the year (Barbeau et al., 2009; Drolet and Barbeau, 2011).

A common behavioural adaptation to cold in infaunal species is to burrow deeper to avoid extreme surface temperatures (Zwarts and Wanink, 1993). We investigated this hypothesis for *C. volutator* and did not see this trend. In fact, over 80% of *C. volutator* were found in the top 1.5 cm of mud, and individuals were found even closer to the surface during the coldest times. Although *C. volutator* were distributed closer to the surface during the coldest part of the winter, this may have been the result of the erosion of top layers of sediment when the surface ice melted two weeks prior, and not a result of a biological response. Regardless of the process, we now know *C. volutator* is exposed to freezing temperatures through the winter.

Since mud does freeze in the winter, and *C. volutator* is found within the column of mud that is subjected to freezing, we wanted to investigate freezing tolerance in *C. volutator*. In our laboratory experiment, there was no significant decline in survival over time at -3°C, although a clear trend was present. This is likely due to the important freezer effect detected, reducing the power of the multiple comparisons. This freezer effect suggests that factors other than temperature might be important in determining survival of *C. volutator*. For example, the effects of initial rate of freezing, temperature cycling, and variation in humidity should be investigated further. With short-term exposure to -8°C, *C. volutator* survival was high but declined with longer exposure periods. It seems likely that *C. volutator* has developed a physiological adaptation that makes them tolerant to freezing, since they apparently do not exhibit avoidance behavior, like migrating or burrowing deeper.

To date, this is the most detailed study of winter ecology of *C. volutator*. We found that densities in the field decline linearly and at a much slower rate than previously thought (the general view had previously been that amphipods probably rapidly died early in the winter following the first freezing events). Behaviour of *C. volutator* is not consistent with the observations made by Zwarts and Wanink (1993) on other burrowing invertebrates; it does not burrow deeper during the winter and is found at highest densities where conditions were the most severe and variable. Although many *C. volutator* died in the freezer over a long exposure period, we have demonstrated that *C. volutator* is tolerant to short-term freezing, and subsequent thawing, which are conditions that it would encounter on the mudflats during the winter. This study has provided researchers with quantitative observations of an ecologically important organism, through a period of high environmental stress.

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Table 1. Results of the mixed-model ANOVA used to measure the change in *Corophium volutator* density (ind. $m^{-2}$) at two sites (Pecks Cove and Grande Anse) in the upper Bay of Fundy, at 3-wk intervals (rounds) over two winters (2009-2010 and 2010-2011).

| Source of Variation | Effect Type | Denominator of F Ratio | df | MS | F | p | Variance Component | Percent of Variation |
|---|---|---|---|---|---|---|---|---|
| Year $Y_i$ | Fixed | $Y_iT_l(S_k)$ | 1 | 2.34E+07 | 3.16 | 0.150 | - | - |
| Round $R_j$ | Fixed | $R_jT_l(S_k)$ | 4 | 8.35E+07 | 16.91 | >0.001 | - | - |
| Site $S_k$ | Fixed | $T_l(S_k)$ | 1 | 2.70E+09 | 318.04 | >0.001 | - | - |
| Year*Round $Y_iR_j$ | Fixed | $Y_iR_jT_l(S_k)$ | 4 | 6.47E+06 | 1.75 | 0.188 | - | - |
| Year*Site $Y_iS_k$ | Fixed | $Y_iT_l(S_k)$ | 1 | 3.17E+08 | 42.87 | 0.003 | - | - |
| Round*Site $R_jS_k$ | Fixed | $R_jT_l(S_k)$ | 4 | 2.47E+07 | 5.00 | 0.008 | - | - |
| Year*Round*Site $Y_iR_jS_k$ | Fixed | $Y_iR_jT_l(S_k)$ | 4 | 1.22E+07 | 3.31 | 0.037 | - | - |
| Transect(Site) $T_l(S_k)$ | Random | $Z_m(Y_iR_jS_kT_l)$ | 4 | 8.47E+06 | 1.20 | 0.313 | 1.78E+04 | 0.48 |
| Year*Transect(Site) $Y_iT_l(S_k)$ | Random | $Z_m(Y_iR_jS_kT_l)$ | 4 | 7.41E+06 | 1.05 | 0.384 | 8.23E+03 | 0.22 |
| Round*Transect(Site) $R_jT_l(S_k)$ | Random | $Z_m(Y_iR_jS_kT_l)$ | 16 | 4.94E+06 | 0.70 | 0.796 | 5.50E+04 | 1.48 |
| Year*Round*Transect(Site) $Y_iR_jT_l(S_k)$ | Random | $Z_m(Y_iR_jS_kT_l)$ | 16 | 3.69E+06 | 0.52 | 0.935 | 1.74E+05 | 4.67 |
| Zone(Year*Round*Site*) $Z_m(Y_iR_jS_kT_l)$ | Random | $e_{n(ijklm)}$ | 240 | 7.08E+06 | 3.18 | >0.001 | 1.25E+06 | 33.41 |
| Residual $e_{n(ijklm)}$ | Random | | 900 | 2.23E+06 | | | 2.23E+06 | 59.75 |

Table 2. Results of a split-plot ANOVA analyzing the vertical distribution of *Corophium volutator* in a column of mud (proportion of individuals in a layer), at different exposure times, days per round and rounds (before, during and after presence of ice) in winter 2009-2010, at the Pecks Cove mudflat in the upper Bay of Fundy.

| Source of Variation | Effect Type | df | MS | Denominator | F | P |
|---|---|---|---|---|---|---|
| Between subject | | | | | | |
| Round $R_i$ | Fixed | 2 | 0.07 | $D_i(R_j)$ | 2.06 | 0.21 |
| Day(Round) $D_j(R_i)$ | Random | 6 | 0.03 | $C_m(D_j(R_i)T_k)$ | 4.43 | <0.001 |
| Time $T_k$ | Fixed | 4 | 0.08 | $D_j(R_i)T_k$ | 1.00 | 0.43 |
| Round*Time $R_iT_k$ | Fixed | 8 | 0.05 | $D_j(R_i)T_k$ | 0.62 | 0.75 |
| Day(Round)*Time $D_j(R_i)T_k$ | Random | 24 | 0.08 | $C_m(D_j(R_i)T_k)$ | 10.14 | <0.001 |
| Core(Day(Round)*Time) $C_m(D_j(R_i)T_k)$ | Random | 180 | 0.01 | Residual | - | - |
| Within Subject | | | | | | |
| Layer $L_l$ | Fixed | 3 | 8.28 | $D_j(R_i)L_l$ | 130.48 | <0.001 |
| Round*Layer $R_iL_l$ | Fixed | 6 | 0.81 | $D_j(R_i)L_l$ | 12.73 | <0.001 |
| Day(Round)*Layer $D_j(R_i)L_l$ | Random | 18 | 0.06 | $L_lC_m(D_j(R_i)T_k)$ | 2.58 | <0.001 |
| Time*Layer $T_kL_l$ | Fixed | 12 | 0.05 | $D_j(R_i)T_kL_l$ | 1.32 | 0.23 |
| Round*Time*Layer $R_iT_kL_l$ | Fixed | 24 | 0.01 | $D_j(R_i)T_kL_l$ | 0.33 | 1.00 |
| Day(Round) *Time*Layer $D_j(R_i)T_kL_l$ | Random | 72 | 0.04 | $L_lC_m(D_j(R_i)T_k)$ | 1.69 | 0.001 |
| Layer*Core(Day(Round)*Time) $L_lC_m(D_j(R_i)T_k)$ | Random | 540 | 0.02 | Residual | - | - |
| Residual $e_{n(ijklm)}$ | Random | 0 | - | | | |

Table 3. Results of an ANOVA on the proportion of *Corophium volutator* surviving at two different cold temperatures (-3 and -8°C) and different exposure times in the laboratory.

| Source of Variation | Effect type | df | MS | Denominator of F ratio | F | P |
|---|---|---|---|---|---|---|
| Exposure $E_i$ | Fixed | 5 | 1.62 | $E_iF_k(T_j)$ | 14.88 | < 0.001 |
| Temperature $T_j$ | Fixed | 1 | 1.36 | $F_k(T_j)$ | 1.02 | 0.37 |
| Freezer(Temperature) $F_k(T_j)$ | Random | 4 | 1.33 | Residual | 113.86 | < 0.001 |
| Exposure*Temperature $E_iT_j$ | Fixed | 5 | 0.29 | $E_iF_k(T_j)$ | 2.70 | 0.05 |
| Exposure*Freezer(Temperature) $E_iF_k(T_j)$ | Random | 20 | 0.11 | Residual | 9.33 | < 0.001 |
| Residual $e_{n(ijk)}$ | Random | 71 | 0.01 | | | |

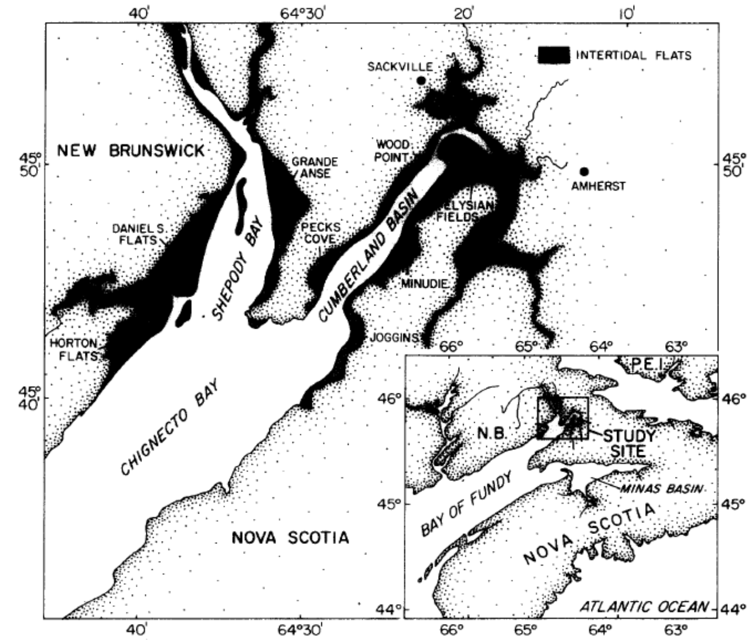


Figure 1. Map of Chignecto Bay in the Bay of Fundy, Canada, showing the location of our two mudflats (Pecks Cove and Grande Anse); taken from Peer et al. (1986).

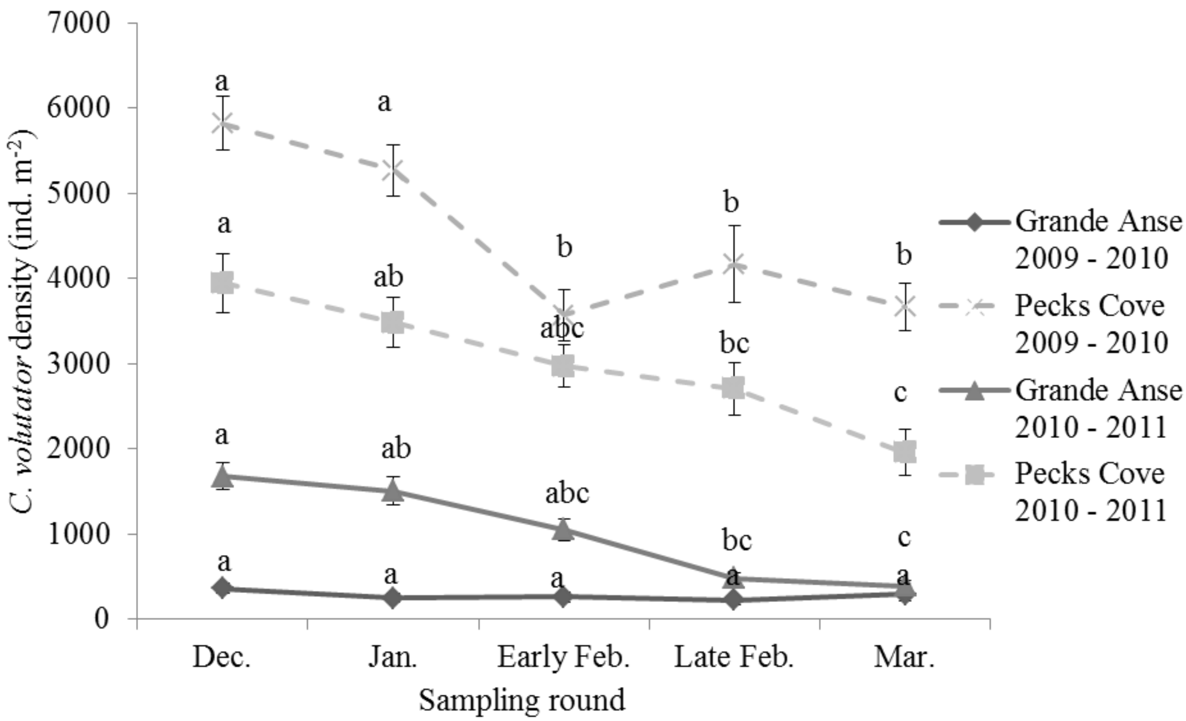


Figure 2. Mean (± SE, n = 56 – 60 cores) density of *Corophium volutator* at Grande Anse and Pecks Cove in the upper Bay of Fundy, through the winters of 2009-2010 and 2010-2011. Results of a Tukey post hoc analysis of the effect of time within each Site x Year combination are presented; sampling rounds not sharing a common letter are significantly different.

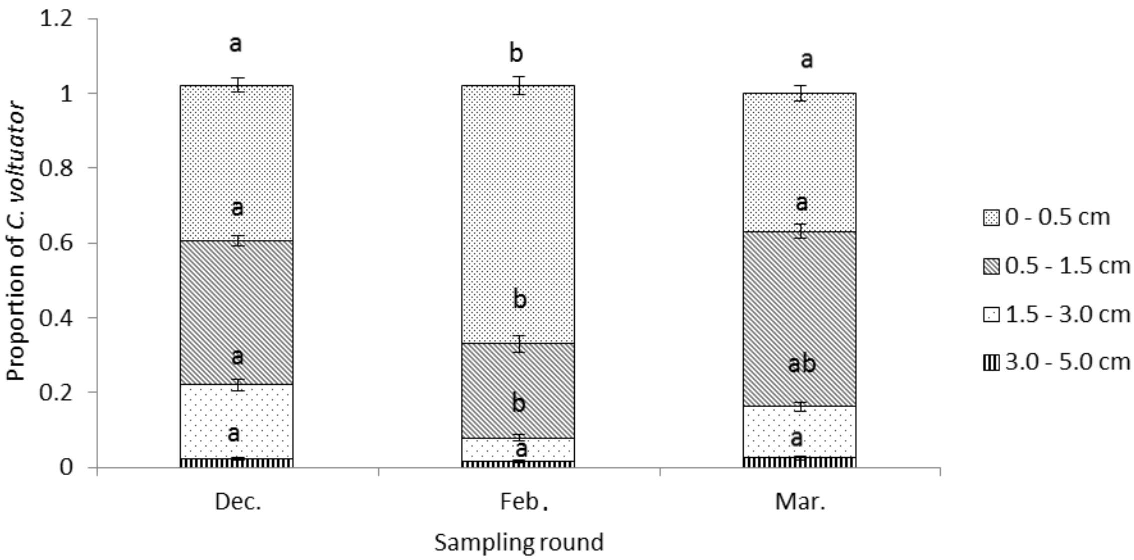


Figure 3. Mean proportion of *Corophium volutator* present per layer of mud (± SE, n = 64 - 74 samples). Samples were collected at three discrete sampling rounds in the winter of 2009-2010, from Pecks Cove, New Brunswick. Results of a Tukey post hoc analysis comparing proportion in a layer between sampling rounds are presented; rounds not sharing a common letter for a particular layer are significantly different.

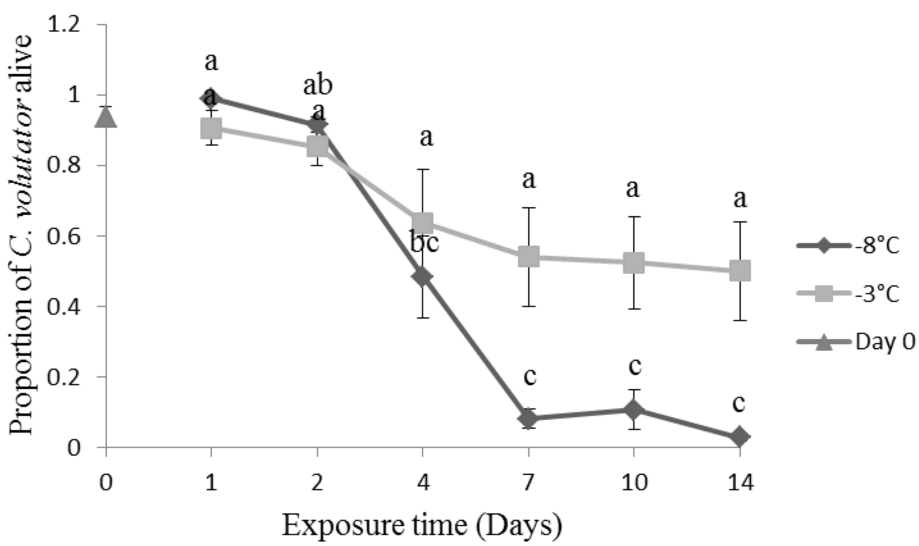


Figure 4. Mean (± SE) proportion of *Corophium volutator* surviving at two temperatures (-3 and -8°C), over a 2-wk period in a laboratory experiment. n = 5 samples for Day 0 and 8 - 9 samples for the other days. Results of a Tukey post hoc analysis comparing survival in each temperature treatment are presented; exposure times not sharing a common letter are significantly different.