Can Salinity-Induced Mortality Explain Larval Vertical Distribution With Respect to a Halocline?

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Abstract.   For the larvae of two echinoderm species that coexist in Atlantic Canada (bipinnaria of the sea star *Asterias rubens* and 4- and 6-arm echinoplutei of the sea urchin *Strongylocentrotus droebachiensis*), we examined the effect of short- and long-term exposure to salinity (ranging from 18 to 35) on the probability of larval survival in laboratory experiments. We also related larval vertical distributions in response to sharp haloclines generated in the laboratory to survival probability in the salinity of different layers in the water column. For both species and developmental stages, survival probability decreased with decreasing salinity, and a salinity range of 24–27 emerged as the critical threshold for larval tolerance. The relationship between the proportion of larvae that crossed a halocline into the top water layer and the survival probability of larvae in the salinity of that layer was significant for both species. Interestingly, the shape of this response was species-specific but not stage-specific for *S. droebachiensis*. Our findings suggest that larval avoidance of low-salinity water layers may be an adaptive behavior that increases survival and indirectly influences larval distribution.

Introduction

The distribution of planktonic organisms has often been attributed to physical forcing and advection. Recent studies, however, suggest that the behavioral interaction between zooplankton and their environment may also be important in regulating their distribution (Metaxas and Young, 1998a, b; Burdett-Coutts and Metaxas, 2004; Gallager *et al.*, 2004; Woodson *et al.*, 2005). Overlying water masses typically vary in strength and direction of currents and in physical and chemical composition, and they are often delineated by salinity gradients. Thus, a behavioral response of organisms to changes in salinity may influence their entrainment into a particular water mass, consequently affecting their dispersal and survival.

Salinity influences osmolality, proportions of solutes, and absorption and saturation of dissolved gases (Kinne, 1966), which, if altered, can cause significant physiological stress on an organism. For many invertebrate larvae, salinity has been shown to strongly influence survival, development, growth, and activity (Hrs-Brenko and Calabrese, 1969; Richmond and Woodin, 1996; Metaxas, 1998; Saranchova and Flyachinskaya, 2001; Qiu *et al.*, 2002), and salinity limits have been identified as being critical to larval survival. For example, larvae do not develop to metamorphosis at salinities of 25 or less for the sea urchin *Strongylocentrotus pallidus* (Roller and Stickle, 1994) and the sea cucumber *Holothuria spinifera* (Asha and Muthiah, 2005), and at 20 or less for the crab *Clibanarius vittatus* (Young and Hazlett, 1978). With few exceptions, most meroplankton osmoconform to their surrounding media (Kinne, 1971). Thus, larvae likely have limited capacity to cope with the physiological stresses associated with salinity changes in their environment, and mechanisms such as avoidance of entrainment in low-salinity waters may increase survival.

Numerous studies have shown that salinity gradients can have a pronounced effect on larval vertical distributions for many species (*e.g.,* Harder, 1968; Tremblay and Sinclair, 1990; Mann *et al.*, 1991; Raby *et al.*, 1994; Metaxas and Young, 1998a, b; Yaroslavtseva *et al.*, 2002), and these responses are often attributed to larval avoidance of low salinity. However, none of these studies have shown behavior to be explicitly related to salinity-induced physiological stress experienced by larvae. In fact, results suggest that whether salinity-induced stress causes behavioral changes in larvae is unclear. For example, aggregation of larvae of the sea urchin *Echinometra lucunter* has been observed at a salinity gradient of 24/33 but not at 27/33 (Metaxas and

Young, 1998b), and short-term (24-h) exposure to salinities of 24 or less significantly reduced survival relative to salinities of 27 and 33 (Metaxas, 1998). In contrast, Yaroslavtseva *et al.* (2002) did not attribute larval aggregation of the sea urchin *Strongylocentrotus intermedius* at a halocline to larval avoidance of deleterious salinities, because aggregation was observed in harmful salinities.

Echinoderms are stenohaline (Stickle and Diehl, 1987), and larvae are likely more sensitive than adults to salinity changes (Kinne, 1971). Avoidance of deleterious salinities would therefore be highly advantageous to their survival. In a previous study, we measured larval vertical distribution for two species of echinoderms, the sea star *Asterias rubens* Linnaeus 1758 and the sea urchin *Strongylocentrotus droebachiensis* Müller 1776, in the presence of haloclines generated in the laboratory (Sameoto, 2007). In this study, we examine whether behavioral changes in meroplankton in response to haloclines are associated with salinity-induced mortality. More specifically, we examine the effect of salinity on the short- and long-term survival of these species and relate observed larval distributions to the probability of larval survival given the salinity structure of the water column. Many studies have observed larval aggregations at salinity gradients, but few have directly addressed the mechanisms for this response. We know of no other study that has explicitly related larval behavior and population-level dispersal response to salinity-induced stress, measured as larval mortality and survival.

Materials and Methods

Fertilization and larval culture

Adults of the sea star *Asterias rubens* and of the sea urchin *Strongylocentrotus droebachiensis* were collected from the subtidal zone near Halifax, Nova Scotia, Canada, in July 2005 and March 2006. Spawning was induced in the laboratory by injecting about 3 ml of 100 μmol l^{-1} 1-methyladenine into the coelomic cavity of *A. rubens* and about 2 ml of 0.55 mol l^{-1} KCl through the peristomial membrane of *S. droebachiensis*. Females were spawned into beakers containing 150 ml of 0.45-μm-filtered seawater, whereas males were dry-spawned to prolong sperm longevity. Fertilization was achieved by mixing sperm and eggs. For *A. rubens*, gametes were mixed from 6 combinations of 5 females and 2 males to generate 6 parental pairs. For *S. droebachiensis*, gametes were mixed from 10 combinations of 6 females and 7 males to generate 10 parental pairs for experiments using 4-arm larvae, while 13 combinations of 7 females and 6 males were used to generate 13 pairs for experiments with 6-arm larvae. (The 4- and 6-arm larvae were not siblings). Fertilization success was 90.6%–97.7% ($n = 67$ to 128) for *A. rubens*, and 91.6%–99.2% ($n = 88$ to 131) and 94.5%–100% ($n = 97$ to 121) for *S. droebachiensis* larvae reared to 4- and 6-arm stage respectively, as indicated by an elevated perivitelline membrane.

Larvae of both species and stages were reared on a high ration (5000 cells ml^{-1}) mixed microalgal diet (*Isochrysis galbana* and *Tetraselmis* sp.) in 4-l glass culture jars containing 0.45-μm-filtered seawater with practical salinity of 34. Cultures were stirred continuously with motorized paddles. All cultures were kept in a temperature-controlled room under a cycle of 12-h light to 12-h dark, and at temperatures of 12 $\pm$ 0.5 °C and 10 $\pm$ 0.5° C for *A. rubens* and *S. droebachiensis*, respectively. The water in the culture jars was changed and microalgae added every other day. For each species, larvae from each parental pair were reared in separate culture jars. However, prior to experimentation, all jars were combined into a single culture from which larvae were used for the experiments.

Effect of salinity on larval survival

We established five salinity treatments for *A. rubens* (18, 21, 24, 27, 35), and seven salinity treatments for *S. droebachiensis* (15, 18, 21, 24, 27, 30, 35), in 200-ml glass beakers containing 150 ml of 0.45-μm-filtered seawater mixed with dechlorinated fresh water. We introduced 100–200 bipinnaria (14 days old) of *A. rubens*, and 50–100 4-arm (8 days old) and 6-arm (22 days old) plutei of *S. droebachiensis* into each of four replicates of each treatment, using a glass Pasteur pipette. For *A. rubens*, the number of live and dead larvae was initially determined after 24 h, and every 2 d thereafter until there were no surviving larvae in salinities lower than 35. For both stages of *S. droebachiensis*, the number of live and dead larvae was determined after 12 h, 24 h, 48 h, and every 2 d thereafter until, for the 4-arm stage, there were no surviving larvae, and for the 6-arm stage, the number of surviving larvae was 3% or less in salinities lower than 35.

Dead larvae sank to the bottom of the beakers and were considered dead if they did not respond to gentle prodding with a glass pipette. When the dead larvae were removed, the water was changed and microalgae (3000 cells ml^{-1} *Isochrysis galbana* and 2000 cells ml^{-1} *Tetraselmis* sp.) were added to each beaker. The experiment was conducted under a cycle of 12-h light to 12-h dark in a temperature-controlled room at 12 $\pm$ 0.5 °C and 10 $\pm$ 0.5 °C, for *A. rubens* and *S. droebachiensis*, respectively.

The effect of salinity on short-term mortality (24 h for *A. rubens* and 12 h for *S. droebachiensis*) was examined using one-way analysis of variance (ANOVA) and *a posteriori* multiple comparisons among means with Student-Newman-Keuls (SNK) tests. ANOVA was done with SPSS 13.0. Data for both stages of *S. droebachiensis* failed to meet the requirement of homogeneity of variances (Levene's test, $P < 0.05$), and percentages were arcsine-transformed to reduce heterogeneity. ANOVA results were considered significant at $\alpha = 0.05$, and adjusted alpha values of $\alpha_{critical} = (0.05/[\text{no. of comparisons}])$ were used to avoid excessive type I error for multiple comparisons.

Long-term effects of salinity were examined using survival analysis. Life tables were constructed from the survival data for each salinity treatment. Since the exact time of death of individual larvae was not known, the survival data were grouped into intervals based on the days when the status of the larvae (dead or alive) was monitored. For example, intervals (in days) for *A. rubens* were (0, 1], (1, 3], (3, 5], *etc.*, where, for example, (3, 5] indicates inclusion of all data associated with the time after 3 days, up to and including 5 days. Larvae that were alive at the termination of the experiment were considered "right censored" for the purposes of statistical analysis—that is, although their exact time of death was unknown, it was greater than the duration of the experiment. Survival functions were compared across salinities with the Wilcoxon test because it weighs early events more than later events (Allison, 1995). It is assumed that larvae would quickly exit a water layer of physiologically stressful salinity and, consequently, early effects of salinities would be more important to larval survival than later effects. Survival analysis was done with SAS 9.1. Adjusted alpha values of $\alpha_{critical} = (0.05/[no. of comparisons])$ were used to avoid excessive type I error for multiple comparisons.

Larval response to haloclines

The vertical distribution of larvae of *A. rubens* and *S. droebachiensis* in response to sharp haloclines was examined in the laboratory using experimentally generated haloclines in rectangular acrylic plastic columns (30 cm height, 10 cm width, 9.5 cm depth). To establish a halocline (~2–3 cm), the column was first filled up to 6 cm with the lower salinity water, then the higher salinity seawater was gravity-fed below the lower salinity layer through a glass tube until the surface of the top water layer reached 16 cm above the bottom. The glass tube was then carefully removed to avoid disrupting the halocline, and water samples were taken at 0.5-cm increments using a Pasteur pipette. Samples were measured using a temperature-compensated handheld refractometer (Fisher Scientific) to establish the salinity structure of the water column.

Four treatments (three haloclines and a control) were used for *A. rubens* and five treatments (four haloclines and a control) for 4- and 6-arm *S. droebachiensis*. The salinity of the bottom layer in all treatments was 35 (ambient). For *A. rubens*, the salinities of the top layer were 21, 24, and 27 (henceforth referred to as 21/35, 24/35, and 27/35); for both stages of *S. droebachiensis*, they were 21, 24, 27, and 30 (henceforth referred to as 21/35, 24/35, 27/35, and 30/35). Control columns filled with seawater of salinity 35 were used to determine larval distribution in the absence of a halocline.

A glass Pasteur pipette was used to introduce about 100–200 larvae of *A. rubens* and 50–100 larvae of *S. droebachiensis* into each column, 3 cm above the bottom. Larvae were allowed to swim for 40 min, then the number of larvae in each 0.5-cm increment was visually recorded,

generating profiles of larval vertical distribution. Columns were covered with a 4-cm-thick polystyrene foam cap to prevent direct light penetration from above, and four replicate columns were run for each treatment.

Survival probability in relation to salinity structure

The relationship between salinity-induced mortality and larval vertical distribution was examined by generating vertical profiles of survival probability that corresponded to the salinity in the profiles from the experimental columns. Short-term survival probabilities (24 h for *A. rubens* and 12 h for *S. droebachiensis*) were calculated directly from life tables for each species and stage at each salinity treatment. For all salinities between 21 and 35 recorded in the haloclines but not tested in the larval survival experiments, survival probabilities were calculated by interpolation. All probabilities were standardized to survival probability at ambient (35) salinity. For each halocline treatment, the mean proportion of larvae found in the top water layer in the halocline study was related to the short-term survival probability associated with the salinity of that top water layer by using regression analysis with the software package Datafit 8.2 (Oakdale Engineering). The proportion of larvae above the halocline was determined as the proportion of larvae above the lower limit of the low-salinity water layer. Since the control of 35 did not have a halocline, we used the average height above bottom of the lower limit of the low-salinity water layer (11 cm) and determined the proportion of larvae above this height.

Results

Short-term effects of salinity on larval survival

Mortality after 24 h for *Asterias rubens* and after 12 h for *Strongylocentrotus droebachiensis* varied significantly among salinities ($F_{4,20} = 22.7$, $P < 0.001$, $F_{6,21} = 235.5$, $P < 0.001$, $F_{6,21} = 61.5$, $P < 0.001$ for *A. rubens*, and 4- and 6-arm *S. droebachiensis*, respectively). For all species and stages, mortality increased with decreasing salinity. For *A. rubens*, mortality did not exceed about 80%, even when salinity was as low as 18. Fewer larvae died in salinities of 27 and 35 than in all other salinities, and there was no difference in mortality between salinities of 18, 21, and 24 (Fig. 1). For both stages of *S. droebachiensis*, mortality was about 50% when salinity was as high as 30, and all larvae (4- and 6-arm) died within 12 h in salinities of 15 and 18 (Fig. 1). Mortality was lowest in salinity of 35, and there was no difference between salinities 21 and 24 for the 4-arm stage, and among all salinities less than 21, for both 4- and 6-arm stages. Although absolute mortality at ambient salinity varied across species and stages, the patterns of mortality with decreasing salinity are consistent between species when standardized relative to mortality at salinity 35.
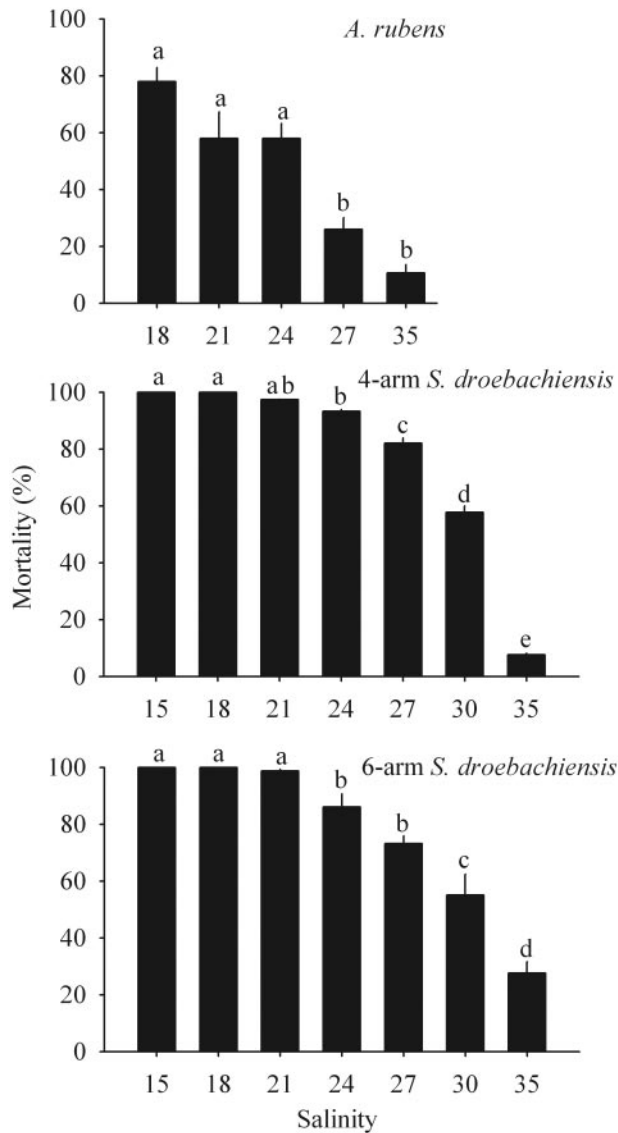
Figure 1. Short-term mortality (mean ± SE, $n = 4$) of 14-day-old *Asterias rubens* bipinnaria, 8-day-old 4-arm *Strongylocentrotus droebachiensis* plutei, and 22-day-old 6-arm *S. droebachiensis* plutei at different salinities. Statistically significantly different means in pairwise comparisons are marked with different letters ($\alpha_{crit} = 0.0062$, $\alpha_{crit} = 0.0025$, $\alpha_{crit} = 0.0026$ for *A. rubens*, 4-arm *S. droebachiensis,* and 6-arm *S. droebachiensis*, respectively).

Long-term effects of salinity on larval survival

For all species and stages, the probability of larval survival decreased with time across all salinities, and with decreasing salinity throughout the duration of the experiment (Fig. 2). For *A. rubens*, the probability of larvae surviving in salinities of 24 or less for more than 5 days was less than 5%, and for more than 11 days it was 0, compared to more than 20 days in salinities 27 or above (Fig. 2). For *A. rubens* and 4-arm *S. droebachiensis*, there was no difference in the probability of larvae surviving in salinities of

21 and 24 (*A. rubens*: $\chi^2 = 2.73$, $P = 0.0988$; [for all other comparisons $\chi^2 > 45$, $P < 0.0001$; $\alpha_{crit} = 0.005$]; 4-arm *S. droebachiensis*: $\chi^2 = 6.47$, $P = 0.0110$; $\alpha_{crit} = 0.0024$). Additionally for 4-arm *S. droebachiensis*, the probability of surviving more than 1 day in salinities 27 or lower was 10% or less, and almost all larvae were dead after 2 days in salinities 24 or lower. There was no difference in survival between salinities 15 and 18 ($\chi^2 < 0.01$, $P > 0.999$; for all other comparisons: $\chi^2 > 9$, $P < 0.0021$; $\alpha_{crit} = 0.0024$). For 6-arm *S. droebachiensis*, the probability of larvae surviving more than 1 day in salinities 27 or lower decreased from about 23% to about 11% to less than 1% for salinities 27, 24, and 21 or lower, respectively (Fig. 2). There was no difference in the probability of larvae surviving in salinities 15 and 18 ($\chi^2 < 0.01$, $P > 0.999$), 15 and 21 ($\chi^2 = 2.08$, $P = 0.150$), and 18 and 21 ($\chi^2 = 3.04$, $P = 0.081$); (for all other comparisons: $\chi^2 > 14.4$, $P < 0.0001$; $\alpha_{crit} = 0.0024$); almost all larvae died within 1 day in these salinities.

Survival probability relative to salinity structure

For all species and stages, the presence of a halocline had a pronounced effect on larval vertical distribution, and the proportion of larvae above the halocline decreased with decreasing salinity in the top layer (Figs. 3, 4, 5). Survival probability also decreased with decreasing salinity in the top water layer. For *A. rubens*, larval aggregation at the halocline occurred when the top-layer salinity was 27, and the highest salinity that larvae did not cross into was 24 (Fig. 3). Short-term survival probability for *A. rubens* decreased with decreasing salinity in the top water layer for salinities 24 and above (Fig. 3). For 4-arm stage *S. droebachiensis*, weak larval aggregation was observed at the halocline when the salinity of the top water layer was 24, but most (~70%) larvae crossed into the upper layer. However, when the salinity of the top water layer was 21, larvae did not cross into this layer (Fig. 4). For this stage, survival probability decreased with decreasing salinity and was about 7% and about 3% in salinities 24 and 21, respectively (Fig. 4). For 6-arm *S. droebachiensis*, most larvae crossed the halocline and aggregated near the surface of the water column when the salinity of the top water layer was 27. However, less than 20% of larvae crossed into salinity 24, and almost no larvae crossed into salinity of 21 (Fig. 5). Short-term survival probability progressively decreased between salinities 30, 27, and 24 (from 64% to 34% to 18%), but was similar between 24 and 21 (Fig. 5).

For all species and stages, a relationship was observed between the proportion of larvae in the top water layer during the halocline experiments and the probability of short-term survival of larvae in salinities associated with that layer. For *A. rubens*, the proportion of larvae that crossed into the top water layer increased linearly with survival probability (Fig. 6). For both stages of *S. droebachiensis*, a logarithmic relationship was observed be-
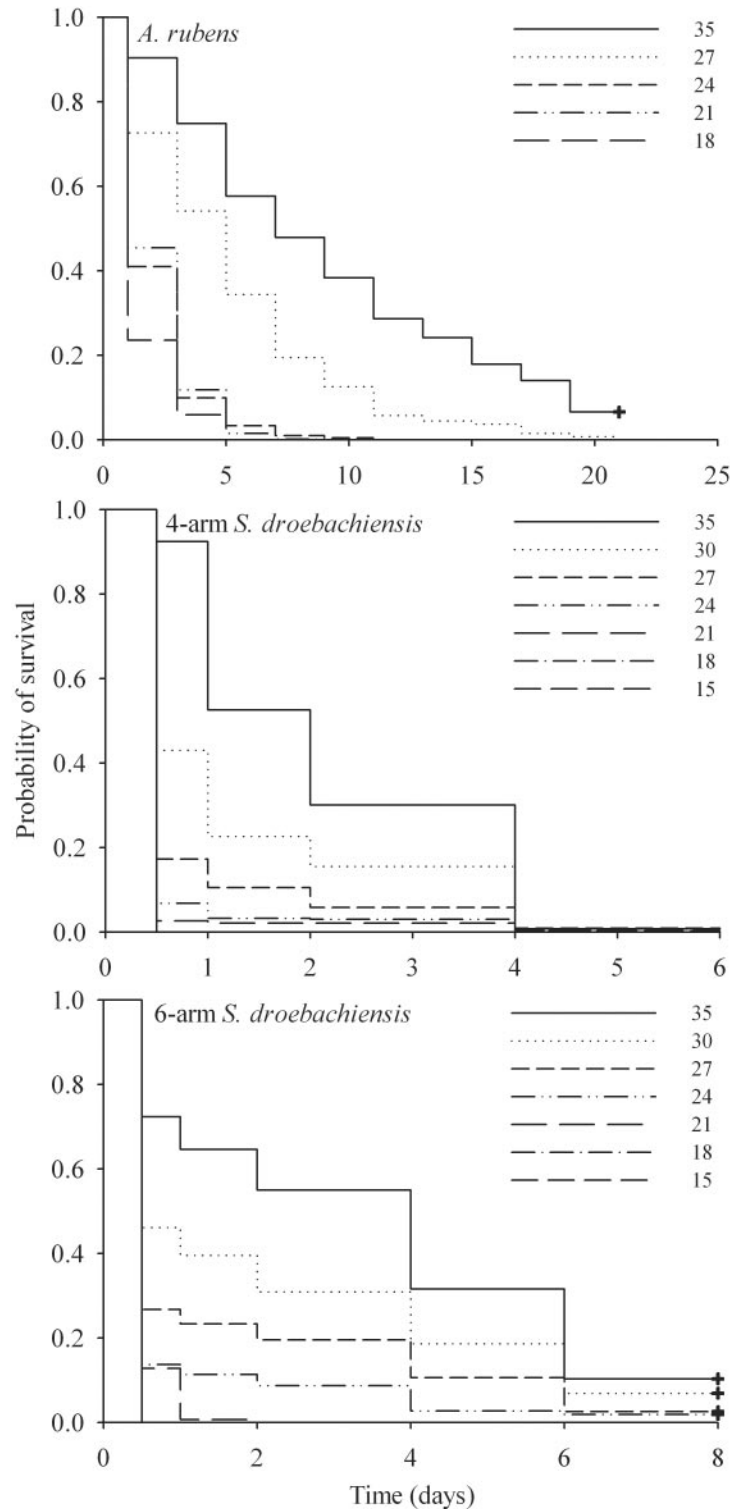
Figure 2. Survival probability over time of bipinnaria of *Asterias rubens* and echinoplutei of *Strongylocentrotus droebachiensis* exposed to different levels of salinity. Time duration varies between species and stages, and crosses indicate censored observations.

tween the proportion of larvae in the top-salinity water layer and survival probability. The proportion of larvae in the top water layer increased more rapidly than a proportional in-

crease in survival probability for salinities 21 and 24; however, at and above a salinity of 27, the relationship decelerated toward an asymptote of 1.0. Interestingly, the
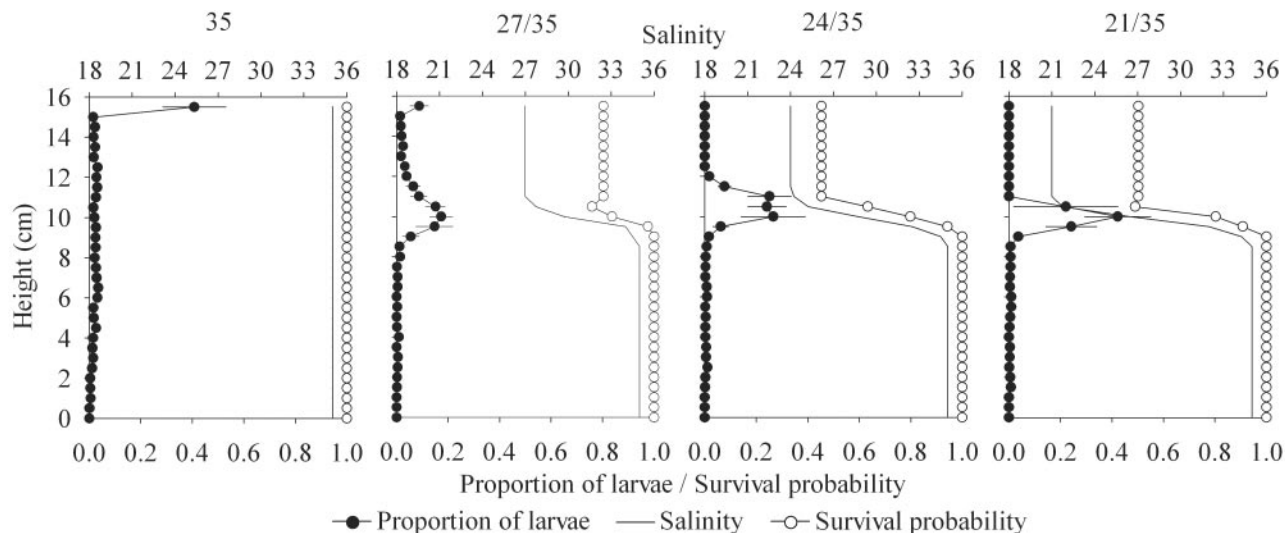
Figure 3. *Asterias rubens*. Vertical distribution (mean ± SE, *n* = 4) in experimental columns of 11-day-old bipinnaria larvae 40 min after being placed in four halocline treatments, and 24-h survival probability associated with the salinity structure of the column.

relationship between the proportion of larvae in the top water layer and survival probability differed between stages. The asymptote was reached at a lower survival probability for the 4-arm (~0.2) than the 6-arm (~0.4) developmental stage.

Discussion

For all species and developmental stages, strong short- and long-term effects of salinity on larval survival were observed. Survival was greatest at ambient salinity (35) and decreased with decreasing salinity. For *Asterias rubens*, short-term mortality did not exceed about 80%, even at salinities as low as 18, suggesting that a proportion of the population may survive short fluctuations of decreased salinities. In contrast, for both stages of *Strongylocentrotus droebachiensis,* mortality was greater than 80% at salinities of 27 and 24, indicating that high mortality could occur even during brief exposure to low salinity.

Similar survival patterns were observed for short- and long-term exposure of larvae to reduced salinities. Interestingly, for *A. rubens*, mortality after 24 h was significantly greater, and larval survival was almost half, in salinities of 24 or less than in 27 or more. This result suggests that the salinity range of 24–27 may be a critical threshold for larval tolerance in this species. In contrast, Saranchova and Flyachinskaya (2001) recorded 100% survival of bipinnaria of *A. rubens* in salinities 14–28. However, population differences in salinity tolerance have been observed for *A. rubens* (Sarantchova, 2001), and the larvae used by Saranchova and Flyachinskaya (2001) were from the White Sea with a salinity range of 22–26.

For both stages of *S. droebachiensis*, short-term exposure to reduced salinities resulted in mortality above 50% in

salinities as high as 30, but long-term exposure suggested that the threshold in salinity tolerance is also 24–27. Similarly, Metaxas (1998) found that larvae of the sea urchin *Echinometra lucunter* failed to develop past the 4-arm stage when reared in salinities of 27 or less, and Roller and Stickle (1993) reported cessation of development of 4-arm larvae of *Lytechinus variegatus* in salinity less than 27.5.

Prior acclimation of either the adults or the larvae to the reduced salinities may have resulted in lower mortality. Sarantchova (2001) showed that acclimation of adult *A. rubens* to low salinity shifted the range of survival of bipinnaria toward lower salinities, and Kashenko (2000) found that normal larval development of the sea cucumber *Apostichopus japonicus* occurred at lowered salinities when earlier developmental stages were acclimated to hyposaline conditions. In contrast, Roller and Stickle (1994) reported that acclimation of adult sea urchins of *S. droebachiensis* and *S. pallidus* does not enhance survival of embryos at reduced salinities. In this study, differences in salinity tolerance were observed between species. However, since adults of both species were collected from the same habitat, it is unlikely that these differences resulted from prior differences in acclimation history.

Salinity-induced mortality may be the selective pressure that influenced the observed larval response to haloclines. For both *A. rubens* and *S. droebachiensis*, larval aggregation at the halocline increased with increasing salinity difference between the two water layers. Fewer larvae passed into the top water layer and mortality was high at salinities that inhibited larvae from entering the top layer. A strong relationship was also observed between the proportion of larvae in the top water layer in the halocline treatments and the probability of larvae surviving in the salinity of that
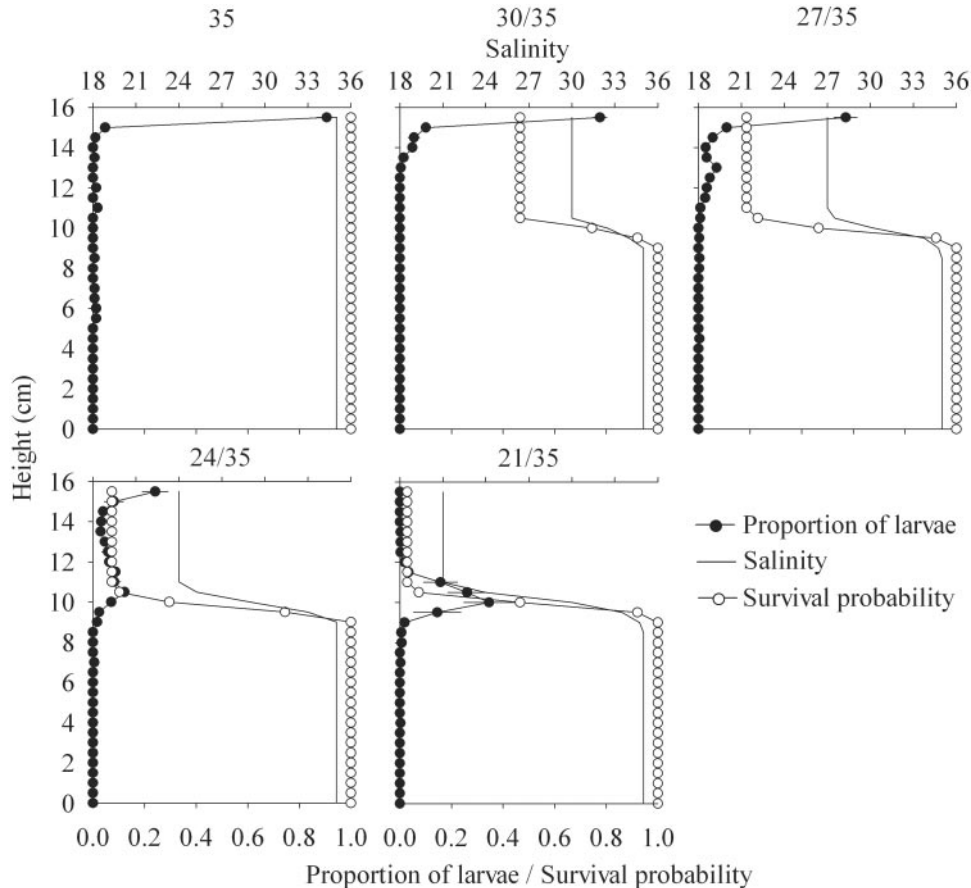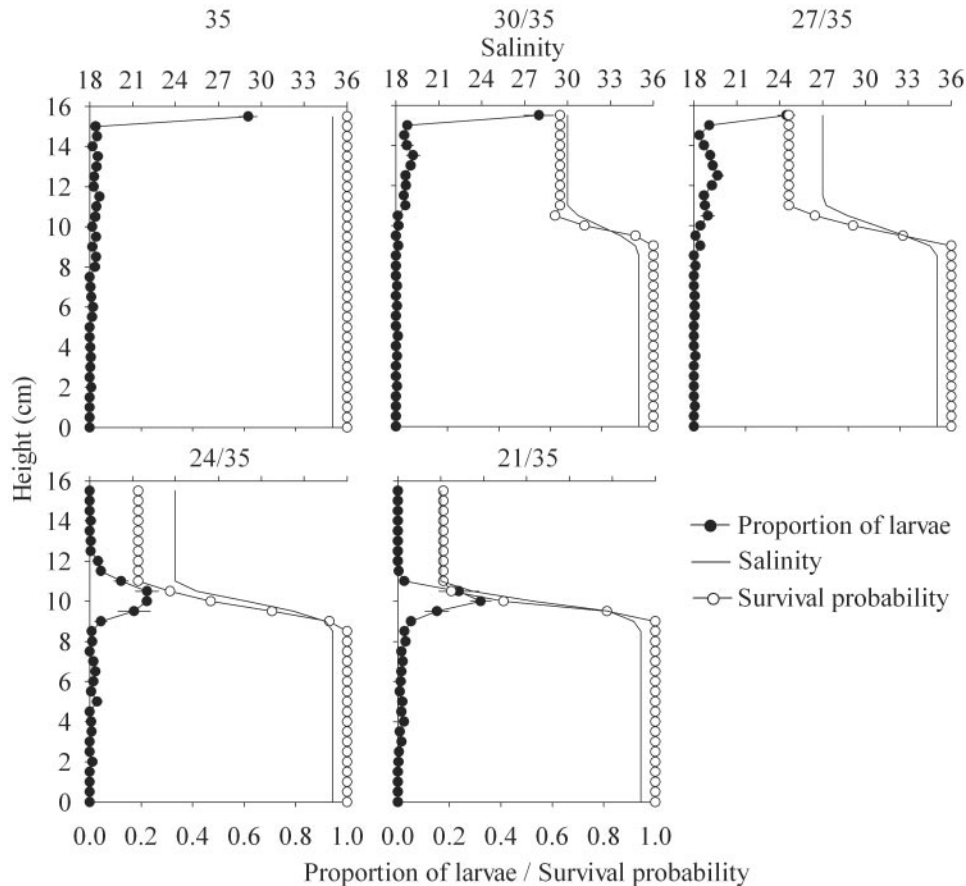
Figure 4. *Strongylocentrotus droebachiensis* (4-arm). Vertical distribution (mean $\pm$ SE, $n = 4$) in experimental columns of 5-day-old echinoplutei 40 min after being placed in five halocline treatments, and 12-h survival probability associated with the salinity structure of the column.

layer. Interestingly, the shape of this relationship varied between *A. rubens* and *S. droebachiensis*, indicating that the functional response of larvae to salinity is most likely species-specific. For *A. rubens*, the number of larvae that crossed into the top-salinity water layer increased proportionally with the survival probability in the salinity of that layer. In contrast, for *S. droebachiensis*, our results suggest the existence of a threshold survival probability below which larvae avoid entering water layers of salinity associated with that probability. It is possible that a threshold also exists for *A. rubens*, but that our experimental treatments did not adequately resolve the range of survival probabilities.

The survival probability associated with the salinity of the top water layer that resulted in a population-wide avoidance response of that layer was greater for *A. rubens* than for *S. droebachiensis*. It is possible that larvae of *A. rubens* may be able to better detect, or avoid, low salinity. Similarly, for *S. droebachiensis*, the survival probability at which no larvae crossed into the top water layer was greater for older larvae than for younger ones. This suggests that the ability to detect low salinities, or the ability of larvae to

regulate a position in the water column relative to environmental discontinuities, may be more developed in older larvae than in younger larvae. A weaker response of 4-arm, relative to 6-arm, stage larvae of *S. droebachiensis* to the same environmental stimulus has also been observed in response to food patches (Burdett-Coutts and Metaxas, 2004).

Differences in the relationship between salinity tolerance and the behavioral response to haloclines observed between stages of *S. droebachiensis* may also be attributed to differences in developmental tradeoffs. Four-arm stage larvae were more sensitive to changes in salinity, but they were less likely than 6-arm stage larvae to respond to low salinity. A position high in the water column would increase dispersal and be favorable for earlier stage larvae, whereas a position lower in the water column may be more advantageous for later stage larvae if it retains them near potential settlement sites. Consequently, for younger larvae, any salinity-induced physiological cost associated with an exposure to lower salinity higher in the water column may be offset by the benefit of dispersal. The length of exposure to low salinity may also influence the tradeoff between dis-

Figure 5. *Strongylocentrotus droebachiensis* (6-arm). Vertical distribution (mean ± SE, *n* = 4) in experimental columns of 18-day-old echinoplutei 40 min after being placed in five halocline treatments, and 12-h survival probability associated with the salinity structure of the column.

persal and physiologically induced stress. George and Walker (2007) showed that larvae of the sand dollar *Dendraster excentricus* were significantly bigger and able to reach metamorphosis when exposed to a 7-day fluctuation in salinity compared with larvae that experienced prolonged and constant exposure to low salinity. Therefore, for 4-arm larvae of *S. droebachiensis*, the benefit of dispersal may be greater than the physiological risks, such as increased mortality, slowed development, or inability to metamorphose, particularly if exposure is likely to be over short periods.

The mechanism of the sensory detection of salinity by larvae is not well known. In adult marine invertebrates, osmoreception has been linked to changes in concentration of solutes. Dufort *et al.* (2001) suggested that adult lobsters, *Homarus americanus*, detect reduced salinities through changes in chloride ion concentration, and Davenport and Wankowski (1973) reported that salinity detection in adult crabs, *Porcellana platycheles*, is likely related to the osmolarity of the solution. Most meroplankton, including larval echinoderms, are probably poor ion regulators, and consequently, in low-salinity waters, their internal ion balance would be easily disrupted by the loss of ions through the cell membrane. Thus, larvae may be sensitive to changes in either ionic concentration or osmolarity. Larvae may also sense an increase in osmotic pressure caused by an influx of water. When larvae are entrained in a reduced salinity, water can diffuse into cells and has been observed to cause cell rupture in mussel eggs (Saranchova and Flyachinskaya, 2001). Additionally, since viscous forces govern larval movement, larvae may detect changes in viscosity associated with changes in salinity; however, this is unlikely in this study, since the maximal difference in dynamic viscosity was only about 2.8% (Dorsey, 1940).

The critical range of salinity for survival (24–27) was similar between species in this study, but larvae of *S. droebachiensis* were more sensitive than those of *A. rubens* to reduced salinities. Adult populations of *A. rubens* and *S. droebachiensis* experience the same environmental conditions since they coexist, and their horizontal and bathymetric distributions overlap in Atlantic Canada. However, since spawning occurs during different seasons (July–August for *A. rubens* and February–April for *S. droebachiensis*), larvae may be exposed to different salinities while in the plankton. Interestingly, the difference between species in salinity sen-
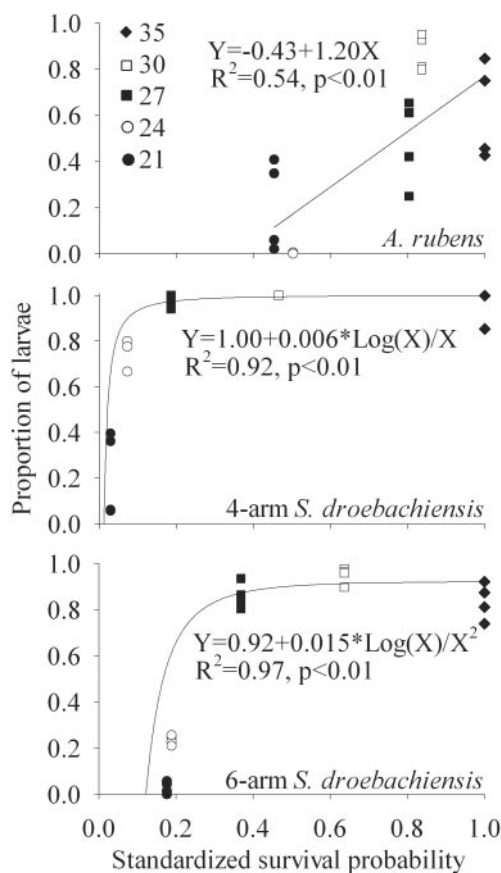
Figure 6. Relationship between the proportion of *Asterias rubens* bipinnaria, 4-arm *Strongylocentrotus droebachiensis*, and 6-arm *S. droebachiensis* that crossed the halocline into the top water layer, and the survival probability associated with that salinity ($n = 4$ for each salinity).

sitivity was not reflected in the response of larvae to haloclines. A lower salinity in the top water layer was required to cause larval aggregation at the halocline for *S. droebachiensis* than *A. rubens*. This suggests that, in addition to salinity-induced mortality, other indicators of salinity-induced physiological stress, such as retarded or arrested development, may also reflect the relationship between larval distribution and salinity gradients. It is also possible that factors unrelated to physiological stress may contribute to larval response to haloclines.

In this study, we suggest that although other factors likely contribute to generating larval aggregations at salinity gradients, the physiological stress associated with salinity, reflected in this study as salinity-induced mortality, may be an important selective pressure that operates on larval behavior. Larval vertical distribution relative to salinity gradients in laboratory experiments can last up to an hour (Metaxas and Young, 1998a; Metaxas and Sameoto, unpubl. data). However, the period that observed aggregations last in the field is unknown, but obviously will be influenced by local hydrodynamics. Nonetheless, a behavioral component to the avoidance of deleterious low-salinity environments would

be an adaptation that increases survival and indirectly influences larval distribution and dispersal in the water column.

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